VIA MEDICA

ORIGINAL ARTICLE

Folia Morphol.
Vol. 69, No. 3, pp. 164–169

ISSN 0015–5659
www.fm.viamedica.pl

Structural organisation of tunica intima in the aorta of the goat

J.A. Ogeng'o, A.A. Malek, S.G. Kiama

Department of Human Anatomy, University of Nairobi, Kenya

[Received 30 March 2010; Accepted 14 May 2010]

The structural organisation of tunica intima in the aorta is important for its integrity, prediction, and diagnosis of atherosclerosis. The goat is a suitable model for cardiovascular studies, but the structure of its tunica intima is scarcely reported. This study, therefore, aimed to describe features of the goat aortic tunica intima by light and transmission electron microscopy.

*Sixteen healthy male domestic goats (*capra hircus*) aged between 6 and 24 months were used: 8 for light and 8 for electron microscopy. The animals were euthanised with sodium pentabarbitone 20 mg/mL and fixed with 3% phosphate buffered glutaraldehyde. For light microscopy, specimens from various regions of the aorta were routinely processed for paraffin embedding and 7 μm sections stained with Mason's trichrome. Those for transmission electron microscopy were post fixed in osmium tetroxide, embedded in Durcupan, and ultrathin sections stained with uranyl acetate and counter stained with lead citrate.*

Endothelium comprises round and squamous cells, linked to the subendothelial material by a simple and sometimes lamellated basement membrane. In the subendothelial zone, a heterogenous population of cells are connected with interlinked collagen and elastic fibres. Both cells and fibres are connected to the internal elastic lamina.

The composite structure and interlinkages in the tunica intima permit unitary function and increase mechanical strength, thus enabling it to withstand haemodynamic stress. (Folia Morphol 2010; 69, 3: 164–169)

Key words: goat, aorta, tunica intima, structural interlinkage

INTRODUCTION

The tunica intima of the aorta varies in its histomorphology depending on blood flow dynamics, age, and animal species [3, 12]. These variations are important in intimal integrity, prediction, and diagnosis of atherosclerosis [13, 22, 27]. The goat is a suitable model for studying vascular disease and for use in experimental cardiovascular surgery because the structure of some of its vessels [16, 28] and its physiological cardiovascular parameters [9, 17, 20] resemble those of humans. The organisation of the tunica intima in its aorta, however, remains largely underreported. The present study, therefore, investigated the characteristics of this layer in the aorta of the goat.

MATERIAL AND METHODS

Sixteen healthy male domestic goats (*Capra hircus*) aged between 6 and 24 months and weighing 10–60 kg were used in this study. The animals were purchased from private livestock farmers in Nairobi. Ethical approval for the study was granted by the

Address for correspondence: J.A. Ogeng'o, BSC Anat., MBChB, PhD, Department of Human Anatomy, University of Nairobi, P.O. Box 00100-30197, Kenya, tel: 254 20442368, e-mail: jogengo@uonbi.ac.ke

Kenya Physiological Society — Animal Research and Ethics Committee. Only animals certified to be healthy by a veterinary doctor and the age of which was known from farm records were used. The animals were euthanised with an overdose of sodium pentabarbitone 20 mg/mL injected intravenously, and fixed by gravimetric perfusion with 3% phosphate buffered glutaraldehyde. Specimens were taken from the ascending, arch, thoracic, and abdominal aortae. Those for light microscopy were routinely processed for paraffin embedding and sectioning, and 7 μm sections were stained with Mason's Trichrome. Those for transmission electron microscopy were post fixed in 1% phosphate buffered osmium tetroxide solution. The sections were cleared in propylene oxide and embedded in 100% Durcupan with catalyst and polymerised in an oven at 60°C for 48 hours. Ultrathin sections were made with Reichert ultramicrotome©, collected on 200 mesh copper grids, stained with uranyl acetate, counterstained with lead citrate, and examined by EM 201 Philips© electron microscope.

RESULTS

In all the segments studied, tunica intima comprises a uniform structure of three layers, namely endothelium and its basal lamina, a variable subendothelial zone, and internal elastic lamina (Fig. 1A). Endothelium consists of a single layer of flat and round cells (Fig. 1B–D). In the flat cells, the cell membranes are pitted with caveolae (Fig. 1C). The generally smooth basal surface is supported on a thin basal lamina, frequently connected to the subendothelial elastic fibres while the nucleus is dented, elongated, and predominantly euchromatic (Fig. 1C). Round endothelial cells, on the other hand, display an irregular luminal surface and a basal surface, bearing long branched processes which project into the subendothelial zone (Fig. 1D). The lateral processes of these round cells also show protrusions into the subendothelial zone. The large preponderantly euchromatic nucleus is generally irregular.

Intercellular junctions between the endothelial cells are of two types, namely a plane angular junction with a single extension anchoring into a cleft (Fig. 1E) and an interdigitation between finger-like lateral extensions of the cell membranes (Fig. 1F). In both cases, adjacent cell membranes show high electron density. The basal surface of the endothelial cell is connected to the basal lamina by focal areas of high electron density (Fig. 1G). Endothelial cells are attached to the subendothelial connective tissue, either through a thin simple basal lamina (Fig. 1E, G) or a lamellated basement membrane (Fig. 1F, H).

The subendothelial zone contains a morphologically heterogeneous population of cells (Fig. 2A–C), divisible into two main categories. The first category of cells is characterised by the presence of remnants of a basal lamina, rough endoplasmic reticulum, and a large euchromatic nucleus (Fig. 2A). These cells vary in morphology, some with regular cell surfaces, and others with highly irregular surfaces. The second category are large with irregular euchromatic nuclei, have a thin rim of cytoplasm, lack remnants of basal lamina, and contain definite rough endoplasmic reticulum. Both cell types are intimately associated with the elastic fibres (Fig. 2B, C). Connective tissue of the subendothelium comprises both collagen and elastic fibres oriented in all directions. The collagen and elastic fibres are frequently interlinked, insert onto the surface of the smooth muscle cells, and connect the basement membrane to the subendothelial cells (Fig. 2D).

The prominent internal elastic lamina is connected to subendothelial smooth muscle cells (Fig. 2E). On its abluminal side, it is often connected to smooth muscle cells, establishing physical contact, through areas of high electron density. In some areas, collagen fibres intervene between the internal elastic lamina and the smooth muscle cell (Fig. 2F).

DISCUSSION

The endothelium comprises morphologically flat and round cells, as reported for the aorta of other mammals [2, 15]. Some of the cells attach to the subendothelial extracellular matrix through a lamellated basement membrane, similar to that in arteries subjected to elevated pressures [14]. The heterogeneity of endotheliocyte morphology, their lateral intercellular junctions, and mode of attachment to subendothelial material are considered to reflect their adaptation to elevated shear and other mechanical stress [4, 12, 21]. This suggests that the tunica intima is designed to withstand haemodynamic forces, to which it is constantly subjected.

Pertinent observations of the current study in support of this suggestion are first that many of the subendothelial cells, similar to literature reports, are typical smooth muscle cells and their modified form or fibroblasts [10, 19]. These cells have been implicated in the synthesis and secretion of extracellular matrix comprising elastic and collagen fibres [24]. Indeed, both cell types are intimately associated with connective tissue fibres which in some cas-

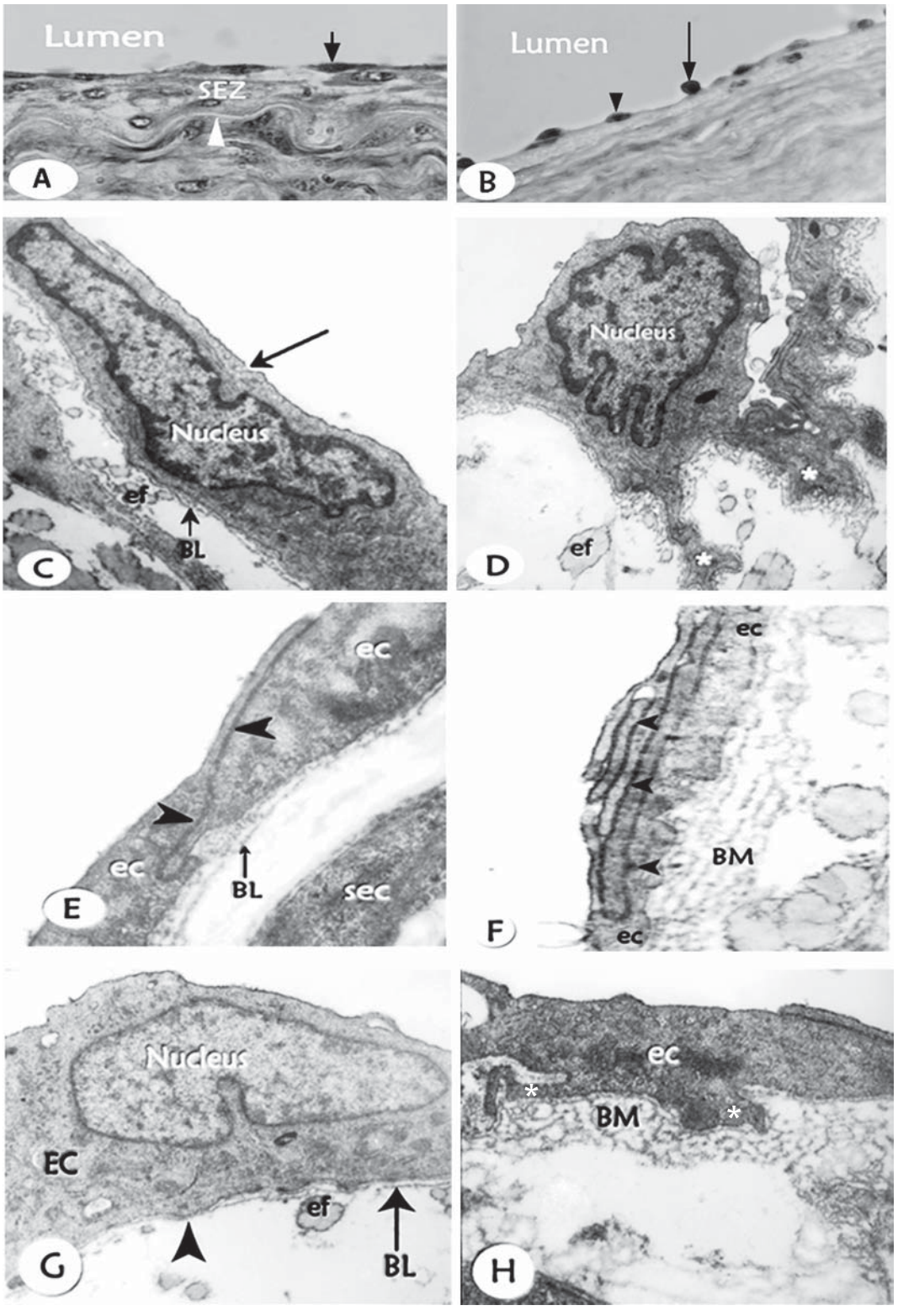

Figure 1. Micrographs of tunica intima of goat aorta showing endothelial cells; **A.** Components of tunica intima, namely endothelium (arrow) and subendothelial zone (SEZ) and internal elastic lamina (arrow head). Mason's trichrome × 250; **B.** Flat (arrow head) and round (arrow) endotheliocytes. Mason's trichrome × 400; **C.** Flat endotheliocyte with caveola (arrow). Note basal lamina (BL) and its association with elastic fibres (ef) × 27,800; **D.** Round endotheliocyte with basal cytoplasmic extensions (stars) into subendothelial zone × 27,800; **E.** Plane intercellular junction (arrowheads) between adjacent endotheliocytes (ec). Note the thin basal lamina (BL) and subendothelial cell (sec) × 27,800; **F.** Interdigitating junction (arrowheads) between two adjacent endothelial cells (ec). Note lamellated basement membrane (BM) × 8,760; **G.** Endothelial cell (EC) with its basal lamina (BL). Note electron dense area of fusion between endothelial cell and basal lamina (arrow head); and intimate association between BL and elastic fibre (ef); **H.** Endothelial cell (ec) with extensions (stars) into lamellated basement membrane (BM).

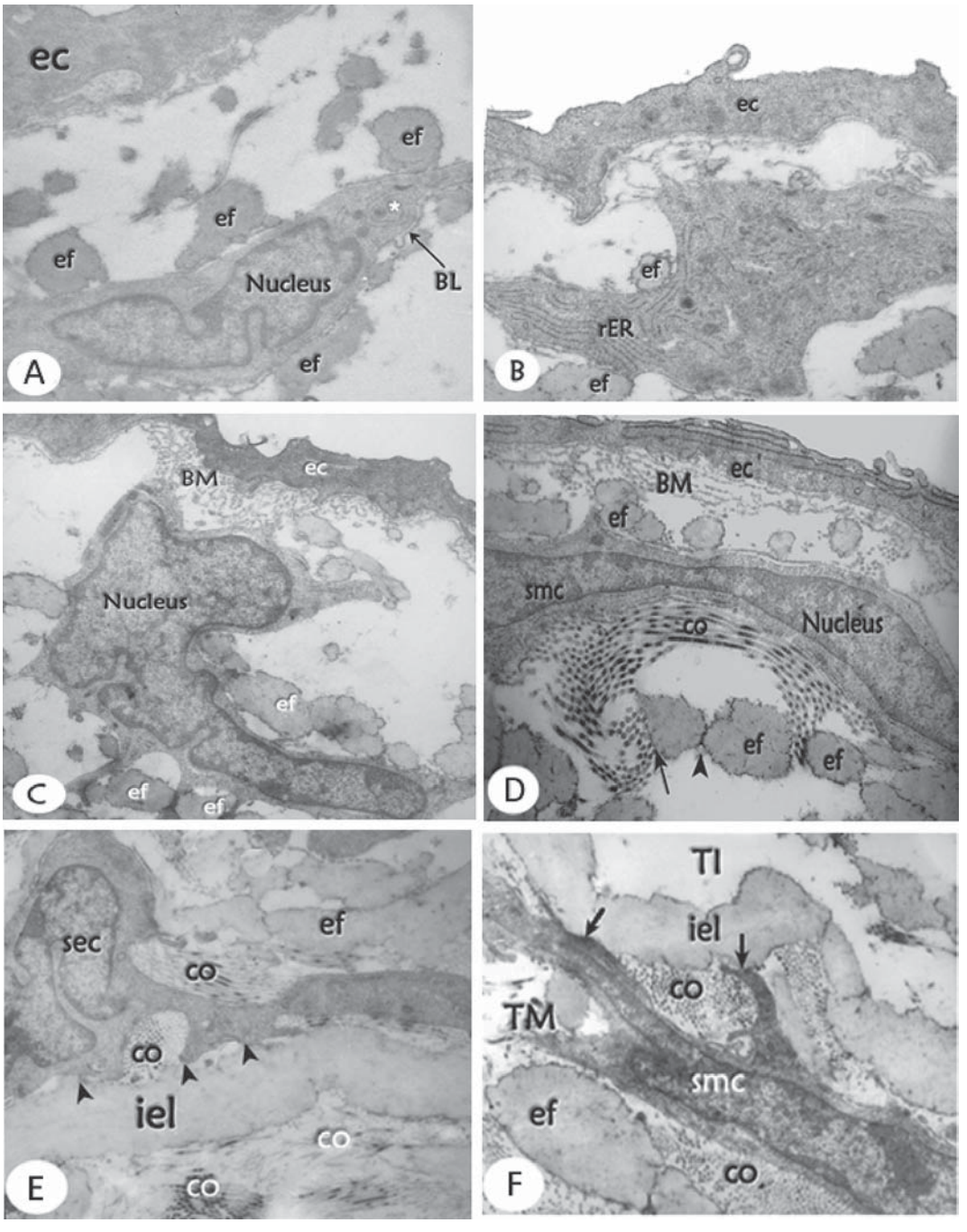

Figure 2. Electromicrographs of subendothelial cells in goat aorta; **A.** Spindle shaped subendothelial cell with a basal lamina (BL) intimately associated with elastic fibres (ef) × 8,760; **B.** Subendothelial rich in rough endoplasmic reticulum (rER) in close association with elastic fibres (ef). Note absence of basal lamina × 27,800; **C.** Subendothelial cell connected to lamellated basement membrane (BM) and elastic fibres (ef) × 8,760; **D.** Subendothelial smooth muscle cell (smc) connected by elastic fibres (ef) to lamellated basement (BM). Note intimate association with collagen (co) and the connection between co and ef [arrows] and the connection between ef (arrowhead) × 27,800; ec — endothelial cell; **E.** Subendothelial cell (sec) in close association with elastic (ef) and collagen (co) fibres. Note connection with internal elastic lamina (iel) × 8,760. **F.** Internal elastic lamina (iel) connected to smooth muscle cell (smc) [arrow] of the tunica media (TM): TI — tunica intima; co — collagen, ef — elastic fibres; × 27,800.

es appear to emanate from them. The presence of the connective tissue fibres oriented in various directions in this layer is important in anchoring the endothelium to the internal elastic lamina, yet allowing its free play during the rhythmic contraction and dilatation of the vessel [5].

The second observation is that elastic and collagen fibres attach onto the smooth muscle cells,

and that the two fibres are interconnected. These are features which have hitherto only been associated with the tunica media and adventitia, for purposes of distributing and withstanding mechanical forces [3, 5, 25]. The tunica intima is thought to yield little, if any, contribution to the structural mechanics of the vessel [11]. Furthermore, it is probable that, like in the tunica media [6], the interlocked structure of endothelium, elastin, muscle, and collagen enables the tunica intima to function as a mechanically homogenous unit [7].

Thirdly, the internal elastic lamina is connected to the smooth muscle cells and elastic and collagen fibres both on the luminal and abluminal side. This suggests that the tunica intima and media are physically interlinked. This linkage may be responsible for structural integration which permits the endothelium, smooth muscles, fibroblasts, and extracellular matrix of the intimomedial layer to act in concert. Such a knit structure enables the vessel wall to withstand the stresses to which it is subjected [25]. The structural linkage between the components of the tunica intima and tunica media through internal elastic lamina suggest that the internal elastic lamina has a more significant mechanical role than previously appreciated. Probably, its interlinkages with other components facilitate its ability to support the forces to which it is exposed [8, 26]. The physical inter-linkage between the components of the subendothelial zone, as observed in the present study, suggests that this layer contributes significantly to the mechanical properties of the aorta.

Heterogeneity of subendothelial smooth muscle and other cells observed in the present study has been reported in the human aortic tunica intima [1, 23]. Transformation of these cells is implicated in atherogenesis [18, 23]. Accordingly, the goat aortic tunica intima may be a suitable model for studying atherosclerosis.

CONCLUSIONS

The composite structure and interlinkages in the tunica intima permit unitary function and increase mechanical strength, thus enabling it to withstand haemodynamic stress.

ACKNOWLEDGEMENTS

To the University of Nairobi for financial support, James Macharia and Jackson Gachoka for technical assistance, and Catherine Chinga for typing the manuscript.

REFERENCES

1. Andreeva ER, Pugach IM, Gordon D, Orckhov AN (1998) Continuous subendothelial network formed by pericyte-like cells in human vascular bed. Tissue Cell, 30: 127–135.
2. Baryshnikova NA, Piatetskii AA, Gusev SA (1989) A quantitative analysis of regional features in the structure of aortal endothelial cells in short-term hypertension. Arkh Patol, 51: 37–42.
3. Bezie Y, Lacolley P, Laurent S, Gabella G (1998) Connection of smooth muscle cells to elastic lamellae in aorta of spontaneously hypertensive rats. Hypertension, 32: 166-169.
4. Davies EC (1994) Immunolocalization of microfibril and microfibril associated proteins in the subendothelial matrix of the developing mouse aorta. J Cell Sci, 107: 727–736.
5. Dingemans KP, Teeling P, Lagendijk JH, Becker AE (2000) Extracellular matrix of the human aortic media: an ultrastructural histochemical and immunohistochemical study of the adult aortic media. Anat Rec, 258: 1–14.
6. Dobrin PB (1999) Distribution of lamella deformations. Implications for properties of the arterial media. Hypertension, 33: 806–810.
7. Dora KA (2001) Cell-cell communication in the vessel wall. Vasc Med, 6: 43–50.
8. Farand P, Garon A, Plante GE (2007) Structure of large arteries: orientation of elastin in rabbit external elastic lamina and in elastic lamellae of aortic media. Microvasc Res, 73: 95–99.
9. Garcia JL, Fernandez N, Garcia-Villalon AL, Gomez B, Dieguez G (1995) Cerebral reactive hyperaemia and arterial pressure in anaesthetized goats. Acta Physiol Scand, 153: 355–363.
10. Glukhova MA, Koteliansky VE eds. (1995) Integrins, cytoskeletal and extracellular matrix in proteins in develo-ping smooth muscle cells of human aorta. In: Molecular and biological responses to the extracellular matrix. Academic Press, San Diego, pp. 37–79.
11. Greenwald SE (2007) Ageing of the conduit arteries. J Path, 211: 157–172.
12. Ingber DE (2002) Mechanical signaling and the cellular response to extracellular matrix in angiogenesis and cardiovascular physiology. Circ Res, 91: 877–887.
13. Jones GT, Jiang F, Mccormick SP, Dusting GJ (2005) Elastic lamina defects are an early feature of aortic lesions in the apolipoprotein knockout mice. J Vasc Res, 42: 237–246.
14. Kimani JK (1981) Subendothelial fibrillar laminae in the carotid arteries of the giraffe (Giraffa camelopardalis). Cell Tiss Res, 219: 441–443.
15. Kolpakov V, Polishchuk R, Bannykh S, Rekhter M, Solovjev P, Romanov Y, Tararak E, Antonov A, Mironov A (1996) Atherosclerosis-prone branch regions in human aorta: microarchitecture and cell composition of the intima. Atherosclerosis, 122: 173–189.
16. Lemson MS, Daemen MJ, Kitshaar PJ, Tordoir JH (1999) A new animal model to study intimal hyperplasia in Av fistula. J Surg Res, 85: 51–58.

17. Manrique M, Alborch E, Delgado JM (1977) Cerebral blood flow and behaviour during bran stimulation in the goat. Am J Physiol, 232: H495–H499.
18. Nicosia RF, Villaschi S (1995) Rat aortic smooth muscle cells become pericytes during angiogenesis *in vitro*. Lab Investi, 73: 658–666.
19. Osawa M, Masuda M, Kusano K, Fujiwara K (2002) Evidence for a role of PECAM-1 in endothelial cell mechanosignal transduction: is it a mechanoresponsive molecule? J Cell Biol, 158: 773–785.
20. Prasinos NN, Galatos AD, Raptopoulos D (2005) A comparison of propofol, thiopental or ketamine as induction agents in goats. Vet Anaesth Analg, 32: 289–296.
21. Ross R (1999) Atherosclerosis-an inflammatory disease. N Engl J Med, 340: 115–126.
22. Sartore S, Chiavegato A, Franch R, Faggin E, Pauletto P (1997) Myosin gene expression and cell phenotypes in vascular smooth muscle during development, in experimental models, and in vascular disease. Arterioscler Thromb Vasc Biol, 17: 1210–1215.
23. Shekhonin BV, Terarak EM (1995) Smooth muscle cell (smc) phenotype in diffuse intimal thickening and atherosclerotic plaques of human aorta. Atherosclerosis, 115: 60–61.
24. Snowhill PB, Foran DJ, Silver FH (2004) A mechanical model or porcine vascular tissue. Part 1: determination of macromolecular component arrangement and volume fractions. Cardiovascular Engineering An Int J, 4: 281–293.
25. Tada S, Tarbell JM (2000) Interstitial flow through the internal elastic lamina affects shear stress on smooth muscle cells in the artery wall. Am J Physiol, 278: H1589–H1597.
26. Xu C, Zarins CK, Glagov S (2001) Aneurysmal and occlusive atherosclerosis of the human abdominal aorta. J Vasc Surg, 33: 91–96.
27. Zarins CK, Xu C, Taylor CA, Glagov S (2004) Localization of atherosclerotic lesions. Vasc Pathol Physiol, 5: 55–65.
28. Zheng JW, Qui WL, Zhang ZY, Lin GC, Zhu HG (2000) Anatomical and Histologic study of the cervical vessels in goats Shangai Kou Qiang Yi Xu, 9: 39–41.