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Strong and Tough Mineralized PLGA Nanofibers for Tendon-to-bone Scaffolds

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Abstract

Engineering complex tissues such as the tendon-to-bone insertion sites require a strong and tough biomimetic material system that incorporates both mineralized and unmineralized tissues with different strengths and stiffnesses. However, increasing strength without degrading toughness is a fundamental challenge in materials science. Here, we demonstrate a promising nanofibrous polymer-hydroxyapatite system in which, a continuous fibrous network must function as a scaffold for both mineralized and unmineralized tissues. It is shown that the high toughness of this material system could be maintained without compromising on the strength with the addition of hydroxyapatite mineral. Individual electrospun poly(lactide-co-glycolide) (PLGA) nanofibers demonstrated outstanding strain-hardening behavior and ductility when stretched uniaxially, even in the presence of surface mineralization. This highly desirable hardening behavior which results in simultaneous nanofiber strengthening and toughening was shown to depend on the initial cross-sectional morphology of the PLGA nanofibers. For pristine PLGA nanofibers, it was shown that ellipsoidal cross-sections provide the largest increase in fiber strength by almost 200% compared to bulk PLGA. This exceptional strength accompanied by 100% elongation was shown to be retained for thin and strongly bonded conformal mineral coatings, which were preserved on the nanofiber surface even for such very large extensions.

Keywords

tissue engineering; bone; tendon-to-bone insertion; electrospinning; mechanical properties

1. Introduction

Musculoskeletal tissue function relies upon matrices of protein nanofibers that often heal poorly. Biocompatible nanofibrous mats produced through electrospinning offer a promising biomimetic, hierarchical platform for engineering of several musculoskeletal tissues [1]. However, their application to tissues such as the attachment of tendon-to-bone has lagged because of fundamental challenges in both physiology and materials science [2]. From the perspective of physiology, the tendon-to-bone attachment involves a gradient of mineral concentration [3–5] that stiffens a continuous underlying protein network by up to 100× [6,7]. From the perspective of materials, stiffening via mineralization must overcome a fundamental challenge in materials science: increasing strength and stiffness without compromising ductility and toughness.

We studied the mechanics of individual unmineralized (pristine) and mineralized nanofibers isolated from nonwoven electrospun poly(lactide-co-glycolide) (PLGA) mats. The experiments revealed mechanisms by which these fibers could be made strong and tough, and could maintain these advantageous properties upon mineralization. These mats can accommodate a graded conformal surface mineral coating analogous to that observed at the attachment of tendon to bone, and are suitable for culturing appropriate cell populations [8]. While the structural features of a mat, such as fiber density, orientation and cross-linking define its stiffness [9,10], the ultimate strength and toughness depend strongly on the properties of the individual nanofibers [11]. Our experiments have shown that mechanical properties of individual nanofibers of amorphous and semicrystalline polymers processed by electrospinning could be significantly different from those of the same polymers fabricated in bulk [12]. Thus, understanding the structural mechanisms of nanofiber deformation at the nanoscale is critical for designing functional materials at the macroscale, as the existing design rules for polymers at the macroscale are not applicable to nanofiber scaffolds.

We found that certain nanoscale structural features endowed fibers with ductility and strain-hardening far in excess of that possible for micron scale and bulk scale PLGA fibers. We identified nanofiber and mineral coating morphologies that could potentially be tailored to retain most of the beneficial elastic-plastic behavior, stiffening and strengthening of pristine PLGA nanofibers after coating with a brittle mineral. Our data show that the strength, stiffness and toughness of PLGA nanofibers are dependent on their cross-sectional and mineral coating morphologies as well as the polymer-mineral interfacial interaction. The data reported in this paper have important implications on the design of novel tissue engineering scaffolds whose functionality, strength and toughness could be tuned by morphological control.

2. Materials and Methods

2.1. Fabrication of Pristine and Mineralized PLGA Nanofiber Scaffolds

The method of electrospinning was applied to produce thick or sparse PLGA nanofibrous mats on metallic collectors. PLGA ($M_w \approx 50\text{--}75\text{k}$, 85:15 lactide/glycolide) was dissolved in an 80/20 mix of dichloromethane (DCM) and dimethylformamide (DMF) to result in a concentration of 25% w/v. This solution was loaded into a 5ml plastic syringe with a 22 gauge needle and was pumped at a flow rate of 0.5ml/hour using a syringe pump. A high voltage power source was used to apply 15 kV between the needle and a flat piece of aluminum foil. After the fiber deposition stabilized, either an aluminum wire mesh or copper TEM grids attached with carbon tape to the surface of a glass slide, were briefly (<10 sec) positioned between the needle tip and the collector. All samples were treated with a plasma cleaner (Harrick Plasma PDC-001) for 8 minutes at 10.5W. The resulting PLGA nanofibers had one of three morphologies: (a) uniform circular cross-section with sparse surface

irregularities, **(b)** uniform ellipsoidal cross-section, and **(c)** non-uniform/rough cross-section in the entire nanofiber length. Therefore, for data analysis the nanofibers were grouped according to their morphology. A subset of nanofiber mats were mineralized according to previously described methods [8]. Mineralized mats were soaked for 30 min in simulated body fluid containing 10 times more calcium and phosphate ions than usual (10SBF) without sodium bicarbonate. The mats were then mineralized in complete 10SBF for 5–30 min, and subsequently washed in an orbital shaker for 6 h with deionized water to remove any soluble ions and crystals not attached to the scaffolds [13]. As previously reported in [8], increasing the time of mineralization led to increased thickness of the mineral coating. The mineral was deposited in two stages: first, the mineral formed a conformal layer around the polymer fibers, and then plate-like mineral crystals began to form on the conformal layer. The morphology of the mineral coatings on the PLGA nanofibers studied in this work could be categorized as: **(a)** thick-platelet mineral coating on top of an inner conformal layer of mineral, **(b)** thick-conformal mineral coating, and **(c)** thin-conformal mineral coating. This morphological classification was followed in the data analysis. 150–250 μm long segments of all of the aforementioned types of PLGA nanofibers were isolated from thick/sparse fiber mats on TEM grids for mechanical testing. Various factors affect the morphology of pristine and mineralized PLGA nanofibers processed by the aforementioned methods. While efforts are underway for better control to synthesize the most mechanically advantageous nanofiber cross-sections and mineral morphologies, in the current study we only take advantage of the inherent variability in morphologies to demonstrate the mechanical consequences of various fiber morphologies.

2.2. Mechanical Testing Methodology

Microscale tension experiments were performed on individual pristine and mineralized PLGA nanofibers using a Microelectromechanical System (MEMS) based method described in [12,14,15], which provided independent, time-resolved, measurements of the applied force and fiber extension with resolutions of ~ 50 nN and 25 nm, respectively. Nanofibers were mounted onto the MEMS devices using an epoxy adhesive and were tested at nominal strain rates of $0.01\text{--}0.03\text{ s}^{-1}$, under an optical microscope at $200\text{--}500\times$ optical magnification. The original and the deformed morphologies of each nanofiber were obtained after completion of each experiment using a field emission scanning electron microscope (FE-SEM, Hitachi S-4800). A thin layer (~ 5 nm) of gold-palladium alloy was sputter deposited onto all fibers after the completion of the mechanical testing to assist during electron microscopy. The original fiber morphology was obtained from a segment of each fiber, which was outside the test gauge section and was not loaded during testing.

3. Results and Discussion

3.1. Mechanical Response of Pristine PLGA Nanofibers

The nanofiber cross-sectional morphology dictated the stress-strain response of pristine PLGA nanofibers. Representative stress-strain curves of nanofibers with uniform circular cross-sections, Figure 1(a), displayed long plateau stress regions and no strain-hardening as pointed by the arrows in Figures 1(a–c). The latter, being the result of molecular entanglements is an advantageous component in the stress-strain curves of glassy polymers, which leads to outstanding mechanical toughening. The elastic modulus and yield strength of the fibers, Table 1, were comparable to that of bulk PLGA [16,17], but unlike bulk PLGA, individual PLGA nanofibers supported unusually large ductilities reaching $\sim 150\%$. In general, bulk PLGA fibers are brittle and fail at ~ 50 MPa stress with elongations smaller than 5% and without strain-hardening [16]. However, it has been reported [17] that micron sized PLGA fibers fabricated by a special high temperature, two-stage drawing process result in a modest improvement in failure strain ($\sim 15\%$) as well as slight increase in failure

strength (~65 MPa) while their yield strength is only 35–40 MPa. In this case, the very limited strain hardening was attributed to the improved molecular orientation and increased crystallite size arising from high temperature drawing.

Compared to nanofibers with uniform circular cross-sections, nanofibers with non-uniform/rough cross-sections not only demonstrated similar ductility, but also supported significant post-plateau hardening, which increased the nanofiber strength by ~50%. Finally, nanofibers with uniform ellipsoidal cross-sections yielded at stress values comparable to nanofibers of the previous two morphologies, but displayed a relatively short stress plateau, followed by extraordinary strain hardening. Clearly, this correspondence between cross-sectional morphology and mechanical response could be exploited to activate distinct deformation mechanisms and tune the nanofiber mechanical response. This is an important outcome, also in view of the consistency of the experimental measurements for each fiber: the mechanical response of individual fibers from the same scaffold was highly repeatable: Figure 1(a) shows the highly consistent stress-strain behavior of four segments of the same PLGA nanofiber with uniform circular cross-section. Two of the nanofiber segments were extended to failure at ~150%, and the other two, were extended to 30% and 65% engineering strain before unloading, following the behavior of the first two closely. Similarly, the stress-strain responses of multiple segments of the same nanofibers belonging to the other two morphologies closely followed each other's strain vs. strain response, as shown in Figures 1(b,c).

The trends of the very intriguing stress-strain curves in Figures 1(a–c) are not due to differences in the constitutive material behavior; rather they are owed to structural and surface morphological effects on nanofiber deformation. Post-testing SEM micrographs, Figure 2(a,b), revealed that the large ductility of PLGA nanofibers with uniform circular cross-sections was due to neck formation and propagation at the plateau threshold stress of ~50 MPa, Figure 1(a). Neck initiation is a *structural* instability, but neck propagation depends on the intrinsic *material* behavior of PLGA. During this instability, deformation (strain) localizes at a small defect in the fiber and the cross-section is reduced while molecular uncoiling takes place. When this process is completed, a stable neck has formed, which then propagates along the fiber, thus reducing its diameter and increasing the true stress. The stable neck propagation observed in pristine PLGA nanofibers with circular cross-sections required sufficient strain-hardening within the neck transition region to stiffen, strengthen, and stabilize the material, hence counteracting molecular flow which would otherwise result in premature failure of the fiber due to unrestricted local thinning at the weakest point while the rest of the fiber cross-section would remain largely intact. Electrospun polymeric nanofibers may undergo molecular orientation during fabrication [18], which limits additional uncoiling and orientation of macromolecules during necking or strain localization, which in turn, provides the necessary resistance to counteract post-yielding molecular flow and stabilize a neck or prevent a local diameter irregularity from rapid thinning and fiber failure. After the stabilized neck propagated over the length of the nanofiber at a constant stress value, i.e. the plateau stress in Figure 1(a), the hardened fiber could fail, Figure 1(a), or harden further, Figures 1(b,c).

Surprisingly, the undesirable plateau stress that limits further strengthening of nanofibers with uniform circular cross-sections could be suppressed by either reducing the *cross-sectional symmetry* or modifying the nanofiber *surface uniformity*. Stress-strain curves from fibers with rough surfaces, Figures 1(c) and 2(c), and uniformly ellipsoidal cross-sections, Figures 1(b) and 2(e), displayed distinct strain-hardening. In addition to nanofibers extended to failure, a segment of the same fibers (when available) was deformed by 30–50% and subsequently unloaded. Nanofibers with non-uniform cross-sections yielded at lower stress than nanofibers with uniform circular or ellipsoidal cross-sections, namely 30–50 MPa vs.

50–60 MPa. They also exhibited a longer plateau region and up to 50% larger elongation at failure compared to fibers with ellipsoidal cross-sections (see Table 1). Although the strength of uniform and non-uniform diameter nanofibers was twice their corresponding yield stress, the *ultimate* strength of non-uniform fibers was ~50% lower than that of fibers with uniform cross-sections. The combination of strength and ductility resulted in comparable energy dissipation for both uniform and non-uniform cross-section fibers. Interestingly, nanofibers with ellipsoidal cross-sections displayed a brief strain-softening regime after yielding followed by unusually strong and consistent strain-hardening, as shown in Figure 1(c). This hardening is highly desirable because it comes to resist a sudden overload in a direct analogy to human muscles.

The deformation mechanisms underlying these stress vs. strain behaviors were further elucidated via SEM imaging of fibers stretched to different strain levels but not failure. For PLGA fibers having non-uniform cross-sections, Figures 2(c,d), a fiber segment stretched by 30% showed strain localization within the initially thinner cross-sections. The pronounced cross-sectional irregularity forced strain localization at multiple sites along the fiber that was similar to the necking instability in fibers with uniform circular cross-sections. However, the lack of necking due to significant localized deformation and hardening within the initially thinner cross-sections prevented extended macromolecular uncoiling, thereby limiting the extent of fiber strain achieved at the point of fiber failure. Instead, these nanofibers with non-uniform cross-sections reached a new hardened material state within the locally thinner sections of the fibers leading to an additional 50% post-yield extension, and also increasing the fiber strength (i.e., engineering failure strength) by up to 100% as compared to fibers with uniform circular cross-sections. Note that all plots are presented in terms of engineering stress and not stretch ratio because, except for the case of nanofibers with ellipsoidal cross-sections, in all other cases the local strain was not constant along the fiber.

Fibers with uniform ellipsoidal cross-sections, Figures 2(e,f), achieved a similar final morphology through a different pathway. Despite the ~100% elongation, their stress-strain behavior shown in Figure 1(c) ruled out the possibility of necking [19]. Instead, the *entire* fiber length was simultaneously subjected to brief post-yield softening and subsequent strain-hardening. Owing to homogenous deformation without necking, the stress-strain curves for these fibers provide the intrinsic material response of electrospun PLGA fibers, including the processes of material yielding, strain-hardening, and failure.

In comparison to bulk PLGA, the electrospun nanofibers had dramatically *greater strength and toughness* due to their enhanced strain-hardening ability of the material, as described by Figure 1(c). Bulk PLGA fibers are relatively brittle, with ~12.5% elongation to failure even under special synthesis conditions [16,17], compared to an overall elongation of $81 \pm 7\%$ for the uniform ellipsoidal PLGA nanofibers in the current study (i.e. ~500% increase). As demonstrated, the nanofiber cross-sectional uniformity could be used to reach very high elongations at the expense of mechanical strength. However, PLGA nanofibers still reached a yield stress of ~60 MPa, which coincides with the upper limit of the ultimate strength of the relatively brittle bulk PLGA (45–65 MPa) [16,17]. In fact, PLGA nanofibers underwent strain-hardening, resulting in a true strength that was 2–4 times that of the bulk material. The reduction in fiber diameter during the entire deformation process was estimated by measuring the diameter near the fracture point from SEM images of fibers stretched to failure. Correspondingly, the “true failure strength” of uniform ellipsoidal PLGA nanofibers was calculated to be 165 ± 9.0 MPa, as noted in Table 1. Accounting for the cross-sectional area of the necked region, which was 60–70% that of the un-necked region as shown in Figure 2(b), pristine PLGA nanofibers with uniform circular cross-sections were also observed to fail at true stress values of 130–165 MPa.

3.2. Mechanical Response of Mineralized PLGA Nanofibers

There are a number of musculoskeletal applications for nanofibers that benefit from mineral coatings (e.g., bone tissue engineering, tendon-to-bone regeneration) as well as added strength and toughness. Therefore, the dramatically improved strength and toughness of pristine PLGA nanofibers must be maintained after mineralization. In general, a well-bonded coherent brittle coating reduces the ductility and strength of a ductile substrate [20]. Our research has shown that the strength and ductility of pristine PLGA nanofibers could be retained even after coating their surfaces with hydroxyapatite mineral layers of different morphologies [8,10], namely (a) *thick-platelet* hydroxyapatite coatings that exhibited dense platelet-like growth atop a thin-conformal coating, shown in Figure 3(a), (b) *thick-conformal* hydroxyapatite coatings with limited platelet-like growth, shown in Figure 3(b), and (c) *thin-conformal* hydroxyapatite coatings, as shown in Figure 3(c).

“Thick-platelet” covered PLGA fibers displayed no strain-hardening after ~100% post-yield elongation at nearly constant flow stress, as shown in Figure 4(a). More importantly, despite the relatively higher elastic modulus (see Table 2), these fibers displayed 50% decrease in yield strength and 75% decrease in engineering failure strength compared to pristine fibers with uniform ellipsoidal cross-sections. Post-testing SEM images (Figure 5) revealed that the longer mineralization times used to synthesize thick-platelet covered nanofibers produced a layered mineral structure that prevented complete fragmentation of the mineral along the entire length of the fiber even after large extensions of the fiber. Thick-platelet nanofibers stretched by 50%, (Figure 5(a)) and 115% (Figure 5(b)) showed: (a) circumferential cracking of the inner conformal mineral layer (that lies beneath the platelets), and (b) fragmentation into thin strips ~200–300 nm wide, in Figure 5(a), or ~150–225 nm wide, in Figure 5(b). The fiber diameter exposed after fragment lift-off was ~60% that of the initial mineralized fiber which implies that the true failure strength (same as the flow stress) was 80–85 MPa. Mineral fragmentation involved the evolution from partial cracking, e.g. arrows pointing to inset (ii) in Figure 5(b), to complete cracking, e.g. arrow pointing to inset (i) in Figure 5(b). A thin, cracked and partially debonded, mineral coating remained after fiber failure as shown in inset (iii) of Figure 5(b) that left exposed segments of the inner PLGA core.

Good adhesive bonding of the relatively stiffer mineral layer to the inner polymer core rendered this layer to be significantly load bearing, leading to cracking and fragmentation as shown in insets (i) and (ii) of Figure 5(b). At sufficiently large fiber extensions, the fragmented inner mineral layer could not elongate synchronously with the polymer core, yet could not crack further and thus began to debond. The plateau stress recorded in the stress-strain plots in Figure 4 (a) is reminiscent of the plateau stress in pristine PLGA nanofibers with uniform circular cross-sections that resulted in neck formation and propagation. In mineralized PLGA nanofibers, the surface mineral layer and the inner polymeric core underwent isostrain deformation that resulted in failure (cracking) of the mineral at its weakest sites. Subsequent to cracking and fragmentation, the PLGA core experienced the entire loading, leading to local yielding as well as *local* post-yielding strain-hardening. The strain-hardening response of the PLGA nanofibers described in the previous section, however, stalled further localized deformation of the polymer. The entire fragmentation process could then start and repeat itself at a different location along the fiber where the conditions for mineral failure and nanofiber yielding were met, thus leading to “propagation of deformation” along the nanofiber. Fragment size saturation, inset (ii) in Figure 5(b), occurred in the mineralized fibers when the width of any fragment was smaller than the minimum length required for effective stress-transfer from the nanofiber to the mineral, based on the Kelly-Tyson [21] or Cox models [22]. At 53% strain, Figure 5(a), fragmentation only occurred along 25% of the deformed fiber length. In contrast, at 115% strain, Figure 5(b), as much as 75% of the deformed fiber had undergone fragmentation.

These observations suggest that the ductility of mineralized fibers with a thick platelet mineral layer could have been much greater than 115% had the entire mineral layer fragmented, which, in turn, would have required a thinner coating. While strain-hardening of the polymer core continues after fragmentation of the mineral layer is completed, interfacial debonding could take place if the interfacial shear strength between the polymer and mineral is low. However, if the interface between the polymer and the mineral is strong, nanofiber failure would occur prematurely at a site of mineral fracture where stress-localization in the polymer takes place. Therefore, a relatively weakly bonded thick coating presents a significant advantage.

Using shorter mineralization times, thick conformal mineral coatings *without the formation of crystal platelets* were deposited onto PLGA fibers. These coatings were nearly twice as thick (75–100 nm) compared to the previously discussed thick platelet covered mineral layer, without accounting for the attached mineral platelets. As shown in Figure 4(b) and in Table 2, these fibers displayed yield strengths similar to fibers covered with thick mineral platelets, but underwent post-plateau strain-hardening and an associated increase in failure strength. A representative undeformed thick-conformal mineralized nanofiber in Figure 6(a) demonstrates the lack of surface platelets. Deformation proceeded by way of cracking and fragmentation, similarly to nanofibers with covered with mineral platelet coats. Figure 6(b) shows the onset of fragmentation, where a ~60% reduction in the overall fiber diameter occurred. A closer look into Figure 6(b) establishes that the mineral coating had a layered structure: after fracture of the outer layer, a thin remnant mineral layer fragmented into ~100–300 nm wide strips, similar to the mineral fragment size in fibers coated with mineral platelets. The outlined regions in the center image in Figure 6, where the mineral layer has cleanly debonded from the polymer core, imply a low mineral-polymer interfacial strength. The latter ensured the continued and unrestricted deformation of the fiber core after mineral debonding, thus resulting in large fiber extensions; a feature that is highly desirable to preserve the integrity of a scaffold subjected to large extensions. Depending on the local mineral layer strength, fragmentation of the entire mineral layer may occur sequentially as described above, and even produce the “necklace” type failure shown in Figure 7 for the case of weak interfacial bonding. This failure mode of the mineral is desirable, as it allows for large deformations, nanofiber strengthening, and significant mineral conservation on the (extended) nanofiber surface.

The thickness of the conformal mineral layer close to the debonded regions in nanofibers with a thick-conformal mineral coating was estimated from Figure 6 to be ~300–350 nm, while the thickness of the fragmented inner mineral layer was 75–100 nm, Figure 6(b). Assuming that the mineral layer is brittle and fragments at low strains (i.e. within the initial linear elastic portion of the fiber stress-strain response), and does not contribute to the fiber load-bearing capacity thereafter, the engineering stress-strain data shown in Figure 4(b) for thick-conformal mineralized PLGA nanofibers were re-plotted in Figure 8 using grey lines wherein only the polymer core diameters were used for the stress calculations (i.e., the core was considered to be the only load-bearing member and the composite fiber radius was accordingly reduced by the undeformed coating thickness of 300 nm). This approach provides an estimate for the load-bearing material cross-section, as the engineering stress measurements in Figure 4(b) continue to account for the thickness of the non-contributing mineral layer after its fragmentation. Under these assumptions, the yield and failure strengths of the mineralized PLGA nanofibers were in agreement with the pristine PLGA nanofibers shown in Figure 1. Therefore, increasing the thickness of the mineral coating does not alter the mechanical properties of individual PLGA nanofibers if the interfacial bonding strength to the polymer fiber is weak.

Control of interfacial adhesion and the mineral coating thickness could result in further mechanical property improvement of the mineralized PLGA nanofibers. As shown in Figure 4(c), very thin conformal mineral coatings on PLGA nanofibers resulted in stress-strain curves analogous to those of pristine nanofibers with uniform ellipsoidal cross-sections, having comparable failure strains of $88 \pm 15\%$ and engineering tensile strength of 136 ± 11 MPa, Table 2. Representative SEM images of deformed and fractured PLGA fibers that had thin conformal mineral coatings, Figures 9(a–c), showed that a thin but strongly bonded and coherent mineral layer led to a high density of lateral cracks in the vicinity of the failure section and less dense, periodic cracks further away. Under isostrain conditions, extension of the composite fiber shown across Figure 9 led to severe fragmentation of the mineral layer, Figure 9(c), leaving the inner PLGA core intact and maintaining its original strain-hardening response, strength, and elongation to failure. Notably, the strong adhesion of the thin mineral layer onto the fiber core resulted in large PLGA fiber deformations, often in the form of crazing, as pointed out in Figure 9(c), where adjacent fragments near the fiber fracture point were bridged by discrete PLGA fibrils that are the result of crazing. The latter is an alternative process to shear failure in polymers, which leads to large deformation at stresses higher than the material yield strength. The existence of such large stresses is supported by Figure 4(c) where the engineering stress in the composite PLGA-mineral fibers exceeded 150 MPa, which is as high as that measured for pristine PLGA nanofibers with ellipsoidal cross-sections. The effect of such large uniaxial stresses is amplified by the constraining effect of the mineral shell, thus resulting in locally 3D stresses that drive the formation of crazes near the fiber surface.

The results for the three types of mineral coated PLGA nanofibers point to the vastly advantageous effect of strongly bonded thin and brittle mineral layers on ductile fibers, which allow for 3 times the strength of bulk PLGA and 100% fiber extensions. Yet, even under such severe stress and strain conditions, the mineralized PLGA nanofibers maintain the functional mineral coating almost intact, thus serving in the best way possible their mechanical role and biophysical functionality.

Previous studies demonstrated that all three mineral coating morphologies investigated herein increase the strength and stiffness of PLGA nanofiber scaffolds [8,10]. The results from the current study indicate that such mineralization-based improvements of nanofiber scaffold mechanical properties were *not* due to improvements in the properties of the nanofibers themselves. Rather, it is likely that mineralization improved on the scaffold properties through network effects, e.g., increased fiber-fiber bonding through mineral cross-bridges. The mechanical property results presented here at the nanofiber level combined with prior results at the scaffold level [8] have important implications for the design of future scaffolds. Improvements in mineralization techniques may enhance the mechanical properties at the nanofiber level, leading to further improvements of the mechanical properties at the scaffold level. These enhancements will be necessary to achieve functional tissue engineering replacements for mineralized tissues, such as bone and the tendon-to-bone attachment.

In terms of mechanical compatibility with tendon-to-bone scaffolds, the elastic modulus, failure strength and ductility of pristine PLGA nanofibers are very similar to those of individual submicron collagen fibrils or their assemblies, such as tendons and ligaments [23–25]. The post-yielding stress evolution of PLGA nanofibers with uniform circular cross-sections resembles the behavior of submicron collagen fibrils that demonstrate an elastic or elastic-perfectly plastic type response, while electrospun PLGA nanofibers with ellipsoidal cross-sections demonstrated significant hardening after yielding. Finally, mineralized PLGA nanofibers with a thin conformal mineral layer resulted in failure strengths similar to primary or secondary bone tissues [23].

4. Conclusions

For the first time, it was shown that the mechanical response of individual pristine and mineralized PLGA nanofibers could be dramatically different than that of bulk PLGA. Control of the pristine nanofiber morphology and bonding interactions between the polymer core and the brittle coating allow for a variety of deformation modes spanning necking, hardening, and homogeneous material deformation. This spectrum of deformation modes has not been shown before for micron or larger sized fibers without modification of the polymer itself. Among the fiber morphologies investigated, pristine PLGA nanofibers with uniform ellipsoidal cross-sections were shown to provide an optimum behavior in terms of strength and ductility, with as much as 500% increase in elongation and 200% increase in failure strength when compared to bulk PLGA. It is noteworthy that, unlike macroscale fibers and materials, surface non-uniformity did not result in early fiber failure. This was due to the significant strain-hardening response supported by the underlying macromolecular network, which, although was two orders of magnitude smaller in length scale than the fiber diameter, it was comparable to the surface non-uniformity. It is, thus, concluded that geometric imperfections in soft materials at the submicron scale are quite benign compared to the macroscale.

When mineralized, nanofibers with ellipsoidal cross-sections and thin conformal mineral coatings supported similar mechanical behavior as the corresponding pristine PLGA nanofibers. Fragmentation and debonding from the PLGA fiber core were shown to be among the most favorable modes of failure of the brittle mineral coatings. Despite fragmentation of the mineral coating, the high strength and toughness of the polymer core properties were still retained. Notably, thin conformal mineral coatings that were strongly bonded on PLGA nanofibers supported a threefold increase in fiber strength compared to bulk while retaining the surface mineral layer, though strongly fragmented, on the PLGA nanofiber after undergoing a 100% elongation. This outstanding result promises to uphold the biological functionality of PLGA fibers with submicron diameters under extreme mechanical environments.

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Figure 1.

Engineering stress-strain curves for pristine PLGA nanofibers with (a) uniform circular, (b) non-uniform and (c) uniform ellipsoidal cross-sections. The entire loading-unloading curves are shown for tests that were interrupted before fiber failure. Fibers with identical diameter are segments of the same long fiber. The 910 nm fiber shown with a solid (black) line in (b) represents an interrupted experiment.

Figure 2.

SEM images of pristine PLGA fibers with different cross-sectional morphologies showing the undeformed and deformed states of the fiber: **(a)** Fiber with *uniform circular cross-section* in its undeformed state, and **(b)** at a point of the stress plateau in Figure 1(a) for 65% strain showing necking. **(c)** Fiber with *non-uniform cross-section* in its undeformed state, and **(d)** at a point of the stress plateau for 30% strain showing several localization points due to initial cross-sectional non-uniformity. **(e)** Fiber with *uniform ellipsoidal cross-section* in its undeformed state, and **(f)** for 50% strain showing no signs of necking. All scale bars are 1 μm long.

Figure 3. SEM images of (a) thick-platelet, (b) thick-conformal, and (c) thin-conformal mineral coatings. The dashed lines represent the estimated interface between the surface mineral and the inner PLGA core. All scale bars are 1 μ m long.

Figure 4. Engineering stress-strain curves of individual PLGA nanofibers with (a) thick-platelet, (b) thick-conformal mineral coating, and (c) thin-conformal mineral coating. The composite fiber diameter was used in all engineering stress calculations.

Figure 5. SEM images of PLGA nanofibers with thick-platelet mineral coatings subjected to **(a)** 53% strain (1275 nm initial fiber diameter), and **(b)** 115% strain (775 nm initial fiber diameter). All scale bars are 1 μm long.

Figure 6. SEM images of PLGA nanofiber with thick-conformal mineral coating. **(a)** Undeformed fiber morphology, and **(b)** mineral fragmentation and debonding from the polymer core. All scale bars are 1 μm long.

Figure 7.
SEM image of 'necklace' type failure of the thick conformal mineral coating due to weak interfacial bonding.

Figure 8.

Engineering stress-strain curves for PLGA fibers with a thick-conformal mineral layer plotted by including the mineral coating in the load bearing fiber diameter (black lines), and by excluding the mineral thickness of 150 nm when the coating fails and does not bear significant load (grey lines)

Figure 9. SEM images of PLGA nanofibers with thin-conformal mineral coating that is strongly bonded onto the fiber. Insets (a–c) are high magnification SEM images depicting the morphology of the mineral layer along the fiber. All scale bars are 1 μm long.

Table 1

Elastic modulus, yield strength, failure strength and failure strain of pristine PLGA fibers (Mean $\pm$ Standard Deviation).

	Uniform Circular	Non-Uniform	Uniform Ellipsoidal
Elastic Modulus (GPa)	1.65 $\pm$ 0.15	1.39 $\pm$ 0.10	2.25 $\pm$ 0.25
Yield Strength (MPa)	55 $\pm$ 5	40 $\pm$ 7	65 $\pm$ 2
Failure Strength (MPa)	140 $\pm$ 15	89 $\pm$ 7	165 $\pm$ 9
Failure Strain (%)	150 $\pm$ 25	122 $\pm$ 7	81 $\pm$ 7

Table 2

Elastic modulus, yield strength, and tensile strength of mineralized PLGA fibers (Mean $\pm$ Standard Deviation).

	Thick Platelet	Thick Conformal	Thin Conformal
Elastic Modulus (GPa)	2.49 $\pm$ 0.18	1.26 $\pm$ 0.23	1.57 $\pm$ 0.17
Yield Strength (MPa)	29 $\pm$ 3	36 $\pm$ 7	48 $\pm$ 10
Failure Strength (MPa)	30 $\pm$ 4	67 $\pm$ 0	136 $\pm$ 11