

## ORIGINAL COMMUNICATION

# HETEROGENEITY OF SMOOTH MUSCLE CELLS IN TUNICA MEDIA OF AORTA IN GOAT (*Capra hircus*)

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## ABSTRACT

Knowledge of the characteristics of vascular smooth muscle cells is important in understanding physicommechanical properties, functions, mechanisms of development, remodelling, regeneration of blood vessels, development and progression of their diseases like atherosclerosis. As the latter diseases become more common, there is need to understand their pathogenesis to inform mitigation strategies. Goat is a suitable model for vascular studies but the organization of vascular smooth muscle cells in its aorta have not been elucidated. This study therefore examined the characteristics of smooth muscle cells in the aorta of goat. Materials were obtained from aortae of six healthy young adult male domestic goats (*Capra hircus*) [age range 12 – 36 months]. Specimens from thoracic aorta were fixed in glutaraldehyde, post fixed in osmium tetroxide and processed for ducurpan embedding. Ultrathin sections stained with uranyl acetate, counterstained with lead citrate were examined by transmission electron microscope. The aortic tunica media contained several phenotypic dispositions of smooth muscle cells; spindle shaped or elongated organelle - poor cells; irregularly shaped cells rich in rough endoplasmic reticulum, mitochondria and a prominent nucleus. The smooth muscle cells also ran in various directions: transverse, oblique and longitudinal. These findings reveal that the smooth muscle cells of the tunica media of goat aorta are phenotypically heterogeneous and run in multiple directions. These characteristics probably confer mechanical strength and functional plasticity to the aortic wall. Designers of aortic substitutes should bear this in mind.

**Key words:** Vascular, Smooth Muscle Cells, Heterogeneity, Aorta

## INTRODUCTION

Features of vascular smooth muscle cells (VSMC) are important for maintaining physicommechanical properties, vascular tone, function and in understanding development, remodelling and regeneration of the vessel wall. They are also critical in elucidating pathogenesis of vascular diseases such as atherosclerosis (Bochaton – Pliat, 2005; Riches et al., 2013; Isayama et al., 2013). Recent studies reveal that medial VSMC are not terminally differentiated, rather they can undergo phenotypic switching in response to fluctuating environmental cues, including during development and progression of vascular diseases like atherosclerosis

(Nemenoff et al., 2011; Gomez and Owens, 2012; Davies et al., 2013; Nguyen et al., 2013). Further, that there are multipotent vascular stem cells, which are phenotypically modulated and can differentiate into diverse varieties (Tonar et al., 2010; Kennedy et al., 2014). As vascular diseases continue to increase, there is need to elucidate the features of VSMC in a variety of animals. Heterogeneity of VSMC has been demonstrated in vessels of rats, pigs and human (Bochaton – Pliat, 2005). There are, however only scanty reports on the detailed organization of smooth muscles in the goat aorta (Ogeng'o et al., 2010). Since goat is

potentially a suitable model for cardiovascular studies (Lemson et al., 1999; Zheng et al., 2000) and bovine aortic vessel substitutes are being advocated (Optiz et al., 2004a, b),

this study aimed at elucidating the features of smooth muscle cells in the tunica of its aorta.

## MATERIALS AND METHOS

Materials for this study were obtained from six healthy young adult male goats (age 12 – 36 months) purchased from livestock farmers in Nairobi. The animals were euthanized with overdose of sodium pentobarbitone and perfused with 3% phosphate buffered glutaraldehyde then post fixed in osmium tetroxide. One millimeter long specimens were obtained from the mid thoracic region (T9), rinsed in sodium phosphate buffer for 15 minutes then dehydrated by passing through increasing concentrations of ethanol (50%; 60%; 70%; 80%; 90%; 95% and 100%) for 30 minutes each, and twice for 1 hour each in absolute ethanol. The sections were then cleared in propylene oxide for 30

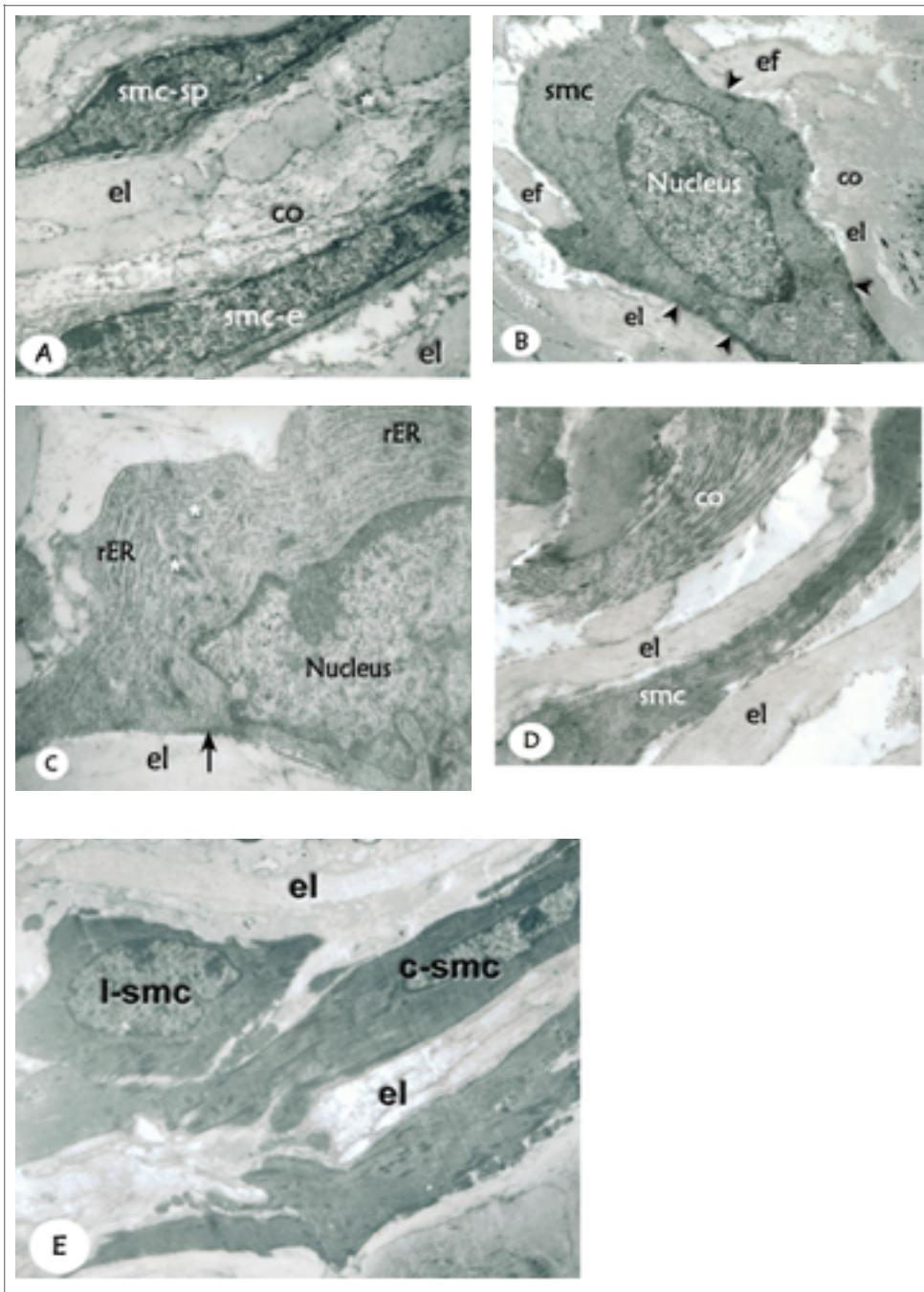
minutes. Subsequently, the sections were infiltrated in catalyst free durcupan mixture I as follows: propylene oxide: durcupan 3:1 – 30 minutes; propylene oxide: durcupan 1:1 – 30 minutes; propylene oxide: durcupan 1:3 – 30 minutes and absolute durcupan at 60°C in oven for one hour. The sections were then embedded in 100% durcupan with catalyst, and polymerized in an oven at 60°C, for 48 hours.

The blocks were trimmed and ultrathin sections made with Reichert ultramicrotome<sup>®</sup>. These sections were collected on 200 mesh copper grids, stained with uranyl acetate, counterstained with lead citrate, and examined by EM 201 Phillips<sup>®</sup> transmission electron microscope

## RESULTS

The tunica media comprised concentric elastic lamellae between which were smooth muscle cells, collagen and elastic fibres. Smooth muscle cells comprised a heterogenous population (Figure 1A-E). Many of them were either elongated or spindle shaped (Figure 1A). These cells hardly contained any synthetic organelles. Other cells were either characterized by irregularity, electron dense areas and close association with elastic lamellae and fibres (Figure 1B), or were large with irregular nuclei, and prominent rough endoplasmic reticulum. These latter cells were also

characterized by close association with elastic lamellae and showed areas of high electron density (Figure 1C). The smooth muscle cells ran in various directions (Figure 1 D,E). Circumferentially oriented cells appeared cut parallel to their long axes giving them an elongated appearance (Figure 1D), while those that ran longitudinally were cut transversely, and appeared round. Occasionally, even in the same interlamellar space, both circumferential and longitudinal smooth muscle cells co-existed (Figure 1E).



**Figure 1A-E: Electron micrographs of tunica media of goat thoracic aorta showing heterogeneity of smooth muscle cells.** A: Spindle shaped (smc-sp) and elongated cells (smc-e). Note the elastic lamellae (el), some interrupted (star), and collagen (co) between the muscle cells. x8,760. B: An irregular cell with large euchromatic nucleus. Note attachment of elastic fibres (ef), elastic lamellae (el) and collagen fibres (co) onto the smooth muscle cell (smc) through areas of high electron density (arrowheads). x27,800. C: An irregular cell with a large

euchromatic nucleus and abundant rough endoplasmic reticulum (rER). Note intimate association of the cell with elastic lamella (el) and areas of high electron density (arrow) x 63,400. D: A circumferential smooth muscle cell (smc). Note the close association with elastic lamellae (el), and adjacent collagen (co). x27,800. E: Longitudinal (l-smc) and circumferential (c-smc) smooth muscle cell between two adjacent elastic lamellae (el). x8,760.

## DISCUSSION

The view generally held is that vascular smooth muscle cells are spindle shaped with a central nucleus oriented in the long axis of the cell (Rhodin, 1980). The present study however, shows that smooth muscle cells in the tunica media of goat aorta display morphological heterogeneity where some cells are spindle shaped, or elongated but most are highly irregular. Spindle shaped and round VSMC have been considered to influence the distribution of mechanical stress by myogenic response (Nakano et al., 2007). The surface irregularities associated with the vascular smooth muscle cells are thought to increase the surface area to volume ratio (Gerrity and Cliff, 1975; Osborn Pelligrin, 1978; Fujiwara and Uehera, 1992) for attachment of connective tissue elements. It is plausible therefore that the irregularity and high cell matrix interaction constitutes a strengthening device that enables the aortic smooth muscle cell to endure the high systolic pressure as has been suggested in the cerebral arteries of hypertensive rats (Fujiwara et al., 1990).

The smooth muscle cells were generally oriented transversely, although some ran longitudinally and obliquely. Studies on the orientation of smooth muscle cells in the wall of the aorta have previously yielded equivocal results, with some reporting oblique (Berry et al., 1974), circumferential (Clark and Glagov, 1979; Rhodin 1980; Todd et al., 1983; Dingemans et al., 2000), and others longitudinal (Wasano and Yamamoto, 1983; Clark and Glagov, 1985). Longitudinal smooth muscles in the aortic wall are thought to strengthen the vessel wall (Osborn-Pelligrin, 1978; Wasano and

Yamamoto, 1983, Clark and Glagov, 1985) and also contribute to regulation of blood flow. It is therefore possible that the longitudinally oriented smooth muscle cells support the arterial wall against longitudinal stress parallel to the muscles (Shiraishi et al., 1986). In cases where smooth muscle is oriented in various directions, it is possible for tension to be developed in different directions in the vessel wall (Osborne-Pelligrin, 1978). The forces produced by each muscle bundle or sheet may be integrated into forces directed transversely with respect to the long axis of the vessel (Fujiwara and Uehara, 1992). The heterogeneity in orientation observed in the current study may therefore be important in influencing the mechanical properties of the aorta (Matsumoto and Nagayama, 2012).

Most of the smooth muscle cells contained many subsarcolemal areas of high electron density. These electron dense areas are of two types, namely those associated with attachment of matrix fibres onto the cell, and those not associated with fibre attachment. The observations of the present study support those on various other mammals (Clark and Glagov 1979, 1985; Dingemans et al., 1981; Bezie et al., 1998). The membrane associated dense plaques of muscle cells are major sites of anchorage of contractile apparatus to extracellular matrix (Clark and Glagov, 1979; Gabella 1984; Small and North, 1995). The mechanical link between muscle cells and elastic or collagen fibres provided by the dense plaques, plays an important role in regulating contractile and elastic tension in stressed vessels. It has

been shown that these focal attachments are stable and have great mechanical strength (Clark and Glagov, 1979; McGuffee and Little, 1996). Bezie et al., (1998) observation that the percentage of cell surface occupied by dense plaques increased in hypertension suggests that these electron dense plaques are involved in strengthening the aortic wall.

Smooth muscle cells were of variable sizes, nuclear morphology and organelle density. These findings support those of reports that vascular smooth muscle cells exist in synthetic, intermediate and contractile forms, which although interchangeable, have different internal features (Chamley-Campbell et al., 1979; Schwartz et al., 1986). This structural heterogeneity is probably a reflection of the functional plasticity within the aortic wall since the VSMC are involved in a wide range of physiological functions and pathological changes in the vessel wall (Lacolley et al., 2012). Further, the heterogeneity is thought to be involved in repair process and early

stages of disease through controlled proliferation, secretion and turnover of extracellular matrix (Riches et al., 2013). The heterogeneity is also a reflection of reversible potential (Wegenseil and Mecham, 2009) and phenotypic switching, which confers ability of VSMC to change in response to various stimuli (Majesky et al., 2011; Gomez and Owens, 2012)

In conclusion, vascular smooth muscle cells in the tunica media of goat aorta are phenotypically heterogenous and run in multiple directions. These characteristics probably confer mechanical strength and functional plasticity to the aorta. Designers of aortic substitutes should bear this in mind.

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## REFERENCES

1. Berry CL, Germain J, Lovell P. 1974. Comparison of aortic lamellar unit structure in birds and mammals. *Atherosclerosis* 19: 47-59.
2. 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.
3. Bochaton – Piallat ML, Gabbiani G. 2005. Modulation of smooth muscle cell proliferation and migration: Role of smooth muscle cell heterogeneity. *HEP*, 170: 645 – 663.
4. Chamley-Campbell J, Campbell GR, Ross R. 1979. The smooth muscle cell in culture. *Physiological Reviews* 59: 1-61.
5. Clark JM, Glagov S. 1979. Structural integration of the arterial wall. I. Relationship and attachments of medial smooth muscle cells in normally distended and hyperdistended aortas. *Laboratory Investigation* 40:587-602.
6. Clark JM, Glagov S. 1985. Transmural organization of the arterial wall. *Arteriosclerosis* 5: 19 – 34.
7. Davies M, Harman J, Yu H, Bennet M, Jorgensen H. 2013. The epigenetic phenotypic switch of vascular smooth muscle cells involved in atherosclerosis. *The Lancet*; 381: S<sub>34</sub>.
8. Dingemans KP, Jansen N, and Becker AE. 1981. Ultrastructure of the normal human aortic media. *Virchows Archives (Pathology and Anatomy)* 392:199-216.

9. Dingemans KP, Teeling P, Legendijk JH, Becker AE. 2000. Extracellular matrix of the human aortic media: an ultrastructural histochemical and immunohistochemical study of the adult aortic media. *Anatomical Record* 258: 1-14.
10. Fujiwara T, Kondo M, Tabei R. 1990. Morphological changes in cerebral vascular smooth muscle cells in stroke-prone spontaneously hypertensive rats (SHRSP). *Virchows Archives* 58:377-382.
11. Fujiwara T, Uehara Y. 1992. The cytoarchitecture of the medial layer in rat thoracic aorta: a scanning electron-microscopic study. *Cell and Tissue Research* 270: 165-172.
12. Gabella G. 1984. Structural apparatus for force transmission in smooth muscles. *Physiological Reviews* 64: 445-477.
13. Gerrity RG, Cliff WJ. 1975. The Aortic Tunica Media of Developing Rat 1. Quantitative Stereologic and Biochemical Analysis. *Laboratory Investigation* 32: 585-600.
14. Gomez D, Owens GK. 2012. Smooth muscle cell phenotypic switching in atherosclerosis. *Cardiovasc Res* 95: 156 – 164.
15. Hao H, Gabbiani G, Bochaton – Piallat ML. 2003. Arterial smooth muscle heterogeneity: Implications for atherosclerosis and restenosis development. *Atheroscler Thromb Vasc Biol*; 23: 1510 – 1520.
16. Isayama N, Matsumura G, Yamazaki K. 2013. Comparison of vascular smooth muscle cells in canine great vessels. *BMC Veterinary Research*, 9: 54.
17. Kennedy E, Hakimjavadi R, Greene C, Mooney CJ, Fitzpatrick E, Collins LE, Loscher CE, Guha S, Morrow D, Redmond EM, Cahill Pan. 2014. Embryonic rat vascular smooth muscle cells revisited: a model for neonatal, neointimal SMC or differentiated vascular stem cells? *Vascular cell*, 6: 6.
18. Lacolley P, Regnaut V, Nicoletti A, Li Z, Michel JB. 2012. The vascular smooth muscle cell in arterial pathology: a cell that can take on multiple roles. *Cardiovasc Res*; 95: 194 - 204.
19. Lemson MS, Daemen MJ, Kitshaar PJ, Tordoir JH. 1999. A new animal model to study intimal hyperplasia in Av fistula. *Journal of Surgical Research*; 85:51-58.
20. Majesky MW, Dong XR, Regan JN, Hognlund VJ. 2011. Vascular smooth muscle progenitor cells: building and repairing blood vessels. *Circ Res*; 108 (3): 365 – 377.
21. Matsumoto T, Nagayama K. 2012. Tensile properties of vascular smooth muscle cells: Bridging vascular and cellular Biomechanics. *J Biomech* 45: 745 – 755.
22. McGuffee I, Little S. 1996. Tunica media remodeling in mesenteric arteries of hypertensive rats. *Anatomical Record* 246: 279-292.
23. Nakano A, Minamiyama M, Seki J. 2007. The three dimensional structure of vascular smooth muscle cells: a confocal laser microscopic study of rabbit mesenteric arterioles. *Asian Biomedicine*; 1: 77 – 86.
24. Nemenoff RA, Horita H, Ostriker AC, Furgeson SB, Simpson PA, van Putten V, Crossno J, Offermanns S, Weiser – Evans MCM. 2011. SDF - 1 $\alpha$  induction in mature smooth muscle cells by inactivation of PTEN is a critical media of exacerbated injury – induced neointima formation. *Arterioscler Thromb Vasc Biol*, 31: 1300 – 1308.
25. Nguyen AT, Gomez D, Bell RD, Campbell JH, Clowes AW, Gabbrani G, Giachelli CM, Parmacek MS, Raines EW. 2013. Smooth muscle cell plasticity: fact or fiction? *Circ Res* 112: 17 – 42.
26. Ogeng'o J, Malek AA, Kiama A. 2010. Interlinkages in the Tunica media of goat aorta. *Int J Morphol* 28: 409 – 414.
27. Opitz F, Schenke-Layland K, Cohnert TU, Starcher B, Halbhuber KJ, Martin DP, Stock UA. 2004a. Tissue engineering of aortic tissue; dire consequence of suboptimal elastic fibre synthesis in vivo. *Cardiovascular Research* 63:719-30.

28. Opitz F, Schenke-Layland K, Richter W, Martin DP, Degenkolbe T, Stock UA. 2004b. Tissue engineering of ovine aortic blood vessel substitutes using applied shear stress, and enzymatically derived vascular smooth muscle cells. *Annals of Biomedical Eng* 32: 212-22.
29. Osborn-Pelligrin MJ. 1978. Some ultrastructural characteristics of the renal artery and abdominal aorta in the rat. *Journal of Anatomy* 125:641-652.
30. Rhodin JAG. 1980. Architecture of the vessel wall, In: Bohr DF, Somlyo AP, Sparks JR (eds). *American Physiological society Bethesda, Maryland* pp 1-31.
31. Riches K, Angelini TG, Mudhar GS, Kaye J, Clark E, Bailey MA, Sohrabi S, Korosissis S, Walker PG, Scott JA, Potter KE. 2013. Exploring smooth muscle phenotype and function in a bioreactor model of abdominal aortic aneurysm. *J Translational Med*, 11: 208.
32. Schwartz SM, Campbell GR, Campbell JH. 1986. Replication of smooth muscle cells in vascular disease. *Circulation Research* 58: 427 – 44.
33. Shiraishi T, Sakaki S, Uehera Y. 1986. Architecture of the media of the arterial vessels in the dog brain: A scanning electron-microscopic study. *Cell and Tissue Research* 243:329-335.
34. Small J, North A. 1995. Architecture of smooth muscle cell: In: *Vascular Smooth muscle cell* (eds Schwartz RM). New York: Academic Press: 169-188.
35. Todd ME, Laye CG, Osborne DN. 1983. The dimensional characteristics of smooth muscle in rat blood vessels. A computer-assisted analysis. *Circulation Research* 53, 319-331.
36. Tonar Z, Witter K, Krizkova V, Eberlova L, Kocova J, Molacek J, Houde KK, Kochova P, Vrxalova J, Topolcan O, Treska V. 2010. Stereological tools for quantitative microscopy of the aortic wall with focus on the abdominal aortic aneurysm microscopy science. *Technology applications and Education. A Mendez – Vilas, J Diaz (Eds):* 926 – 935.
37. Wagenseil JE, Mecham RP. 2009. Vascular extracellular matrix and arterial mechanics. *Physiol Rev*; 89: 957 – 987.
38. Wasano K, Yamamoto T. 1983. Tridimensional architecture of elastic tissue in the rat aorta and femoral artery- A scanning electron microscope study. *J of Elect Microscopy* 32:33-44.
39. Zheng JW, Qiu WL, Zhang ZY; Lin GC; Zhu HG. 2000. Anatomical and Histologic study of the cervical vessels in goats. *Shangai Kou Qiang Yi Xue*; 9: 39 – 41.