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Approaching the compressive modulus of articular cartilage with a decellularized cartilage-based hydrogel

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ABSTRACT

ECM-based materials are appealing for tissue engineering strategies because they may promote stem cell recruitment, cell infiltration, and cell differentiation without the need to supplement with additional biological factors. Cartilage ECM has recently shown potential to be chondroinductive, particularly in a hydrogel-based system, which may be revolutionary in orthopedic medicine. However, hydrogels composed of natural materials are often mechanically inferior to synthetic materials, which is a major limitation for load-bearing tissue applications. The objective was therefore to create an unprecedented hydrogel derived entirely from native cartilage ECM that was both mechanically more similar to native cartilage tissue and capable of inducing chondrogenesis. Porcine cartilage was decellularized, solubilized, and then methacrylated and UV photocrosslinked to create methacrylated solubilized decellularized cartilage (MeSDCC) gels. Methacrylated gelatin (GelMA) was employed as a control for both biomechanics and bioactivity. Rat bone marrow-derived mesenchymal stem cells were encapsulated in these networks, which were cultured *in vitro* for 6 weeks, where chondrogenic gene expression, the compressive modulus, swelling, and histology were analyzed. One day after crosslinking, the elastic compressive modulus of the 20% MeSDCC gels was 1070 ± 150 kPa. Most notably, the stress strain profile of the 20% MeSDCC gels fell within the 95% confidence interval range of native porcine cartilage. Additionally, MeSDCC gels significantly upregulated chondrogenic genes compared to GelMA as early as day 1 and supported extensive matrix synthesis as observed histologically. Given that these gels approached the mechanics of native cartilage tissue, supported matrix synthesis, and induced chondrogenic gene expression, MeSDCC hydrogels may be promising materials for cartilage tissue engineering applications. Future efforts will focus on improving fracture mechanics as well to benefit overall biomechanical performance.

Statement of Significance

Extracellular matrix (ECM)-based materials are appealing for tissue engineering strategies because they may promote stem cell recruitment, cell infiltration, and cell differentiation without the need to supplement with additional biological factors. One such ECM-based material, cartilage ECM, has recently shown potential to be chondroinductive; however, hydrogels composed of natural materials are often mechanically inferior to synthetic materials, which is a major limitation for load-bearing tissue applications. Therefore, this work is significant because we were the first to create hydrogels derived entirely from cartilage ECM that had mechanical properties similar to that of native cartilage until hydrogel failure. Furthermore, these hydrogels had a compressive modulus of 1070 ± 150 kPa, they were chondroinductive, and they supported extensive matrix synthesis. In the current study, we have shown that these new hydrogels may prove to be a promising biomaterial for cartilage tissue engineering applications

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1. Introduction

Arthritis is one of the leading causes of disability among US adults [1]. Some of the current clinical treatments include autologous chondrocyte implantation, mosaicplasty, and microfracture [2,3]. However, not only do these treatments involve high risk of donor site morbidity and/or the need for multiple surgeries, these treatments still lack the ability to regenerate fully functional cartilage tissue [4–6]. Tissue engineering approaches are therefore striving to fully regenerate cartilage tissue by utilizing a bioactive and bioresorbable construct that provides the necessary cues to facilitate cell growth, differentiation, and tissue integration, while providing the mechanical integrity and support to allow the tissue to sustain its load bearing function [3].

Hydrogels have several advantages in cartilage tissue engineering, which include ease of formation, the ability to fine tune mechanical properties, the ability to encapsulate cells, and vast array of conjugation options for degradability, bioactivity, etc [7–9]. Hydrogels can be made from both synthetic (e.g., polyethylene glycol) and natural materials (e.g., collagen, gelatin), where both have their own inherent advantages and disadvantages. Synthetic materials have the advantage of the ability to more readily control the composition and mechanical properties of the hydrogel compared to hydrogels composed of natural materials, but natural materials have the additional advantage of providing biochemical cues and signals to facilitate cell attachment, growth, and differentiation [10].

One such natural material that is gaining attention in tissue engineering approaches is naturally derived extracellular matrix [11]. ECM materials can either be obtained from cell-derived matrices that are secreted during *in vitro* culture or they can be derived directly from native tissue [4,12–16], and often they have been decellularized to remove cellular components and nucleic acids that may have the potential to cause an adverse immunological response [11]. We and other groups have already established that decellularized cartilage has chondroinductive potential [11,13,17–20], and we recently reported the chondroinductive potential of decellularized cartilage (DCC) in pellet culture [11], where we observed increased chondroinductivity of rat bone marrow stem cells (rBMSCs) exposed to DCC as compared to those cells only exposed to TGF-β_3 [11].

Therefore, in this study we endeavored to create a material that was entirely derived from DCC to potentially make the material inherently chondroinductive, and we furthermore endeavored to design a material would have the mechanical properties necessary to be load-bearing. Several studies have made gels entirely out of ECM by first solubilizing the ECM, where the solubilized matrix would form a gel at body temperature [18,21–23]. One group even utilized solubilized cartilage matrix gels for drug delivery, where they noted that the gel maintained enough structural integrity under physiological conditions to be a stable drug depot [24]. We tried using solubilized cartilage hydrogels, but the gels that formed were too compliant and left opportunity for improvement for load-bearing applications. Methods of crosslinking unsolubilized cartilage have been reported, including crosslinking cartilage ECM with genipin, dehydrothermal treatment, ultraviolet irradiation, and carbodiimide chemistry [4,25]. Using these methods, cartilage scaffolds were able to be crosslinked and maintained some mechanical integrity throughout culture where cell mediated contraction was able to be controlled depending on the method of crosslinking. However, the authors of these previous studies noted that the constructs would require additional reinforcements to attain functional biomechanical properties and additionally, a sole ECM content of 10% was used to make the gels. In the current study, we sought to overcome this limitation through solubilizing and further crosslinking cartilage tissue. The rationale for solubilizing the cartilage tissue was to provide more control over mechanical properties through the ability to more finely tune the solid content of the hydrogel. Furthermore, solubilizing the cartilage may free up more reactive sites for crosslinking on the cartilage ECM, which may help reinforce the biomechanical properties of the solubilized cartilage once it is crosslinked. Therefore, based on our experience of functionalizing GAGs such as hyaluronic acid and chondroitin sulfate with glycidyl methacrylate [26,27], which allows the hydrogel to be formed through photocrosslinking, we decided to methacrylate solubilized, decellularized cartilage ECM. Earlier in 2015, one pioneering study reported methacrylating solubilized cartilage matrix to make photocrosslinkable hydrogels, demonstrating for the first time that native tissues can be crosslinked to form hydrogels [28]. However, in that study, the solubilized cartilage matrix was mixed with methacrylated gelatin (GelMA) and the biomechanics of the hydrogels, evaluated via the compressive modulus, still fell short of native cartilage tissue. Garrigues et al. [18] cleverly reinforced solubilized cartilage ECM through combining it with poly(ε-caprolactone) and then electrospinning it into a scaffold. However, the Young's moduli of the cartilage-containing electrospun scaffolds were approximately 10 kPa, which again fall short of the biomechanics of native cartilage tissue. In this current study, the goal was to create the first hydrogel entirely derived from cartilage ECM without additional reinforcements and study its potential for cartilage tissue engineering over a period of 6 weeks, a length of time that should be sufficient to show chondrogenesis and matrix synthesis. We hypothesized that this MeSDCC hydrogel would have a compressive modulus comparable to native cartilage and would be chondroinductive. Therefore, solubilized cartilage hydrogels were photocrosslinked and their mechanics as well as chondroinductive potential were analyzed.

2. Methods and materials

2.1. Tissue retrieval, devitalization, and decellularization

Ten porcine knees obtained from Berkshire hogs (castrated males that were approximately 7–8 months old and 120 kg) were purchased from a local abattoir (Bichelmeyer Meats, Kansas City, KS). Articular cartilage from the knee and hip joints was carefully removed and collected using scalpels. The cartilage was then rinsed twice in DI water and stored at −20 °C. After freezing overnight, the cartilage was thawed and then coarsely ground with dry ice using a cryogenic tissue grinder (BioSpec Products, Bartlesville, OK). Coarse grinding was performed to reduce diffusion distances during the decellularization process. The dry ice was then allowed to evaporate overnight in the freezer, at which point the cartilage was referred to as devitalized cartilage (DVC) [11], and then the DVC was packed into dialysis tubing (3500 MWCO) and decellularized using an adapted version of our previously established method using osmotic shock, detergent, and enzymatic washes [29]. The packets were placed under gentle agitation (70 rpm) in a hypertonic salt solution (HSS) overnight at room temperature. The packets were then subjected to 220 rpm agitation with two reciprocating washes of triton X-100 (0.01% v/v) followed with HSS to permeabilize intact cellular membranes. The tissue was then treated overnight with benzonase (0.0625 KU ml^{-1}) at 37 °C and then with sodium-lauroylsarcosine (NLS, 1% v/v) overnight to further lyse cells and denature cellular proteins. After NLS exposure, the tissue was washed with ethanol (40% v/v) at 50 rpm and then was subjected to organic exchange resins at 65 rpm to extract the organic solvents. The tissue was then washed in

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