**ORIGINAL COMMUNIATION**

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

**Julius A Ogeng'o, Adel K Malek**

Department of Human Anatomy University of Nairobi. Correspondence to Prof. Julius Ogeng'o jogego@uonbi.ac.ke

## ABSTRACT

Knowledge of the characteristics of vascular smooth muscle cells is important in understanding physicomechanical 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 physicomechanical 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 – Piallat, 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 – Pilliat, 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

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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 pentabarbitone 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 $60^0$C in oven for one hour. The sections were then embedded in 100% durcupan with catalyst, and polymerized in an oven at $60^0$c, for 48 hours.

The blocks were trimmed and ultrathin sections made with Reichert ultramicrotome©. These sections were collected on 200 mesh copper grids, stained with uranyl acetate, counterstained with lead citrate, and examined by EM 201 Phillips© 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).

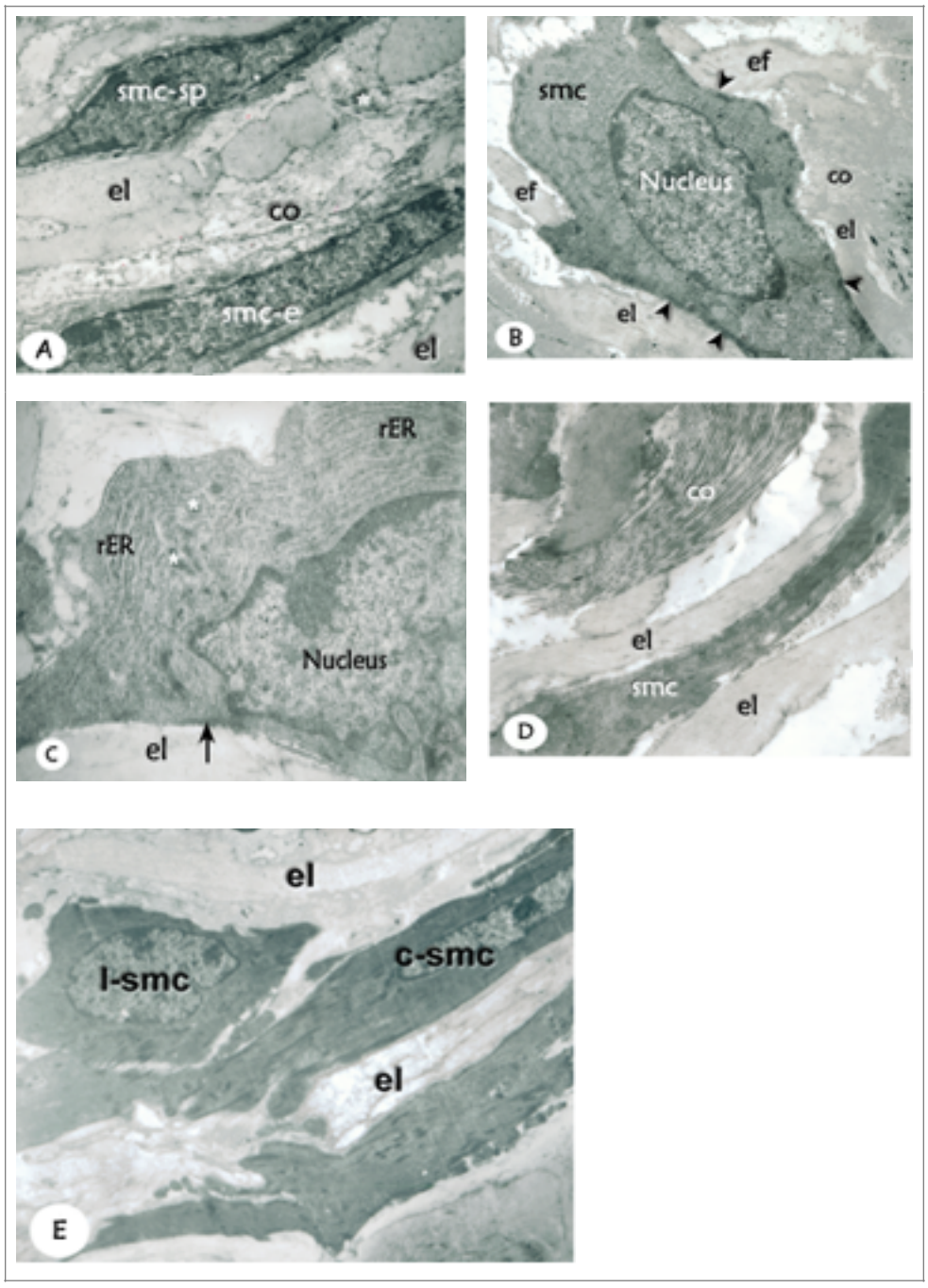


**Figure 1A-E: Electron micrographs of tunica media of goat thoracic aorta showing heterogeneity of smooth muscle cells.** A: Spindle shaped (smc-sp) and elongated cells (smc-e). Note the elastic lamellae (el), some interrupted (star), and collagen (co) between the muscle cells. x8,760. B: An irregular 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; McGufee 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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