A 3-Dimensional Bioprinted Scaffold With Human Umbilical Cord Blood–Mesenchymal Stem Cells Improves Regeneration of Chronic Full-Thickness Rotator Cuff Tear in a Rabbit Model

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Background: Chronic full-thickness rotator cuff tears (FTRCTs) represent a major clinical concern because they show highly compromised healing capacity.

Purpose: To evaluate the efficacy of using a 3-dimensional (3D) bioprinted scaffold with human umbilical cord blood (hUCB)–mesenchymal stem cells (MSCs) for regeneration of chronic FTRCTs in a rabbit model.

Study Design: Controlled laboratory study.

Methods: A total of 32 rabbits were randomly assigned to 4 treatment groups (n = 8 per group) at 6 weeks after a 5-mm FTRCT was created on the supraspinatus tendon. Group 1 (G1-SAL) was transplanted with normal saline. Group 2 (G2-MSC) was transplanted with hUCB-MSCs (0.2 mL, $1 \times 10^6$) into FTRCTs. Group 3 (G3-3D) was transplanted with a 3D bioprinted construct without MSCs, and group 4 (G4-3D+MSC) was transplanted with a 3D bioprinted construct containing hUCB-MSCs (0.2 mL, $1 \times 10^6$ cells) into FTRCTs. All 32 rabbits were euthanized at 4 weeks after treatment. Examination of gross morphologic changes and histologic results was performed on all rabbits after sacrifice. Motion analysis was also performed before and after treatment.

Results: In G4-3D+MSC, newly regenerated collagen type 1 fibers, walking distance, fast walking time, and mean walking speed were greater than those in G2-MSC based on histochemical and motion analyses. In addition, when compared with G3-3D, G4-3D+MSC showed more prominent regenerated tendon fibers and better parameters of motion analysis. However, there was no significant difference in gross tear size among G2-MSC, G3-3D, and G4-3D+MSC, although these groups showed significant decreases in tear size as compared with the control group (G1-SAL).

Conclusion: Findings of this study show that a tissue engineering strategy based on a 3D bioprinted scaffold filled with hUCB-MSCs can improve the microenvironment for regenerative processes of FTRCT without any surgical repair.

Clinical Relevance: In the case of rotator cuff tear, the cell loss of the external MSCs can be increased by exposure to synovial fluid. Therefore, a 3D bioprinted scaffold in combination with MSCs without surgical repair may be effective in increasing cell retention in FTRCT.

Keywords: shoulder; rotator cuff; mesenchymal stem cells; 3D cell-printed construct; scaffold; tissue engineering

Rotator cuff tear (RCT) is often cited as a reason for shoulder pain and dysfunction in patients >40 years of age. Its incidence is up to 30% and 50% in those aged >60 and >70 years, respectively.8 Surgical repair of RCT is one of the most commonly performed orthopaedic surgical procedures.5 However, the failure rate of this procedure has been reported to be between 13% and 94%. Despite considerable advances in operative techniques, optimizing postoperative results and regaining full restoration of rotator cuff function remains difficult. These limitations of current treatments have led to a demand for more fundamental approaches for tendon regeneration.

RCT represents a considerable clinical challenge because of its hostile local environment, which hinders the healing process.35 Biologic adjuvants can be used for the regenerative microenvironment. They may have great potential for improving the healing rate and function of the injured rotator cuff.
Rotator cuff tendons are located between the subacromial bursa and the scapulohumeral joint, with tears frequently entering 1 or both of these synovial cavities. Because the tear is consistently exposed to the synovial environment, spontaneous healing has been shown to be poor. In addition, the exposure of tendon components can induce a strong inflammatory response. Synovial fluid with its fibrinolytic enzymes can prohibit the formation of a blood clot, thus leading to a lack of provisional scaffold bridging the tear site. High initial cell loss (up to 75%) has been reported over 24 hours after intratendinous injection. In the case of RCT, the cell loss of the external MSCs can be increased by exposure to synovial fluid. Thus, regeneration of RCT may be more difficult than that of other extrasynovial tendons. When MSCs are administered to the RCT site, keeping them in the tear region can be a more important issue than having enough exogenous MSCs.

Three-dimensional (3D) bioprinting technology is a manufacturing strategy that can potentially solve current limitations, including inability to spatially distribute MSCs. In addition, it has precise control of the inner microarchitecture. Moreover, this strategy can provide a tissue-specific microenvironment by utilizing various biomaterials, such as synthetic biocompatible and biodegradable polymers (eg, poly(e-caprolactone), polyactic-co-glycolic acid) and extracellular matrix-based hydrogels (eg, collagen, alginate, hyaluronic acid [HA], gelatin). In this regard, a scaffold in the form of a spatially controlled 3D porous structure with high interconnectivity is expected to be able to maximize the retention of seeded MSCs and serve as a structural template to mimic the cells’ natural microenvironment, thus improving cell-to-cell interactions and promoting functionality for tendon regeneration.

In this study, we developed a scaffold augmented with human umbilical cord blood (hUCB)–MSCs used for this study were from a single donor. These MSCs isolated from hUCB were cultured to passage 3. Another 3 passages were needed to collect sufficient numbers of hUCB-MSCs after thawing. The isolation and culture of MSCs were performed as described previously. Consistent with a previous study, these cells expressed the ability to differentiate into bone, cartilage, and tendon cells. In addition, it has precise control of the inner microarchitecture.
CD105 and CD73 but not CD34, CD45, CD14, or HLA-DR. All hUCB-MSCs were at passage 6. HA hydrogel was prepared by dissolving HA (Hyal 2000; LG Life Science) in alpha minimum essential medium (Gibco) to form a composite of hUCB-MSCs and HA. Based on cellular morphological analysis, in vitro viability test, rheological characterization, and cellular proliferation, the optimal density of HA was determined to be 4%. Monolayer culture of hUCB-MSCs and 4% HA were completely mixed.

Fabrication of 3D Bioprinted Scaffold and Scanning Electron Microscopy Imaging

A microextrusion-based 4-head bioprinting system (3DX printer; T&R Biofab) was used for fabricating scaffolds. We used biodegradable polycaprolactone (PCL; MW 43,000, Polyscience) to entrap the HA solution laden with hUCB-MSCs. The 3D bioprinting system used in this study consisted of 3-axis precision stages and 4 individual pneumatic dispensers (3 dispensers for printing cell-laden hydrogels and 1 for biocompatible thermoplastic polymer) (Figure 1A). The dispenser for thermoplastic polymer was controlled by the computerized heating unit. PCL granules were loaded into a 10-mL syringe of the extruder with temperature maintained at 80°C. The nozzle diameter was 150 μm (Figure 1B). After 10 minutes of heating, the molten PCL was extruded with a pressure of 500 kPa, and a 3D scaffold was fabricated at a feed rate of 60 mm/min. The final dimension of the structure was 4 mm in diameter and 1.7 mm in thickness with a concentration of 1 × 10^6 cells per scaffold (total volume, 0.2 mL). Widths of the line and the pitch of the structure were 143 and 713 μm, respectively (Figure 1C). The structural characteristic of the fabricated 3D bioprinted scaffold was examined with scanning electron microscopy (SU660; Hitachi). The scaffold was coated with platinum through a sputter for 20 minutes and examined at an acceleration voltage of 5 kV.

Animal Grouping and Treatment

Inserted tubes were removed at 6 weeks after excisions, and the site of each full-thickness supraspinatus tendon tear was confirmed. At 6 weeks after excision, 32 rabbits were randomly assigned to 4 treatment groups (n = 8 per group). Group 1 (G1-SAL) was transplanted with normal saline. Group 2 (G2-MSC) was transplanted with 0.2 mL of hUCB-MSCs (1 × 10^6 cells) into FTRCTs. Group 3 (G3-3D) was transplanted with a 3D bioprinted construct without MSCs, and group 4 (G4-3D+MSC) was transplanted with a 3D bioprinted construct containing hUCB-MSCs (0.2 mL, 1 × 10^6 cells) into FTRCTs. After treatment, the skin over the induced tendon rupture area was resealed. No medication was administered. Elastic bandage was used to immobilize all rabbits in the equinus position for 2 days after treatment. All 32 rabbits were euthanized by carbon monoxide inhalation at 4 weeks after treatment (Figure 2).

Gross Morphology Examination

After euthanasia, gross morphologic examinations were conducted for each rabbit. Each tendon tear was classified as partial or full thickness. To calculate the size, we positioned a clear plastic ruler near the center of the tear site, took photographs of the gross morphology of the supraspinatus tendon tear and uploaded them into ImageJ software (National Institutes of Health), and traced the outline of the tear edge before treatment and at 4 weeks after treatment (Figure 3).

Histological Examination

Tissue Preparation. The tear site of the tendon was segmented, and the sample was fixed with neutral buffered formalin for 24 hours. Each specimen was embedded in paraffin (Paraplast) and sliced serially into 5 μm-thick sagittal sections. Hematoxylin-eosin (H-E) staining and
Masson trichrome (MT) staining were performed for these specimens. Light microscopy was used for the examination after staining.

**Immunohistochemistry.** The following was used for immunohistochemical staining of tendon sections: mouse anti–collagen 1 monoclonal antibody (COL-1; Abcam) as a marker of collagen fibers (Figure 4, E-H), mouse anti–proliferating cell nuclear antigen monoclonal antibody (PCNA; PC10, Santa Cruz Biotechnology) as a marker of proliferating cells (Figure 4, I-L), and anti–vascular endothelial growth factor polyclonal antibody (VEGF; A-20, Santa Cruz Biotechnology) and anti–platelet endothelial cell adhesion molecule 1 polyclonal antibody (PECAM-1; PC20, Santa Cruz Biotechnology) as angiogenic markers (Figure 4, M-P, Q-T). Paraffin-embedded sections were then cleared, dehydrated, and washed with phosphate-buffered saline (PBS). Antigen was retrieved with citrate buffer (pH 6.0) for 30 minutes at 95°C. Antigen was retrieved with citrate buffer (pH 6.0) for 30 minutes at 95°C. Then cleared, dehydrated, and washed with phosphate-buffered saline (PBS). Antigen was retrieved with citrate buffer (pH 6.0) for 30 minutes at 95°C. Then cleared, dehydrated, and washed with phosphate-buffered saline (PBS). Antigen was retrieved with citrate buffer (pH 6.0) for 30 minutes at 95°C. Sections were then incubated with primary antibodies (1:100-1:200) at room temperature for 2 hours after being washed 3 times with PBS; following this, sections were incubated with secondary antibody (1:100), biotinylated anti-mouse IgG, biotinylated anti-rabbit IgG, or biotinylated anti-goat IgG (Vector Laboratories) at room temperature for 1 hour. After being washed 3 times with PBS, these sections were subjected to a peroxidase reaction with 0.05% Tris-HCl (pH 7.6) containing 0.01% hydrogen peroxide and 0.05% 3',3'-diaminobenzidine (Sigma-Aldrich). Counterstaining was performed with hematoxylin.

**Evaluation of Immunohistochemical Staining.** Thirty fields were randomly selected from each group and photographed with an AxioCam MRC5 interfaced with the Axio-photomicroscope (Carl Zeiss). AxioVision SE64 (Carl Zeiss) was used for analysis. Nuclear or cytoplasmic markers PCNA, VEGF, and PECAM-1 were evaluated according to a semiquantitative scoring system, based on the staining intensity and extent of area. The proportion of positive stained cells was evaluated and scored as 0 (no cells stained positive), 1 (1%-10%, stain-positive cells), 2 (11%-33%), 3 (34%-66%), or 4 (67%-100%). The intensity of COL-1 immunostaining or MT staining was scored as 0 (negative staining), 1 (slight positive staining), 2 (moderately positive staining), or 3 (strongly positive staining).

**Motion Analysis.**

Motion analysis of the rabbits was conducted at baseline (pretreatment) and at 4 weeks after treatment. After 30 minutes of habituation to the open field, motion analysis was performed. Rabbits were allowed to freely explore the arena (3 × 3 m) for 5 minutes. Movements of each rabbit were assessed with a video-tracking system equipped with a camera (SMART 3.0; Panlab) to record the rabbit’s horizontal activity. Measurements on 5-minute walking distance, fast walking time, and mean walking speed were performed. This approach was used for functional evaluation after treatment of RCT in previous studies.

**Statistical Analyses.**

SPSS for Windows (v 25.0; IBM Corp) was used for all statistical analyses. Statistical differences among and between intra- and intergroups were determined with standard descriptive statistical calculations (means and standard deviation), as well as analysis of variance and the Kruskal-Wallis test. When significant differences were found among groups in analysis of variance and the Kruskal-Wallis test, Tukey and Mann-Whitney tests were additionally performed. Mean values were followed by 95% CIs. All data are expressed as mean ± standard deviation. Results were predetermined to be statistically significant at P < .05.

**RESULTS.**

**Gross Morphology.**

In G1-SAL, full-thickness tear was observed in all 8 (100%) rabbits. In G2-MSC, full-thickness tear, partial-thickness tear procedure, and sacrifice.
tear, and nearly complete healing were observed in 2 (25.0%), 4 (50.0%), and 2 (25.0%) rabbits, respectively, at 4 weeks after the treatment. In G3-3D, full- and partial-thickness tears were observed in 3 (37.5%) and 5 (62.5%) rabbits. In G4-3D+MSC, full-thickness tear, partial-thickness tear, and nearly complete healing were observed in 1 (12.5%), 4 (50.0%), and 3 (37.5%) rabbits. There were significant differences in gross morphologic changes between baseline and 4 weeks after treatment in all groups except in G1-SAL (P = .006) (Figures 3 and 5). Gross morphologic mean tendon tear size was 13.79 mm² in G1-SAL, 3.86 mm² in G2-MSC, 4.06 mm² in G3-3D, and 3.78 mm² in G4-3D+MSC at 4 weeks after treatment. There were significant differences in tear size between G1-SAL and the other 3 groups (Table 1, Figure 6).

Histology and Immunohistochemistry

On examination with light microscopy, no signs of inflammation were shown with H-E or MT staining in any group. In G3-3D and G4-3D+MSC, the implanted 3D bioprinted scaffold was clearly evident with H-E and MT staining (Figure 7). Newly regenerated tissue on the periphery and in pores of 3D bioprinted scaffold was identified as fibrovascular tissue. Regenerated collagen fibers were observed inside scaffold pores after MT staining in G3-3D and G4-3D+MSC. Regenerated collagen fibers were observed on MT staining. These regenerated tendon fibers were stained with COL-1 in G2-MSC, G3-3D, and G4-3D+MSC (Figure 4, E-H). The intensity of MT staining revealed numerous MT-stained cells. COL-1-positive cell densities in G2-MSC, G3-3D, and G4-3D+MSC were significantly larger than those in G1-SAL. Significant differences were also found in MT or COL-1 staining results between G2-MSC and G4-3D+MSC (Table 1, Figure 8). Extensive PCNA staining was observed in regenerated collagen fibers in G2-MSC, G3-3D, and G4-3D+MSC (Figure 4, I-L). There were also significant differences in PCNA staining intensities between G2-MSC and G3-3D and between G3-3D and G4-3D+MSC. Immunohistochemistry staining revealed numerous VEGF-positive cells (Figure 4, M-P). There were also significant differences in VEGF staining intensities between G1-SAL and the other groups. However, there were no significant differences in VEGF staining intensities among G2-MSC, G3-3D, and G4-3D+MSC. PECAM-1-positive microvascular densities in G2-MSC, G3-3D, and G4-3D+MSC were significantly higher than those in G1-SAL (Figure 4, Q-T). Based on PECAM-1 staining, there were also significant differences in PECAM-1-positive microvascular densities between G2-MSC and G3-3D and between G3-3D and G4-3D+MSC.

Motion Analyses

On motion analysis, walking distance, fast walking time, and mean walking speed in G2-MSC, G3-3D, and G4-3D+MSC were significantly greater than those in G1-SAL. There were no significant differences in walking distance, fast walking time, or mean walking speed between G2-MSC and G3-3D. In G4-3D+MSC, walking distance, fast walking time, and mean walking speed were greater than those in G2-MSC and G3-3D (Table 1, Figure 9).

DISCUSSION

Findings of this study showed that a tissue engineering strategy based on 3D bioprinted scaffold filled with hUCB-MSCs
could improve the microenvironment for regenerative processes of FTRCT without any surgical repair. When designing this study, we hypothesized that hUCB-MSCs with 3D bioprinted scaffold (G4-3D+MSC) would be more effective in regenerating tendon tear than hUCB-MSC transplantation alone (G2-MSC). In G4-3D+MSC, newly regenerated collagen type 1 fibers, walking distance, fast walking time, and mean walking speed.
Figure 5. Gross morphology of tear site at 4 weeks after treatment. In G1-SAL, FTT was observed in every rabbit. In G2-MSC, PTT, FTT, and CH were observed in 4, 2, and 2 rabbits, respectively. In G3-3D, PTT was observed in 5 rabbits, and FTT was observed in 3 rabbits. In G4-3D MSC, PTT was observed in 4 rabbits, FTT in 1 rabbit, and CH in 3 rabbits. G1-SAL, group 1–normal saline (0.2 mL); G2-MSC, group 2–mesenchymal stem cells (0.2 mL); G3-3D, group 3–3-dimensional bioprinted construct without mesenchymal stem cells; G4-3D+MSC, group 4–3-dimensional bioprinted scaffold with mesenchymal stem cells (0.2 mL). CH, nearly complete healing; FTT, full-thickness tendon tear; PTT, partial-thickness tendon tear.

Figure 6. Supraspinatus tendon tear size at 4 weeks after treatment. Values are presented as mean. *P < .05, 1-way analysis of variance, post hoc Tukey test between 2 groups. There were no gross morphologic changes between pretreatment and 4 weeks after treatment in G1-SAL. There were significant differences in gross morphologic changes between pretreatment and 4 weeks after treatment in G2-MSC, G3-3D, and G4-3D+MSC. G1-SAL, group 1–normal saline (0.2 mL); G2-MSC, group 2–mesenchymal stem cells (0.2 mL); G3-3D, group 3–3-dimensional bioprinted construct without mesenchymal stem cells; G4-3D+MSC, group 4–3-dimensional bioprinted scaffold with mesenchymal stem cells (0.2 mL).

TABLE 1
Tear Size, Semiquantitative Score of Histological Findings, Immunoreactivity of Staining, and Motion Analysis According to Treatment Groups at 4 Weeks After Treatment

<table>
<thead>
<tr>
<th>Group–Injection Regimen, Mean ± SD</th>
<th>G1-SAL</th>
<th>G2-MSC</th>
<th>G3-3D</th>
<th>G4-3D+MSC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gross: tear size, mm²</td>
<td>13.79 ± 1.38</td>
<td>3.86 ± 1.86⁻</td>
<td>4.06 ± 1.40⁻</td>
<td>3.78 ± 1.29⁻</td>
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<tr>
<td>Histological score</td>
<td></td>
<td></td>
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<tr>
<td>MTS</td>
<td>0.33 ± 0.47</td>
<td>2.03 ± 0.62⁻</td>
<td>2.32 ± 0.61⁻</td>
<td>2.56 ± 0.59⁻</td>
</tr>
<tr>
<td>Anti-collagen</td>
<td>0.4 ± 0.62</td>
<td>2.23 ± 0.58⁻</td>
<td>2.49 ± 0.56⁻</td>
<td>2.78 ± 0.66⁻</td>
</tr>
<tr>
<td>PCNA</td>
<td>1.25 ± 1.05</td>
<td>3.57 ± 0.62⁻</td>
<td>3.88 ± 0.63⁻</td>
<td>3.95 ± 0.21⁻</td>
</tr>
<tr>
<td>VEGF</td>
<td>1.40 ± 0.91</td>
<td>2.90 ± 0.84⁻</td>
<td>2.89 ± 0.79⁻</td>
<td>3.22 ± 0.62⁻</td>
</tr>
<tr>
<td>PECAM-1</td>
<td>1.71 ± 0.85</td>
<td>3.16 ± 0.78⁻</td>
<td>3.26 ± 0.85⁻</td>
<td>3.63 ± 0.60⁻</td>
</tr>
<tr>
<td>Motion analysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walking distance, cm</td>
<td>4852.75 ± 137.27</td>
<td>6367.38 ± 154.63⁻</td>
<td>6144.75 ± 201.77⁻</td>
<td>7145.75 ± 201.77⁻</td>
</tr>
<tr>
<td>Fast walking time, %</td>
<td>5.62 ± 1.42</td>
<td>10.11 ± 2.09⁻</td>
<td>8.53 ± 0.58⁻</td>
<td>12.85 ± 1.39⁻</td>
</tr>
<tr>
<td>Mean walking speed, cm/s</td>
<td>6.3 ± 0.57</td>
<td>10.36 ± 2.03⁻</td>
<td>8.95 ± 0.73⁻</td>
<td>13 ± 0.99⁻</td>
</tr>
</tbody>
</table>

⁻P < .05, G1-SAL vs G2-MSC.
⁻⁻P < .05, G1-SAL vs G3-3D.
⁻⁻⁻P < .05, G1-SAL vs G4-3D+MSC.
⁻⁻⁻⁻P < .05, G2-MSC vs G3-3D.
⁻⁻⁻⁻⁻P < .05, G2-MSC vs G4-3D+MSC.
⁻⁻⁻⁻⁻⁻P < .05, G3-3D vs G4-3D+MSC.

*P < .05, 1-way analysis of variance, post hoc Tukey test.
were greater than in G2-MSC based on histochemical and motion analyses. In addition, when compared with G3-3D, G4-3D+MSC showed more prominent regenerated tendon fibers and better parameters of motion analysis. However, there were no significant differences in gross tear size among G2-MSC, G3-3D, and G4-3D+MSC, although these groups showed significant decreases in tear size as compared with the control group (G1-SAL). The gross morphologic mean tendon tear size of each group at 4 weeks after the treatment was 13.79 mm² (G1-SAL), 3.86 mm² (G2-MSC), 4.06 mm² (G3-3D), and 3.78 mm² (G4-3D+MSC). Therefore, a 3D bio-printed scaffold may help with regenerating a higher-quality tendon because tendon regeneration occurs regardless of MSC but the histological quality appears to be better. Whether this leads to a functionally better outcome has not been determined and can be a potential limitation.

Among MSCs from different origins, hUCB-MSCs were used in the present study. hUCB-MSCs have many advantages, such as noninvasive collection, the ability to aim straight toward the injured tissue, low immunogenicity, and extensive secretion profiles. Allogenic UCB-MSCs might have benefits, especially for the elderly and those with multiple comorbidities because the function of autologous MSCs in these populations is impaired. In addition, hUCB-MSCs can be commercially produced in greater quantities. Indeed, hUCB-MSCs are currently available as a novel medication by combining allogenic UCB-MSCs with HA for articular cartilage regeneration in patients with osteoarthritic knees. Many studies have also shown that the use of hUCB-MSCs in animal studies does not show rejection, including inflammation, because of its low immunity.

The efficacy of exogenous MSCs on RCT has been controversial in the literature. For reasons that have not been clarified, MSC transplantation may not lead to complete healing in FTRCT. The inconsistencies of previous findings can be attributed to varied study designs (a human clinical study, an animal model, an animal model with acute or chronic RCT injury), different cell sources with and without scaffold, the type of scaffold used, and the timing of evaluations. Unlike initial expectations on MSCs, the beneficial effects of exogenous MSC transplantation are mainly due to the paracrine action and not entirely to stem cell differentiation.
functional advantages of MSC transplantation for tissue regenerations, many researchers have focused on increasing the total amount of secreted trophic factors and prolongation of the duration of secretion. Meanwhile, a recent study found that, in a porcine model with chronic myocardial infarction, the effect of exogenous MSCs was not proportional to the dose.46 FTRCT is less likely to be regenerated than other extrasynovial tendon injuries because synovial fluid has adverse effects on tendon healing.11 Synovial fluid, with its fibrinolytic enzymes, also prohibits the formation of a blood clot, leading to a lack of provisional scaffold bridging the wound site.35 Therefore, when MSCs are administered for regeneration of FTRCT, cell retention can be regarded as the most important factor rather than the increased dose. In this regard, the use of scaffold in combination with MSCs can be more effective in increasing cell retention in FTRCT.30 However, findings of previous studies on the effect of scaffold were inconsistent.

Using a rat model with acute RCT injury, Lipner et al29 investigated the efficacy of RCT repair augmentation with a nanofiber scaffold seeded with allogenic adipose-derived MSCs as compared with a scaffold without MSCs. Their findings indicated that additional MSC implantation had no beneficial effect on biomechanical or histological outcomes of the repaired RCT. Barco et al3 had a similar conclusion after using a rat model with acute RCT injury. They found no difference between scaffolds with and without MSCs based on biomechanical tests at 4 and 8 weeks after repair. The group with MSCs showed less inflammation at 4 weeks after repair. However, no significant difference was found at 8 weeks.

Given that autograft, allograft, and xenograft scaffolds have many disadvantages, such as donor site morbidity, low availability, inflammatory response, and infection risk, without guaranteeing a full recovery,48 an ideal scaffold should combine macromechanical properties of synthetic scaffolds with micromechanical properties of biologic scaffolds.9,43,45 For tissue and organ regenerations, 3D bioprinted constructs have been regarded as promising biological substitutes.16 In particular, hybrid bioprinting with biodegradable synthetic polymers and cell-laden hydrogels is becoming a promising approach.31 This enables the use of biodegradable synthetic polymers as a framework to support weak cell-laden hydrogel. When fabricating cell-printed constructs, PCL among biodegradable synthetic polymers has been commonly chosen because it has a low melting temperature, which may prevent cells in the printed constructs from being thermally damaged during the fabrication process.23 Synthetic polymers are advantageous as a framework in that (1) they can be fabricated into a regularly controlled architecture with high shape fidelity and (2) they may facilitate the deposition and spatial arrangement of MSCs according to the tissue-specific environment.41 3D bioprinted scaffolds with PCL layers are known to significantly improve the cell viability and proliferation.23 However, developing a large porous scaffold to provide an environment suitable for tendon regeneration remains as a challenge in MSC therapies. Low-porosity scaffolds can impede cellular infiltration into the body of the implant, which may limit their regenerative potential.37 Cellular infiltration into small and dense structures of scaffold is slow, often taking several weeks, thereby limiting seeded cells to the outer region of scaffolds within in vitro and in vivo studies.2,47 In the present study, the 3D bioprinted scaffold resulted in higher levels of porosity, which facilitated the rapid migration of MSCs into the body of scaffolds. In this study, the dimension of the structure was 4 mm in diameter and
1.7 mm in thickness. Because the scaffold was made by 3D printing, the specification was constant and uniform. As a result, 3D bioprinted scaffold in the present work may maintain MSCs within the local microenvironment or niche to properly respond to the repair and arrange a trophic response. It can also protect hUCB-MSCs from exposed synovial fluid. However, this novel scaffold was not made to mimic the orientation of collagen fibril in the tendon or the transition from ranged to random orientation at the tendon-to-bone insertion site. Moreover, even if the scaffold hole is relatively large, it has not been proven to be the most appropriate size for the rotator cuff tendon. Further research is needed to overcome these drawbacks.

Interestingly, there was no significant difference in the rupture size after treatment among G2-MSC, G3-3D, and G4-3D+MSC. However, nearly complete healing rates were 25% in G2-MSC and 37.5% in G4-3D1MSC but were 0% in G3-3D. In the motion analysis, G4-3D+MSC showed better function than G2-MSC and G3-3D. In addition, G2-MSC had better function than G3-3D, although differences between G2-MSC and G3-3D groups were not statistically significant. These findings demonstrated a positive role of MSC administration on tendon regeneration independent of the presence of scaffolds. It is assumed that the scaffold can increase the retention of the MSCs and amplify the regeneration effect of the MSCs. We do not know why groups 2 to 4 did not differ in the rupture size after transplantation. MSCs may help with the quality of the regeneration but not its actual presence. Moreover, in G3-3D, the tear size was unexpectedly reduced. This suggested that a 3D bioprinted scaffold alone can be a useful augment for RCT healing even without MSCs. Although we do not know how this scaffold helps with regeneration, increased recruitment of endogenous stem cells by the 3D bioprinted scaffold might have enhanced tendon regeneration. Endogenous MSC populations would be recruited into the implanted scaffolds, in which moderate inflammation and foreign body responses to the scaffold could produce an angiogenesis-rich microenvironment. The 3D bioprinted scaffold can also provide a tissue-specific microenvironment by utilizing biodegradable PCL and HA. It is expected that the scaffold, in the form of a spatially controlled 3D porous structure with high interconnectivity, will serve as a structural template to mimic cells’ natural microenvironment, thus enhancing endogenous stem-cell homing and tenogenic differentiation. Further research is needed to clarify the exact mechanism.

This study has some limitations. First, we created FTRCTs (5 × 5 mm) just proximal to the insertion site on the supraspinatus tendon; however, we did not evaluate enthesis healing. The tendon-to-bone junction consists of tendon proper, fibrocartilage, mineralized fibrocartilage, and bone. A normal tendon insertion attaches to a bone via fibrocartilage. It acts as a shock absorber by reducing the stiffness gradient between the bone and tendon. A scaffold type should be modified to be suitable for the enthesis. However, as shown in the Results section, increasing cell retention against synovial fluid was also necessary for tendon-to-bone healing. Second, motion analysis, instead of biomechanical testing, was used to evaluate rotator cuff function after treatment. Although motion analysis has not been proven to be a better method than biomechanical testing, it provides important information from histologic examination regarding therapeutic effect on RCT in human and animal studies. Third, it was found that a 3D bioprinted scaffold alone could be a useful augment for FTRCT healing even without MSCs. The local effect of the scaffold is still relatively unknown beyond providing a biomechanical support for tissue growth. Fourth, the inability to demonstrate exogenous MSC retention directly is a limitation. Finally, chronic FTRCT was not completely regenerated. In this respect, it is important to realize that optimal mechanical stimulation is required for regeneration of FTRCT, given that tendons are dynamic tissues capable of responding to mechanical cues by altering the extracellular matrix.

Despite these limitations, this is the first study to show that a tissue engineering strategy based on a 3D bioprinted scaffold filled with hUCB-MSCs can improve the microenvironment for regenerative processes of FTRCT in an in vivo setting. Considering that chronic FTRCT is an extra-articular tendon tear, using 3D bioprinted scaffolds with biodegradable synthetic polymer that have constant, uniform, and larger pores might be more effective in regenerating tendon than the use of MSCs alone.

In conclusion, a 3D bioprinted scaffold improved the microenvironment for regenerative processes of hUCB-MSCs for chronic FTRCT in a rabbit model, although this combination did not reduce the size of torn tendon when compared with scaffold alone or MSCs alone. Such a 3D bioprinted scaffold may help hUCB-MSCs stay longer near the RCT, which would be more effective in regenerating tendon than the use of hUCB-MSCs alone. The findings of this study regarding the combination of hUCB-MSCs and 3D bioprinted scaffold warrant more investigations to overcome limitations of this study.

REFERENCES


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