Engineered Aprotinin Improves Stability of Fibrin Biomaterials
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Introduction
Fibrin can be harvested autologously for creation of biologically active, biocompatible, hydrogel matrices. However, fibrin gels lose utility due to their rapid rate of degradation via the actions of plasmin, especially in vivo. Covalent-binding of a plasmin-inhibitor, such as aprotinin, to the matrix may allow for prolonged stability of fibrin. A recombinant protein, TG-aprotinin, was created by fusing aprotinin to a transglutaminase (TG) substrate sequence which binds fibrin covalently1. TG-aprotinin was then compared to WT aprotinin in a series of in vitro and in vivo fibrinolysis assays.

Materials and Methods
Production and Purification
Aprotinin cDNA (Bayer) was fused to a TG tag and inserted into an E.Coli expression vector. Protein was purified by affinity chromatography. In Vitro Assays
Bioactivity was assessed via plasmin inhibition determined by a fluorogenic plasmin substrate. Fibrinolysis inhibition over time was assessed by exposing TG-aprotinin, WT aprotinin, or control PBS (Ctrl) gels to physiological concentrations of plasmin. Fibrin gel degradation products were assessed by ELISA and western blot. TG-aprotinin, WT aprotinin, and Ctrl gels were also seeded with human dermal fibroblasts (HDFs) and proliferation was assessed over 3 weeks. In Vivo Assay
Mice were subcutaneously implanted with TG-aprotinin and WT aprotinin fibrin gels and were sacrificed at time points. Histology was assessed.

Results
Successful expression of protein of interest was attained, and the identity of TG-aprotinin was verified by PAGE and tandem mass spectrometry. Bioactivity assays indicated that TG-aprotinin inhibits plasmin as much as WT aprotinin at working concentration levels (2.6 M). TG-aprotinin was also found to be more effective at preventing plasmin-mediated fibrinolysis than WT aprotinin (Ctrl) (Fig 1), and more effective at preventing degradation in vivo.

Discussion and Conclusions
We demonstrate that recombinant TG-aprotinin is highly effective in reducing fibrin degradation as compared to WT aprotinin both in vitro and in vivo. By improving their stability, TG-aprotinin may increase the utility of fibrin biomaterials and thus is promising for use in clinical settings.


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Disclosures
The technology has been protected by university patents licensed by Kuros Biosurgery, of which JA Hubbell owns equity.