Journal of Controlled Release xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

# Amphiphilic beads as depots for sustained drug release integrated into fibrillar scaffolds

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ARTICLE INFO

*Article history:*
Received 10 April 2014
Accepted 21 April 2014
Available online xxxx

*Keywords:*
Electrospinning
Drug release
Human mesenchymal stem cells
Fibrous scaffolds
Amphiphilic polymer

ABSTRACT

Native extracellular matrix (ECM) is a complex fibrous structure loaded with bioactive cues that affects the surrounding cells. A promising strategy to mimicking native tissue architecture for tissue engineering applications is to engineer fibrous scaffolds using electrospinning. By loading appropriate bioactive cues within these fibrous scaffolds, various cellular functions such as cell adhesion, proliferation and differentiation can be regulated. Here, we report on the encapsulation and sustained release of model hydrophobic drug (dexamethasone (Dex)) within beaded fibrillar scaffold of poly(ethylene oxide terephthalate)–poly(butylene terephthalate) (PEOT/PBT), a polyether–ester multiblock copolymer to direct differentiation of human mesenchymal stem cells (hMSCs). The amphiphilic beads act as depots for sustained drug release that is integrated into the fibrillar scaffolds. The entrapment of Dex within the beaded structure results in sustained release of drug over the period of 28 days. This is mainly attributed to the diffusion driven release of Dex from the amphiphilic electrospun scaffolds. *In vitro* results indicate that hMSCs cultured on Dex containing beaded fibrillar scaffolds exhibit an increase in osteogenic differentiation potential, as evidenced by increased alkaline phosphatase (ALP) activity, compared to the direct infusion of Dex in a culture medium. The formation of a mineralized matrix is also significantly enhanced due to the controlled Dex release from the fibrous scaffolds. This approach can be used to engineer scaffolds with appropriate chemical cues to direct tissue regeneration.



## 1. Introduction

Native extracellular matrix (ECM) is a complex fibrous structure that provides physical, chemical, and mechanical cues to direct cellular processes [1–5]. A promising strategy to mimicking native tissue architecture is to engineer fibrous scaffolds using electrospinning (ESP) techniques [6]. By incorporating appropriate topographical or therapeutic/bioactive cues within the fibrous scaffolds, various cellular processes can be controlled to facilitate the formation of musculoskeletal tissues [7–9]. For example, these fibrous scaffolds could find applications as bone fillers, in non-load bearing defects such as skull defects, or as bone membranes such as in the case of periosteum regeneration [7–9]. Electrospun scaffolds composed of hydroxyapatite/chitosan have shown to promote new bone regeneration *in vivo* by activating integrin and BMP/Smad signaling pathway [10]. Fibrous membranes composed of gelatin/polycaprolactone have shown to promote *in vitro* and *in vivo* cartilage tissue regeneration [11]. In a similar study, fibrous scaffolds made from poly(L-lactide-co-ε-caprolactone)/collagen (P(LLA-CL)/Col) stimulate differentiation of tendon-derived stem cells when subjected to mechanical stimulation [12].

Even when load bearing applications are considered, electrospun scaffolds can be used in combination with, for example, rapid prototyped scaffolds with mechanical properties matching those of bone [13]. In this respect, the electrospun scaffolds can be useful to deliver biological factors that can augment the regenerative process. Silk fibroin based electrospun scaffolds loaded with bone morphogenetic protein 2 (BMP-2) have shown to promote mineralized matrix formation *in vitro* due to release of BMP-2 [14]. The surface of electrospun fibrous can be functionalized to load appropriate bioactive moieties to

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http://dx.doi.org/10.1016/j.jconrel.2014.04.035
0168-3659/

control cell fate [15–17]. To obtain a 3D porous network, a range of techniques such as use of porogenic materials or water-soluble agents within the polymer solution prior to the electrospinning are proposed [18]. After subjecting the electrospun scaffolds loaded with porogenic materials or water-soluble agents to water, the desired porosity can be achieved [18]. Another technique to enhance the porosity of electrospun scaffolds includes laser ablation [19]. This technique allows incorporation of micromachined pores with predetermined dimension and location to improve the cellular infiltration.

A range of hydrophobic or hydrophilic therapeutic agents can be incorporated within electrospun fibers by blending them with the polymer solution prior to electrospinning [20–23]. The entrapped therapeutic/bioactive molecules can be released *in vitro* and *in vivo* as part of the volumetric or surface matrix or as a soluble factor in a sustained and controlled manner to control cellular behaviors. For example, bioactive agents such as bone morphogenetic proteins (BMPs) [24,25], dexamethasone [26,27], hydroxyapatite [28,29], calcium phosphate [30] and silicate nanoparticles [31–33] are incorporated within polymeric scaffolds to induce osteogenic differentiation of stem cells. The release rate of these bioactive moieties can be modified by altering the fiber morphology, degradation rate, hydrophilicity of polymer and drug loading [9,23,34,35].

Dexamethasone (Dex) is a synthetic member of the glucocorticoid class of steroid drugs and is used in the treatment of severe inflammatory diseases [36]. Dex has a concentration-dependent stimulatory effect on the differentiation of human mesenchymal stem cells (hMSCs) [37,38]. For example, hMSCs treated with Dex show increased levels of alkaline phosphatase (ALP) activity, which is an early marker for osteogenic differentiation [39]. Furthermore, Dex is also known to enhance matrix mineralization of hMSCs in combination with β-glycerolphosphate and ascorbic acid [40]. Although the exact mode of action by which Dex functions is unidentified, it is known that it enters the cell where it binds to specific regulatory proteins thereby activating the transcription of osteoblast-specific genes [26]. Although Dex is known to have a prolonged effect on ALP expression and matrix mineralization even after only a few days of exposure [41], continuous treatment of hMSCs with Dex results in the most efficient induction of differentiation and subsequent matrix mineralization [42].

To control the release of Dex, various strategies such as encapsulation (or entrapped/attached) within poly(lactic-co-glycolic acid (PLGA) microspheres [43], carbon nanotubes [44,45], poly(amidoamine) (PAMAM) dendrimer nanoparticles [46] and hyperbranched polyester hydrogels [47] have been reported. However, limited research has been focused on controlled delivery of Dex from electrospun scaffolds [48–51]. Martins *et al.* showed an increase in ALP expression and matrix mineralization of hMSCs on electrospun polycaprolactone (PCL)/Dex meshes in a basal medium containing β-glycerophosphate compared to the unloaded meshes in an osteogenic medium [48,51]. This study demonstrated that controlled release of Dex is an improvement over normal dexamethasone-in-medium culture conditions [48,51]. However, due to crystalline nature of PCL, the sustained release of Dex over long periods of time was not observed and a plateau phase was reached within 4–5 days. This might be due to the formation of Dex aggregates within the PCL scaffolds over time that results in limited release of entrapped drug. Moreover, the amount of Dex required to induce osteogenic differentiation was not compatible with the standard concentration used in the established osteogenic differentiation protocols. At the same time, it was shown that high concentrations of Dex could impair cell proliferation and trigger the upregulation of adipogenesis in parallel with the osteogenesis (*in vitro*) [52]. Therefore, it is important to tune Dex release rate from any carrier-device according to the strict requirements to obtain an efficient osteogenesis, followed by a robust mineralization.

Recently, Nguyen *et al.* fabricated Dex loaded poly(L-lactic acid) (PLLA) nanofibrous scaffolds [49]. They also observed that the release of Dex from these electrospun fibers induces differentiation of hMSCs over a period of 3 weeks. In a similar approach, Vacanti *et al.* entrapped Dex within electrospun fibers of PLLA and PCL [50]. Entrapped Dex releases from PCL scaffolds within 24 h, whereas from PLLA a sustained delivery for longer time frame was observed. They also demonstrated that the localized *in vivo* delivery of Dex evoked a less severe inflammatory response when compared with only PCL or PLLA fibers.

Although, encapsulation of Dex in hydrophobic polymers such as PCL and PLLA is described, to our knowledge the release of Dex from amphiphilic polymers has not been reported. Amphiphilic block polymers with tailored physical and chemical properties have shown a controlled release profile and linear degradation characteristics that can be used for a range of tissue engineering applications [34,53,54]. We hypothesize that entrapping Dex within bead-like depots in an amphiphilic fibrillar scaffold will result in a sustained release profile over longer duration. Among amphiphilic copolymers, random block copolymers of poly(ethylene oxide) terephthalate and poly(butylene terephthalate) (PEOT/PBT) have been extensively investigated due to their bioactive characteristics [34,55,56]. By varying the molecular weight and polymer composition, a wide range of PEOT/PBT copolymer with the desired mechanical strength, hydration property, degradation profiles and biological characteristics can be obtained [57]. The PEOT/PBT copolymers are biodegradable and have been proposed for osteochondral tissue engineering [58–60]. 3D scaffolds from PEOT/PBT were fabricated by using 3D fiber deposition (3DF) and electrospinning (ESP) and showed to enhance cartilage tissue formation [61]. Due to the amphiphilic nature of PEOT/PBT, it is predicted that hydrophobic drugs (such as Dex) can be entrapped within the polymeric structure and sustained release profiles from the fibrillar structure can be obtained. It is envisioned that such scaffold design can be used for a range of musculoskeletal tissues engineering applications that require control release of hydrophobic drugs to promote tissue regeneration.

In this study, electrospun scaffolds of PEOT/PBT containing different loadings of Dex were prepared. The surface morphologies of these fibers were examined by scanning electron microscopy (SEM). The entrapment of Dex and *in vitro* release kinetics were investigated using spectroscopic and chromatography techniques. The ability of the Dex loaded fibers for controlling hMSC adhesion, proliferation and differentiation on electrospun fibers was also investigated. We hypothesize that hMSCs cultured on Dex releasing scaffolds will show enhanced osteogenic differentiation compared to the direct infusion of Dex in a medium. The proposed approach for directing cellular function by the sustained release of a hydrophobic drug from amphiphilic fibrous scaffolds can be used to engineer a range of biomimetic scaffold for controlled drug delivery and regenerative medicine applications.

## 2. Experimental

### 2.1. Fabrication of PEOT/PBT electrospun scaffolds

PEOT/PBT was obtained from PolyVation B.V. (Groningen, The Netherlands). The composition used in this study was 1000PEOT70PBT30 where, 1000 is the molecular weight in g/mol of the starting poly(ethylene glycol) (PEG) blocks used in the copolymerization, while 70 and 30 are the weight ratios of the PEOT and PBT blocks, respectively. PEOT is a hydrophilic polymer that imparts elastomeric properties, whereas PBT is a thermoplastic crystalline polymer and imparts stiffness to the copolymeric network. The fibrous scaffolds were fabricated by ESP. First, PEOT/PBT (20% *w/v*) was dissolved in a 9:1 ratio of anhydrous chloroform and ethanol. ESP was carried out at 12.5 kV (Glassman High Voltage, INC) using a 21G blunt needle and a flow rate of 2 mL/h. The collector was a circular plate (diameter 6.5 cm) made of aluminum and maintained at a constant distance of 18 cm from the needle. The electrospun scaffolds were dried overnight in vacuum to remove the residual solvent. For the preparation of the Dex loaded PEOT/PBT scaffolds, the drug was dissolved in ethanol (10× the desired final concentration) and then dissolved in 9



Please cite this article as: A.K. Gaharwar, et al., Amphiphilic beads as depots for sustained drug release integrated into fibrillar scaffolds, J. Control. Release (2014), http://dx.doi.org/10.1016/j.jconrel.2014.04.035