Ionic Strength Controls Fibrin Gel Mechanical Properties for Enhanced Osteogenesis

H.E. Davis, S.L. Miller and J.K. Leach.
Department of Biomedical Engineering, University of California, Davis, CA.
School of Medicine, University of California, Davis, CA

Statement of Purpose: Cell delivery with fibrin gels is well documented and viability has been attributed to the final network structure of the gel, usually tailored by modifying the relative concentrations of fibrinogen and thrombin.[1] Stiffer substrates are required to drive osteogenic differentiation of human mesenchymal stem cells (hMSCs), necessitating substantial volumes of fibrinogen. However, patients who suffer from critically sized defects requiring interventions for bone repair are often hemostatically unstable, thus creating a challenge when harvesting clotting proteins in the large quantities necessary to fill a defect. We present an alternative method for tailoring fibrin gel architecture by varying the ionic concentration of the pre-gel solution to produce constructs with suitable mechanical properties for the repair and regeneration of skeletal tissues.

Methods: Human fibrinogen (40 mg/mL in PBS) and 0.00-3.60% (w/v) NaCl were mixed in equal volumes with human thrombin (5 U/mL in 40 mM $CaCl_2$) and allowed to gel for 1 hr at 37ºC. This process resulted in fibrin gels with final fibrinogen, $CaCl_2$, and thrombin concentrations of 20 mg/mL, 20 mM, and 2.5 U/mL, respectively. Changes in optical density (turbidity) were measured at 550 nm directly after mixing all components in order to assess the kinetics of fibril formation. Gelation time was determined by differentiating each curve and ascertaining the maximum values. Compressive moduli of gels were determined after incubation for 1 hr in PBS and then blotted and compressed at 1 mm/min. Equilibrium swelling ratios were determined by incubating the gels in PBS for 24 hr and comparing swollen gel weight to lyophilized weight. Gel degradation was assessed by incubating the constructs in PBS at 37ºC and collecting and replacing the PBS at different time-points. The amount of total protein (*e.g.*, fibrin) was quantified using a Micro BCA Protein Assay Kit. The *in vitro* osteogenic differentiation of hMSCs encapsulated within gels was determined by quantifying alkaline phosphatase (ALP) activity, DNA content, and cell-secreted calcium after culture for 7 and 14 days under osteogenic conditions as described.[2] Constructs lacking salt were not examined, as they were too weak to handle during material characterization. Cell viability was examined at days 1, 3, and 7 during encapsulation using the alamarBlue assay. To assess whether cell cytoskeletal or material contraction forces were primarily responsible for an observed gel geometrical reduction, cell-seeded constructs were incubated in 50 μM of blebbistatin for 7 days. Their resulting diameter was quantified using NIH Image J software and compared to corresponding ionic strength gels without the myosin II inhibitor and gels without cells.

Results: A decrease in turbidity was observed up to a 1.8% NaCl concentration; the addition of further salt resulted in a slight rise of turbidity. Gelation time increased with ionic concentration, in contrast to the trend observed in optical density measurements. Compressive moduli (Fig. 1A) and equilibrium swelling ratios followed a similar trend as optical density with strength peaking at 1.80%, roughly three times the magnitude of gels lacking salt. Gel degradation was primarily linear (R^2 ranging from 0.8975 to 0.9999) for all groups. Metabolic activity was greatest in constructs fabricated with 0.45% salt at days 1 and 3, but by day 7, there were no significant differences between groups. Although we did not detect differences in cell number on day 7 between groups, proliferation and ALP activity was affected at day 14 (Fig. 1B). Gels fabricated with 0.45% salt had significantly increased DNA content compared to all other fibrin groups and corresponding decreased ALP activity. Fibrin constructs fabricated in the least amount of salt resulted in gels with the most cell-secreted calcium at day 7, but these differences were no longer apparent by day 14. Compared to cell-free gels, cell-loaded gels exhibited significant contraction in the presence of 0.45%, 0.90% and 3.60% salt. The addition of blebbistatin increased gel diameter in the 0.45% and 3.60% groups, suggesting that cell contractile forces contribute to gel deformation.

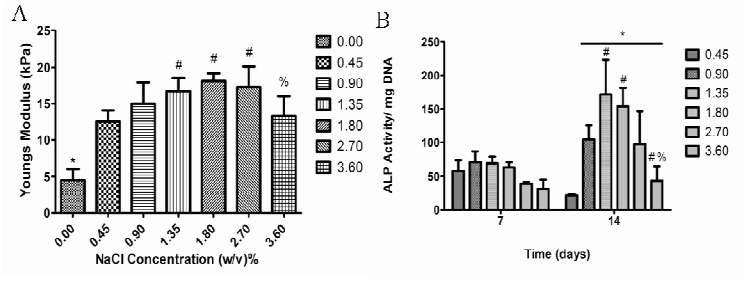

Figure 1. A) Young's modulus of gels (n=5, * $p<0.001$ versus all other gels. # $p<0.05$ compared with 0.45% and % =$p<0.05$ compared with 1.8%). B) ALP activity of encapsulated hMSCs (n=4 * $p<0.01$ compared to 0.45% group, # =$p<0.05$ compared to 0.90% group, % =$p<0.05$ compared to 0.90-2.70% groups). Chart values represent mean ± SD.

Conclusions: These data confirm that simply varying the salt concentration of the pre-gel solution can modulate the material properties of fibrin constructs without additional fibrinogen or thrombin. These data expose the tradeoff between cell viability and differentiation in fibrin gels when varying salt concentration. Furthermore, controlling the ionic strength of the pre-gel solution provides a new approach for generating improved cell delivery vehicles for use in bone tissue regeneration**.**

References:

1. (Catelas I. Tissue Eng 2006; 12(8): 2385-2396.)
2. (Davis HE. J Biomed Mater Res Part A 2009; 90A(4): 1021-1031.)