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# Post-implant evaluation of the anastomotic mechanical and geometrical coupling between human native arteries and arterial cryografts implanted in lower-limb ☆
## Mechanical, histological and ultraestructural studies of implanted cryografts

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ABSTRACT

*Background:* There is an urgent need of vascular substitutes (VS) to be used in lower limb revascularization procedures when autologous veins are not available and synthetic prosthesis are contraindicated. Since the mechanical differences with respect to native vessels are determinants of the VS failure, the substitutes should have mechanical properties similar to those of the recipient vessels. The use of cryopreserved arteries (cryografts) could overcome limitations of available VS. These work aims were to characterize (a) native vessels/implanted cryografts mechanical and geometrical coupling, (b) cryografts capability to ensure mismatch levels lesser than those expected for expanded polytetrafluoroethylene (ePTFE), (c) cryografts functional properties considering their histological and ultra-structural characteristics.

*Methods:* Instantaneous pressure (mechano-transducers) and diameter (B-mode echography) were obtained in implanted femoro-popliteal, ileo-femoro-popliteal and axilo-humeral cryografts ($n = 8$), in femoral arteries from recipients ($n = 8$), recipient-like ($n = 15$) and multiorgan donors-like ($n = 15$) subjects, and in ePTFE segments ($n = 10$). Calculus: (a) Mechanical parameters: elastic modulus, arterial compliance, distensibility and characteristic impedance; (b) Arterial remodeling: diameter, wall thickness, cross-sectional area and wall-to-lumen ratio; (c) Native vessels/VS coupling. Histological and structural analysis were done in explanted femoro-popliteal and axilo-humeral cryografts ($n = 7$).

*Results:* Post-implant the cryografts remodeled. Their stiffness increased and the conduit function diminished. Remodeling resulted in an improvement in native vessels/cryograft coupling, which was always better than native vessels/ePTFE coupling.

*Conclusions:* Post-implant cryograft remodeling improved native vessels/cryografts coupling. Cryografts would have mechanical and geometrical advantages over ePTFE. Anastomotic cryograft remodeling differed from that expected only due to haemodynamic factors. The structural properties of the remodeled cryografts contribute to explain their functional characteristics.



*Abbreviations:* σ, arterial circumferential stress; ε, arterial strain; Γ, coupling factor; AC, arterial compliance; AD, arterial distensibility; ASMA, anti-alpha-smooth muscle actin monoclonal antibody; CSA, cross-sectional area; DBP, diastolic blood pressure; DD, diastolic diameter; $E_{INC}$, incremental elastic modulus; ePTFE, expanded polytetrafluoroethylene; MOD, multi-organ donors; PWV, arterial pulse wave velocity; SBP, systolic blood pressure; SD, systolic diameter; VS, vascular substitutes; Zc, arterial characteristic impedance.

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## Introduction

There is an urgent need of vascular substitutes (VS) to be used in lower limb revascularization procedures when autologous veins are not available and synthetic prosthesis are contraindicated (i.e. in infected fields). Since the mechanical differences with respect to native vessels determine the VS failure, substitutes with properties similar to those of the recipient vessels are necessary [31]. Under arterial haemodynamic conditions, venous grafts and synthetic prosthesis are stiffer than native arteries and show a quasi linear, pure elastic pressure–diameter relationship, instead of the non-linear, viscoelastic arterial behavior [5,37,38]. Looking for overcoming the limitations of the available VS, several alternatives have been proposed. In this context, the use of autologous fresh arteries would be an interesting option. However, except for procedures like coronary bypass, it is difficult to obtain autologous vessels of adequate length and size without significantly altering an organ/tissue blood flow. In the lack of suitable autologous vessels, cryopreserved arteries (cryografts) could be used.

In the past, the reduced availability and problems with preservation/storage limited the cryografts use. However, the recent development of cryopreservation techniques enhanced cryografts availability and value, and renewed the interest in their use. Some issues related with the cryografts and their usefulness as VS remain to be clarified. For instance: (1) *which is the best protocol to cryopreserve arteries?*; (2) *which are the cryopreservation effects on human arteries mechanics?*; (3) *do the cryografts post-implant structural (i.e. histological) changes explain the non-invasive mechanical findings?*; (4) *Do cryografts allow reducing the mechanical and geometrical mismatch generated when venous grafts or synthetic prosthesis are used?*

Several *in vitro* works have been designed to compare cryopreservation protocols, and to evaluate cryopreservation effects on arterial properties [14,17,24,27,30,34,35]. The joint analysis of published data allowed arriving to some consensus related with the best methodologies to cryopreserve/store arteries. However, surprisingly only few works addressed the last two questions stated above.

Our group has demonstrated that (a) the cryopreservation methodologies employed in our national tissue bank maintain the mechanical properties of human saphenous veins [6], and of elastic [7] and muscular human and ovine arteries [4,8], and (b) in some vascular diseases, regardless of their etiology (i.e. hypertension, diabetes, nephropathy), cryografts could allow reducing the mechanical mismatch generated when venous grafts or expanded polytetrafluoroethylene (ePTFE) prosthesis are used (pre-implant studies) [5,2]. In addition, recently using a non-invasive approach, we analyzed the post-implant regional and local mechanical behavior of human cryografts [9]. Continuing our research, in this work we looked for answers to the following questions: (1) *what is the meaning of the cryografts remodeling in terms of their geometrical and mechanical coupling with the native vessels?* (2) *Are the geometrical and mechanical coupling levels between remodeled cryografts and recipient arteries higher than those expected for ePTFE prosthesis?* (3) *Are coupling levels at the anastomotic junction explained only by the recipient hypertensive conditions?*

In this context, this work aims were to:

(1) quantify the mechanical and geometrical coupling between native vessels and implanted cryografts, and to determine if the recipients pressure levels are the coupling levels determinants,
(2) analyze the implanted cryograft capability to ensure geometrical and mechanical mismatch levels lesser than those expected when ePTFE prosthesis are used,
(3) analyze the implanted cryografts mechanical and geometrical properties taking into account the histological and ultra structural characteristics of the cryografts.

## Materials and methods

### *Non invasive mechanical studies*

The study was approved by the Republic University (Uruguay) ethics committee and was done in agreement with the ethical standards of the Declaration of Helsinki. Subjects gave informed consent.

### *Arterial groups*

- *Implanted cryografts:* 8 femoro-popliteal, ileo-femoro-popliteal or axilo-humeral cryografts, implanted in lower limb-salvage procedures, according to General Consent Criteria of the Uruguayan Society of Angiology. The main characteristics were (mean value ± standard deviation, range): length = 45 ± 15, 23–75 cm; cryopreservation time = 98 ± 154, 30–477 days; implant time at study date = 614 ± 211, 368–1088 days; Multi-organ donor's age = 34 ± 9, 21–48 years.

The techniques used to procure arteries, and the cryopreservation, storage and re-warming procedures were previously employed and described in detail [5,4,9]. Briefly, arterial segments were procured from donors during multiple organs and tissue harvesting. Then, the segments were washed with saline solution and stored at 4 °C in a saline solution (NaCl 0.9 g%) with gentamicine (16 mg%), cefuroxime (300 mg%), penicillin G (400.000 IU%), and fluconazol (8 mg%). The warm ischaemia time was 54–63 min (mean = 58 min), and the cold ischaemia was 24–48 h (mean = 32 h). After that, segments were submitted to cryopreservation. To this end, the samples were placed in a sterile bag (volume: 350 cc) containing 85 cc of cryopreservant solution: culture medium (RPMI 1640): 85%; human albumin solution (20%): 5%; and dimethylsulfoxide (DMSO): 10% [10,11]. The bag was sealed hermetically at vacuum (Joisten and Kettenbaum, D51429, Bereisch Gladbach, Mod. 011342) in a laminar flow cabinet (Microflow, Laminar Flow Work Station, MDH Ltd., Wal Worth Road Andover Hants England SP.10.5.AA), and was equilibrated for 30 min at 20 °C. After that, programmed cryopreservation was carried out in a Controlled Rate Freezing System (Model 9000, Gordinier Electronics, Inc., 29975 Parkway, Roseville, MI 48066, USA). For the cooling process, we chose a modified protocol from Pegg's et al. [26]. It consisted in three operative time steps. First, a slow programmed cooling rate with a mean value of −2 °C/min until −40 °C. Second, a slow programmed cooling rate with a mean value of −5 °C/min until −90 °C. Third, a rapid cooling rate obtained by the transference of the bag to the gaseous phase of the liquid nitrogen compartment (−142 °C). The specimens were stored at −142 °C (Mark III, Temperature and Liquid Level Controller, Taylor, Wharton, Theodore, AL, USA) and after the storage period, vessels were re-warmed. In our standard warming protocol, a two-stage process was used, also taking into account Pegg et al. works [26], which highlight the importance of the thawing rate to avoid fractures [13,26,36]. The thawing protocol first step was a slow process, achieved by transferring the bag from the nitrogen gaseous phase to room temperature (20 °C) during 30 min. Then, during the second step, the bag was rapidly transferred to a 40 °C water bath until completely defrost. After thawing, to prevent osmolar stress, the cryoprotectant solution was gradually removed in four 10 min-steps by immersion in tapered concentrations (10%, 5%, 2.5%, and 0% of DMSO) at 20 °C. Finally, arteries were sent in a