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The synergistic effect of nano-hydroxyapatite and dexamethasone in the fibrous delivery system of gelatin and poly(L-lactide) on the osteogenesis of mesenchymal stem cells

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ABSTRACT

Recently, electrospun nanofibrous scaffolds are vastly taken into consideration in the bone tissue engineering due to mimicking the natural structure of native tissue. In our study, surface features of nanofibers were modified through simultaneous electrospining of the synthetic and natural polymers using poly L-lactide (PLLA) and gelatin to fabricate the hybrid scaffold (PLLA/gelatin). Then, hydroxyapatite nanoparticles (nHA) were loaded in electrospun PLLA nanofibers (PLLA,nHA/gelatin) and also dexamethasone (DEX) was incorporated in these fibers (PLLA,nHA,DEX/gelatin) in the second experiment. Fabricated nanofibrous composite scaffolds were characterized via SEM, FTIR spectroscopy, contact angle, tensile strength measurements, DEX release profile and MTT assay. After seeding adipose derived mesenchymal stem cells, osteoinductivity and osteoconductivity of fabricated scaffolds were analyzed using common osteogenic markers such as alkaline phosphatase activity, calcium depositions and gene expression. These results confirmed that all properties of nanofibers were improved by modifications. Moreover, osteogenic differentiation of stem cells increased in PLLA,nHA/gelatin group in comparison with PLLA/gelatin. The sustained release of DEX was obtained from PLLA,nHA,DEX/gelatin which subsequently led to more osteogenic differentiation. Taken together, PLLA,nHA,DEX/gelatin showed significant potential to support the stem cell proliferation and ostogenic differentiation, and can be a good candidates for tissue engineering and regenerative medicine applications.

1. Introduction

Bone tissue engineering has been developed widely in the last decades especially due to the great advances in nanotechnology and nanoscience not only in characterization and fabrication methods of nanomaterials and scaffolds but also in their functional applications (Agarwal et al., 2008; Shin et al., 2012).

Electrospining is one of the methods to fabricate extracellular matrix (ECM) mimicking artificial mats which can be used for cell culture and repair of damaged tissues such as skin, muscle, cartilage, bone and tendon. The ability to produce the ultrafine nanofibers which is the major form of macromolecules in natural ECM, modulating the diameter and other properties of nanofibers according to material features, modifying the scaffolds characteristics via cross-linking pattern with constant architecture properties and also physiological aspects of ECM like adhesion site to cell anchoring are some advantages of this method (Fang et al., 2008; Ma, 2004; Ma and Zhang, 1999).

A variety of biopolymers and biomaterials have been used in tissue engineering as the substrates for scaffolds. Poly(L-lactide) (PLLA) is one of the poly alpha hydroxyl esters which has been taken into consideration in the wide areas of therapeutic applications like bone graft and drug delivery due to the compatibility with immune system, producing the removable natural metabolite through physiological pathways, structural supporting of damaged tissue through the gradual transition pressure to defect site as a result of gradual degradability and the capability of being modified physically, chemically and biologically (Lin et al., 2006). However this polymer cannot provide the osteoindutcive environment due to inappropriate surface

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characteristic and the lack of surface motives participating in the first cellular events. There are a lot of approaches to provide this biocompatibility without altering the bulk properties such as plasma treatment, graft techniques, and the use of natural polymers in the form of composition or coating. Among them, the application of natural polymers becomes a success because they are naturally in the ECM, can mimic that more likely and mediate the cell anchoring and signaling involved in cell spreading, migration, mitosis, differentiation and death (Yang et al., 2002; Ma et al., 2005). However protein linkage with synthetic polymers is limited because the polymer surface needs to be activated by different chemical groups like the amine groups (Kim and Park, 2006). In this study, these limitations were removed through two-nozzle electrospining and actually interweaving and hybridization of electrospun synthetic and natural nanofibers. In this new set up of electrospining, two polymeric solutions localized in two opposite positions and different electric fields. Then the jets of solutions left two nozzles simultaneously to fabricate a hybrid scaffold including nanofibers of synthetic and natural polymers without any chemical interactions.

In addition to the important characteristics of scaffolds for tissue engineering applications such as cell adhesion, differentiation and appropriate tensile properties, an ideal scaffold must keep its integrity while applying in vivo and in vitro. Gelatin is a signaling polymer obtained from the partial hydrolysis of collagen. Although collagen is the most abundant protein in bone ECM which can mimic this matrix more than other proteins, it cannot be electrospun to nanofibers properly due to removing the supra structural properties and also signaling resulted from these properties in applied common solvents for electrospining. A major drawback of natural polymers is their instability under physiological conditions. Actually, the natural polymers such as gelatin are unstable in the culture medium as a result of high hydrophilicity, therefore chemical and physical cross-linking are essential which can fix the mats structurally, control the biodegradability and also affect their thermodynamic behavior. Among these methods, chemical agents are more effective cross-linkers either in the solution or vapor phase; however the application of biological agents is a more preferable method due to their less toxicity (Zeugolis et al., 2008; Mironi-Harpaz et al., 2012; Hayashi and Tabata, 2011; Haugh et al., 2009; Zha et al., 2012; Ratanavaraporn et al., 2010; Lai and Li, 2010). In the present study, different chemical crosslinkers such as EDC and NHS, glyoxal and glutaraldehyde were used to control the gelatin nanofibers degradability and finally the most appropriate crosslinker was selected according to scaffold stability in the culture medium and appropriate nanofiber morphology. Hydroxyapatite nanoparticles (nHA) as the most inorganic materials, have attracted much attention in bone tissue engineering and produce the biological responses resemble to what occur in natural bone reconstruction (Bandyopadhyay-Ghosh, 2008; den Hollander et al., 1991). Actually these nanocrystals affect the ion exchanges, proteins absorption and consequently cell activity, attachment, proliferation and differentiation via their surface topography and chemophysical properties. Several studies reported that the osteoinductivity and osteocoductivity of HA nanoparticles may be related to the surface and geometrical properties which affect the expression of genes such as integrin and alkaline phosphatase (ALP) in the early stages of cell attachment, proliferation and differentiation (Barrere et al., 2003; Lin et al., 2009). However, their high fragility limits the applications especially in the large defects bearing the high mechanical pressure. But incorporating these nanocrystals in the synthetic electrospun nanofibers, provided the chance to fabricate the composites in which the organic and inorganic components interacted chemically. Therefore both the bioactivity of synthetic polymer and mechanical properties of nanoparticles were improved (Kim et al., 2006; Kim and Kim, 2006; Cheung et al., 2007; Hench, 1991).

A variety of factors have been known as the activators involved in signaling during the osteogenic differentiation. Dexamethasone (DEX) is one of these signaling molecules with high osteoinductivity which has been affirmed in addition to the effects on the processes needed for cell growth, mitosis and proliferation (Salgado et al., 2006). Actually, DEX induces the osteogenic differentiation and also conducts the cells toward the late stages of maturation through altering the genes expression (Kim et al., 2005a). DEX can also induce myogenic, cartilogenic, and adipogenic differentiation in different concentrations, therefore the activity of different signaling pathways follows a DEX dose-dependent manner (Zuk et al., 2001; Nguyen et al., 2012). Investigations show that the sustain release of DEX via a releasing system lead to more osteogenic differentiation likely because the cells being exposed to DEX under the more control manner (Martins et al., 2010; Vacanti et al., 2012).

In this study, in order to provide a more appropriate osteogenic environment, DEX was incorporated in PLLA nanofibers in addition to nHA, then these nanofibers hybridized with gelatin nanofibers to fabricate a new releasing system.

The effects of PLLA and gelatin nanofibers, HA nanocrystals, DEX and also the synergetic effects of them were investigated on the adipose derived mesenchymal stem cells (AT-MSCs) behavior. These cells are the promising cell resource especially in medical applications due to their unique properties such as availability, high proliferation rate, high tolerance to apoptosis and nonaggressive isolation method (Peng et al., 2008).

2. Materials and methods

2.1. Fabrication of nanofibrous scaffolds

2.1.1. PLLA mat

To prepare the nanofibrous PLLA scaffold, a 7.3% (w/v) solution of PLLA (M_w = 157000, Sigma–Aldrich) in chloroform and DMF (Merck, Germany) was placed in a syringe which was connected to a needle through an extension tube. Application of 18 kV voltage between the needle and collector which localized in a distance of 17 cm from each other forced the solution droplet to leave the needle tip with the rate of 0.4 ml/h and deposit on the cylinder in the form of nanofibers.

2.1.2. Gelatin mat

To prepare nanofibrous gelatin mat, a 17% solution of gelatin (Type B, Sigma–Aldrich) in acetic acid (Merck, Germany) 40% was filled into a syringe and electrospun on the grounded collector in a distance of 17 cm from the needle with a rate of 0.4 ml/h and application of 17 kV voltage between the needle and collector.

2.1.3. Hybridized PLLA and gelatin

To fabricate a hybrid scaffold, the 7.3% solution of PLLA and the 17% solution of gelatin were prepared and placed in the syringes separately and then electrospun simultaneously by two nozzles in the opposite positions through two independent electric filed. The electrospining options were repeated for every solution with different value in flow rate for gelatin solution which reduced to 0.2 ml/h.

2.1.4. Nanocomposite of PLLA and hydroxyapatite hybridized with gelatin

To fabricate the nanofibrous PLLA,nHA/gelatin mat, the composite of PLLA and nHA (Sigma–Aldrich) was prepared by adding 4.5 ml chloroform to 0.08 g HA, sonication and stirring alternately in order to integrate as much as possible, adding this