Enhancing Integration of Articular Cartilage Grafts via Photochemical Bonding

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ABSTRACT: The integration of osteochondral grafts to native articular cartilage is critical as the lack of graft integration may lead to continued tissue degradation, poor load transfer and inadequate nutrient transport. Photochemical bonding promotes graft integration by activating a photosensitizer at the interface via a light source and avoids negative effects associated with other bonding techniques. We hypothesized that the bond strength depends on photosensitizer type and concentration in addition to light exposure. Photochemical bonding was evaluated using methylene blue (MB), a cationic phenothiazine photosensitizer, and two phthalocyanine photosensitizers, Al(III) phthalocyanine chloride tetrasulfonic acid (CASPc) and aluminum phthalocyanine chloride (AlPc). Exposure was altered by varying irradiation time for a fixed irradiance or by varying irradiance with a fixed irradiation time. MB was ineffective at producing bonding at the range of concentrations tested while CASPc produced a peak twofold bond strength increase over controls. AlPc produced substantial bonding at all concentrations with a peak 3.9-fold bond strength increase over controls. Parametric tests revealed that bond strength depended primarily on the total energy delivered to the bonding site rather than the rate of light delivery or light irradiance. Bond strength persisted for 1 week of in-vitro culture, which warrants further exploration for clinical applications. These studies indicate that photochemical bonding is a viable strategy for enhancing articular cartilage graft integration. © 2018 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res 36:2406–2415, 2018.

Keywords: photochemical bonding; articular cartilage; allograft; cartilage focal defect repair; osteochondral

Focal defects in articular cartilage may be precursors to degenerative osteoarthritis (OA),[1,2] a progressive disorder that is radiographically prevalent in the population over 65 years and affects over 27 million Americans.[3,4] Surgical procedures to repair focal defects such as osteochondral autograft or allograft transplantation[5] may delay OA in younger patients where total knee replacement is undesirable.[6] However, achieving successful graft integration with the surrounding host cartilage remains a persistent obstacle.[2,7–9] Poor integration can lead to impaired load transfer or nutrient transport, fibrocartilage formation, and/or tissue necrosis.[2,10,11] Approaches previously explored to improve graft/implant integration to native tissue include adhesives, enzymatic digestion, suturing, and photothermal tissue welding.[12–15] While offering some advantages, these methods suffer from various limitations including low bonding strength, damage to surrounding tissue, incomplete repair, or introduction of foreign material to the integration site.[12,16]

Photochemical bonding is a promising strategy that employs light-activated photosensitizers to excite and covalently crosslink proteins across the implant-host interface on a short time scale.[16–18] Photochemical bonding occurs when a photosensitizer activated by light at a specific wavelength interacts with the substrate directly (type-I mechanism) or indirectly via singlet oxygen formation (type-II mechanism).[19–21] When collagen is the substrate, radicalization of various amino-acid groups leads to non-native crosslinking.[22,23] Photosensitizers that are activated at higher wavelengths (e.g., 670–690 nm) are advantageous for photochemically bonding cartilage in a clinical setting as higher wavelength light penetrates deeper into the tissue to activate the photosensitizer, potentially achieving a full-thickness bond with greater strength and improved overall graft integration.

Previous studies demonstrated the feasibility of bonding articular cartilage using Al(III) phthalocyanine chloride tetrasulfonic acid (CASPc), a photosensitizer from the phthalocyanine group, and also found that photochemical bonding of cartilage was enhanced by mild enzymatic treatment of the cartilage surface prior to bonding.[11] The aim of this study was to improve various aspects of the photochemical bonding procedure, laying the groundwork for in vivo animal studies and eventual clinical implementation. Specifically, we identified and compared the optimal concentration ranges for three photosensitizers, examined the effects of total exposure on bond shear strength by modifying both irradiance and irradiation time, explored the effect of restricting enzyme and photosensitizer treatment to the implant alone (highly desirable for clinical implementation), and finally evaluated in vitro bond durability through culture.

METHODS

Equipment

Two fiber-coupled lasers with bandwidths of >3 nm were used. An Edmund Optics (Barrington, NJ) 55-370 Fiber-Coupled Laser (670 nm, 0–310 mW) was used for enzymatic treatment, photosensitizer treatment, and low irradiance

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tests, and a Changchun New Industries Optoelectronics Technology (Changchun, China) FC-671 AlGaINP Fiber Coupling Laser System (687 nm, 0–1,200 mW) was used for subsequent photosensitizer studies and higher irradiance studies. A ThorLabs PM100D power meter with S121 sensor (Newton, NJ) was used to preset the required power. Mechanical testing was performed using an Instron 5848 Microtester (Norwood, MA). Biochemical analyses were carried out in a Biotek Synergy HT Microplate reader (Winooski, VT). Live/Dead cell staining was visualized using a Zeiss Axiovert 200M Fluorescence Microscope (Göttingen, Germany). Dye penetration was visualized using a Canon Power Shot G10 camera adapted to a Zeiss Invertoskop 40C microscope.

Materials

Immature bovine stifles were from Research 87 Inc. (Boylston, MA). Low glucose Dulbecco's Modified Eagles Medium (DMEM), Dulbecco's Phosphate Buffered Saline (PBS), gentamicin, glycine, L-ascorbate-2-phosphate, aluminum phthalocyanine chloride (AlPc), and chondroitinase-ABC were from Sigma (St. Louis, MO). Methylene blue (MB) and sodium chloride were from Acros Organics (Morristown, NJ). Al(III) phthalocyanine chloride tetrasulfonic acid (CASPc) was from Frontier Scientific (Logan, UT). HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer solution and antibiotic/antimycotic (AB/AM) were from Corning (Corning, NY). Fetal bovine serum (FBS) and non-essential amino acids (NEAA) were from HyClone (Logan, UT). The Live/Dead viability/cytotoxicity kit was from Invitrogen/Molecular Probes (Eugene, OR). Protease Inhibitor Cocktail Set I was from Calbiochem (San Diego, CA). A 10% formalin, 85 Flex, 95 Flex, 100 Flex, and ClearRite were from Thermo Fisher Scientific (Pittsburgh, PA).

Photochemical Bonding Experimental Layout

Three photosensitizers were selected for these studies based on their efficient ability to transfer electrons at activation wavelengths of approximately 670–680 nm[11,20,21]: Methylene blue (MB), a cationic phenothiazine, Al(III) phthalocyanine chloride tetrasulfonic acid (CASPc), a phthalocyanine used in previous studies of cartilage photochemical bonding, and aluminum phthalocyanine chloride (AlPc), a related phthalocyanine. By performing spectral scans with a microplate spectrophotometer, the peak absorption wavelengths of MB, CASPc and AlPc were determined to be 667, 673, and 685 nm, respectively (Fig. 1), with sufficient spectral overlap with the emission spectrum of the lasers to produce photochemical activation.

To characterize the effects of different enzymatic treatments, photosensitizers and exposures on the strength of bonding in cartilage, a series of benchtop studies and an in vitro study were devised. Four main studies (Fig. 2) were used for benchtop evaluation of the bonding strength: Baseline studies to establish light and photosensitizer penetration; enzymatic digestion studies; photosensitizer treatment studies; and light exposure studies. Bond durability was investigated by determining bond shear strength initially and following 7 days of in vitro culture.

Benchtop Studies

Baseline Studies

To verify sufficient laser light transmission, the net power transmission through cartilage of the Changchun diode laser

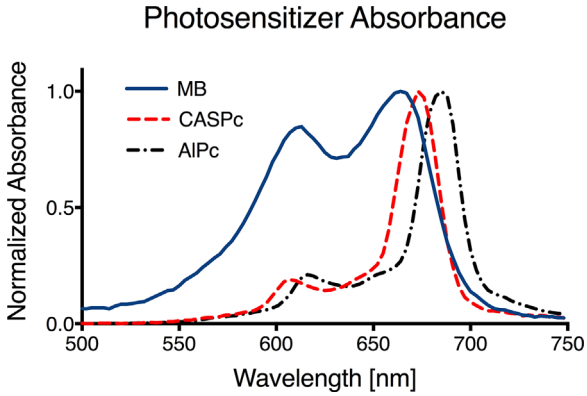

Figure 1. Normalized absorbance vs. wavelength for MB, CASPc and AlPc. Peak absorbance for MB, CASPc, and AlPc occur at 667, 673, and 685 nm, respectively.

was recorded for specimens of varying thicknesses. Eight millimeter full thickness cartilage plugs were extracted from the femoral condyles and patellofemoral grooves of six immature bovine stifles and stored in 1× PBS with protease inhibitors (PBS + PI). Using a microtome, slabs ranging from 50 μm to 5 mm were extracted from each plug, avoiding the superficial and deep zones. Tissue slabs were placed in 8 mm holes in a 3 or 6 mm thick rubber mat glued to glass slides. The laser was coupled to a 40× objective to produce a 5 mm diameter beam with uniform irradiance at the focal point. The power meter was used to set the baseline power intensity to 350 mW. The powers transmitted through the various cartilage slab thicknesses and through the glass slide alone were measured to determine the fractional power transmittance through cartilage. To verify that the superficial tissue does not alter light transmission, tissue samples of varying thickness with an intact articular surface were extracted from one additional stifle and similarly evaluated.

To determine the photosensitizer penetration depth, 4 mm cartilage cores were harvested, trimmed to 2 mm thick (removing superficial and vascularized deep zones) and stored in PBS + PI. The samples underwent a 10 min PBS

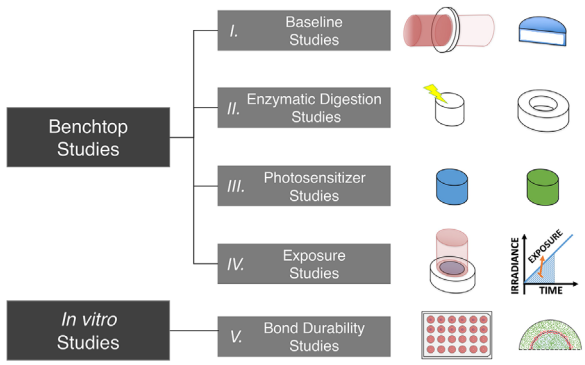

Figure 2. Experimental layout for photochemical bonding studies. I.) Baseline studies include quantifying fractional light transmission through articular cartilage and photosensitizer dye penetration through the tissue. II.) Enzymatic digestion studies quantify the impact digestion time and treated surface (implant or both implant and annulus) have on bond shear strength. III.) Photosensitizer studies examine the bonding capabilities different photosensitizers at different concentrations have on shear strength. IV.) Exposure studies characterize how different irradiance levels and irradiation times affects shear strength. V.) Bond durability studies examained persistent bond shear strength 1 week after culture as well as cell viability.

wash before being enzymatically digested using chondroitinase ABC for 15 min. After digestion, samples underwent a final 10 min PBS wash before being submerged into varying AlPc, CASPc, and MB concentrations (0.5 mM, 15 mM for AlPc and CASPc, and 0.5 and 2.5 mM for MB) for 20 s ($n = 3$). Samples were kept in PBS for less than 30 min before being fixed. Samples were revolved in 10% formalin for 8 h followed by a 45 min 85 Flex submersion, and two 45 min serial baths of each 95 Flex, 100 Flex and ClearRite, before finally being embedded in paraffin. Samples were sectioned at 10 μm ($n = 6$ per plug) and placed on microscope slides. Dye penetration was measured using images taken from the Canon camera attached to the microscope. Several dye penetration locations were measured from each slice and an average was taken as the penetration depth for the dye.

General Protocol for Benchtop Studies II–IV

Sample preparation, surface treatments, exposure, and mechanical testing followed the same procedure for the following benchtop studies. Unless otherwise indicated, the term "surface" refers to the integration surface of the implant and annulus rather than the articular surface.

Sample preparation. Full thickness cartilage plugs (4 and 8 mm diameter) were harvested from the femoral condyles and patellofemoral grooves of immature bovine stifles. Approximately 1 mm of superficial zone was removed as well as the deep zone leaving 2 mm thick discs (Fig. 3A). A defect was created in each 8 mm disc with a 3.5 mm biopsy punch to create a tissue annulus. Samples were stored in PBS + PI at 4°C and tested within 4 days.

Enzymatic and photosensitizer treatment. Enzymatic treatment to reduce proteoglycan content at the integrating cartilage surface has enhanced bonding in various cartilage repair studies.[11,15,24,25] For these photochemical bonding studies, the resultant softening has the additional benefit of ensuring intimate contact that facilitates crosslinking across the interface. To reduce proteoglycan content at the integrating surface and expose collagen before bonding, the annuli and implants were submerged in 1 U/ml chondroitinase-ABC in a 0.01% bovine serum albumin solution at 37°C for 15 min.[11] After chondroitinase digestion, samples were immersed in a 1 ml PBS wash for 10 min to allow diffusion of the enzyme. Following the PBS wash, the 4 mm implants were submerged in photosensitizer for 20 s and patted dry using a Kimwipe to remove excess photosensitizer (Fig. 3B). Immediately afterwards, implants were press-fitted into annuli and irradiated (Fig. 3C, details below).

Interface strength mechanical testing. Samples were equilibrated in PBS for 20 min between irradiation and mechanical testing. Samples were placed in a custom rig and a hemispherical tipped probe (3.24 mm diameter) was used to push out implants at 0.5 mm/s (Fig. 3D).[11] Compressive forces were continuously measured during push-out; the implant and annulus thicknesses were then measured using a resistance micrometer. The bond shear strength was determined as the highest compressive force divided by the implant-annulus interface area ($A_{int} = \pi * d * h$, where $d = 3.75$ mm and h is the height of the implant-annulus overlap) for each sample.

Enzymatic Digestion Study

To determine how the extent of chondroitinase digestion affects cartilage bonding, both annuli and implants were exposed to chondroitinase for 5, 10, or 15 min ($n = 5$). Photochemical bonding parameters, photosensitizer concentration, irradiance, and irradiation time were set constant (see Table 1).

Although previous studies involved enzymatic digestion of both the implant and the annulus,[11] restricting enzymatic treatment to the implant would be clinically advantageous as implant preparation could be performed preoperatively without introducing enzymes to the joint. To address this, the bond shear strength was quantified for surface treatment to the implant alone or to both implant and annulus ($n = 5$). Photosensitizer concentration, irradiance, and irradiation time parameters for these tests are presented in Table 1.

Photosensitizer Study

To identify and compare optimal conditions for the three photosensitizers, the bond shear strengths produced using MB, CASPc, and AlPc over ranges of concentrations were investigated. MB concentration was varied from 0.125 to 2.5 mM while CASPc and AlPc concentrations were varied from 0.05 to 15 mM (Table 2), with $n = 5$–16 samples per group. Enzymatic treatment was applied to both implants and annuli while photosensitizer treatment was limited to the implant. After an implant underwent photosensitizer treatment and was press-fitted into the annulus, the sample was irradiated at 1.579 W/cm^2 for 600 s, incubated in PBS for 20 m and subjected to mechanical push out testing.

As with enzymatic treatment, restriction of photosensitizer treatment to the implant is clinically advantageous. To

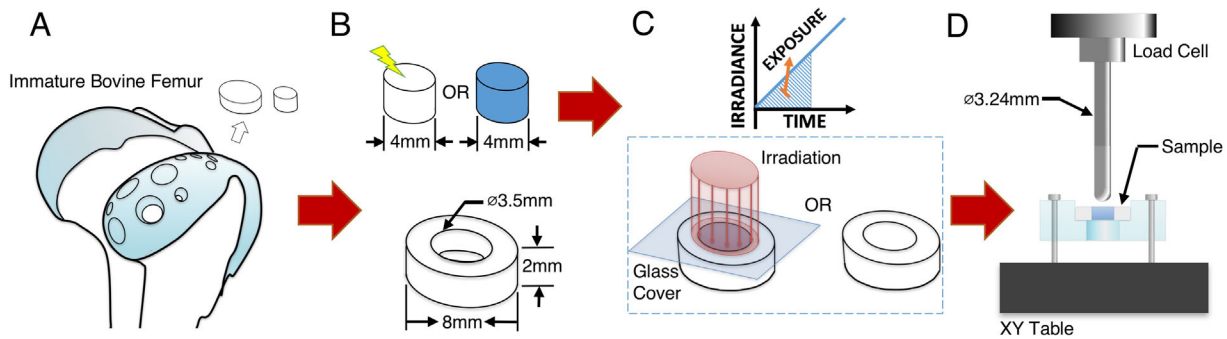

Figure 3. Sample Preparation and Testing. A) Extraction of 4 and 8 mm full thickness articular cartilage plugs. B) Enzymatic surface digestion of cartilage and photosensitizer treatment. C) Laser irradiation of press-fitted sample. D) Mechanical testing of sample to determine bond shear strength.

Table 1. Enzymatic Treatment and Surface Treatment

		Enzymatic Surface Treatment 1 U/ml Chondroitinase		Laser Exposure	
Test Type	Photosensitizer Surface Treatment (AlPc at 5 mM Concentration)	Enzymatic Treatment Time	Enzymatic Surface Treated	Irradiance [W/cm^2]	Irradiation Time [s]
Varied enzymatic time treatment	Implant	5, 10, 15 min	Implant/Annulus	1.579	600
Enzymatic varied surface treated	Implant	15 min	Implant vs. Implant/Annulus	1.579	600
Photosensitizer varied surface treated	Implant vs. Annulus	15 min	Implant/Annulus	1.579	600

Parameters set for baseline and enzymatic digestion studies.

investigate whether the bond shear strength is influenced by which surface is treated, photosensitizer treatment was applied to either the implant or annulus ($n = 6$). Test parameters for this sub-study are described in Table 1.

Exposure Study

The effects of laser exposure on cartilage bond shear strength were investigated using 15 min mild enzymatic surface treatment, 5 mM AlPc and varied irradiation conditions. The average laser exposure can be described by:

$$E = P/A_{\text{beam}} * t \quad (1)$$

where E is the exposure, P is the power emitted by the laser, A_{beam} is the area of the laser beam and t is the time of irradiation. The irradiance is given by P/A_{beam} and reflects the energy flux. Variations in total laser exposure were between 15.8–1,910 J/cm^2 and produced by (i) increasing irradiation time (10–600 s) while keeping irradiance constant (1.58 W/cm^2) ($n = 10$–11/group) or by (ii) increasing irradiance (0.161–3.18 W/cm^2) while keeping irradiation time constant (600 s) ($n = 5$–18/group) (Table 3). After irradiation, samples were equilibrated in PBS for 20 min before mechanical testing.

In Vitro Study

Bond Durability

To determine bond durability in vitro, cartilage implants and annuli were bonded, cultured for 7 days and mechanically tested to determine bond durability. Cartilage plug extraction and trimming were performed as described previously but using aseptic technique within a culture hood. Tissue explants were cultured in serum-supplemented culture medium (DMEM, 10% FBS, 1% NEAA, 10 mM HEPES, 0.5 mg/ml gentamicin, and 50 mg/ml L-ascorbate-2-phosphate) for 1 day prior to treatment. The 4 mm implants were rinsed in 1 ml PBS for 10 min prior to a 15 min chondroitinase digestion, followed by equilibration in PBS for 10 min and random assignment to Control and Bonded groups ($n = 6$). Implants from the Bonded group were submerged in 5 mM AlPc for 20 s, patted dry, and press-fitted into annuli, while Control implants were immediately press-fitted without photosensitizer treatment. In the Bonded group, implant-annulus samples were irradiated using the Changchun laser at 1.579 W/cm^2 for 10 min. Constructs were cultured in 2 ml of culture medium, with media collected and replaced on days 2, 4, and 6. Interface bond shear strength was obtained for groups on day 0 and 7 ($n = 6$ for each group on day 0 and 7).

To determine cell viability, a subsequent study was performed following the same protocol and parameters as in the bond durability study. Live/Dead cell staining was performed approximately 24 h after bonding and 8 days after bonding ($n = 2$). Thin samples (~0.5 mm) were cut perpendicular to the bonded surface and incubated in a plate shaker at 37C in a PBS wash for 30 min before being stained with 4 μM ethidium and 2 μM calcein AM for 45 min under the same conditions. Samples underwent one final PBS wash for 30 min and were sliced to ~0.2 mm slices before imaging with fluorescent microscope.

Data Analyses

Bond shear strength for enzymatic digestion, exposure, and bond durability studies were analyzed via a single factor general linear model, while Photosensitizer type and concentration studies were analyzed via multifactor general linear models. Significance was set at $p < 0.05$ and Bonferroni's test

Table 2. Photosensitizer Concentration

Photosensitizer	Concentration	Enzymatic Treatment	Irradiance	Irradiation Time	Exposure
AlPc	0.05, 0.125, 0.25, 0.5, 1, 5, 15 mM	15 min in 1 U/ml Chondroitinase	1.579 W/cm^2	600 s	947 J/cm^2
CASPc	0.05, 0.125, 0.25, 0.5, 1, 5, 15 mM				
MB	0.125, 0.25, 0.5, 1, 2.5 mM				
Control	0 mM		0 W/cm^2	0 s	0 J/cm^2

Parameters set for photosensitizer studies.

Table 3. Exposure Variation

Photosensitizer	Concentration	Enzymatic Treatment	Irradiance	Irradiation Time	Exposure
AlPc	5 mM	15 min in 1 U/ml Chondroitinase	1.58 W/cm^2	10, 19, 37, 75, 150, 300, and 600 s	15.8, 30, 58, 118, 237, 474, and 947 J/cm^2
AlPc	5 mM		0.161, 0.495, 0.787, 1.08, 1.41, 1.58, 2.22, 2.68, and 3.18 W/cm^2	600 s	97, 297, 472, 647, 848, 947, 1,330, 1,610, and 1,910 J/cm^2
Control	0 mM		0 W/cm^2	0 s	0 J/cm^2

Parameters set for exposure studies.

was used for pairwise comparisons. A power law function was determined via nonlinear regression analysis of combined strength vs. exposure data from the varied irradiance and varied irradiation time studies. Values are presented as mean ± SEM.

RESULTS

Benchtop Studies

Baseline Studies

The fractional power transmission was calculated as the measured power transmission for each slice normalized by the power transmission through the glass alone (Fig. 4). Light transmission decreased with sample thickness due to scattering and absorption, with comparable reduction for samples with and without intact articular surfaces. The fractional power transmission versus cartilage thickness was least-squares fit to both middle zone and intact articular surface samples with a one-phase exponential decay function:

$$\text{Fractional Power} = \text{Ae}^{-\text{thickness}/\Delta} + B \qquad (2)$$

where Δ is the characteristic decay distance. Coefficients for equation (2) were $A = 0.871 \pm 0.032$, $B = 0.112 \pm 0.026$, and $\Delta = 1.39 \pm 0.135$ mm ($R^2 = 0.940$). With cartilage thicknesses ranging from 1.65 to 2.65 mm, corresponding to mean cartilage thickness of adult human femoral condyles,[26] 24–38% of the incident power would be expected to reach the deepest cartilage region, supporting the feasibility of treating full thickness clinical defects.

The photosensitizer penetrated approximately 10 μm into the tissue. Dye penetration was limited to the region where chondroitinase reduced proteoglycan content.

Enzymatic Digestion Study

As previously referenced, mild enzymatic treatment of cartilage surfaces affects the bond shear strength. When varying the enzymatic digestion time, the bond shear strength reached a plateau as the digestion time increased, from 5 min of treatment (109 ± 15 kPa) to 15 min of treatment (135 ± 15 kPa) (Fig. 5). All enzymatic digestions (5, 10, and 15 min) produced bond shear strengths significantly greater ($p < 0.05$, $p < 0.009$, and $p < 0.009$, respectively) than those of controls (68 ± 8 kPa), which primarily reflects the effect of friction and the press fit. Restricting enzymatic digestion to the implant alone did not substantially

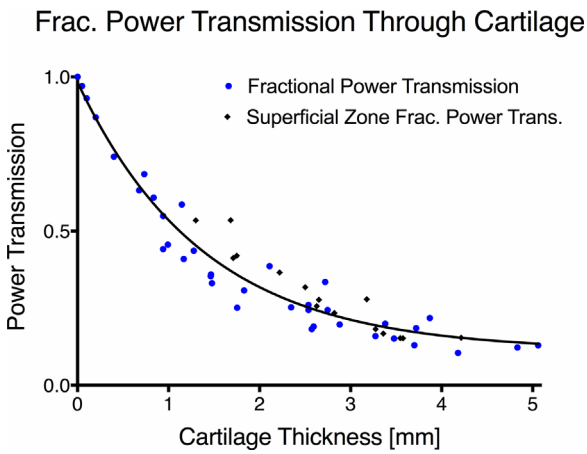

Figure 4. Fractional power transmission of 686 nm light through varying thickness of immature bovine articular cartilage samples. One-phase exponential decay fit: Fractional Power $= 0.871 * e^{(-\text{thickness}/1.39)} + 0.112$ ($R^2 = 0.940$).

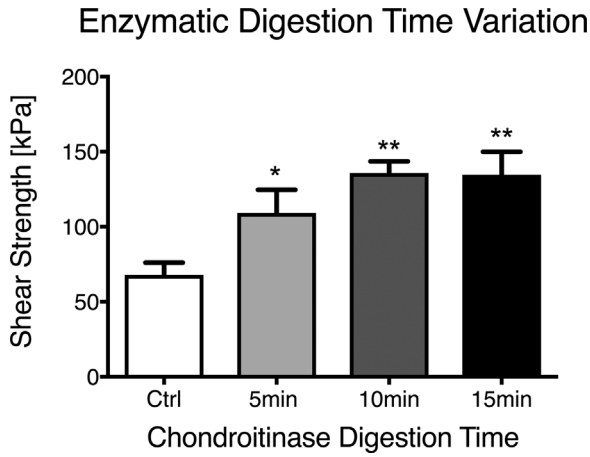

Figure 5. Variation of mild enzymatic digestion time. All enzymatic digestion times produced shear strengths that were significantly greater than that in the control group ($^*p < 0.05$, $^{**}p < 0.01$).

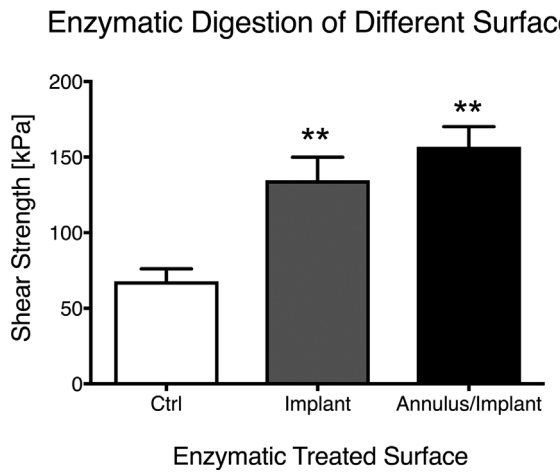

Figure 6. Mild enzymatic treatment of different surfaces. Strengths for different treated enzymatic digestion surfaces (only implant or both annulus and implant) did not significantly differ from each other but both were significantly different from the control group ($^{**}p < 0.01$).

alter the bond strength (Fig. 6). Bond strengths for digestion of the implants alone (135 ± 15 kPa) and for digestion of both implants and annuli (157 ± 13 kPa) were significantly greater than those of controls (68 ± 8 kPa) but did not significantly differ between surface treatment groups.

Photosensitizer Study

There were substantial differences in the peak bond strengths produced by the three photosensitizers (Fig. 7). MB was ineffective at producing bonding over the studied range of concentrations, with no treatments producing significantly greater bond strengths than controls and no significant differences among concentrations. CASPc exhibited a concentration-dependent enhancement of bond strength, with strengths significantly greater than controls at intermediate concentrations (0.25 and 0.5 mM) and a peak strength

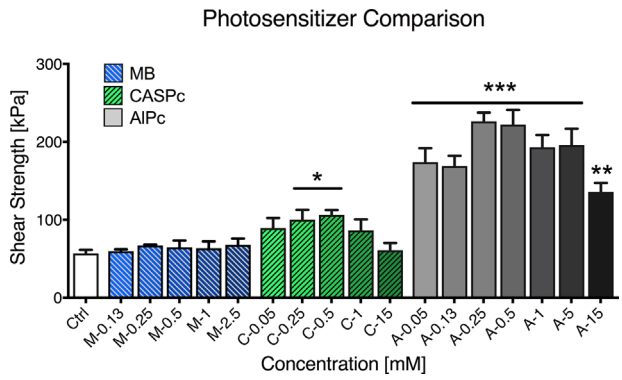

Figure 7. Bond shear strength resulting from three photosensitizers at different concentrations. MB, CASPc, and AlPc were varied from 0.13 to 2.5 mM, 0.05 to 15 mM, and 0.05 to 15 mM, respectively. MB was ineffective at producing bonding. CASPc exhibited shear strengths significantly greater ($^{*}p < 0.05$) than controls at intermediate concentrations. AlPc shear strengths were significantly greater than controls at all concentrations ($^{**}p < 0.01$, $^{***}p < 0.001$).

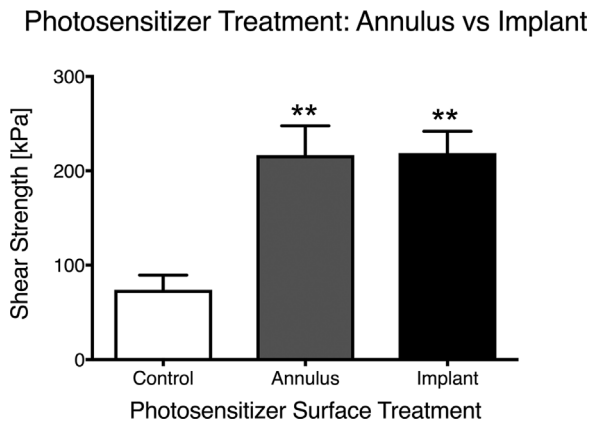

Figure 8. Photosensitizer treatment to either annulus or implant was significantly different from control ($^{**}p < 0.01$) while no significant difference was observed between treatment groups.

of 106.3 ± 6.2 kPa (1.8-fold bond strength increase over control) at 0.5 mM. AlPc also exhibited a concentration-dependent enhancement of bond strength, but produced substantially stronger bonds than CASPc. Bond strength was greater than controls for all AlPc concentrations examined, with a peak strength of 226 ± 11 kPa at 0.25 mM (3.9-fold bond strength increase over control and 1.9-fold bond strength increase over CASPc).

To verify that photochemical bonding depends only on the presence of the photosensitizer and not its point of application, bond strengths were evaluated for AlPc treatment of the annulus or the implant. Bond strengths for annulus and implant treatment were comparable at 217 ± 31 and 219 ± 23 kPa, respectively (Fig. 8). Bond strengths for both groups were significantly greater than controls ($p < 0.003$) but did not significantly differ from each other ($p > 0.999$).

Exposure Study

Effects of varying total light exposure were evaluated for fixed digestion and photosensitizer. Bond shear strength increased with increased irradiation time at a fixed irradiance (Fig. 9A) and with increased irradiance for a fixed exposure time (Fig. 9B). At a fixed irradiance of 1.58 W/cm^2, all irradiation times significantly increased bond strength above controls and the greatest bond shear strength of 203.7 ± 13.8 kPa resulted from the longest irradiation time of 10 min (Fig. 9A). Likewise, for a fixed irradiation time of 10 min, all irradiance levels significantly increased bond strength above controls and the greatest bond shear strength of 289.8 ± 24.0 kPa resulted from the highest irradiance of 3.18 W/cm^2 (Fig. 9B). Combining data from the two studies on a plot of shear strength vs. exposure (Fig. 9C) revealed an apparent power law relationship between shear strength and exposure, with a power law function of $y = 71.57 \times^{0.1577}$ ($R^2 = 0.395$). This consistent relationship indicates that the bond strength is largely determined by the total power delivered to the interface rather than the rate of delivery or beam irradiance.

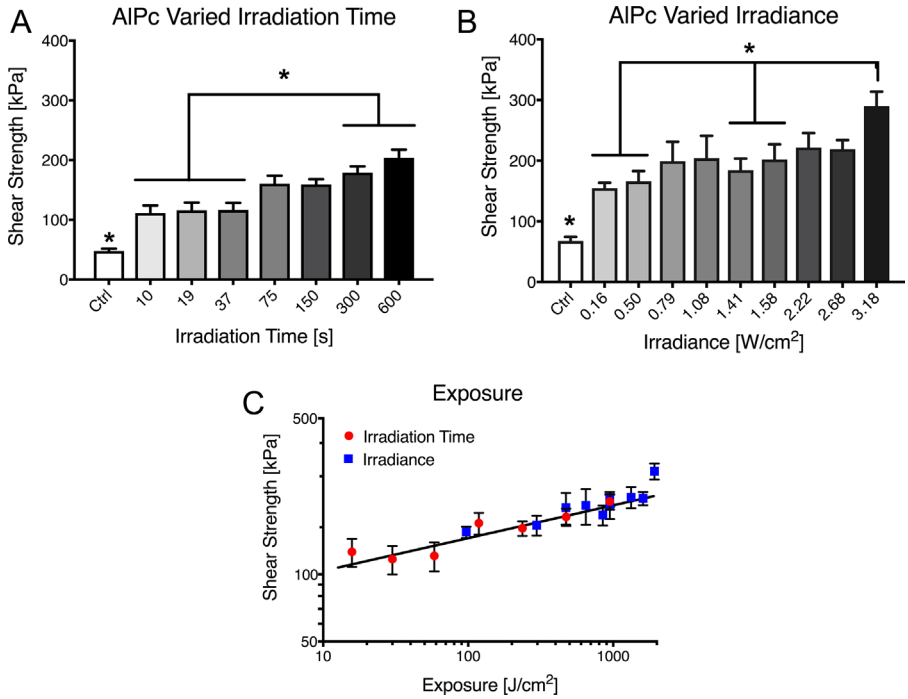

Figure 9. Bond shear strength for varied illumination conditions. Bond shear strength increased A) with increasing irradiation time for a fixed irradiance and B) with increased irradiance for a fixed irradiation time. C) Combined data plotted vs. exposure (product of irradiance and irradiation time) reveals a power law relationship between bond shear strength and exposure, $y = 71.57 \times^{0.1577}$ ($R^2 = 0.395$) ($^{*}p < 0.05$). Control groups were significantly different from all AlPc bonded groups.

In Vitro Study

Bond Durability

Photochemical treatment immediately produced a substantial increase in push-out strength (Fig. 10, 167.7 ± 16.2 vs. 75.4 ± 7.2 kPa, $p < 0.01$). Shear strength in bonded samples remained after 1-week (147.0 ± 16.4 kPa, $p > 0.999$), and remained significantly greater than controls (64 ± 12 kPa, $p < 0.01$).

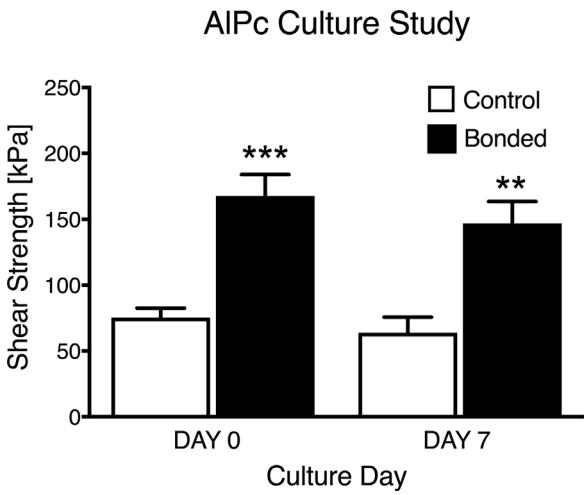

Figure 10. Bond durability 1-week post bonding. Bonded groups were significantly different from control groups on day 0 and day 7 ($^{***}p < 0.001$ and $^{**}p < 0.01$, respectively) with no significant differences between day 0 and day 7 for either Control or Bonded groups.

The bond shear strength did not differ between days 0 and 7 for either Control or Bonded groups ($p > 0.999$). Both initially and after culture, cell death extended ~100–150 μm from the cut surface as previously observed (Fig. 11),[11,27] with no qualitative difference in cell death due to treatment or culture.

DISCUSSION

Bonding between implant and native tissue is essential for successful cartilage integration in an osteochondral transplant procedure. Strong bonds that do not disrupt tissue morphology or cause major cell necrosis but allow for biomechanical load transfer and nutrient transfer are desired for complete tissue integration and repair.[2] Previous studies have shown that poor integration between native and repair tissue have led to incomplete cartilage repair, biomechanical failure, and further degeneration of the tissue.[2,8] In many cases, a gap is left between the implant and native tissue, and it is filled with fibrocartilage tissue that is mechanically inferior to hyaline cartilage.[7] Photochemical bonding provides rapid and durable integration bond strengths between the repair interface without causing adverse effects to surrounding tissue. The pushout model used to quantify the interface shear bond strength in this study provides a reasonable representation of cartilage repair models in vivo where wound/repair edges tend to be normal to the articular surface.[11,25,28,29]

By varying photosensitizer type and dose, treatment conditions and illumination parameters, this

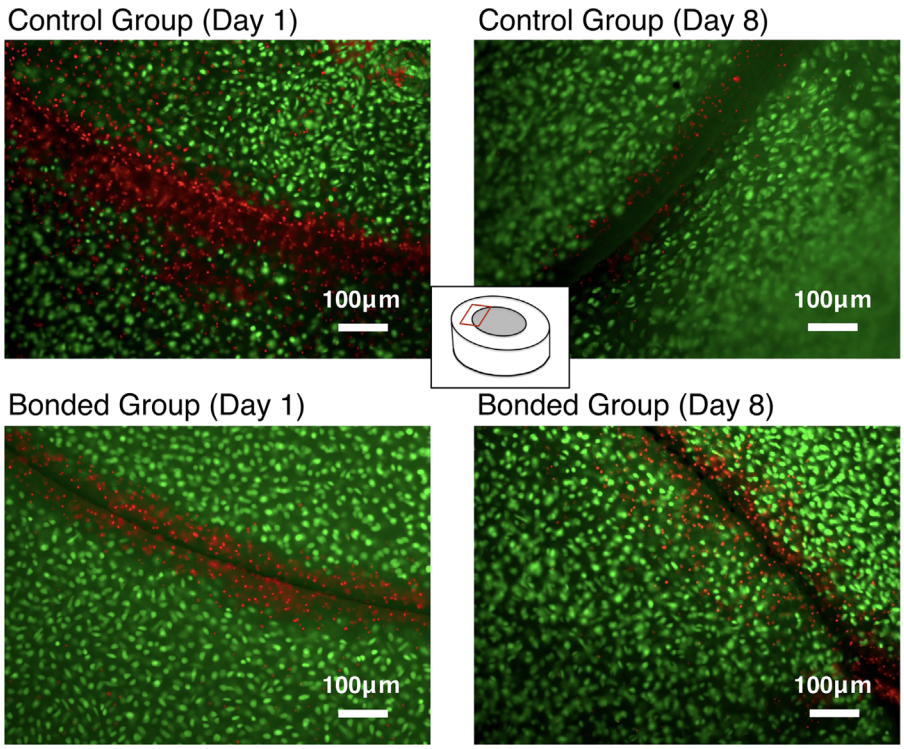

Figure 11. Live/Dead cell staining at the tissue interface in both Control and Bonded AlPc groups. Cell death extended up to ~100–150 μm from interface with no qualitative differences among groups.

study identified conditions producing shear strengths approaching 300 kPa, substantially greater than strengths previously produced by photochemical bonding (50–120 kPa), tissue transglutaminase or fibrin glues (40–50 kPa under ideal conditions) or through 2 weeks of appositional tissue culture (20–100 kPa).[11,14,30] Photochemical bonding has several advantages over other repair enhancement techniques (photo-thermal welding, sutures, fibrin/cyanoacrylate glues) in that it requires a low-powered light to activate a photosensitizer and create non-native crosslinks between proteins across the interface without affecting the surrounding area.[11,22,23,31,32] For covalent crosslinks to form across proteins/microfibrils, the distance between the two proteins needs to be smaller than the photosensitizing reagent.[33,34] Since the extracellular matrix in articular cartilage is high in proteoglycan/sGAG content, a mild enzymatic treatment is needed to allow the proteins across the two interfaces to come into intimate contact for bonding to occur.[29,35] Enzymatic digestion alone may promote integration histologically and biomechanically due to higher matrix connection in the interface area (including collagen fibers crossing the wound area) and higher cell count.[25] Photochemical crosslinking of type I collagen gels results in structural modification including a substantial reduction in pore size,[36] and similar changes might be expected from crosslinking of the compacted type II collagen near the bonded cartilage interface. Non-native crosslinks may also be beneficial in a traumatic or degenerative environment, as they are less susceptible to degeneration.[32]

The choice of photosensitizer strongly impacts the effectiveness of cartilage tissue bonding. MB, a member of the thiazine group, acts through type I (direct) processes where light-activated chromophores generate heat that in turn cause protein (substrate) polymerization and crosslinking.[37,38] MB has been used for photo-thermal tissue welding, protein polymerization, and formation of alkali-labile bonds in nucleic acids,[31,37–39] but was ineffective in inducing implant bonding in this investigation. In contrast, the phthalocyanines CASPc and AlPc act through both types I and II (oxygen-dependent) mechanisms and tend to favor primarily type II mechanisms[39,40] involving singlet oxygen generation, motivating further study of both CASPc and AlPc for photodynamic therapy.[11,20,41,42] Unlike MB, both CASPc and AlPc effectively produced cartilage bonding. Together with previous studies of collagen II gelation,[13] this strongly suggests that type II generation of singlet oxygen is required for cartilage bonding. Singlet oxygen reacts with selected amino acids, primarily histidine, to form covalent crosslinks between the amino acid and neighboring proteins at the tissue's surface.[31] While other photosensitizers such as Rose Bengal and Riboflavin are effective generators of singlet oxygen to covalently crosslink collagen rich tissues, these photosensitizers were not considered due to their low activation wavelength light that yields low light penetration into

the tissue,[17,19,32,35,43] thereby making them suboptimal for cartilage tissue bonding.

Delivery of activating light, photosensitizer concentration and (for type II processes) oxygen availability all influence the extent of interfacial bonding. The exposure delivered to the repair interface influences initial bond strength and the prospects for clinical translation when optimizing for creation of a high bond shear strength at the interface without drying out the tissue (long irradiation time) or overheating the tissue (high irradiance dose). A substantial increase in shear strength occurred as a moderate exposure was reached, and while there was a continual increase, to obtain a comparable increase in shear strength required substantial additional exposure. These results are qualitatively consistent with those of a previous study using a single-lap test and 1,8-naphthalimide photosensitizer (at three concentrations) in combination with high exposures (500–5,500 J/cm^2) to achieve bond shear strengths in the range of 40–130 kPa.[18] Another study using a single-lap test and CASPc produced peak shear strengths of 46 kPa at an exposure of 1,020 J/cm^2.[11] While strength values cannot be directly compared due to different testing modes, photosensitizer, and overall exposure, both display similar trends in shear strength where moderate irradiation times provide significant bonding and a substantial additional irradiation time is required for a comparable increase in shear strength. One possible explanation is the depletion of oxygen (due to type II processes) and its gradual replenishment through diffusion during continued irradiation time.[19,22] Similarly, for a fixed irradiance, a gradual increase of shear strength was observed with increasing photosensitizer concentration up to an intermittent concentration followed by decreased strength with further increases in concentration. This suggests that, at higher photosensitizer concentrations, the substrate is saturated and/or the oxygen is depleted at a higher rate. The observed trend is not consistent with some previous studies reporting increasing shear strengths with increasing photosensitizer concentration,[11,18,35] although the concentration ranges may not have been broad enough to capture the peak and decline that we observed. While limitation by available oxygen might be a concern in a normal synovial environment due to the relatively low oxygen tension in cartilage, in an open surgical procedure the repair surface would be exposed to ambient air and similar oxygen levels to those in this study.

These observations underscore the importance of coordinated selection of photosensitizer, concentration, and exposure conditions to optimize clinical outcome. Both AlPc and CASPc have potential as photosensitizers for integrating articular cartilage at very low concentrations and exposures that are preferred over conditions previously tested. AlPc's ability to maintain bond strength over time is essential for improved repair and integration between repair and native tissue. To improve and optimize the photochemical bonding procedure, it is important to look into other photosensitizer groups that have the potential to crosslink proteins across an interface. The porphyrin photosensitizing group could be explored for possible contenders, as it consists of photosensitizers that act primarily through type II processes and have similar chemical structures to those of the phthalocyanines.[39,44]

Strong initial crosslinks between the two surfaces are beneficial for long term repair; nevertheless, it is important that the initial bonds persist over time without affecting the cells in the vicinity. The culture study revealed that although the bonded group's bond strength decreased over 7 days, it remained significantly stronger than the control group as previously observed.[11] Cell viability remains similar between bond and control group on days 1 and 8 with most cell death attributed to initial cartilage extraction/cutting. While promising, longer term in vitro studies or in vivo evaluations are required to more confidently assess bond durability. Photochemical bonding accompanied by a mild enzymatic digestion of the repair tissue shows promise as an integration enhancement technique to osteochondral/chondral graft transplants in clinical defect repair.

AUTHORS' CONTRIBUTIONS

All authors contributed to study design. Studies were performed by ALA and analyzed by MEL and ALA. IJW performed preliminary bonding studies with AlPc. MEL and ALA participated in data interpretation and in writing the manuscript, and all authors reviewed the final manuscript.

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