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The effect of tendon surface treatment on cell attachment for potential enhancement of tendon graft healing: An ex vivo model

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ABSTRACT

For both tendon allografts and autografts, the surface, initially optimized for gliding, may not be ideal to facilitate tissue integration for graft healing to host tendon or bone. As a prelude to studying tendon–bone integration, we investigated the effect of surface treatments with trypsin or mechanical abrasion on cell attachment to the tendon surface in a canine ex vivo intrasynovial tendon tissue culture model. Intrasynovial tendon allograft surfaces were seeded with cells after the following treatments: (1) no treatment, (2) mechanical abrasion, (3) trypsin, and (4) abrasion and trypsin. The area covered by cells was determined using confocal laser microscopy at one and two weeks. Results were compared to untreated extrasynovial tendon. Additional tendons were characterized with scanning electron microscopy. Tendons with trypsin treatment had significantly more surface coverage with cells than the other groups, after both one and two weeks of culture. In terms of the cellular shape and size, cells on tendons with trypsin treatment spread more and were more polygonal in shape, whereas tendons with mechanical abrasion with/without trypsin treatment contained smaller, more spindle-like cells. Surface roughening can affect cell behavior with topographical stimulation. Trypsin surface digestion exposes a mesh-like structure on the tendon surface, which could enhance cell adherence and, possibly, tendon/bone healing.

1. Introduction

Tendon allografts are used in many musculoskeletal reconstructive procedures, including knee ligament repair [1,2], shoulder rotator cuff repair [3,4] and hand flexor tendon reconstruction [5,6]. The advantages of allografts include ready availability, absence of donor site morbidity, and reduced operating time [7]. In addition, allografts are especially useful in intrasynovial applications, such as in the knee, shoulder, or hand, because intrasynovial autografts are usually not available. Allografts can also be used in an extrasynovial environment, such as the proximal attachment of a tendon allograft to the host muscle-tendon unit. However, while allograft remodeling and incorporation into bone and host tendon are similar qualitatively to autografts [8], they progress much more slowly than in autografts, which can compromise results [9–13]. Enhancement of allograft incorporation could improve clinical outcomes.

In addition to basic issues of host–graft tissue integration, tendon/bone healing is also problematic because of the difficulty of restoring the normal fibrocartilaginous transitional zone of the normal tendon/bone interface [13–16]. This is even more difficult in allograft/bone healing [9], since allografts do not harbor live cells and can elicit an immunogenic response [11]. Thus, when allografts are used, the tendon/bone interface healing mainly relies on the cells from surrounding tissue that migrate into the graft for tissue regeneration. Finally, for both tendon allografts and autografts, a surface initially optimized for gliding may not be ideal to facilitate tissue integration. In addition to other compounds on the surface of gliding tendons, lubricin, a lubricating glycoprotein present on the tendon surface [17,18], is known to inhibit cellular adhesion [19]. When the tendon surface is modified by trypsin digestion, a procedure which removes lubricin and other proteins, the tendon surface becomes visibly rougher, and friction increases [18]. Since lubricin also has anti-adhesive effects, removing lubricin from the graft surface might improve graft incorporation. The roughened surface might also provide a greater surface area for cellular adhesion.

With regard to surface topography, the effects of roughness on cellular behavior have been examined in various artificial materials, such as metals and polymers [20,21]. However, little is known about the effect of roughening of native tissue on the topography of cell attachment.

Our overall goal is to enhance allograft tendon–bone healing. As noted above, one aspect of this process is cellular migration from the living bone host into the recipient allograft tendon. As a prelude to definitive in vivo studies, we wished to study methods that might make the allograft tendon surface more hospitable to migrating

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cells. Therefore, the purpose of this study was to investigate the effect of roughening the tendon surface, either chemically (with trypsin digestion), physically (by mechanical abrasion), or both, on bone marrow stromal cell attachment to the tendon surface in a canine ex vivo intrasynovial tendon model.

2. Materials and methods

Ninety canine FDP tendons of the 2nd, 3rd, 4th and 5th digits from four paws of six dogs were used. FDP tendons and bone marrow were harvested from mixed-breed dogs, which were euthanized for other IACUC approved studies that did not involve or affect the tendons. The tendons were prepared as they would be for allograft use, by applying a repetitive freeze–thawing procedure that destroys the native cells [22], followed by lyophilization, a common procedure used to facilitate allograft storage. The tendons were divided into five groups (Table 1). The tendons used for confocal microscopy were further subdivided into two subgroups, differentiated by culture with bone marrow stromal cells for either one or two weeks. To minimize post mortem effects, the bone marrow cells were harvested while the dogs were anesthetized and tendons were harvested immediately after euthanasia.

2.1. Bone marrow stromal cells (BMSCs) harvest and culture

From each dog, 8.0 mL of tibial bone marrow were aspirated into a 20 mL syringe containing 2.0 mL of heparin solution. The aspirates were centrifuged at 1500 rpm for 5 min at room temperature to remove heparin, and then divided into three equal aliquots. Each aliquot was placed in a 100-mm culture dish with 10 mL of minimal essential medium (MEM) with Earle's salts (GIBCO, Grand Island, NY, USA), 10% fetal calf serum, and 1% antibiotics (Antibiotic–Antimycotic, GIBCO, Grand Island, NY, USA). The bone marrow cells were incubated at 37 °C with 5% CO_2 and 95% air at 100% humidity. After 3 days, the medium containing floating cells was removed and new medium was added to the remaining adherent cells. These adherent cells were defined as BMSCs [23]. The medium was changed every 3 days. After the adherent cells reached confluence, they were released with trypsin from porcine pancreas (Sigma, T-0303, 0.25%) and seeded in new dishes.

2.2. Tendon harvesting, decellularization and lyophilization

Bilateral fore and hind paws were shaved, scrubbed with povidone iodine, and sterilely draped. The 2nd through 5th digit FDP tendons were harvested. After harvest, the tendons were immersed immediately in liquid nitrogen for 1 min and then thawed in warmed saline solution at 37 °C for 5 min. This procedure was repeated five times. This procedure, which kills over 97% of the cells in tendon or ligament tissue [22], also helps to reduce the ability of the graft to incite an immune response in the host. After completing the freeze–thaw cycles, the tendons were frozen in liquid nitrogen and placed into a custom-made lyophilizer for 24 h. Individual tendons were sealed in a plastic bag and stored at room temperature. All tendons were transferred to special plastic bags for gas sterilization one week before use. The tendons were rehydrated in a 0.9% NaCl bath in a closed, sterilized container for 24 h in a 37 °C incubator immediately prior to use.

2.3. Surface treatment

In groups B and D, before rehydration, the lyophilized tendons were abraded 15 times with 180 grit abrasive cloths (3 M, St. Paul, MN, USA) in parallel direction to the tendon. After rehydration, tendons in groups C and D were treated with 0.25% EDTA-trypsin solution for 2 h at 37 °C with 5% CO_2 and 95% air at 100% humidity [18].

2.4. BMSCs seeding of decellularized tendons and culture

Each tendon was rehydrated with saline for 24 h at 37 °C, cut into a 1-cm long portion that was just proximal to distal vincula attachment, and then immersed in minimal essential medium with Earle's salts 10% fetal calf serum, and 1% antibiotics in coculture with BMSCs at a density of 1×10^5 cells/mL, and incubated at 37 °C in a humidified tissue culture chamber with 5% CO_2 for one or two weeks. Culture medium was changed every 3 days. In clinical scenario, the tendon graft buried within bone tunnel for distal tendon/bone attachment repair is roughly 1 cm in length. Thus we used 1-cm segments for this study.

2.5. Observation of cell attachment

A confocal laser microscope (Zeiss 5 Live, Carl Zeiss, Thornwood, NY, USA) was used to assess cell distribution and estimate the cell count on the tendon surface. At the conclusion of the one or two week culture period, the culture medium was replaced with a new medium containing 5 μM calcein AM and 5 μM ethidium homodimer-1 (LIVE/DEAD Viability/Cytotoxicity Kit) (Molecular Probes, Eugene, OR, USA). After 30 min the tendons were removed from the medium and washed with PBS. Four surface photos in each tendon (using C-Apochromat 10×/0.45 W objective lens and 1.0 magnification zoom factor) were captured using the confocal microscope (excitation wavelength: 488 nm; emission wavelength: 505–550 nm for live cells, 568 nm and 635 nm for dead cells, respectively) as shown in Fig. 1. The percentage of the area covered by live cells was determined using image processing software (Zeiss KS400; Carl Zeiss, Thornwood, NY, USA). The mean percentage of four photos was calculated. We initially tried to detach all cells from tendon surfaces by trypsin treatment for cell quantification such as an Alamar Blue assay. However, we could not remove all the cells from the tendon surface by this method. Therefore, we used the mean percentage of cell coverage on the tendon surface to compare cell attachment between the groups. All cell counts were done by an individual who was blinded as to which group the tendons belonged to.

2.6. The strength of cell attachment

After confocal microscopy, all tendons were treated immediately with 0.25% EDTA-trypsin solution for 10 min at 37 °C with 5%

Table 1
Study design (grouping, treatment, measurement and sample size).

Group	Tendon origin	Treatment		Number of tendons used for confocal microscopy	Number of tendons used for SEM
		Mechanical abrasion	Trypsin		
A	Intrasynovial tendon	–	–	16	2
B	Intrasynovial tendon	+	–	16	2
C	Intrasynovial tendon	–	+	16	2
D	Intrasynovial tendon	+	+	16	2
E	Extrasynovial tendon	–	–	16	2