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The Effect of 1-Ethyl-3-(3-Dimethylaminopropyl) Carbodiimide Suture Coating on Tendon Repair Strength and Cell Viability in A Canine Model

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Abstract

Purpose—To determine if impregnating a suture with a cross-linking agent, f 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), improved suture pull-out strength and cell viability.

Methods—Canine flexor digitorum profundus tendons were cut in canine zone D, and a single suture loop was placed in each end, with sutures soaked in either saline or in an EDC solution with a concentration of 1%, 10%, or 50%. Suture pull-out strength, stiffness, and elongation to failure was determined by pulling the loop until failure. Cytotoxicity of the EDC treatment was evaluated by suspending treated sutures over cultured tenocytes.

Results—Mechanical properties for the EDC-treated side were improved over controls when treated with the 10% and 50% EDC solutions. The ratio of dead to live cells was significantly increased at all distances from the suture for the 50%-EDC-treated group.

Conclusions—Suture treated with a 10% EDC solution provided the best combination of mechanical reinforcement and limited toxicity.

Clinical Relevance—Sutures so treated may improve the ability of a tendon repair to sustain early mobilization.

Keywords

cross-linking; repair; suture; tendon

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INTRODUCTION

Early mobilization after flexor tendon repair reduces adhesion formation^{1, 2} improves tendon gliding,³ and enhances healing^{4-8, 9} with success partly dependent on repair strength.^{10, 11} Stronger repairs take better advantage of the strength of the suture material and allow more aggressive rehabilitation¹²⁻¹⁵. However, these alternatives complicate the surgical procedure, may introduce local ischemia that disrupts tendon healing, and create protuberances that increase the work of flexion. The weak link then shifts to the suture-tendon interface, which fail by suture pull-out. We speculated that if the load bearing strength of the tissue surrounding the suture could be increased, an additional advancement in tendon repair strength might be realized.

Zhao et al.¹⁶ showed that direct injection of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) or cyanoacrylate reinforced the tendon-suture interface and improved the pull-out failure strength. EDC is a cross-linking agent that covalently bonds collagen molecules and stiffens the material that comes into contact with it. This creates an eyelet of stiffer material that potentially resists cut-through by the suture. Clinically relevant aspects of the use of EDC for this application, including delivery method, repair techniques, and biological effects on cells, must be studied before advancing this technique.

The objective of this study was to evaluate the effectiveness of using the suture itself as a delivery vehicle for the EDC and to optimize the treatment by evaluating the effect of EDC concentration on mechanical and biological outcomes. The hypotheses were that sutures treated with EDC would have superior retention strength compared to untreated sutures and that cytotoxicity effects would be proportional to EDC concentration. Ideally, optimizing this approach to tendon reinforcement would result in a technique that could be applied surgically with negligible impact to procedure time and markedly increase repair strength without causing toxicity for surrounding cells.

MATERIALS AND METHODS

Suture Treatment with EDC

Three parameters were thought to govern the effectiveness of EDC reinforcement of the tendon tissue with a suture delivery vehicle: suture material, EDC concentration, and soak time. For this study, a 4-0 braided poly-blend suture (FiberWire: Arthrex, Naples, FL) was selected for use based on its reported advantageous properties as compared to other common suture materials¹⁷. Three different concentrations of EDC (Sigma Chemical Co., St. Louis, MO), diluted with saline, were used: 1%, 10% and 50%. A control group treated sutures with a 0.9% saline solution. An effective soak time was unknown. The soaking time needs to be great enough for the suture to absorb a sufficient amount of solution but should not be so long as to adversely affect overall procedure time if applied clinically. Preliminary testing of tendon repairs using sutures treated with an EDC solution showed that 10 minutes of soaking was sufficient to create a measureable increase in repair strength. Therefore, for this study, FiberWire suture was soaked in one of the 4 solutions for 10 minutes.

Tendon Repair Model

A total of 36 flexor digitorum profundus (FDP) tendons were harvested from the 2nd to 5th digits of the forepaws of 5 dogs killed for other studies, which had all been approved by our institutional animal care and use committee. Prior studies were not believed to have affected the mechanical or structural properties of the tendons. After harvest, tendons were immediately frozen at -80°C until use. Twelve tendons were assigned in the order harvested to each of the 3 groups of the 3 different EDC concentrations, testing the lowest concentration group first and progressing to the highest concentration group.

Each tendon was bisected in canine Zone D, functionally comparable to human zone 2^{18, 19}. One transected end of the tendon was sutured with an EDC-treated suture, and the other transected end was sutured with saline-soaked suture as a control. A simplified experimental repair condition was created using a modified Kessler suture penetrating 5 mm into the tendon end, measured with a ruler and forming a loop approximately 40-mm in perimeter (Figure 1). This suture technique was selected because this, along with a circumferential continuous running suture, is one of the most common techniques applied for zone II tendon repair²⁰.

Mechanical Testing

Tendons were connected to a servohydraulic testing machine (MiniBionix 858; MTS Systems, Eden Prairie, MN) using a clamp to secure the tendon, and a rod captured the suture loop (Figure 1). The suture was distracted at a constant rate of 20 mm/min until suture pull-out. Tensile force and displacement was collected at a sample rate of 20 Hz. The peak force was identified to characterize the pull-out strength. Stiffness and elongation to failure were also assessed.

Cell Viability Testing

FDP tendons were harvested immediately from the front limbs of 3 dogs, again used for other approved studies. Tendons were washed in PBS and collectively minced with a sterile scalpel into 1 mm² or smaller pieces and enzymatically digested at 37°C with 0.1% type II collagenase (10 ml per 1 g tissue) (Roche Diagnostics: Basel, Switzerland) for 24 hr. After digestion the slurry was passed through a 40-µm nylon filter (BD Biosciences, San Jose, CA) and centrifuged at 1200 rpm for 5 min. The cell pellet was washed twice, suspended in standard medium and plated onto 100 mm culture dish.

Cultured tenocytes were maintained in high-glucose Dulbecco Modified Eagle Media (Sigma- Aldrich, St. Louis, MO), 10% fetal bovine serum, and 1% antibiotics (Antibiotic-Antimycotic, GIBCO, Grand Island, NY), and incubated at 37°C with 5% CO₂. The medium was changed every 3 or 4 days until reaching confluence. Cells were plated on sterile glass cover slips (Fisher Scientific: Pittsburgh, PA) at a concentration of 2×10^4 cells per slip.

Treated FiberWire suture (control, 1% EDC, 10% EDC, and 50% EDC) was cut into 25-mm lengths. Treated sutures were connected to stainless steel nuts with a mass of approximately 1 gram at each end and then placed into the 35 mm diameter dish, exposing the cultured tenocytes to them for 24 hours (Figure 2). Four specimens of each treatment type were

tested. The sutures and weights were removed and viability was assessed using a LIVE/DEAD cell viability/cytotoxicity kit (Molecular Probes, Eugene, Oregon) following the kit protocol. Tenocytes which had been cultured on sterile glass cover slips were incubated in 1 mL of 2 μ M calcein AM/ 4 μ M ethidium homodimer-1 solution at 37°C with 5% CO₂ for 30 minutes. Cells were allowed to recover for 30 minutes prior to assessment. The cultured cover slips were then inverted and mounted onto a microscope slide. The cover slips were examined with a confocal microscope (LSM510: Zeiss, Germany), and digital photographs were taken at 100 $\times$ magnification near the center of the suture length.

Live and dead cells were identified and counted using Image J software (National Institutes of Health, Bethesda, MD). Each digital photograph was 920 μ m in both height and width. An array of photographs was collected with 3 frames along the suture length (and including the region immediately outside the suture boundary) and 5 frames distant from this line for a total of 15 frames.

The number of live (stained green) and dead (stained red) cells was counted in frames down the middle column. Data for each frame were expressed as a ratio of dead to total cells.

Statistical Analysis

Means and standard deviations of pull-out strength, stiffness, and elongation to failure were assessed. The Student t-test was used to compare pull-out strength and stiffness with and without suture EDC treatment. Based on the mechanical testing of EDC-treated tendons in a previous study¹⁶, a sample size of 12 tendons per treatment group provided 80% power to detect a difference of in tensile strength of 5.75 N. Cell ratio was compared between treatment groups by performing a one-way ANOVA for data at each of the 5 panel positions, followed by the Tukey Studentized range HSD post hoc test. In all cases, $P < 0.05$ was considered significant.

RESULTS

Biomechanical Performance

All tendons failed by the suture cutting through the tendon. In the 10% and 50% EDC groups, the EDC-treated suture repair was significantly stronger than the control ($P < 0.001$ and $P = 0.006$, respectively) (Figure 3A). There were no significant differences in stiffness between the EDC-treated sutures and the control in any group (Figure 3B). Elongation to failure was significantly larger on the EDC-treated side compared to the control side for only the 50% EDC treatment (P -values for the 1%, 10%, and 50% EDC were 0.24, 0.13 and 0.002, respectively), (Figure 3C).

Cell Viability Assessment

Example fields of each of the treated sutures are shown in Figure 4. Cultures in contact with all sutures, regardless of treatment, showed some dead cells lying immediately underneath the suture. Cell ratio was significantly higher in the 50% EDC-treated group at all distances from the sutures (P -values were $P < 0.001$ for all distances). The average dead cell count over the field for each treatment is shown in Figure 5.

DISCUSSION

The results of this study clearly show that the suture can serve as a vehicle for delivery of a stiffening agent such as EDC to the tendon tissue and that this agent can improve the suture fixation strength. Based on the mechanical test data, it is clear that an EDC concentration somewhere between 1% and 10% is required to significantly increase the repair strength in this model. Treating the suture with a 1% EDC solution did not significantly increase the strength, while increasing the EDC concentration to 10% significantly increased the mean repair strength by over 40%. With the effectiveness of the treatment demonstrated, it would be reasonable to treat the suture with other cross-linking agents that could further improve repair strength, be less toxic to cells, or both.

EDC-treated sutures did not significantly affect the repair stiffness. This may indicate that the amount of EDC being delivered is not enough to stiffen the entire tendon at the lacerated ends; rather, only a small amount is stiffened at the suture interface, acting as an eyelet does for a lace.

With regard to the use of EDC as an agent to improve suture holding power, both the strength advantage and threshold for substantial impact to cell viability were achieved with sutures treated with the 10% EDC solution. There was no significant difference in cell ratio in the field surrounding the suture between saline-treated sutures and those treated with 10% EDC. In contrast, the cell ratio was significantly increased for the 50% EDC-treated suture group at all distances from the suture. Previous studies on use of EDC as a cross-linking agent have reported a low cytotoxic effect when used in low doses^{21, 22}. However these studies evaluated the cytotoxicity of the cross-linked end product, whereas in the current study we directly evaluated cytotoxicity of EDC. Another study which evaluated viability of human lymphoma cells when directly exposed to EDC in culture demonstrated that cell viability was inversely proportional to dose, with viability at low doses (~1.6 μ M) being near 100% after 20 hours²³, a finding similar to that of the current study. Since the 10% EDC concentration serves as the point which maximizes the strength advantage and is a threshold for adverse effects on cell viability, it may be reasonable to choose this treatment as a candidate for EDC-reinforced suture repair.

There are several limitations to this study. Strength results are based on a single pull to failure, and no cyclic loading was applied. While based on the data obtained it is reasonable to expect be that the EDC-treated suture would maintain an advantage of untreated suture, the magnitude of the difference may be tempered by the repeated loading experienced in vivo. This study evaluated the strength at time-zero only; and aside from some inferences that can be made from the cell viability testing performed, no conclusions can be drawn about the effect this treatment might have on the repair strength in the long term. Also this study evaluated only one type of suture material using only one repair technique. Different suture materials may differ in their EDC absorption and elution response, and different suture-tissue interactions resulting from different repair techniques may impact the mechanical performance.

Zhao et al previously established the concept of using EDC to reinforce the tissue around a suture to improve the bearing strength ¹⁶. The current study demonstrated a practical method of delivering the EDC to the tendon and showed that there was a limit to the EDC concentration that both provided increased repair strength and limited cytotoxicity.

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Figure 1. Tendons sutured with a modified Kessler loop (A) were mechanically tested using a servohydraulic test system (B).

Figure 2.
Experiment to evaluate tenocyte viability in the presence of an EDC-treated suture in culture.

Figure 3. Comparison between suture treatments of A) mean repair strength, B) mean repair stiffness, C) mean elongation to failure. Mean $\pm$ standard deviation are printed on the bars and whiskers visually represent standard deviations. The * indicates significant differences, $P < 0.05$.

Figure 4. LIVE/DEAD staining of tenocytes extending approximately 4.5 mm from the treated sutures in culture after 24 hour exposure. Live cells are stained green; dead cells, red.

Figure 5.

Dead to total cell ratio as a function of distance from suture evaluated for each suture treatment. Dotted lines represent $\pm$ one standard deviation.