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SYNTHETIC, ENZYMATICALLY DEGRADABLE EXTRACELLULAR MATRICES FORMED FROM RECOMBINANT PROTEIN-(POLY)ETHYLENEGLYCOL

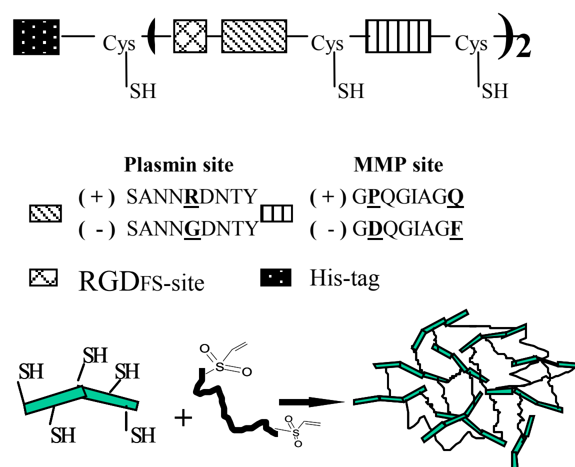
S. C. Rizzi , S. Halstenberg, J. Hubbell [1]

[1] *Institute for Biomedical Engineering, Department of Materials, Swiss Federal Institute of Technology, ETH-Zürich, Switzerland*

INTRODUCTION: Our research addresses the clinical need for synthetic biomatrices as physical supports for the creation of functional tissue and as carrier vehicles for efficient and controlled delivery of drugs and growth factors. Here we describe the development of a novel class of biologically active synthetic hydrogel matrices that form through chemical cross-linking of poly-ethyleneglycol (PEG) with recombinant proteins. The design of our PEG-protein network is motivated by the function of the natural extracellular matrix (ECM) during tissue development and tissue repair. Towards this goal, we generated PEG-protein copolymers that carry some of the key functions of ECM such as the ability to mediate cell adhesion, cellular penetration and migration through proteolytic remodeling, as well as storage and cell-regulated release of growth factors. PEG was chosen for its well known biocompatibility and resistance to protein adsorption. Biological information was conferred to the copolymer backbone by the incorporation of recombinantly derived proteins that carry the cell adhesion motif RGD derived from fibronectin, and substrates for degradation by the cellular proteases plasmin and matrix metalloproteinase (MMP). The copolymer networks form in aqeous solution by spontaneous reaction between thiols of cysteines in the protein backbone and vinylsulfone groups at the termini of PEG. The crosslinking reaction is highly self selective and can be carried out in situ in the presence of cells or tissue.

METHODS: Two artificial proteins of 116 amino acid length were created de novo by recursive PCR methodology using overlapping single stranded DNA fragments as templates. For the present study, two proteins that differ in their sensitivity to degradation by cellular proteases were produced (Fig. 1A). One protein construct was designed for degradibility by plasmin and matrix metalloproteinases (MMPs). A second control protein construct was generated in which cleavage sites for plasmin or MMP were mutated inactive. Both protein constructs contain the cell adhesion peptide motif RGD. Cysteines added within the protein backbone are designated cross-linking sites with PEG divinylsulfone. A thrombin-cleavable histidine tag was added for purification by Ni-affinity chromatography. DNA sequences corresponding to monomeric proteins were dimerized using double non-palindromic restriction sites and were then subcloned into the E.coli expression plasmid PET 14b (Novagen). The resulting protein dimers were recombinantly expressed and purified in E. Coli. Purified proteins are visualized in figure 2, lanes 1 and 5.

Three-dimensional copolymer networks formed spontaneously within minutes under physiological condition by mixing aequeous solutions of recombinant protein and PEG divinylsulfone. A cartoon of the resulting network structure is depicted. The gels form via conjugate addition between thiol groups from cysteines in the protein with unsaturated double bonds present in PEG divinylsulfone (Fig. 1B).



Recombinant Protein + PEG divinyl sulfone = PEG-Protein-co-Polymer

Fig. 1 A and B: A) Generation of artificial recombinant proteins. B) Recombinant protein-PEG hydrogel formation

RESULTS: The principles of this biomaterial scheme were investigated in biochemical and in vitro cell culture assays. Purified recombinant proteins containing active sites for cleavage were specifically degraded by incubation with MMP-1 (Fig 2, lanes 2 to 4). Notably, degradation was almost completely absent in control proteins

containing mutant, inactive cleavage sites (Fig 2, lanes 6 to 8). Cell-derived proteolytic degradation was demonstrated in three-dimensional cell migration assays. Human fibroblasts embedded within degradable PEG-protein copolymers survived within the matrix, and were able to invade the matrix environment through their cellular activities. Cellular invasion was critically dependent on the degradability of the hydrogel (Fig. 3A and B).

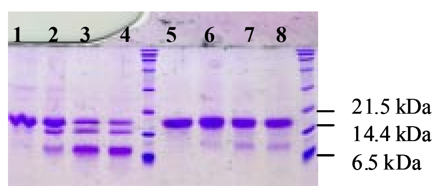


Fig. 2: Degradadation of recombinant protein by MMP-1. Fragmentation of the proteins was analyzed by 20% SDS-PAGE and subsequent Coomassie staining. Lanes 2 to 4 on the left panel show degradation of the MMP sensitive protein after 18h, 72h, 98h. Notably, little degradation was observed for protein containing the mutant, inactive MMP site (right panel, lanes 6 to 8).

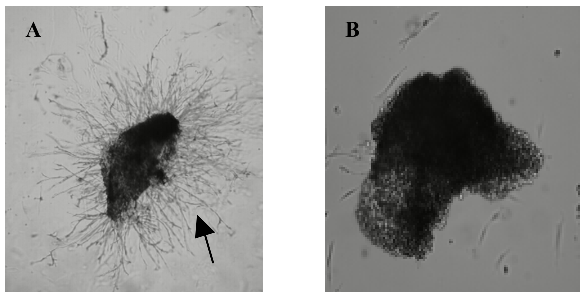


Fig. 3A and B: Cellular invasion of protein-PEG hydrogels is dependent on degradibility of the matrix. Fibrin clots containing human fibroblasts were embedded within three-dimensional degradable (A) or non-degradable (B) protein-PEG hydrogels. Cell migration into the synthetic hydrogel was analysed by phase-contrast microscopy at day 6 of cell culture. Massive cellular invasion was observed for degradable protein-PEG matrix, but was absent in control cultures in non-degradable protein-PEG hydrogels.

CONCLUSIONS: Together, our results have proven the functionality of PEG-protein copolymers as cell ingrowth matrices. Such matrices can be tailored toward specific medical needs and thus become potentially useful for the delivery of drugs and factors for regulated release by cellular activities.