Photochemical Tissue Bonding of Fibrocartilage: A Potential Tool for Enhancing Meniscus Repair

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Introduction: Current meniscal repair techniques suffer from a success rate as low as 25%, with approximately 20% needing additional surgery. Thus there remains a need for improving existing clinical repair methods. Adhesives play an important role in supplementing mechanical closure methods by keeping the tissue connected during the healing phases. Current studies primarily focus on augmenting repair using fibrin glues, despite their limited bond longevity and strength. As an alternative to tissue glues for meniscal repair, photochemical tissue bonding (PTB) produces crosslinking of native proteins across a defect through photochemical reactions. Preliminary studies demonstrated bonding via single-lap shear tests, but bond strength in other physiologically relevant modes has not been explored. This study evaluated the efficacy of PTB of meniscus tissue using both shear and tensile mechanical testing. Furthermore, the study evaluates how bond strength varies with location within the meniscus, defect orientation relative to the primary circumferential fiber bundles, and with surface functionalization. The results indicate a potential for PTB to surpass the strengths achieved with fibrin glues and warrant further studies to evaluate long-term bond durability and implementation in conjunction with standard repair techniques.

Methods: Sample Preparation: Fibrocartilage tissue was harvested from menisci and stored in phosphate buffered saline (PBS) with protease inhibitors. The peripheral region of the meniscus was isolated, frozen and sectioned to the appropriate thickness (push-out 1.74 ± 0.31mm; tensile 1.56 ± 0.33mm). Push-out test specimens (n= 16 per condition, total of 32 samples): After sectioning the fibrocartilage, annuli were produced using a 8mm biopsy punch and central defect was created with a 3.5mm biopsy punch. Oversized implants were made with a 4mm biopsy punch to ensure a press-fit. Tensile test specimens (n=6-18 per condition, total of 71 samples): A template was used to cut 4.85 ± 0.48mm wide by approximately 10mm long pieces from the sectioned peripheral slice. For most tests, samples were cut so that the treated defect was parallel to the circumferential fiber bundles. For examination of orientation effects, an additional group of samples was cut so the treated defect was across the circumferential fiber bundles. Photochemical Tissue Bonding: All samples were treated with a 15mM concentration of aluminum phtalocyanine chloride (AlPc), a pthalocyanine photosensitizer activated at approximately 675nm. For push-out samples, implants only were dipped in AlPc for 20 seconds. For tensile samples, AlPc was applied to the bonding site with a pipette and allowed to sit for 20 seconds before excess was removed. Prior to irradiation, implants were inserted into annuli, and tensile specimens were placed in a fixture that maintained contact at the bond site. Samples were then placed in a petri dish with a moist KimWipe and covered with a glass microslide to help maintain hydration. All bonding sites were irradiated with a 680nm diode laser at ~310mW for 10 minutes, then equilibrated in PBS for 20 minutes prior to mechanical testing. To explore the feasibility of surface functionalization, samples in one additional tensile test group were exposed to 1.5mg/mL of Traut’s
reagent (a thiolation agent) for 10 minutes prior to AlPc application. Untreated controls were assembled without AlPc treatment or laser irradiation. **Mechanical Testing:** Push-out tests to evaluate bond shear strength were conducted by placing the specimen in a confining ring supported from below, but with a hole slightly larger than 4mm to allow the implant to be forced out. Implants were forced out with an indenter at 0.5mm/sec. Tensile specimens were mounted vertically in clamps aligned with a micromanipulator stage, with approximately 2mm between the clamps and the bond site. Samples were distracted at 0.083mm/sec until failure. **Data Analysis:** For push-out samples, shear strength was calculated as the peak compressive force divided by the bond site surface area. For tensile test samples, tensile strength was calculated as the peak force divided by the bond cross-sectional area. As strength data were non-normal, strengths were log-transformed prior to statistical analysis. Strength data were evaluated via general linear models, considering (as appropriate) medial vs. lateral meniscus, circumferential location, sample orientation and surface functionalization. Significance was at p<0.05. Data are presented as mean ± SEM.

**Figure 1. Fibrocartilage sample preparation and testing.**

**Results:** In push-out tests, unbonded control specimens had mean shear strength of 9.88 ± 2.18kPa, reflecting friction and potential interference due to tissue deformation. Photochemically bonded samples exhibited a significantly greater shear strength of 15.4 ± 3.4kPa (Fig. 2A). Unbonded tensile specimens were unattached and were not testable, while photochemically bonded samples had a mean tensile strength of 23.3 ± 6.1kPa (Fig. 2B). Tensile strength was greater for samples from the central region than from anterior or posterior region and was greater for samples from lateral menisci than from medial menisci (Fig. 3A, 3B), perhaps reflecting variations in tissue composition (e.g., collagen content) and structure. Bonding was effective for defects both parallel and perpendicular to the circumferential collagen bundles (Fig. 3C) with no significant difference between orientations, suggesting that photochemical bonding may be suitable for a wide range of tear morphologies. Finally, functionalizing the surface with Traut's reagent prior to AlPc application significantly increased tensile bond strength to 85.9 +/- 24.5kPa (Fig. 2B), substantially exceeding the failure strength of fibrin adhesives.
Discussion: These results show that PTB enhances both the shear and tensile strength across meniscal tissue defects, producing tensile bond strengths on par with that of fibrin glues. However, as this process involves direct chemical cross-linking of proteins, the bond may be substantially more durable than those produced by biological glues. While appropriate for evaluation of cartilage bonding, excessive compliance of isolated meniscal samples makes push-out tests problematic. However, tensile tests clearly establish the efficacy of this bonding approach. Functionalizing the defect surface prior to treatment substantially increased tensile strength, suggesting that this approach may allow the bond to contribute to tissue load bearing in addition to maintaining apposition to facilitate healing. Ongoing work will focus on optimizing bonding protocols, durability in vitro, and evaluation in complex tears in conjunction with mechanical fixation techniques.

Significance: Photochemical bonding provides a promising technique to augment mechanical repair of meniscal tears and may be suitable for treatment of other fibrocartilaginous tissues.

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