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Original Research Article

Chitosan/ Polycaprolactone blend electrospun fibers as a novel biodegradable carrier for dipyridamole

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Abstract: Electrospinning is an efficient method to create biodegradable fibrous structures with promising characteristics that allow the entrapment of biomolecules and pharmaceuticals. Local release of drugs, while decreasing any side effects or toxicity, is still challenging in cardiovascular diseases. Hence, great attention is given to nano/ sub-micron fibers which are formulated by electrospinning, as drug delivery systems (DDSs). The main goal of this study was to formulate electrospun fibers and study their morphological and structural properties, as well as, the release kinetics of the embedded pharmaceutical agent. Dipyridamole (DIP), chitosan (CS), and polycaprolactone (PCL) were dissolved in 2, 2, 2-trifluoroethanol (TFE). DIP was selected as a model anti-coagulant agent that can be utilized in cardiovascular diseases to prevent blood clotting. The morphological properties of the fibers were investigated using scanning electron microscopy (SEM). Fiber diameter was accessed using Image J (NIH). The encapsulation efficiency and total cumulative release of DIP was studied by UV-vis spectrometry using standard curves of concentration versus absorbance. The fibers' mechanical properties were investigated using a uniaxial tensile instrument. Submicron cylindrical fibers with increased mechanical properties and a biphasic release kinetics profile were created. Taken together, blend electrospun fibers exhibited some promising characteristics, highlighting their potential as DDS candidates.
Keywords: Dipyridamole, drug delivery system, electrospinning, fibers, chitosan, polycaprolactone

INTRODUCTION

As a cost-effective and versatile technique for the creation of micro- and nano-scaled structures, electrospinning has received world wide attention from the pharmaceutical development research community over the last two decades [1]. Electrospun fibrous mats showcase interesting characteristics to be exploited as carriers in drug delivery applications, such as increased specific surface area and encapsulation efficiency [2]. Regarding different applications, there are various approaches to produce and functionalize the fiber mats to enhance specific characteristics that are desirable. One successful strategy is to utilize polymers with tailor-made characteristics to have a sustained release profile [3]. Other approaches include the use of electrospinning technique variations, such as coaxial or emulsion electrospinning, where two separate polymeric solutions are used to create phase separation and different compartments [4]. The main aim of this study was to formulate blend electrospun fibers able to entrap a model anti-coagulant drug and investigate the structural and mechanical characteristics of the fibers and the release kinetics profile of the pharmaceutical. Dipyridamole (DIP) is an anti-coagulant and anti-proliferative pharmaceutical agent that has been administrated in patients suffering from cardiovascular diseases [5, 6]. Based on our previous findings, DIP was successfully entrapped int polycaprolactone fibers [3, 4]. In addition, the use of a second, more hydrophilic polymer, proved to be beneficial for some fibers' properties [7]. However, we believe that the use of a secondary hydrophilic polymer of natural origin like chitosan (CS) will further improve the fibers' properties and biocompatibility of the DDS.

MATERIALS AND METHODS

Polycaprolactone (PCL) (Mn: 70000-90000), chitosan (CS) (medium MW) and dipyridamole (DIP)(powder,≥98.0%) were purchased from Sigma-Aldrich. 2,2,2-trifluoroethanol (TFE) was purchased from abcr GmbH & Co.KG. All reagents and solvents were of analytical grade.

DIP, CS and PCL were dissolved in TFE for the blend solution, at concentrations of 15mg/ml, 15mg/ml and 150mg/ml, respectively. Electrospinning

was performed at room temperature and relative humidity at a constant flow rate of 4 ml/h, inside an electrical field of 1kV/cm. The samples were collected in a square aluminum collector (grounded), covered with aluminum foil to facilitate their removal. 30min was the normal electrospinning time for each sample. After been removed, the specimens were kept under vacuum for 24h for the remaining solvent to evaporate.

Morphological and structural investigation was conducted using a scanning electron microscopy instrument (SEM) (S3400N, Hitachi) under high vacuum, constant high voltage of 15kV and at various magnifications. The cumulative release experiments were performed at 37 ^{o}C in a phosphate buffered solution (PBS) under sink conditions, inside a water-bath. The cumulative mass of DIP released in prefixed time points was calculated from the absorbance (291 nm) of DIP diffused in the PBS samples using a UV-vis spectrometer (LIBRA S22, Biochrom).To examine the predominant release kinetics mechanism, the experimental data were mathematically fitted using the following equation [8, 9].

$Q = kt^{n}$

Where,

Q is the drug release percentage,

t is the release time,

k is a constant depended on the characteristics of the particles and

n is the release exponent which indicates the mechanism [10].

Cyclic uniaxial tensile tests were conducted with the help of a tensile testing instrument (LM1 Test bench, TA Instruments), equipped with a 200 N load cell. Rectangular, 15^{x}10mm specimens (n = 5) were carefully removed from the main body of the electrospun samples and were tested at 0 - 30% strain, 1 Hz, at room temperature. The applied force and the local principal strain were monitored, and Young's modulus was calculated.

Statistical analysis was performed using Student's t-test and $p<0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

PCL/ CS blends have been used in the past to create fibers with suitable properties for tissue engineering applications [11]. Blend electrospinning of the aforementioned polymers resulted in smooth, cylindrical sub-micron fibers with an average diameter of 563.1±137.34nm, significantly decreased to 513.61±140.57nm ($p=0.027$) after 9 days of incubation inside PBS (Figures 1A & 1C). A considerable amount of drug aggregates was observed in the surface of the fibers (Figure 1B) while after 9 days in PBS there were significantly fewer visible (Figure 1D). Fiber orientation was random while fiber distribution on the surface of the collector was uniform. The decrease in the average diameter can be explained by the erosion of CS inside PBS. Similar findings were previously described [12]. The combination of PCL, CS and DIP resulted in submicron fibers with an average diameter around 500nm, and therefore to an increased surface-to-volume ratio [13]. Nevertheless, polymeric blend solutions used for electrospinning have to be homogenously mixed and kept under appropriate conditions in order to maintain a stable and repeatable process and produce homogenous fiber mats [14].

The average encapsulation efficiency was calculated at 62.96±6.36%, mainly because of increased drug aggregates in the surface of the fibers, as shown in Figure 1 [4].The obtained results from the *in vitro* release experiments highlighted a bi-phasic kinetics profile of DIP with an initial burst release during the first day and a second, more gradual stage until day 9 (Figure 2). The initial burst phenomenon can be once again explained by the drug molecules adsorbed on the fibers 'surface. The possible loss of mass which would also explain the decrease in the fiber diameter can be attributed to the breakdown of the physical bonds between PCL and CS, leading to the formation of pores in the polymeric matrix [15, 16]. Using the equation of Peppas *et al*. we fitted the experimental data. The release exponent was obtained ($\boldsymbol{n}$ = 0.42, $\mathbf{R^2}$= 0.774).The mathematical fitting was not perfect mainly because of the limited number of time points during the first day and therefore produced a poor correlation coefficient. Nevertheless, for a typical cylindrical formulation, a release exponent less than 0.45 corresponds to Fickian diffusion [8, 10], which is typical for PCL formulations [16]. Therefore, we concluded that the cumulative release of DIP was mainly regulated by the diffusion through the polymeric PCL/ CS matrix during the second release stage and mainly by the erosion of CS and the extended wash-out of the adherent DIP aggregates from the fibers' surface, during the first, burst release stage. In both stages, the degradation of PCL is not at all significant, since PCL has been estimated to last for 2-4 years inside the human body or buffer solutions that simulate body fluids and therefore, we did not expected that it would affect the release kinetics [16].

The average Young's modulus values of the electrospun fibers were calculated by performing tensile mechanical tests. For these experiments we also created fibers that did not have any CS in order to investigate the effect of adding CS in the mechanical properties. The average Young's modulus values for PCL and PCL/CS scaffolds were 183.29±25.18MPa and 212.24±31.48MPa, respectively. The obtained results indicated that the addition of CS in the polymeric blend led to a slight increase in the values of Young's modulus, which was not statistically significant ($p=0.14$). The small increase in the mechanical properties can be correlated with a possible altered

microstructure that the addition of CS might have induced [11].

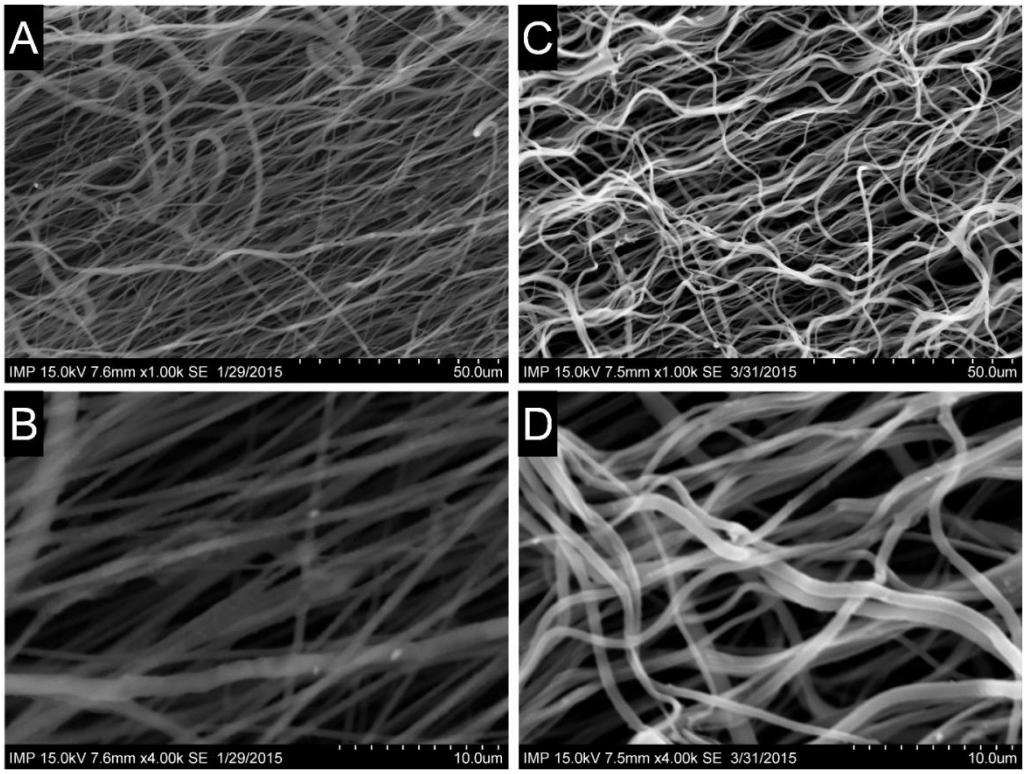

Fig 1(A) Blend electrospun PCL/ CS/ DIP fibers, magnification=1000×, scale bars=50µm; (B) blend electrospun PCL/ CS/ DIP fibers, magnification=4000×, scale bars=10µm; (C) blend electrospun PCL/ CS/ DIP fibers after 9 days in PBS, magnification=1000×, scale bars=50µm; (D) blend electrospun PCL/ CS/ DIP fibers after 9 days in PBS, magnification=4000×, scale bars=10µm.

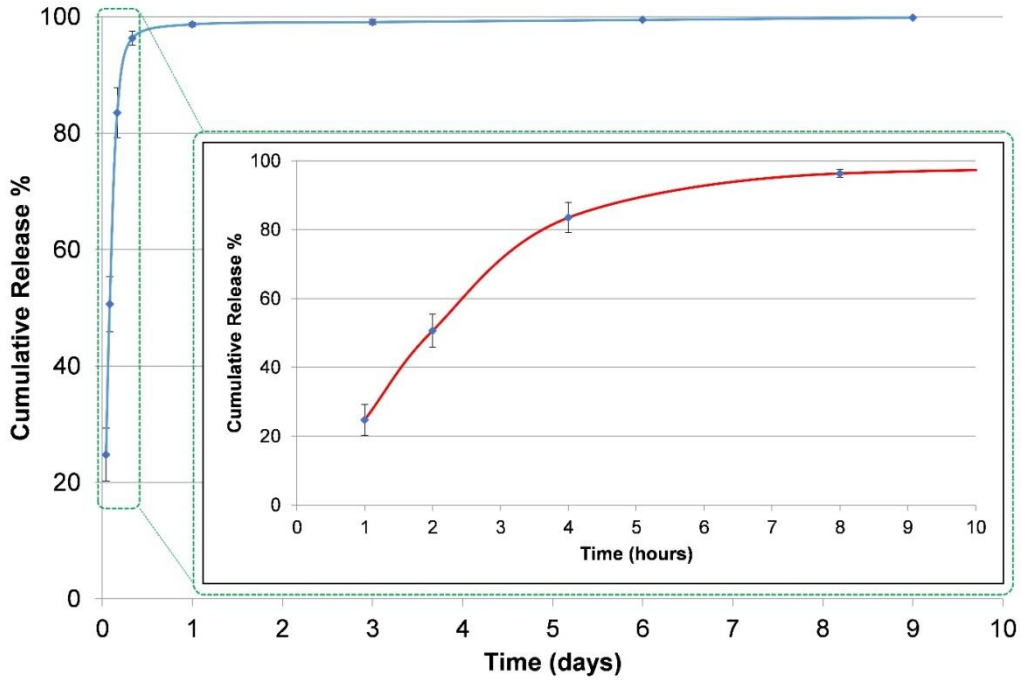

Fig 2: Cumulative in vitro DIP release profiles of PCL/ CS electrospun fibers in PBS (pH 7.4, T=37 ºC) during the first 10h (inset, red) and a total time of 9 days (blue); n=3, mean±SD.

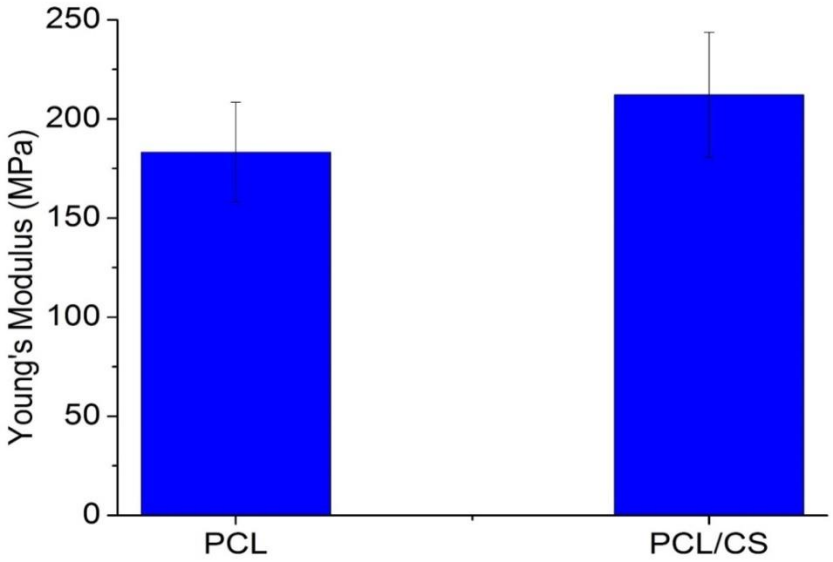

Fig 3: Young's modulus values of PCL and PCL/ CS fibers with DIP; n=5, mean±SD.

CONCLUSION

Blend PCL/CS fibrous mats were fabricated and characterized exhibiting interesting properties as DDS candidates and providing as with useful information for future studies. The PCL/ CS fibers showcased a bi-phasic kinetics profile, determined by CS erosion and Fickian diffusion. In a nutshell, PCL/CS fibrous formulations could be further investigated as a potential DDS for dipyridamole for clinical cardiovascular applications.

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