Engineering Extruded Collagen Fibers for Biomedical Applications

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ABSTRACT: Extruded collagen fibers constitute a promising biomimetic scaffold for tissue engineering applications. In this study, we compared the structural, thermal, and mechanical properties of fibers produced from either NaCl or poly(ethylene glycol) with a number-average molecular weight of 8000 (PEG 8K), the only two coagents that have been used in the fabrication process. As novel, we report the fabrication of fibers with properties similar to native or synthetic fibers using other coagents. NaCl derived fibers were characterized by higher thermal stability ($p < 0.026$), stress ($p < 0.001$), and modulus ($p < 0.0025$) values than PEG 8K, whereas the latter yielded more extendable fibers ($p < 0.012$). Poly(ethylene glycol)s with number-average molecular weights of 200 and 1000 produced fibers with similar mechanical properties ($p > 0.05$) that were thinner ($p < 0.033$), stiffer ($p < 0.022$), and less extendable ($p < 0.0002$) than those of PEG 8K. Poly(vinyl alcohol) (PVA) with a number-average average molecular weight of 9–10,000 and PEG 8K yielded fibers with similar diameters and stress-at-break values ($p > 0.05$); however, the poly(ethylene glycol) derived fibers were more extendable ($p < 0.0003$), whereas the PVA fibers were stiffer ($p < 0.029$). Gum-arabic- and soluble-starch-derived fibers were of similar tensile strength, extendibility, and stiffness ($p > 0.05$). In this *in vitro* study, the thickest ($p < 0.011$) and the weakest ($p < 0.0066$) fibers were produced in the presence of sodium sulfate. J Appl Polym Sci 108: 2886–2894, 2008

Key words: biodegradable; biofibers; biomaterials; extrusion; mechanical properties

INTRODUCTION

The expanding field of tissue engineering demands biocompatible materials that imitate the extracellular matrix. Collagen, a natural material, is favored for biomedical applications because it is received by the body as a normal constituent rather than a foreign matter. In addition, advantages such as high tensile strength and biodegradability, low immunogenicity and antigenicity, and the ability to promote cellular attachment and growth and, consequently, tissue healing and regeneration have established collagen as a superior raw material for biomimetic scaffold fabrication.[1–3]

Extruded collagen fibers, because of their unique and advantageous properties, such as high surface area, softness, absorbency, and ease of fabrication into many product forms, have been used extensively as substrates for nerve regeneration; bone, tendon, and ligament replacement; wound dressing and vascular applications; and suture materials.[4–7] In most cases, acid-soluble collagen has been used; it is a less biocompatible collagen preparation because of the presence of nonhelical telopeptide regions.[2] Thus, in this study, we used pepsin-soluble bovine Achilles tendon collagen, a raw material that has been favored for biomimetic scaffold manufacturing.

Because it is difficult for collagen to fabricate scaffolds on its own, it is usually necessary to combine collagen with other materials,[8,9] referred in this study as *coagents.* Until now, extruded collagen fibers have been produced with either NaCl[4,10] or poly(ethylene glycol) (PEG) with a number-average molecular weight of 8000.[5] Thus, our primary target was to directly evaluate the properties of the fibers produced from these two coagents. As second goal, we aimed to investigate, for the very first time, the influence of other coagents, such as salts, water-soluble polymers, gums, and starches, on the structural, physical, and mechanical properties of the produced fibers.

Recently, many water-soluble polymers have become an attractive field of study because of their good processing characteristics and variable degradation rates.[11] PEG, a low-toxic and low-antigenic polyether diol with

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the general structure HO—$(CH_2CH_2O)_n$—H, has been approved by the U.S. Food and Drug Administration for several medical and food industry applications. It has been reported that PEG could stabilize the porous structure inside collagen sponges, facilitating cell infiltration and tissue ingrowth, which would significantly increase the mechanical stability and enzyme degradation with improved blood compatibility and ability to resist protein adsorption.[12,13] Additionally, it has been shown that the use of PEG for the precipitation of native collagen has a number of advantages over conventional salt-precipitating methods.[14,15] However, a PEG molecule cannot couple with more than two drug molecules; therefore, other polymeric carriers, such as poly(vinyl alcohol) (PVA) with numerous functional groups and capable of covalently coupling several drug molecules, have been introduced.[16] Collagen, which is a hydrogen donor, forms hydrogen bonds with the hydroxyl group from water-soluble polymers.[17]

Polysaccharide–protein interactions are involved in a wide variety of biological functions, including cellular growth, recognition, adhesion, and cancer metastasis.[18] In the presence of polysaccharides, some proteins change their denaturation temperature or modify their ability to form gels, aggregates, or fibers. Depending on their nature, pH, ionic strength, temperature, concentration, and molecular weight, proteins can attract polysaccharides in solution, giving rise to supramolecular soluble complexes.[19] Different approaches have been made to use starch for the production of tailored biodegradable materials.[20] Colloid dispersions of proteins and gums contribute to the structure, texture, and stability of the final product.[21] Films made of protein, starch, and gum retain water and resist oxygen penetration.[22]

EXPERIMENTAL

All chemicals, unless otherwise stated, were purchased from Sigma–Aldrich (Dorset, UK). The bovine Achilles tendons were kindly provided by the BLC Research Centre (Northampton, UK).

Collagen preparation

Typical protocols for the extraction of collagen were used. Briefly, frozen bovine Achilles tendons were minced, washed in neutral phosphate buffers, and suspended in 0.5*M* ethanoic acid in the presence of pepsin (2500 U/mg, Roche Diagnostics, UK) for 72 h at 4°C. Consequently, the collagen suspension was centrifuged (12,000 g at 4°C for 45 min; Gr20.22 Jouan refrigerated centrifuge, Thermo Electron Corp., Bath, Sussex, UK) and purified by repeated salt precipitation (0.9*M* NaCl), centrifugation, and acid solubilization (1*M* ethanoic acid). The final atelocollagen solution was obtained after dialysis (molecular weight cutoff = 8000) against 0.01*M* ethanoic acid and kept refrigerated until use. The collagen purity was determined by sodium dodecyl sulfate/polyacrylamide gel electrophoresis analysis (90% type I), and its concentration was determined by hydroxyproline assay and was subsequently adjusted to 6 mg/mL.

Fiber formation

The procedure for fiber formation was based on previous publications.[4,5,10] Briefly, a 5 mL syringe (Terumo Medical Corp. UK, Ltd., Merseyside, UK) containing the atelocollagen solution was loaded on a syringe pump (KD-Scientific 200, KD-Scientific, Inc., Holliston, MA), which was set to infuse at 0.4 mL/min and extruded through a 1.5-mm internal diameter silicone extrusion tube (Samco Silicone Products, Ltd., Warwickshire, UK) into a fiber formation buffer (118 m*M* phosphate buffer and 20% coagent at pH 7.30–7.50 and 37°C). The different coagents used were NaCl; poly(ethylene glycol)s with number-average molecular weights of 200, 1000, and 8000 (PEG 200, PEG 1K, and PEG 8K, respectively); poly(vinyl alcohol) with a number-average molecular weight of 9–10,000 (PVA 9–10K); soluble starch (American Chemical Society reagent grade); gum arabic (acacia tree, BioChemica grade); sodium sulfate; and glycerol (saturated solutions of starch and gum arabic were obtained; thus, they were filtered before use). After a period of 15 min, the fibers were transferred to the fiber incubation buffer (6.0 m*M* phosphate buffer and 75 m*M* sodium chloride at pH 7.10 and 37°C) for another 15 min. Thereafter, the fibers were transferred into a distilled water bath for another 10 min and finally air-dried under the tension of their own weight at room temperature.

Mechanical testing and structural evaluation

An Instron 1122 universal testing machine (Instron, Ltd., Buckinghamshire, UK) was used for the mechanical tests at an extension rate of 10 mm/min. The gauge length was fixed at 5 cm, and soft rubber was used to cover the inside area of the grips to avoid damage to the fibers at the contact points; fibers that broke at the contact points with the grips were rejected. Each fiber cross-sectional area was calculated with a circular cross-section assumed and by measurement of the diameter at five places along the fiber with a Nikon Eclipse E600 optical microscope (Nikon Instruments, Surrey, UK) fitted with a calibrated eyepiece. Surface observations and failed ends of collagen fibers that were extended to failure were examined with a Hitachi S3000 variable-pressure scanning electron microscope (Hitachi, Berk-

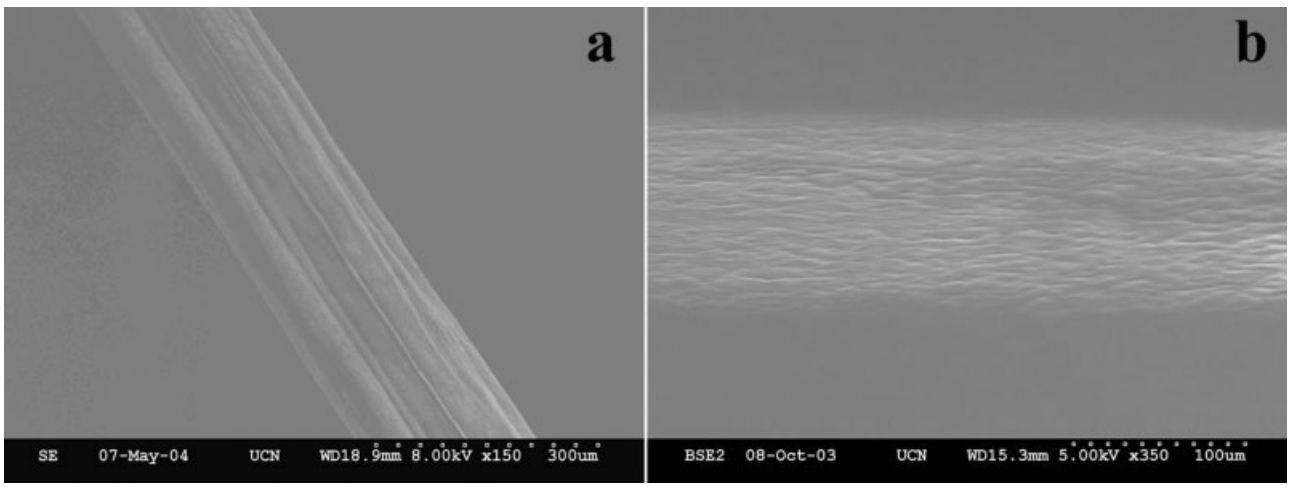

Figure 1 Surface morphology of dry extruded collagen fibers in the presence of (a) 20% PEG 8K and (b) 20% soluble starch.

shire, United Kingdom). The stress at break was defined as the load at failure divided by the original cross-sectional area (engineering stress); strain at break was defined as the change in length divided by the original length; and the modulus was defined as the stress at 2% strain divided by 0.02.

Thermal properties

The shrinkage temperature was determined by differential scanning calorimetry with a 822e Mettler–Toledo differential scanning calorimeter (Mettler–Toledo International, Inc., Leicester, United Kingdom). The reconstituted collagen fibers were hydrated overnight at room temperature in 0.01*M* phosphate-buffered saline at pH 7.4. The wet fibers were removed and quickly blotted with filter paper to remove excess surface water and hermetically sealed in aluminum pans. Heating was carried out at a constant temperature ramp of 5°C/min in the temperature range from 15 to 95°C. Thermal denaturation, an endothermic transition, was recorded as a typical peak, and two characteristic temperatures were measured corresponding to the peak temperature (the temperature of maximum power absorption during denaturation) and onset temperature (the temperature at which the tangent to the initial power versus temperature line crossed the baseline).

Statistical analysis

Numerical data are expressed as mean plus or minus the standard deviation. Analysis was performed with statistical software (MINITAB version 13.1, Minitab, Inc.). A one-way analysis of variance for multiple comparisons and a two-sample *t* test for pairwise comparisons were used after the following assumptions were confirmed: (1) the distribution from which each of the samples was derived was normal (Anderson–Darling normality test), and (2) the variances of the population of the samples were equal to one another (Bartlett's and Levene's tests for homogenicity of variance). Nonparametric statistics were used when either or both of the previous assumptions were violated, and, consequently, the Kruskal–Wallis test for multiple comparisons or Mann–Whitney test for two samples were carried out. Statistical significance was accepted at $p < 0.05$.

RESULTS

Matrix morphology

From the scanning electron microscopy investigation, it became apparent that all fibers, independent of the coagent present in the fiber formation buffer, exhibited the same surface structure, with ridges and crevices running roughly parallel to the fiber longitudinal axis (Fig. 1). Fibers that extended to failure were investigated further, and the interfiber space was found closely packed in all cases (Fig. 2). Typical fracture modes obtained were classified as (1) smooth fracture, (2) rough fracture with the internal structure of the fiber appearing slightly drawn out, (3) longitudinal split fracture, and (4) fibrillation fracture. Although fibers were produced in the presence of glycerol, they were too fragile to handle.

Biomechanical analysis

Uniaxial tensile tests of the dry fibers revealed a typical S-shaped stress–strain curve (Fig. 3), which consisted of a small toe region, a region of increasing stress up to a knee point, and a long region of

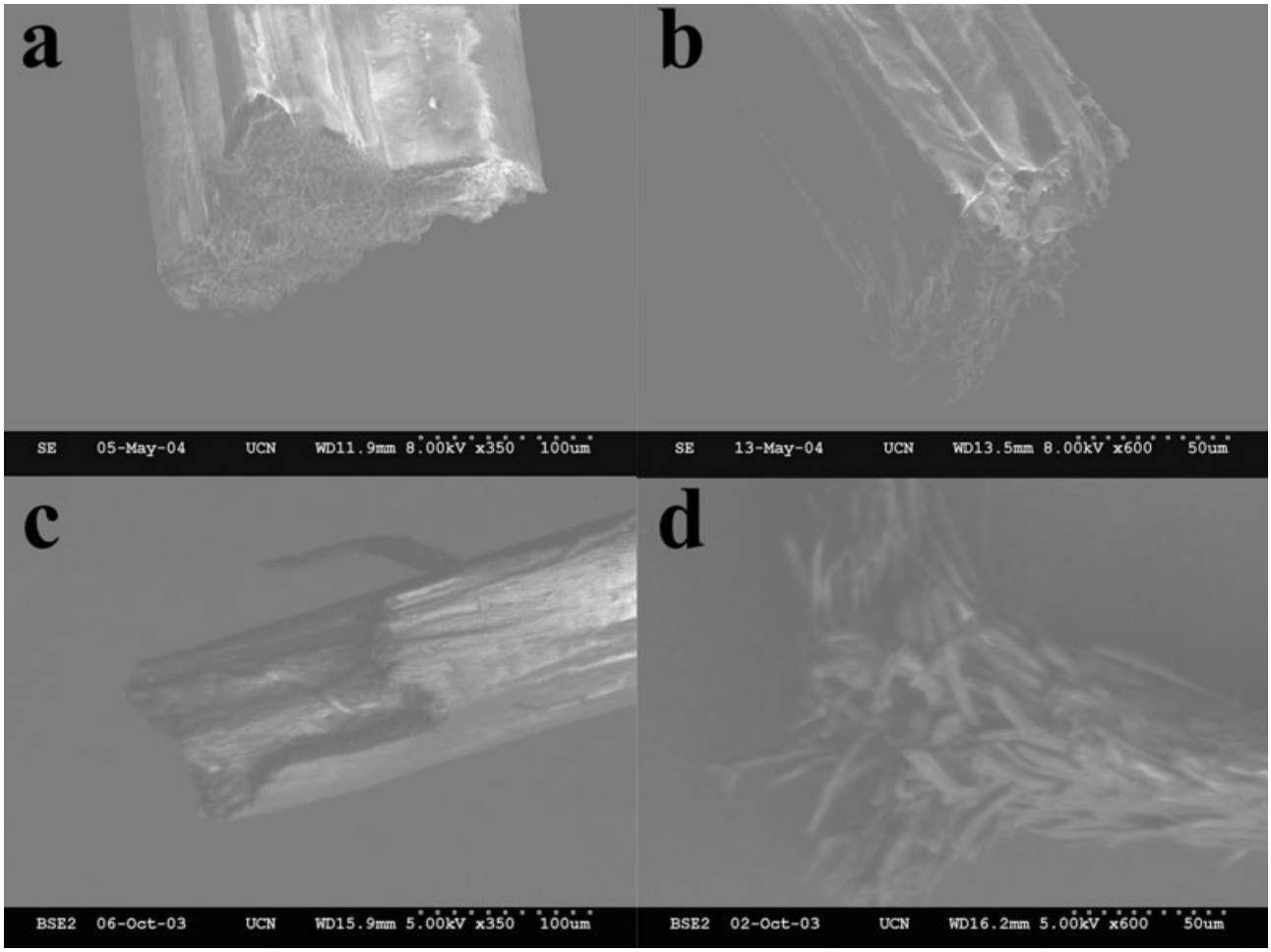

Figure 2 Scanning electron micrographs identifying the different fracture modes of dry extruded collagen fibers: (a) smooth fracture for 20% PEG 8K, (b) rough fracture for 20% PVA 9–10K, (c) split longitudinal fracture for 20% gum arabic, and (d) fibrillation fracture for 20% NaCl.

constant gradient, which persisted until failure. Within each treatment, the stress–strain curves exhibited a fiber diameter dependency; thin fibers showed high-stress/low-strain graphs and a short toe region, whereas fibers of high diameter demonstrated low-stress/high-strain graphs and a long toe region.

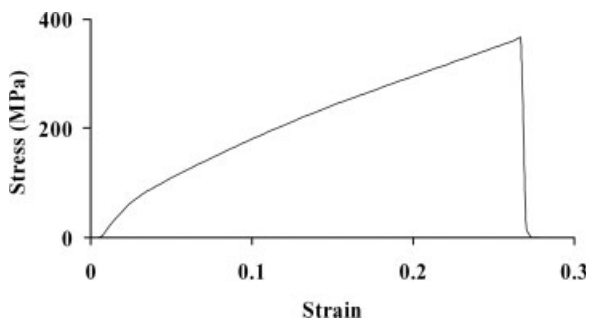

Figure 3 Typical S-shaped stress–strain curve of dry extruded collagen fibers (example from the 20% NaCl treatment).

Influence of PEG 8K and NaCl

Fibers derived in the presence of NaCl exhibited higher ($p < 0.026$) hydrothermal stability (50.1 ± 0.94°C) than those derived in the presence of PEG 8K (47.93 ± 0.51°C). In addition, NaCl derived fibers were characterized by higher stress at break ($p < 0.001$), force at break ($p < 0.0001$), and modulus at 2% strain values ($p < 0.0025$). However, the utilization of PEG 8K yielded more extendable fibers ($p < 0.012$) (Table I). With a linear regression model fitted between stress at break and dry fiber diameter (Fig. 4), the strongest correlation was obtained from the PEG 8K (R^2 values of 0.89 and 0.72 for PEG 8K and NaCl, respectively).

Influence of PEG 200, PEG 1K, and PEG 8K

No significant difference ($p > 0.05$) in mechanical properties was found between the fibers produced in the presence of the low-molecular-weight PEGs. However, both low-molecular-weight PEGs produced fibers that were thinner in diameter ($p <$

TABLE I
Physical and Mechanical Properties of Reconstituted Collagen Fibers in the Presence of 20% Concentrations of Different Coagents in the Fiber Formation Buffer

Treatment	Diameter ± SD (μm)	Stress at break ± SD (MPa)	Strain at break ± SD	Force at break ± SD (N)	Modulus at 2% strain ± SD (GPa)
PEG 200, $n = 14$	98 ± 12	175 ± 59	0.19 ± 0.06	1.27 ± 0.30	1.46 ± 0.64
PEG 1K, $n = 10$	101 ± 15	163 ± 63	0.18 ± 0.03	1.13 ± 0.49	1.52 ± 0.57
PEG 8K, $n = 18$	123 ± 26	136 ± 57	0.36 ± 0.13	1.41 ± 0.17	0.86 ± 0.75
PVA 9–10K, $n = 9$	141 ± 29	155 ± 39	0.20 ± 0.06	2.29 ± 0.39	1.52 ± 0.64
Soluble starch, $n = 7$	125 ± 20	178 ± 77	0.29 ± 0.02	2.00 ± 0.31	0.83 ± 0.71
Gum arabic, $n = 8$	145 ± 24	107 ± 30	0.25 ± 0.10	1.66 ± 0.20	0.62 ± 0.44
Sodium sulfate, $n = 7$	297 ± 111	34 ± 39	0.21 ± 0.08	1.21 ± 0.54	0.29 ± 0.45
NaCl, $n = 25$	119 ± 15	208 ± 57	0.27 ± 0.05	2.23 ± 0.32	1.58 ± 0.49

n is the sample number; SD is the standard deviation.

0.033) and that were found to be stiffer ($p < 0.022$) and less extendable ($p < 0.0002$) than those derived from the PEG 8K (Table I). With a linear regression model fitted between stress at break and dry fiber diameter (Fig. 4), a strong correlation was obtained only for the PEG 8K (R^2 value of 0.89).

Influence of different coagents

The two water-soluble polymers with approximately the same average molecular weights, PEG 8K and PVA 9–10K, yielded fibers with similar diameters and stress-at-break values ($p > 0.05$); however, significant differences in strain ($p < 0.0003$), force ($p < 0.0001$), and modulus ($p < 0.029$) values were obtained (Table I). With a linear regression model fitted between stress at break and dry fiber diameter (Fig. 4), strong correlations were obtained in both cases (R^2 values of 0.89 and 0.83 for PEG 8K and PVA 9–10K, respectively).

The fibers produced in the presence of sodium sulfate were the thickest ($p < 0.011$ for the gum arabic, whereas $p < 0.0038$ for any other treatment) in this *in vitro* study and were characterized by the lowest stress-at-break values ($p < 0.0066$) (Table I). Still, a linear regression model between stress at break and dry fiber diameter (Fig. 4) yielded a strong correlation for the sodium sulfate derived fibers (R^2 value of 0.82).

Fibers derived in the presence of gum arabic and soluble starch were found to be of similar stiffness and extendibility ($p > 0.05$) (Table I). In both cases, fibers with the same diameter and tensile strength were obtained ($p > 0.05$). Once more, high correlations between diameter and stress at break (Fig. 4) were revealed (R^2 values of 0.89 and 086 for gum arabic and soluble starch, respectively).

DISCUSSION

Structural evaluation

All fibers, independent of the treatment, exhibited a rough external surface with ridges and crevices running along the longitudinal axis of the fiber. These morphological characteristics were attributed to the fibrous substructure of the fibers,[23] which manifested itself after drying. Moreover, such unique features have been shown to facilitate cell attachment and fibroblast migration.[24] Detailed scanning electron microscopy of the failed ends of the fibers revealed a consistently filled interfiber space. It has been mentioned that very little free interfiber space occurs in fibers formed *in vitro*.[25] Furthermore, the presence of coagents could have enhanced the close packing of the fibers, as has been shown for composite fibers[26] and elastin-based materials.[27] The fracture modes identified were in accord with previous publications on collagen fibers and native tissues,[28–31] where their relative occurrence was attributed to the handling of the fibers, the strain rate, and possibly to flaws within the fibrous structure. More specific, when the application of load was slow, the fibers tended to break smoothly (smooth fracture). However, the handling of the fibers while still in the wet state improved the alignment and the strength of the outer surface. Upon application of load, failure occurred first within the inner core with the outer aligned layer failing later, which, consequently, left the end appearing drawn out (rough fracture). Split fracture could be initiated from stress concentration at cracks along the longitudinal axis of the fiber. The simultaneous propagation of more than one crack could result in the splitting of the fiber along its axis. Fibrillation fracture indicated that the fiber bundles had sufficient time to split into thinner ones and slip past one another during the application of the load.

Biomechanical evaluation

In the total absence of coagents, although fibers were produced, they were too fragile to handle. It has been shown that it is difficult for collagen to fabricate biomaterials on its own; thus, it is usually necessary to combine it with other materials.[8,9] The

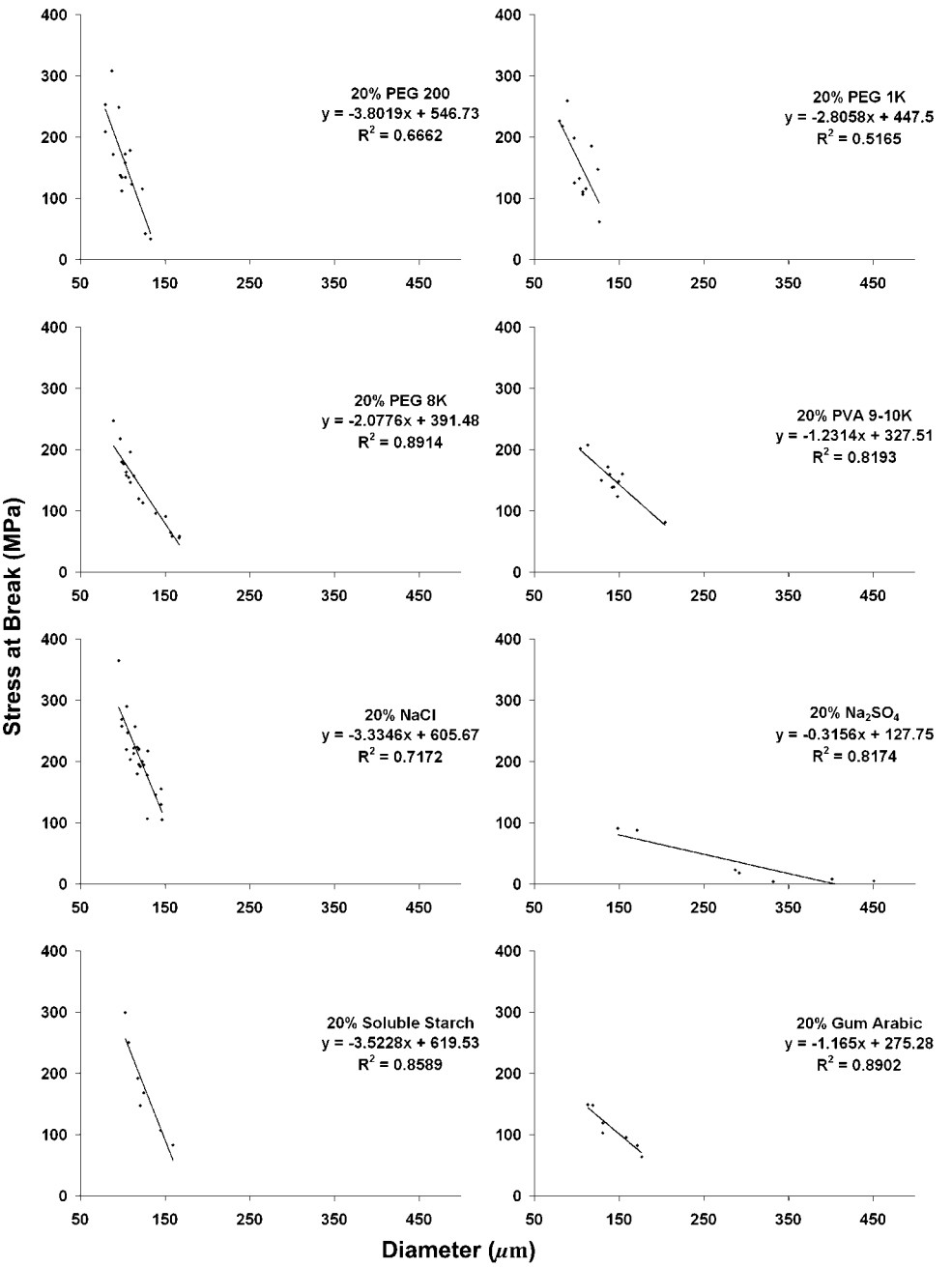

Figure 4 Fitting of a linear regression model between the stress at break and dry fiber diameter. The strongest correlations were obtained from PEG 8K and gum arabic.

utilization of PEG, for example, is essential to the production of collagen gels of sufficient mechanical strength.[32]

Uniaxial tensile tests of dry reconstituted collagen fibers produced stress–strain curves similar to those reported for semicrystalline polymers that yield and undergo plastic flow[33]. Similar curves have been reported previously for native tendon,[34] extruded collagen fibers,[5,35] and even nanofibrous meshes.[36] The yielding mechanism involves some form of flow that occurs within the fiber, possibly interfibrillar slippage, which plays an important role in the tensile deformation of aligned connective tissue such as tendon. As far as the length of the toe region is concerned, we attributed it to the packing of the fibrils within the fibrous structure; the tighter the packing was, the smaller the toe region was, whereas loose fibrillar packing would yield longer toe regions (see later discussion). The lower modulus of the nonlinear toe region in native tissues such as tendons or ligaments has been attributed to the uncrimping of the collagen fibrils and to the initiation of stretching

of the triple helix, the nonhelical ends, and the cross-links.[37]

The strong correlations obtained between stress at break and dry fiber diameter could be explained in two dimensions: (a) the tensile strength increased as the cross-sectional area decreased because there was less chance for defects in thinner sections[38,39] or (b) as the fiber diameter decreased, improved longitudinal alignment took place that enhanced strong interactions between the collagen fibrils.[4,6,35] These strong interactions were illustrated in the stress–strain curves with the short toe region of the thin fibers, whereas the loose interactions were represented by the longer toe regions in the thick fibers. Moreover, the strong interactions would have had an inhibitory effect on the extensibility of the fibers; thus, fibers of low diameter would have exhibited reduced strain-at-break values. Thin fibers, comprised of aligned fibrils, failed at lower loads, which is in accord with previous observations.[6] Modulus values reflect the stiffness or rigidity of the material; the higher its value is, the greater the load would be required to produce a given extension.[40] These results are in agreement with previous work on collagen fibers,[30] composite fibers,[41] and synthetic sutures.[42]

Influence of PEG 8K and NaCl

Maybe the most important aspect of this work was the comparison of the two solely used coagents, namely, PEG 8K[5] and NaCl,[4,10] for the reconstitution of collagen into fibers. Because (1) both NaCl[43,44] and PEG[14,15] were used to precipitate collagen, (2) the *in vitro* self-assembly of collagen molecules into fibrils with periodic patterns[45] was analogous to the precipitation of collagen,[46] and (3) both self-assembly and precipitation are processes driven by electrostatic attraction, hydrogen, and hydrophobic bonding,[47] we tend to believe that either of these coagents is highly suitable for the reconstitution of collagen into fibers.

Collagen fibers derived in the presence of NaCl had higher denaturation temperatures than the PEG-derived ones. We attributed this difference to the better molecular packing that was achieved with a strong electrolyte such as NaCl that could have removed more water from the fibrous structure and resulted in a better interfiber packing. Moreover, the fibers produced in this study exhibited denaturation temperatures ranging from 48 to 50°C, which were higher than any other noncrosslinked collagenous matrix. We identified the coagents present as structure enhancers and, therefore, responsible for the closer packing of the collagen molecules within the fibrous structure, which led to higher denaturation temperatures, whereas in collagen gels, the molecules are more scattered.[48] Salts, for example, affect the thermal stability of collagen in a typical ion-specific way; they alter the structure of the solvent, which, consequently, modifies the solvent–macromolecule interaction involved in the stabilization of the natural conformation.[49] Similarly, it has been shown that PEG promotes the formation of highly ordered fibrils by the removal of free water,[32] whereas polymers increase the shrinkage temperature by steric exclusions.[50]

The PEG 8K derived fibers were more extendable but of lower strength and stiffness than those derived from NaCl. PEG, a linear polymer, is believed to align along the longitudinal axis of the polypeptide chains,[51] as has been shown for PEG in clay films[52] or hydroxyapatite nanocrystals in collagen matrices,[53] hence, the improved extendibility. Similarly, it has been shown in composite fibers that the elongation to break arises from both the collagen and the polymer.[41] On the other hand, smaller and more cumbersome molecules, such as those of Cl^-, Na^+, and Ca^{+2} can easily penetrate into the intrafibrillar compartment[54,55] and, consequently, create a stiffer structure.

Influence of PEG 200, PEG 1K, and PEG 8K

The fibers produced in the presence of PEG 200 and PEG 1K were thinner than those produced in the presence of PEG 8K. As explained previously, the thin fibers were less extendable and, due to better packing density, were stiffer than the thick fibers. These findings are in agreement with previous observations,[56] where it was pointed out that as the molecular weight increased (oxyethylene units), the material became less brittle and could, therefore, resist fracture when subjected to stress. In addition, it has been shown that the deformation to break is dependent on the length of fibrils.[28,30] Therefore, low-molecular-weight PEGs with short chains would yield shorter fibrils than those high-molecular-weight PEGs with longer chains. Moreover, PEG 8K, due to its size, could only align parallel[51] within the limited space between the polypeptide helices,[54,55] which would promote either molecular or fibrillar slippage and imitate, in a way, the glycosaminoglycans in living systems.[32] On the other hand, low-molecular-weight PEGs could populate the available space in a random nonsymmetric order, consequently act as defects, and, therefore, be liable for premature fracture.

It has been shown that the efficiency of protein fractionation increases with the molecular weight of the PEG; at a given polymer concentration on a weight-by-weight basis, polymers of a higher molecular weight are more effective precipitants than polymers of a lower molecular weight, and a range

between 6000 and 8000 has been identified as optimal for PEG.[14,15] The poor correlations between stress at break and dry fiber diameter that were found in this study for the low-molecular-weight PEGs further enhanced the previous observations.

Influence of different coagents

Although the PVA 9–10K and the PEG 8K induced fibers were of similar dry diameter and stress at break values, significant differences were observed in the strain, force, and modulus values, which were attributed to the fact that the multifunctional PVA coupled with more free sides of collagen than the PEG, and, as a result, a stiffer and less extendable material was produced.

The utilization of soluble starch and gum arabic facilitated the fabrication of extruded collagen fibers, which were characterized with proportional mechanical properties. Parallel to our findings, it was shown that starch improved both the mechanical and thermal properties of cellulose fibers.[20] Moreover, the addition of xanthan gum in protein systems enhanced the emulsification process due to network creation,[57] whereas galactomannan gum was retained in collagen fibers, and the triple-helical configuration was conserved.[58]

Fibers derived in the presence of sodium sulfate were found to be the thickest, and, consequently, they exhibited the lowest stress-at-break values. The exceptionally large diameters were attributed to the ionic strength of the solution; it was −19 mV, whereas the ionic strength of all other treatments was in the range −43 to −48 mV. It has been shown that with high ionic strengths, due to the passing of the optimal amount of water removable for fiber stability, the right configuration to build up a rigid and viscoelastic material[57] might be lost[46] and the particle size could be remarkably increased.[59]

The glycerol buffer failed to produce fibers suitable for testing, even after extended incubation in the fiber formation buffer (2 h). It has been reported that glycerol not only inhibits the fibril formation of both acid- and pepsin-soluble collagen type I but also disassembles already formed fibrils.[60,61] However, in later studies, glycerol was shown to stabilize the collagen molecule, which resulted in a moderate increase in the denaturation temperature,[62] and to increase the strength of gelatin gels, which provided stabilization through hydrogen bonding.[63]

Finally, although the testing conditions influenced the mechanical properties of the materials, it was, nevertheless, worthwhile to compare our results with those of other studies. The *in vivo* diameter of collagen fibers, depending on the tissue, has been shown to range from 1 to 300 μm.[64,65] Synthetic, composite, and natural fibers have been shown to have stress and strain-at-break values ranging from 31.8 to 970 MPa and 2.8 to 62%, respectively,[4,26,42,66,67] whereas extruded collagen fibers have been shown to have stress and strain-at-break values ranging from 75 to 224 MPa and 13 to 25%, respectively.[4–6] The extruded collagen fibers produced in this study had a diameter range from 98 to 297 μm, stress and strain-at-break values ranging from 34 to 208 MPa and 18 to 36%, respectively. These results indicate that we can manufacture *in vitro* scaffolds from the natural and highly biocompatible collagen that are able to mimic the properties of not only the extracellular matrix but also synthetic materials.

CONCLUSIONS

Extruded collagen fibers have been shown to comprise a competitive biocompatible scaffold for tissue engineering applications. In this study, for the very first time, we compared the properties of the fibers produced from the two exclusively used coagents, NaCl and PEG 8K. As novel, we demonstrated that such fibers could be produced with other coagents, such as PVA, soluble starch, and gum arabic. The significance of this study lays not only on the fact that new collagen blends were introduced in the quest of the ideal scaffold fabrication but also on the fact that these *in vitro* scaffolds were characterized with properties similar to those of native or even synthetic fibers.

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