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Short Communication

Decellularization of porcine articular cartilage explants and their subsequent repopulation with human chondroprogenitor cells

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ABSTRACT

Engineering tissues with comparable structure, composition and mechanical functionality to native articular cartilage remains a challenge. One possible solution would be to decellularize xenogeneic articular cartilage in such a way that the structure of the tissue is maintained, and to then repopulate this decellularized matrix with human chondroprogenitor cells that will facilitate the reconstitution, maintenance and eventual turnover of the construct following implantation. The overall objective of this study was to develop a protocol to efficiently decellularize porcine articular cartilage grafts and to identify a methodology to subsequently repopulate such explants with human chondroprogenitor cells. To this end, channels were first introduced into cylindrical articular cartilage explants, which were then decellularized with a combination of various chemical reagents including sodium dodecyl sulfate (SDS) and nucleases. The decellularization protocol resulted in a ~90% reduction in porcine DNA content, with little observed effect on the collagen content and the collagen architecture of the tissue, although a near-complete removal of sulfated glycosaminoglycans (sGAG) and a related reduction in tissue compressive properties was observed. The introduction of channels did not have any detrimental effect on the biochemical or the mechanical properties of the decellularized tissue. Next, decellularized cartilage explants with or without channels were seeded with human infrapatellar fat pad derived stem cells (FPSCs) and cultured chondrogenically under either static or rotational conditions for 10 days. Both channeled and non-channeled explants supported the viability, proliferation and chondrogenic differentiation of FPSCs.

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The addition of channels facilitated cell migration and subsequent deposition of cartilage-specific matrix into more central regions of these explants. The application of rotational culture appeared to promote a less proliferative cellular phenotype and led to an increase in sGAG synthesis within the explants. Rotational culture also appeared to promote higher cell viability and led to a more even distribution of cells within the channels of decellularized explants. To conclude, this study describes an effective protocol for the decellularization of porcine articular cartilage grafts and a novel methodology for the partial recellularization of such explants with human stem cells. Decellularized soft tissue explants that maintain their native collagen architecture may represent promising scaffolds for musculoskeletal tissue engineering applications.

1. Introduction

Novel tissue engineering strategies represent an attractive option for articular cartilage repair. Cartilage tissue engineering aims to generate a functional three dimensional cartilaginous graft, through the combination of cells, scaffolds and/or signals, for subsequent transplantation into patients (Chung and Burdick, 2008). In order to thrive within the challenging load-bearing environment of a joint, the ideal engineered graft should mimic the complex depth-dependent structure, composition and mechanical properties of the native tissue (Nagel and Kelly, 2013; Klein et al., 2009). In particular, the collagen network structure of articular cartilage has been shown to be critical to determine the mechanical properties of the tissue (Gannon et al., 2015a, 2015b). While attempts have been made to recapitulate certain aspects of the depth-dependent composition of articular cartilage in tissue engineered grafts (Klein et al., 2003; Kim et al., 2003; Ng et al., 2005, 2009; Nguyen et al., 2011; Thorpe et al., 2013), it has not been possible to engineer cartilage grafts with a collagen structure mimicking the native tissue. For this reason, tissue engineers have yet to develop grafts with mechanical properties that recapitulate those of articular cartilage.

One possible approach to engineer such a tissue would be to decellularize xenogeneic articular cartilage in such a way that the depth-dependent collagen structure of the tissue is maintained, and to then repopulate this decellularized matrix with human stem cells or chondrocytes that will act to reconstitute, maintain and turnover the construct following implantation. Whole organ/tissue decellularization that maintains native tissue structure has been achieved in heart (Ott et al., 2008), lung (Petersen et al., 2010), liver (Uygun et al., 2010) and kidney (Ross et al., 2009). The subsequent recellularization of these organ-derived biological scaffolds has been shown to be feasible, with the resulting constructs displaying certain functionalities mimicking the native organ *in vivo* (Petersen et al., 2010; Uygun et al., 2010).

Over the past decade, a number of studies have explored the potential of decellularized/devitalized cartilage extracellular matrix (ECM) as a scaffold for cartilage tissue engineering applications, showing such biomaterials supporting cellular attachment and chondrogenesis of stem cells and chondrocytes (Yang et al., 2011; Gong et al., 2011; Kang et al., 2014; Cheng et al., 2009, 2011; Diekman et al., 2010; Rowland et al., 2013; Almeida et al., 2014, 2015). Most of these studies however used mincing and/or cyro-milling to particulate the cartilage matrix in order to produce scaffolds that are highly porous, but at the expense of the mechanical functionality and maintaining the collagen architecture of the native tissue.

Only a few studies have explored decellularization of whole cartilage explants and the potential of using such a structurally intact decellularized cartilage scaffold for tissue engineering applications (Kheir et al., 2011; Elder et al., 2010; Schwarz et al., 2012a, 2012b). For example, Kheir et al. (2011) developed a technique to decellularize porcine cartilage–bone constructs, with view to using this as a biological scaffold for joint regeneration. Although accompanied with a significant loss in tissue sGAG content and mechanical properties, the decellularization procedure led to a near-complete removal of genomic DNA, without a noticeable impact on the collagen network architecture of the tissue. While these results are promising, the decellularized matrix exhibited a very low porosity, with limited host cell infiltration (up to 300 μm) into the explants reported 3 month following subcutaneous implantation (Kheir et al., 2011). Therefore novel methods are required for the recellularization of such decellularized cartilage explants, which do not negatively impact the architecture or mechanical functionality of the tissue.

The overall objective of this study was to develop a protocol for the efficient decellularization of porcine articular cartilage grafts and to identify a methodology for the subsequent repopulation of such explants with human chondroprogenitor cells for cartilage tissue engineering applications. First, a previously described decellularization protocol for articular cartilage (Kheir et al., 2011) was modified to include additional steps to increase the porosity of the decellularized matrix. Furthermore, channels were introduced into the cartilage sample before decellularization to facilitate the subsequent recellularization of the explant. The overall effect of this decellularization protocol was assessed histologically, biochemically and mechanically. Next, these decellularized cartilage explants, from here on in termed “scaffolds”, were seeded with human infrapatellar fat pad derived stem cells (FPSCs). The effect of introducing channels, and the application of rotational culture, on the subsequent recellularization of the scaffold was examined. It is hypothesized that the