**Decellularized Tissue Extracellular Matrices: a Potential Source of Biomaterials for Tissue Engineering**

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**Introduction:** Scaffolds made of biomaterials are essential for tissue engineering, as they not only provide a three-dimensional (3D) space for cells to attach and subsequently develop into tissue, but they also encourage vital cellular functions, such as gene expression and matrix production. Recently, various decellularized tissues have been shown to generate functional tissues when organ-specific cells are cultured inside them. Animal studies have shown that decellularized extracellular matrix (dECM) is transplantable and improves the tissue functions of several tissues including the liver and heart (Ott HC. Nat Med. 2008;14:213-221.). However, organs are comprised of multiple cell types and these cells are organized in a hierarchical manner. It is difficult to regenerate such a hierarchical structure from a whole decellularized organ. Here, we present a method by which dECM is mechanically broken down to generate an ECM paste, which can then be reconstituted to make scaffolds with controlled properties.

**Methods:** We used a previously published procedure to produce a dECM (DeQuach JA. PLoS One. 2010;5:e13039.). Briefly, the liver and heart tissues were cut into smaller pieces of < 1 $cm^3$ in volume, and rinsed in deionized (DI) water for at least 30 min to remove the blood and cellular debris. The pieces were transferred into a surfactant solution (1% SDS and 1% penicillin streptomycin in PBS) and were left in it for 3–5 days for their complete decellularization. The surfactant solution was changed daily. The decellularized tissues were then soaked in DI water overnight to remove SDS. The pieces were broken down into fine ECM fibers with a blender and a homogenizer. The resulting samples were centrifuged and then lyophilized. Lyophilized ECM samples were stored at −20°C for future use.

The dECM was solubilized in pepsin solution containing 1 mM hydrochloric acid for 2 days. The dECM solution was crosslinked with 1% glutaraldehyde (GA), transglutaminase (TG), or 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC). The crosslinked dECM solution was cast in molds to create two-dimensional (2D) films. In addition, micropatterned 2D films were created by covering the crosslinked dECM solution on a polydimethylsiloxane (PDMS) micromold and letting it dry overnight.

The surface structures of these films were observed with a scanning electron microscope (SEM). The film samples were dehydrated with ethanol and hexamethyldisilazane (HMDS).

Mechanical testing of the samples was conducted using a mechanical testing machine. dECM film samples of a rectangular shape were used for the tests. Testing was conducted at a strain rate of 0.3%/s, and a 250 N load cell was used. The Young's modulus was defined as the slope of a linear fit to the stress–strain curve.

Statistical differences in the mechanical properties were assessed using one-way analysis of variance (ANOVA) and Tukey's post-hoc test. All statistical tests were two-tailed analyses, with significance set at $p < 0.05$.

Lyophilization was used to create 3D porous ECM scaffolds. Briefly, the dECM solution was poured into molds. After 1 h of crosslinking with TG, the dECM was frozen at −80°C, and then lyophilized. The porous surface structure was observed by SEM.

**Results and Discussion:** The dECM was suspended in water and homogenized, and then processed into 2D films with micropatterns by replica molding (Fig. 1 left). Topographic features on biomaterials have been shown to affect cellular behavior, such as morphology, orientation, and migration. Dai et al. have shown that cells align along the microscale patterns on decellularized tendon tissue surfaces (Dai X. Nanotechnology. 2011;22:494008.). The micropatterned ECM films could facilitate the growth and differentiation of tissue-specific cells.

Young's modulus of dECM films crosslinked with GA was significantly higher than that of the other groups (Fig. 1, right). This result shows that the mechanical properties of dECM can be tuned by changing the types of crosslinker.

The dECM was also processed into 3D porous scaffolds by lyophilization (Fig. 2). It has been shown previously that the porosity and morphology of scaffolds, the variables affecting tissue formation, can be tuned by changing the dECM concentration (Chen GP. Macromol Biosci. 2002;2:67-77.). This fact suggests that the material properties of the decellularized ECM scaffolds could be specifically designed.

**Conclusion:** We created several types of ECM scaffolds from decellularized tissues. Our results show that the properties of dECM can be specifically engineered, and that dECM can be used as a biomaterial for many applications by reconstituting an ECM solution.

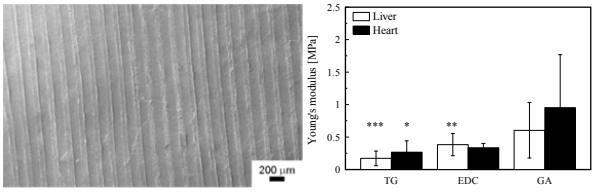


Figure 1. (Left) SEM image of liver dECM films with micropatterns. (Right) Young's modulus of dECM films. *: $p > 0.05$, **: $p > 0.01$, ***: $p > 0.001$, compared with GA.

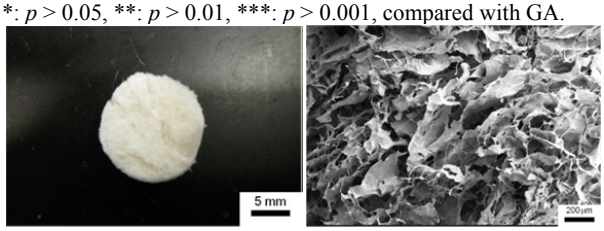


Figure 2. (Left) 3D porous liver dECM scaffold and (Right) its SEM image.