Diffusion-Reaction Models of Genipin Incorporation into Fibrin Networks

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Statement of Purpose: Fibrin is a natural biopolymer featured in many biomedical applications such as haemostatic glues, matrices for controlled release of bioactive molecules and cells, and scaffold materials for both acellular and cell-based strategies in regenerative medicine. Fibrin gels undergo enzymatic degradation through fibrinolysis. The transient nature of fibrin gel is advantageous for natural wound healing, but presents a challenge in some biomedical applications that require stable clots such as embolization treatments. Fibrin clots with enhanced stability could improve the performance of haemostatic materials for minimally invasive occlusion of brain aneurysms[1] or embolization of solid tumors. Genipin is a non-toxic naturally occurring small molecule crosslinking agent that is extracted from the *Gardenia jasminoides*. Genipin is 10,000 times less cytotoxic than glutaraldehyde and is used in tissue fixation, controlled release systems, and peripheral nerve regeneration[2]. A quantitative understanding of genipin incorporation into protein networks could accelerate the design of interventional therapies that use genipin for applications in clot stabilization. This work describes a diffusion-reaction model to identify the rate-limiting step of genipin incorporation into fibrin gels.

Methods: Fibrinogen and thrombin extracted from bovine plasma were dissolved in PBS buffer at concentrations of 20 mg-mL^{-1} and 10 U-mL^{-1} respectively. The concentration of free primary amines within fibrin gels was determined using a ninhydrin assay[38]. Aqueous solutions of genipin (0.4 mL) in PBS were added drop wise to swollen hydrated fibrin gel networks. Fibrin gels were incubated in genipin solutions for 24 hr at 37 ^{o}C with the following molar ratios of genipin:amine in the reaction system: 1:10, 1:20, 1:40, and 1:80. Fibrin gels incubated with genipin were characterized by rheology at prescribed time points. The swelling ratio of crosslinked networks was measured by gravimetry. Briefly, excess water was removed from the surface and the mass of the samples was recorded (W_s). Samples were dehydrated for 60 ^{0}C at 5 Pa for 24 hr and the mass of each sample was recorded (W_d).

Results: The relative rates of genipin diffusion through fibrin gels and the rate of covalent genipin incorporation and crosslinking with fibrin gels can be predicted by calculating the Thiele modulus. The diffusion coefficient of a small molecule in fibrin gel composed of fibers ($D_{genipin\text{-}fibrin}$) is a function of the obstruction factor of diffusion paths, represented by the hydraulic permeability of fibrin gel (κ), and a hydrodynamic factor between the molecule (r_g) and the fiber network (r_f)[43]. The ratio of $D_{genipin\text{-}fibrin}$ to the diffusion coefficient of genipin in water (D_0) can be estimated from the modified Brinkman model. The Thiele moduli calculated for genipin in fibrin gels across a range of values for genipin concentrations. The rate of crosslink formation is limited by the rate of genipin consumption within fibrin gels. The marginal increase of crosslink density by additional genipin crosslinks ($\Delta n_{c,experimental}$) is determined as the difference between the total crosslink density of fibrin gel incubated in genipin solutions ($n_{c,genipin}$) and the crosslink density of pristine fibrin gel ($n_{c,fibrin}$). The temporal evolution of *G'* in fibrin gels incubated in genipin solutions (**Figure 1**) suggests that the modulus increases over time and reaches a maximum after 6 hr.

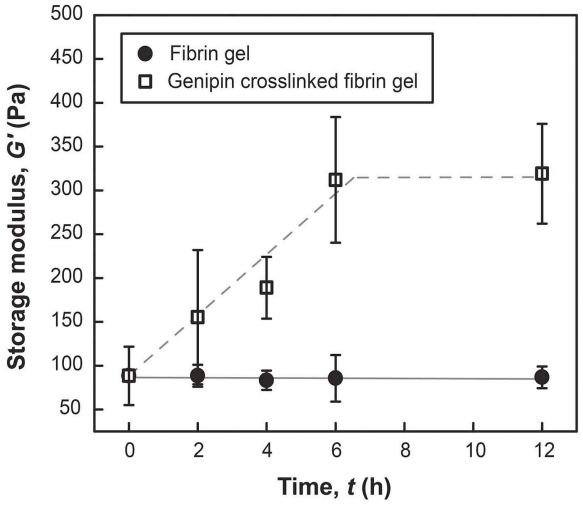

Figure 1. Temporal evolution of *G'* over time of pristine fibrin gel and fibrin gel incubated in genipin solution. The storage modulus of genipin crosslinked fibrin gel reaches a maximum value after 6 hr. Pristine fibrin hydrogel networks disintegrated in PBS buffer after 24 hr, while fibrin hydrogel networks crosslinked with genipin retained mechanical properties (313.29 ± 64.8 Pa).

Conclusions: The efficiency of incorporation is strongly dependent upon the nanoscale morphology of the hydrogel network. Anisotropic structures in fibrin hydrogels limit the overall efficiency of genipin incorporation as crosslinks within the active network. Despite relatively low rates of genipin incorporation into fibrin gels on a stoichiometric basis, genipin crosslinks impart increased mechanical stability and attenuate in vitro disintegration. These observations suggest that genipin could serve as a biologic to improve minimally invasive embolic treatments for various indications. Parameters established here can be applied to engineer system to leverage the rate of genipin supply with the rate of genipin consumption. Crosslinking fibrin gels using genipin is also a potentially effective strategy to stabilize fibrin gel against enzymatic degradation. Knowledge about the relative rates of diffusion-reaction of genipin in fibrillar hydrogels can advance the use of this small molecule biologic in various biomedical technologies.

References: [1] Bederson JB et al. Recommendations for the Management of Patients With Unruptured Intracranial Aneurysms. Circulation 2000. 102 (18) 2300. [2] Bigi et al. Biomaterials 2002. 23 (24) 4827.