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Research Paper

Pore orientation mediated control of mechanical behavior of scaffolds and its application in cartilage-mimetic scaffold design

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ABSTRACT

Scaffolds with aligned pores are being explored in musculoskeletal tissue engineering due to their inherent structural anisotropy. However, influence of their structure on mechanical behavior remains poorly understood. In this work, we elucidate this dependence using chitosan–gelatin based random and aligned scaffolds. For this, scaffolds with horizontally or vertically aligned pores were fabricated using unidirectional freezing technique. Random, horizontal and vertical scaffolds were characterized for their mechanical behavior under compressive, tensile and shear loading regimes. The results revealed conserved trends in compressive, tensile and shear moduli, with horizontal scaffolds showing the least moduli, vertical showing the highest and random showing intermediate. Further, these scaffolds demonstrated a highly viscoelastic behavior under cyclic compressive loading, with a pore orientation dependent relative energy dissipation. These results established that mechanical behavior of porous scaffolds can be modulated by varying pore orientation alone. This finding paved the way to recreate the structural and consequent mechanical anisotropy of articular cartilage tissue using zonally varied pore orientation in scaffolds. To this end, monolithic multizonal scaffolds were fabricated using a novel sequential unidirectional freezing technique. The superficial zone of this scaffold had horizontally aligned pores while the deep zone consisted of vertically aligned pores, with a transition zone between the two having randomly oriented pores. This depth-dependent pore architecture closely mimicked the collagen alignment of native articular cartilage which translated into similar depth-dependent mechanical anisotropy as well. A facile fabrication technique, biomimetic pore architecture and associated mechanical anisotropy make this multizonal scaffold a promising candidate for cartilage tissue engineering.

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1. Introduction

Tissue architecture plays an integral role in enabling tissue function. Of the different functions, mechanical behavior depends heavily on tissue architecture. For this reason, musculoskeletal tissues have attained specialized architectures that allow them to withstand and transmit loads. While isotropic structures can provide for a load bearing function, nature has evolved anisotropic structures for more specialized load bearing functions. For example, tendon's crimped (wave-like) and aligned collagen bundles are nature's optimized anisotropic design for the tissue to bear high tensile loads along the direction of fiber alignment (Lynch et al., 2003). Such structural and consequent functional anisotropy has inspired the design of scaffolds (fibers and hydrogels) with aligned features for musculoskeletal tissue engineering (Chen et al., 2014; Oliveira et al., 2012). Hydrogel based scaffolds provide a porous three dimensional niche for neotissue growth and can be tailored to exhibit high tensile, compressive and shear strengths. Further, hydrogel scaffolds with aligned pores (aligned scaffolds) have been explored for muscle, bone, cartilage and tendon tissue engineering (Oliveira et al., 2012; Kroehne et al., 2008; Jia et al., 2012; Caliari and Harley, 2011). Such aligned scaffolds have been fabricated using various methods such as directional freezing (Kroehne et al., 2008), ionotropic gelation (Yamamoto et al., 2009) and 3D printing (Muehleder et al., 2014).

Directional freezing is a relatively recent adaptation of the conventional freeze drying approach that is based on ice-templated pore formation. In the freeze drying approach, pore characteristics such as size, homogeneity and orientation are dependent on ice crystal nucleation and growth, and can be controlled by modulating the freezing process (Caliari and Harley, 2011; Haugh et al., 2009; O'Brien et al., 2004). While pore size is largely governed by freezing temperature, pore homogeneity is achieved by controlling freezing rate and providing a uniform contact surface (Haugh et al., 2009; O'Brien et al., 2004). On the other hand, pore orientation can be controlled by varying the direction of freezing which in turn controls the direction of ice crystal growth. For example, scaffolds fabricated by unidirectional freezing possess an anisotropic pore architecture with aligned pores (Caliari et al., 2011; Mandal et al., 2013).

While some studies have demonstrated the ability of aligned scaffolds to modulate cell behavior (Pawelec et al., 2015; Downing et al., 2013), there is very limited understanding of their structure–mechanical function relationship. Caliari et al. have previously studied tensile properties of dry collagen–GAG scaffolds with aligned ellipsoidal pores. They demonstrated that aligned scaffolds possess higher tensile modulus along the major axis of pores as compared to isotropic scaffolds (Caliari et al., 2011). Similarly, monotonic compressive properties have also been indicated to be dependent on pore alignment (Wu et al., 2012). While there have been few studies that investigate the tensile and compressive properties of aligned scaffolds with tubular pores, to the best of our knowledge, there are no studies that describe their mechanical behavior and anisotropy under shear and cyclic compressive loading. Hence, we hypothesized that pore orientation governs mechanical behavior of scaffolds under different loading regimes. Since an analysis of this behavior is critical in determining the viability of these scaffolds in musculoskeletal tissue engineering, the first part of the current study presents a comprehensive evaluation of compressive (monotonic and cyclic), tensile and shear properties of chitosan–gelatin (CG) scaffolds with different pore architectures. Chitosan and gelatin were identified as polymers of choice due to their high biocompatibility, and their suitability as scaffold materials for tissue engineering of diverse tissues, including musculoskeletal tissues (Van Vlierberghe et al., 2011).

Several musculoskeletal tissues possess complex architectures where a transition in collagen orientation is observed either across one dimension, like the articular cartilage and the ligament/tendon–bone interface, or more than one dimensions, like the meniscus. For example, the articular cartilage has a layered yet seamless architecture where the horizontally aligned collagen fibers of the superficial zone transit into vertically aligned collagen fibers of the deep zone through a random transition zone. As a consequence, a variety of multizonal or multilayered scaffolds have previously been fabricated and studied for cartilage tissue engineering (Klein et al., 2009). Recently, Levingstone et al. (2014) fabricated a trilayered construct for osteochondral tissue engineering containing a superficial zone composed of collagen—I/II and hyaluronic acid, an intermediate layer composed of collagen —I/II and hydroxyapatite and lower bone layer composed of collagen I and hydroxyapatite. In another work, variation of pore size along depth was achieved using 3D fiber deposition method for osteochondral tissue engineering (Woodfield et al., 2005). While zonal constructs that contain depth-dependent variation in pore size, material composition, fiber alignment, mechanical properties, bioactive molecules or cell type have been reported (Klein et al., 2009), there are no reports where pore architecture has been modulated to mimic collagen organization of the articular cartilage. In the second part of this study, we demonstrate how spatially varying pore alignment can be incorporated in a single scaffold to mimic the transition in collagen orientation that occurs naturally.

The depth-dependent variation in collagen and cellular organization of articular cartilage contributes significantly to its mechanical anisotropy (Halonen et al., 2013; Buckley et al., 2008). Therefore, we hypothesized that mimicking collagen organization through pore orientation in scaffolds can lead to properties similar to those seen due to collagen organization in native articular cartilage tissue. To mimic this structural anisotropy, we report a sequential unidirectional freezing method to obtain a monolithic, multizonal scaffold. This scaffold has been characterized for its physicochemical properties, mechanical properties (compressive and shear) and primary chondrocyte infiltration.

2. Materials and methods

2.1. *Fabrication of random and aligned scaffolds*

CG scaffolds were fabricated using a modification of the freeze-drying technique (Arya et al., 2012). For this, low-viscosity chitosan and gelatin type B (~225 Bloom viscosity) from