Engineered Aprotinin for Improved Stability of Fibrin Biomaterials

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Introduction: Fibrin offers many benefits as a biomaterial, including: autologous availability, favorable viscoelastic properties, bioactivity via integrin binding sites, and excellent biocompatibility. However, a major characteristic drawback of fibrin as a biomaterial is its rapid rate of degradation. Degradation of fibrin can occur via proteolytic cleavage by plasmin, but this mechanism can be inhibited by the small protein, aprotinin. By recombinantly fusing aprotinin to a transglutaminase (TG) substrate sequence tag, the resultant chimeric protein, aprotininTG, can be covalently cross-linked into fibrin during the natural polymerization process. We hypothesize that use of aprotininTG within fibrin matrices may reduce their rate of degradation and therefore further augment their utility as biomaterials.

Methods:

Production and Purification

Using standard cloning techniques, aprotinin cDNA was amplified from plasmid DNA (gift from Bayer) and inserted into expression vector pGEX-4T-1 (GE Life Sciences) with appropriate substrate sequence tag. Optimal conditions were determined for expression of aprotininTG in *E. Coli*. Purification was conducted with glutathione S-transferase (GST) affinity chromatography.

Plasmin Inhibition

Bioactivity of the protein was assessed via a plasmin-inhibition assay. Fluorogenic plasmin substrate was digested by plasmin, and working concentrations (2.6μM) of wildtype aprotinin (Roche) and aprotininTG were compared for their ability to inhibit cleavage of the plasmin substrate over time.

Inhibition of Fibrinolysis

Fibrin gels were exposed to physiological concentration of plasmin in phosphate-buffered saline (PBS) over the course of several days. PBS/plasmin media was collected and refreshed every 8 hours for 3 days, and every 24 hours thereafter. Gel degradation products were assessed by ELISA and western blot with antibody to fibrinogen.

In Vitro Model

Human dermal fibroblasts were seeded within fibrin gels containing PBS, wildtype aprotinin, or aprotininTG. Proliferation was assessed at 7 and 14 days via AlamarBlue®, and gels were then weighed.

Results and Discussion: Successful expression of protein of interest was attained, and the identity of aprotininTG was verified by PAGE and tandem mass spectrometry. Bioactivity assays indicated that aprotininTG (TG) inhibits plasmin as much as wildtype aprotinin (Roche) at working concentration levels (see *Figure 1*). Postive control (Ctrl) indicates conditions without an aprotinin derivative.

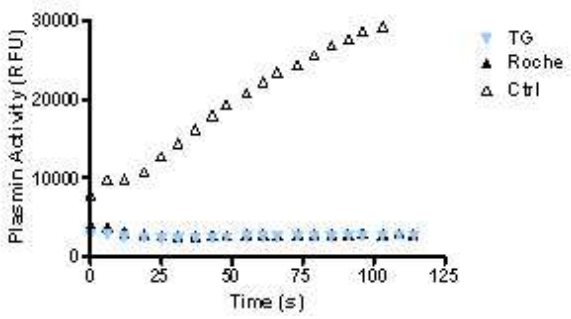

Figure 1. Plasmin inhibition assay.

AprotininTG (TG) was found to be more effective at preventing fibrinolysis than wildtype aprotinin (R) or no aprotinin (Ctrl) when evaluated by plasmin-mediated fibrin gel degradation assays (see *Figure 2*).

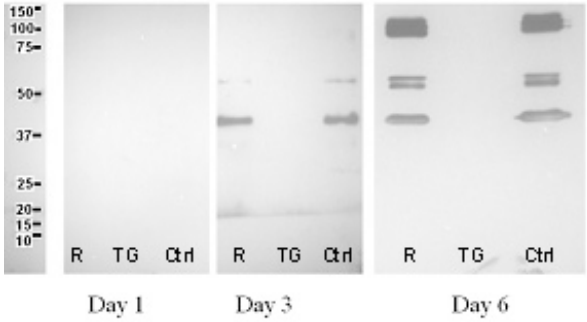

Figure 2. Plasmin-mediated gel degradation assay

In vitro experiments confirmed that aprotininTG-protected gels could support human fibroblast proliferation better after two weeks than fibrin gels with wildtype aprotinin or fibrin gels alone (see *Figure 3*).

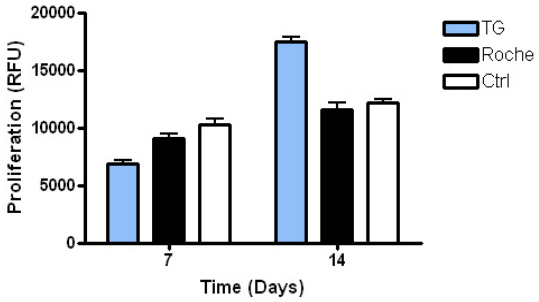

Figure 3. In vitro proliferation assay

Conclusions: Soluble expression and purification of biologically active aprotininTG has been demonstrated and verified. When incorporated into fibrin gels, aprotininTG was found to greatly reduce the amount of fibrin degradation products as compared to wildtype aprotinin. AprotininTG can reduce the degradation rate of fibrin matrices and therefore may increase their utility as biomaterials.