Role of Transglutaminase 2 in vascular remodeling

van den Akker, H.H.O.

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Small artery remodeling:
current concepts and questions

Jeroen van den Akker, Marieke J.C. Schoorl, Erik N.T.P. Bakker, Ed van Bavel

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Abstract

Blood flow regulation by small arteries and arterioles includes adaptation of both vascular tone and structure. It is becoming clear that tone and remodeling of resistance vessels are highly interrelated. Indeed, concepts pointing to continuous resistance artery adaptation and plasticity are emerging. The purpose of this review is to summarize such concepts and approaches related to vascular organization and remodeling, and to point out the missing links and possible directions for future research. We focus on the individual vessel level. Since several relevant studies are based on isolated vessels, we briefly re-iterate the available isobaric and isometric approaches. We further discuss the major elements of the small artery wall and their relation to the passive and active mechanical properties, as important determinants for vascular remodeling. The cytoskeletal elements and actin re-organization during remodeling are discussed, as well as the re-lengthening of smooth muscle cells during prolonged constriction. We then consider tone as major causal factors in remodeling and discuss the role of vessel wall inflammation. Finally we illustrate examples of current quantitative, integrative approaches of small artery mechanosensing and adaptation that may lead to a physiomics description of small artery adaptation in health and in diseases such as hypertension.
**Introduction**

Resistance vessels are the small arteries and arterioles that regulate local perfusion and organ resistance. These typically include vessels of around 200 micron or smaller in diameter. Flow regulation by these vessels occurs at time scales from seconds to weeks, involving adaptation of luminal diameter by both vascular tone and vascular structure. This occurs in response to a wide variety of local mechanical and biochemical stimuli as well as endocrine and neural influences. Flow regulation malfunctions in a variety of cardiovascular and metabolic pathologies. Examples include impaired endothelium-dependent dilation under oxidative stress and inward remodeling of resistance vessels in various hypertensive disorders. Furthermore, resistance vessels may adapt to the presence of flow-limiting stenoses in proximal vessels.

The notions that vascular caliber regulation involves multiple stimuli (e.g. wall tension, shear stress, metabolic factors) in a complex mechanical setting, widely diverging time scales and a distributed-resistance network spanning many generations raises several fundamental questions: How do blood vessels combine responses to pressure, flow, metabolic factors? In a remodeling vessel, how do functional characteristics such as the capacity for active tension generation change? If flow can be controlled by either tone or remodeling, what then determines their balance? I.e. how does a vessel ‘choose’ between deep tone and a wide structural caliber or shallow tone and a narrow caliber? Eventually, an integrative ‘physiomics’ approach could answer such questions. Such an approach would simultaneously consider the mechanical regime, various control loops (e.g. regulation based on wall tension and shear stress), different time domains (tone and remodeling), and the spatial organization of resistance vessels in networks, and ultimately would provide a detailed four-dimensional multi-scale approach of caliber regulation. Such an approach is not yet available, but might evolve from several useful initiatives. Accordingly, the purpose of this review is to summarize current concepts and approaches related to vascular organization and remodeling, and to point out the missing links and possible directions for future research.

We focus on the individual vessel level and on wall plasticity and eutrophic remodeling, i.e. in the absence of a change in wall cross-sectional area, ignoring proliferation and apoptosis in the vascular wall. Since several relevant studies are based on isolated vessels, we briefly re-iterate the classic experimental approaches, isometric wire-mounted and isobaric cannulated vessel segments. We further discuss the major elements of the small artery wall and their relation to the passive and active mechanical properties, as important determinants for vascular remodeling. We then consider cytoskeletal events and tone as major causal factors in remodeling and discuss the role of vessel wall inflammation. Finally we illustrate
examples of current quantitative, integrative approaches of small artery adaptation.

**Isolated small artery techniques**

The contractile properties of intact small arteries are generally studied by either of two methods\(^7\). Segments can be mounted in a wire myograph, where force development is measured at a certain, constant, internal circumference (isometric conditions). Alternatively, vessels are cannulated in a pressure myograph and diameter is measured while pressure is controlled (isobaric measurements).

**Wire myograph**

The wire myograph was first described by Mulvany and Halpern in 1976\(^8\), and was based on a method originally proposed by Bevan and Osher four years earlier\(^9\). This setup is now widely used in routine vascular physiology and pharmacology. In an isometric wire myograph, vessel segments are mounted as ring preparations on two wires, one of which is connected to a force transducer. The other wire is attached to a micrometer, thereby allowing precise control of vascular circumference. An equivalent radius (\(r\)) can be calculated from this circumference, although it should be realized that there is substantial deformation from the normal circular shape. Tension (i.e. force per length) is recorded and based on the presumed parallel arrangement of SMC and extracellular matrix, active tension is calculated from the difference between recorded tension and the passive component obtained during full dilation. After mounting, the vessels are ‘normalized’, i.e. set to the optimal radius for active tension. While this would ideally involve establishment of the full active radius-tension relation, the practical solution is to base the normalization on the passive radius-tension relationship of the vessel\(^{10,11}\). First, the passive radius at an equivalent pressure of 100 mmHg (\(r_{100}\)) is determined from the interception of an exponential fit of the radius-tension relation and the Laplace relation (\(T=P\cdot r\)) at 100 mmHg\(^{11}\). The distension is then usually set to 0.9\(\cdot r_{100}\), since active force production of the vessel is postulated to be maximal at this strain, and kept constant during the experiment. This normalization is based on the frequently used rat mesenteric small arteries. It is not clear whether this would also reflect the optimum for force development in other vessels. For the bulk of studies, this is not relevant, as long as sufficient active tension can be recorded on top of a low passive tension. However, as we explain below, the active radius-tension relation is dynamic, while also the passive relationship changes during remodeling. Therefore, we would suggest including detailed registration of both relations in studies on remodeled vessels, but also in studies that address mechansensing, or those that explicitly compare maximal active tensions between groups.
Pressurized vessels

In 1966, Burg et al. published a technique for the investigation of isolated, pressurized renal tubuli\textsuperscript{12}. This method, in which the wall is vacuum-clamped between two double-barreled pipettes, was adapted by the lab of Duling for arterioles\textsuperscript{13}, and still seems the approach of choice for cannulating the tiniest arterioles. This technique, as well as an alternative double-barreled technique\textsuperscript{14} require complex cannula construction, and a more general approach is the cannulation by two single-barreled pipettes and suturing\textsuperscript{15}. Pressures are generated by hydrostatic height, by roller pumps with pressure feedback, or by electric-pneumatic converters. Diameters are measured manually, using video calipers or off-line analysis of video images, or automatically, using a variety of diameter tracking algorithms\textsuperscript{16}.

An extensive ‘normalization’ protocol such as for wire-mounted vessels is not needed. Rather, the vessel is set to the (assumed) normal pressure, and the passive diameter is recorded, allowing for normalization of subsequent diameter recordings. Studies that include mechanosensing or remodeled vessels almost always include the pressure-diameter relation of the relaxed vessel in order to quantitate the mechanical properties and delineate the span of possible diameters. Axial stretch of cannulated vessels seems less standardized, and this issue seems somewhat ignored. In vivo, vessels are under axial strain due to tethering to the surrounding tissue and the longitudinal stress resulting from the pressure. Vessels thus retract considerably upon isolation. Ideally, one should set the vessel back to its \textit{in vivo} length. Yet, this is difficult to determine, if at all constant. Usually, cannulated vessels are straightened at a standardized pressure. However, this remains a relatively coarse method. Setups including recording of the axial tension are available, possibly allowing better standardization.

There are many reasons for choosing either of the above techniques. Amongst these, the wires allow generally faster mounting and multiple parallel segments, are forgiving with respect to small side branches, and allow easier and much faster data collection. Moreover, the isometric protocols are relatively well standardized. The cannulas provide more realistic mechanical loading and allow the study of flow. Furthermore, spontaneous basal tone and myogenic responses are more easily induced in pressurized vessels. In general, while pharmacological and physiological studies might employ respectively the wires and cannulas, both techniques have shown to be useful for the understanding of tone control as well as vascular plasticity, as will be explained below.
Small artery matrix and passive mechanical properties

The organization of blood vessels in three layers (intima, media, adventitia) was described in detail by Rhodin in the Handbook of Physiology\textsuperscript{17}. Figure 2.1 provides TEM photographs of a mesenteric resistance vessel, showing these layers and their major components. The major components believed to determine the mechanical properties in passive vessels, and thereby the caliber of the vessel, are elastin and collagen. It leaves no doubt that remodeling requires reorganization of at least these components. We therefore discuss their structure, synthesis and embedding, cross-linking and degradation in relation to the mechanical properties of the passive vessel.

Elastin

In large vessels, the large amount of elastin (e.g. 111 mg/g wet weight in the rat carotid artery\textsuperscript{18}) functions to damp pressure pulsations\textsuperscript{19}. Elastin content decreases towards smaller vessels, but despite the ‘muscular’ appearance of the vessels is still abundant (e.g. 15 mg/g in small mesenteric arteries\textsuperscript{19}). This is reflected by the volume occupied by elastic matrix, around 50% in the aorta and 14% in the superior mesenteric arteries. In arterioles, elastin is restricted to the internal elastic lamina. This sheet-like structure is built up by intertwined elastic fibers. Based on electron microscopy, thickness of the internal elastic lamina is in the order of 1 µm\textsuperscript{20}. In somewhat larger resistance vessels, an external elastic media may still be present, as well as some elastic fibers between the SMC\textsuperscript{19}.

Elastin is produced mainly by smooth muscle cells, although also endothelial cells and adventitial fibroblasts are capable of synthesis of tropoelastin\textsuperscript{21,22}. Elastic fibers consist of a core of globular tropoelastin monomers joined by desmosine cross-links\textsuperscript{23,24}. The core of each fiber is surrounded by a sheet of unbranched microfibrils of 10-12 nm in diameter\textsuperscript{25,26}, containing fibrillins as well as an array of other macromolecules.

The incorporation of tropoelastin into elastic fibers is based on self-assembly and ordering (coacervation) and cross-linking. In vivo the coacervation process is guided by fibrillin. Thus, formation of elastic fibers and sheets starts with the assembly of fibrillin molecules at the cell surface\textsuperscript{27} due to transglutaminase induced cross-linking of fibrillin-1 monomers and microfibril-associated glycoproteins in the interbead filaments\textsuperscript{28,29}. These microfibrils form parallel bundles that may be stabilized at inter-microfibrillar regions and serve as scaffolds for deposition of tropoelastin. The resulting elastin is stabilized by lysyl oxidase-derived desmosine cross-links. This process is facilitated by fibulin-4 and -5\textsuperscript{21,30}.

Figure 2.1 (A) Transmission electron microscopic image of a rat mesenteric small artery fixated at 100 mmHg equivalent pressure. (B) Detail of the media and adventitia. EC = endothelial cell; IEL = internal elastic lamina; EEL = external elastic lamina; COL = collagen; SMC = smooth muscle cell; FIBR = fibroblast.
Elastin, once formed by cross-linking, is a remarkably stable protein in the absence of pathologies. Yet, maintenance is required to regulate the local amount of elastic fibers and their physical properties. In particular, remodeling of resistance vessels requires that also the internal elastic lamina is restructured, by a combination of incorporation of new tropoelastin molecules into the existing layer, degradation of part of the layer, and formation and degradation of intra- and intermolecular cross-links. We established a role for Transglutaminase s in small artery eutrophic inward remodeling. While these enzymes are involved in the genesis of elastin fibers, there is currently no evidence that they cross-link mature elastin.

As the name indicates, elastic fibers can easily be distended, to around twice their resting length. Moreover, the incremental elastic modulus (i.e. the slope of the stress-strain relation) is essentially constant over a large part of this range, and is in the order of 600 kPa. The structural base for elastin elasticity is extremely complex and subject of ongoing discussions. Extension of the tropoelastin monomers forms the base of the elasticity of elastin. This extension is based on uncoiling of large dynamic hydrophobic regions. Upon stretch, tropoelastin becomes more ordered, or entropy becomes less, and indeed the elastic nature of elastin is of entropic origin. Hydration of tropoelastin and the presence of bulk water filling the space between the tropoelastin molecules is crucial; without water, elastin is brittle. Various tropoelastin isoforms exist due to alternative splicing. Fundamental elastic properties of these isoforms apart from resting length of the tropoelastin molecule are probably not very different if alternative splicing occurs in the hydrophobic regions, but in other regions this could affect the number of cross-linking sites and thereby affect the degree of cross-linking and the final architecture and stability of the elastin molecule.

Collagen

Like elastin, also collagen content decreases in smaller vessels (e.g. from 124 mg/g in carotid arteries to 67 mg/g in mesenteric vessels). Within small artery networks, collagen content decreases further towards the periphery, e.g. from 20 to 9% of the wall volume over the mesenteric bed. In small arteries, the non-fibrillar collagen IV forms the basement membrane, while collagen I and III are present in an irregular network of small fibrils in the media and in large amounts in the adventitia. Collagen organization and biomechanics in small arteries need more extensive investigation; Information below is therefore mainly derived from tendon, while some studies address collagen in the major arteries.

Collagen is assembled in a multistep-process with distinct steps at specific locations inside the cell and in the extracellular space. The base of the collagen hierarchical organization is formed by the single polypeptide, the central part of which folds into a tight, right-handed \(\alpha\)-helix. Three polypeptides form a left-handed triple helix with a pitch of around 10.4 nm. Collagen type I is a heterotrimer made up of two \(\alpha_1(1)\) and one \(\alpha_2(1)\) peptide; Collagen type III is a
homotrimer containing $\alpha_1$(III). In a so-called D staggered array, 280 nm long trimers are packed into microfibrils at an offset of 67 nm, generating the characteristic striation in fibrillar collagen seen in EM. A wide variety of models for microfibrillar organization has been described, most of them based on a thickness of 5 or more collagen trimers. In tendon collagen I, 5-molecule microfibrils are organized in a regular pattern, with individual collagen trimers traversing between microfibrils. This networked rope design would add to the strength of collagen. Many such microfibrils form fibrils. The fibrils finally form fibers and sheets via interfibrillar proteoglycans. The EM images in Figure 2.1 indicates that the ‘fibers’ in the media of this resistance vessel have only a few fibrils, while some fibrils can be seen in the adventitia that follow the same path and therefore could be considered to form a fiber. The final intra and intermolecular cross-linking within the ECM is mediated by lysyl oxidase.

Two proteolytic systems are responsible for the degradation of many of the ECM components including collagen. The fibrinolytic plasminogen activation system degrades laminin and fibronectin directly. Elastin and collagen are degraded by matrix metalloproteinases (MMPs), some of which can be activated by plasminogen. Possibly, a part of the collagen aggregates is internalized by fibroblasts after an initial extracellular proteolytic event. There, urokinase plasminogen activator receptor associated protein (uPARAP) may cause a further degradation of collagen, although this mechanism is still under debate.

The stress-strain curve of collagen could be based on deformation at many possible levels of integration, ranging from stretch of the individual triple helices to straightening of the complete fibrils, which are known to follow a wavy pattern in the vascular wall (Figure 2.1), and deformation of the interfibrillar connections. Understanding the organizational level at which deformation occurs could provide insight into the link between changes in molecular organization, such as cross-linking, and alterations of the pressure-diameter relation of the blood vessel. The basis for the deformation of collagen has been extensively studied in tendon. The stress-strain curve is characterized by a first region of low stress where macroscopic straightening occurs, followed by a ‘heel’ region where stiffness rapidly increases, where the lateral order of the collagen molecules increases, believed to be caused by a straightening of kinked molecules, and a linear part with high stiffness. Above ~5% strain, stiffness falls again due to irreversible changes. In the linear part, the axial D period (see above) increases with stress. Sasaki and Odajima found fibrillar strain to parallel tissue strain, and estimated a fibrillar stiffness of 430 MPa, but Puxkandl et al. observed that only 10-20% of the tendon strain is associated with strain of the fibrils for slow strain rates. The remaining strain may stem from deformation of the proteoglycan-rich matrix connecting the fibrils. In biaxially loaded bovine pericardium, Liao et al. observed that fibrillar strain only started to occur at 20% macroscopic strain, attributed to...
straightening of the fibers, and above that accounted for only 32% of the strain, the remaining strain was attributed to inter-fibrillar slippage and heterogeneous straightening lengths of the fibrils. In similar experiments on porcine mitral valves, fibrillar strain only occurred at the end of the non-linear region of the stress-strain curve\textsuperscript{55}. The stiffness of the fibrils was estimated to be \(~100\text{ MPa}, as compared to the macroscopic stiffness of 3.5 MPa. These differences were explained on the basis of orientation and alignment of the fibrils. Fibril strain in human aortic adventitia was only 1% for macroscopic strains of 16%; the curvilinear macroscopic stress-strain curve was explained by fiber straightening, fiber reorientation, and finally fiber strain\textsuperscript{56}.

The above findings leave little room for a role of collagen fibril extension in small artery mechanics at relevant blood pressures. Rather, fibrils are expected to remain at rather constant length and straighten towards a less wavy structure during vessel distension. While the waviness indicated in Figure 2.1 suggests room for straightening, possible intrafibrillar cross-linking and attachment to the ground substance may still provide substantial stiffness to these fibers. A further quantitation of collagen architecture in distended vessels will be needed to test whether this is indeed the case. In addition, local visco-elastic properties of collagen could be determined. A possible strategy is based on microrheology\textsuperscript{57} of beads bound to the collagen using antibodies.

**Matrix organization and mechanical properties of the passive wall**

Figure 2.2A depicts a schematic radius-tension relation of a small artery during maximal vasodilation, as would be measured in wire myography. The active curves in Figures 2.2B and 2.2C will be discussed below. Figure 2.2D (‘tone=0’) shows the passive characteristics in a cannulated vessel. Ignoring finite wall thickness and axial distension, passive curves recorded using isometric and isobaric methods are roughly equivalent, and these curves can be converted into each other via the law of Laplace\textsuperscript{58-63}. The passive vessels are characterized by an unloaded diameter and non-linear elasticity reflected by an increasing incremental elastic modulus at higher distortions. This non-linearity is reflected in the wire myograph-based curves and the stabilization of passive diameter at increasing pressures. Physiologically relevant matrix remodeling is reflected by a change of these curves. Thus, in Figure 2.2A, the dotted arrow and grey relation show an inwardly remodeled vessel.

The shape of the passive curves is generally believed to depend on the contribution of elastin and collagen\textsuperscript{13;64;65}, dominated at low strain by elastin, having a low elastic modulus (\(~0.4\text{ MPa}). At higher strains, the stiff (\(~100-1000\text{ MPa}) collagen fibers start to hook on, causing a rapid increase in stress. This ‘hook on model’ (Figure 2.3A) has originally been developed for large vessels\textsuperscript{66-68}. The organization of the mesenteric arterial wall was investigated by scanning electron microscopy in vessels fixed in either relaxed or contracted conditions. Collagen,
elastin and cells were selectively degraded in order to inspect the individual components at a more detailed level. This revealed a pericellular network consisting of irregular collagen fibrils in a network of fibrous elastin. Elastase increased the diameter of cannulated mesenteric vessels, especially at low pressures, but over the entire pressure range in mesenteric arteries of hypertensive rats.

There are concerns with the hook-on model and its relevance for resistance vessels. Thus, in this one-dimensional model, ongoing recruitment of only small fractions of the collagen fibers is needed to explain the gradual stiffening at higher distensions. Final recruitment at the highest pressures can be estimated to be...
It seems not realistic that 95% of the collagen is not involved in the mechanics of the wall at all. Moreover, the fibers that are first recruited will be distended by substantial amounts by the time the vessel has reached its maximal diameter. This is at variance with the strain limit of 3-4% where collagen fibers are known to break. A final concern relates to remodeling: in a theoretical analysis we showed that outward remodeling in the hook-on model requires breakdown of nearly all of the collagen, followed by deposition of new collagen at larger hook-on diameters. In this transition, the vessels would be very vulnerable for mechanical overload. Moreover, there is no histological evidence for such massive degradation of collagen in outward remodeling. We formulated an alternative 1-dimensional conceptual model of vascular wall mechanics (Figure 2.3B) that is based on infinite stiffness of collagen fibers. In this model, elements are arranged in series. Each element has a linear elastin spring and a number of collagen strings, the shortest of which determines the maximal distension of that element. The three-parameter model (elastin stiffness and 2 parameters for distribution of collagen string length) could be fitted to experimental stress-strain curves, obtained on wire-mounted rat mesenteric small arteries. Moreover, the model predicts gradual outward remodeling following collagen degradation, and these predictions could be confirmed by collagenase experiments.

While the 1-dimensional models provide biomechanical concepts, they are clearly oversimplifying. More detailed 2D or 3D models and possibly finite element approaches will be required to quantitate the structural base of vascular biomechanics. Such work has mainly been performed for large vessels, but, provided sufficient anatomical information is available, could also be applied for the understanding of structural remodeling of resistance vessels. The models are generally based on constitutive equations that account for the biomechanical properties of relevant wall structures. The fundamentals of such approaches can be found in the work of Fung. Most models treat the vessel wall as a structural continuum, in which relations are defined between normal and shear stresses and strains on the basis of a mathematical matrix of material properties such as Young’s and shear moduli.

Local stresses may remain in vessels under zero external load. Such residual stresses reduce the stress gradients across the vessel wall under normal load, providing a suitable mechanical environment for SMC contractile function. Residual stresses are of interest for remodeling, since on one hand they are likely to influence local cell behavior, and on the other hand local remodeling processes would underlies the development of residual stresses. Their study could thus provide further insight into vascular plasticity. Elastin appears to have an important role in stress distribution. Enzymatic digestion of elastin, but not collagen digestion or SMC destruction, was shown to reduce residual stress. The circumferential component of residual strain is characterized by the opening angle
Figure 2.3: The hook-on model (A) and serial elements model (B) both explain the nonlinear passive radius-tension curve (C). In the hook-on model, the vessel is represented by a parallel arrangement of elastin and collagen springs. Increasing tension induces distension (grey area) and thereby a recruitment of collagen springs, raising the vessel stiffness. In the serial elements model, collagen is not a spring but a string that can bend but cannot be extended. Each element is an elastin spring in parallel to several of such strings. The vessel wall is represented by many of such elements in series. Upon ongoing tension, the elements become rigid one by one, also leading to a non-linear radius-tension relation. A and B are modified from 1, which also gives a detailed mathematical analysis and experimental test of both models.
following axial cutting of the vessel. This angle was shown to correlate well with the media-to-lumen ratio. In a biomechanical model, smooth muscle contraction is predicted to cause an increase in opening angle, while relaxation results in a decrease\textsuperscript{75}. In general, the opening angle decreases towards the periphery.

**Smooth muscle cells and mechanical properties of small arteries**

**Smooth muscle cells**

SMC content in the media increases with decreasing diameter, up to 85% in small arteries\textsuperscript{18}. Unlike in large vessels, SMCs in most small arteries and arterioles are aligned preferentially circumferentially, with a typical pitch angle smaller than 2°. This configuration has been suggested to provide an optimal resistance against vessel distension\textsuperscript{18,76-78}. The small angle would create a better overlap between cell tips, thereby creating a helical turn of SMCs in the vessel wall\textsuperscript{79}. Considering the current interest in vascular plasticity and re-lengthening of SMC during activation, the normal length of SMC along the resistance vessel tree in the various organs also becomes relevant. McGrath \textit{et al.}\textsuperscript{79} indicate lengths of around 100 micron, allowing the SMC in small arterioles to completely wrap the vessel lumen. Haas and Duling\textsuperscript{80} quantitated the dimensions of vascular cells in various microvascular beds and report SMC length of around 65 micron in dilated rat pial and hamster cheek pouch arterioles of 80-100 micron diameter. Miller \textit{et al.}\textsuperscript{81} report SMC length in rat intestinal arterioles to be 80-90 micron, essentially independent of vessel diameter in the range between 24 and 62 micron arterioles. A single SMC could thus cover the full circumference in small arterioles, but not larger resistance vessels. Although more complex shapes are found in branches and in the precapillary arterioles\textsuperscript{82,83}, it seems fair to generalize that small artery SMC are spindle-shaped, with a length of 60-100 micron that does not depend on the branching order, and a small pitch.

The dense focal adhesions at the membrane and cytosolic dense bodies may be considered to form the mechanical base of the smooth muscle cell. Various actin isoforms span these anchoring points, forming the actin cytoskeleton. Other actins interact with myosin during contractile force development, forming contractile elements analogue to sarcomeres\textsuperscript{84}, but with side-polar rather than bi-polar arrangement, allowing a larger range of shortening. Intermediate filaments surround these structures, while microtubules act as rigid though dynamic struts. We discuss the role of these elements in force development and maintenance of cell organization during activation and remodeling. In addition to studies on small arteries, we include some findings and concepts from non-vascular SMC that may be relevant for the resistance vessels too.
Stimulus-contraction coupling, contractile element signaling and interaction of actin and myosin during contractile activation of smooth muscle have been well reviewed. A common finding is that calcium-dependent activation is followed by calcium sensitization, i.e. myosin light chain phosphorylation at low intracellular calcium, and subsequent maintenance of force in the absence of myosin light chain phosphorylation, allowing the maintenance of intermediate and chronic force at low energy expenditure. Latch bridges, i.e. non-cycling actomyosin bonds in the absence of myosin light chain phosphorylation might explain the state of chronic tension and low energy expenditure of SMC. While it remains unclear whether such bridges exist in small arteries, the concept has recently been used for modeling small artery contraction. Alternatively, recent work on SMC makes clear that both myosin and actin fibers, as well as other intracellular fibers and cytoskeletal elements, form dynamic structures in SMC, whose dynamic organization may underlie adaptation to maintained activation and remodeling.

In a series of studies mainly on airway SMC, Seow and coworkers provide evidence that the contractile apparatus adapts to SMC load. Thus, new ‘sarcomeres’ are formed in series upon maintained lengthening of the cell, causing an extensive broadening of the active length-tension relation (see ). Rapid evanescence of myosin filaments and stabilization of the filaments by myosin light chain phosphorylation underlie this process. Whether such myosin polymerization also occurs in blood vessels, is unknown. Increased actin polymerization has been found during SMC contraction, and is indeed considered essential for such contraction. Recently, Chen et al. demonstrated that myosin phosphorylation triggers such polymerization in rat mesenteric small arteries, indicating that the polymerization concerns the ‘contractile element’ rather than ‘cytoskeletal’ actin. Such polymerization may alternatively reflect longer actin, more parallel actin, or the recruitment of cytoskeletal actin to the contractile apparatus. These possibilities remain to be investigated, e.g. on the basis of altered active force-length relationships.

This dynamic organization of the actin cytoskeleton seems to be of primary importance for maintained tone and vascular plasticity. Gunst and Zhang provide a paradigm for the regulation of smooth muscle cell contraction that could be valid for small arteries too: a tightly regulated polymerization of globular G-actin to fibrillar F-actin is required for tension generation of SMC. Such polymerization does not regulate the cross-bridge cycling, but rather forms an independent process that provides stabilization of the cytoskeleton. The polymerization seems to occur mainly in a submembranous area of the SMC, providing membrane rigidity and adaptation to local forces acting on the dense focal adhesions. It remains to be established whether polymerization occurs also at the actin fibers that interact with myosin. The focal adhesion junctions are not static structures. Rather, contractile stimulation recruits structural proteins such
as alpha-actinin and vinculin that connect actin filaments to these junctions. Both actin polymerization and focal adhesion junction remodeling may be locally controlled by stress. This way, the SMC can adapt its structure in order to optimally carry the forces resulting from activation and pressurization\(^{94}\). Flavahan \textit{et al.} demonstrate that in mouse tail arterioles, actin polymerization occurs in the myogenic response but not in phenylephrine-induced constriction\(^{95}\). At low pressure, F-actin staining was found at the cell periphery, while at high pressure, F-actin increased in the cell interior.

Intermediate filaments in vascular smooth muscle contain vimentin as the most prominent protein, while in smaller vessels also desmin is present\(^ {96;97}\). Vimentin is a substrate for Transglutaminases, which are possibly involved in the dimerization process\(^ {98}\). These filaments extend from the nucleus to the membrane, and also connect to the dense bodies. Vimentin filaments form a dynamic network whose organization is dependent on contractile stimuli. Thus, in airway SMC, serotonin induces a shift from a random network of curved fibers to a network of straight fibers along the long axis of the SMC in 5-15 min\(^ {99;100}\). Phosphorylation of Ser-56 occurs in response to contractile activation. This mediates intermediate filament disassembly\(^ {99-102}\), increasing disassembled fraction of vimentin from around 10 to 20\%\(^ {99;102}\). Vimentin depletion by antisense suppresses force development while signaling remains intact, underlining the requirement of these filaments for contraction\(^ {102;103}\). Likewise, in desmin -/- mice, small artery potential for active force development\(^ {97}\) and phenylephrine-induced tone\(^ {104}\) were impaired. As reviewed by Tang\(^ {105}\), the effect of the vimentin network on contractile properties may stem from several structural and regulatory aspects. The connection to the membrane at desmosomes and to cytoplasmic dense bodies, and thus to the actin fibers provides a base for force transmission. Vimentin organization may further guide the actin network and actin polymerization. Stimulus-induced depolymerization and reorganization of vimentin could thereby mediate the actin reorganization that seems so crucial for prolonged force generation. The vimentin cytoskeleton may also regulate the distribution of p130 Crk-associated substrate (CAS). The CAS family serves as docking station for integrin-mediated signaling\(^ {106}\), and translocation of CAS dissociated from vimentin may facilitate actin polymerization and force development\(^ {100;107}\). Vimentin may also translocate and activate Rho-kinase, which was shown to be involved in small artery basal tone maintenance\(^ {108}\). It remains to be established how critical these processes are in the small arteries. Similarly, the mechanisms by which desmin affects force in small arteries also need to be unraveled.

Microtubuli are made of tubulin dimers that nucleate at microtubule organizing centers such as the centrioles and basal bodies. They are well known to be crucial for mitosis, for dynamic positioning of organelles within cells\(^ {109}\), and for cell motility. Microtubule disruption increases force of SMC\(^ {110}\). A cell signaling
component is present\textsuperscript{111-113}, but increased force or shortening also occurs in maximally active SMC, providing evidence for their role in tensegrity, a model for mechanical balance of cells depending on actin elements act as prestretched fibers and the microtubule as rigid struts opposing the force generated by the actin filaments\textsuperscript{114}. On the other hand, microtubule destruction or stabilization did not affect the unloaded shortening velocity of permeabilized vascular SMC\textsuperscript{115} and intact coronary arteries\textsuperscript{116}. A role for microtubule in migration of vascular smooth muscle cells in neo-intima formation is well recognized and forms the therapeutic base of taxol-eluting stents\textsuperscript{117}. Considering that early eutrophic remodeling of small arteries involves increased overlap and therefore motility of SMC\textsuperscript{118}, it may well be that microtubules play a role in regulation of media architecture and vascular caliber. Clearly, this area needs more research.

\textbf{Mechanical properties of the maximally active small artery wall}

As was the case for the passive steady state mechanical properties, those of the small artery at full activation can be determined using isometric techniques. Figure 2.2B depicts a typical relation. Active tension is determined from subtracting the passive tension from total tension, assuming a parallel arrangement. Arteries display a gradual increase in maximal active tension at larger distension, generally reaching a peak at radii smaller than $r_{100}$, followed by a decline at further distensions. Remodeling of the SMC causes a change in this relation. The grey curve and dotted arrow in Figure 2.2B indicate remodeling towards a smaller optimal diameter.

Peak tension (i.e. force/length) is higher in larger vessels. This is mainly related to a thicker wall, such that active stress is more comparable between vessels of different caliber\textsuperscript{119;120}. We previously obtained isometric radius-pressure curves on cannulated small mesenteric arteries, using radius-driven feedback of the pressure\textsuperscript{121}. We found that the capacity for ‘active pressure’ generation is close to 200 mmHg for these vessels. Translating these curves into radius-tension curves on the basis of the Laplace law reveals a similar shape as found on the wires, indicating that the non-circular shape in wire-mounted preparations has little influence. A consequence of these relations is that at physiological and mildly higher pressures, fully active vessels remain almost closed.

The factors that determine the shape of the active radius-tension relation may include both the organization of the contractile and cytoskeletal elements, as discussed above, and the deformation of the vascular wall during constriction\textsuperscript{119;120}. In constricted vessels, the luminal surface of the vessel folds into ridges, consisting of not only the endothelial cells and internal elastic lamina, but also part of the SMC\textsuperscript{77}. This withdraws part of the SMC from the capacity for tangential force generation. This process of ridge formation has been suggested to depend on dense body organization and to be associated with reorientation of the myofilaments\textsuperscript{122}. Vessel mass luminal to the contractile filaments may furthermore
amplify the effect of contraction on diameter reduction, while steric hindrance between ridges may prevent full closure.

While small artery remodeling is commonly expressed in terms of a change in passive properties, the changes in maximal active radius-tension relations such as indicated in Figure 2.2B have hardly been studied. Yet, it is clear that this relation is tightly controlled. Thus, the optimum radius for active tension is linked to the passive radius. This link is found over many orders of vascular caliber, and apparently is maintained during growth and development. In addition, SMC length is fairly independent of vascular caliber (see above). Therefore, regulation of SMC length, in addition to the regulation of cytoskeletal organization, may underlie shifts in active radius-tension relations during remodeling.

**Small artery SMC and matrix remodeling**

Vascular remodeling reflects any change in vascular structure, including changes in lumen diameter, wall thickness, and wall composition in terms of cellular parameters and amount of extracellular matrix components. In addition, organization of these components may change, such that the functional or mechanical behavior of the vessel has changed. Remodeling is preferentially described in terms of shifts in mechanical characteristics of individual vessels. In addition, describing ‘remodeling’ (or altered modeling) between groups can be based on the wall-to-lumen ratio, or media-to-lumen ratio. This term allows the comparison of vessels from different individuals, irrespective of the anatomical location, size or branching order. To our knowledge, whether the wall-to-lumen ratio is constant over the arterial tree is a question that has not been extensively studied. Data in mice show that the wall-to-lumen ratio is similar in aorta, carotid and mesenteric arteries. However, data from Frobert et al. suggest that porcine coronary arteries show an increase in wall-to-lumen ratio with an increase in branching order. Similar findings have been made by Bevan et al. for human pial arteries. A concern of these studies is that different experimental conditions, such as varying pressure levels, were used. It is not obvious at which pressure, or pressures, these measurements should be made when vessels of different origin are compared. Some general recommendations regarding this issue have been made by Bund and Lee. While the wall-to-lumen ratio is undoubtedly increased in hypertension, it provides little information on the underlying process, which could be an increase in wall mass (hypertrophy or hyperplasia), a decrease in lumen diameter, or a combination of both. A useful graphical representation of the types of remodeling is given by Mulvany et al. where the authors define remodeling on the basis of lumen change (inward or outward) and wall cross sectional area (hypertrophic, hypotrophic or eutrophic). The contribution of each parameter depends on the particular model, but is dominated by eutrophic
remodeling, i.e. a rearrangement of material around a smaller lumen without a change in wall cross-sectional area, in the case of essential hypertension\textsuperscript{128}. However, it should be stressed that SMC proliferation and apoptosis may form part of the remodeling of small arteries\textsuperscript{6}.

**Tone drives remodeling**

We found that chronic vasoconstriction *in vitro* results in the inward remodeling of small arteries\textsuperscript{129;130}. In addition, some arteries show outward remodeling *in vitro* in response to prolonged exposure to vasodilators. These observations have led to the suggestion that tone determines the direction of the remodeling response. This idea is in good agreement with results in hypertensive subjects, where vasodilator treatment, but not blood pressure reduction per se, corrects vascular structure\textsuperscript{1}. This is of importance, since an increased wall-to-lumen ratio is a predictor of cardiovascular events\textsuperscript{131;132}. In models of altered blood flow, changes in tone precede actual remodeling\textsuperscript{133;134}. These changes in tone appear to be an essential intermediary step in the remodeling process, since defective endothelial function and/or flow-induced dilation prevents flow-induced remodeling\textsuperscript{135-138}.

Based on the wall constituents described above and their dynamic structures, tone-remodeling coupling may occur by two pathways, one related to the cytoskeleton and the other to the ‘mold’ that is provided for newly formed extracellular matrix elements. Regarding the first pathway, in a recent review\textsuperscript{139}, Martinez-Lemus *et al.* argue that the boundaries between constriction and remodeling are blurred. They propose that the sequence of events from vasoconstriction, to intracellular reorganization of the cytoskeleton, to cellular repositioning and eventually, a change in the passive vessel diameter, should not be considered as separate events but rather as a continuum. These events rely on overlapping pathways and depend on the same structural elements, which form the cytoskeleton-integrin-extracellular matrix axis. This concept is based on experiments with isolated arteries that are contracted for various periods. Thus, following a 5-minute constriction, vessels fully relax to their original diameter. When constriction is maintained for 4 hrs, removal of the constrictor does not result in complete relaxation\textsuperscript{118}. The prolonged state of constriction is associated with increased overlap of the smooth muscle cells and re-lengthening of the SMC during the maintained vasoconstriction. The lack of full dilation would thus reflect a cytoskeletal brake on distension, and in early remodeling the cytoskeleton would thus take over this function from collagen. The repositioning of SMCs is suggested to redistribute wall stress to non-contractile vessel elements, thereby minimizing SMC energy expenditure\textsuperscript{139}. A re-lengthening of cells in constricted vessels would also cause a leftward shift of the active radius-tension curve, provided that the intracellular organization remains unaffected. We analyzed this relation in small arteries mounted in a wire-myograph setup, which were activated at either low or high distension with endothelin-1\textsuperscript{130}. Here we found that only vessels that are
activated at low distension indeed showed a shift in the active length-tension relationship towards a smaller diameter. Yet, from work by Gunst, Seow and others indicated above on non-vascular smooth muscle cells, it is highly likely that not only the cell length but also the organization of the contractile and cytoskeletal elements changes during such maintained vasoconstriction. Thus, during early remodeling, prior to matrix reorganization, there may be adaptation both of the SMC length and to the SMC length. Figure 2.4 provides a schematic drawing of such SMC plasticity. Following chronic vasoconstriction (Figures 2.4A and 2.4B), re-arrangement of the actomyosin elements would shift the active radius-tension curve leftwards, while reorganization of the cytoskeleton would prevent distension towards the original diameter (Figure 2.4C). Relengthening of the cells and increased overlap would induce similar macroscopic effects (Figure 2.4D). Further work is needed to unravel the nature, balance and reversibility of these processes in early remodeling. It would furthermore be of interest to test whether the myogenic reactivity is shifted after prolonged constriction and re-lengthening of SMC.

An important consequence of the model proposed by Martinez-Lemus is that the traditional view of load-bearing structures under active and passive conditions needs to be reconsidered. Thus, under prolonged deep constriction part of the load is transferred away from active actomyosin cross-bridge cycling, and smooth muscle cells are able to reposition within the vessel wall. After subsequent relaxation, part of the load appears to be carried by cytoskeletal elements, rather than carried by elastin and collagen alone. The question rises whether cytoskeletal elements also limit distension of vessels that are not subjected to prolonged deep constriction. Experiments using cytochalasins indicate that this is not the case. Cytochalasins are widely used to study the cytoskeletal contribution to cell stiffness and motility. By binding to the growing ends of microfilaments, they block both assembly and disassembly of actin monomers. In our hand, cytochalasin D does not increase the passive diameter of freshly isolated vessels (unpublished).

Figure 2.4: Schematic representation of possible SMC plasticity and the consequences on vascular caliber. (A) left: a relaxed, pressurized vessel, consisting of spindle-shaped SMC (yellow) surrounded by distended matrix elements (black). Right: a single SMC, with dense bodies (black), a cytoskeleton (purple) and contractile elements (black actin, green myosin). (B) the same vessel in a deeply contracted state. Actomyosin interaction carries the tension, while matrix and subcortical cytoskeleton become unloaded. Capacity for tension generation becomes less due to reduced amount of effective myosin (green dashed lines). (C) and (D): alternative hypotheses for SMC plasticity during prolonged activation. In C, the reduction of number of actomyosin elements in series would induce a leftward shift of the active radius-tension curve. Reorganization of the cytoskeleton would prevent dilation to the original diameter upon SMC relaxation. In D, possible relengthening and rearrangement of SMC during prolonged activation is shown. Overlap of SMC in the wall increases, the active radius-tension curve would shift leftward, and the cytoskeleton would prevent dilation to the original diameter.
data). Cytochalasin B lowered the pressure for forced dilation of cerebral vessels at normal tone, but did not seem to change the passive diameter at high pressure\textsuperscript{141}.

In desmin-/- mice, passive stresses as well as maximal active stresses were clearly reduced in second order mesenteric resistance vessels. Yet this might reflect altered development rather than a role for desmin in acute passive mechanics\textsuperscript{97}. If indeed in an established structure the extracellular fibers determine the maximal diameter, and considering the link between diameter for optimal active tension and passive diameter, it follows that mechanisms exist that adapt the extracellular matrix to the contractile and cytoskeletal properties of the SMC.

Such a mechanism could reside in the ‘mold’ that is provided by the active vessel diameter (Figure 2.5). If newly formed matrix components are embedded in a constricted blood vessel, it can be envisioned that this would result in a reduction of the passive diameter\textsuperscript{142}. In the reverse case, when new matrix components such as collagen fibers are loosely placed in a vasodilated artery, the maximal diameter may ultimately increase. In this ‘mold’ hypothesis inward remodeling may even occur in the absence of newly formed material, when the existing matrix components are cross-linked by, for example, Transglutaminases. Data from our group and others have shown that members of this family of enzymes can induce small artery remodeling both \textit{in vitro} and \textit{in vivo} in hypertension and flow-induced remodeling\textsuperscript{143-146}. The role of Transglutaminases in remodeling was recently reviewed\textsuperscript{31}. The relationship between tone and remodeling may be further strengthened by the overlap in signaling pathways, which is evident from various studies. Thus, angiotensin II and endothelin-1 are known to induce proliferation and fibrosis in addition to inflammation and vasoconstriction, as reviewed by Intengan and Schiffrin\textsuperscript{6}. The vasodilator signaling of nitric oxide also overlaps with remodeling events, but diverges at the level of cGMP-dependent protein kinase type I\textsuperscript{147}.

\textbf{Inflammation facilitates remodeling}

A specific type of remodeling is the outgrowth of small pre-existing collateral arteries in the face of an arterial obstruction. This process is referred to as arteriogenesis\textsuperscript{148} and may be considered an extreme case of flow-induced remodeling. It is particularly relevant in the case of large artery stenosis which results from atherosclerotic lesions as it provides a natural bypass to alleviate ischemia\textsuperscript{149}. This is an area of intense research, which mainly focused on the stimulation of leukocyte recruitment to the area of vascular remodeling\textsuperscript{150}. These leukocytes, particularly monocytes, but also natural killer cells (a specific lymphocyte with regulatory as well as cytotoxic functions) and CD4+ T cells\textsuperscript{151} facilitate arteriogenesis, probably through the release of metalloproteinases and cytokines. We found that both inward- and outward remodeling in small mesenteric arteries of mice induced by altered blood flow depends on
Figure 2.5: The ‘mold’ hypothesis linking vasoconstriction to inward remodeling. a: part of the relaxed vessel wall, showing a SMC (yellow), matrix fibers (black) and cross-links (blue). b: contraction unloads the existing matrix. C: Matrix turnover. A new matrix element (brown) is embedded that is relatively straight in the constricted vessel. In addition, new cross-links (orange) are formed between and within new and existing matrix. Some old elements have disappeared. D: upon SMC relaxation, the new elements and cross-links prevent dilation to the original diameter.
macrophages. A similar dependence on leukocytes is found in pregnancy induced vascular remodeling. In this case, natural killer cells play a crucial role in the dramatic outward remodeling of uterine arteries, which depends on interferon-γ release. In experimental hypertension, the inward remodeling of small arteries is dependent on proper macrophage function. Our current view is that the inward or outward direction of remodeling is linked to cytoskeletal reorganization and tone as indicated above, while inflammatory mechanisms would facilitate this process through degradation, cross-linking and rebuilding the extracellular matrix. Yet it is clear that this needs further research.

**Pressure-dependent remodeling**

While many stimuli and conditions influence regulation of small artery caliber, pressure plays a special role, since it determines the stresses and strains of the wall elements, it is a direct stimulus for SMC contraction, and since hypertension is one of the most relevant fields for remodeling research. The myogenic response, i.e. the increase in tone and reduction in diameter with pressure, was described by Bayliss in as early as 1902. Johnson pointed out that the myogenic response regulates total wall tension. This concept is still in use. VanBavel et al. and Buus et al. pointed out that total wall tension is influenced by vasoactive agents. Therefore, the myogenic response would not only regulate wall tension against changes in pressure, but also oppose vasoconstrictive influences under pressure-driven conditions (since the vasoconstriction causes a reduction of wall tension) while amplifying them under isometric conditions (since here the activation raises total wall tension). Signaling in the myogenic response has been well reviewed. The response invokes calcium signaling, but also heavily depends on the cytoskeleton and integrin signaling. Pressure-dependent remodeling may relate to such signaling. In addition the strength of the myogenic response may influence the nature of the remodeling. Thus, wall stress in hypertension can be normalized by inward eutrophic remodeling or hypertrophy. On the basis of the 'mold' hypothesis, the eutrophic inward remodeling in small arteries may be the consequence of the strong myogenic response known to exist in these vessels, while large vessels show both a limited myogenic response (i.e. increased diameter at increased pressure) and hypertrophic responses to hypertension. In support of this view, in hypertensive models with compromised myogenic responses, small vessels show hypertrophic remodeling.

**Quantitative approaches to vascular remodeling**

Functional and structural responses of small arteries to mechanical and metabolic stimuli are considered to serve homeostasis of tissue perfusion, wall stress and shear stress. Figure 2.6 indicates tone, plasticity and remodeling as a sequence of events in a possible control loop. Interaction schemes such as this can be refined
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and extended to networks and metabolic variables. Yet the presence of multiple regulated variables, acute and structural effects, and the complexity of network architecture\textsuperscript{166-168} obscures a straightforward insight into the integrated behavior of the resistance vasculature. Several authors built simulation models of vascular regulation in order to obtain a better understanding or to derive new concepts for vascular regulation. To the best of our knowledge, no network-based models have been published that combine tone, remodeling, wall stress, shear stress and metabolic influences in an integrative approach. It is indeed questionable whether such an approach would be insightful at this moment. A more sensible strategy is to take this step by step. Here we suggest a definition of tone that can be used for modeling purposes and we highlight a number of model studies and concepts on diameter regulation of single segments that seem relevant for the progress towards an integrative approach in this area.

How to quantify tone?

‘Vascular tone’ is often defined as the degree of vasoconstriction, expressed as a percentage reduction in diameter. While this definition is fully justified for descriptive and statistical purposes and for the interpretation in terms of vascular resistance, a more fundamental definition may be based on the state of contractile activation of the SMC. Thus, assuming a single SMC compartment parallel to the matrix, tone can also be defined as the actual active tension divided by the maximal active tension at the same diameter. Several experimental\textsuperscript{63;108;121} and model studies\textsuperscript{158;159;169;170} have employed this definition. Figures 2.2C and 2.2D depict radius-tension and pressure-radius curves at various levels of tone according to this definition. As can be seen in Figure 2.2D, radius at any constant tone level increases rapidly with pressure. Vessels therefore need to increase tone with pressure (myogenic response) even to maintain a constant diameter, let alone to obtain a negative myogenic pressure-diameter slope (dash-dotted line in Figure 2.2D).

Tone as a drive for remodeling

The concept that tone drives remodeling was quantitatively analyzed by Jacobsen and co-workers\textsuperscript{159}. These authors built a simulation model based on a wall stress-driven myogenic response in combination with tone-dependent inward or outward eutrophic remodeling. While the authors made specific choices for passive and active radius-stress curves and dynamics of adaptation, the model is in essence a conceptual one, and the conclusions seem valid for a wide array of parameter choices. An increase in pressure resulted in activation and vasoconstriction, as discussed above, but at a longer time scale also induced inward remodeling. This again helps to restore wall stress and causes gradual reduction of tone during the remodeling response. In steady state, at higher pressure the vessel became inwardly remodeled with unchanged basal tone. This model was further tested by simulating vasodilator and vasoconstrictor influences,
Figure 2.6: Vascular tone as a central element in a sequence of interactions regulating local tissue perfusion and peripheral resistance.
from e.g. surrounding tissue and nerve endings. Continuous presence of a vasodilator caused outward remodeling, but tone was restored to original levels. Thus, by having an adaptive response of tissue structure driven by tone, vessels in this model are able to maintain a normal level of activation under a wide variety of chronic conditions. Tone, according to this model, could therefore be regarded as a long-term regulated variable. The exact myogenic responsiveness and the existence or not of a negative slope in the active pressure-diameter relation is not critical in this model. Thus, variations in the myogenic responsiveness, known to exist in the circulation, lead to differences in chronic tone but, possibly counter-intuitively, not to differences in passive vessel caliber.

The model of Jacobsen provides quite a useful approach in understanding integrated regulation of caliber. At the same time, it generates additional questions. The first question is what is actually being regulated in vessels in a network? The Jacobsen model considers regulation of wall stress as the drive for remodeling via tone. Other influences were considered to be simple, uncontrolled offsets in tone, eventually causing structural effects. This all occurs under the condition of controlled pressure, and indeed the authors pointed out that their model simulates an isolated, cannulated vessel under pressure control. However, one might also subject such a vessel to flow control. On the basis of both acute and chronic effects of flow, it has been argued that flow-dependent tone and remodeling act to regulate shear stress. One could construct a tone-remodeling model where tone depends on shear stress. A step increase in flow here would lead to increased shear stress, vasodilation, outward remodeling, reduction in shear stress and recovery of tone to its original level. Other vasoactive factors would lead to changed caliber with unaltered tone in steady state. Sensitivity to shear would influence the chronic level of tone, but not the caliber of the vessel. Such a model would thus be completely analogous to the Jacobsen model, but with pressure exchanged for flow. In a network, alterations in vascular tone and remodeling affect both pressure and flow, while also metabolic influences such as the local oxygen concentration might be considered to form regulated quantities. A necessary further step therefore is to build models combining acute regulation of both wall stress and shear stress with tone-remodeling coupling. These models should initially be analyzed in a setting with constant entrance and exit resistances. Once properly understood, such models could be incorporated in simulations of vascular networks.

**Modeling matrix rearrangement and turnover**

Jacobsen et al. incorporated tone-driven remodeling as a change over time of the unloaded passive diameter, without specification of turnover or rearrangement of individual fibers that underlines such change. A next step could be to include turnover of populations of fibers with dispersion of resting lengths. As an example, Gleason and Humphrey formulated conceptual models of large artery growth...
and remodeling in hypertension based on turnover of vascular elements. The model was based on the assumption that the individual wall elements deform together, but can turnover at different rates, based on the stresses acting on these elements. New elements would have resting lengths matched to the actual diameter of the vessel. Tone was included, but only as a way to maintain the actual diameter against changes in pressure. The authors concluded that stress-dependent turnover ensures normalization of wall stress, through increased wall mass. Differences in turnover rate between elastin, collagen and SMC would cause a stiffening of the vessel during hypertension. Such predictions reflect the hypertrophic rather than eutrophic remodeling of large vessels in hypertension. It needs to be established to what extent known differences in tone control between large and small arteries would affect predictions for remodeling based on such a turnover approach. As indicated above, a further concern in such turnover approaches is that the predictions rely heavily on the arrangement of collagen as parallel or serial elements. Clearly, more experimental evidence is needed for the rates of turnover and arrangement of existing and new fibers.

Conclusions

It is clear that the questions provided in the introduction with respect to integrated regulation of small artery caliber and flow cannot yet be answered. The bottle necks include the need for a better understanding of small artery matrix architecture, maintenance and mechanics, more extensive experimental data on SMC mechanics in remodeling vessels, and a better insight into regulation of SMC cell length and cytoskeletal architecture. We believe that there is much to learn from current progress in non-vascular SMC biophysical research. Considering the size and transparency of small arteries, the rapid progress in molecular and live imaging techniques should provide many new experimental approaches, allowing the bridging of cellular and matrix biophysics and vascular biomechanics. More detailed molecular information will remain needed, but simultaneously we should make sure to ‘see the physiological wood through the molecular trees’, and this requires a combined experimental and modeling approach.
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