INTRODUCTION:
The treatment of intrasynovial flexor tendon injuries presents one of the most challenging problems. Recently we have reported that interpositional tendon sheath grafts accelerate tendon healing. However, synovial tissue 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 synovial patch transplantation by comparing the ability of visceral and parietal cells to migrate into the tendon.

MATERIALS AND METHODS:

Tissue Harvest: The 2nd-5th digit the flexor digitorum profundus (FDP) tendons and synovial tissues were harvested from dogs under sterile conditions after sacrifice. 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). The tendon was divided in its mid portion prior to use.

Tendon Repair and Tissue Cultures: The synovial graft labeled with CellTracker<sup>®</sup> Red CMPT (Molecular Probes, Inc, Eugene, OR) was placed between lacerated tendon ends, with the visceral surface facing either the proximal or distal end of the lacerated tendon. The tendon ends were sutured with two simple sutures of 6-0 Prolene with or without synovial graft. The repaired tendons were cultured with MEM supplemented with 10% FBS and 1% antibiotic-antimycotic solution at 37 °C in a 5 % CO₂ humidified incubator for 10 days. The repaired tendons were removed from the culture dish at day 1, 3, 7 or 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 and 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). 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 (BSA)/0.1% phosphate buffered saline with Tween 20 (PBST) for 1 h before incubation with rabbit polyclonal anti-human α-SMA antibody (Abcam, Inc, Cambridge, MA) 4°C for over night. 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 a Zeiss fluorescent light source microscope (Zeiss Inc., Oberkochen, Germany).

Cell Migration Assay: PureCol bovine dermal collagen (3.1 mg/ml, Inamed Corp., Fremont, CA) was prepared following the company’s instructions. The solution was added with same amount of MEM supplemented with 20% FBS and 2% antibiotic-antimycotic solution. A 1.5 ml aliquot of the solution was added in a 6 well dish and incubated at 37 °C for 1 h. Synovial tissue was cut into 5 mm square sections and put on a cell strainer with the visceral or parietal surface facing to the upper surface of the cell strainer. The synovial piece was saturated at each corner for fixation. The cell strainer was put on the pregelatted gel directly and covered with 3ml MEM supplemented with 10% FBS, 1% antibiotic-antimycotic solution, and incubated at 37 °C in a 5 % CO₂ humidified incubator for 10 days to allow cells to migrate through cell strainer into the collagen gel. 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 resuspended with 200 μl Buffer in DNA Quantity Assay (B-Bridge International, Inc, CA) and homogenized by sonication. DNA was quantified with Picogreen dsDNA Quantitation Kit (Molecular Probes, Inc, Eugene, OR) following the company’s instructions 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: Qualitative observation by microscopy revealed that viable cells were present in synovial grafts at each time point in tissue culture. Partial healing was found in the tendons repaired with synovial graft interposition at day 10 (Figure 1).

Cell Migration Assay: The amount of DNA continued to increase and peaked at day10 in both groups. Notably, the amount of DNA increased exponentially from day 7 to day10, which means that the cells proliferate after migration into the collagen gel. At day 7 and 10 the amount of visceral cell DNA was statistically higher than that of the parietal cells (Figure 3).

DISCUSSION:
In this study we studied the polarity of a synovial graft in order to see the differences in activity of the visceral and parietal surfaces. We found that cell migration was greater for the visceral cells in our tissue culture model and cell migration assay. Although this may be simply due to a difference in the initial number of cells, it is likely that the visceral 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.

SIGNIFICANCE:
This study could serve as a stepping stone to further development of a new technique for the treatment of intrasynovial tendon injury.

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