

PHOTO-CROSSLINKING COLLAGEN GEL FOR TISSUE ENGINEERED CARTILAGE

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INTRODUCTION:

Tissue engineering is a novel approach for regenerating articular cartilage using three-dimensional biomaterials as cell scaffolds. Collagen is one of the ideal natural materials that can be used as a scaffold because of its biodegradability. However, collagen gels can shrink when seeded with cells and may not allow the construct to fit the shape of a cartilaginous defect. Therefore, integrity between the implanted construct and adjacent tissues may not be obtained.

Kochevar et al. reported that type I collagen can be crosslinked using photoreactive dyes, such as Rose Bengal or Riboflavin, 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. Based on these data, the governing hypotheses of this work are: 1) Photochemical crosslinking can be used to generate a hydrogel that permits chondrocyte encapsulation; 2) Chondrocytes in the hydrogels have the capacity to form neocartilage with improved biochemical properties for cartilage repair in the knee; 3) The neocartilage will integrate with existing native tissues forming.

METHODS:

The following experiments were performed using swine (aged 3-6 months) articular chondrocytes as cell source and male athymic mice (aged 6-10 weeks) for implantation. All animal experiments were approved by the IACUC.

Chondrocyte Isolation: Swine cartilage tissues were harvested from the shoulder and knee joints. They were cut into small pieces, washed, and digested for 16 to 18 hours at 37°C in 0.05% collagenase solution.

Experiment I

In order to determine optimal dose of the photo-initiator, Riboflavin, and visible light, *in vitro* cellular viability was performed. Macroscopic pictures were taken to examine whether shrinkage of the collagen gels was suppressed.

Photoencapsulation of Chondrocytes and Culture: Single passaged chondrocytes were resuspended in various test concentrations of Riboflavin solution (0.1–1 mM). Subsequently, the cell suspension was mixed with an equal volume of 0.5% type I collagen solution. The suspension with a final cell concentration of 40×10⁶ cells/ml was poured onto 6 well culture plates and photocrosslinked using various irradiation test doses of visible light. Table 1 shows the combination of different factors that was tested to generate photocrosslinked collagen hydrogels. Control samples were not subjected to irradiation. The constructs were cultured up to 10 days for *in vitro* study using HAM-F12 supplemented with 0.05% ascorbic acid, 10 mM L-glutamine, 0.01 M MEM non-essential amino acids, 1% penicillin-streptomycin, and 10% fetal bovine serum. The *in vitro* constructs were incubated at 37°C in a humid environment with 5% CO₂. Medium was replaced every 2-3 days.

Encapsulated Chondrocyte Viability (N = 5): DNA content analysis was performed as relative cell number using Pico Green fluorescent procedure at day 0, 1, 3, and 7 of the culture. Samples were digested in a papain solution for 15 hours at 60°C to extract DNA. The dye was added to the extracted DNA and fluorescence emission was measured.

Table 1. Combination of factors for photocrosslinked collagen hydrogels

Concentration of Collagen	Photoinitiator	Irradiation Time
Type I collagen (0.5 %)	Riboflavin (0.1 – 1 mM)	Light: λ= 440-500 nm (40 – 300 sec)

Experiment II

Implantation into mice (N=8): The samples were implanted on the dorsum of mice and harvested at 6 weeks to determine whether this novel method would allow the construct to make hyaline cartilage in the *in vivo* environment.

Histological and immunohistochemical evaluation: The samples were fixed in 10% formalin for 24 hours, embedded in paraffin and sectioned. Tissue sections were stained with hematoxylin and eosin to observe chondrocyte morphology and distribution, and Safranin-O was used to study the distribution of GAGs. For immunohistochemistry, sections were stained with primary antibody against swine type II collagen.

Experiment III

To investigate integrity of newly formed tissue to native hyaline cartilage, vital cartilage chips were mixed with the gel and examined histologically and immunohistochemically.

Encapsulation of chondrocytes with native cartilage chips (N=8): Vital native cartilage was minced into 2mm in average diameter. These minced chips were mixed into the gel before the irradiation.

RESULTS:

Experiment I

DNA assay revealed that higher relative cellular number was acquired in the combinations of Riboflavin/irradiation-time at 0.25mM/40 sec. Under this condition, the relative cellular number was 195.9±24.6% at day 7 of culture (Fig 1). Macroscopic pictures demonstrated that this crosslinking technology could prevent shrinkage of the collagen gel.

Experiment II

Figure 2 demonstrates that newly formed tissues included glycosaminoglycan (GAG) and type II collagen, which indicated hyaline

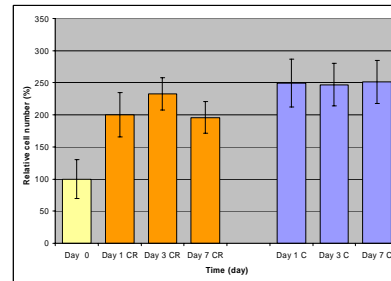


Fig 1: Relative cell number (%) = given cell No×100/initial cell No. CR indicates experimental group. C indicates control group.

cartilage.

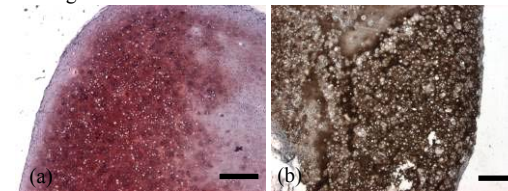


Figure 2: (a) Safranin-O staining; (b) Immunostaining for type II collagen. Bar scale is 200µm.

Experiment III

Figure 3 demonstrates a newly formed tissue was integrated with adjacent native cartilages.

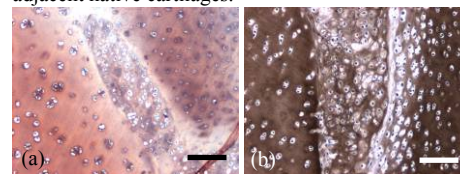


Fig 3: (a) Safranin O staining. (b) Immunostaining for type II collagen. Neocartilage was observed between native tissues. Bar scale is 100µm.

DISCUSSION

This would allow cell-collagen constructs to regenerate hyaline cartilage and to integrate with native cartilage in mice. Additionally, this novel photocrosslinking technology for collagen gel may prevent the gels from shrinking. Although the regeneration occurred in subcutaneous space of mice, which is not a natural environment for articular cartilage, this study encourages further study in large animal joint models.

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