Decellularized Tendon Slices With An Inductive Microenvironment For Enhancing Rat Tendon-derived Stem Cells Proliferation And Tenogenic Differentiation

Liangju Ning, grad student, Yajing Zhang, Yi Zhang, Quan Qing, Yanlin Jiang, Jieliang Yang, Jingcong Luo, Tingwu Qin.
Sichuan University, Chengdu, China.


Introduction: The extracellular matrix (ECM) microenvironment for the stem cell niches, including but not limited to the biochemical composition, matrix topography, and stiffness, is crucial to stem cell proliferation and differentiation. Hence, preservation of native ECM microenvironment during the process of tissue decellularization is highly desirable. Recently, our previous work has developed a thin sheet ECM scaffold: decellularized tendon slices (DTSs), which preserved the elemental mechanical strength, the inherent ultrastructure of native tendon tissue and retained specific proteoglycans and multiple growth factors of tendon ECM [1]. However, it remains an unanswered question whether the DTSs that preserved these native tendon ECM microenvironment cues possess the capacity to promote stem cell proliferation and tenogenic differentiation. The current study aims to investigate the effect of the DTSs with the native tendon ECM microenvironment cues on rat tendon-derived stem cells (TDSCs) viability and proliferation, cell morphology and alignment, and tenogenic differentiation.

Methods: As previously reported [1], the DTSs were fabricated using repetitive freeze/thaw of the intact tendons, frozen section as well as nuclease treatment. Firstly, the surface topography of the DTSs was characterized by scanning electron microscopy (SEM). The average collagen fibril diameter was measured from the high resolution SEM micrographs (at a magnification of 40000×) by Image J software. Then, to characterize the nanomechanical properties of the microenvironment provided by the DTSs, the stiffness (as measured by the Young’s modulus) measurements were performed using an atomic force microscopy (AFM). Finally, the identified TDSCs were cultured on the DTSs and the effect of the DTSs on cell viability and proliferation, cell morphology and alignment, and tenogenic differentiation was determined by LIVE/DEAD assay, alamarBlue® assay, SEM examination and qRT-PCR analysis.

Results: The SEM examination (Fig.1a, b) and AFM measurements (Fig.1c) revealed that the DTSs possess the native tendon ECM microenvironment cues, including the inherent surface topography and similar stiffness to native tendon. When the identified TDSCs were cultured on the DTSs, it can be seen obviously that these cells showed a high rate of viability on the surface of DTSs (Fig.2a). Moreover, alignment of these live cells on the DTSs was apparent from the results of LIVE/DEAD staining (Fig.2a). AlamarBlue® assay revealed that significant higher cell viability of TDSCs was determined when the cells were seeded on the DTSs for 2 d (Fig.2b), as compared with media alone. The SEM micrographs indicated that TDSCs were well attached on the surface of the DTSs and elongated spindle morphology and were aligned along the direction of collagen fibrils at 1d (Fig.2c). As shown in Fig.3, the tendon-specific markers, SCX, TNMD and THBS4, were highly expressed in the TDSCs-DTSs group rather than the pure TDSCs group (Fig.3a, b and c). Additionally, the tendon-related genes, COL I and III, exhibited relatively higher levels in the TDSCs-DTSs group at 7 d and 14 d (Fig.3 d, e).
**Discussion:** Combined with our previous work [1], the DTSs were found to retain the native tendon ECM microenvironment cues, including the inherent surface topography, well-preserved tendon ECM biochemical composition and similar stiffness to native tendon. As expected, when seeded on the DTSs, TDSCs showed homogeneous distribution and alignment along the direction of collagen fibrils of the DTSs, and high cell viability on the DTSs. Also, alamarBlue® assay demonstrated that the DTSs promote the proliferation of TDSCs. These results further imply that those proteoglycans and growth factors retained in the DTSs may have biological activity and play a facilitating role in the growth and proliferation of TDSCs. Another encouragingly, the gene expression results clearly indicated that the DTSs provide an inductive microenvironment for the tenogenic differentiation of TDSCs based on the tendon-specific gene expression.

**Significance:** The present study further supports the use of decellularized tendon ECM as a promising and valuable approach for tendon repair/reconstruction.

![Graph](image)

**Fig.1** (a) SEM characterization of the surface topography. (b) The average collagen fibril diameter (p>0.05). (c) Stiffness measurements (p>0.05).
Fig. 2. (a) LIVE/DEAD assay, (b) AlamarBlue® assay (*, p < 0.05), (c) SEM characterization. The red dotted arrows indicate the orientation of collagen fibers of the DTSs. The yellow solid arrows indicate the elongation and alignment of the TDSCs on the DTSs.

Fig. 3. qRT-PCR analysis of tenogenic-related gene expression of the TDSCs seeded on the DTSs at different time points. **p < 0.01.