Mechanical Properties of Artificial Protein Matrices Engineered for Control of Cell and Tissue Behavior

Kathleen Di Zio[†] and David A. Tirrell*[,‡]

Polymer Science and Engineering Department, University of Massachusetts, Amherst, Massachusetts 01003, and Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California 91125

Received September 9, 2002; Revised Manuscript Received January 2, 2003

ABSTRACT: Genetic engineering methods were used for the preparation of artificial proteins containing sequences designed to reproduce essential features of the extracellular matrix (ECM). The long-term objective of the work is to develop matrices for use in the engineering of small-diameter vascular grafts. The CS5 domain of fibronectin provides binding sites for vascular endothelial cells, while an elastin-like repeat, $[(VPGIG)_2(VPGKG)(VPGIG)_2]$, controls the mechanical properties and includes sites for covalent cross-linking. Bis(sulfosuccinimidyl) suberate and disuccinimidyl suberate were used to cross-link artificial ECM protein films for uniaxial tensile testing. Variation in the amount of cross-linker and protein weight fraction allowed preparation of films with Young's moduli ranging from 0.07 to 0.97 MPa. The weight fraction of protein in the hydrated, cross-linked films was measured to be between 0.2 and 0.4; the molecular weight between cross-links (M_c) varied from 3000 to 38 000. The moduli and M_c of the films span the ranges reported for natural elastins.

Introduction

Diseases of the cardiovascular system are a leading cause of death in the United States, and approximately 550 000 vascular grafts are implanted yearly in the treatment of occlusive arterial disease.[1−4] Although most grafting success has been realized through the use of autologous saphenous vein, the demand for autologous vein greatly exceeds availability.[5,6] Poly(ethylene terephthalate) and expanded poly(tetrafluoroethylene) (ePTFE) are the dominant synthetic alternatives.[5] While these materials can be used for large-diameter vascular grafts in sites characterized by high rates of blood flow, grafts with diameters of less than 6 mm have been plagued by failure through thrombosis and intimal hyperplasia.[5]

Both biological and mechanical considerations are imperative in the design of small-diameter vascular grafts. Establishing a confluent monolayer of endothelial cells has improved graft patency for lower extremity bypasses to 70% at 5 years, rivaling that of saphenous vein.[7] Hemodynamic trauma to the endothelium can be created by compliance mismatch between the graft and native vessel, leading to graft failure.[8−12] The graft must have crush strength and flexibility for cross-joint applications and must maintain physical integrity under long-term pulsatile stress and the load imposed by lumenal pressure.

Natural polymers exhibit properties that are inherently difficult to reproduce in synthetic macromolecules. In recent years, several laboratories have used the biosynthetic machinery of microbial cells to synthesize protein polymers of specific sequence, molecular weight, and functionality.[13−23] Here we examine a set of artificial ECM (aECM) proteins engineered to exhibit the biological and mechanical behavior required of small-diameter vascular grafts.

The design uses domains derived from two naturally occurring ECM proteins (Figure 1). Fibronectin, found in the ECM and in blood serum, contains multiple cell-binding domains. Of special interest in these studies is the CS5 domain, which contains the minimal active sequence REDV and which is known to bind the $\alpha_4\beta_1$ integrin.[24−27] Immobilization of GREDVY on glycophase glass has produced surfaces that support adhesion of human umbilical endothelial cells (HUVEC) but not fibroblasts, smooth muscle cells (SMC), or platelets.[28] The ability of the CS5 domain to support endothelial cell attachment was shown in previous studies of films of recombinantly expressed proteins.[29] In the proteins examined here, we again use the CS5 domain, GEEIQIGHIPREDVDYHYP, to support the adhesion of an endothelial cell monolayer. As a negative control for cell-adhesion studies, the corresponding protein with the REDV sequence scrambled to REVD was also prepared.

Elastin is a fibrous protein found within arterial walls, ligaments, and other connective tissue, where its principal function is to provide elasticity and resilience.[30] In contrast to the modulus of ePTFE (600 MPa), naturally occurring elastin has a modulus of 0.3−0.6 MPa.[31] The sequence VPGVG has been shown to repeat up to 11 times consecutively in mammalian elastin,[32] and the exploration of elastin-like polypeptides has produced cross-linked materials with moduli spanning the range recorded for the arterial wall.[33−35] For the proteins examined here, isoleucine was substituted for valine in the fourth position of the pentapeptide repeat to decrease the lower critical solution temperature (LCST) to below ambient temperature for facile processing.[29,32,34] Within the elastin-like domain, lysine residues were incorporated to allow cross-linking by difunctional electrophiles. A similar elastin-like motif was used by Conticello and co-workers, who prepared elastin-mimetic hydrogels by cross-linking of the protein with bifunctional *N*-hydroxysuccinimidyl (NHS) esters.[36] We have previously reported the tensile properties of aECM protein films cross-linked through N- and

[†] University of Massachusetts.
[‡] California Institute of Technology.

10.1021/ma0256587 CCC: $25.00
Published on Web 02/14/2003

Sequence 1:

M-MASMTGGQQMG-HHHHHHH-DDDDK(LD-GEEIQGHIPREDVYHLYP-G ((VPGIG)$_2$VPGKG(VPGIG)$_2$)$_4$VP)$_3$-KLE

T7 Tag | His Tag | Cleavage site | CS5 Cell-binding Domain | Elastin-like Repeat

Sequence 2:

M-MASMTGGQQMG-HHHHHHH-DDDDK(LD-GEEIQGHIPREVDYHLYP-G ((VPGIG)$_2$VPGKG(VPGIG)$_2$)$_4$VP)$_3$-KLE

T7 Tag | His Tag | Cleavage site | Scrambled CS5 Cell-binding Domain | Elastin-like Repeat

Figure 1. Protein sequences showing normal (**1**) and scrambled (**2**) CS5 domains.

C-terminal lysine residues by treatment with glutaraldehyde,[34] and Urry and co-workers have explored the mechanical behavior of various elastin-like proteins cross-linked by γ-irradiation, dicumyl peroxide, and carbodiimides.[37−39]

Experimental Section

Standard protocols were used for DNA manipulation, bacterial growth, protein expression, sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE), and Western blotting.[40] The antibody used for Western blotting was an anti-T7 tag-horseradish peroxidase conjugate (Amersham).

Protein Expression. DNAs encoding proteins **1** and **2** were each cloned into the pET28 expression plasmid (Novagen, Madison, WI) and induced under control of a bacteriophage T7 promoter by addition of isopropyl-1-β-D-thiogalactoside (IPTG) (Calbiochem, Inc., San Diego, CA) in the expression host BL21(DE3)pLysS (Novagen). Expression was carried out in a 10 L Bioflow 3000 fermentor (New Brunswick Scientific, Edison, NJ) with Terrific Broth (TB) as the expression medium. The medium was supplemented with 25 μL/mL kanamycin (Sigma, St. Louis, MO) and 34 μL/mL chloramphenicol (Sigma). A 400 mL overnight culture was used for inoculation of the fermentation culture. The pH was maintained at 7.2 and the temperature at 37 °C. The culture was induced at an optical density at 600 nm (OD_{600}) of 5−6 with 2.5 mM IPTG and expression was allowed to continue for 2−3 h until OD_{600} reached a value of 13−18. The cells were harvested by centrifugation (15 min, 10 000*g*, 4 °C) and yielded an average of 200 g of wet cell mass per fermentation batch.

Protein Purification. The wet cell mass was redispersed in TEN buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 0.1 M NaCl) at 0.5 g/mL, frozen, and then defrosted at 4 °C with addition of 10 μg/mL deoxyribonuclease 1 (Sigma), 10 μg/mL ribonuclease A (Sigma), and 50 μg/mL phenylmethylsulfonyl flouride (Sigma). The solution was centrifuged (2 h, 35 000*g*, 4 °C) to separate the soluble and insoluble fractions. The supernatant was brought to pH 9 and 1 M NaCl at 4 °C and then allowed to equilibrate to 37 °C before another centrifugation (2 h, 35 000*g*, 37 °C). The pellet was redispersed in distilled water, brought to pH 9, 1 M NaCl at 4 °C, and centrifuged (2 h, 35 000*g*, 4 °C). The supernatant was then warmed to 37 °C and centrifuged (2 h, 35 000*g*, 37 °C). This process was repeated twice with the pellet from this step. The resulting final pellet was redispersed in distilled water, dialyzed for 3 days at 4 °C, and lyophilized to yield the purified product. SDS−PAGE, western blotting, mass spectrometry, and amino acid analysis were used to assess purity and molecular weight. Matrix-assisted laser desorption ionization−mass spectrometry (MALDI−MS) was conducted with a 20:1 ratio of matrix solution (10 mg/mL sinapinic acid in 0.07% trifluoroacetic acid, 30% acetonitrile) to analyte solution (30 mg/mL protein in 4 °C water) on a Voyager DE mass spectrometer (Applied Biosystems). The plate was spotted at 4 °C followed by crystallization at 4 °C for 1 h and 25 °C for 2 h prior to analysis.

LCST Measurement. The LCST of the protein was measured at concentrations of 10, 20, and 30 mg/mL in phosphate buffered saline (PBS), pH 7.4, by increasing the temperature at a rate of 30 °C/h and measuring the percent transmission at 300 nm on an Aviv model 62DS spectrophotometer (Lakewood, NJ).

Protein Cross-Linking. Method I involved cross-linking a solution of protein, bis(sulfosuccinimidyl) suberate (BS3) (Pierce, Rockford, IL), and PBS between two glass plates with 0.45 mm plastic spacers at the edges to set the gap height. This assembly was held together by clamps until cross-linking was complete. Method II cross-linked a dried film of protein cast from anhydrous dimethyl sulfoxide (DMSO) (Sigma) by immersing it in a solution of disuccinimidyl suberate (DSS) (Pierce) dissolved in anhydrous dimethylformamide (DMF) (Sigma).

Residual Lysine Content. Sulfosuccinimidyl-4-*O*−(4,4′-dimethoxytrityl) butyrate (SDTB) (Pierce) was used to determine the residual amine content of cross-linked films.[36,41−43] Films (1−5 mg) were immersed in 0.4 mL of 50 mM $NaHCO_3$, 1 mM SDTB and agitated for at least 2 h at room temperature. Films were removed from the solution, blotted with filter paper, immersed in 50 mL of 50 mM $NaHCO_3$, and allowed to remain at 4 °C overnight. Films were removed from the buffer, blotted with filter paper, and immersed in 1 mL of 88% formic acid. The absorbance at 498 nm was determined with a SpectraMax Plus 384 spectrophotometer (Molecular Devices, Sunnyvale, CA). Calibration curves were made by using the un-cross-linked protein as standard. The protein (3 mg) was dissolved in 0.4 mL of 50 mM $NaHCO_3$, 1 mM SDTB and agitated for at least 2 h at room temperature. The excess SDTB was removed by precipitating the protein with 1 M NaCl, redispersing in 1 mL of 50 mM $NaHCO_3$, and precipitating the protein with 1 M NaCl five times. The resulting precipitated protein was dispersed in 1 mL of 88% formic acid, and the absorbance was determined as described above.

Weight Fraction Protein. The protein content of the films was determined from the difference in weight between dry and wet samples. Wet samples were prepared by equilibration in PBS at a given temperature, followed by the wicking away of excess buffer with filter paper. The films were then placed at 50 °C overnight in a vacuum oven before the dry weights were measured. Film weight was measured again after 1 week at 50 °C under vacuum and showed no further change.

Tensile Testing. Protein films were measured after equilibration in PBS at 37 °C with vernier calipers and a micrometer to determine cross-sectional area. Samples were tensile tested under simulated physiological conditions (PBS, pH 7.4, 37 °C) on an Instron Universal Testing Machine (model 5564). A uniform strain rate of 10% gauge length per minute was used to test samples with an aspect ratio of at least 5 based on ASTM Standard D 882-00. Sample dimensions were length 12 ± 2 mm, width 2 ± 1 mm, and thickness 0.45 ± 0.1 mm. Samples were sandwiched between sheets of filter paper (in the region of the grips) and pressure-clamped in place. Typical elongations to break were 100−200%.

Results and Discussion

Protein Expression and Purification. Expression yields for **1** and **2** were 13 mg/g wet cell mass; nearly 3 g of protein could be isolated from each 10 L batch fermentation.

A distinctive characteristic of elastin-like polymers in aqueous solvents is a lower critical solution temperature (LCST), above which a polymer-rich coacervate forms and below which the polymer is soluble. Cycling crude preparations above and below the LCST provides a

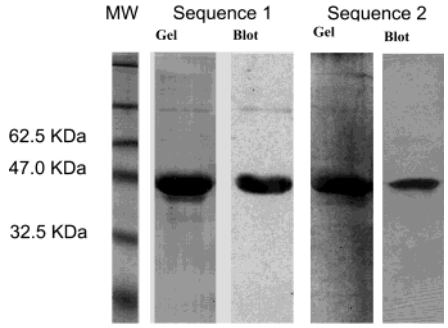

Figure 2. SDS−PAGE and western blots for purified **1** and **2**.

simple method of purification of elastin-like proteins.[17,22,23,33,36,44] Upon thawing the whole cell lysate at 4 °C (below the LCST), the protein remains in solution, allowing separation of insoluble contaminating proteins by centrifugation. Following pH adjustment and addition of salt, raising the temperature to 30 °C (above the LCST) causes the protein to precipitate, and soluble contaminating proteins can be removed by centrifugation.

Purified proteins **1** and **2** were analyzed by SDS−PAGE and western blotting, as shown in Figure 2. Mass spectrometry (Figure 3) shows the molar mass of each protein to lie slightly above the expected mass of 37 120 Da. Amino acid analyses of both proteins were within 1% of the expected results for all amino acids. The analytical results confirm the purity and identity of each target protein.

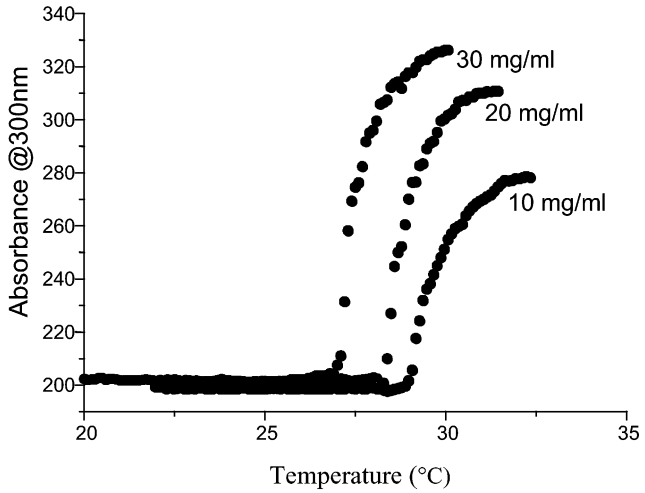

Figure 4. LCST measurements on protein **1** (PBS, pH 7.4) concentrations of 10−30 mg/mL.

The high expression levels and the simple, effective purification process allow multigram quantities of each protein to be obtained easily.

LCST Measurement. Figure 4 shows the LCST behavior of **1** at concentrations of 10, 20, and 30 mg/mL. Sequence **2** shows similar behavior. The onset of the transition occurs at 27 °C at a protein concentration of 30 mg/mL and increases to 29 °C at 10 mg/mL. Similar proteins without the lysine residues in the elastin-like region exhibit an LCST at 12 °C;[29] addition of lysines at the N- and C-termini increases the LCST to 17 °C.[34] The increase in LCST is a result of the

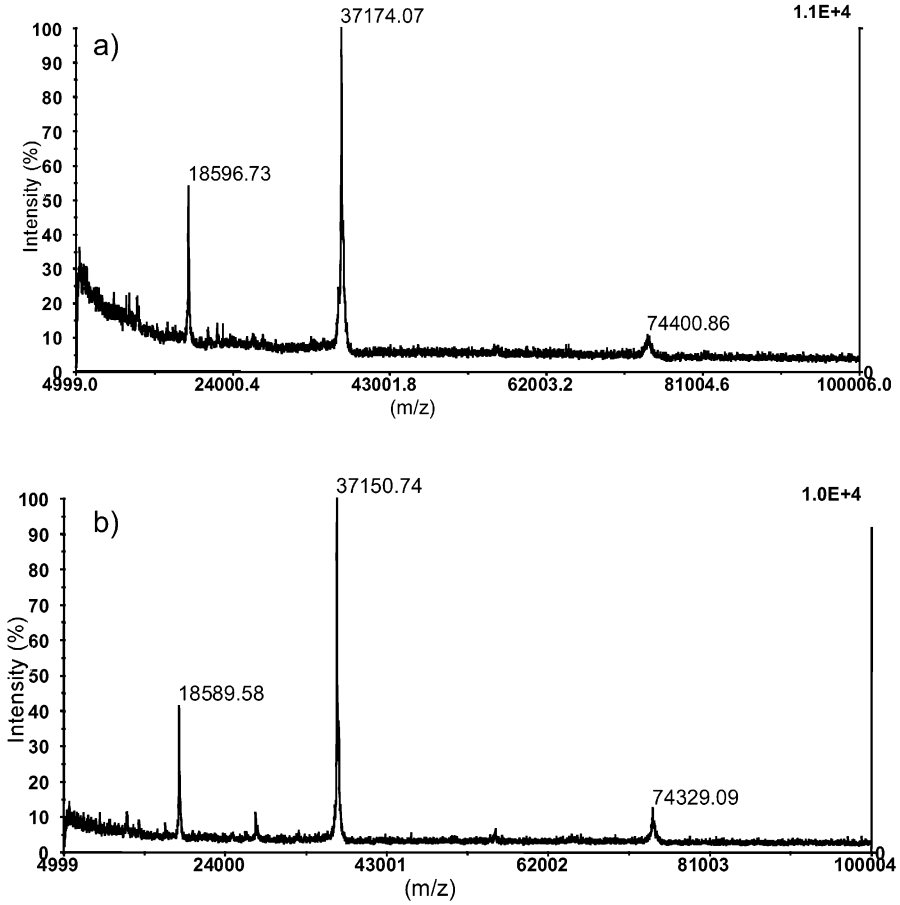

Figure 3. Mass spectra of (a) **1** and (b) **2**. The expected mass of each protein is 37 120 Da. Signals at 18.6 kDa are assigned to doubly charged species and those at 74 kDa to protein dimers.

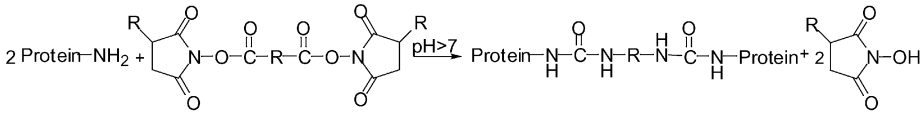

Figure 5. Reaction scheme for cross-linking with bifunctional NHS esters. For DSS, R = H; for BS3, R = SO_3^- Na^+.

Table 1. Physical Properties of Cross-Linked Protein Films

sample	NHS/lysine stoichiometry	protein solution conc (w/v)	reaction temp (°C)	reaction time (h)	wt fraction[a] protein	reacted lysines (%)	$M_c \times 1000$ (g/mol)
Method I: Films Cross-Linked in PBS with BS3							
1	0.5:1	0.4	25	>12	0.30 ± 0.03	42 ± 7	38
2	1:1	0.4	25	>12	0.29 ± 0.02	75 ± 7	14
3	1.5:1	0.4	25	>12	0.25 ± 0.03	76 ± 8	11
4	1:1	0.4	4	>12	0.32 ± 0.02	74 ± 7	15
5	1:1	0.2	25	>25	0.20 ± 0.02	61 ± 15	20
Method II: Films Immersed in DMF/DSS Cross-Linking Solution							
6	5:1	dry film	25	>24	0.32		7
7	10:1	dry film	25	>24	0.37 ± 0.01		4
8	100:1	dry film	25	>24	0.39 ± 0.02	90 ± 7	3
elastin							6

[a] In films equilibrated at 37 °C.

substitution of the hydrophilic lysine residue for isoleucine and is consistent with the extensive studies of Urry and co-workers on the sequence dependence of the LCST.[45]

Preparation of Cross-Linked Films. The reaction scheme for protein cross-linking is shown in Figure 5. By taking advantage of the specificity of the reaction of NHS-esters with amines, cross-linking should be directed to the lysine residues and should leave the cell-binding domains unperturbed. Cross-linked films of **1** and **2** showed similar physical and mechanical behavior; for simplicity, results are discussed for **1**.

Table 1 shows the percentage of lysine residues consumed in each of the cross-linked films. As expected, increasing the molar ratio of activated ester to amine from 0.5 to 1.0 increases the extent of reaction of the protein-bound lysine sites (samples 1 and 2). On the other hand, complete consumption of the lysine side chains is not achieved even by treatment with a 50% excess of the ester (sample 3). More effective cross-linking was accomplished by treatment of dry films with excess DSS in DMF (samples 6–8). The weight fraction of protein in the cross-linked films varied from 0.2 to 0.4 after equilibration in PBS at 37 °C.

Mechanical Properties. Figure 6 shows the stress–strain behavior of films cross-linked in PBS at 25 °C with NHS/lysine ratios of 0.5:1, 1:1, and 1.5:1. The tensile modulus ranges from 0.07 to 0.19 MPa (Table 2). Increasing the amount of cross-linker yields an increase in modulus, consistent with the increased consumption of lysine sites, as discussed above.

For incompressible rubbers, the shear modulus (G) is one-third of the tensile modulus and can be related to the molecular weight between cross-links (M_c) through the relationship

$$G = \rho RT/M_c \qquad (1)$$

where ρ is the protein concentration in g/cm^3, R is the gas constant, and T is the temperature.[31,32,46–48] Equation 1 assumes the network is perfect, in that all chains are effective in giving rise to an elastic restoring force. The use of the protein concentration (ρ) to account for the reduction in the number of elastically effective chains in the hydrated film ignores any elastic effects of swelling. This assumption is appropriate for samples

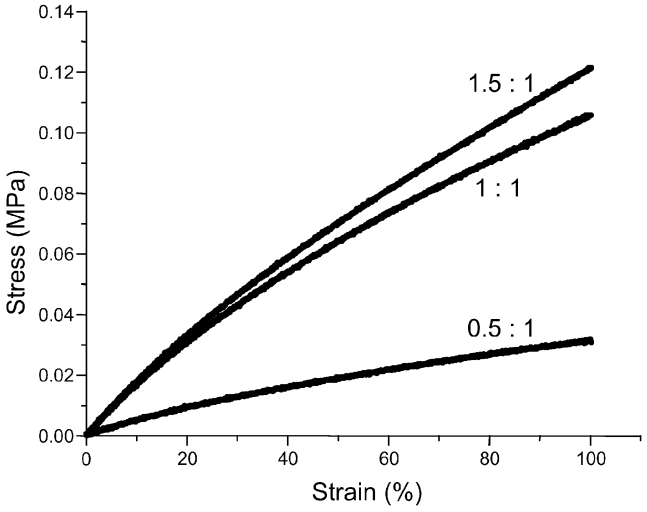

Figure 6. Stress–strain behavior of films cross-linked in PBS at 25 °C with NHS/lysine ratios of 0.5:1, 1:1, and 1.5:1.

Table 2. Mechanical Properties of Cross-Linked Protein Films[a]

sample	no. of samples tested	tensile modulus E (MPa)	shear modulus G (MPa)
Method I: Films Cross-Linked in PBS with BS3			
1	5	0.7 ± 0.03	0.022 ± 0.003
2	8	0.17 ± 0.01	0.055 ± 0.001
3	5	0.19 ± 0.01	0.060 ± 0.001
4	6	0.18 ± 0.02	0.060 ± 0.002
5	5	0.08 ± 0.01	0.026 ± 0.001
Method II: Films immersed in DMF/DSS Cross-Linking Solution			
6	4	0.35 ± 0.31	0.12 ± 0.08
7	6	0.77 ± 0.12	0.25 ± 0.05
8	5	0.97 ± 0.31	0.32 ± 0.10
elastin		0.30 ± 0.06	

[a] Sample numbers correspond to those in Table 1.

cross-linked in PBS[31] but is likely to introduce some error into the estimation of M_c for films cross-linked by immersion in DSS/DMF. Figure 7 shows the tensile properties of films prepared by cross-linking solutions containing 0.2 and 0.4 w/v protein in PBS at 25 °C (Table 1, samples 2 and 5). The modulus of the sample prepared at higher concentration is approximately

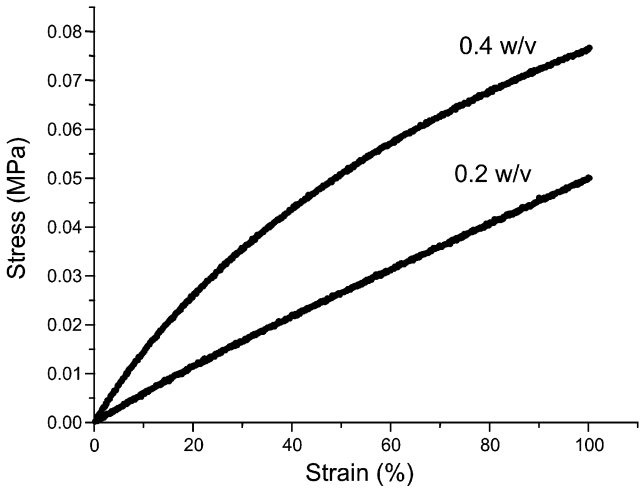

Figure 7. Stress−strain behavior of films cross-linked at 0.4 and 0.2 w/v protein concentrations in PBS with BS3.

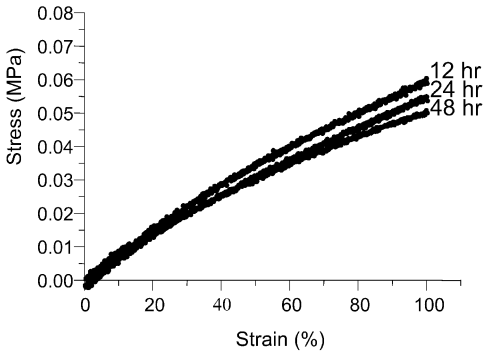

Figure 8. Stress−strain behavior of films cross-linked for 12, 24, and 48 h, showing no evidence of further cross-linking after 12 h.

double that of the film prepared at 0.2 w/v protein, in accord with eq 1 (Table 2).

The effect of cross-linking time on the mechanical properties was explored by treating films for periods of 12−48 h. Figure 8 shows the resulting stress−strain curves; no increase in film stiffness is achieved after 12 h of reaction time.

Films cross-linked in PBS at 25 °C are white and opaque. In contrast, protein films cross-linked at 4 °C are clear and show no visible LCST transition on heating; i.e., they remain transparent at 37 °C. Figure 9 compares the tensile behavior of films cross-linked above and below the LCST. Although the samples differ strikingly in appearance, the modulus is unaffected when measured at 37 °C (Table 2).

Films cross-linked in PBS were softer than desired, with tensile moduli in all cases below 0.2 MPa. Natural elastins exhibit moduli in the range of 0.3−0.6 MPa.[8,31,46] In an attempt to prepare stiffer films, a second cross-linking method was adopted. The protein was dissolved in DMSO and dried to a tacky film, which was immersed in DMF solutions of the cross-linker DSS. In a typical experiment, a 25 mg film of protein was placed into 10 mL of the cross-linking bath, with the NHS/lysine ratio varying from 5:1 to 100:1. Figure 10 shows the mechanical behavior of the resulting films. As the concentration of cross-linker increases, the modulus rises from 0.35 to 0.97 MPa, spanning the targeted range of film stiffness (Table 2).

Theoretical M_c. The M_c calculated for each of the cross-linked films is listed in Table 1. Although the

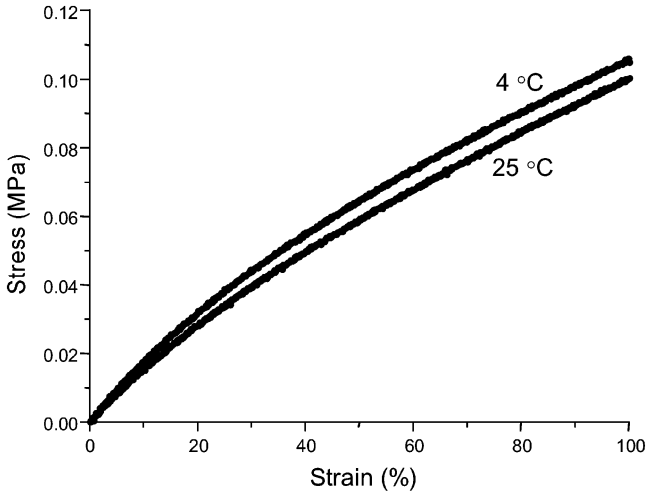

Figure 9. Stress−strain behavior of films cross-linked above (25 °C) or below (4 °C) the LCST, showing little difference in mechanical properties.

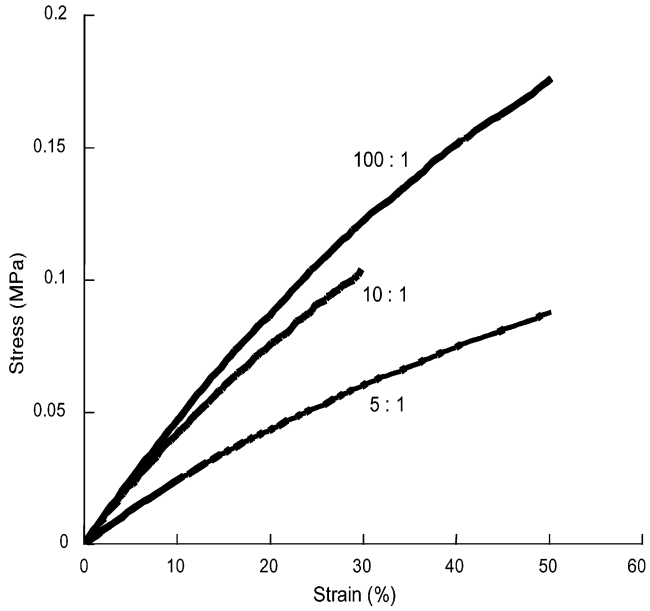

Figure 10. Stress−strain behavior of films cross-linked by immersion in solutions of DMF with DSS at NHS/lysine ratios of 100:1, 10:1, and 5:1.

agreement is less than perfect, the value of M_c calculated from eq 1 varies in the expected manner with the extent of cross-linking estimated from titration of residual amines with SDTB (Table 1). The most heavily cross-linked films are characterized by values of M_c of 3000−7000, comparable to those of natural elastins.[46,49] Complete consumption of the lysine sites would yield an M_c of approximately 2600.

Conclusions

The tensile properties of cross-linked artificial extracellular matrix proteins are suitable for application in small-diameter vascular grafts. Through variation in the conditions of cross-linking, the moduli of aECM films can be tuned to within the range characteristic of native elastins. Ongoing studies address the fatigue behavior, tear strength, and cell-binding behavior of these and related matrices.

Acknowledgment. This work was supported by NIH Grant 5 RO1 HL59987-03 and by NSF Grant BES-

9901648. Mass spectra were recorded in the central facility for mass spectrometry of the Center for the Science and Engineering of Materials at Caltech.

References and Notes

(1) National Heart, Lung and Blood Institute. *Morbidity and Mortality: Chartbook on Cardiovascular, Lung and Blood Diseases*. National Institutes of Health: Bethesda, MD, 1998.
(2) Bos, G. W.; Poot, A. A.; Beugeling, T.; van Aken, W. G.; Feijen, J. *Arch. Physiol. Biochem*. **1998**, *106*, 100−115.
(3) Greisler, H. In *How to Build a Blood Vessel*; Lifeline Foundation Research Initiatives in Vascular Disease Conference, Bethesda, MD, 1997; pp 58−61.
(4) Langer, R. W.; Vacanti, J. *Science* **1993**, *260*, 920−926.
(5) Greisler, H. P. *New Biologic and Synthetic Vascular Prostheses;* R.G. Landes: Austin, TX, 1991.
(6) Musella, R. A.; Willey, E. N. *Microsurgery* **1985**, *6*, 85−91.
(7) Zilla, P.; Deutsch, M.; Meinhart, J.; Fischlein, T.; Hofmann, G. In *How to Build a Blood Vessel*; Lifeline Foundation Research Initiatives in Vascular Disease Conference, Bethesda, MD, 1997.
(8) Abbott, W. M.; Cambria, R. P. In *Biologic and Synthetic Vascular Prostheses*; Stanley, J. C., Ed.; Gurne and Stratton: New York, 1982; pp 189−220.
(9) Greisler, H. P.; Joyce, K. A.; Kim, D. U.; Pham, S. M.; Berceli, S. A.; Borovetz, H. S. *J. Biomed. Mater. Res*. **1992**, *26*, 1449−1461.
(10) Kinley, C. E.; Marble, A. E. *J. Cardiovasc. Surg*. **1980**, *21*, 163−170.
(11) Zwolak, R. M.; Adams, M. C.; Clowes, A. W. *J. Vasc. Surg*. **1987**, *5*, 126−136.
(12) Clowes, A. W.; Reidy, M. A.; Clowes, M. M. *Lab. Invest*. **1983**, *49*, 327−333.
(13) Ferrari, F. A.; Cappello, J. In *Protein-Based Materials*; McGrath, K., Kaplan, D., Eds.; Birkhauser: Boston, MA, 1997; p 37.
(14) Yu, S. M.; Conticello, V. P.; Zhang, G.; Kayser, C.; Fournier, M. J.; Mason, T. L.; Tirrell, D. A. *Nature (London)* **1997**, *389*, 167−170.
(15) Petka, W. A.; Harden, J. L.; McGrath, K. P.; Wirtz, D.; Tirrell, D. A. *Science* **1998**, *281*, 389−392.
(16) Krejchi, M. T.; Atkins, E. D. T.; Waddon, A. J.; Fournier, M. J.; Mason, T. L.; Tirrell, D. A. *Science* **1994**, *265*, 1427−1432.
(17) MacPherson, D. T.; Xu, J.; Urry, D. W. *Prot. Express. Purif*. **1996**, *7*, 51−57.
(18) Szela, S.; Avtges, P.; Valluzzi, R.; Winkler, S.; Wilson, D.; Kirschner, D.; Kaplan, D. L. *Biomacromolecules* **2000**, *1*, 534−542.
(19) O'Brien, J. P.; Fahnestock, S. R.; Termonia, Y.; Gardner, K. H. *Adv. Mater*. **1998**, *10*, 1185−1195.
(20) Kaplan, D. *Nat. Biotechnol*. **2002**, *20*, 239−240.
(21) McGrath, K. P.; Butler, M. M.; DiGirolamo, C. M.; Kaplan, D. L.; Petka, W. A.; Laue, T. M. *J. Bioact. Comput. Polym*. **2000**, *15*, 334−356.
(22) Meyer, D.; Chilkoti, A. *Nat. Biotechnol*. **1999**, *17*, 1112−1115.
(23) Meyer, D.; Chilkoti, A. *Biomacromolecules* **2002**, *3*, 357−367.
(24) Humphries, M. J.; Akiyama, S. K.; Komoriya, A.; Olden, K.; Yamada, K. M. *J. Cell Biol*. **1986**, *103*, 2637−2647.
(25) Mould, A. P.; Humphries, M. J. *EMBO J*. **1991**, *10*, 4089−4095.
(26) Mould, A. P.; Komoriya, A.; Yamada, K. M.; Humphries, M. J. *J. Biol. Chem*. **1991**, *266*, 3579−3585.
(27) Mould, A. P.; Wheldon, L. A.; Komoriya, A.; Wayner, E. A.; Yamada, K. M.; Humphries, M. J. *J. Biol. Chem*. **1990**, *265*, 4020−4024.
(28) Massia, S. P.; Hubbell, J. A. *J. Biol. Chem*. **1992**, *267*, 14019−14026.
(29) Panitch, A.; Yamaoka, T.; Fournier, M. J.; Mason, T. L.; Tirrell, D. A. *Macromolecules* **1999**, *32*, 1701−1703.
(30) Ayad, S.; Humphries, M.; Boot-Handford, R,; Kadler, K.; Shuttleworth, A. *The Extracellular Matrix FactsBook;* Facts-Book Series; Academic Press: San Diego, CA, 1994.
(31) Fung, Y. C. *Biomechanics: Mechanical Properties of Living Tissues*, 2nd ed.; Springer-Verlag: New York, 1993.
(32) Urry, D. W.; Parker, T. M.; Reid, M. C.; Gowda, D. C. *J. Bioact. Comput. Polym*. **1991**, *6*, 263−282.
(33) Urry, D. W.; Luan, C.-H.; Harris, C. M.; Parker, T. In *Protein-Based Materials*; McGrath, K., Kaplan, D., Eds.; Birkhauser Press: Boston, MA, 1997.
(34) Welsh, E. R.; Tirrell, D. A. *Biomacromolecules* **2000**, *1*, 23−30.
(35) Chandran, K. B. *Cardiovascular Biomechanics*; Welkowitz, W., Ed.; University Press: New York, 1992; p 539.
(36) McMillan, R. A.; Conticello, V. P. *Macromolecules* **2000**, *33*, 4809−4821.
(37) Lee, J.; Macosko, C. W.; Urry, D. W. *Macromolecules* **2001**, *34*, 5968−5974.
(38) Lee, J.; Macosko, C. W.; Urry, D. W. *Biomacromolecules* **2001**, *2*, 170−179.
(39) Lee, J.; Macosko, C. W.; Urry, D. W. *Macromolecules* **2001**, *34*, 4114−4123.
(40) Sambrook, J.; Frisch, E. F.; Maniatis, T. *Molecular Cloning*, 2nd ed.; Cold Spring Harbor Laboratory Press: Plainview, NY, 1989.
(41) Guar, R. K.; Gupta, K. C. *Anal. Biochem*. **1989**, *180*, 253−258.
(42) Sarin, V. K.; Kent, S. B. H.; Tam, J. P. Merrifield, R. B. *Anal. Biochem*. **1981**, *117*, 147−157.
(43) Lustenberger, P.; Formstecher, P.; Dautrevaux, M. *J. Chromatogr*. **1980**, *193*, 451−457.
(44) Urry, D. W.; Nicol, A.; McPherson, D. T.; Xu, J.; Shewry, P. R.; Harris, C. M.; Parker, T. M.; Gowda, D. C. In *Encyclopedic Handbook of Biomaterials and Bioengineering, Part A: Materials;* Marcel Dekker: New York, 1995; Vol. 2.
(45) Urry, D. W.; Gowda, D. C.; Parker, T. M.; Luan, C. H.; Reid, M. C.; Harris, C. M.; Pattanaik, A.; Harris, R. D. *Biopolymers* **1992**, *32*, 1243−1250.
(46) Gosline, J. M.; Rosenbloom, J. In *Extracellular Matrix Biochemistry*; Piez, K. A., Reddi, A. H., Eds.; Elsevier Science Publishing Co.: New York, 1984.
(47) Aklonis, J. J.; MacKnight, W. J. *Introduction to Polymer Viscoelasticity;* John Wiley & Sons: New York, 1983.
(48) Young, R. J.; Lovell, P. A. *Introduction to Polymers*, 2nd ed.; Chapman and Hall: London, UK, 1991.
(49) Winlove, C. P.; Parker, K. H. In *Connective Tissue Matrix: Part 2*; Hukins, D. W. L., Ed.; The MacMillan Press Ltd.: New York, 1990.

MA0256587