Biosynthetic Hydrogels Promote 3-D Outgrowth and Dedifferentiation at the Edge of Articular Cartilage Explants in an *in vitro* Model

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Introduction: The distinct biochemical composition of articular cartilage is maintained by highly differentiated cartilage cells called chondrocytes. Chondrocytes have a limited capacity for repairing articular cartilage, and without a vascular supply and limited presence of undifferentiated cells, focal injuries to the joint surface ultimately result in progressive degeneration of the cartilage. This limitation has led to some speculation about the restricted motility of differentiated chondrocytes in their extracellular environment during wound healing. In the present investigation, we explore a cellular ingrowth matrix cartilage repair strategy using photo-polymerizable biodegradable hydrogels made from PEGylated protein polymers. The degradable hydrogels are used to encapsulate articular cartilage explants in order to assess the 3-D outgrowth and cell differentiation of chondrocytes after cartilage injury using an *in vitro* wound repair model. This model is further used to assess the influence of the chondrogenic growth factors IGF-1 and TGF-β on the phenotype of the motile chondrocytes after they enter into and proliferate within the hydrogel matrix.

Materials and Methods: Biodegradable hydrogels were made from PEGylated fibrinogen formed by reacting high excess of poly(ethylene glycol) diacrylate (PEG-DA) with denatured fibrinogen under reducing conditions. The purified PEGylated protein is assembled into a hydrogel by free radical polymerization of unreacted acrylates on the PEG molecules. Proteolytic resistance of the hydrogel is based on the PEG and fibrinogen composition: slow, intermediate, and fast biodegradation is achieved with 1%, 3%, and 5% additional PEG-DA, respectively. The growth factors IGF-1 (50, 100ng/mL) and TGF-β (5, 30ng/mL) were incorporated into the hydrogel precursor solution. Outgrowth studies were performed with slices of articular cartilage explants, isolated from sheep knee joints and immersed in the precursor solution before being polymerized with UV light (365-nm, 700μW/cm^2). The encapsulated explants were cultivated with twice-weekly replenishment of medium and periodic imaging (Fig 1a). Histological staining for proteoglycans (Safranin-O), cell morphology (H&E), and immuno-staining for type I and type II collagen were performed to assess the phenotype of invading chondrocytes and the explanted specimen.

Results: Abundant cellular outgrowth was observed in all hydrogels after several weeks in culture (Fig 1) but not in PEG-only controls. Higher concentrations of PEG-DA significantly slowed down the invasion of chondrocytes into the biosynthetic matrix. Both phase contrast images and histological sections show the invading chondrocytes exhibiting fibrochondrocyte morphology, with spindled cell bodies extending into the hydrogel matrix.

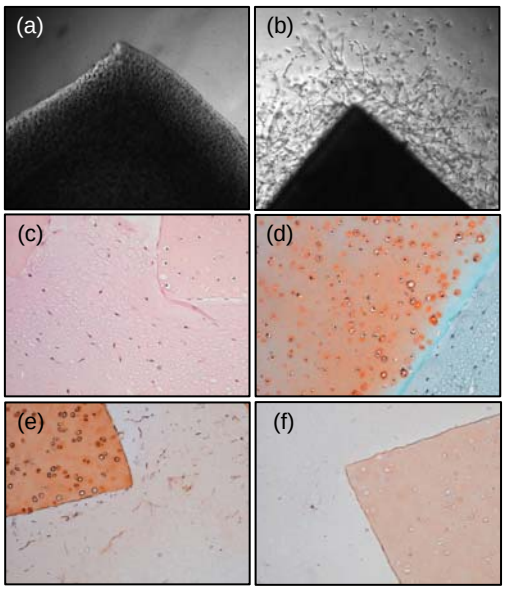

Figure 1: Cartilage explants in hydrogels visualized by phase contrast microscopy (a: day 0; b: day 30) and histologically (c: H&E; d: Safranin-O; e: Col I; f: Col II).

Stained histological sections revealed no expression of proteoglycans and type II collagen in the invaded hydrogel matrix, but some positive staining for type I collagen was detected. Neither IGF-1 nor TGF-β in the hydrogels significantly affected the invasion kinetics, as measured by quantitative morphometric analysis; however, 30ng/mL TGF-β did influence the expression of type II collagen in the cell-invaded matrix.

Discussion and Conclusions: Biodegradable hydrogels that are injected into a cartilage defect and can promote the invasion of chondrocytes from the periphery of a defect site can be used for repairing cartilage by cellular ingrowth. We demonstrated that PEGylated fibrinogen hydrogels enable invasion of chondrocytes from cartilage explants while maintain their physical integrity for several months in culture. The composition of PEG molecules and protein backbone determines the materials' proteolytic responsiveness and structural integrity. In our experiments, invading cells take on the morphology and phenotypic characteristics of fibrochondrocytes; however, this *in situ* polymerizable biomaterial can be modified with growth factors to promote functional articular cartilage repair by the invading host cells.