Evaluating the Utility of Hydrogels and Solubilized Matrix Proteins Derived from Decellularized Synovial Membranes

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Introduction

- Decellularization is the process of removing cellular components from native tissues while retaining the important structural, biochemical, and biomechanical features of the extracellular matrix.[1]
- Whole decellularized tissues are more clinically relevant than synthetic scaffolds but have limitations
  - Fixed geometry and difficulty of cell penetration limit utility of decellularized whole tissue.
  - Modulable hydrogel scaffolds made from decellularized ECM (dECM) may address these shortcomings.[2].
  - Enzymes like pepsin, have previously been shown to be able to create gellable digest solutions.[3]
- Arthritis is one of the most common and costly musculoskeletal disorders.
- Synovial membrane plays a role in the pathogenesis of osteoarthritis and other joint diseases.
  - An *in vitro* model that can recapitulate the biochemical and mechanical factors present in the native environment would be a valuable research tool.

- **Our aims were 1) to develop a process for creating a dECM hydrogel from solubilized decellularized synovium and 2) to assess its utility as a scaffold, coating material, and media supplement.**

Methods

Phase I : Decellularization

- Human synovial membranes obtained from the Musculoskeletal Transplant Foundation were sectioned into 280um sections.
- 1% Triton X-100 was used to remove the cellular components from tissue slices.
- Decellularized tissue were treated with DNase overnight at 37°C.
- Decellularized tissue was washed with water and lyophilized.

Phase II: Preparation of a Hydrogel Precursor

- Digestion: 1mg/mL of a pepsin in 0.01N HCl and 0.1N HCl solutions were used to digest decellularized tissue to make a pre-gel solution.
  - 10mg of lyophilized tissue were digested per mL of pepsin solution.
  - Some dECM samples were subjected to additional washing before digestion.
- BCA assay: To determine the total protein concentration of the digested solutions, we used a bicinchoninic acid assay with a bovine serum albumin standard to determine a standard curve.

Phase III: Applications of Pre-gel solutions

- Acidic pre-gel solutions were neutralized with NaOH and brought to isotonic solute levels before use.
- Scaffold: Synoviocytes ($4x10^5$/mL) were seeded directly into neutralized pre-gel solution.
  - Suspension was incubated at 37C until dECM hydrogel was set.
  - Constructs were grown for 24 hours before live/dead staining.
- Coating: Wells were coated with either collagen or dECM pre-gel solutions at 5ug/cm^2 for 1 hour before synoviocytes were seeded.
- Supplement: Neutralized pre-gel solutions were diluted and used as media supplements at 1, 5, and 10% of total culture volume.
- MTT assay: Metabolic activity of synoviocytes was evaluated using an MTT assay to evaluate the effects of coating and supplementation with solubilized dECM.

Statistics: Data evaluated using two-way ANOVA with Turkey HSD post-hoc tests (α=0.05). Values presented as mean ± standard deviation.

Figure 1. The experimental schematic

Results

Phase I : Decellularization

Based on biochemical and cytotoxicity results, the optimal decellularized method was 1% Triton-X for 2 days. Triton-X also retains Collagen and GAG content relative to control. Residual DNA content post-decellularization was lowered by >80% under Triton-X exposure compared to controls.

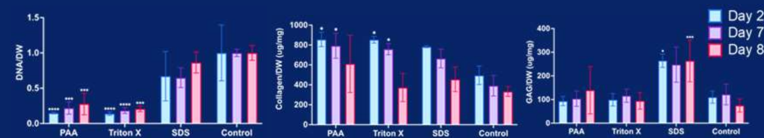
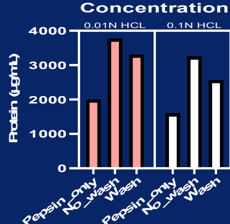


Figure 2. Biochemical content showing remaining relative (A) DNA normalized against controls, (B) COL, and (C) GAG content normalized to respective sample dry weights for each decellularization method, N=3

Phase II: Preparation of a Hydrogel Precursor



- The effectiveness of pepsin digestion in 0.01N HCl and 0.1N HCl solutions was assessed by measuring solubilized protein.
- Both HCl concentrations and washing conditions yielded similar total protein concentration based on a BCA assay.
- dECM soluble protein content of ~1.65mg/mL more than the pepsin solution itself was observed.

Figure 3. Biochemical content showing the total protein solubilization by the Pepsin enzyme solution in HCl across different acid concentrations.

Phase III: Applications of Pre-gel solutions

- 0.1N pepsin digest of decellularized ECM was able to form a hydrogel once pH and salt levels were balanced. (Figure 4A)
- Hydrogels made from 0.1N dECM supported the growth of cells at 24hr. (Figure 4B)
- Groups with 0.1N supplement media had higher metabolic activity than those with 0.01N.(Figure 4.F-G)
- dECM coating and supplementation generally led to lower metabolic activity compared to controls

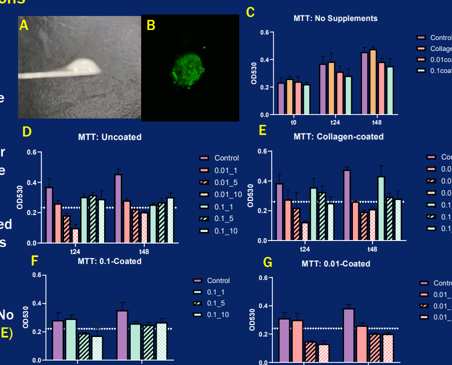


Figure 4. A) Gelled sole decellularized hydrogel (0.1N digest) B) Live Dead stained 0.1N digest hydrogel at D1 C –G) Results of the MTT assay C) No added supplements D) uncoated w/ supplements E) Col coated w/ supplement F-G) 0.1N and 0.01N coated respectively with supplements.

Conclusions

- Pre-gel solutions gel at physiologic temperatures once neutralized and can be used as a scaffold for the culture of synoviocytes.
- Solubilized dECM used as a coating material or media supplement alters cellular metabolism
- Understanding the behavior of cells grown with dECM under these conditions requires further study
- Other reagents (e.g. urea) are known to extract relevant growth factors and proteins from tissues; such extracts may be relevant as media supplements or eventually as therapeutics.

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References

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