Woven Electrochemically Aligned Collagen Scaffold Guides Tenogenesis of Mesenchymal Stem Cells

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Statement of Purpose: Repair and regeneration of injured tendons would benefit greatly from a mechanically robust and bioinductive scaffolds platforms [1]. Previous studies showed that electrochemical processing of collagen solutions generates compact and aligned collagen threads (ELAC) which can induce tenogenic differentiation of MSC's *in the absence* of growth factors [2-4]. While ELAC threads are promising candidates for tendon repair, the next challenge is to weave ELAC threads in an implantable 3D-scaffold form. Weaving process requires production of ELAC threads in sufficient length. To this end-point, the aims of this study were: a) fabricate ELAC threads in continuous length, b) weave such threads as 3D scaffolds with mechanical robustness, c) assess the differentiation and matrix production in woven scaffolds by mesenchymal stem cells (MSCs).

Methods: *Continuous collagen fiber production:* We invented a rotating electrode electrochemical alignment machine (REEAM) to fabricate the fibers. (Fig. 1a). The device consists of a syringe pump and a rotating electrode. Collagen solution applied to the electrode wheel transforms to solid state and collected by a spool. The fibers were treated in phosphate buffered saline and crosslinked in genipin [2]. Three threads (Fig. 1b) were twisted as a yarn (Fig. 1b), and the yarns were woven as scaffolds (5x1.5x15 mm) (Fig. 1c&d). The threads, yarns and scaffolds were subjected to tensile tests to determine the consistency of the strength and stiffness. Scaffolds were woven to match the size of the rabbit infraspinatus which was also tested in tension as a positive control.

Cell culture: Passage 5 hMSCs (Lonza) were seeded on scaffolds (250,000/scaffold). The type of matrix laid by cells was investigated at day 35 by immunohistology for collagen I and tenomodulin, a tendon specific molecule.

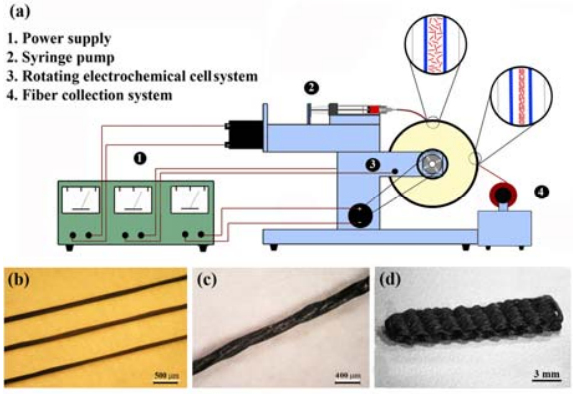

Fig.1. Schematic of scaffold fabrication: component layout of REEAM (a), aligned collagen fibers (b), triple twisted yarn (c), and scaffold woven by using the yarn (d). The dark coloration results from genipin.

Results: Grouping three threads as yarns improved the tensile strength and modulus of collagen threads by 75% and 100%, respectively (Fig. 2a and 2b). The woven scaffold displayed a tensile strength of 60 N (Fig. 2c), a value that was 60% of the intact rabbit tendon's strength. Cells were uniformly distributed across the continuum of the scaffold after 35 days, with actin filaments oriented along the longer axes of collagen threads (Fig. 2a). Immunohistology confirmed that the type-I collagen and tenomodulin were present in the scaffold (Fig. 2b&c), indicating a tendon-like tissue deposition. Cells made two types of collagen matrix: 1) thin fibrils in the pore space (Fig 2b); 2) thick d-banded fibers on ELAC threads (Fig. 3b). The collagen in ELAC threads is organized as thin fibrils (Fig. 3a) and is therefore distinguishable from that made by cells (Fig. 3b).

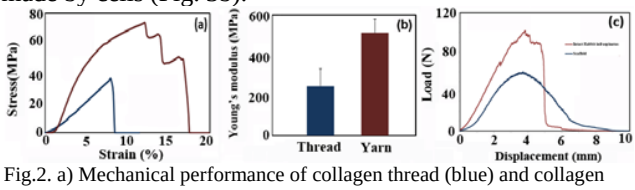

Fig.2. a) Mechanical performance of collagen thread (blue) and collagen yarn (red). b) Young's moduli of single thread (blue) vs. yarn (red). (c) Structural strength of woven scaffold (blue) and intact rabbit infraspinatus tendon (red).

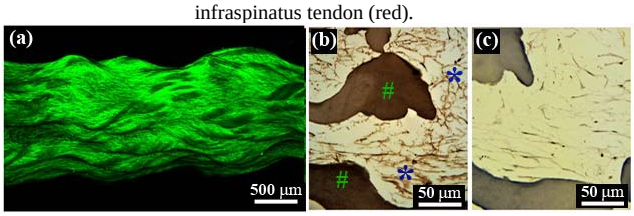

Fig. 3. a) Cellular cytoskeleton at Day 35 illustrated by actin staining. b) Immunohistology for Collagen I (green cross shows collagen thread in scaffold and blue star show collagen made by cells in scaffold holes(b) and tenomodulin (c).

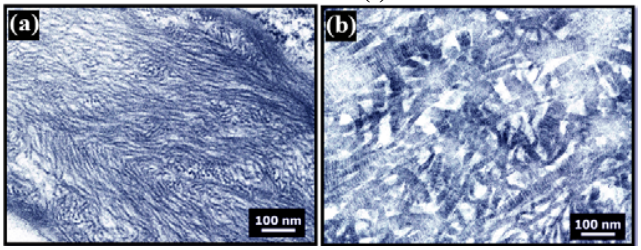

Fig. 4. TEM images of collagen phase in ELAC threads (a) vs. cell made matrix (b).

Conclusions: Electrochemically aligned collagen threads can be woven in a 3D scaffold form whose mechanical behavior converges to that of the real tendon in terms of strength, stiffness, toe-in region and extensibility. The earlier observations on the tenoinductive effect of ELAC at the expression level [4] is confirmed by the present results at the matrix synthesis level. Attaining tenoinduction without using growth factors and on a mechanical competent platform renders this woven scaffold concept a promising candidate for tendon tissue engineering.

References:

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