Extracellular Matrix Engineering: Presentation of therapeutic motifs on fibrin matrices through molecular modification of fibrinogen

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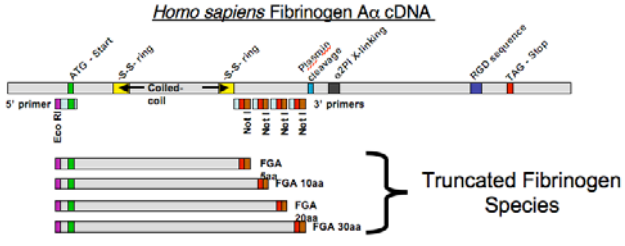
Introduction: Fibrin, a polymer formed following vascular injury to prevent excessive blood loss, has been used for decades as a biological biomaterial. The polymer is formed by the self-assembly of fibrin monomers which are activated by the cleavage of specific residues by the serine protease thrombin. The polymer has many advantages that engineers have used to their advantage. Specifically, fibrin polymer forms rapidly in situ, binds specific growth factors like FGF, is degraded by biofeedback loops linked to the invasion of cells, and the degradation products themselves are bioactive, promoting sustained invasion and proliferation of fibroblasts to enhance the rate of healing. For all the advantages there are equal numbers of disadvantages including poor mechanical stability compared to synthetics and in a highly pure form the incapacity to adequately support cell attachment. Additionally, despite the capacity of a few select growth factors to specifically bind fibrin most growth factors do not. We have developed technology in the past that allows the covalent crosslinking of modified growth factors into fibrin matrices via Factor XIIIa, a transglutaminase. While adequate for the incorporation of growth factors, this technology relies on the enzymatic activity and accessibility of Factor XIIIa and thus the ability to finely control the amount of factor bound versus soluble is problematic in applications requiring in situ polymerization. In this report we demonstrate the capacity to couple therapeutic factors directly to fibrin via genetic coupling and creation of fibrinogen fusion proteins.

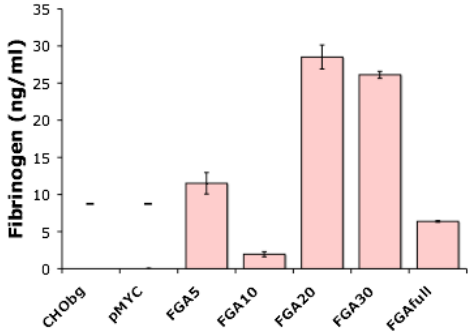
Methods: *Fibrinogen Aα chain truncations and fusions* – We initially truncated fibrinogen Aα chain to determine the minimal sequence required for proper bioassembly and polymer formation. Various truncations of fibrinogen Aα chain were generated via PCR amplification with primers designed to allow subsequent cloning into the pMYCpuro mammalian expression vector. PCR products were purified and vectors cloned and amplified using standard techniques. Creation of fusion protein vectors were initiated similarly but with the insertion of an "in frame" restriction site for subsequent cloning of the fusion species.



Fibrinogen protein production – Fibrinogen was produced in Chinese Hamster Ovary (CHO) cells. Basically, anchorage-dependent, serum-free CHO cell cultures that had been stably transfected with fibrinogen Bβ and γ chain cDNAs were transfected with the mutant Aα chain vectors and positive clones established by antibiotic treatment and clonal selection by exhaustive dilution. Clones expressing high levels of fibrinogen were amplified and fibrinogen production induced with insulin, transferrin, and selenite and protected against proteolysis with aprotinin. Recombinant fibrinogens were purified using a peptide affinity resin.

Polymer Characteristics – Purified fibrinogens will be polymerized with thrombin and polymers will be i) analyzed by SEM following standard preparative methods, ii) measured for permeability through determination of the Darcy constant (Ks), iii) analyzed by torsion pendulum to determine the viscoelasticity, and iv) analyzed for fibrinolysis rate. It is predicted that truncation of the Aα chain will have little effect on polymer formation, but may have an effect on the lateral aggregation of fibers, resulting in "thin" fibers. Similarly we predict that the addition of a secondary structure in place of the Aα chain will have minor effects on the polymer.

Results / Discussion: Initial transient transfections of serum-free CHOβγ cell cultures were performed and the capacity of these cells to assemble and secrete fibrinogen was determined using ELISA on the conditioned media of the cultures. Despite the variability in the expression level of the different truncations, a result common in transient transfection experiments, all truncation of the Aα chain were detectable in the media.



Conclusions: While structural analysis is still to be done, the currents results indicate that recombinant fibrinogen Aα chain fusions can be produced without disturbing bioassembly and secretion. Based on available literature, we hypothesize that the primary alterations in fibrin polymer structure will be in the lateral aggregation of fibers resulting in thinner fibers. The affect of these modifications on cell attachment and migration into the polymer will also be determined and presented.