**An injectable porcine cartilage-derived hydrogel for cartilage repair and regeneration**

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**Introduction:** Cartilage degeneration caused by trauma and osteoarthritis is the major reason of disability in the elderly. Approximately 27 million people in the United States are experiencing such suffer, which significantly reduces the quality of life in such patients. Since cartilage is an avascular tissue and has limited self-renewal capability, it is hard to utilize a common technique to treat such disease. Advanced tissue engineering approach to cartilage reconstruction and regeneration has been investigated for decades and it has shown potential for damaged or diseased tissue replacement. However, it still cannot achieve a new cartilage-like tissue with properties similar with native cartilage tissue. The scaffold as one of the key elements in tissue engineering continues to require improvement for bioactivity and biofunction. Recently, extracelluar matrix (ECM) scaffolds derived from native tissues for tissue regeneration have gained increasing attention due to their high bioactivity and ECM mimetic components [1]. Among them, the injectable ECM hydrogels can be applied for minimally invasive treatment, while facilitating cellular seeding and delivery and provide a biomimetic microenvironment for cell growth for tissue regeneration. Hydrogels derived from native tissues, such as muscle, nerve, bone, and heart, have been prepared for tissue engineering applications [2]. To our knowledge, no report in cartilage-derived hydrogel for cartilage regeneration was found. Here, this work aimed to develop an injectable hydrogel derived from porcine meniscus, and to evaluate its potential for cartilage regeneration.

**Methods:** Porcine meniscus were obtained from adult pigs (weighting 80~100 kg) at a local slaughter house. All reagents were purchased from Sigma and used as received. The meniscus were frozen at -80 $^{o}$C, and then grounded into powder. The powder was rinsed with 1X phosphate buffered saline (PBS), and then decellularized using 1% sodium dodecyl sulfate (SDS) in PBS for 72 h and again using 0.1 % ethylenediaminetetraacetic acid (EDTA) in PBS for 24 h. After that, the sample was washed with deionized water to remove the residual chemicals and freeze-dried overnight to obtain the decelluarized matrix. For hydrogel formation, the decellularized matrix was digested with pepsin in 0.01 M HCL for 48 h at a matrix/pepsin ratio of 10:1 to produce flowable ECM solution. The solution was neutralized using 0.1M NaOH (one-tenth of the digest volume) and 10X PBS (one-ninth of the digest volume), and then diluted to desired ECM concentrations by 1X PBS for a pre-gel solution. The ECM pre-gel solution was transferred into a mold and then placed in a 37 $^{o}$C incubator for 30 min to form a solidified hydrogel.

Decellularization of the sample was examined by hematoxylin and eosin (H&E) and 4', 6-diamidino-2-phenylindole (DAPI) staining and DNA assay. The collagen and glycosaminoglycan (GAG) amounts were measured using Hydroxyproline Assay (Sigma) and Blyscan Sulfated Glycosaminoglycan Assay (Biocolor Ltd.), respectively. Gelation behavior was determined by turbidimetric kinetics study. Compression moduli were measured on an MTS Insight mechanical test workstation. $5*10^5$ Mouse 3T3 fibroblasts/mL were encapsulated into the hydrogel and cultured up to 7 days for toxicity assessment using MTT assay and H&E staining.

**Results:** The cellular nuclei were rarely observed in both H&E and DAPI staining of the decellularized porcine meniscus. Furthermore, the decellularized matrix contained a DNA content of 6.7±0.8 ng/mg dry weight, which is significantly lower than in the native meniscus (115.6±5.3 ng/mg dry weight). The above results indicated an effective decellularization and the decellularized matrix has an acceptable DNA content (<< 50 ng/mg dry weight) [3]. The collagen content of the decellularized matrix and the native meniscus were 78±24% and 42±10%, respectively. However, the GAG content in the decellularized matrix (0.54±0.08 μg/mg dry weight) was much lower than that in the native meniscus (7.75±0.57 μg/mg dry weight), implying that the decellularization may be harsh for the GAG. The formed hydrogel appeared opaque and white with a shape as the mold (**Fig 1 left**). The turbidimetric kinetic study showed that all hydrogels at various ECM concentrations (4 to 10 mg/mL) had a 50% gelation time at approximately 15 min. The compression moduli of hydrogels increased from 224±68 to 997±9 Pa with ECM concentration from 8 to 12 mg/mL (**Fig 1 right**). The 3T3 fibroblasts encapsulated in the hydrogel survived and proliferated as confirmed by MTT assay. H&E staining showed the spread cells uniformly distributed inside the hydrogel.

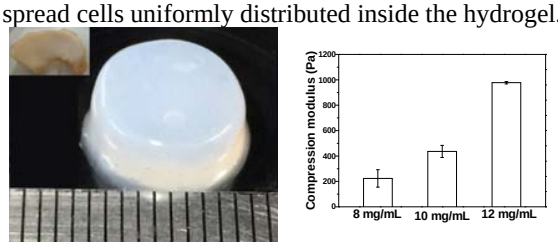


**Figure 1. Left**: Porcine cartilage-derived ECM hydrogel with an original porcine meniscus (inset). **Right**: compression modulus of the hydrogel.

**Conclusions:** Porcine meniscus was successfully processed into an injectable ECM hydrogel through decellularization and enzyme digestion. The cartilage-derived ECM hydrogel with tunable mechanical properties and good biocompatibility has potential to be applied for cartilage regeneration.

**Acknowledgement and References:**
Financial support from UTA new faculty start-up funding.

[1] Badylak SF. Biomaterials 2007;28:3587-93.
[2] Seif-Naraghi SB, et al. Acta Biomater 2012;8:3695-703.
[3] Crapo PM, et al. Biomaterials 2011;32:3233-43.