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REVIEW ARTICLES OF TOPICS

Cell migration after synovium graft interposition at tendon repair site

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Abstract
Background We have recently reported that interpositional synovium grafts from tendon sheath have a potential to accelerate tendon healing when implanted at the repair site. The purpose of this study was to investigate the effect of orientation of the synovium after synovium graft transplantation, by comparing the ability of cells from the visceral and parietal surfaces to migrate into the tendon in a canine tissue culture model.
Methods The synovium graft was placed within a complete tendon laceration, with either the visceral or parietal surface facing the proximal end of the lacerated tendon. The number of migrating cells was quantified by a cell migration assay. Qualitative immunohistochemistry and confocal laser microscopy were also used at day 10.
Results Many labeled synovial cells were observed within the tendon to which the visceral surface of the synovium graft was facing. Migrated cells were also observed on the parietal side, but there were fewer cells compared to visceral surface cells. Migrating cells all expressed α-smooth muscle actin.
Conclusion We found that graft orientation affected cell migration. Whether this finding has clinical significance awaits in vivo study.

Keywords Tendon healing · Synovium graft · Synovial cells · Cell migration

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Background

The treatment of lacerations of the flexor tendons within the finger continues to pose challenges to hand surgeons [4]. One of the major challenges relates to tendon healing, a relatively slow process due to the inherent hypocellular and hypovascular state of the flexor tendons in the fingers [5, 9, 33, 37]. Although advances in the treatment of flexor tendon injuries have led to improved rehabilitation in patients [21, 34, 39, 40], only small improvements have been made in functional outcomes [23]. Therefore, techniques that modulate the biology of tendon healing at the repair site have been a recent research focus [3, 7, 18, 30, 35, 36, 38].

We have recently reported that tendon sheath, interposed between the cut tendon ends at the time of tendon repair, had the potential to accelerate tendon healing in vitro [13]. From the clinical point of view, one of the advantages of synovium graft transplantation is that synovium can be directly harvested from the injury site. In addition, there is no need to harvest and culture cells from other tissues. However, synovium is not uniform, and it was unclear in our previous study whether synovial cell migration was affected by the polarity of the tissue, i.e., the visceral versus the parietal surface. The purpose of this study was to investigate the polarity of synovial cells after synovium graft transplantation by comparing the ability of inside and outside cells to migrate into the tendon.

Materials and Methods

Tissue Harvest

The second to fifth digit flexor digitorum profundus (FDP) tendons were harvested from dogs under sterile conditions

after sacrifice for other IACUC-approved studies. For orientation purposes, the distal edge of the A2 pulley was marked prior to excision. Each tendon was transected 5 mm distal to the previously marked level and shortened by cutting to a standardized length of 30 mm, with the repair site located centrally. There are two collagen bundles at this section of the FDP tendon. The synovium was harvested from proximal part of proximal tendon sheath before FDP tendon harvest (Fig. 1). The harvested tissues were immediately stored in minimal essential medium (MEM) (GIBCO, Grand Island, NY), 10 % fetal bovine serum (FBS) (Mediatech Inc, Manassas, VA), and 1 % antibiotic–antimycotic (GIBCO, Grand Island, NY).

Tendon Repair and Tissue Culture

The cells in the synovium were labeled with CellTracker™ Red CMPTS (Molecular Probes, Inc, Eugene, OR), following the company's instructions. Briefly, staining medium was prepared by adding 2 μL of 1 mM stock solution to 2 ml of serum-free MEM. The synovium graft was incubated in the staining medium for 45 min at 37 °C. After draining off the staining medium, the synovium graft was incubated in the serum-free MEM for 30 min at 37 °C and washed with phosphate-buffered saline (PBS) (GIBCO, Grand Island, NY). The synovium graft was randomly placed between lacerated tendon ends, with the visceral surface of the synovium facing either the distal or proximal cut surface of the lacerated tendon. The tendon ends were sutured with two simple sutures of 6-0 Prolene (Ethicon, Somerville NJ) with or without an interposed synovium graft. The repaired tendons were mounted on a wire mesh designed to maintain the tendons in a straight position without any tension. The mesh was then placed into a 100-mm Petri dish with MEM supplemented with 10 % FBS and 1 % antibiotic–antimycotic solution and incubated at 37 °C in a 5 % CO_2 humidified incubator for 10 days. Culture medium was changed every 3 days. The repaired tendons were removed from culture dish at days 1, 3, 7, and 10 after surgery for H&E staining and fluorescent microscopic examination.

Histological Analysis and Immunohistochemistry

The repaired tendons were fixed in 4 % paraformaldehyde at 4 °C for 24 h. The samples were embedded in Tissue-Tek O.C.T. Compound (Sakura Finetek, Inc, CA). Sections of 15 μm were cut in the sagittal plane using a Leica microtome (Leica Microsystems, Wetzlar, Germany). The sections were mounted on glass slides. For histological analysis, the sections were stained with hematoxylin and eosin and examined for cell distribution in the tendon ends. For immunohistochemistry, the sections were blocked with 2 % bovine serum albumin/0.1 % phosphate-buffered saline with Tween 20 for 1 h before incubation with rabbit polyclonal anti-human α-smooth muscle actin (α-SMA) antibody (Abcam, Inc, Cambridge, MA) 4 °C overnight. Subsequently, sections were stained with Alexa Fluor 488 goat anti-rabbit IgG secondary antibody (Invitrogen, Carlsbad, CA) at room temperature for 1 h. Then, sections were mounted with the Vectashield HardSet Mounting Medium with 4′,6-diamidino-2-phenylindole (DAPI) (Vector Laboratories, Burlingame, CA). Sections were viewed with a confocal microscope (LMS510; Zeiss Inc., Oberkochen, Germany). CellTracker™ Red CMPTS was excited with helium–neon 543 nm laser line, Alexa Fluor 488 with argon-ion 488 nm laser line, and DAPI with ultraviolet light. Images were generated with LMS510 software.

Cell Migration Assay

PureCol bovine dermal collagen (3.1 mg/ml, Inamed Corp., Fremont, CA) was prepared according to the company's instructions. Briefly, 9.75 ml of sterile, chilled PureCol collagen was mixed with 3 ml of sterile 5× MEM, 52.5 μl of sterile 1.75 M NaOH, and 2.25 ml distilled H_2O which adjusted the pH to 7.4±0.2, yielding 15 ml of temporary collagen/MEM solution on ice. Fifteen milliliters of MEM supplemented with 20 % FBS and 2 % antibiotic–antimycotic solution was added to the collagen/MEM solution. A 1.5-ml aliquot of the solution was added to six-well dish and incubated at 37 °C for 1 h. Synovium was cut into 5 mm square sections and put on a cell strainer with the visceral or

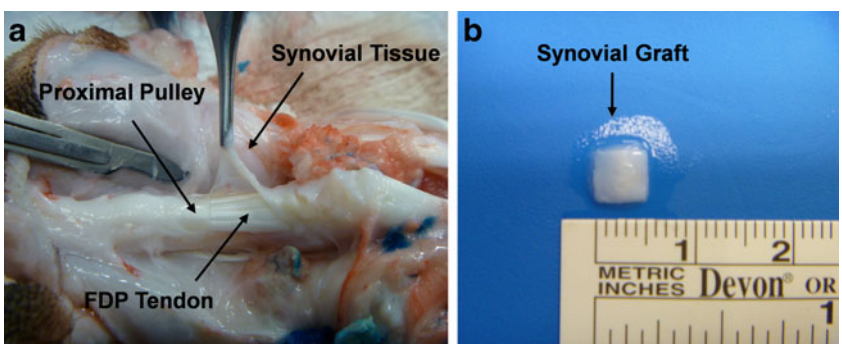

Fig. 1 Dissection of dog FDP tendon and synovium. **a** Harvest of synovium. **b** Synovium graft

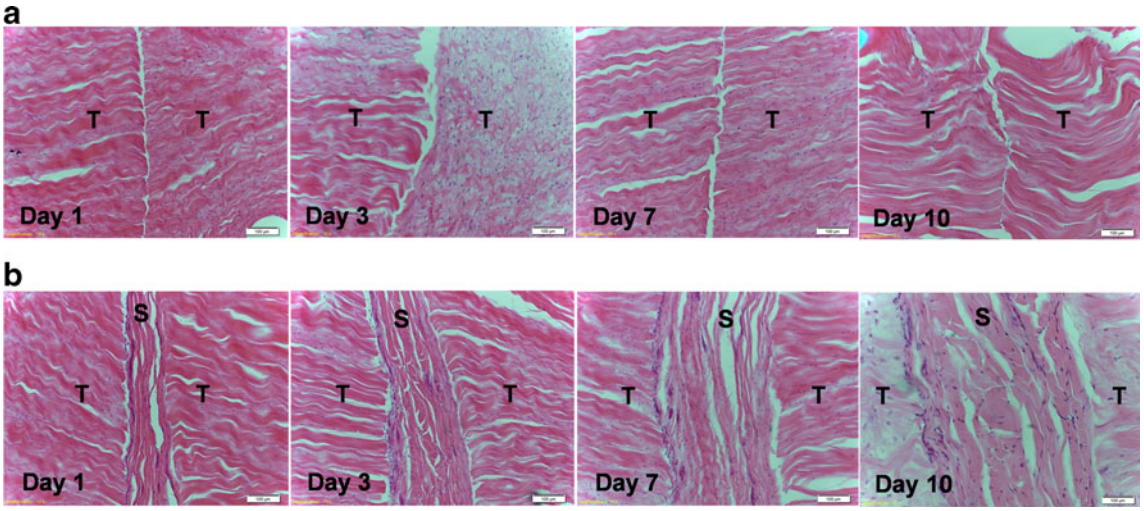

Fig. 2 Histology of suture site at each time point (days 1, 3, 7, and 10) without (**a**) or with (**b**) synovium graft (×10 objective magnification, *scale bar*, 100 μm). Left side is distal end and right side is proximal end. *T*, tendon end; *S*, synovium graft

parietal surface facing to the surface of the cell strainer. The synovium piece was sutured to each corner of the cell strainer for fixation. The cell strainer was put on the gel directly after the gel was covered with 3 ml MEM supplemented with 10 % FBS, 1 % antibiotic–antimycotic solution, and incubated at 37 °C in a 5 % CO_2 humidified incubator for 10 days to allow cells to migrate through the cell strainer into the collagen gel. Culture medium was changed every 3 days. At day 1, 3, 7, or 10 after surgery, the collagen gel with migrated cells was collected in a centrifuge tube and incubated with Collagenase D (Roche Diagnostics, Germany) at 37 °C for 1 h. After digestion, the cells were spun down and the supernatant was removed. The cells were resuspended with 200 μl buffer in DNA Quantity

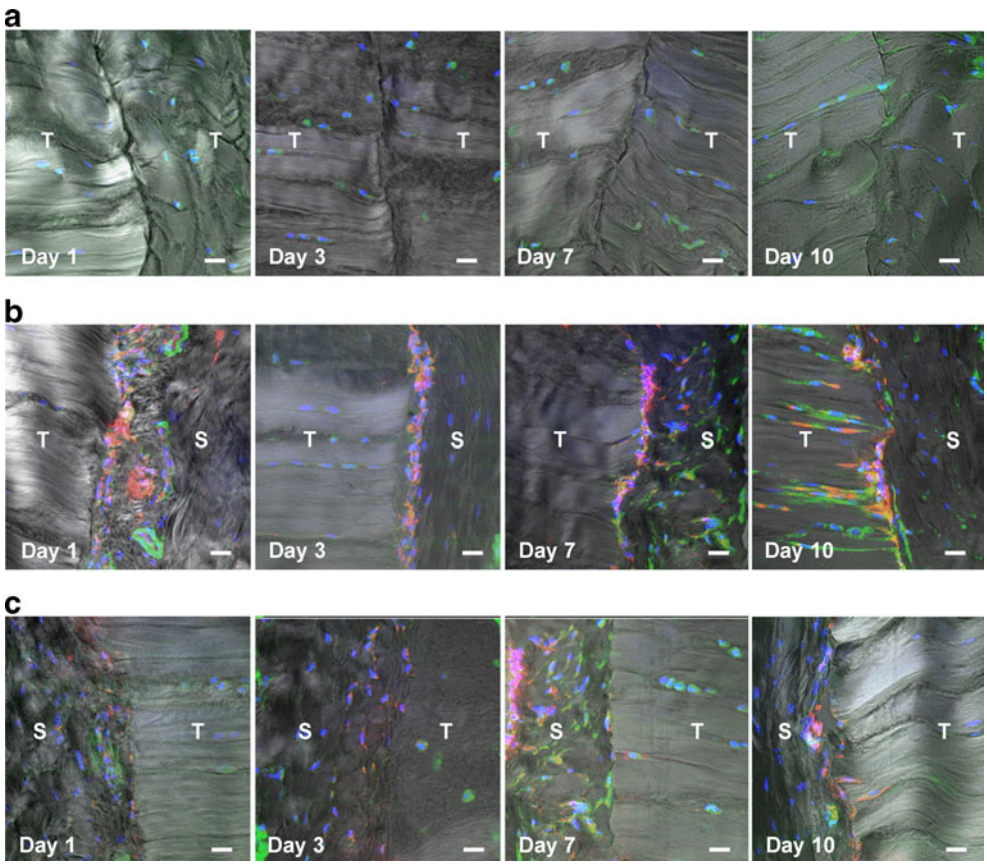

Fig. 3 Confocal laser microscopic images of suture site at each time point, days 1, 3, 7, and 10 (×40 objective magnification, *scale bar* 20 μm). In each time point, **a** top row shows control without synovium graft. **b** Second row shows repair site in which visceral surface of the synovium facing to distal end of tendon. **c** Third row shows repair site in which parietal surface of the synovium facing to proximal end of tendon. *Red color* indicates synovial cells stained with CellTracker. *Green color* indicates α-SMA staining. *Blue color* indicates DAPI staining. *T*, tendon end; *S*, synovium graft

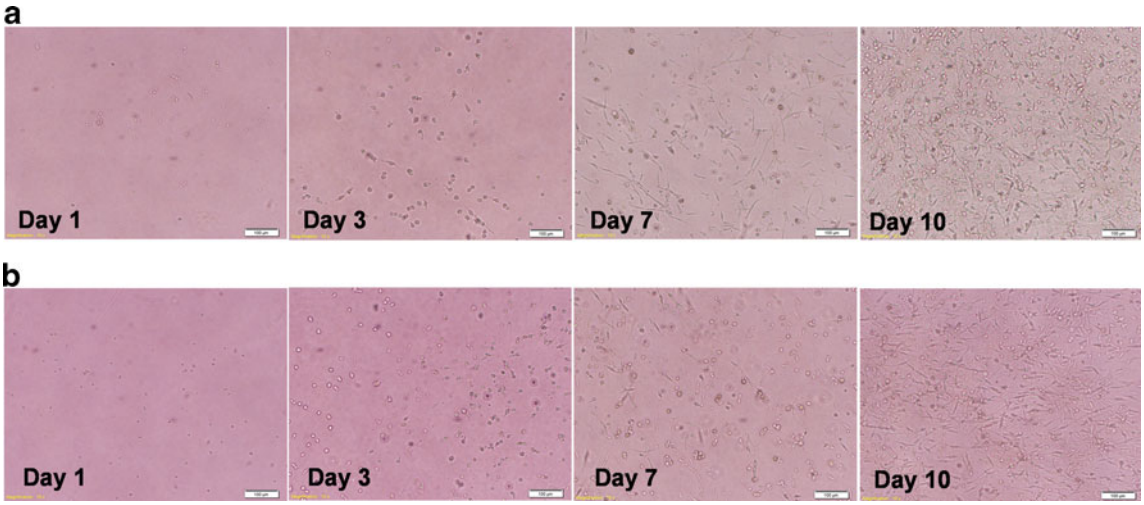

Fig. 4 Microscopic images of migrated cells at each time point, days 1, 3, 7, and 10 (×10 objective magnification, *scale bar* 100 μm). **a** Visceral surface of synovium graft is facing to the bottom. **b** Parietal surface of synovium graft is facing to the bottom

Assay (B-Bridge International, Inc, CA) and homogenized by sonication. A PicoGreen dsDNA Quantitation Kit (Molecular Probes, Inc, Eugene, OR) was used to quantify DNA following the company's instruction using FLUO Star Galaxy (BMG LABTECH GmbH, Germany).

Statistical Analysis

The results of the cell migration assay were analyzed by unpaired *t* test. All results were expressed as means, with the standard deviation in parentheses. A *P* value of 0.05 or less was chosen to indicate significant difference between groups.

Results

Histological Analysis

Viable cells were observed in synovium grafts at each time point in tissue culture with qualitative microscopy. There were no necrotic changes around the lacerated tendon ends in any group. Partial healing was found in the tendons repaired with synovium graft interposition at day 10 (Fig. 2).

Immunohistochemistry

At day 1 and 3, no migrating cells were found on either side in either group. In the synovium graft group, synovial cells were migrating into the tendon on both sides at day 7. At day 10, many migrated cells were observed, with more cells within the tendon end facing the visceral synovial surface. Almost all of migrating cells were spindle shaped and expressed α-SMA (Fig. 3).

Cell Migration Assay

The number of cells that migrated into the collagen gel gradually increased as the days passed in both groups (Fig. 4). The amount of DNA continued to increase and peaked at day 10 in both groups. Notably, the amount of DNA increased exponentially from day 7 to day 10, which means that the cells proliferate after migration into the collagen gel. At days 7 and 10, the amount of the visceral cell DNA was statistically higher than that of the parietal cells (Fig. 5).

Discussion

The concept of extrinsic healing of tendon is that fibroblasts and inflammatory cells migrate into the healing site and

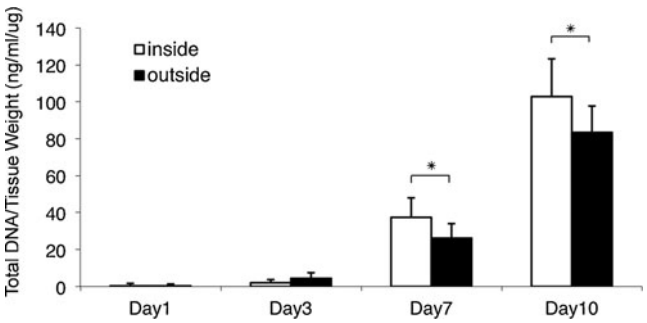

Fig. 5 Cell migration assay. *Inside* indicates that the visceral surface of the synovium graft is facing to the bottom, and *outside* that the parietal surface of the synovium graft is facing to the bottom. Results are presented as mean and standard deviation (SD) of $n=8$. $^*P<0.05$ indicates a significant difference

initiate repair and regeneration of injured tendon [28, 32]. In studies of such healing, the activity of fibroblasts from the synovial sheath is much higher than that of tendon-derived fibroblasts [20, 29]. Based on these studies, we had hypothesized that directly implanted synovium grafts at the repair site of tendons could have a positive effect on tendon healing and we have indeed found that synovium grafts have a potential to accelerate tendon healing in vitro [13].

In this study, we studied the polarity of the synovium graft in order to see if there were any differences in the activity of the visceral and parietal surfaces. We found that cell migration activity was greater for the visceral surface cells in our tissue culture model and cell migration assay. Although this may be simply due to a difference of in the initial number of cells on that surface, it is also possible that the visceral surface cells have a different potential for migration. Nevertheless, cells migrated from both surfaces. We conclude that graft polarity does affect migration, but whether this might limit clinical effectiveness of such a graft to augment tendon healing remains to be demonstrated. Knowledge of graft polarity may be useful in circumstances where one side of a tendon laceration is perceived as having less healing potential than another, for example in an area where a vinculum has been injured [1].

It is well known that tenocytes are responsible for synthesis of extracellular matrix (ECM) proteins and maintenance of ECM structure [2, 8, 31]. The mechanical loads and growth factors induce the synthesis of collagen and other ECM components by tenocytes [16]. Other studies have shown that tendon cells have an intrinsic healing capacity such as proliferation, migration into tendon core [10, 17, 22, 24], and that both intrinsic tenocytes and extrinsic synovial cells expressed a range of inflammatory markers in response to injury [6, 19]. Moreover, fibroblasts become activated to migrate into the damaged tissue and to differentiate into myofibroblasts that contribute to tissue repair during wound healing by cytokines and mechanical microenvironment [12]. Myofibroblasts are characterized by de novo expression of α-SMA in stress fibers and synthesize ECM components; α-SMA has been identified in fibroblastic cells of normal tendons and ligaments [14, 15, 26]. In this study, we showed that the migrating cells expressed α-SMA very strongly. We also found that the synovial cells already expressed α-SMA just after implantation. Some studies have reported that α-SMA-positive fibroblasts are present in synovial tissues of joint and tendon sheath [11, 25, 29]. These studies suggest that myofibroblasts are also involved in the healing process of tendon same as other tissues. Postlethwaite et al. reported that types I, II, and III human collagen can serve as chemoattractants for fibroblasts [27]. This may be one of the mechanisms of cell migration from synovium graft to tendon in which type I collagen is a main component of ECM. However, the relationship between myofibroblasts and tenocytes in the healing process and destination of cells migrating into tendon remains unknown. In a future study, we will study the phenotype of cells after migration by tracking these cells much longer period in order to see the role of migrated cells in tendon healing.

The disadvantage of this study is that it is not an in vivo study, and thus that the effects of some in vivo factors, such as inflammatory response, cannot be addressed. In addition, it is also known that fibroblast migration from synovium results in adhesion formation [20, 29, 41]. In our in vitro tissue culture model, we could not see the effect of these synovium grafts on adhesion formation. Further, in vivo study will be needed to address the effect synovium graft on adhesion formation.

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Conflicts of Interest The authors declare they have no conflict of interest.

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