Development of Injection Formulation for the Engineering of Vascularized Soft Tissue

Young Min Ju, Thomas Shiner, Shaida Moghaddassi, Anthony Atala, James J. Yoo, and Sang Jin Lee
Wake Forest Institute for Regenerative Medicine, Wake Forest University School of Medicine Winston-Salem, North Carolina

Introduction

Soft tissue defects represent a component of injury in a major percentage of military traumas. Development of a clinically useful vascularized soft tissue that can be used as a stand alone therapy or integrated with composite tissue regenerative medicine therapy would be critical for the success of reconstruction. This is especially important in restoring contour in the craniofacial region. In this study we explored the possibility of developing an injectable gel formulation that releases angiogenic factors while maintaining its structural integrity.

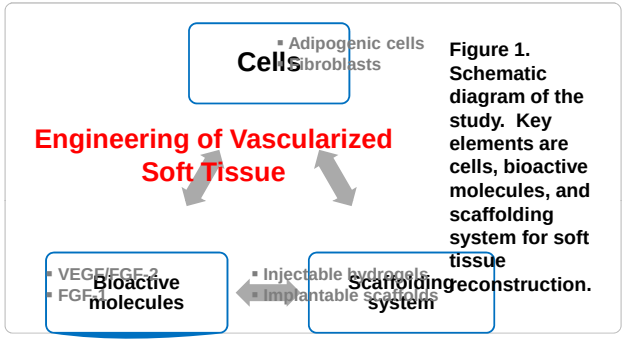


Figure 1. Schematic diagram of the study. Key elements are cells, bioactive molecules, and scaffolding system for soft tissue reconstruction.

Methods

To achieve a injectable gel system that maintains structural integrity, fibrin/collagen and fibrin/HA mixed constructs were prepared in the following ratios of collagen or HA to fibrin (expressed as mg:mg): 1:0.5, 1:1, and 1:2. Dermal fibroblasts were isolated from 6-week old euthanized EGFP-mice (C57BL/6-Tg(CAG-EGFP)1Osb/J, Jackson Lab.) by enzymatic digestion. The cells (2×10^6 cells/mL) were suspended in the fibrinogen solution; subsequently cell-fibrin constructs were prepared adding the same volume of thrombin solution into collagen solution using a double syringe applicator. Hyaluronic acid (HA: 1.0 mg/mL) and fibrin mixed constructs were prepared in a similar fashion. The hydrogels were characterized by measuring the initial mechanical properties with various concentrations and ratio of each component. The hydrogel systems were characterized by measuring initial mechanical properties, cell viability, and *in vitro* and *in vivo* structural stability.

Results

The concentrations and ratios of fibrin/collagen and fibrin/HA were modified for optimizing fabrication conditions for hydrogel systems used in soft tissue engineering. Variation of the fabrication parameters can affect the initial properties of cell-hydrogel constructs. The hydrogel compaction over time in culture decreased with increasing concentration of components but was augmented by the presence of fibrin. The maintenance of structural integrity of the gel was related by the levels of hydrogel compaction, protein density, and cell concentration.

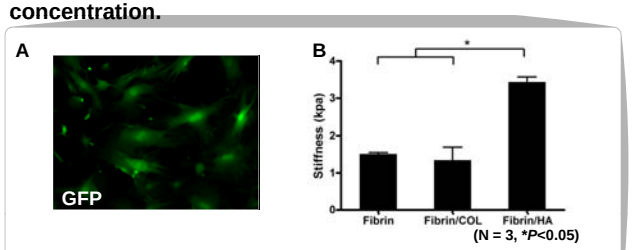


Figure 2. (A) Isolated EGFP-labeled fibroblasts. (B)Initial mechanical properties of fibrin, fibrin/collagen, and fibrin/HA.

Table 1. Four experimental groups were implanted with different formulation of fibrin and collagen or HA.

	Fibringen (mg/mL)	Thrombin (U/mL)	Collagen (mg/mL)	HA	Cell density (cells/mL)
Fibrin	50	10			×
Fibrin	50	10			5×10^6
Fibrin/COL	50	10	5		5×10^6
Fibrin/HA	50	10		10	5×10^6

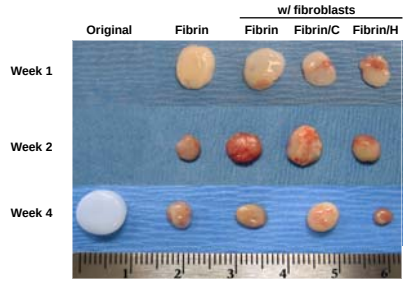


Figure 3. Gross appearance of all experimental groups at 1, 2, and 4 weeks after implantation

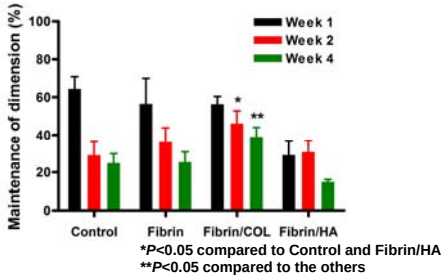


Figure 4. Percentage of maintaining dimension of cell-hydrogel constructs at 1, 2, and 4 weeks after implantation (*, **$P<0.05$).

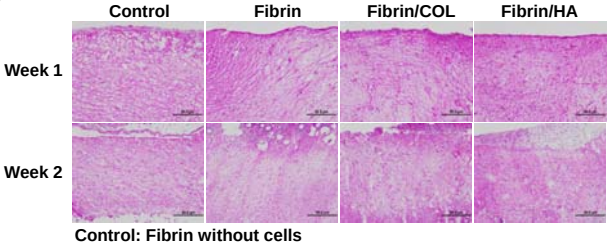
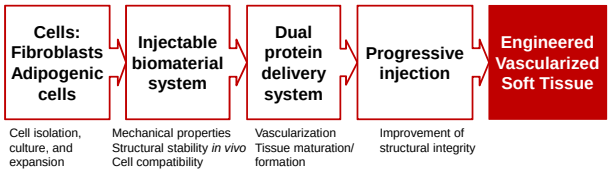


Figure 5. Histological evaluation of the retrieved cell-hydrogel constructs at 1 and 2 weeks after implantation.

Ongoing Studies



Conclusions

The initial mechanical properties of fibrin/collagen, fibrin/HA, and fibrin can be enhanced by varying the quantity and ratio of the components. Further studies are required to demonstrate applicability of the cell-hydrogel constructs for vascularized soft tissue reconstruction.