Review

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Mechanical contribution of vascular smooth muscle cells in the tunica media of artery

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Abstract: The stiffness of arterial wall in response to cardiovascular diseases has been associated with the changes in extracellular matrix (ECM) proteins, i.e., collagen and elastin. Vascular smooth muscle cells (VSMCs) helped to regulate the ECM reorganizations and thus contributed to arterial stiffness. This article reviewed experimental and computational studies for quantifying the roles of ECM proteins and VSMCs in mechanical properties of arteries, including nanostructure and mechanical properties of VSMCs and ECMs, cell-ECM interaction, and biomimetic gels/scaffolds induced contractile properties and phenotype changing of VSMCs. This work will facilitate our understanding of how the microenvironments and mechanotransduction impact and regulate the arterial adaptation.

Keywords: Vascular smooth muscle cell; Contraction; Artery; Collagen; Elastin; Tunica media; Hypertension; Finite element method

1 Load bearing filaments in VSMC cytoskeleton

The cytoskeletal of vascular smooth muscles encompasses filaments and organelles. The density and number of these components can vary with respect to different internal and external signals [1, 2]. The filaments inside cytoskeleton can be classified as actin stress fibers (SFs), microtubules (MTs), and intermediate filaments (IFs), as shown in Figure 1.

These filaments play a principal role in the mechanical properties of vascular smooth muscle cells including proliferation [3], differentiation [4, 5], cell migration [6], and apoptosis [7, 8]. Therefore, mechanical properties of these fibers are critical for the deformation and stability of vascular smooth muscle cells.

1.1 Stress fibers (SFs)

It has been reported that stress fibers (SFs), which mainly alighted in major axis of the cell, are the principal contributor to contractile forces through actomyosin activation [9]. Deguchi et al. [10] performed tensile tests of SFs by isolating these fibers from cultured bovine VSMCs. Each SF is composed of a bundle of actin filaments (AFs). These bundles are held together by the actin-crosslinking protein α-actin. The elastic modulus of SFs was approximately 1.45 MPa which was three orders of magnitude lower than that of single AF (1.8-2.6 GPa) [11]. On the other hand, the breaking force of single AF was determined to be 600 pN, whereas the breaking force of a single SF is approximately...
Microtubules (MTs) have a cylindrical shape with inner and outer diameters of 14 and 25 nm [16]. MTs are rigid filaments with bending stiffness of 100 times higher than that of AFs and with elastic modulus of 1.2 GPa [17]. MTs have a remarkable contribution in stabilization of cells elongation through attaching to the cell membrane via certain capping proteins [18]. The contribution of MTs on cell locomotion and migration by regulating of actin polymerization has been reported [19]. Kato et al. [20] showed that tracheal fusion cells form polarized microtubule bundles oriented towards the leading edge of migrating cells. The function of these microtubules is twofold: to concentrate E-cadherin to the newly contacted cell interface and to initiate the formation of new adherent’s junctions. Microtubule depolymerization enhances isometric contraction of vascular smooth muscle cell, which is not receptor dependent [21]. Besides the principal contribution of SFs in contractility and MTs in migration, MTs are acknowledged to indirectly affect the contractility of VSMCs. Specifically, MTs growth favors dissolution of focal adhesions, whereas disruption of MTs leads to enhanced cell contractility by formation of SFs and focal adhesions [22]. In addition, disruption of the MTs decreased the tensile stiffness of VSMCs by 30% at large strain levels. Insignificant contribution of MTs was observed under small tensile strain which stem from wavy morphology of these fibers [15].

1.3 Intermediate filaments (IFs)

The intermediate filament (IF) network is one of three cytoskeletal systems. IFs are widely distributed from the plasma membrane to nucleus, providing mechanical and structural integrity for the cell [23]. In conjunction with associated proteins, IFs generate networks that serve to generate and support cell shapes. Spatial reorganization of IFs along with the development of SFs make VSMCs able to adjust their contraction/relaxation states. Moreover, the dynamic IFs play a crucial role in regulating various cellular functions including signal transduction; tension development; cell division and migration [24]. The IFs, with the diameter of approximate 10 nm, have been grouped into five types, or sequence homology classes (SHC), on the basis of amino-acid-sequence identity [25]. The most prominent IFs in VSMC cytoskeleton is vimentin, which forms a dynamic network and varies during contraction [26]. The elastic modulus of IFs has been reported in the range of 300-900 MPa [27]. The contribution of IFs in tensile properties of SMCs is remained to be determined even though IFs play important roles in tensile properties of the cells during large deformation [28]. Green et al. [29] speculated that it is impossible to disrupt IFs themselves due to the interaction between IFs and AF structure.

Although the characteristics of each filament in the VSMCs cytoskeleton has been studied separately, the intracellular force balance, contraction, and cell stiffness are strongly influenced by the interaction of cytoskeleton with extracellular matrix (ECM) and signaling pathways as described below.

2 Interaction of VSMCs within the extracellular matrix (ECM)

Structural constituents of ECM, that regulate its passive mechanical behavior, are elastin fibers, collagens, and glycosaminoglycans (GAGs) [30]. Interaction of these structural constituents and VSMCs can trigger significant variations of stiffness of both ECM and VSMCs. The adhesive glycoproteins fibronectin and laminin form connections between ECM and VSMCs via specific integrin receptors. Fibronectin is a multifunctional adhesive protein present in the plasma and also synthesized by vascular cells [31]. VSMCs express both β-1 and β-3 integrins and [32] demonstrated greater functional significance in adhesive processes of β-3 integrin essential for SMC migration. On way to study the interaction between VSMC (and other cells in general) and ECM is to culture the cell on substrate and study the deformations under different circumstances [33]. Adhesion rate, spread area, cytoskeletal assembly, and focal adhesion signaling was evaluated by culturing VSMCs on substrates with different stiffness and coated with fibroactin- or laminin- [34]. When VSMCs were cultured on fibroactin substrates with varied mechanical gradient, it was found out that cells preferentially migrate toward stiffer regions [35, 36]. On the other side, Hartman
**et al.** [37] observed the migration of VSMCs toward the stiffer region of gradient substrate coated with fibroactin, whereas the migration on laminin-coated gradient substrate appeared to be random. This observation indicated that the deformation and migration of VSMCs are not only dependent on the stiffness of ECM but also the type of interacting proteins and the engaged integrins [34].

The ECM stiffness can also affect the phenotype of VSMCs [38]. A stiffer ECM led to synthetic phenotype in the VSMC. Specifically, the VSMC decreases the number of cytoskeletal filaments and exhibits lower stiffness than that of contractile phenotype. Fibronectin drives cells away from the contractile phenotype in vitro, whereas laminin has been shown to conserve it [39]. Cell culture in 2D has been widely used to study the mechanotransduction of VSMCs due to ease of handling, maintenance, and application of mechanical loads [40–42]. However, culturing cells on a 2D substrate affects the cellular deformation, adhesion force and stiffness. To address this issue, engineering 3D gels [43] or scaffolds [44, 45] as the cell culture environment has been suggested.

Artery and its cellular components are continuously exposed to hemodynamic stimuli including cyclic strain, flow shear stress, and blood pressure [46, 47]. These mechanical loadings correlated with VSMC behaviors, ECM remodeling, and vasoregulation [48]. Cyclic mechanical stimulation possesses dual effect on proliferation of VSMC [49], enhance the collagen production [50], and increases the capability of transformation from synthetic SMC phenotype into contractile phenotype [51]. A cyclic tensile strain of 5% reduced SMC proliferation [52]. Conflicting variation of VSMCs phenotype with respect to the level of cyclic loading has been reported [53], whereas overexpression of contractile phenotype proteins has been observed [54–58]. Solan et al. [59] showed that cyclic strain had a direct impact on increasing collagen content and organization in ECMs. Bono et al. [60] studied the effects of cyclic strain (7%) on the VSMCs behavior which were cultured on 2D substrates and in 3D matrix composed of type I collagen. It was demonstrated that in the 3D culture environment there are more VSMCs aligned in the direction of strain (nearly 60%). Additionally, the level of SM α-actin in VSMCs cultured in the 3D collagen matrix was higher than that cultured on the monolayer 2D substrate. This research indicated that in 3D culture environment and under cyclic loading the density of contractile proteins inside VSMC’s cytoskeleton increases remarkably. It is worth mentioning that in the cardiac cycle VSMCs are cyclically stretched by ~10% with a 25-50% mean strain, and their mechanical properties should be evaluated over a large range of deformations [61].

It was noted that the ECM mechanical properties including its heterogeneity are the key factors to impact the 3D VSMC contractility [62]. Novel hydrogels have been developed to resemble the composition of ECM and thus in vivo mechanical environment [63, 64]. Ding et al. [65] developed a biomimetic fibrous hydrogel with tunable structure and stiffness. The developed ECM array consisted of collagen I, III, IV, fibroactin, and laminin. The effect of ECM deposition and stiffening during vascular disease progression on VSMCs contraction/relaxation was investigated. Although, the developed hydrogel encompassed the composition of ECM components, the challenges lie in the control of the architecture and alignments of collagen fibers. It has been illustrated that fibers orientation affect their load sharing contribution to the media tunica [66]. Phillipi et al. [67] reported that a remarkable variation of collagen fiber orientation distribution exists in the diseased aortic media. Considering the limitation in reproducing a complex in vivo ECM environment, the load sharing of VSMCs with respect to these structural components of ECM remained to be explored.

### 3 Arterial constituents

The artery wall exhibits three major layers: Intima, media and adventitia. The intima layer is predominantly populated with endothelial cells (ECs), which synthesize proteins, such as collagen IV and laminin, to create basal lamina. Its main function is to transmit signals that control vascular tone. It has a minimal contribution to the artery’s mechanical properties. The adventitia mainly consists of fibroblast and a collagen-rich ECM. Adventitial fibroblasts respond to a variety of chemical and mechanical cues. For example, hypertensive environments result in increased fibroblast proliferation and collagens I and III synthesis. Adventitia bears over half of the load at abnormal pressure due to collagen’s role as structural reinforcement [68]. The media is the thickest layer, between the intima and adventitia layers. It serves as the primary load bearing components. The media are composed by multiple lamellar units (LU), which consists elastic lamellae encompassing smooth muscle cells (SMC), interposed with collagen fiber network, as shown in Figure 1.

The LU was comprised of approximately 29% elastin, 24% SMCs, and 47% collagen and ground substance [69]. The volume of a single medial SMC was 1630±640 μm³. The healthy aortic media SMC was in the shape of ellipse. The average length of minor and major axis is 3.1±0.8 μm and 19.0 ±3.3 μm, respectively. The average aspect ratio, *i.e.*,...
major/minor axis is 6.2±1.4. At the relaxed state, the elastic modulus of the rat aortic VSMC in the major and minor direction is 14.8 KPa and 2.8 KPa, respectively [70]. Upon contraction, the elastic modulus in major and minor direction is 88.1 KPa and 59 KPa, respectively. The average density of SMCs within the media is 3.7±0.6 ×10⁵ cells/mm³ [69]. Between lamellae, the major axis of each nucleus aligned in the circumferential direction with a 19±3° radial tilt, resulting in cytoplasmic ends directed toward top and bottom of the lamellae. Collagen type I is the most abundant within blood vessels and had been proposed as the primary determinant of tensile properties [71]. Collagen was organized as bundles of fibers (numbering 24±15 fibers per bundle), thinner bundles or individual fibers. Collagen fibers aligned preferentially circumferential in the media but showed random orientation in the adventitia. The LU thickness ranges 13-15 µm [66] with an elastic lamellar thickness of 1.0-2.2 µm. The number of LUs of the media layer is established during arterial development and is directly related to the tension in the wall. It was noted that the tension per lamellar unit is constant across mammalian species and throughout the arterial tree [72].

Elastic modulus of elastin and collagen fibers was reported as approximately 0.6 MPa and 1 GPa, respectively [73]. Collagen fibers have a wavy nature and low contributions to mechanical behaviors at low pressure load. This is due to the waviness of collagen fibers [74], which was gradually straightened under pressure. Only 6-7% of collagen fibers are engaged at physiological pressure [69]. Microscopy studies on male adult rats revealed that collagen fibers aligned in the longitudinal-circumferential plane of the media layer of aorta. On the other hand, elastin fibers tended to align in the circumferential direction in SML, but often formed a longitudinally network structure in Els [69]. Collagen fibers were observed more in ELs than in SMLs, and ELs comprise elastin and collagen fibers. Collagen fibers have a diameter of 3 µm and average segment length of 13-17 µm. The diameter of elastin fibers is measured around 0.1 µm which placed in ELs with an interconnecting, fenestrated network.

### 4 Arterial stiffness

The stiffness of artery is directly related to the function of each component in the LU. Due to their higher elastic modulus, elastin and collagen fibers were classically considered as the main load bearing elements in LU. At physiological pressure, arterial stiffness was predominantly determined by elastin fibers, while wavy collagen fibers, without being straightened yet, did not bear much load. Then, the abnormally large mechanical load could straighten the collagen fibers, which were able to carry more load than elastin fibers. These sequential participation of elastin and collagen fibers in arterial stiffness led to non-linear stress-strain response of the arterial wall, while it was suggested that VSMCs have no contribution in the mechanical response of the artery [75], as illustrated in Figure 2.

![Figure 2: Representative circumferential stress–stretch relationship for the mouse ascending aorta.](image)

Increased arterial stiffness is correlated with a larger collagen/elastin ratio in LU. Aging is associated with the defragmentation and discontinuity of elastin fibers. The damaged elastic fibers are generally not replaced, because elastin expression is turned off in adult species. This damage alone will weaken the artery. Then the arterial remodeling lead to more collagen fibers production, and usually increase the arterial stiffness [76, 77]. It has been reported that blood pressure and arterial stiffness are inversely related to elastin’s amount in the media layer [72, 78–81]. Many cardiovascular disease, specifically hypertension, are related to high stiffness of artery induced by elastin reduction and collagen fiber production [72]. Advanced glycation end-products (AGEs), which accumulate slowly with normal aging or in diabetes at a faster rate, has been considered as a major index factor for arterial wall stiffening [82, 83]. This was attributed to the increased protein–protein crosslinks on the collagen molecule [84, 85] and implied that collagen/elastin components alone are not the only inclusive parts to determine the arterial stiffness in certain situations. Using hypertensive rat models, several groups observed minimal changes in collagen content of artery [86–89]. Instead, reduced collagen content were reported in some cases [90, 91]. Hu et al. [92] monitored
over 8 weeks of ECM content in a coarctated mini-pig aorta. They observed that relative collagen content was increased at 2 weeks of hypertension, stayed at this high level for 4 weeks, and then declined to the baseline level at 6 weeks. The relative elastin content decreased at 2 weeks and remained at a similar level thereafter. The incongruous observations in the literature might be due to the variations in experimental protocols, including measurement methods of arterial collagen content, the hypertension degree, and the location of harvested artery [88].

Apart from the variation of collagen/elastin fibers content and ECM in general, VSMCs might have a contribution to arterial wall stiffness. Sehgel et al. [93] suggested to look into the contribution of VSMC to arterial stiffness since variation in elastin density was not enough to alter a major change in aortic stiffness. Animal studies (spontaneously hypertensive monkeys [94, 95] and rats [96]) showed that VSMC in the aortic media layer is stiffer due to hypertension or aging. These observations indicated that VSMC alone might contribute to arterial stiffness but has not been measured yet.

5 VSMC

Stiffness measurement of vascular smooth muscle cells are challenging due to its sensitivity to phenotypic switching in response of the environment. It has been reported that cultured VSMC on substrate might change their phenotype to synthetic [97]. VSMCs are aligned circumferentially in the media layer and undergo large deformations in physiological conditions. When the artery enlarges due to the hemodynamic pressures, VSMCs stretch along their major axis. However, AFM technique is only able to measure the elastic properties of local regions of cells under small deformations and cannot provide enough information associated with the tensile properties of whole VSMC in physiological strain range (median strain of 25-50%). Due to the aforementioned reasons, it is vital to obtain tensile properties of the cells freshly isolated from the artery wall. In this regard, different methods for gripping the VSMC and performing tensile test have been suggested. Knotting [98], aspiration [99], adhesion on pipette [100], plate [101] and micropillar array substrate [14] are among the popular cell gripping methods for the tensile testing of VSMCs.

6 Mechanical contribution of arterial constituents

6.1 Experimental studies

There is a range of techniques to quantify the mechanical behaviors of cells, such as Atomic Force Microscopy (AFM) [88, 93, 95, 96]. The contraction response of VSMC can be measured directly by AFM tests, or in an indirect way by comparing the expression of primary SMC-specific contractile markers such as SM α-actin. It is well known that by phenotype changing of VSMCs to synthetic type, the number of stress fibers decreases, and the number of organelles increases which prepare the cell to proliferate and generate ECM proteins. These changes in the cytoskeleton decrease contractility and stiffness of VSMCs (by one-third or one-fourth). Thus, the initiation of cell proliferation can be counted as an indicator of relaxed VSMCs. Hu et al. [92] reported that cell proliferation occurred at 2, 4, and 6 weeks, but not at 8 weeks of hypertension. The highest proliferation rate was captured at 2 weeks of hypertension. Xu et al. [102] found that proliferation of medial VSMCs was induced rapidly within 3 days after acute coarctation of the rat aorta and continued for 2 weeks. In addition, in addition, fluctuations in VSMCs stiffness was detected over 8 weeks of high tension loading of rat aorta [103]. Tosun and McFetridge [104] used cardiac output to define frequency profile of cyclic stretch of human VSMCs which was against with the previous in vitro models which were stimulated with constant pulse frequencies. It was revealed that the phenotypic outcome may be more dependent on the variation in the stimuli, rather than specific amplitude of change.

These studies indicate that VSMC’s stiffness could decrease sharply at the early stages of hypertension because of their dedifferentiation. However, it is reported that medial VSMCs expressing contractile proteins could also proliferate and actively synthesize ECM proteins [92]. On the other hand, the dedifferentiated cells express low levels of contractile markers and high levels of signaling molecules associated with cell growth, migration, fibrosis, and inflammation [105]. Matsumoto et al. [103] investigated the effects of hypertension on morphological, contractile and mechanical properties of rat aortic VSMCs. They found that the density of SFs and the stiffness of each SF may dependent on the intensity and duration of hypertension. The contraction and stiffness of VSMCs increased to its maximum at 8 weeks of hypertension and decreased thereafter. However, these observations were not correlated with the previous studies. The potential explanation could
be the level of hypertension, measurement techniques, level of VSMCs tension and VSMCs alignment.

The mechanical properties of elastin fiber network in the media layer were evaluated under uniaxial or biaxial tension [106–110]. Weisbecker et al. [111] compared the mechanical behavior of elastase and collagenase treated media from human thoracic aorta to untreated control specimens. VSMCs were still visible after elastase treatment and it was noted that their passive response might slightly affect the anisotropy of the tissue. One limitation of this work was neglecting the dependency of the mechanical properties on age or on the location of the artery. Martinez and Han [112] showed that collagenase treatment (collagen content decreased by 15%) caused an enhancement in the axial deformation but not in the circumferential deformation. This was explained by the dominating circumferential alignment of collagen in the vessel wall. While collagenase treatment may equally break the collagen fibers aligned in both the axial and circumferential directions, the ratio of change in the circumferential direction would be much smaller due to the large amount of collagen at the baseline [112]. However, Dorbin et al. [113] observed a considerable reduction in the arterial wall stiffness in the circumferential direction of collagenase treated dog arteries. The difference might be associated with the type of species, the density of collagenase used, or the implemented testing conditions [114]. Moreover, compared to elastase treatment, collagenase treatment seemed had less effect on the physiological pressures as that collagen is not fully engaged in the bearing arterial wall stresses. Reportedly, a decrease VSMC content by 11±7% in porcine carotid arteries was associated with enlargement of arterial wall at pressures up to 120 mmHg and mechanical stiffening of the arterial wall at higher pressures [115]. Despite the valuable results, the conducted researches had limitations such as being performed under static loading conditions, and the collagen fibers or VSMCs were partially removed in the treated specimens.

Although there have been many experiments to quantify the contribution of medial fibrous matrix in mechanical properties of the artery, the load sharing capacity of VSMCs has been underestimated. Previous studies about determining the stiffness and contraction of VSMCs in hypertension provided valuable information but sometimes are inconsistent which makes it difficult to evaluate the mechanical contribution of VSMCs in normotension and hypertension arteries. In addition, the load sharing capacity of VSMCs in LU is still not clear. Heterogeneity of LU and different mechanical properties of each component are the problematic issues to determine the portion of load taken by each constituent when the artery is exposed to hemodynamic pressures.

6.2 Computational methods

Numerical simulations have been implemented for many years to study the mechanical behavior of arteries. In the previous developed models, the arterial wall has been modeled as a single layer [116], two or three layers [117,118]. The applied constitutive relations to the arterial wall have been formulated by hyperelastic material with orthotropic, transverse isotropic, and isotropic behavior [119–123]. The main concern about these models was to predict the macroscopic mechanical properties of the artery and evaluate its deformation [124–127]. Considering the highly heterogeneous microstructure of the arterial layers has been challenging in these studies.

Furthermore, micromechanical modeling approach has been employed to include clearly distinguishable constituents inherited different material properties. The goal was to predict the anisotropic response of the heterogeneous material on the basis of the geometries and properties of the individual constituents, a task known as homogenization [128]. Application of micromechanical modeling in arterial mechanics is vast. Capturing the responses of hyperelastic tissues with multiple families of collagen fibers [129], elucidating the interaction between collagen and non-fibrillar matrix [130], strain hardening of collagen-I gel and realignment of the network [131] can be counted as the micromechanical modeling applications associated with the behavior of fiber matrix. Thunes et al. [66] developed a micromechanical model to detect the stress field of the fiber matrix after collagen recruitment. The VSMCs were simplified and replaced by a homogenous medium as the non-fibrous part.

In order to study the VSMC contraction effects in the media tunica and stress distribution through the thickness of artery, Lukes and Rohan [132] proposed a 3D micromechanical model, which consisted of a hyperelastic matrix (ECM), an incompressible inclusion (VSMC), and contractile bars (SFs). The micro-scale model was then coupled with a 2D macro-scale model of the arterial wall consisted of two layers of tunica media and tunica adventitia.

Nakamachi et al. [51] constructed a multi-scale FE model for stress and strain evaluation of VSMC of the human artery. Their micro-scale model was based on a Representative Volume Element (RVE) model and consisted of a VSMC embedded in a homogenous matrix, Figure 3. Despite of the novelty of the developed model, the simplified ECM structure and neglecting the distribution of col-
lagen/elastin fibers could be influential on the obtained results. Moreover, there were lack of discussion about mechanical contribution of the constituents in the arterial wall.

![Figure 3: Macro scale model of the arterial wall with three layers (right); arterial VSMC and RVE model (left)](image)

It has been found that the microstructure of ECM can vary by some diseases. Collagen disposition and cross-link disruption has been observed in the arteries with Marfan syndrome [133]. Moreover, Marfan aortic samples are histologically characterized by the fragmentation of elastic laminae (almost 50% lower [134–136]), which leads to the formation of aneurysms. Therefore, considering the heterogeneous structure of ECM will allow to detect the ongoing mechanisms behind the arterial disease which change the properties of ECM and VSMC state.

7 Sumamry

This review summarized the mechanical contribution of VSMCs to the arterial stiffness with focus on the load sharing of collagen/elastin fibers and contracted/relaxed VSMCs in the media layer of artery. In view of VSMCs cytoskeleton, it was noted that stress fibers have the major contribution in VSMCs contraction, however, microtubules and intermediate filaments can indirectly affect contractility of the cells. In addition, the cytoskeleton responses are strongly related to the interaction of integrin receptors and extracellular matrix.

VSMCs alter their proliferation and contractility or change their phenotype with respect to the mechanical environment, such as 2D or 3D ECM, and level of cyclic strains. Specifically, the cultured VSMCs change their phenotype compared with in vivo conditions. The responses of VSMCs subjected to cyclic loading is dependent on the time period of the applied load.

The mechanics of VSMCs could be better delineated using numerical simulation. The interaction between collagen and non-fibrillar matrix, alignment and recruitment of collagen fibers and induced stresses in VSMCs during extension have been elucidated. However, the load sharing capacity of VSMCs in Lamellar unit as well as the influence of phenotype changing on the VSMCs contribution in arterial stiffness remained to be determined.

The focus of this review paper was on the tunica media. However, the contribution of tunica adventitia and intima should not be neglected. Adventitia prevents the arterial wall from overexpansion. The most abundant cell type in adventitia is the fibroblast with a stiffness ranging 1-27 kPa, which synthesize the extracellular matrix and collagen fibers. Endothelial cells in intima have a relative lower stiffness of 1-2kPa, but plays an important role in contraction and relaxation of VSMCs and arterial stiffness.

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