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Viscoelastic evaluation of fetal umbilical vein for reconstruction of middle cerebral artery

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Research Highlights

(1) This study used middle cerebral arteries from human cadavers and fetal umbilical veins vested after spontaneous delivery, and explored the feasibility of using fetal umbilical vein for reconstruction of middle cerebral artery. The stress relaxation and creep mechanical properties of the middle cerebral artery and fetal umbilical vein were compared.
(2) Fetal umbilical vein has appropriate stress relaxation and creep properties for transplantation. Its viscoelasticity is similar to that of middle cerebral artery, which is beneficial for reconstruction of injured middle cerebral artery.

Abstract

The transplantation of artificial blood vessels with < 6 mm inner diameter as substitutes for human arterioles or veins has not achieved satisfactory results. Umbilical vein has been substituted for artery in vascular transplantation, but it remains unclear whether the stress relaxation and creep tween these vessels are consistent. In this study, we used the fetal umbilical vein and middle cerebral artery from adult male cadavers to make specimens 15 mm in length, 0.196–0.268 mm in nica media thickness, and 2.82–2.96 mm in outer diameter. The results demonstrated that the stress decrease at 7 200 seconds was similar between the middle cerebral artery and fetal umbilical vein specimens, regardless of initial stress of 18.7 kPa or 22.5 kPa. However, the strain increase at 7 200 seconds of fetal umbilical veins was larger than that of middle cerebral arteries. Moreover, the stress relaxation experiment showed that the stress decrease at 7 200 seconds of the fetal umbilical vein and middle cerebral artery specimens under 22.5 kPa initial stress was less than the decrease in these specimens under 18.7 kPa initial stress. These results indicate that the fetal umbilical vein has appropriate stress relaxation and creep properties for transplantation. These properties are advantageous for vascular reconstruction, indicating that the fetal umbilical vein can be transplanted to repair middle cerebral artery injury.

Key Words

neural regeneration; neural plasticity; middle cerebral artery; fetal umbilical vein; stress relaxation properties; creep properties; viscoelasticity; transplantation; biomaterial; biomechanics; neuroregeneration

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Conflicts of interest: None declared.

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INTRODUCTION

Intracranial large arterial and extracranial carotid stenoses are common causes of ischemic stroke. Chinese young and middle-aged patients commonly suffer from intracranial artery stenosis, especially middle cerebral artery stenosis[1]. Because of the progress and maturity of microsurgical techniques, as well as the diversification of intracranial blood monitoring and perfusion evaluation methods, extracranial-intracranial bypass for treatment of ischemic cerebrovascular disease has obtained positive outcomes[2-5]. Currently, autologous blood vessels are used as a substitute in clinical vascular remodeling[6-7]. Autologous blood vessels are characterized by good compatibility and convenient specimen collection, but their limited availability forces clinical physicians to use artificial blood vessels as substitutes. Large diameter artificial blood vessels have succeeded as substitutes for large human arteries, but the use of artificial blood vessels with < 6 mm inner diameter as substitutes for human arterioles or veins has not achieved satisfactory results. Induction of thrombosis and intimal thickening typically leads to obstruction of artificial blood vessels, necessitating the rapid development of arteriole and vein substitutes[8].

Numerous reports have detailed empirical studies and clinical applications of umbilical veins for artery transplantation[9-13]. Li *et al* [14] found that human umbilical vein is a good substitute for artery grafts, and that physicians should pay attention to the relationship between its compliance and fetal age, as well as the matching of umbilical vein and host artery to improve the long-term patency rate of transplanted blood vessels. Sun and colleagues[15] thought that the compliance of umbilical vein was best at a fetal age of 37–40 weeks. Hayashi *et al* [16] confirmed that the elastic modulus and rigidity of the artery increased with increased blood pressure and increased distance from the heart. This finding is associated with the ratio of elastic to collagen fibers, the number of smooth muscles, and different microstructures. Many scholars also investigated the mechanical properties of fetal umbilical vein and middle cerebral artery[17-24]. Wang *et al* [25] established functional equations for stress relaxation and creep of the common carotid artery and middle meningeal artery of normal rats. Yu *et al* [26] found that maximum load, maximum stress, maximum displacement, and maximum strain of cerebral arteries of rats with atherosclerosis were lower compared with normal rats. Moreover, the stress-strain curve was exponentially altered. Middle cerebral artery showed different tensile mechanical properties between atherosclerotic and normal rats. The arterial vessels in atherosclerotic rats cannot extend as large as in normal rats. Previous studies focusing on the mechanical properties of middle cerebral arteries and fetal umbilical veins typically measured the pressure-volume relationship and uniaxial tension. Similar to other biological materials, middle cerebral artery and fetal umbilical vein are elastic bodies. Their viscoelasticity is mainly shown by stress relaxation and creep. Since fetal umbilical vein could be transplanted to repair middle cerebral artery injury, it is necessary to characterize any differences in stress relaxation and creep between them. Thus, we compared the viscoelasticity of middle cerebral arteries from fresh normal Chinese cadavers and fetal umbilical veins to explore the feasibility of fetal umbilical vein transplantation for middle cerebral artery injury, and to provide a basis for the clinical application of umbilical vein as a middle cerebral artery graft.

RESULTS

Stress relaxation curves for middle cerebral artery and fetal umbilical vein specimens were similar

As shown in Figure 1, the stress decrease at 600 seconds of middle cerebral artery and fetal umbilical vein specimens under 18.7 kPa initial stress was large and then diminished slowly over time. The stress relaxation curve at 7 200 seconds was horizontal, and this curve varied logarithmically. The stress of middle cerebral artery specimens was 18.7 kPa at

0 seconds and 14.9 kPa at 7 200 seconds, a reduction of 3.8 kPa. The stress of fetal umbilical vein was 18.7 kPa at 0 seconds and 14.8 kPa at 7 200 seconds, a reduction of 3.9 kPa. The decrease of both specimens was similar at 7 200 seconds (*P* > 0.05). Statistical analysis of the stress relaxation data was performed, and the mean value and standard deviation are shown on the curve.

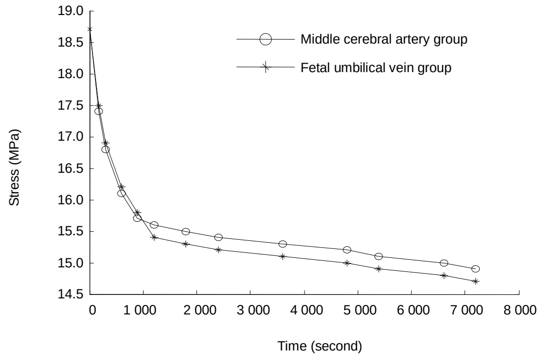

Figure 1 Stress relaxation curve of middle cerebral artery and fetal umbilical vein specimens under 18.7 kPa stress.

The stress decrease tendencies of both specimens were similar at 7 200 seconds (*P* > 0.05; paired *t*-test).

As shown in Figure 2, the stress decrease at 600 seconds of middle cerebral artery and fetal umbilical vein specimens under 22.5 kPa stress was large and then diminished slowly over time. The stress relaxation curve at 7 200 seconds was horizontal, and this curve varied logarithmically. The stress of middle cerebral artery specimens was 22.5 kPa at 0 seconds and 19.4 kPa at 7 200 seconds, a reduction of 3.1 kPa. The stress of fetal umbilical vein was 22.5 kPa at 0 seconds and 19.46 kPa at 7 200 seconds, a decrease of 3.04 kPa. The decrease of both specimens was similar at 7 200 seconds (*P* > 0.05).

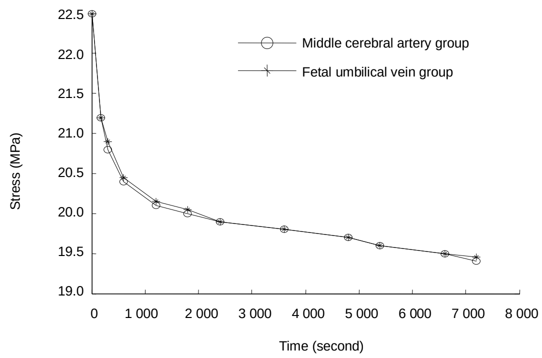

Figure 2 Stress relaxation curve of middle cerebral artery and fetal umbilical vein specimens under 22.5 kPa stress.

The stress decrease tendencies of both specimens were similar at 7 200 seconds (*P* > 0.05; paired *t*-test).

These results demonstrated that the stress decrease at 7 200 seconds was similar between middle cerebral artery and fetal umbilical vein specimens, regardless of initial stress of 18.7 kPa or 22.5 kPa (*P* > 0.05). The stress decreases at 7 200 seconds of the fetal umbilical vein and the middle cerebral artery specimens under 22.5 kPa stress were less than the decrease in these specimens under 18.7 kPa stress (*P* > 0.05). With the increase inconstant strain, initial stress increased, but stress at 7 200 seconds decreased, indicating that the stress decrease followed a logarithmic relationship. The stress relaxation curves of fetal umbilical vein and middle cerebral artery specimens were similar.

Stress relaxation function curves of middle cerebral artery and fetal umbilical vein specimens were similar

As shown in Figure 3, under 18.7 kPa initial stress, the normalized stress relaxation function value of middle cerebral artery and fetal umbilical vein specimens was 1 at 0 seconds. The stress relaxation function values of middle cerebral artery and fetal umbilical vein specimens were 0.798 and 0.808, respectively, at 7 200 seconds. Moreover, the magnitude of decrease at 7 200 seconds in the stress relaxation function was similar for both specimens (*P* > 0.05).

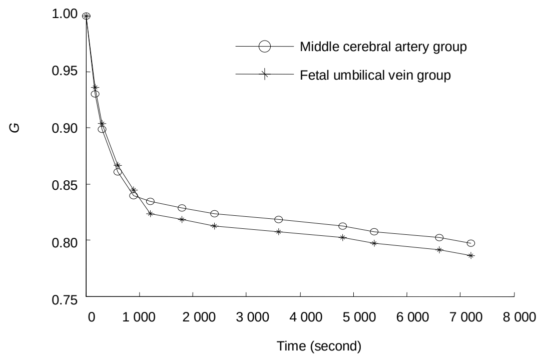

Figure 3 Normalized stress relaxation function curves of middle cerebral artery and fetal umbilical vein specimens under 18.7 kPa stress.

G stands for experimental value of stress relaxation function. The G value was calculated as σ(*t*)/σ(0). The stress decrease at 7 200 seconds in the stress relaxation functions was similar for both specimens (*P* > 0.05; paired *t*-test).

As shown in Figure 4, under 22.5 kPa initial stress, the normalized stress relaxation function value of middle cerebral artery and fetal umbilical vein specimens was 1 at 0 seconds. The stress relaxation function values of middle cerebral artery and fetal umbilical vein specimens were 0.862 and 0.864, respectively, at 7 200 seconds. Moreover, the magnitude of decrease at 7 200 seconds in the stress relaxation function was similar for both specimens (P > 0.05).

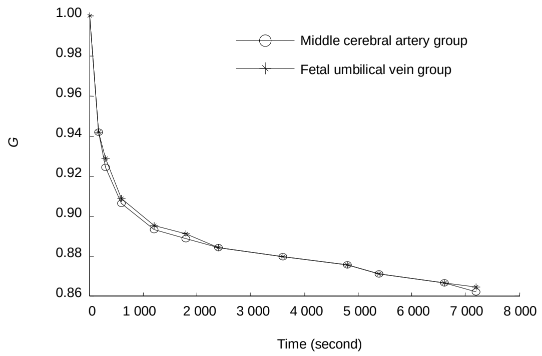

Figure 4 Normalized stress relaxation function curves of middle cerebral artery and fetal umbilical vein specimens under 22.5 kPa stress.

G stands for experimental value of stress relaxation function. The G value was calculated as σ(t)/σ(0). The stress decrease at 7 200 seconds in the stress relaxation functions was similar for both specimens (P > 0.05; paired t-test).

Creep curve characteristics of fetal umbilical vein specimens were better than those of middle cerebral artery

The creep response of middle cerebral artery and fetal umbilical vein specimens was recorded at 18.7 kPa stress, and their creep curves varied exponentially. The strain increased rapidly for 600 seconds and then continued to increase, but more gradually. By 7 200 seconds, the creep curves were horizontal. The strain of middle cerebral artery was 25.2% at 0 seconds and 27.3% at 7 200 seconds, an increase of 2.1%. The strain of fetal umbilical vein was 26.1% at 0 seconds and 29.3% at 7 200 seconds, an increase of 3.2%, which was significantly greater than that of middle cerebral artery (P < 0.05; Figure 5).

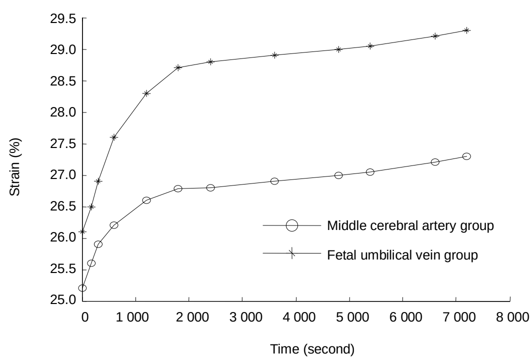

Figure 5 Creep curves of middle cerebral artery and fetal umbilical vein specimens under 18.7 kPa constant stress.

The strain increase at 7 200 seconds of fetal umbilical vein was greater than that of middle cerebral artery (P < 0.05; paired t-test).

The creep response of middle cerebral artery and fetal umbilical vein specimens was recorded at 22.5 kPa stress, and their creep curves also varied exponentially. The strain increased rapidly for 600 seconds and then continued to increase, but more gradually. By 7 200 seconds, the creep curves were horizontal. The strain of middle cerebral artery was 32.6% at 0 seconds and 34.2% at 7 200 seconds, an increase of 1.4%. The strain of fetal umbilical vein was 33.8% at 0 seconds and 36.1% at 7 200 seconds, an increase of 2.3%, which was significantly greater than that of middle cerebral artery (P < 0.05; Figure 6). Moreover, the strain increase at 7 200 seconds of middle cerebral artery under 22.5 kPa constant stress was less than that under 18.7 kPa constant stress (P < 0.05).

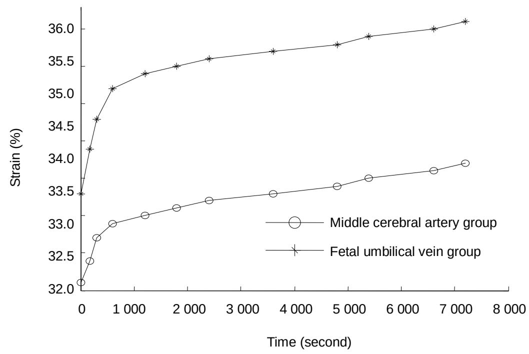

Figure 6 Creep curves of middle cerebral artery and fetal umbilical vein specimens under 22.5 kPa constant stress.

The strain increase at 7 200 seconds of fetal umbilical vein was greater than that of middle cerebral artery (P < 0.05; paired t-test).

Under the 18.7 and 22.5 kPa initial stress, the strain increase at 7 200 seconds of fetal umbilical vein was greater than that of middle cerebral artery specimens (P < 0.05). The increase at 7 200 seconds under 22.5 kPa initial stress was lower than that under 18.7 kPa initial stress (P < 0.05). With the increase in constant stress, initial strain increased, but the strain at 7 200 seconds was increased disproportionately. These results indicated that the strain increase of middle cerebral artery altered exponentially. Moreover, the changes in the creep curve of fetal umbilical vein resembled those changes in the middle cerebral artery creep response.

The normalized creep function curve of fetal umbilical vein specimens were better than those of middle cerebral artery

Under 18.7 kPa initial stress, the normalized creep function values of middle cerebral artery and fetal umbilical vein were 1 at 0 seconds. The creep function value at

7 200 seconds of fetal umbilical vein specimens was greater than that of middle cerebral artery specimens (1.29 *vs*. 1.27; $P < 0.05$; Figure 7).

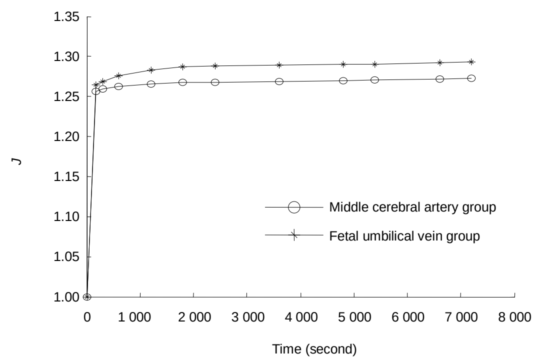

Figure 7 Normalized stress creep function curves of middle cerebral artery and fetal umbilical vein specimens under 18.7 kPa stress.

J stands for experimental value of creep function. J = L(*t*)/L(0). The creep at 7 200 seconds of fetal umbilical vein specimens was greater than that of middle cerebral artery specimens ($P < 0.05$; paired *t*-test).

Under 22.5 kPa initial stress, the normalized creep function values of middle cerebral artery and fetal umbilical vein were 1 at 0 seconds. The creep function value at 7 200 seconds of fetal umbilical vein specimens was greater than that of middle cerebral artery specimens (1.35 *vs*. 1.33; $P < 0.05$; Figure 8).

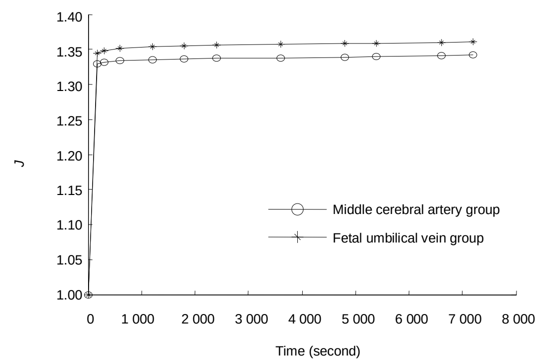

Figure 8 Normalized stress creep function curves of middle cerebral artery and fetal umbilical vein specimens under 22.5 kPa stress.

J stands for experimental value of creep function. J = L(*t*)/L(0). The creep at 7 200 seconds of fetal umbilical vein specimens was greater than that of middle cerebral artery specimens ($P < 0.05$; paired *t*-test).

DISCUSSION

The results of this study demonstrated that the stress decrease at 7 200 seconds was similar between middle cerebral artery and fetal umbilical vein specimens, regardless of initial stress of 18.7 kPa or 22.5 kPa. The stress decrease at 7 200 seconds of both specimens was smaller under 22.5 kPa stress than that under 18.7 kPa stress. With increased constant strain, initial stress increased, but the stress decrease at 7 200 seconds was reduced, indicating that the stress decrease of middle cerebral artery specimens varied logarithmically under the condition of constant strain. Under 18.7 kPa and 22.5 kPa initial stress, the strain increase at 7 200 seconds of fetal umbilical vein specimens was larger than that of middle cerebral artery specimens. The stress decrease and increase in stress relaxation at 7 200 seconds were smaller under 22.5 kPa initial stress than those under 18.7 kPa initial stress. With increased constant stress, initial strain increased, but the strain increase at 7 200 seconds was reduced, suggesting that the strain increase of middle cerebral artery specimens varied exponentially under constant stress.

Stress relaxation and creep curves changed the most within the first 600 seconds, and then the stress diminished slowly. By 7 200 seconds, the stress relaxation curve was horizontal. The stress relaxation and creep of fetal umbilical vein and middle cerebral artery specimens altered rapidly at the beginning of the tests, suggesting that the inherent oncotic pressure of the specimens was less than local pressure. The speed of water exudation from the tissue was rapid. With continued loss of fluids, the difference between oncotic and local pressures was reduced. Therefore, long-time stress slowly decreased, strain slowly increased, and the stress relaxation and creep curves tend towards horizontal. The patterns of changes in stress relaxation and creep curves of fetal umbilical vein resemble that of middle cerebral artery.

The viscoelasticity of vessel wall meets the requirements for physiological function of blood vessels. The tensile stress relaxation and creep properties of fetal umbilical vein are important to determine as they affect the tension and elongation of stoma after transplantation for middle cerebral artery injury. The stress relaxation of fetal umbilical vein under constant tensile strain helps to lighten the tension on the stoma after transplantation. The creeping ability of fetal umbilical vein was large under constant physiological stress, which could help limit opening and displacement of the stoma after transplantation. Recently, vascular tension has been discussed as a manner to make artificial blood vessels for vascular regeneration[27-28]. A previous study verified that transient rapid tension causes endothelial cell detachment, and promotes injury to the internal elastic membrane[29]. Thus, gradual vascular tension is considered a better method. The reconstruction of normal adult vascular tissue is slow, but suitable longitudinal tension possibly accelerated this process[30]. However, there was no effective method for successfully extending blood vessels[31].

The inner membrane of umbilical vein is very thin, and the internal elastic membrane is intact. The media is composed of 14–23 layers of smooth muscle. The outer membrane is thinner than the media. The external elastic membrane is not visible. There are collagen fibers, a few smooth muscle cells, and some longitudinal smooth muscle. The umbilical vein has some similar properties as vein, but more closely resembles medium-sized arteries. Taken together, the structural similarities suggest umbilical vein could be used as a substitute for artery transplantation. The intact internal elastic membrane of umbilical vein could prevent smooth muscle cells from entering the inner membrane, avoiding vascular obstruction and improving the patency rate[32]. Experimental results confirmed that the viscoelasticity indexes of fetal umbilical vein are better than those of middle cerebral artery. Thus, its stress relaxation and creep properties are suitable for the transplantation in the treatment of middle cerebral artery injury.

The specimens used in the 22.5 kPa stress relaxation and creep tests were also used in the 18.7 kPa stress tests, after 24 hours of restoration. This was possible because the stress relaxation and creep tests are non-destructive tests. The applied stress did not exceed normal systolic pressure 18.7 kPa. Both kinds of specimens were restored for 24 hours after 18.7 kPa stress relaxation and creep tests. Thus, is the results are comparable. In summary, fetal umbilical vein has similar stress relaxation and creep properties to middle cerebral artery, and could be a beneficial graft for vascular reconstruction after middle cerebral artery injury.

MATERIALS AND METHODS

Design

Contrast observational study.

Time and setting

Experiments were performed at the Mechanics Experiment Center, Jilin University, China from August 2009 to May 2011.

Materials

A total of 20 normal fetal umbilical veins (gestational age of 38–40 weeks) after spontaneous delivery by 24–30 year old healthy parturients were provided by the China-Japan Friendship Hospital, Jilin University, China. All parturients signed the informed consent agreement. Fetal umbilical veins were placed in saline in accordance with a previously published method[33]. Middle cerebral artery specimens were obtained from 20 normal fresh adult male Chinese cadavers aged 28–32, which were provided by the Jilin University School of Medicine, China. Middle cerebral artery specimens were obtained from the cadavers by dissection within 24 hours after death. In accordance with a previously published method[33], the specimens were placed in saline until further use. The protocols were conducted in accordance with Administrative Regulations on Medical Institutions, formulated by the State Council of China[34].

Methods

Preparation of fetal umbilical vein and middle cerebral artery specimens

Twenty fetal umbilical veins and twenty middle cerebral artery specimens were stored in saline for 1 day, and then cut into specimens with an outer diameter of 2.82–2.96 mm, length of 15 mm, and media thickness of 0.196–0.268 mm using a S-5 aseptic plastic-handled scalpel (Huaian Lianhe Yikang Medical Supplies Co., Ltd., Xuyi County, Jiangsu Province, China).

Stress relaxation experiments on fetal umbilical vein and middle cerebral artery specimens

Following previously described methods[25-26], ten fetal umbilical vein specimens and ten middle cerebral artery specimens were randomly selected and pretreated with an electronic universal testing machine (Shimadzu, Tokyo, Japan). To simulate human body temperature, the temperature was maintained at 36.5 ± 0.5°C. The two kinds of specimens were clamped in a fixture, and then exposed to strain at 50%/min. Under 18.7 kPa stress[35], the strains in the middle cerebral artery group and fetal umbilical vein group were 25.2% and 26.1%, respectively. Thereafter, the strain was kept constant, and the stress reduced with time. The duration of the stress relaxation experiment was 7 200 seconds, and 100 sets of experimental data were collected. We maintained specimen hydration by dousing them in saline.

After the 18.7 kPa stress relaxation experiment, specimens were allowed to rest for 24 hours in saline. Then, a second stress relaxation test following the above protocol was performed, with both kinds of specimens exposed to

22.5 kPa stress. Under 22.5 kPa stress, the strains in the middle cerebral artery specimens and fetal umbilical vein specimens were 32.6% and 33.8%, respectively.

As described previously[33], curve fitting was done using a computer with the data from 100 sets of experiments with 18 time points from 10 specimens for each group. The time points for collecting data were 0, 60, 120, 180, 300, 420, 600, 900, 1 200, 1 500, 1 800, 2 400, 3 000, 3 600, 4 800, 5 400, 6 600 and 7 200s seconds.

We obtained 18.7 and 22.5 kPa stress relaxation function G(t) values for the middle cerebral artery and fetal umbilical vein specimens. The G(t) value was calculated as $\delta(t)/\delta(0)$[36]. Following a previously described method[33], curve fitting was done with normalized stress relaxation functions for both kinds of specimens at both applied stress levels.

Creep experiments of fetal umbilical vein and middle cerebral artery specimens

Ten fetal umbilical vein specimens and ten middle cerebral artery specimens were randomly selected. The clamping protocol, experimental temperature, data collection and sample pre-treatment for the creep test were identical to those in the stress relaxation test. The rate of stress increase was 0.5 MPa/min for the creep experiments. When the strains in the middle cerebral artery specimens and fetal umbilical vein specimens reached 25.2% and 26.1%, respectively, under 18.7 kPa stress, the stress should remain constant. However, the strain increased with time. We maintained specimen hydration by dousing them in saline.

After the 18.7 kPa creep experiment and the specimen restoration for 24 hours, a second creep experiment was performed following the above protocol but exposing both kinds of specimens to 22.5 kPa stress. When the strains in the middle cerebral artery specimens and fetal umbilical vein specimens reached 32.6% and 33.8%, respectively, under 22.5 kPa stress, the stress should remain constant.

As described previously[33], curve fitting was done with the data from the middle cerebral artery and fetal umbilical vein specimens at both stress levels over the same 18 time points as before.

We obtained creep function J(t) values for the middle cerebral artery and fetal umbilical vein specimens. The J(t) value was calculated as $J(t)=L(t)/L(0)$[36]. Following a previously described method[33], curve fitting was done with the normalized creep function data for both kinds of specimens at over each time point.

Statistical analysis

The experimental data were compared with SPSS 11.0 software (SPSS, Chicago, IL, USA) using paired t-test. A value of $P < 0.05$ was considered statistically significant. Stress relaxation and creep data from both kinds of specimens were processed using normalization analysis.

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