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Metformin and silymarin loaded onto poly(caprolactone)/chitosan polymeric nanofiber based pads for diabetic wound healing

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Diabetes is one of the most prevalent genetic-metabolic diseases worldwide, affecting a significant number of individuals from diverse communities. One of its major complications is diabetic foot ulcers, resulting from several factors including peripheral vascular damage and an impaired immune system. In recent years, various approaches have been proposed for the treatment of diabetic foot ulcers. Among the innovative strategies, the utilization of advanced drug delivery systems has gained considerable attention. Nanofibers based on biocompatible and biodegradable polymers have been extensively studied for drug delivery and tissue engineering applications. Chitosan/poly(caprolactone) nanofibers have been investigated in various studies for targeted drug delivery, including in the context of diabetes. In this research, chitosan/poly(caprolactone) nanofibers loaded with metformin and silymarin were prepared and evaluated for their physicochemical properties and cellular toxicity. The nanofibers exhibited a size of less than 200 nanometers and possessed sufficient mechanical strength. The synergistic effects of metformin and silymarin encapsulated within chitosan/poly(caprolactone) nanofibers were studied for the treatment of diabetic foot ulcers. Drug release studies demonstrated an initial burst release followed by sustained and controlled release over an extended period. Cellular toxicity results indicated the biocompatibility of the nanofibers, making them suitable candidates for animal and clinical studies. Overall, chitosan/poly(caprolactone) nanofibers exhibited desirable physicochemical characteristics, and their biocompatibility and biodegradability properties enhance their potential for clinical applications.

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Introduction

Diabetes, a chronic metabolic disorder characterized by elevated blood glucose levels, affects a substantial portion of the global population.[1] It arises due to either insufficient insulin production or ineffective utilization of insulin by the body. The two primary types of diabetes, namely type 1 and type 2, exhibit distinct etiologies but share the common feature of impaired glucose regulation.[2,3] Prolonged hyperglycemia in diabetes leads to widespread systemic complications, including cardiovascular diseases, neuropathy, nephropathy, and retinopathy. Among these complications, the development of diabetic wounds presents a significant challenge, as they are often chronic, non-healing ulcers that afflict individuals with diabetes.[4–7] These wounds result from a combination of factors, including impaired blood circulation, peripheral neuropathy, and compromised immune response. Understanding the underlying mechanisms of diabetic wound healing and developing effective therapeutic interventions is crucial in order to mitigate the burden of this debilitating condition.

Diabetic wounds pose a significant challenge in the field of healthcare due to their high prevalence and complications. Diabetic wounds, commonly known as diabetic ulcers, are

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chronic and non-healing wounds that occur in individuals with diabetes mellitus. These wounds develop as a result of several interrelated factors, including impaired microcirculation, neuropathy, and compromised immune response.[8] The presence of elevated blood glucose levels further exacerbates the healing process by impeding various cellular and molecular mechanisms involved in tissue repair. Diabetic wound healing is a complex biological process that involves a series of coordinated events, including inflammation, angiogenesis, extracellular matrix deposition, re-epithelialization, and remodeling.[9,10] Impaired wound healing in individuals with diabetes can lead to severe consequences, such as infection, gangrene, and amputation, thereby significantly impacting the quality of life for affected patients. Hence, understanding the underlying mechanisms of diabetic wound healing and developing effective therapeutic strategies to accelerate and enhance this process is of utmost significance in clinical practice.[11–14]

Diabetic wounds require specialized treatment strategies to promote effective healing and prevent complications. A range of innovative approaches have emerged in recent years, offering promising solutions for managing these challenging wounds. One such approach involves the use of advanced dressings as part of wound care. Non-adherent dressings with low adhesive properties provide a gentle and protective environment for the wound bed. Hydrocolloid dressings facilitate moist wound healing and promote granulation tissue formation. Hydrogel dressings maintain a moist environment, enhance autolytic debridement, and support tissue regeneration.[15] Foam dressings offer excellent absorbency and fluid retention capabilities, while alginate dressings provide hemostatic properties and facilitate wound exudate management. Additionally, iodine-impregnated dressings and silver-containing dressings demonstrate antimicrobial effects, aiding in infection control. Other treatment modalities, such as ozone therapy, maggot therapy, light therapy, leech therapy, vacuum therapy, and PRP (platelet-rich plasma) therapy, have shown promise in enhancing the healing process of diabetic wounds. The comprehensive understanding and utilization of these diverse treatment options can significantly improve outcomes in managing diabetic wounds.[16]

Drug delivery plays a pivotal role in modern medicine by ensuring the efficient and targeted administration of therapeutic agents to specific sites within the body. It encompasses various techniques and systems designed to enhance drug efficacy, minimize side effects, and optimize patient outcomes.[17–22] One such approach is the use of polymeric wound dressings as drug delivery systems.[23–25] These dressings, specifically designed for diabetic wound management, offer a unique platform for localized drug delivery.[26–28] The polymers used in these dressings possess desirable properties such as biocompatibility, biodegradability, and controlled release capabilities.[29] Through the incorporation of pharmaceutical agents into the polymer matrix, the dressings can release the drugs in a controlled manner directly at the site of the wound.[30] This localized drug delivery approach allows for high drug concentrations at the target site, maximizing therapeutic benefits while minimizing systemic side effects. Furthermore, the polymeric wound dressings provide a protective barrier, maintaining a moist environment and promoting wound healing. Overall, the use of polymeric wound dressings as drug delivery systems represents a promising strategy in diabetic wound management, offering the potential for enhanced healing outcomes and improved patient care.

Chitosan, a naturally occurring polysaccharide derived from chitin, has gained significant attention in the field of drug delivery due to its remarkable properties. Its biocompatibility, biodegradability, non-toxicity, and mucoadhesive nature make it an attractive material for various pharmaceutical applications. In drug delivery, chitosan has demonstrated the ability to loading and deliver a wide range of therapeutic agents, including small molecules, proteins, peptides, and nucleic acids. Its positively charged amino groups allow for interactions with negatively charged drug molecules, facilitating controlled drug release.[31–36] Chitosan-based drug delivery systems have been utilized in diverse routes of administration, including oral, nasal, transdermal, and ocular delivery. In wound care, chitosan has shown immense potential as an active ingredient in wound pads. These pads, fabricated from chitosan and other polymers, can provide a conducive environment for wound healing.[37–40] Chitosan's antibacterial properties aid in infection prevention, while its ability to promote cell adhesion and proliferation enhances tissue regeneration. Additionally, chitosan-based wound pads can offer sustained release of therapeutic agents, such as growth factors or antimicrobial agents, directly at the wound site, facilitating accelerated healing. The versatility and effectiveness of chitosan in drug delivery make it a promising material for developing advanced wound pads that can significantly improve the management of diabetic wounds.[41]

Metformin and silymarin are pharmaceutical agents with potential applications in diabetic wound healing. Metformin, commonly used for managing type 2 diabetes, possesses anti-inflammatory properties, improves angiogenesis, and enhances collagen synthesis, all of which are crucial for wound healing. Silymarin, derived from the milk thistle plant, exhibits antioxidant, anti-inflammatory, and immunomodulatory activities. It promotes angiogenesis, inhibits collagen degradation, and has antimicrobial effects. These properties make both metformin and silymarin intriguing candidates for topical application in diabetic wound treatment. However, further research is necessary to understand their precise mechanisms and optimize their delivery methods for optimal therapeutic efficacy.[42–44] Both polymers are biodegradable and have complementary properties that make them suitable for various biomedical applications. Studies have demonstrated that chitosan and PCL can form compatible blends with improved mechanical properties, biocompatibility, and degradation rates compared to individual polymers. Polymers with similar chemical structures are more likely to be compatible. For example, chitosan and PCL have some similarities in their chemical compositions, making them more compatible than polymers with vastly different structures. Compatibility can be influenced by intermolecular interactions such as hydrogen

bonding, van der Waals forces, and electrostatic interactions between polymer chains. Strong intermolecular interactions can promote compatibility and improve the blending of polymers.[45–49]

This research presents a novel approach to enhance diabetic wound healing. The use of polymeric nanofibers, specifically composed of poly(caprolactone) and chitosan, serves as a unique platform for the controlled delivery of metformin and silymarin directly to the site of the diabetic wound. This combination of materials offers several positive aspects. Firstly, poly(caprolactone) provides structural integrity and controlled release properties, while chitosan brings inherent biocompatibility and mucoadhesive characteristics. The mucoadhesive attributes inherent in chitosan bear significant relevance for wound healing applications. Mucoadhesion denotes the capability of a substance to attach to mucosal surfaces, including those present in the gastrointestinal tract or on the skin. In wound healing management, the mucoadhesive properties of chitosan play a pivotal role in facilitating its adherence to the wound site, thereby establishing a protective shield and fostering the process of wound healing. Chitosan's utility in diverse wound dressings and bandages stems from its adeptness in adhering to the wound surface, regulating hemorrhage, and stimulating tissue regeneration.[50] Secondly, the incorporation of metformin and silymarin offers a synergistic effect, combining the anti-inflammatory, angiogenic, collagen-promoting, and antimicrobial properties of these agents. Furthermore, the topical application of the polymeric nanofibers enables targeted delivery, ensuring high drug concentrations at the wound site while minimizing systemic side effects. Overall, our work presents a promising and innovative strategy for diabetic wound treatment, addressing the need for effective localized drug delivery and providing a foundation for future advancements in wound healing research and clinical practice.

Results and discussion

In this study, we conducted a series of experiments and evaluations to investigate the properties and performance of chitosan/poly(caprolactone) (PCL) nanofibers loaded with metformin and silymarin. The nanofibers were fabricated by electrospinning technique, ensuring the attainment of desired morphology. The resulting nanofibers were then subjected to exact characterization through meticulous analysis. The loading of metformin and silymarin were performed and the release profile of the mentioned drugs were studied.

SEM images

The scanning electron microscopy (SEM) technique was employed to investigate the morphology and structural features of the chitosan/poly(caprolactone) (PCL) nanofibers in this study. The SEM results are presented in Fig. 1. As depicted in the SEM image, the chitosan/poly(caprolactone) nanofibers exhibited a highly interconnected network structure without the presence of any noticeable bead-like structures. It should be noted that for the samples PCL/Cs-25(8%) : 75(1%), the size of the fibers is large, resulting in their agglomeration or sticking together. In contrast, this ratio (75% polycaprolactone to 25% chitosan) yields finer fibers and reduced fiber adhesion. This finer fiber structure is beneficial for various applications,

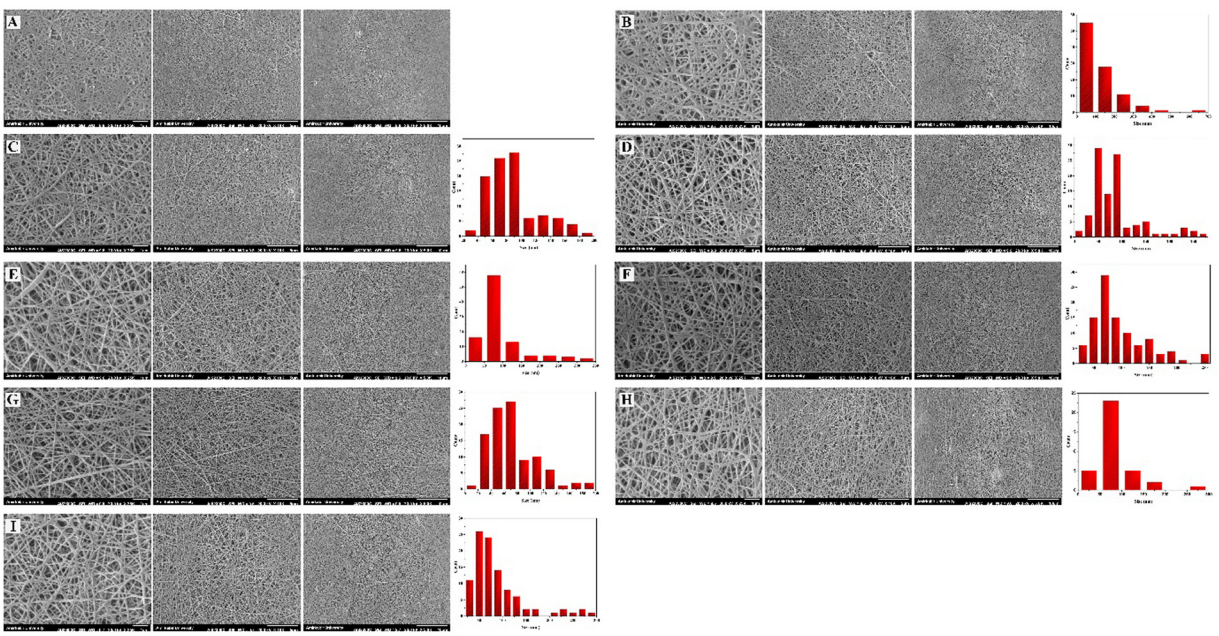

Fig. 1 Morphology of nanofibers using SEM-acquired images. (A) chitosan/PCL nanofibers with a ratio of 25 to 75 (PCL/Cs-25(8%) : 75(1%)); (B) ratio of 50 to 50 (PCL/Cs-50(8%) : 50(1%)); (C) ratio of 75 to 25 (PCL/Cs-75(8%) : 25(1%)); (D) nanofibers containing 1% metformin (PCL/Cs75 : 25-Met 1%); (E) containing 2% metformin (PCL/Cs75 : 25-Met 2%); (F) containing 5% metformin (PCL/Cs75 : 25-Met 5%); (G) containing 1% silymarin (PCL/Cs75 : 25-Sil 1%); (H) containing 2% silymarin (PCL/Cs75 : 25-Sil 2%); and (I) containing 5% silymarin (PCL/Cs75 : 25-Sil 5%).

Table 1 The average size of the nanofibers, extracted from SEM results

Nanofibre	Average size (nm)
PCL/Cs-25(8%) : 75(1%)	Aggregation occured
PCL/Cs-50(8%) : 50(1%)	128 nm ± 17
PCL/Cs-75(8%) : 25(1%)	88 nm ± 8
PCL/Cs75 : 25-Met 1%	88 nm ± 14
PCL/Cs75 : 25-Met 2%	91 nm ± 7
PCL/Cs75 : 25-Met 5%	97 nm ± 11
PCL/Cs75 : 25-Sil 1%	73 nm ± 9
PCL/Cs75 : 25-Sil 2%	86 nm ± 6
PCL/Cs75 : 25-Sil 5%	86.5 nm ± 9

including drug delivery, as it provides a larger surface area and an enhanced surface-to-volume ratio. The absence of beads along the nanofibers indicates a homogeneous distribution of the polymer components, ensuring the uniform release of loaded drugs. The average diameter of the nanofibers, as provided in the Table 1, is an important parameter that influences the drug loading and release behaviour. The precise control of the nanofiber diameter is crucial for achieving the desired drug delivery profiles, as it affects the diffusion rate, surface area, and overall drug loading efficiency.[51] The nanofibers in this study exhibited a diameter within the desired range, indicating their suitability for effective drug loading and release. Overall, the SEM results confirmed the successful fabrication of chitosan/poly(caprolactone) nanofibers with a uniform and interconnected structure.[52] These morphological characteristics, combined with the appropriate diameter, make the nanofibers well-suited for drug loading experiments at different weight ratios. The SEM analysis provided valuable insights into the structural features of the nanofibers, laying the foundation for further investigations on drug loading, release kinetics, and their potential applications in the field of diabetic wound healing.

Wettability and swelling results

Wettability, a critical parameter characterizing the interaction between nanofiber networks and liquids, was evaluated by measuring the contact angle of water droplets on the surface. Fig. 2 depicts the arrangement of water droplets on poly(caprolactone)/chitosan nanofiber networks, illustrating the impact of loading varying concentrations of metformin and silymarin on nanofiber wettability. Notably, increasing silymarin concentration led to reduced hydrophilicity, while higher metformin concentrations resulted in enhanced wettability, underscoring the significant influence of drug loading on nanofiber wettability properties. Wettability, essential for understanding liquid–solid interactions, reflects a material's ability to form a thin film over a solid surface. It is influenced by factors such as the surface tension of the liquid, the surface energy of the solid, and the contact angle formed between the liquid and the solid surface. Hydrophilicity, a related concept, denotes a material's affinity for water or polar substances, with hydrophilic surfaces fostering strong attractions to water molecules, thus promoting the formation of a thin water film. It should be noted that silymarin has a hydrophobic nature and increasing its

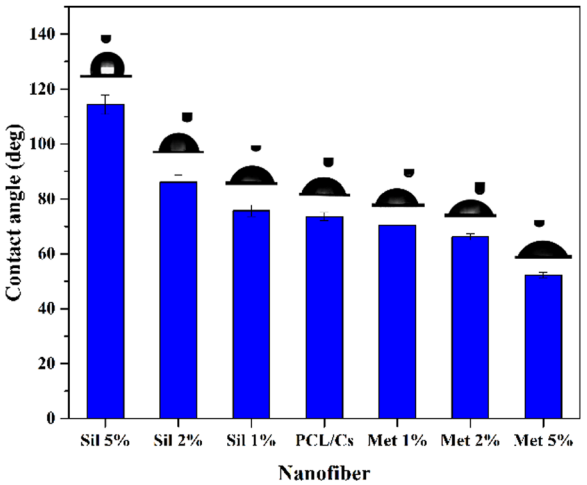

Fig. 2 The wettability results of various nanofibers with different doses of metformin and silymarin.

percentage increases its hydrophobic properties of nanofiber. This agrees with the observed results in Fig. 2. Although metformin is a hydrophobic drug, when this drug is converted into a salt form, it can increase its solubility in water making it more water-soluble and hydrophilic compared to the pure hydrophobic metformin molecule making it easier to dissolve in water and absorb water as a result increase the wettability of the nanofibers.

Tensile results

The evaluation of nanofiber tensile strength was conducted utilizing an Instron mechanical testing machine (model 5566, England), with the findings depicted in Fig. 3. Notably, an increase in silymarin concentration correlated with higher tensile strength, peaking at over 40 MPa in nanofibers loaded with 5% silymarin. Conversely, elevating metformin concentration resulted in a slight decrease in tensile strength. These observations underscore the role of silymarin in enhancing nanofiber mechanical properties, while metformin incorporation exhibited a minimal effect. The attained tensile strength values highlight the potential utility of these drug-loaded

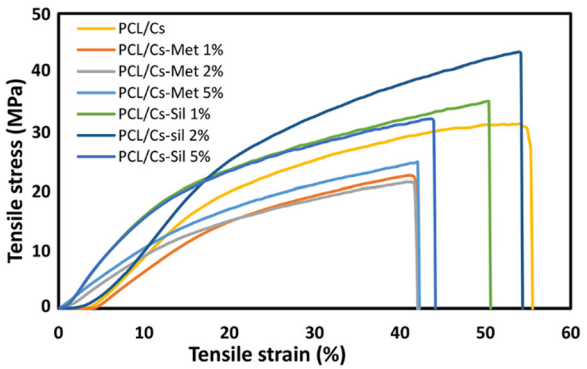

Fig. 3 The tensile properties of various nanofibers for studying the effect of the loading of different doses of metformin and silymarin.

nanofibers in applications necessitating adequate mechanical stability.

Furthermore, factors influencing the enhancement of nanofiber tensile strength were explored. While no significant difference in nanofiber diameter was observed, nanofibers containing silymarin exhibited reduced diameter, potentially mitigating defects and augmenting the surface area-to-volume ratio, thereby contributing to increased tensile strength.

Silymarin emerges as a primary contributor to enhanced tensile strength in nanofibers, acting as a cross-linking agent that fosters stronger interactions among polymer chains within the nanofiber matrix. This mechanism holds promise for improving mechanical properties, including heightened tensile strength.

Conversely, the presence of metformin hydrochloride led to a decrease in the tensile strength of nanofibers composed of poly(caprolactone) and chitosan. This phenomenon may be attributed to larger nanofiber diameters and an accelerated degradation rate associated with metformin, thereby underscoring its contrasting impact on nanofiber mechanical properties.

Water uptake results

The water uptake and swelling behaviour of the nanofiber scaffolds were investigated by immersing the nanofibers in deionized water for 24 hours. The percentage of water uptake was calculated by measuring the weight of the nanofibers after immersion (W_1) and comparing it to the initial weight (W_0). The results of the study are presented in the figure. It was observed that the addition of metformin increased the water uptake due to its hydrophilic nature, leading to greater water absorption by the nanofibers. Conversely, the incorporation of silymarin, which exhibits hydrophobic properties, resulted in reduced water absorption and decreased swelling of the nanofibers. These findings highlight the influence of drug loading on the water uptake and swelling behaviour of the nanofibers, which can have implications for their performance in biomedical applications where controlled water absorption and swelling are desired. The uptake results are presented in Fig. 4.

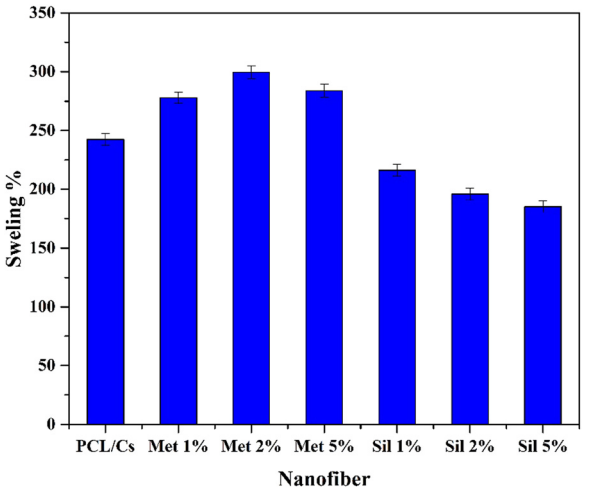

Fig. 4 The water uptake properties of the fabricated nanofibers.

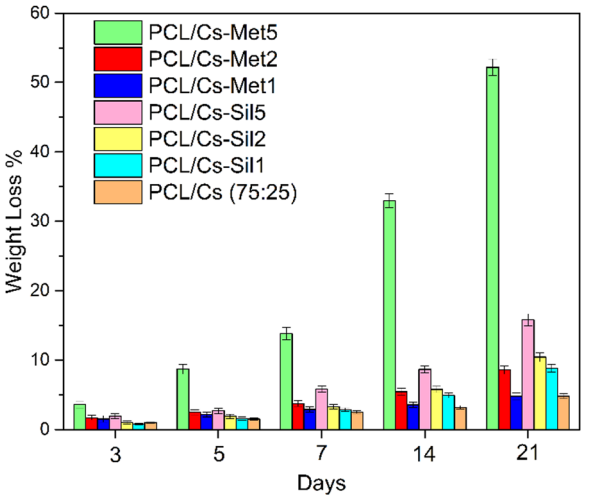

Fig. 5 The results of the degradation of the fabricated nanofibers after various times.

Degradation results

The degradation of the nanofiber samples was evaluated and the results are shown in Fig. 5, the degradation test was conducted on all prepared nanofiber samples after 3, 5, 7, 14, and 21 days. The results indicated that the nanofibers without any drug loading exhibited the least amount of degradation throughout the test duration. Furthermore, the nanofibers loaded with silymarin experienced less degradation compared to those loaded with metformin.

Release profile results

The drug release from the nanofibers was evaluated using the immersion method, and the results are presented in Fig. 3–5 and 3–6. As depicted in Fig. 6, the release of metformin increased with an increase in the drug concentration within the nanofibers. Furthermore, the drug release results indicated that the majority of the drug was released within the initial 2 hours from the nanofibers. Similar behavior was observed for the nanofibers loaded with silymarin, showing a comparable drug release pattern to metformin. These findings demonstrate the release capability of the nanofibers, where the drug release can be tailored by adjusting the drug loading concentration. Such behavior is crucial for achieving the desired therapeutic efficacy and sustained drug release for effective treatment in biomedical applications.

Cytotoxicity results

To assess the cytotoxicity, L929 cell line, which is a mouse fibroblast cell line, was employed. The cytotoxicity results are depicted in Fig. 7. As evident, an increase in drug concentration led to an increase in cellular toxicity. Additionally, the drug-free nanofibers exhibited acceptable cell viability, and after 7 days, a significant difference in cytotoxicity was observed between the drug-free nanofibers and those loaded with drugs. These findings

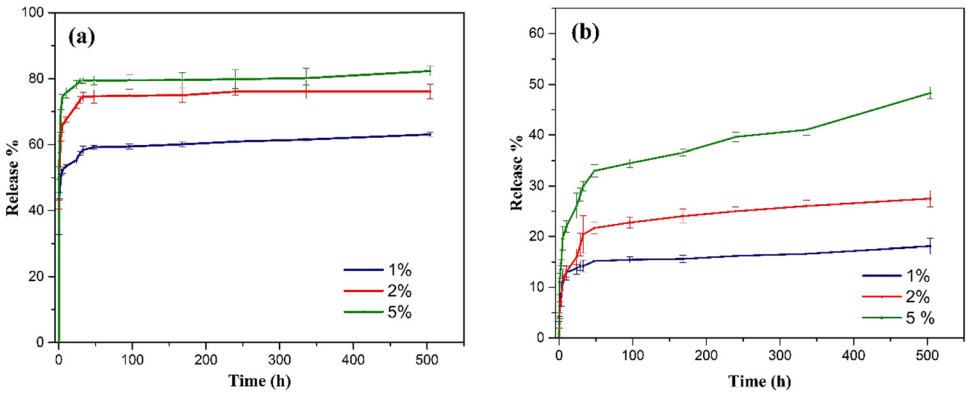

Fig. 6 The release profile of (a) metformin and (b) silymarin from the fabricated nanofibers after various times with different drug contents.

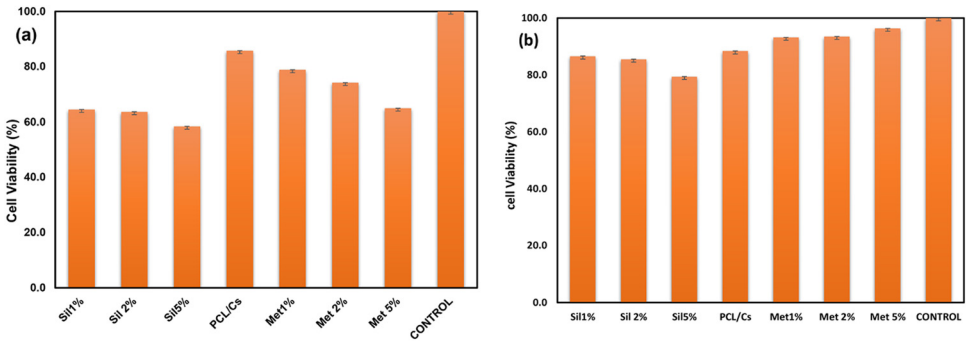

Fig. 7 The MTT results of the fabricated nanofibers after (a) 7 days and (b) 3 days.

highlight the importance of considering the cytotoxic effects when designing drug-loaded nanofibers for biomedical applications, as maintaining cell viability is crucial for ensuring biocompatibility and promoting tissue regeneration.

Generally, diabetic foot ulcers remain one of the most common complications of diabetes. Due to impaired blood circulation and compromised immune system in diabetic individuals, they are susceptible to both skin and systemic infections. In recent years, the use of advanced drug delivery systems such as nanofibers has provided promising therapeutic options for the management of such wounds. In this study, chitosan/poly-caprolactone nanofibers were utilized for loading and delivering metformin and silymarin in the treatment of diabetic foot ulcers. The electrospinning method was employed to prepare the nanofibers, which is a simple, cost-effective, and widely applicable technique for a variety of polymers. The choice of electrospinning over more complex methods like bubbfil spinning or centrifugal spinning was based on the suitability of the polymers used in the study. Chitosan and poly-caprolactone, the two polymers used in this research, have been extensively studied and widely used in similar applications. Chitosan, being biodegradable and biocompatible, has a high potential for its hydrophilic nature and the cell adhesion, and the potent antimicrobial properties of this polymer, especially hydrophilic ones. However, its high molecular weight and inherent viscosity require the presence of another polymer to reduce the solution's viscosity. Poly-caprolactone, as the second component within the chitosan chains, prevents the aggregation and chain entanglement of chitosan, thereby enhancing its workability.[53] Moreover, poly-caprolactone's favourable biocompatibility does not hinder the medical application of chitosan.[54,55] The resulting poly-caprolactone nanofibers had an average size of less than 200 nanometers, which was further reduced by the addition of chitosan. This reduction in size was attributed to the increased chain density of chitosan caused by the presence of poly-caprolactone within the chains.[56] Nanofibers, which contribute to tissue regeneration and the repair of damaged tissues, must possess requisite structural integrity for effective integration into the host tissue. The mechanical characteristics of nanofibers, such as tensile strength, are contingent upon the nature and concentration of the loaded pharmacological agent. Nanofibers incorporating silymarin demonstrated significantly enhanced strength in comparison to drug-free nanofibers. Moreover, nanofibers imbued with metformin exhibited suitable strength, as they are intended for use as wound dressings on the skin, where exceedingly high strength is not imperative, albeit currently lacking a specific reference for this assertion.[57,58] In neutral solutions, including physiological environments, silymarin has a weak acidic property and becomes negatively charged. The weak bonds formed

between silymarin's negative charges and the positive charges of the amine groups in the chitosan network contribute to the enhanced structural strength. In contrast, metformin, being positively charged in physiological environments, causes repulsion between the chitosan chains, resulting in increased fiber fragility. The pK_a value of metformin is 12.4, indicating its significant protonation in physiological conditions. The contact angle test is used to determine the hydrophilicity of the nanofibers. As observed from the results, nanofibers containing metformin exhibited higher hydrophilicity than drug-free and silymarin-loaded nanofibers. Increasing the metformin ratio led to higher hydrophilicity, while increasing the silymarin content decreased the hydrophilicity of the nanofibers. This can be attributed to the structural characteristics of these two drugs. Although silymarin has several active functional groups that become ionized, imparting hydrophilic properties to the molecule, the overall presence of bulky hydrophobic groups in the molecule renders it relatively hydrophobic in nature. This hydrophobicity limits the solubility and bioavailability of silymarin, making it a challenging compound to formulate for pharmaceutical and nutraceutical applications. However, researchers have explored various approaches to improve its solubility and bioavailability. Some strategies include formulating silymarin in lipid-based formulations, employing nanotechnology-based delivery systems, and enhancing its water solubility through complexation with cyclodextrins or other hydrophilic carriers. Lipid-based formulations, such as self-emulsifying drug delivery systems (SEDDS) or nanoemulsions, have been investigated to enhance the solubility and absorption of silymarin.

Materials and methods

General remarks

The chemicals used in this study were purchased from reputable suppliers, including Sigma-Aldrich, Merck, Acros Organics, and Scharlau. The chemicals were used as received and used without further purifications. Metformin hydrochloride was purchased from Mahban chemi Co. silymarin,poly-(ε-caprolactone) (PCL; M_n = 80 KDa), chitosan (Cs; M_n = 200 kDa, degree of deacetylation: 75–85%), acetic acid glacial (AcOH), and formic acid (HCO_2H) were purchased from sigma-aldrich and used without further purification. 3-(4,5-Dimethylthyazolyl-2)-2,5-diphenyl tetrazolium bromide (MTT) was purchased from ICN Biomedicals Inc. Dulbecco's Modified Eagle's Medium (DMEM), RPMI1640 medium, fatal bovine serum (FBS) and trypsin–EDTA were obtained from Gibco/Life Technologies.

Several instruments and equipment were utilized in this study to support the characterization and analysis of the fabricated polymeric nanofibers loaded with metformin and silymarin. A Universal Tensile Testing Machine was applied for recording the tensile measurements. The ELISA reader (Bio Tek 80) was employed in this study. Scanning electron microscope (TESCAN 4006) was used for the stusy of the microstructure, surface topography, and structural integrity and the morphology of the samples.

Preparation of solutions

A 10% poly(caprolactone) (PCL) solution and a 1% chitosan solution were prepared by dissolving the polymers in a 1 : 1 mixture of glacial acetic acid and formic acid. The desired solutions were obtained by mixing the two solutions at different mass ratios of chitosan to PCL, including 75 : 25, 50 : 50, and 25 : 75. To achieve this, the desired volume of PCL was placed in a container and simultaneously mixed with a magnetic stirrer. Subsequently, the desired volume of chitosan solution was gently added to the container, and during the mixing process, the solution was stirred using a magnetic stirrer. After preparing the desired ratio solution, it was transferred into a syringe for electrospinning.

Electrospinning

The electrospinning setup consisted of a high-voltage power source. A stationary collector plate (aluminium foil measuring 15 × 15 cm), a digital syringe pump with a minimum injection speed of 0.1 mL h^{-1}, and a nozzle were used. The syringe containing the mixed polymer solution was attached to the syringe pump, and the flow rate was set at 0.1–0.7 mL h^{-1}. The distance between the nozzle and the collector (an aluminium foil measuring 15 × 15 cm) was set at 15–20 cm. The collector plate was set to rotate at a speed of 250 to 600 rpm. A voltage of 15–17 kilovolts was applied to two electrodes connected to the nozzle and the collector. In this manner, the solution was subjected to electrospinning, and the nonwoven nanofiber network was collected on the collector plate.

In this study, a series of samples were prepared by varying the ratio of poly(caprolactone) (PCL) to chitosan (Cs) and by loading different drugs into the nanofibers. In addition, different loading rations were studied. The first three samples, namely PCL/Cs-25(8%) : 75(1%), PCL/Cs-50(8%) : 50(1%), and PCL/Cs-75(8%) : 25(1%), represent the PCL to Cs weight ratios of 25 : 75, 50 : 50, and 75 : 25, respectively. In the solutions are prepared from solution with PCL content was 8% and Cs content was 1%. These variations in the PCL to Cs ratio allow for the exploration of different polymer compositions and their effects on the physicochemical properties of the nanofibers. Furthermore, additional samples were prepared with specific drug loadings. The samples labeled PCL/Cs-75 : 25-Met 1%, PCL/Cs-75 : 25-Met 2%, and PCL/Cs-75 : 25-Met 5% denote the nanofibers with a PCL to Cs ratio of 75 : 25 loaded with metformin at 1%, 2%, and 5% weight ratios, respectively. Similarly, the samples labeled PCL/Cs-75 : 25-Sil 1%, PCL/Cs-75 : 25-Sil 2%, and PCL/Cs-75 : 25-Sil 5% represent the nanofibers loaded with silymarin at 1%, 2%, and 5% weight ratios, respectively. By systematically varying the PCL to Cs ratio and drug loading, we aim to investigate the influence of these parameters on the physicochemical characteristics, drug release profiles, and potential therapeutic effects of the prepared nanofibers. This comprehensive approach allows for a thorough understanding of the relationship between composition, drug loading, and the performance of the nanofibers, paving the way for optimized formulations for diabetic wound healing applications.

Cell culture

L929 cell lines (mouse fibroblasts) were obtained from the Royan Institute for Biotechnology Research Cell Bank (Tehran, Iran). The cells were cultured in Cell culture flasks containing minimal essential medium (MEM) (Sigma, Germany) supplemented with 10% fetal bovine serum (FBS), 1% antibiotic–antimycotic solution (penicillin–streptomycin), and 1% non-essential amino acids (NEAA). The cell culture flasks were maintained at 37 degrees Celsius in a humidified incubator with 5% CO_2. Daily microscopic examination was performed to monitor cell growth and confluency. Passage of cells was carried out when approximately 80% of the flask surface was occupied by cells.

Loading of silymarin and metformin

For loading silymarin and metformin into the nanofibers, prior to the electrospinning process, each drug was separately added to a specific weight ratio of 25 : 75 of chitosan to poly(caprolactone) polymer solution. The mixture was stirred using a magnetic stirrer overnight and the remaining steps were repeated as previously described.

Swelling capacity

The swelling capacity were evaluated by immersing the nanofibers in deionized water at 37 degrees Celsius for 24 hours. To measure the percentage of the swelling capacity of the nanofibers, the samples were weighed initially (W_0) and weighed again after 24 hours (W_t). The swelling capacity was calculated according to the eqn (1):

$$\text{Swelling capacity} = \frac{W_t - W_0}{W_0} \times 100 \quad (1)$$

Degradation of nanofibers

Initially, a sample of the fabricated nanofiber scaffold (50 mg) was cut and placed in a phosphate buffer solution with a pH of 7.4 (25 mL) at 37 °C for 21 days with agitation at 100 rpm. After this period, the samples were removed from the buffer solution and dried in a desiccator. The percentage weight loss was determined by comparing the initial and final weights of the fibres, and the changes in structure and morphology were examined using scanning electron microscopy (SEM). The degradation was calculated by the eqn (2):

$$\text{Percentage degradation} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100 \quad (2)$$

Drug release

The release of drugs from the nanofibers was investigated using an immersion method. Approximately 10 mg of nanofibers were placed in dialysis bags with a molecular weight cut-off of 12 kDa, and then 2 mL of a 0.2 M phosphate buffer solution (PBS) was added to each bag as the dissolution medium. The dialysis bags were immersed in 100 mL of PBS as the receiving medium and placed on a magnetic stirrer at a temperature of 37 °C. At predetermined time intervals, 1 mL of the receiving medium was withdrawn and replaced with an equal volume of fresh buffer solution. The concentration of the samples was analysed at a wavelength of 283 and 324 nm for silymarin and 232 nm for metformin.

Cytotoxicity evaluation of the samples

To assess the biocompatibility of the nanofibers with L929 cells, the indirect MTT method was employed as per established protocols. This investigation adhered to the guidelines outlined in the ISO10993-5 standard for such assessments. To execute the study, scaffolds with a defined area of 3.5 ± 0.5 cm^2 were meticulously sterilized. Subsequently, these prepared scaffolds were introduced into 1 mL of the designated culture medium. Following a cultivation period of 7 days, cellular extracts were meticulously isolated for subsequent analysis *via* the MTT assay technique. This approach allowed for a comprehensive evaluation of the potential interactions between the nanofibers and L929 cells, shedding light on their biocompatibility in accordance with recognized scientific procedures.[59]

Conclusion

In conclusion, this study focused on the fabrication of drug-loaded nanofibers using the electrospinning technique and investigated their physicochemical properties and biological performance. The results demonstrated that the nanofibers possessed uniform morphology, appropriate mechanical strength, and desired drug release characteristics. The SEM analysis revealed the interconnected network structure of the nanofibers, facilitating drug loading and release. The contact angle measurements indicated the tuneable hydrophilic and hydrophobic nature of the nanofibers by adjusting the drug concentrations. The mechanical testing showed that the nanofibers exhibited favourable tensile strength, with the highest strength observed in the nanofibers loaded with 5% silymarin. Moreover, the drug release studies confirmed the sustained and controlled release of both metformin and silymarin from the nanofibers over time. The cytotoxicity evaluation demonstrated concentration-dependent toxicity, emphasizing the importance of carefully selecting drug concentrations to ensure biocompatibility. Collectively, these findings highlight the potential of drug-loaded nanofibers as promising candidates for various biomedical applications, such as drug delivery systems and tissue engineering scaffolds.

Conflicts of interest

There are no conflicts to declare.

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