**IN VITRO TENDON ENGINEERING USING TENOCYTES AND POLYGLYCOLIC ACIDS**

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**Introduction:** Tendon defect remains a major concern in plastic and hand surgery, due to the lack of tissue source for tendon grafting. We have previously demonstrated that tendon tissues that resemble natural tissue in gross and histological structures and biomechanical properties could be generated in a hen model using polyglycolic acid (PGA) and isolated tenocytes [1]. This study explored the possibility of engineering tendon in vitro using PGA and hen tenocytes.

**Methods:** Tenocytes were isolated from flexor tendons of Leghorn hens by 0.25% trypsin and 0.1% collagenase II digestion, and in vitro expanded to passage 2. The cells were then collected and resuspended in the culture medium. Total 2.0 × 10^7 cells were seeded onto unwoven PGA fibers that were shaped into a cord-like structure to form a cell-scaffold construct followed by culturing in DMEM plus 20% FBS. One week later, both ends of the constructs were fixed on a U-shaped spring as the experimental group (n=16), and the distance between two ends of the springs was carefully adjusted to match the exact length of the construct. Thus, a physiologically static strength was allowed to generate inside the construct by the contraction of cell-extracellular matrix during tendon formation. Media were changed three times a week. In control group, the PGA constructs (n=3) were generated and cultured in vitro similarly to the experimental group except that no cells were seeded. Specimens of the experimental group were harvested at 4 (n=6), 6 (n=7) and 10 (n=3) weeks respectively for HE and Masson staining, and mechanical analysis. Specimens of 4 weeks were also evaluated with immunohistochemistry (IHC) analysis. Group t-test was used for statistical study and p<0.05 was considered as significant.

**Results:** Specimens of the experimental group demonstrated that neo-tendon tissue was formed at 4 weeks. HE and Masson staining revealed collagen fiber formation along with partially degraded PGA fibers. IHC also showed positive staining of type I collagen. At 6 weeks, neo-tendons became a little thinner than those of 4 weeks (2.2mm ± 0.39mm vs 2.5mm ± 0.28mm in diameter). Histology showed tenocytes and collagen fibers were longitudinally aligned, and more PGA fibers were degraded than before. At 10 weeks, however, the diameter of the specimens (2.0mm ± 0.34mm in diameter) was similar to that of 6 weeks. HE staining demonstrated that the tissue structure of engineered tendon was similar to that of normal tendon, including similar tenocyte/collagen ratio and a wave-like, longitudinal pattern of collagen fibers. In addition, PGA fibers were mostly degraded at 10 weeks. In contrast, PGA cord-like structures in the control group were broken down weeks, and completely degraded at 10 weeks. Biomechanically, the tensile strength (Newton/mm²) of the experimental specimens of 6 weeks was stronger (0.473 ± 0.162) than that of 4 week specimens (0.281±0.107, P < 0.05), but not significantly different from that of 10 week specimens (0.486 ± 0.201, P > 0.05).

**Discussion and Conclusions:** The advantages of in vitro tendon engineering are: (1) avoiding implant GPA fibers in vivo directly, which can cause localized pain and aseptic inflammation by the acidic degraded product; (2) in vitro engineered tendon is stronger than the cell-scaffold construct and therefore, is better for tendon repair and functional recovery.

The results show that in vitro engineered tendons resemble natural tendon grossly and histologically. Nevertheless, they remain relatively weaker than in vivo engineered tendon [1], suggesting that a cyclic strain rather than the static strain may potentially increase the tensile strength of in vitro engineered tendon.


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**Biomechanical analysis of in-vitro engineered tendon, indicating statistical significance between 4 weeks and 6 weeks specimens (P < 0.05), while no statistical significance between 6 weeks and 10 weeks specimens (P > 0.05).**