Fibrinogen and Thrombin Concentrations Modulate the Diffusion and Flow Rates of Fibrin Matrices

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Statement of Purpose: Fibrin is a natural biopolymer derived from the coagulation cascade, where the interaction of fibrinogen and thrombin forms a provisional 3D matrix during wound healing. Biochemically, fibrin has been shown to promote cell growth and proliferation in a variety of cell model systems[1]. The success of a cell supportive composition is highly dependent on the attributes of the fibrin scaffold. Scaffold pores must have controlled size in order to balance the amount of cellular nutrient exchange and proliferation with the mechanical integrity of the scaffold. The purpose of this study is to determine the effects of different fibrinogen and thrombin concentrations on the diffusivity, flow rates, and burst release rates of neutrally charged dextran microparticles through 3-D fibrin matrices.

Methods: Fibrin matrices comprising of different formulations of fibrinogen and thrombin (Tisseel®, Baxter Bioscience), were formed in modified vertical column apparatus. Flow assessment was determined by eluting serum-free DMEM through the pre-formed matrices. Elution data was interpreted using Darcy's law of fluid dynamics in porous structures[2] to ascertain the average effective pore size of each fibrin matrix. Diffusion and burst release assessments were determined on fibrin matrices preformed on Transwell® cell culture inserts. The diffusion and burst release rates of fluorescent labeled dextran microparticles (FITC 3,000 MW, Rhodamine 7,000 MW) through the fibrin matrices were examined at various time points. Agarose gels with known pore sizes were used as controls to rule out possible interactions and partitioning effects between dextran particles and fibrin.

Results: Darcy flow assessment showed that at constant thrombin concentration of 2 IU, increasing the fibrinogen component would correlate to a decrease in flow rate of liquid media; thereby, translating to smaller pore radii ($p<0.05$). This corresponding decrease in flow rate was observed in fibrin compositions containing < 15 mg/ml of fibrinogen. At fibrinogen concentration greater than 15 mg/ml, the flow rate was too slow to discern statistically significant differences. Diffusion rate of dextran microparticles through 3-D fibrin matrices was shown to be affected by particle size and fibrin composition. In figure 1, increasing the fibrinogen concentration resulted in a corresponding decrease in the diffusivity of particles and that the smaller Fitc-dextran particles diffused more readily through the 3-D matrices than the larger Rhodamine-dextran particles. Figure 2 showed that increasing the thrombin concentration, at constant fibrinogen concentration, resulted in a decrease in the rate of particle release or burst release from the 3-D fibrin matrices.

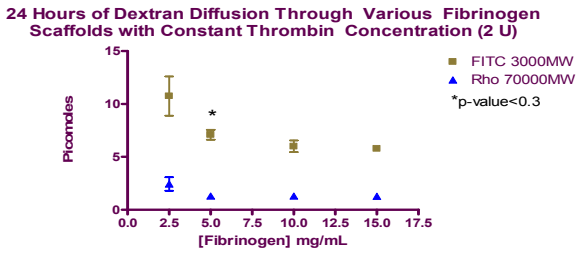

Figure 1

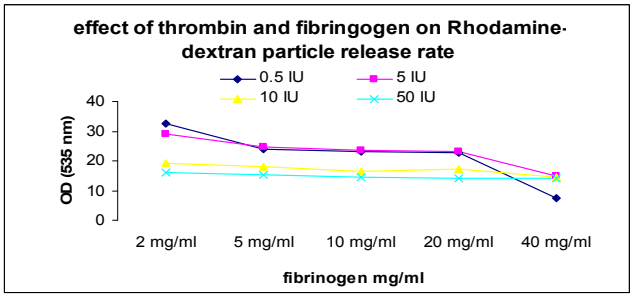

Figure 2

Conclusions: We have previously shown that fibrinogen concentration has significant effects on cell proliferation and function.[3] In this project, we've shown that increasing either the fibrinogen or the thrombin composition of the final 3-D fibrin matrices directly affected mass transport and pore dimensions, which may correlate to influences on nutrient uptake, waste removal, and delivery of micro particles to entrapped cells within the 3-D fibrin matrices. The data showed that fibrin matrix composition can be modulated (through fibrinogen and thrombin composition) to control the diffusion, flow, and release rates of molecules of different sizes. Future studies will compare experimental nutrient concentration profiles with predicted gradients from mathematical models, and use that information to design optimal fibrin matrices for efficient cell delivery and transplantation as well as delivery of therapeutic micro-particles.

References

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