INTRODUCTION

Flexor tendon injuries in the hand are common. Functional outcomes of these injuries have improved, but complications such as adhesions and repair ruptures remain problematic in some cases. These complications are believed to be related to an inherent weakness in the healing capacity of tendons, which are hypocellular, and for which the usual mode of healing involves recruitment of extratendinous fibroblastic cells.

To overcome the problems associated with tendon, bone marrow derived stromal cells (BMSCs) have been used as a source of additional cells at the tendon repair site. BMSCs are able to differentiate into a spectrum of cell types including fibroblasts and tenocytes.

During last decade, investigators have started to focus on a natural packet of multiple growth factors, the platelet, delivered in the form of platelet-rich plasma (PRP) to improve tissue healing. PRP is used for both bone and soft tissue healing enhancement, including tendon. PRP also enhances proliferation of BMSC, and some growth factors known to be present in PRP, such as IGF-1 and TGF-β, augment the effect of BMSC on tendon.

Therefore, the purpose of the current study was to test the hypothesis that the use of BMSCs and PRP at the tendon repair site would accelerate tendon healing, using an in vitro canine tendon tissue culture model. We further hypothesized that PRP would enhance the effect of BMSC on tendon healing site and increase tendon healing strength, beyond the effect of BMSCs alone.

MATERIALS AND METHODS

Tendon Harvest: A total of 192 flexor digitorum profundus (FDP) tendons from the 2nd to 5th digits of both forepaws and paws were immediately harvested from 12 dogs after sacrifice for other, IACUC approved, studies.

PRP Preparation: Whole blood was withdrawn into a sterile syringe containing citric acid-citrate-dextrose anticoagulant (ACD-A). PRP preparation from blood was carried out using the GPS III System (Biomet Biologic, Warsaw, IN), according to the manufacturer’s directions. A solution of 1000 units of bovine thrombin (BioPharm, Alpine, UT) per milliliter of 10% calcium chloride (Sigma, St. Louis, MO) was used to activate the PRP. This mixture was then left at room temperature for one hour to lyse the platelets and release the growth factors. The solutions were centrifuged for 5 minutes at 1500 rpm and the supernatant was collected. Platelets within whole blood and the PRP were counted according to the method of Brecher and Cronkite.

BMSC Harvesting and Suspension: Bone marrow was harvested from mixed-breed dogs and centrifuged. The supernatant was removed and the bone marrow cells were cultured in α-MEM (Gibco, Grand Island, NY) containing 10% fetal calf serum and 1% antibiotics (Gibco) at 37°C with 5% CO₂. The adherent cells were defined as BMSCs.

Preparation of Cell-Seeded Collagen Gel: PureCol bovine dermal collagen (Inamed Corporation, Fremont, CA) was prepared following the company’s instructions. The amounts of collagen and cell density were adjusted to a final collagen concentration of 0.5 mg/ml and initial cell density 1.0 x 10⁶ cells/ml. After incubating at 37°C in a 5% CO₂ humidified incubator for two days for gelation, the BMSC-seeded patch was cut to a similar cross-sectional shape as the tendon ends (roughly 2x4 mm), and used. For the PRP group, 1 ml of PRP supernatant was added to each gel solution.

Tendon Repair and Tissue Culture: The tendons were randomly assigned to one of four treatment groups: 1) repaired tendon without patch; 2) repaired tendon with PRP mixed gel patch without cells; 3) repaired tendon with cell-seeded gel patch; and 4) repaired tendon with cell-seeded PRP mixed gel patch. Each tendon was cut centrally at the zone II D level. The gel was placed between the lacerated tendon ends. Then the tendon ends were apposed with two simple loop sutures of 6-0 Prolene (Ethicon, Somerville, NJ). Tendons were cultured for 2 or 4 weeks in α-MEM (Gibco) with 10% fetus bovine serum and 1% antibiotics (Gibco).

Biomechanical Testing: A single loop suture was placed at each end of the cultured tendon to connect the tendon to a custom-designed micro-tester for mechanical evaluation. Before testing, the tendon apposition sutures were cut at both side of the tendon, without disrupting the repair site, in order to assess the strength of the healing tissue rather than the suture strength. The specimen was then distracted at a rate of 0.1 mm/second till failure. The failure was confirmed by two points, complete loss of attaching force among appositioned tendon ends detected by load transducer and disruption of connecting tissues under gross observation. The displacement and force measured by the transducer were recorded for data analysis.

Cell Viability Assessment: The BMSCs were labeled with PKH26 red fluorescent cell linker (Sigma, St. Louis, MO) before seeding in the patch. After culture, tendons were frozen immediately on dry ice and mounted with O.C.T. compound (Tissue-Tek; Sakura Finetek, Torance, CA). Sections of 6 μm were cut in the sagittal plane using a cryostat (Leica, Bannockburn, IL). Acquired specimens were observed using laser scanning confocal microscopy (LSM510; Zeiss, Thornwood, NY).

Histology: Cultured tendons were collected and fixed in 10% neutral buffered formalin for 24 hours. The samples were mounted with O.C.T. compound (Tissue-Tek; Sakura Finetek, Torance, CA). Sections of 6 μm were cut in the sagittal plane using a cryostat (Leica, Bannockburn, IL). The sections were stained with hematoxylin and eosin (H&E). The morphology was evaluated with light microscopy.

Sample Size and Power: Based on preliminary data, the standard deviation of the maximum strength was calculated to be 25 mN. Assuming similar variability in the proposed study, a sample of 160 tendons provides 80% power to detect a difference of 25 mN in maximum strength between any two experimental conditions ($\alpha = 0.05$).

RESULTS

Platelets Count: Mean platelet counts were 5.41-fold greater in the PRP compared to whole blood (PRP 243 ± 49 x 10³/µl, whole blood 1316 ± 263 x 10³/µl, p=0.0006).

Maximum Strength and Stiffness of the Healing Tendons: Analysis using a 2-factor ANOVA with repeated measures showed that the repaired method had a significant effect on both maximum strength and stiffness of the healing tendons. The effect of time was not significant in either maximum strength or stiffness. Since the interaction between repair method and time was not significant, the comparison between each patch method was tested using the Tukey-Kramer post-hoc test.

The maximum breaking strength and stiffness of the repaired tendons with a BMSC-seeded PRP patch was significantly higher than the repaired tendon without patch and with a BMSC-seeded patch (Fig. 1).

Cell Viability and Histology: The labeled viable BMSCs were present among the repair site in both the BMSC-seeded patch and BMSC-seeded PRP patch group after 4 weeks of culture (Fig. 2). Under light microscopy, every group showed partial connection of collagen bundles between the opposed tendon ends after 4 weeks of culture. No difference was found between each group.

DISCUSSION

In this study, the BMSC-seeded gel patch with PRP improved maximal strength and stiffness of the tissue between the tendon ends in vitro. Though further studies to understand its mechanism are needed, this data suggests that a BMSC-seeded patch with PRP has the potential to enhance flexor tendon healing.

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