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Mechanical properties of rat thoracic and abdominal aortas

N. Assoul[a,b], P. Flaud[c], M. Chaouat[a,d], D. Letourneur[a,b,*], I. Bataille[a,b]

[a]*INSERM, U698, Bio-ingénierie Cardiovasculaire, Hôpital X. Bichat, 75018 Paris, France*
[b]*BPC, Institut Galilée, Université Paris 13, 93430 Villetaneuse, France*
[c]*Matière et Systèmes Complexes, CNRS UMR 7057, Université Paris 7, 75013 Paris, France*
[d]*Service de Chirurgie Plastique, Hôpital Rothshild, 75012 Paris, France*

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Abstract

Mechanical properties of abdominal and thoracic arteries of 2 mm in diameter were determined from adults Wistar rats. A tensile testing instrument was used to obtain stress/strain curves with arteries immersed in physiological buffer at 37 °C. A displacement was applied on all arteries with various frequencies (1–7.5 Hz) and strains (5–60%). From each curve a Young modulus was obtained using a mathematical model based on a nonlinear soft tissue model. No influence of frequency on modulus was evidenced in the tested range. Abdominal aortas, which were found slightly thicker than thoracic aortas, were characterized by a higher modulus. Due to the interest of decellularized biological materials, we also used SDS/Triton treated arteries, and found that the chemical treatment increased modulus of thoracic arteries. Tensile tests were also performed on thoracic aortas in the longitudinal and transversal directions. Longitudinal moduli were found higher than transversal moduli and the difference could be related to the longitudinal orientation of collagen fibers. These data and mathematical model seem useful in the design of new vascular synthetic or biological prostheses for the field of tissue engineering.

Keywords: Mechanical properties; Rat arteries; SDS/Triton treatment; Tensile tests; Young modulus

1. Introduction

Most studies concerning biomechanical aorta evaluation deal with compliance calculation by means of pressure/artery dimensions relationship (Mourlon-Le Grand et al., 1993; Van Gorp et al., 1995; Bézie et al., 1998; MacWilliams et al., 1998; Zhao et al., 2000; Labat et al., 2001). Although these methods are interesting because pressure and artery diameter may be measured in situ, no correlation between biomechanical properties and rheological law is evidenced. Indeed, no universal value of Young modulus is obtained, mainly because of its dependence to measurement conditions. For example, Young modulus obtained by the use of a tensile testing instrument is given for two strain conditions (Katsuda et al., 2002), or stress/stretch curves are directly given (Papadopoulos and Delp, 2003). Mathematical model by curve fitting is also rarely described (Orosz et al., 1999).

Information about mechanical behavior of small caliber arteries like rat aortas is interesting to obtain, as a first approach to the design of small artificial arteries. Indeed, cardiovascular diseases constitute the first cause of mortality in the industrialized countries and patients may suffer a vessel wall thickening that causes sudden death or heavy surgical intervention. When the vascular material of the patient becomes insufficient for autologous transplantation, the damaged artery has to be replaced by an artificial vessel. For vessels larger than 6 mm diameter, artificial substitutes provide satisfying results. The problem remains for small calibers due to thrombosis and intimal hyperplasia (Kannan et al., 2005; Kakisis et al., 2005; Salacinski et al., 2001). Indeed, small caliber flexible prostheses presenting a non-thrombogenic internal surface remain to be designed. Among the possible ways, tissue

*Corresponding author at: INSERM, U698, Bio-ingénierie Cardiovasculaire, Université Paris 13, 93430 Villetaneuse, France.
Tel.: +33 1 49 40 40 90; fax: +33 1 49 40 30 08.
E-mail address: didier.letourneur@galilee.univ-paris13.fr (D. Letourneur).

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engineering is an emerging field to find cell-compatible tubular synthetic or natural matrices as vascular substitutes (Chaouat et al., 2006; L'Heureux et al., 2006). These substitutes are designed to possess similar mechanical properties as native arteries (Salacinski et al., 2001; Laflamme et al., 2006; L'Heureux et al., 2007). In order to facilitate the design of tubular material that would be first implanted in the rat aorta, the main features of the native material have to be known (Zidi and Cheref, 2003).

In addition, chemically treated arteries also constitute an interesting biological material for vascular replacement. Both native and treated rat aortas were thus evaluated because of the potential use of treated aortas as vascular scaffolds (Gomes et al., 2001; Allaire et al., 1997; Touat et al., 2006). Treated cadaveric human arteries were envisaged as possible sources but their low availability limited their widespread use (Dahl et al., 2003). From treated porcine aorta, matrix components were selectively removed to obtain two scaffold types, namely elastin and collagen scaffolds. Compared to elastin scaffolds and fresh aorta, aortic collagen scaffolds exhibited lower tensile moduli and strain at rupture (Lu et al., 2004). Porcine treated arteries (Dahl et al., 2003) were also used as a scaffold onto which vascular cells were seeded. In order to develop small-diameter vascular grafts, dog carotids (internal diameter = 3 mm) were treated and then seeded with host bone-marrow-derived cells (Debes and Fung, 1995). After 8 weeks, the vascular grafts evidenced the regeneration of the three elements of artery, namely endothelium, media and adventitia (Cho et al., 2005). These works emphasized the possible use of chemically treated arteries as biocompatible scaffolds. However, a good knowledge of their physical properties is thus required.

This study aims at evaluating the mechanical properties of small-diameter arterial materials immersed in physiological buffer at 37 °C, using a tensile testing instrument and a curve fitting method. Using native and treated rat arteries, we studied the influence of tensile rate on Young moduli by varying the frequency. Arteries are also known to be anisotropic due to smooth muscle cells and matrix orientation. For example, biaxial tensile tests of square pieces of porcine coronaries evidenced artery anisotropy and a marked nonlinear elastic behavior in both directions (Prendergast et al., 2003; Lally et al., 2004). For this reason, tensile tests were carried out here in longitudinal and transversal directions. We also investigated the variation of mechanical properties along the vasculature by comparing Young moduli of thoracic and abdominal arteries.

2. Experimental part

2.1. Artery preparation

Rats were adult male Wistar rats (Breeding Center René Janvier). A total of 30 rats were used of this study. The procedure and the animal care complied with the Principles of Laboratory Animal Care formulated by the Institute of Laboratory Animal Resources. The studies were carried out under authorization number 006235 of the Ministère de l'Agriculture, France. General anesthesia was obtained by intra-peritoneal injection of pentobarbital (0.1 ml/100 g), which allowed a major sedation with spontaneous ventilation. For aorta removal, the abdominal wall was incised, the pericardium was pushed aside, and then aorta leaving the left ventricle and going down along the spinal column was located. For the thoracic arteries, a segment of about 3 cm was cut 2 cm below the aortic arch. For the abdominal arteries, a segment of about 4 cm was centered on the middle of the aorta and was cut. In order to estimate the in vivo strain of the artery, each section was seized between two forceps and measured before and after its removal. The in vivo strain ε_v was then defined as follows:

$$\varepsilon_v = \frac{L_{before} - L_{after}}{L_{after}} \times 100. \quad (1)$$

L_{before} and L_{after} are respectively the lengths of the sample before and after sampling. Taking into account this in vivo longitudinal strain, the strain values presented in Section 3 will most often be higher than 15%. The in vivo transversal strain being estimated around 7% (Maurice et al., 2005), low strains are presented for transversal assays.

The removed arteries were left in a 0.9% NaCl solution (CDM Lavoisier Laboratories) in order to avoid drying. Forceps and scalpels were used to remove periadventitial tissue around the vessel, then a segment of approximately 1.5 cm was cut and placed in culture media (DMEM; Gibco) until tensile tests. A viability test using MTT (3-(4,5-dimethyldiazol-2-yl)-2,5-diphenyl tetrazolium bromide; Sigma) was used to evaluate the cell viability. We found that human vascular cells were functional for 24 h. In our experiments, mechanical tests were all performed within the 6 h after removal from the animals. The chemical treatment protocol was based on previous studies from our laboratory (Allaire et al., 1997) and was slightly modified. Successive baths were performed at 37 °C: 0.3% sodium dodecylsulphate (SDS) overnight bath, Phosphate buffer saline (PBS) for 30 min (repeated twice), 2.5% Triton bath for 30 min (repeated twice), PBS for 3 h (repeated three times), and PBS overnight.

2.2. Measurements

The measurement device (Fig. 1A) is intended to test various soft materials and biomaterials in physiological conditions, namely immersed in physiological buffer at 37 °C. The nominal displacement range is ±9 mm and the force range is 1 N. The force sensor (FGP instrument: nominal extent 1 N) is linked to the superior jaw while the displacement sensor (Schlumberger: nominal extent ±9 mm) is joined to the inferior jaw. Longitudinal and transversal stretching were performed both on native and treated arteries. For longitudinal tests, the arteries were