Regeneration of Achilles Tendon: The Role of Dynamic Stimulation for Enhanced Cell Proliferation and Mechanical Properties

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Statement of Purpose: Tendons and ligaments showed closely packed collagenous bundles oriented parallel to the longitudinal axis of each structure, which was considered as viscoelastic materials. Recently, the application of cyclic tensile stimulation using a bioreactor has attracted much interest in the tendons and ligaments tissue engineering as one of the essential factors. In this study, tenocytes were seeded on rubber-like elastic PLCL [poly(L-lactide-co-ε-caprolactone)] scaffolds and cultured under the static and dynamic conditions. The effect of dynamic tensile stimulation was assessed.

Methods: Rod-type PLCL scaffolds (5 mm in diameter, length 5 cm) with porosity of 83% were prepared by an extrusion-salt leaching method. Tenocytes from New Zealand White rabbits were seeded at a density of 1×10^6 cells/scaffold. Scaffolds were loaded on a bioreactor providing cyclic tensile extension for the dynamic culture, whereas scaffolds were also cultured in Petri dishes for the static culture. After 2 and 4 weeks, tissues were harvested and characterized in terms of cell proliferation, mechanical properties, and cellular or ECM distribution.

Results: The DNA content was quantified to evaluate cell proliferation. The DNA concentration of cultured PLCL scaffolds increased with culture time (Figure 1). The level of DNA amount elevated dramatically in the dynamic culture system, compared with the static culture system with a statistically considerable difference. This enhanced cell proliferation in dynamic culture should be only attributed to the effect of dynamic mechanical stimulation.

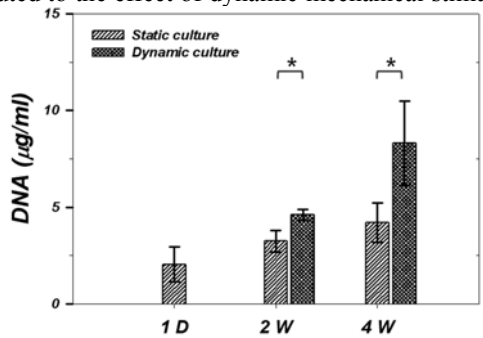

*Fig. 1. Tenocyte proliferation under static or dynamic culture (1 day, 2 weeks, and 4 weeks), *P<0.05*

The ultimate tensile stress under static or dynamic conditions decreased progressively with culture time (Figure 2) due to the degradation. The scaffolds from the static culture exhibited all the stress values smaller than the controls did indicating the contribution of cells for degradation. However, in the dynamic culture larger amount of ECM and neo-tissue produced by more cells compensated the mechanical reduction to some extent.

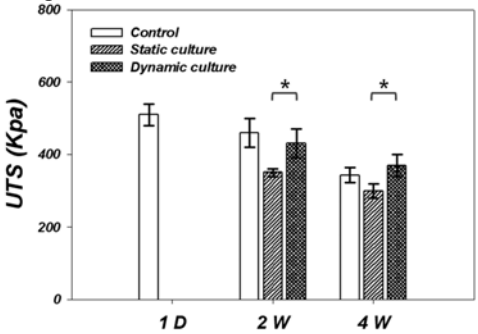

*Fig. 2. Ultimate tensile stress of PLCL scaffolds before and after cell culture, *P<0.05*

H&E staining revealed the numbers and distribution of tenocytes cultured in the scaffolds (Figure 3). The specimens from the dynamic culture system exhibited cells more densely and more widely, compared to those from the static culture. Therefore, the advantage using mechanical stimulation during cell culture was confirmed.

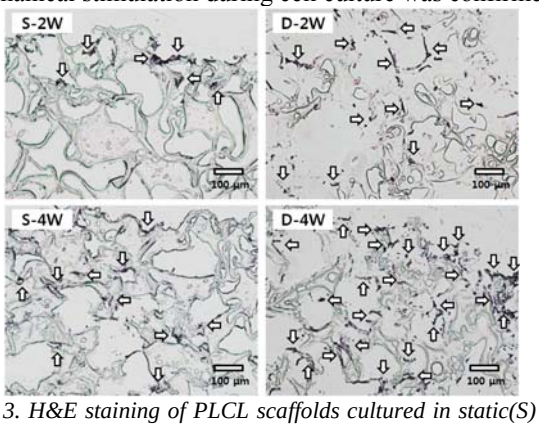

Fig. 3. H&E staining of PLCL scaffolds cultured in static(S) or dynamic(D) system for 2 and 4 weeks (Arrows indicate the cluster of cell/ECM constructs.)

Conclusions: The mechanical stimulation played a crucial role in the regeneration of tendon tissue with respect to the enhanced cell proliferation, increased secretion of ECM and mechanical properties. In conclusion, the dynamic tensile stimulation appeared to be an essential factor in tendon / ligament tissue engineering and elastic PLCL copolymer would be very beneficial to conduct such an investigation.

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