Enzymatic Resistance and Mechanical Stability of Genipin-Crosslinked Films

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Statement of Purpose: Genipin is a natural biocompatible crosslinking agent extracted from the Gardenia jasminoides. It is 10,000 times less cytotoxic than it's more commonly used counter agent, glutaraldehyde [1]. Fibrin is natural biopolymer useful in natural wound healing, yet continues to have limitations in some biomedical applications that require stable clots, such as treating intracranial aneurysms. Fibrin clots with enhanced stability could improve the performance of embolization treatments. In our study, fibrin hydrogel networks are stabilized via covalent crosslinking with genipin. Fibrin gels undergo enzymatic degradation through fibrinolysis. The mechanical stability and enzymatic resistance is evaluated through the incorporation of streptokinase, a bacterial enzyme derived from group C (beta)-hemolytic streptococci. It indirectly breaks down blood clots via the activation of plasminogen [3]. This work describes the identification of enzyme kinetics to observe the mechanical stability of genipin-crosslinked fibrin gel networks.

Methods: Streptokinase was dissolved in PBS buffer at concentrations of 100 U/mL, 50 U/mL, 25 U/mL, and 10 U/mL. Fibrinogen and thrombin extracted from bovine plasma were also dissolved in PBS buffer at concentrations of 20 mg/mL and 10 U/mL, respectively. Fibrin gels were synthesized by combining 0.1 mL thrombin solution with 0.4 mL of fibrinogen solution in each well of a 24 well plate. The gels were incubated at 37°C for at least 12 hrs on a rotary shaker prior to the addition of genipin. Genipin solutions at concentrations of 1 mg/mL, 3.5 mg/mL, 5 mg/mL, 10 mg/mL, and 25 mg/mL were added to hydrated fibrin gel networks and incubated for 24 hrs at 37°C on a rotary shaker. Fibrin gels of each condition, pristine fibrin gel or genipin-crosslinked fibrin gel, were characterized by rheology at prescribed time points. Streptokinase solution at 100 U/mL was added to the crosslinked fibrin gels and the enzymatic degradation was characterized over time. Streptokinase solutions at 50 U/mL, 25 U/mL, and 10 U/mL were added to pristine fibrin gels to measure the rates of enzymatic degradation over time. The mechanical stability of each sample was determined by the storage modulus at each time point.

Results: The storage modulus of each sample was measured using the rheometer at prescribed time points throughout the experiment. The pristine fibrin gels depict lower mechanical stability (92.92 ± 15.37 Pa) as compared to genipin-crosslinked fibrin gels (133.95 ± 6.68 Pa for genipin concentration of 25 mg/mL). The addition of streptokinase (SK) under controlled conditions demonstrates degradation kinetics on the fibrin gels. The assessment of streptokinase kinetics involves evaluating plasminogen activation leading to the degradation of the fibrin gel. At 2 hrs, pristine fibrin gels exposed to streptokinase at 50 U/mL had fully degraded. The pristine fibrin gels with 25 U/mL completely dissolved after 4 hrs while the genipin-crosslinked fibrin gels maintained a steady decline in mechanical integrity over time in incubation. We observed a 41.04 % decrease in pristine fibrin gels after 2 hr of incubation. Additionally, we observed an 11.83% decrease in the 25 mg/mL genipin crosslinked fibrin gels over a 24 hr period.

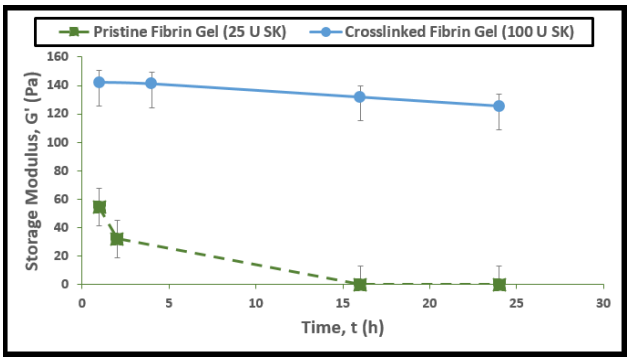

Figure 1. Enzyme degradation over time of pristine fibrin gel and genipin-crosslinked fibrin gel network. The storage modulus of genipin-crosslinked fibrin gel depicts a steady decrease over time losing integrity due to dehydration at 24 hrs (125.33 ± 20.73 Pa). Pristine fibrin gel fully degraded after 4 hrs of incubation at 37°C.

Conclusions: We have developed an in vitro experimental model that closely resembles the clinical conditions of endovascular treatments. Using this model, we can predict efficient enzymatic resistance and stability of genipin-crosslinked fibrin gel networks. These networks depict significantly higher mechanically stability than pristine fibrin gels. The expression of such kinetic attributes of streptokinase depends on the ability of the plasminogen activators. The generalization of the Brownian diffusion model of enzyme dynamics also allows for further prediction of the behavioral mechanisms in fibrin gels. The increased timescale in the stability of fibrin gels crosslinked with genipin provides an enhanced system. These observations suggest that genipin could serve as a biologic to improve minimally invasive embolic treatments.

References: [1] Bigi, A., G. Cojazzi, S. Panzavolta, N. Roveri, and K. Rubini. "Stabilization of Gelatin Films by Crosslinking with genipin." Biomaterials 23.24 (2002): 4827-832. [2] Chang WH, Chang Y, Lai PH, Sung HW. A genipin-crosslinked gelatin membrane as wound-dressing material: In vitro and in vivo studies. J Biomater Sci Polym Ed.2003;14:481–495. [3] Sikri N, Bardia A. A history of streptokinase use in acute myocardial infarction. Tex Heart Inst J.2007;34:318–27.