CRYOGRAFTS IMPLANTATION IN HUMAN CIRCULATION WOULD ENSURE A PHYSIOLOGICAL TRANSITION IN THE ARTERIAL WALL ENERGETICS, DAMPING AND WAVE REFLECTION

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Short title: Arterial cryograft and native arteries: functional matching

SUMMARY

Each artery conduces blood (conduit function, CF) and smoothes out the pulsatility (buffering function, BF), while keeping its wall protected against the high oscillations of the pulse waves (damping function, ξ). These functions depend on each segment visco-elasticity and capability to store and dissipate energy. When a graft/prosthesis is implanted, the physiological gradual transition in the visco-elasticity and functionality of adjacent arterial segments is disrupted. It remains to be elucidated if the cryografts would allow keeping the physiological biomechanical transition. Aim: to evaluate the cryografts capability to reproduce the functional, energetic and reflection properties of patients' arteries and fresh-homografts. Common carotid's pressure, diameter and wall-thickness were recorded *in vivo* (15 patients) and *in vitro* (15 cryografts and 15 fresh-homografts from donors). Calculus: elastic (E_{pd}) and viscous (V_{pd}) indexes, CF, BF, dissipated (W_D) and stored (W_{PS}) energy and ξ. The graft-patient's artery matching was evaluated using the reflection coefficient (Γ) and reflected power (W_Γ). Cryografts did not show differences in E_{pd}, V_{pd}, BF, CF, W_D, W_{PS}, and ξ, respect to fresh-homografts and patients' arteries, ensuring a reduced Γ and W_Γ. Cryografts could be considered alternatives in arterial reconstructions since they ensure the gradual transition of patients' arteries biomechanical and functional behavior.

Keywords: Arterial wall, visco-elasticity, cryopreservation, buffer function, conduit function

INTRODUCTION

The large systemic arteries can be analyzed as a transmission line, that consist of cylindrical segments that distribute the blood to the different tissues (conduit function, CF), at the time they smooth out the pressure and flow pulsatility (buffer function, BF), caused by the intermittent ventricular ejection (Nichols and O'Rourke 1998). The arteries' function also entail their role as determinants of the left ventricular afterload, and in keeping the wall segment protected against the structural injuries that could be caused by the high frequency components of the pressure and flow waves (wall damping or auto-protection function, ξ) (Armentano *et al.* 2006; Armentano *et al.* 2007; Nichols and O'Rourke 1998). All these functions depend on the arterial geometrical and visco-elastic properties, determined by the individual contribution and the arrangement among passive (elastin and collagen fibers) and active (smooth muscle cells) components of the arterial wall, which in turn, modulate the wall capability to store, transfer and dissipate energy (Barra *et al.* 1993; Armentano *et al.* 1995A; Shadwick 1999; Wells *et al.* 1999; Bia *et al.* 2005A; Armentano *et al.* 2006, Armentano *et al.* 2007). Normally, there is a gradual change in the large arteries properties, with an increase in the arterial impedance, elasticity and viscosity towards the periphery, without great differences between consecutive segments (Nichols and O'Rourke 1998; Bia *et al.* 2005C; Li 2000). Hence, in physiological conditions the impedance and the visco-elastic matching between consecutive segments is high (Li 2000). The gradual change in the arterial geometry and mechanical/functional behavior is advantageous for the cardiovascular system since it prevents: (1) the generation of wave reflection sites near the heart, keeping the ventricle afterload reduced, and (2) the generation of local vascular disturbs that have been associated to the development of vascular alterations (i.e. luminal obstruction by intimal hyperplasia) (Li 2000; Cabrera *et al.* 2005).

The importance of the gradual changes in the mechanical properties of consecutive arterial segments is also recognized in the vascular surgical field, since a non-physiological mechanical matching between the native arteries and the vascular grafts or prosthesis has been associated to the vascular by-pass or reconstruction failure (Tai *et al.* 1999; Seifalian *et al.* 2002; Cabrera *et al.* 2005). Consequently, the ideal arterial substitute must have identical visco-elastic and impedance levels respect to the patient's artery, to reduce the local mechanical mismatch, the pulse wave amplification and the generation of wave reflections (Tai *et al.* 1999; Seifalian *et al.* 2002; Cabrera *et al.* 2005). However, unfortunately the biomechanical properties of the current venous or synthetic prostheses are very different to that of the native artery, and they continue to fail at high rates (Cabrera *et al.* 2005; Abbott *et al.* 1987). Additionally, it is difficult to obtain an autologous medium or large artery of adequate length and size to be used in arterial by-pass or reconstruction, without altering the perfusion of an organ or essential part. In lack of suitable autologous arteries, mainly two kinds of preserved arterial segments are used as vascular substitutes: fresh arteries (fresh-homografts), stored during a short term at low temperatures (i.e. 4ºC), and cryopreserved arteries (cryografts), stored at lower temperatures (i.e. –142ºC) and thawed at the time to be used. Fresh-homografts have theoretical advantages over cryografts since their visco-elastic properties would not be altered by the cryopreservation/thawing process (i.e. cryoprotectants agents toxicity, temperature levels and rates). On the contrary, the key advantage of cryopreservation is that it allows long-term banking of blood vessels for elective use in surgeries (Albertini *et al.* 2000; Kreienberg *et al.* 2002; Pascual *et al.* 2004). Anyway, considering the described potential damages to the arterial components during cryopreservation (Rosset *et*

al. 1996; Rigol *et al.* 2000), it must be evaluated if the cryografts would allow keeping the gradual transition of the mechanical and functional properties of the arteries, when used as vascular substitutes.

An adequate mechanical evaluation of cryografts requires considering several methodological aspects. First, although the mechanical studies of fresh-homografts and cryografts are frequently performed in rings or blades (Pascual *et al.* 2004; Vischjager *et al.* 1996; Adham *et al.* 1996), the use of vascular segments allows a better analysis of the mechanical properties in a dynamic way, reproducing the *in vivo* hemodynamic and geometrical conditions and preserving the shape and integrity of the arterial wall (Blondel *et al.* 2000; Langerak *et al.* 2001; Bia *et al.* 2005B). Second, considering the pressure and frequency-dependence of the arterial wall properties isobaric, isofrequency and dynamic analysis are necessary to perform an appropriate vascular mechanical evaluation (Armentano *et al.* 1995A; Bia *et al.* 2005A,B). Finally, when cryografts are used for clinical purposes, they are interposed between patients' arteries, so the evaluation of the cryograft's usefulness requires comparing them with the patients' arteries.

In this context, this work's main aim was to evaluate the capability of human arterial cryografts to reproduce the functional, energetic and reflection properties of patients' native arteries. Additionally, considering that fresh-homografts have been recently considered the most adequate vascular grafts in small arteries surgeries, we compared the patients' arteries and cryografts with fresh-homografts, so as to evaluate if the cryografts could be considered as an alternative when fresh-homografts are not available. To fulfill the aims we employed an original isobaric, isofrequency and dynamic *in vitro/in vivo* approach, and we quantified the arterial conduit and buffer function, the capability to store/transfer and dissipate energy and to protect the wall components (damping function). Additionally, we calculated the reflection properties expected to be found if cryografts or fresh-homografts were sutured to the patient's arteries.

METHODS

The study comprised the recording of common carotid artery (CCA) pressure and diameter: a) *in vivo*, obtained non-invasively in normotensive patients (n=15), and b) *in vitro*, in fresh-homografts (n=15) and cryografts (n=15) from donors.

Non-invasive study

Fifteen normotensive male patients (51±11.5 years old, body mass index 24±1 kg/m^2) were included. After given written consent, the subjects were examined in a quiet room with controlled temperature (20±1°C), while in the recumbent position, after 10 minutes of rest. The same physician, trained in these vascular examinations, performed all the measurements.

Echographic studies were performed with a real-time B-mode ultrasound imager (ATL HDI 5000, Miami Lakes, USA). The left CCA was examined, at 3 cm proximal to the bifurcation, with a 7.5 MHz probe. The sound beam was adjusted perpendicularly to the arterial surface of the far wall of the vessel to obtain two parallel echogenic lines corresponding to the lumen-intima and media-adventitia interfaces along at least 1 cm of the segment to be measured. A fixed image (end-diastolic electrocardiogram triggering) and a sequence of images were acquired to assess the intima-media thickness (IMT) and to determine the instantaneous arterial diameter, respectively (Armentano *et al.* 1998). To quantify the diameter, the image analysis involved the automatic detection of the anterior and posterior walls interfaces (Iôtec System, Paris, France), using a

procedure previously validated against the sonomicrometric technique (Graf *et al.* 1999).

The CCA pressure waveform was recorded with a tonometer (Millar Instruments Inc.) at the same site of the diameter measurement (Armentano *et al.* 1998; Armentano *et al.* 1995B). The instantaneous pressure waveforms were digitized every 1 ms. The CCA pressure signal was calibrated by assigning the diastolic pressure measured by brachial sphygmomanometry to the minimum value obtained in the CCA, and the mean pressure (calculated from the sphygmomanometric values as a 1/3 of pulse pressure plus diastolic pressure), to the average (Armentano *et al.* 1995B). During both, the diameter and pressure measurement, the electrocardiogram was acquired. These signals were interpolated in time in order to obtain the same number of data points, calculating the averaged cycle (Armentano *et al.* 1998; Armentano *et al.* 1995B).

In vitro study

The CCAs to be used were procured with surgical aseptic techniques, from 15 donors in brain death conditions. Donors mean age was 29.2 years old (range 23–44). All procedures of vascular tissue procurement and processing took into account ethical and safety concerns for therapeutic use, and included consent documentation according to legal ruler Nº 14.005 and Nº 17.668 of Uruguay. General and particular exclusion criteria agreed with the International Standards on Tissue Banks issued by the International Atomic Energy Agency, and with the Standards of the Spanish Association of Tissue Banks, respectively (Bia *et al.* 2005B). Each CCA was carefully cleared from surrounding tissues after the removal of the sternocleidomastoideus muscle. Before being taken away, a CCA segment (5-cm in length) was marked in situ with two sutures in the adventitia. After the segments were harvested, both CCA (right and left) were washed with saline solution. The complete procedure (warm ischemia) lasted 55-68 minutes (mean 60.6 min). All segments were temporarily stored during 24-48 hours (cold ischemia) at 4°C in saline solution with antibiotics. Finally, the procured segments assigned to the fresh-homograft group were submitted to biomechanical tests, while the segments of the cryograft group were submitted to the cryopreservation procedure.

Cryopreservation and thawing

The CCA segments were immersed during 30 minutes in a final volume of 0.085 l cryopreservant solution at 20ºC [RPMI 1640 medium: 85%; 20%-human albumin solution: 5%; and dimethilsulphoxide (DMSO): 10%] in a cryoresistant bag (Joisten & Kettenbaum, Bergisch Gladbach, Germany), thermally sealed under laminar flow conditions (Microflow Laminar Flow Work Station, MDH Ltd., Hants, UK). A programmed cryopreservation was carried out in a controlled rate freezing system (Model 9000, Gordinier Electronics, Inc., Roseville, Michigan, USA). To establish the cooling rate we modified a protocol from Pegg *et al.*, used in rabbit CCAs (Pegg *et al.* 1997). Our protocol consisted in two operative time steps: first, a slow programmed cooling rate with a mean value of 1ºC/min until -90ºC; second, a rapid cooling rate obtained by the immediate transference of the bag to the gas phase of liquid nitrogen compartment (-142ºC). The arterial segments were stored during 30 days at -142ºC (Temperature and Liquid Level Controller, Taylor-Wharton, Theodore, Alabama, USA) and after the storage period, they were thawed. Our standard thawing protocol comprised a two-stage process, obtained by modifying Pegg *et al.* method (Pegg *et al.* 1997). First, a slow warming was achieved by transferring the bag from the nitrogen gas phase to the room temperature (20ºC) during 30 minutes. Second, a rapid warming was achieved by transferring the bag to a 40ºC water bath until completely defrost. To prevent the cellular damage, once the segments were thawed, the cryoprotectant solution was

gradually removed (at 20ºC) in four steps of 10 min, by immersion in tapered concentrations of DMSO in saline solution (10%, 5%, 2.5%, and 0%). Finally the cryografts were sent in saline solution to be biomechanically tested.

In vitro biomechanical tests

Each CCA segment was non-traumatically mounted on specifically designed cannula of the flow circuit loop (Figure 1), immersed and perfused with oxygenated Tyrode's solution (37ºC, pH=7.4). The perfusion line consisted in polyethylene tubing and a Windkessel chamber, powered by a pneumatic pump, regulated by an air supply machine that allowed fine adjustments of the pump rate, pressure values and waveforms (Cabrera *et al.* 2005; Bia *et al.* 2005A,B). Pressure was measured with a microtransducer (1200 Hz frequency response, Konigsberg Instruments, Inc., Pasadena, CA, USA) laterally placed in the proximal cannula. Arterial diameter was measured with a pair of ultrasonic dimension gauges (5MHz, 2 mm) sutured to the vessel. The transit time of the ultrasonic signal (1580 m/s) was converted into distance by means of a sonomicrometer (1000 Hz frequency response, Triton Technology Inc. San Diego, CA, USA).

Once placed in the organ chamber, all segments were allowed to equilibrate for a period of 15 minutes under a steady flow (0.40-0.45 l/min) and stretching rate (~80 beats per minute). The Windkessel chamber and tubing resistance were regulated to generate an intra-vascular pulse pressure of ~ 5.32 kPa (40 mmHg) and a mean pressure of ~11.31 kPa (85 mmHg). By modifying the circuit loop controls we reproduced *in vitro* the pressure and pump rate levels, and the pressure waveforms observed *in vivo*, so as to obtain physiological pressure-diameter loops (Figure 2). Pressure and diameter signals of 10-20 consecutive beats were sampled every 5 ms.. At the end of the test, the segment was weighted to estimate the wall thickness as:

$$\text{Eq. 1.}\quad \text{CCA wall thickness} = R_e - \sqrt{R_e^2 - \frac{V}{\pi \cdot L}}$$

where R_e is the external radii measured by sonomicrometry, V is the segment volume (calculated using the weight of the CCA segment and assuming a tissue density of 1.055 g/mL), and L its *in vivo* length (Barra e*t al.* 1993; Armentano *et al.* 1995A).

Calculations

Arterial viscous and elastic indexes, and conduit and buffer functions

The visco-elastic properties of the arterial wall were studied using a Kelvin-Voigt model (spring-dashpot). Accordingly, the pressure developed in the wall (P_{total}) to resist stretching is separated into viscous ($P_{viscous}$) and elastic ($P_{elastic}$) components (Bauer *et al.* 1979; Armentano *et al.* 1995A; Bia *et al.* 2005A):

$$\text{Eq. 2.}\quad P_{total\ (t)} = P_{elastic\ (t)} + P_{viscous(t)} = E_{pd} \cdot D_{(t)} + V_{pd} \cdot \frac{dD_{(t)}}{dt}$$

where E_{pd} and V_{pd} are the pressure-diameter elastic and viscous indexes, respectively; D is the arterial diameter, and dD/dt is the first derivative of the diameter with respect to time. As the $P_{viscous}$ is proportional to dD/dt, the $P_{elastic}$ can be expressed as:

$$\text{Eq. 3.}\quad P_{elastic} = P_{total} - V_{pd} \frac{dD}{dt}$$

In order to quantify the viscous properties, the viscous term was subtracted from P_{total}, through the criterion of disappearance of the hysteresis loop (Bauer *et al.* 1979; Armentano *et al.* 1995; Recchia *et al.* 1999). To calculate V_{pd}, based on the mentioned method, the diameter signal was shifted to fit the pressure signal foot, avoiding the phase delay due to the pressure and diameter measurements' distance (Bia *et al.* 2004). Increasing values of V_{pd} were given by visually inspecting the reduction of the hysteresis loop area. When the area reached a minimum (considered as the value that preserved the clockwise course of the loop), the iterative process was stopped and the corresponding value of V_{pd} was considered as the viscous index value (Bauer *et al.* 1979; Armentano *et al.* 1995). The diastolic pressure-diameter relationship was used to calculate the elastic index (E_{pd}) as the slope of a linear regression fit (Armentano *et al.* 1995A; Bia *et al.* 2004; Bia *et al.* 2005A). Almost, two considerations should be done. First, despite of the recognized non-linearity of the arterial pressure-diameter relationship, we used a linear fit to calculate the elastic index since the pressure-diameter relationship shows a linear behavior in our study range of pressure (below the break point, normally localized between 120-130 mmHg) (Armentano *et al.* 1991; Armentano *et al.* 1995A). Second, to calculate the elastic index we considered only the diastolic phase of the pressure-diameter loop, since its lower harmonic content matches the pure elastic pressure-diameter or stress-strain relationship (Cabrera *et al.* 2002; Armentano *et al.* 1995A). In fact, the use of the diastolic phase of the pressure-diameter relationship (also named "purely elastic relationship") allows studying the elastic component of the arterial wall dynamic behavior, avoiding (or at least minimizing) the contribution of the wall viscous or frequency-dependent mechanical behavior (Bia *et al.* 2004, Armentano *et al.* 1995A). This approach, was proposed by Bauer *et al.* (Bauer *et al.* 1979), and used by us and other groups in the arterial wall visco-elastic characterization (Recchia *et al.* 1999; Cabrera *et al.* 2002; Cabrera *et al.* 2005; Bia *et al.* 2004).

In agreement with previous works, the arterial wall BF was determined by the V_{pd}/E_{pd} quotient (Westerhof & Noordergraaf 1970; Armentano *et al.* 1995A; Bia *et al.* 2005A). An elevated quotient represents a high buffering effect, with an increased attenuation of pressure oscillations. Additionally, the local CF was evaluated as the inverse of the arterial characteristic impedance (Zc). The arterial Zc is defined as the impedance to flow at the site of measurement, in the absence of reflected waves. It correlates directly with the elastic properties and inversely with the arterial cross sectional area (or diameter). A high Z_C implies high resistance to the blood flow, resulting in a reduced capacity to conduct blood. To ensure an adequate mean arterial pressure (necessary to overcome the peripheral resistance) and to minimize the ventricle work and the wave reflections, large and medium arteries must present low Zc to the ventricle pulsate flow (Cholley *et al.* 2001; Pepine *et al.* 1982). Therefore, the capability to conduce blood (the CF) was analyzed as $1/Z_C$. The Z_C was calculated as:

$$\text{Eq. 4.} \quad Z_C = \frac{\rho_b \cdot PWV}{\text{ACSDA}}$$

where ρ_b is the blood density (assumed equal to 1.055 g/mL), ACSDA is the arterial cross sectional diastolic area (assuming a cylindrical geometry: $\pi.R^2$, where R is the mean diastolic radii), and PWV is the pulse wave velocity (Nichols and O'Rourke 1998). The PWV was calculated using the Moens-Korteweg's equation (Nichols and O'Rourke 1998):

$$\text{Eq. 5.} \quad PWV = \sqrt{\frac{1334 \cdot E_{pd} \cdot h_m}{2 \cdot R \cdot \rho_a}}$$

where h_m is the mean wall thickness, R is the mean diastolic radii, and ρ_a is the wall density (assumed equal to 1.055 g/mL).

Arterial wall energetic and damping

The pressure-diameter hysteretic loop of the arterial wall evidences that, although most of the strain energy is recovered during the diastolic elastic recovering, there is also a viscous lost of energy. While the stored and recovered energy is important to ensure the blood flow when the ventricle is in diastole, the energy lost due to the wall viscosity helps to avoid transmitting the high-frequency components (harmonics) of the arterial pressure and/or flow waves to the diameter wave, also allowing to attenuate the traveling pressure pulses and preventing reflected pressure waves from resonating in the arterial system (Shadwick 1999; Armentano *et al.* 2006). To characterize the functional contribution of the elastic and viscous properties to the arterial wall energetic, the viscous or dissipated energy (W_D, related to the area of the pressure-diameter loop), and the energy stored in the arterial wall in systole and recovered completely during the arterial unloading in diastole (W_{PS}, peak-strain energy), were calculated as (Armentano *et al.* 2006):

$$\text{Eq. 6. } W_D = \frac{\omega \cdot V_{pd} \cdot ACSP}{\pi} \qquad \text{Eq. 7. } W_{PS} = \frac{2 \cdot E_{pd} \cdot ACSP}{\pi}$$

where ω is the angular frequency ($2 \cdot \pi \cdot$heart rate) and ACSP the arterial cross-sectional pulsatility, calculated as the difference between the arterial systolic (or maximal) and the diastolic (or minimal) cross-sectional areas (Armentano *et al.* 2006).

Vibrations due to high-frequency harmonic components produce structure injuries (Armentano *et al.* 2006; Armentano *et al.* 2007). The aim of wall damping is to reduce accelerating oscillations, to exert an auto-protective effect against the high oscillatory frequencies present in the physiological pressure and flow waves. An optimal damping requires ensuring an optimal energy dissipated/stored-recovered ratio (Thakrar *et al.* 2006; Armentano *et al.* 2006). Consequently, similar to previous works the arterial wall damping, ξ, defined as the ratio of energy dissipated per beat in a given segment to the peak-strain energy stored in the same segment was calculated (Armentano *et al.* 2006):

$$\text{Eq. 8. } \xi = \frac{W_D}{W_{PS}}$$

Reflection coefficients and reflected energy

Wave reflections play an important role in determining the pressure and flow waveforms and levels, and the left ventricle afterload. Despite wave reflection sites exist all over the arterial system (i.e. due to geometric and elastic non-uniformities, branching and impedance mismatch at arterial junctions) the main sites of wave reflections are the peripheral resistances (arterioles) (Li 2005). However, the interposition of a rigid vascular graft (or stent) could determine new reflection sites, and consequently an increased wave reflection related to a reduction in the local vascular protection and the peripheral perfusion efficiency, and to an increase in the ventricular afterload (Dobson *et al.* 2006; Morita *et al.* 2002; Tai *et al.* 2000). Hence, we evaluated the capability of cryografts and fresh-homografts to reproduce the physiological wave reflections levels, by calculating the reflection coefficient and the reflected power for the graft-patients' arteries. The patients' native artery-fresh-homograft ($\Gamma_{NA\text{-}FH}$) and native artery-cryograft ($\Gamma_{NA\text{-}CG}$) reflection coefficient were quantified, using the mean values of Zc, as

(Li 2000; Nichols and O'Rourke 1998):

Eq. 9. $$\Gamma_{NA-FH} \equiv \frac{Z_{C\ native\ artery} - Z_{C\ fresh\,hom\,ograft}}{Z_{C\ native\ artery} + Z_{C\ fresh\,hom\,ograft}}$$

Eq. 10. $$\Gamma_{NA-CG} \equiv \frac{Z_{C\ native\ artery} - Z_{C\ cryograft}}{Z_{C\ native\ artery} + Z_{C\ cryograft}}$$

A Γ=0 indicates optimal matching, a condition in which the local impedance differences or gradients, and consequently the wave reflections are inexistent (Hirayama *et al.* 1997). In contrast, a $\Gamma \neq 0$ (range: 1 to -1) indicate mismatch between the patients' native artery and the graft. Finally, the percentage of the incident power that is reflected (reflected power, W_Γ) in a hypothetical junction native artery-graft was calculated as: $(\Gamma_{NA\text{-}FH})^2$ and $(\Gamma_{NA\text{-}CG})^2$ (Li 2000; Nichols and O'Rourke 1998).

Statistical analysis: All data are reported as mean ± standard deviation. Significant differences were assessed using ANOVA for repeated measures followed by Bonferroni test. A $p<0.05$ was adopted as significative difference. All calculations were performed with SPSS software (version 10.0, Statistical Package for the Social Sciences).

RESULTS

Hemodynamic parameters for all the groups are shown in Table I. There were not differences in pressure and heart or pump rate among the groups, enabling isobaric and isofrequency comparisons for all the parameters listed in Table II.

Table II shows E_{pd}, V_{pd}, buffer and conduit function values. The elastic and viscous indexes were similar among groups. The buffer and conduit function, calculated from *in vitro* measurements, for both, cryografts and fresh-homografts were similar to those calculated non-invasively for the patients' CCA. The similarity between cryografts and fresh-homografts' buffer and conduit functions indicate that our cryopreservant and thawing procedure did not alter the arterial functional capability.

Figure 3 shows the W_D, W_{PS} and ξ calculated for the patients' arteries, cryografts and fresh-homografts. There were non-significant differences among the groups, neither in their capability to dissipate and store/transfer energy, nor in their auto-protective capacity.

The $\Gamma_{NA\text{-}FH}$ and $\Gamma_{NA\text{-}CG}$ values were 0.035 and 0.033 respectively, and consequently the reflected power was lesser than 1% for both grafts (0.13 % and 0.11% for fresh-homografts and cryografts, respectively).

DISCUSSION

The following discussion focuses on the two central aspects of this study, related to the cryograft functional capability:

a) The CCA cryografts showed conduit, buffer, energetic and damping capabilities similar to those observed *in vivo* in normotensive patient's CCAs and *in vitro* in fresh-homografts.

b) The use of a cryograft would minimize the impedance mismatch and the generation of non-physiological wave reflections, ensuring the gradual transition of the arterial visco-elastic, functional, energetic and damping properties, as expected for a fresh-homograft.

Visco-elasticity, and buffer and conduit functions

Our data showed that cryografts' elasticity and viscosity were similar to those of the fresh-homografts, suggesting that the cryopreservation/thawing procedure did not modify the arterial biomechanical properties. Elastin, collagen and smooth muscle cells mainly determine the arterial wall visco-elastic properties. Hence, our results could indicate that during the cryopreservation/thawing procedure there were no changes, neither in the structure, nor in the fibrillar/cellular organization, that could impair the arterial wall mechanics.

Cryografts and patients' arteries showed no differences in the elastic index. Under physiological hemodynamic conditions (as was the case of our study), the wall elasticity is determined mainly by the elastin fibers, with a lesser contribution of the collagen and smooth muscle. In fact, the elastic index values found in this work and the elastin elastic module reported previously are in the same order (Table II) (Barra *et al.* 1993; Armentano *et al.* 1995A). It is to note that the described age-dependent elastic differences were not evidenced in our work (Hansen *et al.* 1995). This could be explained by at least three factors. ***First***, the study of arteries from individuals of different age at the same mean and pulse pressure conditions could have reduced the potential pressure-dependent differences in the elastic properties. In this sense, a consideration should be done related to the difference between patients and multiorgan donors' age. In our study, donors mean age was 29 years old, while patients averaged 51 years old. Generally, arteries to be considered vascular grafts, according to the international protocols and rules belong to young donors. However, patients requiring vascular grafts are frequently older. Hence, we reproduced this difference by selecting patients averaging 50 years old. ***Second***, in arteries evaluated *in vitro* the elastic module observed was higher than that of *in vivo* arteries, which has been attributed to experimental handling and/or to potential changes in the vascular wall control systems (Wells *et al.* 1999; Cabrera *et al.* 2006). ***Third***, according to tissue banking protocols, the harvested arteries were immersed in an antibiotic solution before being mechanically tested, and during this period the mechanical properties could have been modified.

Taking into account that the smooth muscle tone was expected to be lesser *in vitro* than *in vivo*, we also expected a higher viscous level in the patient's arteries. However, when analyzing the arterial wall viscosity, we found that the cryografts' and fresh-homograft viscous behavior was similar to that of the patients' arteries. This could be explained by the fact that the arteries studied *in vitro* were from individuals younger than the patients. Therefore, the described decrease in viscosity with age could have counterbalanced the tone-dependent viscous differences between *in vitro* and *in vivo* (Wells *et al.* 1999).

Other studies analyzing the elastic behavior of fresh and cryopreserved arteries showed results similar to ours. In those studies the mechanical properties of the elastin and collagen fibers, the collagen recruitment point, as well as the breaking stress, remained unchanged after cryopreservation (Adham *et al.* 1996; Blondel *et al.* 2000; Langerak *et al.* 2001; Pukacki *et al.* 2000). On the contrary, our results disagree with those of Rosset *et al.* (Rosset *et al.* 1996) who found changes in several mechanical parameters (i.e. compliance, stiffness index, mean relative arterial pulsatility) of human carotid arteries during cryopreservation. The dissimilar results between our study and those of Rosset *et al.* could be at least partially ascribed to the works' differences in methodological factors related to tissue injury during cryopreservation/thawing. We found not works related to the cryopreservation/thawing effects on wall viscosity. In this sense, despite of Rosset *et al.* described a reduction in

the area of the cryopreserved CCA pressure-diameter loop (related to the wall viscous behavior), they did not calculate the wall viscosity or indexes related to the pressure-diameter hysteretic area.

Our results showed similar values among cryografts, fresh-homografts and patients' arteries functional behavior. Thus, it could be said that the used cryopreservation/thawing procedures did not modify the buffer capability of the cryografts, which as well showed a buffer capacity similar to that of potential recipients. Additionally, at least in theoretical terms, the implantation of our cryografts in the carotid region would not reduce the conduit capacity of the vascular circuit; hence they would keep the arterial conduction in physiological levels.

Energetic, damping and reflection properties

Our results showed that the wall capability to store/transfer and to dissipate energy was similar among cryografts, fresh-homografts and patient's arteries (Figure 3). Consequently, the employed cryopreservation/thawing procedure allowed obtaining a cryograft with energetic properties identical to those of the patients' arteries. So, at least theoretically, a cryograft implanted in the carotid circulation would keep the arterial wall energy dissipation, avoiding the increase in the ventricle afterload, as well as the reduction in the wall protection related, respectively, to augmented and reduced energy dissipation levels. In the same way, since the cryografts energy storage/transfer capability was similar to that of the patient's native arteries, the graft implantation would not change the energy necessary to strain the artery during the systolic distension, and returned to the system during the diastolic recoil. Hence, when used as large arteries substitutes, the cryograft would allow keeping in physiological levels the arterial-dependent determinants of the left ventricle afterload and would ensure an adequate supplementary blood pumping action of the arterial wall during the diastolic recoil.

When evaluating the wall damping we found that the auto-protective capability was similar among the cryografts, fresh-homografts and patients' arteries. The arterial wall remodeling (i.e. intimal hyperplasia, medial hypertrophy) during hypertension and the venous graft remodeling have been interpreted as a compensatory mechanism to increase the damping and consequently the wall auto-protection (Armentano *et al.* 2006; Armentano *et al.* 2007; Zócalo *et al.* 2006). Although, the remodeling would be beneficial for the vascular wall itself, it could determine the lumen occlusion and consequently the graft failure. Hence, taking into account our results it could be said that the cryografts would ensure a physiological damping level, avoiding the remodeling process and their consequent detrimental effects on the blood flow and graft patency.

Finally, our results showed that the interposition of a cryograft in the patients' arterial system would ensure a high graft-native artery impedance matching, avoiding new proximal source of wave reflection (low reflection coefficient and reflected power). Consequently, contrary to previous works in which the effects of stiff prosthesis were evaluated (Dobson *et al.* 2006; Morita *et al.* 2002), finding an increase in wave reflections, the interposition of a cryograft would determine both, reduced haemodynamics alterations in the graft-native artery junction and reduced ventricle afterload (avoiding the early return of reflected waves to the heart).

Applied Physiology

An ideal biomaterial to be used as arterial graft has been a subject of intense investigation. Although many substantial

progresses have been reached, the "optimal or ideal" arterial substitute remains elusive. Among the proposed vascular substitutes are the homografts from multiorgan donors. However, there have been controversies related to the most adequate method of preservation (i.e., fresh or cryopreserved). The cryografts advantages are their major protection against infection transmission and the longer storage-period (Albertini *et al.* 2000). Additionally, the progresses reached during the last few years, in minimizing the tissue damage due to the cryoprotectant agents used and the temperatures levels and rates, determined an increase in the cryografts use (Albertini *et al.* 2000; Pascual *et al.* 2004).

In this work, we found that if our arterial cryografts were to be used as arterial substitutes, the physiological levels of the arterial visco-elastic behavior, conduit and buffer function, energetics and auto-protective (damping) capability would be preserved. Moreover, all parameters were similar to those of fresh-homografts, considered as the "most adequate grafts" in the surgical field. Hence, the cryograft could be considered a safety graft that would ensure the physiological gradual change in the arterial mechanical and functional properties between adjacent native arteries. So, the surgeon could be able to use a graft that overcomes the limitations of the available, related to their differences in the wall properties, respect to the native arteries.

Recently, Jacot et al. employed duplex ultrasound scan to quantify the temporal changes in elastic properties and wall dimensions in lower-extremity vein grafts after implantation (Jacot *et al.* 2004). However, as the same author stated, a more accurately method to determine quantitative changes in the vascular thickness and visco-elastic properties over time, is needed. In this context, the non-invasive method used in this work to evaluate the arterial visco-elasticity, wall thickness, conduit and buffer function, energetics and damping properties, could be considered an alternative approach to analyze the homograft functionality after implantation. This methodology could also help to follow the cryograft adaptation over time and to evaluate its response to physiological and pathological haemodynamic states.

CONCLUSION

The conduit and buffer function, energetics, damping and wave reflection properties of arterial cryograft, fresh-homografts and patients' arteries were characterized, on the basis of the analysis of pressure and diameter waveforms recordings. Cryografts showed similar visco-elasticity, conduit and buffer function capabilities, wall energetics, damping and reflection properties respect to fresh-homografts and patient's arteries. The cryografts implantation in human circulation would ensure the physiological transition in the arterial visco-elasticity, buffer and conduit function, energetics, damping and wave reflection.

ACKNOWLEDGEMENTS

This work was performed within a cooperation agreement between Universidad de la República (Uruguay)–Universidad Favaloro (Argentina) and it was partially supported by PEDECIBA-BIOLOGIA and CSIC-UdelaR (Uruguay), and the René Favaloro University Foundation (Argentina). The authors gratefully acknowledge the technical assistance of Mr. Elbio Agote.

FIGURE LEGENDS

Figure 1. Experimental apparatus for arterial segment testing. PP: Pneumatic pump. R: Resistance modulator. Chamber: thermally regulated chamber containing Tyrode's solution at 37°C. Reservoir: reservoir containing oxygenated Tyrode's

solution at 37°C. PM: Pressure microtransducer. D-D': Diameter sonomicrometric gauges. Thick arrows indicate flow direction. Insert shows the arterial segment instrumentation.

Figure 2. Left: Cryograft pressure (thick line) and diameter (thin line) waves. Note the physiological pressure levels and waves forms, generated in the segment. Right: Pressure-diameter loop of the same cryograft. Note the loop hysteresis and the quasi-linear diastolic decay, related to the viscous and the purely elastic properties of the arterial wall, respectively.

Figure 3. Wall energetic and damping capabilities (mean value±standard deviation) of patients' arteries, cryografts and fresh-homografts. W_D and W_{PS}: energy dissipation and peak-strain energy, respectively. Non-significant differences were found.

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TABLES

TABLE I: *IN VIVO* AND *IN VITRO* HEMODYNAMIC PARAMETERS

	Patients' arteries (n=15)	**Fresh-homografts** (n=15)	**Cryografts** (n=15)
Systolic pressure (10^3 N/m^2) [mmHg]	14.50±0.93 [109±7]	14.36±0.53 [108±4]	14.36±0.40 [108±3]
Diastolic pressure (10^3 N/m^2) [mmHg]	8.38±1.06 [63±8]	8.51±0.53 [64±4]	8.11±0.53 [61±4]
Pulse pressure (10^3 N/m^2) [mmHg]	5.85± [44±10]	5.72±0.53 [43±4]	5.85±0.40 [44±3]
Mean pressure (10^3 N/m^2) [mmHg]	10.51±0.80 [79±6]	10.37±0.40 [78±3]	10.24±0.40 [77±3]
Systolic diameter (10^{-3} m)	7.95±0.80	8.55±0.19 [a]	8.48±0.30
Diastolic diameter (10^{-3} m)	7.50±0.89	8.16±0.21 [a]	7.98±0.21
Pulse diameter (10^{-3} m)	0.43±0.13	0.40±0.05	0.43±0.17
Mean diameter (10^{-3} m)	7.64±0.86	8.29±0.20 [a]	8.13±0.23
Heart or pump rate (Hz) [b.p.m.]	1.33±0.11 [80±7]	1.33±0.07 [80±4]	1.35±0.05 [81±3]

Values are: Mean ± SD. Significance: a: $p<0.05$ between Patients' arteries and Fresh-homografts; b: $p<0.05$ between Patients' arteries and Cryografts; c: $p<0.05$ between Fresh-homografts and Cryografts (ANOVA and Bonferroni test).

TABLE II. ARTERIAL VISCOELASTIC AND FUNCTIONAL PARAMETERS

	Patients' arteries (n=15)	**Fresh-homografts** (n=15)	**Cryografts** (n=15)
E_{pd} (10^3 kPa/m) [mmHg/mm]	15.79±2.41 [119±13]	15.06±1.14 [113±9]	15.27±1.03 [113±10]
V_{pd} (10^2 kPa.s/m) [mmHg·s/mm]	4.43±0.58 [3.40±0.60]	4.08±0.24 [3.07±0.18]	4.06±0.37 [3.06±0.27]
Buffer function (10^{-3} s)	28.2±6.1	27.2±3.9	27.1±2.9
Conduit function (cm^3/dyn·s)	59.5±6.1	63.1±3.8	65.9±4.1

Values are: Mean ± SD. E_{pd} and V_{pd}: elastic and viscous indexes, respectively. Significance: a: $p<0.05$ between Patients' arteries and Fresh-homografts; b: $p<0.05$ between Patients' arteries and Cryografts; c: $p<0.05$ between Fresh-homografts and Cryografts (ANOVA and Bonferroni test).

Figure 1

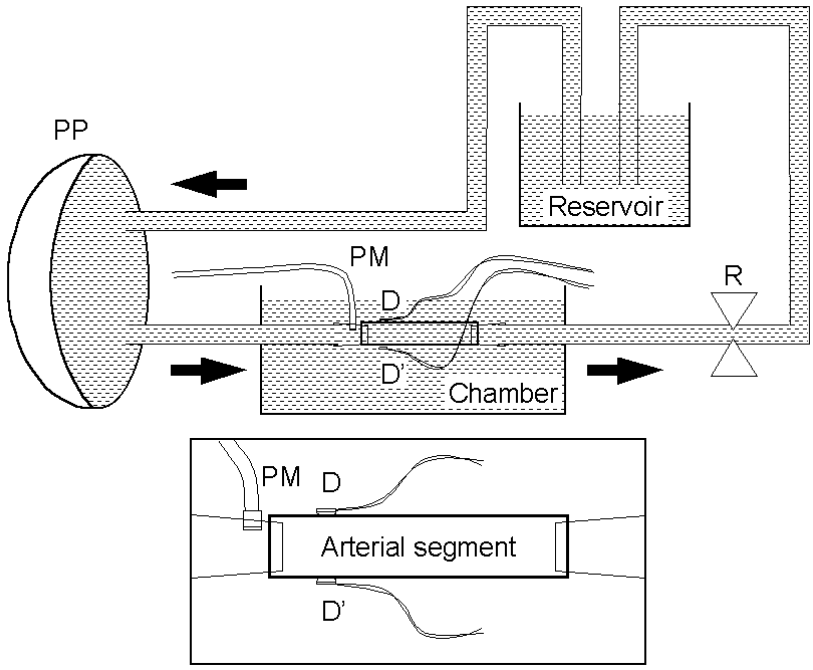


Figure 2

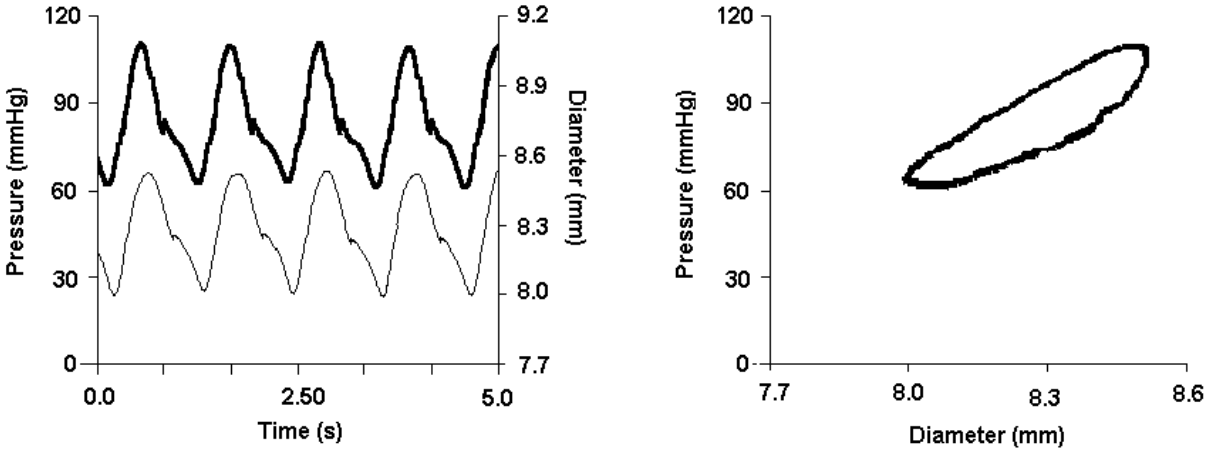
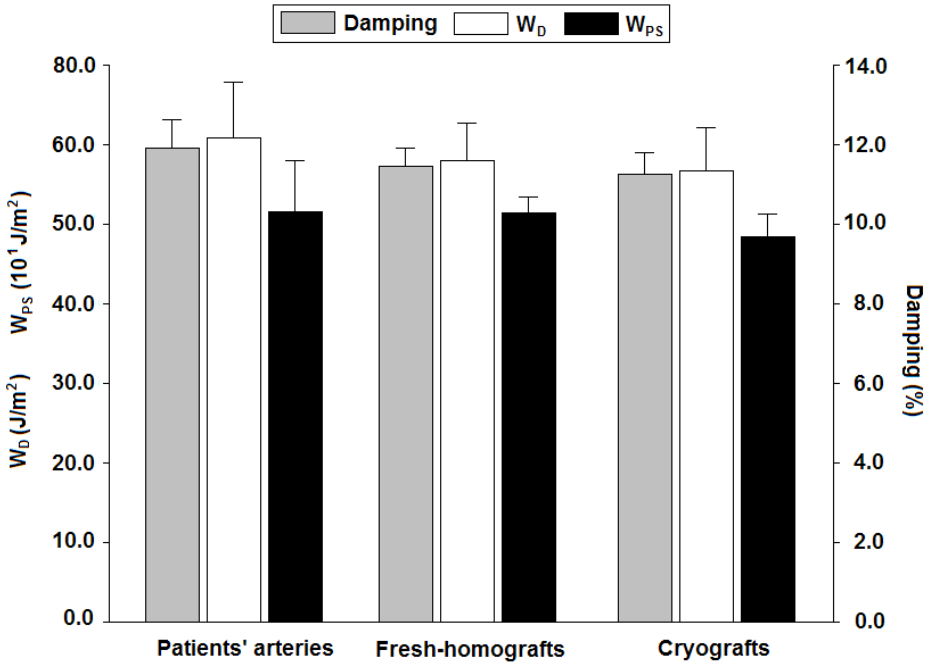


Figure 3


Damping
W_D
W_{PS}
80.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
W_{PS} (10^1 J/m^2)
W_D (J/m^2)
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Damping (%)
Patients' arteries
Fresh-homografts
Cryografts