Materials Science and Engineering C 45 (2014) 446–454

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Elasticity assessment of electrospun nanofibrous vascular grafts: A comparison with femoral ovine arteries

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ARTICLE INFO

Article history:
Received 17 March 2014
Received in revised form 29 July 2014
Accepted 11 September 2014
Available online 16 September 2014

Keywords:
Vascular grafts
Electrospinning
PLLA
Mechanical properties
Pressure–diameter loop

ABSTRACT

Development of successful small-diameter vascular grafts constitutes a real challenge to biomaterial engineering. In most cases these grafts fail in-vivo due to the presence of a mechanical mismatch between the native vessel and the vascular graft. Biomechanical characterization of real native vessels provides significant information for synthetic graft development. Electrospun nanofibrous vascular grafts emerge as a potential tailor made solution to this problem. PLLA-electrospun nanofibrous tubular structures were prepared and selected as model bioresorbable grafts. An experimental setup, using gold standard and high resolution ultrasound techniques, was adapted to characterize in vitro the poly(L-lactic acid) (PLLA) electrospun structures. The grafts were subjected to near physiologic pulsated pressure conditions, following the pressure–diameter loop approach and the criteria stated in the international standard for cardiovascular implants-tubular vascular prostheses. Additionally, ovine femoral arteries were subjected to a similar evaluation. Measurements of pressure and diameter variations allowed the estimation of dynamical compliance (%C, 10^{-2} mm Hg) and the pressure–strain elastic modulus ($E_{P\varepsilon}$, 10^6 dyn cm^{-2}) of the abovementioned vessels (grafts and arteries). Nanofibrous PLLA showed a decrease in %C (1.38 ± 0.21, 0.93 ± 0.13 and 0.76 ± 0.15) concomitant to an increase in $E_{P\varepsilon}$ (10.57 ± 0.97, 14.31 ± 1.47 and 17.63 ± 2.61) corresponding to pressure ranges of 50 to 90 mm Hg, 80 to 120 mm Hg and 100 to 150 mm Hg, respectively. Furthermore, femoral arteries exhibited a decrease in %C (8.52 ± 1.15 and 0.79 ± 0.20) and an increase in $E_{P\varepsilon}$ (1.66 ± 0.30 and 15.76 ± 4.78) corresponding to pressure ranges of 50–90 mm Hg (elastin zone) and 100–130 mm Hg (collagen zone). Arterial mechanics framework, extensively applied in our previous works, was successfully 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. The specific design and construction of the electrospun nanofibrous PLLA vascular grafts assessed in this work, showed similar mechanical properties as the ones observed in femoral arteries, at the collagen pressure range.

1. Introduction

Development of successful small-diameter vascular grafts constitutes a real challenge to biomaterial science and engineering. Lack of adequate dimension availability (autografts), the presence of immunogenic response (allografts and xenografts) and thrombosis, stenosis and occlusion phenomena (synthetic grafts) represent serious limitations. Mechanical properties are a critical issue in vascular scaffold designing, due to the cyclic activity of the cardiac muscle and its influence on the behavior of tissues or cells [1]. Surgical placement of a vascular graft disturbs the local hemodynamics as well as stress distribution in the arterial wall, mainly at the anastomotic sites. Even more, the presence of the graft imposes mechanical constraints affecting the host artery deformation at the anastomosis, during the cardiac cycle [2]. Nowadays, poly(ethyleneterephthalate) (PET) and expanded (poly(tetrafluoroethylene)) (ePTFE) are the only synthetic materials approved by U.S. Food and Drug Administration (FDA) as a replacement for peripheral arteries. However, in most cases, synthetic grafts fail in vivo due to the presence of a mechanical mismatch between the

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http://dx.doi.org/10.1016/j.msec.2014.09.016
0928-4931/

conduit and the native vessel [1]. Mismatch at the anastomosis causes intima hyperplasia and a reduction in patency rate [3]. Elevated occlusion rates are observed especially for diameters less than 6 mm and, due to the material high stiffness, extremely thin walls would be required to match artery compliance [2]. In this sense, compliance mismatch between native artery and the artificial graft has been long discussed as a cause of failure over prolonged periods of implantation, particularly for small diameter conduits [4]. As a result, neither geometrical nor pulsatile strains and stresses matching can occur during the entire pulsatile cycle [3]. In terms of the 'biomimetic' approach, major requirements concern mechanical strength, compliance and suture retention strength comparable to physiologic values [6]. Additionally, poor biocompatibility at the blood–biomaterial interface leads to graft failure and recurrent disease [5].

Electrospun nanofibrous vascular grafts emerge as a potential tailor made solution to the mechanical mismatch problem. The electrospinning technique has gained attention in the last decade as an attractive and versatile method to obtain nanofibrous structures for several applications such as catalysis, nanofluidics, sensors, medicine, energy, environmental engineering, biotechnology, defense and security, and healthcare [8,9]. Particularly, electrospun structures mimic the extracellular matrix of natural tissues and therefore are being implemented as scaffolds for tissue engineering applications [10–12]. In this sense, different nanofibrous structures and morphologies can be developed depending on the electrospinning technique [8,9,13]. The obtained synthetic structures possess a nanofibrous morphology, elevated interconnected porosity and high surface to volume ratio. Additionally, multilayered structures, that mimic the wall structure of artery segments, could be also generated.

Simultaneous measurement of pressure and diameter of blood vessels and/or synthetic arterial grafts has a significant role in cardiovascular research. Acquisition of vascular diameter response to internal pressure waveforms constitutes the basis of the hysteresis stress–strain loops construction, which can be perfectly used to obtain the mechanical properties of the conduit [7,14,15]. In this sense, biomechanical properties can be obtained in vitro, by means of an experimental setup, in which near-physiological hemodynamic conditions can be reproduced [16,17]. To this end, an international standard for cardiovascular implants-tubular vascular prostheses [18] establishes the criteria for the execution of experimental measurements and the evaluation of properties of vascular grafts. In some studies, dynamic pressure–diameter relationship was obtained in order to evaluate electrospun vascular grafts [19–22]. However, to the best of our knowledge, the use of sonomicrometry as a tool to measure the diameter variation was not reported, being laser or video measurements the techniques implemented instead. Only the sonomicrometry technique enables the implementation of the measurements in vivo.

The aim of this study was to extend the established approach developed in our previous works [7,14,23] to electrospun vascular graft characterization. In the present work, poly(L-lactic acid) (PLLA) electrospun nanofibrous tubular structures, as a model bioresorbable grafts, were obtained and subjected to pulsated pressure conditions, following criteria stated in the abovementioned standard as well as our framework of arterial mechanics. Graft dynamic response was obtained from experimental measurements by applying an ultrasonic gold standard method (pressure–diameter relationship) and high-resolution ultrasound techniques. Similarly, femoral arteries were explored in order to relate its mechanical performance to the one observed in the synthetic grafts.

2. Materials and methods

2.1. Inner pressure measurements

The in-vitro measurement of internal pressure in vascular grafts and ovine femoral arteries was performed by means of solid state microtransducers (Konigsberg Inc., PA, USA) (Fig. 1a). These micro transducers have an elevated frequency response that usually cannot be achieved with common fluid-catheter transducers (like Sthatham pressure transducer). The microtransducer selected for the experimental protocol (model P2.5, 1200 KHz bandwidth) has a sensing surface designed to be in direct contact with blood flow or physiological solutions. Before the in-vitro measurements, the pressure measurement chain (microtransducer, amplifiers and post processing) was calibrated using a reference digital manometer.

2.2. Diameter variation and sample thickness measurements

The in-vitro temporal variation of the external diameter of vascular grafts and ovine femoral arteries was measured using Sonomicrometry technique. Since its first implementation in 1956 [24] by Franklin and Rushmer, this procedure has been improved and refined, becoming a gold-standard for in-vivo physiological research. Sonomicrometry determines instantaneous external vessel diameter by means of measuring the flight time of an ultrasound burst between a pair of small transducers. These transducers (small ultrasound crystals) are fixed diametrally opposed on vessel external wall. Small 5 MHz sonomicrometry ultrasound transducers built at our laboratory (Fig. 1b) and a Triton Technology System 6 Mainframe (Triton Technology INC., SD, USA) equipped with sonomicrometer module were used to obtain the external diameter measurements. The system was calibrated using its internal time–diameter reference before each in-vitro measurement.

Wall thickness and internal diameter of each sample were measured in-vitro by means of high resolution ultrasound techniques. An equipment constituted by an ultrasound probe (Panametrics INC., Massachusetts, USA), a graduated mechanical positioning system and a set of cross correlation processing algorithms (A-Scan system) [25] were used.

2.3. Materials for the construction of the vascular grafts

Poly(L-lactic acid) (PLLA) (PLA2002D Mw = 287.9 Kg mol^{-1}) was obtained from Natureworks (MN, USA). Dichloromethane (DCM) and

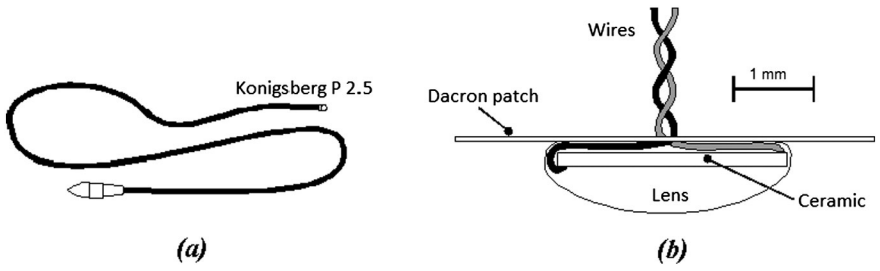

Fig. 1. Solid state microtransducer, Konigsberg P2.5 (a). Monocrystal utilized in diameter variation measurements. Piezoelectric ceramic is soldered to a bipolar wire, covered by a polyurethane resin small lens (b).

N,*N*-dimethylformamide (DMF) were acquired from Anedra (BA, Argentina).

2.4. Construction of vascular grafts: electrospinning procedure

Small-diameter vascular grafts were obtained using the electrospinning technique. A PLLA solution (10% wt/v) in DMF/DCM solvent mixture (40/60 ratio) was injected through the needle. The electrospinning setup consisted of a high voltage power supply (Gamma High Voltage Research Inc., Ormond Beach, Florida, USA), a blunt stainless steel needle (18 gauge, Aldrich®), a syringe pump (Activa® A22 ADOX, Ituzaingó, Argentina), and a rotating collector with a 5 mm diameter mandrel.

Based on previous studies [26], the processing parameters were selected (flow rate (f) = 0.5 mL/h, needle-collector distance (d) = 15 cm, applied high voltage (V) = 13 kV, and rotation speed (r) = 1000 rpm). A grounded electrode was placed under the rotating mandrel. The electrospinning process was carried out for 2 h, while the needle position was shifted every 15 min to achieve a uniform thickness along the graft length. The graft was removed from the collector, and extensively dried under vacuum at room temperature. Tubular specimens of 8–11 cm length were obtained.

2.5. Vascular graft morphology

The electrospun tubular structures were characterized by scanning electron microscopy (SEM) (JEOL Model JSM-6460LV). Samples were mounted on a glass holder, sputter coated with gold in an argon-purged chamber evacuated to 500 m Torr, and examined using SEM with an accelerating voltage of 15 kV. Fiber mean diameter and diameter distribution were measured using an image processing software (Image Pro Plus, Media Cybernetics Inc., USA). An amount of 100 nanofibers per sample was measured in order to obtain a meaningful statistical value.

2.6. Obtention of samples of ovine femoral arteries

Four healthy male Merino sheep, weighing 25 to 35 kg, were included in this study. All protocols were conducted in accordance with Guide for the Care and use of Laboratory Animals [27]. All animals were vaccinated and treated for skin and intestinal parasites. During 30 days before surgery, they were appropriately fed, and assessed for optimal clinical status. General anesthesia was induced with intravenous administration of pentobarbital (35 mg/kg). The respiration was maintained with a positive respirator (Dragger SIMV Polyred 201, Madrid, Spain). Respiratory rate, tidal volume, and the inspired oxygen fraction were adjusted to maintain arterial pCO_2 at 35–45 mm Hg, pH at 7.35–7.4, and pO_2 above 80 mm Hg. Femoral arteries were selected in order to evaluate their biomechanical properties. Vascular conduits were exposed and dissected, and a 6 cm length segment was in situ measured and marked with two suture references in the adventitia.

2.7. Dynamic mechanical characterization

2.7.1. Biomechanical framework

Arterial conduit elastic behavior is usually studied using linear elastic theory. However, nonlinearity of the stress–strain relationship and the anisotropy of the wall represent major constraints for this approach [7]. Nevertheless, the deformation results relatively small (less than 5%) when pressure varies from the systole to diastole, and the pressure–diameter relationship becomes virtually linear [23]. 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 [4]. This pressure–diameter relationship is termed the "J" curve, and can be observed in Fig. 2. Diameter–pressure loop evolution (beat to beat study) is also visualized [7].

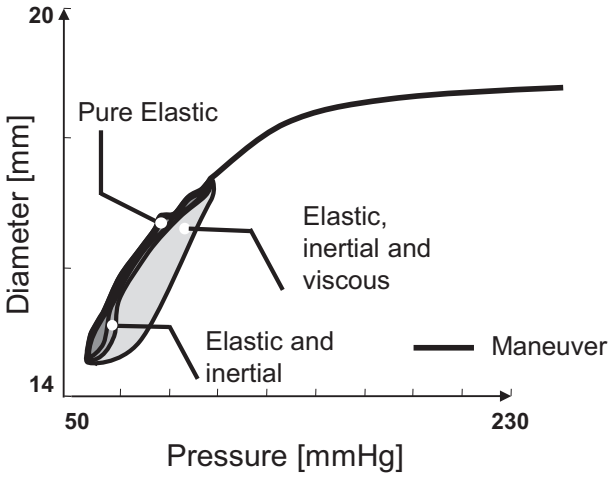

Fig. 2. Schematic diameter versus pressure loop, where its elastic, inertial and viscous components are presented. A high pressure maneuver is also showed (solid line).

Arterial wall properties depend on the mechanical role developed by passive components (elastin and collagen fibers) and active components (smooth muscle cells). These components determine the elastic, viscous and inertial properties of the vessel, being the inertial component negligible [7]. Accordingly, differentiated evaluations may be performed in order to identify the contribution of each component to the overall elastic response (Fig. 2). Values of Young's modulus are extremely different for elastin and collagen fibers, contributing individually to the whole arterial elasticity. In addition, elasticity changes are observed under smooth muscle cell activation and the recruitment of collagen fibers supporting the wall stress [14]. Concerning this, mechanical properties can be quantitatively analyzed using instantaneous pressure–diameter recordings. To our knowledge, pressure–diameter or stress–strain loop determination constitutes the most appropriate technique to assess a dynamic behavior of the in vivo and ex vivo as well as vascular grafts.

2.7.2. Experimental setup

We used a hemodynamic work bench simulator (HWBS) [16,17,26], designed to measure instantaneous pressure and diameter in blood vessels and scaffolds. The simulator consists basically of a specially designed programmable pump that impulses fluid into a hydraulic closed circuit, and a fluid pool where samples are placed in an adjustable sample fixing system and immersed in a physiological solution. The closed circuit is basically composed by silicone tubes, variable constrictions, the sample (vessel or graft) and a fluid reservoir (Fig. 3a). Variable constrictions are used to adjust peripheral resistance and pressure reflected waves [16]. Pulse frequency can be adjusted in order to mimic the pulse rate of a normal adult human which is generally comprised between 60 and 80 beats per minute, considering basal conditions.

The samples were placed in the adjustable fixing system and the pool was filled with Tyrode's solution, with a pH of 7.4. Temperature was controlled, using a set-point of 37 ± 2 °C. Sample pressure was measured using a Konigsberg micro transducer, placed near the center of each sample. External diameter was obtained using the gold-standard sonomicrometry technique descripted in Section 2.2. Ultrasound transducers were placed as close as possible to the pressure transducer in order to avoid any possible undesired viscous behavior. Additionally, the A-scan system, also detailed in Section 2.2, was combined with the HWBS for wall thickness assessments (Fig. 3b). Diameter

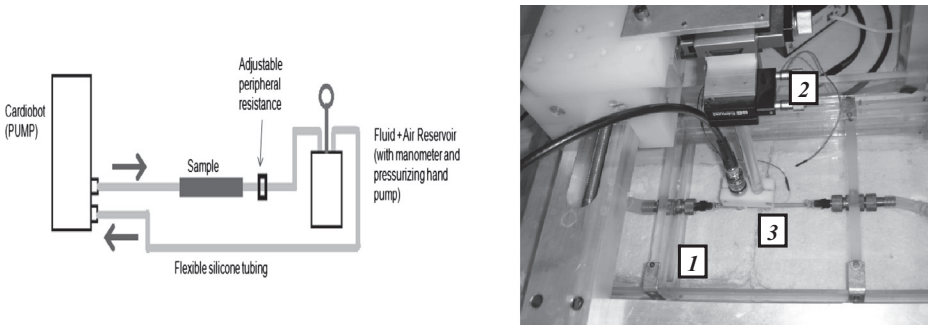

Fig. 3. Experimental setup schematic diagram. Compliant silicone tubing is used for the circuit (a). (1) Pool and (2) graduated mechanical positioning system. (3) Sample placed in adjustable fixing system during wall thickness measurements (b).

and pressure signals were acquired at 2000 samples per second using a 12-bit data acquisition module (NI USB-6009) during a 15 s interval. Acquisition and signal processing were performed through a specially developed Matlab application (Mathworks Inc., San Diego, USA). Every experiment was tried four times to get statistically reliable values.

2.7.3. Experimental protocol: vascular grafts measurements

Firstly, electrospun nanofibrous vascular graft samples were placed in the experimental setup adjustable sample fixing system, one at a time. A longitudinal preload of 0.5 N was applied to each sample. Subsequently, the fixing system was locked, imposing an isometric condition. Diameter–pressure analysis was performed following the criteria stated at ISO7198 standard [18]. Obtained D–P loops were evaluated at 80–120 mm Hg range, which corresponds to physiological human blood pressure condition. Ranges from 50 to 90 mm Hg and 110 mm Hg to 150 mm Hg were also measured in order to fulfill the requirements established in the standard. Posteriorly, a hydraulic maneuver was performed. Intraluminal pressure was gradually increased from 50 mm Hg to 150 mm Hg while it was simultaneously acquired with external diameter variation.

2.7.4. Experimental protocol: femoral artery measurements

Femoral arteries were placed in the fixing system and a longitudinal preload was applied in order to recover the original segment length (measured in-vivo before sample extraction). Once placed in the experimental setup, the arterial segments were allowed to equilibrate for a period of 10 min under a steady state of flow and pressure at a stretching rate of 108 bpm. Obtained D–P loops were evaluated at 60–90 and 100–130 mm Hg pressure ranges.

2.7.5. Elasticity assessment

Action of internal pressure determines different types of wall stress [7]. In this sense, the corresponding circumferential mean value (σ_M) was approximated according to:

$$\sigma_M = P_M \cdot (R_M/h) \quad (1)$$

where P_M is the internal applied pressure, R_M is the corresponding mean radius and h is the wall thickness. In addition, vessel (artery or graft) elastic response was evaluated by means of two parameters. Firstly, dynamic compliance was calculated as defined in [18] as follows:

$$\%C = \left[(R_S - R_D).10^4/R_D\right]/(P_S\text{-}P_D) \quad (2)$$

where P_S is the highest pressure value (systolic, mm Hg) and P_D is the lowest pressure value (diastolic, mm Hg). R_S and R_D are the corresponding internal radii (mm). The circumferential compliance as calculated above is expressed as a percentage of the diameter change per 100 mm Hg. Wall was assumed to be elastic and incompressible, following the linear theory. Moreover, and because estimated arterial diameter could be characterized as continuous function of pressure by means of an interpolating method, the compliance pressure curve was calculated by deriving the diameter pressure curve (dD/dP) to determine compliance for a given value of blood pressure. In all cases, the curve was obtained over pressure range ranging from 50 to 150 mm Hg, allowing comparison with other studies.

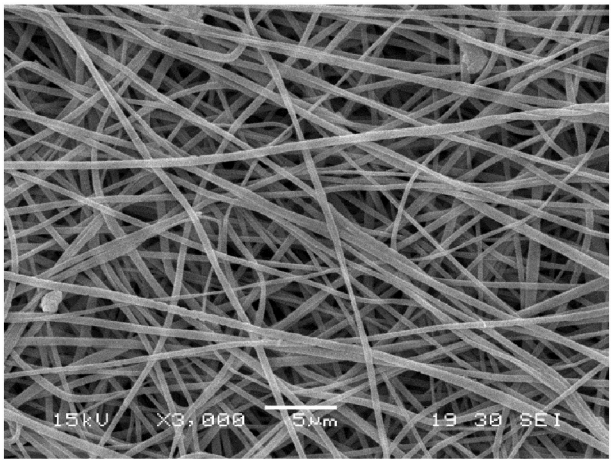

Fig. 4. SEM micrograph showing the nanofibrous morphology of PLLA-based grafts.

Table 1
Ultrasound (A-Scan system) wall thickness (WTh) and static internal diameter (SID) measurements for PLLA vascular grafts.

PLLA Sample	WTh (mm)	SID (mm)
#1	0.0393 ± 0.0015	4.9127 ± 0.0013
#2	0.0410 ± 0.0010	4.9294 ± 0.0022
#3	0.0385 ± 0.0022	4.9271 ± 0.0018
#4	0.0374 ± 0.0017	4.9132 ± 0.0017
#5	0.0375 ± 0.0030	4.9324 ± 0.0012

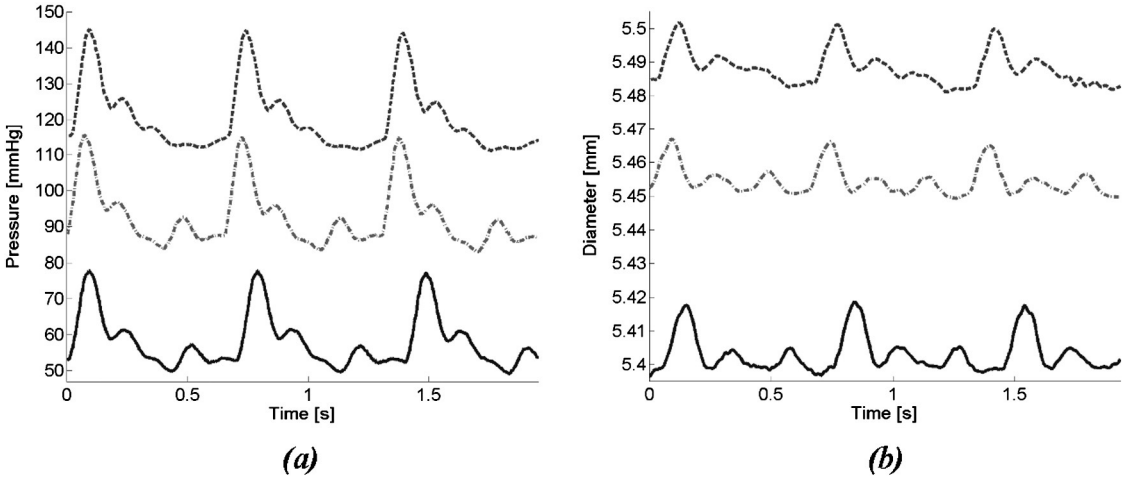

Fig. 5. Applied pressure waveforms for 50–90 mm Hg (solid), 80–120 mm Hg (dashed dotted) and 110–150 mm Hg ranges (dashed). Frequency: 60 ± 5 beats per minute (a). Measured diameter waveforms for 50–90 mm Hg (solid), 80–120 mm Hg (dashed dotted) and 110–150 mm Hg ranges (dashed) (b).

Secondly, incremental elastic modulus ($E_{P\varepsilon}$) was estimated at mean pressure, for each of the pressure ranges, according to [25]:

$$E_{P\varepsilon} = dP/d\varepsilon_{\text{mean preasure}} \quad (3)$$

$$\varepsilon = D/D_0 \quad (4)$$

where P constitutes the transmural pressure and ε is the corresponding strain, obtained by referencing the diameter dynamic changes (D) to its unstressed value (D_0). Additionally, diameter–pressure relationship for the entire set of vascular grafts was assessed.

2.8. Statistical analysis

Result data were expressed as means ± standard deviation. Statistical analysis was carried out using the unpaired Student's t-test. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Vascular graft morphological characterization

Five different PLLA tubular samples obtained from a 5 mm diameter rotational collector (electrospinning technique, Section 2.4) were evaluated in the present work. The grafts exhibited randomly distributed bead-free nanofibrous morphology, as observed by SEM (Fig. 4) and the obtained fiber mean diameter was 383 ± 103 nm.

Subsequently, wall thickness and internal diameter of PLLA conduits were estimated by means of high-resolution ultrasound techniques (A-Scan system, Section 2.2), at low intraluminal pressure (low tangential stress, HWBS, Section 2.7.2). The obtained results are shown in Table 1.

3.2. Vascular graft mechanical properties

Dynamic compliance and elastic modulus were assessed for electrospun vascular grafts according to Eqs. (2), (3) and (4), depicted in Section 2.7.5. To this end, instantaneous internal pressure and external diameter were measured for each PLLA sample, at each

Table 2
Obtained dynamic measurements of poly(L-lactic acid) (PLLA) developed vascular grafts, for each pressure range (R1, R2 and R3). PR: pressure range. SP: systolic pressure, DP: diastolic pressure. SD: systolic diameter, DD: diastolic diameter. σ_M: mean circumferential wall stress. Values are expressed as mean ± standard deviation.

PLLA Sample	PR (mm Hg)	SP (mm Hg)	DP (mm Hg)	SD (mm)	DD (mm)	σ_M (10^6 dyn/cm^2)
#1	R1: 50–90	77.75 ± 0.49	49.75 ± 0.48	5.418 ± 0.001	5.396 ± 0.001	5.42 ± 0.04
	R2: 80–120	114.67 ± 0.45	83.41 ± 0.52	5.466 ± 0.001	5.449 ± 0.001	8.69 ± 0.05
	R3: 110–150	144.10 ± 0.77	111.64 ± 0.42	5.500 ± 0.001	5.482 ± 0.001	11.41 ± 0.04
#2	R1: 50–90	104.18 ± 0.16	51.09 ± 0.22	5.360 ± 0.002	5.329 ± 0.001	5.98 ± 0.01
	R2: 80–120	127.22 ± 0.28	73.84 ± 0.26	5.378 ± 0.001	5.352 ± 0.001	7.99 ± 0.02
	R3: 110–150	154.41 ± 0.28	100.52 ± 0.32	5.379 ± 0.001	5.356 ± 0.001	10.34 ± 0.02
#3	R1: 50–90	84.98 ± 0.43	24.96 ± 0.21	5.317 ± 0.001	5.264 ± 0.001	4.11 ± 0.02
	R2: 80–120	136.69 ± 0.41	76.43 ± 0.13	5.385 ± 0.001	5.358 ± 0.001	8.97 ± 0.02
	R3: 110–150	165.94 ± 0.47	105.53 ± 0.23	5.419 ± 0.001	5.398 ± 0.001	11.77 ± 0.02
#4	R1: 50–90	103.53 ± 0.54	53.02 ± 0.69	5.410 ± 0.001	5.371 ± 0.001	6.71 ± 0.04
	R2: 80–120	138.06 ± 0.64	86.63 ± 0.70	5.513 ± 0.001	5.490 ± 0.001	10.17 ± 0.05
	R3: 110–150	170.27 ± 0.49	119.47 ± 0.34	5.560 ± 0.001	5.542 ± 0.001	13.50 ± 0.03
#5	R1: 50–90	99.20 ± 0.53	42.23 ± 1.04	5.346 ± 0.001	5.305 ± 0.001	5.79 ± 0.07
	R2: 80–120	127.92 ± 1.37	75.39 ± 0.22	5.393 ± 0.001	5.361 ± 0.001	8.88 ± 0.03
	R3: 110–150	160.89 ± 1.21	105.78 ± 0.55	5.435 ± 0.001	5.410 ± 0.001	11.97 ± 0.06
Mean ± SD	R1: 50–90	95.44 ± 10.02	43.69 ± 10.80	5.366 ± 0.038	5.327 ± 0.046	5.62 ± 0.91
	R2: 80–120	130.25 ± 7.63	78.74 ± 5.12	5.423 ± 0.055	5.398 ± 0.058	8.96 ± 0.75
	R3: 110–150	160.53 ± 8.47	108.30 ± 6.83	5.455 ± 0.066	5.433 ± 0.069	11.83 ± 1.08

Table 3
Mechanical evaluation of poly(L-lactic acid) (PLLA) vascular grafts. PR is the pressure range (R1, R2 and R3), %C is the compliance and $E_{P\varepsilon}$ is the pressure–strain elastic modulus. Values are expressed as mean ± standard deviation.

PLLA Sample	PR (mm Hg)	%C (10^{-2} mm Hg)	$E_{P\varepsilon}$ (10^{6} mm Hg)
#1	R1: 50–90	1.44 ± 0.08	10.38 ± 0.58
	R2: 80–120	0.99 ± 0.06	13.85 ± 0.83
	R3: 110–150	1.06 ± 0.05	13.02 ± 0.66
#2	R1: 50–90	1.09 ± 0.05	11.56 ± 0.49
	R2: 80–120	0.90 ± 0.01	15.04 ± 0.22
	R3: 110–150	0.80 ± 0.02	17.30 ± 0.45
#3	R1: 50–90	1.70 ± 0.01	11.21 ± 0.09
	R2: 80–120	0.85 ± 0.02	15.15 ± 0.38
	R3: 110–150	0.65 ± 0.03	20.00 ± 0.91
#4	R1: 50–90	1.36 ± 0.06	9.10 ± 0.43
	R2: 80–120	0.78 ± 0.03	15.46 ± 0.53
	R3: 110–150	0.61 ± 0.04	20.01 ± 0.44
#5	R1: 50–90	1.33 ± 0.01	10.50 ± 0.19
	R2: 80–120	1.13 ± 0.06	11.87 ± 0.55
	R3: 110–150	0.83 ± 0.05	15.86 ± 0.81
Mean ± SD	R1: 50–90	1.38 ± 0.21b, c	10.57 ± 0.97b, c
	R2: 80–120	0.93 ± 0.13a, c	14.31 ± 1.47a, c
	R3: 110–150	0.76 ± 0.15a, b	17.63 ± 2.61a, b

a, b, and c indicate $p < 0.05$ compared to 50 to 90 mm Hg, 80 to 120 mm Hg and 110 to 150 mm Hg, respectively.

pressure range (Section 2.7.3). Pressure variations were imposed by HWBS. Obtained waveforms are illustrated in Fig. 5 for an individual case.

Results for dynamic measurements of the selected PLLA samples are shown in Table 2, corresponding to each pressure range (R1: 50–90 mm Hg, R2: 80–120 mm Hg and R3: 110–150 mm Hg). Maximal (systolic) and minimal (diastolic) dynamic variations of internal pressure and external diameter were obtained. Mean circumferential stress values are also presented.

Assessments of dynamic compliance and pressure–strain elastic modulus for each vascular graft are presented in Table 3, for each pressure range. Compliance is observed to decrease significantly in most cases, as transmural pressure increases. Same pressure dependent behavior is suggested by the increase of pressure–strain elastic modulus, denoting a stiffening of the vascular graft.

3.3. Femoral artery elasticity evaluation

The procedure described for the mechanical characterization of PLLA vascular grafts was replicated for the set of femoral arteries. Assessed dynamic compliance and pressure–strain elastic modulus values are tabulated in Table 4. Collagen range is observed to be significantly stiffer (a higher elastic modulus and a lower compliance) than the elastin range.

Table 4
Mechanical evaluation of femoral artery samples. PR is the pressure range (R1 and R2), %C is the compliance and $E_{P\varepsilon}$ is the pressure–strain elastic modulus. Values are expressed as mean ± standard deviation.

Femoral sample	PR (mm Hg)	%C (10^{-2} mm Hg)	$E_{P\varepsilon}$ (10^{6} mm Hg)
#1	R1: 60–90	8.69 ± 1.64	1.85 ± 0.36
	R2: 100–130	0.61 ± 0.15	18.77 ± 5.19
#2	R1: 60–90	8.25 ± 0.52	1.70 ± 0.13
	R2: 100–130	0.89 ± 0.24	10.61 ± 1.42
#3	R1: 60–90	7.42 ± 0.33	1.78 ± 0.13
	R2: 100–130	0.74 ± 0.24	19.19 ± 3.76
#4	R1: 60–90	9.75 ± 0.44	1.29 ± 0.03
	R2: 100–130	0.86 ± 0.12	14.44 ± 1.04
Mean ± SD	R1: 60–90	8.52 ± 1.15	1.66 ± 0.30
	R2: 100–130	0.79 ± 0.20[a]	15.76 ± 4.78[a]

[a] Indicates $p < 0.05$ compared to 100 to 130 mm Hg.

3.4. Vascular grafts diameter–pressure and compliance–pressure curves (maneuvers)

Diameter–pressure and compliance–pressure curves for the entire set of vascular grafts (Section 2.7.3) are shown in Fig. 6. A pressure range of 50 to 150 mm Hg was obtained by means of the development of a slow hydraulic maneuver (Section 2.7.5). Femoral artery dynamic compliance (collagen range, 100–130 mm Hg) is observed to be closer to the graft compliance pressure–curve. Compliance assessments of similar materials such as PLCL (at 100 mm Hg) [1] and ePTFE (at 100 mm Hg) [28] are also depicted in the figure.

3.5. Vascular grafts and femoral artery pressure vs. diameter loop evaluation

Fig. 7 shows the measured pressure versus diameter loops for both femoral artery and vascular graft. These loops exhibit the mechanical response for the entire physiological range. As can be seen, the conduit stiffness (artery or graft) can be determined as the slope of the pressure vs. diameter loop, at a particular pressure value or range. As was expected, elastic modulus corresponding to the elastin range (60–90 mm Hg) was lower than the collagen range value in femoral artery evaluation. In this sense, PLLA vascular graft mechanical behavior is observed to be extremely closer to the elastic response corresponding to the collagen range.

4. Discussion

In the present work, we applied a well-known approach [7] since it allows the in vitro dynamic characterization of synthetic and biologic vascular prostheses as well as native arteries, under different simulated near-physiological conditions. This framework enables to assess the mechanical properties of arteries, vein and synthetic prostheses under controlled conditions of pressure, flow, frequency and temperature, taking into account the theoretical basis of the pressure–diameter relationship. This permits to mimic in vitro the purely elastic parameters observed in vivo, whereas viscous and inertial behaviors are determined by the dynamic conditions of the system. Indeed, arterial wall mechanical properties produce nonlinear pressure–diameter relations, particularly when vessel structure is exanimated over a wide range. Total elastic modulus can be decomposed, where contribution of elastin, collagen and smooth muscle can be quantified [7]. The function of elastin and collagen fibers is to maintain a steady tension to hold the wall against the transmural pressure present in the vessel, while the activation of smooth muscle alters both viscosity as well as elasticity of the vessel wall [14]. An artery can largely expand and contract elastically mainly due to elastin fibers, when low pressure variations are applied, whereas collagenous fibers remain unstretched [4]. Furthermore, the amount of elastic components differs greatly between different types of normal vessels. The mathematical approach allows the individual characterization assuming a three-element Maxwell model [14]. The activation of smooth muscle indicates a greater expenditure of energy in the pulsatile expansion of the vessel with each heartbeat. Under physiological conditions, the artery can be considered essentially viscoelastic. This behavior can be perfectly appreciated in the hysteresis loop, evidenced by the pressure–diameter relationship [7]. In this sense, elastic deformation is proportional to the potential energy stored during systole that will be yielded to the system during the diastole. On the other hand, viscosity quantifies the absorption of energy by the vessel wall.

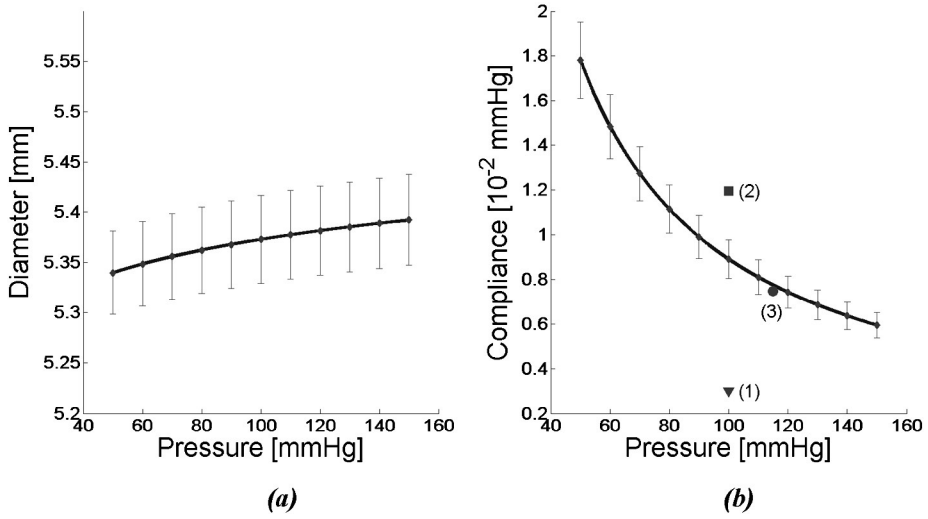

Fig. 6. Diameter versus pressure (a) and compliance pressure (b) curves obtained as a result of the hydraulic maneuver over a pressure range of 50 mm Hg to 150 mm Hg. Values are expressed as mean ± standard deviation. (1) PLCL (100 mm Hg) [1], (2) ePTFE (100 mm Hg) [28] and (3) femoral artery (collagen range 100–130 mm Hg) dynamic compliance values.

Characterization of mechanical properties using pressure–diameter loops provides insight into the structural alterations of the vessel walls, allowing the assessment of its individual mechanical behavior. This methodology provides accuracy and reproducibility on a long time basis because of the high frequency and linear response of the transducers [14]. Moreover, it can be performed during in vitro experiences, ex vivo and mainly in vivo, in conscious animals [7]. It becomes clear, that this advantage over other known methods allows the study of vascular grafts both as individual conduits as well as when they are surgically implanted, by means of chronic instrumentation. The potential of the technique becomes indisputable when multilayer conduits have to be characterized. In this sense, the mechanical behavior of PLLA electrospun vascular grafts developed in the present work was successfully characterized. Both dynamic compliance and pressure–strain elastic modulus obtained values were observed to be repetitive and comparable to the results achieved in previous studies [1,28].

PLLA was chosen as a representative bioresorbable polymer, commonly used in biomedical applications, such as drug delivery systems, tissue engineering, and biomedical devices. PLLA is a FDA approved biodegradable polyester that exhibits semicrystalline structure and good electro-spinnability [29–32]. The degradation kinetics of PLLA was studied in vitro, showing a degradation period (into its monomers) of 6 to 12 months. This rate of in vitro and in vivo biodegradation can be adjusted by manipulating the crystallinity and copolymerization of isomers or other monomers [33]. The obtained results reinforce the findings in previous works in which it is demonstrated that PLLA possesses an elevated elastic modulus, required to withstand high pressure and flow without collapse or degradation until tissue develops and matures in vivo. It also presents a mechanical behavior similar to collagen [34].

It is noteworthy that the scaffolds provided mechanical support and showed sufficient strength for arterial vascular applications. However, it has to be noted that the matching of mechanical, structural and biological properties with those of native vessels is a critical requirement for any small diameter vascular graft design. Mechanical mismatch has been associated with perturbation in the local

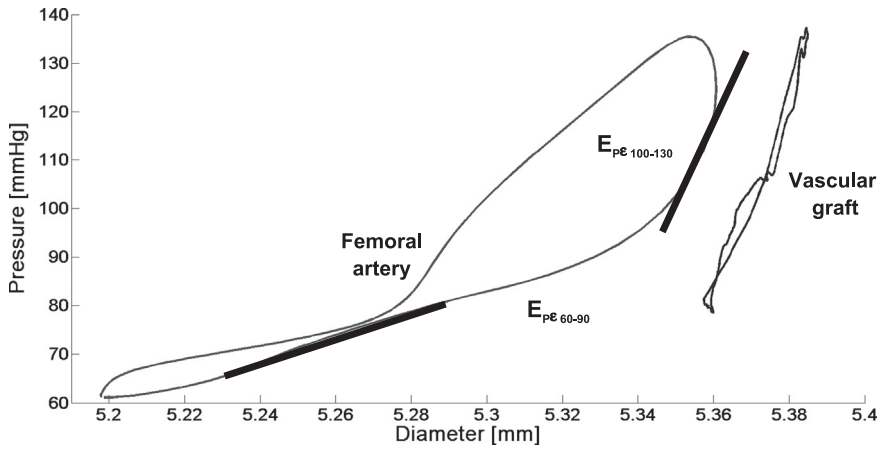

Fig. 7. Diameter versus pressure loop of a femoral artery (solid, left) and a vascular graft (solid, right) for an typical case. Elastin ($E_{P\varepsilon\ 60\text{–}90}$) and collagen ranges ($E_{P\varepsilon\ 100\text{–}130}$) pressure–strain elastic modulus slopes are also presented (dashed).

hemodynamics, establishing a basis for restenosis and graft failure [35]. It is a well-known fact that poly(glycolic acid), poly(L-lactic acid), and their copolymers are generally much stiffer than native soft tissues [20]. The artery damping properties should be taken into account in the development of vascular prosthesis with appropriated mechanical properties. The compliance of the native artery absorbs the energy of pulsatile flow and lowers shear stress, and decreases turbulent flow.

Results obtained from the experimented samples revealed a significant variation of compliance and pressure–strain elastic modulus over the considered pressure ranges with negligible viscous and inertial behaviors. Interestingly, it can be observed that as internal pressure increases, the vascular graft showed a stiffness increase. Native arteries present elevated compliance values at low pressures which becomes low as pressure increases [1]. This behavior is attributed to collagen fiber recruitment and smooth muscle cell activation [7]. Compared to femoral arteries, vascular electrospun nanofibrous PLLA graft mechanical characterization can be associated to the collagen range (100–130 mm Hg). This behavior can be visualized both in the pressure–diameter loops (Fig. 7) as well as in the compliance–pressure curve (Fig. 6), jointly with previous work findings. These results confirmed the assumption that PLLA behaves mechanically like collagen. As a result, mechanical behavior of viscoelastic segmented poly(ester urethanes) (SPEU) and polymer blend electrospun grafts could be also characterized. Even more, an in vivo characterization in conscious animals could also be performed.

5. Conclusion

In this work, arterial mechanics framework using sonomicrometry, extensively applied in previous works, has been used to characterize electrospun vascular grafts in vitro. This technique allows extending its application directly to in vivo experiences, in conscious and chronically instrumented animals. Sonomicrometry has the unique ability to measure diameter variation under physiologically minimally invasive conditions. These results are promising for the characterization of different electrospun vascular grafts obtained from a variety of polymeric formulations, such as other polyesters, biopolymers, bioresorbable elastomeric polyurethanes, including their blends. Furthermore, the mechanical response developed of multilayer grafts could be optimized, based on their in vitro and in vivo pressure–diameter evaluation.

Acknowledgments

This work was supported by the National Agency of Innovation and Research (ANII; grants: FCE-1-2011-1-7172; Uruguay) and PEDECIBA-BIOLOGIA (Uruguay), the Buenos Aires Regional Faculty of National Technological University (PID 25/c131), the Argentinean National Agency of Scientific and Technological Promotion (PICT 224 grant), the National Research Council (PIP 522 grant), and the National University of Mar del Plata (15/G359 grant). F.M.B. thanks CONICET for the fellowship awarded.

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