Scholars Academic Journal of Biosciences (SAJB) **ISSN 2321-6883 (Online)**
Sch. Acad. J. Biosci., 2016; 4(2):149-153 **ISSN 2347-9515 (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com DOI : 10.36347/sajb.2016.v04i02.012

Case Report

PCL/PEG coaxially spun fibers as a drug delivery system for anti-thrombotic pharmaceutical agents

Alexandros Repanas[1*], Fedaa AL Halabi[1], Sofia Andriopoulou[2], Sotirios Korossis[2], Birgit Glasmacher[1]
[1]Institute for Multiphase Processes, Leibniz Universität Hannover, 30167 Hannover, Germany
[2]Department of Cardiothoracic, Transplantation and Vascular Surgery, Hannover Medical School, 30625 Hannover, Germany

***Corresponding author**
Alexandros Repanas
Email: repanas@imp.uni-hannover.de

Abstract: Electrospinning is an effective technique to prepare non-woven fibrous formulations with interesting properties that enable the encapsulation of various pharmaceutical agents. Targeted delivery of drugs, while preventing any side effects or toxicity, still remains a challenge, especially in the cardiovascular field and therefore, nano/ sub-micron fibers created by electrospinning have been investigated as drug delivery system (DDS) candidates. The aim of the current study was to create coaxially spun fibers and investigate their morphological and biological characteristics, as well as, the release kinetics of the encapsulated pharmaceutical agent. Dipyridamole (DIP), polycaprolactone (PCL), and polyethylene glycol (PEG) were dissolved in 2,2,2-trifluoroethanol (TFE). DIP was chosen as a model anti-thrombotic agent that could be used in cardiovascular diseases to prevent thrombosis. The structural properties of the fibers were assessed with scanning electron microscopy (SEM). The cumulative release of DIP was assessed by UV-vis spectrometry using standard curves of absorbance versus concentration. Biocompatibility experiments were conducted using murine L-929 fibroblasts (Passage 7). Smooth, cylindrical sub-micron fibers were fabricated, providing a sustained bi-phasic release kinetics profile of DIP during an extended period of more than 3 months with an initial burst phenomenon during the first 8h and a subsequent gradual diffusion through the polymeric matrix. No change in the metabolic activity of the fibroblasts in the presence of the fibers extracts was observed from the cytotoxicity assay. In a nutshell, coaxially electrospun fibers exhibited some interesting features, indicating their potential as DDS candidates.
Keywords: Dipyridamole, drug delivery system, electrospinning, fibers, polyethylene glycol, polycaprolactone.

INTRODUCTION

As a cost-efficient and facile method for the production of micro- and nano-structured formulations, electrospinning has gained widespread attention from the biomedical research community over the last decade [1-2]. Electrospun fibers exhibit unique properties to be used as formulations in drug delivery applications, including high porosity and encapsulation efficiency [3]. Depending on the specific application, there are different approaches to create and modify the fibers to have the desired characteristics. One approach is to use polymers with determined properties in order to achieve a suitable release profile [4]. Another approach is to use a specific variation of the electrospinning technique, known as coaxial electrospinning, where two different solutions are used so as to create a core-shell structure [5]. The pharmaceutical can be incorporated only in the solution that form the core compartment to control its release kinetics. The main objective of the current study was to fabricate coaxially spun fibers from a blend of polymers, able to encapsulate a model anti-thrombotic agent and study the properties of the scaffolds as well as the release kinetics of the drug, while investigating any possible toxicity effects of the fibers. Dipyridamole (DIP) is an anti-thrombotic and anti-proliferative agent that has been used in patients suffering from cardiovascular diseases [6 – 8]. Based on previous findings, DIP was successfully entrapped into particle formulation for sustained release [9]. However, only few studies have focused on the fabrication and characterization of the fibrous formulations to encapsulate this pharmaceutical agent [4, 10].

CASE REPORT

Polycaprolactone (PCL) (Mn: 70000-90000) and dipyridamole (DIP)(powder,≥98.0%) were purchased from Sigma-Aldrich. Polyethylene glycol (PEG) (Mw: 10000) was purchased from Fluka. 2,2,2-trifluoroethanol (TFE) was purchased from abcr GmbH & Co.KG. RPMI 1640 (cell culture medium) was purchased by Merck Millipore. All reagents and solvents were of analytical grade.

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

TFE for the shell solution at concentrations of 100 mg/ml and 50 mg/ml, respectively. Coaxially spun fibers without DIP were also created as a control. Electrospinning was performed at room temperature and relative humidity at a constant flow rate of 4 ml/h (1 ml/h core solution and 3 ml/h shell solution), inside an electrical field of 1kV/cm.

Structural and morphological analysis was performed using a Scanning Electron Microscope (SEM) (S3400N, Hitachi) under high vacuum at various magnifications. The drug release experiments were conducted at 37 ^{o}C in a phosphate buffered solution (PBS) under sink conditions. The cumulative amount of DIP released in predetermined time points was calculated from the absorbance (291 nm) of PBS samples using a UV-vis spectrometer (LIBRA S22, Biochrom).

In order to determine the electrical conductivity of the solutions a conductometer (SevenMulti, Mettler Toledo AG) was used. All measurements (n = 5) were carried out at 25 ^{o}C.

To determine the release kinetics mechanism, the experimental data were mathematically fitted using the following equation [11, 12].

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

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].

The cytotoxicity experiments were performed according to the ISO10993-12:2012 with L-929 murine fibroblasts (Passage 7) to assess the biocompatibility of the fibers. An extract cytotoxicity assay was performed in six replicates using cell culture medium supplemented with 80% DMSO as the positive and complete culture medium as the negative control.The metabolic activity of the fibroblasts [evaluation of the adenosine triphosphate (ATP) content] was determined using the ATPlite kit (Perkin Elmer) according to the manufacturer's instructions.

Statistical analysis was performed (One-way ANOVA with post-hoc Tukey, $p<0.05$ statistical significance).

DISCUSSION

Blending polymers with different properties can be very effective in electrospinning to fabricate formulations with desired characteristics. Coaxial electrospinning resulted in smooth, cylindrical sub-micron fibers with an average diameter of 503.48 ± 136.36 nm, significantly decreased ($p<0.001$) compared to fibers without DIP(727.76 ± 284.63 nm) (Figure 1). The fibers had random orientations and were uniformly distributed on the surface of the collector. The decrease in the average fiber diameter could be explained by a possible increase in the polymeric solution electrical conductivity because of the incorporation of DIP that resulted in extended jet elongation inside the electrical field and thus, to thinner fibers. Similar findings were previously described by several groups [14 - 16]. In fact the electrical conductivity of the core polymeric solution significantly increased from 0.521 ± 0.02 μS/cm for PCL-only solution to 18.81 ± 0.06 μS/cm for the solution with DIP ($p<0.001$). The combination of PCL, PEG and the encapsulated drug resulted in very thin fibers with average diameters near 500 nm, thus increased surface-to-volume ratio. However, combining polymers for electrospinning is not always simple and it is quite important that a homogenous solution is prepared to achieve a stable and repeatable process [17].

The results from the cumulative release of DIP revealed a bi-phasic release profile of the drug with an initial burst phenomenon during the first hours and a subsequent gradual sustained release until day 96 (Figure 2). The initial burst phenomenon can be explained by the DIP molecules close or on the surface of the fibers as well as by the possible presence of pores through the polymeric matrix due to PEG erosion inside PBS [9, 13, 18, 19]. The possible loss of mass due to the breakdown of the esteric bonds between PCL and PEG, leads to increased levels of hydrophilicity of the polymeric matrix through time [22]. After fitting the experimental data using the appropriate equation the release exponent ($\boldsymbol{n}$ = 0.42, $\mathbf{R^2}$= 0.928) was obtained; A release exponent $n \leq 0.45$ for a cylindrical formulation corresponds to the Fickian diffusion [11 - 13]. Therefore, the release of DIP was primarily regulated by the diffusion through the polymeric matrix and secondarily by the polymer (PEG) erosion, especially in the first stage, through a biphasic kinetics profile. The degradation of PCL is not significant during this period, and therefore does not affect the release kinetics [20].

In order to investigate any potential cytotoxic effects of the fibers *in vitro*, an extract cytotoxicity assay on fibroblasts was performed. The fibrous mats were previously disinfected using 70% ethanol. The ATP levels of all of the specimens (with/ without DIP) were significantly higher compared to the positive control ($p < 0.05$). Thus, indicating the specimens did not have any cytotoxic effect on the metabolic activity of the L 929 murine fibroblasts (Figure 3).

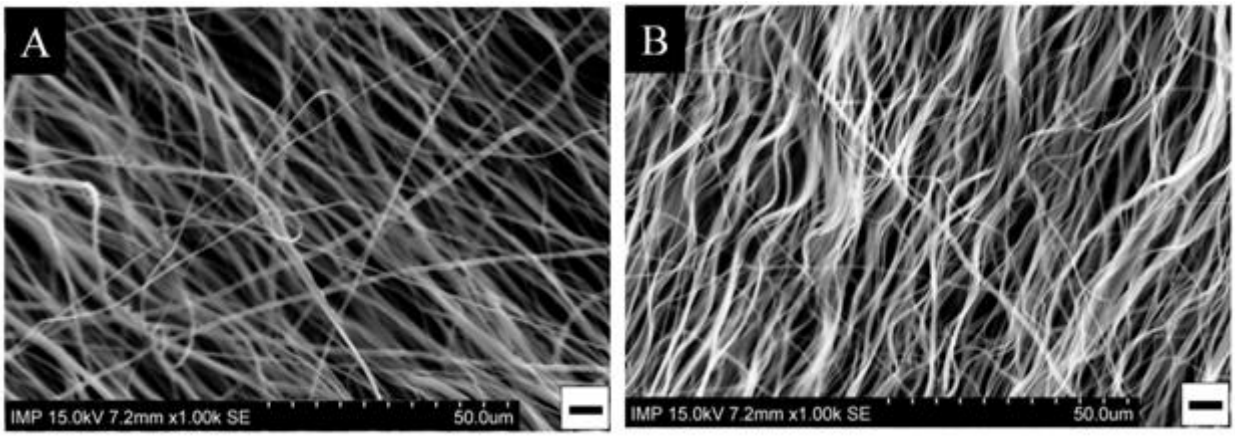

Fig-1:Coaxially spun empty PCL/ PEG fibers (A); coaxially spun PCL/ PEG DIP fibers (B); magnification = 1000×, scale bars = 10 µm.

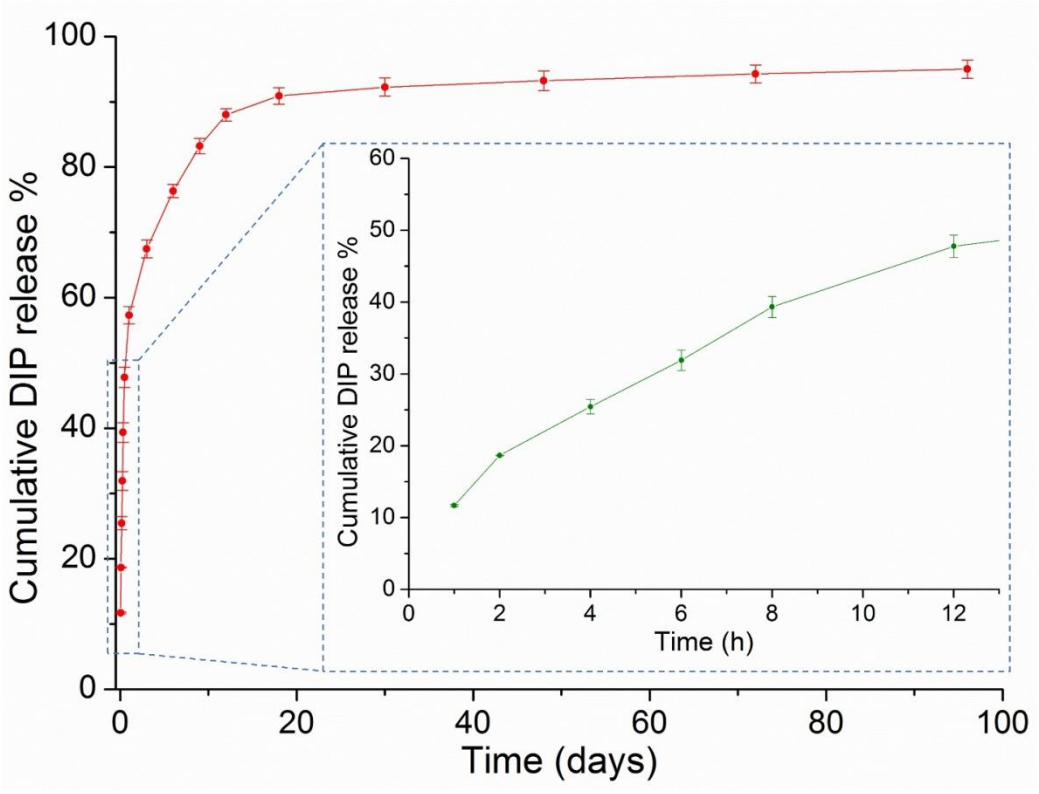

Fig-2: Cumulative in vitro drug release profiles of PCL/ PEG coaxially spun fibers in PBS (pH 7.4, T = 37 ^{o}C) during the first 12h (inset, green) and a total time of 96 days (red); n =3, mean ± SD.

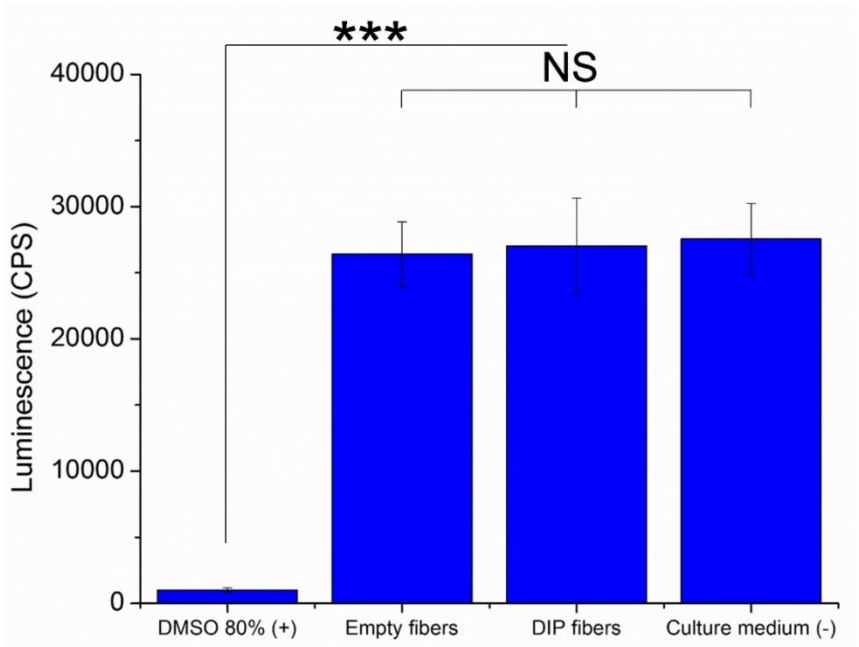

Fig-3: Extract cytotoxicity assay (ISO 10993-12:2012) for PCL/ PEG fibrous scaffolds, with and without DIP; Control (+) = RPMI 1640 supplemented with 80% v/v DMSO; Control (-) = complete culture medium; L-929 murine fibroblasts were used (Passage 7); n = 6, mean ± SD, *** = p<0.001, NS = not significant.

CONCLUSION

Composite PCL/PEG fibers were designed and characterized as candidates for DDSs, through a period of 3 months exhibiting *in vitro* biocompatibility and providing useful input for future studies of these fibers as drug carriers. The coaxially spun fibers exhibited a bi-phasic release kinetics profile, primarily governed by Fickian diffusion, as well as PEG erosion during the initial hours. Taken together, PCL/PEG fibers could be further studied as potential DDSs of anti-thrombotic agents for clinical applications in the cardiovascular field.

Acknowledgements

The authors would like to acknowledge the contribution of Sonia Zia, M.Sc. from the Department of Cardiothoracic, Transplantation and Vascular Surgery, Hannover Medical School, to the cytotoxicity experiments. This study was granted by the Center of Pharmaceutical Process Engineering (PVZ) via the SynFoBIA research project.

REFERENCES

1. Szentivanyi A, Zernetsch H, Menzel H, Glasmacher B; A review of developments in electrospinning technology: New opportunities for the design of artificial tissue structures. International Journal of Artificial Organs, 2011;34(10):986–97.
2. Szentivanyi A, Chakradeo T, Zernetsch H, Glasmacher B; Electrospun cellular microenvironments: Understanding controlled release and scaffold structure. Advanced Drug Delivery Reviews, 2011; 30;63(4–5):209–20.
3. Repanas A, Andriopoulou S, Glasmacher B; The significance of electrospinning as a method to create fibrous scaffolds for biomedical engineering and drug delivery applications. Journal of Drug Delivery Science and Technology, 2016;31:137-146
4. Repanas A, Glasmacher B; Dipyridamole embedded in Polycaprolactone fibers prepared by coaxial electrospinning as a novel drug delivery system. Journal of Drug Delivery Science and Technology, 2015;29:132-142
5. Repanas A, Wolkers WF, Gryshkov O, Kalozoumis P, Müller M, Zernetsch H, *et al*.; Coaxial Electrospinning as a Process to Engineer Biodegradable Polymeric Scaffolds as Drug Delivery Systems for Anti-Inflammatory and Anti-Thrombotic Pharmaceutical Agents. Clinical and Experimental Pharmacology, 2015;5:192-199.
6. Begandt D, Bader A, Gerhard L, Lindner J, Dreyer L, Schlingmann B *et al*.; Dipyridamole-related enhancement of gap junction coupling in the GM-7373 aortic endothelial cells correlates with an increase in the amount of connexin 43 mRNA and protein as well as gap junction plaques. Journal of Bioenergetics and Biomembranes, 2013;45:409–419.
7. Forbes CD; Secondary stroke prevention with low-dose aspirin, sustained release dipyridamole alone and in combination. ESPS Investigators. European Stroke Prevention Study. Thrombosis Research, 1998; 92:S1–S6.
8. Sacco RL, Diener HC, Yusuf S, Cotton D, Ounpuu S, Lawton WA *et al*.; Aspirin and extended-release dipyridamole versus clopidogrel for recurrent

stroke. The New England Journal of Medicine, 2008;359:1238–1251.
9. Zhu W, Masaki T, Bae YH, Rathi R, Cheung AK, Kern SE; Development of a sustained-release system for perivascular delivery of dipyridamole. Journal of Biomedical Materials Research. Part B, Applied biomaterials, 2006;77(1):135–143.
10. Punnakitikashem P, Truong D, Menon JU, Nguyen KT, Hong Y; Electrospun biodegradable elastic polyurethane scaffolds with dipyridamole release for small diameter vascular grafts. Acta Biomaterialia 2014;10(11):4618–4628.
11. Ritger PL, Peppas NA; A simple equation for description of solute release I. Fickian and non-fickian release from non-swellable devices in the form of slabs, spheres, cylinders or discs. Journal of Controlled Release, 1987;5:23–36.
12. Ritger PL, Peppas NA; A simple equation for description of solute release II. Fickian and anomalous release from swellable devices. Journal of Controlled Release 1987;5:37–42.
13. Siepmann J, Peppas NA; Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC). Advanced Drug Delivery Reviews, 2001;48:139–157.
14. Sill TJ, von Recum HA; Electrospinning: Applications in drug delivery and tissue engineering. Biomaterials. 2008;29(13):1989–2006.
15. Cramariuc B, Cramariuc R, Scarlet R, Manea LR, Lupu IG, Cramariuc O; Fiber diameter in electrospinning process. Journalof Electrostatics, 2013;71(3):189–98.
16. Okutan N, Terzi P, Altay F; Affecting parameters on electrospinning process and characterization of electrospun gelatin nanofibers. Food Hydrocolloids, 2014;39:19–26.
17. Pelipenko J, Kocbek P, Kristl J; Critical attributes of nanofibers: Preparation, drug loading, and tissue regeneration. International Journal of Pharmaceutics, 2015;484(1–2):57–74.
18. Kim TG, Lee DS, Park TG; Controlled protein release from electrospun biodegradable fiber mesh composed of poly(ε-caprolactone) and poly(ethylene oxide). International Journal of Pharmaceutics 2007 29;338(1–2):276–83.
19. Zweers MLT, Engbers GHM, Grijpma DW, Feijen J; Release of anti-restenosis drugs from poly(ethylene oxide)-poly(dl-lactic-co-glycolic acid) nanoparticles. Journal of Controlled Release 2006; 114(3):317–324.
20. Woodruff MA, Hutmacher DW; The return of a forgotten polymer—Polycaprolactone in the 21st century. Progressin Polymer Science, 2010; 35(10):1217–56.