Similarities of Arterial Collagen Pressure-Diameter Relationship in Ovine Femoral Arteries and PLLA Vascular Grafts

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Abstract— **<u>Introduction</u>: *In-vivo* implanted vascular grafts fail due to the mechanical mismatch between the native vessel and the implant. The biomechanical characterization of native vessels provides valuable information towards the development of synthetic grafts. <u>Materials and Methods</u>: Five samples of electrospun nanofibrous poly(L-lactic acid)(PLLA) tubular structures were subjected to physiological pulsating pressure using an experimental setup. Four ovine femoral arteries were also tested in the experimental setup under the same conditions. Instantaneous diameter and pressure signals were obtained using gold standard techniques, in order to estimate the dynamic pressure-strain elastic modulus ($E_{P\varepsilon}$) of both native vessels and grafts. <u>Results</u>: Synthetic grafts showed a significant increase of $E_{P\varepsilon}$ (10.57±0.97 to 17.63±2.61 10^6 dyn/cm^2) when pressure was increased from a range of 50-90 mmHg (elastin-response range) to a range of 100-130 mmHg (collagen-response range). Furthermore, femoral arteries also exhibited a significant increase of $E_{P\varepsilon}$ (1.66±0.30 to 15.76±4.78 10^6 dyn/cm^2) with the same pressure variation, showing that both native vessels and synthetic grafts have a similar behavior in the collagen-acting range. <u>Conclusion</u>: The mechanical behavior of PLLA vascular grafts was characterized *In vitro*. However, the procedure can be easily extrapolated to *In vivo* experiences in conscious and chronically instrumented animals.**

***Key words*— vascular grafts, PLLA, mechanical properties, pressure-diameter loop, femoral arteries**

I. Introduction

The simultaneous measurement of pressure and diameter variations in blood vessels has a significant role in cardiovascular research. The basis of the stress-strain relationship is the measurement of the response of the arterial diameter to the internal pressure waveforms. This relationship is an essential factor in scaffold designing, due to the activity of the cardiac muscle and its influence on the behavior of tissues or cells [1,2,3,4]. The placement of a vascular graft in the pathway of a vessel disturbs the local hemodynamics as well as the stress distribution in the arterial wall, especially at the anastomotic sites. Furthermore, it constrains the natural deformation of the host vessel during the cardiac cycle [5]. The synthetic grafts currently used as a replacement for peripheral arteries are made of polyethylene terephthalate (polyester) and polyester (*ePTFE*). However, in most cases, synthetic grafts fail *In vivo* as a consequence of the mechanical mismatch that exists between the graft and the native vessel [4].

The biomechanical characterization of native vessels is essential for synthetic graft development, which includes the selection of suitable grafts to surgically replace vascular segments. In order to do so, the values of mechanical strain, vessel distensibility and suture retention strength must be comparable to physiological conditions [6]. Hence, the simultaneous measurement of internal pressure and diameter (i.e., the obtaining of the pressure-diameter loop) can be used to quantify the elastic response of the native vessel, and then compare it to the synthetic vascular graft [1]. This analysis can be performed *In vitro*, using the experimental setup in which near-physiological hemodynamic conditions can be reproduced [7,8]. However, the technique could be extended to *In vivo* experiments, including conscious and chronically instrumented animals.

In this work, five samples of biocompatible and bioresorbable polyester poly(L-lactic acid) (*PLLA*) tubular structures were subjected to pulsating pressure conditions using an experimental setup. The dynamic response of the grafts was determined by obtaining the pressure-diameter relationship (a gold standard technique) using high-resolution ultrasound. Similarly, four native femoral arteries that were obtained from sheep were analyzed, in order to compare their mechanical behavior to the one observed in the synthetic grafts.

II. Materials and Methods

A. Materials

Five vascular grafts were constructed using the electrospinning technique. The device consists of a rotating collector with a 5 mm diameter mandrel, a syringe pump (Activa® A22 ADOX, Ituzaingó, Argentina), a high voltage power supply (Gamma High Voltage Research Inc., Ormond Beach, Florida, USA) and a blunt metal needle (18 gauge, Aldrich®) to be used as an injection tip.

B. Experimental Setup

An experimental setup, including a circulating loop for measuring instantaneous pressure and diameter in blood vessels and scaffolds, was used. It basically consists of a

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specially designed programmable pump that ejects a fluid into a hydraulic closed circuit, and a fluid pool with an adjustable sample fixing system [9]. The circuit is made of silicon tubes where the sample is to be attached and a fluid reservoir (see Fig.1). The pumping frequency can be adjusted in order to mimic the normal pulse rate for an adult (60 to 80 beats per minute). Both grafts and native vessel samples were fixed to the loop and submerged in a bath of tyrode's solution, with a pH value of 7.4 and 100% oxygen saturation. The temperature was controlled using a 37 ± 2°C set point. Internal pressure was measured using a high frequency response solid-state pressure sensor (Konigsberg P2.5S, 1200 Hz, Pasadena, USA) that was placed proximally to the diameter measurement site. Then, the external diameter was measured using the gold standard sonomicrometry technique (System 6 mainframe with sonomicrometer module, Triton Technology Inc., San Diego, CA, USA). See Fig. 2. The optimal positioning of the dimensional gauges was assessed by means of an oscilloscope. Finally, diameter and pressure signals were acquired at 2000 samples per second, using a 12/bit data acquisition module (NI USB-6009) during a 15-second interval, using a specially developed MatLab application (Mathworks Inc., San Diego, USA), also used to process the signals. Each experiment was repeated four times in order to ensure statistically reliable results.

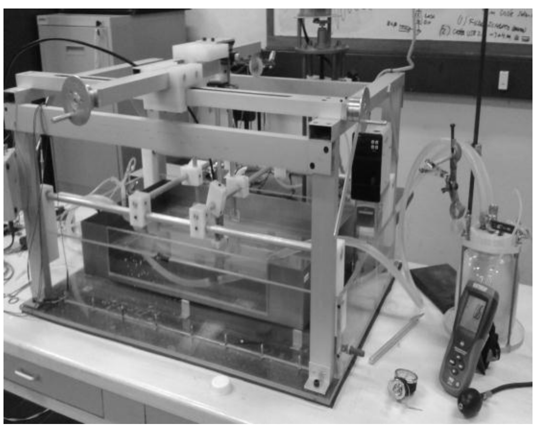

Fig. 1. Experimental setup used for elastic response evaluation of poly(L-lactic acid) vascular grafts and arteries.

C. *Femoral Arteries Surgical Procedure*

Five healthy male Merino sheep, weighing 25 to 35 kg, were included in this study. All protocols were conducted following *The Guide for the Care and Use of Laboratory Animals* guidelines [12]. All animals were vaccinated and treated for skin and intestinal parasites. During 30 days before surgery, they were properly fed and assessed for optimal clinical status. General anesthesia was induced with intravenous administration of pentobarbital (35 mg/kg). Respiratory -maintained with a positive respirator (Dragger SIMV Polyred 201, Madrid, Spain)-, tidal volume, and the inspired oxygen fraction were adjusted to maintain arterial pCO_2 at 35-45 mmHg, pH at 7.35 - 7.4, and pO_2 above 80 mmHg. Femoral arteries were selected in order to evaluate their biomechanical properties. Vascular conduits were exposed and dissected, and a 6 cm length segment was measured *in situ* and marked with two suture references in the adventitia. Once they were placed in the experimental setup, the segments were allowed to equilibrate for a period of 10 minutes under a steady state of flow and pressure at a stretching rate of 80 beats per minute.

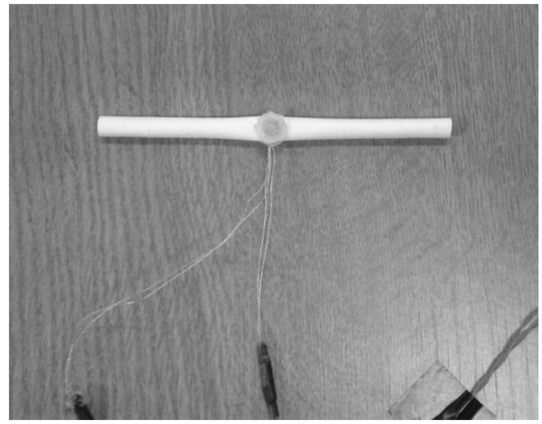

Fig. 2. Placement of ultrasonic sensors in the poly(L-lactic acid) vascular graft for recording of distensibility variations.

D. *Elastic behavior assessment*

The elastic behavior of arterial conduits is usually studied using linear elastic theory. Arterial wall properties depend on the mechanical role developed by passive components (elastin and collagen fibers) and active components (smooth muscle cells). These elements determine the elastic, viscous and inertial properties of the vessel, although the inertial contribution is generally considered negligible. Particularly, the nonlinearity of the stress-strain relationship and the anisotropy of the wall represent major constraints for this approach [1]. Native arteries exhibit a marked increase in distensibility in low-pressure regions, followed by a gradual reduction in pressure-dependent distensibility in the physiological pressure regions, and little distension is observed in high-pressure regions [11]. Accordingly, differentiated evaluations may be performed in order to identify the contribution of each component to the overall elastic response. Values of Young's modulus are extremely different for elastin and collagen fibers, contributing individually to the whole arterial elasticity (see Fig. 3). In addition, stiffness changes are observed under smooth muscle cell activation and the recruitment of collagen fibers supporting the wall stress [2]. For this reason, mechanical properties have to be quantitatively analyzed using instantaneous pressure-diameter recordings. To the best of our knowledge, the evaluation of the pressure-diameter (*P-D*) or stress-strain loops constitutes the most appropriate technique to assess the dynamic behavior of the *In vivo*, *Ex vivo* as well as vascular grafts. In this sense, the incremental elastic modulus ($\boldsymbol{E_{P\varepsilon}}$) was estimated at mean pressure according to [9]:

$$E_{P\varepsilon} = \left.\frac{dP}{d\varepsilon}\right|_{mean\ pressure} \qquad \varepsilon = \frac{D - D_0}{D_0} \tag{1}$$

where $\boldsymbol{P}$ is the transmural pressure and $\boldsymbol{\varepsilon}$ is the corresponding strain, obtained by referencing the diameter dynamic changes ($\boldsymbol{D}$) to its unstressed value ($\boldsymbol{D_0}$). The *P-D* loops obtained in both vascular grafts and in native vessels were evaluated at two pressure ranges, where the $\boldsymbol{E_{P\varepsilon}}$ modulus was assessed. Firstly, a 50-90 mmHg range was studied, predominantly dominated by the elastin fiber response ($\boldsymbol{E_{P\varepsilon\,50\text{-}90}}$). Secondly, a blood pressure condition of 110-130 mmHg was considered, where the collagen fiber intervention introduces a significant stiffness increase ($\boldsymbol{E_{P\varepsilon\,100\text{-}130}}$).

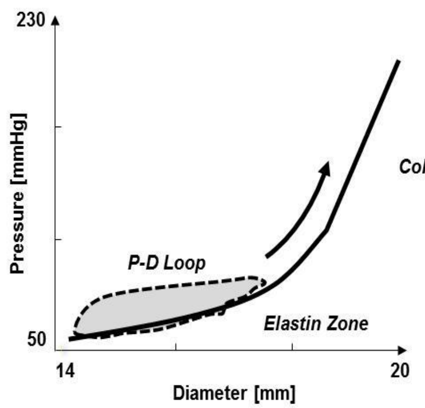

Fig. 3. Native arteries pressure-diameter loop behavior. Values of Young's modulus are extremely different for elastin (low pressure) and collagen (high pressure) fibers.

E. Statistical analysis

Results were obtained from repeated measurements. Data were expressed as mean value ± standard deviation. In order to ensure the normal distribution of the data, a Shapiro-Wilk test (small sample size) was performed. Statistical analysis was carried out using the unpaired Student's t-test. A value of $p<0.05$ was adopted as statistically significant.

III. Results

Instantaneous internal pressure and external diameter were measured for each sample (grafts or vessels), at each pressure range. Pressure waveforms were imposed by means of the programmable circulating system. Conduit stiffness was obtained by means of the pressure-strain relationship, within the mentioned pressure variations (see Fig. 4).

Assessed $\boldsymbol{E_{P\varepsilon}}$ values for grafts and femoral arteries are described in Table 1. The samples in the collagen range, limited by 100-130 mmHg, are observed to be significantly stiffer (i.e., a higher pressure-strain slope) than in the elastin range (50-90 mmHg). Compared to PLLA vascular grafts, $\boldsymbol{E_{P\varepsilon\,100\text{-}130}}$ showed similar values while $\boldsymbol{E_{P\varepsilon\,50\text{-}90}}$ differed by approximately one order of magnitude.

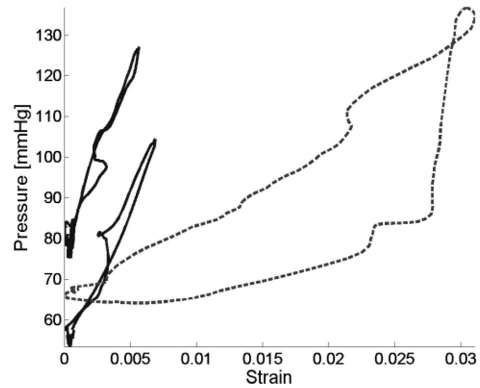

Fig. 4. Pressure-strain relationship evaluation. **Dashed Line**: Femoral artery loop behavior. **Solid Line**: Vascular graft loop behavior (50-90mmHg and 100-130mmHg variation). Elastic modulus ($\boldsymbol{EP_\varepsilon}$) was assessed for each pressure range ($\boldsymbol{EP_{\varepsilon\,50\text{-}90}}$ and $\boldsymbol{EP_{\varepsilon\,100\text{-}130}}$) in both vessels types.

IV. Discussion

In this work, a complete assessment of the mechanical properties of synthetic vascular substitutes was carried out, in order to compare the graft-vessel dynamic behavior, under different physiological conditions.

TABLE I
ELASTIC MODULUS ASSESSMENT [10^6 DYN/CM^2] FOR BOTH POLY(L-LACTIC ACID) VASCULAR GRAFTS AND NATIVE ARTERIES. EVALUATION WAS PERFORMED FOR 50 TO 90 MMHG AND 100 TO 130 MMHG PRESSURE RANGES.

Vascular Segment	$E_{P\varepsilon\,50\text{-}90}$	$E_{P\varepsilon\,100\text{-}130}$
Femoral Arteries	1.66±0.30	15.76±4.78*
Electrospun PLLA Grafts	10.57±0.97	17.63±2.61*

*P<0.05 was considered as significant different. Values are expressed as mean ± standard deviation

It is a well-known fact that arterial wall mechanical properties produce nonlinear pressure-diameter relationships, particularly when the vessel structure is examined over a wide pressure range. A decomposition of the entire elastic modulus may be performed, where the contribution of elastin, collagen and smooth muscle can be individually quantified [1]. The function of elastin and collagen fibers is to maintain a steady tension, in order to hold the wall against the transmural pressure inside the vessel, while the activation of smooth muscle alters both the viscosity as well as the elasticity of the vessel wall [2]. An artery can expand and contract elastically to a great extent mainly due to elastin fibers, within low pressure variations, whereas collagenous fibers remain unstretched [11]. Furthermore, the amount of each elastic component differs greatly between different types of normal vessels and, under physiological conditions, the artery can be considered essentially viscoelastic. The presence of a hysteresis loop when observing the pressure-diameter relationship is the evidence of the viscoelastic behavior of arteries [1]. For this reason, the characterization of mechanical properties using pressure-diameter loops provides an insight into the structural alterations of the vessel walls, allowing the assessment of the elastic response under different pressure conditions. Additionally, this methodology ensures accuracy and reproducibility in the long term, due to the high frequency and linear response of the transducers [2]. Moreover, this technique can be used both *In vitro* and *ex vivo,* and mainly during *In vivo* experiences, in conscious animals [1]. The self-evident advantage of this technique versus other methods is the ability of studying vascular grafts both *Ex vivo* and *In vivo*, the latter by using chronic instrumentation. The great potential of this technique becomes certain when analyzing multilayered conduits.

Considering the above, electrospun nanofibrous PLLA grafts where evaluated dynamically by applying the described vascular analysis technique. All samples were placed in the experimental setup and observed under near-physiological pulsating pressure conditions, where specific pressure ranges were selected. Additionally, ovine femoral arteries were studied using the same methodology. The elastic modulus was calculated using the collected experimental data, where incompressibility and the elastic linear theory were assumed, following a protocol developed for *In vitro* arterial studies [1]. The elastic modulus was calculated as the slope of the pressure-strain curve, which, in theory, describes the inherent stiffness of a vessel, independently of its geometry [2].

It is noteworthy that the PLLA grafts provided mechanical support and showed sufficient strength for arterial vascular applications. However, it should be noted that the matching of mechanical, structural and biological properties with those of the native vessels is a critical requirement for any small diameter vascular graft. Mechanical mismatch has been associated with perturbation in the local hemodynamics, establishing a basis for restenosis and graft failure [13]. Results obtained from the experimented samples revealed a significant variation of $\boldsymbol{E_{P\varepsilon}}$ under the different pressure ranges. Interestingly, it can be observed that as the internal pressure rises, the vascular graft shows a significant stiffness increase. When comparing femoral arteries to vascular electrospun nanofibrous PLLA grafts, both mechanical characterizations can be associated when in the collagen range (100-130 mmHg). This behavior can be observed in the variations of the pressure-strain loops. However, considering the low number of samples evaluated in the present study, further studies are necessary to improve the preliminary findings. In addition, although grafts showed curves with small dissipative effects, the presence of hysteresis due to these effects was not taken into account for the present study.

In summary, arterial mechanics evaluation, which has been extensively used in our previous works, was used to characterize PLLA vascular grafts *In vitro*, although its application can be directly extended to *In vivo* experiences, in conscious and chronically instrumented animals.

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