

PHOTO-CROSSLINKING COLLAGEN GEL FOR TISSUE ENGINEERED CARTILAGE

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INTRODUCTION: Numerous hydrogels have been developed as three-dimensional scaffolds for chondrogenesis. Solubilized collagen is an ideal natural material that can be used as a scaffold because of its biocompatibility and biodegradability. Although spontaneously forming collagen gels can conform to a cartilage defect, they are soft and unstable. Kochevar et al. reported that type I collagen can be crosslinked using photoreactive dyes, such as riboflavin (vitamin B2), and exposure to visible light. *In situ* gel crosslinking could induce molecular interactions with the native cartilage surrounding the lesions to stabilize the gel during cartilage formation. The governing hypotheses of this work are: 1) photochemical crosslinking can be used to generate stable collagen hydrogels; 2) Chondrocytes encapsulated in the hydrogels form neocartilage; 3) the neocartilage will integrate with existing cartilage.

METHODS: Chondrocytes were suspended in 4 test concentrations of riboflavin solution (0.1–1 mM). The cell suspension was mixed with an equal volume of 0.5% type I collagen solution. The suspension with a final cell concentration of 40×10^6 cells/ml was poured onto 6 well culture plates and photocrosslinked using 4 irradiation test doses of visible light. Control samples were not subjected to irradiation. The constructs were cultured up to 10 days to evaluate cell survival with live-dead assay.

Implantation of photocrosslinked constructs (n=8) was performed to determine whether this novel method would allow the construct to make hyaline cartilage in the *in vivo* environment. Specimens were evaluated histologically (Safranin-O and immunohistochemically for COL 1 and COL 2) and biochemically (collagen and GAG content). To evaluate the ability of the gel to permit cartilage formation and integration with the surrounding native cartilage, photocrosslinked gels with cells were placed between discs of knee cartilage and implanted in mice.

RESULTS: Cell viability remained high with short irradiation times at all concentrations of

riboflavin. Increased irradiation and riboflavin concentration had a negative effect on cell viability (Fig 1). Specimens placed in mice showed neocartilage formation as evidenced on specimens stained with Toluidine blue (Fig 2a) and Safranin-O and produced GAG (Fig 2b) and type 2 collagen (Fig 2c) Neocartilage between cartilage discs formed tight bonds with existing cartilage (Fig 2d).

Figure 1

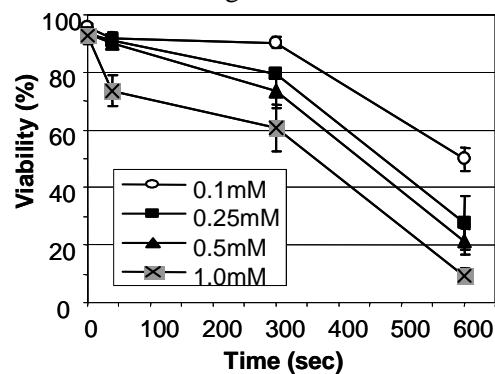
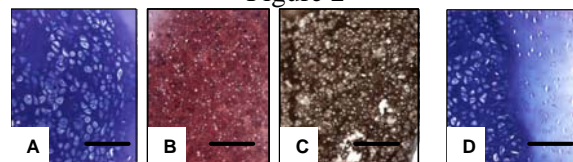


Figure 2



DISCUSSION & CONCLUSIONS: Crosslinking collagen into hydrogels can be achieved using benign light sensitive photoreactive dyes like riboflavin and visible light. Photochemically crosslinking the collagen solutions containing chondrocytes permits cell survival and neocartilage formation. As such, collagen containing chondrocytes could be injected into a defect site and polymerized *in situ*. The crosslinking process could stabilize the hydrogel in the defect and permit new cartilage formation to restore the joint surface. The results from this study encourages further study in large animal joint models.

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