Skin Research and Technology 2001; 7: 18–23
Printed in Denmark. All rights reserved

Skin Research and Technology
ISSN 0909-752X

Viscoelastic properties of human skin and processed dermis

Frederick H. Silver[1], Joseph W. Freeman[1] and Dale DeVore[2]

[1]*Division of Biomaterials, Department of Pathology and Laboratory Medicine, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ and* [2]*Collagenesis, Inc., Beverly, MA, USA*

Background/aims: The purpose of this work is to attempt to determine the elastic spring constant for collagen and elastic fibers (elastin) in skin and to detemine if the values of these elastic constants are similar to those reported for other tissues.
Methods: We studied the viscoelastic mechanical properties of human skin and dermis by measuring the incremental stress-strain behavior. Elastic stress-strain curves were used to obtain the elastic spring constant of elastin and collagen while the collagen fibril length was obtained from the slope of viscous stress-strain curves.
Results: Our results suggest that the elastic spring constant for elastin is about 4.0 MPa while that for collagen is about 4.4 GPa. The former value is similar to that calculated for ligamentum nuchae while the latter value is about 70% of the value found for tendon and self-assembled type I collagen fibers. The differences between the elastic constants for collagen molecules in tendon and skin is hypothesized to reflect the higher molecular tilt angle and lower D period found in skin compared to tendon as well as a shorter fibril length.
Conclusion: The differences in the collagen types present in skin and tendon may influence collagen self-assembly and the resulting viscoelastic properties.

Key words: skin – dermal allograft – viscoelasticity – collagen – elastic tissue

Accepted for publication 27 July 2000

One of the primary functions of skin is to protect internal organs and tissues from mechanical trauma. Skin is viscoelastic and as a result the mechanical response to loading involves both a viscous component associated with energy dissipation and an elastic component associated with energy storage (1). Energy applied to skin is partially dissipated through viscous sliding of collagen fibrils during alignment with the force direction (2) while the elastic behavior of skin is important in ensuring shape recovery after deformation (3). Changes in the collagen fibril orientation during deformation of the dermis are critical to maintaining the large extensibility of human skin (3, 4). The mechanical properties of skin are related to the structure and properties of the collagen and elastic fibers and the proteoglycans that are found in the skin (2).

Skin is a multilayered material with well-defined anatomical regions (5, 6). The superficial epidermis forms an uninterrupted barrier that ranges in thickness from 0.07 mm to 0.12 mm over much of the body surface (1) and contributes little to the mechanical properties (2). Below the epidermis is the dermis, which is between 1 mm and 4 mm thick (5); the dermis is composed of the papillary and reticular layers. The papillary dermis forms the upper layer, which comprises about 10% of the full dermal thickness. It contains thin collagen fibrils 20–40 nm in diameter (5) that are packed into thicker collagen fibers 0.3–3.0 μm in diameter (7). Below the papillary layer is found the reticular layer, which contains collagen fibrils 60–100 nm in diameter. Reticular collagen fibrils are composed primarily of type I collagen (5) and have fiber diameters between 10 μm and 40 μm (5). Type III collagen accounts for only about 15% of the dermal collagen, much of which is found in the papillary layer (5).

In the young adult, the collagen in the papillary dermis appears as a feltwork of randomly oriented fine fibers while that in the reticular dermis consists of loosely interwoven, large, wavy, randomly oriented collagen bundles (8). Increased collagen fiber density is observed with increased age, which is reported to reflect a decrease in the spaces between individual bundles (8). The mean fractional volumes of collagen fibers determined from stereological data is reported to be relatively constant for both papillary and reticular dermis (9) and is between about 66% and 69% for all age groups studied (9). Collagen fibers are observed to be more compact with increased age and appear to unravel (8).

Elastic tissue in skin consists of superficial thin bundles of microfibrils that become associated with

progressively larger amounts of amorphous elastin and increase in size from the papillary to reticular dermis (9). The relative volume of elastic fibers increases from about 0.7% to about 2.5% while the diameters increase from about 1 μm to 2 μm, going from the papillary to the reticular dermis (10). The volume fraction increases with age up to about 1 year, then appears to plateau. The density of elastic fibers in the papillary dermis decreases from about 2.5% to about 2% for individuals older than 10 years. Elastic fibers from skin of older individuals appear to fray and contain holes (8).

Biomechanical studies suggest that the initial portion of the stress-strain curve of skin is highly viscoelastic with a high viscous dissipation occurring during collagen fibril alignment (3). The stress-strain behavior of skin is composed of three phases (1). Up to strains of about 0.3 the collagen network offers little resistance to deformation and the behavior is dominated by the elastic fibers (11). Between strains of about 0.3 and 0.6 the collagen fibrils begin to offer resistance to deformation. During the linear portion of the stress-strain curve, the elastic component dominates the deformation (3) and appears to involve stretching of the flexible regions of cross-linked collagen molecules (12–15). In tendon, the viscous component of the stress-strain behavior is associated with fibrillar slippage (12–15). In the yield and failure region (strains above 0.6), fibril defibrillation occurs. While it is clear from inspection of stress-strain curves that the behavior of skin and tendon are quite different (1), it is not clear whether the elastic spring constant for collagen molecules in tendon (7 to 8 GPa) is different from that for collagen in skin (15).

The purpose of this paper is to analyze the viscoelastic properties of human skin and dermis in an attempt to determine if the elastic spring constant for collagen molecules in skin and tendon are the same. In addition, we will attempt to calculate the elastic spring constant for elastic fibers (elastin). Differences in the elastic spring constant for collagen between skin and tendon may reflect differences in the collagen fibrillar structure and give additional insight into the relationship between collagen composition and mechanical properties of connective tissue.

Material and Methods

Data for the viscoelastic behavior of human skin was obtained from Dunn & Silver (3). Skin used in that study was obtained from the thoracic and abdominal areas from patients 47–86 years of age and tested within 7 days of autopsy. All samples were tested at a strain rate of 10%/min using a gauge length of 2 cm. All stress data for human skin obtained from Dunn & Silver (3) were multiplied by a factor of 1.0+ strain to correct for changes in cross-sectional area during testing.

The mechanical behavior of different decellurized collagenous substrates were determined in this study. Samples of Alloderm® Acellular Dermal Graft were obtained from LifeCell Corporation (Woodlands, TX, USA) while processed human dermis (Dermaplant™ Dermal Allograft) was obtained from Collagenesis, Inc. (Beverly, MA, USA). Dermaplant™, which will be referred to subsequently in this paper as processed dermis, was prepared in the following fashion. Skin was procured from tissue banks complying with all applicable federal and state regulations and with American Association of Tissue Banks standards. Skin was cryopreserved for storage prior to processing using standard skin bank procedures. After frozen skin was removed from storage and thawed, it was processed by treatment with solutions to remove the epidermis and other cellular materials, virally inactivated, and extensively washed to remove residuals of decellurization and viral inactivation solutions. The processed dermis was then frozen and freeze-dried prior to mechanical testing.

Strips of either Alloderm or processed dermis (5 cm×1 cm×1 to 2 mm thick) were rehydrated in phosphate buffer solution (PBS) at room temperature for a minimum of 30 min prior to mechanical testing. Sample dimensions were measured by observation through the calibrated eyepiece of a Leitz Pol microscope (Leitz, Rockville, NJ, USA). The dimensions were measured in three places along the strip and the average cross-sectional area was obtained. It was assumed that the sample cross section was rectangular.

Standard viscoelastic testing was conducted on wet samples at room temperature, as previously described (12, 13). Strips were clamped into the Instron grips, and samples were stretched in tension at a strain rate of 10%/min using an Instron Model 1122 testing device. The gauge length used was 2 cm. All strains were determined from the displacement of the crosshead. Specimens were subjected to strain increments starting at a strain of 0.10, resulting in an incremental stress-versus-strain curve (Fig. 1). All stresses were multiplied by 1.0+the strain to correct for changes in the cross-sectional area during deformation. This assumes that deformation occurs with no change in volume. After the sample was stretched to each strain increment, the stress was allowed to decay to equilibrium before an additional strain increment was added. The elastic component of the

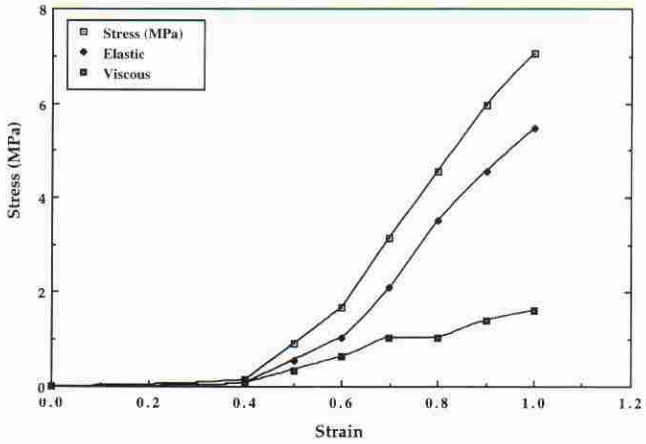

Fig. 1. Engineering stress-strain curves for skin. Total (unfilled squares, top), elastic (filled diamonds, middle) and viscous (filled squares, bottom) curves for human skin obtained from Dunn & Silver (3). Total stress was obtained from the initial stress observed at a strain rate of 10%/min while elastic stress was obtained from the equilibrium value of the stress at a fixed strain. Viscous stress was defined as the difference between the total and elastic stresses.

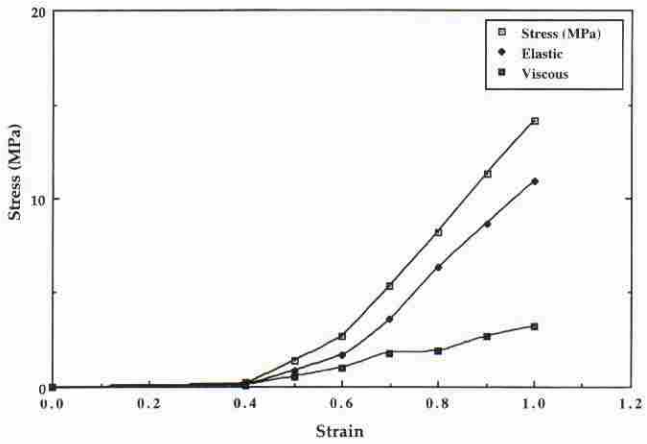

Fig. 2. True stress-strain curves for human skin. Total (unfilled squares, top), elastic (filled diamonds, middle) and viscous (filled squares, bottom) curves for human skin obtained from Dunn & Silver (3) after correction for cross-sectional area changes that occur during deformation. Engineering stresses shown in Fig. 1 were multiplied by the strain +1.0 to give the true stress and then plotted versus strain. Total stress was obtained from the initial stress observed at a strain rate of 10%/min while elastic stress was obtained from the equilibrium value of the stress at a fixed strain. Viscous stress was defined as the difference between the total and elastic stresses.

stress was defined as the stress at equilibrium while the viscous component was calculated from the difference between the total stress and the elastic stress component. Total, elastic and viscous stress-strain curves were approximated by straight lines using a curve fitting program within Cricket Graph.

Lines representing the viscous stress-strain curves obtained from incremental stress-strain curves were converted into fibril lengths based on estimation of axial ratios and shape factors, as described previously (12, 13). Viscous stress-strain equations were approximated by straight lines as discussed above and the equations were divided by the strain rate (0.1/min) to give an equation that represented the extensional viscosity versus strain in MPa-s. The shear viscosity as a function of strain was then approximated from the extensional viscosity by dividing by a value of 3.0, which is equivalent to the relationship between shear modulus and tensile modulus for isotropic materials with Poisson's ratio equal to 0.5. Shear viscosity as a function of strain was converted into shape factor, V, by dividing by the solvent viscosity (8.23×10^{-4} MPa-s) for water and by the volume fraction of polymer. The volume fraction of collagen was estimated from the wet and dry sample weights and was about 0.17 for processed dermis. The axial ratio, Z, was estimated from equation (1) where k is 0.1395 for collagen.

$$Z = (V/k)^{0.552} \qquad (1)$$

Collagen fibril lengths were calculated from equation 1 using the estimated value of the average fibril diameter (80 nm) in the reticular layer reported by Smith (5).

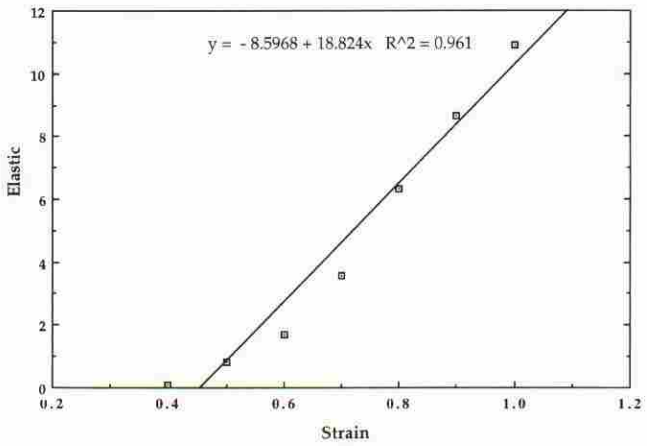

Fig. 3. Determination of the elastic spring constant for human skin in the linear region. A plot of the slope of the elastic true stress-strain curve in the linear region for human skin is shown. The equation shown is the best linear fit to the experimental stress-strain curve. Also shown is the correlation coefficient R^2. The elastic spring constant was determined to be 18.8 MPa.

Results

Total, elastic and viscous stress-strain curves for human skin are shown in Fig. 1. When this data was replotted after the stress was converted into true

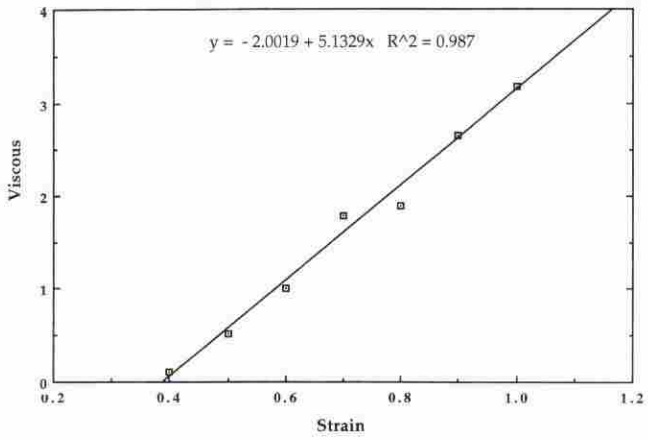

Fig. 4. Determination of the viscous slope for human skin in the linear region. A plot of the slope of the viscous true stress-strain curve in the linear region for human skin is shown. The equation shown is the best linear fit to the experimental stress-strain curve. Also shown is the correlation coefficient R^2. The viscous slope is used to calculate the fibril length using equation 1.

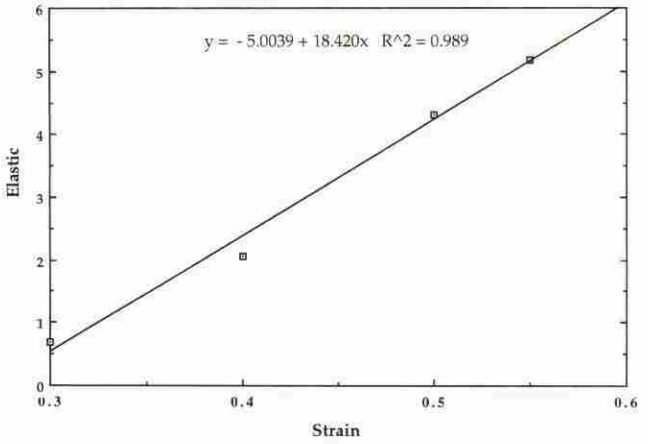

Fig. 5. Determination of the elastic spring constant for Alloderm® in the linear region. A plot of the slope of the elastic true stress-strain curve in the linear region for human skin is shown. The equation shown is the best linear fit to the experimental stress-strain curve. Also shown is the correlation coefficient R^2. The elastic spring constant was determined to be 18.4 MPa.

stress from engineering stress, the stress-strain curves shifted upward (Fig. 2). The true stress was obtained by multiplying the engineering stress by 1.0 + the strain. The total and elastic stress-strain relationships rapidly increase after an initial low slope region. The viscous stress-strain curve has a small initial slope that increases almost linearly after a strain of about 0.4. The elastic stress-strain curve can be broken into two straight lines. The slope of the initial portion of the curve is about 0.100 MPa while that of the high strain region is about 18.80 MPa (Fig. 3). The slope of the viscous stress-strain curve above a strain of 0.4 is about 5.13 MPa (Fig. 4)

Stress-strain data for Alloderm and processed dermis are shown in Table 1. Values for the initial slope are all about 0.100 MPa. The value is only approximate since the slope in this region is much lower than that of the second portion of the curve. The slopes of the high strain elastic stress-strain curves were 18.8 and 17.55 for Alloderm (Fig. 5) and for processed dermis, respectively.

TABLE 1. Slopes of the incremental stress-strain curves

Sample	Slopes (MPa)			Fibril length (μm)
	Initial elastic	Final elastic	Viscous	
Human skin	0.10	18.8	5.13	54.8
Alloderm®	0.10	18.4	7.05	63.7
Processed dermis	0.10	17.6	4.35	48.8

* Calculated using a fibril diameter of 80 nm.

The slopes of the linear portion of the viscous stress-strain curves were 5.37, 7.05 and 4.35 for human skin, Alloderm and processed dermis, respectively. Fibril lengths calculated from the slopes of the viscous stress-strain curves in the region of strain above 0.4 are listed in Table 1 and are 54.8 nm, 63.7 nm and 48.8 nm for human skin, Alloderm and processed dermis, respectively.

Discussion

We previously reported that the elastic spring constant for type I collagen fibers in tendon and for self-assembled cross-linked type I collagen fibers is between 5 and 7.9 GPa after correction for the collagen volume fraction and the ratio of macroscopic deformation to molecular deformation (a factor of 10) that is associated with a specific macroscopic strain (13, 14). The slope of the viscous stress-strain curve was determined to be about 160 MPa for rat tail tendon fibers (13, 14) and 41 to 84 MPa for turkey tendons from 12- to 16-week-old animals (14) at a strain rate of 10% per min. The fibril length for rat tail tendon calculated using equation 1 and a fibril diameter of 250 nm was 880 μm while turkey tendon fibril lengths were between 414 and 616 μm using a fibril diameter of 264 nm (13, 14).

In the present study, we have determined that the slope of the elastic stress-strain curve for skin and dermis for strains up to 0.4 is 0.100. The slope increases above a strain of 0.4 to 18.8 MPa for human

skin, to 18.4 MPa for Alloderm and to 17.55 MPa for processed dermis. It is believed that the initial slope of the stress-strain curve represents the contribution of elastic fibers to the stress-strain behavior of skin (3, 11). If we correct the initial elastic slope by the approximate volume fraction of elastic fibers (2.5%), the slope becomes 4.00 MPa. The slope of the stress-strain curve of elastin from ligamentum nuchae corrected for true stress is 2.04 MPa (16); assuming a volume fraction of elastin of about 0.45 at 23°C (17), this gives a corrected slope for elastic fibers of 4.53 MPa in ligamentum nuchae. This value is close to the measured value for skin. The value of 4.00 MPa for the elastic constant of elastin is higher than the previous estimate (2) and does not change after removal of the cellular components since the slope for Alloderm and processed dermis was identical to that of intact skin.

The value of the slope of the elastic stress-strain curve at strains above 0.4 reflects the contribution of the collagen fibrils (3). For human skin, the value obtained in this study is 18.8 MPa. When this value is corrected for the volume fraction of polymer (0.17) and the ratio of the macroscopic strain to the molecular deformation, an elastic constant for collagen molecules in skin is obtained. If it is assumed that the ratio of the macroscopic strain to the molecular strain is a factor of 10 as previously estimated for tendon (13, 14) and that this ratio must be corrected by the length of the linear region for skin (0.4) divided by that of the length of the linear region in tendon (0.1), then the corrected elastic constant for collagen in skin becomes about 4.4 GPa. This value is about 60% of that calculated for tendon. The difference between the elastic constants for collagen molecules in tendon and skin may reflect differences in the D periods of these tissues (67 nm in tendon and 65 nm in skin), which reflect differences in molecular tilts. It has been modeled that cross-link differences in tendon and skin require that the collagen molecules in skin have a molecular tilt of 16° compared to a tilt of 7° in tendon (18). The effect of a molecular tilt of 16° would be to lower the effective elastic constant of collagen.

The stiffness of fiber reinforced composites falls off rapidly as the angle between the fiber axis and the direction of the applied stress increases. The increased angle of tilt of collagen molecules with respect to the fibril axis in skin could explain the apparent differences in elastic spring constants of collagen molecules in skin and tendon. Another factor that may explain the decreased elastic spring constant of skin with respect to tendon may be the type III collagen content. Type III collagen is found in skin in combination with type I molecules. Results of a recent modeling study suggest that the type III collagen molecule is more flexible than that of type I collagen (15), suggesting that the lower elastic spring constant may reflect the addition of more flexible collagen molecules. Removal of the cellular components from skin did not change the effective elastic constant of collagen since the slope of the high strain region was not significantly changed for Alloderm and processed dermis in comparison to intact human skin. This result was expected, as discussed previously (2).

Collagen fibril lengths were calculated from the slope of the viscous stress-strain curve and from equation 1. For human skin the value of the fibril length was found to be about 55 μm, which is about an order of magnitude below that found for tendon (12–14). The fibril lengths were similar for Alloderm and processed dermis, suggesting that removal of the cellular components did not appear to affect collagen fibril length. The shorter fibril lengths calculated for skin and processed dermis compared to tendon suggest that the decreased fibril length may lead to the decreased elastic spring constant for collagen in skin compared to tendon.

These results suggest that the mechanical behavior of skin differs from that of tendon in several ways (15). The initial portion of the stress-strain curve involves stretching of the elastic fibers, which have an elastic constant of about 4.0 MPa. During this portion of the stress-strain curve, the collagen fibrils and fibers are presumably folded so that deformation involves little collagen molecular stretching and slippage. During subsequent skin deformation, collagen molecular stretching and slippage occur. The decreased D period of the collagen molecules in skin and the shorter fibril lengths result in increased fibrillar slippage and energy dissipation, effectively lowering the elastic constant of collagen. These results suggest that differences in the types of collagen found in skin and tendon may explain the differences in the mechanical properties of these tissues. Increased tilt angles of the collagen molecules in skin with respect to the fibril axis are consistent with the apparent decrease in the elastic spring constant of collagen and lower moduli of skin with respect to tendon (1). Differences in the collagen composition of skin with respect to tendon may also reflect differences in collagen fibril lengths and lead to changes in the elastic component of the viscoelastic behavior. In this manner small modifications in the self-assembly of collagen single molecules in different tissues may lead to large differences in the mechanical behavior.

References

1. Silver FH. Biological materials: structure, mechanical properties, and modeling of soft tissues. New York: N. Y. U. Press, 1987: 75–79, 164–195.
2. Silver FH, Kato YP, Ohno M, Wasserman AJ. Analysis of mammalian connective tissue: relationship between hierarchical structures and mechanical properties. J Long-term Effect Med Implants 1992: 2: 165–198.
3. Dunn MG, Silver FH. Viscoelastic behavior of human connective tissues: relative contribution of viscous and elastic components. Connect Tissue Res 1983: 12: 59–70.
4. Dunn MG, Silver FH, Swann DA. Mechanical analysis of hypertrophic scar tissue: structural basis of apparent increased rigidity. J Invest Dermatol 1985: 84: 9–13.
5. Smith LT, Holbrook KA, Byers PH. Structure of the dermal matrix during development and in the adult. J Invest Dermatol 1982: 79: 93s–104s.
6. Ramshaw JA. Distribution of type III collagen in bovine skin of various ages. Connect Tissue Res 1986: 14: 307–314.
7. Brown IB. Scanning electron microscopy of human dermal fibrous tissue. J Anat 1972: 113: 159.
8. Lavker RM, Zheng P, Dong G. Aged skin: a study by light, transmission electron, and scanning electron microscopy. J Invest Dermatol 1987: 88: 44s–51s.
9. Cotta-Pereira G, Rodrigo G, Bittencourt-Sampaio S. Oxytalan, elaunin, and elastic fibers in the human skin. J Invest Dermatol 1976: 66: 143–148.
10. Vitellaro-Zuccarello L, Cappelletti S, Poozo Rossi VD, Sari-Gorla M. Stereological analysis of collagen and elastic fibers in the normal human dermis: Variability with age, sex, and body region. Anat Rec 1994: 238: 153–162.
11. Oxland H, Maschot J, Vidiik A. The role of elastin in the mechanical properties of skin. J Biomech 1988: 21: 213–218.
12. Silver FH, Christiansen DL, Snowhill PB, Chen Y. Transition from viscous to elastic-dependency of mechanical properties of self-assembled collagen fibers. J Appl Polymer Sci (in press).
13. Silver FH, Christiansen DL, Snowhill PB, Chen Y. Role of storage on changes in the mechanical properties of tendon and self-assembled collagen fibers. Connect Tissue Res 2000: 41: 155–164.
14. Silver FH, Christiansen DL, Snowhill PB, Chen Y, Landis WJ. The role of mineral in the viscoelasticity of turkey tendons. Biomacromolecules 2000: 1: 180–185.
15. Silver FH, Freeman JW, Seehra GP. Collagen self-assembly and the development of tendon mechanical properties. J Biomech (in press).
16. Mukherjee DP, Kagan HM, Jordan RE, Franzblau C. Effect of hydrophobic elastin ligands on the stress-strain properties of elastin fibers. Connect Tissue Res 1976: 4: 177–179.
17. Lillie MA, Gosline JM. The effects of hydration on the dynamic mechanical properties of elastin. Biopolymers 1990: 29: 1147–1160.
18. Mechanic GL, Katz EP, Henmi M, Noyes C, Yamauchi, M. Locus of a histidine-based, stable trifunctional, helix to helix collagen crosslink: stereospecific collagen structure of type I skin fibrils. Biochemistry 2000: 26: 3500–3509.

Address:
Frederick H. Silver
Department of Pathology and Laboratory Medicine
UMDNJ-Robert Wood Johnson Medical School
Piscataway, NJ 08854
USA

Tel: 732 235 4027
Fax: 732 235 4825
e-mail: silverfr@umdnj.edu