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Dextran hydrogels incorporated with bioactive glass-ceramic: Nanocomposite scaffolds for bone tissue engineering

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ABSTRACT

A series of nanocomposite scaffolds comprised of dextran (Dex) and sol–gel derived bioactive glass ceramic nanoparticles (nBGC: 0–16 (wt%)) were fabricated as bioactive scaffolds for bone tissue engineering. Scanning electron microscopy showed Dex/nBGC scaffolds were consisting of a porous 3D microstructure with an average pore size of 240 μm. Energy-dispersive x-ray spectroscopy illustrated nBGC nanoparticles were homogenously distributed within the Dex matrix at low nBGC content (2 wt%), while agglomeration was observed at higher nBGC contents. It was found that the osmotic pressure and nBGC agglomeration at higher nBGC contents leads to increased water uptake, then reduction of the compressive modulus. Bioactivity of Dex/nBGC scaffolds was validated through apatite formation after submersion in the simulated body fluid. Dex/nBGC composite scaffolds were found to show improved human osteoblasts (HOBs) proliferation and alkaline phosphatase (ALP) activity with increasing nBGC content up to 16 (wt%) over two weeks. Owing to favorable physicochemical and bioactivity properties, the Dex/nBGC composite hydrogels can be offered as promising bioactive scaffolds for bone tissue engineering applications.

1. Introduction

There are many topics today that attract attention to bone tissue engineering, such as the ever-increasing life expectancy, prevalence of bone defect and issues associated with biological grafts including extra surgery, increase the risk of morbidity, blood loss, sepsis and pain (in autografts) and increasing risk of rejection due to carrying histocompatibility antigens different from the host (in allografts and xenografts) (Horner et al., 2010). Bone tissue engineering, as an alternative therapeutic method for reconstructing native tissue inside the body, uses a temporary and porous 3D scaffold for the delivery and integration of cells and/or growth factors at the repair site (Khojasteh et al., 2017; Mozafari, Mehraien, Vashaee, & Tayebi, 2012; Razavi et al., 2014). These scaffolds are usually fabricated from bioresorbable and bioactive materials, which have the potential to support and stimulate the regeneration of living tissue (Jazayeri et al., 2017; Razavi et al., 2014; Yazdimamaghani et al., 2013). Additionally, key characteristics for the scaffold design include biodegradability, biocompatibility, ideal mechanical properties and the ability not to produce any immunogenic responses in the body (O'brien, 2011). A variety of materials for the scaffold preparation have been reported, including metals (Yazdimamaghani et al., 2015), ceramics (Hench, 1991), and polymers, both natural and synthetic (Deepthi, Venkatesan, Kim, Bumgardner, & Jayakumar, 2016; Dhandayuthapani, Yoshida, Maekawa, & Kumar, 2011; Liu & Ma, 2004; Patel, Bonde, & Srinivasan, 2011; Yazdimamaghani et al., 2014). The corrosive nature of metals results in loosening of implants, corrosion products with unwanted side effects, high density and hardness compared with natural tissues, low biocompatibility, inactive connections with tissue, and allergic reactions, which make them unfavorable substitutes (Murugan & Ramakrishna, 2005). Ceramics are fragile, have a low flexural strength and lack resiliency. These undesirable qualities of metals and ceramics attracted attention toward using other materials for tissue engineering purposes, including biodegradable polymers (*e.g.*, polylactic acid), polysaccharides (*e.g.*, chitosan, alginate, cellulose, hyaluronic acid and dextran) (Van Vlierberghe, Dubruel, & Schacht, 2011) and proteins

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(*e.g.*, elastin, collagen) (Jonker, Löwik, & van Hest, 2012). In particular, polymeric hydrogels that are biocompatible and hydrophilic (Hoffman, 2012) are beneficial in various applications, specifically in medical fields to study cell growth for production of tissue-equivalent constructs (Drury & Mooney, 2003).

Among the natural polymers, dextran, a hydrophilic carbohydrate biopolymer that degrades in certain physical environments without any effect on the cell viability, has attracted attention in biological systems (Heinze, Liebert, Heublein, & Hornig, 2006). Cross-linked dextran hydrogels (CDH) are known to provide favorable extracellular matrix (ECM) conditions with high water content and biocompatibility. However, some limitations to these hydrogels have also been demonstrated, such as poor mechanical properties, low load-bearing capacity and the ease of deformation, which restrict their use in applications of hard tissue engineering. The *in vitro* capacity of dextran for culture and proliferation of cells were reported previously (Deux et al., 2002; Fricain et al., 2013; Liu & Chan-Park, 2009). Nevertheless, these cells were shown to attach and proliferate in clumps, rather than spreading onto the surface of the scaffolds, indicating the constricted capability of the pristine dextran to provide cell adhesion and spreading (Liu & Chan-Park, 2009). In order to improve the bioactivity and obtain better mechanical properties, some inorganic materials, like hydroxyapatite (HA), has been incorporated with dextran matrix. Varoni et al. synthesized nanostructured hydroxyapatite-dextran composite scaffolds (Varoni et al., 2010), while Fricain et al. recently reported a nano-hydroxyapatite-pullulan/dextran polysaccharide composite macroporous material for bone tissue engineering (Fricain et al., 2013).

Among inorganic materials, bioactive glass ceramics (BGC) containing SiO_2–CaO–P_2O_5 networks have gained much attention due to their biocompatibility, bioactivity and osteoconductive properties. Through the formation of surface hydroxy carbonate apatite (HCA) layer, these ceramic bonds to both hard and soft tissues (Lizzi et al., 2017; Salinas & Vallet-Regí, 2013; Siqueira et al., 2017). Taking into account the previous art, bioactive glass ceramic affects the cell adhesion, proliferation, differentiation and colonization on the surface of implants (Kargozar et al., 2017; Shamsi et al., 2017; Wu, Wu, Xue, Li, & Liu, 2017; Wu, Zhou, Chang, & Xiao, 2013). Various composite hydrogels based on the bioactive glass have been synthesized recently, using biodegradable natural polymers including:

- α-chitin/nBGC composite scaffolds (Peter et al., 2009).
- nBGC disseminated into chitosan matrix (Peter, Binulal, Soumya et al., 2010).
- Chitosan–gelatin/nBGC composite scaffolds for alveolar bone tissue (Peter, Binulal, Nair et al., 2010).
- Porous gelatin/BG for bone tissue engineering (Mozafari, Rabiee, Azami, & Maleknia, 2010; Nadeem, Kiamehr, Yang, & Su, 2013).
- Collagen hydrogels incorporated with surface-aminated mesoporous nBGC (El-Fiqi, Lee, Lee, & Kim, 2013).
- nBGC-reinforced gellan-gum hydrogels for bone tissue engineering (Gantar et al., 2014).

To the best of our knowledge, CDH-nBGC scaffolds for tissue engineering applications have not been reported before. The preparation of CDH-nBGC scaffolds was carried out in this work to verify the possibility of improving the mechanical properties and the bioactivity of the dextran hydrogels using nBGC particles, such that it would extend the application range of the dextran to include bone tissue engineering. The reinforcement effect of nBGC particles was evaluated by the analysis of the microstructure, the mechanical properties and the ability to form HA in simulated body fluid. In addition, *in vitro* studies were performed by means of culturing normal human osteoblast (HOB) cells onto the composite scaffolds. To this end, the viability, morphology and alkaline phosphatase (ALP) activities were assessed *in vitro* for up to two weeks. We hypothesize that the fabricated biocompatible CDH-nBGC constructs provide valuable scaffolding properties, such as favorable cell adhesion and proliferation, as well as enhanced mechanical properties.

2. Materials and methods

2.1. Materials

Dextran (Dex) (40000 g mol^{-1} weight average molecular weight) was purchased from Pharmacosmos A/S, tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$) and triethyl phosphate ($C_6H_{15}O_4P$) were purchased from Merck Chemicals, Dusseldorf, Germany. Nitric acid (> 65% purity) was purchased from Sigma-Aldrich, Steinheim, Germany. Simulated Body Fluid (SBF) was purchased from Pardis Pajhohesh, Yazd, Iran. Ethanol (> 99% purity) was purchased from Hamoon Teb Markazi Medicinal Chemical Industrial Co, Zarrandiyyeh, Iran. All chemicals were of analytical grade and used as supplied, without further purification. Deionized water (high-performance liquid chromatography grade) was prepared in-house using reverse osmosis technique (AquaMax 311, YoungLin Instruments, Anyang, South Korea) and used in the preparation of solutions.

2.2. Synthesis of nano bioactive glass ceramic particles (nBGC)

Bioactive glass–ceramic (molar composition 64% SiO_2–31% CaO–5% P_2O_5) was synthesized by a sol–gel method, as reported by Ravarian et al., with some modification (Ravarian et al., 2010). Briefly, 13.33 g (0.064 mol) of tetraethyl orthosilicate was added to 30 mL of 0.1 M nitric acid; the solution was stirred for 30 min in order to complete the acid hydrolysis of TEOS. The following reagents were added in sequence, with about 45 min provided for each reagent to react thoroughly: 0.91 g (0.005 mol) triethyl phosphate (TEP) and 7.32 g (0.031 mol) of calcium nitrate tetrahydrate. To allow completion of the hydrolysis reaction, mixing was continued for 1 h after the last addition. The solution was cast in a cylindrical Teflon container and kept sealed for 10 days at room temperature to allow the hydrolysis and polycondensation reactions to take place until the gel was formed. The gel was kept in a sealed container and heated at 70 °C for 3 days. To remove the remaining water, a small hole was contrived in the lid to allow the leakage of gasses during heating of the gel to 120 °C for 3 days. The dried gel was then heated for 24 h at 700 °C to first, stabilize the glass, and second, to eliminate the residual nitrate. The obtained glass after the thermal process was in the form of clumps of nanoparticles. Then, in order to decrease the size and suitable dispersion in dextran solution, milling operations were fixed for 5 h by a fast mill (FMD-2 M, Sanat Ceram, Iran). Alumina vial and balls were utilized. The process was performed at a ball-to-powder weight ratio (BPR) of 10:1. Absolute ethanol was used to prevent extensive agglomeration during the process.

2.3. Preparation of the nanocomposite scaffolds

CDH-nBGC composite scaffolds have been prepared *via* a chemical crosslinking technique previously reported by Imren et al. (Imren, Gümüşderelioğlu, & Güner, 2006) with some modifications (Kenari, Alinejad, Imani, & Nodehi, 2013; Kenari, Imani, Dashtimoghadam et al., 2013; Kenari, Imani, & Nodehi, 2013). To this end, polymer solutions with a constant concentration (37.5 w/v%) were prepared by dissolving a specific amount of dextran in 1.2 M sodium hydroxide aqueous solution. Different percentages of nBGC, specifically 2, 4, 6, 8 and 16 wt%, were dispersed in distilled water then sonicated for 10 min. The suspended nBGC were added to dextran solution and stirred (with a magnetic stirrer at 1200 rpm for one hour) in order to prepare the CDH-nBGC composite. The resultant solution was subjected to sonication for 30 s to achieve a homogeneous dispersion. Epichlorohydrin (10 v/v%) was added as a crosslinker. After 30 s of sonication, the resultant mixtures were poured into the containers and