Dehydration-Induced Strengthening, but Not Viscoelasticity, is Retained in Rehydrated Collagen Fibers

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Statement of Purpose: Because collagen plays an important structural role in many natural tissues, collagen-based biomaterials are being developed for a variety of tissue engineering applications. Collagen fibers, when incorporated within cell-seeded collagen gels, limit cellular contraction of the gels, increasing permeability and cell viability [1,2]. Including a dehydration process during the production of collagen fibers increases fiber strength [3]. Characterizing how fabrication procedures change collagen fiber properties (modulus, yield strength, and viscoelastic characteristics) is important for optimizing the creation of fibers with desired mechanical and structural properties.

Methods:

Fiber formation: A 1% (w/v) solution of Type I bovine Achilles collagen in 0.005 HCl was extruded into a polymerization buffer to form fibers [1-4]. Fibers were soaked in the buffer for 45 minutes, soaked in a 1:1 (w/v) solution of buffer and 95% ethanol for 4 hours, and cross-linked for 24 hours in a 1% (w/v) solution of 1-ethyl-3(3-dimethyl aminopropyl carbodiimide) in water. Half of the prepared fibers were air-dried in tension via simple conduction for 24 hours and then rehydrated for 12 hours before use; in this report these fibers are called "rehydrated." The remaining ("hydrated") fibers were not dehydrated, and were instead stored in phosphate-buffered (PBS) saline at 22°C for an average of 48 hours before use. Both rehydrated and hydrated fibers were obtained from a single batch of fibers to eliminate possible effects of batch-to-batch variation.

Tensile tests at variable strain rates: The ends of fibers of both rehydrated and hydrated fibers were affixed to Lexan tabs using cyanoacrylate, and kept hydrated with PBS (n = 6 of each type). The tabs were clamped into an Instron materials testing machine such that there was no measurable slack or tension within the fiber. The diameter and length of each fiber were recorded. The fibers were pulled to failure at two rates (50 mm/min and 1000 mm/min) and measurements were recorded. Fibers which failed at a point of adhesion to a tab were discarded from the data set. The resulting modulus and yield strength were calculated for each fiber.

Stress relaxation: Fibers of both types were prepared and physical measurements were recorded in the same manner as tensile testing. The clamped fibers were extended to about 10% elongation at 50 mm/min and held for 60 minutes at 22°C. Hydration with PBS was maintained throughout testing. Changes in the load carried by the fibers were recorded and used to determine how stresses in the fibers varied over time.

Results/Discussion: Tensile tests of the fibers provided evidence that the yield strength and modulus of rehydrated fibers were significantly ($P < 0.0001$ and $P=0.009$, respectively) higher than the strength and modulus of hydrated fibers. The properties of rehydrated fibers assessed in this study were not significantly rate-dependent; however, the yield strength and modulus of hydrated fibers depended on tensile test rate.

Mean Properties of Rehydrated Fibers

	1000 mm/minute	50 mm/minute	Rate Dependent?
Modulus (MPa)	1051.87	738.15	No *(P = 0.636)*
Yield Strength (MPa)	100.81	118.65	No *(P = 0.736)*
% Elongation at Yield	13.5%	12.1%	No *(P = 0.707)*

Mean Properties of Hydrated Fibers

	1000 mm/minute	50 mm/minute	Rate Dependent?
Modulus (MPa)	13.41	0.057	Yes *(P < 0.001)*
Yield Strength (MPa)	0.121	0.010	Yes *(P < 0.001)*
% Elongation at Yield	1.3%	22.6%	Yes *(P < 0.001)*

Hydrated fibers displayed stress relaxation curves characteristic of viscoelastic biological materials, indicating that hydrated collagen fibers have intrinsic viscoelastic behavior. Rehydrated fibers did not display this behavior, suggesting that in contrast to dehydration-induced strengthening, viscoelastic behavior is permanently removed in the dehydration process.

Conclusions:

Including a dehydration step in the collagen fiber fabrication process increased yield strength by approximately 800 times and increased stiffness by roughly 75 times, even though fibers were thoroughly rehydrated before testing. Rehydration did not, however, reinstate observed property rate-dependence or viscoelastic stress relaxation behavior. This information can be used in establishing collagen fiber fabrication and storing procedures to allow the production and use of fibers with predictable, desired mechanical properties.

References:

[1] Gentleman *et al.*, *Tissue Engineering* 10:421-7, 2004. [2] Gentleman *et al.*, *Biomaterials* 24:3805-13, 2003.
[3] J. Haarer, M.S. thesis, Rose-Hulman Institute of Technology, 2006.
[4] Kato *et al.*, *Biomaterials*, 10:38-42, 1989.