Cellular Engineering

Mechanical and dimensional adaptation of rabbit carotid artery cultured *in vitro*

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Abstract—*The effects of the mechanical environment on arterial walls were investigated in rabbit common carotid arteries, cultured for six days under three different intraluminal pressures (0, 80 and 160 mmHg) in a perfusion culture system. The mechanical responses following the culture were examined using a quasi-static pressure–diameter test. Specimen viability was determined by smooth muscle contraction induced with KCl. Eighteen out of 21 cultured segments showed a peak reduction in diameter of more than 10% and were used for the analysis. The arterial segments cultured at 0 mmHg had a significantly smaller diameter than those cultured at other pressures. The segments cultured at higher pressure had lower incremental elastic moduli at 20 and 80 mmHg and higher moduli at 160 mmHg. The walls of the cultured segments were thicker in groups with higher pressure. These results indicate that, even in culture, the mechanical environment is a major determinant for the mechanical property and dimensions of the arterial wall. Arterial walls may respond to their mechanical environment even if other factors, such as hormonal environment and nervous stimuli, are kept unchanged.*

Keywords—*Mechanical adaptation, Smooth muscle cells, Arterial wall mechanics, Tissue culture*

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1 Introduction

HYPERTENSION CAUSES various changes in the artery wall, such as wall thickening and changes in mechanical properties. Many of these changes can be considered to be the adaptation processes of soft biological tissues to increased load. For example, wall thickening occurs as if the wall maintains its intramural hoop stress at a normal level; a decrease in the incremental elastic modulus compared at the same pressure suggests the restoration of elastic properties under physiological conditions (BERRY and GREENWALD, 1976; MATSUMOTO and HAYASHI, 1994; VAISHNAV *et al.*, 1990; WOLINSKY, 1972). These responses are, however, also attributable to other factors, such as hormonal changes and altered nervous stimuli. In fact, there have been several papers, including LIU *et al.* (1988), that have demonstrated that renal hypertension causes wall thickening of distal arteries, even if the intraluminal pressure is kept within a normal range by a constriction being placed proximal to them. It is very difficult to exclude such factors as long as mechanical adaptation is studied *in vivo*.

Recent advances in cell and tissue culture technology enable us to maintain blood vessel viability for more than a week in culture (BARDY *et al.*, 1995; 1996; FRY *et al.*, 1990; FRY and PAP, 1992; LABADIE *et al.*, 1996). If you culture arterial segments under various mechanical conditions to discover their changes, then you can evaluate physical factors separately from other physiological factors. In this study, we have developed a perfusion system to culture blood vessels under controlled mechanical conditions and have observed morphological and mechanical changes following the culture.

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2 Materials and methods

2.1 *Blood vessel culture apparatus*

Fig. 1 shows the apparatus developed for artery culture. As the first step, we used steady flow and pressure. The specimen was cultured in aerated Dulbecco's Modified Eagle Medium supplemented with 20% fetal bovine serum and penicillin-streptomycin (1 unit ml^{-1}). The medium, driven by a roller pump, goes through a depulsator, perfuses into the specimen and goes up through a water column, the top of which is open to air. Intraluminal pressure corresponding to the column height applies to the specimen. The medium then overflows the column and perfuses the outside of the specimen. The entire set-up was placed in a warmer box kept at 37°C. Flow rate and pressure can be set independently by adjusting the rotation of the pump and the column height, respectively.

2.2 *Specimen*

Common carotid arteries of 21 male Japanese white rabbits, weighing 3250 ± 40 g (mean ± SEM), were used as the specimen. Tubular segments of the arteries were excised from the

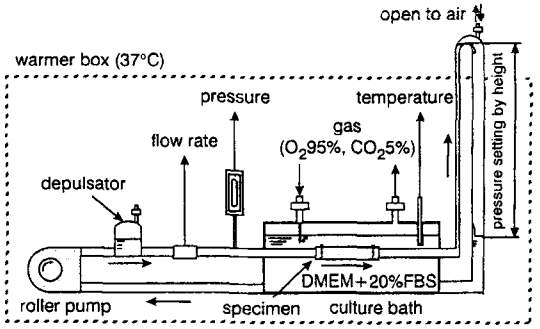

Fig. 1 *Schematic representation of blood vessel culture apparatus. DMEM = Dulbecco's Modified Eagle Medium; FBS = fetal bovine serum*

animals under sterilised conditions, after the animals had been sacrificed by an overdose of sodium pentobarbital. The segments were then cultured for 6 days under three different mechanical environments, mimicking normotension (denoted as Normo), hypertension (Hyper), and hypotension (Hypo). Specimens in groups Normo and Hyper were incubated in the blood vessel culture apparatus after being stretched to 1.4 times their no-load length, which is an average longitudinal stretch ratio of rabbit common carotid arteries obtained from our preliminary experiments. The flow rate of the medium was set at 80 ml mm^{-1} to apply physiological shear stress (1 Pa) to the specimens. Referring to a precise blood pressure measurement in Japanese white rabbits (SAITO *et al.*, 1986), intraluminal pressure was set at 80 mmHg for group Normo and 160 mmHg for group Hyper. A specimen in group Hypo was stretched to 1.4 times its no-load length with a special jig in a Petri dish and cultured with the medium in an incubator. Following the 6 day culture, specimens were used for the mechanical test. Specimens obtained from the contralateral artery were used just after excision as a non-cultured control and were referred to as Normal.

2.3 *Mechanical test*

The quasi-static pressure–diameter relationship of each specimen was obtained with an experimental apparatus similar to that used by TAKAMIZAWA and HAYASHI (1987). The specimen was transferred to a tissue bath filled with aerated Krebs–Ringer solution at 37°C, while its axial length was maintained with a special jig. It was then distended and collapsed, with the solution changing the intraluminal pressure between 0 and 200 mmHg at a rate of about 3 mmHg s^{-1}. The external diameter D_o was monitored with a TV system, and the intraluminal pressure P_i was monitored with a pressure transducer. The distension and collapse were repeated 5–8 times until the pressure–diameter (*P–D*) loop became reproducible. The ascending limb of the last stable loop was used for the control *P–D* curve. Subsequently, 100 mM of KCl was added to the bath, while keeping $P_i = 30$ mmHg. After change in the vessel diameter ceased, P_i was decreased to 0 mmHg, and the vessel was distended once to 200 mmHg to obtain the *P–D* curve under smooth muscle contraction.

2.4 *Data analyses*

After these mechanical tests, each specimen was removed from the apparatus to measure its no-load length after culture. The no-load length ratio λ_{NL}, which is the ratio of the no-load length of the specimen after incubation to that before incubation, was calculated. A ring-like segment was then obtained from the specimen, for the measurement of its inner and outer diameters in the no-load state (d_i and d_o, respectively). The inner diameter of a pressurised specimen D_i was calculated, assuming the incompressibility of the wall material,

$$D_i = \sqrt{D_o^2 - \frac{1}{\lambda_z}(d_o^2 - d_i^2)} \tag{1}$$

where λ_z is the longitudinal extension ratio (= [length during testing]/[no-load length]). The mechanical properties of the specimens were evaluated with an incremental elastic modulus H_{00} (HUDETZ, 1979), obtained from the no-load diameters and the slope of the *P–D* curve at each pressure

$$H_{00}(P_i) = 2\left(\frac{\Delta P_i}{\Delta D_o}\frac{D_o D_i^2}{D_o^2 - D_i^2} + \frac{P_i D_o^2}{D_o^2 - D_i^2}\right) \tag{2}$$

where ΔD_o is the increment of outer diameter with the pressure increase ΔP_i. The incremental elastic modulus represents the inherent elastic properties of the wall material.

The viability of the specimens was determined by smooth muscle contraction induced with KCl. The diameter change due to smooth muscle contraction $\%\Delta D(P_i)$ was calculated for every 10 mmHg, as follows:

$$\%\Delta D(P_i) = \frac{D_o(P_i) - D_{KCl}(P_i)}{D_o(P_i)} \times 100 \tag{3}$$

where $D_o(P_i)$ and $D_{KCl}(P_i)$ are the outer diameters at P_i before and after KCl administration, respectively.

2.5 *Histological observation*

After the mechanical test, the ring-like segments, whose inner and outer diameters had been measured, were fixed with formalin under no-load condition, sliced, and stained with haematoxylin-eosin or elastica-Masson for histological observation.

2.6 *Statistical analysis*

Data are expressed as mean ± SEM. Differences were analysed by a one-way ANOVA and were considered significant when $p < 0.05$.

3 Results and discussion

Fig. 2 shows the change in diameter after KCl administration. The reduction in diameter has its peak generally in a physiological pressure range. In this study, three out of 21 cultured specimens showed a peak response smaller than 10% and were omitted from the analysis. The rest of the specimens constricted significantly even after the 6 day culture, although their peak response was significantly lower than that of the normal one. The difference between the cultured segments was not significant. This suggests that smooth muscle viability was maintained sufficiently during the incubation period.

Pressure–diameter curves obtained from each group are summarised in Fig. 3. The arterial wall cultured at 0 mmHg (Hypo) was less distensible than the others. The outer diameter was significantly smaller in this group than the others at 80 and 160 mmHg. Another difference is the pressure at a 'bending point'. Generally speaking, carotid arteries are distensible in a low-pressure region and become abruptly stiff over a certain point. We call this point the bending point and defined it as shown in Table 1. The pressure at this point P_b was also significantly lower in the group Hypo (Table 1).

No significant difference was found in the pressure–diameter relationship between the groups Normo and Hyper and between the groups Normo and Normal. The hypertensive

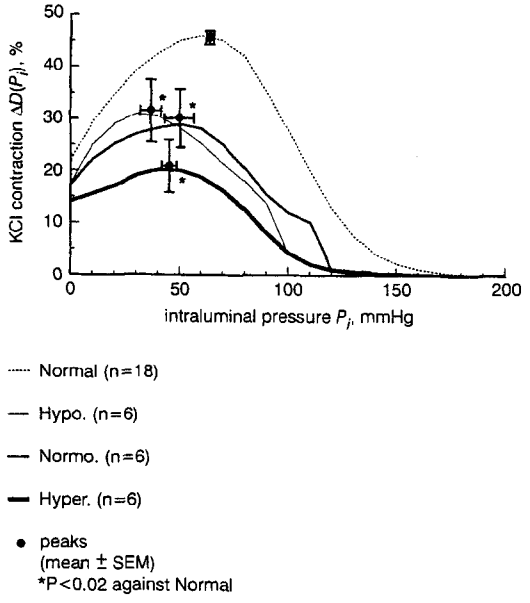

Fig. 2 *Contraction responses of rabbit common carotid arteries cultured for six days at 0 mmHg (Hypo), 80 mmHg (Normo) and 160 mmHg (Hyper) to KCl.*

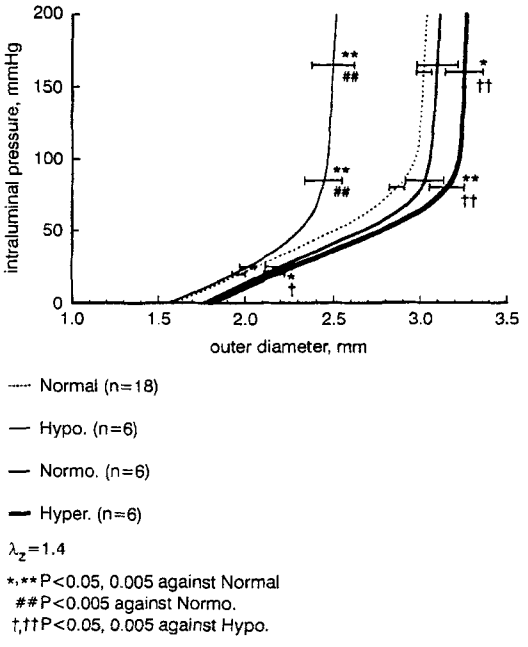

Fig. 3 *Change in pressure–diameter relationship of rabbit common carotid arteries following six-day culture at 0 mmHg (Hypo), 80 mmHg (Normo), and 160 mmHg (Hyper). Normo, non-cultured control. Difference in outer diameter among groups was evaluated at 20, 80, and 160 mmHg.*

group had a significantly larger diameter than the normal one. Incremental elastic moduli calculated from pressure–diameter curves are shown in Table 1, along with P_b and the no-load length ratio λ_{NL}. Segments cultured at higher pressure had lower moduli at 20 and 80 mmHg and higher moduli at 160 mmHg, although the difference was not significant between Normo and Hyper at 80 mmHg, and Hypo and Normo at 160 mmHg. The elastic moduli of segments cultured at normal pressure did not differ significantly from those of normal segments, except at 80 mmHg. These results imply that the pressure–diameter relationship and elastic property of arterial segments in culture change in response to mechanical load, and a normal load is necessary to keep mechanical properties unchanged.

Changes in the dimensions of cultured segments are shown in Table 1 and Fig. 4. Longitudinal length increased significantly in the group Hyper, as indicated by λ_{NL} greater than unity (Table 1). The length of the cultured segments in the other two groups did not change significantly. Fig. 4 shows arterial wall dimensions at 10 mmHg in the four groups. We chose this pressure because the variation in diameter among the groups was smallest at this condition, and thus the wall thickening could be evaluated without being affected by the difference in diameter.

Inner diameter was not significantly different among the groups. Even though the diameter difference was minimised at this pressure, the outer diameter was significantly smaller in the group Hypo than in the groups Normo and Hyper. Compared with Normal, the outer diameter and wall thickness increased significantly in Normo and Hyper. The walls of the cultured segments were thicker in groups with higher perfusion pressure.

These results suggest that the arterial wall in culture may thicken or become thinner in response to intraluminal pressure elevation and reduction, respectively. A diameter change due to the wall thickening may be more noticeable in the outer wall than in the inner wall. These changes are qualitatively similar to the mechanical changes observed *in vivo* (MATSUMOTO and HAYASHI, 1994).

Histological sections of the control and cultured segments are shown in Fig. 5. Sections of the cultured segments were similar to that of the non-cultured control. As they were fixed under a no-load condition, their elastic lamellae (black in this Figure) are wrinkled. The number of lamellar units per wall thickness was largest in group Normo (8.8 ± 0.3, $n = 12$) and smallest in group Hypo (7.9 ± 0.4), and was statistically similar among the four groups. Thus, changes in the inter-lamellar distance following culture were similar to those of wall thickness. These changes are also qualitatively similar to the changes observed *in vivo* (MATSUMOTO and HAYASHI, 1996).

Table 2 summarises the effects of the perfusion pressure on the mechanical properties and wall dimensions of cultured

Table 1 Change in mechanical parameters in arterial segments following six-day culture (mean ± SEM)

Group	n	P_{b2} mmHg	Incremental elastic modulus H_{00} at 20 mmHg, kPa	80 mmHg, kPa	160 mmHg, MPa	λ_{NL}
Normal	18	74 ± 1	69.6 ± 2.7	662 ± 38	11.72 ± 1.28	–
Hypo	6	50 ± 5**t, ‡, ††	85.2 ± 7.6*, ‡	2306 ± 520*, ‡	8.25 ± 1.44	1.004 ± 0.014
Normo	6	70 ± 2	65.0 ± 1.9	853 ± 88*	8.86 ± 0.91	0.976 ± 0.013
Hyper	6	72 ± 2	53.8 ± 3.9**, ‡, ††	646 ± 75†	13.46 ± 1.17‡, †	1.045 ± 0.007‡, †, ¶

P_b = pressure at bending point, defined as intersection of two tangents to pressure–diameter curve at ~20 and ~180 mmHg. λ_{NL} = no-load length ratio (=[no-load length after culture]/[no-load length before culture]). *,**$p < 0.05$, 0.01 against Normal; ‡$p < 0.05$ against Normo; †, ††$p < 0.05$, 0.01 against Hypo, respectively. ¶$p < 0.001$ against artificial data equivalent to unity (1.000 ± 0.000, $n = 6$). Normal, non-cultured control; Hypo, Normo and Hyper, segments cultured at 0, 80 and 160 mmHg, respectively

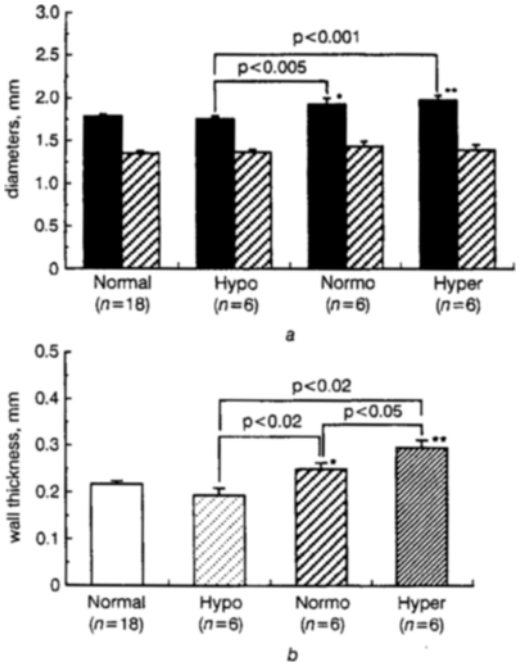

Fig. 4 *Change in wall dimensions of rabbit carotid arteries cultured at 0 mmHg (Hypo), 80 mmHg (Normo), and 160 mmHg (Hyper). Normo, non-cultured control. $P_i = 10$ mmHg, $\lambda_z = 1.4$. *,**$p < 0.05, 0.002$ against Normal (mean ± SEM). (a) (▨) inner and (■) outer diameters. (b) wall thickness*

arteries. The effects of hypertension on rat and dog thoracic aortas are also shown for comparison. Qualitatively speaking, the effects of high perfusion pressure on wall dimensions, such as wall thickness, inner diameter and axial length, are similar to those of hypertension *in vivo*. The changes in parameters such as diameter at the same pressure and the elastic modulus due to high perfusion pressure were opposite to those observed *in vivo*. It is interesting to note this difference, because such a difference may be attributable to factors that are not present in this system, such as pulsatility and hormonal and nervous stimuli.

Although the changes in the dimensions of the cultured arteries were qualitatively similar to those observed *in vivo*, some of them are not quantitatively similar. For example, the amount of wall thickening due to high perfusion pressure was smaller than that expected *in vivo*. It has been pointed out that the hypertensive aortic wall thickens so as to keep the circumferential wall stress in a normal range (MATSUMOTO and HAYASHI, 1994; VAISHNAV *et al.*, 1990; WOLINSKY, 1972). In this experiment, the mean circumferential stress of the normal segment at 80 mmHg was 110 kPa. The circumferential stress of the segments cultured under hypertensive condition (160 mmHg) was estimated to be 250 kPa, at the beginning of the culture, and became 200 kPa at the end of the culture. Although the wall thickening reduced the circumferential stress by 20% in 6 days, its amount was not enough to compensate the effect of pressure increase. Part of these quantitative differences may be attributable to the loss of smooth muscle viability, as indicated by the decreased KCl contraction (Fig. 2). Further investigation is necessary to improve smooth muscle viability.

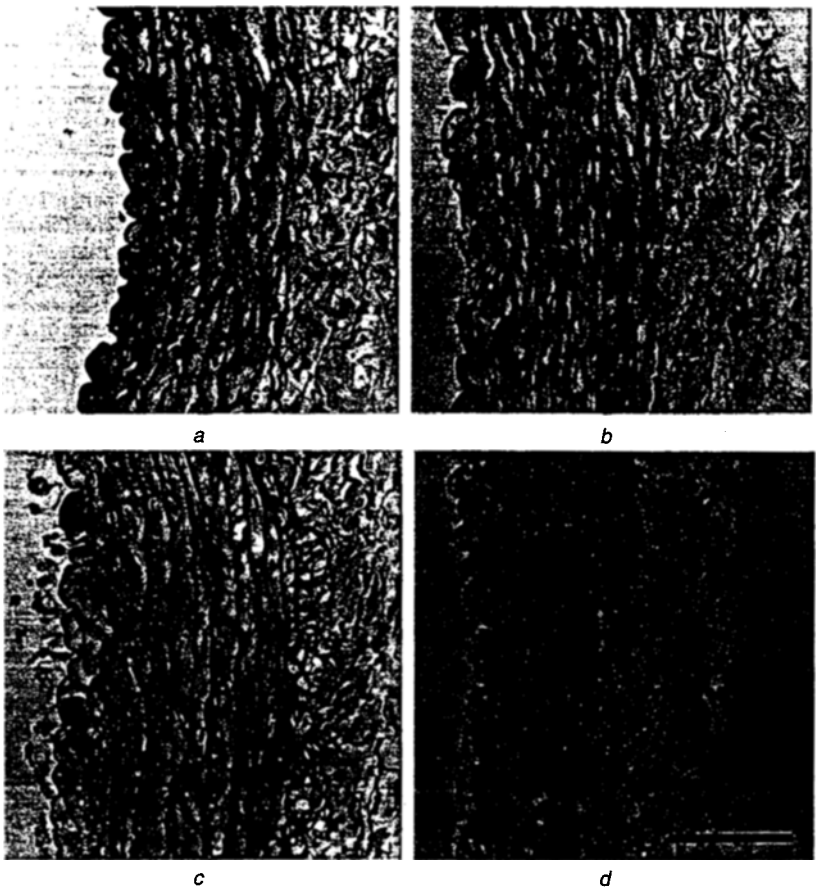

Fig. 5 *Micrographs of rabbit carotid arteries cultured at (a) 0 mmHg, (b) 80 mmHg, and (c) 160 mmHg and (d) their non-cultured control fixed under no-load condition. Elastica-Masson stain. Sections are perpendicular to longitudinal axis of vessel. Intimal surfaces face left. Length marker (100 μm) shown in (d) applies to all*

Table 2 Summary of changes in mechanical parameters of cultured artery: comparison with in vivo results

Parameter	Pressure Lo⟷Hi		Hypertensive aorta*	Similar to *in vivo?*
P–D relationship:				
diameter at same pressure	↓	(↑)	(↓)	no
P_b	↓	(↑)	↑	yes
Elastic modulus:				
H_{00} (20 and 80 mmHg)	↑	↓	↑	no
H_{00} (160 mmHg)	(↓)	↑	↓	no
Wall dimensions:				
wall thickness	↓	↑	↑	yes
inner diameter	–	–	–	yes
axial length	–	↑	↑	yes

P_b = pressure at bending point (see Table 1 for definition)
*Summarised from BERRY and GREENWALD (1976) and MATSUMOTO and HAYASHI (1994) for rat, and VAISHNAV *et al.* (1990) for dog

The changes during culture may include passive processes such as swelling and creep of the tissues. To consider this possibility, we also cultured arterial segments whose cells had been killed by rapid freezing with liquid nitrogen, and found no significant change, either in dimensions or mechanical properties during the 6 day culture. Furthermore, histological observation of the cultured specimens showed no evidence of tissue swelling. These results suggest that the mechanical response observed in the cultured arteries are attributable to active remodelling of the arterial wall caused by the vascular cells. Details of these experiments will be reported elsewhere.

Endothelial cells may have a marked effect on the changes in the cultured arteries. Owing to technical difficulties during experimental handling, it was hard to maintain the entire endothelial cell monolayer in this series of experiment. To investigate the effect of endothelial cells on the changes during culture, we need to improve the method of specimen excision and attachment to the apparatus.

In conclusion, the present results indicate that the mechanical environment is a major determinant for the mechanical properties and dimensions of artery walls, even in culture. The arterial wall may change its dimensions in response to its mechanical environment, even if other factors, such as hormonal environment and nervous stimuli, are kept unchanged.

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Author's biography

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