Decellularized Liver Tissue Based Hydrogel for Repair and Regeneration

Ramon E. Coronado[1,2], Shanmugasundaram Natesan[1], Washburn W. Kenneth[2], Glenn A. Halff [2], Robert J. Christy[1]

US Army Institute of Surgical Research, Fort Sam Houston, TX 78234[1]
University of Texas Health Science Center at San Antonio, San Antonio, TX 78229[2]

Introduction: Hydrogel based bioscaffolds developed using decellularized extracellular matrices (ECM) from specific tissues and organs are gaining increasing importance in regenerative medicine (**1**). Specifically, the matrix must be able to mimic the *in vivo* microenvironment of the tissue to repair large surface area-to-volume injuries. These ECM-based matrices have been shown to retain and deliver tissue-specific biochemical and molecular cues facilitating the integration and differentiation of cells, as well as restore tissue function (**2**). We have developed a novel procedure to produce liver tissue ECM based hydrogels and evaluate its ability to support liver stem cell attachment, viability and maintenance of hepatocyte specific phenotype *in vitro*. Ultimately, these ECM based hydrogels when combined with other liver-specific cell types can be used for liver repair *in vivo*.

Materials and Methods: Porcine liver tissue slices (3mm) were decellularized using modifications from a previously published protocol (**3**). Briefly, tissue slices were incubated with trypsin-EDTA (0.02% $^{v}/_{v}$ trypsin to 0.05% $^{v}/_{v}$ EDTA) with agitation (120 rpm) at 37°C for 1 hour. Tissues were washed with deionized water and incubated with 3% $^{v}/_{v}$ Triton X-100 (1 hour), washed with deionized H_2O and incubated with 4% $^{v}/_{v}$ sodium deoxycholate (2 hours) at 120 rpm at room temperature. Following this treatment, tissues were washed in phosphate buffered saline (1x) and deionized water (3x) for 15 minutes with agitation (120 rpm) at room temperature. Finally, the decellularized tissue was treated with 0.1% $^{v}/_{v}$ peracetic acid, 4% $^{v}/_{v}$ ethanol in deionized water for 2 hrs and washed with PBS and deionized water, as above. For analysis, tissues before and after decellularization were compared. To visualize nuclei, tissue sections were stained with 4',6-diamidino-2-phenylindole (DAPI) and residual DNA content, was quantified using a PicoGreen dsDNA Assay (Life Technologies) according to manufacturer's instructions. Hepatocyte growth factor (HGF), basic fibroblast growth factor (bFGF) and epithelial growth factor (EGF) were quantified using Quantikine ELISA kits (R&D Systems). Hydrogels were prepared by digesting milled decellularized liver ECM (420 µm) with pepsin (10:1 w/w per ml of 0.5M acetic acid). The homogenous ECM solution was then gelled by neutralizing with 1N NaOH and incubating at 37°C for 30 minutes. *In vitro* cell attachment and viability was evaluated using of human induced pluripotent hepatocyte cells (CellularDynamics).

Results: The decellularization protocol successfully reduced DNA from 3019 ± 1945 ng/mg in the native porcine liver to 43.4 ± 10.9 ng/mg in the decellularized extracellular matrix. Analysis of the growth factors HGF, bFGF, and EGF, which are native to liver tissue, were retained after decellularization to levels of 1078 ± 489 pg/mg, 40 ± 6 pg/mg, and 0.7 pg/mg, respectively. In addition, after pepsin digestion of milled decellularized liver ECM we were able to prepare stable hydrogels (Figure 1). Most importantly, the hydrogel-based scaffold supported attachment and viability of human derived hepatocytes indicating the ability of the hydrogels to support liver-specific differentiated cell phenotypes.

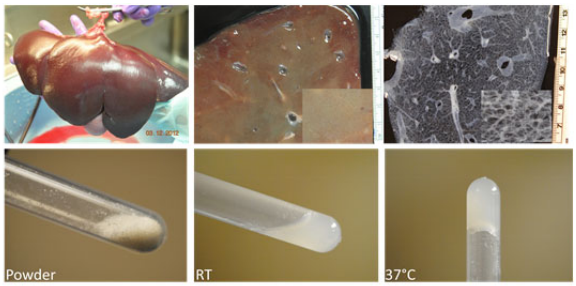

Figure 1. Representative images of porcine liver tissue decellularization and subsequent ECM-based hydrogel formation.

Conclusion: In this study we have developed a method to prepare decellularized liver ECM. The decellularized ECM can be used to prepare a hydrogel-based matrix that supports liver cell specific attachment and viability. Most importantly, these results show that organ-specific ECM based hydrogels can be used to study liver cell-tissue specific ECM interactions *in vitro* and act as a tissue engineering platform for liver repair and regeneration.

References:

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