

ENGINEERING THE EXTRACELLULAR MATRIX: SYNTHETIC PROVISIONAL HYDROGELS FOR WOUND REGENERATION

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INTRODUCTION: The extracellular matrix (ECM) of connective tissue plays an essential role in the regulation of cell behaviour and tissue formation, providing both biophysical and biochemical cues for the cells in contact. The remodelling and repair of tissues - involved in many physiologic and pathologic situations - is highly cell-controlled. Cells locally express or activate proteases that cleave the surrounding macromolecules thereby degrading the ECM.

The goal of this project was to design synthetic extracellular matrices that can be used as alternatives for naturally occurring matrices such as fibrin or collagen, which require difficult purification procedures and carry the risk of disease transmission. The poly(ethylene glycol)- (PEG) based hydrogels described herein contain a combination of biological signals, namely ligands for cell adhesion and migration and susceptibility to cell-secreted matrix metalloproteases (MMPs), which allow them to undergo cell-mediated remodelling.

MATERIALS AND METHODS: Branched PEG's (Shearwater Polymers, USA; 4arm, Mw: 10, 15 and 20kD) were end-functionalised with vinylsulfone. Crosslinker peptides with different enzymatic activity (kcat/Km) were designed bearing a cysteine (for X-linking reaction with vinylsulfone under physiologic conditions) on both ends of a matrix metalloproteinase (MMP) substrate sequence [1], e.g. GCRD-GPQGIAGQ-DRCG. Hydrogels in contact with cells were also functionalised with pendant adhesion peptides (RGDSP). Cell-induced proteolytic degradability of the gels was analysed as a function of time by measuring the radial distance that human foreskin fibroblasts migrated through the networks from an embedded cell-fibrin cluster. Gels with different network architectures (Mw of PEG) as well as various adhesivity and enzymatic degradability were tested.

RESULTS AND DISCUSSION: Fibroblasts cultured inside adhesive and MMP-sensitive hydrogels migrated over large distances (Fig. 1). Cell migration did not occur when a) a peptide without enzymatic activity was incorporated, b) nonadhesive peptides (RDGSP) were used or c) in the presence of an MMP inhibitor (GM6001, Chemicon, USA). Moreover, the cell invasion speed responded to the protease substrate activity (Fig. 2), the adhesion site density or the network architecture.

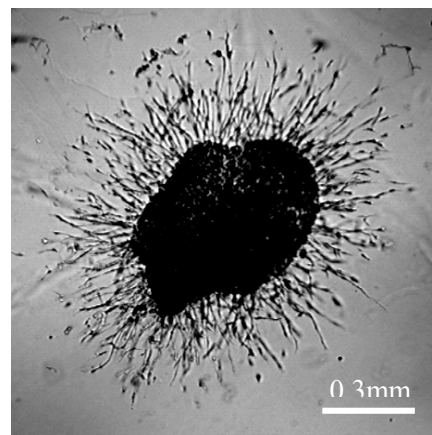


Figure 1. Within networks containing adhesion sites and MMP-sensitive peptides, fibroblasts were able to migrate over long distances.

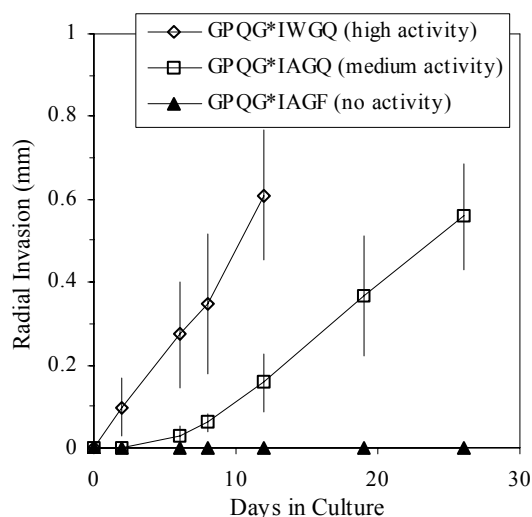


Figure 2. Cell invasion rate can be tailored by the activity (Km/kcat) of the incorporated MMP substrate.

CONCLUSIONS: The key characteristics of natural extracellular matrices can be rationally engineered into a synthetic material, allowing cells to migrate in response to the action of cell-secreted MMPs. Moreover, the migration speed can be tailored through several characteristics of the matrix. We believe that such hydrogels have a strong potential as provisional scaffolds to guide tissue reconstruction.

REFERENCES: [1] H. Nagase and G.B. Fields (1996), *Biopolymers (Peptide Science)*, **40**: 399-416.