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 to enhanced structural cohesion and tissue viability. Current methods for achieving cartilage bonding involve fibrin-based adhesives and sutures. Standard chemical crosslinking methods and photothermal soldering approaches 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 characterized by indirect photo-oxidation of a substrate via formation of singlet oxygen ($^{1}\text{O}_2$); protein oxidation is a secondary event. Proteins are typically inefficient generators of $^{1}\text{O}_2$ by direct sensitization, requiring the presence of an exogenous photosensitizer. 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, seal urethral tissue, and seal scleral incisions in vitro. Of the type-II photosensitizers, Rose bengal was found to bond partial-thickness, cryopreserved porcine skin grafts while preserving collagen organization and tissue viability. 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. Subsequent studies qualitatively observed similarly bonded meniscal and articular cartilage tears in sheep. 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. Chloro-aluminum phthalocyanine tetrasulfonic acid (CASPc), an anionic, hydrophilic phthalocyanine that may sensitize via type-I and type-II processes, was therefore selected for this study.

The overall goal of this study was to explore the feasibility of bonding live cartilage tissue through photochemical
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 (Eugene, OR). Ascorbate, kanamycin, chondroitinase-ABC and hyaluronidase were from Sigma (St. Louis, MO). Collagenase (type 2) was from Worthington Biochemicals (Lake-wood, NJ). Fetal bovine serum (FBS) was from HyClone (Logan, UT). Imma-
ture bovine stifle were from Research 87 (Boyleston, 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 stifle 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 × 3.5 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 and other studies of integrative repair. 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 initial 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. 2a), 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/cm² and an exposure of 1020 J/cm². 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 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.
### Table I

<table>
<thead>
<tr>
<th>Treatment protocol</th>
<th>Enzyme treatment</th>
<th>CASPc concentration</th>
<th>Irradiance</th>
<th>Exposure</th>
<th>Tissue type</th>
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<tr>
<td>COL-15</td>
<td>50 U/ml collagenase (type 2)</td>
<td>15 min</td>
<td>15 mM</td>
<td>1.7 W/cm²</td>
<td>1020 J/cm²</td>
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<tr>
<td>CH-15</td>
<td>1 U/ml Ch-ABC</td>
<td>15 min</td>
<td>15 mM</td>
<td>1.7 W/cm²</td>
<td>1020 J/cm²</td>
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<tr>
<td>HY-15</td>
<td>10 U/ml hyaluronidase</td>
<td>15 min</td>
<td>15 mM</td>
<td>1.7 W/cm²</td>
<td>1020 J/cm²</td>
</tr>
<tr>
<td>CHHY-SEQ</td>
<td>1 U/ml Ch-ABC for 4 min then</td>
<td>16 min</td>
<td>10 U/ml hyaluronidase for 4 min, then repeat</td>
<td>16 min</td>
<td></td>
</tr>
<tr>
<td>CH-20</td>
<td>1 U/ml Ch-ABC</td>
<td>20 min</td>
<td>15 mM</td>
<td>1.7 W/cm²</td>
<td>1020 J/cm²</td>
</tr>
</tbody>
</table>

AC = articular cartilage, MFC = meniscal fibrocartilage.

### PARAMETRIC VARIATIONS FOR ARTICULAR CARTILAGE

Variations from the baseline protocol (Table II) included altered photosensitizer concentration, irradiance, or exposure ($n = 8$ per group). All solutions for these studies were maintained at 37°C, for greater physiologic relevance and where chondroitinase-ABC exhibits greater activity. CASPc concentrations of 7.5 and 3.25 mM were investigated holding all other parameters constant. For other test groups either irradiance (1.7, 1.1, or 0.9 W/cm²), exposure (1530, 1020, or 510 J/cm²), or both were varied. Tissue harvested from five stifles was randomly distributed among the groups as before.

To investigate functional modification of the tissue surface, one group was treated with Traut’s reagent. Following enzymatic treatment, the end 3.5 mm of both slices were immersed for 10 min in 1.5 mg/ml of Traut’s reagent in PBS, followed by a PBS rinse prior to photosensitizer treatment using 7.5 mM CASPc. A final test group investigated physical debridement by abrading the tissue with 320-grit sandpaper in place of the enzymatic treatment. Controls were as described above.

### SINGLE-LAP MECHANICAL TESTING

Bonded lap specimens were hydrated in PBS for 10 min prior to tensile extension to failure using a modified single-lap test in which the bond area was supported laterally to restrict rotation of the bond (Fig. 3). Numerical and experimental analyses indicate that this modification reduces both the peak shear and peel stresses near the bond edges, bringing the actual shear stress along the bond closer to the nominal shear stress calculated as the force divided by the bond area²³.

Mechanical testing was performed at room temperature on an ELF3200 (Enduratec, Minnetonka, MN) using a 25 N load cell (Interface, SMT1-5.6). A 12X linear translation stage (Thorlabs, Newton, NJ) controlled horizontal positioning of the upper grip fixture, bringing the test specimen into contact with both supports. The end 5 mm of each tissue strip was gripped using wire-cut steel secured by screws. The upper grip fixture was raised at 0.5 mm/min while sample hydration was maintained with a PBS drip. Upon failure, tissue bonds completely lost integrity. Nominal shear strength was defined as the maximum force measured prior to failure divided by the interfacial bond area.

### CARTILAGE DEFECT MODEL PREPARATION

The durability of photochemical bonding and the effects on cell viability were examined using an in vitro cartilage defect model²⁴. Cylindrical articular cartilage cores were harvested from the femoral condyles and patellofemoral grooves of two immature bovine stifles using 8 mm and 4 mm biopsy punches. Cores were trimmed to a 2 mm thickness that excluded the superficial and calcified zones before storage in serum-free medium (DMEM, 0.5 µg/ml gentamicin, 100 µg/ml kanamycin, 1% AB/AM) at 37°C for 12 h. Annuli were created by removing a 3.5 mm core from the center of each 8 mm disc, while the 4 mm discs were used as oversized inserts. Annuli and inserts were rinsed in PBS and randomly paired with no consideration of harvest location.

### DEFECT BONDING AND CULTURE

All surface treatments were conducted via immersion without agitation at 37°C. Annuli and inserts for all treatment groups ($n = 12$ per group) were first immersed in 1 U/ml chondroitinase-ABC for 15 min and then in PBS for 10 min. A control group underwent no additional treatment. Annuli from the second group were immersed for 20 s in 15 mM CASPc. Both annuli and inserts from the third group were immersed for 10 min in 1.5 mg/ml of Traut’s reagent/PBS and annuli were then immersed for 20 s in 7.5 mM CASPc. Annuli and inserts were blotted dry prior to being press-fit together.

Only the photosensitizer-treated groups were irradiated. Constructs from these groups were seated underneath a piece of cover glass on sterile, PBS-soaked cotton to avoid dehydration. Irradiation was centered on the construct and incident normal to the disc surface (Fig. 2(b)) as a 5 mm spot at 1.1 W/cm² for 10 min (667 J/cm²). To mimic potential clinical application to an articular surface, only one surface was irradiated. All constructs were immersed in PBS for 20 min, then transferred to serum-supplemented medium and cultured for up to 7 days. Medium was changed every 48 h.

### DEFECT INTERFACE STRENGTH TESTING

Using the system and load cell described above, the bond strength was assessed via a slow rate push-out test as previously described²⁴. Samples were randomly selected for push-out testing immediately, after 3 days, or after 7 days in culture ($n = 4$ per group per culture duration). A sample was seated in a custom sample holder while a 3.45 mm diameter plunger pushed the insert out of the annulus through a 5 mm diameter hole in the support at a rate of 0.5 mm/s. Failure stress was defined as the peak force divided by the lateral area of the insert using measured dimensions at the time of testing (to account for any tissue swelling). Separated discs and annuli were immediately returned to culture and qualitatively assessed for cell death using the

### Table II

<table>
<thead>
<tr>
<th>Treatment designation</th>
<th>Surface treatment</th>
<th>CASPc concentration</th>
<th>Irradiance</th>
<th>Exposure</th>
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<tr>
<td>CH-15</td>
<td>Ch-ABC</td>
<td>15 mM</td>
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<td>1020 J/cm²</td>
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<td>Varied photosensitizer concentration</td>
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<tr>
<td>CH-15-PSH</td>
<td>Ch-ABC</td>
<td>7.5 mM</td>
<td>1.7 W/cm²</td>
<td>1020 J/cm²</td>
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<td>CH-15-PSQ</td>
<td>Ch-ABC</td>
<td>3.25 mM</td>
<td>1.7 W/cm²</td>
<td>1020 J/cm²</td>
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<td>Varied irradiation parameters</td>
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<tr>
<td>CH-15-IR15</td>
<td>Ch-ABC</td>
<td>15 mM</td>
<td>1.7 W/cm²</td>
<td>1530 J/cm²</td>
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<tr>
<td>CH-15-IR5</td>
<td>Ch-ABC</td>
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<td>1.7 W/cm²</td>
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<td>CH-15-IR15P2/3</td>
<td>Ch-ABC</td>
<td>15 mM</td>
<td>1.1 W/cm²</td>
<td>1020 J/cm²</td>
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<tr>
<td>CH-15-IR10P1/2</td>
<td>Ch-ABC</td>
<td>15 mM</td>
<td>0.9 W/cm²</td>
<td>510 J/cm²</td>
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<td>Alternative surface treatments</td>
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<tr>
<td>CH-15-PSH-TR</td>
<td>Ch-ABC + Traut’s reagent</td>
<td>7.5 mM</td>
<td>1.7 W/cm²</td>
<td>1020 J/cm²</td>
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<tr>
<td>Debride-PT</td>
<td>Sandpaper abrasion</td>
<td>15 mM</td>
<td>1.7 W/cm²</td>
<td>1020 J/cm²</td>
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</tbody>
</table>
Results

TISSUE AND ENZYME COMPARISONS

Articular cartilage strips did not bond when treated only with photosensitizer and irradiated, supporting the need for some surface treatment to expose proteins for photochemical bonding. Treatment with collagenase or hyaluronidase alone prior to photosensitizer treatment and irradiation also failed to produce cartilage bonding. No control cases maintained sufficient structural integrity during hydration to allow mechanical testing. In contrast, pre-treatment with chondroitinase-ABC consistently produced stable photochemical bonding. The CH-20 protocol produced a 51.2 ± 4.8 kPa bond strength, which was significantly stronger (P < 0.01) than the CH-15 treatment at 28.2 ± 3.2 kPa. The sequential enzymatic treatment, CHHY-SEQ, resulted in a 61.3 ± 4.6 kPa bond strength, significantly stronger (P < 0.01) than all treatment groups except CH-20.

In contrast to articular cartilage, meniscal fibrocartilage bonded without enzymatic modification. Meniscal tissue samples treated with photosensitizer and irradiation but not chondroitinase-ABC had a bond strength of 36.0 ± 3.8 kPa. Compared to 40 ± 4.5 kPa and 32 ± 4.3 kPa for the CH-20 and CHHY-SEQ protocols, respectively, there were no significant differences between the groups (Fig. 4). Specimens irradiated but not treated with CASPc did not bond.

PARAMETRIC VARIATIONS FOR ARTICULAR CARTILAGE

At 37°C where the chondroitinase-ABC was more active, the baseline protocol CH-15 produced a nominal shear strength of 45.9 ± 3.6 kPa. Control groups all fell apart during hydration and were not testable. A general trend toward decreasing shear strength was found with decreasing photosensitizer concentration. The bond strength was significantly lower with 3.25 mM CASPc than with 15 mM CASPc (Fig. 5).

Within the range investigated, higher power density had a stronger effect on interface strength than total exposure. Holding exposure constant while reducing irradiance reduced bond strength. Similarly, for exposures of 1020 J/cm² or less, holding irradiance constant and reducing exposure decreased bond strength. The baseline combination of irradiance and exposure was found to produce the highest bond strength within the investigated ranges (Fig. 6).

Functionalizing the surface using Traut’s reagent after enzymatic treatment produced the highest bond strength of all treatment protocols at 92.1 ± 9.3 kPa, roughly twice that of the baseline case. Abrasion of the cartilage tissue in place of enzymatic treatment produced a mean bond strength of only 1.4 ± 1.4 kPa.

DEFECT MODEL PUSH-OUT TESTS

Samples treated with chondroitinase-ABC only exhibited a fairly constant push-out strength across days 0, 3 and 7 in culture with a mean nominal shear strength of 60 ± 11 kPa (Fig. 7). Specimens further treated with photosensitizer and irradiated exhibited significantly higher failure strength (P < 0.05) than for the enzyme-only group, with a mean strength of 104 ± 16 kPa across all time points.
Additional treatment with Traut’s reagent produced the highest shear strength ($P < 0.01$), averaging $279 \pm 52$ kPa (with less consistency across time points). Intact cartilage had a substantially higher strength of $5.37 \pm 0.70$ MPa, although this represents a punching failure of the tissue rather than failure at an existing interface.

In Live/Dead images, non-treated, non-press-fit specimens showed a region of cell death adjacent to the cut surface, while those exposed to photosensitizer showed a similar though slightly increased necrosis region. For specimens from the press-fit annuli/insert constructs, a high overall viability was observed with no apparent increase in cell death at the photosensitizer-treated and irradiated interface over the enzyme-only group. On day 3 of culture, both press-fit groups were qualitatively observed to exhibit a necrosis region at the annulus/insert interface of approximately twice that of the non-press-fit samples. The only difference observed was that the photosensitized samples exhibited a slightly increased necrosis region at the outer annulus surface compared to the enzyme-only samples, similar to the findings for non-press-fit specimens. Traut’s reagent increased cell death near the interface and induced dispersed cell death throughout the center of the sample, indicating that alternative functionalizing reagents should be explored. Results after 7 days of culture were similar (Fig. 8).

Discussion

This study investigated a means of linking collagen fibrils in adjacent cartilage tissues to provide a structural connection in a clinically relevant procedural time span with hope that the initial bonding will lead to enhanced integration and repair. Without extensive cell death and with bond stability in culture, results demonstrated the potential of combining enzymatic surface modification with photodynamic techniques to directly bond cartilage tissues. Clinical experience and other studies have found that lack of lateral support and bonding to native tissue is a critical factor leading to graft complications and failure\textsuperscript{25–27}. Both the single-lap and disc/annulus tissue models in this study were thus designed to be representative of bonding of an implant to host tissue within a chondral defect or repair of vertical fissures.

Crosslinks in proteins induced by $^{1}$O\textsubscript{2} primarily involve side chain amino acid residues, though only cysteine, histidine, methionine, tyrosine, and tryptophan react with $^{1}$O\textsubscript{2} at physiological pH at rates fast enough to be significant\textsuperscript{7,15,28}. The specific nature of the crosslinking mechanisms and side groups involved has been debated\textsuperscript{29,30}, but reactions are often byproducts of oxidized residues reacting with other residues\textsuperscript{7,8,31}. Studies have shown that photo-oxidized collagen forms aggregates and crosslinks\textsuperscript{32–34}, and that $^{1}$O\textsubscript{2} treated collagen behaves like pepsin-treated collagen when solubilized, failing to associate into physiologic fibrils although no molecular denaturation was seen\textsuperscript{35}.

Photochemical bonding without an exogenous layer to promote adhesion, as performed in this study, is an example of zero-length crosslinking where a bond is created that contains no intermediate molecules adding to the final ultrastructure\textsuperscript{36,37}. Crosslinks between adjacent collagen microfibrils to form interfibrillar bonds are possible only if the distance between the two microfibrils is smaller than the length of the crosslinking agent introduced. Considering the scale of individual collagen molecules and fibrils, an approximate distance of 1.3–1.7 nm becomes the critical parameter for
crosslinking in a zero-length process. This partly explains why the combination of enzymatic surface treatment (to remove proteoglycans and expose the collagen) with intimate contact between the tissue sections was required to form a photochemical bond for articular cartilage. Protocols for the enzymatic treatments used in this study were taken directly from previous efforts, which were shown to remove surface proteoglycans to a depth on the order of a few microns without significant disruption of the collagen matrix and found no long-term negative impacts in a rabbit model over a period of 6 months. The majority of specimens in this study thus used the CH-15 protocol, though other variations to this treatment may improve interfacial bonding strength.

The most apparent critical factor presenting itself in these experiments is the need for enzymatic surface modification prior to achieving a photochemical bond for articular cartilage. Meniscal tissue, with its significantly lower proteoglycan content, required no such modification. Whether or not the difference in collagen type is also contributory could not be ascertained here. Interestingly, while chondroitinase-ABC treatment was consistently effective, treatment with hyaluronidase did not facilitate tissue bonding. The enzymatic effect on bond strength may therefore be either a consequence of the enzyme site specificity or of the ability of the enzyme to penetrate to its targeted site in the amount of time allowed; further optimization may improve results. Additionally, the removal of proteoglycans to promote oxidative photochemical bonding of collagen fibrils in cartilage may be intrinsically required as proteoglycans and glycosaminoglycans have been found to act as antioxidants.

Previous single-lap tests examining integrative cartilage repair have produced strengths on the order of 30–35 kPa after 14–21 days in culture in a non-supported single-lap configuration using the same test rate and similar geometry as in this study. Other studies using a naphthalene dye investigated meniscal tissue bonding using 2.7 W/cm² mercury lamp irradiation and obtained a shear strength of approximately 21 kPa using the standard, non-supported lap configuration at a significantly higher displacement rate of 0.25 mm/s (15 mm/min). In the present study, the baseline treatment protocol produced a mean failure strength of 45.9 ± 3.6 kPa using the modified single-lap configuration and a displacement rate of 0.5 mm/min. Differences in test configuration and test rate make direct comparisons difficult. However, testing of baseline protocol samples using the standard (non-supported) single-lap configuration produced a failure strength of 30.3 ± 1.9 kPa, while testing in the modified configuration at a higher rate (0.5 mm/s) produced a failure strength of 162 ± 24 kPa. The combined enzymatic digestion and

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Fig. 8. Cell viability images. All images show representative tissue sections at the inner edge of annuli after 7 days of culture and push-out testing. (a) Location of images; (b) sample treated with enzyme and press-fit only; (c) sample press-fit and photochemical bonding; (d) sample treated Traut’s reagent and photochemical bonding. An increase in cell death was consistently noted near the surface treated with Traut’s reagent.
photochemical bonding strategy explored in this study is thus able to produce bond strengths that are at least comparable to those reported using other strategies.

Push-out testing of native explant insert/annulus defect models using similar dimensions, but without a press-fit as in this study, produced strengths of 28.5 ± 8.7 and 33.3 ± 7.0 kPa at 20 and 40 days, respectively. In another tissue insert/annulus study, interfacial push-out strength at 56 days was found to be approximately 155 kPa for both untreated and 1% trypsin-treated assemblies. Although this particular agent is not ideal due to its cytotoxicity, the concept of functionalizing the surface with agents that serve to enhance the photochemical process was also validated.

In conclusion, this study has demonstrated the potential of combining enzymatic surface modification with photodynamic namic techniques to directly bond cartilage tissues, and that differences in the cartilage tissue type can necessitate different treatments to enable such bonding. Of particular note, the bond strength achieved with a 10 min irradiation was on the order of that previously achieved through several treatments to enable such bonding. Further enhancement of push-out strength was achieved by additional treatment with Traut’s reagent, which reacts with primary amines to free sulfhydryl groups that may enable disulfide bonds when oxidized.

Conflict of interest

The authors have no conflicts of interest to disclose.

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