

Hyaluronan-Fibrin Gel System for Cartilage-Mediated Bone Regeneration

Paizy E. Mikael^{1,2} and Syam P. Nukavarapu^{1,2,3}

¹Institute for Regenerative Engineering, ²Mat Sci. and Eng., ³Orthopaedic Surgery, University of Connecticut

Statement of Purpose: Most strategies currently being developed for the reconstruction and repair of long segmental bone defect support intramembranous ossification. However, long-bone development occurs through cartilage-mediated ossification where bone formation takes place through cartilage matrix formation, matrix vascularization and finally ossification. Here we aim to engineer a hydrogel system which has the intrinsic capacity to guide progenitor cell source through endochondral ossification. In this study, we are proposing to develop an IPN like gel system comprising of varying amounts of hyaluronan (HA) and fibrin (FB). The optimal combination will be determined through their regenerative potential to guide human bone marrow MSCs through the endochondral route by mimicking the natural microenvironment of cartilage formation and the subsequent mineral deposition.

Methods: Fresh human bone marrow aspirate (BMA) was purchased (Lonza, Walkersville MD); 35ml of BMA was concentrated (cBMA) to a final volume of 5ml using an automated cell separator, Magellan[®] (Arterioocyte Medical Systems, Hopkinton MA). Cells were encapsulated in either hyaluronan (BioTime Inc, Alameda CA), Fibrin gel (Sigma-Aldrich), or different HA:FB combinations: 90:10, 80:20, 70:30, 60:40, and 50:50. Cultures were maintained in Chondrogenic media for 14 days. Histological sections stained with Alcian Blue to determine cartilage matrix formation. Storage modulus, G' , was measured for each gel between 1 and 10^2 strain units at 0.5 Hz oscillating frequency at 37°C.

Results: In the current gel system, fibrin is expected to exert the contractile force necessary for cell condensation¹, which is a prerequisite for MSC chondrogenesis. The incorporation of hyaluronan is expected to maintain the shape and volume of the hydrogel complex. In addition, HA is a natural component of the cartilage matrix that has the ability to promote cell attachment and proliferation¹. Together, the FB-HA gel system may have the characteristics required to support cartilage-mediated bone regeneration.

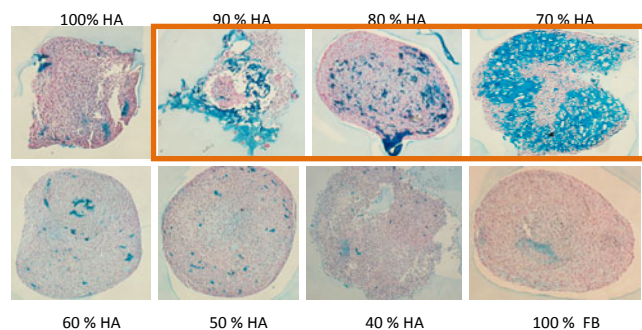


Figure 1: Sulfate GAGs staining pattern images of HA-FB constructs containing enriched hBMSCs.

Here we examine whether the different combinations of HA:FB hydrogels will support endochondral ossification. The Alcian blue staining shows HA:FB combinations

expressing higher GAG formation than either 100% HA and 100% FB. This study clearly demonstrate that optimal HA:FB combinations for enhanced glycosamioglycan formation are the 70, 80, and 90% HA to 30,20,10% FB gel, respectively (figure 1). To better understand the physical behavior of these hydrogels, we examined the gel's resistance to deformation (G'), figure 3. While most combinations showed little differences in stiffness for the range shown; they all maintained their structure. In contrast, the deformation for 100% HA occurred between 10 and 100 strain units.

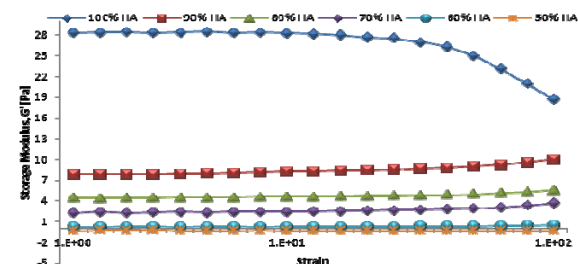


Figure 2: Rheological characterization of hyaluronan (HA) and fibrin (FB) combinations. Raw data showing storage modulus, G' , from 1 strain unit to 10^2 , for 6 combinations: 100% HA, 90%HA-10%FB, 80%HA-20%FB, 70%HA-30%FB, 60%HA-40%FB, and 50%HA-50%FB.

In order to examine the relative contribution of the elastic and viscous component of each gel, the phase angle δ was determined. The higher δ is, the more viscous the material is. In this study, 90%, 80% and 70% HA show higher phase shift and therefore can be characterized as more viscous than other 100%, 60% and 50%.

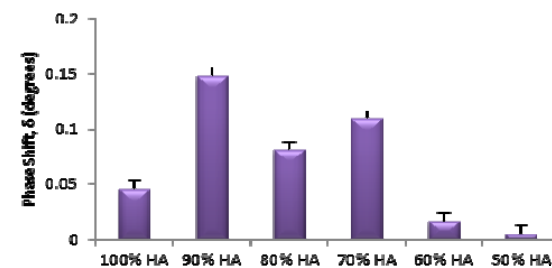


Figure 3: phase shift measurement for each hydrogel combination from 1 to 100 strain units.

Conclusions: Scaffold systems designed to provide the necessary micro-environment is essential for successful tissue regeneration. Through this study, we have created a novel IPN like gel system with variable stiffness suitable to support the stages of cartilage-mediated bone regeneration. The developed gel system combined with a weight-bearing scaffold and enriched human bone marrow aspirate, we propose to develop a novel strategy for segmental bone defect repair via endochondral ossification route.

References:

1. Lee et al. Acta Biomater. 2013; 9: 5149-5152.