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Photochemical approaches for bonding of cartilage tissues

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Summary

Objective: The objective of this study was to evaluate photochemical bonding as an approach for adhering live cartilage tissues across a repair interface in a manner that may lead to enhanced integration.

Design: Photochemical bonding of both meniscal fibrocartilage and articular cartilage was explored using an anionic, hydrophilic phthalocyanine photosensitizer. Variations on surface preparations and irradiation parameters were explored using overlapped tissue strips and tested using a modified single-lap shear test. Durability of the photochemically induced bonds and cellular viability were examined in an *in vitro* cartilage defect model for up to 1 week in culture, with bond strength assessed *via* push-out test.

Results: Meniscal tissue strips bonded with no surface treatment, but cartilage strips required enzymatic treatment with chondroitinase-ABC to effectively bond. More aggressive removal of glycosaminoglycans at the interface led to increased bond strengths. Bond strength achieved with a 10 min irradiation of treated tissue was on the order of that previously achieved through several weeks of culture. In the defect model, photochemical bonds between a tissue annulus and a press-fit tissue core were maintained for 1 week in culture without substantial increases in cell death near the bonded interface.

Conclusions: With appropriate treatment parameters, photochemical bonding rapidly produced a stable structural interface between cartilage tissue samples and may be a promising strategy for enhancing initial attachment in cartilage repair strategies.

Key words: Cartilage, Fibrocartilage, Photochemical bonding, Interface.

Introduction

In cartilage repair, initial integration between the host and repair tissues is desirable for nutrient transport, molecular deposition to enhance integration, and eventual stress transmission across the tissue interface. Effective transport and crosslinking of newly synthesized collagen molecules across a repair site may be vital to the process of integrative repair, and experiments have correlated collagen deposition with strength of integrative repair *in vitro*[1,2]. Current methods for achieving cartilage bonding involve fibrin-based adhesives and sutures. Standard chemical crosslinking methods[3] and photothermal soldering approaches[4] do not lend themselves to adhesion of living cartilage tissues due to excessive cellular necrosis.

Photochemical methods are one option that may achieve bonding across a repair interface without producing a barrier to tissue integration and with minimal thermal effects. Often the primary photochemical reaction is only a precursor to secondary reactions that cause polymerization or crosslinking of molecules. Type-I photochemical processes are distinguished by direct photo-oxidation of protein side chains or bound chromophores due to electron/hydrogen transfer directly from the substrate. Type-II processes are characterized by indirect photo-oxidation of a substrate *via* formation of singlet oxygen ($^1O_2$); protein oxidation is a secondary event[5,6]. Proteins are typically inefficient generators of $^1O_2$ by direct sensitization, requiring the presence of an exogenous photosensitizer[7,8]. The effects of photochemical treatment on a protein depend strongly on both the class of photosensitizer used and the amino acid residues present in that particular protein.

Several photochemical approaches using argon lasers for excitation have been investigated for tissue bonding. Riboflavin-5-phosphate, a dominantly type-I chromophore, has been used to stiffen the cornea[9,10], seal urethral tissue[11], and seal scleral incisions *in vitro*[12,13]. Of the type-II photosensitizers, Rose bengal was found to bond partial-thickness, cryopreserved porcine skin grafts while preserving collagen organization and tissue viability[14]. A patented 1,8-naphthalimide dye has been used to bond previously frozen and strongly debrided articular cartilage and meniscal fibrocartilage strips subjected to impulse-style loading[15]. Subsequent studies qualitatively observed similarly bonded meniscal and articular cartilage tears in sheep[16,17]. Experiments using collagen gels found that the presence of a type-II (but not the absence of a type-I) photochemical pathway was required to affect collagen-II in a manner enhancing structural coagulation[18]. Chloro-aluminum phthalocyanine tetrasulfonic acid (CASPc), an anionic, hydrophilic phthalocyanine that may sensitize *via* type-I and type-II processes[19], was therefore selected for this study.

The overall goal of this study was to explore the feasibility of bonding live cartilage tissue through photochemical

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collagen crosslinking. Photochemical bonding of both meniscal fibrocartilage and articular cartilage were initially investigated, with shear strength assessed using a modified single-lap test. Enhancements to the technique through mild enzymatic treatment of the tissue surface or functionalization of the tissue surface with more photoreactive groups were also investigated. Durability of the photochemically induced bonds and cellular viability were examined in an *in vitro* cartilage defect model for up to 1 week in culture, with shear strength assessed using a push-out test. These studies demonstrated that rapid initial adhesion of cartilage tissue may be achieved through photochemical bonding, offering the potential for effective attachment and subsequent biological integration.

Method

MATERIALS

High glucose Dulbecco's Modified Eagles Medium (DMEM), antibiotic/antimicotic (AB/AM), gentamicin, HEPES buffer solution, non-essential amino acids (NEAA) and Dulbecco's phosphate buffered saline (PBS) without calcium and magnesium were from Invitrogen (Carlsbad, CA). Ascorbate, kanamycin, chondroitinase-ABC and hyaluronidase were from Sigma (St. Louis, MO). Collagenase (type 2) was from Worthington Biochemicals (Lakewood, NJ). Fetal bovine serum (FBS) was from Hyclone (Logan, UT). Immature bovine stifles were from Research 87 (Boylston, MA). CASPc was from Frontier Scientific (Logan, UT). Traut's reagent was from Pierce (Rockford, IL), and the Live/Dead kit (L3224) was from Invitrogen/Molecular Probes (Eugene, OR).

SINGLE-LAP SPECIMEN PREPARATION

Initial investigation of various protocols for photochemical bonding involved tissue strips (or laps). Full-thickness articular cartilage slabs were harvested from the femoral condyles of immature bovine stifles cultured for 1–4 days in serum-supplemented medium prior to preparation as test strips. Culture medium (DMEM, 10% FBS, 1% NEAA, 10 mM HEPES, 0.5 μg/ml gentamicin, and 50 μg/ml ascorbate) was changed every 48 h. Harvested cartilage was removed from culture and sectioned into full-thickness slices 0.5 mm thick with a Microm HM-450 sliding microtome. Slices were immediately immersed in PBS, then cut to dimensions of 3 mm wide and 10 mm long using custom cutting jigs, removing both the superficial and deep zones (Fig. 1). Radial slices of the medial meniscus were similarly prepared and cut to the same final dimensions (10 mm × 3 mm × 0.5 mm) while excluding the vascular zone. Tissue strips were kept in PBS until treatment for bonding and tested within 3 h of removal from culture. Strips were selected at random for bonding pairs with no attempt to match adjacent tissue sections.

ENZYMATIC TREATMENT AND PHOTOCHEMICAL BONDING OF LAP SPECIMENS

As multiple variations on the bonding protocol were explored, one treatment protocol was selected as a baseline to which others would be compared. Enzymatic treatment parameters for this group were chosen to provide similarity to previous work investigating mild enzymatic degradation to enhance cartilage bonding using an adhesive[20] and other studies of integrative repair[21,22]. In this protocol, designated as CH-15, the end 3.5 mm of each strip in a bonding pair was submerged in 1 U/ml chondroitinase-ABC in PBS for 15 min. Strips were then immersed in PBS for 10 min before one specimen from each pair was immersed for 30 s in 15 mM CASPc/PBS. Both strips were blotted dry and arranged in apposition to create an overlap of 3.5 mm × 3 mm. Moistened tissue paper was used to back and overlay the specimen without covering the overlap. Clear plastic wrap was then placed over the sample, and the specimen was placed between two microscope slides constrained by clamps to maintain intimate contact. Characterization studies found no significant effect of pressure magnitude once intimate contact was produced. For irradiation, a 667 nm fiber-coupled diode system was coupled to a 40× objective to produce a 4 mm diameter beam with nearly uniform power density (irradiance). Directed perpendicularly to the bond area through the top specimen [Fig. 2(a)], the beam covered approximately 95% of the overlap.

TISSUE AND ENZYME COMPARISONS

Initial studies evaluated articular cartilage and meniscal fibrocartilage as well as the efficacy of different enzymes with the photochemical bonding process for the single-lap constructs. All solutions for this portion of the study were at room temperature (25°C). One group ($n = 9$) for each tissue type was treated with photosensitizer and irradiated but not enzymatically treated. Enzymatic treatments employed chondroitinase-ABC, hyaluronidase, or collagenase ($n = 9$/group) at varying concentrations and durations as described in Table I. Samples were irradiated at 1.7 W/cm^2 and an exposure of 1020 J/cm^2. Tissue harvested from a total of five stifles was randomly distributed across the groups such that each group included tissue from at least two joints with no more than half from a given joint. Similarly, no more than half of the samples in each group were cultured for the same amount of time. Control groups for each tissue included each enzymatic treatment with no photosensitizer or irradiation, enzymatic treatment and irradiation but no photosensitizer, and enzymatic treatment and photosensitizer but no irradiation.

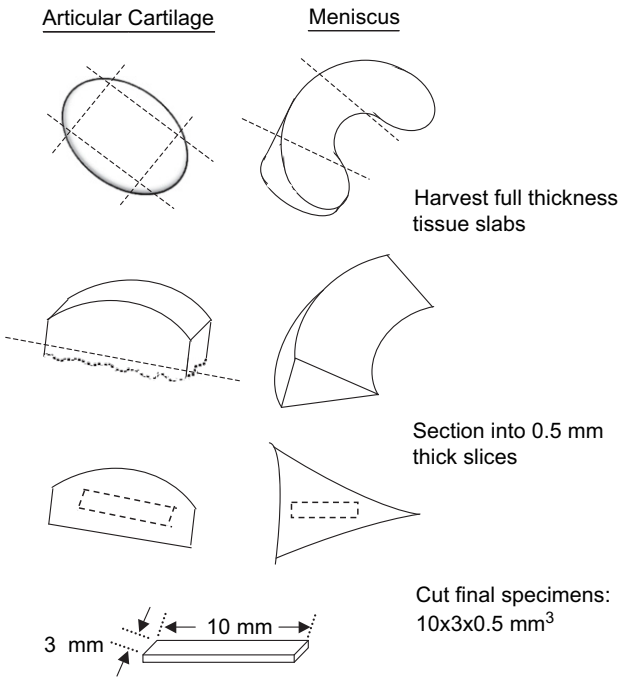

Fig. 1. Preparation of tissue strips for lap bonding studies. Full-thickness tissue blocks were isolated from immature bovine femoral condyles and medial menisci. Tissue blocks were sectioned to produce 0.5 mm thick slices, and 3 mm wide, 10 mm long strips were removed with custom cutting jigs.

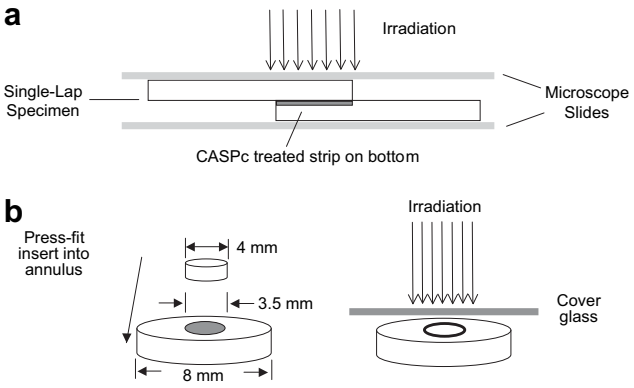

Fig. 2. Specimen irradiation geometry. (a) Single-lap specimens were irradiated perpendicular to the bond surface through a glass slide with the photosensitizer-treated lap on the bottom. (b) Defect model specimens were irradiated through a glass cover slip parallel to the irradiated surface of the insert and annulus.