Controlling the Degradation Rate of PEGylated Fibrin Gels for Cell Delivery

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Statement of Purpose: Cell therapy promises to help heal ischemic and other tissue damaged by disease or trauma. A mechanism for site-specific delivery that will degrade and be remodeled by biological processes is needed. Fibrin gels fulfill these requirements. As part of the normal wound healing process, fibrinogen is cleaved by thrombin resulting in a three-dimensional fibrin gel. Proteolic enzymes present during wound healing eventually digest the gel as cellular ingrowth occurs.

At a relatively low concentration of 10mg/ml, fibrinogen is conducive to cell proliferation, but degrades more quickly than desired for many cell therapies. Current fibrin sealants containing protease inhibitors such as aprotinin or ε-amino caproic acid slow the degradation rate, but have been shown to have deleterious affects on the healing process[1].

We propose a PEGylated fibrin clot that degrades at a slower rate without the addition of protease inhibitors. Our previous data show that cellular proliferation is increased by PEGylating fibrinogen prior to cleavage by thrombin (Fig. 1).

Methods: Fibrinogen (porcine, Sigma) was added to tris buffered saline, pH 7.8 (TBS, Sigma) and mixed thoroughly. PEG derivatives at varying concentrations (Fig. 1) were added to TBS. (PEG-$(SMB)_2$, Nektar or PEG-$(NPC)_2$, SunBio). Each solution was sterile filtered and used immediately.

Equal volumes of the PEG and fibrinogen solutions (or TBS as a control) were mixed and allowed to incubate for 5 minutes at 37°C. 200µl of each mixture was added to a cloning cylinder over a coverslip (Thermanox, Nunc) in a Petri Dish or on a well plate. An equal volume of thrombin (human, Sigma) diluted to 100U/ml in 40mM $CaCl_2$ was added to the above fibrinogen mixture. The gel was allowed to crosslink for 5 minutes at 37°C and the cloning cylinder was removed. Serum free media (DMEM, Sigma) plus 1% Antibiotic-Antimycotic (Gibco) was added. At least 5X the volume of the gel was used and in all cases enough media was added to ensure the gels were covered. The gels were incubated (37°C, 5% CO_2) and then removed at various time points for analysis.

Analysis was performed by removing the media, removing the gel from the coverslip or well plate, and placing it on labeled filter paper. The gel was rinsed and then desiccated for at least 10 hours before weighing.

Results / Discussion: After 2 days, the fibrin only and both PEGylated fibrin gels at a 10:1 molar ratio of PEG to fibrinogen retained their rough morphology and size. The PEGylated fibrin mixture at a 100:1 ratio using PEG-$(SMB)_2$ never formed a viable gel. The 100:1 mixture using PEG-$(NPC)_2$ did form a gel, though the morphology made it difficult to visually assess. At day 4, the fibrin only gel was mostly dissolved and the two 10:1 gels retained most of their original volume. The mass loss of the gels was assessed during the initial degradation period. All data are normalized to the initial theoretical weight of the starting fibrinogen and PEG based on full conversion. Both the non-PEGylated gel ($P<0.01$) and the 100:1 PEG-$(NPC)_2$ gel ($P<0.05$) were significantly reduced from day 2 to day 4 while both 10:1 gels were not significantly reduced.

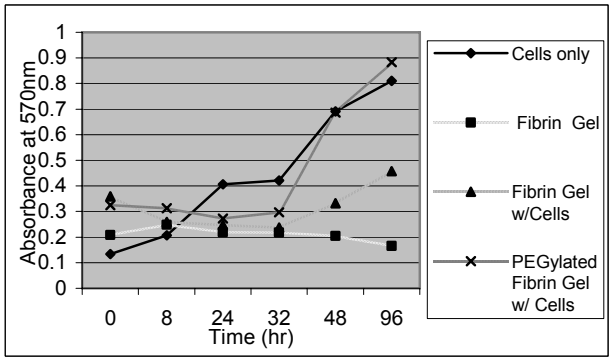

Figure 1: Cell Proliferation in PEGylated Fibrin Gels

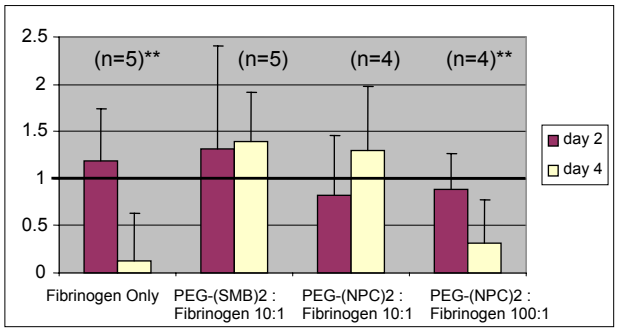

Figure 2: Mass change of PEGylated Fibrin Gels

In addition to the work above, cell viability of bone marrow mononuclear cells was assessed using an MTT assay after trypsinization of the gel (Fig. 1). These data suggest cells in the PEGylated fibrin gels demonstrate significantly increased proliferation compared to the fibrin gel alone. Also, rheology was studied using a small-strain oscillatory shear experiment with a plate-plate geometry. Dynamic oscillating measurement were performed at 0.5Hz so that storage and loss moduli (G' and G'') could be recorded.

Conclusions: Degradation control and cell viability are core qualities needed for a tunable, biodegradable cell therapy matrix. We have shown that cellular proliferation is increased and the longevity of the gel is increased when using a 10:1 molar ratio of PEG:Fibrinogen. Interestingly, increasing the PEG:Fibrinogen ratio to 100:1 significantly reduces the degradation time. Future studies are necessary to assess the utility of this device as a viable cellular delivery methodology

References:

[1] Krishnan L, Lal AV, Shankar PR, Mohanty M. Fibrinolysis inhibitors adversely affect remodeling of tissues sealed with fibrin glue. Biomaterials 24 (2003) 321–327