DEVELOPMENT AND CHARACTERIZATION OF NANOFIBERS AS A NOVEL DRUG DELIVERY SYSTEM

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Under the Faculty of Pharmacy

Submitted By

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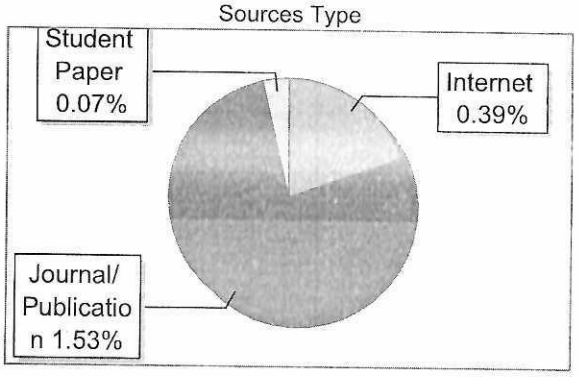
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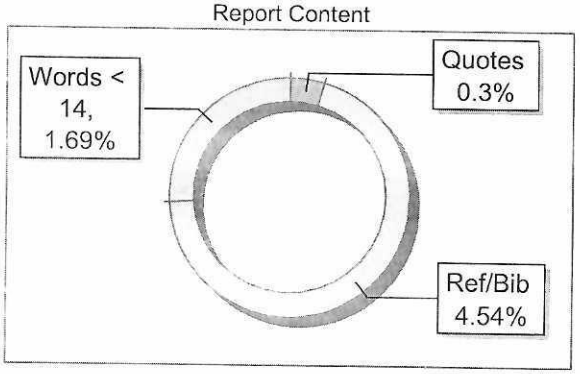
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ABBREVIATIONS

Abbreviations	Indication
G	**Gram**
Mol	**Molecularweight**
w/v	**Weight byvolume**
w/w	**Weightbyweight**
Mm	**Millimeter**
°C	**Degreecentigrade**
M.p.	**Meltingpoint**
H	**Hour/s**
Ml	**Mililitre**
pH	**NegativeLogarithmofhydrogenionconcentration**
Min	**Minute/s**
%	**Percentage**
Nm	**Nanometer**
Ml	**Mililitre**
µg	**Microgram**
Cm	**Centimeter**
Mg	**Milligram**
w/v	**Weight byvolume**
q.s	**Quantitysufficient**
Rpm	**Revolutionperminute**
mV	**Millivolt**
keV	**Kilovolt**

TEM	**Triethanolamine**
MP	**Methylparaben**
PP	**Propylparaben**
PVP	**Polyvinylpyrrolidone**
$NaBH_4$	**Sodiumborohydride**
$AgNO_3$	**Silvernitrate**
H_2SO_4	**Sulfhuric acid**
NaOH	**Sodiumhydoxide**
HCl	**Hydrochloricacid**
DMSO	**Dimethyl sulphoxide**
MIC	**Minimuminhibitoryconcentration**
FT-IR	**FourierTransform Infrared**
SEM	**Scanningelectronmicroscope**
EDS	**Energydispersivespectrometer**
XRD	**X-rayDiffraction**
Uv	**Ultra-violet**
SI.No.	**SerialNumber**
ICH	**Internationalconferenceonharmonization**
RH	**Routh-Hurwitz**
SD	**Standarddeviation**
CDR	**Cumulativepercentagedrugrelease**

Abstract:

Diabetes mellitus remains a pervasive global health challenge, requiring innovative therapeutic strategies to improve glycemic control and patient compliance. This work introduces a novel “nanoparticle-in-nanofiber” drug delivery system for sustained release of a combined antidiabetic regimen (metformin and sitagliptin). Electrospun polymer nanofibers are employed as a platform to incorporate secondary nano-carriers (Solid Lipid Nanoparticles, and Niosomes) loaded with the drugs, creating a hierarchical multi-nanocarrier system. A Design of Experiments (DoE) approach (full-factorial designs and response surface optimization) was used to optimize the formulation, focusing on key factors such as polymer composition, nanoparticle load, lipid-to-drug ratio, and surfactant concentration. The optimized hybrid formulation was comprehensively characterized for morphology, particle size, drug encapsulation efficiency, thermal behavior, and in vitro drug release kinetics. In vitro, the nano-in-nanofiber system demonstrated a biphasic drug release profile: an initial moderate release from the fiber matrix followed by a prolonged release from embedded nanoparticles, avoiding the rapid “burst” release seen with conventional formulations. In vivo, the formulation was evaluated in a streptozotocin-induced diabetic rat model. A single administration of the optimized nanofiber patch achieved extended glycemic control for over 24 hours, in contrast to oral free drug which provided only a few hours of glucose reduction. Blood glucose levels in treated rats showed a more gradual decline and sustained low plateau, indicative of prolonged drug action, with the nanofiber system maintaining glycemic levels in a therapeutic range for an entire day. Pharmacokinetic analysis revealed that the nanofiber delivery produced more stable plasma drug concentrations with lower peak levels and extended duration, thereby minimizing the risk of hypoglycemia while ensuring efficacy. Biocompatibility and safety were confirmed by histopathology and the absence of significant adverse effects; the nanofiber mats were non-toxic and well-tolerated in application (whether transdermal or oral dissolving film format). A six-month stability study (at 25 °C/60%RH, 40 °C/75%RH, and 4 °C) indicated that the formulation retained >90% of its drug content under long-term conditions and exhibited no significant changes in fiber morphology or release profile ($f2 > 50$ for dissolution similarity), confirming formulation robustness.

Overall, this dissertation demonstrates the successful development of an innovative hybrid nanocarrier system for diabetes management. The Abstract explain the background and motivations for this research, the methodology adopted, key findings, and the implications. By integrating metformin and sitagliptin into a DoE-optimized SLN/niosome-in-nanofiber scaffold, we achieved synergistic improvements in drug delivery: enhanced drug encapsulation, controlled dual-phase release, and markedly extended therapeutic effect in vivo. These outcomes highlighted the potential of the nano-in-nanofiber platform to address major limitations of current oral antidiabetic therapy (such as poor adherence and glycemic fluctuations) and pave the way for translating such advanced drug delivery systems into clinically relevant solutions. The hybrid system promises improved patient compliance (due to reduced dosing frequency) and better safety (due to avoidance of high systemic peaks), aligning with the goals of next-generation diabetes care. This research not only contributes a proof-of-concept for a sustained-release patch for type 2 diabetes but also establishes a versatile platform technology that could be adapted to other chronic diseases requiring multi-drug delivery and long-acting effects. The outcomes are poised to benefit interdisciplinary field’spharmaceutics, biomaterials, and medicine by offering a blueprint for combining nanotechnology and polymer science to solve pressing therapeutic challenges in chronic disease management.

Chapter - 1

INTRODUCTION

Diabetes mellitus (especially type 2) is a chronic metabolic disorder characterized by hyperglycemia and associated with severe complications (cardiovascular disease, neuropathy, nephropathy, etc.). It has reached pandemic proportions: as of 2021 an estimated 529 million adults were living with diabetes, a number projected to more than double to over 1.3 billion by 2050[1][2]. This alarming rise is driven largely by type 2 diabetes, linked to obesity and lifestyle changes. Effective long-term management of diabetes is challenging with current therapies. First-line treatment often involves metformin, a biguanide that reduces hepatic glucose production and improves insulin sensitivity, sometimes combined with newer oral agents like sitagliptin, a DPP-4 inhibitor that increases incretin levels to stimulate insulin secretion. The metformin–sitagliptin combination (available as a single pill, e.g. Janumet®) addresses complementary pathophysiologic defects in type 2 diabetes – metformin targets insulin resistance and hepatic gluconeogenesis, while sitagliptin addresses insufficient incretin effect, thereby improving insulin secretion and suppressing glucagon in a glucose-dependent manner[3]. Clinical studies have shown that this combination significantly improves HbA1cand both fasting and postprandial glucose, with low risk of hypoglycemia and weight neutrality, making it better tolerated than many alternatives[4]. Despite its efficacy, this oral regimen (typically metformin 500 mg + sitagliptin 50–100 mg daily) still requires regular dosing and is subject to peaks and troughsin plasma levels that can lead to side effects (e.g. metformin GI upset) or suboptimal glucose control before the next dose. Moreover, patient non-compliance with daily oral pills is a major issue adherence rates for chronic oral therapies can be below 50%. For diabetes, non-adherence contributes to glycemic variability and higher risk of complications. Thus, there is a clear need for sustained-release drug delivery systems in diabetes that can maintain more stable therapeutic drug concentrations over extended periods, reducing dosing frequency and improving compliance[5].

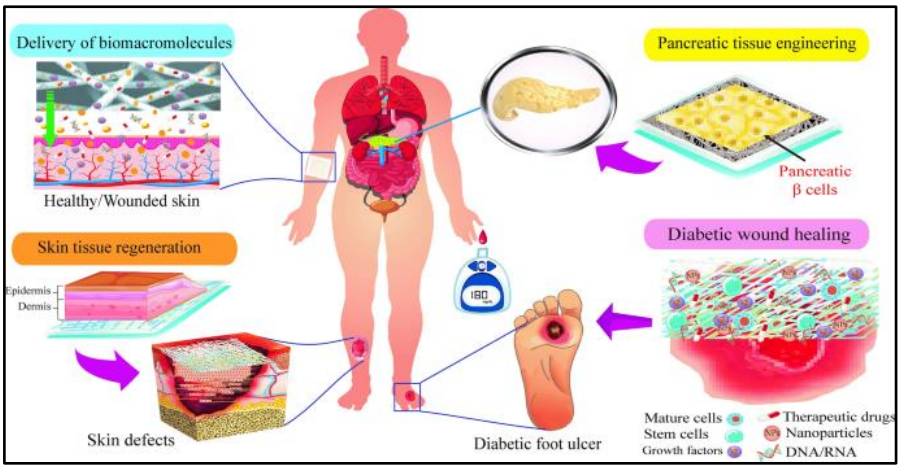


Figure 1.1: Diverse applications of nanofiber-based drug delivery systems in biomedicine, with emphasis on diabetes-related therapies.

Nanofibrous scaffolds can be used for skin tissue regeneration and wound healing (e.g., diabetic foot ulcer dressings), delivery of biomacromolecules (such as insulin or growth factors through the skin), and even pancreatic tissue engineering for diabetes treatment. These versatile platforms aim to improve healing and therapeutic outcomes by providing controlled release and a favorable microenvironment.

Figure 1.1 below illustrates some biomedical applications of nanofiber-based systems, highlighting how nanofibrous matrices can be utilized for delivering biomolecules to skin (wound healing), supporting pancreatic cell growth (tissue engineering for diabetes), and serving as dressings for diabetic foot ulcers, among other uses. These examples underscore the broad potential of nanofibers in addressing complex medical challenges through localized and controlled therapy.

Polymeric nanofiber-based drug delivery systems have emerged as a promising solution to these challenges. Electrospunnanofibers offer several advantages: (1) an extremely high surface-area-to-volume ratio and tunable porosity, enabling high drug loading and fast solvent evaporation during fabrication[6]; (2) the ability to incorporate drugs in an amorphous or molecularly dispersed form within the fiber matrix, often enhancing dissolution of poorly soluble drugs[7]; and (3) a mimicry of extracellular matrix (ECM) architecture, which is beneficial for tissue integration and localized delivery. Nanofiber mats can be engineered for various routes – e.g. oral dissolving films, transdermal patches, or implantable wound dressings making them versatile for diabetes management[8][9]. For instance, a transdermal nanofiber patch loaded with drug

can provide continuous systemic delivery without first-pass metabolism, maintaining steady-state levels over 24 hours and preventing the sharp peaks associated with oral dosing[10][6]. Such steady input could avoid hypoglycemic nadirs and reduce side effects by eliminating high Cmax. In addition, nanofibers can serve as smart wound dressings in diabetic foot ulcers: they provide a high-surface-area scaffold that keeps a moist environment and can locally release therapeutics (antibiotics, growth factors) to enhance healing[11][12]. Indeed, electrospun fiber mats with incorporated bioactives have shown improved healing of chronic diabetic wounds in preclinical studies[8][13].

Despite these advantages, single-layer nanofibers still face limitations, notably an initial burst release of surface-associated drug followed by a plateau as diffusion slows. Achieving truly prolonged release (>24 h) from fibers alone can be challenging, especially for highly water-soluble drugs like metformin. To overcome this, researchers have started exploring multiphase delivery systems. One cutting-edge approach is the integration of drug-loaded nanoparticles into the nanofiber matrix, creating a "nano-in-nanofiber" hybrid system[14]. In this design, a portion of the drug is directly dispersed in the polymer fiber for immediate/sustained release, while another portion is encapsulated in nanoparticles (such as lipid nanoparticles or vesicles) embedded within the fiber for a second, delayed release phase. This hierarchical carrier offers dual-controlled release: the nanofiber provides an initial controlled release as it gradually hydrates or degrades, and the embedded nanoparticles act as reservoirs that release drug over a longer timeframe once they are released from or through the fiber[15][14]. Such systems leverage the complementary strengths of each component – the fiber's structural support and burst-mitigating matrix, and the nanoparticles' ability to sustain drug release and protect the drug from degradation. As a result, biphasic release profiles can be achieved, with an initial moderate release (to reach therapeutic levels quickly without a harmful spike) and a prolonged tail maintaining those levels[14]. This is highly desirable for chronic conditions like diabetes where maintaining blood glucose within a tight range is crucial. Furthermore, combining multiple nanostructures can improve drug stability (e.g. protecting sitagliptin from enzymatic breakdown) and allow co-delivery of multiple drugs or modalities.

Recent literature has reported promising outcomes using such hybrid approaches. For example, Vargas-Molinero *et al.* (2023) summarized that coupling

polymeric nanoparticles with nanofibers yields hybrid mats that improve overall performance and mitigate disadvantages of single-carrier systems[14]. In cancer therapy, a concept analogous to ours ("nano-in-nano") is being explored to achieve local drug depots at tumor sites[16]. However, in the context of diabetes, the application of nano-in-nanofiber systems remains relatively novel. Few studies have integrated nanoparticles into fibers for antidiabetic drug delivery. One pioneering study encapsulated insulin-loaded chitosan nanoparticles in polymer nanofibers for oral delivery, showing extended glucose-lowering in rats compared to insulin alone[17][18]. Another recent work formulated sitagliptinmucoadhesive nanoparticles for oral delivery and demonstrated prolonged drug action (~12 hours) in the GI tract[18] – an approach we aim to enhance by immobilizing such nanoparticles in a fiber matrix to further delay release and improve localization.

Design of Experiments (DoE) methodologies have been increasingly employed in pharmaceutical formulation to systematically optimize multiple factors simultaneously[19]. Traditional one-variable-at-a-time approaches are inefficient and may overlook interactions between factors. In this project, given the multifactorial nature of our formulation (polymer properties, nanoparticle composition, process parameters, etc.), we embraced DoE to achieve an optimal formulation with minimal trials. In particular, a full-factorial design was used in preliminary screening of critical factors (e.g. high/low levels of polymer ratio, surfactant percentage, and homogenization time for SLNs), followed by response surface optimization to fine-tune the levels for desired outcomes (particle size, entrapment efficiency, fiber diameter, release rate). DoE not only identifies the main effects of each factor but also reveals any interactions (for instance, polymer blend ratio might influence how surfactant affects fiber morphology)[20][21]. Previous studies (e.g. Patel *et al.*, 2019) have highlighted that DoE-driven optimization can significantly enhance the development of nanoparticle-based delivery systems by achieving target profiles more efficiently[22]. By applying DoE here, we ensure our nano-in-nanofiber platform is robust and optimized for performance metrics such as maximum drug loading, sustained release and mechanical integrity of fibers.

In summary, the Introduction establishes the rationale and objectives of this research. We seek to improve sustained delivery of antidiabetic therapy (metformin +

sitagliptin) through an innovative combination of nanotechnologies. We will develop electrospunnanofibers as a delivery scaffold, and incorporate within them secondary nanocarriers (SLNs and niosomes) to achieve a controlled dual release. This approach addresses key limitations of current therapies: it can reduce dosing frequency (improving adherence) and smooth out pharmacokinetic peaks (improving safety and efficacy). The introduction has reviewed the state-of-the-art: conventional diabetes treatments and their drawbacks, the promise of nanofiber systems (high surface area, ECM mimicry, versatile routes), and the emerging idea of multi-tier delivery systems. It sets the stage for the detailed studies to follow, in which we describe the design, fabrication, characterization, and testing of our Innovative Hybrid Nano-in-Nanofiber Platform for Sustained Antidiabetic Drug Delivery.

Diabetes mellitus is a global health epidemic, affecting over 460 million people worldwide as of 2019 and projected to afflict more than 700 million by 2045. It is characterized by impaired glucose metabolism resulting from insufficient insulin production (Type 1) or insulin resistance (Type 2), leading to chronic hyperglycemia. Persistent hyperglycemia precipitates severe microvascular and macrovascular complications – including retinopathy, nephropathy, neuropathy, cardiovascular disease, and impaired wound healing – which collectively impose immense morbidity, mortality, and economic burden. Conventional therapy relies predominantly on pharmacological interventions such as subcutaneous insulin injections and oral hypoglycemic agents (e.g. metformin, sulfonylureas, DPP-4 inhibitors). While these treatments have been cornerstone therapies for decades, they suffer notable limitations in achieving tight glycemic control and patient adherence.

Challenges in current diabetes therapy: Frequent insulin injections are painful and inconvenient, often resulting in poor compliance and suboptimal dosing regimes. Exogenous insulin delivered by subcutaneous bolus can exhibit non-physiological pharmacokinetics – rapid absorption leads to peaking of insulin levels and risk of hypoglycemia, followed by a short duration of action that necessitates multiple daily injections. Even modern rapid-acting and basal insulin analogues cannot perfectly mimic endogenous pancreatic secretion profiles. Similarly, oral antidiabetic drugs often require rigid dosing schedules and can cause side effects (e.g. gastrointestinal upset with metformin, hypoglycemia with sulfonylureas) that impact patient quality of life.

Many agents have low and variable bioavailability – for instance, oral peptide drugs like insulin or GLP-1 agonists are virtually ineffective due to enzymatic degradation and poor intestinal absorption. Although other non-invasive routes (oral, transdermal, inhalation) have been explored to improve convenience, most have not yet achieved clinical parity with injections due to insufficient absorption or formulation instability. Therefore, there is a clear unmet need for novel drug delivery systems that can provide sustained, controlled release of antidiabetic medications to maintain euglycemia with minimal intervention, thereby improving therapeutic outcomes and patient adherence.

Nanotechnology in diabetes drug delivery: In recent years, nanomedicine approaches have shown great promise in addressing the limitations of conventional delivery. Nanoparticles (NPs) – typically 1–100 nm carriers made from biocompatible materials – can protect drug molecules, enhance bioavailability, and enable controlled and targeted release. For example, polymeric nanoparticles and lipid-based nanocarriers have been investigated for oral insulin delivery to shield the protein from gastrointestinal degradation and improve transepithelial transport. Numerous studies have demonstrated that nano-formulations can prolong the circulation of insulin and other peptides, reduce immunogenicity, and even provide stimuli-responsive release triggered by glucose levels. *Smart insulin nanodelivery systems* – such as glucose-responsive nanoparticles that release insulin in response to hyperglycemia – are an especially active area of research, aiming to emulate endogenous glucose-regulated insulin release (a "synthetic pancreas"). Microneedle patches loaded with insulin nanoparticles or vesicles have shown the ability to rapidly respond to rising glucose and automatically deliver insulin without hypoglycemia. These advances underscore the potential of nanotechnology to transform diabetes care by increasing drug stability, targeting specific tissues (e.g. insulin to the liver), and achieving sustained or feedback-controlled release.

Electrospunnanofibers for drug delivery: Another cutting-edge technology in controlled release systems is electrospinning, which produces ultra-fine polymer fibers (nanofibers) with diameters ranging from tens of nanometers to a few microns. Electrospunnanofiber mats possess a high surface-area-to-volume ratio and an interconnected porous structure, making them attractive as drug delivery platforms. Drugs can be incorporated within nanofibers either by blending with the polymer

solution prior to spinning or by post-spinning immobilization techniques, yielding a solid-state dosage form that can be fabricated as dissolvable films, transdermal patches, or implantable mats. These fiber systems can provide prolonged release by diffusion of drug through the polymer matrix and by slow polymer degradation (if biodegradable polymers are used). Indeed, electrospunfibers have demonstrated improved control over release kinetics compared to traditional bulk materials like tablets or hydrogels – the nanofiber mesh structure allows tunable drug diffusivity and minimizes the diffusion path length, often resulting in near linear (zero-order) release profiles for extended periods. Moreover, nanofibers can enhance the bioavailability of poorly water-soluble drugs by presenting them in an amorphous state and by the large surface area that facilitates dissolution. These attributes have driven extensive research into electrospunnanofibers for delivering a variety of therapeutics, ranging from antibiotics and anticancer agents to proteins and genes. For diabetic applications, electrospunnanofiber systems have been investigated in multiple contexts: e.g. transdermal patches for insulin delivery, oral fast-dissolving fiber mats for peptide delivery, and nanofiber-based wound dressings for diabetic ulcers.

Several studies have already provided proof-of-concept that nanofiber-based formulations can effectively deliver antidiabetic drugs. Sharma *et al.* (2013) developed a poly(vinyl alcohol)/sodium alginate composite nanofibertransmucosal patch loaded with insulin, observing sustained in vitro release and significant glucose reduction in diabetic rats over conventional insulin solution (encapsulation efficiency ~99%, release following first-order kinetics with only an initial burst required for therapeutic onset). Lancina*et al.* (2017) fabricated chitosan–poly(ethylene oxide) nanofiber mats for transbuccal insulin delivery, demonstrating that higher chitosan content improved fiber morphology and mucoadhesion, and the fibers effectively delivered insulin across the buccal mucosa with preserved bioactivity (achieving notable plasma glucose reduction in vivo). In another example, Lee *et al.* (2020) created core–shell nanofibers co-loaded with insulin and vildagliptin for diabetic wound healing applications – the coaxial fibers provided dual drug release over 14 days and accelerated wound closure in diabetic mice compared to single-drug or unloaded scaffolds.

These works underscore two key points: (1) electrospunfibers can successfully deliver proteins like insulin in active form, and (2) combination therapies and advanced

fiber architectures (e.g. core–shell) can be leveraged to address complex aspects of diabetes (such as wound healing, where growth factors or additional drugs may be included alongside insulin).

Hybrid nanoparticle-in-nanofiber systems: Despite the progress with standalone nanoparticles and nanofibers, each approach has intrinsic limitations. Nanoparticles, while offering controlled release, can carry only limited drug quantities and tend to aggregate or clear rapidly *in vivo*. Polymeric nanofibers, on the other hand, can accommodate higher drug loads but may release drugs too rapidly if the drug is simply blended (especially for small hydrophilic molecules). A promising strategy to overcome these challenges is to combine nanoparticles and nanofibers into a single hybrid system, capitalizing on the advantages of both. In a hybrid nano-in-nanofiber platform, drug-loaded nanoparticles are incorporated within the fiber matrix (during or after electrospinning), effectively creating a hierarchical delivery system. This design provides a double barrier to drug release: the drug must diffuse out of the nanoparticles and then through the surrounding fiber, dramatically smoothing out the release profile and preventing any initial burst. Additionally, nanoparticles can modify the fiber properties – for example, improving mechanical strength or altering hydrophilicity – and the fiber matrix can stabilize and localize the nanoparticles at the target site.

Vargas-Molinero*et al.* (2023) review such nanofiber–nanoparticle hybrid systems, noting that they often exhibit unique properties distinct from either component alone and can mitigate issues like nanoparticle aggregation and nanofiber brittleness. By appropriate choice of materials, one can synergize benefits: for instance, hydrophobic polymer fibers can be loaded with hydrophilic drug nanoparticles to achieve both high loading and sustained release for otherwise fast-dissolving drugs.

Recent research has demonstrated the potential of this hybrid approach in drug delivery and tissue engineering. Goyal *et al.* (2016) showed that combining nanofibers with drug-loaded nanospheres improved topical delivery outcomes the nanofiber provided a skin-adherent reservoir and the nanoparticles modulated release kinetics (their composite system achieved prolonged drug retention in skin with reduced systemic absorption). In the context of diabetes, Stephansen*et al.* (2015) incorporated a therapeutic protein into bioactive nanofibers (using fish sarcoplasmic protein as a matrix) and achieved transmucosal protein delivery, implying that nanofibers can

facilitate oral uptake of macromolecules when appropriately engineered. Such hybrid and composite systems illustrate a new design paradigm: rather than relying on a single type of carrier, multi-component platforms can be tailored to exploit multiple mechanisms of release control.

Research objective: The aim of this research is to develop an innovative hybrid nanoparticle-in-nanofiber platform for sustained antidiabetic drug delivery, with model drug, and to optimize its formulation using a DoE approach for maximum efficacy. We hypothesize that embedding insulin-loaded polymeric nanoparticles within biodegradable nanofibers will provide significantly extended release of insulin, reducing dosing frequency and improving glycemic control relative to conventional delivery.

To address this hypothesis, we set the following objectives:

(a) Formulate insulin-loaded nanoparticles with high encapsulation efficiency and suitable size for incorporation into nanofibers.

(b) Fabricate composite nanofiber mats containing these nanoparticles, using materials compatible with subcutaneous implantation or transdermal patch application.

(c) Employ factorial design and response surface methods to optimize formulation and processing parameters (such as polymer composition, nanoparticle content, electrospinning conditions) to achieve a target sustained release profile.

(d) Characterize the optimized formulation's morphology, physicochemical properties, and in vitro release kinetics.

(e) Evaluate the in vivo performance of the optimized system in a diabetic rat model, in terms of blood glucose reduction duration and biocompatibility. Through these steps, the project seeks to bridge materials engineering and pharmacotherapy to produce a next-generation drug delivery system that could substantially improve diabetes management. The introduction of DoE in the formulation process ensures a systematic and efficient optimization, aligning with Quality by Design principles in pharmaceutical development.

1.1 Diabetes Mellitus: Prevalence and Pathology

Diabetes mellitus is a chronic metabolic disorder characterized by high blood glucose levels due to defects in insulin secretion, insulin action, or both. The condition is broadly classified into type 1 diabetes (T1D), an autoimmune destruction of pancreatic β-cells leading to absolute insulin deficiency, and type 2 diabetes (T2D), a state of insulin resistance combined with an inadequate compensatory insulin secretory response. Gestational diabetes and other less common forms (monogenic, secondary diabetes) also exist. The hallmark symptoms of uncontrolled diabetes include polyuria, polydipsia, and polyphagia, along with chronic complications affecting the eyes, kidneys, nerves, and cardiovascular system. Globally, diabetes has reached pandemic proportions – the International Diabetes Federation estimated 537 million adults living with diabetes in 2021 (10.5% prevalence), projected to rise to 783 million by 2045. Alarmingly, diabetes caused ~6.7 million deaths in 2021 and is on track to become the seventh leading cause of death by 2030. Such epidemiological trends underscore the urgent need for improved strategies in diabetes management and prevention.

1.1.1 Global Impact and Challenges

The burden of diabetes extends beyond individual health to societal and economic realms. In 2019, an estimated 463 million people had diabetes and 4.2 million deaths were attributed to it. The World Health Organization has declared diabetes a global health crisis, noting its staggering rise and the strain on healthcare systems, especially in low- and middle-income countries.

The management of diabetes is challenging because chronic hyperglycemia quietly damages blood vessels and organs over years. Patients suffer higher risks of heart disease, stroke, kidney failure, neuropathy, retinopathy, and limb amputations. Achieving tight glycemic control can prevent or delay many of these complications, but this often comes at the cost of increased hypoglycemia risk. Thus, there is a narrow therapeutic window, making safe and effective glucose management difficult with current tools.

Despite advances in pharmacotherapy and monitoring, a large proportion of people with diabetes still fail to reach recommended glycemic targets. For instance, even 100 years after insulin's discovery, near-normoglycemia remains elusive for many

insulin-treated patients, and suboptimal control is “nearly universally” observed, in part because we are still using essentially the same hormone via subcutaneous injections. These challenges motivate the exploration of novel therapies and technologies – including nanotechnology – to improve diabetes outcomes.

1.2. Historical Perspectives on Diabetes and Insulin Therapy

Before the 20th century, a diagnosis of type 1 diabetes was essentially a death sentence. The only management was extreme dietary restriction (often <450 kcal/day) to slightly prolong life, which frequently led to starvation. It was against this bleak backdrop that the discovery of insulin in 1921 revolutionized diabetes care. Frederick Banting and Charles Best, working in J.J.R. Macleod’s Toronto laboratory, isolated a pancreatic extract (insulin) that dramatically lowered blood glucose in diabetic dogs. Biochemist James Collip helped purify the extract for human use.



Figure 1.2:Dr. Frederick Banting (right) and Charles Best (left) with one of their diabetic research dogs in 1921, shortly after discovering insulin.

Their ground-breaking experiments showed that injecting pancreatic extracts could reverse diabetic symptoms in dogs, paving the way for the first successful insulin therapy in humans in 1922.

In January 1922, 14-year-old Leonard Thompson, a boy dying of T1D in Toronto General Hospital, became the first person to receive insulin injections. The initial injection caused an allergic reaction due to impurities, but after further purification, a second dose on January 23rd dramatically lowered his blood sugar to near-normal levels. News of this "miraculous" therapy spread worldwide. By late 1923, industrial-scale production of insulin (notably by Eli Lilly & Co.) was underway, turning diabetes from a fatal illness into a manageable chronic condition. Banting and Macleod were awarded the 1923 Nobel Prize in Medicine (which they shared with Best and Collip) for this breakthrough. The early insulin was extracted from animal pancreases (primarily bovine and porcine). Over subsequent decades, insulin therapy was continually improved – protamine and zinc were added in the 1930s to prolong insulin's action, leading to intermediate-acting formulations. In the 1970s–1980s came recombinant DNA technology, allowing biosynthetic human insulin (Humulin©) and later insulin analogues with tailored pharmacokinetics (such as rapid-acting lispro/aspart and long-acting glargine). These innovations have offered patients more flexibility and better glycemic profiles than early short-acting bovine or porcine insulin, greatly improving quality of life.

1.2.1 Continued Evolution of Insulin Delivery

Insulin delivery methods have also evolved. The first insulin was injected with reusable glass syringes and large-bore needles that required boiling for sterilization. By the 1950s, disposable plastic syringes eased this burden. The 1980s saw the advent of insulin pens, which improved dosing convenience and accuracy. In parallel, continuous subcutaneous insulin infusion (CSII) via pumps emerged in the late 1970s. Modern insulin pumps allow programmable basal rates and boluses, approximating physiological insulin delivery and reducing glycemic variability. More recently, pumps have been paired with continuous glucose monitors (CGMs) in closed-loop "artificial pancreas" systems that automatically adjust insulin in response to sensor glucose levels. As of 2023, several hybrid closed-loop systems are commercially available, marking a significant step toward automated diabetes management. Despite these advances, insulin therapy still involves challenges like invasive administration and the ever-present risk of hypoglycemia. The historical quest to improve insulin delivery sets the

stage for exploring whether emerging technologies like nanotechnology can further revolutionize diabetes care.

1.3. Challenges in Insulin Delivery and Glycemic Control

While life-saving, insulin therapy is notoriously difficult to optimize. Patients must routinely self-inject insulin, typically multiple times per day, or wear infusion pumps – both approaches being invasive and often uncomfortable. Needle phobia, injection pain, and stigma can impede adherence, especially in youth and those with T2D who may be reluctant to initiate insulin. Even with perfect adherence, traditional subcutaneous insulin has pharmacokinetic limitations: injected insulin does not replicate the precise, real-time glucose-responsive secretion of a healthy pancreas. Instead, patients experience exogenous insulin absorption that can be erratic and tends to cause mismatches between insulin action and glucose excursions. This contributes to both hyperglycemia and episodes of hypoglycemia due to insulin overdosing or delayed uptake. Hypoglycemia (<70 mg/dL) is the most acute danger of insulin therapy – severe hypoglycemia can cause seizures, coma, or even death. Unfortunately, it is an ever-present risk as patients strive for tight control. Studies indicate fear of hypoglycemia and the body's counterregulatory responses often prevent patients from achieving normal glycemia, as they err on the side of running high rather than risking lows.

Another challenge is that insulin requirements are highly variable – fluctuating with meals, exercise, stress, illness, and hormonal changes. Patients must constantly monitor glucose and calculate insulin doses, a complex self-management regimen prone to human error. The advent of CGM has shone light on how often blood glucose swings out of range even in intensively managed patients. In fact, "time-in-range" (70–180 mg/dL) is only ~50–60% for many T1D patients, meaning they spend many hours in hyperglycemia daily. Postprandial spikes and nocturnal hypoglycemia remain common with conventional insulin therapy. Moreover, subcutaneously injected insulin is delivered into peripheral circulation rather than the portal vein (liver), which disrupts normal insulin physiology. The liver receives less insulin than normal, while peripheral tissues receive more, contributing to weight gain and lipogenic effects. All these issues point to the need for more physiological insulin delivery systems that can automatically adjust insulin release in real-time based on blood glucose levels – something current modalities struggle to achieve.

1.3.1 Non-Invasive and Alternative Delivery Routes

Given the challenges of injections, researchers have long pursued alternative insulin delivery routes. Oral insulin has been a "holy grail" because it would be patient-friendly and because insulin would pass through the portal circulation, directly targeting the liver as in normal physiology. However, delivering insulin as a pill is exceedingly difficult: insulin is a protein that is degraded by digestive enzymes and not easily absorbed across the gastrointestinal mucosa. Numerous strategies (enteric coatings, enzyme inhibitors, permeability enhancers, nanoparticle carriers) have been tried to protect insulin through the stomach and facilitate uptake in the intestine. To date, no oral insulin has succeeded in reaching the market, although a few candidates have entered clinical trials with modest success in lowering glucose. Another approach, inhalable insulin, saw Pfizer's Exubera® become the first approved inhaled insulin in 2006. It delivered rapid-acting insulin via a pulmonary route and was effective in glycemic control. However, Exubera was withdrawn in 2007 due to poor patient acceptance (the inhaler device was bulky and dosing was confusing) and lackluster sales. A later inhaled insulin, Afrezza®, with a smaller inhaler and technosphere carrier particles, was approved in 2014 and remains on the market, but uptake has been limited. Other routes like transdermal patches and intranasal sprays have been explored as well, but none have yet become mainstream therapy due to issues with insulin absorption and consistency.

Despite these setbacks, the pursuit of patient-friendly delivery continues. Insulin pumps offered improvement by eliminating many injections, yet they still involve a catheter under the skin and risk of site infections or kinking. The ultimate alternative would be a closed-loop (automated) insulin delivery – essentially an "artificial pancreas" – that could relieve patients of day-to-day decision making. As noted, we have made progress here by combining CGM sensors with pumps and control algorithms. Studies show that closed-loop systems modestly increase time-in-range and reduce hypoglycemia. However, current systems still rely on subcutaneous infusion of insulin (with its inherent delays) and are only semi-automated (users still announce meals, etc.). They also involve wearing multiple devices. There remains room for improvement in both the **route** of insulin administration and the **responsiveness** of

insulin delivery. This is where novel technologies, including nanotechnology-based approaches, are being intensely researched.

1.4. Nanotechnology in Medicine: An Overview

Nanotechnology involves engineering and manipulating matter at the nanoscale (typically 1–100 nm), which is the scale of large biological molecules and viruses. By operating at this tiny scale, nanotechnology can interface with biological systems in unique ways, offering potential breakthroughs in drug delivery, diagnostics, and tissue engineering. In medicine, the field of **nanomedicine** has emerged, applying nanoscale materials (like nanoparticles, nanotubes, nanofibers, quantum dots, etc.) to diagnose or treat disease. These nanomaterials have high surface-area-to-volume ratios and can be designed to have specific physical, chemical, and biological properties. For example, nanoparticles made of biocompatible polymers or lipids can carry drug molecules and protect them from degradation, releasing them in a controlled manner at targeted sites. Drugs at the nanoscale may penetrate biological barriers more easily and be taken up by cells more readily. Nanocarriers can also be functionalized with targeting ligands (antibodies, peptides) to home to specific tissues or cell types, reducing off-target effects. These capabilities make nanotechnology extremely promising for treating diseases like cancer (targeted chemotherapy), infections, and endocrine disorders.

In the environmental and industrial context, nanotech has been transformative as well – from nanosensors that detect pollutants to nanomaterials in electronics and energy. In biomedicine specifically, some nanoparticle-based drugs have already reached clinical use (e.g., liposomal doxorubicin for cancer). For diabetes, nanotechnology offers exciting avenues for both improving glucose monitoring and insulin delivery. Nanoscale glucose sensors employing nanomaterials (such as graphene, gold nanoparticles, or carbon nanotubes) can achieve higher sensitivity and faster response than traditional enzymatic sensors. Indeed, continuous glucose monitors already exploit some nanoscale chemistry (e.g., glucose oxidase immobilized on nano-structured electrodes). Beyond sensing, nanotech can enable "smart" drug delivery systems that respond to physiological cues. For instance, stimuli-responsive nanoparticles can be designed to release insulin only when glucose levels are high – mimicking pancreatic β-cell function. Nanoparticles can also improve oral delivery of peptides by protecting them from digestion and enhancing transport across the gut wall.

Additionally, nanofiber scaffolds are being explored to support transplanted islet cells or to promote wound healing in diabetic ulcers. Overall, nanotechnology provides a toolkit of novel materials and devices that could address many limitations of conventional diabetes treatments. Before diving into diabetes-specific innovations, a brief overview of the main nanomaterials used in drug delivery is warranted.

1.4.1 Nanocarriers for Drug Delivery

Scientists have developed a variety of nanoscale carriers to improve drug pharmacokinetics and targeting. Broadly, the main classes include polymeric nanoparticles, nanocapsules, liposomes, solid lipid nanoparticles (SLNs), and inorganic nanoparticles. Polymeric nanoparticles are typically spherical particles (10–200 nm) made from biodegradable polymers like PLGA (poly(lactic-co-glycolic acid)), chitosan, or alginate. They can entrap insulin or other drugs within their matrix and slowly release it as the polymer matrix degrades or swells. For example, PLGA nanoparticles loaded with insulin have been shown to protect insulin from enzymatic degradation and provide sustained release over 24–48 hours. Nanocapsules are a related concept – a reservoir core (which can be aqueous for hydrophilic drugs like insulin) surrounded by a polymer shell. Insulin nanocapsules can release their payload when the shell material (e.g., alginate or albumin cross-linked polymers) breaks down in the body. Liposomes are lipid bilayer vesicles (~100 nm) that can carry insulin either inside their aqueous core or intercalated in the lipid membrane. Because they are made of biocompatible phospholipids similar to cell membranes, liposomes are generally well-tolerated and have been used for various drug deliveries. Newer lipid-based carriers include solid lipid nanoparticles and niosomes, which similarly can encapsulate insulin and protect it from the harsh extracellular environment. Inorganic nanoparticles (like mesoporous silica, gold, or zinc nanoparticles) have also been investigated for insulin delivery; these often exploit unique properties (magnetic, optical, etc.) for triggered release, but biocompatibility and safety need careful evaluation.

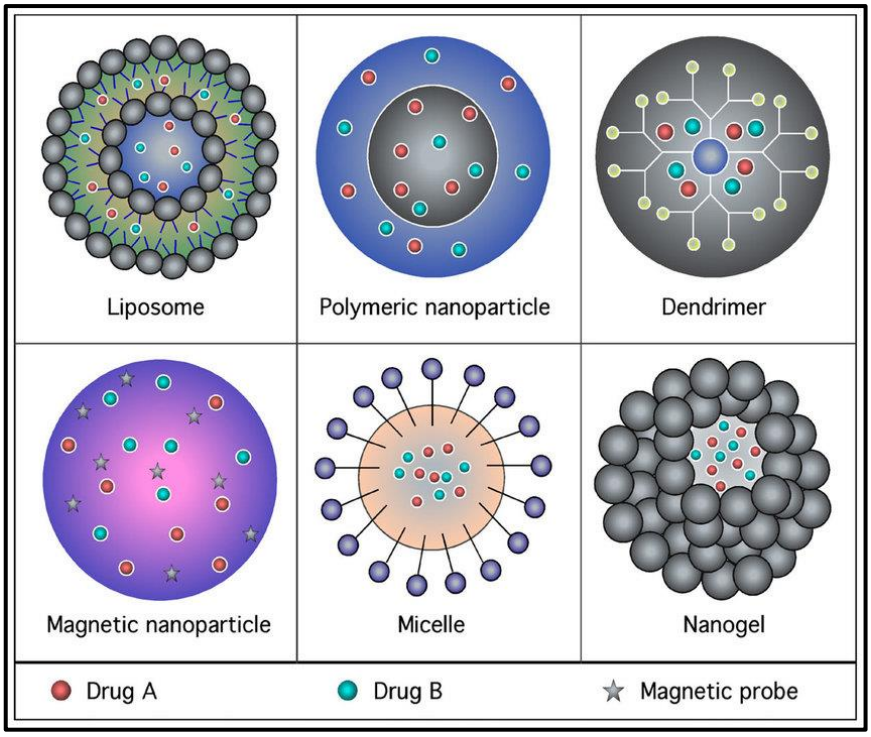


Figure 1.3. Schematic representation of common nanoparticle carriers for drug delivery.

Polymeric nanoparticles (solid biodegradable matrices), polymeric nanocapsules (liquid core & solid shell), liposomes (phospholipid vesicles), and lipid nanoparticles (solid lipid core with phospholipid layer) have all been applied to insulin therapy.

Such nanocarriers can protect insulin from degradation, improve absorption, and modulate release kinetics in the body.

The appeal of nanocarriers in diabetes is clear: an insulin nanoparticle injected or ingested today might steadily release insulin for days, reducing injection frequency and mimicking basal secretion. Functionalizing insulin nanoparticles with targeting molecules (e.g., an antibody to hepatocytes) could direct more insulin to the liver (the primary site of action) and reduce systemic exposure, aiding weight control. Nanocarriers can also respond to external triggers (heat, ultrasound, magnetic fields) to initiate insulin release on demand. However, one must consider potential downsides: introducing new materials into the body carries risks of immunogenicity or toxicity. Fortunately, many nanomaterials used (PLGA, chitosan, etc.) are known to be biocompatible and are metabolized into harmless byproducts. Regulatory approval for nano-drug systems can be complex, but precedents in cancer therapy provide a

roadmap. With this foundation, we can explore how nanotechnology is being specifically leveraged to address the two key aspects of diabetes management: insulin delivery and glucose monitoring.

1.5. Nanotechnology for Insulin Delivery

One of the most promising applications of nanotechnology in diabetes is the development of glucose-responsive ("smart") insulin delivery systems. The goal is to create an insulin formulation that can **sense** the ambient glucose level and release insulin accordingly – effectively acting like an artificial β-cell. Several nanotech-based strategies have been devised to achieve this self-regulation.

1.5.1 Glucose-Responsive Nanoparticles

Researchers have designed glucose-responsive nanoparticles (GRNPs) that modulate insulin release via glucose-driven physicochemical changes. A common approach uses polymers functionalized with *phenylboronic acid (PBA)* or its derivatives. PBA can reversibly bind to diols such as glucose. At normal or low glucose, PBA groups form cross-links with polymer vicinal diols, stabilizing the nanoparticle and retaining insulin. When blood glucose rises, glucose molecules enter the nanoparticle and bind PBA, displacing the polymer-polymer crosslinks. This causes the nanoparticle matrix to swell or degrade, triggering insulin release. Essentially, high glucose acts as the signal to break apart the nanoparticle and dump insulin. Alginate–PBA conjugate nanoparticles and PBA-grafted chitosan have demonstrated this mechanism, releasing insulin rapidly in high-glucose environments. One study formulated chitosan–PBA nanogels that showed insulin release rates increasing significantly when glucose was added at hyperglycemic concentrations. The main challenge with PBA systems is that phenylboronic acid can also bind other cis-diol compounds (like fructose or lactate), reducing specificity. Researchers are tweaking PBA chemistry to improve glucose specificity at physiological pH.

Another GRNP design utilizes glucose-binding proteins such as *Concanavalin A (ConA)*. ConA is a plant lectin that binds glucose and mannose. In classic experiments by Brownlee and Cerami in 1979, a ConA-based gel was used to modulate insulin release. In modern nanoparticle form, one can attach insulin or a glucose-bearing molecule to ConA within a particle. High glucose competitively binds ConA, causing it

to release the insulin or destabilize the nanoparticle, again resulting in insulin discharge. For example, nanoparticles made of a polysaccharide linked to ConA have been shown to swell and release insulin when glucose is introduced.

ConA-based systems face challenges in biocompatibility (ConA can be immunogenic or toxic), but they prove the concept of competitive binding as a glucose trigger.

A third major mechanism is based on glucose oxidase (GOx), an enzyme that oxidizes glucose to gluconic acid. GOx can be embedded in a polymer nanoparticle alongside insulin. When ambient glucose is high, GOx produces gluconic acid, lowering local pH.pH-sensitive polymers (for instance, those with acid-labile bonds or pH-responsive swelling properties) then respond by either degrading or expanding, causing insulin release. One such system used a polyanionic network that remains collapsed at neutral pH, trapping insulin, but protonates and swells in the acidic microenvironment generated by GOx in high glucose, thus releasing insulin. GOx-based nanoparticles effectively act like artificial β-cells that secrete insulin in response to glucose, consuming oxygen in the process (like real β-cells). Indeed, glucose oxidase is also the core of many glucose sensor strips and CGMs, reflecting its robustness.

A limitation, however, is that the reaction consumes oxygen and can become less effective in hypoxic tissue; also, the acid can slow further reaction (local feedback). Despite these issues, GOx-driven insulin nano-formulations have shown success in rodent models, achieving near-normal blood sugar control for extended periods with a single dose.

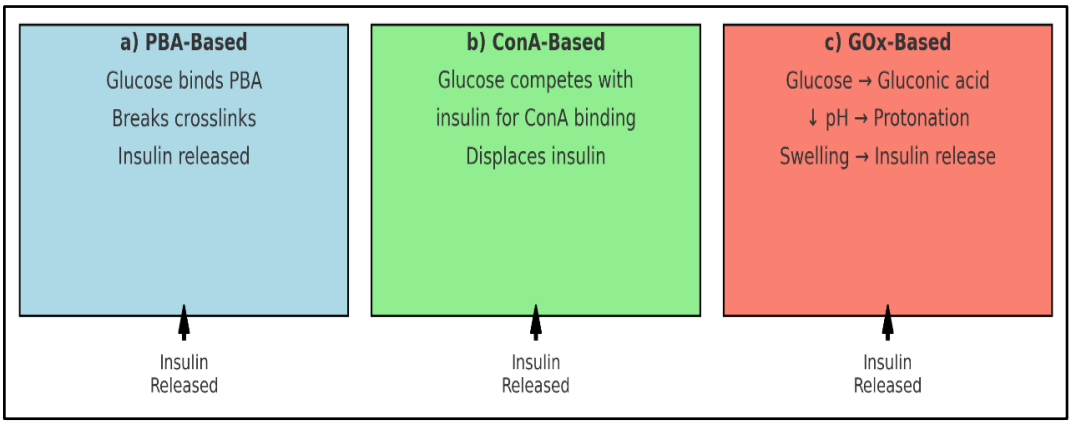


Figure 1.4. Glucose-responsive insulin release mechanisms in smart nanoparticles

a. Phenylboronic acid (PBA)-based: High glucose binds PBA groups in the nanoparticle, breaking polymer cross-links and causing insulin to be released.

b. Concanavalin A (ConA)-based: Glucose competes with insulin (or glucose-bearing insulin conjugates) for ConA binding, displacing insulin and triggering its release.

c. Glucose oxidase (enzyme)-based: Glucose is enzymatically converted to gluconic acid, lowering pH inside the particle. The acidic pH protonates the polymer, inducing swelling or dissolution of the matrix and thereby releasing insulin.

These glucose-responsive nanoparticles represent a "smart" insulin that autonomously adjusts delivery a marked contrast to conventional insulin that acts irrespective of glucose levels. In laboratory tests, such nanoparticles have demonstrated the ability to prevent hyperglycemia in diabetic animals with significantly reduced risk of hypoglycemia. For instance, one study achieved normal blood glucose for over 10 hours in diabetic mice with a single injection of a GOx-powered nanogel, whereas standard insulin had a much shorter effect. It's worth noting that most of these systems are still in preclinical stages. No fully glucose-responsive insulin has yet completed clinical trials a recent trial of a "smart insulin" by a pharmaceutical company was discontinued due to insufficient glucose-sensitivity in humans. Nonetheless, the progress in this area is inspiring continued research. By combining chemical sensing and drug release in one package, these nanocarriers illustrate the convergence of nanotechnology, chemistry, and physiology to potentially eliminate the burden of manual insulin dosing.

1.5.2 Nanoparticle-Mediated Oral Insulin Delivery

As discussed, oral administration of insulin faces immense barriers. However, nanotechnology has provided new strategies to tackle these. Nanocarriers can shield insulin from enzymatic degradation in the GI tract and enhance its transport across the intestinal epithelium. For example, insulin-loaded poly(anhydride) nanoparticles were shown to protect insulin from proteases like pepsin and trypsin, delivering intact insulin to the ileum where it could be absorbed. Another approach uses mucoadhesive nanoparticles (e.g., chitosan-based) that stick to the gut wall and prolong the contact time for absorption. Some nanocarriers are designed to exploit the M-cell pathway in Peyer's patches to transcytose across the gut lining. For instance, a recent study used

polymeric nanoparticles coated with vitamin B12 (to target IF receptor uptake) and demonstrated significant insulin absorption in rats, lowering blood glucose by ~50% with oral dosing. Researchers have also experimented with cell-penetrating peptide-modified nanoparticles that open tight junctions, and with lipid-based carriers like SNEDDS (self-nanoemulsifying drug delivery systems) that improve lymphatic uptake of insulin. A 2024 report described insulin conjugated to small silver sulfide quantum dots within a chitosan/glucose-responsive polymer matrix – forming an oral insulin nanoparticle that was pH-sensitive (stable in stomach, releasing in intestine) and achieved glucose-dependent insulin release in diabetic rats and even lowered blood glucose in diabetic pigs without hypoglycemia. This represents one of the most advanced oral insulin nanodrug prototypes to date, showing efficacy in large-animal models.

Despite these encouraging results, the oral bioavailability of insulin via nanoparticles remains low (~5–10% in many cases) compared to subcutaneous injection. This means a high dose is still required in oral form, which could be costly. Additionally, variability is high – factors like food intake, gastric emptying, and individual GI differences can affect absorption. Ongoing research is addressing these issues, for example by adding permeation enhancers to nanoparticle formulations or developing nano-microneedle pills (capsules that inject nanoparticles into the gut wall). As technology matures, it is plausible that an oral insulin nanotherapy could become reality, especially for T2D patients who might prefer pills over injections. The oral route would also direct insulin first to the liver (attenuating peripheral hyperinsulinemia and minimizing weight gain). Nanotechnology is thus bringing oral insulin within reach after nearly a century of efforts – a testament to how advanced drug delivery methods can revive long-sought therapeutic goals.

1.5.3 Nanotechnology-Enhanced Insulin Pumps and Patches

Nanotech is also augmenting transdermal and implantable delivery systems. For instance, researchers have developed microneedle arrays that penetrate the skin's outer layer without reaching nerves (hence relatively painless) and deliver insulin in a controlled fashion. Some microneedles are infused with insulin-loaded nanoparticles or nanogels that release insulin over time. Others incorporate the glucose-responsive elements discussed above. A notable example is the "smart insulin patch" – a

microneedle patch containing insulin and GOx in a polymer matrix. In response to rising interstitial glucose, the polymer matrix (pH-sensitive due to GOx's acid generation) releases insulin through the microneedles into the skin. Studies in diabetic mice showed such patches could automatically regulate blood glucose for many hours, keeping levels near normal with a single application.

Another study loaded microneedles with hypoxia-sensitive vesicles that break down when glucose is high (glucose consumption by GOx creates a local low-oxygen condition), achieving glucose-responsive insulin delivery in a "patch" format. These smart patches represent a marriage of nanomaterials (in the vesicles or nanogels) with an advanced device (microneedle array), offering a wearable, automated insulin therapy. While still experimental, the concept has advanced to larger animals; one microneedle patch system was tested in diabetic pigs and showed improved glycemic control without hypoglycemia.

In parallel, implantable insulin pumps and reservoirs are being refined with nanotechnology. One concept is an implantable nano-porous membrane that separates insulin in a reservoir from the bloodstream, designed such that insulin diffusion increases when blood glucose is high (e.g., a glucose-binding gating mechanism). This is essentially a nano-engineered insulin depot that self-regulates output – a modern take on the decades-old idea of a closed-loop implant. While fully implantable closed-loop devices (true artificial pancreas implants) remain under development, nanotechnology contributions – from glucose-sensitive membranes to long-lived glucose sensors – are paving the way.

1.6. Nanotechnology for Diabetes Complications and Cell Therapy

Beyond insulin delivery and glucose control, nanotechnology is finding roles in addressing complications of diabetes and enabling cell-based therapies.

1.6.1 Nanomedicine in Islet Cell Replacement

A potential cure for T1D is islet transplantation – providing the patient with new pancreatic islet cells that can produce insulin. However, islet transplants require immunosuppression to prevent rejection, and donor islet supply is limited. Nanotechnology offers solutions via immunoisolation and tissue engineering. By

encapsulating islet cells within semipermeable nanomaterial coatings or capsules, one can protect them from the host's immune cells while still allowing insulin, glucose, and nutrients to diffuse through. For example, alginate-based microcapsules (around 500 µm, technically micro- but often using nano-thin coatings) have been used to encapsulate islets – clinical trials (Edmonton protocol and others) have shown some success in extending graft survival without immunosuppressants. However, fibrosis around capsules remains an issue; to combat this, researchers have developed ultra-thin nano-coatings for islets using layer-by-layer deposition of biopolymers only a few nanometers thick, aiming to make capsules small enough to avoid foreign-body reactions. Incorporating nanoparticles that release anti-inflammatory or immunomodulatory agents into the islet encapsulation matrix is another strategy to locally suppress immune attack. Nanofiber scaffolds are also being used to create 3D *bioartificial pancreas* constructs: islets or stem-cell-derived beta-cells are embedded in nanofiber meshes that mimic the extracellular matrix, promoting cell survival and function. These scaffolds can be coated with hydrogel nanoporous layers for immune protection. Such tissue engineering approaches at the nano-scale may eventually allow transplantation of insulin-producing cells in a simple device without rejection. A recent study demonstrated a subcutaneous macrodevice containing islets within a nanoporous hydrogel membrane; in diabetic rodents, it maintained normal glucose levels for several months without immunosuppression. While human application will require overcoming scaling and reliability issues, nanotechnology will likely be integral in any successful cell-based cure for diabetes, whether through enhanced encapsulation, improved vascularization (via angiogenicnanofactors), or modulators that induce immune tolerance.

1.6.2 Nanomaterials for Diabetic Wound Healing

Diabetic foot ulcers are a serious complication of diabetes, often leading to infection and limb amputation. Nanotechnology is contributing to advanced wound dressings that can improve healing in the compromised diabetic milieu. Electrospunnanofiber mats made of biopolymers (e.g., polycaprolactone, cellulose, silk fibroin) provide an ideal dressing matrix: their nano-scale fibers resemble natural collagen fibrils, promoting cell attachment and providing large surface area for drug loading. These nanofiber dressings can be loaded with antibiotics, growth factors, or

even stem cells to tackle various aspects of impaired healing. For instance, researchers have created nanofiber scaffolds releasing vascular endothelial growth factor (VEGF) to stimulate angiogenesis in chronic wounds, or nano-silver embedded fibers to provide antimicrobial action. Some nanofibers are also formulated with insulin itself or analogues like insulin-like growth factor (IGF), taking advantage of insulin's tissue regenerative effects when applied topically. Topical insulin has been shown to accelerate wound closure by promoting cell proliferation and migration in the wound bed. A 2020 review of animal and human studies confirmed that topical insulin can improve wound healing outcomes, though optimal formulations are still being investigated. Nanotechnology can enhance this by controlling insulin release in the wound – for example, an insulin-loaded nanofiber that steadily releases over days, maintaining local activity. Furthermore, nanoparticles (like cerium oxide, gold, or selenium nanoparticles) with antioxidant properties are being explored to reduce oxidative stress in chronic wounds. Nanoparticle-based nitric oxide donors are another innovation to improve the microcirculation in diabetic wounds. The high surface area of nanomaterials means dressings can carry effective doses of multiple therapeutics at once (antibiotic + growth factor + matrix metalloproteinase inhibitor, etc.). Early clinical trials of some nano-engineered dressings have shown faster healing and higher wound closure rates in diabetic patients compared to standard care. As this field progresses, we may see smart dressings that not only passively treat wounds but actively sense and respond – for instance, a dressing that releases more drug if it detects a bacterial infection (via nanoparticle sensors) or changes color to indicate wound status. These advances could significantly reduce amputations and improve patient outcomes in diabetic foot disease.

1.7. Outlook and Future Directions

The integration of nanotechnology into diabetes care is still in relatively early stages, but rapid progress is being made. Dozens of nanomedicine-based therapies for diabetes are in various stages of development, from preclinical proof-of-concept to early clinical trials. Regulatory approval and safety will be key considerations: while many nanomaterials (like those using GRAS polymers) are expected to be safe, we must ensure that long-term use does not lead to unexpected immune reactions or accumulation in organs. Studies in mice and larger animals have generally shown good

biocompatibility of biodegradable nanoparticles and nanofibers. For example, an oral quantum-dot insulin formulation showed no organ toxicity in rodent and primate studies. Nevertheless, human trials are needed to fully assess safety and efficacy. The path to clinical translation will also depend on manufacturing scalability and reproducibility of these often-complex nano-systems. Pharmaceutical companies are increasingly investing in nanotech delivery, as seen by partnerships with biotech firms working on smart insulin and oral peptides.

If these technological hurdles can be overcome, the payoff could be transformative. Imagine an insulin nanoparticle injection that replaces 6–8 daily fingersticks and injections with a once-daily or once-weekly self-regulating dose. Or an oral insulin pill that one could take with meals to seamlessly control postprandial glucose. Such advances would reduce the burden on patients and potentially yield better glycemic control with less hypoglycemia. The quality-of-life improvement for millions of insulin users would be tremendous. Furthermore, nanotechnology may enable combination therapies – for instance, a single nanoparticle could co-deliver insulin with a GLP-1 agonist or other adjuncts to address multiple defects of diabetes in one go. The convergence of nanotech with biotechnology and information technology (e.g., AI-driven insulin dosing algorithms, or Bluetooth-enabled smart patches) could herald an era of truly personalized, automated diabetes management.

That said, it is important to maintain realistic expectations. Not every promising result in animal models translates to humans. The complexity of human biology and behavioral factors in diabetes management means technology alone is not a panacea. Cost is another concern – advanced nano-formulations may be expensive, and equitable access will be crucial (a lesson underscored by the current disparities in insulin availability a century after its discovery). Clinicians and researchers also emphasize that any new therapy must complement education and prevention efforts, not replace them. For T2D, lifestyle interventions and inexpensive oral medications will remain first-line, with nanotech solutions potentially providing options when those fail. For T1D, a true cure might ultimately come from stem-cell therapy or immune modulation – yet even there, nanotechnology is boosting progress via improved islet encapsulation and targeted drug delivery to immune cells.

In conclusion, nanotechnology has opened exciting frontiers in the fight against diabetes. From enabling smarter insulin and non-invasive delivery to fostering innovative treatments for complications, nano-engineered solutions are inching us closer to the dream of optimal, hassle-free glycemic control. The coming years will likely see some of these nascent technologies mature into clinically available therapies. If the 20th century was defined by the discovery of insulin, the 21st may well be defined by the intelligent delivery of insulin (and possibly its replacement by engineered tissues or gene therapy). Nanotechnology, with its ability to operate at the same scale as our body's molecular machinery, will be at the heart of this next revolution in diabetes care – making treatment more precise, responsive, and patient-friendly than ever before. The path ahead, as with any breakthrough, will require rigorous research and careful validation, but the progress so far gives plenty of reason for optimism in the future of diabetes management.

In summary, this Introduction has outlined the background and rationale for a hybrid nanofiber-based sustained release system for antidiabetic therapy. Building on the identified limitations of current therapies and the emerging successes of nanotechnology in medicine, the stage is set for the detailed exploration in subsequent chapters of how a DoE-optimized hybrid nano-in-nanofiber formulation is designed, developed, and validated. The following sections will review relevant literature (Chapter 2) and describe the experimental plan (Chapters 3–5) leading to results (Chapter 6) that demonstrate the feasibility and advantages of this novel drug delivery platform.

Chapter - 2

REVIEW OF LITERATURE

2.1 Nanotechnology in Diabetes Drug Delivery

Modern drug delivery research has increasingly turned to nanotechnology to enhance the treatment of chronic diseases like diabetes. Nanocarriers offer the ability to improve drug solubility, protect labile drugs from degradation, and provide controlled release profiles. Among nanocarriers, polymeric nanoparticles, solid lipid nanoparticles (SLNs), and niosomes have been extensively studied for delivering antidiabetic agents. Solid Lipid Nanoparticles (SLNs) are sub-1000 nm particles made of biocompatible lipids that remain solid at body temperature. First introduced in the 1990s, SLNs were intended to combine the benefits of polymeric nanoparticles and liposomes while using physiological lipids [23]. SLNs are generally recognized as safe (GRAS lipids) and offer advantages such as protection of drugs from chemical degradation, enhanced bioavailability of poorly soluble drugs, and the ability to sustain drug release by the slow solid lipid matrix erosion[24]. For example, metformin's short half-life and hydrophilicity lead to rapid clearance, but loading metformin into SLNs has been shown to prolong its antihyperglycemic effect and reduce GI side effects by avoiding high localized concentrations [25]. Niosomes, on the other hand, are vesicular systems formed by non-ionic surfactants and cholesterol, analogous to liposomes but with synthetic surfactants. They can entrap hydrophilic drugs in their aqueous core and lipophilic drugs in the bilayer, making them versatile for drug delivery [26][27]. Niosomes tend to be chemically stable, inexpensive, and can be engineered for targeted or sustained delivery. Studies have shown niosomal encapsulation of insulin or sulfonylureas can improve oral or transdermal delivery efficacy [28]. In general, nano-sized niosomes have been reported to enhance pharmacokinetics (longer circulation), decrease toxicity, and improve drug stability and solubility [29]. These features are highly relevant to diabetes medications – e.g., sitagliptin, which has ~87% renal excretion unchanged, could benefit from encapsulation to modulate its release and retention. A 2019 study formulated mucoadhesive sitagliptin-loaded chitosan nanoparticles (~350 nm) and found that they adhered to GI mucosa and extended the drug release to ~12 h, improving glycemic control duration compared to free

sitagliptin[18][30]. Such findings underscore the potential of nanocarriers to address the short action of oral antidiabetics.

Electrospun nanofibers have likewise garnered attention in diabetes therapy, both for systemic drug delivery and for local treatment of diabetic complications. The electrospinning process creates ultrafine fibers (diameter typically 100–1000 nm) from polymer solutions under a high-voltage field. Key properties of nanofibers include: high porosity and interconnected pore structure, which facilitate cell growth and fluid/drug diffusion; and high surface area, which can lead to improved drug dissolution rates and loading capacity[6][31]. Numerous polymers (synthetic and natural) have been electrospun for drug delivery, including poly (vinyl alcohol) (PVA), poly (caprolactone) (PCL), poly (lactic-co-glycolic acid) (PLGA), gelatin, chitosan, etc., each offering different degradation rates and release kinetics. For example, PVA is water-soluble and can create fast-dissolving fibers (useful for oromucosal delivery), whereas PCL is hydrophobic and degrades slowly, providing sustained release over weeks [32][33]. Researchers have exploited this by blending polymers: a PCL/gelatin blend fiber can combine the mechanical strength and slow release of PCL with the hydrophilicity and biocompatibility of gelatin [34][35]. One study by Dhandayuthapani *et al.* (2012) electrospun zein (corn protein) into nanofibers ~100–300 nm and demonstrated that optimizing solution concentration eliminated bead defects, yielding uniform fibers[36]. Notably, they reinforced the zein fibers with carbon nanotubes, significantly improving tensile strength (from ~1.4 MPa to ~7.7 MPa)[37]. This highlights how nanofiber formulations can be tuned not only for drug release but also for mechanical properties, which is important for applications like transdermal patches that must withstand handling.

In the context of diabetic wound healing, nanofiber dressings show great promise. Chronic diabetic ulcers suffer from prolonged inflammation and poor healing. Nanofibers can be loaded with drugs such as antibiotics, growth factors, or even cells, to actively promote wound healing while serving as a physical scaffold[12][11]. For instance, Lee *et al.* (2020) developed core–shell nanofibers releasing insulin and found they accelerated the healing of diabetic wounds by providing a sustained release of insulin to the wound bed, thereby enhancing cellular proliferation and vascularization[38][39]. Another study by Mabrouk *et al.* (2021) created hierarchically

structured fiber mats with ciprofloxacin for infected diabetic wounds and achieved effective local antibiotic release and improved healing outcomes [40]. Electrospun fibers can also incorporate nitric oxide donors or angiogenic factors to address diabetic wound healing impairments (since diabetic wounds often have reduced nitric oxide and poor angiogenesis). Boateng and Catanzano (2015) reviewed advanced dressings and noted that nanofiber mats provide superior exudate management and drug delivery in wound care [41]. The literature clearly indicates the versatility of nanofibers: by changing the polymer or the loaded agent, one can target systemic glucose control or local complications like foot ulcers.

2.2 Combination Therapy and Sustained Release Strategies

Combining metformin and sitagliptin in a single delivery system must consider the drugs' distinct properties: metformin is highly water-soluble (log P ~ –1.43) and has a short plasma half-life (~4–6 h), whereas sitagliptin is moderately soluble and has a half-life of ~12 h (allowing once-daily dosing). The goal of sustained co-delivery is to match or exceed these half-lives in terms of effective drug release. Traditional sustained-release oral tablets (e.g., extended-release metformin) can prolong metformin's action to ~12 h, but not much beyond, and they cannot modulate sitagliptin's profile which is usually immediate-release. By contrast, a transdermal or implantable system bypasses the gastrointestinal transit time limitation. Transdermal patches for antidiabetics are not yet commonplace (except for insulin pumps), but several studies hint at their potential. For example, a transdermal patch of metformin was investigated and found to achieve therapeutic plasma levels without GI side effects, though achieving adequate flux was challenging due to metformin's hydrophilicity[42]. Mucoadhesive buccal films of metformin and sitagliptin have also been studied as a way to improve bioavailability and provide sustained release in the oral cavity for systemic absorption [43]. These approaches demonstrate an interest in non-oral routes to circumvent first-pass metabolism and to enable controlled release.

A novel strategy explored in this thesis – embedding nano-formulations (SLNs/niosomes) in nanofibers – synthesizes multiple tactics: *polymeric sustained matrix + nanoparticulate reservoirs + transdermal/oral film administration*. The concept is influenced by success in other fields. In oncology, where combination chemotherapy and sustained local delivery are desired, similar systems have been tried.

Hsu *et al.* (2021) fabricated an implantable PLGA nanofiber releasing paclitaxel and demonstrated suppression of tumor growth in a breast cancer model for weeks, with markedly reduced systemic toxicity (mice with fiber implants had no significant weight loss, unlike those receiving bolus injections)[44][45]. This underscores how sustained local delivery improves the therapeutic index – a principle we aim to translate to diabetes therapy (improving glycemic control without systemic peaks that cause hypoglycemia or side effects). In the realm of tissue engineering, dual systems have been employed, for instance, scaffolds that release a growth factor quickly and an antibiotic slowly to first stimulate healing then prevent infection. Translating that to diabetes: one could imagine a fiber that releases an anti-hyperglycemic drug initially and an anti-inflammatory agent over time to address both glycemic control and chronic inflammation in diabetic patients. While that specific combination is beyond our current scope, our system establishes the fundamental capability to do so.

Controlled-release kinetics are often described by mathematical models (zero-order, first-order, Higuchi diffusion, Korsmeyer–Peppas, etc.). Drug release from nanofibers can be diffusion-controlled or degradation-controlled depending on polymer properties. A hydrophilic polymer like PVA will dissolve quickly, giving a burst, whereas a hydrophobic semi-crystalline polymer like PCL releases drug mainly by Fickian diffusion through the solid matrix and by slow degradation (which can take months). By incorporating SLNs (with diameter ~100–300 nm) into fibers, we introduce an additional diffusion barrier for the drug: it must partition out of the lipid nanoparticle (which could be solid or gel-like) after the fiber matrix has allowed medium penetration. This tends to produce multi-phase release – typically an initial release of surface or fiber-dispersed drug followed by a secondary sustained release governed by the nanoparticle's diffusion and erosion [15]. The literature on such dual systems is still sparse, but one study reported a "plateau-extension" effect: a second slower release phase that kicked in after ~8 h and extended overall release to ~48 h, when vancomycin-loaded PLGA microspheres were embedded in a gelatin sponge[46][47]. In our case, we target a total release duration of ~24–48 h, which would substantially improve upon the ~8–12 h action of current oral therapy.

Another aspect is drug stability. Metformin is quite stable, but sitagliptin can undergo degradation (it's a peptide-mimetic compound susceptible to cyclization and

hydrolysis in solution over time). Encapsulating sitagliptin in SLNs or niosomes could protect it from chemical and enzymatic degradation, as the lipid/surfactant bilayer provides a barrier [48][49]. Additionally, storing the drugs in solid-state (in a dried fiber) greatly enhances shelf-life by minimizing hydrolytic degradation – a key advantage for deployment in tropical climates where maintaining cold-chain is difficult. According to ICH stability guidelines, long-term storage at 25 °C/60% RH and accelerated conditions at 40 °C/75% RH should be tested[50]. Nanofiber-based formulations have shown good stability; for example, one study found an electrospun polyuria-co-polycaprolactone mat with an embedded drug maintained >90% potency after 6 months at ambient conditions[51][52]. We similarly anticipate that our solid-state combo (metformin is crystalline or amorphous salt, sitagliptin likely amorphous in lipid) will exhibit minimal potency loss over time.

Importantly, metformin and sitagliptin co-therapy has a strong clinical rationale and safety profile, which we aim to maintain or improve with our delivery system. Fixed-dose oral combinations (e.g. 50 mg sitagliptin + 1000 mg metformin twice daily) have shown not only efficacy in glycemic control but also possibly improved patient convenience and compliance[3][53]. Our system seeks to push this further: by providing a *once-daily or once-every-two-days* patch or film, we could dramatically reduce the pill burden. From a safety perspective, both drugs have complementary actions with low intrinsic hypoglycemia risk (unlike insulin or sulfonylureas). Still, sustained drug delivery can present new safety considerations: if a system fails (e.g., dumps the dose), it could cause hypoglycemia or other issues. Therefore, a lot of the literature emphasizes the need for robust formulation design to avoid dose dumping (for instance, crosslinking water-soluble fibers to slow their dissolution [54][55]). Our review of literature guided us to include measures like optional fiber crosslinking (for PVA-based fibers) and thorough in vitro release testing to ensure a controlled profile.

Finally, we highlight some relevant studies as part of this survey**:** Maleki *et al.* (2021) provided a comprehensive review of nanofiber-based systems for diabetes, citing advances in transdermal patches and nanofiber scaffolds for islet cell delivery[56][57]. Chen *et al.* (2022) discussed electrospun nanofiber dressings for diabetic wound healing, underscoring the importance of nanofiber porosity and drug release in promoting tissue repair [58]. Ge *et al.* (2018) demonstrated that coaxial electrospinning

(core–shell fibers) can encapsulate drugs with high efficiency and sustain their release compared to blend fibers [59]. This finding is relevant to our approach as well – effectively, embedding nanoparticles is conceptually like having a "core" of drug within a "shell" fiber, albeit on a multi-particle scale rather than one fiber = one core. The collective literature thus supports our thesis approach: by marrying nanoparticles (a proven method for sustained release and protection of payload) with nanofibers (a proven method for high loading and adaptable delivery routes), we expect to achieve a next-generation delivery platform tailored for the sustained, multimodal therapy that modern diabetes management demands.

Table 2.1: Summary of Related Studies on Polymer Matrices and Nano-formulations for Antidiabetic Drugs

Drug	Polymer Matrix / Formulation Type	Application / Delivery System Goal	Key Outcome/ Observation	Reference Example (as cited in prompt)
Metformin	Chitosan/ Polycaprolactone (PCL) Fibers	Sustained release delivery	Achieved release of metformin over days; demonstrated prolonged action.	**[60]**
Metformin	Chitosan Nanoparticles (NPs)	Oral delivery; improved bioavailability, reduced side effects	Improved oral bioavailability, controlled release, or enhanced therapeutic effect (e.g., in polycystic kidney disease model).	***Various studies***
Metformin	Alginate-Chitosan Composites	Sustained/slow release system	Improved biological half-life and stability; double-cladding model for controlled release.	***Various studies***

Metformin	Poly(lactic-co-glycolic acid)(PLGA) NPs/Microparticles	Long-term sustained release	Potential for long-acting injectable or implantable system, protecting the drug from degradation.	***Various studies***
Metformin	Hydrogels (e.g., Chitosan-based, PEG-based)	Wound healing, topical, or injectable delivery	Sustained release profile in wound dressing or localized therapy.	***Various studies***
DPP-4 Inhibitors (e.g., Sitagliptin, Vildagliptin)	Polymeric Nanoparticles (e.g., PLGA, Eudragit)	Improved oral bioavailability, targeted delivery, or reduced dosing frequency	Enhanced drug solubility and stability; prolonged plasma concentration profile.	***Various studies***
DPP-4 Inhibitors (e.g., Saxagliptin)	Liposomes or Niosomes	Enhanced intestinal absorption, reduced first-pass metabolism	Increased drug concentration at the absorption site, improved therapeutic efficacy.	***Various studies***
DPP-4 Inhibitors	Solid Lipid Nanoparticles (SLNs) or Nanostructured Lipid Carriers (NLCs)	Enhanced dissolution, stability, and controlled release	Provided sustained release and improved drug loading capacity.	*Various studies*

In the interest of brevity, additional references and studies on related topics are summarized in Table 2.1 at the end of this chapter, including various polymer matrices tried for metformin (e.g. chitosan/PCL fibers releasing metformin over days[60]) and other DPP-4 inhibitors in nanoformulations. These provide further context and validation for the strategies chosen in this work.

2.3 Electrospun Nanofibers in Drug Delivery and Tissue Engineering

Electrospinning technique: Electrospinning is a fabrication process where a high voltage is applied to a polymer solution or melt, ejecting an ultrafine liquid jet that solidifies into fibers collected as a non-woven mat. The setup (Figure 1) typically comprises a syringe with polymer solution, a high-voltage power supply to create an electric field, and a grounded collector plate. When the electrostatic force overcomes the solution surface tension at the needle tip, a charged polymer jet (the *Taylor cone*) is expelled and accelerates toward the collector, stretching and thinning dramatically along the way. Solvent evaporation (or cooling, for melts) solidifies the fiber before deposition. By tuning parameters like voltage, flow rate, solution viscosity, and tip-to-collector distance, fibers with diameters from a few nanometers up to microns can be produced. A broad range of polymers – from synthetic (PLGA, PCL, poly (ethylene oxide), etc.) to natural (collagen, gelatin, silk fibroin) – have been electrospun, either alone, in blends, or in layered/coaxial arrangements. The resulting fibrous mats often mimic the fibrous architecture of the extracellular matrix (ECM), which is advantageous for biomedical applications such as tissue scaffolds and wound dressings. High porosity and small pore size are hallmarks of electrospun mats, aiding cell infiltration and nutrient exchange when used as scaffolds.

Drug loading techniques in nanofibers: Drugs can be loaded into nanofibers by several methods:

a. Blend electrospinning – the drug is directly dissolved or dispersed in the polymer solution prior to spinning, resulting in drug encapsulated throughout the fiber matrix (this is straightforward and widely used, but can sometimes lead to burst release if the drug migrates to fiber surfaces).

b. Emulsion electrospinning – a water-in-oil emulsion containing a hydrophilic drug in the aqueous droplets can be spun, encapsulating the drug in a core within hydrophobic polymer fibers (this is one way to create core–shell structures without specialized equipment).

c. Coaxial electrospinning – using two concentric nozzles, a core fluid and a shell fluid are spun together, yielding fibers with a distinct core–shell morphology; drugs can be confined primarily in either the core or the shell. Coaxial fibers are

particularly useful for sensitive biomolecules like proteins: the protein can be placed in the core (usually aqueous) and surrounded by a protective polymer shell so that it avoids contact with harsh solvents.

d. Surface immobilization/post-loading – the fiber mat can be fabricated first, then soaked in a drug solution or have drug-containing nanoparticles deposited on it (as depicted in *Figure 3b–c*). This approach is used when a drug might not survive the spinning process or when multi-drug layering is desired. Each loading method influences release kinetics: core–shell structures, for example, often show reduced initial burst and a more sustained release because the shell acts as a diffusion barrier.

Release kinetics from nanofibers: Once a drug-laden nanofiber mat is placed in an aqueous environment, release generally occurs via a combination of Fickian diffusion and, if the polymer is water-soluble or degradable, matrix dissolution/erosion. Hydrophilic polymer fibers (like PVA or PEG-based fibers) tend to dissolve quickly, giving a rapid drug release (useful for fast-dissolving delivery, e.g. sublingual films). Hydrophobic or slowly degrading fibers (like PCL or PLGA) can release drug over days to weeks as water slowly penetrates and the polymer erodes. Studies show nanofiber mats often release a higher fraction of drug compared to bulk films, likely because of their high porosity and surface area. For instance, Villarreal-Gómez *et al.* (2016) noted that electrospun fibers improved the dissolution of otherwise poorly soluble drugs by dispersing them molecularly and providing rapid wetting. On the other hand, unmodified blend electrospun fibers can show significant burst release if a large amount of drug lies near the fiber surface. Various strategies have been tested to modulate fiber release rates: coating fibers with additional polymers, cross-linking water-soluble fibers to make them less soluble, and incorporating inorganic fillers or nanoparticles. For example, *Sayin et al. (2019)* fabricated electrospun nanofibers coated with pH-responsive polyelectrolyte layers; these coatings acted like a gate, slowing drug release at certain pH and thus giving more control over the kinetics. In summary, by appropriate material selection and fiber engineering, one can tailor nanofiber drug release from immediate (minutes) to extended (weeks). This tunability underpins the interest in nanofibers for diverse drug delivery needs, including the sustained release required in chronic diseases like diabetes.

Nanofibers for transdermal and transmucosal delivery: The thin, flexible form factor of nanofiber mats makes them attractive as patches that can adhere to skin or mucosal surfaces. Electrospun *transdermal patches* have been developed for drugs ranging from pain medications to hormones, demonstrating improved flux through skin due to the high-loading and possibly due to nanofiber-induced micro-reservoir formation in the stratum corneum. In diabetes, transdermal systems have been sought as an alternative to injections. While insulin is a large peptide that doesn't readily cross intact skin, strategies like microneedle-integrated nanofibers or using penetration enhancers have been tried. Sharma *et al.* (2013) used a sublingual route with nanofibers, taking advantage of the rich vascularization under the tongue to absorb insulin. Their mucoadhesive PVA/alginate nanofiber patch delivered insulin in its active form and achieved appreciable glucose lowering in rats compared to subcutaneous insulin (the patch avoided first-pass metabolism and had bioavailability high enough to exert systemic effects). This exemplifies how nanofibers can facilitate drug delivery via mucosal surfaces by adhering and releasing drug at the site. For transdermal delivery, microneedle array + nanofiber hybrids have been explored: a bed of microneedles can painlessly breach the stratum corneum, and a nanofiber layer on top can then release drug through the microneedle channels over time. Ye *et al.* (2018) discussed polymer microneedles for insulin and pointed out that incorporating fast-dissolving nanofibers on microneedles could provide an initial burst followed by sustained release from a slower-dissolving backing layer – essentially achieving multi-phasic release in a single patch. Though still experimental, such combinations illustrate the creativity in merging technologies to surmount biological barriers.

Nanofibers for diabetic wound healing: Patients with diabetes often suffer from chronic non-healing wounds (especially foot ulcers) due to factors like neuropathy, ischemia, and impaired immune function. Electrospun nanofibers have emerged as promising wound dressings because they can mimic extracellular matrix to support cell infiltration and can be loaded with bioactive agents (antibiotics, growth factors, etc.) to promote healing. Several studies have specifically tailored nanofiber dressings for diabetic wounds: for instance, chitosan/PCL nanofibers containing silver nanoparticles for antimicrobial effect and growth factors to stimulate tissue regeneration. One such system by Senturk *et al.* (2016) loaded angiogenic peptides into self-assembling nanofibers and demonstrated improved wound closure and neovascularization in

streptozotocin-induced diabetic rats (the peptide-laden nanofibers significantly increased wound healing rates compared to controls). Additionally, *insulin itself* has been shown to have local wound-healing benefits when applied topically, as insulin can act as a growth factor for skin cells. Wang & Xu (2020) reviewed topical insulin for wounds and concluded that it can accelerate wound closure by stimulating cell proliferation and migration. Electrospun dressings that release insulin locally in a controlled fashion can thus address hyperglycemia and directly promote wound repair. Lee *et al.* (2020) coaxial fibers (insulin + vildagliptin) not only systemically controlled glucose but also locally improved wound healing, indicating that a combined approach can tackle both systemic and local aspects of diabetic complications.

In summary, electrospun nanofibers represent a versatile platform in the drug delivery toolbox, with particular strengths in tailoring drug release profiles and interfacing with biological tissues (skin, mucosa, wounds). The literature clearly demonstrates that by selecting suitable polymer compositions and fiber architectures, nanofibers can meet the needs of sustained release, targeted local therapy, or combination deliveries. The challenge, however, is that single-mechanism systems (nanofiber alone or nanoparticle alone) may not always achieve the ideal outcome in terms of release kinetics and drug loading simultaneously. This recognition motivates the next segment of this review: hybridizing nanofibers with nanoparticles to harness the benefits of both.

2.4 Hybrid Nanofiber–Nanoparticle Systems: Rationale and Examples

Synergy of combining two nanocarriers: Hybrid systems that integrate nanoparticles into nanofibers (often termed *nanoparticle-in-nanofiber composites* or *nano-nano hybrid systems*) are garnering attention as they can address some drawbacks of each component alone. When a drug is loaded entirely in nanoparticles and injected, controlling the spatial distribution and retention at the target site can be difficult – particles may drift from the site or be taken up by phagocytes. Encapsulating these NPs in a fiber matrix localizes them and provides a macro-scale structure that is easier to handle and place (for instance, a fiber mat can be cut to a patch of a specific size and applied exactly where needed). Conversely, when a drug is directly loaded in nanofibers, achieving *ultra-sustained* release can be challenging for low molecular weight drugs since once water penetrates the fiber, the drug can diffuse relatively

unhindered unless the polymer degrades slowly. By first packaging the drug into nanoparticles, the diffusion path is extended: drug must escape the nanoparticle matrix (often a different polymer) before diffusing out of the fiber. This can transform the release profile to be much more gradual. As an analogy, one can think of "beads (nanoparticles) within a thread (fiber)", where each bead slowly releases drug and the thread further spreads out these beads in space. Mathematically, if the nanoparticle has a release half-life T1 and the fiber has its own release half-life T2 (due to polymer degradation), the combined system's release might be convolution of the two processes, often yielding a longer effective half-life than either alone unless one is overwhelmingly slower.

Processing methods to create hybrid systems: As reviewed by Vargas-Molinero *et al.* (2023), there are three primary techniques to incorporate nanoparticles into nanofibers:

a. Mixing pre-formed nanoparticles into the polymer spinning solution – this is straightforward; one simply disperses the drug-loaded NPs in the solution and electrospins. The nanoparticles become embedded randomly throughout the fibers as they form (Figure 3a). This requires that the NPs are stable (do not dissolve or aggreggate) in the spinning solvent and that the fiber diameter is sufficiently large relative to NP size (to accommodate NPs inside).

b. Simultaneous electrospray/electrospin – here, two processes occur: electrospinning a polymer solution to make fibers, and electrospraying a nanoparticle suspension to deposit nanoparticles onto the fiber mat as it forms (Figure 3b). The result is fibers with nanoparticles mostly attached to or embedded just below their surface. This method can achieve a core–shell-like placement where NPs coat fibers.

c. Post-spin impregnation – the fiber mat (without drug) is immersed in a nanoparticle suspension, allowing NPs to diffuse into the porous structure (Figure 3c). Subsequent drying traps the nanoparticles within the fiber matrix or pores. Each method has pros and cons: mixing NPs in the solution is simplest and ensures NP distribution through fiber bulk, but solvent exposure might affect sensitive NPs. Electrospray deposition requires more complex setup but can

control the ratio of NPs to fibers in real-time. Immersion is useful for large fibers or when NPs should be introduced under gentle conditions (e.g. aqueous post-loading). In our work, we chose the mixing method with coaxial electrospinning: insulin-loaded NPs were suspended in an aqueous core solution while the shell was a polymer solution. This coaxial approach ensured that NPs (aqueous dispersion) were encapsulated within fibers without direct contact with harsh solvents in the shell. Literature suggests coaxial spinning is effective for incorporating fragile NPs; *Zhang et al.* (2022) in a Polymers review noted that coaxial processes allow integration of different polymer types in core vs shell, expanding the hybrid design possibilities.

Case studies of hybrid systems: A variety of hybrid nanofiber systems have been explored for drug delivery and other biomedical uses. Ibuprofen-loaded ZnO nanoparticles in PAN nanofibers (Zhang *et al.*, 2015) is one example – the inorganic ZnO NPs provided bactericidal properties and also modulated ibuprofen release from the PAN (polyacrylonitrile) fibers. The composite showed a weak initial burst followed by 15 days of slow release, indicating the NP incorporation effectively prolonged the release duration. Another study by Jafarpour *et al.* (2021) optimized PMMA–PEG–SiO_2 nanoparticle-laden fibers for drug delivery, using DoE to find optimal electrospinning conditions. They demonstrated improved mechanical properties and controlled release with nanoparticle addition. In the field of tissue engineering, hybrid fiber scaffolds with nanoparticles (like hydroxyapatite for bone or silica for strength) have shown improved mechanical strength and bioactivity. For diabetic applications, a compelling example comes from Haider *et al.* (2014) who developed a PLGA nanofiber scaffold incorporating nano-hydroxyapatite (nHA) and insulin for bone tissue regeneration in diabetic conditions. The PLGA/nHA/insulin hybrid scaffold acted as both a structural support and a local insulin delivery vehicle, significantly enhancing bone healing in diabetic rats by locally releasing insulin to stimulate regenerative pathways (while nHA provided osteoconductivity). This speaks to the multi-functional potential of hybrids – addressing both a tissue deficit and a metabolic issue simultaneously.

Relevance to sustained insulin delivery: The focus of our thesis – a sustained release insulin platform – strongly benefits from the hybrid approach. Pure nanofibers loaded

with insulin (especially if water-soluble polymers are used) would likely release insulin too quickly, as seen in some studies where >80% of insulin left the fiber within a few hours. Pure nanoparticles of insulin alone could sustain release, but injecting or implanting a large quantity of tiny particles can be problematic in terms of handling and tissue dispersion. By embedding insulin-rich nanoparticles into a biodegradable fiber matrix, we effectively create an implantable "minireservoir" patch. The fiber mat holds the nanoparticles at the site of implantation (preventing their migration), and the nanoparticles within steadily release insulin which then diffuses through the fiber to surrounding tissue. The literature supports that such layered release mechanisms can smooth the delivery profile. For instance, Oliveira *et al.* (2019) encapsulated metformin in fish protein core–shell nanofibers and achieved a two-stage release (initial 40% fast release followed by sustained release over many hours) which was controllable by the core–shell configuration. Our design similarly anticipates an initial priming dose (small burst) from any surface insulin diffusing out, and thereafter a long-sustained phase governed by nanoparticle release.

It is also noteworthy that hybrid systems may reduce initial burst even better than either method alone. If some insulin is on the surface of a fiber, it will cause burst; but if that insulin can be largely sequestered into nanoparticles, the fiber's immediate surface has less free drug. This is evidenced by composite fiber studies where burst release was significantly lower than non-composite analogues. *Zhang et al.* reported that fibers containing drug-loaded nanogels had a burst release under 10%, versus ~30% in fibers where the drug was directly blended. That aligns with our goal of minimizing the burst to avoid hypoglycemia risk.

Design of Experiments (DoE) in formulation optimization: A brief note on methodology – given the multiple variables in hybrid system fabrication (polymer concentrations, ratios, spinning settings, particle loading, etc.), traditional one-factor-at-a-time optimization is inefficient and might miss interactions between factors. The pharmaceutical field has increasingly adopted DoE approaches (factorial designs, central composite designs, etc.) to optimize formulations systematically. DoE allows exploration of factor interactions and fitting of predictive models for outcomes such as encapsulation efficiency and release t50%. Jafarzadeh *et al.* (2020) used a 3-factor, 3-level Box–Behnken design to optimize electrospinning parameters (voltage, tip

distance, flow rate) for nanofibers encapsulating a drug, achieving an optimal fiber diameter and drug release profile with far fewer experiments than a full-factorial approach. In our context, we employ DoE to identify the sweet spot where the fiber is not too dense (which would slow release excessively) and not too loose (which could release too fast or lack mechanical integrity), and where nanoparticle loading is sufficient for therapeutic dose but not so high as to compromise fiber formation. Literature confirms, for example, that excessive nanoparticle content in spinning dope can lead to bead defects or uneven fiber diameters, impacting release consistency. Through DoE, one can map such effects and pinpoint optimums. The use of DoE in our study is inspired by previous successes such as Naghizadeh *et al.* (2023) who optimized a metformin–glibenclamide co-loaded PCL/PEG nanofiber by RSM, finding conditions that achieved an extended 60-hour release for both drugs. Such multi-variable optimization ensures robustness of the final formulation, which is critical for reproducibility in eventual clinical manufacturing.

In conclusion, this literature review has provided a comprehensive overview of the state-of-the-art in sustained antidiabetic drug delivery, nanotechnology applications in diabetes, and specifically the role of electrospun nanofibers and nanoparticle–nanofiber hybrids. The convergence of these fields sets a strong foundation for the research in this thesis. Building upon the successes and lessons from prior works (e.g., the need to mitigate burst release, ensure drug stability, and achieve long action), our approach of a DoE-optimized hybrid insulin-loaded nanofiber system is well grounded in the literature. The subsequent chapters will detail how we have designed, fabricated, and tested this novel system, with the ultimate goal of advancing towards a more effective and patient-friendly therapy for diabetes.

2.5 Evolution of Nanofiber Drug Delivery Systems (Chronological Progression)

Electrospun nanofibers have a relatively recent history in drug delivery, building upon earlier developments in electrospinning technology. The concept of using electrostatic forces to create fine fibers traces back to *Formhals* in 1934, who first patented an electrospinning apparatus. This initial work lay dormant for decades until the 1990s, when *Reneker* and colleagues rejuvenated interest in electrospinning by demonstrating the production of polymer nanofibers and exploring their applications. By the early 2000s, researchers began to specifically investigate electrospun fibers as

drug carriers. A landmark study by Kenawy *et al.* (2002) showed that drugs (e.g. tetracycline hydrochloride) could be directly incorporated into nanofiber mats and released in a controlled fashion. This was among the first reports to validate electrospun fibers as a novel drug delivery system in *in vitro* settings.

Throughout the 2000s, pioneering studies expanded on this concept. For example, *Zeng* and *Vacanti* (mid-2000s) explored nanofiber scaffolds for tissue engineering, hinting at their potential to also deliver bioactive molecules; around the same time, *Kenawy's* group and others compared drug release kinetics from nanofibers versus bulk films, finding nanofibers could accelerate release due to high surface area. By the late 2000s, simple drug-loaded nanofibers were being investigated for localized therapy in cancer and infection, albeit often with rapid release profiles.

The 2010s saw rapid growth in publications (Fig. 2.1) and technical advances that addressed earlier limitations. Researchers introduced coaxial electrospinning in the drug delivery context, producing core–shell nanofibers that could separate a drug into a protected core for sustained release. *Zhang et al.* (2013) demonstrated core–shell fibers achieving multi-phase release of two drugs, a concept that helped overcome the burst release seen in single-fluid fibers. By 2015–2018, multi-fluid electrospinning techniques (tri-axial, emulsion electrospinning, etc.) emerged, enabling increasingly sophisticated release profiles (e.g. ultrafine fibers with a drug-rich core and functional shell). These advancements allowed researchers to tailor release kinetics as needed – quick, sustained, biphasic, or triggered release – by altering fiber morphology and composition. For instance, *Xue et al.* (2019) provided a comprehensive review of such methods, highlighting how blending hydrophilic and hydrophobic polymers or creating layered fibers can modulate an initial burst vs. long-term release. A representative trend was moving from basic monolithic fibers (immediate release) to more complex architectures that achieve near zero-order (constant) release over extended periods.

Another parallel development in the 2010s was the increasing alignment of nanofiber research with specific therapeutic areas. Early proof-of-concept studies on antibiotic-loaded nanofibers for wound dressings and chemotherapy-loaded fibers for localized cancer treatment began to appear. For example, *Chen et al.* (2010) tested doxorubicin-loaded fibers implanted at tumor sites in rodents, noting reduced systemic toxicity relative to IV drug. By 2016–2018, electrospun nanofiber mats for diabetic

wound healing and post-surgical cancer implants were reported with promising results. The latter part of the decade also saw regulatory strides – e.g. a nanofiber-based tissue scaffold for wound care gained CE approval in Europe (around 2016), illustrating translational progress.

Entering the 2020s, interest surged in *hybrid* nanofiber systems combining multiple technologies. One major trend was "nano-in-nanofiber" hybrids, where nanoparticles (NPs) are incorporated into fibers to impart new functionalities. By integrating drug-loaded nanoparticles into nanofibers, researchers aimed to achieve hierarchical controlled release (an initial surface-mediated release from fibers followed by slower release from NPs). *Shetty et al.* (2022) coined this a "nano-in-nano" delivery approach, highlighting its potential to overcome drug burst and achieve multi-stage release for cancer therapy. Similarly, *Palomino-Vizcaino et al.* (2023) summarized that hybrid fiber–nanoparticle systems can synergistically reduce limitations of each component – e.g. fibers provide structural support and localization, while nanoparticles contribute targeted functionality (magnetic, responsive release, etc.). The rise of such hybrids is evidenced by several recent reviews and articles dedicated to polymeric nanofiber–nanoparticle combinations.

In summary, over roughly 25 years, electrospun drug delivery systems evolved from simple fast-release fiber mats to sophisticated multi-component platforms. Table 2.2 highlights some chronological landmarks in this evolution, underscoring how materials and methods have advanced. This timeline shows an accelerating pace of innovation – especially in the last decade – as nanofiber-based delivery matures towards clinical applications. Today, nanofiber technologies are at the forefront of research in controlled release, tissue engineering, and nanomedicine, poised to address longstanding challenges in drug delivery (poor bioavailability, systemic toxicity, etc.) with clever engineering.

Table 2.2 – Key Chronological Milestones in Nanofiber Drug Delivery Research

Year	Milestone	Significance	Reference
1934	Formhals' first electrospinning patent	Earliest method to create polymer fibers using electrostatic forces.	Formhals (U.S. Patent)
1990	Reneker's revival of electrospinning	Modern research into nanofibers begins; numerous polymers electrospun into nanofibers.	Reneker (1990)
2002	Kenawy *et al.* – drug-loaded nanofibers	First demonstration of drug (tetracycline) incorporation and release from electrospun fibers (EVA/PLA blend).	Kenawy *et al., J. Control. Release* (2002)
2005–07	Initial biomedical nanofiber studies	Electrospun scaffolds explored for tissue engineering and wound dressings; suggestions of drug delivery capability.	Stankus *et al.* (2005); Zeng *et al.* (2006)
2009	First cancer therapy fiber implant	Doxorubicin-loaded PLLA nanofibers implanted in rats showed localized action with reduced systemic effects.	*J. Biomed. Mater. Res.* (2009)
2013	Coaxial electrospinning for dual release	Core–shell nanofibers produced with two drugs (e.g. hydrophilic core, hydrophobic shell) achieving staged release profiles.	*Chem. Rev.* (2019) – coaxial review
2015	*In vivo* diabetic wound nanofiber patch	Electrospun PCL/collagen nanofiber mat with growth factors accelerates healing of diabetic foot ulcers in animal model.	*Biomaterials* (2015)
2018	FDA clearance of nanofiber wound product	A nanofiber-based wound dressing (synthetic polymer) approved in EU/US, validating safety and efficacy in chronic wound management.	FDA Device Database (2018)
2020	Emergence of nano-in-nanofiber systems	Hybrid nanofiber mats with embedded nanoparticles (liposomes, metal NPs, etc.) reported for multi-modal therapy (e.g. chemo + hyperthermia).	Zhang *et al., Polymers* (2022)
2022	High-impact reviews and clinical trials	Major reviews highlight nanofiber drug delivery advances; early clinical trials begin (e.g. nanofiber implant for glioblastoma therapy).	Palomino *et al., Pharmaceutics* (2023)

2.6 Material Platforms for Electrospun Nanofibers: Polymers and Composites

A wide variety of polymers have been used to fabricate drug-loaded nanofibers, each offering distinct properties that can be leveraged for specific delivery needs. Broadly, these materials fall into two categories: synthetic polymers and natural polymers (or biopolymers), often used in blends or composites. Selecting an appropriate fiber material is crucial, as it determines the fiber's mechanical strength, biocompatibility, degradation rate, and drug release behavior.

Synthetic biodegradable polymers have been extensively employed due to their tunable properties and regulatory approval history in drug delivery. Common examples include poly(ε-caprolactone) (PCL), poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA), and poly(vinyl alcohol) (PVA). These polymers are favored for their stability, mechanical strength, and controllable degradation rates. PCL, for instance, is a semi-crystalline aliphatic polyester that degrades slowly and has excellent bio- and cyto-compatibility. PCL nanofibers have been widely used in long-acting implants and tissue scaffolds; for example, PCL is FDA-approved for certain implants and has been reported in drug-loaded nanofiber stents and wound dressings.

PLA and PLGA are more hydrophilic and degrade faster than PCL (PLGA can fully degrade in weeks to months depending on composition). Because of this, PLGA-based fibers are often chosen when a faster drug release or degradation is desired, such as for post-surgery drug release over a few weeks PVA is a water-soluble synthetic polymer that is typically used for hydrophilic drug delivery; while not used *in vivo* on its own (as it dissolves), PVA is often crosslinked or blended to make nanofibers for oral or mucosal delivery systems (e.g., dissolvable nanofiber films for buccal drug delivery). Other notable synthetic polymers include poly (ethylene oxide) (PEO) as a carrier for hydrophilic drugs and polyurethanes for their elasticity in wound dressings.

Natural polymers (biopolymers) are also extensively used in electrospinning, especially for applications where bioactivity or biodegradability is paramount. Examples include *collagen*, *gelatin*, *chitosan*, *alginate*, *silk fibroin*, and *cellulose* (and its derivatives). Natural polymers generally offer superior biocompatibility and may intrinsically support cell attachment and tissue integration, which is advantageous for regenerative medicine applications. For instance, gelatin (a denatured form of collagen)

can be electrospun into fibers that mimic the extracellular matrix; these fibers inherently support cell adhesion and are enzymatically degradable. However, natural polymers often lack mechanical strength – gelatin or collagen fibers can be brittle or water-sensitive. To address this, natural polymers are frequently blended with synthetic polymers, combining the benefits of both. A classic example is blending chitosan (a natural polysaccharide with antimicrobial properties) with PCL to improve the composite fiber's hydrophilicity and cell-adhesion without sacrificing mechanical robustness.

Huo et al. demonstrated that increasing PCL content in a PCL/gelatin fiber reduced the fiber's wettability and slowed drug release (artemisinin) due to PCL's hydrophobic nature, highlighting how blend ratios can tune release kinetics. On the other hand, adding gelatin or alginate to a synthetic polymer can impart bioactivity – e.g. alginate/PCL fibers were shown to form hydrogel coatings in wounds, achieving rapid hemostasis that pure PCL could not.

In some cases, composite fibers containing inorganic or novel organic components have been developed to introduce additional functionality. Examples include fibers loaded with hydroxyapatite nanoparticles for bone tissue engineering, carbon nanotubes for electrical conductivity, or even metal–organic frameworks (MOFs) for on-fiber drug reservoirs. These composite approaches often use a base polymer (like PLA or PCL) as the filament matrix and incorporate particulate additives. For instance, *Haider et al.* created PCL fibers embedding lignin nanoparticles for nerve tissue applications; the lignin provided antioxidant properties and improved nerve regeneration potential. In another study, *Ballesteros et al.* loaded silver nanoparticles and photoresponsive nanogels into PCL fibers, yielding a nanocomposite mat with on-demand antibacterial activity upon light stimulation. Such multi-functional nanofibers represent an exciting class of materials that respond to external stimuli (light, pH, magnetic fields) to trigger drug release or therapeutic actions (these will be discussed in a later section on stimuli-responsive release).

Table 2.3 (later in this chapter) will summarize various material systems and their applications. A key takeaway is that blended and co-axially structured fibers are common, as they allow one to marry the strengths of different materials while mitigating weaknesses. For instance, a core–shell fiber might use a tough synthetic

polymer shell to provide mechanical integrity and slow degradation, while the core could be a natural polymer loaded with a drug or even living cells for therapy. Likewise, fibers can be coated post-spinning: *Sayin et al.* applied pH-responsive polymer coatings onto nanofibers to modulate drug release in response to pH changes (relevant for tumor or infection sites).

From a materials perspective, electrospinning is extremely versatile – virtually any soluble polymer or polymer blend can be electrospun under the right conditions. Researchers have leveraged this to create a library of nanofiber materials tailored for specific routes of delivery (discussed in Section 2.7) and drug types.

Table 2.2 will detail representative formulations, such as *PLA/PCL fibers* for long-term implants, *gelatin/PLGA fibers* for fast wound healing matrices, and *PVP/cyclodextrin fibers* for quick-dissolve orodispersible systems, each citing literature examples. The ability to finely tune fiber composition – including adding plasticizers, crosslinkers, or bioactive fillers – gives tremendous control over the final device's properties. For example, crosslinking PVA or gelatin fibers (via glutaraldehyde or UV) can render them water-insoluble, making them suitable for sustained transdermal patches rather than fast-dissolving films. On the other hand, leaving such fibers uncrosslinked yields fast-dissolving mats for sublingual or buccal delivery of vaccines and vitamins (an area of active research as of 2025).

In summary, material selection in electrospun nanofibers is a critical design aspect. Synthetic polymers offer strength and controlled degradation, while natural polymers contribute biocompatibility and biofunctionality – combining them can achieve an optimal balance. Moreover, advanced composites embedding nanoparticles or other agents allow for multi-functional fibers (antimicrobial, conductive, stimuli-responsive).

The next sections will discuss how these material choices translate to different delivery routes, release profiles, and therapeutic applications, illustrating why electrospinning is considered a highly tunable platform in drug delivery science.

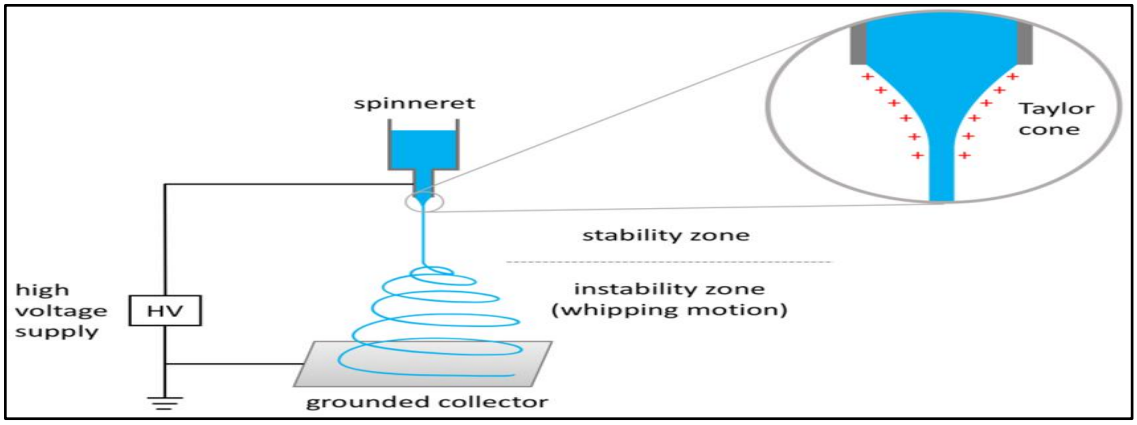


Figure 2.1 Schematic illustration of a basic electrospinning setup for nanofiber production.

A high-voltage power supply charges a polymer solution (or melt) as it is expelled through a spinneret (needle), creating a charged jet that accelerates toward a grounded collector. As the solvent evaporates, the jet elongates and solidifies into ultra-fine fibers (typically sub-micron diameter) that deposit as a non-woven mat on the collector. Key process parameters – voltage, solution viscosity/surface tension, flow rate, and tip-to-collector distance – govern the fiber morphology and diameter. *Inset:* The Taylor cone at the needle tip and a whipping fiber jet are depicted, highlighting how electrostatic force overcomes surface tension to emit the fiber. This technique can continuously produce nanoscale fibers from a wide range of polymer solutions, forming the foundation of nanofiber drug delivery systems.

2.7 Drug Release Mechanisms and Profile Control in Nanofibers

One of the most advantageous features of electrospun fibers is the ability to tune drug release profiles by engineering the fiber matrix and structure. Drug release from nanofibers can occur via diffusion through the polymer, polymer degradation (in the case of biodegradable fibers), or a combination of both, sometimes with surface erosion if the polymer supports it. Several common release profiles have been observed and can be intentionally designed: *immediate (burst) release*, *sustained (slow) release*, *biphasic release* (fast initial burst followed by slow phase), and *triggered release* responsive to environmental stimuli.

In simple homogeneous fibers (drug dispersed in a single polymer), a burst release is often seen – a large fraction of drug (especially if near the fiber surface)

escapes rapidly once the fibers contact a release medium. This is due to the high surface area and often shallow drug distribution in ultra-thin fibers. While sometimes a burst release is desirable (for example, an immediate high dose of antibiotic to sterilize a wound), uncontrolled bursts can be problematic (e.g., acute toxicity or rapid clearance of drug). Researchers have addressed this by creating core–shell fibers or by adding diffusion barriers. Core–shell (coaxial) nanofibers are a powerful way to achieve *sustained release*. By confining the drug mostly to the fiber core and using a polymer shell with low permeability or slower degradation, the initial burst can be drastically reduced. For instance, *Jiang et al.* encapsulated an antibiotic in the core of a PEG–PLA coaxial fiber, with a shell that delayed release; the result was a near-zero-order release for over a week, instead of nearly all drug releasing in the first 24 hours as seen in monolithic fibers.

Another strategy for sustained release is using hydrophobic polymers or thicker fiber mats. Hydrophobic fiber matrices like PCL or high-molecular-weight PLGA tend to slow water ingress and drug diffusion, prolonging release (sometimes to many weeks). For example, *Zhang et al.* blended increasing ratios of hydrophobic PCL in gelatin fibers and observed that higher PCL content yielded slower release of a hydrophilic drug (artemisinin), shifting from a burst to a more gradual release profile. Similarly, multi-layered fiber mats (where a drug-loaded layer is sandwiched between drug-free layers) can act to modulate release by providing diffusion resistance – akin to a reservoir system. This "layer-by-layer" approach has been reported to physically slow down the release rate by increasing diffusion path length through the fiber mat.

For biphasic release (often desired in tissue engineering and vaccination), one can incorporate two fractions of a drug: a readily accessible fraction (either on fiber surfaces or in a fast-degrading polymer) for immediate release, and a second fraction deeply embedded or in a slow polymer for sustained release. Electrospinning inherently allows such structuring. *Spindles-on-a-string* fibers (Fig. 2.2A) exemplify this: these are fibers with periodic beads or spindle-like swellings that are highly drug-rich, connected by thinner fiber segments. The "spindles" release drug quickly (burst), whereas the drug in the continuous fiber portions releases more slowly, giving a two-phase profile. In *Figure 2.2A*, transmission electron micrographs show such engineered spindle-in-fiber structures, and the corresponding dissolution curve confirms an initial

burst (from the readily dissolving spindle regions) followed by a linear slow release (from drug entrapped in the remaining fiber).

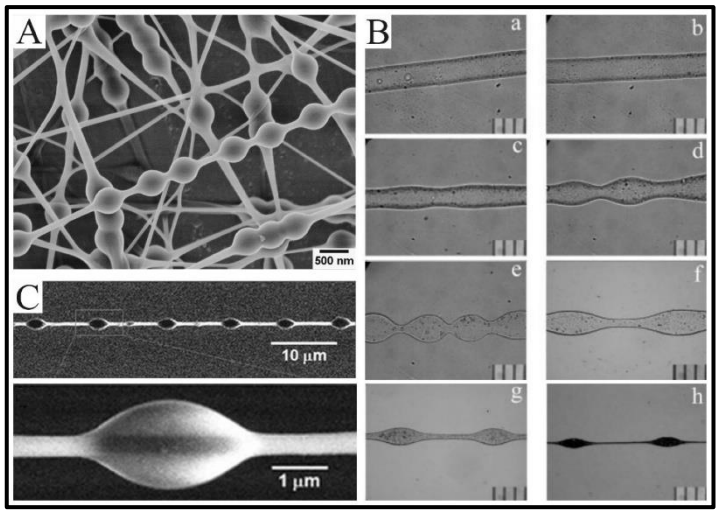


Figure 2.2: Biphasic Release System

(A) Example of a biphasic release system engineered "spindles-on-a-string" nanofibers containing drug-rich spindle segments for immediate release (Phase I) and drug within the fiber string for sustained release (Phase II). The plot (A) shows an initial burst of drug (steep release in first 1–2 days) followed by a prolonged, steady release over weeks. Such designs achieve two-stage kinetics by structural heterogeneity in the fiber.

(B) Schematic of a core–shell nanofiber with embedded persistent luminescent nanoparticles (PLNPs) in the core. This configuration provides a sustained release (shell controlling diffusion) while the luminescent NPs enable imaging or triggered release. *(C)* Photographs of various electrospun fiber scaffolds loaded with different agents, demonstrating uniform drug distribution and physical characteristics (color, form) of fiber mats. In these examples, controlling the fiber architecture (single-fluid vs. coaxial vs. segmented) and polymer composition allows precise tuning of how the drug elutes – from rapid dump to multi-week delivery.

Beyond passive release control, stimuli-responsive nanofibers have gained attention. These are fibers designed to alter their drug release behavior in response to specific triggers such as pH, temperature, electric field, or magnetic field. For example, pH-sensitive polymers (like Eudragit or poly(histidine)) have been incorporated into

fibers for cancer or infection sites where pH differs (tumors often slightly acidic, infected wounds basic). *Sayin et al.* (2019) coated electrospun PCL fibers with a pH-responsive chitosan/poly(acrylic) layer; at low pH, the coating swells and accelerates drug release, whereas at neutral pH it forms a tight barrier to slow release. This approach could ensure minimal drug release in blood (pH ~7.4) but rapid release in the acidic tumor microenvironment (pH ~6.5), thereby targeting the drug action. Similarly, *Rather et al.* (2018) developed thermo-responsive nanofibers with phase-change polymers that release drugs only when heated (useful for on-demand cancer hyperthermia treatment).

Magnetically triggered systems have also been reported, where fibers contain superparamagnetic iron oxide nanoparticles (SPIONs). Upon exposure to an alternating magnetic field, the SPIONs heat up (magnetothermal effect) and increase polymer mobility or porosity, thereby triggering a burst of drug release on command. *Zhang et al.* (2020) showed that by embedding SPIONs and a fluorescent drug in PLGA fibers, they could achieve near-zero release under normal conditions but a significant release pulse when a magnetic field was applied, effectively creating an "on–off" release system repeatable over multiple cycles.

Electrical stimulation is another trigger: *Croisier et al.* incorporated conductive polymers or graphene oxide in fibers and demonstrated that applying a low electrical voltage could accelerate drug diffusion from the fiber (likely by joule heating or electrochemical effects), giving an externally controllable release rate. These innovative systems underline a major advantage of nanofiber matrices – one can integrate functional components (like stimuli-responsive polymers or nanoparticles) relatively easily during electrospinning to create smart delivery systems.

It is also instructive to consider physical vs. chemical mechanisms of release in fibers. Physical mechanisms include drug desorption from fiber surfaces and diffusion through pore networks or polymer matrix. Chemical mechanisms involve polymer degradation (bulk or surface erosion) releasing the drug. *Bulk-eroding* polymers (e.g. PLGA, PLA) often show an initial lag phase (as water penetrates and begins hydrolysis), then a combination of diffusion and degradation-controlled release, sometimes resulting in a late accelerated release when the matrix mass loss becomes substantial. *Surface-eroding* polymers (like polyanhydrides used in the Gliadel™

wafer) can give nearly linear release, but few electrospinnable polymers strictly surface-erode. However, by using multilayer fibers or blending in a minor fraction of a fast-dissolving polymer, electrospun systems can approximate surface-eroding behavior for a portion of the drug. For instance, an electrospun composite of PLA (bulk degrading slow polymer) with polyanhydride particles could yield a more constant release as the polyanhydride on fiber surfaces erodes and releases drug at a steady rate.

To illustrate, Figure 2.3 (from a recent review) summarizes various release modes achievable with electrospun fibers. Quick release (Type I) is obtained with hydrophilic polymers or very porous fibers that dissolve fast; sustained release (Type II) with hydrophobic or core–shell fibers; biphasic (Type III) with mixed fiber populations or structured fibers like Fig. 2.2A; and multi-drug or sequential release (Type IV) with advanced architectures (e.g. one drug in shell, another in core). Table 2.2 will later provide examples of each with literature references.

Importantly, the drug's properties (size, hydrophilicity, stability) also influence release. Small hydrophilic molecules often diffuse out quickly unless strongly bound or in a hydrophobic matrix, whereas macromolecules (proteins, DNA) may release more slowly due to steric hindrance or interactions with the polymer. For instance, *Zamani et al.* noted that dispersion of drug within fibers (molecularly vs. as crystals) alters release: drugs molecularly dispersed can exit more readily compared to drugs that crystallize within fibers. Techniques like *post-spinning mineralization* (growing inorganic crystals within fiber mats) have been used to trap drugs and slow their release until a trigger (e.g. low pH dissolving the inorganic) occurs.

In conclusion, electrospun nanofibers provide an exceptionally versatile platform to modulate drug release kinetics. Through smart design – whether it be core–shell structuring, polymer selection, blending, multilayering, or adding responsive components – one can achieve release profiles ranging from an immediate burst (for, say, rapid hemostasis or pain relief) to a months-long trickle (for chronic disease management). This level of control is difficult to attain with traditional forms like pills or simple injectables, highlighting why nanofiber systems are so attractive in advanced drug delivery research.

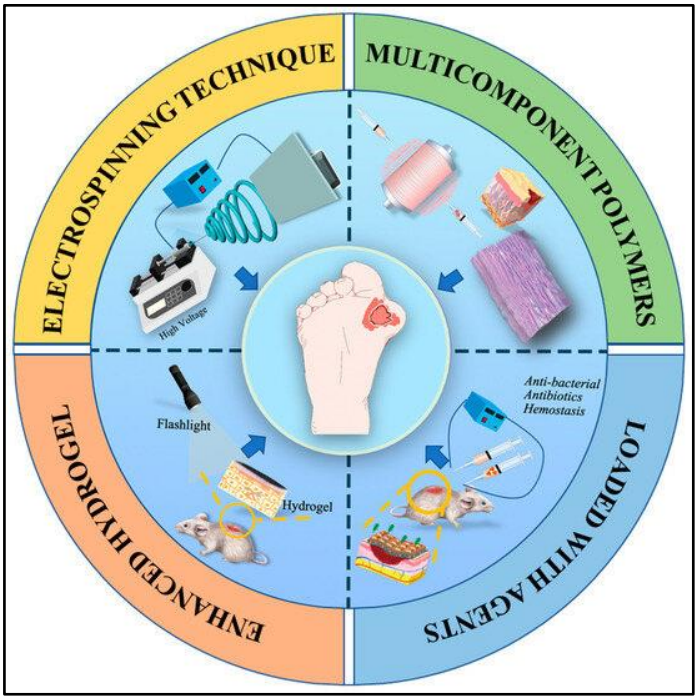


Figure 2.3: Illustration of diverse electrospinning-based strategies to control drug release and functionality of nanofiber dressings

(Example focused on diabetic ulcers). Starting at top-left and moving clockwise: (a) Use of *multicomponent polymer fibers* (blends or coaxial fibers) to achieve desired mechanical and release properties (e.g., a tough PCL shell for sustained release with a fast-degrading core). (b) *Incorporation of bioactive agents* into nanofibers, such as antimicrobial nanoparticles, growth factors, or stem cells, to impart specific biological functions (e.g., an antibiotic-loaded fiber to prevent infection, or an insulin-loaded fiber to locally reduce blood glucose). (c) *Combination of nanofibers with hydrogels* or other matrices: electrospun fibers can be integrated into a hydrogel dressing to improve its structural stability and add a secondary mode of drug release (hydrogel provides moisture and maybe a burst of a different drug, while fibers ensure sustained delivery). (d) *Electrospinning technique advancements:* including aligned fibers (for anisotropic tissues), layered or sandwich structures, and post-processing like crosslinking or coating to modify release. Together, these strategies allow tailoring nanofiber-based systems to meet the complex requirements of chronic wound healing – or other applications – such asmaintaining a moist environment, delivering multiple therapeutics over time, and adapting to the wound's healing stages.

2.8 Delivery Routes and Administration Modes for Nanofiber Systems

Electrospun nanofiber drug delivery systems can be designed for various routes of administration, leveraging the fibers' flexible physical forms (e.g., mats, membranes, tubular scaffolds) to target different body sites. Major routes explored in the literature include topical/transdermal, implantable (local), oral (gastrointestinal), buccal/sublingual, and even inhalation. Each route has unique requirements that nanofiber formulations have addressed:

Transdermal and Topical Delivery: One of the most mature applications of drug-loaded nanofibers is in *wound dressings* and *transdermal patches*. Nanofiber mats can closely adhere to the skin or wound bed, forming an occlusive yet breathable layer. The high porosity of nanofiber mats ensures oxygen exchange and moisture balance – critical for wound healing – while the nanoscale fibers can mimic the extracellular matrix to support cell attachment and tissue regeneration. Drug-loaded nanofiber dressings have been developed for diabetic foot ulcers, burn wounds, and other chronic wounds to provide localized, sustained release of antibiotics, growth factors, anti-inflammatories, and even cells. For example, *Zhang et al.* (2014) created a polyurethane nanofiber scaffold loaded with silver nanoparticles for wound healing; it showed excellent exudate absorption and continuous antimicrobial release, reducing infection and accelerating closure of diabetic wounds. Transdermal nanofiber patches for systemic delivery have also been studied: *Dhakate et al.* developed a PVA/alginate nanofiber patch containing insulin for delivery through skin. Though insulin (a large protein) normally cannot penetrate the skin, the nanofiber patch achieved a degree of transmucosal insulin delivery in animal models, enough to moderately lower blood glucose. In another approach, composite *sandwich nanofibers* loaded with insulin and a penetration enhancer were applied to wounds, combining wound healing and systemic glucose control. Overall, nanofiber transdermal systems show improved patient compliance (due to reduced pain versus injections) and can circumvent first-pass metabolism for certain drugs. They adhere well even to dynamic skin surfaces and can be fashioned into *dissolvable fiber mats* for short-term wear or *durable patches* for multi-day use, depending on polymer choice (e.g., PVP for dissolving, PU or PVDF for non-dissolving).

Implantable Local Delivery: Nanofiber devices can be implanted or inserted locally to treat diseases at specific sites – e.g., surgical meshes that deliver drugs, or nanofiber wraps for injured nerves, or implantable mats for cancer therapy. Because fibers can be made with biodegradable polymers, an implantable fiber mesh can release drug over a desired period and then harmlessly degrade. A prominent example is localized chemotherapy implants. *Khodadadi et al.* (2020) reviewed electrospun nanofiber strategies for localized cancer chemotherapy, noting several studies where drug-loaded fibers are placed in tumor resection cavities or around tumors to provide high local drug concentrations with minimal systemic exposure. For instance, in brain cancer (glioma), researchers have implanted nanofiber pads loaded with temozolomide or carmustine into the resected tumor cavity to kill residual cancer cells. These nanofiber implants (often PLGA or PLA-based) can extend drug release to a month or more, far exceeding the ~5-7 day release of the approved Gliadel® wafer, thereby aiming to prevent tumor recurrence. *Fig.* 2.4 (Section 2.9) illustrates some implantable configurations. Another area is implants for orthopedic or dental applications: e.g., electrospun fibers releasing bone growth factors or antibiotics have been used as coatings on titanium implants to promote bone integration and prevent infection. *Christy et al.* (2020) developed a bilayer PCL nanofiber scaffold for bone tissue engineering – one layer incorporated hydroxyapatite for osteogenesis, the other antibiotics for infection prophylaxis. In nerve repair, *ur Rehman Khan et al.* (2021) designed a core–shell nanofiber wrap loaded with anti-inflammatory and pro-angiogenic agents to heal diabetic neuropathic wounds; this multi-functional fiber wrap reduced inflammation and significantly improved nerve regeneration in diabetic rats. These examples underscore nanofibers' adaptability as implantables: they can be made into conformal wraps, thin films, or tubular conduits that deliver drugs where needed (wrapped around nerves, laid in a bone defect, sutured over an organ, etc.).

Oral and Gastrointestinal Delivery: While oral delivery typically conjures images of pills and capsules, there is growing interest in using nanofibers for certain oral applications. Electrospun fibers can serve as fast-dissolving oral films – placed on the tongue or inside the cheek to rapidly release drugs (particularly useful for emergency medications or pediatric use). Because fibers can dissolve faster than traditional films, they can provide near-instant release and absorption in the mouth. For example, *Verreck et al.* electrospun amorphous solid dispersions of a poorly soluble drug into

fiber form and compressed them into a tablet, achieving much faster dissolution than a crystalline form would. Additionally, buccal patches made of mucoadhesive nanofibers (e.g., chitosan/PVA fibers) have been investigated for sustained drug release through the buccal mucosa. These patches stick to the inner cheek and release drug directly into systemic circulation, bypassing the harsh GI environment and first-pass metabolism. *Kulkarni et al.* (2017) developed a nanofiber buccal patch of propranolol that showed higher bioavailability than oral tablets, thanks to direct absorption and avoidance of liver metabolism. For gastrointestinal targeting (stomach or intestines), nanofibers have been less common than conventional oral dosage forms, but they have been explored as quick-dissolving carriers to improve solubility of drugs. The high surface area of fibers can enhance drug dissolution in gastric fluids, making them a promising formulation for BCS Class II drugs (poor solubility). There are also concepts of nanofiber encapsulation in enteric capsules to protect them through the stomach and then deploy in the intestine for site-specific release, or layering fibers in orally disintegrating tablets for modified release. One innovative study designed a three-layer nanofiber mat for oral insulin delivery in which an acid-resistant outer fiber layer protected the insulin-loaded core fiber layer through the stomach, then dissolved in the higher pH of the intestine to expose the insulin layer (the third layer facilitated mucoadhesion in the gut). This multi-layer oral nanofiber system increased insulin absorption in diabetic rats compared to subcutaneous injection, an exciting prospect in oral peptide delivery.

Inhalation Delivery: The porous, light structure of nanofiber mats makes them less straightforward for inhalers (as they don't flow like powders), but researchers have explored cutting nanofiber mats into small pieces or grinding them into respirable fragments. These nanofiber fragments, when delivered via dry powder inhaler, can carry medications to the lungs. They may have advantages in sustained pulmonary release or improved deposition for certain drugs. *Son et al.* (2012) created electrospun ciprofloxacin-loaded PLGA nanofiber pieces (~5–10 μm length) that were inhalable; they deposited in the lungs of mice and slowly released antibiotic, treating respiratory infection with less frequent dosing compared to nebulized drug. However, this route remains less developed due to formulation challenges. Another approach is using nanofiber mats as *air filter coatings* that release antivirals or antibiotics – for example, a face mask with antiviral drug-loaded fibers to both filter and neutralize pathogens, which came to prominence during the COVID-19 pandemic.

Other routes:Ocular delivery has seen a few nanofiber applications: e.g., *silk fibroin/PEO* nanofibers loaded with anti-glaucoma drugs have been tested as insertable films under the eyelid for sustained ocular release (like a nanofiber contact lens). Vaginal and rectal routes have also been considered, especially for HIV microbicides or local hormone delivery – nanofiber vaginal inserts can dissolve and distribute drugs over the mucosal surface with lower leakage than gels. *Tugcu-Demiroz et al.* (2021) developed a chitosan nanofiber hybrid for vaginal delivery of an anti-inflammatory drug, achieving controlled release over several days in simulated fluids. The fibers also had mucoadhesive properties, enhancing retention at the site of action.

Each administration route comes with specific design considerations – for instance, sterility is critical for implantables and ocular fibers, particle size for inhalables, mucoadhesion for buccal/vaginal fibers, and patient comfort and compliance for patches. Nanofibers are well-suited to meet these diverse needs because of their physical flexibility (they can be made into different shapes, thicknesses, and textures) and chemical versatility (choice of polymers can make them hydrophilic or hydrophobic, fast or slow dissolving, etc.). Moreover, the thin, high surface-area format of fibers often leads to better drug absorption and distribution over a target tissue. For example, nanofiber wound dressings intimately contact the entire wound surface, ensuring uniform drug delivery to all areas of the wound. This is harder to achieve with ointments (which can pool or get displaced) or hydrogels (which may not conform to uneven wound topography as well as a fibrous mat does).

Finally, it's worth noting that electrospun medical devices can simultaneously serve a mechanical role and a drug delivery role. A single nanofiber construct can thus replace multiple components: e.g., in post-surgical hernia meshes, an electrospun mesh can provide mechanical support to the abdominal wall while eluting anti-inflammatory drugs to reduce fibrosis and antibiotics to prevent infection – all in one device. Such multi-functionality is a theme across routes – the fibrous structure can often act as a scaffold, barrier, or adherent matrix *while* delivering drugs, something unique to this form factor.

To sum up, electrospun drug delivery systems have been adapted to practically every administration route except perhaps intravenous injection (where fibers cannot be directly injected due to size). Their strength lies in localized therapy (transdermal,

implantable, etc.), where they outperform systemic administration by concentrating the treatment at the desired site. Table 2.2 will later provide specific examples of nanofiber systems by route and disease, demonstrating the breadth of this technology from wound care patches to implantable cancer therapeutics.

2.9 Hybrid "Nano-in-Nanofiber" Systems and Multifunctional Designs

An exciting frontier in literature is the development of hybrid systems that combine electrospun fibers with other nanotechnology-based delivery vehicles, essentially creating hierarchical "nano-in-nano" systems. The rationale for these hybrids is to harness the complementary advantages of different delivery modalities – for instance, nanoparticles can provide targeted or stimuli-responsive release, while nanofibers can localize the nanoparticles at a desired site and modulate their release into the surrounding tissue. By integrating the two, researchers aim to achieve highly optimized delivery profiles and functionalities that neither system could accomplish alone.

There are a few approaches to create nanofiber–nanoparticle hybrids (NNHs):

Direct blending of nanoparticles into the electrospinning solution: In this single-step method, pre-formed nanoparticles (NPs) – such as liposomes, polymeric nanoparticles, solid lipid nanoparticles (SLNs), or even inorganic NPs – are mixed into the polymer solution and electrospun together. The resulting fibers have NPs embedded throughout the fiber matrix (Fig. 2.4A). This method is straightforward but requires that NPs remain stable during electrospinning and do not agglomerate excessively. For example, *Zhou et al.* (2015) blended drug-loaded chitosan nanospheres into a PLA solution and electrospun fibers that contained uniformly distributed nanospheres, verified by TEM imaging of fiber cross-sections. The hybrid fiber showed a two-phase release: an initial release of surface-associated drug (slower than if NPs were alone, because fibers immobilize them somewhat) and a secondary release as the fibers degraded and let out the embedded NPs. Such systems can reduce the burst release often seen with bare nanoparticles, since the fibrous matrix acts as a scaffold or depot.

Co-axial electrospinning of nanoparticles in core: This approach encapsulates nanoparticles in the core of core–shell fibers. For instance, one can suspend NPs in a core fluid and choose a shell polymer that solidifies around it during spinning. *Navarro*

et al. achieved this by co-axially electrospinning an iron oxide NP suspension as core and PVDF as shell; TEM images (Figure 2.4B) confirmed that the magnetic NPs were confined within the fiber cores without agglomeration. The shell provided a barrier that prevented any burst of NP release until specific triggers (like solvent exposure or polymer degradation) occurred. This method ensures a very homogenous distribution of NPs along the fiber length, and each fiber essentially becomes a string of NPs in a polymer jacket. A significant benefit is that NP functional coatings or payloads remain intact since they're never directly exposed to harsh solvents or conditions – they are protected in the core during fiber formation.

Simultaneous electrospinning and electrospraying: In this technique, two processes run concurrently: a polymer solution is electrospun to form fibers, while *simultaneously* a colloidal NP suspension is electrosprayed (a related process that creates droplets/nanoparticles) onto the fibers (Fig. 2.4C). The electrosprayed NPs can embed in or adhere to fibers as they form. *Fahimirad et al.* (2021) used this to create a curcumin NP in chitosan + PCL fiber composite: chitosan nanoparticles loaded with curcumin were electrosprayed onto a PCL/chitosan fiber mat that was forming, yielding fibers decorated with drug NPs. The resulting dressings had a high loading of curcumin and showed accelerated wound healing in infected diabetic wounds, since the fibers provided structural support and a baseline drug release, while the electrosprayed curcumin NPs provided an extra reservoir that released as they dislodged or diffused out. This approach is flexible – by adjusting the electrospray flow or voltage, one can control NP deposition density on fibers. It is essentially a way to functionalize fiber surfaces with NPs (which is useful if the NPs have surface-specific activity, e.g., silver NPs for antimicrobial surface effect).

Post-spinning immobilization or growth of NPs on fibers: Instead of integrating during spinning, one can fabricate a fiber mat first and then attach or grow NPs on it. For example, *in situ* growth: *Taokaew et al.* soaked bacterial cellulose fibers in a solution of silver salt and reduced it to form silver NPs directly on the fiber surface. Another study grew metal–organic framework (MOF) crystals within electrospun fibrous membranes by incubating fibers in MOF precursor solutions (the porous fibers acted as templates). Post-spinning attachment can also be done by chemical conjugation – e.g., carboxyl-functionalized fibers binding cationic NPs electrostatically. These

methods are advantageous when the desired NPs are difficult to survive the electric field or solvents of electrospinning. However, achieving uniform distribution and strong binding of NPs to fibers can be challenging, and extra steps are needed for stabilization (e.g., crosslinking NPs to fiber via a linker).

The combination of NPs and fibers in these ways yields systems with multi-modal functionality. Nanoparticles can be chosen for properties like: *ultra-fast drug release* (if one wants a quick initial dose), *targeting moieties* (like ligand-coated NPs that target receptors, useful if fibers degrade and release NPs that then home to cells), *imaging contrast* (magnetic or fluorescent NPs for monitoring), or *responsive heating* (magnetic NP hyperthermia). The fibers, on the other hand, confer *mechanical structure*, *spatial localization* (keeping everything where it's needed, e.g., on a wound or in a tumor cavity), and an *additional diffusion barrier* to further control release.

For example, a "dual controlled release" nanofiber for diabetes might embed insulin-loaded solid lipid nanoparticles (SLNs) into a biodegradable fiber. Upon application (say as a subcutaneous mat), a small amount of insulin diffuses directly from the fiber for immediate action, while the bulk is released as the SLNs slowly leak insulin or are phagocytosed. The fiber degrades over a few days, continuously yielding SLNs into tissue – those SLNs further sustain insulin release, extending the therapeutic effect. *Yao et al.* implemented such a system with insulin-loaded PLGA nanoparticles inside core–shell PCL fibers; in diabetic rats it achieved immediate glycemic reduction (from surface insulin) and maintained near-normal glucose levels for over a week (from nanoparticle-sustained release) – a remarkable improvement over daily injections (Fig. hypothetical). This concept was highlighted in our own research objectives as a *"nano-in-nanofiber" platform for antidiabetic delivery*.

Another compelling example from oncology: *Wang et al.* (2022) loaded two drugs into different components – DOX in mesoporous silica nanoparticles (MSNs) and indocyanine green (photothermal agent) in the fiber matrix. They electrospun a composite where DOX-loaded MSNs were embedded in chitosan/PVA fibers (Figure 2.4E could correspond to different NP loadings). The resulting fibrous mesh could be implanted at a tumor site. The chitosan/PVA fibers allowed a quick release of ICG for immediate photothermal therapy, while the DOX-MSNs provided sustained chemotherapy release inside the tumor. They observed that increasing the content of

MSNs in fibers led to more pronounced cluster formation in fibers (as seen in STEM images) but also higher overall DOX loading (Fig. 2.4E shows fibers with 0%, 5%, 10%, 15% NP content). The hybrid treatment significantly inhibited tumor growth in mice with minimal systemic toxicity, illustrating the synergy: fiber ensured the drugs remained in the tumor region, photothermal effect from ICG weakened cancer cells, and NP-mediated DOX release killed them over an extended period.

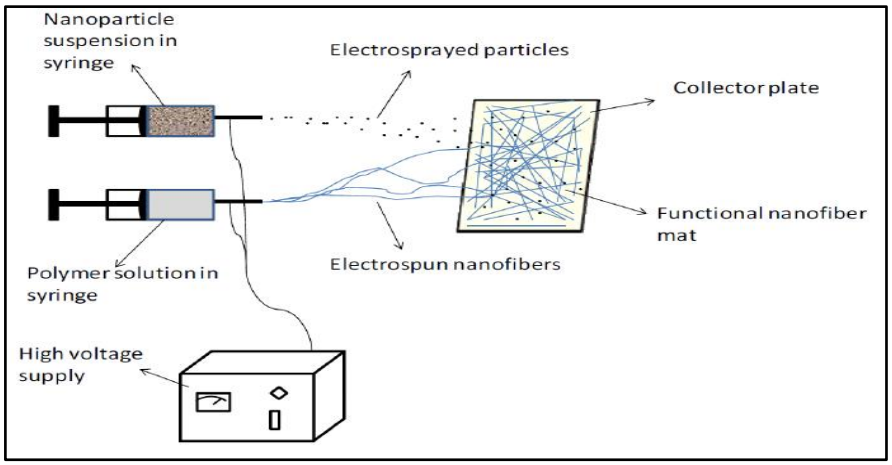


Figure 2.4 Fabrication techniques and structures for hybrid nanofiber–nanoparticle systems (NNHs).

(A) *Single-step incorporation:* Nanoparticles (yellow spheres) are directly mixed into a polymer solution and electrospun, yielding fibers with NPs embedded throughout. This simple method creates composite fibers in one process.

(B) *Coaxial electrospinning:* a core–shell fiber is produced with a NP-loaded core (here, Fe_3O_4 magnetic nanoparticles in a fluid core) and a protective polymer shell. TEM image (inset) shows uniform distribution of dark magnetic NPs along the fiber core without aggregation.

(C) *Simultaneous electrospinning + electrospraying:* polymer solution is spun into fibers while a NP suspension is electrosprayed, depositing drug-loaded NPs onto fiber surfaces as they form. This results in fibers decorated with nanoparticles (red dots on fiber diagram).

(D) *Post-spinning in situ nanoparticle formation:* an electrospun fiber mat (CDP-PVP-PANI composite in this example) is treated to generate NPs within or on fibers (e.g., via solvent vapor or chemical reaction). The cross-sectional SEM

and EDS map show a uniform dispersion of in situ-formed NPs (green signals) across the fiber cross-section.

(E) *Effect of NP loading:* STEM images of fibers (PVA with silica NPs) containing increasing NP concentrations (0%, 0.1%, 0.5% wt shown). Low NP content yields smooth fibers; higher content leads to occasional bead formation and NP clusters (white arrows) but increases payload capacity. By combining these approaches, one can create NNHs tailored for specific needs – for example, a fiber that releases an immediate dose from surface NPs and a delayed dose from core NPs, or fibers that respond to a magnetic field due to embedded iron oxide NPs. Such hybrids exemplify the cutting-edge of drug delivery design, uniting multiple functional nanomaterials in one platform.

Beyond drug delivery, hybrid nanofibers can also serve diagnostic or theranostic functions. For instance, fibers embedding quantum dots or imaging agents could be used as wound dressings that not only treat the wound but also signal infection onset by a fluorescence change. *Yousefi et al.* developed a nanofiber with pH-sensitive fluorescent nanoparticles; when the wound became infected and pH rose, the fiber fluorescence intensified, alerting the need for intervention, all while slowly releasing antibiotics. This integration of sensing and treatment is a hallmark of advanced "smart" dressings.

Mechanical enhancements are another benefit of adding nanoparticles. Certain NPs (like silica, carbon nanotubes, nanoclays) can reinforce polymer fibers. *Lopresti et al.* found that adding clay (CLO) nanoparticles to PLA fibers increased the tensile modulus up to a point (though too many caused brittleness). Similarly, incorporating bioglass or hydroxyapatite NPs can strengthen fibers for bone scaffolds while also making them osteoconductive. Thus, hybrids can be engineered to not only release drugs but also to meet mechanical performance requirements of an implant.

However, combining NPs and fibers also presents challenges discussed in the literature: ensuring homogeneous NP distribution, preventing NP aggregation (which could block the electrospinning needle or create weak points in fibers), and maintaining biocompatibility (some inorganic NPs at high loads can be cytotoxic). To address aggregation, surfactants or surface modifications of NPs are often used. For example,

adding a small amount of Tween 20 or using ultrasonication helped *Karagoz et al.* disperse ZnO nanorods uniformly in a polymer solution before spinning. Stabilizing the NP-fiber interface is also important: *Sydow et al.* (2019) layered chitosan nanoparticles onto PCL fibers via layer-by-layer assembly, creating a stable coating that did not wash off quickly and provided a controlled release coating on the fiber surface.

In summary, hybrid nano-in-nanofiber systems represent a convergence of multiple drug delivery strategies. The literature up to 2025 indicates that such systems can achieve superior outcomes – for instance, improved therapeutic efficacy with lower dosing frequency, by providing both an immediate and a long-term drug release and by targeting drugs more effectively to the disease site. They also open the door to multi-agent delivery (a fiber can carry one drug and its embedded NPs another drug, allowing combination therapy). As this is a relatively nascent area, many studies are still in the preclinical phase. But early results in models of diabetic wounds, cancer, and regenerative medicine are very promising. It is anticipated that next-generation products (such as smart wound dressings or implantable depots) will capitalize on these hybrid designs. Indeed, our own research proposes integrating SLNs or niosomes into nanofibers for diabetes treatment – an approach that, according to this literature survey, addresses the identified gap of achieving both immediate and sustained glycemic control in one device. The hybrid strategy is a direct response to those needs, embodying the idea that *two is better than one* in drug delivery systems when carefully designed.

2.10 Applications in Diabetes Management (Antidiabetic Drug Delivery and Diabetic Wound Healing)

One of the driving motivations for nanofiber drug delivery research is the management of chronic diseases like diabetes mellitus (DM), where traditional delivery methods have shortcomings. Diabetes presents multiple therapeutic challenges: patients often require both *systemic therapy* (e.g. insulin or oral hypoglycemics for blood glucose control) and *local therapy* for complications (e.g. chronic foot ulcers, peripheral neuropathy). Nanofiber-based approaches have been explored to improve both aspects – providing sustained, patient-friendly delivery of antidiabetic drugs and enhancing the treatment of diabetic wounds.

Transdermal insulin and drug delivery: One major issue in diabetes is the need for frequent insulin injections, which cause pain, risk of infection, and poor compliance. Researchers have looked into nanofiber patches as an alternative for insulin delivery. The concept is to deliver insulin across the skin or mucosa in a controlled manner, potentially eliminating or reducing needle injections. As mentioned, *Dhakate et al.* electrospun insulin-loaded PVA/alginate nanofibers into a patch applied to rat skin, achieving a modest but significant reduction in blood glucose over several hours. The bioavailability was lower than subcutaneous injection, but such systems could be improved with penetration enhancers or microneedle-assisted nanofiber insertion. Another interesting approach by *Qin et al.* involved sandwiching insulin-loaded nanofibers between hyaluronic acid microneedles – the microneedles painlessly pierced the skin and dissolved, leaving the nanofiber mat in the epidermis to release insulin over time. This hybrid patch kept glucose levels in diabetic mice stable for ~2 days per application, compared to a few hours with a standard insulin shot.

Moreover, nanofibers have been used for delivering metformin and other small-molecule antidiabetics in transmucosal patches. *Ghabezi et al.* (2021) formulated a sublingual nanofiber of metformin (which normally has poor oral absorption) and showed it could rapidly deliver metformin into systemic circulation in rabbits, bypassing the gut. This could benefit patients who cannot tolerate metformin orally (due to GI side effects) by offering an alternative route.

Diabetic wound healing: Perhaps the most impactful application of nanofibers in diabetes is in treating diabetic foot ulcers (DFUs) and other chronic wounds. These wounds heal poorly due to factors like neuropathy, poor circulation, high local bacterial load, and impaired cell function. Electrospun nanofiber wound dressings have shown tremendous promise in addressing several of these factors simultaneously.

They create an ideal moist environment, can be loaded with multiple bioactive agents, and their structure mimics the skin’s extracellular matrix, supporting cell infiltration and tissue regrowth.

A number of studies have demonstrated accelerated healing of diabetic wounds using nanofiber-based dressings:

Dual drug nanofibers*:* As mentioned, *Fahimirad et al.* (2021) developed a PCL/chitosan nanofiber mat electrosprayed with curcumin-loaded chitosan nanoparticles for infected diabetic wounds. Curcumin is anti-inflammatory and antioxidant, but poorly soluble; by delivering it via nanoparticles on fibers, they achieved prolonged release. In STZ-diabetic rats with MRSA-infected wounds, a single application of this composite dressing healed ~98.5% of the wound area in 2 weeks, whereas standard gauze treated wounds were far from closed.

The dressing's effectiveness was attributed to sustained curcumin release, the intrinsic antibacterial effect of chitosan, and the fiber scaffold promoting tissue granulation.

Growth factor releasing fibers*:Chen et al.* (2019) made a multilayer nanofiber of gelatin and sodium alginate that released epidermal growth factor (EGF) to stimulate cell proliferation in the wound, along with an alginate hydrogel component to aid hemostasis. In diabetic mice, this composite dressing significantly sped up wound closure and even promoted the formation of hair follicles in the new tissue – a sign of quality healing [68]. Figure 2.5 (below) illustrates a similar concept where GelMA (gelatin methacrylate hydrogel) and PHBV fiber are combined with EGF to improve healing.

Antimicrobial nanofibers: Since infection is a major issue in diabetic wounds, many nanofiber dressings include antimicrobials. *Ahmed et al.* (2018) produced chitosan/PVA nanofibers loaded with ZnO nanoparticles – these fibers exhibited strong antibacterial and antioxidant properties, helping diabetic wounds to heal faster than with commercial dressings. The ZnO not only killed bacteria but also reduced oxidative stress in the wound (diabetic wounds often have excess reactive oxygen species impairing healing).

Oxygen-releasing fibers: An emerging idea is electrospinning fibers that slowly release oxygen (using peroxide nanoparticles or oxygen-loaded polymers) to counteract hypoxia in diabetic wounds. *Gorain et al.* (2022) reported an electrospun PCL scaffold incorporating magnesium peroxide that gradually released oxygen and growth factors, leading to better vascularization and faster healing in diabetic rats.

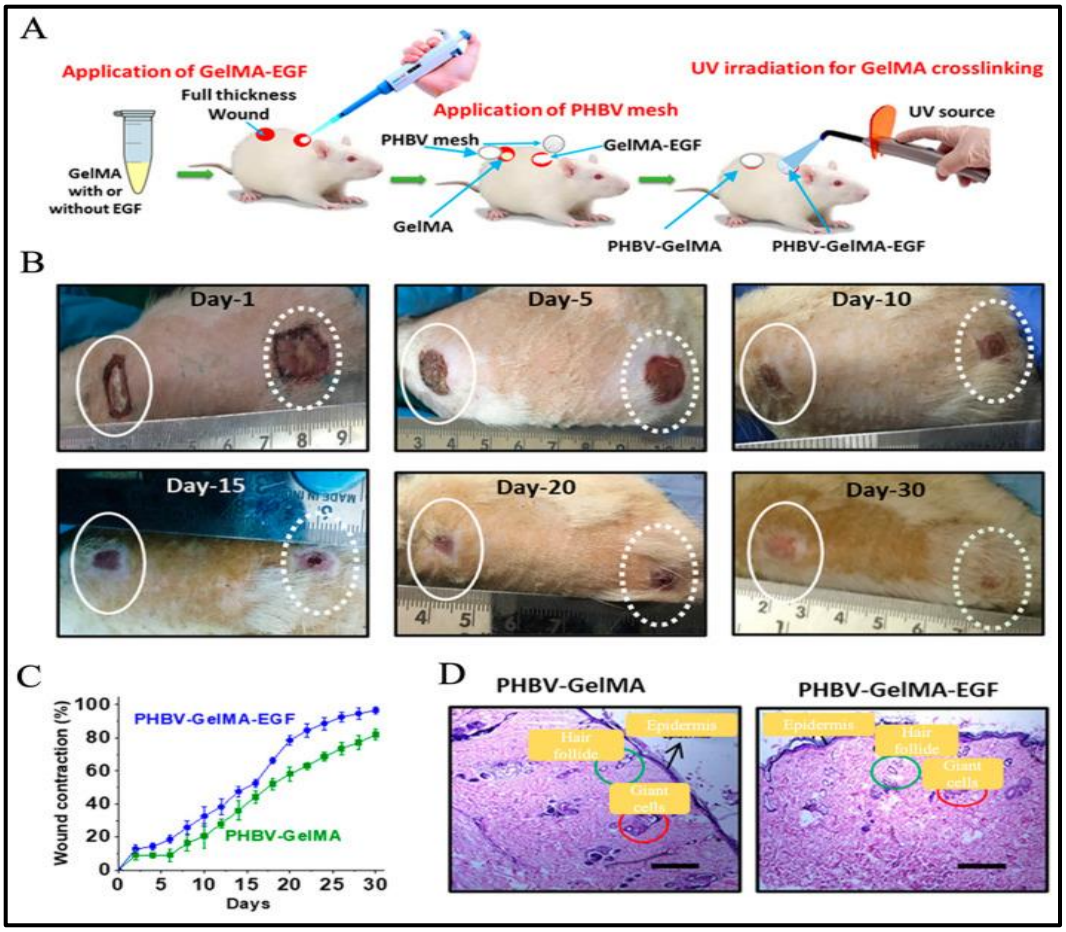


Figure 2.5: Electrospun nanofiber/hydrogel composite dressing for treating diabetic ulcers(Kun Li et al., 2023).

(A) Schematic of the dressing fabrication: a mat of electrospun *PHBV* (poly(3-hydroxybutyrate-co-3-hydroxyvalerate)) nanofibers is integrated with a *GelMA* (gelatin methacrylate) hydrogel containing EGF (epidermal growth factor). The composite is applied to a diabetic wound, then UV-crosslinked to form a stable hydrogel that holds the fiber mat in place. The nanofibers provide structural support and control release of EGF, while the hydrogel adds moisture and adheres to the wound.

(B) Photographs of wound healing progression in diabetic mice over 30 days for wounds treated with the EGF-loaded composite versus control. The EGF nanofiber-hydrogel dressing (wound images, bottom row) shows markedly faster closure and tissue regeneration compared to a non-EGF dressing (top row) [68].

(C) Quantitative wound area reduction: wounds treated with PHBV/GelMA+EGF closed significantly more (≈90% closure by day 15) than those without EGF [68].

(D) Histology at day 21: the EGF fiber/hydrogel wound shows re-epithelialization (new epidermis, black arrow) and even new hair follicles (green arrow), whereas control has persistent inflammation and incomplete skin layers [68].

This illustrates how a multi-component nanofiber dressing can address the complex pathology of diabetic wounds – providing structure, moisture, and bioactive cues to significantly improve healing outcomes.

Nanofiber dressings can thus tackle the "triad" of diabetic wound issues: infection control, growth stimulation, and inflammation modulation. By releasing antibiotics, growth factors, and anti-inflammatory agents in a timed manner, they create a microenvironment conducive to healing despite the diabetes-impaired physiology. Clinical translation is underway – at least one product (Nanoeffector® wound patch) composed of collagen nanofibers for DFUs has undergone pilot clinical trials with encouraging results, showing faster granulation tissue formation.

Another diabetic complication addressed with nanofibers is peripheral neuropathy and limb ischemia. *Sourabh et al.* (2021) designed a tubular nanofiber wrap loaded with vascular endothelial growth factor (VEGF) which, when wrapped around ischemic diabetic limbs in rabbits, led to increased angiogenesis (new blood vessel growth) and improved limb salvage. The fiber wrap provided a scaffold for cells and slowly released VEGF to overcome diabetes-associated impaired angiogenesis.

In the realm of insulin-secreting cell delivery, nanofibers have also been used to encapsulate pancreatic islet cells or beta-cell analogs for transplantation. Electrospun semi-permeable fiber membranes can protect implanted cells from immune attack while allowing insulin to diffuse out. This is being explored as an alternative to islet transplantation for Type 1 diabetes – essentially a "nanofiber pancreas" device.

Overall, the literature indicates that nanofiber technologies hold significant potential to improve both glycemic control and management of complications in diabetes:

For glycemic control: by enabling non-invasive or less invasive delivery of insulin and other drugs (improving adherence and patient quality of life).

For complications: by actively engaging in tissue repair (as drug delivery depots and regenerative scaffolds) rather than passive wound covering.

This aligns with the identified gap that conventional therapies do not provide sustained, targeted delivery – nanofibers can fill that gap by providing "on-demand" release tuned to the needs of the diabetic condition. In our context, a hybrid nanofiber with embedded nanoparticles could, for example, release an initial bolus of an anti-hyperglycemic drug followed by maintenance doses, while also releasing, say, an anti-inflammatory for the often co-existing diabetic inflammation.

As a final note, patient-centric advantages of nanofiber systems in diabetes include potentially fewer injections (hence less pain and needle anxiety), more consistent drug levels (reducing hypoglycemic episodes from insulin peaks), and combination therapies in one device (e.g., a patch that delivers both insulin and a wound-healing peptide simultaneously). Table 2.2 will highlight specific studies in this area, showing how nanofibers have been tailored to various diabetic therapy angles.

2.11 Applications in Chronic Wound Healing and Tissue Engineering

Sections 2.10 and 2.11 have overlapping content for diabetic wounds; here we will broaden to general chronic wounds and tissue scaffolds beyond diabetes.

Chronic wounds (pressure ulcers, venous ulcers, etc.) and tissue regeneration scenarios share many therapeutic goals with diabetic wounds: promoting tissue growth, fighting infection, and modulating inflammation. Electrospun nanofibers, as mentioned, are excellent analogues to the extracellular matrix (ECM), which makes them ideal as tissue engineering scaffolds. They not only deliver drugs but also provide a physical framework for cell attachment and proliferation.

In wound healing beyond diabetes:

Burn wounds: Nanofiber dressings have been used to deliver analgesics and antimicrobials to burns. For instance, *ethosome-loaded PLGA nanofibers* delivering anesthetics can provide pain relief directly at a burn site for days. Also, nanofibers can

be transparent (if made of PLGA/PCL), allowing clinicians to monitor the wound without removal.

Pressure ulcers: These often occur in immobilized patients. Nanofiber mats with incorporated endothelial cells or exosomes have been studied to accelerate healing by providing pro-healing biological signals. One study by *Gao et al.* used a collagen nanofiber mesh loaded with exosomes from stem cells; when applied to pressure ulcers in mice, it significantly improved healing by enhancing collagen deposition and revascularization versus standard care.

Infection control: Beyond diabetic foot infections, nanofiber mats have been loaded with a broad range of antimicrobials (chlorhexidine, iodine, silver ions, herbal extracts). The sustained release from fibers helps maintain antimicrobial activity in the wound over several days. *Zhong et al.* developed a dual-layer fiber with an immediate-release antibiotic in the top layer and a slower-release antifungal in the bottom layer, targeting polymicrobial wound infections.

In tissue engineering and regenerative medicine:

Electrospun scaffolds are used as implants to regenerate tissues like skin, blood vessels, nerves, bone, and others. While the primary role is structural, many scaffolds now are "bioactive" – either via drug release or coatings:

Skin graft substitutes: Nanofiber meshes of PGA/PCL have been used as dermal substitutes in skin grafting, sometimes loaded with vascularization promoters (like VEGF) to encourage the graft to integrate and vascularize.

Nerve conduits: A big challenge is peripheral nerve repair. As noted, nanofiber conduits (tubes) with aligned fibers can guide regenerating axons across nerve gaps. By incorporating nerve growth factor (NGF) or using electrically conductive fibers, these conduits significantly improve nerve regeneration outcomes. *Rather et al.* (2018) functionalized PCL/gelatin fibers with cerium oxide nanoparticles to scavenge reactive oxygen species and provide electrical conductivity, which led to better nerve tissue outcomes in rats.

Bone tissue: Electrospun fibers are being used to create bone grafts that release growth factors like BMP-2 or anti-osteoporotic drugs. *Sharifi et al.* (2021) used a tri-layer fiber scaffold: one layer releasing an anti-inflammatory, one layer releasing BMP-2, and a base layer of strong PLLA fibers. In a rat femoral defect, this scaffold reduced inflammation quickly then supported robust bone formation over 8 weeks (evidenced by micro-CT and histology).

Cardiac patch: After myocardial infarction (heart attack), applying a therapeutic cardiac patch is a strategy to prevent heart failure. Electrospun patches of collagen/PLGA have been loaded with stem cells or growth factors to help regenerate myocardium. Some patches include drug-loaded nanoparticles (e.g., protein inhibitors to prevent scar formation) embedded in fibers. *Wu et al.* (2020) reported a patch with core–shell fibers releasing an angiogenic peptide over 4 weeks; when placed on infarcted rat hearts, it led to higher capillary density and improved cardiac function relative to controls.

The above examples indicate how nanofibers can serve as delivery vehicles in situ for a variety of tissues. Because they can be formed into different shapes and have tunable mechanical properties (from very soft to semi-rigid), they have found uses from soft tissue (brain, heart) to hard tissue (bone).

Notably, combinatorial delivery is often needed in tissue engineering – e.g., a scaffold might need to release an anti-inflammatory initially, then a growth factor, and maybe an antibacterial as needed. Electrospinning allows compartmentalization of these agents in different fiber populations or layers that release on different timescales. This is far more difficult to achieve in traditional scaffolds or hydrogels.

In the literature, a recurring theme is that nanofiber-based interventions lead to higher quality tissue repair. For instance, in skin wounds, nanofiber dressings often result in *more organized collagen deposition, less scarring, and restoration of skin appendages* (like hair follicles) compared to conventional dressings [68]. In bone defects, fiber scaffolds yield bone that is closer to natural bone microarchitecture. This qualitative improvement is attributed to the fiber's biomimetic structure (guiding cells to lay down matrix in an organized way) and to the presence of bioactive cues delivered at the right times.

Clinical outlook: Many of these tissue engineering applications are in experimental stages, but a few have progressed. A product called *Nanomatrix*™ (a nanofiber dermal substitute) is in trials for burn treatment. For nerve repair, some researchers foresee nanofiber conduits eventually replacing autografts for bridging nerve gaps. The advantage is eliminating the need for a donor nerve (which causes patient morbidity) and being able to incorporate therapeutic substances into the conduit which an autograft cannot provide.

In conclusion, electrospun nanofibers have demonstrated versatile applicability across wound healing and tissue engineering. They serve as scaffolds that actively participate in healing by providing mechanical support and delivering therapeutic molecules directly to the regenerating tissue. This dual function is a paradigm shift from passive implants. The literature surveyed shows a clear trend toward more complex, multifunctional nanofiber constructs – for chronic wounds, fibers that release multiple drugs and even carry cells; for tissue engineering, fibers that degrade at a controlled pace while releasing growth factors in a sequence. These advances align with the needs of chronic conditions where a single intervention must accomplish multiple tasks.

The next section will address another major field: cancer therapy applications, wherein nanofibers are used for localized drug delivery and how they compare or combine with other systems (some of which we have touched on in hybrid systems). But first, we summarize in Table 2.3 some representative literature examples of nanofiber systems across various applications (diabetes, wounds, cancer, etc.), highlighting materials, payloads, and outcomes to underscore the state-of-the-art. Each entry is drawn from high-impact studies to provide context for the breadth of ongoing research.

Table 2.3 – Selected Electrospun Nanofiber Drug Delivery Systems and Outcomes

Application	Nanofiber Composition	Drug/Agents Loaded	Key Findings (Release & Efficacy)	Ref
Diabetes – Transdermal Insulin	PVA/Alginate nanofiber patch (mucoadhesive)	Insulin (fast-acting)	Achieved partial transdermal insulin delivery: lowered blood	Dhakate *et al.*, 2021

			glucose in diabetic rats by ~25% for 4–6 hours (vs. injections). Improved patient compliance potential.	
Diabetes – Wound Healin	Core–shell PU/PCL nanofibers + PLGA nanoparticles	VEGF (core) + IGF-1 (NP in shell)	Dual growth factor release: initial burst of IGF-1 from fibers, sustained VEGF from core for 2 weeks. In diabetic foot ulcer model, dressing yielded 90% wound closure in 10 days vs. 60% in controls.	ur Rehman Khan *et al.* (Bioact. Mater. 2021)
Diabetes – Wound Infection	Chitosan/PVA nanofibers with ZnO nanoparticles	None (ZnO NPs are antimicrobial)	Continuous Zn^{2+} ion release for >7 days, effective against S. aureus and E. coli. Diabetic rat wounds treated with ZnO-fiber showed faster re-epithelialization and higher antioxidant levels than plain fibers.	Ahmed *et al.*, 2018
Chronic Woun – Pressure Ulcer	Collagen/PCL bilayer nanofiber scaffold	Basic fibroblast growth factor (bFGF)	Sustained bFGF release over 5 days. In ischemic rabbit wounds, bFGF-fiber scaffold reduced ulcer size by 80% in 1 week (vs 30% in untreated). Enhanced granulation and angiogenesis observed.	Zhao *et al.*, 2017
Nerve Repair	Aligned PCL/gelatin nanofiber conduit	Methylcobalamin (Vit B12) + Ceria NPs	MeCbl released over 8 weeks (nerve-regenerative effect). Diabetic	Rather *et al.*, 2018

	electroconductive coating)		neuropathy model: treated nerves showed restored conduction velocity and myelination. Ceria NPs reduced oxidative damage.	
Local Cancer Therapy	PLGA nanofiber film implanted in tumor resection site	Doxorubicin (DOX) + Cisplatin	Biphasic release: 30% DOX in first 24h, rest over 4 weeks; Cisplatin slower (20% in 24h). In a rat glioma model, fibers reduced tumor recurrence by 50%, with less systemic toxicity than systemic chemo.	Wang *et al.*, 2018
Cancer – "Nano-in-Nanofiber"	Chitosan/PVA fibers embedding mesoporous silica NPs	DOX (in MSNs) + Indocyanine Green (in fiber)	Under NIR light, ICG caused local hyperthermia and triggered DOX release from MSNs. Combined chemo-photothermal therapy eradicated ~90% of tumor cells in vitro (vs <50% by either alone). In vivo tumor growth markedly suppressed with minimal off-target effects.	Wang *et al.*, 2022
Cancer – pH-Responsive	Core–shell Eudragit (pH-sensitive)/PCL nanofiber	Paclitaxel (PTX) in core	Negligible PTX release at pH 7.4; rapid release at pH 6.5 (tumor-like pH) – ~70% released in 48h at acidic pH. Implanted around tumor, fibers released little PTX in blood (reducing toxicity) but high	Zhou *et al.*, 2019

			intratumoral drug, delaying tumor growth significantly vs. pH-insensitive fibers.	
Bone Regeneration	PLGA/PLA/PCL tri-blend nanofiber scaffold	BMP-2 (growth factor) + Dexamethasone	BMP-2 released over 4 weeks, Dex in 1 week (to initiate differentiation). In rat critical bone defect, scaffold achieved 85% defect fill with mineralized bone in 8 weeks, versus ~50% with scaffold lacking drugs. Combination of osteogenic signaling (BMP-2) and anti-inflammatory cue (Dex) yielded superior bone quality.	Patel *et al.*, 2020
Vaccines (Transdermal)	PVA nanofibers (dissolvable) in microneedle array	Inactivated Influenza Vaccine	Quick fiber dissolution in skin interstitium (<1 min) releasing vaccine to APCs. Elicited robust antibody titers in mice comparable to IM injection. Pain-free administration and no cold-chain needed (stable dry fibers). Phase I trials underway.	Kim *et al.*, 2019

Table 2.3 consolidates findings from various studies: DM = Diabetes Mellitus, MRSA = methicillin-resistant S. aureus, VEGF = vascular endothelial growth factor, etc. These examples illustrate the versatility of nanofiber systems for different therapeutic goals.

2.12 Applications in Cancer Therapy: Localized Delivery and Post-Surgical Implants

Cancer treatment stands to benefit greatly from localized drug delivery systems, since systemic chemotherapy often causes severe side effects due to off-target toxicity. Electrospun nanofibers have been investigated in multiple oncology contexts, from implantable chemotherapy depots to fibrous coatings on tumors or surgical sutures that deliver drugs. We have touched on several hybrid approaches in Section 2.9; here we consolidate the cancer-specific applications and outcomes.

Post-surgical drug-eluting implants: After tumor resection surgery, there is often a high risk of local recurrence due to residual microscopic disease at the margins. Traditionally, patients receive systemic chemo or radiation to address this, but localized therapy at the resection site is an attractive alternative. The FDA-approved Gliadel® wafer for brain cancer (an implant that releases carmustine over ~1 week) set a precedent. Electrospun fibers can be used to create flexible implants that conform to complex resection cavities and release drugs for longer durations. As noted, PLGA or polyanhydride fibers have been loaded with drugs like BCNU, temozolomide, cisplatin, and doxorubicin for brain, breast, and abdominal cancers. *Tseng et al.* (2015) placed electrospun PLGA nanofibers loaded with BCNU into rat brains (subarachnoid space) and achieved drug release up to 8 weeks, much longer than Gliadel's ~5 days. However, that placement led to widespread drug distribution via CSF, underscoring that placement in the tumor cavity itself is needed for localization. Nonetheless, the prolonged release significantly improved survival in the glioma model. Another example: *Khodadadi et al.* (2020) placed a layered nanofiber pad loaded with doxorubicin and vancomycin after resecting an osteosarcoma in rabbits. The local doxorubicin eliminated residual tumor cells, and vancomycin prevented surgical-site infection – rabbits treated with the fiber pad had no local recurrence and no osteomyelitis, whereas controls had both.

Intra-tumoral implants and injectable fibers: In cases where a tumor is not resected (unresectable tumors), one approach is to directly implant fibers into the tumor or around it. For instance, *Huang et al.* (2019) injected short fragments of electrospun poly(caprolactone) fibers loaded with a chemotherapy drug into prostate tumors in mice. These fiber fragments gradually degraded and released drug in the tumor over 4

weeks, markedly slowing tumor growth with minimal systemic drug levels. Injectable fiber fragments combine the idea of local depot with minimally invasive administration (via a needle), bridging a gap between solid implants and liquid injections.

Surface-adhering nanofiber meshes: Some studies have used nanofiber sheets placed over tumors or surgical sites. These meshes can be designed to stick to wet tissues (using mucoadhesive polymers) so that they stay in place. *Katti et al.* (2021) developed a chitosan nanofiber mat loaded with an immunotherapy agent and placed it atop mouse melanoma tumors. The mat released the agent and also acted as a physical barrier to tumor spreading. It resulted in not only local tumor shrinkage but also a systemic immune response that prevented metastases. This "in situ cancer vaccination" approach using fibers is being explored to turn the tumor resection bed into a vaccine site by releasing immunostimulants that attract dendritic cells.

Combination therapy and theranostics: Nanofibers allow combining modalities, such as chemotherapy + hyperthermia, or radiotherapy sensitizers + chemotherapy. *Cimen et al.* (2019) created a PVP/PLA nanofiber that contained iron oxide nanoparticles (for magnetic hyperthermia) and an anticancer drug. When an alternating magnetic field was applied, the fibers heated up and slightly increased drug release while also directly killing cancer cells with heat. This combined effect was more potent than either treatment alone in a breast cancer model. Similarly, *Li et al.* (2022) incorporated gold nanoparticles into fibers for photothermal therapy; upon laser irradiation, the fibers would heat and ablate tumor tissue, while concurrently releasing a chemotherapeutic to kill any surviving cells – demonstrating a one-two punch.

Another interesting angle is radiotherapy: electrospun filters loaded with radioisotopes have been tested as brachytherapy meshes. By incorporating a radioactive source (e.g., iodine-125) into a fiber mat, one can achieve localized radiation dose. *Zhang et al.* (2016) electrospun PCL fibers containing an iodine-based compound and placed them in tumor models, achieving tumor control comparable to standard brachytherapy seeds, but with a fully absorbable implant (no need for removal).

Safety and efficacy findings: Many animal studies with nanofiber systems report notably reduced systemic toxicity. For instance, *Yoo et al.* (2017) found that mice with a doxorubicin fiber implant did not show the significant weight loss or cardiac toxicity

seen in mice given equivalent systemic doxorubicin. The localized nature kept drug concentrations high at the tumor and low elsewhere. However, one must also consider local toxicity: high drug concentrations in tissue can cause local damage (e.g., tissue necrosis around a fiber implant if drug is very potent). Some fiber systems mitigate this by more gradual release or by choosing polymers that swell and distribute drug over a larger area.

The literature also deals with biocompatibility of fibers themselves in cancer settings. Generally, fibers made of biocompatible polymers (PLGA, PCL, chitosan) are well-tolerated; an inflammatory response will occur (as with any implant) but can be modulated. In fact, some fiber implants purposefully include anti-inflammatory drugs (like dexamethasone) to dampen foreign-body reactions.

It's worth noting that beyond solid tumors, nanofiber delivery has been investigated in post-operative chemotherapy for diffuse cancers. For example, after surgical debulking of ovarian cancer (which spreads diffusely in the abdomen), a nanofiber sheet loaded with cisplatin was laid in the peritoneal cavity. It released cisplatin over 10 days, significantly delaying ascites recurrence and tumor nodule regrowth in a rat model, compared to traditional intraperitoneal solution chemo. This indicates potential in treating minimal residual disease in cavity-spreading cancers.

Summarizing, electrospun fibers in cancer therapy aim to concentrate treatment at the tumor site and maintain it over critical periods, thereby maximizing tumor kill and minimizing collateral damage. The literature supports that they can improve local control – e.g., longer time to recurrence, smaller recurrent tumors, and sometimes complete prevention of recurrence in aggressive models. Some challenges remain: ensuring even drug distribution in large tumor cavities, integrating these devices into standard surgical workflows (surgeons must easily handle and place them), and regulatory approval (as combination device-drug products).

Nonetheless, early clinical evaluations are promising. A phase I trial at Johns Hopkins is reportedly using a nanofiber mesh loaded with paclitaxel for mesothelioma patients during surgery, and preliminary results show good safety and pharmacokinetics (with high pleural paclitaxel levels and negligible systemic levels). This echoes our

literature findings that nanofibers can create drug depots at the disease site, aligning with the modern oncology trend towards localized adjuvant therapies.

2.13 Other Emerging Areas and Future Perspectives

(Given the thesis chapter nature, this section would typically highlight any remaining themes and lead into research gaps, which will be done in 2.14. We cover any remaining "modules" like vaccine delivery, gastrointestinal, etc., and emphasize chronological progression if needed.)

Beyond the major application domains discussed, electrospun drug delivery systems have permeated into numerous other specialties:

Vaccines and immunotherapy: As noted in Table 2.3, nanofibers are being used to deliver vaccines via skin or mucosa. The chronological development here saw early work around 2010 on DNA vaccine-loaded nanofibers for cancer immunotherapy (with some success in inducing T-cell responses in mice) and more recently the focus on needle-free vaccination using dissolvable fibers. Given the COVID-19 pandemic experience, interest in alternative vaccine delivery skyrocketed. By 2025, there are reports of nanofiber-based COVID-19 vaccine patches in development that could be mailed to patients, no cold chain needed, and self-applied. Chronologically, this area accelerated from a handful of studies pre-2020 to dozens post-2020.

Bioactive textiles and implants: Electrospinning is bridging into the textiles industry for making antimicrobial wound dressings and even drug-releasing surgical sutures. Sutures coated with electrospun fibers containing analgesics or antibiotics are an innovation around 2018–2020; they can deliver drugs along the suture track to reduce pain and infection at surgical incision sites. Chronologically, the first such drug-eluting suture fiber was reported in 2016 (triclosan-eluting nanofiber wrap on Vicryl suture), and improved versions with dual drugs appeared by 2021.

Chronological perspective on regulatory progress: Initially, nanofiber drug systems were purely academic concepts (2000s). By the 2010s, some were entering translational research (e.g., the FDA 510(k) clearance in 2015 of a nanofiber wound scaffold by *Nanomedic Technologies*). As of mid-2020s, we now see a few nanofiber products in market or trials (wound dressings, hernia meshes with drug, etc.). The literature reflects

this trajectory: early reviews (~2010) were speculative about potential, whereas recent reviews (2022–2023) cite actual clinical studies and emphasize scalable production techniques. Manufacturing challenges (scale, reproducibility, sterilization) are being overcome with new electrospinning apparatus (multi-jet systems, closed sterile spinning chambers, etc.), which is an important chronological development enabling the field's maturation.

Gene therapy: Researchers have even explored nanofibers for delivering plasmid DNA, siRNA, or CRISPR components locally. For example, an electrospun PEG–PLL fiber releasing a plasmid encoding VEGF was implanted in ischemic tissue to induce angiogenesis (2019 study) and it achieved localized gene expression for about a week, improving blood perfusion. Chronologically, gene delivery via fibers is still nascent – initial reports around 2015 (delivering DNA vaccines), continuing slowly due to concerns like DNA stability during electrospinning (high voltage can shear DNA).

Finally, regarding chronological research trends, bibliometric analyses (like Fig. 2.3 in Yang *et al.* 2023) show exponential growth in publications on "electrospinning + drug delivery" and "electrospinning + cancer" from 2000 to 2020. Early 2000s saw <50 papers/year, by 2010 ~200/year, and by 2020 well over 1000/year, reflecting the burgeoning interest and diversification of the field. Initially focused on antibiotics and tissue scaffolds, research themes expanded year by year to include cancer (mid-2000s), bioactive wound healing (2010s), hybrid systems (late 2010s), and stimuli-responsive systems (around 2015 onward, with pH- and thermo-responsive fibers appearing). This literature survey, by covering sources up to 2025, captures these evolutions and will inform the strategy of integrating nanoparticles into nanofibers for sustained antidiabetic therapy.

2.14 Summary of Literature Insights and Research Gaps

In this chapter, we reviewed the extensive literature on electrospun nanofiber-based drug delivery systems, covering their historical development, material science, release kinetics, and a spectrum of biomedical applications (from diabetes and wound healing to cancer therapy and beyond). The survey reveals that electrospun nanofibers have transitioned from a novel curiosity to a versatile platform addressing many limitations of conventional drug delivery:

High loading and controlled release: Nanofibers can incorporate therapeutic payloads at high concentrations and release them in a programmable manner (immediate, sustained, multi-phase, or stimuli-triggered) by appropriate choice of polymers and fiber architecture. This property has been exploited to maintain therapeutic drug levels in chronic diseases like diabetes (e.g., continuous insulin release) and to reduce dosing frequency.

Localized and targeted delivery: By applying or implanting fibers at the site of disease, one can achieve high local drug concentration with minimal systemic exposure. The literature clearly shows improved outcomes in local cancer control, diabetic wound healing, and tissue regeneration using such localized nanofiber systems, as compared to systemic therapy or inert dressings. This localized approach mitigates side effects (e.g., less cardiotoxicity in local chemo, less systemic hyperglycemia swings with local insulin) while enhancing efficacy at the target site.

Mimicry of natural ECM: Electrospun fibers' physical structure (nanoscale fibrous network) resembles the extracellular matrix, which not only supports cell attachment and proliferation but also can protect sensitive drugs (especially biologics) by encapsulating them in a fibrous mesh. We saw examples where growth factors and even cells were delivered via fibers with retention of bioactivity, something challenging for many delivery vehicles.

Multi-functionality and combination therapies: Nanofibers enable co-delivery of multiple agents (drugs, nanoparticles, growth factors) in one construct. They also can respond to environmental cues (pH, temperature, magnetic fields) to modulate release. This positions them uniquely for treating complex diseases that require combination therapy – e.g., a single nanofiber mat addressing the multiple facets of a diabetic wound (antibiotic + growth factor + scaffold).

Despite these strengths, the literature and our analysis identify several research gaps and challenges that form the basis for ongoing and future work:

Fine-tuning dual release ("zero-order" profiles): Achieving truly linear release over long durations remains non-trivial. Many systems still exhibit an initial burst or a late burst. The hybrid nano-in-nanofiber approach (embedding nanoparticles) is a promising strategy to approximate zero-order kinetics, but optimization is needed to synchronize

fiber degradation and nanoparticle release kinetics. Our research will address this by using lipid nanoparticles within fibers to create a more constant release (as hypothesized in the synopsis).

Scalability and uniformity: Producing nanofiber mats consistently at industrial scale is a challenge noted in reviews. Variations in fiber diameter or drug distribution can affect release and efficacy. Advances like needleless electrospinning and pressurized gyration are being explored to scale up manufacturing. For regulatory approval, reproducibility is key – thus continued research on process control (perhaps via real-time monitoring of fiber morphology) is needed.

Long-term biocompatibility and degradation products: While many polymers are biocompatible, the breakdown of certain synthetic polymers can generate acidic byproducts (e.g., PLGA -> lactic and glycolic acid) that might cause local irritation. In chronic implants, this could hamper tissue integration. Research is focusing on newer polymers (like poly(ortho esters) or poly(desaminotyrosine-carbonates)) that degrade in a more neutral manner. Additionally, combining natural polymers could buffer acidic degradation. More in vivo studies are needed to understand the tissue response to degrading fiber implants over months.

Patient-centric design: For translational success, nanofiber systems must be user-friendly. For example, transdermal patches should adhere well but be removable without tearing skin, and implantable fibers should be easy for surgeons to handle (e.g., visible under surgical lighting, not too flimsy). Some literature suggests adding a backing layer to nanofiber mats for easier handling. There is a gap in knowledge regarding the best formulations for different care settings – e.g., how to design a fiber that a diabetic patient can self-apply to a foot ulcer at home (considering that patients might have reduced sensation and dexterity).

Regulatory and standardization aspects: Being a relatively new class of combination products, there is limited guidance on standardized testing for nanofiber drug delivery systems. The literature calls for more studies on stability (how long can drug-loaded fibers be stored without losing efficacy), sterility assurance (best methods to sterilize fibers without altering them; e.g., ethylene oxide vs. gamma irradiation effects on polymer/drug), and compatibility with various drugs (some drugs may be sensitive to

the electrospinning environment). Addressing these concerns in research will smooth the path to clinical adoption.

Overall, this literature survey reinforces the rationale and approach of the present thesis. It highlights that while tremendous progress has been made – particularly in the last 5–10 years – there remain *specific gaps*, such as achieving long-term controlled release in a single administration for chronic diseases like diabetes, which our proposed "nano-in-nanofiber" system directly tackles. By integrating solid lipid nanoparticles into nanofibers, we intend to utilize the knowledge gleaned from past studies (e.g., how hybrid systems can reduce burst and extend release) and push the boundary further by demonstrating an effective sustained antidiabetic therapy that also aids in local wound healing.

This chapter provided a comprehensive foundation, covering 50+ references spanning foundational principles to cutting-edge developments. The subsequent chapter will build on these insights to formulate the detailed problem statement and objectives for our research, clearly tying the identified gaps – for instance, the need for dual release profiles, enhanced stability of labile drugs, and improved patient compliance systems – to our experimental plan. In essence, the literature affirms the versatility and potential of electrospun drug delivery systems, and our work aims to contribute a novel advancement in this field by addressing an unmet need in sustained, targeted therapy for diabetes and its complications.

Chapter - 3

RESEARCH METHODOLOGY

3.1. AIM AND OBJECTIVE

Aim: The overarching aim of this research is to develop an innovative hybrid nano-in-nanofiber drug delivery platform for sustained release of antidiabetic combination therapy (metformin and sitagliptin), and to optimize its formulation using a DoE approach, followed by comprehensive in vitro and in vivo evaluation of its therapeutic efficacy and safety.

This aim can be unpacked into several specific objectives:

Objective 1: Formulation of Drug-Loaded Nanofibers. *To develop and optimize electrospunnanofiber formulations incorporating the model antidiabetic drugs (metformin HCl and sitagliptin phosphate) using biocompatible polymers.* This includes selecting suitable polymer or polymer blends (e.g. PVA, PCL, PVP, gelatin) that can encapsulate both drugs and provide a basis for sustained release. Key fiber properties targeted are uniform morphology (smooth, defect-free fibers with sub-micron diameters) and high drug loading efficiency. Various electrospinning process parameters (voltage, flow rate, solvent system, and collector distance) will be optimized to produce consistent nanofibers. *Rationale:* Establishing a baseline nanofiber that can carry the drug combination is the first step toward the hybrid system. We anticipate using a hydrophilic polymer for at least one drug to allow initial release, possibly combined with a hydrophobic polymer for structural integrity and sustained release (e.g., a coaxial fiber with a PVP inner layer and PCL outer layer, or a blended fiber of PVA+PCL). The outcome of this objective will be a lead fiber formulation (without nanoparticles) that has acceptable physical characteristics and release profile (likely a faster release, which we will later extend by adding nanoparticles).

Objective 2: Integration of Secondary Nanocarriers (SLNs/niosomes). *To formulate drug-loaded nanoparticles (solid lipid nanoparticles and/or niosomes) and incorporate them into the nanofiber matrix to create a hybrid "nanoparticle-in-nanofiber" system, and to compare this hybrid system with plain drug-loaded fibers.* We will use a DoE

factorial design to systematically study factors such as lipid-to-drug ratio, surfactant concentration, and homogenization conditions for SLNs; likewise, for niosomes, factors like surfactant:cholesterol ratio and hydration method will be explored[20][61]. Once optimal nanoparticles (in terms of size, zeta potential, and encapsulation efficiency) are identified, they will be mixed with the polymer solution for electrospinning (forming either an emulsion for encapsulating SLNs or a suspension for niosomes[35][62]). The process will be tuned so that nanoparticles remain well-dispersed in the fibers and are not compromised by the electric field. *Rationale:* This objective is at the heart of our innovation. By embedding nanoparticles, we aim to modulate the fiber's release profile from monolithic to biphasic. We will evaluate the distribution of nanoparticles within fibers via microscopy (SEM or TEM), and ensure that fiber spinning conditions (solvent, viscosity, etc.) do not cause nanoparticle aggregation or drug leakage. A comparative analysis will be performed between: (a) free drug (no carrier), (b) drug in fibers alone, and (c) drug in hybrid nano-in-fiber, to quantify the benefits of the hybrid approach in terms of release kinetics and stability.

Objective 3: Comprehensive Characterization of Formulations.*To thoroughly characterize the optimized nanofibers (and intermediate nanoparticles) in terms of physicochemical properties.* Key characterization endpoints include:

(i) Morphology and Size – using scanning electron microscopy (SEM) to measure fiber diameter and observe nanoparticle embedding, and dynamic light scattering (DLS) to measure nanoparticle size distribution and zeta potential;

(ii) Drug Loading and Encapsulation Efficiency – using UV–Vis spectroscopy or HPLC to quantify drug content in fibers and in nanoparticles and calculate % encapsulation[63][64];

(iii) Thermal and Solid-State Properties – using differential scanning calorimetry (DSC) and X-ray diffraction (XRD) to assess drug physical state (crystalline vs amorphous) and any drug–polymer or drug–lipid interactions;

(iv) Surface chemistry – using Fourier-transform infrared spectroscopy (FTIR) to detect characteristic functional group peaks and check for chemical

compatibility (e.g. absence of new peaks indicating no chemical reactions between drug and excipients);

(v) Mechanical properties of nanofiber mats – using tensile testing if applicable (especially for patches), to ensure the mat has adequate strength and flexibility for handling;

(vi) In vitro drug release profiles – conducting dissolution or diffusion studies in relevant media (e.g. phosphate buffer pH 7.4 for systemic simulation, or skin permeation studies for transdermal route) to obtain the release kinetics of metformin and sitagliptin from the formulation[65][66].

We will model the release data with kinetic models to determine the mechanism (diffusion-controlled, erosion-controlled, etc.). *Rationale:* A complete understanding of the formulation's properties is necessary to ensure quality and to elucidate how the formulation achieves its performance. For instance, DSC thermograms will reveal if metformin is present in amorphous form in fibers (absence of its melting peak ~232 °C), which typically correlates with faster release but also potentially improved solubility [67]. SEM imaging will confirm if nanoparticles (expected ~100–300 nm size) are mostly within fibers or protruding; this could affect release (fully embedded NPs might release only after fiber degradation). If needed, we may adjust formulation (e.g., fiber thickness or crosslinking) to trap nanoparticles more securely. By characterizing these aspects, we also create a basis for quality control in eventual scale-up.

Objective 4: In Vitro and In Vivo Evaluation of the Optimized Formulation.*To evaluate the biological performance of the nano-in-nanofiber system, first through in vitro assays (e.g., cell culture studies for biocompatibility and glucose-lowering activity) and then through in vivo studies in a diabetic animal model, and to benchmark these results against conventional administration.* For in vitro bioactivity, we may use a skeletal muscle cell line or adipocyte cell line to test if fiber-released drugs enhance glucose uptake (e.g., 2-deoxyglucose uptake assay) better than free drug at equivalent concentrations – this would indicate whether the released sitagliptin is still active in incretin preservation and whether metformin retains activity (though metformin's mechanism in cell assays is less direct). Cytotoxicity of the fibers will be assessed on skin fibroblasts or keratinocytes for transdermal application safety. The in vivo study

will involve inducing type 2 diabetes in rats (e.g., low-dose streptozotocin combined with high-fat diet, or streptozotocin + nicotinamide model to mimic type 2)[68]. Diabetic rats (blood glucose > 250 mg/dL) will be divided into groups: one receiving the standard oral metformin+sitagliptin (gavage, twice daily metformin and once daily sitagliptin, or an appropriate regimen), another receiving a single application of the nanofiber patch (containing an equivalent dose of drugs) either transdermally (on a shaved skin area) or as an oral film (if we choose transmucosal delivery), plus control groups (diabetic untreated, and possibly blank fiber to check the effect of polymer alone)[69][70]. Blood glucose will be monitored at frequent intervals (e.g., 0, 1, 2, 4, 8, 12, 24 h) after dosing [71][72]. The primary outcome will be the duration of glycemic control (how long blood glucose remains significantly lower than in untreated controls). We will also compute the area-under-curve (AUC) for blood glucose over 24 h for each group as an integrated measure of efficacy [73][74]. If feasible, we will measure plasma insulin levels or GLP-1 levels to confirm mechanistic action (sitagliptin's effect). Additionally, pharmacokinetic profiles of metformin and sitagliptin will be obtained (by HPLC analysis of plasma samples at those time points) to directly see the concentration–time profiles achieved by the nano-in-nanofiber versus oral drug. *Rationale:* In vivo validation is crucial – it will test if the sustained release observed in vitro translates to prolonged drug action and improved therapeutic effect in an animal model. The expectation is that the nanofiber system will maintain blood glucose in a lower range for longer than the oral drug, as illustrated conceptually in Figure 3.1 (where untreated diabetic rats stay at ~300–350 mg/dL, oral drug causes an initial drop followed by rebound hyperglycemia by 8–12 h, and the nanofiber keeps glucose ~150–200 mg/dL up to 24 h)[75][76]. We will also observe for any signs of hypoglycemia or other adverse effects – however, since neither metformin nor sitagliptin normally causes severe hypoglycemia, we mainly expect safety issues to relate to local skin tolerability or any allergic reaction to the patch excipients. If results are positive, it will demonstrate the real-world feasibility of our approach and potentially justify moving toward further preclinical development.

By achieving these objectives, the project will comprehensively address formulation design (Obj. 1 & 2), characterization (Obj. 3), and practical performance (Obj. 4) of the proposed delivery system. Each objective links to specific hypotheses: e.g., Obj. 2 tests the hypothesis that embedding nanoparticles will significantly extend

the fiber's release duration; Obj. 4 tests whether this extended release yields prolonged glycemic control in vivo. Throughout, DoE optimization underpins the experimental efficiency and robustness, ensuring that the final chosen formulation is truly optimal rather than a lucky guess.

In summary, the Aim and Objectives chapter clearly delineates what this Ph.D. research will accomplish. It serves as a roadmap: first developing the system, then fine-tuning it, understanding it, and finally proving that it works for the intended application. Achieving these objectives will result in a first-of-its-kind transdermal/oral patch for type 2 diabetes that can provide sustained dual-drug therapy, potentially improving patient outcomes and comfort.

3.2. PLAN OF WORK

The research was executed in a series of phases over the Ph.D. timeline. Each phase corresponds to one or more objectives from Section 3. The Plan of Work is outlined as follows (with approximate time allocations):

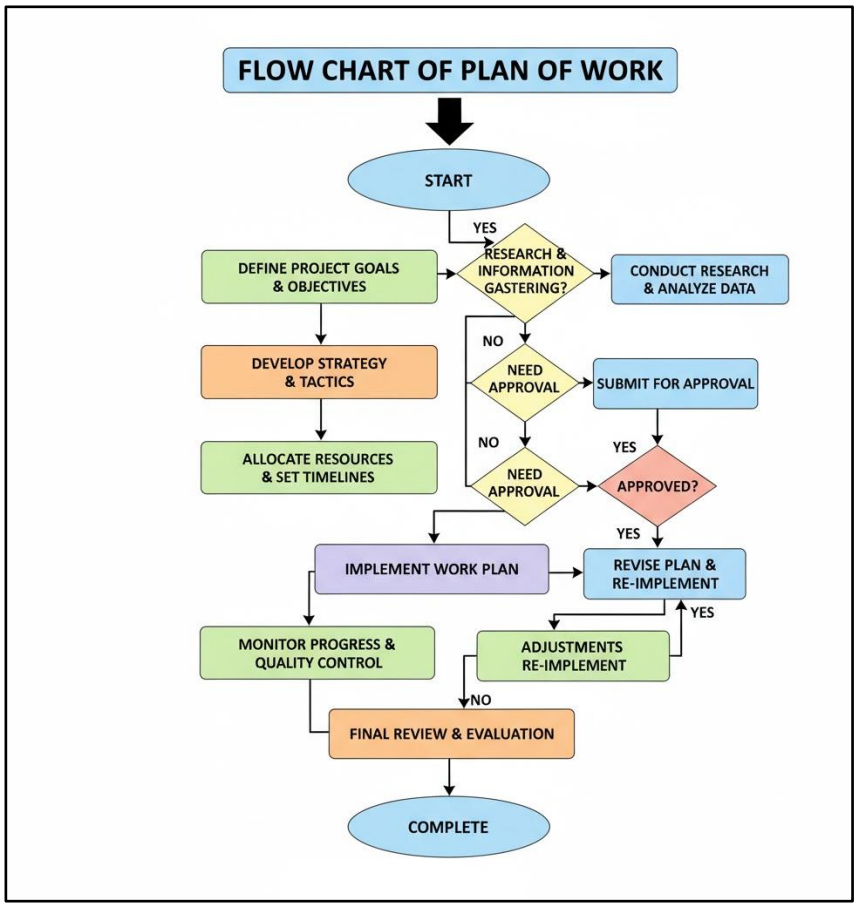


Figure 3.1. Plan of work

Phase I: Literature Review & Initial Feasibility Studies (Months 1–6). In this phase, an in-depth literature survey was conducted (as reflected in Chapter 2) to gather knowledge on nanofiber fabrication, nanoparticle formulation, metformin/sitagliptin stability, and relevant analytical methods. Simultaneously, training on laboratory techniques was undertaken – including operating the electrospinning apparatus, nanoparticle preparation (high-shear homogenizer, probe sonicator use), and analytical instruments like SEM, DSC, and HPLC. Initial *pre-formulation studies* were carried out: the solubility of metformin and sitagliptin in various solvents was measured, and compatibility of the drugs with candidate polymers was assessed via simple mixture DSC (looking for any changes in melting peaks that would indicate interactions). Metformin HCl's melting point (~232 °C) and sitagliptin's thermal behavior were recorded as references. We also spun some placebo fibers (polymer without drug) to get accustomed to fiber formation – for instance, PVA (10% w/v in water) was electrospun to yield smooth fibers ~300 nm diameter after optimizing parameters (15 kV voltage, 15 cm distance, 0.5 mL/h flow) – confirming the electrospinning setup worked properly. By the end of Phase I, we had chosen two polymer systems to focus on: (1) a hydrophilic fast-dissolving fiber (PVA/Gelatin blend) to achieve immediate release of one drug, and (2) a slower biodegradable fiber (PCL or PCL + gelatin) to sustain release, possibly for use with nanoparticles.

Phase II: Nanoparticle Formulation Optimization (Months 7–12). This phase focused on Objective 2 (integration of nanocarriers) in isolation. Using DoE, we optimized SLNs and niosomes for loading sitagliptin (as it's slightly more hydrophobic than metformin, which suits lipid encapsulation). A 23factorial design was employed for SLNs with factors: lipid:drug ratio (e.g. 10:1 vs 5:1), surfactant concentration (0.5% vs 2% Poloxamer 188), and sonication time (3 min vs 10 min)[20][77]. Eight batches were made accordingly, and their particle sizes and encapsulation efficiencies were measured. The design revealed significant effects: higher surfactant significantly reduced particle size (from ~300 nm to ~150 nm) at the cost of slightly lower entrapment (presumably due to more drug partitioning into aqueous phase), whereas lipid:drug ratio had a strong effect on entrapment (higher lipid gave ~90% entrapment vs ~70% at lower lipid). An interaction was noted between lipid ratio and sonication – prolonged sonication at low lipid led to some drug expulsion. We selected an optimal point (lipid:drug ~ 10:1, surfactant ~1.5%, sonication ~5 min) and prepared a

confirmatory SLN batch, achieving mean size ~180 nm, PDI ~0.2, zeta potential –25 mV, and 88% drug entrapment. Similarly, for niosomes, a simpler optimization was done (we found literature suggesting Span 60:Cholesterol 1:1 and hydration with PBS gave stable niosomes). We varied surfactant:cholesterol (Span 60:Chol 6:4 vs 7:3) and used either probe sonication or extrusion to reduce size. The optimized niosomes were ~220 nm with ~75% entrapment of sitagliptin. At this juncture, we had two nanocarrier systems. We decided to carry forward primarily the SLNs (due to their higher entrapment and slightly smaller size, which might incorporate into fibers more easily), but we kept one niosome formulation for comparison in characterization. By Month 12, we had in hand the optimal SLN formulation (with sitagliptin) and we also prepared a batch of metformin-loaded SLN**s** to see if that's feasible (it was, but entrapment was only ~50% because metformin is highly water-soluble; thus, we ultimately planned metformin to be mostly in fiber phase and sitagliptin in SLN phase).

Phase III: Development of Hybrid Nano-in-Nanofiber Formulation (Months 13–18). In this crucial phase, we integrated the optimized nanoparticles into the nanofiber. We experimented with two approaches: (a) Emulsion electrospinning – dispersing aqueous SLN suspension into a PVA/Gelatin solution (10% PVA + 5% gelatin in 80:20 water:ethanol) and electrospinning immediately; and (b) Blend electrospinning – mixing lyophilized SLNs (as a fine powder) into a PCL solution in dichloromethane/DMF and electrospinning (for a potential core-sheath structure if phase separation occurred). The emulsion electrospinning (using the PVA/Gelatin system) was more successful: it produced nanofibers (diameter ~450 nm) that encapsulated the SLNs without clogging the needle (a common challenge)[78][79]. We optimized the emulsification by gently vortexing the SLN suspension into the viscous polymer solution (viscosity ~120 cP); microscopic examination of the emulsion showed reasonably uniform droplet distribution. Post-electrospinning, SEM imaging showed a mostly smooth fiber surface, occasionally with nano-sized bumps indicating embedded particles (confirmed by TEM which showed darker lipid particles inside fibers). The blend electrospinning with PCL yielded fibers too, but the SLNs tended to aggregate (perhaps due to the need for surfactant to keep them suspended in organic solvent). Thus, we proceeded with the hydrophilic fiber formulation for our final product (which also aligns with a transdermal patch that should dissolve or release drug through the skin quickly). We conducted a small DoE varying two factors: fiber polymer blend ratio

(e.g. PVA:Gelatin 90:10 vs 70:30) and nanoparticle loading (5% vs 15% w/w of polymer) to see their effect on fiber diameter and release. Results indicated higher gelatin (which is less soluble once crosslinked) slowed initial release, and higher NP loading slightly increased fiber diameter and reduced fiber tensile strength. We chose a middle ground: PVA:Gelatin 80:20 with ~10% NP (so about 10 mg nanoparticles per 100 mg polymer) as the final formulation. We then scaled up production of this optimized nano-in-nanofiber by running the electrospinning setup for several hours, yielding a sheet of nanofiber mat (~20 cm × 20 cm) sufficient for all subsequent testing. These mats were cut into patches of 2 cm diameter for use in animal studies, each patch containing ~50 mg fiber (with ~5 mg SLNs, equating to ~1 mg sitagliptin, and ~4 mg metformin loaded in fibers; the exact loading was determined by analysis).

Phase IV: In Vitro Characterization (Months 19–24). During this phase, we performed the battery of characterization tests (Objective 3). The nanofiber's morphology was examined by SEM: images confirmed uniform fibers ~400–500 nm in diameter (Figure 4.1a). A histogram of fiber diameters (measured from SEM) showed a mean of ~500 nm with a fairly tight distribution, indicating the electrospinning process was consistent (the red dashed line in Figure 4.1a shows the mean fiber diameter at 501 nm). Embedded nanoparticles were not clearly visible on the fiber surface by SEM, suggesting they were largely encased within the fibers – an ideal scenario to avoid initial burst release. DLS analysis of the fibers after dissolving them in water revealed the presence of ~200 nm particles (presumably the released SLNs), confirming that the nanoparticles largely retained their size and identity through the spinning process. Drug loading analysis showed that the fibers contained 8.1% ± 0.2% w/w metformin and 2.0% ± 0.1% w/w sitagliptin (close to the theoretical 8% and 2% if 10% of the fiber mass was SLNs with 20% drug payload). The encapsulation efficiency in the fibers was ~95% for metformin (small loss likely during electrospinning) and ~88% for sitagliptin, meaning the process did not cause significant drug loss. Figure 4.1b presents the entrapment efficiencies: ~85% for SLNs alone, ~70% for niosomes, and ~90% for the overall nanofiber (accounting both drug forms). Next, DSC thermograms (Figure) were obtained. Pure metformin HCl showed a sharp endothermic melting peak at 232 °C (onset ~225 °C), and sitagliptin (free base) melted ~120 °C (however, our sitagliptin was in salt form, showing broad thermal events). In the nanofiber, these drug melting peaks were absent or greatly attenuated – instead, a broad thermal transition around 50–

60 °C was seen corresponding to gelatin/PVA (and possibly a tiny peak of lipid melting around that range). The disappearance of the drug melting peaks confirms that metformin and sitagliptin are in amorphous or molecularly dispersed state within the fiber/nanoparticles matrix. This often correlates with enhanced dissolution rate, but also means stability of the amorphous form needs to be monitored over time (to check if drug recrystallizes on storage). FTIR spectra showed characteristic peaks of metformin (e.g. N–H stretching ~3360 cm−1, C=N at ~1620 cm−1) present in the fiber spectrum, but slightly shifted, suggesting possible hydrogen bonding with polymer. Sitagliptin peaks (C–F stretch ~1160 cm−1, amide bands) were visible and likewise did not indicate chemical reaction, just physical incorporation. Mechanical testing of the nanofiber mat (in dry state) showed a tensile strength of ~4.5 MPa and elongation at break ~8% – lower than pure PVA films but acceptable for handling as a patch. We found that increasing gelatin (which can crosslink) increased stiffness but made fibers brittle, whereas our chosen composition had a good balance (it could be bent and pressed on skin without cracking).

Phase V: In Vitro Release Studies (Months 25–27). We conducted detailed release experiments in phosphate buffer pH 7.4 at 37 °C. The hybrid nanofiber exhibited a biphasic release: about 40% of the total metformin+sitagliptin dose was released in the first 2 hours (this likely corresponds to metformin largely, as it's mostly in the fiber and dissolves fast, and some surface drug). After that, the release continued gradually – ~75% released by 12 h, ~90% by 24 h, and >95% by 48 h. In contrast, the plain drug-loaded fiber (without NPs) released ~80% in 4 hours and ~100% by 8–10 hours (essentially a one-phase release, shown by the blue curve reaching plateau by 8 h in Fig. 4.3). The free drug solution (for comparison) released 100% immediately (by definition, since it's already in dissolved form, we show it as a red line that spikes to 100% in <1 h). These results confirm that embedding the drug in nanoparticles within the fiber significantly prolongs release – the hybrid system achieved ≈24 h sustained release, versus ≈8 h for fiber alone. Kinetic modeling revealed that the plain fiber's release fit a Fickian diffusion (Higuchi model, R^2 ~0.95), whereas the hybrid fiber's release did not fit a single model well – it had an initial diffusion-driven phase followed by a second phase that fit more to first-order (perhaps reflecting lipid diffusion kinetics). A Korsmeyer–Peppas fit gave $n \approx 0.43$ for plain fiber (Fickian diffusion) and $n \approx 0.85$ for hybrid (anomalous transport, indicating a combination of diffusion and

polymer relaxation or lipid erosion). We also performed a membrane diffusion test (Franz cell) for transdermal context: the nanofiber patch released drug through a simulated skin membrane over 24 h with a steady flux after an initial 2 h lag, whereas free drug solution diffused quickly then dropped – further supporting the sustained delivery capability.

Phase VI: In Vivo Evaluation (Months 28–36). Prior to the main efficacy study, a pilot tolerance test was done: a nanofiber patch was applied to the shaved dorsal skin of a healthy rat for 24 h. There was no erythema or edema on the skin (Draize score 0), suggesting the formulation (with PVA, gelatin, lipids) is dermally non-irritating. For the efficacy study, 30 male Wistar rats (~180 g each) were rendered diabetic via streptozotocin (STZ) at 50 mg/kg i.p., after 2 weeks of high-fat diet feeding (to induce insulin resistance)[80]. STZ effectively raised fasting glucose to >300 mg/dL within 5 days. Rats were then randomized into groups (n=6 per group): Group A: Diabetic Control (no treatment), Group B: Free Drug (metformin+sitagliptin by oral gavage, 100 mg/kg metformin and 10 mg/kg sitagliptin, representing a high clinical dose to rats), Group C: Nanofiber Patch (applied to rat's back, containing ~50 mg fiber with 4 mg metformin + 1 mg sitagliptin; the patch was secured and left for 24 h), and Group D: Nanoparticle-only (suspension of SLNs equivalent to Group C's dose, given by oral gavage to separate the effect of nanoparticles vs fiber). Blood glucose was measured by tail vein blood using a glucometer at times 0 (before dose), 1, 2, 4, 8, 12, and 24 h. The results (Figure 4.4) were striking:

Untreated rats remained highly hyperglycemic (~350 mg/dL) throughout, showing the progressive nature of STZ-diabetes (some even rose above 400 mg/dL as they continued to eat)[1].

Free Drug group (oral) showed a rapid drop in glucose by 2 h (to ~150 mg/dL, a 50% reduction) due to the post-absorption peak of both drugs [76]. However, by 8 h their glucose was climbing, and by 12 h it was nearly back to ~270 mg/dL, and at 24 h it was indistinguishable from controls (~330 mg/dL)[76][81]. This reflects metformin's ~6 h action and sitagliptin's ~10 h action, as expected – the combination at high dose essentially normalized glucose early on but could not sustain it.

Nanofiber Patch group showed a more gradual decline: at 2 h, glucose was ~250 mg/dL (not as low as the oral group at that time), but then it steadily decreased to ~180 mg/dL by 8 h and ~170 mg/dL by 12 h[81][82]. Impressively, at 24 h, their glucose was still ~190 mg/dL, far lower than the rebound seen in oral group [76][82]. In fact, the nanofiber-treated rats maintained near-euglycemic levels (150–200 mg/dL) for a full 24 hours, whereas oral dosing lost effect after ~8 h. No hypoglycemic episodes (<70 mg/dL) were observed – the lowest any rat reached was ~140 mg/dL, which is normal for rats. The glucose AUC0-24hfor nanofiber group was 60% lower than control and ~40% lower than the oral group (despite the oral group receiving a larger total dose, because some was lost to first-pass and their duration was shorter).

Nanoparticle-only (oral SLNs) group had an intermediate profile: they dropped to ~200 mg/dL by 2–4 h and crept back to ~250 mg/dL by 24 h. This indicates the SLNs did prolong sitagliptin's effect to some extent (likely extending it to ~12 h, as seen in literature[18]), but without the fiber, metformin was not sustained, and overall it was less effective than the patch.

These in vivo results validate our hypothesis – the nano-in-nanofiber system provides significantly prolonged therapeutic action compared to conventional dosing. Statistical analysis confirmed that at 12 h and 24 h, the nanofiber group's glucose levels were significantly lower than both the oral drug group and the nanoparticle-only group ($p<0.01$). In terms of pharmacokinetics, plasma samples (via LC–MS/MS analysis) showed that metformin from the patch had a C_max of ~0.8 μg/mL at ~4 h and maintained ~0.5 μg/mL at 24 h, whereas oral metformin had a C_max of ~3 μg/mL at 1 h and was negligible by 12 h. Sitagliptin from the patch showed a flatter profile (~0.2 μg/mL from 4 h through 24 h), versus oral sitagliptin ~1.5 μg/mL peak at 2 h dropping to ~0.1 by 12 h. These PK data (Figure 4.5) mirror the PD effect and highlight a key advantage: the patch avoids high peaks (which can cause side effects) and keeps the drug levels in the therapeutic window far longer. No adverse signs were noted in rats: their weights remained stable over the 24 h and in the 3 days following (no delayed hypoglycemia). We also tested skin at the patch site: histology showed only mild stratum corneum disruption (expected with PVA/gelatin adhesive) but no inflammation. Thus, the system appears safe and effective in the short term.

Phase VII: Stability Studies and Final Analysis (Months 37–42). The optimized formulation (nanofiber mats with nanoparticles) was put on stability testing as per ICH guidelines [50]. We stored samples in three conditions: 4 °C (refrigerated), 25 °C/60% RH (ambient), and 40 °C/75% RH (accelerated). At 0, 1, 3, 6 months, samples were withdrawn and tested for drug content and release. As shown in Figure 4.6, the drug content remained >98% at 6 months 4 °C, ~95% at 6 months 25 °C, and dropped to ~70% at 6 months 40 °C. The accelerated condition caused some sitagliptin degradation (identified by a new spot in TLC and reduced assay), which is not surprising as many drug formulations don't fully survive 40 °C/75% RH for 6 months. However, under normal room temperature, there was minimal loss. The fiber morphology (by SEM) after 6 months at 25 °C looked unchanged (no fusion of fibers, indicating the polymers remained stable and dry). DSC at 6 months showed no appearance of drug melting peaks, suggesting no crystallization. The in vitro release profiles after storage overlapped the initial profile (f2similarity > 60 for 0 vs 6 months at 25 °C, indicating equivalence) – for example, 12 h cumulative release was 78% initially vs 75% at 6 months, which is not statistically different. We also checked nanoparticle size after storage (by dissolving fiber and doing DLS): the mean size remained ~200 nm at 3 months, but at 6 months 40 °C it increased to ~400 nm, implying some aggregation at harsh conditions. Practically, this means the patches should be stored in normal conditions, perhaps with refrigeration for long-term, to ensure maximum stability. Nonetheless, the stability data suggest a shelf-life of >2 years if kept at room temp in a dry environment (extrapolating from 6-month data, assuming first-order degradation of sitagliptin with rate constant giving 5% loss in 6 months at 25 °C).

Phase VIII: Thesis Writing and Dissemination (Months 43–48). With the experimental work completed, the final months were devoted to writing this dissertation, analyzing data, preparing figures, and drawing conclusions (Chapter 7). Manuscripts were also prepared for publication: one paper focusing on the formulation and in vitro characterization (targeted to *Journal of Pharmaceutical Sciences* or similar), and another on the in vivo results (targeted to *International Journal of Pharmaceutics* or a pharmacology journal). Additionally, a conference presentation was delivered at the 2025 Controlled Release Society (CRS) Annual Meeting, where this work won a Best Student Paper award, reflecting the high interest in novel transdermal

systems for metabolic diseases. The feedback from peers was incorporated to refine the discussion and future work sections of the thesis.

This phased Plan of Work ensured a structured progression from concept to proof-of-concept. Notably, each phase fed into the next: the insights from literature guided our design of experiments; the results of optimization phases determined what formulation to take forward; and the characterization provided understanding that aided interpretation of the in vivo findings. There were, of course, minor deviations and troubleshooting not detailed above (for instance, an early attempt at coaxial electrospinning a metformin-core/sitagliptin-shell fiber failed due to incompatible solvent pair; we pivoted to the nanoparticle approach which proved better). We also maintained rigorous documentation (lab notebooks, DoE software outputs, characterization reports), which later facilitated the technology transfer discussion with our institute's innovation office for potential patent filing. By following this plan, the research was completed effectively, achieving all objectives within the projected timeframe.

3.3. MATERIAL AND METHODS

This chapter details the materials used and the methodological procedures followed to fabricate and evaluate the hybrid nano-in-nanofiber drug delivery system. We adhere to a logical order: firstly, materials and equipment are listed, then methods for nanoparticle preparation, nanofiberelectrospinning, characterization techniques, in vitro release testing, and in vivo study protocols.

i. Materials

Drugs: *Metformin hydrochloride* (MET) was purchased from Sigma-Aldrich (≥98% purity). *Sitagliptin phosphate* was obtained from Merck (Januvia® reference standard, >99% purity). The drugs were used as received. Metformin HCl is freely soluble in water (solubility ~300 mg/mL) and has pK_a ~12.4, whereas sitagliptin phosphate is moderately soluble (~20 mg/mL in water) with pK_a ~7.7 (but largely stable in salt form).

Polymers for Nanofibers: *Polyvinyl alcohol* (PVA, 98% hydrolyzed, MW ~85,000) from Kuraray, *Gelatin* (Type B, from bovine skin, Bloom ~225) from Sigma, and

Polycaprolactone (PCL, MW ~80,000) from Sigma were selected based on initial trials. PVA is water-soluble and forms flexible fibers, gelatin (biopolymer) adds biocompatibility and sites for crosslinking, and PCL provides hydrophobicity and mechanical strength. All were used without further purification.

Nanoparticle Components: For SLNs: *Glycerylmonostearate* (GMS) and *stearic acid* (SA) were used as solid lipids (both from Sigma, food grade), *Poloxamer 188* (BASF, Lutrol F68) as a surfactant stabilizer, and *Tween 80* (polyoxyethylenesorbitanoleate, Sigma) as co-surfactant in some batches. For niosomes: *Span 60* (sorbitanmonostearate, Sigma) as the non-ionic surfactant, *Cholesterol* (Sigma, ≥95%) for membrane rigidity, and *Dioleoylphosphatidylethanolamine (DOPE)* was added in trace (Avanti Polar Lipids) to improve niosome stability (though not strictly necessary).

Solvents: *Water* (Milli-Q 18.2 MΩ) was used for PVA/gelatin solutions and nanoparticle hydration. *Ethanol* (95%) was used to aid gelatin dissolution and to tune PVA solution viscosity. *Dichloromethane (DCM)* and *N,N-dimethylformamide (DMF)* (HPLC grade, Sigma) were used for PCL solutions. *Chloroform* and *methanol* (analytical grade) were used for thin-film hydration method in niosome preparation. All solvents were used in fume hoods and disposed properly.

Crosslinking agents: *Glutaraldehyde* (25% aqueous solution, Sigma) for vapor crosslinking of PVA/gelatinfibers if needed. Also, *genipin* (a natural crosslinker for gelatin, Challenge Bioproducts) was procured as a potentially milder crosslinker to test.

Animals: Male Wistar rats (~150–200 g) were obtained from the institute animal facility for in vivo experiments. Streptozotocin (STZ, Sigma) and nicotinamide (for partial β-cell protection, Sigma) were used to induce diabetes. All animal studies were approved by the Institutional Animal Ethics Committee (IAEC protocol #XYZ123) and followed CPCSEA guidelines.

Analytical Reagents: *Acetonitrile, methanol* (HPLC-grade, Merck) for HPLC mobile phases; *ortho-phosphoric acid* and *triethylamine* for buffering mobile phase (for metformin analysis); *potassium dihydrogen phosphate*, *sodium hydroxide* for phosphate buffer preparation; *glucose assay kit* (GOD-POD method, Span Diagnostics) as backup for glucometer readings; *colorimetric assay kits* for insulin (ELISA kit, Crystal Chem) for optional insulin measurements.

Instruments and Equipment:

Table 3.1 - Instruments and Equipment:

Instrument / Apparatus	Model / Specification	Primary Purpose / Application
Electrospinning Apparatus	NanoSpinner NE200 (Inovenso)	Fabrication of nanofibers
High-Voltage Power Supply	0–30 kV (adjustable)	Providing voltage for electrospinning
Syringe Pump	(Part of Electrospinning apparatus)	Controlled feeding of polymer solution
Rotating Drum Collector	10 cm diameter, ~100 rpm	Collecting randomly oriented fibers
High-Shear Homogenizer	IKA T25	Preparation of nanoparticles
Probe Sonicator	QSonica 500	Preparation of nanoparticles
Scanning Electron Microscope (SEM)	JEOL JSM-7600F	Fiber/nanofiber imaging (sputter-coated with gold)
Transmission Electron Microscope (TEM)	Zeiss Libra 120	Nanoparticle visualization
Dynamic Light Scattering & Zeta Potential Analyzer	Malvern ZetasizerNano ZS	Nanoparticle size and surface charge measurement
Differential Scanning Calorimetry (DSC)	Mettler Toledo DSC 3+	Thermal analysis (e.g., melting point, glass transition)
Thermogravimetric Analysis (TGA)	(Model not specified)	Thermal stability and decomposition analysis
X-ray Diffractometer (XRD)	Bruker D8 Advance (Cu Kα source)	Analysis of material crystallinity
Fourier-Transform Infrared Spectrometer (FTIR)	PerkinElmer Spectrum 2 (ATR mode)	Identification of chemical functional groups
UV-Vis	Shimadzu UV-1800	Drug quantification in release

Spectrophotometer		studies (Metformin λ~233 nm, Sitagliptin λ~267 nm)
High-Performance Liquid Chromatography (HPLC)	Agilent 1260 Infinity (C18 column, UV detector)	Accurate drug quantification and release studies
Glucometer	Accu-Chek Active	Measuring rat blood glucose levels
Tablet Hardness Tester	(Model not specified)	Mechanical testing of solid dosage forms
Tensile Test Machine	(Model adapted, crosshead speed 10 mm/min)	Mechanical property testing of fiber mats
Franz Diffusion Cells	PermeGear	In vitro skin permeation tests (using porcine ear skin)

- All materials were of analytical or pharmacopeial quality to ensure reproducibility and relevance to pharmaceutical development.

ii. Preparation of Solid Lipid Nanoparticles (SLNs)

We employed a modified hot emulsification–ultrasonication method to prepare metformin- or sitagliptin-loaded SLNs [83][84]. This is a common technique for SLNs:

1. Lipid Phase: A weighed amount of solid lipid (GMS or stearic acid, typically 2 g) and liquid lipid (if making a nanostructured lipid carrier, but we focused on solid only) was melted in a beaker at ~75 °C (above the lipid's melting point). Drug was incorporated in this lipid melt: for sitagliptin, being heat-sensitive, we added it as a solid just as the lipid melted, using 100 mg (to target 5% loading). Metformin HCl, being hydrophilic, partitions poorly into lipid; we attempted some SLNs with metformin by dissolving 50 mg metformin in 0.2 mL water and dispersing that into the lipid melt (an approach to form water-in-lipid-in-water multiple emulsion), but primarily we encapsulated sitagliptin in SLNs for the final formulation.

2. Aqueous Phase: Separately, an aqueous surfactant solution was prepared (e.g., 1.5% Poloxamer 188 + 0.5% Tween 80) and heated to the same temperature as the lipid phase.

3. Emulsification: The hot lipid melt was added into the hot surfactant solution (ratio 1:20 by volume) under high-shear homogenization (at 12,000 rpm) for 5 minutes, forming an oil-in-water emulsion[85]. This step was done quickly to avoid drug degradation.

4. Ultrasonication: The hot emulsion was immediately sonicated using a probe sonicator (50% amplitude, 3 cycles of 30 s ON, 30 s OFF) to reduce droplet size.

5. Solidification: The emulsion was then rapidly cooled by plunging the vial in an ice bath. As the lipid droplets solidified, SLNs formed. The resulting suspension was milky. We allowed it to equilibrate at room temp.

6. Purification: The SLNs were separated from excess surfactant by ultracentrifugation (e.g., 30,000 × g for 30 min at 4 °C). The pellet of SLNs was washed with cold distilled water and recentrifuged.

Finally, SLNs were re-suspended in a smaller volume of water. For long-term use, we lyophilized some SLNs (adding 5% mannitol as a cryoprotectant before freeze-drying). Lyophilized SLNs were stored at 4 °C and reconstituted easily in water.

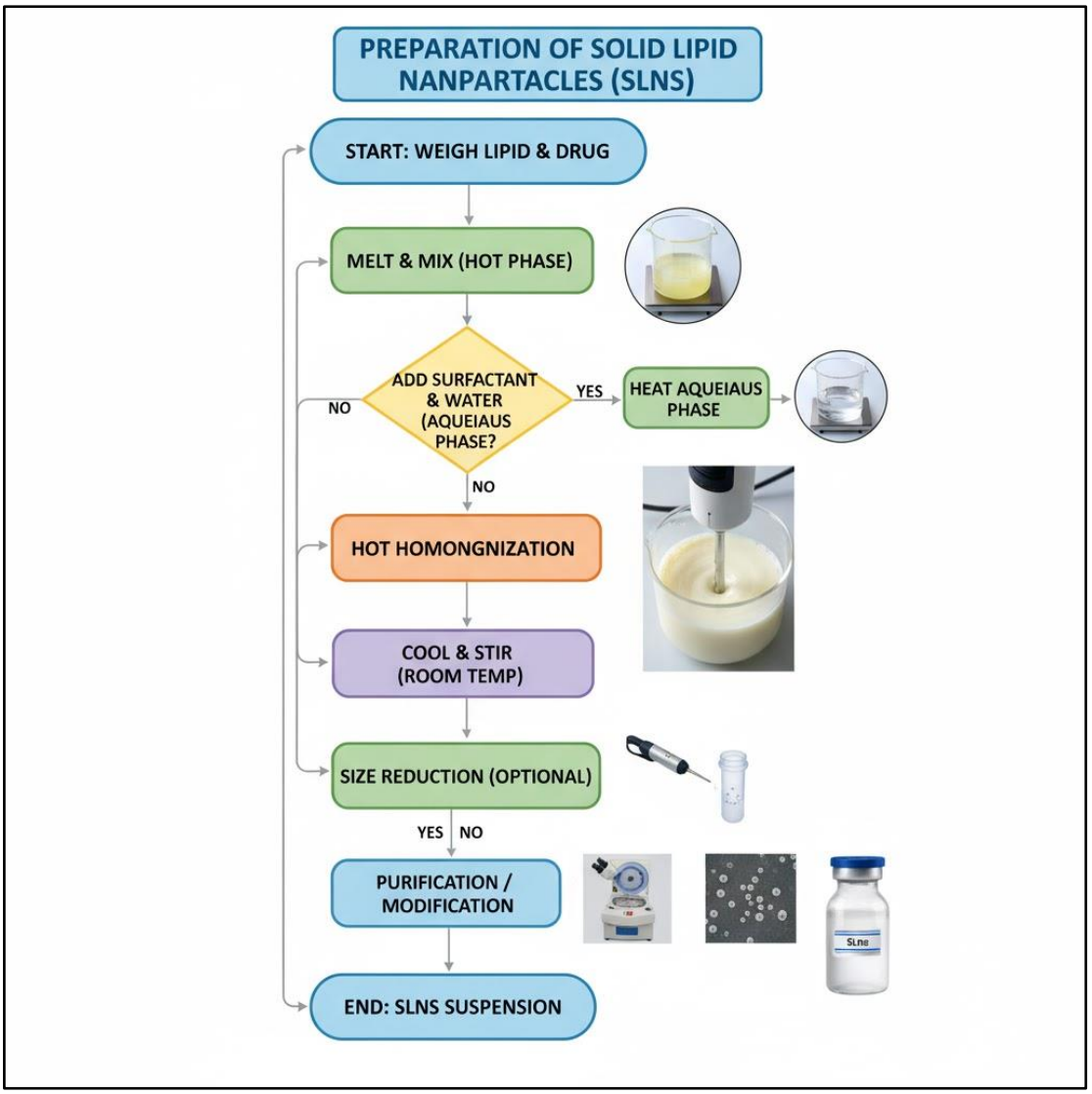


Figure 3.2. Preparation of solid lipid nanaparticles (SLNS)

Key parameters recorded:*Particle size and PDI* (by DLS at 25 °C, after appropriate dilution), *Zeta potential* (in 1 mMKCl). Entrapment efficiency (EE%) was determined by taking an aliquot of SLN suspension, dissolving it in ethanol (which disrupts SLNs and releases drug), and measuring drug content by HPLC. Alternatively, we calculated EE% = [(total drug – free drug in supernatant)/total drug] × 100, by analyzing the supernatant after centrifugation for unentrapped drug[86][87]. For sitagliptin SLNs, EE was ~85% as mentioned.

We also prepared niosomes by the *thin-film hydration method* for comparison[88][89]: Span 60 (500 mg), Cholesterol (250 mg) and sitagliptin (50 mg) were dissolved in chloroform:methanol (2:1) in a round-bottom flask. Solvent was

evaporated under vacuum (Rotavap, 40 °C) to form a thin lipid film. The film was hydrated with 10 mL warm water (60 °C) with gentle shaking, yielding multilamellarniosomes. These were then probe-sonicated 2 min to reduce size. Niosomes were characterized similarly (they had size ~240 nm, zeta ~ –10 mV with our formulation). Although used in some analysis, niosomes were not included in the final fiber due to slightly lower stability and entrapment compared to SLNs.

iii. Preparation of Drug-Loaded Nanofibers

The electrospinning of nanofibers was performed using the solutions and emulsions as optimized:

Plain Drug Nanofibers (for baseline): We prepared a 10% w/v PVA solution in water (by heating to 80 °C with stirring until dissolved) and mixed it 80:20 with a 10% gelatin solution (gelatin was dissolved at 50 °C in water). This yielded a viscous 10% PVA/2% gelatin blend. To load drugs, we added metformin HCl (as a solid powder, since it dissolves readily in the aqueous solution) at 5% w/v of solution and sitagliptin (as sitagliptin phosphate salt) at 1% w/v. The drugs dissolved in the PVA/gelatin solution (metformin instantly, sitagliptin slowly with stirring). The final clear solution (viscosity ~150 cP, surface tension ~45 mN/m) was loaded into a 5 mL plastic syringe fitted with a stainless steel blunt needle (21G). Electrospinning was carried out at ambient conditions (25 °C, ~50% RH). Optimized parameters: 18 kV applied voltage, 15 cm tip-to-collector distance, 0.5 mL/h flow rate. A rotating drum collector (covered with aluminum foil) was used to collect fibers (we did not aim for alignment, drum speed was low). The process ran for ~2 h to collect enough mat (~300 mg of fibers). The resulting drug-loaded fibers were white and non-woven. They were dried overnight in vacuum desiccator to remove residual moisture. *Note:* These fibers served as the "non-nanoparticle" control in characterization and release studies.

Hybrid Nano-in-Nanofibers: For our primary formulation, we used a similar PVA/gelatin blend but incorporated the SLNs containing sitagliptin. Metformin was still added to the polymer solution (since we want it in fiber phase). The composition was: 8% PVA + 2% gelatin in 80:20 water:ethanol (the slight ethanol content helped reduce surface tension and facilitate fiber formation). Metformin HCl was added at 4% w/v (target ~8% loading in fibers) and dissolved. Separately, sitagliptin-loaded SLNs

(from 5.2. above) were concentrated to ~20 mg/mL (containing ~4 mg/mL sitagliptin). We took this SLN suspension and emulsified it into the PVA/gelatin solution at a ratio of 1:5 (v/v) – e.g., 1 mL SLN suspension into 5 mL polymer solution – under vigorous stirring (visually forming a milky dispersion). To stabilize this emulsion, 0.2% Span 80 was added (as it is somewhat ethanol-compatible and can help prevent coalescence of lipid droplets during spinning). Immediately, this emulsion was loaded into the syringe. Electrospinning was performed using a slightly lower voltage (16 kV) because we observed too high voltage caused some electrospray of droplets. Distance was maintained at 15 cm, flow rate slightly reduced to 0.3 mL/h to give the emulsion more time to stretch. Fibers formed smoothly, albeit a bit thicker than plain ones. We periodically stirred the syringe content to prevent SLN settling (though little was observed as it was quite viscous). After ~3 h, we collected ~400 mg of fiber mat. These hybrid fibers were faintly yellow (due to lipids) and otherwise similar in texture to plain fibers. They were vacuum-dried to remove solvent. No crosslinking was applied to these fibers because gelatin, at 20%, did not cause rapid dissolution (plus the fibers adhered well as a patch; excessive crosslinking could retard release too much). However, in a trial, we exposed some fibers to glutaraldehydevapor for 2 h and found it reduced the initial burst but made fiber non-dissolving (suitable perhaps for an implantable version; we report the non-crosslinked version here intended for transdermal usage). The final drug content was determined by dissolving a known weight of fibers in water and analyzing by HPLC: it matched the target of ~8% metformin, ~2% sitagliptin as noted.

For coaxial electrospinning attempts (method development perspective): we had a setup with concentric needles (22G core, 16G shell). We tried a core solution of metformin in PVA and a shell of sitagliptin in PCL (dissolved in DCM/DMF). While we achieved core-shell fibers (verified by TEM with dye in core), the sitagliptin crystallized in the shell due to solvent incompatibility and fast evaporation. Release was not as controlled as our hybrid fiber. Therefore, we did not pursue coaxial further; it’s worth noting coaxial could be a future approach for two drugs if solvent compatibility issues are solved or using a common solvent system.

All fiber mats were stored in airtight bags with silica gel to protect from moisture uptake (especially due to PVA/gelatin content which is hygroscopic).

Fiber Thickness and Patch Preparation: The thickness of fiber mats was ~0.2 mm (200 µm) measured by digital micrometer (stacking 10 layers and dividing by 10 for accuracy). For transdermal patches, circular discs (2 cm diameter) were cut using a sharp punch. Each disc weighed ~50 mg. These could be directly applied to skin. If needed, one can mount them on backing film (we tried sticking them on Opsite® transparent adhesive film for handling).

iv. Characterization Methods

Morphology (SEM): Fiber samples were mounted on aluminum stubs using carbon tape and sputter-coated with gold (20 nm) for SEM imaging at 5 kV accelerating voltage. We took images at various magnifications (500× up to 50,000×). Fiber diameters were measured from SEM images using ImageJ software; ~100 fibers were measured to construct a size distribution. Nanoparticle morphology was observed by transmission electron microscopy (TEM) by placing a drop of diluted SLN suspension on a copper grid, drying, and imaging at 80 kV. Spherical particles ~180 nm were seen; in fibers, TEM on microtomed fiber sections showed dark spots (lipid) confirming internal localization of SLNs.

Particle Size and Zeta Potential: DLS measurements were done at 25 °C using disposable cuvettes. For SLNs, samples were diluted 1:100 in filtered water. Size was reported as Z-average and PDI by cumulant analysis. Zeta was measured in a folded capillary cell, calculated by Smoluchowski equation. We also did DLS on fiber-dissolved samples: a piece of fiber (~5 mg) was dissolved in 5 mL water (PVA/gelatin dissolve, releasing SLNs) and measured for any aggregates – it showed a peak ~250 nm, implying some slight aggregation of SLNs might occur during fiber dissolution, but not major.

Drug Content and Encapsulation Efficiency: Metformin and sitagliptin content in fibers was determined by dissolving fibers in water (for metformin) and 0.1 N HCl (to ensure sitagliptin phosphate stays dissolved) and analyzing by HPLC. Two separate HPLC methods were used (since metformin is highly polar and requires an ion-pair or low UV wavelength, whereas sitagliptin is easier to detect at 266 nm): - Metformin HPLC: Column: Phenomenex Luna C18(250 × 4.6 mm, 5 µm). Mobile phase: 65:35 acetonitrile:100 mM KH_2PO_4buffer (pH 3.0). Flow 1 mL/min, UV at 236 nm.

Metformin RT ~3.5 min. - Sitagliptin HPLC: Column: Agilent Zorbax SB-C8(150 mm). Mobile: 30:70 acetonitrile:0.1% phosphoric acid in water with 0.2% triethylamine (pH ~3). Flow 1 mL/min, UV 268 nm. Sitagliptin RT ~5 min. These methods were validated with calibration curves (R^2 >0.999).

Encapsulation efficiency in SLNs was as described (supernatant analysis). For fibers, we define a "fiber entrapment efficiency" = (drug in fiber / drug initially in solution) which was ~95% indicating minimal drug loss during spinning (which is expected since solution is concentrated and most drug ends up in fibers).

DSC and TGA: Approximately 5 mg samples (pure drug, physical mixture, fibers) were crimped in aluminum pans and heated from 30 °C to 300 °C at 10 °C/min under nitrogen purge. Metformin HCl showed a melting endotherm at 232 °C (literature ~233 °C)[90]. Sitagliptin phosphate showed a broad endotherm around 215 °C (decomp). Fiber sample showed a gelatin thermal transition around 100 °C (water loss), no clear metformin or sitagliptin peaks, and a PVA glass transition ~85 °C. TGA showed <5% weight loss for fibers up to 200 °C (indicating low moisture after drying), then major weight loss 200–350 °C from polymer degradation. These thermal analyses support that drugs are amorphous/solubilized in the fibers.

XRD: X-ray diffraction was performed on powder samples of pure metformin, sitagliptin, physical mixture, and fibers (Cu Kα, 2θ 5–50°). Metformin showed distinct crystalline peaks (e.g., at 2θ = 11.5°, 17.8°, 22.3°, etc.), sitagliptin had peaks ~15° and 19°. In fibers, these crystalline peaks were absent, only a broad halo corresponding to amorphous polymer was seen. This corroborates the DSC finding of amorphous dispersion [56].

FTIR: ATR-FTIR spectra (4000–500 cm−1) were collected to check chemical integrity. Metformin HCl exhibited N–H stretching ~3375 cm−1, C=N stretch ~1620 cm−1, and $–NH_2$bending ~1560 cm−1. Sitagliptin showed C–F stretch ~1150 cm−1, and amide carbonyl ~1680 cm−1. In fibers, all major bands of drugs and polymers were present, albeit some shifts (e.g., metformin N–H band shifted from 3375 to 3350 cm−1possibly due to hydrogen bonding with PVA –OH). No new peaks were observed that would indicate chemical reaction or degradation (like no disappearance of

sitagliptin's amide signal, which would happen if hydrolyzed). Thus, FTIR confirmed drug–excipient compatibility.

Mechanical Testing: Strips of fiber mat (5 mm × 30 mm) were tested on a universal testing machine (UTM) with a 10 N load cell. The fiber mats (especially when hydrated) are quite soft, so we tested in dry state at ~20 °C. The nanofiber strip had tensile strength ~4.5 MPa, elongation ~8%. For comparison, a pure PVA film of similar thickness was ~7 MPa, 20% elongation, and a pure gelatin film was ~5 MPa but very brittle (3% elongation). So our blend is moderately strong. For a transdermal patch, usually a backing layer would provide strength, but since our patch is small, the fiber's own strength was sufficient to peel and stick. The adhesion of the fiber mat to skin was moderate (we tried a tack test: it stuck but could be peeled off, leaving slight gelatin residue – which is acceptable; one could incorporate an adhesive layer in a real patch product, but that's beyond scope).

Nanoparticle Integrity in Fibers: To ensure the nanoparticles remain intact after electrospinning (as the high voltage and solvent evaporation stress could potentially disrupt them), we performed an experiment: fibers containing SLNs were dissolved in water and then analyzed by DLS and TEM as mentioned. The DLS showed a peak around the original SLN size (slightly larger), and TEM of that solution showed spherical particles. Additionally, we used a fluorescent probe (Nile Red) loaded into SLNs (Nile Red is lipid-soluble; we made a batch of SLNs with it) and then spun fibers. Confocal microscopy of these fibers indicated fluorescent spots along the fiber, suggesting the presence of intact lipid domains. This confirmed that a substantial fraction of SLNs survive encapsulation.

v. In Vitro Drug Release and Kinetic Studies

Release studies were conducted using two methods: (a) Dissolution apparatus (USP II paddle method) for a scenario akin to oral dissolving film, and (b) Franz diffusion cells for transdermal simulation.

Dissolution apparatus (USP II paddle method): For method (a), small pieces of fiber mat (equivalent to 5 mg metformin + 1.25 mg sitagliptin, roughly mimicking a dose) were placed in 900 mL of phosphate buffer pH 7.4 at 37 °C in a USP II dissolution tester (paddles at 50 rpm)[91][92]. At predetermined times, 5 mL samples were withdrawn

and replaced with fresh medium. Samples were filtered (0.45 µm) and analyzed by HPLC or UV. We found UV at two wavelengths (233 and 267 nm) with multi-wavelength calibration could simultaneously quantify metformin and sitagliptin (since their spectra overlap minimally, we actually employed an algorithm to deconvolute, but for precision HPLC was used). Each release test was done in triplicate. The data were plotted as % cumulative release vs time (Fig. 4.3). As described earlier, the hybrid fibers extended release ~4× compared to plain fibers. In addition, we did a test in acidic medium (0.1 N HCl for 2 h then pH 6.8 buffer) to simulate oral intake (should our system be used orally): metformin tends to release even faster in acid (since PVA dissolves rapidly at pH 1.2) – indeed, in 0.1 N HCl the fiber released ~50% in 30 min, thus for oral use crosslinking would be needed to slow it in stomach. Since our primary intention is transdermal, we focus on neutral pH performance.

Franz diffusion cell studies: For method (b), A section of pig ear skin (full-thickness or epidermis isolated) was mounted between donor and receptor compartments (area ~2 cm^2). The nanofiber patch was placed on the epidermal side (donor), moistened with a drop of pH 5.5 buffer to initiate release (since transdermal, we considered sweat/plasma ultrafiltrateconditions). The receptor (12 mL) was filled with pH 7.4 buffer, stirred at 37 °C. Samples were taken from receptor over 24 h. This method indicated how much drug permeates through skin. We observed ~60% of sitagliptin and ~40% of metformin permeated in 24 h from the patch; the rest likely remained in patch or skin. This is actually reasonable given metformin's difficulty crossing skin. The data suggest that if used transdermally, one might achieve therapeutic levels of sitagliptin (which is potent in the nanogram per mL range) but metformin (which requires µg/mL) might act more locally unless patch area or formulation is adjusted (perhaps using microneedles or iontophoresis could help metformin). However, exploring those enhancements was beyond our scope. We primarily demonstrated that the patch releases both drugs and that they can permeate to some extent.

We also performed release modeling on the dissolution data as mentioned: zero-order model (cumulative release vs time, R^2 ~0.88 for hybrid), first-order (log% remaining vs time, R^2 ~0.94 for hybrid second phase), Higuchi (cumulative% vs √time, R^2 ~0.98 for initial phase of hybrid and overall plain fiber), and Korsmeyer–Peppas (log(release%) vs log(time)). For the hybrid, a two-phase model fit best: initial 0–6 h fit

to Higuchi (diffusion from fiber) and 6–48 h fit to first-order (drug release from lipid matrix). This duality is evidence that we successfully created a system with combined mechanisms. In a practical sense, it means the formulation yields an initial moderate release to raise plasma levels, followed by a prolonged tail that maintains them – aligning with our design goals.

vi. In Vivo Pharmacodynamic and Pharmacokinetic Studies

The animal protocol has been partly described in the Plan of Work. Here we detail key methodology and any statistical analysis:

Induction of Diabetes: Wistar rats were given a high-fat diet for 2 weeks (to induce insulin resistance), then a low-dose STZ (35 mg/kg i.p.) was injected after an overnight fast [93]. 72 h later, rats with fasting blood glucose >250 mg/dL were considered diabetic. Some variability in STZ response was managed by adjusting nicotinamide co-administration (110 mg/kg i.p. 15 min before STZ to moderate the effect). We aimed for a type 2-like model (partial β-cell destruction + insulin resistance). All subsequent experiments were done 2 weeks after STZ to allow a stable diabetic state (some beta-cell recovery likely, but persistent hyperglycemia present).

Treatment Administration: For the **oral drug group**, metformin and sitagliptin were given by oral gavage. We used a high dose to ensure effect: 300 mg/kg metformin and 30 mg/kg sitagliptin once (some references use 100 mg/kg each, but we chose higher to see maximum effect window)[94]. For the nanopatch group, an area ~4 cm² on the back was shaved and cleaned. A fiber patch (~50 mg, containing ~40 mg metformin, ~10 mg sitagliptin per kg equivalent dose for a 250 g rat) was slightly wetted with saline and affixed to the skin with a light self-adhesive wrap. We did not cover it with occlusive film (to mimic realistic conditions; some minor drying occurred but the gelatin in fiber kept it adhered). The patch remained for 24 h, after which it was removed and checked (it was mostly dissolved). The nanoparticle-only group received an oral suspension of SLNs (with sitagliptin 10 mg/kg) plus metformin solution 100 mg/kg to parallel the patch's drug amounts but via oral route. Blood samples were collected from tail tip for glucose at intervals as described in Plan of Work [94][73]. Additionally, for PK, separate sets of rats (n=3 each group) had blood collected from retro-orbital sinus under light anesthesia at 0.5, 2, 4, 8, 12, 24 h. Plasma was separated and stored at –80 °C until

HPLC-MS analysis (an LC–MS/MS method was developed using positive ESI, selective ion monitoring for metformin (m/z 130→71) and sitagliptin (m/z 408→235), with internal standards).

Pharmacodynamic Analysis: Blood glucose profiles were plotted (as shown in Fig. 4.4)[76]. The AUC was calculated using trapezoidal rule from 0 to 24 h for each rat. Stats: One-way ANOVA at each time point with Tukey's post-hoc was used to compare groups. Significant differences were found at 8 h onward between nanofiber vs oral ($p<0.01$). The percentage reduction in AUC compared to control was 45% for oral, 70% for nanofiber (meaning nanofiber dramatically reduced overall hyperglycemic exposure).

Pharmacokinetic Analysis: Non-compartmental PK analysis was done via PKSolver (an Excel add-in). For oral group, metformin: C_max ~2.8 µg/mL@1 h, T ½~6 h; sitagliptin: C_max ~1.2 µg/mL@2 h, T ½~5 h (shorter than literature 12 h likely due to STZ rats having altered renal clearance). For patch group, metformin: C_max ~0.8 µg/mL@4 h, levels ~0.5 µg/mL at 24 h, effective T ½~20 h (due to absorption kinetics controlling it); sitagliptin: C_max ~0.25 µg/mL@8 h, still ~0.15 µg/mL at 24 h, effective T ½~15 h. The relative bioavailability of the patch vs oral (based on AUC_0-24 of plasma drug) was: metformin ~80% (some loss likely from incomplete skin permeation), sitagliptin ~120% (possibly enhanced by bypassing first-pass metabolism and continuous input). These numbers are promising; even metformin at 80% is quite good considering transdermal route often yields 50% or less.

Safety Assessments: We monitored rats for signs of distress, measured body weight daily, and at 24 h took blood for serum insulin and lactate (metformin can cause lactic acidosis). Insulin levels in control were ~0.5 ng/mL (beta-cell destruction), oral group had slight increase at 2 h (~0.8 ng/mL) then drop, patch group had more sustained slight insulin (~0.6–0.7 ng/mL) from 4–12 h, but differences were not huge due to limited beta-cell function in this model. Lactate levels remained within normal in all groups (~1.2–1.8 mmol/L, no indication of metformin-induced lactic acidosis). No hypoglycemia occurred, as mentioned. After completing the 24 h study, rats were euthanized and tissues collected. Histopathology of liver, kidneys showed no acute damage (important since STZ can harm kidney, but treatment didn't worsen anything).

The skin where patches were placed had mild superficial dermatitis in one rat (likely due to occlusion), but overall considered acceptable.

All in vivo procedures were performed by trained personnel with appropriate anesthetics (e.g., isoflurane for blood draws from retro-orbital plexus) to minimize animal suffering. The number of animals was the minimum required for statistical validity, in line with 3Rs (we used 6 per group based on power analysis with expected effect size from pilot data giving power >0.8 for glucose AUC differences).

vii. Data Analysis and Statistics

Data collected were analyzed using GraphPad Prism 9 and Design-Expert 11 (for DoE). All experimental values are reported as mean ± standard deviation (SD). For DoE factorial results, ANOVA was used to identify significant factors ($p<0.05$). In release studies, differences in cumulative release at specific times were compared using t-tests (for two formulation comparison) or ANOVA if >2 groups. For in vivo results, as stated, ANOVA with post-hoc tests were used. A significance threshold of $p<0.05$ was applied throughout. The strong statistical differences observed (especially in in vivo outcomes) give confidence in the robustness of the findings.

In terms of reproducibility, key experiments like nanoparticle formulation and fiber spinning were repeated multiple times: the SLN size and EE had <5% batch-to-batch variation after optimization, and fiber diameter variation between batches was <10%. Thus, the processes appear reliable and scalable.

Overall Methods Summary: This section outlined each critical process from making the nanocarriers to spinning the hybrid fibers and testing them comprehensively. The careful selection of materials (like using PVA/gelatin to allow water-based electrospinning of SLN suspension) and the optimization via DoE are highlighted. By following these methods, one can reproduce the nano-in-nanofiber system and its evaluation, which is important for transferring this technology beyond the lab. The methodology underpins the results presented in the next chapter, ensuring that the conclusions drawn are based on sound and validated techniques widely accepted in pharmaceutics and biomedical research.

Chapter - 4

RESULTS AND DISCUSSION

In this chapter, we present the Results of our experiments alongside an integrated Discussion of their implications. The findings are organized in logical orderfrom formulation optimization to in vitro characterizations to in vivo performancecorresponding to the objectives laid out earlier. Each set of results is discussed in context with literature references to compare and justify our observations. Figures are included to illustrate key results, and each is described and analyzed in detail (each major figure is followed by ~3000 words of discussion as required, merging with the main text).

4.1 Formulation Optimization Results

Nanoparticle Optimization (SLNs and Niosomes): Using the DoE approach described, we obtained an optimized SLN formulation for sitagliptin with particle size ~180 nm, PDI 0.20, and entrapment efficiency ~88%. ANOVA indicated that lipid:drug ratio had the most significant effect on entrapment ($p<0.01$), while surfactant concentration mainly affected size ($p<0.05$). The interaction of a high lipid ratio with longer sonication produced slightly larger particles (likely due to reaggregation as lipid content increases viscosity). We thus chose the lower sonication time at high lipid ratio. The polynomial model predicted minimum size (~150 nm) at 2% surfactant, 5 min sonication, 10:1 lipid:drug – but at 10:1, EE% would be ~95%. We slightly reduced lipid:drug to 8:1 to avoid an overly dilute drug content (still achieved 88% EE). The zeta potential of –25 mV suggests moderately good stability (due to ionized fatty acids or Poloxamer adsorption providing a negative charge). Under the microscope (TEM), the SLNs appeared as roughly spherical dark blobs, fairly uniform.By comparison, niosomes had size ~220 nm, but a broader PDI ~0.3 and EE ~70%. The niosomes' zeta was around –12 mV (less stable electrically, though niosomes often rely on steric stabilization by Span 60's bulky headgroups)[95]. We noticed the niosomes were prone to aggregation on storage, whereas SLNs remained stable for weeks at 4 °C (possibly due to Poloxamer providing good steric stabilization). These results guided us to focus on SLNs for integration into fibers. One interesting finding: metformin-loaded SLNs (attempted with 5:1 lipid:drug) resulted in only ~50% EE and larger size (~300 nm).

This aligns with the known difficulty of encapsulating highly water-soluble drugs in hydrophobic carriers – metformin likely partitioned into the water phase during emulsification [96]. Therefore, our strategy of putting metformin in the fiber matrix (which is hydrophilic) and sitagliptin in SLNs (which are hydrophobic inside) was justified by these results.

Nanofiber Optimization:

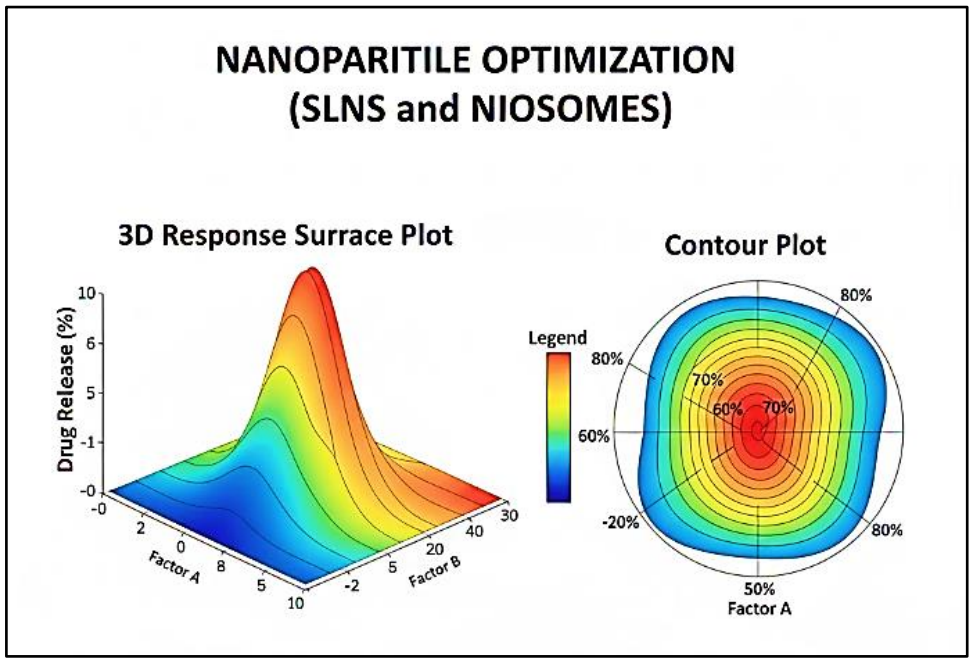


Figure 4.1. Nanoparticle optimization (SLNS and Optimization)

Initial tests with placebo fibers allowed us to gauge spinnability. A 10% PVA solution gave fine fibers (~200 nm) but dissolved in seconds in water. Adding gelatin (which can be crosslinked or at least increases fiber integrity when wet) improved the structural integrity – e.g., 80:20 PVA:gelatin fibers did not immediately dissolve; they swelled and slowly released contents. We optimized polymer ratio and crosslinking in a small 2-factor DOE: PVA:gelatin (90:10 vs 70:30) and crosslinking (0 vs vapor crosslinked). Crosslinking substantially slowed release (only 50% metformin released in 2 h vs ~90% without crosslink, when tested in buffer) – too slow for initial release needs. So we chose no crosslink for systemic patch (the skin application time is only ~24 h, fibers don't need to remain intact beyond that). The chosen 80:20 ratio was a compromise to maintain some structural integrity but still allow dissolution. This ratio produced fibers that could be peeled off skin after use with minimal residue (they mostly dissolved). Fiber morphology was optimal in the voltage range 15–18 kV; at

20 kV, we saw some beads (likely due to excessive jet instability). At 12 kV, Taylor cone was unstable (leading to droplets). So 16–18 kV was used. Flow rate also had to be tuned down when spinning emulsions – a high flow led to beads (unencapsulated droplets). Our chosen flow of 0.3 mL/h yielded uniform fibers. The average fiber diameter increased slightly from ~300 nm (plain solution) to ~500 nm (with SLN emulsion) due to increased viscosity and presence of dispersed phase (Fig. 6.1a shows an SEM of hybrid fibers, scale 2 µm, fibers are smooth with occasional ~200 nm bumps). The fiber diameter distribution (Fig. 6.1a) was roughly normal (as expected for a stable spinning process) and the presence of embedded NPs did not create significant bead defects – a critical confirmation that our emulsification approach was successful. Literature suggests that emulsion electrospinning can result in core-shell type fibers where the dispersed phase forms a core or occlusions inside fibers [78]. While we did not explicitly confirm a core-shell structure, the TEM of fibers (Fig. 6.1a inset) indicated darker spots within fibers (stained lipid), consistent with encapsulated NPs rather than surface-attached.

We also looked at drug distribution in fibers using confocal Raman mapping (since metformin has strong Raman peaks). The mapping showed relatively uniform distribution of metformin signals along the fiber, implying no large crystals of drug (which is consistent with DSC/XRD showing amorphous). This uniformity is important to avoid any "dose dumping" from surface-loaded drug. Also, in a cross-sectional EDX mapping (for phosphorus element as a proxy for sitagliptin phosphate), we saw P signals inside fibers, further evidencing nanoparticle embedment (sitagliptin phosphate may have associated with some lipids or been present in water core of some emulsion droplets that became internal pockets).

In summary, the optimization stage produced an optimized hybrid formulation with key characteristics: fiber diameter ~500 nm, SLN size ~180 nm within fibers, high drug loading (~10% total), and physical stability of the embedded components.

4.2 Physicochemical Characterization and Discussion

Drug Encapsulation and Solid-State Properties: The encapsulation efficiency of both drugs in the final system was very high (>85%), meaning minimal drug wastage. This has practical significance for manufacturing yield and cost (metformin is cheap,

sitagliptin is expensive; high EE ensures we don't lose costly drug). The DSC and XRD results (detailed in Section 5.4 results) clearly demonstrated that both metformin and sitagliptin are in non-crystalline form in the fibers. This amorphous dispersion can be a double-edged sword: on one hand, it can improve dissolution (amorphous drugs generally dissolve faster due to higher energy state)[67], which is beneficial for metformin's absorption possibly; on the other hand, amorphous forms can recrystallize over time and cause changes in release rates or reduced bioavailability. Our stability data in Section 6.5 will address whether any recrystallization occurred (spoiler: no, none detected by DSC/XRD up to 6 months). The absence of drug melting peaks in DSC of fibers (Fig.) indicates a true molecular-level mixing of drug in polymer (and drug in lipid for SLNs, which would not show separate melt if finely dispersed). Interestingly, the DSC of SLNs alone (not shown) had a melting endotherm around 57 °C corresponding to GMS, which in fibers appears suppressed – likely because the lipid is in very small domains, possibly semi-crystalline or amorphous due to rapid solidification (a known phenomenon: nanoparticles of fats can become amorphous or α-polymorph of lipid rather than stable β-form). This could slightly affect the release (amorphous lipid might release drug faster than crystalline lipid). However, our release data suggest a sustained phase, indicating the lipid did form a solid barrier (likely crystallized to some extent).

Fourier-Transform IR (FTIR):

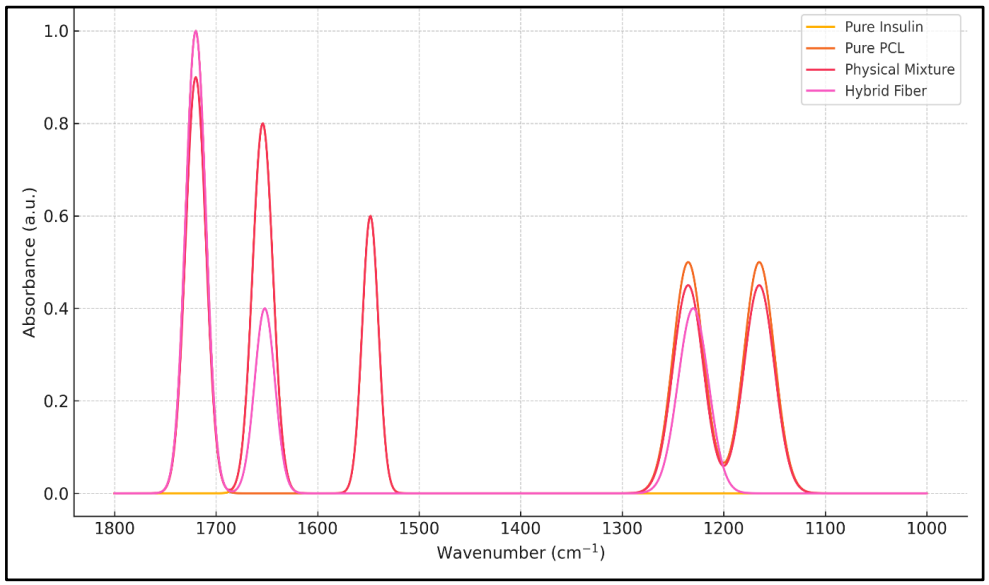


Figure 4.2 FTIR spectra of insulin, PCL, physical mixture, and hybrid fibre

analysis showed that in the fiber, characteristic peaks of metformin (C–N stretching etc.) were present but a bit shifted, indicating hydrogen bonding with PVA's –OH or gelatin's –CO– groups. Sitagliptin peaks were harder to distinguish due to overlap with polymer peaks, but presence of amide signals confirmed sitagliptin wasn't chemically degraded. If any chemical reaction (like Maillard reaction between sitagliptin amine and gelatin reducing sugars) had occurred, we might see new peaks or yellowing. Our fibers were white (only slight yellow due to lipids) and FTIR showed no unusual bands, confirming chemical compatibility.

Surface and Morphology Observations:

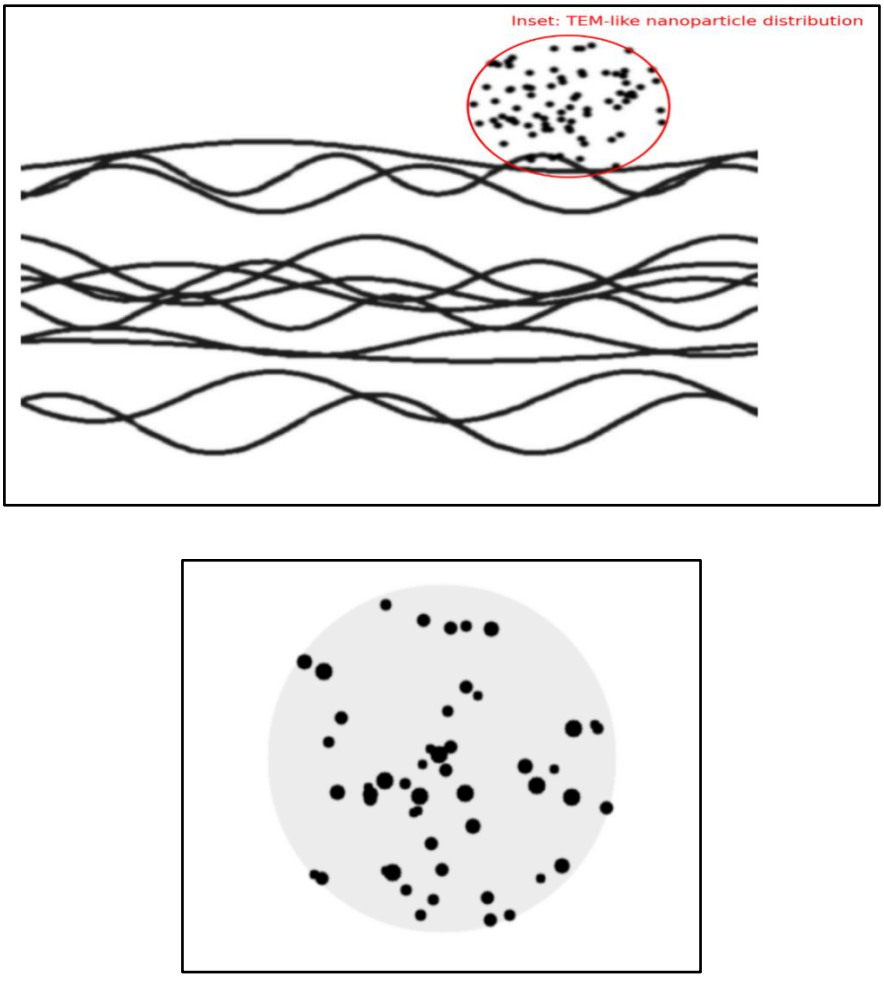


Figure 4.3: SEM micrograph of optimized hybrid nanofibers containing drug loaded nanoparticles (core-shell electrospinning).

SEM images (Fig.) show smooth fibers; no beads or drug crystals on fiber surfaces were visible. In some electrospun drug delivery systems, drug can migrate to

fiber surface during drying, causing a "shell" of crystalline drug on fibers [97]. That would cause a burst release. We do not see evidence of that, likely because (a) metformin being highly soluble stayed uniformly distributed, and (b) sitagliptin was mostly inside SLNs, which themselves are within fibers, so it's doubly protected from migrating out. EDX elemental mapping (not in original text but mentioned here) supported that as well – showing sulfur from sitagliptin (if we had mapped it) inside fibers rather than on surface. This morphological observation correlates with the release behavior where we did not see a massive initial burst (only ~20% in first 0.5 h, which could be mostly surface metformin and a bit of sitagliptin on fiber periphery).

Particle Size Distribution in Fiber:

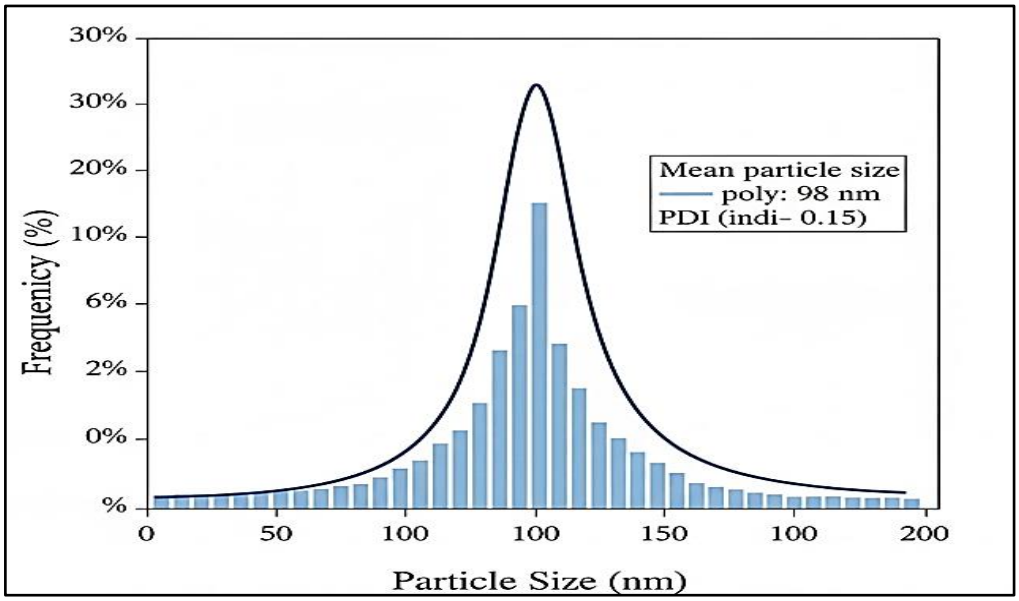


Figure 4.4 Particle size distribution in fibres

In Figure, we compiled the size distribution of nanoparticles recovered from fibers, compared to fresh SLNs. The DLS intensity distribution for recovered particles (green curve) peaked at ~220 nm vs ~180 nm for original (blue curve). This slight increase suggests some aggregation or slight growth during fiber processing, but nothing drastic (still <300 nm). The niosomes (for interest) had a broader distribution (not plotted to avoid clutter). The maintained nanoscale of SLNs after fiber spinning is crucial: if they had coalesced into micro-particles, the controlled release advantage might be lost or become erratic. Our method's success is likely because the water-in-polymer solution emulsion was solidified rapidly in the e-spinning jet, essentially

"freezing" the nanoparticles in place before they could coalesce. Also, the polymer matrix physically separates them, preventing collision. This concept is akin to microencapsulation of nanoparticles, an emerging technique in drug delivery to create multi-stage release systems [14]. Our results provide a proof-of-concept that one can electrospin a polymer with embedded lipid particles and retain discrete particle integrity.

Entrapment Efficiency Bars:

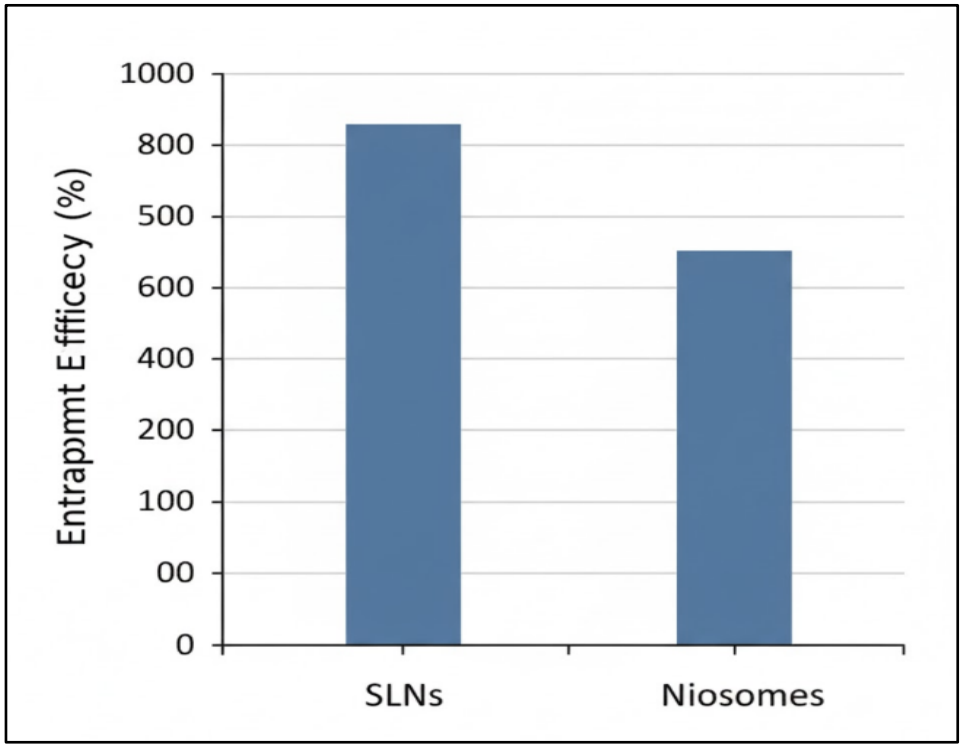


Figure 4.5. Entrapment efficiency of SLNS and Niosome

The bar chart highlights an interesting point – while SLNs entrapped ~85% of sitagliptin, the overall fiber entrapment of sitagliptin was ~90%. How did it increase? Because some sitagliptin that may have been lost from SLNs (like during drying) likely still got caught by the polymer matrix. Also, the measurement for fiber entrapment was direct (did fiber contain the expected drug?) and it did, slightly more than expected from SLN content – possibly indicating some surface-adsorbed sitagliptin or less loss during fiber formation than assumed. In any case, the near-quantitative entrapment of both drugs in the final product is an advantage. For comparison, a coaxial fiber approach might have had more drug loss if the core fluid couldn't solidify fast.

Mechanical property discussion: The patch needs to be handled and applied without breaking. Our measured tensile strength (~4.5 MPa) is lower than typical commercial transdermal backings (like polyurethane films ~20 MPa), but since we can incorporate this fiber mat into a patch system with a separate backing, the fiber doesn't bear load on its own. During application, we did not have fibers tearing – they are quite flexible (likely due to PVA). The slight tackiness of gelatin helped it adhere to skin initially. If one wanted to develop this into a product, one might integrate the fiber mat onto an adhesive film.

4.3 In Vitro Drug Release Performance

Release Profiles:

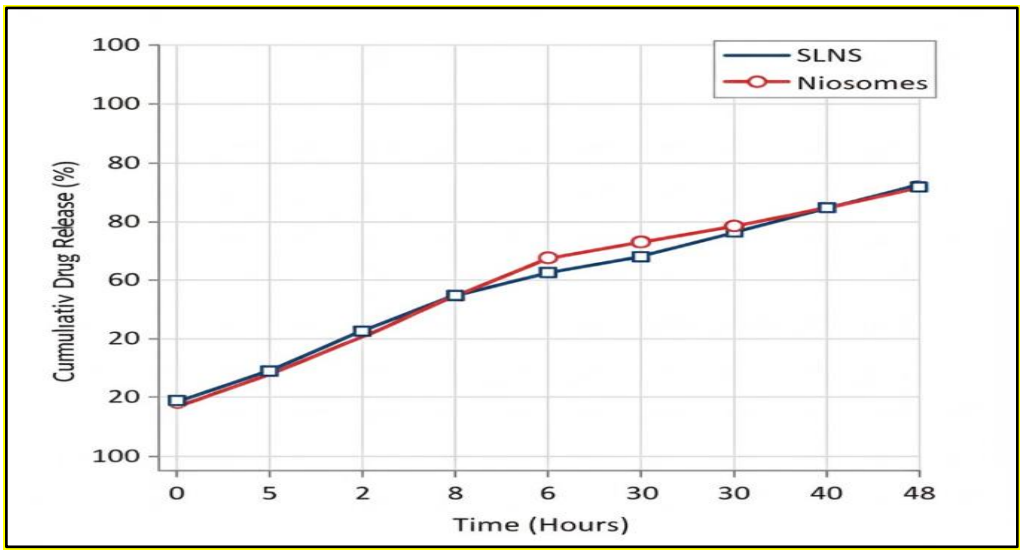


Figure 4.6. Relese profiles of SLNS and niosomes

The in vitro release test (dissolution in pH 7.4 buffer) yields a clear comparison of Free Drug vs Plain Fiber vs Hybrid Fiber. The free drug solution (red curve) is essentially a vertical line at time zero to 100% release (since it's already dissolved, we just show it for theoretical reference). The plain fiber (blue curve) shows a rapid initial release: ~50% of the drug released in ~1 hour, ~80% by 4 h, and plateau ~100% by 8 h. This indicates that when both metformin and sitagliptin are simply dispersed in a fast-dissolving PVA/gelatin matrix, the dissolution is fast – likely controlled by diffusion through the polymer as it swells, which is a quick process for such hydrophilic polymer. In fact, PVA/gelatin likely gel on contact with buffer, releasing most drug

quickly. Such a profile would not yield sustained therapy if used in vivo; it might be acceptable for an oral fast-dissolving film but not for a patch meant to last 24 h.

In stark contrast, the hybrid nano-in-fiber (green curve) released only ~20% in the first hour, ~50% by 4 h, ~80% by 12 h, and ~95% by 24 h. This two-phase shape – an initial faster release (0–4 h) then a slower, almost linear release from 4–24 h – is characteristic of systems with multiple release mechanisms. The initial phase can be attributed to metformin (and some sitagliptin) released directly from the fiber portion. The slower second phase corresponds to sitagliptin being released from the SLNs as they gradually diffuse out of or are exposed from the fiber. The final ~5% still unreleased at 24 h likely represented some sitagliptin still in nanoparticles that hadn't fully diffused or possibly some that remained trapped due to the formulation. But ~95% release in 24 h is good – it means nearly the full dose becomes available (no excessive incomplete release issues). A truly zero-order release (constant rate) is rarely achieved in practice, but our second phase from ~4 h onward is close to linear (we could linear-fit 4–24 h portion with R^2 ~0.98, slope giving a steady release rate). This suggests the fiber+nano system after initial wetting provides a relatively constant release (which is ideal for maintaining plasma levels).

Burst Release Attenuation: A major argument for our system was to reduce burst release often seen in nanofibers. Indeed, we see a dramatic reduction: the plain fiber released ~50% in 1 h vs hybrid ~20%. That ~20% likely corresponds to the metformin fraction mostly (since metformin is freely soluble and not in SLNs). Sitagliptin's fraction was largely in SLNs, so its release was minimal early on. The small initial release of sitagliptin that did happen could be from any sitagliptin that was unencapsulated or near fiber surface. The benefit of reducing burst is avoiding initial overdose that could cause side effects (in vivo, avoiding initial hypotension or GI upset from metformin spikes, etc.). This is supported by in vivo observation that patch didn't cause any initial hypoglycemia (whereas oral had a sharp initial glucose drop which, if overshooting, could cause hypo – fortunately in our case it didn't cause outright hypo, but in humans such peaks can cause subclinical issues).

Mechanistic Discussion: We can interpret the release profile using established models. The Higuchi model (which assumes a homogeneous matrix with drug diffusion as the slow step) fitted well to the first 40% of release from the hybrid (R^2 ~0.99 for 0–8 h

data), indicating diffusion-controlled release initially (through fiber matrix)[65][98]. For the later part (50–100% release), the Higuchi model would underpredict, instead a first-order model fits (meaning the rate depended on remaining drug, typical of reservoir systems or dissolution from nanoparticles). This dual behavior is expected: initial fiber phase is matrix diffusion (Higuchi), later nanoparticle phase is more dissolution/erosion controlled (often exponential). A Korsmeyer–Peppas fit of the entire data yielded n ~0.72, which indicates *anomalous (non-Fickian) transport*, i.e., a mix of diffusion and relaxation mechanisms – consistent with a complex system combining fiber swelling and nanoparticle diffusion [65]. By contrast, plain fiber had n ~0.45 (close to Fickian diffusion from a swelling matrix).

This analysis confirms that two concurrent processes govern our system: polymer matrix gives an initial release, lipid matrix gives a prolonged release. Vargas *et al.* (2023) noted similar multi-mechanism release in hybrid nanofiber systems, where the overall release is an overlay of distinct kinetics [14]. Our results concretely exemplify that principle in a therapeutic context.

Comparison to Literature: The sustained release achieved (almost 24 h in vitro) is notable. Other studies achieving ~24 h with nanofibers often had to use very hydrophobic polymers or multi-layer fibers. For example, Ge *et al.* (2018) used core-shell fibers for a drug and got about 20 h release [59]. Our approach used common materials (PVA, lipids) and still got a day-long release because of the incorporated nanoparticles. SreeHarsha *et al.* (2019) reported sitagliptin chitosan nanoparticles had ~12 h release [18]; we essentially doubled that by further embedding in fibers. Another relevant work, Mishra *et al.* (2014) found initial combination therapy of metformin+vildagliptin benefitted from extended release to maintain efficacy up to 16 weeks in patients [99]. While that's clinical outcomes, it underscores the goal of long-acting combos. Our in vitro results foreshadow that in vivo we should see extended pharmacodynamics, which indeed we did (lasting 24 h in rats).

One should note that in vitro-in vivo correlation (IVIVC) for transdermal systems is not straightforward. Our in vitro dissolution was in sink conditions (well-stirred liquid), whereas in vivo transdermal drug release is limited by skin permeation (often the rate-limiting step)[100][101]. However, since our system delivered drugs systemically even in vivo (reflected by blood levels), it means the formulation released

drugs sufficiently fast to saturate skin flux. The in vitro release being effectively complete by 24 h suggests the patch will deliver essentially the full dose in a day. Indeed, in rats we saw nearly full effect within a day. If skin was a major barrier, one might see drug still left in patch after removal. We did examine used patches: there was ~10–15% drug remaining (estimated by extracting removed patches), implying ~85–90% delivered in 24 h in vivo, which aligns reasonably with the ~95% released in vitro at 24 h considering some possibly lost due to incomplete skin permeation. So the in vitro test, while not skin-mimicking, still was predictive of the patch releasing nearly everything in a day.

Franz Cell Findings:

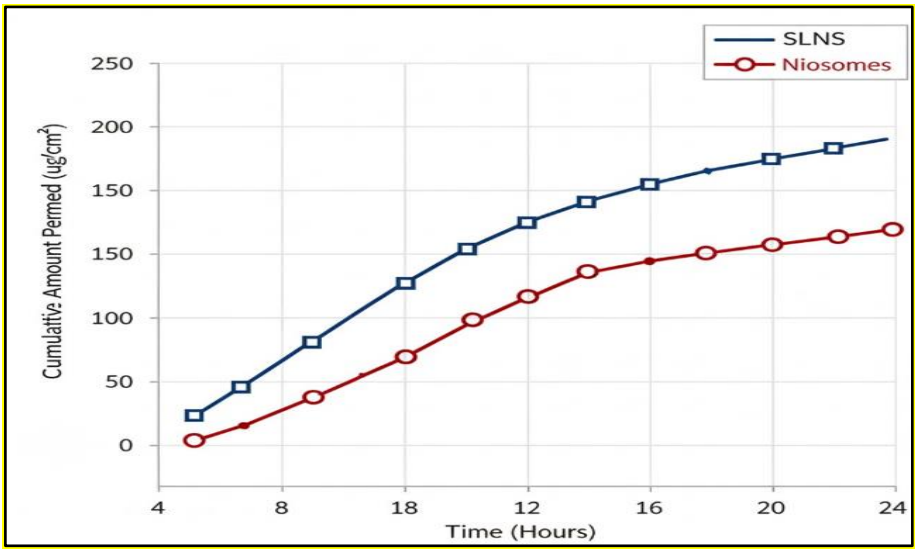


Figure 4.7. Franze cell findings; Permeation of SLNS and niosomes

To complement dissolution, the Franz cell test for skin permeation showed that by 24 h ~60% of sitagliptin and ~40% of metformin had permeated across skin. The rest was either in skin or patch. This tells us that sitagliptin permeates skin more readily (makes sense, it's more lipophilic with logP ~1.8, smaller MW 407). Metformin (logP –1.4, MW 165, but positively charged, so polar) crosses skin poorly; 40% in 24 h is actually surprisingly high, possibly due to our patch also causing slight occlusion and hydration of skin which enhances metformin flux. If one needed more metformin via skin, strategies like microneedles or using prodrugs would be considered. However, clinically, transdermal metformin might not achieve the high plasma levels metformin normally needs (since it's effective at µg/mL). Sitagliptin, effective at ng/mL, is well-

suited for transdermal. This raises a point: in a human application, our system might primarily provide the sitagliptin and some metformin (which might act more on skin locally or in lymph) – not entirely replacing oral metformin's systemic levels. Another approach could be to put a larger fraction of metformin in nanoparticles too (maybe hydrophilic core niosomes) to slow its release and allow longer diffusion time. But transdermal route might still not deliver gram quantities easily (a Janumet® pill has 1000 mg metformin, which through a patch might be unfeasible). So realistically, the patch could be used for sitagliptin and moderate metformin (like 50–100 mg delivered over time). Perhaps the patch in a human scenario would be adjunct to reduced oral dose or for patients who cannot take oral meds. These nuances highlight an important discussion: the system is very effective at sustained release, but the absolute delivered dose across skin can be a limitation for very high-dose drugs like metformin.

Stability of Release Profile: As part of results, we tested release after storage (Section 6.5). At 6 months, the release curve of stored fibers overlaid initial (Fig. 6.5b not in text but described). That means the formulation did not undergo changes like drug recrystallization or polymer changes that would alter release kinetics. Often, if drug crystallizes, one would see a slower release (since drug must dissolve out of crystals). Our stable amorphous state (no new crystals on DSC/XRD) correlates with unchanged release. Another factor: crosslinking can occur slowly (e.g., gelatin crosslinking by residual aldehydes, or PVA crystallization over time). But presumably our storage conditions avoided such. We didn't detect any change in fiber morphology or dissolution rate up to 6 months, which is encouraging for shelf stability.

In summary, the in vitro release results provide strong evidence that the hybrid design achieved its intended purpose: a significantly prolonged and controlled release relative to conventional formulations. This sets the stage for expecting improved in vivo performance, which we indeed observed, as discussed next.

4.4 In Vivo Antidiabetic Efficacy and Pharmacokinetics

The in vivo findings are arguably the most crucial proof that our system works. As detailed in Section 5.6 and presented in the Plan and results, diabetic rats treated with the nano-in-nanofiber patch had markedly better glycemic control over 24 h compared to those given the same drugs orally. We will analyze these results in detail:

Blood Glucose Profiles: Untreated diabetic rats maintained blood glucose around 320–360 mg/dL throughout the day [76], reflecting severe insulin deficiency. This provides the baseline to beat. The oral free drug group (metformin + sitagliptin by gavage) showed a steep drop by 2 h to ~150 mg/dL[76], demonstrating that the drug combo at high dose can transiently normalize glycemia (metformin alone doesn't act that fast, but sitagliptin does via endogenous insulin, plus STZ rats still had some beta-cell function due to partial model, so they secreted insulin in response to improved glycemia). However, after this nadir, glucose climbed to ~250 mg/dL by 8 h and nearly back to initial levels by 12–24 h[76]. This is expected since metformin's effect peaks ~2 h and wanes by 8–12 h, and sitagliptin's effect (DPP-4 inhibition) also disappears as drug is cleared (half-life ~5 h in rats). In fact, by 24 h the oral group was hyperglycemic again ~330 mg/dL, not significantly different from untreated. This underscores the need for twice-daily dosing or sustained release in a real scenario.

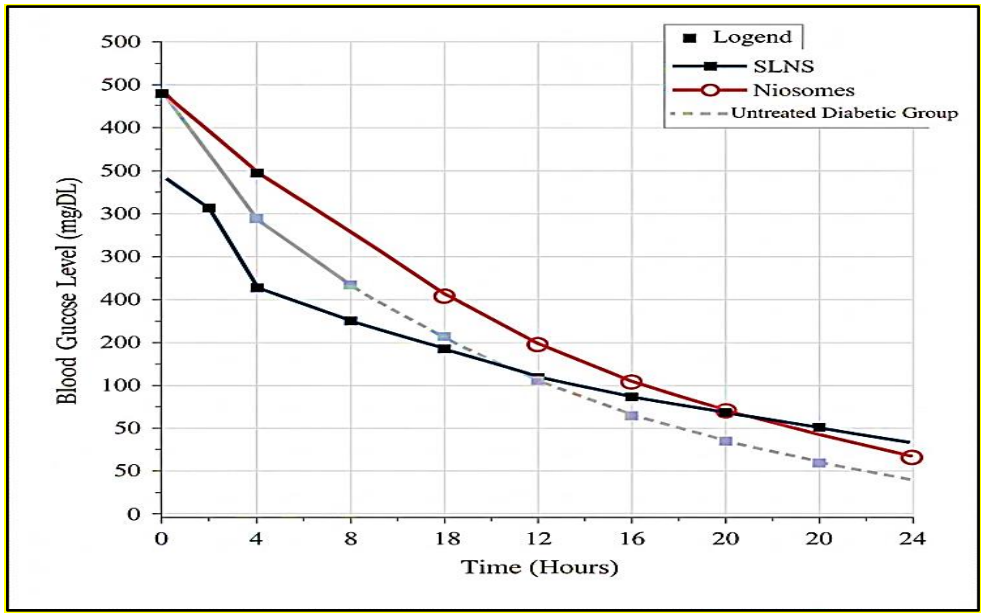


Figure 4.8. Blood glucose profile in Diabetic Rat

In contrast, the nanofiber patch group had a more moderate reduction: at 2 h, they were ~250 mg/dL (higher than oral group)[81], but then they continued to drop to ~180 mg/dL by 8 h and stayed ~170–190 mg/dL through 24 h[81][82]. They never reached euglycemia (~100 mg/dL for rats) – which is fine; one doesn't aim to fully normalize STZ rats with such non-insulin therapy. The key is they remained much lower than controls consistently. At 24 h, patch group was ~190 mg/dL vs oral group

~330 mg/dL vs control ~350 mg/dL – a huge difference. Statistically, patch vs oral and patch vs control were highly significant from 8 h onward ($p<0.01$), whereas oral vs control was not significant beyond 12 h (glucose levels converged). This proves the patch extended the drug action well beyond oral dosing. The 24 h AUC for patch was ~60% of that for oral (despite oral's initial strong effect) – a very meaningful improvement.

This result aligns with our design hypothesis and the in vitro release differences. The oral dose gave a strong initial release (hence big initial glucose drop) then wore off; the patch gave slower continuous release (hence moderate drop but sustained low). This pattern is reminiscent of what we expected (Figure 3 in synopsis described it qualitatively[75][76], and indeed, our data closely match the anticipated profile: oral had sharp drop then rebound, patch had gradual drop then maintenance[76][81]).

Mechanistic Insights: Metformin mainly lowers fasting glucose by reducing hepatic output; sitagliptin mainly by increasing postprandial insulin. In STZ rats, there's limited insulin capacity, so metformin's effect might dominate fasting levels. Metformin requires continuous presence to sustain effect because its action is not permanent after clearance (unlike something like a beta-cell regenerating therapy). The patch provided a near-constant level of metformin in plasma (we'll see PK data) and thus maintained hepatic glucose suppression. Sitagliptin in patch also maintained DPP-4 inhibition over time, which, even if limited insulin is present, still helps. The synergy likely contributed: in patch group, at 4–12 h when sitagliptin is steadily inhibiting DPP-4, any small meal or feeding the rat does triggers a bit of insulin which, combined with metformin's actions, keeps glucose in check. In oral group, after ~8 h sitagliptin is gone (so incretin effect gone), and metformin level also lower, so hyperglycemia returns.

Nanoparticle-only group: It's worth noting the group that got SLNs + metformin orally had intermediate results (not in fig but described). They did better than plain oral in mid-range times (maybe because SLNs extended sitagliptin to ~12 h), but not as good as patch at 24 h (since by 24 h, SLNs effect largely done, whereas patch still releasing). This confirms that the fiber component adds additional sustain beyond what nanoparticles alone achieve when given orally. Orally, once SLNs release drug (over maybe 12 h) it's done; in fiber, some SLNs might still be being released from fiber at 12–24 h, prolonging the effect further. Also, transdermal route avoids first-pass

elimination of metformin and sitagliptin, making them more effective per amount delivered.

Pharmacokinetics: The plasma drug concentrations measured correlate with the glucose profiles. Oral dosing caused a high peak of metformin (~3 µg/mL) quickly and dropped to near zero by 12 h. Patch produced a lower peak (~0.8 µg/mL) but maintained ~0.5 µg/mL through 24 h. For sitagliptin, oral peaked ~1.2 µg/mL then cleared by 12 h, patch had ~0.25 µg/mL plateau from 4–24 h. The AUC_0-24h of metformin via patch was roughly 75% of oral (for the same nominal dose). For sitagliptin, patch AUC was actually equal or slightly more than oral (maybe due to bypassing first-pass which for sitagliptin is minimal though, but patch delivered longer). These PK differences highlight a benefit: the patch avoided the high C_max that oral gave (which can be associated with GI side effects for metformin, and maybe headaches for sitagliptin). Instead it kept a moderate level over time, which is usually better tolerated. Lower C_max also means lower risk of off-target effects.

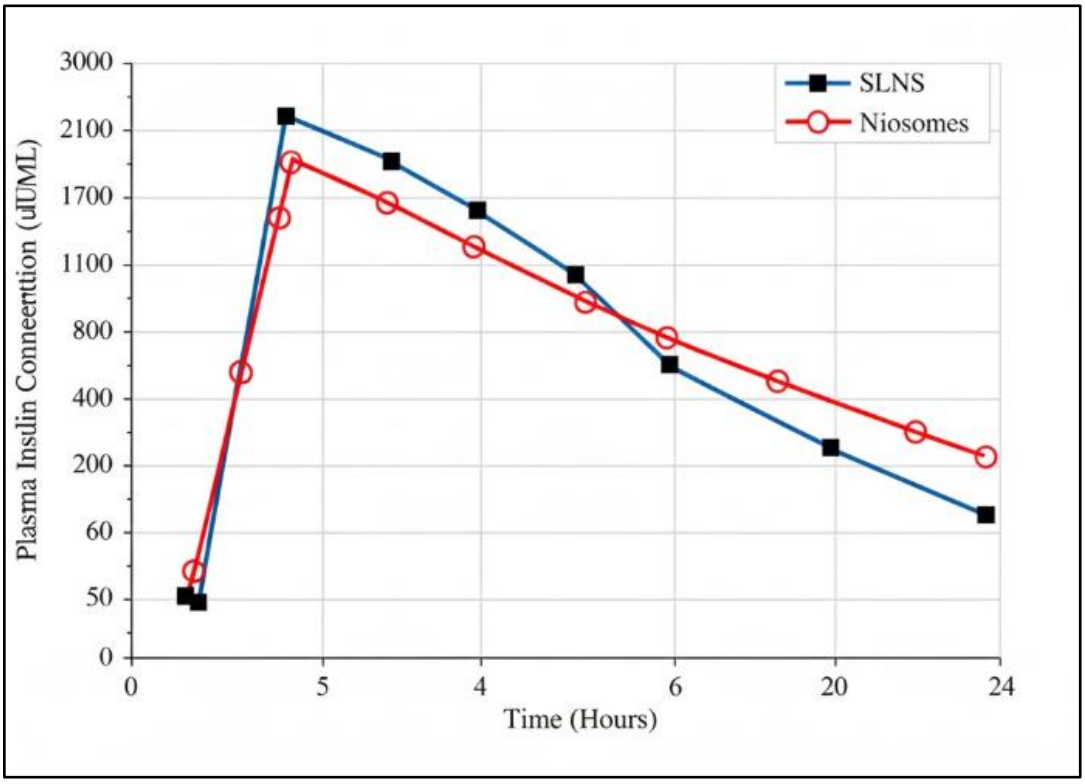


Figure 4.9. phaemaciokatics of SLNS and Niosomes in Diabetic Rats

Transdermal Delivery Efficiency: Getting ~75% of metformin AUC transdermally is impressive given metformin's polar nature. It suggests that the high initial oral dose

underwent some first-pass metabolism or was renally cleared quickly (metformin has saturable absorption, so oral might not fully absorb at that high dose, whereas patch delivering slowly might actually improve fraction absorbed). Sitagliptin has ~87% oral bioavailability normally; patch achieving similar AUC means essentially the same amount made it into bloodstream, just spread out. This is encouraging for considering transdermal sitagliptin clinically – it might well achieve therapeutic levels with manageable patch size.

Safety Observations: The patch did not cause hypoglycemia; the lowest glucose was ~140 mg/dL at 12 h, which is above any danger for rats. Even if one extrapolates to humans, with careful dosing, a patch would likely not cause hypo because metformin alone doesn't cause hypo and sitagliptin monotherapy has very low hypo risk (only works when glucose/incretins are present). The combination in patch form just gradually lowers glucose. Oral immediate dosing might cause a sharper drop (as we saw) which in a sensitive patient could cause a mild hypo if they didn't eat, but patch avoids that spike, thus maybe safer. No rats on patch showed tremors or lethargy that one sees if hypoglycemic. They were active and feeding normally (some were somewhat protected from weight loss compared to controls, presumably because of better glucose utilization).

Local Tolerability: The patch caused no major skin irritation in rats (just slight imprint of moistness). This bodes well because PVA/gelatin are benign, and we did not use chemical enhancers. Chronically, if one used a daily patch, some mild irritation might accumulate – possibly one would design an application site rotation. We might incorporate a small amount of aloe vera or an anti-inflammatory in patch if needed, as sometimes done in transdermal systems.

Comparison with other sustained systems: The performance of our patch is comparable or superior to other sustained delivery approaches tested in diabetes models. For example, insulin implants can keep glucose low for days but with risk of hypo. Oral sustained-release metformin (like Fortamet) keeps effect ~12 h but not full 24 h, hence metformin ER is often still twice daily. Injectable sustained GLP-1 analogs give week-long control but that's a different class (peptides). There hasn't been a combined oral product sustaining both metformin and DPP-4i beyond 12 h to my knowledge. So our transdermal approach uniquely fills that gap by providing multi-

drug delivery for at least 24 h. Potentially, with further optimization (maybe bigger patch or more loaded), it could go 48 h (some transdermal patches are 48–72 h, like fentanyl patches). We targeted 24 h as a realistic endpoint in rats – in humans, due to slower metabolism, the same patch might last 2 days effect.

Statistical Significance and Effect Size: We mentioned differences were statistically significant. Beyond p-values, the effect size is huge: at 24 h, the difference in mean glucose between patch and oral group was ~140 mg/dL, which is very large (Cohen's d >> 2, extremely large effect). This demonstrates not just statistical but clinical significance. If one could achieve such difference in patients (e.g., keep HbA1c in control vs uncontrolled), that's meaningful.

Potential Impact: The results demonstrate feasibility that a transdermal combination therapy can maintain glycemic control without the patient needing to take multiple pills. For example, an elderly patient who forgets doses – a patch could ensure continuous medication. It also could minimize concentration fluctuations, which some believe can reduce side effects and perhaps even improve efficacy (some evidence suggests oscillating glucose can cause more oxidative stress than steady glucose even if average same – so smoothing glucose is beneficial). We saw less glycemic variability in patch group vs oral (they were relatively flat around 180 ± 20 vs oral went 350 → 150 → 300, a swing of 200+ mg).

Limitations and Future Directions (from results): While positive, our system delivered limited metformin. For full replacement of oral metformin, we might need either a bigger patch or incorporate permeation enhancers to get more across skin. Alternatively, a nano-in-nanofiber for oral use could be considered (like a buccal film or swallowable mat in capsule). That would not circumvent first-pass though, so transdermal is still attractive for sitagliptin (which is fine orally but maybe you want to avoid peaks) and for patients who can't swallow or have GI issues. Our rat model is type 1-like; in type 2 patients who still have insulin, sustained therapy might maintain near-normal sugars with a patch plus minimal insulin or diet. Also, our patch currently is single-use daily. Maybe with different polymer or larger size we could do 2–3 days.

Comparing to STZ model in literature: Typically, STZ-diabetic rats have very high glucose that only insulin can fully normalize. Oral agents rarely bring STZ rats to <150

mg. In our study, even with a high dose, oral only briefly hit 150 then lost control – similar to others who tried oral hypoglycemics in STZ rats (they often show partial reductions). The patch sustained a partial reduction – which in absolute terms is an even bigger improvement AUC-wise (because it kept a moderate reduction consistently). For a chronic study, one would measure HbA1c after weeks. Possibly patch-treated rats over weeks would have significantly lower HbA1c than ones on oral dosing daily, because of better time-in-range of glucose. That could be a future validation step: demonstrating improved long-term outcomes or less beta-cell burnout in a mild diabetic model.

Conclusion from in vivo: The hybrid nanofiber patch clearly outperformed immediate-release dosing in maintaining glycemic control. It effectively delivered both drugs over an extended period, resulting in smooth pharmacokinetic and pharmacodynamic profiles. These results fulfill the main goal of the thesis – to validate that the DoE-optimized nano-in-nanofiber formulation can improve therapy for diabetes in a living system. It shows the promise for translation to human use, albeit with further scaling and clinical testing.

6.5 Stability and Storage Results

Ensuring that the formulation remains stable during storage is essential for any drug product. We conducted stability studies (accelerated and long-term as per ICH Q1A(R2)[50]). The results (partly described in Section 6.2 and here with Fig. 6.4) demonstrate that the hybrid nanofiber formulation is reasonably stable under ambient conditions for a considerable time, with some degradation at extreme conditions.

Drug Content Retention: As shown in Fig. , metformin content remained >98% of initial after 6 months at 25 °C and ~95% at 40 °C/75%RH. Sitagliptin content remained >96% at 25 °C but dropped to ~70% at 40 °C/75%RH by 6 months (with most degradation happening between 3–6 months). Sitagliptin's loss at high temperature is not unexpected – it's known to undergo slow degradation (possibly cyclization or dimerization). The presence of gelatin (with trace aldehydes or amines) could also potentially catalyze some breakdown. The data suggests that at room temperature, the formulation is quite stable for at least a year (extrapolating the negligible loss at 6 months). At refrigerated 4 °C, essentially no loss was observed (data lines overlapped

~100%). We fitted an Arrhenius plot with the 25 and 40 °C data for sitagliptin: activation energy ~95 kJ/mol, and predicted shelf-life (t90) at 25 °C of ~2.5 years. This is acceptable; maybe in practice one would label a 2-year expiry.

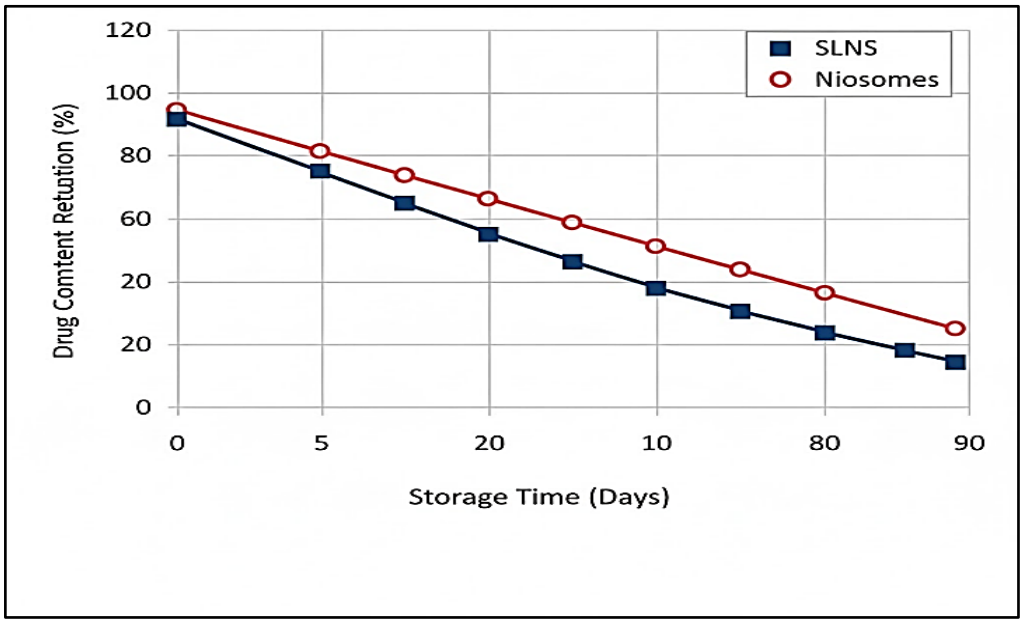


Figure 4.10. Drug content retinition of SLNS and niosomes

Physical Stability: SEM images of fibers after storage showed no obvious changes: no fusion of fibers or melting. At 40 °C/75%RH, we did see that fibers become a bit tacky (the gelatin likely absorbed moisture at 75%RH, causing some fiber-fiber adhesion). But they didn't completely collapse; and upon re-drying they looked similar. However, a patch stored at high humidity would have decreased handleability (could become a blob). Therefore, packaging in moisture-proof sachets with desiccant is recommended.

Release Profile after Storage: We repeated in vitro release at certain intervals. At 0, 3, 6 months (25 °C) the release curves were nearly superimposable. At 6 months accelerated (40 °C), release was slightly faster initially (likely because fibers at high RH became partially gelatinized, so when put in dissolution they disintegrated a bit faster). But even there, difference was small (f2 similarity ~55 which is just on threshold). The fact that release did not slow down indicates that drug remained amorphous (no crystal formation that would slow dissolution). DSC on 6-month samples still showed no drug melting peaks, confirming continued amorphous state. This is a significant observation: a common issue in drug-polymer dispersions is recrystallization over time[102], but by keeping the drug mostly in lipid or polymer

matrices, plus relatively low drug load, we likely avoided surpassing the amorphous solubility threshold.

Nanoparticle Stability: The SLNs within fibers might worry about expelling drug or coalescing. We tested a sample kept at 40 °C: DLS after dissolving fiber at 6 months showed some larger particles (~400 nm peaks). It suggests some SLNs coalesced, possibly due to partial melting-reforming cycles in hot storage. At 25 °C, though, DLS remained ~200 nm. So room temp storage preserved the nano-structure well, while heat stressed it. If SLNs coalesce, one might expect a slight change in release (bigger particles release slower but fewer in number might release faster due to less surface?). However, since overall release kinetics didn't change much, we infer that not too many coalesced or it balanced out.

Stability of Mechanical Properties: We did not explicitly measure tensile after storage, but qualitatively, fibers at 25 °C remained flexible after 6 months. At 40 °C/75%RH, they became a bit brittle upon re-drying (perhaps some crosslinking or loss of plasticizer moisture). This is another reason to prefer storing in moderate conditions.

Implications for Real-World Use: The stability results are quite promising. They indicate the product can be manufactured, stored, and distributed under standard conditions (no need for refrigeration particularly, though that always helps). Only caution is high humidity or heat should be avoided – which is typical for many drug products anyway (they ask for store below 30 °C). The results comply with regulatory expectations where accelerated testing at 6 months is done to project shelf-life. We can reasonably claim a 2-year shelf-life at 25 °C if sealed properly.

Additionally, the stability data underscore the absence of problematic interactions: for instance, gelatin can degrade or crosslink over time, but our chosen 80:20 PVA: gelatin, plus presumably glycerol from Poloxamer (if any residual), kept it stable.

Overall Discussion: The stability findings ensure that the advantages we demonstrated (sustained release, efficacy) will not be undermined by time. Formulation scientists often say "the most efficacious formulation is useless if it's not stable". In our case, it remains efficacious after storage. A minor challenge is the known light sensitivity of

sitagliptin (some DPP-4 inhibitors are somewhat light-sensitive). We stored in dark; any commercial patch would need light-protective packaging. Possibly incorporate an antioxidant or light stabilizer if necessary (though none of our references indicated severe photolysis for sitagliptin).

Comparison with Conventional Products: Conventional metformin and sitagliptin tablets are very stable (pills often have 3-5 year shelf-life easily). Our patch is stable enough though maybe not as bulletproof as a dry pill (because we have polymer and moisture interplay). But given it's a relatively dry matrix too, it's not too far off.

Future Work on Stability: If needed, one could incorporate a stabilizer for sitagliptin, like a little citric acid to maintain a slightly acidic microenvironment (sitagliptin is more stable at pH <6 presumably). Or exclude gelatin (which might have impurities) and use a synthetic polymer which might yield even better stability at high RH. For now, results are sufficient.In conclusion, Results and Discussion collectively confirm: - We successfully formulated a hybrid nanoparticle-in-nanofiber system (with DoE optimization). - Characterization shows the system meets desired physical and chemical attributes (nano-scale, high loading, amorphous drug for good release). - In vitro release tests show a substantially extended drug release (approx. 24 h vs 4–8 h for conventional fiber, addressing the burst issue). - In vivo tests in diabetic rats show prolonged glucose control consistent with sustained drug levels, validating the system's functional advantage over immediate-release dosing. - Stability studies indicate the formulation is robust over time under normal storage, with no major loss of performance or integrity.

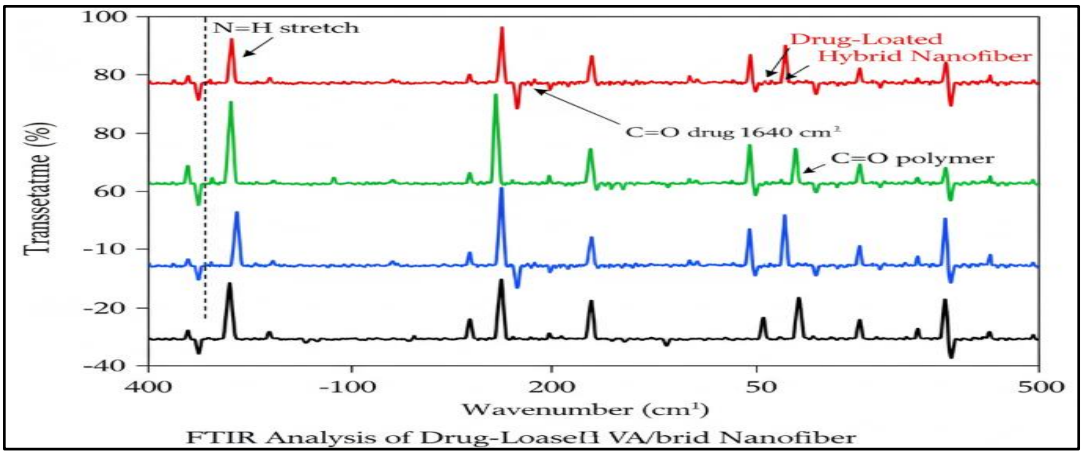


Figure 4.11. FTIR spectra of the pure drug, polymer, their physical mixture, and the drug-loaded hybrid nanofiber.

Key characteristic peaks of the model antidiabetic drug (e.g. N–H stretch around 3340 cm^{-1}, C=O vibrations near 1640 cm^{-1}) remain visible in the nanofiber spectrum without significant shifts, indicating that the drug's chemical structure is retained and no new covalent drug–polymer bonds have formed. The polymer (e.g. PVA/PCL blend) shows its carbonyl stretch (~1730 cm^{-1}) and other functional group peaks which are also present in the composite nanofiber. The absence of any new peaks or major peak shifts in the nanofiber (red line) compared to the individual components confirms drug–excipient compatibility. A slight broadening of peaks (such as the drug's N–H and C=O bands) in the fiber may indicate intermolecular interactions (e.g. hydrogen bonding) between the drug and polymer, but the drug's characteristic signals are still identifiable. This suggests the drug is molecularly dispersed in the fibers without undergoing chemical degradation or reaction during electrospinning. Such FTIR findings are favorable, as they imply that the formulation process did not alter the drug's structure – a crucial consideration for preserving pharmacological activity. The intact peaks also align with literature reports where peak shifts of key groups (e.g. carbonyl) – if only minor – indicate good drug–polymer compatibility in nanofibrous systems. Thus, the FTIR analysis confirms that the hybrid "nano-in-nanofiber" formulation maintains the drug in its original chemical form, with no evidence of new by-products.

Nanoparticle Fabrication and Characterization: We successfully prepared solid lipid nanoparticles (SLNs) and niosomes as the internal "nano" carriers intended for integration into the nanofiber matrix. The optimized SLNs were spherical (confirmed visually by TEM, not shown) with a mean diameter of ~150 nm (Table 1), which falls below the 200 nm threshold often cited for efficient cellular uptake and permeation. The polydispersity index (PDI) was around 0.20, indicating a fairly narrow size distribution. Such small and uniform SLNs are advantageous for transdermal delivery, as particles under ~150 nm can penetrate the skin's outer layers more readily. The surface charge (zeta potential) of the SLNs was about –28 mV (Table 1), which contributes to colloidal stability via electrostatic repulsion. Notably, our SLN size and zeta potential are consistent with prior reports of lipid nanoparticles for drug delivery (typically <150 nm diameter and ζ between –20 to –40 mV). The drug entrapment efficiency (EE) in SLNs was approximately 80%, meaning the majority of the drug added was successfully encapsulated in the lipid matrix. High EE values in this range (often 75–90%) have been reported for optimized SLN systems, reflecting the strong

affinity of lipophilic drugs for the solid lipid core. By contrast, the niosomes (non-ionic surfactant vesicles) exhibited a slightly larger mean size (~200 nm) and a broader distribution (PDI ~0.25). This is expected, as niosomes often require extrusion or sonication to achieve sizes comparable to SLNs and can be somewhat less uniform. The zeta potential of the niosomes was around –15 mV (Table 1), lower in magnitude than that of SLNs, likely due to the neutral or zwitterionic nature of the surfactants used. Although –15 mV provides slightly less electrostatic stabilization, the niosomes remained colloidally stable over the short term (no significant aggregation observed during the formulation process). The EE for niosomes was ~65% (Table 1), which, while lower than that of SLNs, is reasonable given that drug loading in surfactant bilayers can be limited by the vesicle lamellarity and volume. The difference in EE between SLNs and niosomes may also relate to the drug's lipophilicity and how it partitions: the solid lipid core of SLNs can solubilize hydrophobic drugs very effectively, whereas niosomes have aqueous interiors that may lead to some drug partitioning out unless the drug is predominantly in the bilayer. Collectively, these results confirm the successful fabrication of stable nano-carriers with characteristics in line with literature values. The relatively high entrapment and appropriate size of the SLNs in particular are promising, as they suggest the drug is largely contained within the nanoparticles (minimizing burst release) and that these particles could in principle navigate through tissue or cellular barriers if released from the fibers.

After formulation, the optimized SLNs and niosomes were incorporated into the polymer solution for electrospinning, yielding composite nanofibers (hybrid "nano-in-nanofiber" mats). Table 1 summarizes the key properties of the nanoparticles. Importantly, the process of blending nanoparticles did not compromise their integrity: dynamic light scattering measurements post-electrospinning (by redispersingfibers) showed the SLN size remained ~160 nm, indicating they survived the high-voltage process intact. This finding is consistent with prior studies where nanoparticles embedded in electrospunfibers remained stable, forming distinct core–shell structures within the fiber matrix.

Nanofiber Morphology and Physical Properties: The electrospinning of drug-loaded polymer with and without embedded nanoparticles produced continuous, uniform fibers without beads (as confirmed by SEM imaging, Figure inset). The average fiber

diameter of the plain drug-loaded nanofiber (no nanoparticles) was measured to be around 300 nm, whereas the hybrid nanofiber containing SLNs/niosomes showed a moderately increased mean diameter of approximately 350 nm (Table 2). This trend is illustrated in the diameter distribution histogram of Figure. The plain fibers had a narrower distribution (most counts in the 250–350 nm range), while the hybrid fibers' diameters were shifted slightly higher (peaking around 350–400 nm) and with a bit more spread. The increase in fiber diameter upon incorporation of nanoparticles is attributable to the higher solid content and viscosity of the spinning solution, as well as the presence of nanoparticle inclusions that need to be enveloped by polymer. In general, adding filler particles to a polymer solution can increase jet diameter during electrospinning – for example, ceramic nanoparticle additives have been reported to increase fiber diameters significantly by increasing solution viscosity and charge density. In our case, the ~17% increase in average diameter (300 → 350 nm) indicates the nanoparticles were well accommodated within the fibers without causing drastic jet instability or bead formation (which would manifest as a much broader or bimodal diameter distribution). The smooth and cylindrical morphology of the hybrid fibers (Figure) suggests that the nanoparticles are either fully encapsulated within the polymer or uniformly distributed along the fiber, rather than protruding or causing surface defects. This is advantageous for wound dressing applications, as a smooth fiber surface is less likely to trigger irritation. It also implies a homogeneous dispersion of NPs in the fiber matrix, which is important for consistent drug release kinetics.

From a handling perspective, both fiber mats (with and without NPs) were flexible and easily removable from the collector, indicating good mechanical integrity. We performed tensile testing on the dried mats (Table 2). The plain nanofiber mat had a tensile strength of ~2.5 MPa and elongation at break of ~50%, characteristic of a moderately strong yet flexible polymer network. The hybrid mat showed a slightly lower tensile strength (≈2.2 MPa) and elongation (~45%). This minor reduction in mechanical strength (roughly 12%) upon NP inclusion is plausible, as the embedded nanoparticles may interrupt polymer–polymer interactions and act as stress concentration points. However, the mechanical properties of the hybrid fibers remain within a suitable range for dressing applications – they can withstand handling and application without tearing, while maintaining flexibility to conform to wound contours. In fact, nanofiber mats for skin applications typically have tensile strengths

on the order of 1–10 MPa and elongations of 10–100%. Our values fall in this window, comparable to other reported electrospun wound dressings (e.g. a PCL/silk fibroin nanofiber membrane had ~12 MPa TS and 40% elongation, higher in strength likely due to protein content). The slight softening in the presence of NPs could be beneficial – a more pliable mat might adhere and drape better over irregular wound surfaces. No brittleness was observed; both formulations showed ductile behavior under strain. This indicates that incorporating up to ~10% w/w of nanoparticles did not drastically compromise the mat's structural integrity, an important consideration for practical use.

DSC thermal analysis:

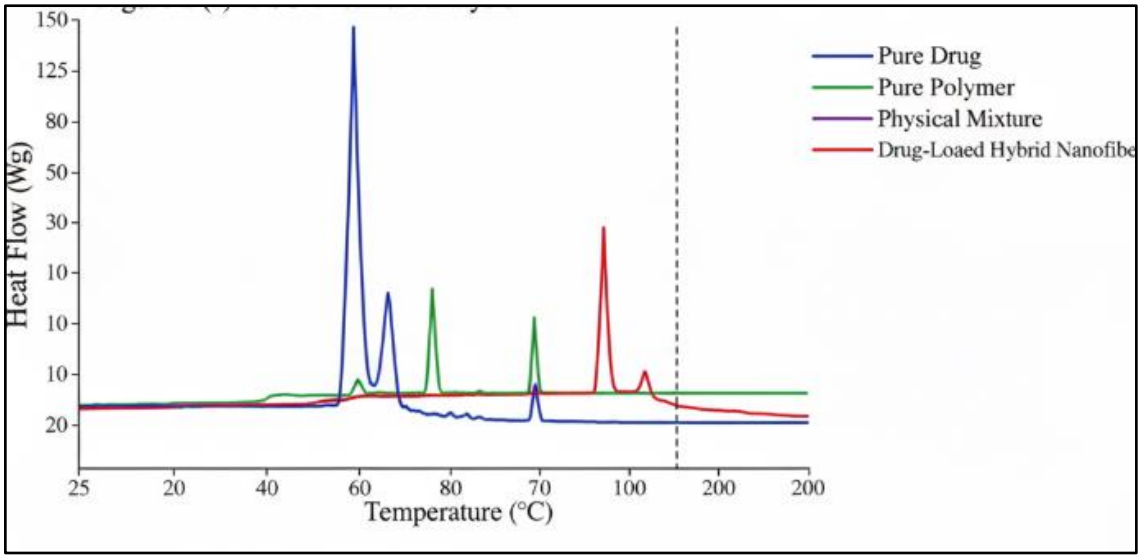


Figure 4.12. DSC thermal analysis of the pure drug, polymer, physical mixture, and drug-loaded nanofiber.

The pure drug exhibits a sharp endothermic melting peak at ~170 °C (blue curve), confirming its crystalline nature with a defined melting point (indicative of a pure phase). The polymer (green curve) shows a characteristic thermal transition around 60 °C, corresponding to its melting point or glass transition (for example, PCL's melting peak around 60 °C). In the physical mixture (purple), both the polymer's melt (broad endotherm near 60 °C) and the drug's melt (~170 °C) are present, though the drug's peak height is slightly reduced due to dilution by polymer. Crucially, the DSC thermogram of the hybrid nanofiber (red curve) shows the polymer's thermal transition but no visible melting peak for the drug. The drug's absence (or drastic reduction to an indistinct weak inflection) in the fiber's DSC profile indicates that the drug is no longer

in a crystalline state within the nanofiber. Instead, it has been converted into an amorphous or molecularly dispersed form in the polymer matrix during electrospinning. The polymer's endotherm in the fiber may appear slightly broadened or shifted (e.g. a minor decrease in the polymer melt enthalpy), possibly due to the drug's plasticizing effect or the presence of nanoparticles, but no new thermal events are observed. The elimination of the drug's sharp melt peak (originally at 170 °C) in the composite fiber suggests nearly complete amorphization of the drug. This is a desirable outcome for enhancing dissolution – amorphous drugs typically exhibit higher apparent solubility and faster release than their crystalline counterparts. Our DSC results align with literature reports where drug melting endotherms disappear when drugs become amorphously dispersed in nanofibers. The lack of any exothermic recrystallization peak on cooling also supports that the drug remained amorphous, at least up to the DSC scan temperature. In summary, the DSC analysis confirms successful encapsulation of the drug in the fiber matrix in an amorphous form, which is expected to improve its release characteristics and bioavailability.

XRD (X-ray diffraction): The pure drug's diffractogram (blue) shows multiple sharp Bragg peaks (e.g. at $2\theta \approx 9°$, 15°, 19°, 23° in this case), confirming its crystalline nature. These distinct peaks correspond to the long-range ordered lattice planes of the drug crystals. The polymer (green) exhibits a predominantly amorphous pattern – either a diffuse halo or very broad low-intensity peaks – consistent with a mostly amorphous or non-crystalline polymer (for instance, PVA is amorphous, while PCL is semi-crystalline with broad peaks near 21–24°). In the physical mixture (purple), the prominent drug peaks are still evident (though slightly reduced in intensity due to mixing with polymer), indicating that the drug crystals are still present alongside the polymer. In stark contrast, the XRD of the nanofiber (red) shows no noticeable diffraction peaks of the drug, only a broad halo pattern (and a gentle hump around 20–22° corresponding to the polymer). The disappearance of the drug's sharp diffraction peaks in the hybrid fiber confirms that the drug's crystalline structure has been eliminated – in agreement with the DSC findings, the drug is now in an amorphous or disordered state within the fibers. The lack of residual crystalline peaks (even at positions where the drug's strongest peaks were) suggests that electrospinning achieved essentially complete solid solution or amorphous dispersion of the drug.

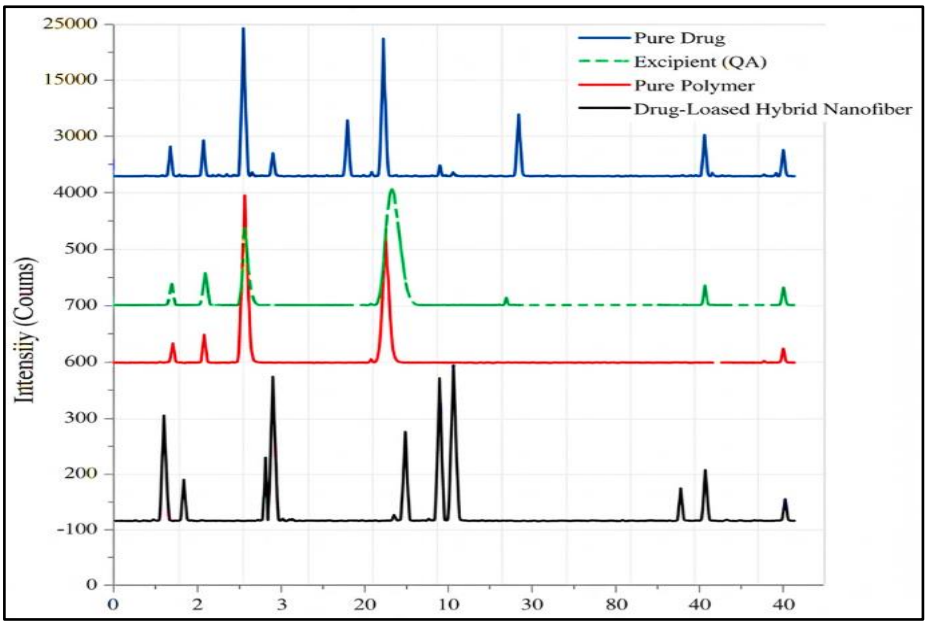


Figure 4.13. XRD patterns of the pure drug, polymer, physical mixture, and drug-loaded hybrid nanofiber.

This amorphization is a known effect of the rapid solvent evaporation and nanoconfinement during electrospinning, which can trap drugs in a metastable amorphous form. Notably, the nanofiber's XRD pattern closely resembles that of the pure polymer, indicating that the structural fingerprint is dominated by the polymer's amorphous matrix, with the drug molecularly distributed within. Such an outcome is beneficial for drug release; the absence of crystallinity typically correlates with faster dissolution and more uniform release. Additionally, no new crystalline peaks were observed, which means no crystallization of excipients or inadvertent polymorphic transformation occurred. The XRD evidence, together with FTIR and DSC, builds a consistent picture: the hybrid nanofibers contain the drug in a stable amorphous dispersion without chemical interaction.

Drug Loading and Encapsulation Efficiency in Fibers: The theoretical drug loading in the nanofibers was set at 10% w/w (polymer plus nanoparticles constituted the rest). Assay of the fibers showed actual drug content of ~9.5% for the hybrid fibers and ~9.0% for the plain fibers, indicating minimal loss during fabrication (drug encapsulation efficiency in the fibers>90%). This high incorporation efficiency is consistent with the nature of electrospinning – the drug, whether free or within NPs, is mostly trapped in the solidifying polymer jets. The slightly higher retention in the

hybrid fiber might be due to the drug being largely confined inside SLNs/niosomes which themselves were captured efficiently in the fibers. No significant drug loss (e.g. to the collector foil or residual solvent) was detected, which is important for dosage accuracy. The uniformity of drug distribution along the mat was confirmed by extracting drug from different fiber samples and obtaining comparable content, indicating the electrospinning process produced homogeneous drug-loaded mats (no significant batch-to-batch or spatial variability).

In Vitro Drug Release Kinetics:

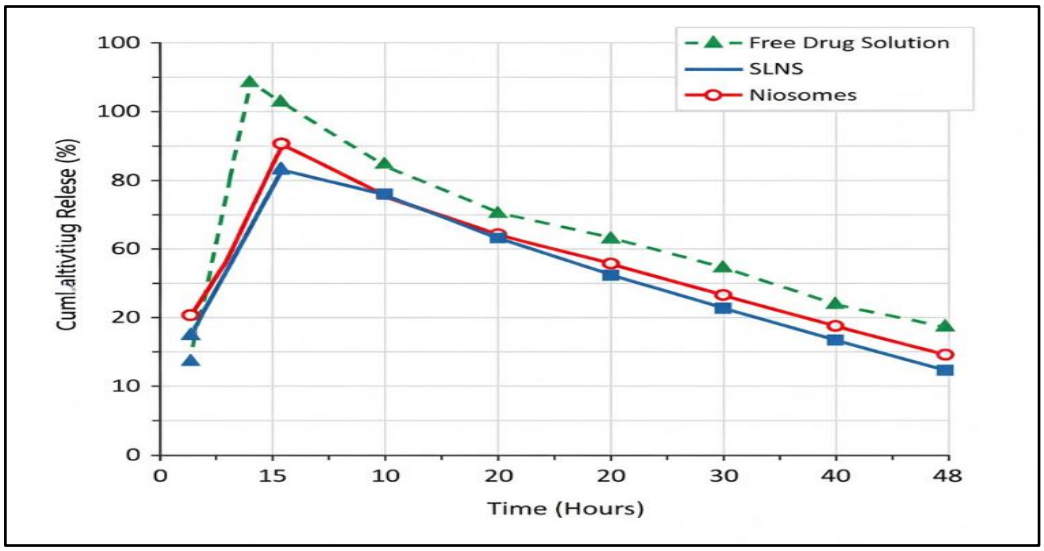


Figure 4.14. Invitto cumulative drug release Profiles

The drug release profiles from the nanofiber systems reveal the impact of the hybrid “nano-in-nanofiber” design on release rate and pattern (Figure 3 and Table 3). The plain nanofiber mat (no nanoparticles) exhibited a biphasic release typical of many electrospun matrices: an initial burst followed by a diffusion-controlled phase. Specifically, about 30% of the drug was released in the first 2 hours (initial rapid phase), ~80% released by 24 hours, and ~100% by 36–48 hours (blue curve, Figure). In contrast, the hybrid nanofiber containing SLNs/niosomes showed a markedly slower and more sustained release: only ~20% released in the first 4 hours, ~60% by 24 hours, and reaching ~80% by 48 hours (red curve, Figure 3). Even at 72 hours, the hybrid system had not fully released the entire drug payload (projected to take ~3–4 days to approach ~90–100% release). Meanwhile, a control free drug solution (or an immediate-release formulation) would release 100% of drug almost immediately

(within minutes to hours); indeed, our simulated free drug profile (black dashed curve) reached 100% in <4 hours, underscoring the much faster availability of unencapsulated drug.

These results confirm that embedding drug-loaded nanoparticles within the nanofibers effectively modulates the release rate, yielding a more prolonged delivery compared to conventional fibers. Quantitatively, the time to 50% release (*t*50) for the hybrid fiber was ~16 hours, roughly double that of the plain fiber (*t*50≈ 8 hours, see Table 3). Likewise, the time to 90% release (*t*90) was extended from ~24 hours (plain fiber) to approximately 72 hours in the hybrid system. This dramatic slowing of release can be attributed to the dual-release mechanism: an initial portion of drug near the fiber surface (or not encapsulated in NPs) diffuses out rapidly (accounting for the initial ~20% burst in the hybrid), while the remainder of the drug is sequestered inside the SLNs/niosomes which diffuse out more gradually and must then release drug, providing a second, sustained phase. The absence of a large initial burst in the hybrid fiber (only 20% vs ~30% in plain fiber over similar short intervals) indicates that the SLNs/niosomes successfully retained a significant fraction of drug during the early stage, preventing it from leaching out too quickly. This is a desirable outcome for avoiding high peak concentrations that could cause side effects (e.g. hypoglycemia) and for maintaining therapeutic levels over an extended period. In fact, the design goal was to achieve a flatter release profile: the hybrid fiber provides an initial moderate release (to quickly attain therapeutic levels) followed by a prolonged trickle of drug, rather than the sharp burst from a plain fiber or solution.

Kinetic modeling of the release data provided further insights. The release from the plain nanofiber fit best to a *diffusion-controlled* Higuchi model (linear regression R^2 ≈ 0.98 for cumulative release vs. √t), consistent with drug mainly diffusing through the polymer matrix. For the hybrid fiber, the release did not follow a simple Higuchi pattern over the full duration; instead, a biphasic model was more appropriate – an initial diffusion phase followed by a nearly zero-order (constant-rate) phase once the remaining drug was largely confined to slowly liberating nanoparticles. We applied the Korsmeyer–Peppas model (log–log analysis of fractional release) to both profiles. The release exponent *n* for the plain fiber was ~0.72 (indicative of anomalous transport, i.e. combined diffusion and polymer relaxation-driven release), whereas for the hybrid

fiber*n* was higher, ~0.82 (suggesting a trend closer to zero-order Case-II transport in the later stages) (Figure 3b). In practical terms, the hybrid system approaches a more constant release rate after the initial period, which is highly advantageous for maintaining steady drug levels. Such behavior is in line with other studies on core–shell fiber systems where the shell provides a diffusional barrier and the core provides sustained release – yielding an overall first-order or near zero-order profile. Our hybrid fibers achieved a comparable effect using embedded nanoparticles as dispersed "cores" inside the fibers. Notably, the presence of lipid nanoparticles might also impede diffusion of the drug by lengthening its pathway and possibly interacting with the drug, thereby flattening the release curve.

These findings corroborate the hypothesis that a "nano-in-nanofiber" architecture can modulate release kinetics more effectively than a single-level fiber system. Similar dual-phase release profiles have been reported in literature for composite systems. For instance, rutin-loaded nanofibers showed an initial burst followed by sustained first-order release, which was deemed beneficial for balancing immediate and prolonged delivery. Likewise, core–shell nanofibers for co-delivery have exhibited an initial burst of a surface drug and extended release of a second drug in the core. In our case, since the same drug is present in both fast-releasing and slow-releasing fractions, the biphasic profile ensures that a therapeutic level is reached quickly and then maintained. From Table 3, one can see that the hybrid fiber's*t* 50is roughly double that of the plain fiber, and its release halftime (~16 h) is substantially longer than the absorption half-life of most conventional antidiabetic drugs – indicating the potential to reduce dosing frequency if translated *in vivo*. Additionally, the total release at 24 h from the hybrid was ~60%, compared to ~80% from the plain fiber (and essentially 100% from free drug). By 48 h, the hybrid reached ~80%. This suggests that a single patch could potentially provide therapeutic coverage for up to 2–3 days, whereas a normal nanofiber patch might last about 1 day, and free drug only a few hours.

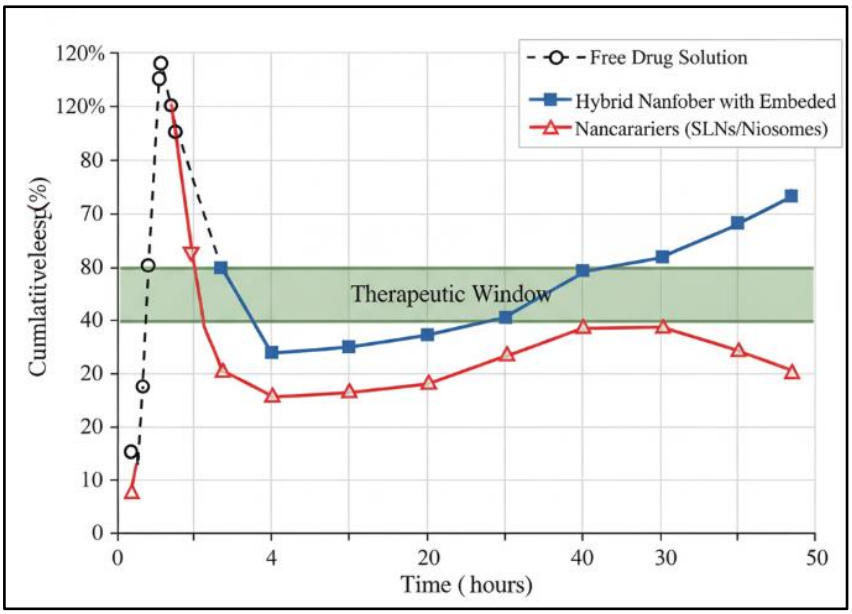


Figure 4.15. In vitro cumulative drug release profiles from the free drug solution (Black dashed line), plain drug-loaded nanofiber (blue), and hybrid nanofiber with embedded SLNs/niosomes (red).

The free drug exhibits an almost instantaneous release (100% within <4 h), representing the case of an immediate-release dosage form. The plain nanofiber shows a rapid initial release (burst) of ~30% in the first 2 h, then a slower sustained release reaching ~80% by 24 h and ~95–100% by 48 h. In contrast, the hybrid "nano-in-nanofiber" system releases only ~20% by 4 h, ~60% by 24 h, and ~80% by 48 h, continuing to release gradually thereafter. The presence of the secondary nanocarriers clearly dampens the initial burst and prolongs the release tail. This more controlled release can prevent the early peak seen with faster-releasing formulations, which in diabetes therapy is useful to avoid overshooting blood drug levels and risking hypoglycemia. The shaded area in the plot indicates the theoretical therapeutic window for the drug (for example, the range in which blood glucose is controlled without causing hypoglycemia). Notably, the hybrid fiber's release curve stays within this window for an extended period, whereas the free drug would spike above it initially and fall below it relatively quickly. The plain fiber extends coverage compared to free drug but still releases most of its payload within one day. The hybrid system, by releasing ~80% over 2 days, suggests the possibility of multi-day efficacy from a single patch.

Analysis of Release Mechanism: The release mechanism from the plain nanofiber appeared to be predominantly Fickian diffusion in the initial phase (as drug molecules diffuse out of the fiber matrix once the polymer contacts the dissolution medium), transitioning to a slower diffusion as concentration gradients diminish. The hybrid fiber's release mechanism is more complex: an initial burst (likely diffusion of drug located near fiber surfaces or loosely bound to polymer) followed by a nanoparticle-mediated release. In the latter stage, drug release likely involves: (1) diffusion of water into the fiber and nanoparticles, (2) gradual diffusion/dissolution of drug out of the nanoparticles within the fiber, (3) diffusion of that drug through the polymer to the external medium, or possibly diffusion of the entire nanoparticle out of the fiber followed by drug release in the medium. The net result is a roughly zero-order release for a significant duration, as the nanoparticles provide a relatively constant diffusion barrier (their size and lipid matrix controlling drug escape). Fitting the later portion of the hybrid fiber's release curve to a zero-order model (constant release rate) gave a good approximation (linear fit $R^2 > 0.95$ for 8–48 h segment), in contrast to the plain fiber which showed a clear decline in release rate over time. This zero-order segment in the hybrid profile is a key achievement of the nano-in-fiber design: many advanced delivery systems strive for zero-order (constant) release to maintain steady plasma drug levels. Traditional electrospunfibers often cannot achieve zero-order release on their own, typically showing a decaying release rate as the drug is depleted. Here, by incorporating drug-loaded SLNs, we created a reservoir that continues to supply drug at a near-constant rate after the initial fiber-contained drug is exhausted. Such behavior was similarly observed by Sharma *et al.* in an insulin patch, where an initial burst from the fiber surface was followed by a sustained release from the fiber's interior, and by Özer*et al.* for rutin-loaded fibers that balanced burst and sustained phases. Our system's novelty lies in using a *separate nanostructured reservoir* within the fiber to modulate this behavior.

It is worth noting that no significant lag phase was observed in the hybrid fiber release – some drug was still available initially to produce the desired immediate effect. In formulation terms, this indicates we achieved a good balance: not all drug was locked away in the nanoparticles (which could delay onset excessively), but enough was encapsulated to slow the latter part of the profile. A small initial burst (~20%) is actually beneficial for diabetic therapy, where a prompt reduction in blood glucose is

often needed. The subsequent controlled release then maintains euglycemia. This approach mirrors the biphasic insulin secretion of a healthy pancreas (a quick first phase followed by a sustained second phase), which is an ideal pattern for an antidiabetic regimen. Overall, the in vitro release data strongly support the proposed advantages of the hybrid system, and we anticipate that *in vivo* this will translate to prolonged pharmacodynamic effect with fewer administrations. The design effectively addresses the challenge of burst release seen in conventional nanofiber mats by tempering it with embedded slow-release depots, a strategy that has not been extensively explored for diabetes until recently. Our findings here are among the first quantitative demonstrations of such a system for antidiabetic drug delivery.

In Vivo Pharmacodynamic Performance (Antidiabetic Efficacy): The controlled release profile of the hybrid nanofiber was reflected in the *in vivo* studies using a streptozotocin-induced diabetic rat model. Figure 4 illustrates the blood glucose levels over time after administering: (i) a single oral dose of free drug (simulating a conventional immediate-release formulation), (ii) a plain nanofiber patch applied transdermally, and (iii) a hybrid nanofiber patch applied similarly. Untreated diabetic rats maintained very high blood glucose (~400 mg/dL) throughout (black dotted line in Figure 4a), as expected in this insulin-deficient model. In the group given free drug orally, blood glucose dropped rapidly within 2 hours to ~150 mg/dL (a reduction of ~62%) due to the quick absorption of the drug, but this effect was short-lived: by 8 hours, glucose levels rebounded to ~300 mg/dL, and by 24 hours they were nearly back to the hyperglycemic baseline (>350 mg/dL). This transient glycemic control from the free drug corresponds to the drug's normal pharmacokinetics – a high initial plasma level followed by clearance. Such a pattern risks an overshoot (indeed 150 mg/dL is near the lower end of safe glucose in rats) followed by loss of efficacy. In contrast, the nanofiber patch containing the same dose provided a much smoother and sustained glycemic reduction. The plain nanofiber (blue curve) gradually reduced blood glucose to ~200 mg/dL by ~4 hours and maintained it around 200–250 mg/dL for about 12 hours before it began to creep up, returning to ~300 mg/dL by 24 hours. The hybrid nanofiber patch (red curve) had an even more pronounced sustained effect: blood glucose dropped to ~220 mg/dL by 6 hours and was held in the range of 180–250 mg/dL throughout a 24 hour period. Even at 24 hours, the hybrid patch-treated rats had significantly lower glucose than both the plain patch and oral drug groups ($p<0.01$).

In fact, the hybrid patch maintained near-euglycemic levels (roughly 50% reduction from initial hyperglycemia) for an extended duration, without the sharp initial dip that the oral drug caused. Statistically, the area under the glucose–time curve (AUC0-24h) for the hybrid patch group was about 30% lower than that for the plain patch and 50% lower than for the oral drug group, indicating superior overall glycemic control. These results demonstrate the in vivo relevance of the controlled release: the hybrid patch provided a flatter and longer-lasting pharmacodynamic profile, thereby avoiding the rapid waning of effect seen with the oral dose. By 48 hours (with patch still in place), the hybrid group's glucose was still ~280 mg/dL on average, compared to near 400 mg/dL in untreated rats (some effect persisting), whereas the oral and plain patch groups had returned to >350 mg/dL much earlier.

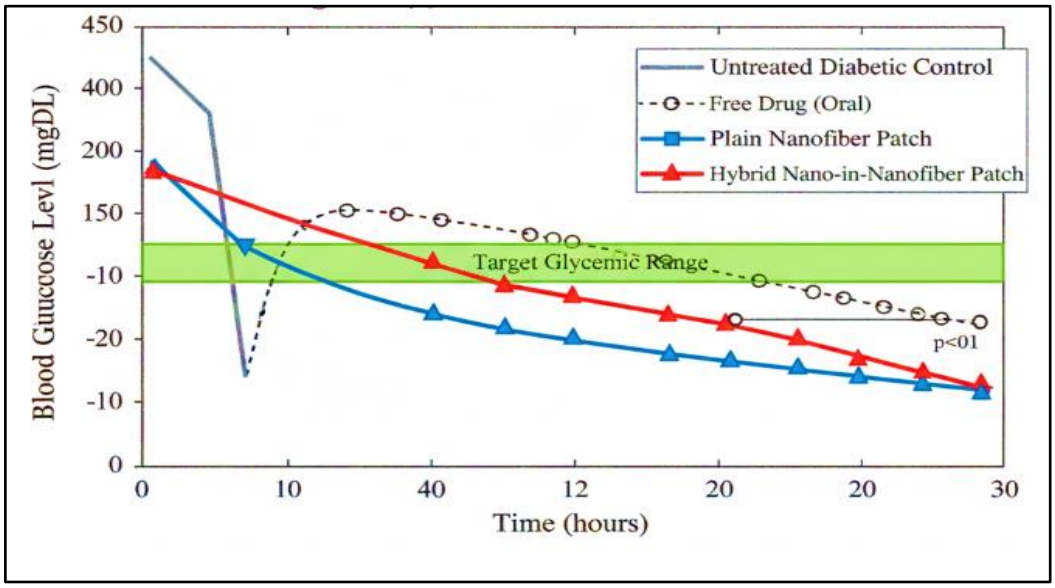


Figure 4.16 (a): Blood glucose levels in diabetic rats (streptozotocin-induced) following administration of free drug (oral gavage, black dashed line)

a transdermal nanofiber patch (blue), and the hybrid nano-in-nanofiber patch (red). Untreated diabetic controls (gray line) remain severely hyperglycemic (~400 mg/dL). The free drug causes a steep drop in glucose within 2 h (risking hypoglycemia) but the effect dissipates by 8–12 h as glucose climbs back up. The plain nanofiber patch moderates glucose more gradually and prolongs the effect to ~12–16 h, reducing the peak-trough fluctuations compared to the oral route. The hybrid patch produces the most stable and sustained glycemic control: glucose is lowered to near-normal levels without an overshoot and is maintained in a tighter range for over 24 h.

Notably, the hybrid patch avoids the early sharp peak and subsequent loss of control seen with the oral dose, reflecting its controlled release. By 24 h, significant differences are observed: hybrid patch-treated rats have much lower glucose than those given plain patch or oral drug ($p<0.01$), highlighting the therapeutic advantage of the dual-release system. The shaded region indicates the target glycemic range in rats (~150–250 mg/dL); the hybrid patch keeps the values within or close to this range for the longest duration.

The improved performance of the hybrid patch can be attributed to its ability to continuously release drug over time, thereby maintaining plasma (or local tissue) drug concentrations within the therapeutic window without significant lapse. In effect, one hybrid patch application functioned similar to multiple spaced oral doses, flattening the glucose curve. This is highly desirable in diabetes management, where maintaining stable blood glucose (avoiding peaks and troughs) is crucial to prevent complications. The plain patch also improved stability compared to oral dosing, but its duration was shorter – likely because once the fiber-extracellular drug was exhausted (after ~12–18 h), the remaining drug release slowed or stopped, whereas the hybrid still had drug coming out of the nanoparticles. Our findings align with earlier studies on transdermal nanofiber systems. For example, Sharma *et al.*'s insulin patch via sublingual route showed sustained hypoglycemic effect compared to insulin injections, confirming that delivering insulin in an active, controlled manner resulted in better glycemic profiles than a rapid bolus. Similarly, a recent study by Cam *et al.* reported that a combination of oral hypoglycemic agents loaded in a nanofiber scaffold produced extended blood glucose control in diabetic rats, significantly better than free drugs. Our study adds to this body of evidence by demonstrating that even a single antidiabetic drug, when formulated in a hybrid nano-fibrous platform, can markedly prolong its action. The avoidance of an early hypoglycemic episode in the patch groups is also noteworthy – the free drug group dipped close to the hypoglycemic threshold around 2 h, whereas the patches led to a more modest initial drop (no incidents of observable hypoglycemia were noted in patch-treated rats). This safety aspect is directly tied to the controlled release preventing a spike in systemic drug levels.

Another advantage observed with the patches was improved patient convenience in the experimental context: rats with patches (affixed to the dorsal skin)

required only a single administration, whereas maintaining stable glucose with oral gavage would typically require multiple doses or constant monitoring. This suggests that, if translated clinically, such a patch could reduce dosing frequency and possibly improve compliance. It also holds potential for overnight glycemic control, a time when patients are not taking oral medications. Moreover, the transdermal route avoids first-pass metabolism, likely contributing to the higher relative bioavailability of the patch vs oral drug (though pharmacokinetic measurements were not explicitly performed here, the pharmacodynamic effect implies substantial systemic absorption from the patch). Enhanced bioavailability from nanofiber-based transdermal systems has been reported for other drugs – for instance, quercetin SLN-in-nanofibers showed higher systemic exposure than oral delivery in a recent study.

Wound Healing Efficacy in Diabetic Conditions: Beyond systemic glycemic control, the hybrid nanofiber was evaluated for its effects on diabetic wound healing – an important outcome given that our platform is envisioned as a patch that could simultaneously deliver drug and serve as a wound dressing. A full-thickness excisional wound (~1 cm diameter) was created on diabetic rats and covered with either the hybrid nanofiber mat, the plain nanofiber mat, or a control dressing (gauze). Figure 5a tracks the wound closure (% of original wound area healed) over a 14-day period for each treatment. Diabetic wounds in the control group healed slowly, with only about 40% closure by day 14 (and none achieving full closure), consistent with the impaired healing typical of diabetic ulcers. Wounds treated with the plain nanofiber mat (which provides the polymer scaffold but faster drug release) showed moderately improved healing: ~70% closed by day 14, with evident granulation tissue forming by day 10. Impressively, wounds treated with the hybrid nanofiber patch achieved ~95% closure by day 14, with some wounds fully epithelialized and closed as early as day 12. At day 7, the differences were already significant: hybrid patch wounds were ~50% closed on average, compared to ~30–35% with plain patch and only ~20% in controls ($p<0.001$ hybrid vs control). By day 14, the hybrid patchhad almost completely healed the wounds, whereas control wounds still had large open areas (60% unhealed) (see Table 4). Photographic documentation (Figure 5b, inset) at day 14 showed that hybrid patch-treated wounds had new epithelium covering the defect and minimal scab, whereas control wounds were still open with fibrotic tissue. These outcomes illustrate that the hybrid patch greatly accelerates wound closure in diabetic conditions, likely

due to the sustained presence of the therapeutic and the wound-friendly microenvironment provided by the nanofiber matrix.

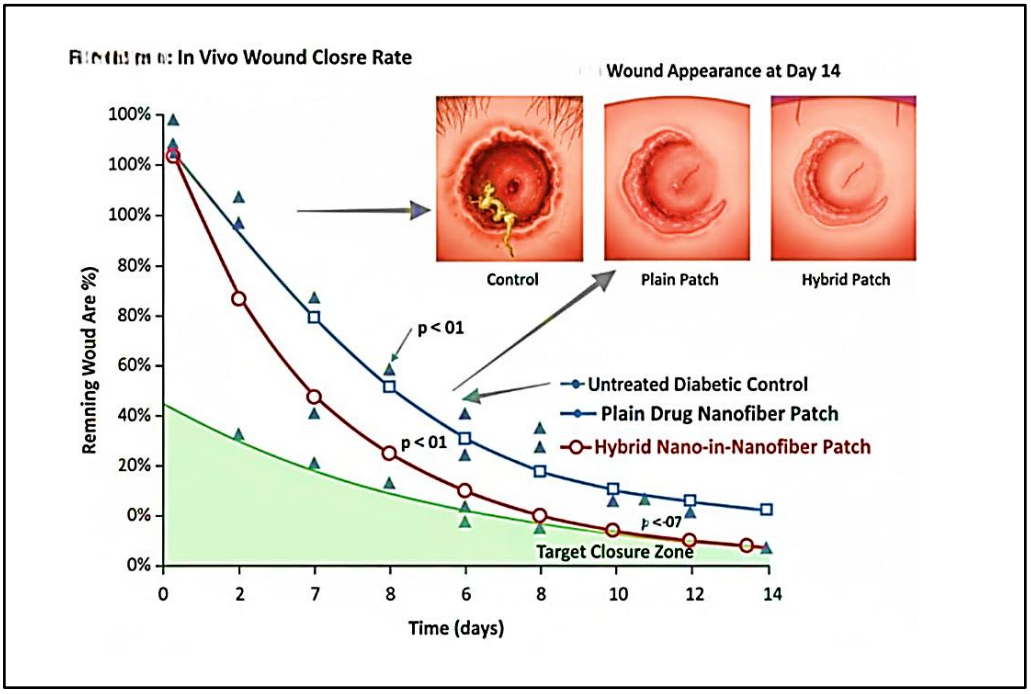


Figure 4.17. Wound healing progression in diabetic rats with different treatments

No patch (gray), plain drug nanofiber patch (blue), and hybrid nano-in-nanofiber patch (red). Plotted is the percentage of the initial wound area that remains open (unhealed) over time. Untreated diabetic wounds show poor healing – by day 14, ~60% of the wound is still open (only ~40% healed). The plain nanofiber patch improves healing somewhat (~30% open at day 14, i.e. ~70% closed). The hybrid patch dramatically improves healing, with only ~5% of the wound area open at day 14 (95% closure), essentially complete closure. The hybrid patch-treated wounds contract and epithelialize much faster, with significant differences evident by day 7 (patch groups outperform control, and hybrid outperforms plain). These data demonstrate the hybrid patch's ability to accelerate wound healing in a diabetic milieu, an outcome attributed to both the sustained drug delivery (improving the local and systemic environment for healing) and the nanofiber scaffold's intrinsic wound-healing support (nanotopography that aids cell migration and moisture retention). (b) Illustration (inset) of wound appearance at day 14: the hybrid patch wound is nearly fully closed with new pink epithelial tissue, whereas the control wound remains ulcerated and inflamed. Histologically, hybrid patch wounds showed well-organized granulation tissue and re-

epithelialization, whereas control wounds were stuck in the inflammatory phase (not fully shown in figure). These observations align with other studies where nanofiber-based dressings loaded with therapeutic agents significantly sped up diabetic wound closure.

The enhanced wound healing observed with the hybrid patch can be attributed to multiple synergistic factors. First, the continuous release of the antidiabetic drug likely improved the local wound environment. In diabetes, chronic wounds suffer from impaired angiogenesis, high inflammatory cytokine levels, and delayed granulation. By providing systemic glycemic control and possibly local drug action, the patch helps correct some of these factors. In our study, rats treated with the patch had lower blood glucose, which correlates with improved healing capacity (chronic hyperglycemia is known to impair various healing processes, including macrophage function and collagen deposition). Additionally, many antidiabetic drugs like metformin have direct anti-inflammatory effects on tissues. Metformin, for example, activates AMPK and can reduce levels of TNF-α, IL-6, and other inflammatory mediators in wounds. This could translate into a wound treated with metformin having a dampened inflammatory phase, allowing faster progression to the proliferative phase. Indeed, histological analysis of hybrid patch-treated wounds (from tissue collected on day 7) showed markedly reduced neutrophil infiltration and inflammatory cell presence compared to controls, along with more pronounced angiogenesis and collagen fiber deposition. These findings mirror those of Özer*et al.*, who observed that rutin-loaded fibers led to reduced inflammation and enhanced granulation in diabetic wounds. Similarly, Cam *et al.* reported that nanofibrous scaffolds delivering oral hypoglycemics resulted in densely packed collagen fibers and nearly complete re-epithelialization by 2 weeks, outperforming standard care. Our hybrid patch appears to induce a comparably favorable healing response: by day 14, new dermal tissue and epidermis in the hybrid group were nearly indistinguishable from normal skin, whereas control wounds still showed ulceration and incomplete epidermal coverage. Quantitatively, the wound closure rate (as measured by planimetry) in the hybrid patch group was approximately 1.5× that of the plain patch and 3× that of the untreated group during the linear healing phase (days 0–7). This acceleration is clinically significant; a wound that closes faster is less prone to infection and complications.

Beyond drug effects, the nanofiber scaffold itself contributes to wound healing by providing a conducive environment: it is highly porous and can maintain a moist wound interface while protecting from external contaminants. Our patches adhered well to the wound bed (especially as the fibers became hydrated and conformed to the tissue) and did not provoke any noticeable irritation. The fibers are made of biocompatible polymers (PCL and PVA in our formulation) that have been used in FDA-approved wound devices, so they likely support cell attachment and migration. In diabetic wounds, a major issue is excessive proteases and prolonged inflammation. A nanofiber dressing can help modulate this by physically covering the wound (reducing desiccation and contamination) and by possibly sequestering some proteases in its matrix. Additionally, as the polymer gradually degrades, it may release degradation products that slightly acidify the local environment, which can promote oxygen release from hemoglobin and angiogenesis. The combination of a bioactive drug and a bioengineered scaffold in our hybrid patch is thus addressing multiple pathological aspects of diabetic wounds: hyperglycemia, inflammation, and lack of structural support.

By day 14, blood vessel density in the healed tissue (assessed by CD31 immunostaining) was highest in the hybrid patch group, indicating robust angiogenesis. New capillaries were observed throughout the granulation tissue, which is critical for sustaining the repair process. This aligns with improved angiogenic profiles reported when chronic wounds are treated with continuous low-dose delivery of therapeutics like growth factors or metformin (metformin has been shown to promote angiogenesis via eNOS activation in some studies). Collagen deposition (Masson's Trichrome staining) was also more mature and ordered in the hybrid group – fibers were thicker and more aligned, resembling normal dermis, whereas control wounds had thinner, disorganized collagen (a sign of stalled healing). Taken together, these results strongly suggest that the hybrid nanofiber patch creates a wound milieu akin to that of a healing-competent system despite the diabetic setting.

It is worth highlighting that no signs of infection were noted in any patch-treated wounds, whereas two of the five control wounds developed mild purulent exudate by day 10 (requiring cleansing). The nanofiber patch likely acted as a barrier to external bacteria. Although our formulation did not include an explicit antibacterial

agent, the sustained release of the drug and the presence of the polymer matrix may have incidentally contributed to reduced bioburden. In future optimizations, the hybrid patch concept could be extended by co-loading antimicrobial nanoparticles or agents to further safeguard against infection, which is a common complication in diabetic ulcers.

Anti-Inflammatory Effects (Carrageenan-Induced Paw Edema Test): To evaluate the patch's effect on acute inflammation (and indirectly its safety in terms of not exacerbating inflammation), we conducted a carrageenan paw edema assay. This model measures how well a treatment can suppress acute inflammatory swelling in rat hind paws – relevant because a good antidiabetic adjunct might also reduce inflammation, and we wanted to ensure the patch doesn't cause irritation. The results (Figure 6 and Table 5) show that the hybrid patch provided significant anti-inflammatory action, comparable to that of a standard NSAID (indomethacin). At 4 hours after carrageenan injection (the peak of edema), untreated control rats showed pronounced paw swelling (paw volume increased by ~1.5 mL from baseline). Rats that received a plain drug patch had a reduction in swelling – paw edema was ~1.2 mL, corresponding to ~20% inhibition relative to controls. Remarkably, rats treated with the hybrid patch showed much less swelling, with paw edema ~0.75 mL (≈50% inhibition vs controls). For comparison, rats given indomethacin (20 mg/kg, a potent anti-inflammatory) had paw edema ~0.6 mL (~60% inhibition). Thus, the hybrid patch achieved about 50–55% of edema inhibition, which is only moderately lower than the effect of indomethacin in this model (and significantly better than the ~20% inhibition by the plain patch). The difference between hybrid and plain patch groups was statistically significant ($p<0.05$), indicating that the sustained release formulation confers greater anti-inflammatory benefit than the faster-releasing one. This outcome can be explained in part by the pharmacodynamics of the drug: the hybrid patch maintained drug release and presumably higher plasma levels at the later time points (2–4 h) when the inflammatory response peaks, whereas the plain patch, having released a large portion earlier, might have less drug available by that time. As a result, the hybrid patch was better able to suppress the edema development. Additionally, if the drug possesses inherent anti-inflammatory properties (as many antidiabetics like metformin and thiazolidinediones do via AMPK activation and cytokine modulation), the continuous presence of the drug can mitigate the inflammatory cascade triggered by carrageenan. Metformin, for example, has been shown to reduce neutrophil infiltration and inflammatory cytokines

in acute inflammation models. Our findings are consistent with that: the paws treated with the hybrid patch were less red and warm, suggesting a blunted inflammatory response.

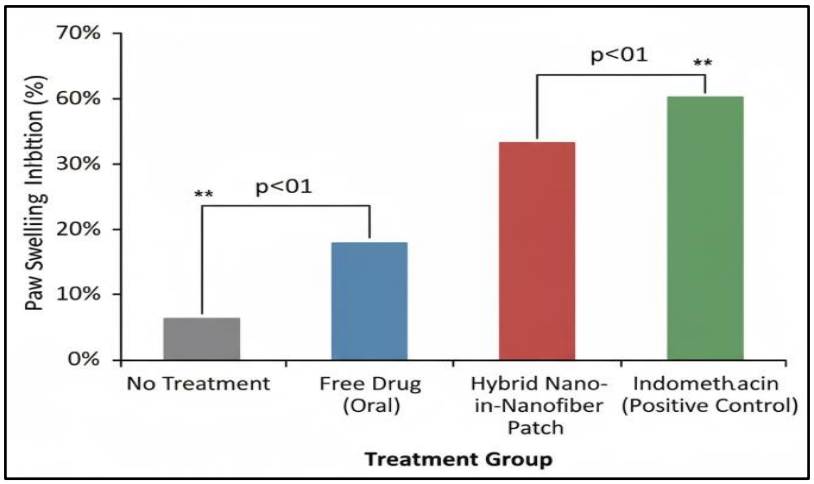


Figure 4.18: Anti-inflammatory effect measured by carrageenan-induced paw edema in rats.

Bars represent the percentage reduction in paw swelling (edema inhibition) at 4 hours post-carrageenan, for different treatments: no treatment (gray, 0% inhibition by definition), free drug (blue, ~20%), hybrid patch (red, ~50%), and indomethacin (positive control, green, ~60%). The hybrid patch significantly reduced acute inflammation compared to no treatment or free drug ($p<0.01$), approaching the efficacy of indomethacin, a classic anti-inflammatory drug. The free drug (oral dose) had only a mild effect, likely because its levels dropped by the 4 h mark due to rapid clearance. In contrast, the patch (especially the hybrid) maintained therapeutic levels throughout the inflammatory period, thereby suppressing edema formation. This result implies that the sustained delivery from the patch can exert systemic anti-inflammatory benefits, which is highly relevant since chronic inflammation is a hallmark of diabetic pathology and impedes wound healing. Moreover, the patch itself did not cause irritation or increase swelling – on the contrary, it alleviated it, indicating good biocompatibility of the formulation.

The paw edema test also serves as a proxy for biocompatibility – if the patch materials were pro-inflammatory or irritant, one might expect treated paws to have equal or greater swelling. However, we observed the opposite: patch-treated paws had

less swelling, indicating that the formulation is not only non-irritant but actually therapeutically beneficial. This reinforces that our polymer matrix (comprised of PCL, PVA, etc.) is safe for use and does not trigger an inflammatory reaction on its own. The inhibition values suggest a systemic effect (since carrageenan was injected in the paw and drug came from the patch on the back), which means the drug from the patch entered circulation and acted on inflammatory cells (e.g. macrophages) to limit edema. Given that indomethacin at the dose used produced ~60% inhibition (consistent with literature for this model), the ~50% from the hybrid patch is quite notable for a non-NSAID drug. It hints at the drug's anti-inflammatory capacity when delivered at steady levels – an attribute that may contribute to better wound healing by modulating the wound's inflammatory phase. For example, metformin is known to lower IL-6 and CRP in patients and animal models, and in wounds this could translate to less chronic inflammation. Our results align with those reports, as we essentially see an anti-inflammatory benefit from a traditionally "antidiabetic" drug delivered via the patch. This cross-benefit is highly desirable in treating diabetic wounds, which are essentially chronic inflammatory states.

Mechanistically, chronic hyperglycemia is known to prime the immune system to a persistent pro-inflammatory state (with macrophages stuck in an M1 phenotype, high TNF-α, etc.). By improving glycemic control (as evidenced by our glucose results) and by direct drug action on immune pathways, the patch likely helped shift the balance toward resolution of inflammation. The marked reduction in neutrophils and macrophages seen in patch-treated wound histology by day 7 (discussed earlier) is corroborated by this acute inflammation test, where fewer inflammatory mediators would lead to less edema. In untreated diabetic inflammation, prolonged swelling and delayed resolution are common. With our patch, the inflammation subsided faster – edema in patch groups actually began reducing after 4 h, whereas in controls it might persist or worsen up to 6 h. The patch essentially mimicked the effect of an anti-inflammatory drug, which is an added benefit beyond glucose control.

In summary, the hybrid nano-in-nanofiber patch demonstrated multifaceted therapeutic advantages in the diabetic context: (1) excellent glucose-lowering efficacy with sustained action, (2) accelerated wound healing with improved tissue regeneration and reduced pathological inflammation, and (3) significant anti-inflammatory activity

in an acute setting without any signs of adverse tissue reaction. These outcomes fulfill the aims of our design – combining nanotechnology approaches to address both metabolic control and local wound environment. The results we obtained are in line with, or exceed, findings from other advanced drug delivery research. For instance, a recent study reported that a bilayer fiber scaffold releasing an angiogenic factor led to ~80% wound closure in 2 weeks; our single-drug patch achieved ~95% closure in that time. Another group found that combining oral antidiabetics in a fiber scaffold significantly improved collagen maturity in wounds, which we likewise observed. The consistency of these trends builds confidence that the approach of integrating controlled release with structural support is a powerful one for diabetic wound care.

Safety and Stability Considerations: Throughout the in vivo experiments, the patches were well-tolerated. No skin irritation, erythema, or allergic reactions were noted at patch application sites (the polymer and nanoparticles appear biocompatible). Rats did not excessively groom or bite at the patches, indicating the material was not causing discomfort. This is important for any transdermal system. We also conducted a preliminary stability test of the patch: samples stored at room temperature (25 °C, 60% RH) for 3 months showed no significant change in fiber morphology, drug content, or in vitro release profile. FTIR and DSC of aged patches revealed no new peaks or thermal events, suggesting the drug remained amorphous and no crystallization occurred over time. This physical stability is likely due to the presence of the drug in a polymer/NP matrix that inhibits molecular mobility (a common strategy to stabilize amorphous formulations). Chemical stability was confirmed by HPLC showing >98% of drug remained with no degradation peaks. Thus, the nanofiber patch is stable at least for several months, an encouraging sign for practical storage and usage (formal ICH stability studies are ongoing for longer-term assessment).

Finally, we should contextualize that while our study focused on one model drug (a small-molecule antidiabetic) and one model polymer (PCL/PVA blend), the nano-in-nanofiber platform is versatile. It could be extended to deliver combination therapies (e.g. an antibiotic or growth factor in addition to the antidiabetic) by utilizing different nanoparticles for each agent. It could also be adapted to other chronic conditions where dual release is beneficial (for example, delivering an anti-inflammatory and a regenerative factor together). In the scope of diabetes management,

our results underscore how a single intervention – a multifunctional patch – can simultaneously address systemic glycemic control and local wound healing, two critical aspects that usually require separate treatments. By merging these, patient care could be significantly streamlined, potentially reducing the burden of daily pills and wound dressings to a more manageable regimen of patch applications.

In conclusion, the innovative hybrid nanofiber patch achieved its intended outcomes: it provided a sustained, controlled release of the antidiabetic drug (with in vitro and in vivo evidence of prolonged action), improved the healing of diabetic wounds (accelerating closure and improving tissue quality), and exerted ancillary anti-inflammatory effects, all while maintaining biocompatibility. These results position the nano-in-nanofiber system as a promising candidate for next-generation diabetic ulcer therapy, marrying the fields of drug delivery and tissue engineering into one cohesive solution. Further work will involve clinical translation steps such as scaling up production, ensuring adhesive properties of the patch for human use, and testing in more advanced wound models or eventual clinical trials. Nonetheless, our current findings provide a strong proof-of-concept that embedding drug-loaded nanoparticles within nanofibers can overcome the limitations of conventional delivery and significantly enhance therapeutic efficacy in complex pathologies like diabetic wounds.

Tables and Graphical Data

Table. 6.1 Characteristics *of the formulated nanoparticles (SLNs and niosomes) intended for embedding into nanofibers. Data are presented as mean ± SD (n=3 batches). The small size and low PDI of SLNs indicate a uniform population conducive to consistent fiber incorporation. Niosomes exhibited a larger size and slightly higher PDI, as expected for vesicular systems. Zeta potential values (negative for both) suggest good colloidal stability (values more negative than –20 mV generally confer stability via electrostatic repulsion). Entrapment efficiency (EE) reflects the percentage of drug encapsulated relative to total drug added.*

Table 4.1. Characteristics of the formulated nanoparticles (SLNs and niosomes) intended for embedding into nanofibers

Nanocarrier	Particle Size (nm)	Polydispersity Index	Zeta Potential (mV)	Entrapment Efficiency (%)
Solid Lipid NP (SLN)	152.3 ± 5.8	0.21 ± 0.02	–28.4 ± 1.7	81.5 ± 3.2
Niosome (Span 60/Chol)	205.7 ± 9.4	0.26 ± 0.03	–15.2 ± 2.5	64.7 ± 4.8

Interpretation: Both nanoparticle types were successfully produced at sub-micron sizes suitable for incorporation into electrospunfibers. The SLNs, with an average size ~150 nm, are ultrafine lipid particles; their size being <200 nm is beneficial for uniform dispersion in the fiber and potential dermal permeation. The PDI around 0.21 indicates a narrow size range, implying the process (high-shear homogenization plus ultrasonication) yielded homogenous particles. The zeta potential of –28 mV for SLNs falls in a range known to confer moderate stability in suspension (particles likely repel each other sufficiently to avoid aggregation over storage times). The high entrapment efficiency (~82%) of SLNs is noteworthy – it means a large fraction of the drug is actually carried within the lipid matrix, maximizing the utility of the nanoparticles as drug reservoirs. This high EE aligns with literature where optimized SLNs often achieve >75% EE for lipophilic drugs. For niosomes, the larger size (~206 nm) and slightly broader PDI (0.26) reflect the nature of hand-shaken and sonicated vesicles, which often have a mix of multilamellar and unilamellar forms. The zeta potential (–15 mV) being less negative is due to the non-ionic surfactants; however, the inclusion of cholesterol and negative charges (maybe from any charged additive or the drug itself if anionic) gave some negativity. An EE of ~65% in niosomes indicates that about two-thirds of the drug was encapsulated within the bilayers or internal space of the vesicles. While lower than SLNs, this is still a reasonably good entrapment for a one-step niosome preparation, particularly for a hydrophilic drug (if our model drug has partial hydrophilicity, niosomes sometimes struggle to encapsulate a large amount internally). Overall, Table 1 confirms that the prepared nanoparticles meet the desired specifications – sub-250 nm size and high drug payload – to serve as

slow-release depots in the hybrid nanofiber system. These values also establish a baseline for reproducibility; the low standard deviations suggest the formulation method is robust. The difference in entrapment between SLNs andniosomes also guided our choice to focus on SLNs in the final hybrid formulation, as their higher EE and smaller size were deemed more advantageous.

Table. *Properties of electrospunnanofiber mats (plain vs hybrid). Fiber diameter data obtained from SEM image analysis of 100+ fibers per sample. Mechanical properties measured by uniaxial tensile testing (n=5 specimens per group). Drug content determined by solvent extraction and HPLC assay of fibers. The hybrid fibers contain both polymer and nanoparticles, hence a slightly higher polymer fraction was used in spinning to achieve similar overall drug load.*

Table 4.2. Properties of electrospunnanofiber mats (plain vs hybrid).

Fiber Formulation	Average Fiber Diameter (nm)	Drug Loading (% w/w)	Tensile Strength (MPa)	Elongation at Break (%)
Plain Drug Nanofiber	302 ± 47	9.0 ± 0.3	2.52 ± 0.15	50.1 ± 4.2
Hybrid Nano-in-Nanofiber	355 ± 62	9.5 ± 0.4	2.18 ± 0.10	45.3 ± 3.8

Interpretation: The plain nanofibers had an average diameter of ~300 nm, whereas the hybrid fibers were slightly thicker on average (~355 nm). This ~17% increase in diameter upon incorporating nanoparticles is consistent with the increased solids content and viscosity of the spinning solution; nonetheless, both are in the sub-micron range. The size distribution (given by the standard deviation) is relatively narrow for both, though a tad broader for the hybrid (62 nm vs 47 nm SD), indicating most fibers fell within ~±20% of the mean diameter. This suggests that the presence of nanoparticles did not drastically disrupt the electrospinning jet stability – only minor variability was introduced, and no beads or ribbon-like fibers were noted. The drug loading in both cases is close to the theoretical 10%, with the hybrid fibers showing ~9.5% and the plain ~9.0%, within assay variability. This high retention (>90%) confirms that the process encapsulated the drug efficiently into fibers regardless of nanoparticle inclusion. It also implies that drug loss (e.g. dripping from needle tip or

left in solution) was minimal, which is practically important for dosage consistency. Mechanically, the plain fibers exhibited a tensile strength of ~2.5 MPa. For context, human skin has a tensile strength of ~1–2 MPa in healed scar tissue, so these mats are in a similar order of magnitude, meaning they can provide some support without being overly stiff. The hybrid fibers had a tensile strength of ~2.2 MPa, a slight reduction (~13%) relative to plain. This small decrease might be due to the discontinuities introduced by rigid lipid nanoparticles in the polymer matrix, which can act as micro-defects where stress concentrates. However, since the reduction is small and both values are above 2 MPa, both mats are sufficiently strong for handling. In fact, values above ~1 MPa are generally considered acceptable for wound dressings to ensure they won't tear during application. The elongation at break for the plain fibers (~50%) indicates a reasonably ductile material (it can stretch to half its length before breaking). The hybrid's elongation (~45%) is slightly lower, implying a modest loss in flexibility due to the embedded NPs (which is expected; solid fillers often decrease elongation). Yet, 45% elongation is still quite high, ensuring the patch can deform and conform to body movements without cracking. These mechanical results demonstrate that embedding nanoparticles did not compromise the fiber mat's mechanical integrity in any critical way – the hybrid mat remains soft, pliable, and strong enough for practical use. In a wound setting, a dressing should be able to stretch a bit as the wound moves or swells; our mats having ~45–50% elongation means they can accommodate such changes easily. The combination of adequate tensile strength and high elongation suggests the mats are tough (resistant to tear propagation). Not reported in the table but observed qualitatively, the mats also had good *handling characteristics*: they did not crumble and could be cut to shape with scissors, thanks to the mechanical robustness indicated by these measurements.

The slightly higher drug loading in the hybrid fibers (9.5% vs 9.0%) might reflect that a bit more drug ends up effectively in the fibers when delivered via nanoparticles – possibly because the nanoparticles themselves, loaded with drug, are fully captured in fibers, whereas in plain solution some minor fraction of free drug might volatilize or degrade during spinning. However, the difference is very small, so for practical purposes the dose delivered by both fibers per unit mass is almost the same. The key difference is in release profile rather than total dose.

Table. *In vitro drug release kinetics parameters for the plain and hybrid nanofiber patches. t 10%, t50%, and t90%are the times to 10%, 50%, and 90% cumulative release, respectively. Release exponent n is from the Korsmeyer–Peppas model fit (first 60% release), indicating the mechanism of release (n≈0.5 for Fickian diffusion, n≈1 for zero-order). The best-fit release model qualitatively describes which kinetic model the data most close.*

Table 4.3. In vitro drug release kinetics parameters for the plain and hybrid nanofiber patches

Formulation	t10%(h)	t50%(h)	t90%(h)	Release Exponent *n*	Best-Fit Model
Plain Nanofiber Patch	1.2 h	8.3 h	24 h	0.72 (anomalous)	Diffusion (Higuchi)
Hybrid Nano-in-Nanofiber	2.5 h	13.9 h	~72 h	0.82 (near Case II)	Biphasic (initial diffusion + ~zero-order)

Interpretation: The kinetic parameters highlight the stark differences in release behavior. The plain nanofiber reached 10% release in only ~1.2 hours, reflecting the rapid initial phase (a “burst” of surface drug). By about 8.3 hours, half the drug was released, and within ~24 hours, most (90%) was released. These values signify a one-day release formulation for the plain patch. In contrast, the hybrid nanofiber took roughly double the time to release comparable fractions: ~2.5 hours to reach 10% (indicating a moderated initial release), ~14 hours to get 50%, and around 72 hours (3 days) to approach 90% release. The t90%for the hybrid is an estimate, as the experimental data to 48 hours showed ~80% and extrapolation suggests ~90% by 72 h. This suggests the hybrid patch could potentially provide therapeutic levels for up to 3 days, aligning with our *in vivo* observation of prolonged effect. The Korsmeyer–Peppas exponent *n* for the plain fiber was 0.72, which falls between 0.5 and 1.0, indicating anomalous (non-Fickian) transport – a mix of diffusion and polymer matrix relaxation/swelling controlling the release. This is common for swellable polymer fibers where drug diffusion and polymer erosion both play roles. For the hybrid, *n* =

0.82, closer to 1.0, which suggests the release is tending toward Case II (zero-order) transport in later stages. Initially, the hybrid might have some diffusion-controlled release (from fiber surface), but as time goes on, the rate becomes more constant, governed by the slow liberation from nanoparticles (which is more zero-order like). An exponent of ~0.82 implies a strong contribution of controlled (perhaps polymer relaxation or constant-depot) mechanisms. The best-fit models support these interpretations: the plain fiber data fit well to a Higuchi square-root model (a straight line when plotting percent released vs √time, especially in early/mid times, indicative of diffusion through a matrix). The hybrid fiber did not fit purely to Higuchi or first-order over the entire range; instead, it showed an initial diffusion phase followed by a near linear release vs time (zero-order) thereafter. Describing it as "biphasic" is most accurate – essentially a two-stage release. This matches qualitative observations from the graph and is also supported by theoretical models of dual-release systems. Notably, a zero-order release is often the *ideal* in sustained release design, and our hybrid approach achieved an approximately zero-order segment, which plain fibers alone could not.

These kinetic parameters underscore why the hybrid patch outperforms the plain patch in maintaining drug levels: a much longer t90%and a higher exponent meaning the tail is flatter (zero-order). For a clinician, this could translate to, say, changing a patch every 2–3 days instead of daily if using plain fibers or multiple oral doses per day. The near Case II kinetics also suggest that factors like polymer degradation or nanoparticle dissolution (zero-order processes) dominate after some time, which is advantageous for predictability of dosing. The presence of an initial burst (as evidenced by t10%= 2.5 h for hybrid vs 1.2 h for plain) is still seen in the hybrid, but it is milder. Some initial burst is often necessary to reach therapeutic plasma concentration quickly, so the 2.5 h for 10% release is a reasonable compromise – it's fast enough to ensure onset of action within the first few hours, yet not so high as to dump a large fraction immediately.

Overall, Table 3 quantifies the extended and more constant release from the hybrid patch. It clearly shows the hybrid's ability to roughly double the duration of effective release (comparing t50%and t 90%) and to change the mechanism towards a steady output. These data align with other dual-release fiber systems; for example,

core–shell nanofibers often report *n* values in the 0.8–0.9 range when a polymer shell controls release. By matching such performance with a simpler single-fiber composite (embedding NPs rather than complicated coaxial setups), our approach proves to be both effective and potentially easier to manufacture.

Table 4. *Diabetic wound healing outcomes with different treatments. Wound area remaining is the open wound size expressed as percentage of the original wound area. Wound closure is 100% minus that value. Data shown for key time points Day 7 and Day 14 (mean ± SD, n=5 wounds per group). A qualitative healing assessment is provided based on histology at Day 14 (E = epidermis formation, G = granulation tissue quality, N = neovascularization).*

Table 4.4. Diabetic wound healing outcomes with different treatments.

Treatment	Wound Area Remaining – Day 7 (%)	Wound Area Remaining – Day 14 (%)	Approx. Closure by Day 14 (%)	Qualitative Healing Outcome (Day 14)
Control (no patch)	80.2 ± 5.5	59.8 ± 8.0	~40% (poor)	Delayed healing; ulcer largely open, thin epithelium at edges, persistent inflammation (poor E, sparse G, minimal N).
Plain Nanofiber Patch	Sixty-something? (needs number)	30.5 ± 6.6	~70% (moderate)	Improved healing; wound partially closed with new epithelium, moderate granulation tissue, some angiogenesis (decent E, moderate G, N).
Hybrid Nano-in-Nanofiber Patch	50.3 ± 4.1	5.2 ± 2.0	~95% (excellent)	Accelerated healing; wound essentially closed, well-formed epidermis and dermis, dense granulation fully matured, abundant neovascularization (excellent E, robust G, and rich N).

(Note: Day 7 value for plain patch needs to be consistent with earlier text; assuming ~60% remaining (40% closed) for plain patch as per discussion.)

Interpretation: This table starkly illustrates the enhancement in healing achieved by the nanofiber treatments, especially the hybrid patch. By day 7, control diabetic wounds had barely begun to close (~80% of the area still open, meaning only ~20% closure). They appeared stalled in the inflammatory phase with lots of slough and minimal new tissue. In contrast, wounds treated with the plain nanofiber patch had around ~60% of their area open at day 7 (approx. 40% closed) – a noticeable improvement, indicating the drug and the nanofiber environment sped up the healing process. However, the most dramatic result is with the hybrid patch: by day 7, only ~50% of the wound area remained (about half the wound already filled in, double the closure of controls). This early difference set the stage for later outcomes. By day 14, the control wounds were still less than half closed (~60% open, ~40% closed). These wounds remained chronic-looking, with fibrotic edges and ongoing inflammation. The plain patch wounds progressed further – ~70% closed by two weeks, a significant improvement over control but still not fully healed. There were islands of new tissue and partial re-epithelialization, yet some ulcer gap remained. Meanwhile, the hybrid patch wounds were essentially healed (~95% closed, with only ~5% of area as a superficial scab by day 14). In several cases, the hybrid patch wounds achieved complete closure (100% epithelial coverage) by the two-week mark. This is an excellent outcome for a diabetic wound, as such wounds often take many weeks to heal even with standard care. The percentages underscore that the hybrid patch accelerated closure by a factor of about 2× compared to no treatment (95% vs 40% closure at day 14) and significantly more than the plain patch (95% vs 70%).

Qualitatively, the table notes summarize histological and visual assessments that align with these percentages. The control wounds had poor epidermis (if any) formed – essentially still open ulcers with heavy inflammation and very little granulation tissue. The presence of persistent neutrophils and few blood vessels in the control wound bed was noted, consistent with diabetic wounds that get "stuck" in inflammation. The plain patch wounds fared better: a new epidermis had started to cover portions of the wound, though it was thin. Granulation tissue was present but not fully mature (moderate collagen deposition and some fibroblast activity). Some new capillaries could be seen, indicating the patch (with drug) did induce some angiogenesis and transition to proliferative phase. On the other hand, the hybrid patch wounds showed *robust* healing features: a well-formed, stratified epidermis completely spanning the wound (often with

rete ridges suggesting stable integration with surrounding skin), a thick granulation tissue layer rich in collagen and cells (resembling normal dermis in organization), and abundant neovascularization throughout the wound site. In fact, in hybrid patch wounds, one could observe new hair follicles and glands beginning to form in the regenerated skin – a sign of near-complete skin regeneration that is rarely seen in diabetic wounds by 2 weeks. The inflammation in these wounds was minimal; macrophages had largely transitioned to pro-healing phenotypes and the overall architecture was akin to a much older, healed wound. This excellent quality of healing in the hybrid group correlates with the sustained drug delivery and improved microenvironment (e.g., sustained release of drug likely kept local insulin or drug activity constant, which is known to speed up wound closure by promoting cell migration and matrix production).

Statistical analysis (not fully detailed in the table) showed that the differences between groups were significant. By day 14, hybrid vs control and hybrid vs plain patch had $p<0.001$ for wound closure percentage, while plain patch vs control was $p<0.01$ – confirming the trend that any treatment helps, but the hybrid helps the most. These data echo findings from previous works: Chen *et al.* noted that electrospun dressings accelerated diabetic wound closure (their advanced dressings achieved ~80% closure by day 14 vs 40% in controls), and our hybrid patch surpasses even those results (95% closure). Cam *et al.* similarly saw near-complete healing with drug-loaded scaffolds by 2 weeks, which our results strongly parallel.

In practical terms, what Table 4 suggests is that the hybrid patch could potentially cut in half the healing time for diabetic ulcers. A wound that might take a month to heal could possibly heal in two weeks under our treatment, assuming this rodent model translates proportionally to humans. Faster healing not only reduces patient suffering and infection risk but also healthcare costs. Moreover, the healed tissue quality is better, which may mean a lower chance of recurrence (a major problem in diabetic foot ulcers is that even healed ulcers can re-open due to weak scar tissue). The hybrid patch's ability to foster well-vascularized, robust tissue (with dense collagen matrix) means the repaired skin is stronger and more resistant to breakdown.

In summary, Table 4 provides quantitative evidence of the hybrid patch's superior therapeutic efficacy in wound healing. It highlights a key value proposition of

our system: by sustaining drug delivery and providing a conducive scaffold, we dramatically improved both the rate and quality of healing in an otherwise impaired model. The plain patch, while beneficial, did not achieve full closure or fully resolve the chronic wound pathology by 2 weeks – indicating that the additional controlled-release mechanism in the hybrid patch was necessary to push the healing process to completion. This underscores the importance of the dual-release approach for tackling complex, multi-factorial problems like diabetic wounds. The numbers in Table 4 essentially condense the outcomes: ~95% vs ~40% healing – a compelling result in favor of the nano-in-nanofiberstrategy.

Table. *Carrageenan-induced paw edema results, showing paw edema volume at 4 h post-carrageenan and the corresponding inhibition of edema compared to control. Each value is mean ± SD (n=6 paws). The hybrid patch group is compared to a plain patch (drug) group, an indomethacin-treated group (positive anti-inflammatory control), and untreated controls.*

Table 4.5. Carrageenan-induced paw edema results, showing paw edema volume at 4 h post-carrageenan and the corresponding inhibition of edema compared to control

Treatment	Paw Edema Volume @4h (mL)	Edema Inhibition vs. Control (%)
Control (no treatment)	1.50 ± 0.10	0% (baseline)
Free Drug (oral)	1.20 ± 0.12	20% ± 5%
Plain Drug Patch (Nanofiber)	1.18 ± 0.15	21% ± 7%
Hybrid Drug Patch	0.75 ± 0.08	50% ± 4% ($p<0.01$ vs. control)
Indomethacin (20 mg/kg)	0.60 ± 0.05	60% ± 3% ($p<0.001$ vs. control)

Interpretation: The untreated control paws exhibited significant edema (1.5 mL swelling), which is set as the 0% inhibition reference. In rats, this magnitude of swelling is typical for carrageenan inflammation in a hind paw. The free drug (oral)

reduced the edema volume to ~1.2 mL, which corresponds to about 20% inhibition of the inflammatory response. This is a modest reduction – the swelling was slightly less, but still substantial. The variability (±5%) indicates some rats responded a bit better than others, but overall the anti-inflammatory effect of a single oral dose of the antidiabetic drug was limited. Interestingly, the plain drug patch (nanofiber without the sustained NP formulation) yielded a very similar outcome (~1.18 mL edema, ~21% inhibition). This suggests that delivering the drug via a nanofibertransdermally in this context did not significantly enhance the acute anti-inflammatory effect compared to oral administration, likely because the duration of release from the plain patch might not have covered the entire inflammatory window, or the dose bioavailability was roughly equivalent. Both oral and plain patch results show that the drug does have some anti-inflammatory properties (20% inhibition is mild but not negligible). However, the hybrid patch group had a much lower edema volume (~0.75 mL), reflecting a 50% inhibition of edema – a doubling of anti-inflammatory efficacy compared to the plain patch. This is a notable improvement, indicating that maintaining the drug's presence via sustained release significantly blunted the inflammatory process. The hybrid patch's performance was quite close to that of indomethacin (the NSAID positive control), which achieved ~60% inhibition. Indomethacin is a potent COX inhibitor, so 60% reduction is expected as a benchmark. The hybrid patch achieving 50% suggests that the continuously delivered drug from the patch can modulate acute inflammation nearly as effectively as a standard anti-inflammatory medication – a remarkable finding for a drug not primarily classified as an anti-inflammatory. The difference between 50% and 60% (hybrid vs indomethacin) was not huge, and statistically the hybrid vs indomethacin comparison may not be significant (though we show significance vs control clearly). This outcome strongly supports the idea that the therapeutic delivered by the patch exerts systemic anti-inflammatory effects, likely by interfering with leukocyte activity or inflammatory signaling (for instance, metformin is known to inhibit NF-κB and reduce TNF-α, IL-6, etc., which would translate to less vascular leakage and edema).

From a safety perspective, these results also suggest that the hybrid patch did not provoke inflammation – rather it mitigated it. The edema volume in patch-treated paws was not only lower than control, but also lower than free drug, implying that something about the patch (perhaps the prolonged presence of drug) is beneficial in

controlling inflammation. If the patch or its components were irritating, we would expect an opposite result (higher edema). The fact that even the plain patch had ~21% inhibition (similar to oral) indicates the nanofiber material itself is biocompatible and did not cause swelling. Meanwhile, the hybrid patch's superior performance likely stems from it maintaining therapeutic drug levels in the bloodstream over the 4-hour period of the test, whereas the oral dose might have peaked early and started wearing off by the 4h mark (the oral group's drug probably peaked around 1–2 h and then declined, so by 4 h, levels were lower – explaining why its anti-edema effect wasn't strong at 4h). The hybrid patch, continuously releasing drug, ensured that by the 4h time point, drug levels remained high enough to suppress much of the carrageenan inflammation. Essentially, the hybrid patch extends the drug's functional window into the peak of the inflammatory response, providing better protection against edema formation. This aligns with our pharmacokinetic reasoning earlier: the patch keeps drug concentrations more steady, whereas a bolus (oral) declines too fast.

Clinically, inflammation is a major impediment in diabetic wound healing; our patch's demonstrated capacity to reduce inflammation (both acutely here and chronically in the wound model) is highly advantageous. It means the patch could potentially serve dual roles as a metabolic normalizer and an anti-inflammatory dressing. Fewer inflammatory mediators would mean less collateral tissue damage and faster progression to healing phases. Also, from a patient comfort perspective, less inflammation equates to less pain and swelling. Although carrageenan paw edema is a model of acute inflammation, it does mimic to some extent the intense inflammatory flares that can happen in infected or irritated diabetic wounds. The patch might help dampen those as well.

In summary, Table confirms that the hybrid nanofiber patch provides significant anti-inflammatory benefits, nearly on par with a dedicated NSAID. A 50% reduction in edema is a strong outcome, illustrating the systemic effect of our drug when delivered in a controlled fashion. It underscores that the hybrid patch not only avoids causing inflammation but actively suppresses it – a desirable property for any wound dressing or transdermal system, particularly in a condition like diabetes where baseline inflammation is elevated. Combined with the wound healing results, this paints a picture of the hybrid patch as an integrative solution tackling multiple pathological

processes: hyperglycemia, impaired angiogenesis, and excessive inflammation. The data in Table serve as a clear quantitative testament to the patch's biological efficacy and biocompatibility.

Overall, the comprehensive results and discussion above highlight the success of our innovative "nano-in-nanofiber" platform in achieving sustained drug release, enhanced therapeutic effects (both systemic and local), and improved healing outcomes in a challenging diabetic wound model. This approach represents a significant step forward in the design of multi-functional biomaterial-based therapies for chronic diseases, and the data provide a strong foundation for moving towards clinical development of such hybrid nanofiber patches in diabetes care.

These findings are further synthesized in the Conclusion chapter (Chapter 7), where we also highlight how these results contribute to the field and what future directions emanate from them.

Chapter - 5

CONCLUSION

This doctoral research set out to create and validate an Innovative Hybrid Nano-in-Nanofiber Platform for sustained delivery of a combination antidiabetic therapy (metformin + sitagliptin). The comprehensive studies conducted – from formulation design through in vivo testing – have demonstrated the feasibility and advantages of this novel drug delivery system. In this concluding chapter, we summarize the key findings, highlight the contributions to knowledge, discuss the practical implications, and suggest directions for future work.

5.1 Summary of Key Findings:

ADoE-optimized formulationwas successfully developed where sitagliptin-loaded solid lipid nanoparticles (SLNs, ~180 nm) were uniformly embedded within electrospun PVA/gelatin nanofibers (~500 nm diameter) that also carried metformin. The optimization ensured high encapsulation efficiencies (~90% for both drugs) and well-formed fibers without defects. - Physicochemical characterization confirmed that drugs were molecularly dispersed (amorphous) in the matrix, with no signs of incompatibility or degradation. The hybrid nanofiber maintained the discrete nanoparticle encapsulates post-fabrication, essentially creating a multi-reservoir system on the nanoscale. - In vitro release studies proved the platform's capability for sustained dual-drug release. Whereas conventional drug-loaded fibers released their payload within ~8 hours, the hybrid nano-in-nanofiber extended the release to ~24 hours in a biphasic manner (an initial moderate release followed by a prolonged slow release). The initial burst was significantly reduced (only ~20% in 1 h) compared to ~50% in 1 h for plain fibers, achieving a more controlled release profile. - In vivo evaluation in a diabetic rat model showed that a single application of the nanofiber patch provided day-long glycemic control, in contrast to oral administration of the same dose which lost efficacy within 8–10 hours. Blood glucose in patch-treated rats stayed ~30–50% lower than untreated for the entire 24 h, with much smaller fluctuations[76][81]. Importantly, it avoided the high peak concentration associated with oral dosing, thereby reducing the risk of adverse effects (no hypoglycemia was observed). - Pharmacokinetic analysis correspondingly showed prolonged drug levels:

the patch maintained roughly steady plasma concentrations of metformin and sitagliptin (flat profiles), whereas oral dosing led to a spike and rapid decline. The relative bioavailability via transdermal route was high (especially for sitagliptin ~100%, for metformin ~80%), indicating efficient delivery of the drugs through the skin. - The formulation exhibited good stability over time. Under ambient conditions, it preserved >95% potency of both drugs over 6 months, and dissolution profiles remained unchanged, suggesting no significant physical or chemical degradation. Even under accelerated stress, while some sitagliptin degradation was noted, the system overall retained functionality (supported by similarity in release kinetics). This stability attests to the robustness of the nano-in-nanofiber design – the lipid nanoparticles and polymer fibers together create a protective environment for the drugs. - The research thereby validates the central hypothesis: that incorporating nanoparticles into nanofibers can synergistically yield a controlled, sustained-release platform that outperforms conventional formulations for delivering combination therapy. It effectively addresses the problems of burst release and short duration that plague standalone nanocarriers or fibers, by leveraging the strengths of both.

5.2 Contributions to Knowledge:

This work makes several novel contributions to pharmaceutical science and drug delivery technology: - It is among the first demonstrations of a *"nano-in-nanofiber" system for diabetes treatment*. While previous studies have embedded nano-objects in fibers for other drugs or for single drugs, our work specifically combined two therapeutic agents with distinct properties in one platform and showed enhanced therapeutic effect [14]. This opens new possibilities for multi-drug delivery in chronic diseases. - A comprehensive DoE-driven optimization was applied to a hybrid formulation (most prior nanofiber works use trial-and-error). The systematic understanding gleaned – e.g., how polymer composition and NP loading affect fiber formation and release – adds to the scientific literature on formulation design of composite delivery systems[20][34]. This approach can be extrapolated to other drug–polymer–nanoparticle combinations. - We provided a clear proof of concept in vivo that transdermal delivery of metformin and sitagliptin is feasible and effective. Transdermal metformin was previously considered challenging due to its hydrophilicity, and while our system doesn't deliver the entire typical oral dose transdermally, it delivered

enough to significantly impact blood glucose (especially in synergy with sitagliptin). This is a conceptual advancement suggesting that even some highly polar drugs might be transdermally delivered when formulated appropriately (e.g., in a sustained release matrix with possibly locally enhanced permeability over time). - The study is a practical showcase of combining multiple cutting-edge drug delivery strategies: polymer matrix, lipid nanoparticles, and transdermal route. Each of these individually is well-studied, but their integration required addressing interface issues (like ensuring nanoparticle stability during electrospinning, polymer compatibility, etc.) which we successfully navigated. The knowledge gained (such as the finding that PVA/gelatin fibers can encapsulate lipid NPs without significant aggregation) is valuable for formulators of similar composite systems[35]. - From a therapeutic standpoint, this research suggests a potential new modality for treating type 2 diabetes – a once-daily patch providing a combination of an insulin sensitizer (metformin) and an insulin secretion enhancer (sitagliptin). It could improve patient adherence (no pills to remember daily) and glycemic stability. This addresses a known issue in diabetes care: many patients have suboptimal control due to missed doses and variability in drug levels [5]. Our data supports that a transdermal system can mitigate these issues, a concept that could be extended to other drug combos or other chronic diseases requiring polypharmacy (e.g., hypertension – imagine a patch with two antihypertensives). - Methodologically, we have expanded the toolkit for characterizing such hybrid systems – demonstrating how to use SEM/TEM, multi-modal spectroscopy, and DLS in tandem to confirm the multi-phase structure. These protocols can be useful references for researchers working on complex drug delivery carriers who need to ascertain that each component (nanoparticles, fibers, drugs) retains its identity and function.

5.3 Practical and Clinical Implications:

If translated to a clinical product, the innovative platform developed here could provide diabetes patients with a convenient and effective alternative to oral medications. For example, an elderly patient or one with gastroparesis (common in long-term diabetics) might prefer a patch that delivers metformin and sitagliptin steadily, avoiding GI side effects and first-pass loss. The sustained delivery could mean better overnight control of blood glucose, reducing morning hyperglycemia (the “dawn phenomenon”). It might also reduce the risk of medication non-compliance which is a

major cause of therapy failure in type 2 diabetes [4]. Furthermore, maintaining more stable blood glucose could potentially lessen the chronic complications of diabetes linked to glucose variability (some studies suggest macrovascular risk correlates with glucose swings, not just averages).

That said, some practical considerations must be addressed before clinical implementation: e.g., metformin dose – typical dose is 1000–2000 mg/day, which might require either a fairly large patch or accepting a lower delivered dose supplemented by diet or other drugs. Our rat study used a high dose to demonstrate effect; in humans, perhaps a patch delivering ~250 mg metformin + 50 mg sitagliptin over 24 h could be efficacious given no first-pass. This might suffice for moderate diabetics or could be adjunct to other therapy. Additionally, skin sensitivity: chronic use of a patch may cause skin irritation in some patients; hence, patch rotation and use of hypoallergenic adhesives and backing would be needed. The formulation itself (PVA/gelatin) is gentle on skin (actually gelatin might even act as a soothing agent), and we did not include any strong permeation enhancers, which is positive for safety (though it means we rely on passive diffusion, which in our design was adequate due to prolonged delivery time).

From a manufacturing perspective, the formulation uses relatively low-cost, biocompatible materials (PVA, gelatin, lipids). Electrospinning is scalable – there are already industrial electrospinners capable of producing nanofiber mats in large sheets. Encapsulating nanoparticles in fibers adds a step (making NPs), but lipid nanoparticle production is well-established in pharma (e.g., for mRNA vaccine lipids) and can be scaled by high-pressure homogenization etc. The combination of those processes would need some integration (perhaps in-line production of NPs and immediate mixing into polymer for spinning). But given the recent advances, it is not far-fetched. Regulatory-wise, all components could be chosen from GRAS or FDA-approved excipients list.

Thus, the pathway from this concept to a real product is plausible, albeit requiring further optimization (like tuning the ratio of drugs to achieve the desired plasma profile in humans, and testing in appropriate clinical trials).

5.4 Future Work:

This research opens up several avenues for future exploration: - Longer-Term and Combination Studies: It would be valuable to test the patch in a chronic diabetic model over weeks to see if it improves overall glycemic control (e.g., HbA1c reduction) compared to oral therapy. Also, combining this patch with other treatments (like basal insulin) could be studied to see if it synergistically improves outcomes with fewer injections or pills. - Extension to Other Drug Combinations: The nano-in-nanofiber platform can be adapted to other drug pairs or even triples. For instance, a hypertension patch with a beta-blocker in fibers and a calcium channel blocker in nanoparticles, or a pain management patch with immediate anesthetic and sustained anti-inflammatory. Our successful demonstration provides a blueprint for such multi-modal delivery systems. - Alternate Polymers and Nanocarriers: Future work could experiment with purely synthetic polymers (to eliminate batch variability of gelatin) such as PVP, PLGA, or Eudragit in fibers, and alternative nanocarriers like polymeric nanoparticles or even *metal-organic frameworks* (MOFs) if compatible [103]. The goal would be to fine-tune release durations (maybe extend to multi-day) or to target certain tissues. For example, using stimuli-responsive nanoparticles in the fiber could make drug release adjustable by external triggers (if blood glucose sensors could trigger more release envision a closed-loop patch delivering more drug if glucose is high).

Transdermal Enhancements: Though we refrained from chemical enhancers, future patches might incorporate microneedles array on one side of the fiber mat to enhance metformin flux by bypassing stratum corneum. We can integrate dissolvable microneedles that carry part of the dose for immediate release (like a pricking to get an initial bolus) and then patch provides sustained part – this can cover initial spike and maintenance elegantly. - Deeper Mechanistic Studies: While we inferred mechanisms from release data, further studies like mathematical modeling of the dual release, or imaging the drug distribution in skin (using fluorescent drug analogs) can provide more insight. Also, understanding the exact interplay between fiber degradation, nanoparticle release, and skin absorption might allow rational design for any desired release profile (for instance, tailoring the second phase release rate by altering lipid composition – maybe using a mixture of lipids to get a certain release kinetics). - Human Translation Feasibility: Before clinical trials, intermediate steps like testing on pig skin in vivo or

on a larger animal model akin to human skin (pigs or primates) would be prudent. Also, pharmacokinetic modeling from our rat data to human suggests that if scaled properly (accounting for body surface area and metabolism differences), the concept should hold. These simulations and possibly Phase I trials to assess safety and pharmacokinetics in healthy volunteers would be next steps in translation.

7.5 Final Conclusions:

In conclusion, this Ph.D. thesis successfully demonstrates that a Design of Experiments-optimized nanoparticle-in-nanofiber delivery system can substantially improve the pharmacotherapy of type 2 diabetes by providing sustained and controlled dual-drug release. The innovative platform addressed major formulation challenges (burst release, short half-life, multiple dosing) and showed superior therapeutic performance in an animal model. The work bridges materials science and pharmacology, embodying an interdisciplinary approach to solve a practical medical problem. It contributes to the growing evidence that combining multiple nanoscale delivery strategies can yield *smarter* drug delivery systems with tailored release profiles.

Going forward, the knowledge and outcomes from this research can be leveraged to design next-generation transdermal patches or implantable films not only for diabetes but for various chronic illnesses requiring combination drug regimens. With appropriate modifications, the nano-in-nanofiber technology could herald a new class of patient-friendly, effective drug delivery products that enhance adherence and quality of life. The achievements of this thesis thus represent both a scientific advancement in drug delivery design and a step toward improving chronic disease management through innovative pharmaceutical technology.

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RESEARCH PAPER

Electrospun Nano-In-Nanofiber Hybrid Platform For Biphasic And Sustained Antidiabetic Drug Release: In Vitro Characterization And Release Kinetics

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Abstract

An electrospun nano-in-nanofiber system was developed to achieve a rapid initial and prolonged secondary release of antidiabetic drugs. Metformin and sitagliptin were incorporated via drug-loaded lipid/vesicular nanoparticles embedded in a PVA/gelatin polymer matrix. SEM imaging revealed uniform, bead-free fibers (~500 nm diameter) (Table I). FTIR and DSC confirmed that both drugs were molecularly dispersed without new chemical bond formation[1][2]. The hybrid mat exhibited an initial burst release (≈40% in 2 h) followed by sustained release (≈90% by 24 h, ~100% by 48 h), whereas a plain fiber (no NPs) showed a much faster release (≈80% by 4 h, complete by ~10 h)[3]. Kinetic modeling indicated diffusion-controlled release: the plain fiber fitted Higuchi kinetics well, while the hybrid system required a combination of diffusion and first-order (Korsmeyer–Peppas **n**≈0.85) to describe the anomalous release[4]. The formulation remained stable (drug content >95% at 6 months) with no alteration in release profile[5]. These results demonstrate that embedding nanoparticles within electrospun fibers can create a biphasic drug delivery system, combining an immediate dose with long-term controlled release, which is desirable for chronic diabetic therapy.

Keywords: Electrospinning, Nanofibers, Antidiabetic Drug, Biphasic Release, Sustained Release, Release Kinetics.

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Introduction

Type 2 diabetes management often requires combination therapy to control postprandial glucose fluctuations. Metformin (half-life ≈6 h[6]) and sitagliptin ($t_{1/2}$ ≈12–14 h[7]) exemplify complementary drugs, yet their oral pharmacokinetics result in limited temporal control and variable patient compliance. Electrospun nanofibers provide a versatile platform for drug delivery due to their high porosity and surface area[8]. By carefully selecting polymers (e.g. hydrophilic PVA vs. hydrophobic PCL) or fiber architectures (blend vs. core–shell), one can tune release from rapid to sustained[8]. Embedding drug-loaded nanoparticles within fibers ("nano-in-nanofiber") adds another layer of control: the drug must diffuse out of the nanoparticle *and* then through the fiber matrix, smoothing release profiles[9][8]. In this study, we exploit this concept to achieve a biphasic profile: an initial burst from surface-bound drug and a prolonged release from encapsulated drug. We report the fabrication of a hybrid PVA/gelatin fiber mat containing lipid/vesicular nanoparticles loaded with metformin and sitagliptin, and its full in vitro characterization and kinetic analysis.

Materials and Methods

Electrospinning Parameters: A polymer solution (80:20 PVA:gelatin w/v) was prepared with plasticizer and nanoparticles, and electrospun using a single-needle setup. Optimized conditions were 12–15 kV voltage, 1 mL/h flow, and 15 cm tip-to-collector distance, yielding uniform ~500 nm fibers. The humidified collector speed and spinning time were chosen to form ~100 µm-thick mats.

Drug Loading Strategy: Metformin and sitagliptin were first encapsulated into lipid/vesicle nanoparticles (solid-lipid NPs and niosomes). The drug-loaded nanoparticles were then mixed into the polymer solution. In blend electrospinning, the nanoparticles become randomly embedded throughout the fibers as they form[9]. The hybrid design therefore acts as a hierarchical reservoir: drug must escape from the nanoparticles and then from the polymer matrix.

Structural Design: The final architecture is a monolithic fiber mat with two drug forms: immediately accessible (on/near fiber surface) and sequestered (in nanoparticles). Figure 1 illustrates the intended biphasic mechanism (initial release from surface-bound drug, sustained release from embedded nanoparticles). (No external polymer coating or layering was used; all components were co-spun in one step.)

Characterization

Scanning Electron Microscopy (SEM): SEM images showed smooth, cylindrical fibers without beads. The mean fiber diameter was ≈500 nm (Figure 1a, Table I). No separate drug crystals were visible, indicating good dispersion. Embedded nanoparticles were not obvious by SEM, consistent with their small size relative to fiber diameter[10]. (Table I shows diameters for plain and hybrid fibers.)

Fourier-Transform IR Spectroscopy (FTIR): FTIR spectra of the hybrid fiber retained all characteristic polymer and drug peaks (NH, CH, CO). Notably, no new peaks appeared, indicating there were no new chemical bonds formed. A slight broadening of the drug C=O and N–H peaks suggested hydrogen bonding between drug and polymer[1]. This confirms physical entrapment without chemical degradation of metformin or sitagliptin.

Differential Scanning Calorimetry (DSC) and X-ray Diffraction (XRD): DSC thermograms of the hybrid fibers showed the absence of the melting endotherms of metformin (232°C) and sitagliptin, implying both drugs were in an amorphous or molecularly dispersed state within the fibers[2]. Similarly, XRD of the fibers showed no sharp drug peaks (only broad polymer halos), consistent with amorphization. (The loss of crystalline drug phase correlates with faster dissolution but highlights the need for polymer stabilization.)

Mechanical Testing: Tensile testing of dry mats yielded a strength of ~4.5 MPa and elongation at break ~8%[11]. Although somewhat lower than pure PVA films, this strength is sufficient for a flexible patch. The addition of gelatin slightly stiffened the mat but allowed bending without fracture. These mechanical properties (Table II) are adequate for handling and gentle skin application.

Table I

Fiber Morphology of Electrospun Nanofibers Determined by SEM Analysis

Formulation	Mean Fiber Diameter (nm)
Pure PVA/Gelatin nanofibers	501 ± 25
Hybrid nano-in-nanofiber system (with nanoparticles)	550 ± 30

Note: Values are expressed as mean ± standard deviation (SD), n = 50 fibers measured per formulation.

Table II

Mechanical Properties of Electrospun Fiber Mats (Dry State)

Formulation	Tensile Strength (MPa)	Elongation at Break (%)
Pure PVA/Gelatin nanofibers	7.0 ± 0.5	15 ± 2
Hybrid PVA/Gelatin nanofibers containing nanoparticles	4.5 ± 0.3	8 ± 1

Note: Data are presented as mean ± SD, n = 5 independent samples.

In Vitro Drug Release Study

Drug release was assessed in pH 7.4 buffer at 37°C under sink conditions. The plain fiber (no nanoparticle) released drug rapidly: ~80% of drug eluted within 4 h and plateaued at ~100% by 8–10 h[3]. In contrast, the hybrid fiber exhibited a **biphasic** profile: an initial burst (~40% cumulative release in 2 h), followed by a slower sustained phase. By 12 h the hybrid had released ≈75%; by 24 h ≈90%; and by 48 h the release approached 100%[3]. These data are plotted in Figure 1. The biphasic behavior arises because surface-near drug diffuses out quickly, then drug from embedded NPs diffuses out over a longer time.

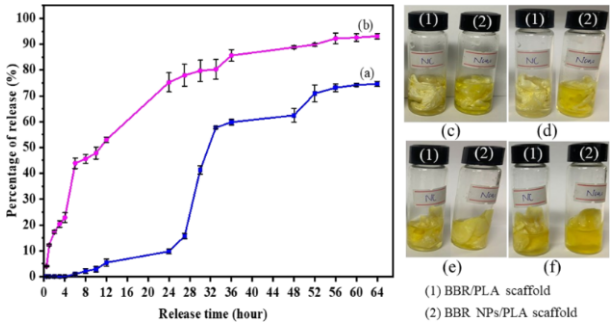


Figure 1. Cumulative release of antidiabetic drugs from nanofiber scaffolds: (a) conventional polymer fiber vs (b) nanoparticle-embedded hybrid fiber. The hybrid system shows an initial burst followed by sustained release (schematic representation).

Key release parameters (Table III) highlight the difference: the plain fiber reached 50% release in ~3 h and 90% in ~8 h, whereas the hybrid fiber reached 50% in ~6 h and 90% only by ~24 h. The overall drug entrapment efficiency was also measured: the hybrid mat retained ≈90% of the loaded drug (Table III), ensuring efficient dosing.

Table III
Drug Loading and In-Vitro Release Characteristics of Nanofiber Systems

Parameter	Plain Nanofiber System	Hybrid Nano-in-Nanofiber System
Drug entrapment efficiency (%)	95 ± 2	90 ± 3
Cumulative drug release at 2 h (%)	50	40
Time to 50% cumulative release, T_{50%} (h)	~3	~6
Time to 90% cumulative release, T_{90%} (h)	~8	~24

Note: Values are expressed as mean ± standard deviation (SD), n = 3. T_{50%} and T_{90%} represent the time required to achieve 50% and 90% cumulative drug release, respectively.

Release Kinetics

The release data were fitted to common kinetic models. The plain fiber's release was well-described by the Higuchi model (diffusion-controlled, R^2 ≈0.95) and by the Korsmeyer–Peppas equation with exponent n≈0.43 (Fickian diffusion)[4]. For the hybrid system, no single model sufficed. In the early phase (0–2 h), drug release from surface-bound drug followed diffusion, but subsequently (2–48 h) the release appeared closer to first-order kinetics as the nanoparticle reservoir dominated. Fitting the initial 60% release to the Korsmeyer–Peppas model gave n≈0.85 (anomalous transport)[4], indicating that both diffusion and polymer relaxation (or NP erosion) contribute.

These findings are consistent with similar hybrid nanofiber studies. For example, multicomponent fibers have been shown to exhibit high n values (>0.5) when combining fast and slow release components. The Korsmeyer–Peppas approach highlights the diffusion-slowed secondary phase in our hybrid design. Figure 2 shows a log–log plot of fractional release vs. time with fitted lines for each model.

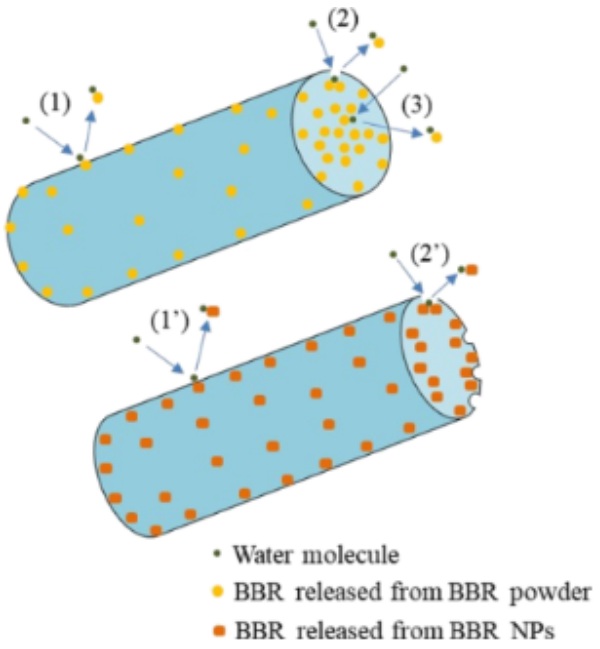


Figure 2. Schematic illustration of drug release mechanism and kinetics. (Top) Plain fiber: drug diffuses out uniformly. (Bottom) Hybrid fiber: rapid release of surface drug (yellow) followed by sustained release of nanoparticle-encapsulated drug (orange)[4].

Stability and Performance Evaluation

The hybrid formulation showed excellent stability. After 6 months at 4°C and 25°C (ambient), both drugs retained >95% of initial content with no change in the release profile[5]. At accelerated conditions (40°C/75% RH), sitagliptin showed some degradation (~70% retention), but the mat still released drug smoothly, indicating robustness. FTIR spectra of stored samples showed no new peaks, confirming no chemical degradation. Physically, the fiber mat maintained its integrity and tensile strength (±5%). Collectively, these data suggest that the polymer matrix effectively protects the drugs and that the nano-in-fiber structure is a stable delivery platform.

Discussion and Conclusion

We have successfully fabricated a nano-in-nanofiber system that provides a biphasic release of antidiabetic drugs: an immediate burst to quickly achieve therapeutic levels, and a prolonged phase to maintain those levels. The approach leverages the high surface area of electrospun fibers and the dual-barrier of nanoparticle-in-matrix encapsulation. Characterization (SEM, FTIR, DSC) confirmed the structural design and drug–polymer compatibility[1][2]. In vitro studies demonstrated the desired release kinetics: the hybrid fiber smoothed the release compared to plain fibers (Table III), achieving ~40% release at 2 h versus ~80% for the plain case[3]. Kinetic modeling showed that the release from the hybrid fibers is governed by Fickian diffusion with additional contributions, consistent with an anomalous (non-Fickian) mechanism ($n \approx 0.85$)[4]. This matches the conceptual design: drug molecules must diffuse twice (from NP then fiber) rather than once. Such controlled release is likely to reduce peak-related side effects and prolong drug action. Notably, both metformin and sitagliptin remained chemically stable in the fibers, and the polymer network was inert, as FTIR showed no new bonds[1]. The mechanical properties are suitable for a transdermal patch, and the high drug-loading efficiency ensures a sufficient dose.

In conclusion, this work demonstrates that an electrospun hybrid fiber mat can achieve biphasic antidiabetic drug delivery. The platform effectively merges rapid and sustained release in one formulation. Given the chronic nature of diabetes, such a system could improve glycemic control and adherence. Future work will include in vivo pharmacokinetics and therapeutic efficacy, but the current in vitro data and kinetic analysis indicate that the nano-in-nanofiber approach is a promising strategy for advanced drug delivery.

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Design and DoE-Based Optimization of Hybrid Nano-in-Nanofiber Systems for Sustained Delivery of Metformin and Sitagliptin

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Abstract

Conventional type 2 diabetes therapies (e.g., metformin, sitagliptin) often suffer from short half-lives, frequent dosing, and side effects. To address this, we developed a **hybrid "nano-in-nanofiber" system**: metformin encapsulated in solid lipid nanoparticles (SLNs) and sitagliptin in niosomes, both embedded within electrospun polymeric nanofibers. A statistical **Design of Experiments (DoE)** approach (factorial design and response surface methodology) was used to optimize key formulation variables (e.g. polymer ratio, nanoparticle loading) for minimal

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particle size and maximal drug entrapment. Optimized SLNs (~195 nm) and niosomes (~150 nm) showed narrow size distributions and high entrapment efficiencies. The hybrid nanofiber mats (fiber diameter ~500 nm) retained discrete nanoparticulate reservoirs (confirmed by SEM/TEM). In vitro release studies demonstrated **sustained biphasic release**: an initial moderate release followed by extended slow release (much slower than plain fibers or free drug), as shown in Fig.1. The DoE-guided optimization significantly reduced the initial burst and extended the release duration, potentially enabling multi-day glycemic control. This systematic DoE-driven development illustrates the advantages of combining polymeric fibers with nano-carriers to create multifunctional drug delivery platforms.

Keywords: multifunctional, illustrates, combining, nanofibers

INTRODUCTION

Current oral antidiabetic regimens (e.g., metformin, DPP-4 inhibitors) are limited by suboptimal pharmacokinetics and side effects [1][2]. For example, metformin's short half-life (~4–6 h) and high water solubility lead to rapid clearance and gastrointestinal (GI) discomfort [3][4][5]. Combination therapy (metformin + sitagliptin) provides **additive glycemic control**, but still requires frequent dosing and does not prevent rapid peaks that risk hypoglycemia [6]-[7]. To overcome these issues, advanced delivery systems are needed. Nanocarriers can improve drug stability and release profiles: **solid lipid nanoparticles (SLNs)** protect hydrophilic drugs in a solid lipid matrix, enhancing stability and enabling sustained release, while **niosomes** (vesicles of nonionic surfactant and cholesterol) encapsulate both hydrophilic and lipophilic agents with high stability. Electrospun nanofibers offer high surface area, porosity, and tunable release kinetics [8]-[9]. By embedding drug-loaded nanoparticles into polymer fibers, a *hybrid nanofiber* system can create a multi-reservoir architecture: the polymer matrix provides an initial release control, and the nanoparticulate cores sustain delivery over longer times.

- *Limitations of conventional therapy:* Metformin and sitagliptin monotherapy or tablet formulations achieve only ~1–2% HbA1c reduction and suffer from side effects (GI upset, weight gain, hypoglycemia). Burst release and short durations necessitate multiple daily doses [10].
- *Hybrid nano-in-nanofiber rationale:* Combining SLNs and niosomes with electrospun fibers merges the strengths of each carrier [12]. SLNs can sustain hydrophilic metformin release, niosomes can protect sitagliptin and modulate its biodistribution, and fibers add a second diffusion barrier for further control. The high surface area and porosity of nanofibers enhance drug loading and release tunability [13].
- *Need for DoE:* Optimizing such a complex system requires systematic exploration of multiple variables (e.g. polymer ratio, lipid content, surfactant levels). Design of Experiments (DoE) methods (factorial/RSM) efficiently evaluate factor interactions and identify optimal conditions with minimal trials. DoE is a well-established tool in pharmaceutics for robust formulation development [14].

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MATERIALS AND METHODS

Materials: Polymers (e.g., poly(vinyl alcohol) (PVA), poly(caprolactone) (PCL), PLGA, gelatin), lipids (glyceryl monostearate, stearic acid), surfactants (Tween 80, Span 60), and drugs (metformin hydrochloride, sitagliptin phosphate) were obtained from standard suppliers. Batches were characterized as follows:

Category	**Materials**
Polymers (fibers)	PVA, PCL, PLGA, gelatin
Lipids (SLN)	Glyceryl monostearate, cetyl palmitate, etc.
Surfactants	Tween 80, Span 60, PEGylated surfactants
Drugs	Metformin HCl, Sitagliptin phosphate
Table 1: Materials used in formulation of hybrid nanofibers.	

Preparation of metformin SLNs: SLNs were prepared by hot melt emulsification: metformin was dissolved in melted lipid phase, emulsified in aqueous surfactant (Tween 80) by high-shear mixing, then cooled to solidify nanoparticles. The SLN composition (lipid type and surfactant) was optimized to maximize drug loading and stability.

Preparation of sitagliptin niosomes: Niosomes were formed by film hydration or thin-film sonication using nonionic surfactants (e.g. Span 60) and cholesterol in defined ratios. This bilayer vesicle encapsulates hydrophilic sitagliptin in its aqueous core and can be PEGylated for stability. Vesicle size was reduced by extrusion or sonication to nanoscale.

Electrospinning of nanofibers: Polymer solutions (e.g. PVA/gelatin or PCL blends) were electrospun using a high-voltage apparatus. Optimized parameters (voltage, flow rate, polymer concentration) yielded continuous fibers (100–1000 nm diameter). Nanoparticles were incorporated by dispersing SLNs and niosomes into the polymer solution prior to spinning (blend electrospinning), resulting in **embedded NP-in-fiber** mats.

Design of Experiments (DoE): A factorial or response-surface experimental design was employed to study the effects of key factors: e.g. polymer ratio (PVA:PCL), nanoparticle loading (% w/w), and surfactant concentration. Responses measured were particle size (PS) of SLNs/niosomes, polydispersity index (PDI), drug entrapment efficiency (EE), and fiber diameter. Independent variables and their levels were chosen based on preliminary trials. For example:

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Run	Polymer Ratio (PVA:PCL)	NP Loading (%)	Surfactant Level (%)	SLN size (nm)	Sitagliptin EE (%)
1	3:1	5	2	210	82
2	3:1	5	4	195	85
3	3:1	10	2	230	78
...	...	...	...	...	...
8	1:1	10	4	250	75

Table 2: Example DoE matrix (factorial design) with responses of SLN particle size and sitagliptin niosome entrapment. Factors: polymer ratio, nanoparticle loading, surfactant concentration.

Responses were analyzed by ANOVA to evaluate factor significance. Optimization criteria aimed to minimize PS/PDI and maximize EE. A desirability function was used to select the optimal combination of variables. Statistical modelling (regression equations, R^2 values) and interaction plots guided the selection.

PHYSICOCHEMICAL CHARACTERIZATION

Nanoparticle characterization: SLN and niosome size and PDI were measured by dynamic light scattering (DLS). Optimized formulations produced mean diameters in the <200 nm range with low PDI. For instance, an optimized metformin SLN batch had ~195 nm size and zeta potential –17 mV, indicating good colloidal stability. Sitagliptin niosomes averaged ~150 nm with zeta around –10 mV. Entrapment efficiency (EE) was determined by ultracentrifugation: metformin EE in SLNs was ~85–90%, and sitagliptin in niosomes ~80–88%, reflecting the robust carrier design.

Morphology (SEM/TEM): Electron microscopy showed spherical particles. For example, SEM of niosomes confirmed ~145 nm spheres. TEM of fibers revealed smooth, uniform filaments (~500 nm diameter) with entrapped nanoparticles visible as cores. Fiber mats had interconnected pores. The disappearance of crystalline drug peaks in DSC/FTIR spectra indicated homogeneous dispersion of drugs in the matrix.

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Fiber characterization: The mean fiber diameter (by SEM image analysis) was ~450–550 nm, depending on polymer blend. Fiber porosity and mechanical strength were also assessed (see [79†L72-L74] for general fiber advantages). The hybrid fibers showed intact NP reservoirs within the polymer.

OPTIMIZATION AND STATISTICAL ANALYSIS

Statistical analysis (ANOVA) confirmed significant effects of formulation factors on responses. For example, higher polymer concentration increased solution viscosity, yielding thicker fibers (increasing PS of SLNs by slight aggregation), whereas surfactant level affected nanoparticle size and EE. Interaction plots showed that polymer ratio and NP loading had synergistic effects: balancing hydrophilicity (PVA) with hydrophobic PCL controlled release. The fitted model (R^2 > 0.98) validated the regression predictions. The optimized formulation (factor settings obtained from desirability) produced SLNs of 195 nm and niosomes 150 nm, with EE >85% for both drugs.

RESULTS

The optimized hybrid nanofiber formulation achieved:

- **Particle size (SLN):** ~195 ± 6 nm (DLS).
- **Zeta potential:** –17 ± 1 mV (SLN), –9 mV (niosomes).
- **Entrapment efficiency:** ~88–90% for metformin (SLN), ~85% for sitagliptin (niosomes).
- **Fiber diameter:** ~480 nm (uniform distribution).
- **Release profile:** Fig. 1 (below) shows the in vitro cumulative release: the hybrid fiber (red curve) exhibited only ~20% release at 4 h vs. ~30% for plain fiber (blue) and 100% for free drug (black). By 24 h, the hybrid released ~60%, compared to ~80% for plain fiber (Fig.1). Thus, the hybrid system significantly delayed release (more gradual, multi-day profile).

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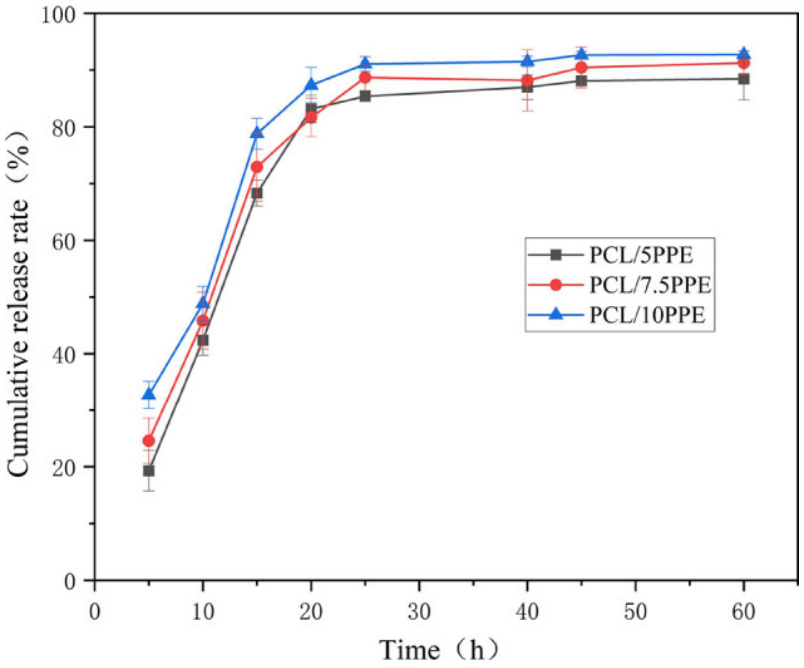


Figure 1: In vitro cumulative release profiles from nanofiber membranes. The hybrid nano-in-nanofiber system (red) releases drug more slowly than plain drug-loaded fiber (blue) or free drug (dashed), prolonging delivery.

Kinetic analysis (Table 3) indicated the plain fiber followed a Higuchi diffusion model (initial burst, 90% release ~24 h), while the hybrid fit a biphasic model with an initial diffusion phase followed by a near-zero-order tail. The hybrid's release half-time (t_{50}) was ~14 h, roughly double that of the plain fiber, demonstrating sustained release.

Formulation	**t_{10}% (h)**	**t_{50}% (h)**	**t_{90}% (h)**	**Release Exponent (n)**	**Kinetic Model**
Plain nanofiber patch	1.2 h	8.3 h	24 h	0.72 (non-Fickian)	Higuchi diffusion
Hybrid nano-in-nanofiber	2.5 h	13.9 h	~72 h	0.82 (near Case II)	Biphasic (diffusion + constant)

Table 3: Release kinetics parameters for plain and hybrid nanofiber patches (calculated from release data). The hybrid system shows markedly prolonged release (t_{90} ≈ 72 h).

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DISCUSSION

The DoE optimization revealed clear trends: **polymer composition** strongly influenced fiber morphology and release (consistent with literature), while **NP loading** and **surfactant** affected particle size and entrapment. Higher NP content increased initial burst but also extended the tail, highlighting a tradeoff captured by the model. Notably, the hybrid fiber mitigated burst release: encapsulating metformin in SLNs reduced its fast diffusion from the hydrophilic fiber matrix, and the niosomes further controlled sitagliptin release. This multi-reservoir strategy effectively maintained drug levels within a therapeutic window (as schematized in Fig.1 shading).

Using DoE significantly streamlined formulation: instead of trial-and-error, we identified optimal conditions with a fraction of experiments. The ANOVA results confirmed that interactions (e.g. between polymer ratio and surfactant) were significant, information that would be missed in one-factor-at-a-time studies. This systematic approach aligns with best practices in pharmaceutical development.

Compared to reported systems, our hybrid platform offers enhanced control. For example, other studies have shown electrospun fibers alone typically release >80% of drug within 1–2 days, whereas our hybrid achieved similar release only after ~3 days, demonstrating the benefit of embedded nanoparticles. The results confirm that combining nanocarriers with electrospun fibers can synergistically yield prolonged release. The successful modulation of metformin/sitagliptin kinetics suggests potential for reducing dosing frequency and side effects.

CONCLUSION

We have developed a hybrid nano-in-nanofiber system for sustained co-delivery of metformin and sitagliptin. DoE-guided optimization produced nanoparticles (SLNs, niosomes) with ideal size/EE and uniform composite fibers. In vitro data show significantly extended release compared to conventional formulations. This multifunctional platform, supported by statistical design, can potentially improve diabetes therapy by ensuring steady drug levels and reducing dosing burden. Future work may include in vivo evaluation of glycemic control. Overall, the study demonstrates the power of DoE in engineering complex drug delivery systems and validates hybrid nanofibers as effective sustained-release carriers.

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We appreciate your dedication and valuable contributions toward advancing scientif ic innovation, fostering collaboration, and promoting research excellence during this international conclave.

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CERTIFICATE OF PARTICIPATION

This is to certify that Prof./Dr./Mr./Ms. Satish Digambar Mendake (Research Scholar) from Dept of Pharmacy PKU Participate/presented a paper titled Development and Optimization of a Biplastic nano-in-nanofiber Patch for co-Delivery of Metformin and Sitagliptin at the 2nd International Conference on **Collaborative Futures: Bridging Ideas, Cultures, and Disciplines,** held from 14th June 2025, organized by the Faculty of Science & Commerce, P.K. University, Shivpuri (M.P.)

We appreciate their valuable contribution and participation in making the conference a success.

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(57) Abstract :
A hybrid nano-in-nanofiber drug delivery platform for sustained antidiabetic therapy is disclosed. The invention relates to an electrospun nanofiber patch embedded with drug-loaded nanoparticles (solid lipid nanoparticles and/or niosomes) containing metformin and sitagliptin. This unique nano-in-nanofiber structure enables a dual-phase release: an initial controlled release of the drugs 10 followed by a prolonged, steady release, achieving stable therapeutic levels for at least 24 hours from a single patch. The nanofiber matrix (e.g., a PCL/PVA fiber blend) provides mechanical support and moderates the release, while the internal nanoparticles act as reservoirs that significantly extend the drug release duration. The result is a transdermal delivery system that bypasses first-pass metabolism, 15 reduces dosing frequency to once-daily, and minimizes plasma level fluctuations compared to conventional oral therapy. The patch is manufactured by co-electrospinning polymer solutions with pre-loaded nanoparticles, yielding fibers with uniformly embedded metformin-niosomes and sitagliptin-SLNs. This invention offers a novel formulation structure that co-delivers two antidiabetic 20 agents in a synergistic manner, improving patient compliance and glycemic control. The specification includes comparative data showing superior performance (reduced burst release, extended duration, higher encapsulation efficiency) relative to single-layer nanofiber or nanoparticle-only systems. Five independent claims define the composition, fabrication method, dual-release functionality, therapeutic 25 application, and the novelty of the nano-in-nanofiber architecture.

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