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Research Paper

Designed composites for mimicking compressive mechanical properties of articular cartilage matrix

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ABSTRACT

Collagen, chitosan–polycaprolactone (CH–PCL) copolymer with PCL content of around 40 wt% and chondroitin sulfate (CS) were mixed together at various ratios to prepare collagen/CH–PCL/CS composites and the resulting composites were used to build stratified porous scaffolds that are potentially applicable for articular cartilage repair. The ternary composites were designed in such a way that collagen content in the scaffolds decreased from the top layer to the bottom layer while the content of CH–PCL and CS altered in a reversed trend in order to reach partial similarity to cartilage matrix in the composition of main components. Porous structures inside collagen/CH–PCL/CS scaffolds were constructed using a low-temperature deposition processing technique and graded average pore-size and porosity for the scaffolds were established. Such produced scaffolds were further crosslinked using 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide under optimized conditions, and the obtained scaffolds showed well-defined elastic compressive properties. Compressive modulus (*E*) and stress at 10% strain (σ_{10}) of full scaffolds in wet state reached about 2.8 MPa and 0.3 MPa, respectively, and meanwhile, *E* and σ_{10} of layers inside hydrated scaffolds changed in a gradient-increased manner from the top layer to the bottom layer with significant differences between contiguous layers, which partially mimics compressive mechanical properties of cartilage matrix. In addition, in vitro culture of cell–scaffold constructs exhibited that scaffolds were able to well support the ingrowth and migration of seeded cells, and cells also showed relatively uniform distribution throughout the scaffolds. These results suggest that the presently developed collagen/CH–PCL/CS scaffolds have promising potential for applications in articular cartilage repair.

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1. Introduction

Articular cartilage is noted for a limited capacity for self-repair upon damage caused by traumatic injuries or degenerative diseases, especially for those defects correlated to relatively large areas of cartilage surface (Chiang and Jiang, 2009). The difficulty in repairing articular cartilage defects can be mainly ascribed to its avascular characteristic and relatively low cellular metabolic activity (Jackson et al., 2001). Clinical treatments for articular cartilage repair commonly involve microfractures, subchondral bone drilling, arthroscopic lavage with or without corticosteroids, abrasion arthroplasty, mosaicplasty and total joint replacement (Marsano et al., 2007; Thiede et al., 2012). Although improved short-term results from clinical therapies have been reported, these strategies have limited success in which they are deficient in long-term repair or lead to the formation of mechanically inferior fibro-cartilaginous tissue in the cartilage defect void (Hunziker, 2002; Shapiro et al., 1993). Many efforts have thus been made to search for alternative therapies that can yield correctly reparative tissue in cartilage defects, and on the other hand, achieve long-term recovered function of regenerated articular cartilage (Jackson et al., 2001; Chiang and Jiang, 2009). Nowadays, tissue engineering, which usually involves in combination of cells, porous scaffolds and bioactive agents, has emerged as a new method for generating functional new tissues to replace damaged articular cartilage (Oliveira et al., 2007; Tan et al., 2007).

It is known that articular cartilage has a stratified structure and its composition and properties appear to change in an apparently anisotropic manner. Histological examinations point out that articular cartilage has four diacritical layers, generally being named as superficial layer, intermediate layer, deep layer and calcified layer (Castro et al., 2012; Leong et al., 2008). From the superficial zone to the calcified region, the content of water and type-II collagen progressively decreases whereas proteoglycans show an inverse trend, and meanwhile, collagen fibers and chondrocytes are organized in a certain oriented manner in different layers, which results in varied compressive properties between layers. In addition, the diameter of type-II collagen and the compressive mechanical modulus of articular cartilage matrix also change from the superficial layer to the calcified layer. Although tissue engineering strategies have potential for the repair of articular cartilage lesions, fabrication of satisfactory scaffolds faces various degrees of difficulties owing to hierarchical structures and anisotropic properties of cartilage matrix. Nevertheless, to date, many attempts have been made to build scaffolds with various compositions and structures that are approximately similar to that of cartilage matrix in order to achieve improved results for articular cartilage repair (Dormer et al., 2010; Harley et al., 2010a, 2010b; Kim et al., 2012; Leong et al., 2008; Levingstone et al., 2014).

Many types of biodegradable materials, including naturally occurring and synthetic polymers or their combinations, have been investigated for fabrication of scaffolds that have designed compositions, structures and functions (Guo et al., 2012; Miao and Sun, 2010). In the case of articular cartilage tissue engineering, type-II collagen has received specific attention because it is a key component in cartilage matrix which plays important roles in supporting chondrogenesis and maintaining chondrocytic phenotype (Chang et al., 2006; Levingstone et al., 2014; Moutos et al., 2007). Up to now, type-II collagen has indeed been extensively investigated for articular cartilage repair (Chiang and Jiang, 2009; Jackson et al., 2001). However, collagen alone seems not to be adequately competent for repairing articular cartilage lesions because of its high swelling, fast degradation and poor wet-state mechanical strength even though doable crosslinkers have been utilized (Berthiaume et al., 2011; Tierney et al., 2009).

Chondroitin sulfate (CS) is a type of natural glycosaminoglycans (GAGs) mainly found in connective tissues such as bone, skin, and cartilage (Pieper et al., 1999). As a main GAG in cartilage matrix, CS is responsible for water retention due to its charged feature (Servaty et al., 2001), and it also plays a role in intracellular signaling, cell recognition and connecting different matrix components to the cell-surface glycoproteins and collagen (Vazquez et al., 2013). Taking into account the importance of CS, a logical strategy seems to build scaffolds for articular cartilage repair by directly using CS. But in fact, CS is usually employed as an accessory component in different types of scaffolds because it accounts for a relatively low percentage in native cartilage matrix as compared to type-II collagen (Chung and Burdick, 2008; Vazquez et al., 2013), and additionally, it generally shows inferior wet-state mechanical strength.

Besides CS, another natural polysaccharide, chitosan, has also been widely studied for applications in articular cartilage repair because it has good ability to support chondrogenic activity and cartilage matrix expression by chondrocytes besides its structural similarity to GAGs (Suh and Matthew, 2000). Despite various advantages for biomedical applications, unmodified chitosan also shows poor wet-state mechanical properties (Wan et al., 2004a) and has fast in vivo degradation (Wan et al., 2010).

One of effective approaches to regulating degradation and mechanical properties of natural polymers is to use them together with some other biodegradable polyesters in the form of blends or composites (LaPorta et al., 2012; Seidi et al., 2011; Smith et al., 2005; Wan et al., 2008). Of biodegradable polyesters, polycaprolactone (PCL) has been extensively investigated because it has adjustable mechanical strength and shows soft- and hard-tissue compatible properties (Wu et al., 2010). PCL is a semi-crystalline, linear and aliphatic polyester with a low melting point (ca. 60 °C), which allows easy processing. Apart from certain applications in sutures, wound dressings and drug delivery vehicles, PCL has also been commonly used for articular cartilage repair (Martinez-Diaz et al., 2010; Matsiko et al., 2013).

Grafting is also a versatile means to modify natural polymers in addition to blend or composite-based physical modification (Bhattacharya and Misra, 2004). Grafting polycaprolactone side chains onto chitosan backbone can generate some chitosan-polycaprolactone (CH–PCL) copolymers that would have tailorable mechanical and degradation properties as well as potential solubility in aqueous media, depending on its PCL content (Wan et al., 2010). Therefore, it would be feasible to blend collagen, CH–PCL and CS together to produce applicative composites and further to process