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Association between Mechanics and Structure in Arteries and Veins: Theoretical Approach to Vascular Graft Confection

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Abstract—**Biomechanical and functional properties of tissue engineered vascular grafts must be similar to those observed in native vessels. This supposes a complete mechanical and structural characterization of the blood vessels. To this end, static and dynamic mechanical tests performed in the sheep thoracic and abdominal aorta and the cava vein were contrasted with histological quantification of their main constituents: elastin, collagen and muscle cells. Our results demonstrate that in order to obtain adequate engineered vascular grafts, the absolute amount of collagen fibers, the collagen/elastin ratio, the amount of muscle cells and the muscle cells/elastic fibers ratio are necessary to be determined in order to ensure adequate elastic modulus capable of resisting high stretches, an adequate elastic modulus at low and normal stretch values, the correct viscous energy dissipation, and a good dissipation factor and buffering function, respectively.**

I. Introduction

TISSUE engineered vascular grafts must exhibit biomechanical and functional properties similar to those observed in native vessels. For this purpose, mechanical properties of arteries and veins, and the association with their structural composition should be completely characterized.

Mechanical properties of blood vessels are largely determined by the relative proportions of its constitutive main components: elastin, collagen, and vascular smooth muscle. Elastin determines the elastic properties of the vessel wall at low stretch values. At great stretches exists a greater recruitment of collagen fibers, which in over all gives an elastic non linear behavior [1]. In addition, smooth muscle cells are the principal source of viscosity and energy dissipation [2][3]. In the particular case of central veins (cavas and/or pulmonar) that arrive to the right and left atriums, an additional component is the cardiac muscle. The proportion of these components varies among the different vessels and through the whole circulatory system, having in consequence different specific mechanical responses. In general, elastin fibers have low values of elastic modulus, whereas collagen fibers are stiffer. In consequence, the relative proportion of these two elastic components for a given vessel will determine its global elastic response. The proportion of muscle cells will determine the viscosity of the wall and its capacity to dissipate energy.

For a complete characterization of the mechanical behavior of soft tissues, the static and dynamic responses must be evaluated. The static mechanical behavior is traditionally studied with a tensile test. The dynamic mechanical behavior can be studied by means of a dynamic test in which a complex modulus of the material in the frequency domain is obtained. This complex modulus (E^*) has real and imaginary parts. The former is related to the elastic response of the material (i.e. storage modulus E') and the latter (i.e. loss or dissipation modulus E'') to its viscous behavior.

In this study, we explore three different blood vessels looking forward to establish the theoretical basis to be considered in the vascular graft engineering. To this objective, we investigated the relationship between the mechanical responses of the vessels whit their corresponding compositions.

II. Materials and Methods

A. Segments acquisition and preparation

Experiments were conducted on blood vessels obtained from three healthy male Merino sheep weighing 25- 35 kg. All protocols were approved by the Research and Development Council of the participant institutions, and were conducted in agreement with the Guide for the Care and use of Laboratory Animals (U. S. N. R. Council, Guide for the Care and use of Laboratory Animals. Washington, DC: National Academy Press, 1996).

For each sheep, two arteries and one vein were selected to evaluate their biomechanical properties: a) the proximal descending thoracic aorta, b) the distal abdominal aorta, and c) the anterior cava vein. A 5-6 cm length segment of each vessel was excised. All procedures were identical to those used in previous works [4]. From each segment a small ring was obtained to be analyzed using histological techniques. After that, the segments, immersed in criopreservant solutions were sent for biomechanical testing [5].

B. Histology studies

Vascular specimens were fixed by immersion in 10% formaldehyde and embedded in paraffin to obtain 7-µm-

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thick transverse sections perpendicular to the longitudinal axis of the vessel. Specimens were deparaffinated and hydrated, and finally stained with the Cajal-Gallego method, which differentially stains muscle cells (yellow-green), elastin (dark red) and collagen (blue). Histological images were digitized on a square frame (630 ×1024 pixels) with an optical microscope at magnification 400×. To quantify the relative amount of each component, a procedure previously proposed by Kawasaki et al. [6] was used. In brief, after eliminating the pixels that do not correspond to vascular tissues, the elastin, collagen and smooth muscle relative contents were determined as the ratio of the number of pixels that were stained dark red (elastin), blue (collagen) and yellow-green (smooth muscle), respectively, to the total number of pixels for each image.

C. Uniaxial tensile tests

Standard bone-shaped tensile specimens were obtained from each vessel in the circumferential direction. Dimensions of the central section were 10mm x 2mm x h, being h the thickness of the wall measured for each segment with a thickness gage (Mitutoyo). Specimens were fixed to the grips of an electromechanical tensile testing machine (Instron 5866) and immersed in an organ bath of PBS solution at 37ºC. The axial force was measured with the tensile testing machine by means of a 10N load cell (Instron 2519-101, precision > 5mN) and the elongation by the internal LVDT sensor (precision > 10μm). All specimens were mechanically preconditioned to remove the initial stress relaxation effect and to yield a stable response. Loading rate was 0.03 mm/s. A detailed description of the process is found in [7] .

D. Dynamic tests

Dynamic tests were performed with Nanobionix (MTS, USA). For each vessel, a bone-shaped segment adjacent to the one used in the tensile test was used (the segment was also cut in the circumferential direction). The mounting and preconditioning of the segment is similar to that described for uniaxial tensile tests. Again, the segments were immersed in a PBS solution bath at 37°C. In each test, the sample was elongated at a 0.03 mm/s rate until a preselected load was achieved, and then returned to the original position at the same rate. Simultaneously, the equipment imposes a sinusoidal load of 4.5 mN at a selected frequency, and measures the resulting sinusoidal displacement. From the relation of amplitude and the phase difference between both sinusoidal signals, the equipment calculates E' and E'' at the given frequency. By repeating this test at different selected frequencies, the complex modulus E^* for a certain frequency range is obtained, defined as:

$$E^* = \frac{\sigma(\omega)}{\varepsilon(\omega)} = E' + iE'' \quad (1)$$

where σ is the stress, ε the strain, and ω the angular frequency.

This test was performed for every vessel segment at frequencies of 1, 2, 3, 4, 5, 10, 20, 40 and 60 Hz.

E. Data processing

For the tensile tests, the load-displacement curves were converted to stress(σ)-stretch(λ) as:

$$\sigma = \frac{F}{A_0} \quad (2); \quad \lambda = \frac{L}{L_0} = \varepsilon + 1 \quad (3)$$

where L is the length of the segment and L_0 its initial value, A_0 is the cross sectional area, and F is the load developed in the specimen.

From the σ-λ curves, the initial elastic modulus (E_{low}) corresponding to low stretch values, and the final elastic modulus (E_{high}) corresponding to the high stretch values were determined as the slope of the linear fit of the corresponding dots. In the case of E_{low}, the fit was performed from the beginning of the test until the beginning of the transition region. For E_{high}, the fit was performed from the end of the transition region up to the beginning of the rupture of the segment [7] (Fig. 1). The transition region was determined by the change in the first derivative of stress respect to stretch. More details of the procedure can be found in [7].

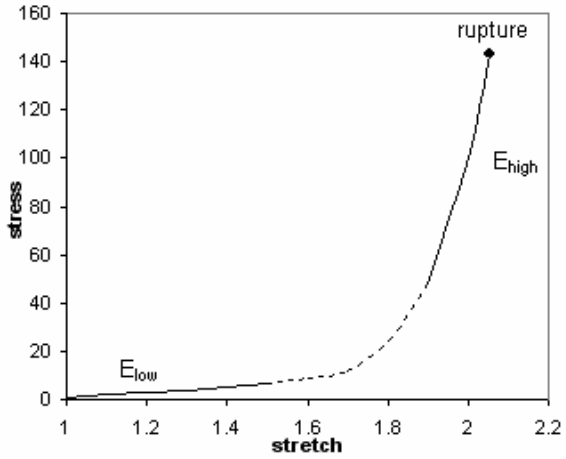

Fig. 1. Example of a tensile test. Doted curve corresponds to the transition region.

For the dynamic tests, since E^* depends of stress (and consequently of strain) we computed for all segments E' and E'' for every frequency at a selected stress of 70 KPa, which corresponds to a particular working point. Therefore, the plots of E' and E'' as a function of frequency were obtained. For quantification and comparison of the moduli, their average values were calculated for the frequency range of 5-60 Hz [8].

The results are reported as mean ± standard deviation.

III. Results

In Fig. 2, the σ-λ curves for each vessel are shown (solid line corresponds to the thoracic aorta, dashes line to the abdominal aorta and doted line to the cava vein segments). Differences in their static mechanical behavior are evident.

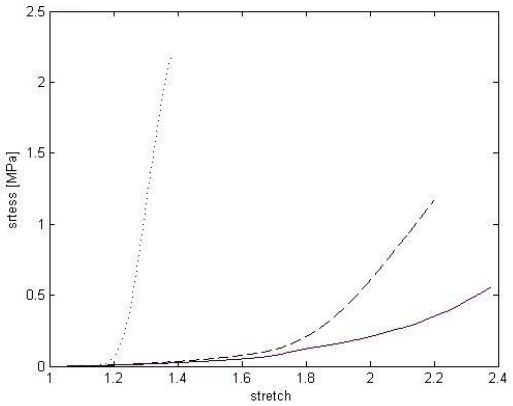

Fig. 2. Stress-stretch curve of the tensile test for one of the segments of each vessel. Solid line: thoracic aorta, dashed line: abdominal aorta, doted line: cava vein.

A close relationship (R^2=0.874) between mean value of E_{low} and the ratio between mean relative content of collagen (%C) and mean relative content of elastin (%E) was found (Fig. 3).

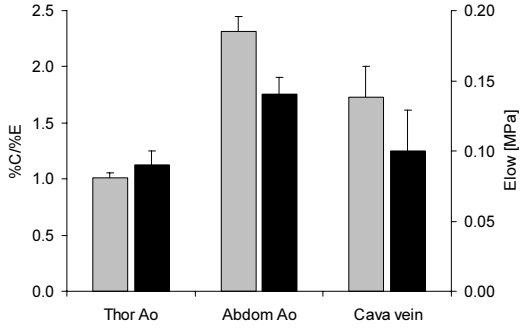

Fig. 3. Relationship between E_{low} (black) and the ratio relative collagen content-relative elastin content %C/%M (grey) for each vessel.

A way to estimate a nondimensional absolute content of each component is by multiplying the relative content by the vessel diameter and the vessel thickness. Fig.4 shows the strong relationship (R^2=0.998) between the estimated absolute content of collagen *CA* and E_{high}.

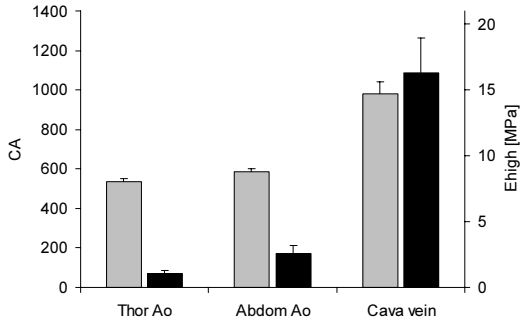

Fig. 4. Estimation of absolute collagen content *CA* (grey) and E_{high} (black) for each vessel.

In Fig. 5 and 6, *E'* and *E''* as a function of frequency for each vessel are shown respectively.

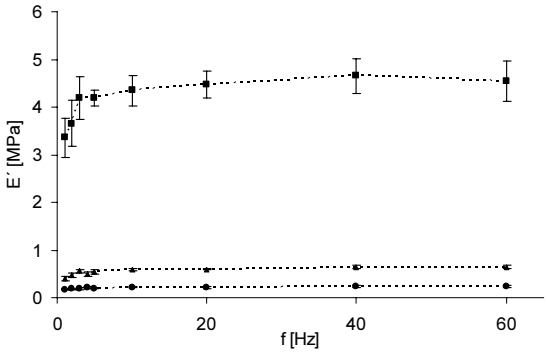

Fig. 5. *E´* as a function of frequency for each vessel. Circles correspond to thoracic aorta, triangles to abdominal aorta and squares to cava vein.

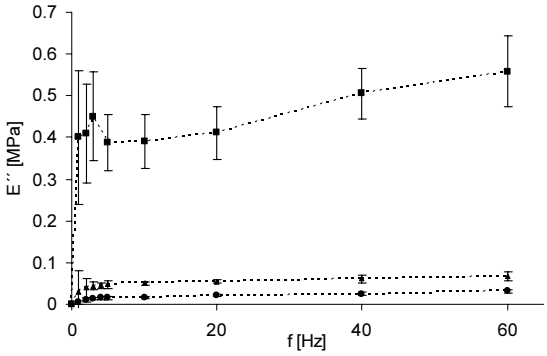

Fig. 6. *E´´* as a function of frequency for each vessel. Circles correspond to thoracic aorta, triangles to abdominal aorta and squares to cava vein.

The loss modulus *E''* characterizes the viscous dissipation of the material. Since there is evidence that muscle cells are the principal responsible of the viscous behavior of the vessels[2][3], we compared the average value of *E''* in the 5-60 Hz frequency range for each vessel with the corresponding mean relative content of muscle cells (%M). Results are shown in Fig. 7 (R^2=0.997).

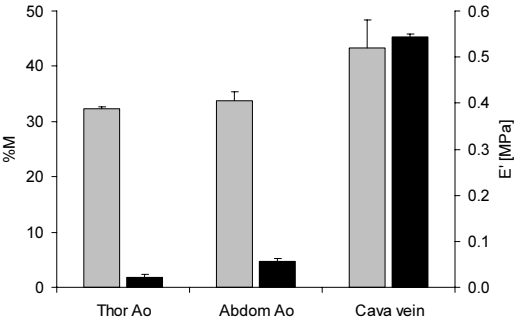

Fig. 7. Relationship between mean *E´´* in the 5-60 Hz frequency range (black) and mean relative content of smooth muscle % M (grey)for each vessel.

An important measure of the arterial mechanics is the ratio between storage modulus and loss modulus (*E'/E''*), which is related with the dissipation factor. A close relationship (R^2=0.892) between this variable and the ratio between %M and %F (%F=%C+%E is the relative content of total elastic fibers) was found (Fig. 8).

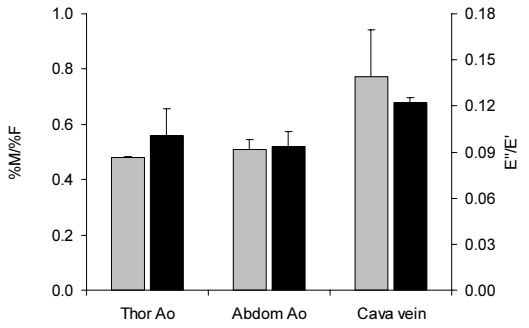

Fig. 8. Relationship between mean *E´/E´´* (black) and the ratio muscular cells - elastic fibers %M/%F (grey) for each vessel.

IV. Discussions

The static elastic moduli obtained are consistent with the bibliography [1]. Differences in these moduli between the three vessels were significant. The expected biphasic nonlinear behavior was obtained in all cases.

The close relationship found between E_{low} and the ratio of %C and %E can be explained by the fact that for the low stretch region, both collagen and elastin fibers are actuating. Therefore, the proportions of these two fibers will determine the elastic modulus of the segment in this particular region (more collagen fibers or less elastin fibers will determine a stiffer vessel, and vice versa).

The estimated absolute content of collagen for each vessel segment showed a good relationship with E_{high}, confirming previous results and hypothesis that at high stretch values, the main component actuating is the collagen [1].

In the cava vein the great amount of muscle was consistent with their high value of *E''*. An explanation could be that central veins such as the cava are highly muscularized in order to more efficiently dissipate the pulsatile waves exerted backwards by the auricles in their contraction.

For the three vessel, the close relationship found between muscle content and *E''* confirms the hypothesis that muscle cells are the main viscous dissipating element [2][3].

Finally, the close relationship between the ratio *E''/E'* and the ratio %M/%F confirms that collagen and elastin fibers determine the elastic response of the vessel, whereas muscle cells, the energy dissipation. Also, *E''/E'* (which is often called the dissipation factor) showed similar values for the arteries, whereas was larger for the vein, according to previous studies [8][10].

V. Conclusion

In this study, a complete mechanical and structural characterization of arteries and veins is shown. Direct relationships between both aspects were found.

In order to be able to construct engineered vascular grafts that reproduce native vessels functions and properties, our study indicates that these aspects must be considered:

a) The absolute amount of collagen fibers to ensure an adequate elastic modulus capable of resisting high stretches.

b) The collagen/elastin ratio to ensure an adequate elastic modulus at low and normal stretch values (reducing mechanical mismatch between the graft and native vessels at normal conditions).

c) The amount of muscle cells to ensure correct viscous energy dissipation.

d) The muscle cells/elastic fibers ratio to ensure a good dissipation factor and buffering function.

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