

Mechanical Characterization of Fibrous Composite Material Made of Collagen and Elastin

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INTRODUCTION: Main structural components of most of load-bearing soft tissues are collagen and elastin. For example, in tendon and ligament, collagen demonstrates a high elastic modulus (~1 GPa) and accounts for approximately 90% of the tissue dry weight, and its fibers are aligned to the long axis of the tissue. On the other hand, elastin demonstrates a lower elastic modulus (~0.6 MPa) but a greater extensibility and accounts for several percent of the tissue dry weight, and elastin fibers are found between collagen fibers as well as in the matrix between collagen fiber bundles. With these mechanical and structural characteristics, tendons and ligaments exhibit their characteristic viscoelastic mechanical properties. Because these soft tissues only possess limited healing capability, artificial tissue replacements are a promising option for surgical repair. To date, many experimental studies were conducted on the development of collagen-based biomaterials. However, only few studies have reported the fabrication of composite biomaterials made of collagen and elastin but failed to form fibrous composite materials. Therefore, the present study has been performed to develop a method to fabricate fibrous composite materials made of collagen and elastin that benefit from mechanical behaviors of both collagen and elastin.

METHODS: Fibrous composite materials were fabricated by the integration of elastin fiber tissues made by electrospinning and collagen gel through chemical cross-linking. Elastin electrospinning was carried out with soluble elastin powder (Extracellular Matrix Laboratory, Japan), dissolved in 1,1,1,3,3,3-Hexafluoro-2-propanol at 15% (W/V), using NANON-3 electrospinner (MECC, Japan). Fabricated elastin microfiber sheet was rolled into a cylindrical shape with approximately 1 mm diameter and cross-linked using hexamethylene diisocyanate. This elastin fiber tissue was immersed in neutralized collagen sol (Koken, Japan) for 1 h at 4°C, followed by 37°C incubation for 1 h in order to complete the gelation of collagen sol. The construct was incubated in PBS containing 40% polyethylene glycol supplemented with 10 mM genipin as a chemical crosslinker at 37°C for 24 h (E+C group). Along with this construct, elastin fiber tissue only (E group), and collagen only (C group) were also prepared. Tensile strength, tangent modulus, failure strain, load relaxation rate, and hysteresis loss of the constructs were determined by quasi-static and cyclic stretching tests in a humidified condition at 37°C using a tensile tester (CellScale, Canada). The latter was performed with 10% strain amplitude at 0.5 Hz for 100 cycles. Load relaxation rate was determined from the peak load of the 1st and 100th cycle (100% equals to no relaxation). Hysteresis loss was determined as an average of the loss during single cycle calculated from the 91st to 100th cycles. Tissue fiber structure was assessed via scanning electron microscopy. Statistical analysis was performed to examine if each of the properties of E+C construct was different from E or C construct, respectively, using Welch's t-test. Statistical significance was set at $P < 0.05$.

RESULTS: Elastin microfiber sheet was successfully fabricated by electrospinning; the average diameter of microfiber was approximately 2 μm . When this was integrated with collagen gel, collagen fibers penetrated into elastin fiber network (Fig. 1). However, the integration of collagen and elastin fibers were only evident near the outer surface of the rolled elastin tissue; no collagen fibers were observed in the middle of the rolled elastin tissue. Mechanical characterization demonstrated that collagen-elastin composites (E+C group) showed significantly higher tangent modulus and lower failure strain than elastin only construct (E group), and there was no statistically significant difference in tensile strength (Fig. 2). When compared to collagen only construct (C group), the strength of E+C group were significantly lower but tangent modulus and failure strain were not significantly different. Hysteresis loss in E+C construct was not significantly different from E group but was markedly smaller compared to C group ($P = 0.06$). There was no significant difference in load relaxation rate between E and E+C and between C and E+C, respectively, although E+C was higher than C but lower than E.

DISCUSSION: We successfully fabricated fibrous composite materials made from elastin and collagen fibers, resulting in improvement of stiffness and hysteresis loss compared to the construct made by elastin only and collagen only, respectively. Therefore, the mechanical behaviors of the composite material benefit from the stiffness of collagen fibers and the elasticity of elastin fibers. However, it is one of limitations that collagen fibers were not formed in the entire elastin tissue, possibly due to the viscous nature of collagen sol. It was aimed that E+C construct would benefit more from both collagen and elastin, demonstrating high strength and stiffness as well as low hysteresis loss and load relaxation. The properties of E+C construct was still strongly influenced by the properties of elastin fibers. To achieve our aim, the amount of collagen in the composite material should be increased and there is an ongoing study to tackle this issue.

SIGNIFICANCE/CLINICAL RELEVANCE: (1-2 sentences): We created an original method to fabricate fibrous composite material made from collagen and elastin. This technique can be used to develop artificial load-bearing soft tissue such as tendon and ligament.

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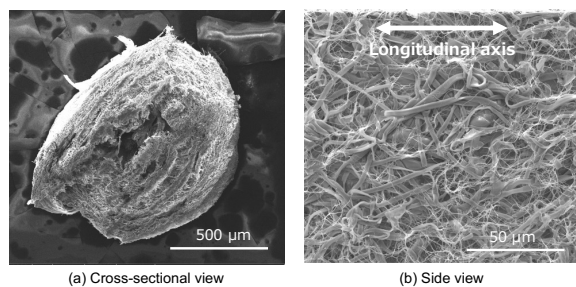


Figure 1 Photomicrographs of fibrous composite material of collagen and elastin (E+C construct) taken with scanning electron microscope. (a) Cross-sectional view and (b) side view. Elastin fibers were formed by electrospinning and showed a flattened-shape, whereas thin collagen fibers were formed via self-organization (gelation).

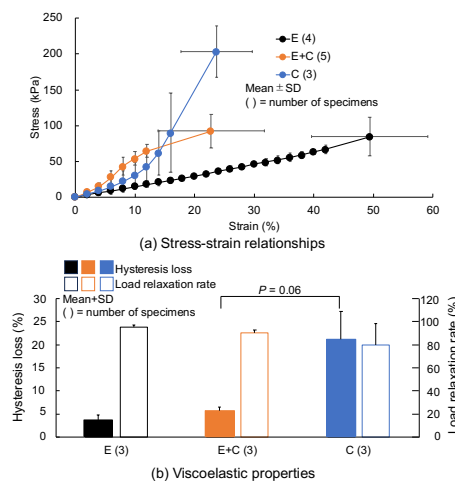


Figure 2 Mechanical properties of the constructs. (a) Stress-strain relationships obtained by quasi-static tensile tests. (b) Viscoelastic properties obtained by cyclic stretching tests.