Preparation and characterization of decellularized tendon slices for tendon tissue engineering

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Abstract: To develop a naturally derived tendon tissue engineering scaffold with the preservation of the native ultrastructure, tensile strength, and biochemical composition of the tendon extracellular matrix (ECM), decellularized tendon slices (DTSs) were prepared using repetitive freeze/thaw of the intact Achilles tendons, frozen section, and nuclease treatment. The DTSs were characterized in the native ultrastructure, mechanical properties, biochemical composition, and cytocompatibility. Histological examination and DNA quantification analysis confirmed that cells were completely removed from tendon tissue by repetitive freeze/thaw in combination with nuclease treatment 12 h. The intrinsic ultrastructure of tendon tissue was well preserved based on scanning electron microscopy examination. The tensile strength of the DTSs was retained 85.62% of native tendon slice. More than 93% of proteoglycans (fibromodulin, biglycan) and growth factors (TGF-β1, IGF-1, VEGF, and CTGF) inherent in tendon ECM were preserved in the DTSs according to ELISA analysis. Furthermore, the DTSs facilitated attachment and repopulation of NIH-3T3 fibroblasts in vitro. Overall, the DTSs are sheet scaffolds with a combination of elemental mechanical strength and tendon ECM bioactive factors that may have many potential applications in tendon tissue engineering. © 2012 Wiley Periodicals, Inc. J Biomed Mater Res Part A 2012:100A:1448–1456.

Key Words: tendon, decellularization, scaffold, proteoglycans, growth factors


INTRODUCTION
The self-repair of injured tendon is difficult due to the poor blood supply and low regenerative capacity. In spite of the use of stem-cell therapy and gene therapy, tissue engineering has become one of the promising approaches for tendon repair/reconstruction, especially for large tendon defects.1–3 An important area of tissue engineering is to develop improved scaffolds that more nearly recapitulate the biological properties of native extracellular matrix (ECM).4 The use of ECM derived from decellularized tissue as a biologic scaffold is increasingly frequent in regenerative medicine and tissue engineering strategies.5 In the absence of methods for de novo construction of a true ECM mimic from purified components, the decellularized native tendon tissue may be an ideal source for tendon tissue engineering scaffolds. Decellularization techniques provide methods to extract cells from tissues, thereby isolating the ECM components. The ECM provides a natural scaffold for structural support of tissues and harbors a complex assembly of biochemical cues comprised of proteins, glycosaminoglycans (GAGs), proteoglycans, and growth factors.6 Hence, preservation of the native ultrastructure and biochemical composition of tendon ECM during the process of tissue decellularization is highly desirable. However, acellular tendon ECM has not been widely used due to its unique tissue structure. It is well known that tendon is a dense connective tissue, which is difficult to remove the cell component. Different decellularization methods, including physical, chemical and enzymatic treatments,7–12 have been used to decellularize intact tendon/ligament tissues. However, reseeding of the decellularized intact tendon scaffold is still a problem that the reseeded cells were limited to distribute only throughout the scaffold’s surface, with far fewer cells in the core of the scaffold in spite of the use of a tissue bioreactor.13 If cells do not efficiently migrate into the decellularized matrix, the structure may eventually collapse in the body with degradation of the matrix.13 Whitlock et al.14 increased the porosity and pore size of the tendon scaffold using a combined solution of TritonX-100 and peracetic acid. Although cells could be removed by such treatment and the infiltration of reseeded cells could be increased, the original structure and ECM microenvironment of the tendon have been destroyed.

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Recently, a novel scaffold with multilayer tendon slices was reported, but the tendon slices with the thickness of 50 μm would not be expected to have enough initial mechanical strength to withstand early motion and rehabilitation. More recently, our previous work has confirmed that tendon slices with 300 μm or more in thickness had similar mechanical properties to the intact tendon bundle. However, no research has been carried out to simultaneously consider the efficiency of decellularization, preservation of the native ultrastructure and mechanical properties, and retention of tendon ECM biochemical composition when the tendon ECM scaffold was prepared. Besides, the cytocompatibility of the tendon ECM scaffold is also critical for a successful tendon repair, because the formation of new tendon tissue largely depends on the interactions of the scaffold with the transplanted or in-growing cells.

To date, several research groups have succeeded in preparing acellular tendon/ligament using detergent and/or several enzymes. They reported that the matrix structure and/or mechanical properties of decellularized tendon were preserved compared with that of native tendon. However, care must be taken to flush residual chemicals from ECM after decellularization, particularly detergents such as SDS that penetrate into thick or dense tissues. Cytotoxicity is possible even at reduced agent concentrations and will inhibit or completely negate the beneficial properties of a cell-free ECM scaffold. In the present study, decellularized tendon slices (DTSs) were prepared using repetitive freeze/thaw of the intact Achilles tendons, frozen section, and nuclease treatment (including DNase and RNase from bovine pancreas). Then, the characteristics of the DTSs, including the native ultrastructure, mechanical properties, biochemical composition, and cytocompatibility were investigated in detail.

MATERIALS AND METHODS

Achilles tendon harvest

The Achilles tendons were harvested from six cadavers of adult beagle dogs (15–20 kg) within 4 h of euthanasia, which were collected from the West China Zoological Experiment Center-affiliated Academia Sinica (Chengdu, China). The Achilles tendon was exposed by opening the skin, and the tendinous portion between the insertion to the bone and the muscle–tendon junction was harvested. The harvested tendons were frozen at −80°C until processing.

Preparation of the DTSs

A total of 36 frozen Achilles tendons were thawed at room temperature and trimmed into segments roughly 40 mm in length. The 24 segments were used to prepare the DTSs according to the following procedure. Briefly, the tendon segments were subjected to repetitive freeze/thaw treatment for five times and fixed to the cutting base plate of a cryostat (Leica CM3050S, Nussloch, Germany) with O.C.T. compound (polyvinyl alcohol and polyethylene glycol; Tissue-Tek®; Sakura Finetek USA, Torrance, CA). The tendon segments were longitudinally cut into tendon slices with a thickness of 300 μm according to the previous study. Then, following washing three times in cation-free phosphate buffered saline (PBS) for 30 min each, the tendon slices were incubated in nuclease solution (including RNase 100 μg mL⁻¹ and DNase 150 IU mL⁻¹), (Roche Diagnostic, Indianapolis, IN) at 37°C. To optimize the incubation time, four different time points at 0, 4, 8, and 12 h were applied. The tendon slices underwent the repetitive freeze/thaw in combination with nuclease treatment were used to obtain DTSs. The rest 12 segments were directly performed to longitudinally cut into tendon slices with a thickness of 300 μm, as the native tendon slices (NTSs). Then all tendon slices were rinsed for 30 min in PBS at room temperature with gentle agitation. The rinsing was repeated three times.

DNA quantification assays

The NTSs (n = 13) were frozen-dried under −70°C with a lyophilizer (CHRIST GAMMA2-16 LSC, Germany) for 24 h and then weighed, minced, and placed into 2.0 mL microcentrifuge tubes. These processes were repeated for the DTSs subjected to different incubation times (n = 13 for each time point). A total of 1.5 mL papain in phosphate buffer (0.2 mg mL⁻¹ papain) was added in each tube, as described previously with some modifications. Subsequently, all samples were digested for 48 h at 60°C. Digested samples were centrifuged at 21,000g for 5 min at room temperature. The supernatant of each sample was removed to a fresh tube and immediately submitted to the PicoGreen DNA assay (Invitrogen) following manufacturer’s instructions and adjusted for dry weight and normalized for blank samples.

Histology and scanning electron microscopy (SEM)

The NTSs and DTSs were examined with histology and SEM. For histology, the samples (n = 3 for each group) were fixed in 4% paraformaldehyde for 24 h at room temperature and embedded in paraffin, and then longitudinally cut into 5 μm thick sections. The sections were mounted on slides and stained using hematoxylin and eosin (H&E, Sigma) as well as 4,6-diamidino-2-phenylindole (DAPI, Sigma) to identify the cellular components and collagen fibrous structure. For the SEM, the specimens (n = 2 for each group) were fixed in 2.5% glutaraldehyde for 2 h at 4°C. After dehydration in graded ethanol, the specimens were conducted to critical point drying, gold sputter coating. SEM images were taken using a FEI Inspect F50-SEM (Netherlands) with a 30 kV acceleration voltage.

Tensile mechanical testing

The mechanical properties of NTSs and DTSs (n = 8 for each group) were analyzed on a mechanical testing machine (INSTRON 5567, USA), as previously described. Tensile testing was applied along the longitudinal axis at a rate of 5 mm min⁻¹. Ultimate tensile strength (UTS, MPa) at break was calculated from the maximal load recorded during each test. The strain at UTS was calculated using the displacement data recorded by the instrument and initial length of the specimen measured with a caliper after placement of
each sample in the grips of the apparatus. Elastic modulus (MPa) and stiffness (N mm⁻¹) were calculated from the linear portion of the stress–strain curves and load-displacement curves obtained from the testing, respectively. Only specimens that failed in the midsustance region of the tendon slices were included for calculations. Specimens that failed at the grips were excluded from calculations.

**Analysis of retained biochemical composition**

Two critical proteoglycans, including fibromodulin (Fmod) and biglycan (Bgn), and four different growth factors (TGF-β1, IGF-1, VEGF, and CTGF) retained in the DTSs were quantified by ELISA analysis. Briefly, frozen-dried samples were minced and pulverized for 2 min at 25 Hz by a Mixer Mill (Retsch, MM400, Germany) after precooled in liquid nitrogen for 5 min. Then, the powder was collected and homogenized with a tissue homogenizer in 1.5 mL ice-cold PBS. The extracted lysates were centrifuged at 1000g for 20 min and the supernatant was collected. The NTSs were used as control.

ELISA analysis of the extracted lysates was performed (n = 6 for each group) according to the instructions from the kits’ manufacturers (Bgn and Fmod, GB System, USA; IGF-1, Westtang Biotec, Shanghai, China; TGF-β1, R&D Systems, Minneapolis, USA; VEGF and CTGF, Cusabio Biotec, Wuhan, China). All samples were normalized for dry weight prior to pulverization.

**In vitro cytotoxicity and cell repopulation evaluation**

To determine the cytotoxicity of the DTSs that might result from the residual reagents of processing procedure, 10% alamarBlue® (Invitrogen) testing was performed to assess cell viability. Briefly, the sterilized DTSs (n = 10, 0.03 cm² per well) were placed in the center of subconfluent murine NIH-3T3 fibroblasts monolayers in 96-well plates, which covered one-tenth of the surface area, according to the established standards. Cells not exposed to the DTSs served as the control (n = 10). The cell-DTSs contact was maintained for 24, 48, and 72 h, at 37°C with 5% CO₂. At the end of the incubation, the DTSs were removed and cell viability was measured. Briefly, 10 μL of alamarBlue® was added to each well. After a 3 h incubation at 37°C, 50 μL of 3% SDS was directly added to 100 μL of cells in alamarBlue® reagent to stop the reaction. Then, the absorbance of alamarBlue® was monitored at 570 nm, using 600 nm as the reference wavelength (normalized to the 600 nm value).

For cell repopulation evaluation, the sterilized DTSs (n = 12) were washed three times with 10 mL of PBS and incubated overnight using serum-free DMEM at 37°C with 5% CO₂. NIH-3T3 fibroblasts were cultured to 80% confluence in 75 cm² tissue culture flasks in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin. The fibroblasts were trypsinized and labeled with the red fluorescent dye PKH26 (Sigma) according to the manufacturer’s protocol. Then, the labeled cells were seeded onto the incubated DTSs at a density of 10⁶ cells mL⁻¹ by adding 1 mL 3T3 cell suspension to each of the 24-well tissue culture plates. The medium was changed every 2 days for up to 4 days, and cells-seeded DTSs were rinsed with PBS and processed for histology and SEM examination as previously described.

**Statistical analysis**

All values were recorded as mean ± the standard deviations (SD). A one-way analysis of variance (ANOVA) with a Tukey’s post hoc test was performed to determine the statistical differences between the different groups in DNA quantification. A two-tailed Student’s t test was used to evaluate the statistical differences of mechanical properties, biochemical and cytotoxicity analysis. Data were analyzed using the SPSS software ver.16.0 (SPSS, USA). Differences were considered significant at p < 0.05.

**RESULTS**

**DNA quantification**

To evaluate the efficacy of removal of all cellular contents from tendon slices, we first conducted DNA quantification analysis of different nuclease treatment time after repetitive freeze/thaw. As shown in Figure 1, the results exhibited that nuclease treatment after repetitive freeze/thaw significantly reduced the DNA content compared with that of the control or repetitive freeze/thaw without nuclease treatment (p < 0.05). Furthermore, nuclease treatment 12 h after repetitive freeze/thaw significantly reduced the DNA content compared with that of nuclease treatment 8 or 4 h (p < 0.05). According to these results, repetitive freeze/thaw in combination with nuclease treatment 12 h was determined as the decellularization method of preparing the DTSs. Therefore, the decellularized tendon slices with repetitive freeze/thaw in combination with nuclease treatment 12 h (hereinafter referred to as the DTSs) were performed the following studies.

**Histology**

The protocol for decellularization of the tendon slice was effective in removing cellular and nuclear material. H&E staining of the NTSs and DTSs demonstrated that...
preservation of the native collagen structure while removing the cellular components [Fig. 2(a,b)]. DAPI staining also showed that few nuclear materials were visible in the DTSs [Fig. 2(d)] whereas abundant nuclear materials were evident in the NTSs [Fig. 2(c)].

SEM
SEM examination confirmed that there were no substantial differences between micrographs of the surface structure of the NTSs [Fig. 3(a)] and DTSs [Fig. 3(b)]. In addition, high magnification micrographs revealed that the characteristic banding patterns of native collagen were well preserved after decellularization treatment [Fig. 3(c,d)].

Tensile mechanical testing
Tensile mechanical testing demonstrated that the UTS of the DTSs (29.68 ± 6.73 MPa) was retained 85.62% of the control (34.47 ± 3.47 MPa) and the difference was not statistically significant (p > 0.05, Table I). The percent strain at UTS of the DTSs (19.16% ± 4.58%) was 123.61% of the control (15.50% ± 3.12%) and no statistical significance was found between two groups (p > 0.05, Table I). Elastic modulus was reduced in the DTSs (210.68 ± 46.43 MPa) to 70.29% of the control (299.71 ± 41.67 MPa) and the difference was statistically significant (p < 0.05, Table I). The stiffness (N mm⁻¹) of the DTSs was 27.40 ± 8.66 N mm⁻¹ and similar to that of the control at 25.50 ± 3.71 N mm⁻¹ (p > 0.05, Table I).

Analysis of retained biochemical composition in the DTSs
The results of two critical proteoglycans and four different growth factors retained in the DTSs by ELISA assays are shown in Table II. No significant differences were found in the effect of repetitive freeze/thaw in combination with nuclease treatment 12 h on the contents of Fmod, Bgn, as well as TGF-β1, IGF-1, VEGF, and CTGF in lyophilized DTSs when compared to the control (p > 0.05). Compared to NTSs, the content of Fmod in the DTSs decreased to 96.83%, Bgn to 93.75%, TGF-β1 to 97.28%, IGF-1 to 93.74%, VEGF to 96.28%.

In vitro cytotoxicity and cell repopulation of the DTSs
Compared with exposed to media alone (n = 10, control), significant higher cell viability of NIH-3T3 fibroblasts exposed to the DTSs was determined using alamarBlue®
(absorbance at $\lambda = 570$ nm) when the cell-DTSs contact was maintained for 24 and 48 h ($p < 0.05$). Although no significant difference was found at 72 h incubation, higher cell viability was also observed in DTSs group when compared with the control (Fig. 4).

As shown in Figure 5(a,d), the SEM analysis revealed that fibroblasts attached well to the surface of the DTSs after only 5 h of incubation. It is interesting that the fibroblasts seeded on the DTSs showed good proliferation after the longer period of incubation [Fig. 5(b,c,e,f)]. Especially, the fibroblasts formed dense cell sheets on the surface of the DTSs at the end of 4 days of incubation [Fig. 5(c,f)].

The NIH-3T3 fibroblasts labeled with PKH26 were observed as red under the phase contrast fluorescence inverted microscope (Olympus, Japan) [Fig. 6(b)]. Coupled with the nuclei staining of DAPI [Fig. 6(a)], exogenously added fibroblasts [Fig. 6(c)] were easily distinguished from the DTSs [Fig. 6(d)]. Although most fibroblasts were distributed throughout the surface of the DTSs [Fig. 6(e,f)], a few cells were found within the DTSs as well [Fig. 6(f)]. Obviously, repetitive freeze/thaw in combination with nuclease treatment 12 h of the tendon slices facilitated fibroblasts attachment, adhesion and repopulation of the DTSs.

**DISCUSSION**

Biologic scaffolds, composed of ECM, have been increasingly used in regenerative medicine and tissue engineering. Theoretically, decellularized ECM derived from the same type of tissue is the ideal scaffold for engineering a new tissue. Various decellularization methods have been investigated for preparing acellular tendon scaffolds.7–12,14–16 Minimal

**TABLE I. Tensile Testing: NTSs Versus DTSs (mean ± SD)**

<table>
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<th>NTSs</th>
<th>DTSs</th>
<th>% of NTSs</th>
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<td>UTS (MPa)</td>
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<td>Elastic modulus (MPa)</td>
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<td>210.68 ± 46.43*</td>
<td>70.29</td>
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<tr>
<td>Stiffness (N mm⁻¹)</td>
<td>25.50 ± 3.71</td>
<td>27.40 ± 8.66</td>
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* Signifies a $p$ value of $<0.05$ as compared to NTSs.
disruption of tendon ECM ultrastructure and composition during tendon tissue processing is typically desirable. In addition, one of the critical problems is that after the cell component was completely removed, the reseeded cells of the intact decellularized tendon scaffold were limited to the scaffold’s surface and difficult to migrate into the intact acellular tendon scaffold.\(^{10}\) The studies on multilayer tendon slices and sandwich model of engineered cartilage provided a good strategy for addressing this problem.\(^{13,15}\) However, the tendon slices of 50 \(\mu\)m in the multilayer composite may not be able to withstand early motion and rehabilitation after implantation.\(^{15}\) Our previous study on the mechanical characteristics of native tendon slices with different thickness found that a tendon slice of 300 \(\mu\)m was the thinnest to conserve the mechanical properties of normal tendon bundles.\(^{16}\) In this study, we focused on the tendon slices of 300 \(\mu\)m and successfully developed a decellularized tendon slice scaffold, in which all cellular and nuclear materials were efficiently removed while the intrinsic ultrastructure and mechanical properties of the native tendon tissues were basically preserved. More importantly, the DTSs retained specific proteoglycans and growth factors of the native tendon ECM. Furthermore, the DTSs were confirmed to have excellent cytocompatibility and facilitate attachment and repopulation of NIH-3T3 fibroblasts. Overall, DTSs are sheet scaffolds with a combination of elemental mechanical strength and tendon ECM bioactive factors that may have many potential applications in tendon tissue engineering.

Multiple freeze/thaw treatment has been utilized to produce a variety of decellularized biologic scaffolds, including nerve,\(^{22}\) dermis,\(^{23}\) embryoid bodies,\(^{19}\) and tendon,\(^{7}\) and so forth. Those studies demonstrated that physical cell disruption was a mild decellularization treatment that preserves tissue components for successful tissue repair, but used alone, such method was not capable of completely removing cellular materials.\(^{19,24}\) The results of the current study indicated that repetitive freeze/thaw alone did not allow the efficient removal of DNA (removing only 28.79% of DNA content in Fig. 1), and increasing nuclease treatment time significantly reduced the remaining DNA content (\(p < 0.05\)). Hence, repetitive freeze/thaw in combination with nuclease treatment 12 h was selected as a decellularization method for tendon slices. H&E and DAPI staining also showed that such treatment can effectively remove the cellular and nuclear materials. The results of histology and SEM analysis confirmed that such treatment can preserve the dense collagen structure of native tendon tissue. Furthermore, the resulting mechanical integrity of DTSs was found to have mechanical properties similar to NTSs, with the exception of elastic modulus. Especially, the tensile strength of DTSs was retained 85.62% of NTSs. Relatively high retention (70.29% of NTSs) of elastic modulus of DTSs was obtained and the actual value of elastic modulus was much higher than that of gel/sponge scaffolds in other studies.\(^{25,26}\) According to our in vivo results, the current mechanical properties of DTSs were sufficient for the needs of tendon repair (unpublished data).

Encouragingly, in the current study, the DTSs did preserve more than 93% Fmod and Bgn as well as four growth factors of the NTSs. We selected Fmod and Bgn as aforementioned four growth factors for analysis because they may play a very important role in tendon tissue engineering, although the mechanisms are not completely understood. Bi et al.\(^{27}\) demonstrated that Fmod and Bgn as two critical components of the tendon stem cell niche, their absence could alter the fate of the tendon stem/progenitor cells from tenogenesis to osteogenesis. That study reminds us that the preservation of Fmod and Bgn in the acellular tendon ECM plays a crucial role for the development of stem cells-based tendon tissue engineering. In addition, we believe that the preservation of growth factors in the acellular tendon ECM is also very important. Involved in almost every stage of tendon healing process, growth factors stimulate cellular proliferation, differentiation, ECM deposition as well as tissue ingrowths.\(^{28}\) For instance, TGF-\(\beta\)1, IGF-1, VEGF, and CTGF all can promote cellular migration and proliferation as well as collagen synthesis.\(^{29-32}\) Also, TGF-\(\beta\)1, VEGF, and CTGF can promote angiogenesis during the

<table>
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<th>Group (n = 6)</th>
<th>Proteoglycans</th>
<th>Growth Factors</th>
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<tr>
<td></td>
<td>Fmod (ng mg(^{-1}))</td>
<td>Bgn (pg mg(^{-1}))</td>
</tr>
<tr>
<td>NTSs</td>
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<tr>
<td>DTSs</td>
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**TABLE II. Effect of Repetitive Freeze/Thaw in Combination With Nuclease Treatment 12 h on the Contents of Two Proteoglycans and Four Growth Factors in Lyophilized NTSs (mean ± SD)**

**FIGURE 4.** The viability of NIH-3T3 fibroblasts cultured in direct contact with the DTSs at 24, 48, and 72 h as determined by the alamarBlue® assay. *, signifies a value of < 0.05 as compared to the control.
FIGURE 5. SEM micrographs of NIH-3T3 fibroblasts attachment to the surface of the DTSs after 5 h (a and d), 1 day (b and e) and 4 days (c and f) of culture. Scale bar = 200 μm in images a–c; Scale bar = 50 μm in images d–f.

FIGURE 6. Micrographs of PKH26 labeled NIH-3T3 fibroblasts attachment and repopulation of the DTSs after 2 days of culture in vitro. (a) Fibroblasts were stained with DAPI. (b) Fibroblasts were labeled with PKH26. (c) Overlap of (a) and (b). (d) The DTSs were observed by phase contrast inverted microscope. (e) Overlap of (c) and (d). Additionally, the cell-seeded DTSs were stained with H&E to demonstrate successful repopulation after 4 days of culture in vitro (f). Solid arrow indicates most fibroblasts attached to the surface of the DTSs, and dotted arrow indicates a few cells ingrew to the DTSs. Scale bar = 50 μm in images a–e; Scale bar = 100 μm in image f. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
remodeling process. Additionally, previous studies found that TGF-β1 binds to both Bgn and Fmod, and the decreased sequestration of TGF-β1 within the ECM of Bgn -/-/Fmod -/- mandibular condylar chondrocyte lead to overactive TGF-β1 signal transduction, which can induce chondrogenesis and ECM turnover. In fact, these growth factors are a diverse group of signal molecules whose effects are intricate and overlapping, and whose action is often dependent on dose, temporal expression, interaction with other growth factors, and even spatial distribution at the injury site. The present study indicated that repetitive freeze/thaw in combination with nuclease treatment 12 h has slight effect on the contents of these growth factors. Compared to the NTSs, the content of TGF-β1 in the DTSs decreased to 97.28%, IGF-1 to 93.74%, VEGF to 96.28%. Interestingly, the content of CTGF after decellularization treatment unexpectedly rose, which may be caused by the process of pul- verization and homogenate or unavoidable measurement error in test procedure. Although there were no relevant data to support our biochemical results obtained in the present study, we believe our findings have provided some new important information to the field of tendon tissue engineering.

Another encouragingly, the results of cytotoxicity and cell repopulation evaluation showed the DTSs have excellent cytocompatibility. An unexpected finding is that the current DTSs showed no cytotoxic effect on fibroblasts and facilitated the proliferation of NIH-3T3 fibroblasts, as seen by cytotoxicity and SEM assay. A reasonable explanation is that the DTSs scaffold retained proteoglycans and growth factors, which may play a facilitating role in the growth and proliferation of fibroblasts. They were expected to play greater roles in the future studies of stem cells-based tendon tissue engineering.

Although the present study was conducted upon single tendon slice, these DTSs not only can be used to construct multilayer tendon slice scaffold for tendon tissue engineer- ing with the variability in size and the feasibility of allowing for much more cell seeding or ingrowth than an intact decellularized tendon scaffold, but also may work well as a biological healing material for surgical repair due to its ele- mental mechanical strength and well-preserved tendon ECM bioactive factors.

CONCLUSIONS
DTSs are thin sheet scaffolds with elemental mechanical strength that preserve the inherent ultrastructure of native tendon tissue and retain specific proteoglycans and multiple growth factors of tendon ECM. These growth factors are present in the DTSs including TGF-β1, IGF-1, VEGF, and CTGF which may promote remodeling of the scaffold mater- ial when applied for tendon tissue engineering. The DTSs also have excellent cytocompatibility and facilitate attach- ment and repopulation of NIH-3T3 fibroblasts. Further study regarding the potential of this scaffold for tendon tissue engineering, including the bioinductive property in vitro/in vivo and the performance to tendon tissue repair in situ should be explored in the future.

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