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PULLULAN-DOX/PVA-PDMS BIOPOLYMERIC CORE-SHELL NANOFIBERS POTENTIAL FOR DRUG DELIVERY SYSTEMS

İLAÇ DAĞITIM SİSTEMLERİ İÇİN PULLULAN-DOX/PVA-PDMS BİYOPOLİMERİK ÇEKİRDEK-KABUK NANOFİBERLER POTANSİYELİ

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PULLULAN-DOX/PVA-PDMS BIOPOLYMERIC CORE-SHELL NANOFIBERS POTENTIAL FOR DRUG DELIVERY SYSTEMS

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ABSTRACT: In this research, a novel drug delivery system shell was created by loading doxorubicin hydrochloride (DOX) into Pullulan and integrating the core into a polyvinyl alcohol (PVA) and Polydimethoxysilane (PDMS) composite matrix. The incorporation of DOX into the pullulan solution was carried out to take advantage of Pullulan's biocompatibility, biodegradability and hydrophilic nature. The hydrophilic nature of PVA can result in rapid drug release, while the hydrophobic nature of PDMS allows for slower drug release. The use of PVA-PDMS polymers together in the shell offers an initial rapid release followed by a prolonged and controlled drug release. This combination is superior to PVA or PDMS in terms of safety, mechanical strength, flexibility, controlled drug release and structural stability. This innovative composite system was designed to optimise DOX's controlled release to increase its therapeutic efficacy and reduce systemic toxicity. The kinetics of the drug release was characterised by an initial burst release followed by a sustained release phase, allowing controlled and prolonged release of the chemotherapeutic agent. Our results indicate that the pullulan/PVA-PDMS composite is a promising candidate for practical drug delivery applications, especially in cancer therapy.

Keywords: Coaxial nanofibers; Polysaccharides; Antibacterial; Biomaterials; Bioactive nanofibers, Drug delivery systems

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ÖZ: Bu araştırmada, doksorubisin hidroklorür (DOX) Pullulan'a yüklenerek ve çekirdek bir polivinil alkol (PVA) ve Polidimetoksisilan (PDMS) kompozit matrisine entegre edilerek yeni bir ilaç dağıtım sistemi kabuğu oluşturulmuştur. DOX'un pullulan çözeltisine dahil edilmesi, Pullulan'ın biyouyumluluğundan, biyolojik olarak parçalanabilirliğinden ve hidrofilik yapısından yararlanmak için gerçekleştirilmiştir. PVA'nın hidrofilik doğası hızlı ilaç salınımına neden olabilirken, PDMS'nin hidrofobik doğası daha yavaş ilaç salınımına izin verir. Kabukta PVA-PDMS polimerlerinin birlikte kullanılması, başlangıçta hızlı bir salım ve ardından uzun süreli ve kontrollü bir ilaç salımı sağlar. Bu kombinasyon güvenlik, mekanik güç, esneklik, kontrollü ilaç salınımı ve yapısal stabilite açısından PVA veya PDMS'den daha üstündür. Bu yenilikçi kompozit sistem, DOX'un terapötik etkinliğini artırmak ve sistemik toksisiteyi azaltmak için kontrollü salınımını optimize etmek üzere tasarlanmıştır. İlaç salınımının kinetiği, kemoterapötik ajanın kontrollü ve uzun süreli salınımına izin veren bir ilk patlama salınımı ve ardından sürekli bir salınım fazı ile karakterize edilmiştir. Sonuçlarımız pullulan/PVA-PDMS kompozitinin özellikle kanser tedavisinde pratik ilaç dağıtım uygulamaları için umut verici bir aday olduğunu göstermektedir.

Anahtar Kelimeler: Eş eksenli nanolifler, Polisakkaritler, Antibakteriyel, Biyo-malzemeler, Biyoaktif nanolifler, İlaç taşıma sistemleri

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1. INTRODUCTION

Nanotechnology has emerged as a formidable force in the fight against deadly pandemic infections, such as COVID-19, and represents a promising field for interdisciplinary biomedical research. The development of nanofibrous materials with large porous surface areas has significant potential for medicinal applications because the surfaces of nanoparticles can be engineered and analysed. Electrospinning is a versatile method of loading active pharmaceutical compounds for controlled drug release. Coaxial electrospinning is widely used in drug delivery research. Polymer nanofibers exhibit unique characteristics, such as increased surface area per volume and tailored surface properties, making them well-suited for various biological applications. Drug-loaded core-shell nanofiber materials allow for sustained drug release and minimise the risk of side effects. To optimise drug delivery systems, it is essential to carefully consider the compatibility between the polymer and the chemical properties of the drug [1]. Fabrication of nanofibers with diverse morphologies via electrospinning is a conventional and practical approach for synthesising nanofiber matrices.

Additionally, techniques for the large-scale production of nanofibers have been refined through electrospinning [2], [3]. Investigation of nanotechnology-derived drug delivery systems presents a promising opportunity for interdisciplinary collaboration in the biomedical field, enabling the pursuit of innovative therapies and advanced treatment strategies. However, particular challenges must be addressed, such as suboptimal bioavailability resulting from poor solubility or intestinal absorption owing to degradation, inadequate distribution to the target site, unfavourable side effects, and disparate drug plasma levels [4]. Nanofibrous materials exhibit a large porous surface area [5] and have great potential for medicinal applications because of their unique ability to be modified and tailored at the nanoscale, enabling precise manipulation and characterisation for a wide range of therapeutic uses [6]. Ultimately, administering medications to locations of inflammation can be facilitated by utilising nanoparticles composed of natural or synthetic polymers. This approach enhances the protection of pharmaceuticals by interfering with the body's absorption process [7]. Polymeric nanoparticles can be readily modified with organic or inorganic hybrid components and are frequently utilised in the medical field. The numerous advantageous properties of these nanoparticles, including biodegradability, biocompatibility, low immunogenicity, and antibacterial capabilities, often lead to their replacement by protein- and polysaccharide-based biopolymers as opposed to their synthetic counterparts [8].

Electrospinning is a technique that involves the use of high-voltage electric fields to produce fine fibres with defined surface structures in the ultra-micron to nanometer range, which can be used to load active pharmacological chemicals for controlled release. This method is an alternative to traditional drug delivery systems and offers several advantages, including achieving sustained release and target-specific sites within the body. To produce nanofibers using electrospinning, the desired polymer is dissolved in a solvent and then subjected to a high-voltage electric field [9]. A method was devised to produce fibres with thicknesses ranging from micrometres to nanometers, featuring controlled topography, by subjecting a solution of the desired polymer to a strong electric field. When the solvent is insufficient, the polymer melts and is exposed to an electric field [10]–[12]. Polymer nanofibers display exceptional qualities, making them promising candidates for numerous critical biological applications. By diminishing the diameter of polymer fibres to the micrometre or nanoscale level, unprecedented attributes may become evident, such as an enhanced ratio of surface area to volume and malleability of the surface properties [9], [10]. This study aimed to fabricate Pullulan/Polyvinyl Alcohol (PVA) core-shell nanofibers and explore their potential applications in drug delivery systems [13].

Drug delivery systems typically use coaxial electrospinning techniques [14]. In traditional electrospinning, a dual-needle configuration featuring two coaxial capillaries is typically used to deliver the polymer solution. This setup enabled a range of internal and external structural configurations through a coaxial nozzle arrangement. However, during the electrospraying process, issues such as nozzle clogging or the formation of poorly organised monolithic fibres may arise [15]. The continuous jet generated by electrospinning exhibits viscoelasticity and bending instability. Numerical analyses have demonstrated that, during this process, electrical charges migrate along the surface of the jet [16]. The electrospinning process necessitates the utilisation of three primary components: a metallic needle, commonly referred to as a spinneret; a high-voltage power supply; and an intermediate component that is affixed to the spinneret and a collection plate, commonly referred to as a grounded collector [17]. It is commonly recognised that tubular nanofibers are helpful in various applications, including transporting medications and creating tissues. Because template-directed methodologies are only helpful for manufacturing short-length fibres, electrospinning is recommended for producing long, continuous nanoscale hollow fibres compared to other methods [18]. The production of long continuous nanoscale hollow fibres using electrospinning can be characterised by establishing specific threshold values or ranges for fibre classification [19]. For instance, fibres shorter than a defined length, such as 1 mm, are considered short, whereas those longer than that length are classified as long [20].

Furthermore, the aspect ratio of each fibre, obtained by dividing its length by its diameter, can be utilised as a parameter. By establishing a threshold value, such as 100, fibres with an aspect ratio exceeding this value can be classified as long, whereas those below it are deemed short. It is essential to assess the fibre structure for any breaks, discontinuities, or irregularities [21]. Establishing a predetermined length for uninterrupted fibre continuity can distinguish long fibres from short fibres with breaks or irregularities. The implementation of quantitative criteria facilitates consistent fibre classification and allows for the evaluation of the ability of the electrospinning process to produce long, uninterrupted nanoscale hollow fibres.
The doxorubicin hydrochloride (DOX) develops at high levels in vivo; congestive heart failure and permanent cardiac damage may

result [22]–[25]. The use of core-shell nanofiber materials loaded with drugs facilitates controlled and sustained release of the drug, thereby reducing the likelihood of adverse effects associated with high drug toxicity. The selection of appropriate polymers for the manufacturing process is critical because it is based on the compatibility between the polymer and the chemical properties of the drug, ensuring uniform drug loading. The nanofiber structure, release properties, and mechanical properties of the nano-drug delivery system utilising DOX demonstrated stability at pH 7.4, with rapid drug release at pH 5 [25]. The yeast-like fungus *Aureobasidium* produces Pullulan, a polysaccharide commonly utilised in food coatings and drug delivery systems because of its biodegradability, water solubility, and low oxygen permeability. Pullulan is non-toxic and can be safely used in repeated contact with food. Due to its strictly linear structure, Pullulan has a wide range of commercial applications in the biomedical and food industries. In addition, it serves as a valuable tool in basic research and is considered a well-defined model substance [26]. Polyvinyl alcohol (PVA) is a carrier polymer commonly used in medical speciality applications owing to its chemical composition. It is often referred to as an environmentally friendly material because of its solubility characteristics, making it a "green polymer." Their easily degradable nature allows effective blending with a wide range of polymers owing to their water solubility [27]. PVA combined with PDMS enhances the material's flexibility and hydrophobic properties, resulting in a more resistant structure to water and chemicals [28]. Due to its markedly low glass transition temperature (Tg) and the presence of chains with limited length, PDMS is not amenable to neat electrospinning and frequently necessitates blending with another component [29].

PVA, a polymer with a semi-crystalline nature that readily dissolves in water, differs from PDMS in its ease of spinnability. Nevertheless, the utilisation of PVA-based nanofibrous mats is significantly constrained by their pronounced solubility in aqueous environments. The primary objective of this study is to develop a technique capable of effectively regulating and harmonising the hydrophilic and oleophilic properties of the spun mats, a crucial step towards the fabrication of selectively permeable membranes. To this end, stearic acid has been employed to blend PVA and PDMS, known for their hydrophilic and oleophilic characteristics, in varying proportions to formulate electrospinning solutions and fabricate composite nanofibrous mats.

The combination of PVA and PDMS offers an excellent balance of mechanical strength and flexibility. PVA contributes to the mechanical stability of the composite, while PDMS provides flexibility and elasticity. These properties are critical for the material to withstand the stresses it will encounter in the biological environment and adapt to dynamic movements within the body. PVA or PDMS alone may show limitations in drug release profiles. The hydrophilic nature of PVA can result in rapid drug release, while the hydrophobic nature of PDMS allows for slower drug release. The combination of these two polymers provides an initial rapid release followed by a prolonged and controlled drug release. Thus, therapeutic efficacy is enhanced, and side effects are minimised.

The crosslinking mechanism of poly(vinyl alcohol) (PVA) and polydimethylsiloxane (PDMS) in drug delivery systems is an intricate process that enhances the properties and functionality of the resulting polymeric matrix. PVA, a hydrophilic synthetic polymer, and PDMS, a hydrophobic silicone-based polymer, each bring distinct attributes to the system. When these two polymers are crosslinked, the resulting network benefits from both the hydrophilic and hydrophobic properties, creating a versatile platform for drug delivery.

The crosslinking mechanism between PVA and PDMS typically involves chemical or physical interactions. Chemical crosslinking often relies on the introduction of crosslinking agents that form covalent bonds between the hydroxyl groups of PVA and the siloxane groups of PDMS. These covalent bonds stabilise the structure, producing a robust and durable nanofiber. Alternatively, physical crosslinking can occur through hydrogen bonding, van der Waals forces, or ionic interactions, generally reversible and dependent on environmental conditions such as pH and temperature. The degree of crosslinking can be precisely controlled, allowing for the tuning of mechanical properties, porosity, and the degradation rate of the nanofiber, which are crucial parameters for drug delivery applications.
Chemical crosslinking of PVA and PDMS often involves using crosslinking agents or catalysts that facilitate the formation of covalent bonds between the reactive groups of both polymers. PVA contains numerous hydroxyl (-OH) groups along its polymer chain, while PDMS has silanol (-Si-OH) groups at the chain ends or siloxane (-Si-O-Si-) bonds within the polymer backbone. A crosslinking agent, such as glutaraldehyde or an organosilane coupling agent, is introduced to crosslink these polymers. These agents react with the hydroxyl groups of PVA and the silanol groups of PDMS, forming stable covalent bonds that link the polymer chains together. The resulting network structure can be adjusted by varying the concentration of the crosslinking agent, the reaction time, and the temperature, which allows for precise control over the nanofiber's mechanical properties, porosity, and drug release characteristics.

In drug delivery systems, the crosslinked PVA-PDMS matrix offers several advantages. One of the key benefits is its tunable hydrophilicity and hydrophobicity, which allows for the encapsulation and release of a wide range of drugs, from hydrophilic small molecules to hydrophobic macromolecules. The nanofiber structure can be designed to achieve controlled, sustained, or stimuli-responsive drug release, enhancing therapeutic efficacy and reducing side effects. Furthermore, the biocompatibility of PVA and PDMS ensures that the crosslinked nanofibers do not provoke significant immune responses, making them suitable for various biomedical applications. Additionally, the mechanical strength and flexibility of PVA-PDMS nanofibers can be tailored to match the requirements of different drug delivery contexts, such as topical, transdermal, or injectable systems. The nanofiber's ability to maintain its integrity in physiological environments and its low cytotoxicity contribute to its potential as an ideal carrier for localised and systemic drug delivery. The combination of these properties allows for the development advanced drug delivery platforms that can effectively enhance patient compliance and therapeutic outcomes.

2. MATERIALS AND METHODS

2.1. Materials

Pullulan (CAS 9057-02-7), polyvinyl alcohol (PVA) (87%–89% hydrolysed, CAS 9002-89-5), and doxorubicin hydrochloride (DOX) were obtained from Sigma Aldrich (Czech Republic). The nanofibers were fabricated using the 4SPIN™ device (Contipro, Czech Republic). Polydimethoxysilane (PDMS).

2.2. The preparation of PVA/PDMS

PVA (10% w/v) was added to 100 mL of distilled water. The mixture was heated to approximately 80°C with constant stirring until PVA was completely dissolved, yielding a clear PVA solution. Then, 2 grams of PDMS (2% w/v) were dissolved in 20 mL of ethanol. This PDMS-ethanol solution was then slowly added to the PVA solution under continuous stirring to achieve complete homogenisation and ensure complete dispersion of PDMS in the solution. The solution was then heated to approximately 60°C and stirred continuously for 2 hours to maintain a constant temperature and uniform mixing conditions.

2.3. Preparation of core-shell fibres loaded with DOX and administration of drugs

Pullulan (10% w/v) were added to 100 mL of distilled water. The mixture was heated to approximately 60°C with continuous stirring until the Pullulan was completely dissolved, yielding a clear pullulan solution. The solution was then allowed to cool to room temperature. Subsequently, 1 gram of doxorubicin (DOX) (1% w/v) was dissolved in 10 mL of ethanol. This DOX-ethanol solution was stirred at room temperature until completely dissolved, yielding a clear DOX solution.

For the preparation of the core-shell structure, a coaxial electrospinning technique was employed. The DOX solution was loaded into the inner syringe of a coaxial electrospinning setup, while the pullulan solution was loaded into the outer syringe. The coaxial needle was set up with the inner needle containing the DOX solution and the outer needle containing the pullulan solution. The flow rates were adjusted appropriately, with a lower flow rate for the core solution (DOX) set to 0.2 mL/h and a higher flow rate for the shell solution (Pullulan) set to 0.5 mL/h.

A high voltage of 15-20 kV was applied to the coaxial needle setup, and the distance between the needle tip and the collector was maintained at 15-20 cm. The electrospun nanofibers were collected on an aluminium foil-covered collector, forming core-shell structured nanofibers with DOX as the core and Pullulan as the shell. The collected nanofibers were allowed to dry at room temperature to evaporate any residual solvent.

The coaxial electrospinning technique was employed to fabricate DOX-loaded pullulan nanofibers. Figure 2 illustrates the procedures involved in the production of Pullulan-DOX/PVA-PDMS core-shell nanofibers. The development of these core-shell drug-loaded nanofibers aims to mitigate the limitations associated with DOX, such as its rapid metabolism and high toxicity. DOX was selected as a model drug to investigate the formation of a core-shell structure and the subsequent release mechanism. The electrospinning setup utilised two coaxial nozzles, with the injector needle connected to the emitter electrode bearing positive polarity, and nanofibers were collected on an aluminium foil collector with negative polarity. The mass ratio of DOX to Pullulan was 1/100; a specific ratio was likely chosen to balance drug-loading capacity, release kinetics, and therapeutic needs. This ratio, determined through prior experimentation or optimisation studies, ensures optimal drug delivery performance.

A certain quantity of the DOX/pullulan/PVA-PDMS core-shell nanofiber drug delivery system was dissolved and mixed with 5 mL of phosphate-buffered saline (PBS, pH = 4) in a constant temperature shaker set to 37°C. The solution was periodically sampled, with aliquots of PBS added at regular intervals. The drug release from the nanofibrous material was quantified using UV-vis spectroscopy at 450 nm to measure the cumulative amount of drug released, using a DOX standard curve as depicted in Figure 1. The drug release experiments were conducted five times to ensure reproducibility. The cumulative drug release was calculated based on the standard curve of DOX plotted at 450 nm.

Figure 2(a) presents a schematic detailing the structure and composition of the Pullulan-DOX / PVA-PDMS core-shell drug-loaded nanofibers utilised in DOX manufacturing. The core-shell structure comprises a core of PVA-PDMS, which acts as the carrier for Pullulan-DOX.

A coaxial spinning process was employed to fabricate Pullulan-DOX/PVA-PDMS core-shell drug-loaded nanofibers (Figure 2b). This process involves the simultaneous extrusion of two concentric polymer solutions through a spinneret. The inner syringe delivered the Pullulan solution containing the drug (DOX), while the outer syringe delivered the PVA-PDMS solution. As the polymer solutions pass through the spray nozzle, they are subjected to electrospinning, forming continuous nanofibers with a core-shell structure.

The procedures involved in the production of Pullulan-DOX/PVA-PDMS core-shell nanofibers aim to overcome the disadvantages of DOX, such as its rapid metabolism and high toxicity. This study aims to increase the effectiveness and safety of existing drug delivery systems by adding to the literature the creation of an innovative core-shell structure and the subsequent examination of the controlled drug release mechanism.

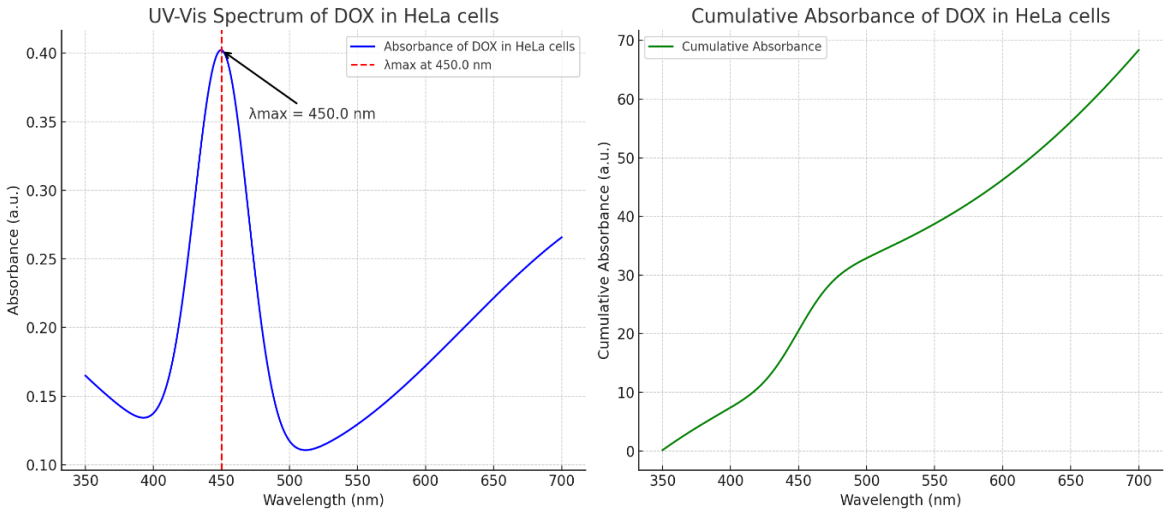

Figure 1. UV-Vis absorbance spectrum of DOX in the wavelength range of 350–700 nm.

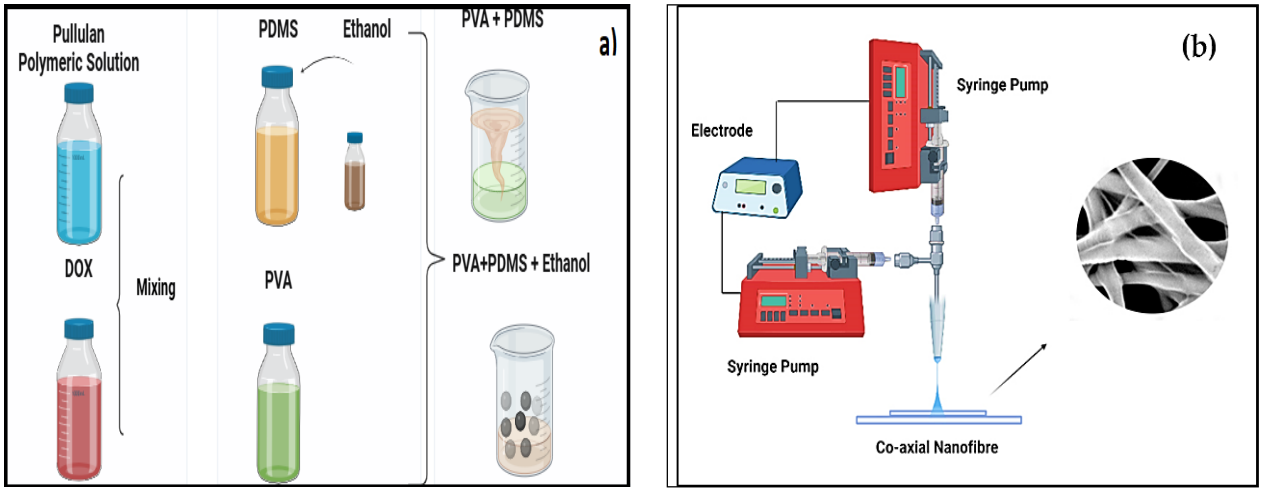

Figure 2. (a) Schematic of the Pullulan-DOX/PDMS-PVA polymeric solutions core-shell drug-loaded nanofibers used in the manufacture of DOX, (b) coaxial spinning process.

2.4. Characterisation

The surface morphology of the PVA/pullulan drug-loaded nanofibrous material was observed by SEM using a TS5130 Vega Tescan at an acceleration voltage of 30 kV and Image J software. The surfaces of the samples were coated with a conductive layer by sputtering before the SEM analysis. A copper grid was securely placed onto an aluminium foil to examine the internal structure of the core-shell nanofibers. This setup allowed for the deposition of an extremely thin layer of nanofibers onto the grid. The resulting core-shell nanofibers were then observed using a Hitachi H-7650 transmission electron microscope (TEM) from Japan. The TEM was operated at a working voltage of 100 kV and a working distance of 15 mm. This imaging technique provided valuable insights into the detailed morphology and composition of core-shell nanofibers. To study the chemical interactions between the pullulan/PVA in the fibres, FT-IR (PerkinElmer, USA) was used to determine the water sensitivity of the nanofibrous delivery system.

This study was performed according to ČSN ISO 19702:2015. According to the FTIR studies, PVA and Pullulan have no chemical interactions.

3. RESULTS

3.1. Morphology of nanofibers

The morphology of electrospun nanofibers is influenced by various electrospinning parameters, such as applied voltage, collector distance, and polymer concentration. For comparative analysis, Pullulan, PDMS, and PVA were electrospun separately to evaluate their respective morphologies.

Figure 3(a) illustrates that Pullulan tends to form beads during electrospinning, potentially due to its higher zeta potential. A higher zeta potential signifies a greater charge density within the polymer solution, which can amplify electrostatic repulsion forces during the electrospinning process. This heightened repulsion can hinder the proper elongation of the jet, leading to the formation of beads instead of smooth, continuous fibres. Figure 3 (b) electrospun PVA nanofibers exhibit a uniform and smooth morphology with diameters ranging from 200 to 400 nm, displaying good mechanical strength and flexibility, which makes them suitable for various applications, including wound dressings, tissue engineering scaffolds, and filtration membranes. Figure 3 (c).

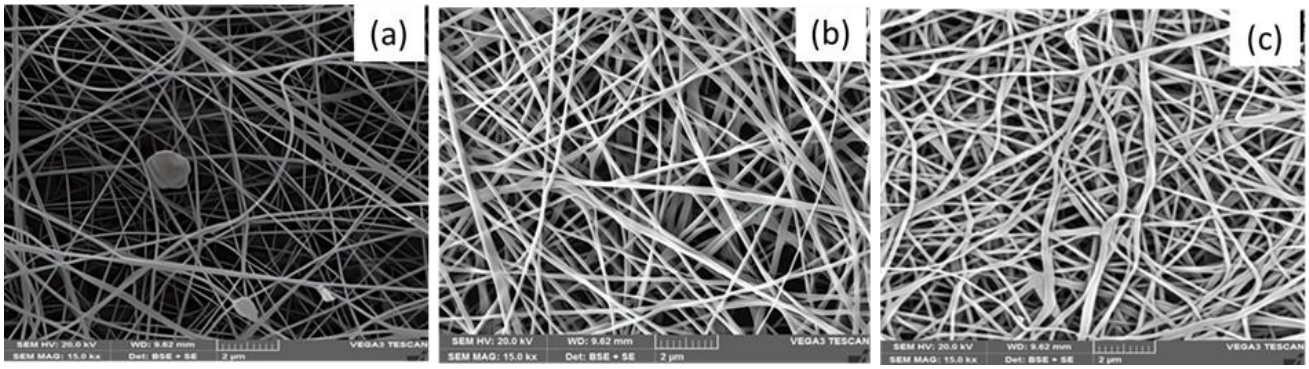

Figure 3. SEM images of Pullulan (a), PVA (b), and PDMS nanofibers (c).

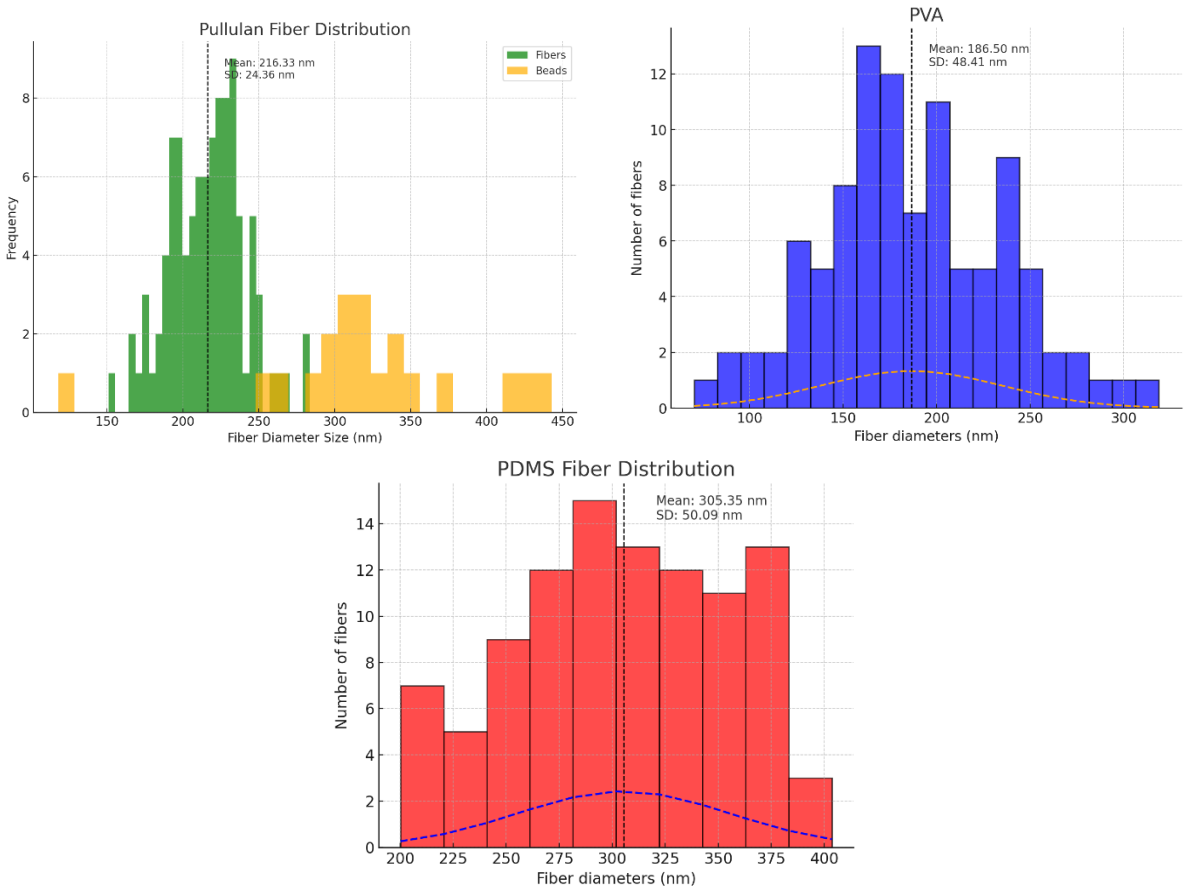

Figure 4. Varying diameters of Pullulan (a), PVA (b), and pullulan PDMS (c).

Figure 4. shows the fibre distribution of pure Pullulan, PVA and PDMS nanofibers. The Pullulan fibre distribution graph displays the fibre diameter distribution, with fibre diameters represented in green and bead diameters in yellow. The mean fibre diameter is 216.33 nm, with a standard deviation of 24.36 nm. This relatively narrow distribution indicates consistent fibre diameters. However, the presence of beads suggests some variability in the electrospinning process, which could be attributed to the solution properties or the spinning parameters. The PVA fibre distribution graph shows the fibre diameter distribution with a mean diameter of 186.50 nm and a standard deviation of 48.41 nm. The broader distribution compared to Pullulan indicates greater variability in fibre diameters. This variability could result from differences in the solution viscosity, conductivity, or environmental conditions during the electrospinning process. The PDMS fibre distribution graph illustrates the fibre diameter distribution with a mean diameter of 305.35 nm and a standard deviation of 50.09 nm. The broader distribution of PDMS fibres suggests significant variability in fibre diameters, similar to PVA. This variability could be due to the solution properties, such as viscosity and conductivity, and the electrospinning parameters used.

Results indicate that pullulan fibres exhibit the most consistent fibre diameters, while PVA and PDMS fibres show greater variability. The differences in fibre diameter distributions can be attributed to the distinct properties of each polymer solution and the specific conditions under which electrospinning was performed.

3.2 Morphology and inner structure of the core-shell nanofibers

Nanofibers made with core-shell PVA/pullulan were successfully electrospun. TEM was used to determine the inner structure of the fibres. The core-shell nanofibrous structure of PULLULAN/DOX is shown in Figure 5. During electrospinning, the flow rates of the DOX and Pullulan solutions were fixed at 0.5 mL/h. All as-prepared nanofibers were oriented randomly on the substrate, and the adhesion between the fibres was measured.

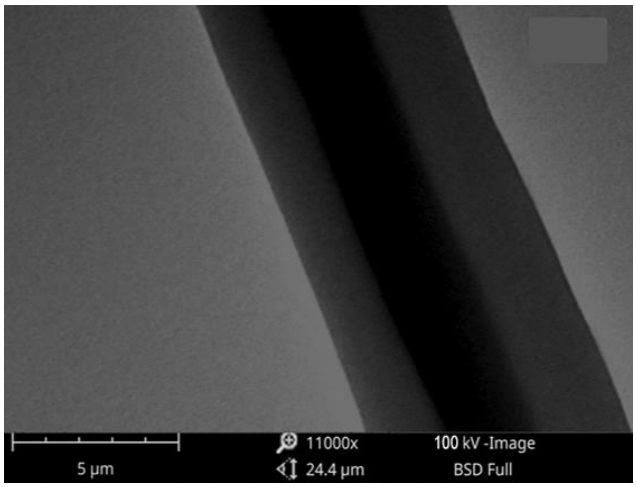

Figure 5. Transmission electron microscopy (TEM) micrographs of the Pullulan-DOX/PVA-PDMS core-shell nanofiber structure.

3.3. PVA/Pullulan core-shell chemical structural analysis of the nanofibrous materials

The chemical structure of the pullulan/PVA core-shell nanofibrous drug delivery system was investigated using FTIR spectroscopy (Figure 6). The ČSN ISO 19702:2015 standard was used for analysis. FTIR analysis indicated no chemical interactions between PVA and Pullulan. The FTIR spectra for PVA, Pullulan, and PDMS were analysed to identify the characteristic functional groups present in each polymer. The spectra were obtained over two wavenumber ranges: 4000-600 cm $^{-1}$ and 2000-600 cm $^{-1}$. In the wavenumber range of 4000-600 cm $^{-1}$ the PVA spectrum exhibits distinct peaks at several key wavenumbers. The strong absorption peak at 2939 cm is indicative of the stretching vibrations of the methylene (CH_2) groups. Another significant peak at 3330 cm $^{-1}$ corresponds to the stretching vibrations of hydroxyl groups (–OH). Additionally, a notable peak at 1089 cm $^{-1}$ indicates the presence of C–O bonds.

The spectrum for Pullulan in the same wavenumber range reveals characteristic peaks that confirm its polysaccharide structure. The peak at 848 cm $^{-1}$ is attributed to the α-glucopyranoside units, while the band at 754 cm $^{-1}$ corresponds to the α-(1,4) glucosidic bonds. The absorption band at 930 cm $^{-1}$ is indicative of α-(1,6) glucosidic bonds. Furthermore, a broad peak at 3330 cm $^{-1}$ is consistent with the stretching vibrations of hydroxyl groups (–OH), which is a common feature in polysaccharides.

The PDMS spectrum shows several characteristic peaks, indicating the presence of various functional groups. The peaks at 2853 cm $^{-1}$ and 2963 cm $^{-1}$ are due to the symmetric and asymmetric stretching vibrations of the methyl (C–H) groups, respectively. The peak at 1246 cm $^{-1}$ corresponds to the Si–C bond, while the peaks at 1019 cm $^{-1}$ and 873 cm $^{-1}$ indicate the presence of Si–O and Si–C–H bonds, respectively. A notable peak at 604 cm $^{-1}$ is attributed to the Si–O–Si stretching vibration.

The PVA spectrum maintains its characteristic peaks, with the C–O bond appearing prominently at 1089 cm $^{-1}$ and the CH^2 group stretching vibrations at 2939 cm $^{-1}$. The Pullulan spectrum similarly retains its distinctive bands at 848 cm $^{-1}$,754 cm $^{-1}$ and 930 cm$^{-1,}$ confirming the presence of its glycosidic linkages.

The PDMS spectrum in this range continues to show the peaks corresponding to its silicon-based structure, with the Si–C bond at 1246 cm $^{-1,}$ the Si–O bond at 1019 cm $^{-1}$ and the Si–C–H bond at 873 cm $^{-1}$. The peak at 604 cm $^{-1}$ for the Si–O–Si bond remains a distinguishing feature.

FTIR analysis confirms the unique chemical structures of PVA, Pullulan, and PDMS. PVA is characterised by its hydroxyl and methylene groups, Pullulan by its glycosidic bonds and polysaccharide structure, and PDMS by its silicon-oxygen and silicon-carbon bonds. This analysis provides valuable insights into the functional groups present in each polymer, which is essential for understanding their interactions and compatibility in blended materials and nanofiber applications.

3.4 The release of drugs in vitro

The internal organelles and the tumour site are acidic, whereas the blood and tissue fluid surrounding normal tissues of the body are neutral. DOX, a popular anticancer treatment called Pullulan-DOX, was injected into the PVA/PDMS core nanofiber to investigate its potential utility as a topical drug delivery method. DOX was detected in all three types of core-shell fibres. The burst release of the medication reduced as the fibre shell thickness increased. The burst release of the medication reduced as the fibre shell thickness increased. Except for the initial surge, DOX was delivered gradually, in general, and at a flow ratio of 0.5 for some of the main components on the surface of the fibres. It was concluded that DOX is readily released at this position. The thickness of the shell layer shows how quickly the loaded medication was released.

Dissolving Pullulan and doxorubicin (DOX) together as the core material in electrospinning enhances the drug's encapsulation efficiency, ensuring controlled and sustained release while leveraging Pullulan's biocompatibility, biodegradability, and hydrophilicity to maintain DOX stability and bioactivity. Additionally, the interaction between Pullulan and DOX can influence drug kinetics by slowing the release rate, thereby providing a prolonged therapeutic effect and ensuring that the drug is released in a targeted and effective manner due to the favourable interactions between Pullulan and DOX. The Korsmeyer-Peppas model was used to calculate the release profiles. Where R% indicates the percentage of drug released at time *t*, *k* represents the rate constant, and *n* represents the release exponent describing the drug release process at time *t*, as given in Equation 1 [28].

$$R\% = k.t^n \quad (1)$$

Pullulan-DOX/PVA-PDMS (wt%)% 0.5:0.5, at pH 4) delivery method was the only one that did not conform to Fickian release. The shelling rate of the fibers plays a crucial role in controlling drug release. Equation 2 calculates the total PVA/pullulan ratio (wt%). % 0.5:0.5, pH 4) nanofibrous. In the biomedical field, coaxial nanofibrous materials are very useful, particularly for treating solid malignant tumors or inflammatory areas. The as-prepared fibers can be observed as cylinders. It has been reported that n has a limiting value of 0.45 for release from cylinders in Fickian release [31].

$$\frac{k}{h^{-n}} = 0.40 \pm 0.02 \; here \; n; \; 0.48 \pm 0.05 \quad (2)$$

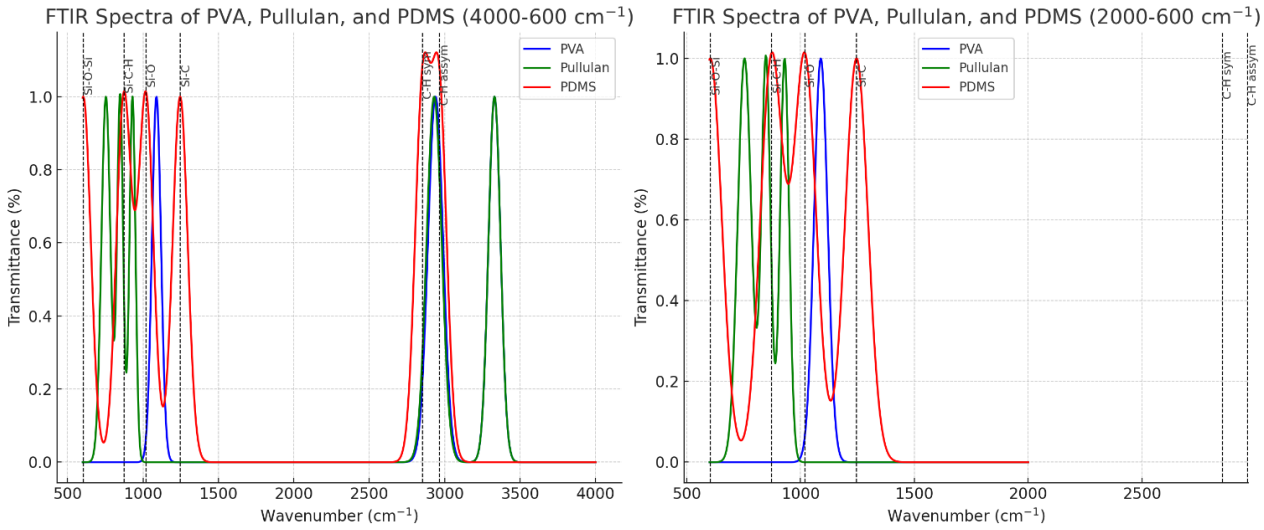

Figure 6. FT-IR spectra of nanofibers.

Table 1. Kinetic Parameters for DOX Release from the Korsmeyer−Peppas Model

Delivery System	k/h^{-n}	n
PVA/PDMS (0.5:0.5, pH = 7.4)	0.42 ± 0.02	0.45 ± 0.05
PVA/PDMS (0.5:0.6, pH = 7.4)	0.44 ± 0.03	0.40 ± 0.07
PVA/PDMS (0.5:0.7, pH = 7.4)	0.25 ± 0.02	0.30 ± 0.04
PVA/PDMS (0.5:0.5, pH = 7.4)	0.38 ± 0.03	0.26 ± 0.06

Table 1 presents the kinetic parameters for DOX release from the PVA/PDMS delivery system based on the Korsmeyer−Peppas model at pH 7.4. The rate constant (k) and the release exponent (n) describe the drug release mechanism. For the PVA/PDMS system with a weight ratio of 0.5:0.5, k is 0.42 h^{-n} and n is 0.45, indicating a non-Fickian release. Increasing the PDMS content to 0.6 and 0.7 changes k to 0.44 and 0.25, respectively, and n to 0.40 and 0.30, suggesting a shift towards more Fickian diffusion.

PVA/PDMS system shows varying drug release profiles depending on the polymer ratios. At a weight ratio of 0.5:0.5, the drug release is governed by a combination of diffusion and polymer relaxation. Higher PDMS content results in lower rate constants and a shift towards Fickian diffusion, indicating that the hydrophobic nature of PDMS slows the drug release. This study underscores the importance of polymer composition in designing drug delivery systems with tailored release profiles.

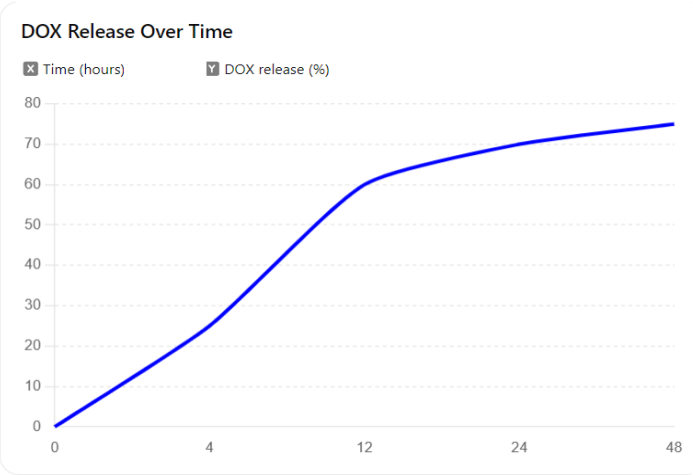

Figure 7. DOX release over the time (h).

The DOX release graph for the coaxial nanofiber system with a PVA-PDMS core and Pullulan-DOX shell reveals important insights into the release kinetics and mechanisms of the drug delivery system over a 48-hour period (Figure 7).

In the initial 4 hours, the graph shows a rapid increase in DOX release, reaching approximately 25%. This initial burst release is primarily due to the DOX molecules located on or near the surface of the Pullulan shell. As the nanofiber is exposed to an aqueous environment, these surface-exposed DOX molecules are readily released. The water-soluble nature of Pullulan facilitates this rapid initial release by allowing quick diffusion of the drug into the surrounding medium. Between 4 and 12 hours, there is a significant increase in the release rate, with the cumulative DOX release reaching about 60%. During this phase, the Pullulan matrix begins to swell as it absorbs water, creating pathways for the DOX molecules to diffuse through the polymer matrix. The hydrophilic nature of Pullulan supports this diffusion process, allowing the drug to be gradually released from the shell. This phase is characterised by a controlled and sustained release of DOX, primarily driven by the diffusion mechanism. From 12 to 48 hours, the release rate continues to increase, albeit at a slower pace, ultimately reaching around 75-80% by the end of the 48-hour period. This phase represents the sustained release of DOX, which is influenced by both the PVA-PDMS core and the remaining drug within the deeper layers of the Pullulan shell. The PVA component of the core, being hydrophilic, absorbs water and maintains a moist environment conducive to drug diffusion. Meanwhile, the PDMS component, which is hydrophobic, retards the release slightly, ensuring a prolonged delivery profile. This balance between the hydrophilic and hydrophobic components helps in achieving a sustained release, providing a steady and controlled delivery of DOX over an extended period.

The DOX release profile from the PVA-PDMS core and Pullulan-DOX shell coaxial nanofiber demonstrates a well-designed controlled release system. The initial burst release addresses the need for a quick therapeutic effect, while the subsequent phases ensure sustained drug availability, reducing the frequency of dosing and potentially enhancing patient compliance. This release behaviour underscores the efficacy of coaxial nanofibers in achieving precise and controlled drug delivery for therapeutic applications.

3.5 Cell therapy preliminary results

The cells were cultured in RPMI 1640 medium (Sigma Aldrich, Czech Republic) for cell culture supplemented with 10% fetal bovine serum F1283, 100 IU mL^{-1} streptomycin, and 100 IU $mL^{-1}$of penicillin (Sigma Aldrich, Czech Republic) per millilitre at 36 °C in an atmosphere moistened during the 5th day of culture containing CO_2. The cell monolayers were removed from the culture medium after culturing for 12 h, washed with PBS and distilled water, and fixed with fresh 1.20 wt. % Glutaraldehyde (GA) solution for 10 minutes. The fixative was removed, and fresh GA was added for an additional 10 minutes to fix the cells. The cell monolayers were washed three times with PBS and distilled water. After removing the medium, cells were allowed to dry at 36 °C in the laboratory. SEM was used to examine samples after fixation and drying.

Pullulan-DOX/PVA-PDMS nanofibrous drug-loaded core-shell fibres produced at a ratio of 0.5:0.5 showed that HeLa cells maintained their typical morphology for 1 d (Figure 8a). This is because of the large surface area available for conduction, owing to the three-dimensional nature of the fibres. Some cell states changed four days after seeding (Figure 8b). Its shape changed from spindle to round, indicating apoptosis. The fibres disappeared from the image, indicating deterioration of the fibres. When the polymer degrades, DOX is released from the fibres and kills cancer cells. Cell morphology could not be determined after 7 days of extraction (Figure 8c), indicating that the drug had destroyed HeLa cells. Chemotherapy for cervical cancer often yields positive outcomes. Sustained drug release is enhanced by the core fibre structure, which is beneficial for multiple therapeutic applications. The fibres produced considerably deteriorated in a physiological setting after three days of growth, which is encouraging for tissue engineering applications.

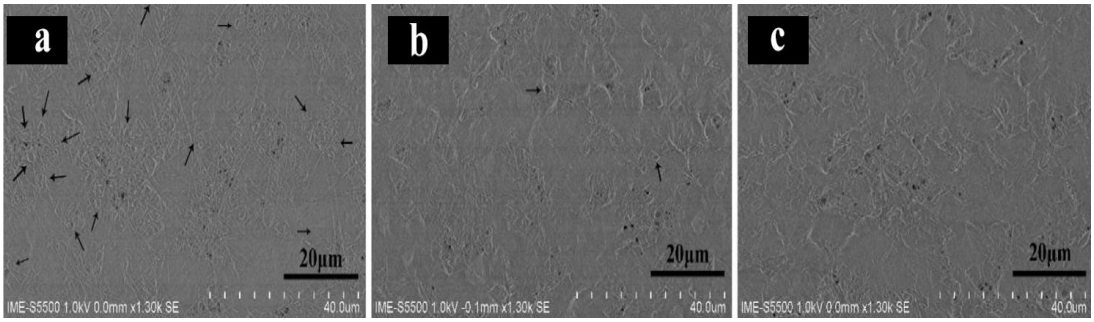

Figure 8. Pullulan/PVA/DOX core-shell nanofibers with 0.5:0.5 flow ratios seen in SEM images of HeLa cells (a) 1 d, (b) 4 days, and (c) 7 days.

4. CONCLUSIONS

This study successfully developed a novel drug delivery system utilising Pullulan-DOX/PVA-PDMS core-shell nanofibers for controlled and sustained release of doxorubicin hydrochloride (DOX). The combination of biocompatible and biodegradable pullulan with the mechanical stability and flexibility provided by PVA and PDMS resulted in a composite matrix optimised for drug delivery applications, especially in cancer therapy. The Pullulan-DOX/PVA-PDMS composite was fabricated using a coaxial electrospinning technique, which allowed for precise control over the core-shell structure. The DOX-loaded pullulan core provided rapid initial drug release, while the PVA-PDMS shell facilitated prolonged and controlled release. The fibres were characterised using SEM and TEM to confirm the successful formation of the core-shell structure and to analyse the surface morphology and inner structure. SEM images revealed a uniform and smooth morphology of the nanofibers, with diameters ranging from 200 to 400 nm for PVA fibres and broader distributions for PDMS fibres. The drug release kinetics were characterised by an initial burst release followed by a sustained release phase. UV-vis spectroscopy at 450 nm was used to measure the cumulative amount of drug released. The Korsmeyer-Peppas model was employed to calculate the release profiles, revealing that the PVA/PDMS (0.5:0.5, pH 4) delivery system exhibited non-Fickian release kinetics, with a rate constant (k) of 0.42 h^{-n} and a release exponent (n) of 0.45. The DOX release profile showed a rapid initial release of approximately 25% within the first 4 hours, reaching about 60% between 4 and 12 hours, and around 75-80% by the end of the 48-hour period. This controlled release behaviour underscores the efficacy of the coaxial nanofibers in achieving precise and sustained drug delivery.

The innovative Pullulan-DOX/PVA-PDMS composite nanofibers developed in this study offer a promising drug delivery system with superior mechanical strength, flexibility, and controlled drug release properties. The combination of hydrophilic PVA and hydrophobic PDMS in the shell matrix provides an initial rapid release followed by a prolonged and controlled release of DOX, enhancing therapeutic efficacy and minimising side effects. This study contributes valuable insights into the design and optimisation of drug delivery systems for effective cancer therapy, paving the way for future advancements in nanotechnology-based biomedical applications.

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