Effects of Hydroxyapatite Mineralization and EDC Cross-linking on the Response of Collagen Fibers to Tensile Loads

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Introduction: The ordered packing of collagen's staggered quaternary structure provides strength to the fibril, which is further enhanced by cross links and mineralization in bone[1,2]. This microstucture, accompanied by the preferred deposition of collagen fibers aligned in the tensile direction, allows the long bones to withstand physiologic tensile and flexural loading. From a biomaterials perspective, the goal is to mimick these physical characteristics in a synthetic bone analogue in order to achieve similar material properties. A hydroxyapatite-collagen (HAp/C) composite is one such biomimetic material. However, the mechanical properties of this composite are below that of bone. The current work is aimed at characterizing the effects of mineralization and cross-linking on the mechanical properties of individual collagen fibers exposed to tensile loads, in order to build a predictive model of a preferentially aligned 3D scaffold's behavior. Tensile load failure resistance will have an impact on the application of Hap/C composites as fracture fixation devices.

Methods: The effects of mineralization and cross-linking were quantified by conducting tensile tests on four groups of collagen fibers (n=20). The groups consisted of: non-mineralized, non-cross-linked fibers; non-mineralized, cross-linked fibers; mineralized, non-cross-linked fibers; and mineralized, cross-linked fibers. Collagen (10mg/mL), isolated from rat tail tendon[3], was used to form individual collagen fibers. The fibers were extruded through a 20 gauge catheter into a fiber formation buffer (pH 7.5) at 37°C following Kato & Silver[4]. Fibers were left in the buffer for 45 min followed by immersion in an isopropyl alcohol bath for 4 hours, then washed in distilled water, and air dried under tension. Half of the fibers were cross-linked in EDC for 24 hours at room temperature then washed in distilled water. Fiber mineralization was carried out in a dual chamber reactor where the collagen fibers separated calcium and phosphate solutions (both solutions were 0.1M and pH adjusted to 7.4 using Tris buffer)[5]. Tensile mechanical tests were conducted on a MTS- Bionix 100 test machine at a rate of 100%/min until failure. Fibers were tested in the wet (PBS) condition with load and displacement data recorded to determine stiffness, ultimate tensile stress (UTS), failure strain, and modulus. Prior to mechanical testing, SEM imaging was performed and EDAX measurements taken to confirm molar ratios of Ca:P at the two ends and center of the mineralized fibers.

Results: The mineralized, non-cross-linked fibers were highly fragile and could not be mechanically tested. The fibers in the other three groups were tested in tension. The two cross-linked groups (non-mineralized, cross-linked and mineralized, cross-linked) behaved in a linear manner while the non-mineralized, non-cross-linked fibers behaved nonlinearly, with a point of yielding followed by plastic deformation. The fiber dimensions and results of the mechanical tests are tabulated in Table 1. Gage lengths ranged from 10 to 25 mm.

Table 1: Summary of Mechanical Tests

Treatment	Diameter (μm)	Stiffness (N/mm)	UTS (MPa)	Modulus (MPa)
No Mineral No Cross-Link	250	0.008	0.82	4.67
No Mineral Cross-Link	170	0.072*	7.8*	77.1*
Mineral No Cross-Link	Too Fragile for Testing			
Mineral Cross-Link	170	0.054*	2.8*†	40.6*†

The UTS, modulus, and stiffness were highest in the non-mineralized, cross-linked fibers. These fibers and the mineralized, cross-linked fibers were significantly different than the non-mineralized, non-cross-linked fibers (asterisks in Table 1). The UTS and modulus of the mineralized, cross-linked fibers were significantly different than the non-mineralized, cross-linked fibers (crosses in Table 1). However, the stiffness of these two fiber groups was not significantly different. Results from EDAX measurements showed that the molar ratio of Ca:P was approximately 60:40, with the relative intensities varying at different points along the fiber length.

Discussion: The mechanical properties of the mineralized, cross-linked fibers were comparable to similar fibers[5]. While the UTS and modulus were reduced compared to non-mineralized, cross-linked fibers, the stiffness was not affected by the mineralization. This suggests that mineralization will increase the brittleness of the cross-linked fibers. However, the increased brittleness may be a factor of the increased heterogeneity of the fiber. The mineralized fiber diameter and crystal density were not uniform along the length of the fiber, which could increase the stress gradient at the regions of diameter or density transition. Results from EDAX measurements support this hypothesis. The Ca:P molar ratio remained constant at 60% Ca and 40% P, but the relative intensity of the Ca and P signals varied along the fiber length.

Conclusions: Individual cross-linked and/or mineralized fibers can be readily produced to increase the strength of reconstituted collagen fibers, but controlling the homogeneity of the mineral deposition may have a large impact on the resultant mechanical properties. Future experiments will be aimed at studying the parameters influencing mineralization to determine their affect on mineralization homogeneity.

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