REVIEW ARTICLE

Quantitative Vascular Evaluation: From Laboratory Experiments to Point-of-Care Patient (Experimental Approach)

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Abstract: This paper illustrates the evolution of our knowledge of arterial mechanics from our initial research works up to the present time. Several techniques focusing on this topic in terms of our experience are discussed. An interdisciplinary team composed by different institutions from Argentina, Uruguay, France and Spain was created to conduct research, to train human resources and to fulfill the inevitable social role of gaining access to technological innovation to improve public health.

1. INTRODUCTION

The systemic circulation plays a significant role, in which blood vessels (arteries) contribute in the regulation of blood flow, which is periodically imposed by cardiac ejection. In order to understand this complex mechanism, a partnership between the French National Institute of Health and Medical Research (INSERM) and the National Scientific and Technical Research Council of Argentina (CONICET, Argentina) was initiated in 1986, to develop research activities in the field of arterial mechanics.

The approach adopted by our group encouraged the creation of an interdisciplinary team to conduct research, to train human resources and to fulfill the inevitable social role of gaining access to technological innovation to improve public health. It was principally constituted by: Universidad de la República (Uruguay), the Jean le Rond D'Alembert Institute (Sorbonne University, France), the Biomedical Polymers Division Research Institute of Materials Science and Technology (Universidad Nacional de Mar del Plata, Argentina), the Universidad Politécnica de Madrid (Spain) and the Georges-Pompidou European Hospital (Paris Descartes Faculty of Medicine, Paris, France). Working together with these institutions, we have been doing research for the development of techniques to measure arterial dynamic properties locally and accurately. The strength of this joint work lies in the extensive experience that the members of each of the four teams have in the fields of biomedical engineering, physiology, biomechanics, biomaterials, numerical methods and noninvasive measurement techniques.

The proposed methodology is supported by the conceptual framework of Cardiovascular Engineering, which integrates elements from biology, engineering, mathematics and physics to describe and understand the cardiovascular system. Its objective is to develop, verify and validate a predictive and quantitative detailed comprehension of the cardiovascular system and to apply such concepts to the solution of various diseases. This platform allows us to develop a higher level of specificity to address the study of the phenomena related to the arterial wall-blood interface, where the main causes of cardiometabolic diseases emerge.

In addition to enhancing the creation of technology through innovative developments in basic sciences, this interaction offers the possibility of using national technology in one of the world's most renowned centers for the diagnosis and treatment of cardiovascular diseases (Universidad Favaloro and its University Hospital). The multidisciplinary approach taken in this project makes it possible to complete virtually all the stages of a modern scientific research process: mathematical modeling, numerical simulation, "customized" equipment, physiopathology and technological innovation, laying the foundations to constitute a team oriented to molecular biology and tissue engineering.

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This paper illustrates the evolution of our knowledge of arterial mechanics from our initial research works up to the present time. Several techniques focused on this topic evaluation in terms of our experience are discussed.

2. GENERAL OVERVIEW

Up to now, several studies focused on the mechanical properties of the descending thoracic aorta have been conducted in conscious animals, using different vasoactive drugs (antiotensin II, nitroglycerin, adrenaline and noradrenaline), under various pharmacological blocking conditions (atropine, propranolol and atropine associated to propranolol) and considering normal physiological conditions [1, 2].

With respect to the research in pathophysiologic mechanisms of disease, there has always been the need of using *in vivo* models [3]. To study the basic biological processes and the mechanisms related to human disease, traditional small animal models (*e.g.*, rats, mice, guinea pigs, hamsters, and rabbits) have provided access to the scientific community to physiological *in vivo* models. In this context, closer the *in vivo* assessment parallels a clinical situation, the more clinically applicable are the results of the assessment. Particularly, the sheep model is quite relevant in cardiovascular research, as it is comparatively less complicated to control several variables. Also, the hemodynamic and metabolic parameters of sheep often relate closely with the human condition, especially with respect to thrombogenicity [3].

Our first relevant results appeared in 1991, when studies carried out in chronically instrumented conscious animals demonstrated that the relationship between stress and strain of the aortic wall is essentially nonlinear and such relation is given by the elastic characteristic of the elastin fibers (which withstand the resistance to strain at low pressure levels), the elastic characteristic of collagen fibers (which withstand the resistance to strain at very high pressure levels) and a nonlinear function, which represents the recruitment of collagen fibers as the artery distends [4].

The effects of vitamin D3-induced experimental calcinosis on the viscoelastic properties of the arterial wall were studied in conscious dogs, revealing that severe and accelerated calcinosis, which produces significant calcium deposits along the entire arterial tree, is paradoxically accompanied by a reduction in aortic wall stiffness, mainly due to structural and functional changes experienced by collagen fibers. This phenomenon has important physiopathological implications in relation to the development of aneurysms [5]. Experiments in animals with renovascular hypertension showed that an angiotensin converting enzyme inhibitor affects the elasticity of the arterial wall by distending it until normal levels are restored, in addition to the already known decrease in arterial pressure. Such inhibitor mainly acts on the elastic behavior of the elastin and vascular smooth muscle (*VSM*) fibers [6].

In [7], the elastic contribution of the *VSM* was also studied in conscious animals, using a modified three-element Maxwell model. Two years later, the total characterization of the mechanical properties observed in the arterial wall (as in any physical system, -elasticity, viscosity and inertia) was then performed in chronically instrumented animals [8]. A set of procedures was developed to measure the hysteresis presented by the arterial wall pressure-diameter relationship in terms of viscosity and inertia. Having identified all the wall mechanical properties, a model was developed to better understand arterial physiology and physiopathology.

The influence of endothelium on the biomechanical behavior of the arterial wall has been characterized throughout the study of the endothelial dependence of arterial elasticity, modulated by blood viscosity and heart rate [9-11]. These works showed that wall elasticity in arteries is influenced by the rheological conditions of blood, probably due to the presence or absence of endothelial relaxation factors. A direct action of shearing forces on the vascular smooth muscle through endothelial cells increases elasticity. On the other hand, an increase in arterial stiffness was also observed after adventitia removal, thus proving the influence of the adventitial function [12-14]. Reversal blood flow component was found to be a determinant factor in the arterial functional capability in [15]. Aortic flow, pressure and diameter were measured in sheep under baseline and high reversal and oscillatory flow conditions. Evidence of a smooth muscle contraction pattern was noted during high reversal flow rate conditions, with an increment in the buffer function and a decrement in the conduit function.

In order to study the viscoelastic properties of arteries and determine the dynamic behavior of the wall, simplified models were developed to simultaneously obtain the elastic, inertial and viscous moduli, and Young's complex modulus was evaluated in relation to frequency in conscious animals. Linear autoregressive models were applied to the single beat pressure-diameter data to assess the arterial system dynamics, under baseline conditions and after *VSM* activation. In phenylephrine-induced activation, the three moduli increased significantly, suggesting a reduction in the damping function [16, 17]. As a result, this methodology was posteriorly implemented in [18, 19] to evaluate the role of vascular damping *in vitro* measurements applied to common carotid arteries. It was concluded that *VSM* cells, as smart viscoelastic spring-dampers, exert a protective effect against high-frequency stretching, adjusting energy dissipation. The results suggested that during hypertension, this protective vascular action (modulated by the inhibition of the angiotensin-converting enzyme) reduces extra load to the heart and maintains enhanced arterial wall damping.

The mechanical behavior of the arterial wall was also modeled as a fractal network of viscous springs and dashpots. This fractal order, which may be understood as an infinite distribution of elements in a tree or a staircase shape, led to the design of the so-called fractional models [20]. In this sense, the fractal genesis of the entire arterial tree is unquestionable. For this reason, the time behavior of its associated hemodynamic variables was also evaluated, applying processing methodologies (time series fractal analysis) in line with such conception. The characterization of the morphological changes suffered by the arterial pressure (*AP*) waveform resulted in the coinage of the term '*unwrinkling*' [21].

3. ARTERIAL WALL DYNAMICS

3.1. Chronically Instrumented Animals

A chronically instrumented animal is an animal which has undergone a surgical procedure, under strict asepsis standards, during which measurement instruments were implanted (pressure transducers, intravascular or intra-cardiac catheters, dimension sensors, devices for altering or interrupting blood flow through a certain vessel, *etc.*) at the first stage, and which, after a postoperative recovery period, is studied using such instruments [7]. All the procedures followed to carry out this kind of work are performed pursuant to the ethics rules of our institution, which are in accordance with the guidelines of the American Physiological Society and the Helsinki Declaration related to the care and use of laboratory animals published by the US National Institute of Health [22].

Since instrumentation is performed in advance, the researcher has direct and immediate access to the variables to be studied without the need to anesthetize the animal, thus allowing the studies to be performed while the animal is conscious. A conscious animal is defined as an animal which completely maintains its relation with its environment, without any sedation and in a state of wakefulness, that is, whose central nervous system and autonomous nervous system are functioning normally during experimental sessions. This is very important when studying the cardiovascular system given the active participation of the nervous system in hemodynamic regulation. It is important to stress that this is an integrative physiology model, in which the physiological compensatory mechanisms are operating and intact [7]. Although this impairs "variable control", it facilitates the extrapolation of the results to the clinical situation.

3.1.1. Intravascular Pressure and Cardiovascular Dimensions Measurements

A way of measuring pressure in the cardiovascular system is through solid state microtransducers. This kind of pressure microtransducers have the advantage that the sensing part is in direct contact with blood and thus have a higher frequency response than a fluid column catheter system. The size of these microtransducers ranges between 2.5 and 7 mm and they have been designed so that they can remain inside the body for long periods of time. The main setbacks of intravascular microtransducers are their difficult calibration, their thermal instability and in some cases, their fragility. Since once they are implanted in the body they cannot be recalibrated, it is convenient to place a fluid column catheter next to the intravascular microtransducer to check calibration. There is another kind of solid state intravascular microtransducer which can be used by catheterism but which cannot remain chronically implanted in an animal. It consists of a catheter with a pressure sensor at one end placed laterally in relation to the longitudinal axis. A variety of this type of solid state catheters has two pressure sensors separated by a 10 cm distance. However, it can be calibrated *in vitro* immediately before being used (Fig. **1**).

Sonomicrometry is a technique that measures the distance between pairs of small transducers implanted in the muscle or in similar tissues. Franklin and Rushmer were the first to describe this technique in 1956 and since then, the technique has been improved and refined [7]. The distance to be dynamically measured is determined by measuring the ultrasound transit time between the pair of ultrasonic microcrystals. To validate the measurement, the sound velocity in the medium must be known and should be constant, irrespective of time, temperature, and fiber orientation. The velocity of sound in the cardiac muscle and in blood is known to be 1580 m/s approximately [7].

The enormous advantage of this technique is that it can be used to study cardiovascular dimensions in chronically instrumented closed-chest animals. The microcrystals are manufactured in our laboratory with round-shaped lead zirconate titanate ceramics, with an emission frequency of 3 or 5 MHz and a 2 to 7 mm diameter. The piezoelectric ceramic has a welded wire on each of its sides, which are coated with a semi-spherical polyurethane resin, like a lens. The morphology of the microcrystal pairs depends on the distance and the tissue for which they have been created (Fig. **1**).

The device that activates the emitter crystal and receives the signal captured by the receptor is called sonomicrometer. Such device (Triton Technology, San Diego, California) has different channels, allowing several signals to be measured simultaneously. Each channel has an independent calibration module capable of calibrating each signal in linear millimeters within a 2 to 123 mm.

3.1.2. Acquisition and Storage: Physiological Signal Integrated Module

An ad-hoc system is in charge of adjusting the signals coming from invasive high frequency response pressure sensors (Konigsberg Inc., 1200Hz), electrocardiography signals, Doppler velocity signals (center line velocity) and arterial diameter signals provided by piezoelectric crystals (sonomicrometry). The module allows offset and gain adjustments to be made, for each kind source. The acquired information can be pre-processed, prior to being transferred to the storage module. Relevant electrical standards are followed and the noise level is within the limits established for medical tests and experimentation. Processing, visualization and elaboration of results are directly performed through a specially designed software, which embraces the required features for each particular case. An embedded database system allows data to be sent in standard formats for exchange of remote data.

3.2. *In Vitro* Experiments: Circulating Loop

Vessel wall mechanics can be evaluated *in vitro*, by means of a closed circulating system that allows conduits to be submitted to physiological values of pressure, stretching rates and blood hematocrit values. The specimen is first immersed and perfused with oxygenated Tyrode's solution. The perfusion line consists of polyethylene tubing and a reservoir chamber, powered by an electronically regulated hydraulic pump allowing fine adjustments of pumping rate and pressure values [23]. This experimental system deals with waveforms, controlling mean and diastolic values, with the objective of maintaining controlled mean blood flow. As a result,
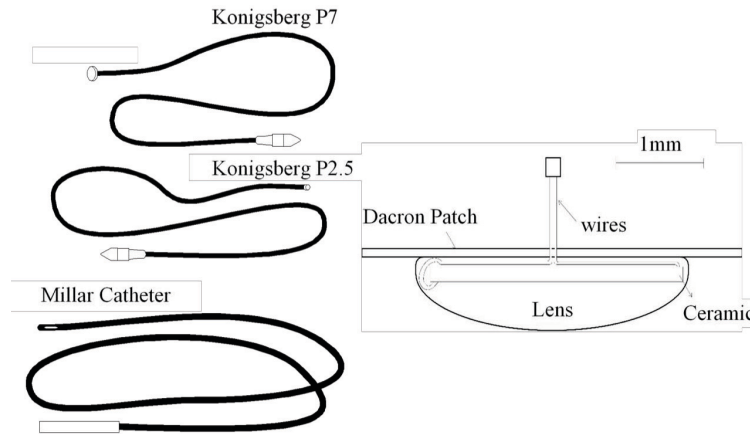
Fig. (1). Left panel: Types of solid state microtransducers (Konigsberg Inc. & Millar Inc.) Right panel: Microcrystal used to measure vascular diameters. In all cases, the piezoelectric ceramic is welded to a bipolar cable and coated with a polyurethane resin lens [7].

the conditions observed *in vivo* can be reproduced, for preserving mechanical "homeostasis". Internal arterial pressure is measured by pressure transducers while vascular distension during the experiments is assessed by means of the sonomicrometry technique [23, 24].

3.3. Bioreactors

A bioreactor is a device used for animal cell culture. This platform can establish temperature, shear stress, PH and pressure conditions, whose correct setting is essential to ensure the viability of the culture cells. The design is supported by computerized numerical processing for the dynamic simulation of fluids inside the bioreactor flow chamber and the simulation of the thermal conduction phenomenon in its constitutive material. The latter process ensures a constant temperature in the culture area. In order to analyze the results, fluorescence and phase contrast microscopy is generally applied. This technique can be used to study cellular orientation, differentiation and response to different kinds of stimuli, thus providing a characterization of the evolution of the tissue in time [25, 26].

3.4. Aortic Surgical Instrumentation

For 30 days prior to a surgery, large animals (in general Merino sheeps and mongrel dogs) have to be clinically controlled. During this period, they receive treatment against endo and ectoparasites, as well as immunizations against diseases typical of the species. Finally, through blood and urine tests, the optimal health condition of the animals is verified, before subjecting them to surgical intervention.

The instrumentation surgery is performed under general anesthesia, induced with (20 mg/kg intravenous) sodium thiopental. After performing the endotracheal intubation, the anesthesia is maintained with enflurane (1.5% in oxygen at 4 L/min) through a Bain tube connected to a Brid Mark VIII respirator (Palm Springs, California). A thoracotomy at the level of the fifth intercostal space provides visualization of the heart, surrounded by the pericardium; the aortic arch and its branches; and the descending thoracic aorta, after moving the left lung towards the diaphragm.

The measurement of the external diameter of the aorta is taken through microcrystals of 5 MHz, whose lens is relatively flat. On top of the lens, the microcrystal has a dacron patch attached, through which it is sewn to the vascular wall with non-perforating stitches. The implantation is performed in the upper third of the descending thoracic aorta, which is minimally released from its surrounding tissues in order to prevent the proliferation of scar tissue. Once the first microcrystal has been attached to the aortic wall surface, it is mapped with the second crystal until an optimal quality of the signal provided by the sonomicrometer is obtained. In this case, the maximum distance is sought in order to ensure that the diameter is being measured and not a string of the vascular circumference. Aortic pressure is measured using a pressure microtransducer (Konigsberg Inc., Pasadena, USA), calibrated against a fluid-filled polyvinyl chloride catheter. The transducer is implanted in the descending thoracic aorta through a stab wound in the left brachial artery [4, 7].

3.5. Constitutive Equation of the Arterial Wall

The development of a model representing the behavior of the arterial wall requires the correct identification of the parameters governing its function. In the analysis of the vascular mechanical response, *AP* is the phenomenon responsible for the excitation of the model, while the variation in the arterial diameter (*AD*) is the associated response. The classic approach proposes the use of basic components with a dominant characteristic, so that their combined response allows the observed behavior to be estimated [27]. The analysis of stress-strain loop path reveals the presence of purely elastic (ideal springs), purely inertial (ideal masses) and purely viscous (ideal dampers) components. Their combination will represent the stress exerted by the wall in order to oppose the stretching or mechanical demand applied on it [8].

Ideal springs are characterized by their elastic constant E, as expressed by Hooke's Law. This law implies a linear relation between the applied stress (σ) and the experimented longitudinal strain (ε). In the case of ideal dampers, the passive damping constant (η_P) is represented by the relationship between σ and the time change rate of ε. The elastic compo-
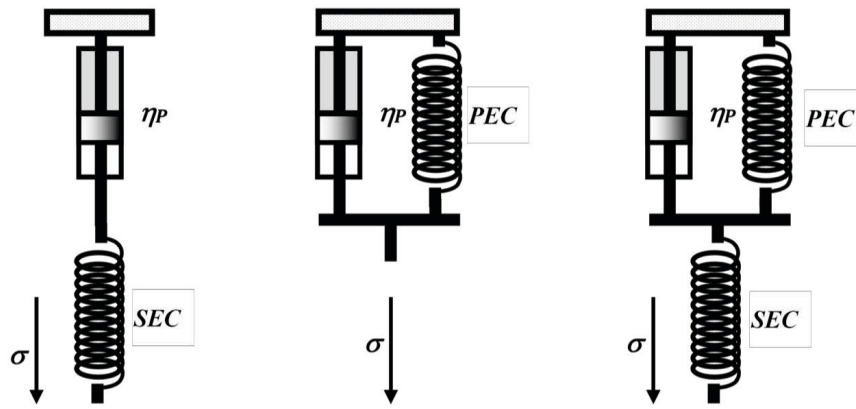
Fig. (2). Modeling of the arterial wall based on elastic (E) and viscous passive (η_P) components according to the stress applied (σ). SEC is Serial Elastic (E) Component. PEC is Parallel Elastic (E) Component (a) Maxwell Model (b) Voight Model (c) St. Venant Model.

nents react to the strain magnitude, while the viscous components react to the velocity at which such strain occurs. In view of this, the inertial components (***M***) react at the velocity change rate. These components are usually neglected due to their poor contribution. The resulting expressions for the abovementioned components are summarized below [27]:

A Maxwell element is defined as the combination of an elastic and a viscous component arranged in series. In contrast, the Kelvin-Voigt element uses the same components but arranged in parallel. A third combination is known as St. Venant element, where an elastic component is added in series to the Kelvin-Voigt element. This last combination is the one that most closely reflects, in principle, the viscoelastic behavior shown by arterial conduits (Fig. **2**). The mission of the elastic component is to absorb the impacts produced by the characteristic pulsatility of the *AP* wave. It accumulates kinetic energy during systole, which will be returned during diastole, when there is no stimulus. The objective of the viscous damper is to control the intensity in the return of such energy, protecting the elastic element from abrupt reactions which may lead the wall to enter a permanent oscillation regime, when impacting against the fluid. In short, the function of the damper is to dissipate part of the energy delivered by the elastic element as heat [18, 27]. It should be noted that the model adopted to represent the behavior of the arterial wall should consider its ability to actively regulate its damping effect. Vascular tissue behaves as a smart material, with the ability to adjust the values of the elastic and viscous components, in order to optimally adapt to the diverse pulsatility or oscillation conditions, whether in the short, medium or long term [18].

$$\sigma_E = E_H \varepsilon \qquad \sigma_\eta = \eta_P \frac{d\varepsilon}{dt} \qquad \sigma_M = M \frac{d^2}{dt^2}\varepsilon$$

The behavior of the passive elastic response (represented by the elastin-collagen set) can be represented as the combination of two elastic moduli, one of them for the elastin fibers (E_E) and the other for the collagen fibers (E_C). The latter is affected by a nonlinear function (f_C) which represents the amount of collagen fibers withstanding the stress [4], and it can be expressed as follows:

$$E_{PEC} = E_E + E_C f_C$$

At low *AP* levels, the stress-strain curve shows a linear behavior, exclusively dominated by the action of the elastin fibers. This means that the collagen fibers only act once an excitation threshold related to stringent strains has been passed. Physically, elastin fibers are modeled considering an elastic behavior. In turn, collagen fibers are modeled in a similar way, but differ in that they are recruited according to the stress level reached. This recruitment can be associated to a series of elastic components which take place (activate) in relation to the level of strain experienced by the arterial wall. As a result, a gradual opposition (Fig. **3**) to the stretching imposed by AP can be observed in the interior of the conduit lumen, with a nonlinear response [7].
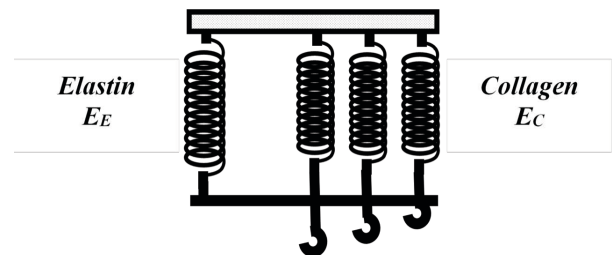
Fig. (3). Modeling of the arterial wall and incorporation of the recruitment function of the collagen fibers. The latter activate (hook) according to the level of stress exerted on the wall, showing a nonlinear response.

The recruitment function of the collagen fibers can be expressed through a normal morphology function, whose coefficients can be obtained by means of nonlinear adjustments. At low stress levels, f_C tends to zero, which is consistent with a purely elastic response, exclusively associated to elastin fibers [4]. In relation to the *VSM*, which is not actually considered a passive elastic but an active elastic, the model proposed by Kelvin is more appropriate to represent it [8]. It is composed of an elastic component placed in series with a contractile component (generating force) with viscous properties. Then, a viscous component only present during activation (η_A) is placed parallel to it. Similarly to the collagen fibers, the response developed by the *VSM* is governed by an elasticity modulus (E_{SM}) affected by a nonlinear activation function (f_{SM}) [8].
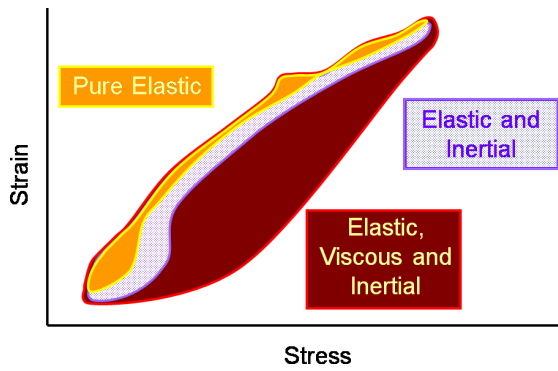
Fig. (4). Individual contribution of the elastic, viscous and inertial properties of the mechanical behavior of the arterial wall, evaluated in the stress-strain relationship.

As a result of this, the behavior of the wall can be expressed as a combination of elastic, viscous and inertial responses defined in the preceding paragraphs (Fig. **4**). Parameter E combines the elastic behavior of the action of the collagen, elastin and smooth muscle fibers, in the activation state. The physical effects related to strain velocity are considered in the viscous parameter that models the smooth muscle during rest (η_P). The effects related to the acceleration of the deformation are represented by the inertial behavior typical of the wall (M). In short, the governing equation of the arterial stress-strain relationship can be expressed as follows:

$$\sigma = E\varepsilon + (\eta_A + \eta_P)\frac{d\varepsilon}{dt} + M\frac{d^2\varepsilon}{dt^2}$$

$$E\varepsilon = E_E(\varepsilon - \varepsilon_0) + E_C f_C \varepsilon + E_{SM} f_{SM} \varepsilon$$

where each term shows the behavior of the elastin, collagen and smooth muscle cell fibers (E_E, E_C and E_{SM} respectively), the inertial and viscous effects (M and η_P) represented in the Kelvin-Voigt model, and ε_0 represents the arterial deformation state prior to excitation.

By definition, the purely elastic stress-strain relationship develops following the same geometric place, both for an increase or decrease in deformation. As a result, an algorithm to find the purely elastic relationship was developed, removing the viscous and inertial stresses until finding an optimal value, using the hysteresis loop disappearance criterion developed in the previous equation. At the first stage, M was considered null and the η values were increased from zero until reaching a value at which the stress-strain relationship area finds found a minimum without altering the clockwise circulation. Once that viscosity value is established, M is increased until the hysteresis loop has completely disappeared.

3.6. Smart Smooth Muscle Spring-Dampers

High frequency vibrations tend to produce mechanical structure injures. In [18], it was suggested that vascular **VSM** cells, as smart viscoelastic spring-dampers, exert a protective effect against high-frequency stretching, adjusting energy dissipation. Human common carotid arteries were evaluated in an *in vitro* system, as the one described in previous sec-

tions. Adaptive modeling techniques were applied to the obtained pressure and diameter measurements, in order to calculate mechanical impedance, and creep and stress relaxation time constants. It was demonstrated that **VSM** acted as smart spring-dampers, dissipating high-frequency components that might have damaging effects. During hypertension, energy dissipation is highly increased, protecting arterial wall but producing an additional load on the heart. Besides, the **VSM** modulates its degree of activation and vessel wall remodeling, thus preventing high frequency vibrations from damaging the wall constituents.

3.7. Viscoelastic Mapping of the Arterial Tree and Numerical Modeling

In [28-30], a comparison of the biomechanical behavior of veins and arteries from different regions and sizes under arterial conditions was carried out. *In vitro* pressure and diameter were measured in four different veins and three different ovine arteries using an *in vitro* system. A diameter-pressure transfer function was designed, and compliance, viscous, and inertial indexes, as well as viscous energy and buffering function were calculated. Regional differences in vein mechanical behavior and energy dissipation were found. Veins and arteries varied in their mechanical properties and buffering, but the differences were less significant when considering the smallest artery.

For the evaluation of their biomechanical properties, eleven arteries from different regions were selected in [31, 32]: carotid, brachiocephalic trunk, ascending aorta, proximal, medial and distal descending aorta, and femoral artery. Pressure waveforms were imposed by the *in vitro* circulatory system, which were assessed internally using solid-state micro transducers inserted in each artery through a tiny incision. Sonomicrometry technique was applied to evaluate the external diameter variations. The use of a simple viscoelastic model such as the Kelvin model was studied in this work for the prediction of simultaneous measurements of cross-sectional area and arterial blood pressure, illustrating how the elastic and viscoelastic properties are modified across proximal and distal vessels. Nonlinear optimization was used to develop a mathematical model, minimizing the differences between measured and computed values of the cross-sectional area. It was illustrated that the viscoelastic model captured essential features of the data, significantly better than a traditional elastic wall model. This study also showed that peripheral vessels are more rigid than proximal vessels, whereas the viscoelastic relaxation times did not reveal significant differences across the seven sites. Similarly, the Quasilinear Viscoelasticity theory was then applied in [33], in measurements from the thoracic descending aorta and the carotid artery obtained from human and ovine arteries.

A 1D fluid dynamics arterial network model with 14 vessels was developed to assimilate *ex vivo* 0D temporal data for pressure-area dynamics in individual vessel segments from 11 male Merino sheep [34]. The model was implemented to assess the inflow profile and total peripheral resistance and compliance values for the downstream network, contemplating the presence of viscoelastic behavior evidenced in the arterial wall. Lastly, the evaluation of the ex-

tent to which vessel wall parameters estimated from *ex vivo* data can be used to realistically simulate pressure and area in a vessel network was performed. According the performed simulations, elastic wall parameters were found to yield pressure-area relationships across all vessel locations, in ranges comparable to those in the *ex vivo* data [34]. Recently, viscoelasticity was described by a nonlinear Kelvin-Voigt model in which the coefficients were fitted using experimental time series of pressure and radius measured on a sheep's arterial network [35]. The viscoelastic relaxation time (defined by the ratio between the viscoelastic coefficient and Young's modulus) was found to be nearly constant throughout the network. In addition, the damping effect of viscoelasticity on the high-frequency waves was clear especially at the peripheral sites. Using a similar approach, pulse wave reflection of different endovascular repair techniques in abdominal aortic aneurysm was evaluated using 1D patient-specific models [36].

3.8. Cryopreservation Studies

An arterial graft should ideally exhibit the identical functional properties of the host artery. In this respect, expanded polytetrafluoroethylene prosthesis (*ePTFE*) or saphenous vein (*SV*) grafts are usually used to surgically reconstruct the common carotid artery in several clinical situations. As a consequence, the use of fresh or cryopreserved (defrosted) arteries poses an interesting alternative. In [37], the functional properties of fresh and cryopreserved carotid and femoral arteries, and also the same for the venous and synthetic grafts were evaluated. Fresh and cryopreserved carotid and femoral arteries showed similar behaviors both at the viscoelastic and functional levels on the contrary with *ePTFE* and *SV* grafts.

While the situation of tissue donation and transplantation differs between Latin American and European countries, a common problem is tissue deficiency. Consequently, the feasibility to establish an intercontinental network for tissue exchange was evaluated by studying the distensibility of ovine arteries divided into three groups: intact (*in vivo* tests, conscious animals), fresh control (*in vitro* tests immediately after the artery excision, Uruguay), and cryografts (*in vitro* tests of cryopreserved-transported-defrosted arteries, Spain). As showed by the comparison between fresh control and cryografts, neither the cryopreservation nor the exchange network impaired the distensibility, despite the expected histological changes found in the cryografts. As a result, cryografts would be capable of ensuring a reduced biomechanical mismatch [38].

4. ENDOTHELIUM DYNAMICS

The dynamics of the arterial system involves the analysis of its principal components: the arterial walls, blood flow and their interrelation, which may be related to the complex processes resulting in the arterial diseases [9, 11].

The aim of this section is to address the difference in wall elasticity, in terms of blood shearing over the endothelial layer. To this end, the biomechanical behavior of intact and de-endothelized bovine arterial segments (brachiocephalic trunk) was characterized, using different values of blood viscosity and keeping flow levels constant. Essentially,

changes in the mean arterial diameter caused by an increase in blood viscosity were more pronounced in intact arteries than in arteries without an endothelium. For the same levels of blood viscosity, the variations in the elastic modulus in relation to its baseline value, before and after the endothelium removal, were significantly different. These results show that the elasticity of the arterial wall determined by means of its incremental modulus was strongly affected by blood viscosity (and therefore shear stress), probably due to the presence or absence of endothelium relaxing factors. Arterial smooth muscle activation caused by direct shear stress when the endothelium cells were removed can be understood as another factor. This physiological effect might protect the integrity of the ventricular-arterial coupling and might suggest that in the presence of endothelial dysfunction, the increase in blood viscosity might involve changes in muscle tone and arterial stiffness, thus impairing the left ventricular function as an adaptive pressure pump [9, 11].

5. APPLIED NONLINEAR DYNAMICS

5.1. Application of Fractional Models

Viscoelastic models are useful to better understand arterial wall mechanics in physiological and pathological conditions. As stated before, the simple models composed by elastic and viscous elements usually represent the behavior of viscoelastic materials, which may be linear springs and dashpots. Fractional viscoelastic models can be taken one step further, since they include at least one fractional element, also known as spring-pot. A correspondence between a *'fractal network'* of springs and dashpots can be established in order to construct similar fractional order dynamic models for the biomechanical properties of tissues.

The spring-pot element represents an intermediate behavior between a linear spring and a linear dashpot, proven to be efficient in describing rheological tissues. Two fractional alternative models were proposed in [20], with one and two spring-pots, tested in an anesthetized sheep in a control state and during smooth muscle activation. Only the model with two spring-pots correctly followed the activation state with the best performance. Stress relaxation of human arteries was then described with this methodology in [39] and its numerical approach was introduced in [40].

5.2. Complexity of the Arterial Tree: Arterial Pressure Waveform Analysis

Fractional order components can be also related to fractal-like structures that might be associated to complex collagenous arrangements, present in arterial tissues [41]. This fractal concept, strongly associated to the genesis of the arterial network, gave rise to a 'holistic approach of fractal dimension variations throughout the arterial network', based on *AP* waveform complexity analysis, both in health and disease condition.

The conception of the arterial system as a single close-ended conduit, with constant or variable properties along its length, has yielded acceptable results in relation to low frequency perturbations [42]. However, the anomalies are evident in the case of high frequencies. It is justified by the fact that although the arterial system is not a single conduit, it is

made up of a set of tubular branches. Also the attenuation effect produced by arterial wall viscosity must be considered. One of the most related issues is the existence of an inverse relationship between frequency and wavelength since wavelengths corresponding to high frequencies would produce significant phase differences between the waves coming from different reflection sites. For this reason, the overall behavior of the arterial system is affected by the stiffness gradient together with the distributed nature of the terminal branches [42].

Explicit references to the creation of vascular networks from fractal rules can be found in the literature [43], which are in line with the observations made by Taylor in [42]. A fractal is a structure composed by sub-units in multiple levels or scales (self-similarity), which resemble the structure of the whole object, and has a fractional dimension, thus breaking the integer dimension values defined for Euclidean objects (1, 2 or 3). The fraction obtained (Fractal Dimension, *FD*) describes the existence of a highly-detailed structure whose representation is absolutely ignored by traditional geometry. Several patterns found in nature (and particularly in biology) have a fractal behavior. They can be observed in arterial and venous branches, cardiopulmonary structures, bile ducts and also physiological time series such as heart rate and blood pressure.

The effect of arterial tree structure on arterial pressure fractal behavior was assessed on laboratory animals in [21, 44].

To quantify waveform morphology complexity (or roughness) of aortic *AP*, firstly, *FD* was applied. Following that, the stiffness of aortic arterial wall was assessed using the first derivative of the pressure-strain relationship while the effect of wave reflection was estimated from augmentation index (A_{IX}) measurements. Then, a pneumatic silicon-rubber-made cuff occluder was implanted around the descending thoracic aorta, proximally to the *AP* and *AD* transducers (piezoresistive and ultrasonic, respectively) to eliminate the peripheral wave reflections.

Pressure-diameter loops were used to study aortic stiffness in both basal and occlusion states (activation of the cuff and removal of total reflection). A biphasic model was implemented, in which the low-pressure slope was related to the elastin elastic response whereas the high-pressure slope illustrated the recruitment of collagen fibers. A significant decrease in aortic pressure waveform complexity (pointed out by a *FD* diminution) was noted as a result of the occlusion maneuver concomitant to aortic stiffening and an increment of A_{IX}. This condition was also dealt in [45], while evaluating the effects of arterial cross-clamping (a common strategy used in vascular surgery) on arterial stiffness, in humans. The *AIx* (normalized to 75 beats-per-minute, *AIx@75*) and *FD* values were determined from radial arterial pressure tracings during surgery. In both aortic and iliofemoral interventions, after arterial clamping, median *AIx@75* rose and *FD* dropped significantly; the opposite occurred after arterial unclamping.

5.3. Modeling Arterial Pressure by Solitary Waves

Pulse pressure propagation was represented as a combination of solitons (a solitary wave packet that maintains its shape while it propagates at a constant velocity) due to the fact that many of the phenomena observed during that kind of propagation is observed along the systemic circulation [46]. A compartmental model was designed in order to evaluate the pressure pulse amplification variation linked to arterial aging. Soliton waves were used to synthesize arterial blood pressure waveform propagations, followed by validation with the same obtained from individuals from different age groups. Morphological changes were identified in the synthesized blood pressure waveforms as a consequence of aging (*i.e.* due to the increment in arterial stiffness) [46].

5.4. Tissue Engineering

Tissue Engineering emerges as a futurist alternative which is starting to gain relevance, moving from the laboratory to the patient, and which has created Regenerative Medicine. Tissue engineering is defined as the application of principles and methods of the engineering and life sciences to the understanding of the structure-function relationships under normal or pathological conditions [47]. This multidisciplinary technology is currently being used to develop biological substitutes to repair or regenerate functional tissues and organs. The three pillars of Tissue Engineering are cellular biology, the engineering sciences and clinical medicine. Such disciplines face the following challenges: identifying tissues and organs which can be replaced; collecting both embryonic and adult stem cells; understanding cell signaling; conducting *in vitro* and *in vivo* vascularization; building matrices and developing bioreactors. The preliminary studies of our group towards this direction were based on the evaluation of the mechanical properties of electrospun nanofibrous vascular grafts (monolayered and bilayered), to be used in tissue engineering and as vascular replacements [47-49].

CONCLUSION

When modeling blood circulation, the heart is usually considered the main element, the only one which has an actual relevance in the operation of the system, thus neglecting blood vessels, which are considered simple conduits that connect the cardiac pump with the organs. Such a basic approach underestimates the prominent role shown by blood vessels in general and by arteries in particular (especially the aorta) in the regulation of the blood flow periodically imposed by cardiac ejection. The study of the arterial system is therefore very important, be it to understand its intrinsic operation or to evaluate the conditions for optimal coupling with the heart. Fig. (**5**) schematizes a 'holistic view' of vascular dynamics that defines the characteristics of this 'arterial hydraulic load': the 'continent' is visualized on left side of the scheme, represented by the arterial wall (deeply studied by our group). In arteriosclerosis, arteries become stiffer due to the rupture of elastin fibers, as a consequence of aging. On the other hand, atherosclerosis emerges as an focalized arterial disease and its related to the formation of atheromatous plaques [50]. Despite being a heterogeneous, non-Newtonian fluid and thus difficult to study, blood (the arterial 'content') provides extremely useful information [51]. Blood viscosity depends on fluid velocity conditions, which in turn depend on the location of the erythrocyte in the vessel axis [9] (right side of the scheme). As a result, the
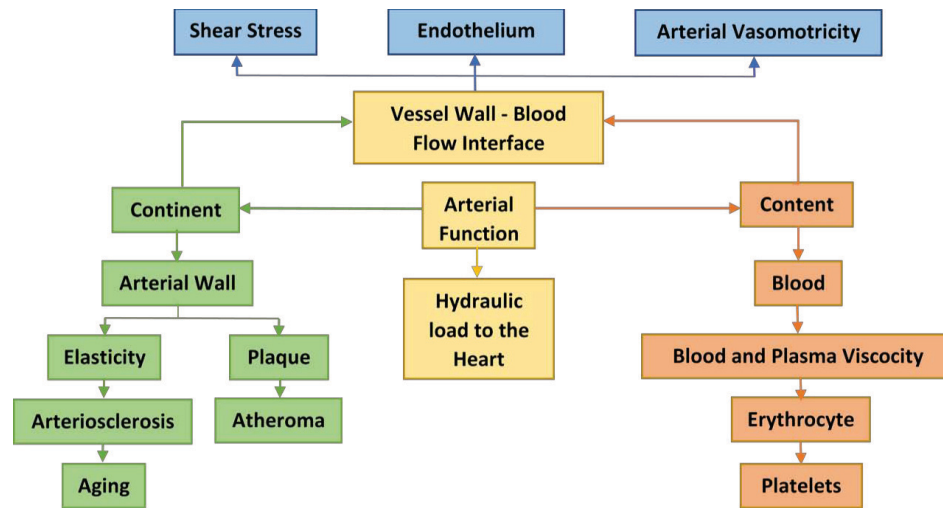
Fig. (5). Holistic view of vascular dynamics: Continent (arterial wall), content (blood) and their interaction.

'arterial function' can be defined in virtue of the existing relationship between continent and content, named 'fluid-structure interaction'. Endothelium layer plays a key role in this scenario, continuously sensing the flow conditions (by evaluating the wall shear stress) and acting in consequence, in order to stablish the 'arterial vasomotricity'. This interaction was also evaluated by our interdisciplinary team, as expressed in previous paragraphs (upper part of the scheme).

Although arterial resistance has the most significant effect on ventricular ejection, it is well known that a rise in arterial stiffness results in a less efficient coupling between the heart and the arteries, which is concomitant to an inefficient use of the energy delivered by the left ventricle. In particular, a drop in arterial capacitance might cause an increase in systolic pressure [52] and thus impose a supplementary load on the heart [53].

In the second part of this review, the experimental knowledge addressed by our group about arterial dynamics give rise to a clinical approach, whose methods can be used in clinics in the early diagnosis of vascular disease, acute events prevention and cardiovascular risk stratification.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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