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Biodegradable bead-on-spring nanofibers releasing β-carotene for bone tissue engineering

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ABSTRACT

Bead-on-string mats based on poly(lactide-*co*-glycolide) (PLGA) releasing β-carotene (βC) as a natural osteogen were fabricated and used for bone tissue engineering. Mesenchymal stem cells (MSCs) seeded on the scaffolds successfully differentiated to osteoblasts without using any a differential medium. The mats showed a small burst of β-carotene (24–27%) during the first day and a sustained slow release up to 21 days. The MTT and SEM results indicated good attachment and proliferation of MSCs on the scaffolds. Calcination of scaffolds and expression of *RUNX2*, *SOX9*, and osteonectin genes approved the differentiation of seeded MSCs to osteoblasts without using any external osteogenic differential agent. The scaffold loaded with 4% β-carotene not only induced the early phase of osteogenesis but also advanced the differentiation to the osteoblast maturation phase. Thus, these bead-on-string scaffolds can be used as a substrate for direct bone tissue engineering.

1. Introduction

The appearance of beads on fibers is usually considered as a negative defect during electrospinning process, whereas almost all of the researchers try to optimize the spinning conditions for fabricating uniform and smooth fibers. For example, lower mechanical properties have been reported for beaded mats due to the concentration of stress between the beads and fibers [1, 2]. However, the presence of beads on fibrous mats, known as bead-on-spring structure [3], can provide unique properties such as higher surface roughness and hydrophobicity [4–6], selective wettability [7, 8], encapsulating drugs or other biomolecules for sustained release [1, 2, 9–12] as well as applicability in photonics [3] and microelectronics [13].

Recently, beaded nanofibers have attracted many interests as drug releasing scaffolds for tissue engineering application. The size of beads is in micro-range, bigger than that of the nanofibers, thus they can act as reservoirs for bioactive cues [1, 10]. Gaharwar et al. [2] have used a beaded fibrous mat of poly(ethylene oxide terephthalate-*b*-butylene terephthalate) loaded with dexamethasone for the osteogenic differentiation of human mesenchymal stem cells (hMSCs). The beads acted as reservoirs for the sustained release of dexamethasone. More recently, Ding et al. [14] studied the synergistic effect of dexamethasone release and surface nanoroughness of beaded nanofibers on the osteodifferentiation of rat bone marrow MSCs. Results showed that the gradual release of dexamethasone into the culture medium promoted the differentiation of cells to osteoblasts, while the surface nanoroughness played a supporting role. Li et al. [1] have also entrapped bovine serum albumin (BSA)-loaded dextran particles within bead-on-string nanofiber scaffolds made of poly(lactide-*co*-glycolide) (PLGA) for tissue engineering. *In vitro* release data showed a more sustainable release profile with less initial burst release.

Bioactive cues encapsulated within nanofibers might facilitate the appropriate environment for cell attachment and proliferation [15–17]. β-carotene (βC) is the most abundant precursor of vitamin A in the human diet found in dark green and orange fruits and vegetables [18, 19]. It is a natural antioxidant used for the treatment of cancer and cardiovascular diseases and osteoporosis [20]. Due to the conjugated double bonds, β-carotene is electrical activity [21, 22] (Fig. 1a). The ability of β-carotene for the osteogenic differentiation of stem cells has been reported [23–25]. Consequently, it is expected that the encapsulation of this bioactive component in scaffolds can facilitate the osteogenic differentiation of stem cells on them [26].

This study aims to fabricate bead-on-spring fibrous mats releasing β-carotene as scaffolds for bone tissue engineering with ability to self-differentiate MSCs to osteoblasts without adding any differential agent. Poly (lactide-*co*-glycolide) (PLGA) was chosen as an FDA-approved biodegraded polymer for fabricating the scaffolds due to its degradability and no cytotoxicity [1, 27, 28]. The morphology and drug

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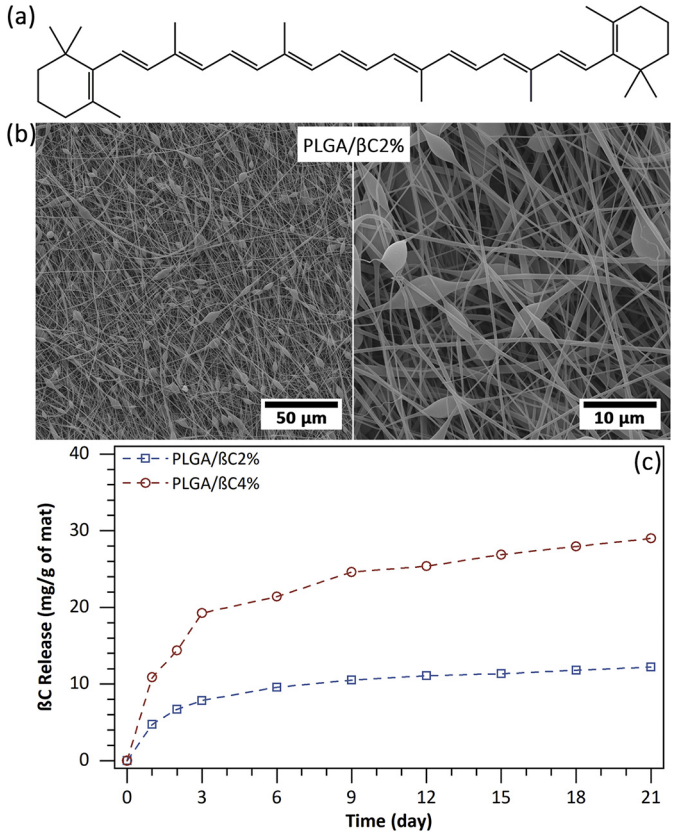

Fig. 1. (a) The molecular structure of β-carotene. (b) SEM images of PLGA/βC2% mat. (c) *In vitro* release profiles of β-carotene from the mats in PBS (pH = 7.4) at 37 °C.

release profile of the fabricated bead-on-spring mats was evaluated. The biological activity of the β-carotene releasing scaffolds for the proliferation and differentiation of MSCs to osteoblasts was studied.

2. Experimental

All experimental details including materials, electrospinning process, instruments, and biological assays are provided in the electronic supplementary information (ESI).

3. Results and discussion

3.1. Fabrication of scaffolds

PLGA beaded fibrous mats containing 0, 2 or 4 wt% (called as PLGA, PLGA/βC2% and PLGA/βC4%, respectively) were prepared *via* electrospinning technique. The PLGA benefit lies in the fact that scaffolds will be degraded as the seeded cells proliferate and secrete extracellular matrices [1]. The fabricated mats consisted of oval-shaped beads with an average size of 3.0 ± 1.0 µm on nanofibers with an average diameter of 450 ± 250 nm with smooth surfaces (Fig. 1b). The fibers were randomly oriented and connected the beads. The bead density of mats defined as the number of beads on fiber mats in 100 μm^2 of SEM images [11] was in the range of 1.2–1.8.

The conventional electrospun mats have limitations from initial burst release [1, 29] that can have a harmful effect on the body. The formation of beads on a fibrous network makes the possibility of using these beads as drug reservoirs to regulate the releasing profile [1, 2, 9–12]. The *in vitro* release profile of β-carotene from mats (PLGA/βC2% and PLGA/βC4, 5 × 5 mm^2, thicknesses < 0.1 mm) in phosphate buffered saline (PBS, pH = 7.4) at 37 °C was monitored over 21 days using high-performance liquid chromatography (HPLC) as the detection method. PBS contained butylated hydroxyl toluene (0.22 mM) to stabilize the released β-carotene [30]. Since the ratio of width/length to thickness of samples is > 10, it can be assumed that the release occurs in a one-dimensional way. The β-carotene release of both mats showed a two-step profile (Fig. 1c); A burst release (24–27% of total loaded) during the first day and a sustained slow release up to 21 days. The initial burst is related to releasing of drug molecules encapsulated on or near the surface of beads and fibers. The sustained release can be due to the drug diffusion out or PLGA degradation, or a combination of both. To find out the mechanism of β-carotene release from mats, the release data was fitted with the well-known Korsmeyer-Peppas model ($M_t/M_\infty = Kt^n$) [31], where M_t and M_∞ are the amounts of released drug at time t and final, respectively, K is the release constant and n is the diffusion exponent indicating the drug release mechanism. The results (Fig. S1 in ESI) showed a good fitness (R^2 = 0.96) with diffusion exponent values of 0.29 and 0.31, respectively, indicating Fickian diffusion as the major mechanism for releasing of β-carotene from the mats. The release constant values were also 0.41 and 0.43 1/day, respectively, showing that the release rates are not significantly different. It is worth to mention that, even after 21 days, all loaded β-carotene was not released, which is due to its degradation within mats or in the release media before detection.

PLGA is a biodegradable polymer undergoing hydrolysis through cleavage of ester linkages on its backbone in the aqueous media [32]. The hydrolysis process is through diffusion-in of water molecules into the polymer and uniform bulk degradation of the matrix, which depends on the lactic acid/glycolic acid ration molecular weight of PLGA, as well as, crystallinity and T_g of the polymer [33]. Drugs loaded in PLGA carriers are released through either diffusion-out from bulk, or surface-erosion, or a combination of both mechanisms [33]. The PLGA (Resomer® RG 504, acid-terminated, L/G: 50/50, M_w 38–54 kD) used for the preparation of scaffolds does not show a significant mass loss in aqueous media till the first month [34, 35]. Thus, the main mechanism of β-carotene release during the first 21 days is mainly diffusion-out and not surface-erosion, which is in agreement with the results of Korsmeyer-Peppas modeling.

3.2. Biocompatibility

Biocompatibility is the vital property of a scaffold for the attachment and proliferation of cells on it [36]. Thus, the viability of MSCs (10^4 cell/well) on the fabricated scaffolds (0.5 × 0.5 cm^2) was evaluated. The optical microscope images (Fig. 2a) showed that MSCs have proliferated and moved toward the scaffold. The MTT results (Fig. 2b) also revealed no cytotoxicity effects for the scaffolds during 72 h. Loading of β-carotene within scaffolds did not significantly change ($p > 0.05$) the viability of seeded MSCs. The cell proliferation of MSCs was considerably improved ($p < 0.05$) on the third day for all scaffolds that shows the suitability of scaffolds for the attachment and growth of MSCs on them.

SEM images (Fig. 2c) displayed a good attachment of MSCs on the scaffold after 48 h, where they have well developed the cell-cell and cell-matrix interactions. The nuclei of MSCs on scaffolds was studied by DAPI assay. The fluorescence microscope images (Fig. 2d) represented