# Anisotropic *In Vitro* Vessel Model Using Poly(vinyl Alcohol) Hydro Gel and Mesh Material

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**ABSTRACT:** In the present study, the authors fabricated straight multilayer hybrid tubular *in vitro* vessel models (inner diameter $D_{in} = 10$ mm; thickness $T = 4$ mm) composed of poly(vinyl alcohol) hydrogel (PVA-H) and anisotropic mesh materials. The authors performed tensile, stress-relaxation and cyclic-tensile tests using axial and circumferential test pieces as well as pressure-diameter (P-D) tests using tubular test piece. In the tensile and stress-relaxation tests, the anisotropic and nonlinear mechanical properties and hysteresis characteristic of the *in vitro* models were confirmed. The *in vitro* models also showed behavior qualitatively similar to that of native arteries in cycle-tensile and P-D tests. These results demonstrate that the mechanical properties of native vessels can be duplicated in an *in vitro* model by controlling the components of the mesh material, the orientation of elastic fibers in the mesh material, and the concentration and thickness of PVA-H layers. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 116: 2242–2250, 2010

**Key words:** mechanical properties; nonlinear polymers; viscoelastic properties; relaxation; water-soluble polymers

## INTRODUCTION

Experimental studies concerning the development and progression of cardiovascular diseases have been actively conducted, in which *in vitro* vessel models with realistic geometry have been used for experiments.[1–4] Artery models that mimic mechanical properties (e.g., low skin friction, viscoelasticity, and anisotropy) of the native vessels have a high degree of usability for experimental researches concerning the diseases. Acrylic resin and silicone rubber have been used as materials for vessel models in previous studies.[1–4] They are easy to handle, and their high transparency enable optical measurements, e.g., particle image velocimetry (PIV) and laser-doppler velocimetry (LDV). However, acrylic resin is rigid and a silicone rubber tube has ~10 times the stiffness of a native vessel. Also, their skin friction is relatively high. On the basis of this background, the authors have developed *in vitro* vessel models using poly(vinyl alcohol) hydro gel (PVA-H). The transparency of PVA-H is quite high and the acoustic characteristic is near the living tissue, thus optical observation and ultrasound measurement are possible. In addition, the elasticity and viscosity of PVA-H can be adjusted by changing the concentration, polymerization degree, and crystallinity degree of PVA, and the authors have previously reproduced the elasticity and viscosity of PVA-H approximating those of native vessels.[5] Because blood flow is deeply influenced by the moving of the vessel wall, this is very useful for reproducing the cardiovascular system in an *in vitro* system. These indicate that PVA-H is a very useful material for *in vitro* models.

Recently, PVA-H has also received attention due to its potential as soft tissue substitute. Because the compliance-mismatch between native tissues and the present clinical artificial grafts is a reason for a low patency rate after implantation, especially in small diameter arteries (<6 mm),[6–8] researches of vascular tissue engineering using PVA-H are conducted.[9,10] Therefore, the fabricating method of tissue-mimicking *in vitro* models will be helpful for the development of artificial vessels. Unlike native tissues, the mechanical properties of general PVA-H displayed isotropic behavior. A method for inducing anisotropy in PVA-H has been developed by applying a certain strain to rectangular gel samples during the freeze-thaw cycles.[11] However, this method is difficult to apply to a cylindrical model, especially in the circumferential direction. In addition, the mechanical properties of native arteries are nonlinear. To reproduce these properties with an *in vitro* model are also important.

In this study, the author fabricated straight multilayer tubular *in vitro* vessel models with the anisotropic and nonlinear mechanical properties, utilizing PVA-H and anisotropic mesh materials. To confirm the mechanical properties of these models, the author performed tensile, stress-relaxation, and

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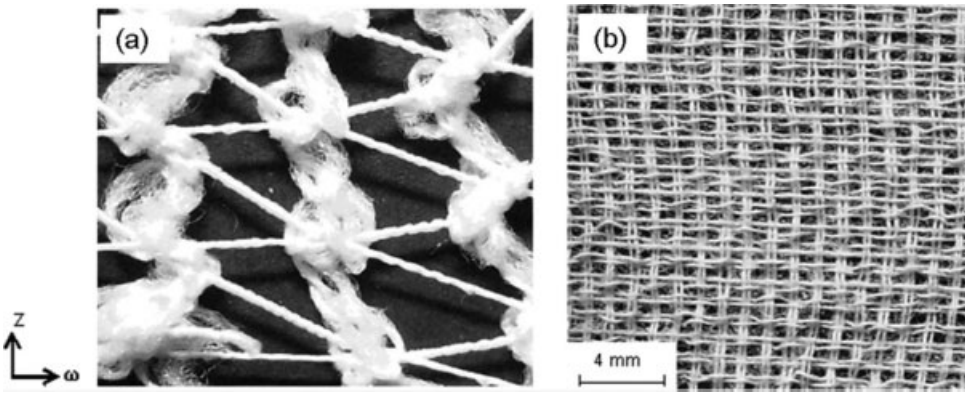


**Figure 1** Close up picture of mesh materials: (a) and (b) show HA and HB, respectively, and the mesh materials were stretched twice as the original length in the circumferential direction (ω).

cyclic-tensile tests using longitudinal (z) and circumferential (ω) test pieces as well as pressure-diameter (*P-D*) tests using tubular test pieces.

## METHODS AND EXPERIMENT

### PVA-H vessel model

Two types of mesh material were used in this study to fabricate *in vitro* vessel models, i.e., commercial stretch medical bandages using cotton and rubber. One of these is a bandage for fingers (a tubular bandage with rough mesh; NICHIBAN, ELASTIC NET BANDAGE 1) and the other is an Adaptic dressing (a finely meshed bandage which is stretchy in the longitudinal direction, but has little elasticity in the vertical direction; NIPPON EIZAI, SELAOBI). The close up picture of these mesh materials is shown in Figure 1. We term our *in vitro* model using the former "hybrid a (HA)" and the one using the latter "hybrid b (HB)." An *in vitro* model using only PVA-H was also constructed for the control experiment, and is hereafter termed "control." The fabrication process of the vessel models is described later. In this figure, the mesh materials were stretched twice the original length in the circumferential direction (ω).

*In vitro* models HA and HB were composed of three layers, the mesh material being sandwiched between two PVA-H layers. Commercial PVA powder with a molecular weight of 1700 (JAPAN VAM and POVAL, JF-17) was used in this study. We made PVA solution (10 wt %) using a hot stirrer with PVA powder and solvent, which was a mixture of 20 wt % water and 80 wt % dimethylsulfoxide (DMSO). After cooling the PVA solution to room temperature, a stainless steel cylinder bar ($L$ = 500 mm, $D_{out}$ = 10 mm) was inserted into an acrylic tube ($L$ = 500 mm, $D_{in}$ = 14 mm) and the solution flowed into the space. Both ends of the space between the stainless steel cylinder bar and the acrylic tube were then sealed by two short silicone rubber tubes ($L$ = 25 mm, $D_{in}$ = 10 mm, thickness $T$ = 2 mm). The solution in the acrylic tube was placed in a freezer at −30°C for 24 h for gelation and the first layer of PVA-H ($L \fallingdotseq$ 450 mm, $D_{in}$ = 10 mm, $T$ = 2 mm) was obtained. The PVA-H tube was then pulled out with the stainless steel cylinder bar and

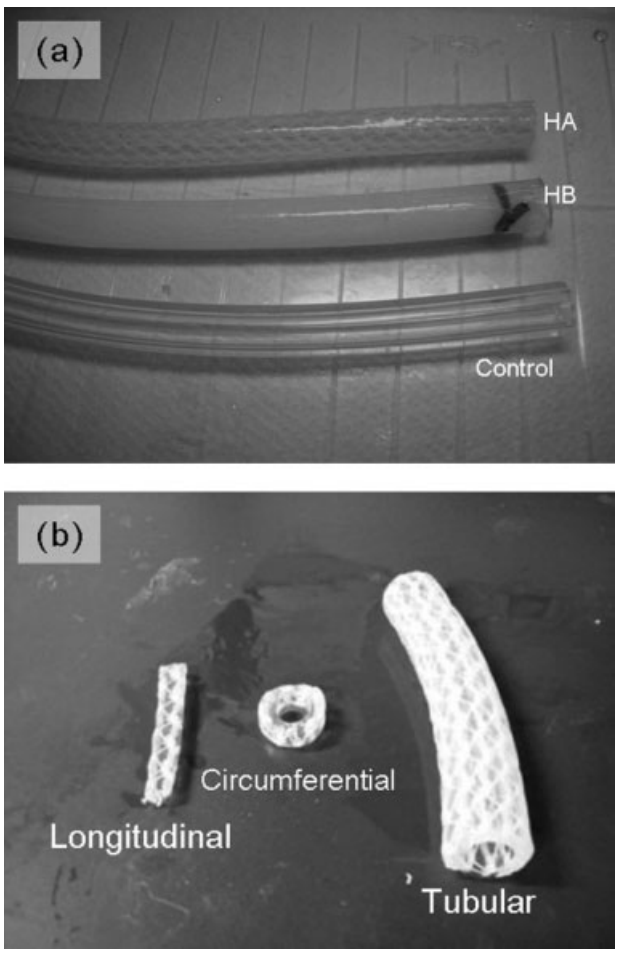


**Figure 2** (a) Shows *in vitro* vessel models and (b) shows three types of test pieces of HA.

**TABLE I**
**Experiment Description Using PVA-H *In Vitro* Vessel Models**

| Type of the test | Contents of the test |
|---|---|
| Tensile test | The test pieces ($L = 30$ mm, $W = 5$ mm) were stretched at a constant rate of 20 mm/min by the PC-controlled testing machine (SHIMADZU, EZ-S) to strain $\varepsilon = 1.0$ (only HB_$z$* was limited to 0.3) and returned to the original length to confirm the anisotropy and hysteresis curves. Three pieces of each *in vitro* model and each direction ($z$ and $\omega$) were tested. |
| Stress-relaxation test | The test pieces were stretched at a constant rate of 500 mm/min to strain $\varepsilon = 1.0$ (only HB_$z$ was limited to 0.3) and held at that state for 5 min to confirm the relaxation and anisotropy. One piece in each *in vitro* model and each direction ($z$ and $\omega$) was tested. |
| Cyclic-tensile test | The test pieces were stretched at a constant rate of 20 mm/min by the same PC-controlled testing machine mentioned in previous sections to $\varepsilon = 1.0$ (only HB_$z$ was limited to 0.3) and returned to the original length for three cycles. In these sequences, we confirmed the change of the hysteresis curve. One piece of each *in vitro* model and each direction ($z$ and $\omega$) were tested. |
| *P-D* test | The tubular test piece ($L = 50$ mm) was tied to at the experiment system shown in Figure 3 and inner pressure $P$ was applied by increasing the hydraulic head from 0 cm to 200 cm by increments of 20 cm ($P$: 200 cm$H_2O$ = 147 mmHg). In the steady state under each pressure, the B-mode measurement of the ultrasonic device (GE Healthcare, LOGIQ7, research pilot unit) was used to measure outer diameter $D_{out}$. The ultrasonic probe was kept from touching the *in vitro* model and the probe was inserted into the water by about 10 mm. |

* The results obtained with longitudinal ($z$) and circumferential ($\omega$) test pieces are represented with subscripts "_$z$ and _$\omega$," respectively.

wrapped over the mesh materials. After the mesh materials were fixed, the PVA-H tube with the mesh material was inserted into an acrylic tube which had a large diameter ($L = 500$ mm, $D_{in} = 18$ mm), and the second layer of PVA-H was created using a method similar to that used for the first one. The *in vitro* model was then soaked in ethanol for 24 h. Following this, we replaced the solvent with water. By this process, vessel models HA and HB ($L \fallingdotseq 450$ mm, $D_{in} = 10$ mm, $T = 4$ mm) were obtained. The control vessel model was made using a stainless steel cylinder bar ($L = 500$ mm, $D_{in} = 10$ mm) and an acrylic tube ($L = 500$ mm, $D_{in} = 18$ mm). *In vitro* models are shown in Figure 2(a). The vessel model is cut into strip specimens ($L = 30$ mm, $W = 5$ mm, $T = 4$ mm) in circumferential and longitudinal directions for the tensile and relaxation tests. Also, the tubular test pieces ($L = 50$ mm) for the *P-D* tests are prepared. A set of test pieces of HA is shown in Figure 2(b).

### Experiment

Three types of tests were performed to confirm the mechanical properties of the *in vitro* models: the tensile test, the stress-relaxation test, and the *P-D* test. Details of each test are shown in Table I. Note that the results obtained in the longitudinal ($z$) and circumferential ($\omega$) test pieces are represented with subscripts "_$z$ and _$\omega$," respectively.

## RESULTS

### Tensile, stress-relaxation, and cyclic-tensile tests

In this study, we define the stress $\sigma$ (load/initial area of the test piece) and strain $\varepsilon$ (change in length/original length) for the analysis. Figure 4 shows the results of the tensile tests (hysteresis curves). It was impossible to stretch the longitudinal test piece of HB (HB_$z$) to strain $\varepsilon = 1.0$ because it ruptured near $\varepsilon = 0.4$ in a pilot study. For this reason, in the case of HB_$z$, the pieces were stretched until a strain of 0.3. In each figure, the lines show the average value of three tests and the error bars show the maximum and minimum values. As a comparison, we investigated the mechanical properties of mesh materials which were used in this study by performing a tensile test. The test pieces were stretched at a constant rate of 20 mm/min by the PC-controlled testing machine (SHIMADZU, EZ-S). One piece of each mesh material and each direction ($z$ and $\omega$) were tested. Figure 5 shows the relationship between strain $\varepsilon$ and the load per unit length $\sigma_l$.

The results of the relaxation test are shown in Figure 6. We define the maximum stress at strain $\varepsilon = 1.0$ (at 0.3 in the case of HB_$z$) as $\sigma_0$ and the stress at $t$ second after the stress reached the peak as $\sigma_t$. Relaxation strength $Relax_t$ is defined in the following equation.

$$Relax_t = (\sigma_0 - \sigma_t)/\sigma_0 \times 100 \quad (1)$$

The results of relaxation strength at $t = 1$ and $t = 300$, i.e., $Relax_1$ and $Relax_{300}$, are shown in Figure 7(a,b). Discussion of these results can be found in the next section.

Figure 8(a–f) shows the result of the cyclic-tensile test. It was confirmed that the effect of viscosity in hysteresis curves was prominent in the first cycle; however, it was decreased dramatically as the cycle increased. The degree of decreasing varied with the vessel model and

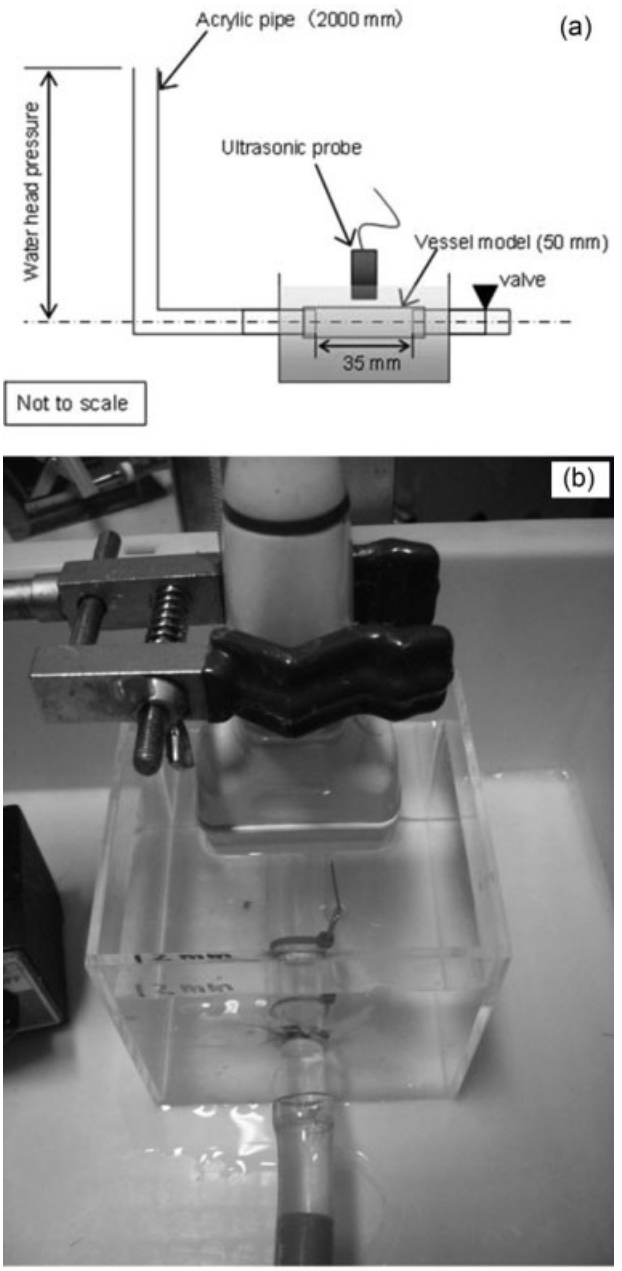


**Figure 3** (a) Schematic diagram of the *P-D* test system; (b) measured section and the ultrasound probe.

direction. The area enclosed by a hysteresis curve is termed as a loop area ($A$). The loop areas of the second and third cycle are normalized by that of the first cycle ($A_0$). The changing of a normalized loop area in each vessel model is shown in Figure 9(a–c).

### *P-D* test

Figure 10(a–c) shows ultrasonic measurement image samples of the vessel models in the *P-D* tests. In Figure 10, the boundary of the vessel model can be clearly confirmed. The relationships between the inner pressure $P$ and outer radius $R_{out}$ are shown in Figure 11(a). The unit of pressure is transformed from $cmH_2O$ to mmHg in the figure for comparison.

## DISCUSSION

It has previously been reported that the loop areas of hysteresis curves of native arteries (carotid, aorta, etc.) are generally small in the longitudinal direction

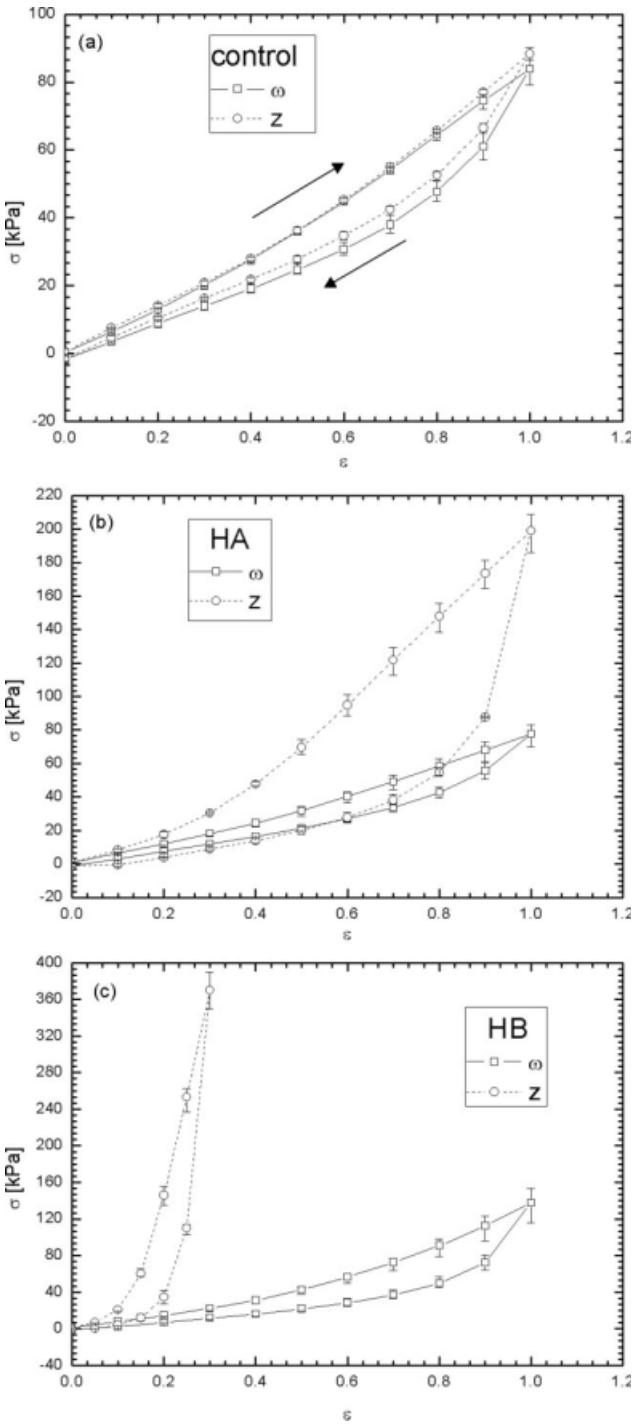


**Figure 4** Results of the tensile test for (a) control, (b) HA, and (c) HB. (Lines show the average value and the error bars show the maximum and minimum values in the test).

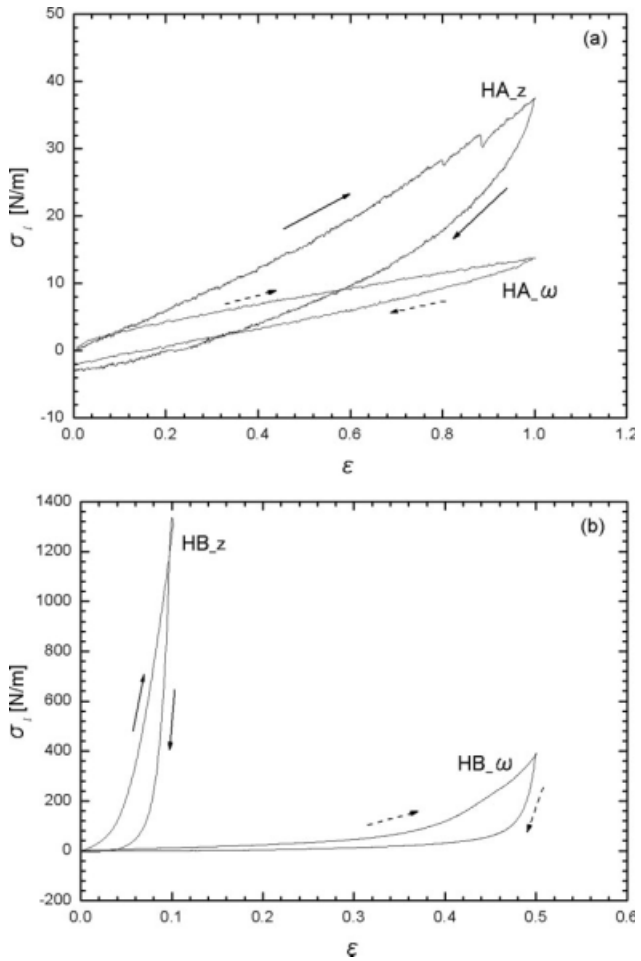


**Figure 5** Results of the tensile test of mesh materials: (a) and (b) show the result of HA and HB, respectively.

and large in the circumferential direction.[13] As shown in Figure 4(a), there was little difference in the hysteresis curves of the control vessel models between the longitudinal and circumferential directions, inducing that the control vessel model was nearly isotropic. By contrast, loop areas in the hysteresis curves of HA and HB [Fig. 4(b,c)] varied between these directions and anisotropy was confirmed. It should be noted that the loop area in the case of HA_*z* was much larger than that of HA_ω. Also, it was confirmed that the stress in HB_ω was slightly larger than those in the other cases against the same strain, whereas in the longitudinal direction, the HA model showed twice the strength of the control vessel model against the same strain and the HB model showed about 10 times the strength. In Figure 5, the plastic deformation of HA was confirmed in both direction and the tension strengths were quite different between HA and HB. This resulted in the nonlinear and anisotropic mechanical properties in the hybrid *in vitro* vessel models by comparing the results and Figure 4.

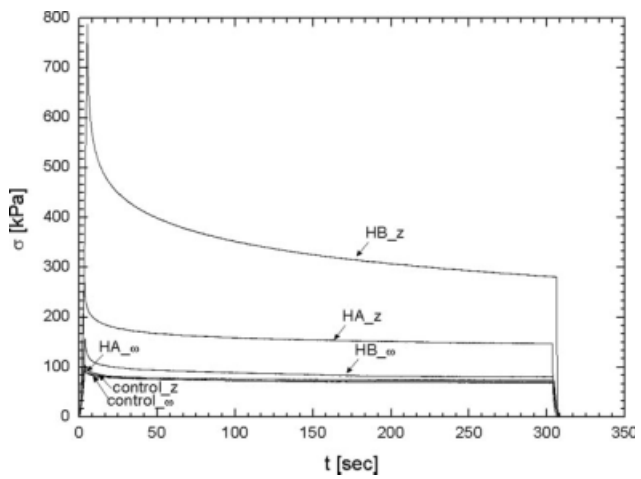


**Figure 6** Result of the stress-relaxation test in each case.

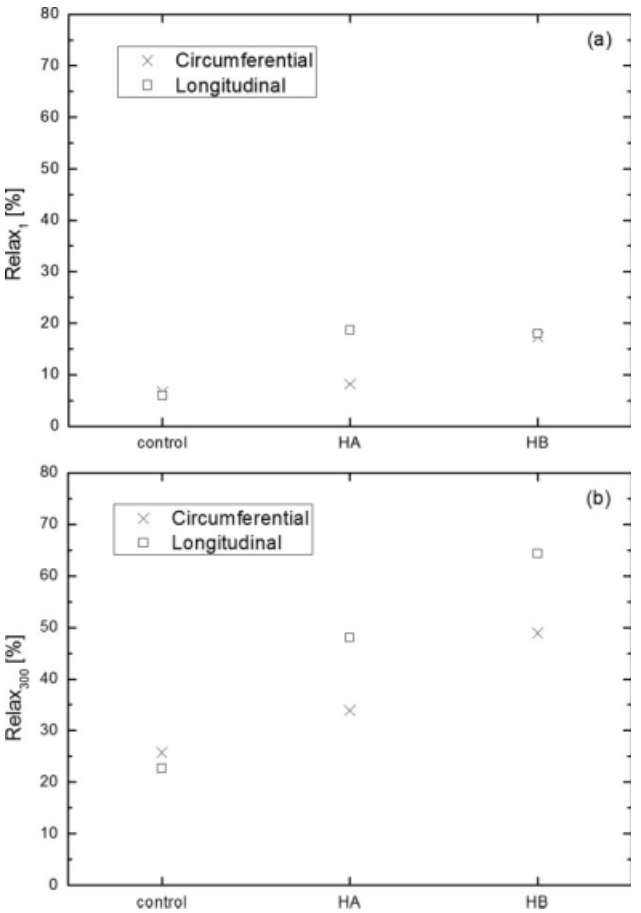


**Figure 7** Relaxation strength ($Relax_t$) at (a) $t = 1$ (s) and (b) $t = 300$ (s) of each model in relaxation test.

Among the native vessel components, elastin, collagen, and smooth muscle play roles in the mechanical properties.[14] The mesh materials used in this study were mainly composed of cotton and rubber. It was confirmed that the elastic strength and hysteresis were different between HA and HB because of the type of the mesh materials, i.e., the orientation of cotton and rubber (Fig. 1). Controlling components of the mesh material and the orientation of elastic

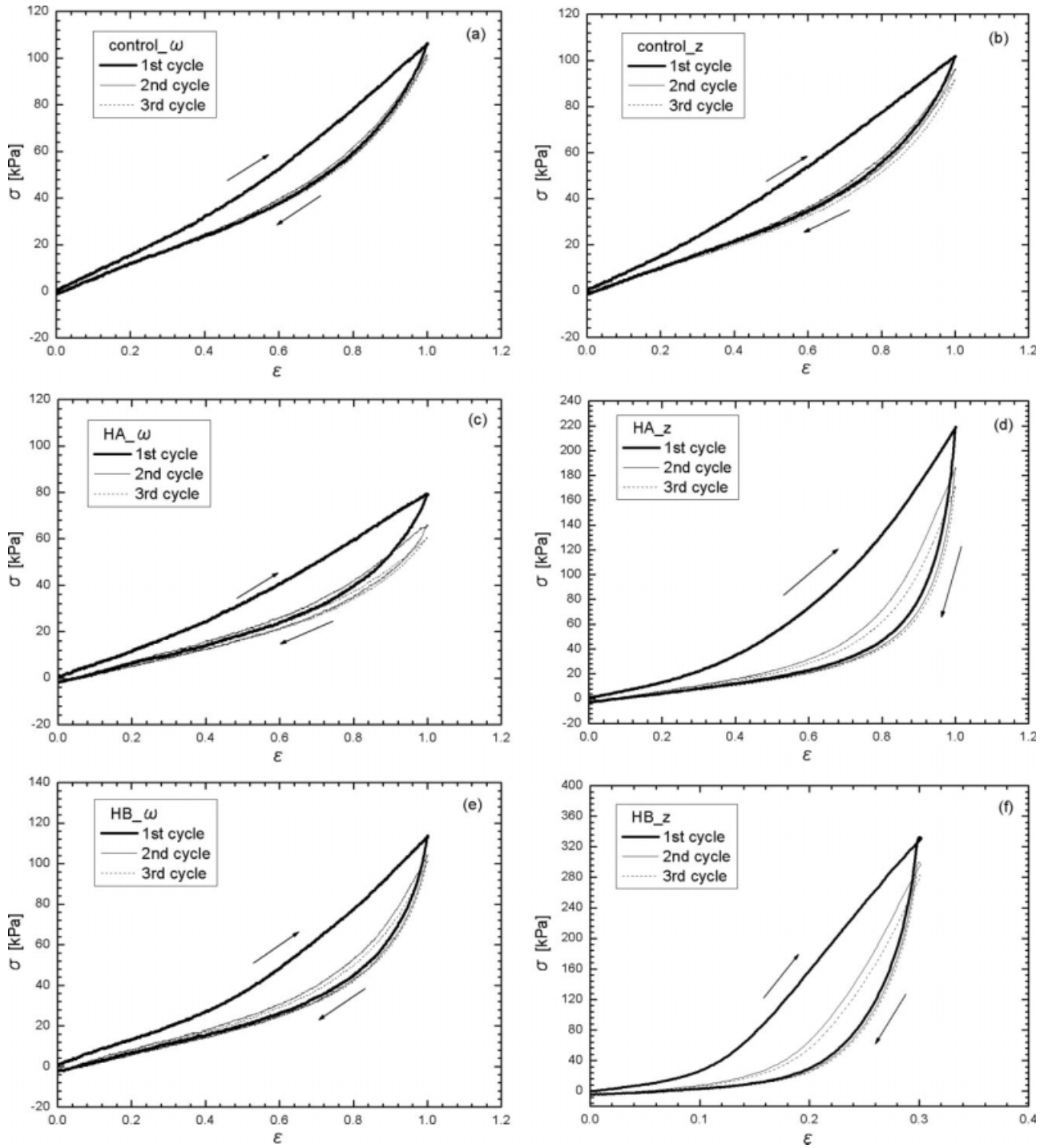


**Figure 8** Result of cyclic-tensile test: (a) and (b) show the results of control, (c) and (d) show results of HA, and (e) and (f) show those of HB.

fibers in the mesh material is the key for the reproduction of the mechanical properties of native vessels. Studies on this will be conducted in the near future.

In Figure 7(a), relaxation strength $Relax_1$ significantly differs between the longitudinal and circumferential directions in HA. Meanwhile, in Figure 7(b), relaxation strength $Relax_{300}$ significantly differs in both of HA and HB. The results of HA and HB show a direction-dependent relaxation strength. The results also indicate that the mode of relaxation of these vessel models is different after the stretch. In contrast, no significant direction-dependent difference is found in the control model. The directional character and strength of relaxation in Figure 7(b) are different from those of the previous study using

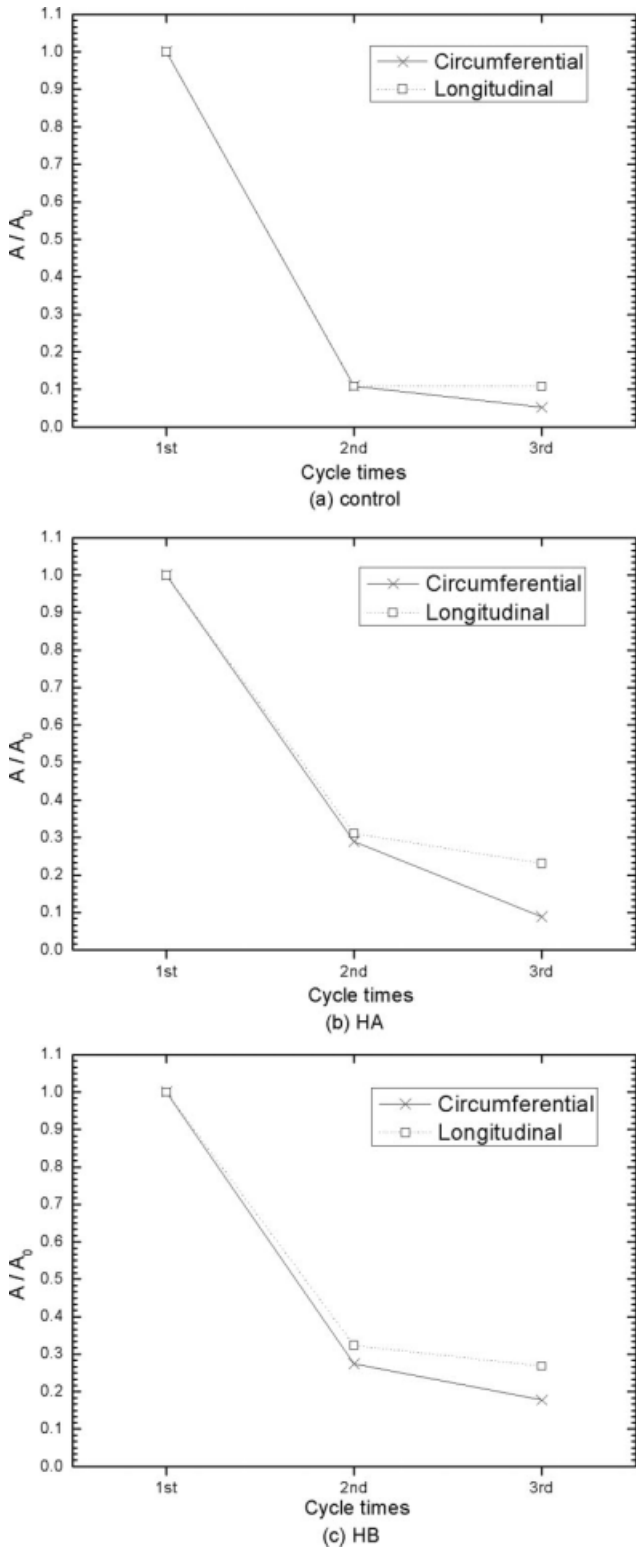


**Figure 9** The changing of normalized loop area in cycle-tensile test: (a–c) show the result of control, HA, and HB, respectively.

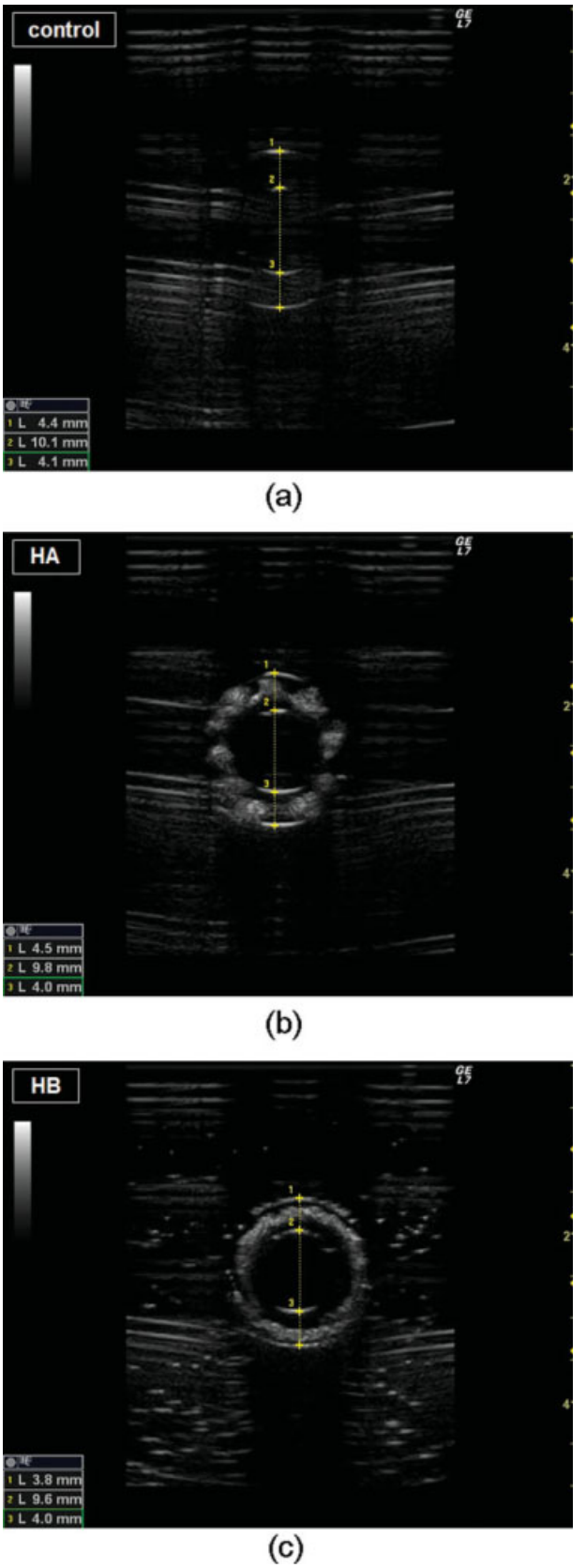


**Figure 10** Ultrasound measurement images in the *P-D* test: (a–c) show the result under the no load condition

native arteries which have performed by Azuma and Hasegawa.[13] Meanwhile, as we have mentioned earlier, it is possible to control the direction-dependency and strength of the relaxation of the *in vitro*

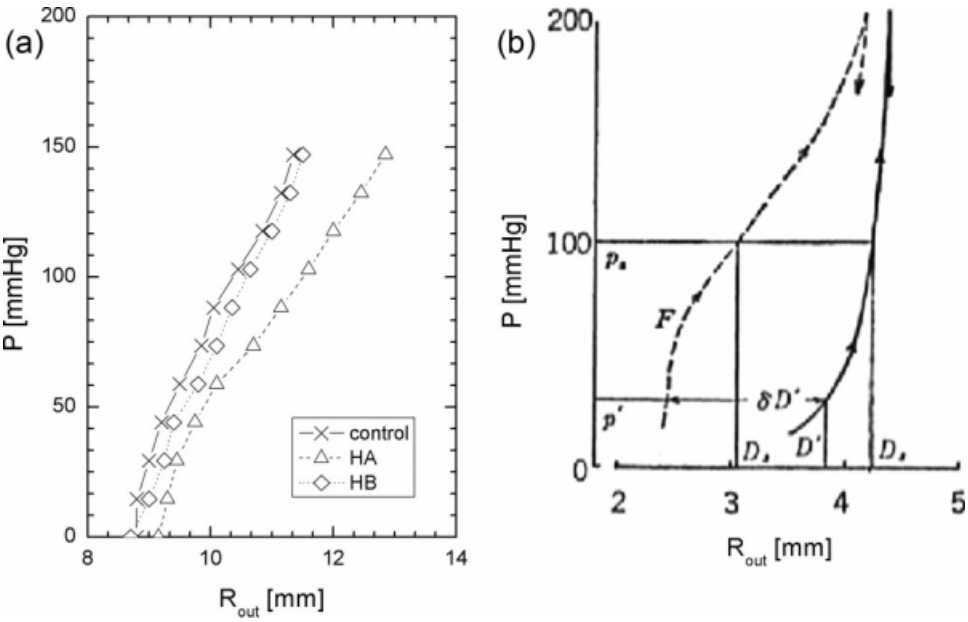


**Figure 11** Comparison of the *P-D* test: (a) The result in the present study; (b) the result of the previous study using vertebral artery. Broken line shows the result with the activation of the smooth muscle and the solid line shows the result without activation.[12]

vessel model by coordinating the direction and the material of the mesh material. In addition, because PVA-H is a kind of viscoelastic material, it can be presumed to play a great role in the relaxation. This result indicates that the thickness of the PVA-H layers is also a control factor for the reproduction of the mechanical properties of native vessels.

In cyclic tensile, loop areas of hysteresis curves were prominent in the first cycle, whereas it was decreased dramatically as the cycle increased. The loop area corresponds to the energy loss in a sample associated with a stretch cycle. The hysteresis in native arteries is mainly caused by viscous effect of the native vessel component, e.g., principally the smooth muscle. Because the mass and the active level of smooth muscle varied among the native arteries, the loop area in native arteries shows region dependency.[13,15] In this study, the degree of decreasing varied with vessel model and direction. This is considered to be partially caused by the mesh material (i.e., material of meshes and the orientation of them) as we mentioned earlier. Also, the entanglement between mesh materials and PVA-H and the slip along the boundary are also considered to have influence on this phenomenon. In previous work, Goto and Kimoto performed this type of cyclic-tensile test using various native vessels.[15] The effects of rate, grade, and interval of repetitive stretches on the change of hysteresis curves were investigated in the study. The results obtained in this study was qualitative similar to those of previous study.

The present results of the *P-D* test were compared with those of a previous study performed by Nagasawa et al.[12] It was confirmed that *in vitro* models, especially HA, showed behavior qualitatively near that of the native vessel (vertebral artery) with activated smooth muscle [Fig. 11(b)].

Vessel models were tubular in this study, and this technique is also applicable to a three-dimensional vessel model. The size of the *in vitro* vessel models depends on the thickness of the mesh materials. Thus, a model in the order of a coronary artery ($D_{in}$ = 3 mm, $T$ = 1 mm) at the minimum is producible at this time. The advantage of PVA-H, transparency was lost because of the existence of the mesh materials, especially in HB. Weaving a mesh material with transmissive elastic fibers may be a way of resolving this problem and is also future work.

## CONCLUSIONS

In this study, we fabricated straight multilayer *in vitro* tubular vessel models composed of PVA-H and anisotropic mesh materials. We performed tensile, stress-relaxation, and cycle-tensile tests using axial and circumferential test pieces as well as a *P-D* test using tubular test pieces. In the tensile and stress-relaxation tests, we confirmed anisotropic and nonlinear mechanical properties and hysteresis characteristic of the *in vitro* models. The *in vitro* models also showed behavior quantitatively similar to that of the native vessels in cycle-tensile and *P-D* tests.

These results indicate that the mechanical properties of native vessels can be duplicated in an *in vitro* model by controlling some factors of PVA-H (e.g., the concentration and thickness of the PVA-H layers) and those of the mesh material (e.g., the components of the mesh material and the orientation of the elastic fibers in the mesh material). These are issues to be addressed in our future work. Besides the relationship between mesh materials and PVA-H, e.g., entanglement and slip along the boundary, is also considered to have influence on the mechanical properties of *in vitro* vessel models. In addition, a rule-of-mixtures (ROM) approach is considered to be helpful for predicting the behavior of the materials using in the *in vitro* vessel model. These issues will be investigated in detail in future work.

## References


1. Papathanasopoulou, P.; Zhao, S.; Köhler, U.; Robertson, M. B.; Long, Q.; Hoskins, P.; Xu, X. Y.; Marshall, I. J Magn Reson Imaging 2003, 17, 153.
2. Zhao, S. Z.; Papathanasopoulou, P.; Long, Q.; Marshall, I.; Xu, X. Y. Ann Biomed Eng 2003, 31, 962.
3. Tateshima, S.; Murayama, Y.; Villablanca, J. P.; Morino, T.; Takahashi, H.; Yamaguchi, T.; Tanishita, K.; Viñuela, F. J Neurosurg 2001, 95, 1020.
4. Flora, H. S.; Talei-Faz, B.; Ansdell, L.; Chaloner, J. E.; Sweeny, A.; Grass, A.; Adiseshiah, E. J Endovasc Ther 2002, 9, 665.
5. Kosukegawa, H.; Mamada, K.; Liu, L.; Kuroki, K.; Hayase, T.; Ohta, M. J Fluid Sci Technol 2008, 3, 533.
6. Brett, C. I.; Chrysanthi, W.; Robert, T. T. Circ Res 2006, 98, 23.
7. Snady, F. C. S.; Donald, J. L. J Biomech 1992, 25, 297.
8. William, M. A.; Joseph, M.; Jonathan, E. H.; Gilbert, L.; David, F. W. J Vasc Surg 1987, 5, 376.
9. Mathew, D. T.; Birney, Y. A.; Cahill, P. A.; McGuinness, G. B. J Biomed Mater Res B Appl Biomater 2008, 84, 531.
10. Mathew, D. T.; Birney, Y. A.; Cahill, P. A.; McGuinness, G. B. J Appl Polym Sci 2008, 109, 1129.
11. Millon, L. E.; Mohammadi, H.; Wan, W. K. J Biomed Mater Res B Appl Biomater 2006, 79, 305.
12. Nagasawa, S.; Handa, H.; Okumura, A.; Naruo, Y.; Moritake, K.; Hayashi, K. Biorheology 1981, 18, 97.
13. Azuma, T.; Hasegawa, M. Jpn J Physiol 1971, 21, 27.
14. Sato, M.; Hayashi, K.; Niimi, H.; Moritake, K.; Okumura, A.; Handa, H. Med Biol Eng Comput 1979, 17, 170.
15. Goto, M.; Kimoto, Y. Jpn J Physiol 1966, 15, 169.