CS-Blood Hydrogel Adhesive for the Expansion and Controlled Differentiation of Stem Cells

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Statement of Purpose: Clinically, there exists a need for adhesive biomaterials. Fibrin glue exhibits outstanding biocompatibility, but it lacks the desired adhesive strength and degrades rapidly. The aim of this study was to design a substitute to fibrin glue with improved adhesive properties as well as controlled degradation.

Methods: Chondroitin sulfate succinimidyl succinate (CS-NHS) in PBS was mixed with human whole blood (hWB), causing the proteins in blood to reacted with CS-NHS to form a hydrogel (Figure 1A). A modification of ASTM standard F2255-05 was used to quantify tissue adhesion. Briefly, two pieces of porcine skin were glued together and pulled apart at 0.05 mm/sec using a mechanical testing device. The stress at which the bonding failed was designated as the adhesive strength of the material. Hydrogels containing 50% (v/v) hWB and 5, 10 or 15% CS-NHS (w/v) were tested and compared to fibrin glue and Dermabond®.

Human mesenchymal stem cells (hMSCs) were encapsulated in the hydrogel and were stained for 30 minutes using 4 μM calcein AM and 4 μM ethidium homodimer-1 (EthD-1) so that the cytoplasm of live cells fluoresced green and the nuclei of dead cells fluoresced red, respectively. The cells were washed 3 times with PBS and imaged using a fluorescent microscope. Cell spreading as well as viability inside the hydrogels were quantified using these stains.

The hydrogel formulation was varied to investigate cell spreading and chondrogenesis. For cell spreading experiments the hydrogels contained 5% (w/v) CS-NHS and 60 or 75% (v/v) hWB. For the chondrogenic experiments, hydrogels contained 5% (w/v) CS-NHS and 50% (v/v) blood. Chondrogenesis was evaluated using biochemical assays, histology, fluorescent staining and real time PCR.

Results: CS-blood hydrogels supported hMSC proliferation and spreading. Viability of hMSCs encapsulated in CS-blood hydrogels was 98.4 ± 2.7% following 2 weeks of culture. Increase in cell spreading with increasing blood content of the hydrogel was observed due to the higher concentration of integrin binding peptide motifs as well as the increase in porosity of the hydrogel. In the absence of chondrogenic factors, hMSCs maintained their fibroblast-like morphology (figure 1B), but as soon as chondrogenic factors were added, the cells aggregated and assumed a round chondrocyte-like morphology (Figure 1C). Thus, the cells can be expanded in the hydrogels without differentiation.

Following exposure to chondrogenic factors, hMSCs underwent chondrogenesis. Biochemical results and gene expression profiles demonstrated collagen and aggrecan synthesis. Additionally, collagen type II was the predominant type of collagen synthesized, which is the type of collagen found in articular cartilage. Gene expression results also showed an elevated expression of Sox-9. Collagen synthesis was also observed via histology with Masson's trichrome stanining.

The adhesive strength of the material is 5.6 times that of fibrin glue and ~0.6 times that of the cyanoacrylate Dermabond® (Figure 1D). As the concentration of CS-NHS increased from 5 to 10% (w/v), the adhesive strength increased from 8.7 to 19.6 kPa, while the modulus of the material remained constant. Furthermore, after increasing the CS-NHS concentrations from 10% to 15% (w/v) both the modulus and adhesive strength of the material decreased. Therefore, the adhesive strength was dependent both on the modulus of the adhesive as well as the concentration of crosslinker (CS-NHS) used.

Cells from the surrounding tissue infiltrated acellular hydrogels that were injected subcutaneously in a rat model (Figure 1E). The cells deposited collagen in the bulk of the material. Additionally, neovasculature was observed in the bulk of the material. This demonstrated that cells can migrate through the CS-blood adhesive and also remodel it.

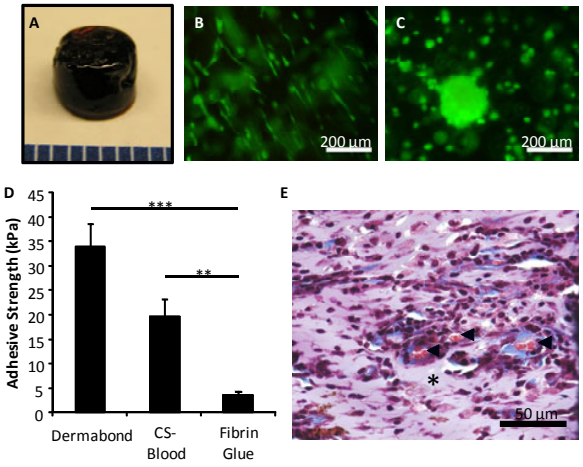

Figure 1. (A) Gross image of a CS-Blood hydrogel. hMSCs in CS-blood hydrogels cultured in (B) expansion medium followed by (C) chondrogenic medium. (D) The adhesive strength of CS-blood is between that of Dermabond® and fibrin glue. (E) Masson's trichrome staining of CS-blood gels injected subcutaneously in a rat model. asterisk = hydrogel; arrow head = neovasculature.

Conclusions: The CS-blood material can support cells and can be remodeled by cells. Its physical properties in regards to tissue and cell adhesion make it a good delivery vehicle for both tissue regeneration and repair. The ability to control differentiation in the material could prove useful in applications where secretion of growth factors by stem cells is desired as well as applications where the cells are needed for new tissue formation.