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Fabrication and biocompatibility of agarose acetate nanofibrous membrane by electrospinning

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ABSTRACT

In the present paper, agarose acetate (AGA) nanofibrous membranes containing different weight percentages of β-tricalcium phosphate (β-TCP) were successfully developed through electrospinning. The fibers in the nanofibrous membranes had a rough surface due to the β-TCP particles which were uniformly dispersed within or on the surface of AGA fibers. Rat-bone marrow-derived mesenchymal stem cells (rBMSCs) were cultured on the AGA based nanofibrous membranes while showed a good adhesion and proliferation. It was found that more rBMSCs were differentiated to osteoblast-like cells on the β-TCP containing nanofibrous membranes compared with the single AGA membrane, and more alkaline phosphatase (ALP) and mineralized matrix could be detected when rBMSCs were cultured on the β-TCP containing nanofibrous membranes. The nanofibrous membranes were implanted into Sprague-Dawley (SD) rats for biocompatibility test. Gross examination and histological analysis of the AGA based nanofibrous membranes results showed that there was less inflammatory response. All of experimental results suggested that the AGA based nanofibrous membranes had the great potential application in bone tissue engineering.

1. Introduction

Polysaccharides have drawn much attention to its outstanding degradability, excellent biocompatibility, and have glorious biomedical capabilities to promote cell adhesion, proliferation, and tissue regeneration in recent years (Garcia-Gonzalez, Alnaief, & Smirnova, 2011; Rinaudo, 2008). Numerous different polysaccharides have been evaluated as tissue engineering scaffold, such as alginate, starch-based materials, cellulose, dextran and chitosan (Bhattarai, Li, Edmondson, & Zhang, 2006; Fuchs et al., 2009; Jiang et al., 2008; Peter et al., 2010). Agarose, a polysaccharide consisting of D-galactose and 3, 6-anhydro-L-galactopyranose derived from the cell walls of red algae, has been investigated extensively for chondrocyte culture and cartilage regeneration (Benya & Shaffer, 1982; Khanarian, Haney, Burga, & Lu, 2012; Rahfoth et al., 1998). Bone has a very close structure to the cartilage. These features of agarose might appropriate for bone regeneration. Agarose based gels were also proved have a good biocompatibility for osteoblast adhesion and proliferation (Alcaide et al., 2009; Hu et al., 2016). The bio-inert, non-toxic and low inflammatory response of agarose play very important roles in these studies. However, the numerous hydroxyl groups in agarose caused a strong hydrophilicity of agarose, which resulted in the poor processing properties, and mechanical weakness and less porosities of agarose based scaffold which are unfavorable in bone defect repair. Thus, hydrophobic agarose by modifying is a key issue to broaden its application in tissue engineering. A kind of hydrophobic agarose derivative named agarose acetate (AGA) was synthesized successfully by acylation reaction in our previous work in order to improve processability for the further biomedical application (You, Zhao, Chen, & Tang, 2011). Similar work has also been reported such as synthesis of β-1, 3-glucan esters and its nanofibers used in skin tissue engineering (Wu et al., 2016; Wu et al., 2013). AGA was more hydrophobic than agarose, and favorable to protein adsorption or cell adherence. AGA could dissolve in many organic solvents and could be fabricated by multiple forming processes. For example, AGA can be spun, and its fibers maintain stability in the water instead of swelling which is favorable to construct porous tissue engineering scaffold (You et al., 2011).

Various processing techniques have been used to process different polymers (PLA, PCL, chitosan, collagen etc) into applicable scaffolds to satisfy the fundamental requisites, such as phase-separation, freeze-drying, self-assembly and salt-particle leaching etc (Borzacchiello et al., 2011; Narayanan, Vernekar, Kuyinu, & Laurencin, 2016). Electrospinning is widely considered as a promising polymeric processing technique for the fabrication of fibrous scaffolds (Liang, Hsiao, & Chu, 2007; Narayanan, Aguda et al., 2016; Norouzi, Boroujeni, Omidvarkordshouli, & Soleimani, 2015). As the natural extracellular

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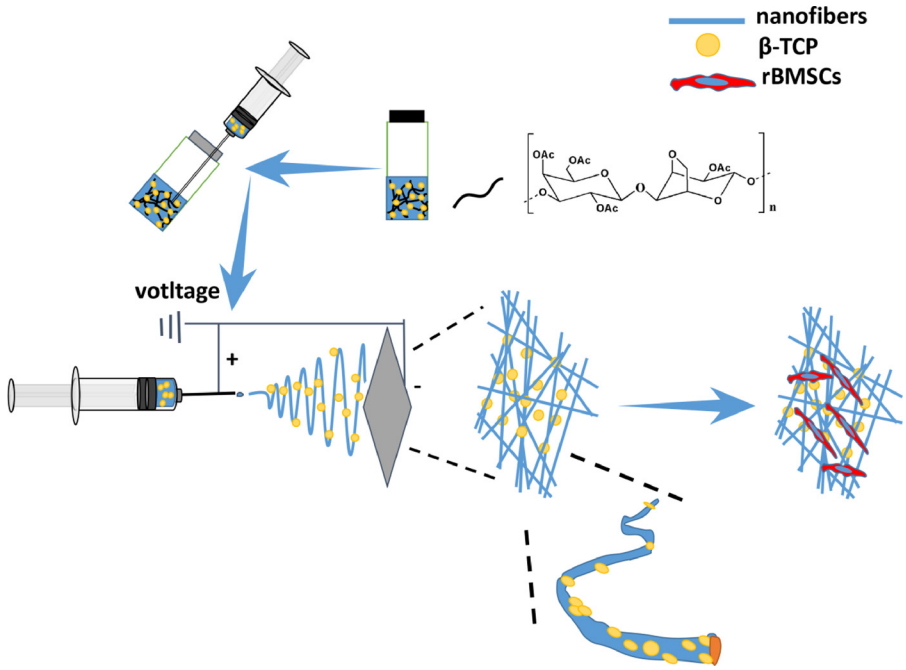

Fig. 1. Illustration of the electrospinning process of the AGA/β-TCP nanofibrous membrane.

matrix consists of fibrous collagens and proteoglycans, and the collagen fibers are organized in a 3D porous architecture, some of other forming processes cannot mimic the extracellular matrix structure perfectly (Heydarkhan-Hagvall et al., 2008). An electrospun fibrous scaffold is commonly composed of fibers with nanometer diameter resulting in the high surface area to volume ratio and the high porosity, thus it is very suitable for replicating the physical structure of ECM (Bhardwaj & Kundu, 2010; Mironov, Kasyanov, & Markwald, 2008; Tamayol et al., 2013). Furthermore, scaffolds consisting of electrospun fibers can be functionalized for enhanced cellular activities by incorporating bioactive compounds (Li, Vepari, Jin, Kim, & Kaplan, 2006). Thus, electrospun nanofibers have been used widely for regeneration of human tissues such as skin, bone, nerve, and vessels (Ghasemi-Mobarakeh, Prabhakaran, Morshed, Nasr-Esfahani, & Ramakrishna, 2008; Hasan et al., 2014; Ladd, Lee, Stitzel, Atala, & Yoo, 2011; Yoshimoto, Shin, Terai, & Vacanti, 2003).

BMSCs are currently among the best characterized stem cells isolated from various tissue sources such as fat, muscle, and bone. These cells are able to differentiate into bone, cartilage, muscle tissue, and neurons and were ultimately reported in almost all cell lineages (Maietta et al., 2018; Narayanan, Bhattacharjee, Nair, & Laurencin, 2017). The application of appropriate biomaterials in combination with BMSCs has attracted the attention of the research community for bone regeneration. Various synthetic biomaterials have been employed to support the adhesion and accelerate the osteogenic differentiation in MSC-based regenerative therapy for treating bone impairment.

Bone is a hybrid system of hydroxyapatite (HA) and collagen type I fibers, is assembled in a complex and organized porous structure (Rogers & Daniels, 2002; Wang et al., 2012). In bone tissue engineering studies, calcium phosphate materials have demonstrated osteoinductivity (Dorozhkin & Epple, 2002; LeGeros, 2008),and are commonly added to the scaffold to improve the osteoinductivity (Li et al., 2014). β-TCP has been found to possess sufficient biodegradability, good biocompatibility and osteoinductivity, which is used in bone tissue engineering (Thomas et al., 2007). Such inorganic particles mixed with polymers will also improve the scaffolds mechanical properties (Maietta et al., 2018). A composite scaffold composed of AGA and β-TCP may combine the advantage of both in bone regeneration.

In the current study, AGA combining β-TCP nanofibrous membranes were fabricated by electrospinning. The properties of the AGA nanofibrous membrane and the effects of β-TCP on average diameter, surface morphology, wettability, mechanical stability, osteogenesis of the nanofibrous membranes were assessed. In addition, the biocompatibility of the AGA based scaffolds was tested by subcutaneous implantation. All of these experiments are for evaluating the potential of AGA based nanofibrous membranes as bone tissue engineering scaffold.

2. Materials and methods

2.1. Materials

Agarose (98%, MW = 306.26) was purchased from Shanghai Aladdin Bio-chem Technology Co., LTD. AGA was synthesized by a previous method (You et al., 2011). β-TCP (98%), acetone and dimethylacetamide (DMAC) was purchased from Macklin (Shanghai, China). All chemicals were of analytical grade and were used without further purification.

2.2. Fabrication of AGA/β-TCP nanofibrous membrane

AGA, AGA/20%β-TCP (w/w) and AGA/40%β-TCP (w/w) nanofibrous membranes were electrospun by a mixture solution of 6% (w/v) AGA acetone/DMAC (2/1 v/v) with homogeneous dispersing β-TCP powders. Electrospinning was carried out in the syringe with a flat needle having an inner diameter of 0.5 mm, at a voltage of 20 kV, and flow rate 2 ml/h, and rotating mandrel speed of 3000 rpm, and receiving distance of 20 cm. The produced fibrous membranes were dried in a vacuum to remove residual acetone and DMAC and cut into a desired shape for further characterization. The schematic representation of electrospinning setup is shown in Fig. 1.

2.3. Characterization of AGA/β-TCP fibrous membranes

The crystal phases of the fibrous membranes were examined by X-ray power diffraction (XRD, Rigaku, Japan) and IR spectra of the fibrous membranes were detected using an ATR-FTIR (Nicolet 8700, USA) method. Thermogravimetric analysis (TGA) of the nanofibrous