Optimizing decellularization detergents for meniscus-derived injectable hydrogel

Jinglei Wu[1,2], Qing Ding[1,2], Ahana Dutta[1,2], Yi Hong[1,2]*.

1. Department of Bioengineering, University of Texas at Arlington, Arlington, TX 76019, USA
2. Joint Graduate Program in Biomedical Engineering, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA

Statement of Purpose: Injectable hydrogel derived from meniscus is promising for meniscus repair and regeneration. Biochemical and mechanical properties of extracellular matrix (ECM) hydrogel were determined by the decellularized ECM, which was largely influenced by decellularization detergents [1]. In the current study, we treated porcine menisci using three detergents, including sodium dodecyl sulfate (SDS), Triton X-100 and peracetic acid, and then assessed morphology, DNA, collagen and glycosaminoglycan (GAG) contents of the obtained decellularized meniscal ECMs (DM-ECMs). Furthermore, the DM-ECMs were processed into soluble ECM solutions, which formed meniscus-derived hydrogels at 37ºC. Their gelation behaviors, morphology and mechanical properties were measured to determine the optimal detergent.

Methods: Porcine menisci were harvested from adult pigs (80~100 kg) at a local slaughterhouse. The menisci were decellularized with 1% SDS, 1% Triton X-100, or 0.1% peracetic acid for 3 days. The solutions were changed every day. Then menisci were treated with 0.1% ethylenediaminetetraacetic acid for 24h and rinsed with deionized water to remove residual chemicals. The samples were frozen and freeze-dried to obtain DM-ECMs. Their morphology was observed using a scanning electron microscope (SEM). The DNA, collagen and GAG contents of the DM-ECMs were evaluated using PicoGreen DNA assay (Invitrogen, Life Technologies, Inc), hydroxyproline assay (Sigma) and Blyscan Sulfated Glycosaminoglycan assay (Biocolor, UK), respectively.

The DM-ECMs were further digested using pepsin/0.01 M HCl solution with at 1:10 pepsin/ECM mass ratio for 48 h. The obtained solutions were then neutralized with the supplement of 10 % of 0.1 M NaOH and 10% of 10X PBS in an ice bath. Then the neutralized solution was injected to cylinder molds and incubated at 37 °C for 30 min to form solid hydrogels. The morphology of the hydrogels was observed using a SEM [2]. Gelation behavior was determined by turbidimetric kinetics study. Compression stresses and moduli were measured on a MTS Insight mechanical test workstation.

Results: SEM images showed that both the SDS and Triton X-100-treated DM-ECMs partially broke down to fibrils. The peracetic acid-treated DM-ECM exhibited a well-preserved architecture of organized pores and hollows without fibrils (**Fig. 1A**). The Triton X-100 and peracetic acid-treated DM-ECMs had DNA contents of 0.15 ± 0.02 and 0.10 ± 0.04 ng/mg, respectively, which were significantly lower than that of the SDS-treated DM-ECM. The total collagen contents of the DM-ECMs treated by SDS, Triton X-100 and peracetic acid were 78 ± 22%, 77 ± 10% and 76 ± 9%, respectively. However, the peracetic acid treated DM-ECM had a significantly higher GAG content of 6.19 ± 0.50 μg/mg compared to SDS (0.54 ± 0.08 μg/mg) and Triton X-100-treated (0.82 ± 0.36 μg/mg) DM-ECMs (**Fig. 1B**).

All DM-ECM hydrogels showed similar sigmoidal curves for hydrogel formation. In addition, the ECM hydrogels had a fibrous morphology with an average fiber diameter of approximately 400 nm (**Fig. 1C**). No significant difference in fiber diameter was observed among the three hydrogels. The peracetic acid-treated DM-ECM hydrogel had significantly higher peak compression than the SDS and Triton-X 100 treated DM-ECM hydrogels (**Fig. 1D**). At low ECM concentrations of 6 and 8 mg/mL, no significant difference was found in initial moduli between Triton X-100 and peracetic acid-treated meniscal ECM hydrogels. However, the initial moduli of the peracetic acid-treated meniscal ECM hydrogel with high ECM concentrations (10 and 12 mg/mL) were significantly greater than the Triton X-100 treated DM-ECM hydrogels.

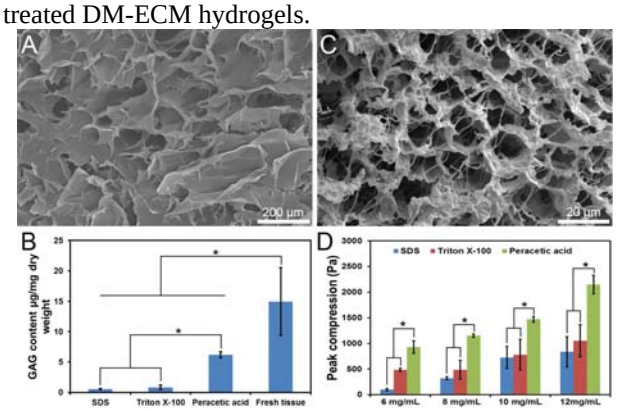

Figure 1. (A) The SEM image of peracetic acid-treated DM-ECM. (B) GAG contents of the decellularized meniscal ECMs (C) SEM image and (D) peak compression of the DM-ECM hydrogels. *:$P < 0.05$.

Conclusions: Three detergents were utilized to treat meniscus decellularization. The preacetic acid was considered as an optimal detergent for meniscus decellularization in terms of high GAG and great mechanical properties. The meniscus-derived hydrogel may find opportunities to be applied as a tissue-specific scaffold for meniscal tissue repair and regeneration.

Acknowledgement
We greatly appreciate the support from UTA start-up fund.

References:

[1] He M, et al. *Tissue Eng Part B*, 2013;19:194-208.
[2] Wolf MT, et al. *Biomaterials*, 2012;33:7028-38.