Tendon-Derived Stem Cells (TDSCs) Promote Tendon Repair in a Rat Patellar Tendon Window Defect Model

Ming Ni,[1,2] Pauline Po Yee Lui,[1,2,3] Yun Feng Rui,[1,2] Yuk Wa Lee,[1,2] Yuk Wai Lee,[1,2] Qi Tan,[1,2] Yin Mei Wong,[1,2] Siu Kai Kong,[4] Pui Man Lau,[4] Gang Li,[1,2,3] Kai Ming Chan[1,2]

[1]Department of Orthopaedics and Traumatology, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China, [2]The Hong Kong Jockey Club Sports Medicine and Health Sciences Centre, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China, [3]Program of Stem Cell and Regeneration, School of Biomedical Science, The Chinese University of Hong Kong, Hong Kong SAR, China, [4]Programme of Biochemistry, School of Life Sciences, The Chinese University of Hong Kong, Hong Kong SAR, China

Received 27 March 2011; accepted 30 August 2011
Published online 16 September 2011 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jor.21559

ABSTRACT: Injured tendons heal slowly and often result in the formation of mechanically and functionally inferior fibrotic scar tissue or fibrous adhesions. This study investigated the use of tendon-derived stem cells (TDSCs) for tendon repair in a rat patellar tendon window defect model. Fibrin glue constructs with or without GFP-TDSCs were transplanted into the window defect. The patellar tendons were harvested for histology, ex vivo fluorescent imaging and biomechanical test at various time points up to week 4. Our results showed that TDSCs significantly enhanced tendon healing as indicated by the increase in collagen production as shown by hematolxylin stain-ability of the tissue, improvement of cell alignment, collagen fiber alignment and collagen birefringence typical of tendon. The labeled cells were observed at weeks 1 and 2 and became almost undetectable at week 4. Both the ultimate stress and Young's modulus were significantly higher in the TDSCs group compared to those in the fibrin glue group at week 4. In conclusion, TDSCs promoted earlier and better repair in a rat patellar tendon window defect model. J Orthop Res 30:613–619, 2012

Keywords: tendon-derived stem cells (TDSCs); tendon injury; tendon regeneration or repair; tendon tissue engineering

The patellar–patellar tendon–bone autograft is commonly used in anterior cruciate ligament (ACL) reconstruction.[1] After acute tendon injury, tendons do not heal by a regenerative process but via formation of a fibrotic scar or fibrous adhesions, with poor tissue quality and mechanical properties.[2] Therefore, healing at the donor site remains an important clinical issue because of its high morbidity.[3] Tissue engineering is a promising approach for tendon regeneration. Previous studies have shown positive effects of bone marrow-derived mesenchymal stem cells (BMSCs),[4–6] dermal fibroblast,[7] tenocytes,[8] and embryonic stem cells-derived MSCs (ESC-MSCs)[9] for tendon repair. However, these cell sources have some limitations including dedifferentiation, inferior proliferation, differentiation, and collagen production abilities of tenocytes[10–14]; risk of teratoma formation of ESCs as well as risk of ectopic bone[4] and tumor induction[15] of BMSCs. It is known that MSCs from different cell sources showed different yield, proliferation and differentiation potential.[16] Recently, stem cells have been isolated from tendons.[17,18] Bi et al.[17] showed that the stem/progenitor cells isolated from tendon could form tendon tissue in a nude mice model and the donor origin of these cells within the neo-tendon tissue was confirmed by the green fluorescent protein (GFP) label carried by the tendon stem/progenitor cells. As stem cells isolated from tendon, they may be used for tendon tissue engineering. This study aimed to investigate the effect of tendon-derived stem cells (TDSCs) in tendon repair using a rat patellar tendon window defect model.

MATERIALS AND METHODS

Isolation and Culture of Rat GFP-TDSCs

All experiments were approved by the Animal Research Ethics Committee of the authors' institution. Four- to 6-week-old male outbred GFP Sprague–Dawley rats, (SD-Tg (CAG-EGFP) Cz-004Osb), weighting 150–220 g were used for TDSC isolation as described previously.[18] The mid-substance of patellar tendons were excised from healthy rats overdosed with 2.5% sodium phenobarbital i.p. (1.0 ml/400 g). Care was taken that only the mid-substance of patellar tendon tissue, but not the tissue in the bone–tendon junction, was collected. Peritendinous connective tissue was carefully removed and the tissue was stored in sterile phosphate-buffered saline (PBS). This minimized the chance that TDSCs were contaminated by stem cells from the surrounding tissues. The tissue was minced, digested with type I collagenase (3 mg/ml; Sigma-Aldrich, St. Louis, MO) and passed through a 70 μm cell strainer (Becton Dickinson, Franklin Lakes, NJ) to yield single-cell suspension. The released cells were washed in PBS and resuspended in low glucose Dulbecco's modified Eagle's medium (LG-DMEM) (Gibco BRL Life Technologies, Invitrogen Carlsbad, CA), 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine (complete culture medium) (all from Invitrogen Corporation, Carlsbad, CA). The isolated nucleated cells were plated at an optimized low density (500 cells/cm^2) for the isolation of TDSCs from rat patellar tendon (Supplementary Information S1) and cultured at 37°C, 5% CO_2 to form colonies. At day 2 after initial plating, the cells were washed twice with PBS to remove the non-adherent cells. At days 7–10, they were trypsinized and mixed together as passage 0 (P0). TDSCs were subcultured at 4000 cells/cm^2 when they reached 80–90% confluence. Medium was changed every 3 days. Cells at passage 4 were used for all experiments. The

Additional Supporting Information may be found in the online version of this article.
M. Ni and P.P.Y. Lui are co-first authors of this work. Lee, Y.W. Lee, Q. Tan, and Y.M. Wong contributed equally to this work.
Correspondence to: Pauline Po Yee Lui (T: 852-2632-3072; F: 852-2646-3020; E-mail: pauline@ort.cuhk.edu.hk)

clonogenicity and multi-lineage differentiation potential of these cells were confirmed before being used for the experiments in this study using standard assays as described previously and the results were shown in Supplementary Information S1.[18] Our previous study on rat patellar GFP-TDSCs isolated by the same method showed protein expression of CD90 and CD73 as well as mRNA expression of *Oct-4* (unpublished results). A previous study showed that tendon stem cells, but not tenocytes, showed colony-forming ability and processed multi-lineage differentiation potential.[14] Tendon stem cells were also reported to express Oct-4, SSEA-4, and nucleostemin, whereas tenocytes expressed none of these markers.[14,19] Since our TDSCs exhibited colony-forming ability and showed multi-lineage differentiation potential (results shown in Supplementary Information S1) as well as expressed *Oct-4* mRNA (results not shown), they were not tenocytes.

Animal Surgery

Thirty-eight outbred Sprague–Dawley male rats (7–8 weeks, body weight of 250–320 g) were used in this study. To create the tendon defect, the central portion of the patellar tendon (~1 mm in width) was removed from the distal apex of the patella to the insertion of the tibia tuberosity without damaging the fibrocartilage zone with two stacked sharp blades as described in the previous study.[20] The operated rats were divided into 2 groups with 19 rats in each group: (a) fibrin glue-only group; (b) allogeneic TDSCs in fibrin glue group.

To prepare the TDSCs-fibrin constructs for in vivo transplantation, TDSCs were trypsinized after confluence and washed with PBS. Fibrin glue solution (Beriplast P Combi-Set, CSL Behring, King of Prussia, PA), which contained human fibrinogen, human coagulation factor XIII, bovine aprotinin, human thrombin, and calcium chloride, was used for cell delivery. Sixty microliters of 1.6×10^7 cells were mixed with 370 μl of fibrin glue solution A. Afterwards, the cell suspension was mixed with fibrin glue solution B (370 μl, 1:64 diluted with $CaCl_2$ buffer). Eight hundred microliters of the mixture were plated in a 35 mm culture dish (8 cm^2) and allowed to gel for 5 min. TDSCs-fibrin constructs of dimension 1 mm (thickness) × 10 mm (length) × 2 mm (width) were then cut from the dish for transplantation. Each construct thus contained 4×10^5 cells in 20 μl fibrin glue and one 35 mm dish could provide 16 constructs for 16 animals. Constructs at the edge of the dish were not used and we have checked the number of cells in each construct using Alamar blue assay and found that the cell number was relatively constant across different constructs in the dish (results not shown). Constructs prepared in the same way without cells were used for transplantation in the control group.

The fibrin glue construct with or without TDSCs was placed in the tendon defect horizontally with the length and width of the construct fitted into the length and width of the wound, respectively. The wound was then closed in layers. The animals were allowed to have free-cage activity until euthanasia. The construct remained in the window wound as shown by ex vivo fluorescent imaging as described in the result section. At weeks 1, 2, and 4 after surgery, three animals in each group were killed and the patellar tendons were harvested for ex vivo fluorescent imaging of GFP signal, followed by histology for the examination of cellularity and vascularity of the regenerated tissue by H&E staining and collagen fiber alignment by polarization microscopy. At week 4, another 10 animals from each group were euthanized and both patellar tendons (injured and intact) were harvested for biomechanical test. References were made to the adjacent normal tendon tissue and samples of normal patellar tendon tissue in studying the effect of TDSCs in histology.

The SD rats that were used in the present study were outbred animals. The transplantation of TDSCs from GFP or wild-type SD rats to other wild-type SD rats is an allogeneic transplantation because outbred animals are genetically different individuals. The transplantation of tails from one outbred strain to another was also regarded as allogeneic in another study.[21] Having said that, the degree of allogeneity depends on the colony size. The animal house of our university bred the animals according to the Poiley's system with maximum avoidance of inbreeding (Supplementary Information S2). As a result, the SD genome is quite variable both within a colony and especially between colonies. In addition, GFP SD rat in our animal house is maintained by mating a SD female rat with a male GFP SD rat. Hence the allogeneity would be even higher because the GFP founder carries genetic pool different from the wild-type SD rats.

Histology

The patellar tendon was fixed in buffered formalin, embedded in paraffin, cut longitudinally to 5-μm thick sections and mounted on 3-aminopropyl-triethoxy-silane (Sigma-Aldrich) coated slides as described in previous studies.[20] After deparaffination, the sections were stained with hematoxylin and eosin and examined under light microscopy (DMRXA2, Leica Microsystems Wetzlar GmbH, Wetzlar, Germany). Collagen fiber alignment was examined under polarized light using the same microscope. The samples were assessed by two independent assessors.

Biomechanical Test

We followed the procedures as described in our previous study.[22] The patellar–patellar tendon–tibia composite was first isolated. The regenerated tissue in the window wound connected to the bony ends was then isolated by excising the medial and lateral healthy tendon using two stacked blades similar to the creation of tendon defect. The composite was fixed on a custom-made testing jig with two clamps. The lower one was used to fix the tibia shaft and plateau while the upper one was used to fix the proximal patella, the quadriceps muscles and its tendons without creating mechanical stress to the junction and the mid-substance. The whole construct was then mounted onto the Hounsfield H25KS mechanical testing machine (Tinius Olsen Ltd, Salfords, UK). The test to failure was performed at a testing speed of 40 mm/min and preload of 0.1 N using a 50-N load cell. The sample was kept moist with 0.9% saline irrigation. The load-displacement curve of the healing tendon tissue was recorded. The ultimate stress (N/mm^2) was calculated based on the ultimate load divided by the cross-sectional area at the rupture site measured by the animal ultrasound system with images taken immediately prior to the biomechanical test. The Young's modulus (N/mm^2) was calculated from the linear slope of a stress-strain curve. The percentage of testing values in the regenerated tissue to the central portion of the contralateral healthy patellar tendon created similarly with two stacked blades was also reported.

Ultrasound Imaging

The harvest knee was scanned before the biomechanical test using the animal ultrasound system (Vevo-770 High Resolution

In-Vivo Micro-Imaging System from VisualSonics, Toronto, Ontario, Canada). The ultrasound system is equipped with a rat handling platform, a 3-D motor, a 3-D mode imager and a RMV^{TM} (real-time micro visualization) 711 scan head at 55 MHz. The resolution of the ultrasound system is 30 μm. Briefly, the harvest knee was shaved and placed on the handling platform in a supine position with knee flexion of approximately 100°. The limb was fixed with modeling clay and coupling gel was added to cover the whole knee. After determining the best position for imaging (Supplementary Information S3), the 3D image of the patellar tendon was scanned for 12 mm with step size of 0.032 mm. The tendon contour corresponding to the rupture site in the biomechanical test was drawn and the cross-sectional area was measured using the system software.

Ex Vivo Fluorescence Imaging

Ex vivo fluorescence imaging of GFP signal in the transplanted cells in the tendon defect at the time of sample harvest was done by IVIS 200 imaging system (Xenogen, Alameda, CA). The harvested patellar tendons were placed in the light-tight specimen chamber of the IVIS 200 imager. Grayscale reference images were obtained with low-light illumination. Fluorescence images were then acquired in complete darkness with a GFP excitation/emission filter set at 3.0 s exposure time, binning: small, F/stop: 8, field of view: C, Lamp level: High, with a cooled charge-coupled device (CCD) camera. The pseudocolor image (indicating light intensity, blue least and yellow most intense) was superimposed over the grayscale reference image to form a composite image. The IVIS 200 imager is calibrated against a NIST traceable source.

Data analysis

Data were shown in boxplots. The biomechanical test data were reported as mean ± SD. Comparison of biomechanical parameters of the two groups was done using Mann–Whitney *U*-test as the data were not normally distributed. All the data analysis was done using SPSS software (SPSS, Inc., Chicago, IL, version 16.0). $p \leq 0.050$ was regarded as statistically significant.

RESULTS

Gross Observation

The vascularity over the injury site was high in gross observation of the knee just before sample harvest in both groups at week 1 (Fig. 1A and D). However, the vascularity was lower in the TDSCs group compared to that in the control group at week 1 (Fig. 1A and D). The vascularity decreased at week 2 and week 4 in both groups (Fig. 1B–F). More knee swelling was observed in the control group, compared to that in the TDSCs group, at all time points. The window gap could be observed in both groups but became less distinctive with time in both groups. The window gap was less visible in the TDSCs group compared to that in the control group at week 2 and week 4 (Fig. 1B vs. E, C vs. F). After dissecting the surrounding tissue, the patellar tendon looked glittering white in the TDSCs group while it looked transparent in the control group at week 2 (Fig. 1G vs. I) and week 4 (Fig. 1H vs. J).

Histology and Polarization Microscopy

The fibrin glue degraded at week 1 after transplantation. The cellularity and vascularity (Fig. 2A, arrows) increased at week 1 compared to the region outside the window wound in the fibrin glue controls (Fig. 2A). The cellularity reduced, with some extracellular matrix production as shown by the hematoxylin stainability of the extracellular matrix, at week 2 (Fig. 2B). More extracellular matrix production was observed at week 4 (Fig. 2C). The healing tendon cells were round and randomly oriented at both week 1 and week 2 (Fig. 2A and B, *), but some cells became more elongated at week 4 (Fig. 2C, *). For the TDSCs in fibrin glue group, the fibrin glue degraded at week 1. The cellularity was high and was higher than that in the control group at week 1, due to TDSCs transplantation (Fig. 2D). The cellularity inside wound was reduced at week 2 (Fig. 2E) and was reduced further at week 4 compared to that at week 1 (Fig. 2F). Extracellular matrices were produced at week 1 (Fig. 2D) and more matrices were produced at week 2 (Fig. 2E vs. B) and week 4 (Fig. 2F vs. C) in the TDSCs group compared to those in the control group, as shown by the hematoxylin stain-ability of the extracellular matrix. The cells were round and randomly oriented at week 1 in the TDSCs group, similar to that in the control group (Fig. 2D, *). However, the healing tendon cells became elongated, though remained randomly oriented, at week 2 (Fig. 2E, *). At week 4, longitudinally arranged spindle-shaped healing tendon cells were observed embedded between parallel collagen fibers (Fig. 2F, *). The tissue adjacent to the window defects in both groups was normal, with spindle-shaped tendon fibroblasts embedded in dense parallel collagen fibers as in normal tendon (result not shown). The collagen birefringence was low at week 1 and increased with time in both groups (Fig. 3). At week 4, collagen fibers with birefringence typical of tendon, was observed in TDSCs group, but not in the control group (Fig. 3C′ vs. F′).

Biomechanical Test

The ultimate stress ($p = 0.013$; 11.73 ± 2.52 N/mm^2 vs. 16.42 ± 3.61 N/mm^2) (Fig. 4A) and the Young's modulus ($p = 0.002$; 71.67 ± 17.35 N/mm^2 vs. 129.91 ± 33.57 N/mm^2) (Fig. 4B) were significantly higher in the TDSCs group compared to those in the control group at week 4. TDSCs transplantation restored the ultimate stress to $48.53 \pm 10.12\%$ of the contralateral control at week 4 compared to only $35.69 \pm 6.90\%$ in the control group ($p = 0.025$). TDSCs transplantation restored the Young's modulus to $57.36 \pm 13.13\%$ of the contralateral control at week 4 compared to only $30.44 \pm 7.89\%$ in the control group ($p = 0.001$).

Ex Vivo Fluorescent Imaging

The transplanted cells were observed in the window wound at week 1 (Fig. 5D). The signal decreased at

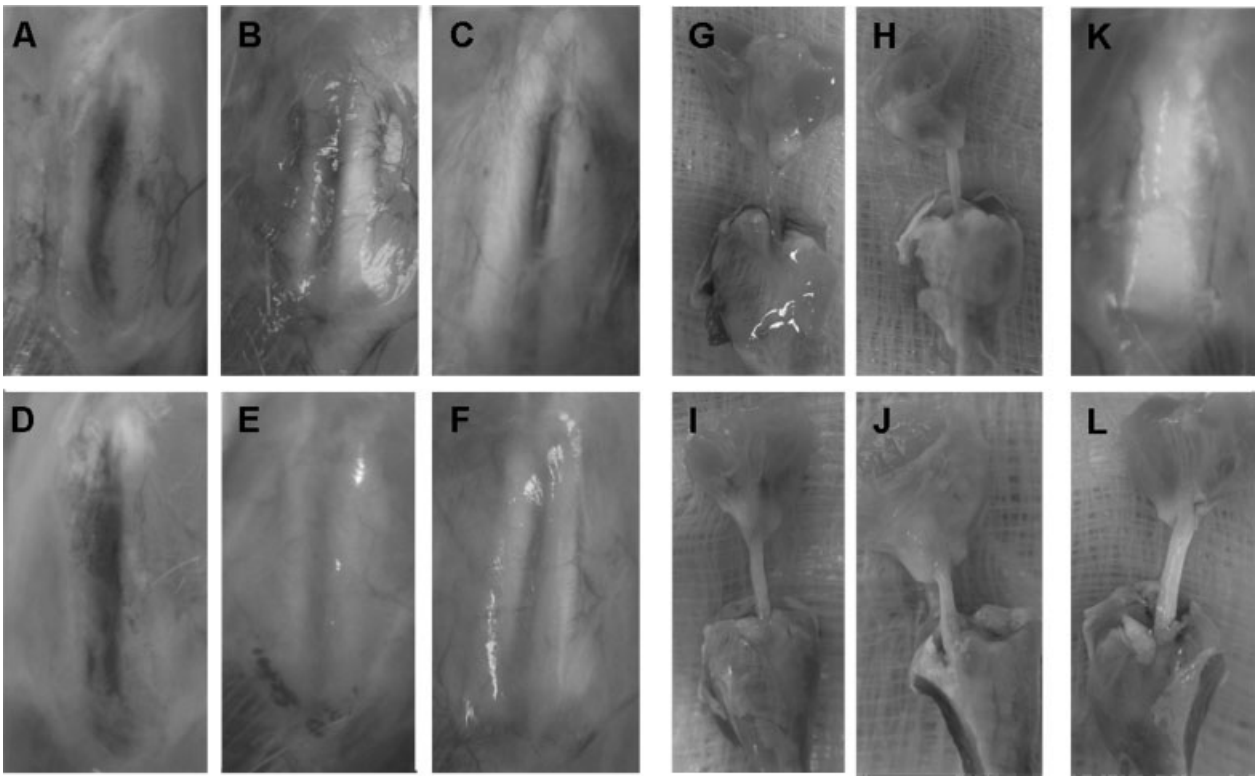

Figure 1. Photographs showing the gross view of the knee (A–F) and the patellar tendon (G–J) in fibrin glue control group (A–C, G–H) and TDSCs in fibrin glue group (D–F, I–J) at week 1 (A, D), 2 (B, E, G, I) and 4 (C, F, H, J) after injury. Panels K and L are the gross views of the intact knee and the intact patellar tendon, respectively. Panel K has the same magnification as panel (A–F) while panel L has the same magnification as panel (G–J).

week 2 (Fig. 5E) and became almost undetectable at week 4 (Fig. 5F). Only one sample in the TDSCs group showed some weak signal at week 4. No fluorescent signal was observed in the fibrin glue control group at all time points (Fig. 5A–C).

DISCUSSION

Our result showed that TDSCs significantly enhanced early stage of tendon healing compared to the fibrin glue-only controls as indicated by (1) the increase in collagen production as shown by hematoxylin stain-ability

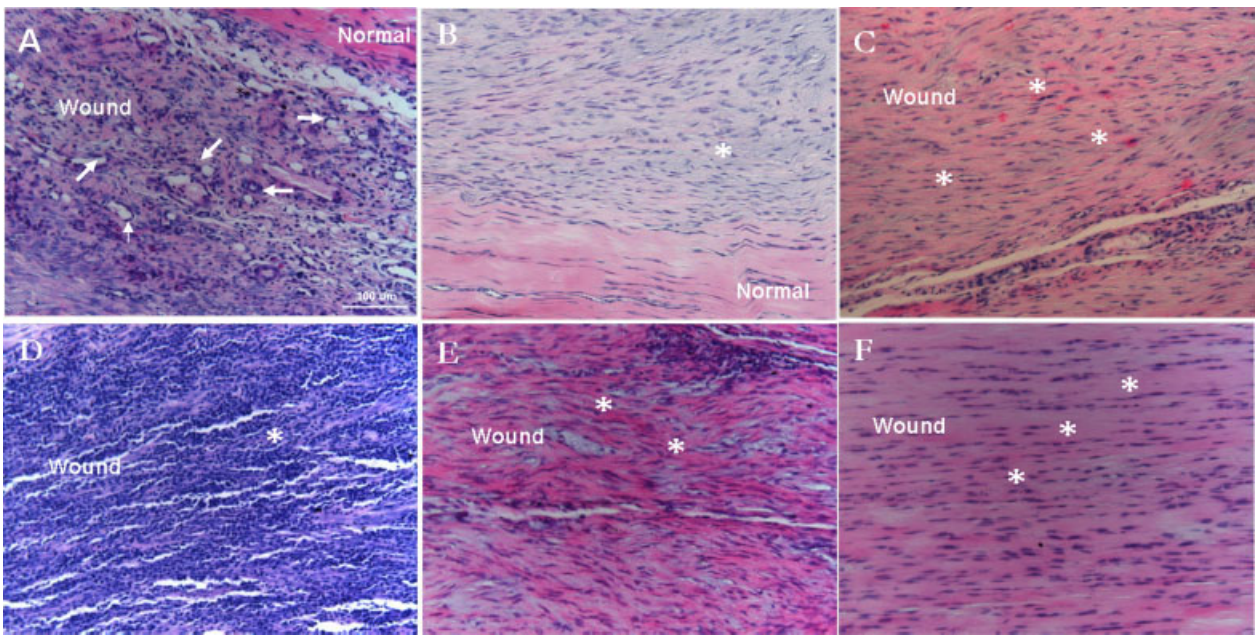

Figure 2. Photomicrographs showing the histology of the window defect in the fibrin glue control group (A–C) and TDSCs in fibrin glue group (D–F) at week 1 (A, D), 2 (B, E), and 4 (C, F) after injury. Stain: hematoxylin and eosin; magnification: 200×; scale bar: 100 μm; Arrows: blood vessels; *: healing tendon cells.

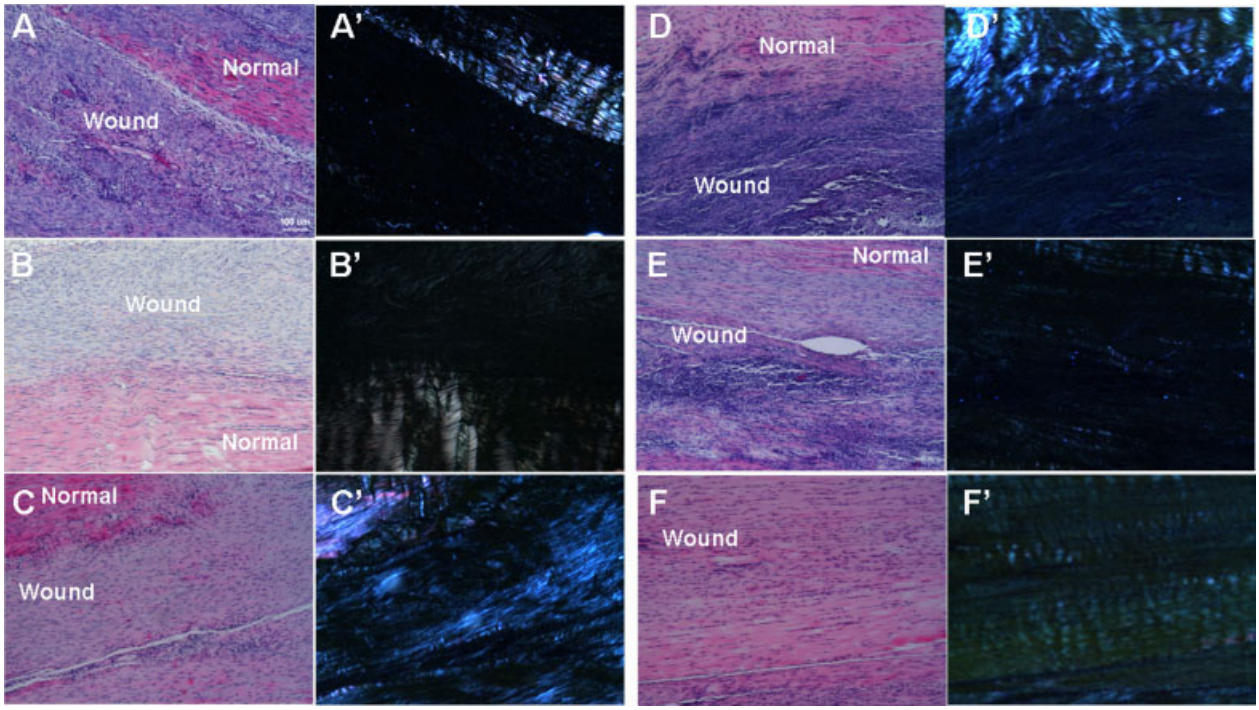

Figure 3. Photomicrographs showing the histology (A–F) and the corresponding polarized image (A′–F′) in the window defect in the fibrin glue control group (A–C, A′–C′) and the TDSCs in fibrin glue group (D–F, D′F′) at week 1 (A, A′, D, D′), 2 (B, B′, E, E′), 4 (C, C′, F, F′) after injury. Magnification: 100×; scale bar: 100 μm; Stain: hematoxylin and eosin.

of the tissue; (2) improvement of cell alignment, collagen fiber alignment, and collagen birefringence typical of tendons; and (3) increase in the biomechanical properties of the regenerated tissue. We followed up tendon healing after TDSC transplantation up to 4 weeks because our previous study showed that the ultimate stress and pyridinoline content peaked at day 30 and plateau afterwards after removal of the central one-third of the patellar tendon in a rat model.[22] The promotion of early stage of tendon healing may allow earlier rehabilitation for the promotion of earlier and better clinical outcome after injury. The long-term effect of TDSCs on tendon repair awaits further investigation. Fibrin glue is commonly used as a carrier for stem cells in tendon tissue engineering.[5] It degraded in less than 1 week in this study. The production of extracellular matrix might help to prevent the dispersion of cells as no fluorescence signal was found in other tissues (see below).

Using ex vivo imaging of GFP label, we showed that TDSCs were present in the wound and the cell number decreased with time. The decrease in fluorescent intensity is unlikely to be due to silencing of the GFP construct as GFP signal is constitutively expressed in the cells under the control of ubiquitous CAG promoter and the fluorescent intensity was proportional to the cell number in vitro (unpublished results). We checked the GFP signal in other organs including liver, heart,

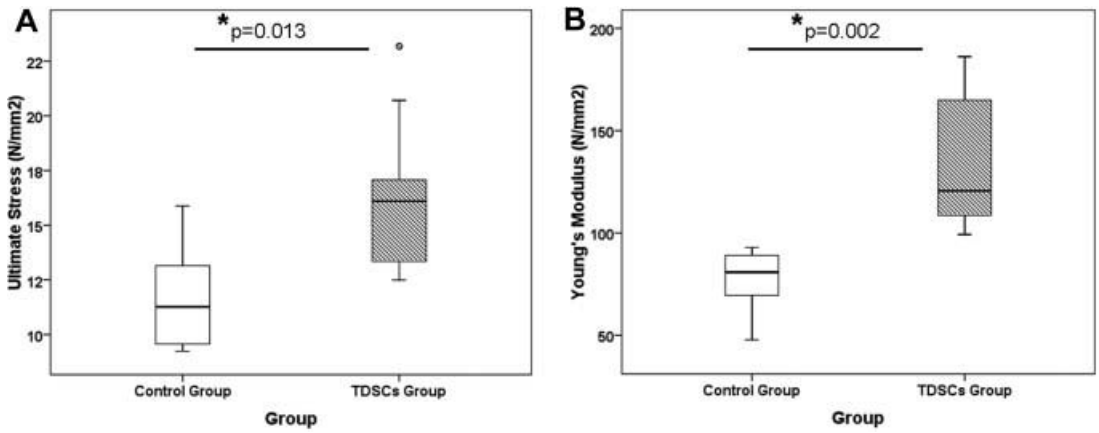

Figure 4. Boxplots showing (A) the ultimate stress and (B) the Young's modulus in the fibrin glue control group and the TDSCs in fibrin glue group at week 4 after injury. $^{*}p \leq 0.05$.

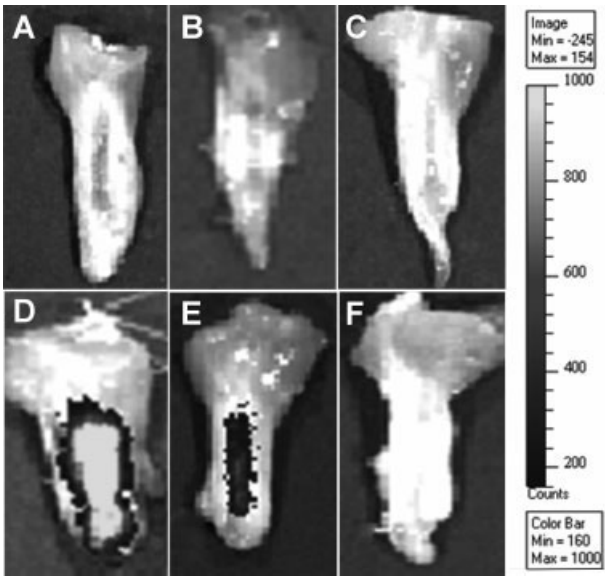

Figure 5. Photographs showing the GFP signal from the transplanted TDSCs in the tendon defect at (D) week 1, (E) week 2, and (F) week 4. (A–C) Background signal in the fibrin glue control group at weeks 1, 2, and 4, respectively.

and lung and found no fluorescent signal in these organs and hence ruled out the migration of the transplanted cells to other organs (unpublished result). Our result was consistent with the hypothesis of natural clearance of the excessive cells at the end stage of the healing process, similar to that reported previously,[20] as there was no visible cell-mediated immune response to allogeneic TDSCs in our samples. Ouyang et al.[23] also reported that the labeled transplanted BMSCs diminished with time and the authors suggested that to be related to the remodeling process. As we observed almost complete absence of GFP signal in the TDSCs group at week 4, it seemed that the transplanted cells contributed to tendon healing by producing tropic paracrine factors that promoted healing, rather than direct differentiation to tenocytes. The host cells played a role in the repair and remodeling of the tendon defect. A previous study showed that seeded allogenic BMSCs remained visible within the tendon wound for at least 8 weeks and became tenocyte-like 5 weeks after implantation in a rabbit window defect model.[23] Integration of GFP-labeled mesenchymal progenitor cells into crimp pattern of adjacent healthy areas of tendon was reported in small lesions created in superficial digital flexor tendon in horses at day 10 or 34.[24] Allogeneic BMSCs were also reported to remain viable and present at the repair site up to week 6, becoming more diffuse at later time-periods, in a rabbit Achilles tendon transection model.[5] It is important to note that rats have shorter lifespan compared to rabbits and horses. Hence the fate of stem cells observed in previous studies should be compared with the present data with care. Future study should track the fate of TDSCs after allo-transplantation with co-immunohistochemical staining of GFP signal and tenomodulin, scleraxis or Tbhs4 which were reported to be tendon-specific markers at the tissue level, proliferating cell nuclear antigen (PCNA) or terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay to confirm the data of ex vivo fluorescent imaging and our hypothesis.[11,25,26]

A previous study has shown that BMSCs could avoid immunorejection and hence an allogeneic source could be used for allogeneic transplantation.[27] Ouyang reported no apparent lymphocyte infiltration with the use of allogeneic BMSCs for tendon repair,[6,23] consistent with our observations. The use of allogeneic TDSCs is essential for future clinical application of this cell type for tendon tissue engineering because of donor site morbidity and the need of multiple operations with the use of autologous cell source. Residual tendon tissue, which can be used for the isolation and banking of allogeneic TDSCs, is readily available during routine tendon/ligament surgery such as the residue tissue during tendon graft harvesting in ACL reconstruction procedure.

In conclusion, our results showed that TDSCs could promote earlier and better recovery after tendon injury. TDSCs therefore can be used as a new cell source for tendon repair.

ACKNOWLEDGMENTS

This work was supported by equipment/resources donated by the Hong Kong Jockey Club Charities Trust and the General Research Fund (project number: 471411) from the Research Grants Council of the Hong Kong Special Administrative Region, China.

REFERENCES

1. Reinhardt KR, Hetsroni I, Marx RG. 2010. Graft selection for anterior cruciate ligament reconstruction: a level I systematic review comparing failure rates and functional outcomes. Orthop Clin North Am 41(2):249–262.
2. Hampson K, Forsyth NR, El Haj A, et al. 2008. Tendon tissue engineering. In: Ashammakhi N, Reis R, Chiellini F, editors. Topics in tissue engineering, 4: pp 1–21. Chapter 3. http://www.oulu.fi/spareparts/ebook_topics_in_t_e_vol4/index.html
3. Marumoto JM, Mitsunaga MM, Richardson AB, et al. 1996. Late patellar tendon ruptures after removal of the central third for anterior cruciate ligament reconstruction. A report of 2 cases. Am J Sports Med 24(5):698–701.
4. Awad HA, Butler DL, Dressler MR, et al. 2003. Repair of patellar tendon injuries using mesenchymal stem cells and collagen scaffolds. J Orthop Res 21(3):420–431.
5. Chong AK, Ang AD, Goh JC, et al. 2007. Bone marrow-derived mesenchymal stem cells influence early tendon-healing in a rabbit Achilles tendon model. J Bone Joint Surg Am 89(1):74–81.
6. Ouyang HW, Goh JC, Thambyah A, et al. 2003. Knitted poly-lactide-co-glycolide scaffold loaded with bone marrow stromal cells in repair and regeneration of rabbit Achilles tendon. Tissue Eng 9(3):431–439.
7. Deng D, Liu W, Xu F, et al. 2009. Engineering human neo-tendon tissue in vitro with human dermal fibroblasts under static mechanical strain. Biomaterials 30(35):6724–6730.

8. Chen JM, Willers C, Xu J, et al. 2007. Autologous tenocyte therapy using porcine-derived bioscaffolds for massive rotator cuff defect in rabbits. Tissue Eng 13(7):1479–1491.
9. Chen X, Song X, Yin Z, et al. 2009. Stepwise differentiation of human embryonic stem cells promotes tendon regeneration by secreting fetal tendon matrix and differentiation factors. Stem Cells 27(6):1276–1287.
10. Almarza AJ, Augustine SM, Woo SL. 2008. Changes in gene expression of matrix constituents with respect to passage to ligament and tendon fibroblasts. Ann Biomed Eng 36(12): 1927–1933.
11. Jelinsky SA, Archambault J, Li L, et al. 2010. Tendon-selective genes identified from rat and human musculoskeletal tissues. J Orthop Res 28(3):289–297.
12. Schwarz R, Colarusso L, Doty P. 1976. Maintenance of differentiation in primary cultures of avian tendon cells. Exp Cell Res 102(1):63–71.
13. Yao L, Bestwick CS, Bestwick LA, et al. 2006. Phenotypic drift in human tenocyte culture. Tissue Eng 12(7):1843–1849.
14. Zhang J, Wang JHC. 2010. Characterization of differential properties of rabbit tendon stem cells and tenocytes. BMC Musculoskelet Disord 11:10.
15. Tasso R, Augell A, Carida M, et al. 2009. Development of sacromas in mice implanted with mesenchymal stem cells seeded onto bioscaffolds. Carcinogenesis 30(1):150–157.
16. Sakaguchi Y, Sekiya I, Yagishita K, et al. 2005. Comparison of human stem cells derived from various mesenchymal tissues: superiority of synovium as a cell source. Arthritis Rheum 52(8):2521–2529.
17. Bi Y, Ehirchiou D, Kilts TM, et al. 2007. Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche. Nat Med 13(10):1219–1227.
18. Rui YF, Lui PPY, Li G, et al. 2010. Isolation and characterization of multi-potent rat tendon-derived stem cells. Tissue Eng Part A 16(5):1549–1558.
19. Zhang J, Pan T, Liu Y, et al. 2010. Mouse treadmill running enhances tendons by expanding the pool of tendon stem cells (TSCs) and TSC-related cellular production of collagen. J Orthop Res 28(9):1178–1183.
20. Lui PPY, Cheuk YC, Hung LK, et al. 2007. Increased apoptosis at late stage of tendon healing. Wound Repair Regen 15(5):702–707.
21. Huggins CB, Anderson KP. 1976. Growth of transplanted tails of infant rats in adolescent allogeneic recipients. Proc Natl Acad Sci USA 73(9):3283–3287.
22. Chan BP, Fu SC, Qin L, et al. 1998. Pyridinoline in relation to ultimate stress of the patellar tendon during healing: an animal study. J Orthop Res 16(5):597–603.
23. Ouyang HW, Goh JCH, Lee EH. 2004. Viability of allergenic bone marrow stromal cells following local delivery into patellar tendon in rabbit model. Cell Transplant 13(6):649–657.
24. Guest DJ, Smith MR, Allen WR. 2008. Monitoring the fate of autologous an allogeneic mesenchymal progenitor cells injected into the superficial digital flexor tendon of horses: preliminary study. Equine Vet J 40(2):178–181.
25. Schweitzer R, Chyung JH, Murtaugh LC, et al. 2001. Analysis of the tendon cell fate using Scleraxis, a specific marker for tendons and ligaments. Development 128(19):3855–3866.
26. Shukunami C, Takimoto A, Oro M, et al. 2006. Scleraxis positively regulates the expression of tenomodulin, a differentiation marker of tenocytes. Dev Biol 298(1):234–247.
27. Ryan JM, Barry FP, Murphy JM, et al. 2005. Mesenchymal stem cells avoid allogeneic rejection. J Inflamm (Lond) 2:8.