Biomimetic gradient scaffold promotes tendon-to-bone healing in a rabbit model of rotator cuff tear

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INTRODUCTION:

Rotator cuff tear is one of the most prevalent shoulder disorders affecting nearly 13% of people over 50 years of age [1]. Although surgical techniques for rotator cuff repair have been advanced, the postoperative retear rate still remains high which is mainly attributed to inferior tendon-to-bone healing. Previously, we have developed a novel decellularized tendon-fibrocartilage-bone composite (dTFBC) composite and demonstrated its efficacy in augmenting rotator cuff healing when combined with bone marrow-derived mesenchymal stem cell sheet (BMSCS) [2]. However, the transplantation of BMSCS was conducted during the surgery which requires sophisticated manipulation and prolongs the operative time. Pre-recellularization of the dTFBC would facilitate the surgical procedure and benefit future clinical translation. Therefore, a biomimetic gradient scaffold was constructed by seeding BMSCS on the dTFBC and co-culturing for a week. We then validated its effects on enhancing tendon-to-bone healing in a rabbit model of acute rotator cuff tear. It was hypothesized that the dTFBC-BMSCS scaffold would promote tendon-to-bone healing more effectively than repair with single dTFBC or sutures.

METHODS:

All the animal experimental procedures were approved by our IACUC. A total of 36 male New Zealand White rabbits (2.8-3.2 kg) were randomly allocated into 3 groups (n=12 per group): control group, dTFBC group, and dTFBC-BMSCS group. Bilateral infraspinatus tendon tears were created with complete debridement of the fibrocartilaginous tissues. The torn tendons were subjected to suture repair (control), dTFBC repair or dTFBC-BMSCS repair in a transosseous manner. Rabbits were sacrificed 12 weeks after surgery. Four rabbits without any intervention were served as normal controls. The isolation of BMSCs and preparation of BMSC sheet were based on previous protocols [2]. P2 BMSCs were seeded in 6-well plates at a density of $1 \times 10^6 \, cells/cm^2$. The formation of sheet was induced by adding 50 µg/ml L-ascorbic acid (Sigma) to the culture medium. Allogenic rabbit patellar tendons with its tibial attachment were harvested and decellularized to fabricate dTFBC [2]. Surgical procedures were shown in **Fig.1A-F**. Histological analysis was performed on sections stained with hematoxylin and eosin, Masson's trichrome, and Safranin O. Semiquantitative scoring (lower scores suggest better morphology) was used to evaluate the healing quality of the tendon-to-bone interface (TBI). Biomechanical testing was conducted to assess the ultimate failure load and stiffness of the repaired tendons using a universal testing machine (AGS-X). All data are presented as mean ± SD, tested for normality. Comparisons between the groups were performed with one-way ANOVA followed by *post hoc* Tukey tests. The Fisher's exact test was used to test for differences in mode of failure. Statistical analysis was conducted using GraphPad Prism 9 (GraphPad Software), and P values < 0.05 were considered statistically significant.

RESULTS:

There were no infection or apparent detachment at the repair site in all three groups. While abundant scar-like tissues were observed at the footprint in the controls, less fibrous tissue with a clearer delineated footprint was noticed in the dTFBC and dTFBC-BMSCS groups. SEM scanning revealed that the BMSCS closely attached to the scaffold with cells migrated inward (**Fig.2**). Histological examination showed that there were immature and disorganized fibers at the TBI in the control group, whereas well organized and oriented collagen fibers were seen in the other two groups (**Fig.3**). In addition, more mature chondrocytes and more newly formed fibrocartilage were observed in tendons repaired with dTFBC-BMSCS scaffold. The total histologic score of the dTFBC-BMSCS group was significantly lower than in the other groups ($P < 0.001$). The subcomponents of the score including fiber structure and fiber arrangement were significantly better in the dTFBC and dTFBC-BMSCS groups than in the controls ($P < 0.001$). The dTFBC-BMSCS group also had better scores in nuclei roundness and vascularity compared to the controls ($P = 0.0016$ and $P < 0.001$, respectively) and the dTFBC group ($P < 0.001$ and $P < 0.001$, respectively). There was a trend of lower insertional failure in dTFBC-BMSCS group (33%) compared to the control group (70%) and the dTFBC group (70%), though no significant differences were noted ($P = 0.310$). Although the ultimate failure load was not significantly different among groups (115.60 ± 38.51 N, 124.11 ± 48.64 N, and 112.51 ± 21.29 N for control, dTFBC, and dTFBC-BMSCS, respectively), dTFBC-BMSCS repair exhibited higher stiffness compared the control group and the dTFBC group ($P = 0.0320$ and 0.0175, respectively). Compared with controls, the cross-sectional area was significantly increased in the dTFBC group ($P = 0.0041$), but not in the dTFBC-BMSCS group.

DISCUSSION:

In this study, we found that the dTFBC-BMSCS scaffold enhanced histological and mechanical outcomes of the repaired infraspinatus tendon compared with the group receiving suture repair and the group receiving dTFBC alone. In contrast to our previous study [2], the implantation of BMSCS on the dTFBC was achieved 7 days prior to the surgery, which not only allows a good integration between the cell and scaffold, but also provides a readily available bioscaffold shortening the operation time. Although the dTFBC-BMSCS scaffold demonstrated promising results and translational potential, there are several limitations worth noting. First, the rabbit model of acute rotator cuff injury may not replicate the real clinical scenario. Future research using a preclinical large animal model of chronic tear is required. Second, the postoperative evaluation was only performed at 12 weeks, more time points are needed to assess the long-term efficacy of dTFBC-BMSCS scaffold. Finally, the underlying mechanism of dTFBC-BMSCS scaffold on enhancing tendon-to-bone healing warrants further study.

SIGNIFICANCE: The dTFBC-BMSCS scaffold holds a great potential of clinical translation as an off-the-shelf product for treating rotator cuff tear.

REFERENCES:

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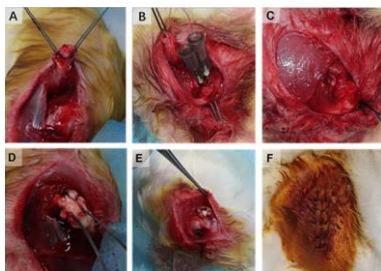
Fig 1. (A) The detached tendon was sutured using modified Mason-Allen stitch. (B) Two bone tunnels were created at the footprint. (C) The sutures were passed through tunnels and tied in the controls. (D) dTFBC or dTFBC-BMSCS scaffold was bridged with the torn tendon by interrupted stitches. (E) The whole construct was reopposed to the footprint in the same manner as the controls. (F) The wound was closed in layers.
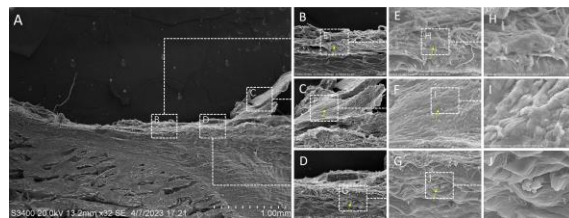

Fig 2. Scanning electron microscope images of the cross-sectional surface of dTFBC-BMSCS scaffolds at low (A-D) and high (E-J) magnification. (A) After 7 days of culture, the BMSCS integrated well with the scaffold. (B, E, H) BMSCs grew well on the collagen fibrils. (C, F, I) Large amount of extracellular matrix was distributed on the surface of the scaffold. (D, G, J) A few BMSCs migrated into the collagen fibrils. Yellow arrow indicates cells.
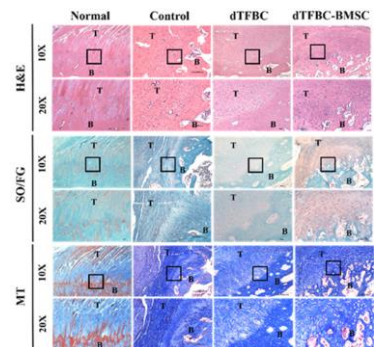

Fig 3. Representative images of the TBI with hematoxylin and eosin staining (H&E), Safranin O (SO), and Masson's trichrome staining (MT) at 12 weeks postoperatively (Scale bars: 200 µm). The boxed areas in the upper images are shown at a higher magnification in the lower images. (T, tendon; B, bone.)