# The Biomechanical Function of Arterial Elastin in Solutes

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*Elastin is essential to accommodate physiological deformation and provide elastic support for blood vessels. As a long-lived extracellular matrix protein, elastin can suffer from cumulative effects of exposure to chemical damage, which greatly compromises the mechanical function of elastin. The mechanical properties of elastin are closely related to its microstructure and the external chemical environments. The purpose of this study is to investigate the changes in the macroscopic elastic and viscoelastic properties of isolated porcine aortic elastin under the effects of nonenzymatic mediated in vitro elastin– lipid interactions and glycation. Sodium dodecyl sulfate (SDS) was used for elastin–lipid interaction, while glucose was used for glycation of elastin. Elastin samples were incubated in SDS (20 mM) or glucose (2 M) solutions and were allowed to equilibrate for 48 h at room temperature. Control experiments were performed in 1 × Phosphate buffered saline (PBS). Biaxial tensile and stress relaxation experiments were performed to study the mechanical behavior of elastin with solute effects. Experimental results reveal that both the elastic and viscoelastic behaviors of elastin change in different biochemical solvents environments. The tangent stiffness of SDS treated elastin decreases to 63.57 ± 4.7% of the control condition in circumference and to 58.43 ± 2.65% in the longitude. Glucose treated elastin exhibits an increase in stiffness to 145.06 ± 1.48% of the control condition in the longitude but remains similar mechanical response in the circumferential direction. During stress relaxation experiments with a holding period of half an hour, elastin treated with SDS or glucose shows more prominent stress relaxation than the untreated ones.* [DOI: 10.1115/1.4006593]

## 1 Introduction

Elastin is one of the major extracellular matrix (ECM) assemblies that endow blood vessels with critical mechanical properties such as flexibility and extensibility, which are essential to accommodate deformations encountered during physiological functions [1]. Elastin also presents in other connective tissues such as lung, skin, and ligament, and serve important roles for physiologic elasticity of these organs. It is well known that elastin is remarkably long lived, and it can suffer from cumulative effects of exposure to chemical damage. Arterial elastin is an extremely hydrophobic protein, which makes it an attractive site for the deposition of hydrophobic ligands such as lipids that could potentially alter elastin's mobility [2]. The lipid content of the human aorta increases with age [3], and might contribute to the gradual stiffening of arteries [4]. Nonenzymatic glycation is magnified in diabetic patients. It is one of the main mechanisms of ageing of the long-lived elastin protein, and has been shown to correlate with the severity of diabetic complications [5,6].

Elastin network contains hydrated elastin molecules that rearrange when subjected to external stresses [7]. Molecular probe testing has revealed that elastin fibers contain a network of water-filled pores accessible to solutes with molecular weights below 1000 Da. The water spaces between and around fibers are accessible to much larger solutes [8]. The intra- and extrafibrillar spaces can be modified by mechanical stresses and biochemical environments [8,9]. Relaxation time required for the rearrangement is directly related to the free volume available to the elastin chains to move.

Sodium dodecyl sulfate (SDS) is an anionic hydrophobic ligand that is widely used in tissue engineering and biochemistry [10]. According to earlier study by Gunstone et al. [11], SDS interacts with protein much the same way as lipids do; thus, it is chosen here to study the possible lipid-induced changes in the mechanical function of arterial elastin. SDS has been used as a lipid model to identify the possible lipid-induced changes in the viscoelastic behavior of arterial elastin [2]. SDS has an alkyl chain that is long enough to interact hydrophobically with elastin. Also, its negative charge allows possible electrostatic interaction with elastin. A previous study has also shown that SDS can alter the mechanical properties of elastic fibers [12]. Glucose, also known as blood sugar, is a very important carbohydrate in biology and its effect on the mechanical properties of blood vessels is magnified in diabetic patients [9,13]. Nonenzymatic glycation, by which glucose can directly condense with amino groups of proteins, is one of the main mechanisms of aging of the long-lived elastin protein [14]. Side chain modifications from elastin–lipid interactions and glycation will compromise the mechanical properties of elastin. Changes in mechanical properties of elastin have important medical and physiological consequences. In a few previous studies the mechanical loading has been limited to simple uniaxial stretching [2,9,12,13], which ignore the multiaxial loading state under physiological conditions. A planar biaxial tensile test with independent control of load in both perpendicular directions, although it cannot replicate the physiological loading conditions, is sufficient in elucidating the anisotropic stress-strain responses, which is important to fully characterize the mechanical behavior of soft biological tissues.

We have previously studied the elastic and viscoelastic behavior of aortic elastin in PBS using combined experimental and modeling approaches [15,16]. Biaxial tensile testing and biaxial stress relaxation experiments were performed to effectively characterize the orthotropic hyperelastic and viscoelastic properties of elastin. These experimental approaches establish a foundation for our current study and will be adopted. Moreover, we will focus on the effects of immediate biochemical environments on the elastic and viscoelastic properties of elastin. In this study, the elastic and viscoelastic behaviors of isolated aortic elastin were studied under equibiaxial tension with the combined effects of mechanical loading and immediate biochemical environments. Biaxial stress relaxation experiments were performed to study the time-dependent behavior of porcine aorta and its elastin. Possible intra- and extrafibrillar changes under solvent effects on the mechanical behavior of arterial elastin will be discussed.

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## 2 Materials and Methods

**2.1 Sample Preparation.** Porcine thoracic aortas were harvested from a local abattoir and transported to the laboratory on ice. Before experiment, the aortas were cleaned of adherent tissues and fat, rinsed in distilled water, and cut into squares of about 20 mm × 20 mm. All samples were taken from the midpoint of the thoracic aorta to minimize changes of mechanical properties with aortic longitudinal position. Purified aortic elastin was obtained using a cyanogen bromide (CNBr) treatment [17]. Briefly, aortic samples were treated in 50 mg/mL CNBr (Acros Organics) in 70% formic acid (Acros Organics) solution with gentle stirring for 19 h at room temperature. They were then stirred in the same solution for 1 h at 60 °C, followed by 5 min boiling. Both aorta and elastin samples were kept in 1 × phosphate buffered saline (PBS) before further experiment.

**2.2 Elastin–Lipid Interactions and Glycation.** To study the effects of immediate biochemical environments on the structure and function elastin, 20 mM SDS and M glucose solutions were used for in vitro elastin–lipid interactions and glycation, respectively [9,12,13]. Sample sizes before and after chemical treatments were measured. The side length was measured using a digital caliper. Thickness was determined by averaging measurements taken using a digital caliper at nine positions over the sample. The tissue samples were then transferred to SDS or glucose solutions and were allowed to equilibrate for 48 h at room temperature [2,9,13]. These tissue samples (SDS treated, n = 8; glucose treated, n = 7) were maintained in the corresponding SDS or glucose solutions during mechanical testing in order to keep chemical balance. All control experiments were performed in 1 × PBS.

**2.3 Mechanical Testing.** Biaxial tensile tests were performed before and after the chemical treatments to study the elastic behaviors of elastin with the effects of immediate chemical environment. A more detailed description of the biaxial tensile test of elastin was presented in our previous study [15,16]. Briefly, tissue samples of about 2 cm × 2 cm were sutured at the sides and an equibiaxial membrane tension was applied to the sample. All mechanical testing of elastin was performed at room temperature. Tissue samples were preconditioned for eight cycles with 15 s of half cycle time to obtain repeatable material response. Each sample was then subjected to biaxial tensile test to the peak membrane tension varied from 40 N/m to 100 N/m. Every test trial consisted of ten cycles to allow the sample exhibiting repeatable loading and unloading curve.

Biaxial stress relaxation tests were performed on elastin samples with or without chemical treatment. After the preconditioning cycles were completed, elastin samples were loaded to another series of equibiaxial tension state with the same half cycle time of 15 s. Immediately after the last cycle of the test, the tissue sample was quickly loaded to the target equibiaxial membrane tension with a rise time of 2 s by simply repeating the required displacements during the final equibiaxial tensile test. The elastin sample was then held at the constant stretch for 1800 s. The force and stretch in both directions were recorded during the holding period. Our previous study shows that stress relaxation preconditioning is essential to achieve repeatable stress relaxation behavior in soft biological tissues [16]. Five cycles of stress relaxation were applied to elastin samples in different aqueous solutions and the result of the fifth cycle of stress relaxation was used to study the viscoelastic behavior of elastin. Cauchy stress and Green–Lagrange strain were calculated and used to describe the elastic and viscoelastic behavior of elastin. Unless otherwise specified, the chemically treated but unloaded state was used as a reference state in these calculations.

**2.4 Statistical Analysis.** The sample dimensions, biaxial tangent modulus, and maximum stress relaxation of elastin samples with and without biochemical treatments were averaged over a number of samples. Averaged results are expressed as mean ± standard error of the mean. Statistical analysis was performed using one-way analysis of variance (ANOVA) and two-tail two-sample t-test assuming unequal variances. Differences are considered statistically significant when $p < 0.05$.

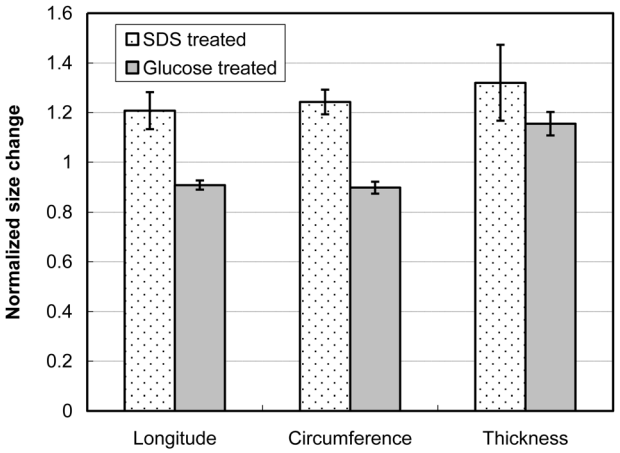


**Fig. 1 Average size changes of elastin and standard deviation (shown in error bars) due to elastin-lipid interactions (n = 8) and glycation (n = 7) in the longitude, circumference, and thickness direction of the elastin sample. Data were normalized to measurements in 1 × PBS.**

## 3 Results

Cells, collagen fibers, and other ECM components were removed in the purified aortic elastin. Interested readers are referred to our previous work for detailed histology and microscopy validation [15,16]. Figure 1 shows the normalized size changes of elastin samples after treatments with SDS (n = 8) and glucose (n = 7). To obtain the normalized size changes, the longitudinal and circumferential length and thickness of the elastin samples after solute treatments were divided by their dimensions measured before the treatments. After treatment with glucose, the side lengths of the elastin network decrease to about 90.4 ± 2.1% of its original dimension, however, the thickness increases to about 115 ± 4.7%. The swelling effect of SDS is prominent. After treatment with SDS, the side lengths increase to about 122 ± 0.66% of its original dimension and the thickness increases even more, to about 132 ± 0.15% of its original dimension. All size changes are statistically significant ($p < 0.05$).

The elastin–lipid interactions and glycation have effects on both the elastic and viscoelastic properties of elastin. Representative comparison curves of the Cauchy stress versus Green–Lagrange strain responses from the SDS treated and nontreated samples are shown in Fig. 2. We chose to use Cauchy stress Green–Lagrange strain to describe the mechanical behavior of elastin to be consistent with previous studies on elastin mechanics [18–21]. For the SDS treated elastin network, it becomes softer in both longitudinal and circumferential directions. Figure 3 shows the representative stress-strain responses of elastin after glycation. Glucose treated elastin stiffens in the longitudinal direction. However, its stress-strain responses remain similar in the circumferential direction.

To quantitatively compare the effects of elastin–lipid interactions and glycation on the elastic behavior of elastin, tangent modulus in the circumferential and longitudinal directions was calculated by differentiating the stress-strain curve. Figure 4*a* shows the normalized tangent modulus of elastin treated with SDS. To obtain the normalized modulus, the tangent modulus of elastin treated in solutes was divided by the tangent modulus of the corresponding elastin sample tested in 1 × PBS. SDS

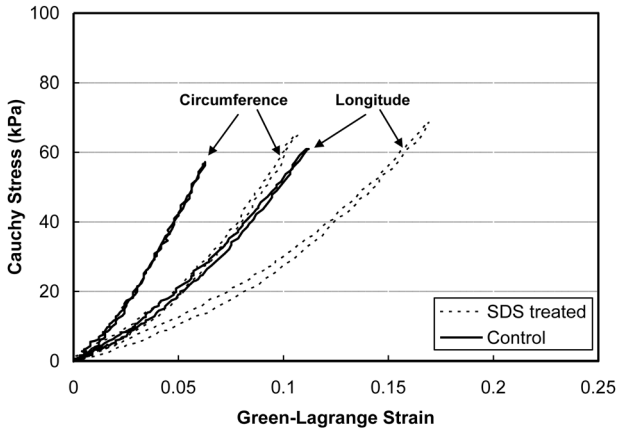


**Fig. 2 Representative Cauchy stress versus Green–Lagrange strain curves from equibiaxial tensile test of aortic elastin before and after SDS treatment**

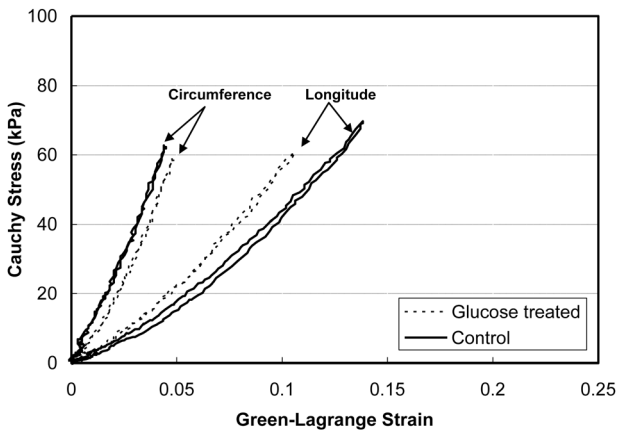


**Fig. 3 Representative Cauchy stress versus Green–Lagrange strain curves from equibiaxial tensile test of aortic elastin before and after glucose treatment**

treatment causes significant decreases in the tangent modulus. In the circumferential direction the tangent modulus decreased to 59.77–64.93% ($p < 0.05$) of the control condition, while in the longitudinal direction the tangent modulus decreased to 55.59–63.98% ($p < 0.05$). Figure 4*b* shows the normalized tangent modulus after glucose treatment. Glucose treated elastin becomes stiffer in the longitudinal direction, which is manifested by a significant increase in normalized tangent modulus to 142.69–147.53% ($p < 0.05$) but remains similar in elastic responses in the circumferential direction ($p = 0.63$).

Figure 5 shows the effects of chemical treatments on the stress relaxation responses of elastin network. All samples were tested at the initial stresses of $67.81 \pm 7.48$ kPa to eliminate the effect of initial stress levels on the rate of stress relaxation [16]. In order to compare the time-dependent responses of different tissue samples, the stresses were normalized to that at the beginning of the holding period. Elastin samples treated with SDS show more stress-relaxation than in $1 \times$ PBS but less than that in glucose. Elastin samples treated with glucose show the most stress-relaxation of the three. Elastin samples tested in $1 \times$ PBS exhibited stress relaxation to an average of $91.39 \pm 1.03\%$ ($n = 6$) of initial stresses. SDS treated elastin samples relaxed stresses to $87.90 \pm 2.18\%$ ($n = 5$) and glucose treated ones to $83.96 \pm 5.3\%$ ($n = 5$) of the initial stresses. Statistical analysis shows that the differences of stress relaxation among those three kinds of elastin samples are significant ($p < 0.05$).

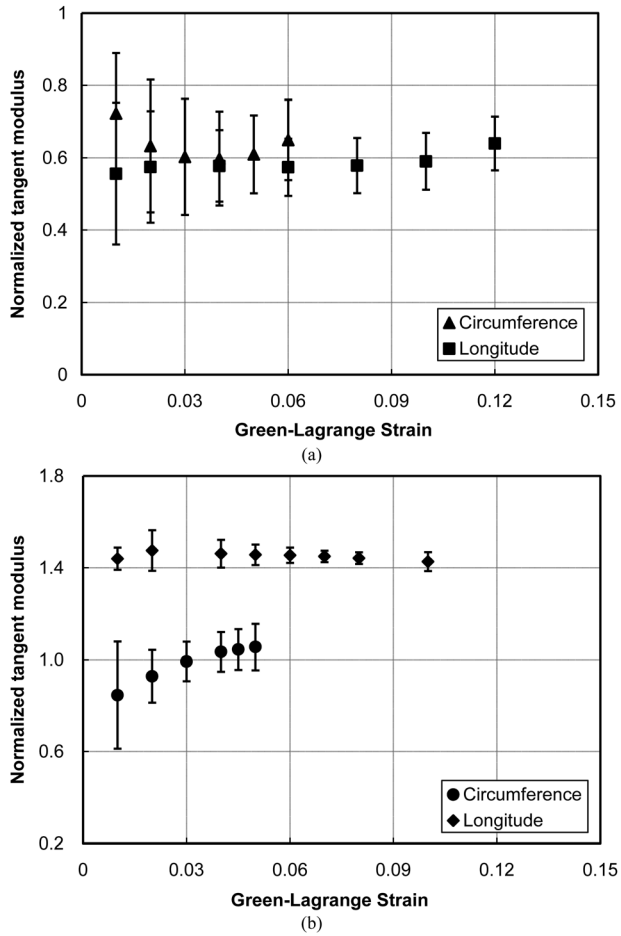


**Fig. 4 Normalized biaxial tangent modulus of aortic elastin after (*a*) SDS and (*b*) glucose treatments. Data were normalized to measurements in $1 \times$ PBS. Calculations were based on sample dimensions after solute treatments.**

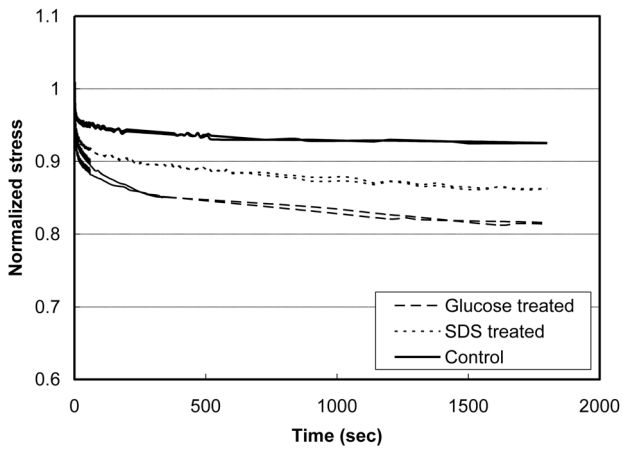


**Fig. 5 Representative stress relaxation curves of elastin samples tested in $1 \times$ PBS, 20m M SDS, and 2 M glucose**

## 4 Discussion

The mechanical properties of elastin are closely related to its microstructure and the immediate biochemical environments. In the present study, SDS and glucose were used to study the effects of physiological relevant intra- and extrafibrillar changes on the elastic and viscoelastic behavior of arterial elastin. The swelling effect of SDS and the deswelling effect of glucose on isolated

elastin network are significant. Previous studies have shown that the swelling and deswelling effect on elastin is influenced by temperature and solution concentration [9,22]. Lillie and Gosline [2] found that the amount of SDS incorporated into the elastin network increases as the SDS concentration increases up to a concentration of 10 mM and then becomes stable. In the present study, the SDS concentration was chosen to be 20 mM to achieve a stable network swelling effect. Earlier studies also indicate that elastin samples reach equilibrium in SDS after 48 h of incubation [2,12,22]. The swelling length ratio of elastin was reported to be around 1.27 under 20 mM SDS treatment [2]. Bush et al. [12] observed that the swelling length change of elastin in 0.1 M SDS is 1.4. The cubed length ratio of elastin under SDS content of 0.32 g/g (g SDS/g elastin), which is equivalent to 20 mM concentration of SDS, is around 2.0 at 23 °C [22]. Our results (Fig. 1) show that the swelling length ratios of elastin at room temperature are around $1.22 \pm 0.06$ for both side lengths and $1.32 \pm 0.15$ for sample thickness that lead to the volume change to be 1.9, which corresponds with previous literature. Lillie and Gosline [9] studied the deswelling effect of elastin in 0–5 M glucose solution. The mean length deswelling ratio of elastin in 2 M glucose at 20 °C is around 0.95. Winlove and Parker -[13] used 2 M glucose solution and 48 h of incubation time to allow elastin samples to reach equilibrium. Their results show that elastin in 2 M glucose deswells by $11.5 \pm 2.5\%$ of its original length. Our results show that 2 M glucose treated elastin deswells to $90.4 \pm 2.1\%$ of its side lengths; however, the thickness increases to about $115.5 \pm 4.7\%$. The thickness increase of glucose treated elastin samples has not been reported before. Such unique dimensional alterations in elastin indicate preferential microstructural changes caused by elastin glycation. More studies are demanded to reveal the molecular mechanisms in order to explain this unusual size change for glucose treated elastin.

The stiffness of SDS treated elastin samples decreases in both longitudinal and circumferential directions (Figs. 2 and 4*a*). Bush et al. [12] examined the mechanical properties of arterial elastin in 0.1 M SDS solution using a ring stretch test and used elastin in distilled water as control. They found that SDS treated elastin shows decreases in modulus when the stress was calculated based on sample dimensions after SDS treatment. They proposed that the changes of elastin's mechanical behavior were influenced by the swelled macroscopic dimensions of SDS treated elastin samples. If the strains and modulus values were corrected based on elastin's dimensions before the SDS treatment, elastin treated with SDS would become stiffer and less extensible than elastin under control condition [12]. In the present study, in order to eliminate the effect of sample size changes caused by SDS swelling, the normalized tangent modulus was also obtained from stress-strain curves calculated based on the sample dimensions measured before SDS treatment, i.e., without the swelling effect, and the results are shown in Fig. 6*a*. The modified stresses based on the unswelled dimensions are largely increased. However, even using the untreated dimensions of elastin sample, the normalized tangent modulus is still less than one demonstrating that SDS treated elastin becomes softer than that in 1 × PBS in both circumferential (decreases to $74.69 \pm 5.51\%$, $p < 0.05$) and longitudinal (decreases to $70.88 \pm 3.19\%$, $p < 0.05$) directions. The discrepancy between our results and the study by Bush et al. [12] possibly comes from the differences in mechanical testing methods and control conditions. A uniaxial tensile test was used in a study by Bush et al. [12]. Arterial elastin has been shown to process anisotropic mechanical properties [15], which is also demonstrated by our current results in Figs. 2 and 3. Thus biaxial tensile testing is necessary in order to fully characterize the mechanical behavior of elastin. In addition, 1 × PBS was used in our study as control condition instead of distilled water used by Bush et al. [12]. The effect of water swelling on the mechanical properties of elastin is unknown and may also contribute to the discrepancy.

Swelling alone does not cause the change of mechanical response of SDS treated elastin [12]. Murkherjee et al. [23] proposed that SDS disrupts the hydrophobic interactions between the polypeptide chains of elastic fibers and, thus, reduces the strength and stiffness of elastin. But this was disapproved by a later study by Kawazoye et al. [10]. They studied the SDS-binding capacity with bovine aortic elastins, the binding regions, and the rate of SDS release under repeated washing. They found that SDS changes the mechanical properties of elastic fibers by affecting the interfiber interactions and outer surfaces instead of binding to the internal polypeptide chains folded within the fibers. Accordingly, Lillie and Gosline [2] studied the responses of elastin in different concentrations of SDS and suggested that SDS causes no conformation change of elastin. They suggested that the mechanical behavior of SDS treated elastin could be altered by interfiber network spaced SDS displacing the elastin's hydration water molecules that are required for mobility.

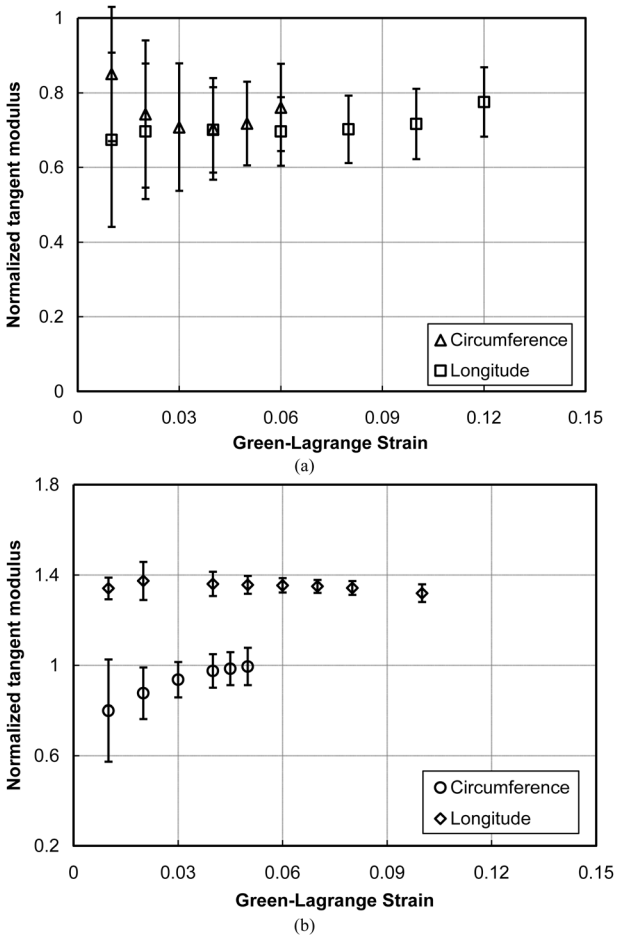


**Fig. 6 Normalized biaxial tangent modulus of aortic elastin after (*a*) SDS and (*b*) glucose treatments. Data were normalized to measurements in 1 × PBS. Calculations were based on sample dimensions before solute treatments.**

Glucose mediated in vitro nonenzymatic glycation is known to have significant effects on the biomechanical properties of collagen-rich soft tissues [24]. It has also been shown to affect the mechanical responses of elastin [9,13,25]. Significant increase in the stiffness of arterial elastin was observed following glucose treatment [25]. Increased tensile storage and loss modulus were also shown by several previous studies [9,25] in arterial elastin treated with glucose. Our results show that glucose treated elastin becomes stiffer in the longitudinal direction, but the changes are not significant in the circumferential direction (Figs. 3 and 4*b*). The anisotropic glycation modulations in the longitudinal and circumferential directions following glucose treatment are interesting and have not been shown before. The cause of this may be

related to the differences in the structure of the elastic lamellae in the two directions. Previous microscopic studies have shown that in the circumferential direction, the elastic lamellae are very wavy and corrugated, while in the longitudinal direction the lamellae sheets are fairly flat [26,27]. Thus, the effect of crosslinking caused by in vitro glycation might be more marked in the longitudinal than in the circumferential direction; however, such speculations need to be confirmed. We have ongoing experimental efforts on using spectroscopic analysis to provide molecular level insight into the interesting phenomena.

Similarly as for SDS treated elastin, normalized tangent modulus of glucose treated elastin was also obtained from stress-strain curves calculated based on sample dimensions before the glucose treatment. Figure 6*b* shows that using different sample dimension measures does not change the results significantly. The tangent stiffness in the longitudinal direction is higher than that of the control group (increases to $134.98 \pm 1.61\%$, $p < 0.05$), however, the changes in the circumferential direction are not significant ($p = 0.067$).

The glycation effects we observed are the results of short-term exposure to glucose, which might not cause preferential interactions between elastin and glucose [9]. Winlove et al. [25] performed biochemical analyses to investigate the charge-structure and ion-binding properties. They suggested that long-term incubations glycation are associated with the appearance of advanced glycation end (AGE) products. The interactions of elastin with sugar are not only due to the changes in the charge profile but also in the conformation of the molecules resulting from glycation of the charged lysine and arginine side-chain residues. Lysine later becomes involved in the formation of the desmosine crosslinks of elastin network. Such effects on the physical properties of elastin are of physiological significance.

SDS and glucose treatments have significant influences on the viscoelastic behavior of elastin as well (Fig. 5). Elastin fibers contain a network of water-filled pores accessible to solutes with molecular weights below 1000 Da [8]. Solutions with small molecular weights, such as SDS (288.38 Da) and glucose (180 Da) [28] may change the spaces within the elastin fibril network [8]. This suggests that chemical solutes might change the free volume in the elastin network and, thus, the relaxation time for molecules chains to rearrange during stress relaxation. Winlove and Parker [13] studied the change in dynamic behavior of elastin in glucose solution with the composition of the intrafibrillar fluid. They suggested that the viscous solution penetrates the intrafibrillar space of elastin and becomes an important determinant of its time-dependent mechanical properties. Our study shows that glucose and SDS treated elastin exhibits more stress relaxation responses than untreated elastin during the same holding period. Several previous studies attributed the changes in the viscoelastic properties of elastin to the changes in network volume and hydration. Lillie and Gosline [2] investigated chemical composition and interfibrillar space of the elastin network under different concentrations of SDS solvents and proposed that SDS causes no significant change in elastin's conformation, but displaces some of the water molecules closely associated with elastin. Lillie and Gosline [29] also tested the viscoelastic behavior of elastin under the effects of several polar solutes, including glucose. They observed that the viscoelastic behavior of elastin in glucose solution was similar to that of dehydrated elastin. Such similarity suggests that glucose only interacts with elastin indirectly. They concluded that glucose solutes exert both an external and an internal osmotic stress to the elastin network as the solutes are small enough to penetrate the network. It is noted that these results of the glucose effects on elastin are of short-term glycation, and long-term effects of increasing blood sugar level still need further exploration.

There are several limitations in the current study calling for further investigations into the issue. The aortic elastin was studied at the tissue level and the mechanical properties measured are results from the crosslinked three-dimensional fiber networks. With more understanding on the multiscale mechanics and microstructure, tissue-level measurements could potentially provide information on the intrinsic mechanical properties of elastin fibers. The purification method can affect the elastin and the removal of other proteins and glycoproteins may affect the way elastin interacts with SDS and glucose, which may cause the present results to be less relevant physiologically. Although SDS was used as a lipid model in this study, however, it might interact with elastin in different ways to the lipids in vivo. The concentrations of SDS and glucose and incubation time used in this study were chosen based on earlier studies to achieve the resulted effects on elastin.

## 5 Conclusions

We studied the changes of the elastic and viscoelastic properties of elastin with the effects of in vitro nonenzymatic mediated short-term elastin-lipids interactions and glycation using biaxial tensile and stress relaxation tests. Elastin with SDS and glucose treatments shows significantly changes in both the elastic and viscoelastic properties, demonstrating that the mechanical behavior of elastin is highly related to its microstructure and the external chemical environments. Biaxial tensile test is necessary to reveal changes in the anisotropic mechanical behavior of elastin in solutes. More studies to investigate the fibrillar and subfibrillar microstructures of elastin network in physiological meaningful aqueous agents, dose-dependent, and time-course responses are required to further elucidate the effect of the immediate biochemical environment on elastin's mechanical behaviors. Such knowledge is integral to understanding the performance of elastin in a biological system.

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