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Research Article

A dual drug delivery system made from PCL/ PLA electrospun fibers for the encapsulation of anti-coagulant agents

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Abstract: Treatment costs of cardiovascular-related diseases have been predicted to surpass $3\times10^{11}$$ by 2030, according to the American Heart Association. New clinical approaches have been implemented to engineer fibrous materials that can provide efficient local delivery of various pharmaceutical agents, such as anti-coagulants. Electrospinning is a cost-efficient technology that can fabricate biomimetic structures as suitable candidates for drug delivery systems (DDS). A clinically successful combination of anti-coagulants is acetylsalicylic acid (ASA) and dipyridamole (DIP). Aim of this study was to formulate and characterize a dual drug delivery system made by biocompatible and biodegradable polymers, encapsulating the aforementioned pharmaceuticals. Three distinct types of fiber mats were fabricated. ASA only, DIP only and ASA+DIP fibers had an average diameter of 0.84 ± 0.27μm, 1.05 ± 0.34μm, and 0.64 ± 0.2μm, respectively. The cumulative drug release of both pharmaceuticals showcased a bi-phasic profile with an initial burst release and a secondary gradual phase that lasted for more than 90 days. Both anti-coagulants followed a Fickian diffusion release mechanism that was confirmed after fitting of the experimental data. In a nutshell, the obtained results indicate that electrospun PCL/PLA fibers could be used as a DDS in cardiovascular diseases.
Keywords: Acetylsalicylic acid, dipyridamole, DDS, electrospinning, polycaprolactone, polylactic acid.

INTRODUCTION

Life-threatening thrombosis incidents after a stroke or myocardial infarction are very frequent, especially for patients that underwent a severe cardiovascular event and are afterwards recovering from surgery [1]. Novel strategies have been implemented to develop biomimetic materials that can provide local delivery of anti-coagulant and other pharmaceutical agents to the site of need [2, 3]. Electrospinning is an electro-hydrodynamic technology that can engineer non-woven structures, which beyond other possible applications can also be used as drug delivery systems (DDSs) [4, 5]. A successful combination therapy with anti-thrombotic drugs is acetylsalicylic acid (ASA) and dipyridamole (DIP) [6]. The design and characterization of a dual delivery system made by polycaprolactone (PCL) and polylactic acid (PLA), able to encapsulate a model anti-thrombotic agent duo, was the aim of this study. The properties of the fibers as well as the release kinetics of the drugs were examined. Electrospun fibers exhibit unique properties to be used as formulations in drug delivery applications, including high porosity and encapsulation efficiency [7].Based on some of our previous findings, DIP and ASA were successfully entrapped into fibrous formulation for sustained release [8-10]. However, no studies so far have focused on the fabrication and characterization of fibrous structures to encapsulate this combination of anti-coagulants.

EXPERIMENTAL SECTION

Polycaprolactone (PCL) 15wt%, polylactic acid (PLA) 5wt%, Acetylsalicylic acid (ASA)1.5wt% and Dipyridamole (DIP) 1.5wt%, were dissolved in 2,2,2-Trifluoroethanol (TFE). PCL, PLA, ASA and DIP were purchased from Sigma-Aldrich, while TFE was purchased from abcr GmbH & Co.KG. All reagents and solvents were of analytical grade. Polymeric solutions with only one of the pharmaceuticals were also prepared to serve as controls. Electrospinning was performed at a flow rate of 4 mL/h and at an electrical field of 1kV/cm at room temperature. The structural and morphological characteristics of the fibers were studied using a Scanning Electron Microscope (S3400N, Hitachi) under high vacuum at various magnifications.

In order to determine the surface hydrophilicity of the fiber mats an optical contact angle instrument (FM40 Easy drop, Krüss) was used. Square pieces of $1\times1cm^2$ were punched out from each of the different fiber mats to investigate the static contact angles of water droplets on the surface. Measurements were taken at 0s after a single droplet of bi-distilled water (1μL) got in contact with the surface of the

samples, as previously described [9]. All measurements (n=5) were carried out at room temperature.

In vitro drug delivery experiments were performed in PBS (pH=7.4, T=37^{o}C) using a UV-Vis spectrophotometer (LIBRA S22, Biochrom) under sink conditions. The cumulative release of both drugs was calculated and the release mechanism was determined as it was previously described [8, 9].To determine the release kinetics mechanism, the experimental data from the drug release experiments were mathematically fitted using the following equation [11, 12].

$$\mathbf{Q = kt^n}$$

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 [13].

Statistical analysis was performed using one-way ANOVA with post-hoc Tukey via the software Origin Pro 8.5 (Origin Lab Corporation).

DISCUSSION

Blend electrospinning resulted in smooth, cylindrical fibers with average diameter of 0.84 ± 0.27μm, 1.05 ± 0.34μm, and 0.64 ± 0.2μm,for ASA only, DIP only and ASA+DIP fibers. The fibers had random orientations and were uniformly distributed on the surface of the collector (Figure 1). The distribution of values, average and standard deviation of the fiber diameters are depicted in Figure 2. The values from the three distinct fiber mats differed statistically ($p<0.005$), but all followed a normal distribution. The decrease in the average fiber diameter for the ASA+DIP specimens could be explained by a possible increase in the polymeric solution's electrical conductivity because of the incorporation of both DIP and ASA that resulted in extended jet elongation inside the electrical field and thus, to thinner fibers. Similar findings, correlating fiber diameter and solution electrical conductivity, were previously described by several groups [14-16]. The combination of PCL, PLA and the encapsulated drugs resulted in sub-micron scale fibers with average diameters between 500nm and 1.5μm, thus to increased surface-to-volume ratio [5]. Nevertheless, combining polymers with different properties for electrospinning is not always trivial and it is very crucial that a homogenous solution is maintained to achieve a stable and repeatable process [17].

In order to study whether the differences between fibers with only one of the drugs or both encapsulated, or if the different structural properties affected the hydrophilicity of the fiber mats, static water contact assay was performed. No significant difference ($p>0.05$) was detected between the contact angle values for the different specimens, with 104.14 ± 4.31^o, 104.38 ± 4.42^o, 107.8 ± 5.5^o, for fiber mats of ASA, DIP and ASA+DIP, respectively (Figure 3). The hydrophilicity of the fiber mats can influence the rate through which the aqueous media is absorbed by the polymeric matrix during the drug delivery experiments, and therefore influence release kinetics [17, 18]. It was thus important to determine this property.

The results from the cumulative release of ASA and DIP from the three different fiber mats revealed a bi-phasic kinetics profile of both pharmaceuticals, with an initial burst phenomenon during the first hours and a following, more gradual diffusion during more than 90 days (Figure 4). The observed burst phenomenon during the first hours could be explained by the drug molecules close or on the surface of the fibers [8, 9, 13, 18].The bi-phasic kinetics profile is also very typical for PCL formulations [19].After fitting the experimental data from the ASA+DIP specimens, the release exponents (**ASA**: **n** = 0.36, $\mathbf{R^2}$= 0.962; **DIP**: **n** = 0.44, $\mathbf{R^2}$= 0.946) for both drugs were obtained; A release exponent $n \leq 0.45$ for a cylindrical formulation corresponds to Fickian diffusion [11-13]. Therefore, the release of DIP was mainly regulated by the diffusion through the blend polymeric matrix of PCL and PLA. The degradation rates of both PCL and PLA are not significant during this period and therefore do not affect the release kinetics of the drugs [19, 20].

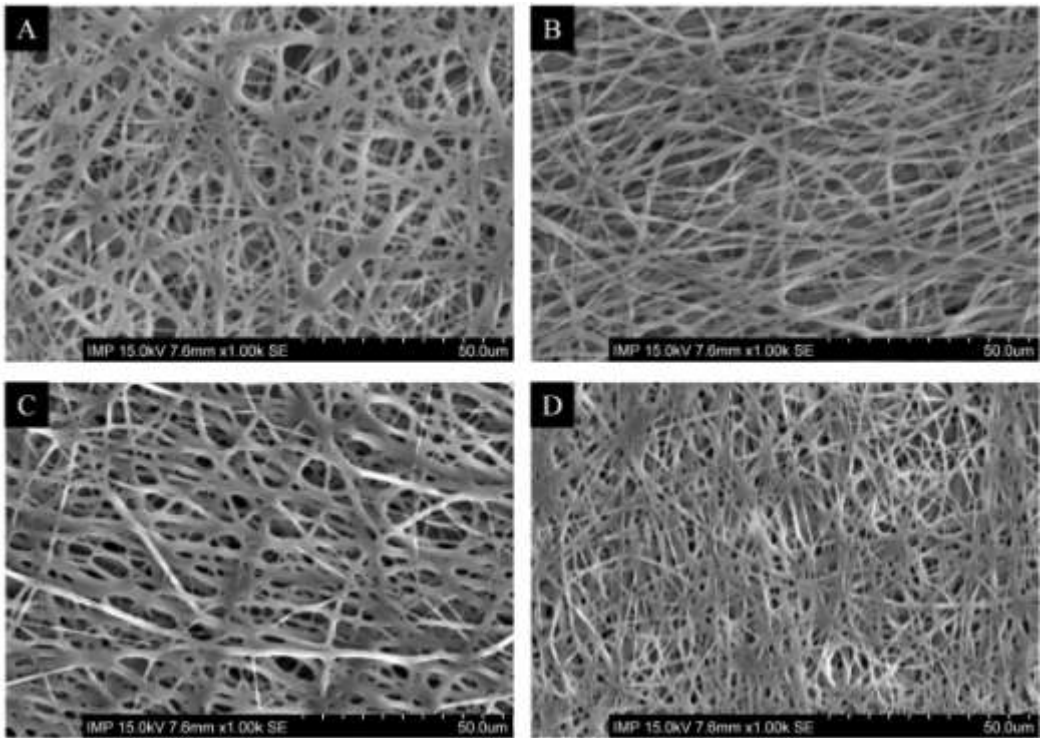

Fig 1: Electrospun empty PCL/ PLA fibers (A); PCL/ PLA fibers with ASA (B); PCL/ PLA fibers with DIP (C);PCL/ PLA fibers with ASA+DIP (D);magnification = 1000×, scale bars = 50µm.

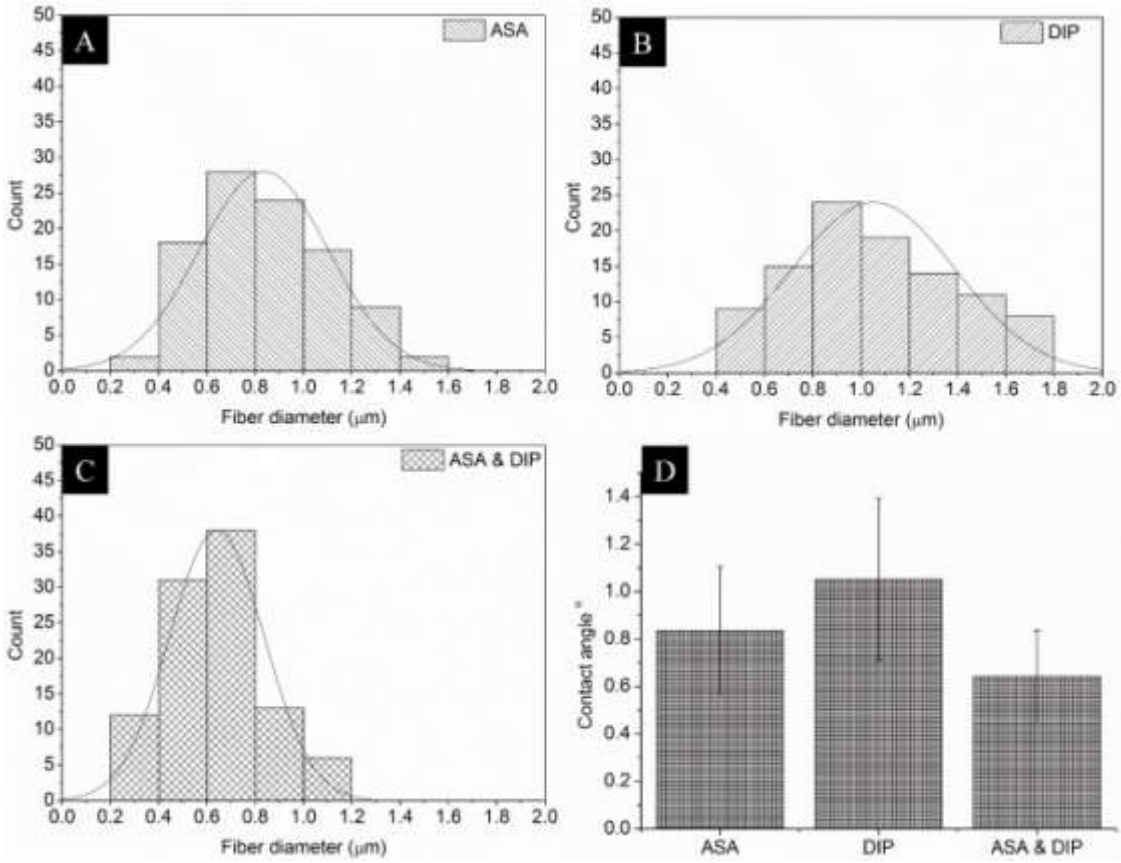

Fig 2: Average fiber diameter and distribution of values for PCL/ PLA fibers with ASA (A); PCL/ PLA fibers with DIP (B); PCL/ PLA fibers with ASA+DIP (C); comparative bar graphs of all specimens (D); n=100, mean ±SD.

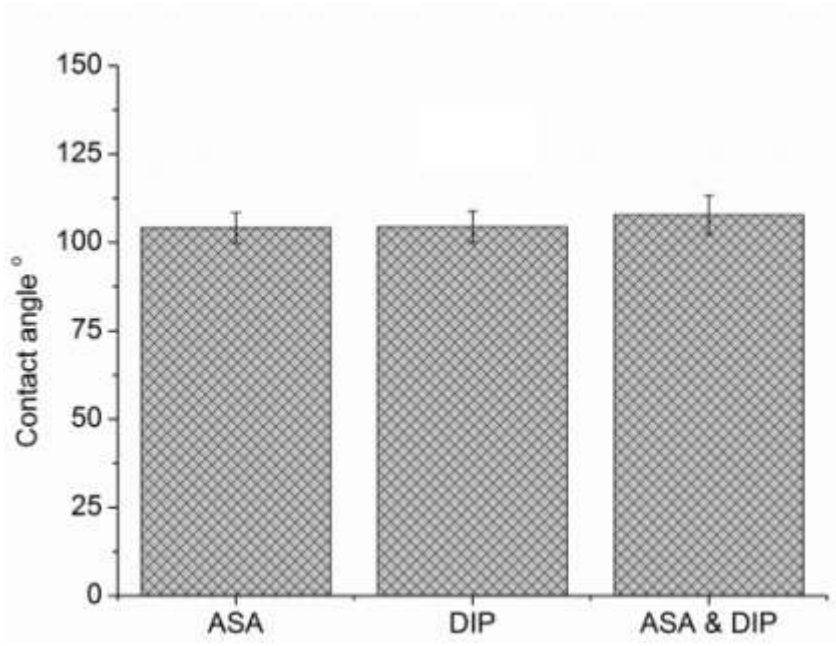

Fig 3: Static water contact angle assay for PCL/ PLA fibrous mats, with ASA, DIP and both drugs; n=5, mean ± SD.

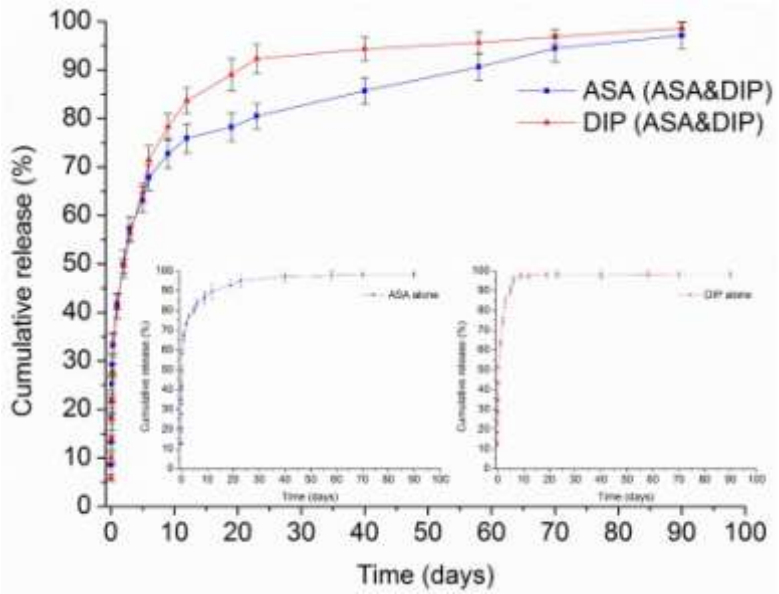

Fig 4: Cumulative in vitro drug release profiles of ASA (blue) and DIP (red) from PCL/ PLA electrospun fibers incubated in PBS (pH=7.4, T=37 ^{o}C) during a total period of 90 days; n =3, mean ± SD.

CONCLUSION

Composite PCL/PLA fibers were designed and characterized as a DDS candidate, through a period of 90 days providing useful input for future studies of these fibers as carriers. The electrospun fibers showcased a bi-phasic release kinetics profile for both ASA and DIP, primarily governed by Fickian diffusion. Taken together, PCL/PLA fiber scould be further studied as potential DDSs of anti-thrombotic agents for clinical applications in the cardiovascular field.

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