Cryopreserved Human Veins Reduces the Vascular Access Biomechanical Mismatch in Chronically Hemodialyzed Patients

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Introduction
Patients in chronic severe renal failure usually require hemodialysis that depends on a proper function of the vascular access (VA), which is performed in order to provide an adequate blood flow during each dialysis session. For a long term treatment, the autologous vessels are used for VA confection. However, native vessels (NV) of chronically hemodyalized patients (CHDP) are not always an option for VA. Then, synthetic conduits have been utilized, being the expanded polytetrafluoroethylene (ePTFE) prostheses the substitute most largely chosen [Hofstra, 1994].

The hemodialysis VA failure is a very important cause of morbidity in hemodialyzed patients, being the thrombosis the main undesirable event that is usually preceded by intimal hyperplasia [Roy-Chaudhury, 2002]. It has been reported that intimal hyperplasia formation is determined by the elastic mismatch between the prosthetic graft and the NV [Cabrera-Fischer, 2005; Haruguchi, 2003]. Intimal hyperplasia decreases the intra vascular lumen causing severe access dysfunction [Haruguchi, 2003; Hofstra, 1995].

As was aforementioned, biomechanical mismatch (BM) between NV and ePTFE is a source of VA failure. In order to reduce the BM, the use of fresh or cryopreserved human veins (FHV and CHV, respectively) obtained from human donors, could be an alternative. We have recently demonstrated that the cryopreservation methodology used in our tissue bank keeps the biomechanical properties of human veins unchanged [Bia, 2007]. However up to now, no studies were performed to determine if the BM observed between NV and ePTFE could be reduced using FHV or CHV.

Purpose
To compare the biomechanical behavior of NV in healthy subjects and in CHDP with that of ePTFE, FHV and CHV.

Material and methods

1. Non-invasive human studies:
Healthy subjects (3 males, 3 females), 35±3 years old, body mass index 24.3±1.5 kg/m² (Group “H”) and patients in chronic severe renal failure (3 males, 3 females), 45±4 years old, body mass index 25.5±1.8 kg/m² (Group “CHDP”, chronically hemodyalized patients) were included in this study. All subjects gave written consent for this non-invasive clinical research.

In all the subjects, the carotid–humeral pulse wave velocity (PWV) was measured following international recommendations [Laurent, 2006].

2. In vitro studies:
Donor criteria selection and tissue procurement: All procedures agreed with ethical and safety
concerns for therapeutic use. Documented consent was obtained according to Nº 14005 and Nº 17668 legal rules of República Oriental del Uruguay. Exclusion criteria agreed with the International Atomic Energy Agency (IAEA, International Standards for Tissue Banks) and the American Association for Tissue Bank (AATB). In 13 multiorgan donors (age: 31±3 years) the left and right great saphenous vein were dissected. Then, vascular segments (5 cm-length) were marked with suture references. After harvesting the segments were washed with saline solution. The venous segments, randomly assigned to the fresh-control group (Group “FHV”), were biomechanically tested. The other venous segments were assigned to the cryopreserved group (Group “CHV”) and were biomechanically tested after 30 days of cryopreservation.

**Cryopreservation procedure:** After incubation in saline and antibiotic solution 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%. 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 minutes at 20 ºC. Then, the programmed cryopreservation was carried out in a Controlled Rate Freezing System (Model 9000, Gordinier Electronics, Inc. 29975 Parkway, Roseville, Michigan 48066 U.S.A.). For cooling we chose a three operative steps protocol [Bia, 2007]. First a slow-programmed cooling rate (2 ºC/min) until –40 ºC. The second step also consisted in a slow cooling rate (5 ºC/min) until –90 ºC. Finally, in the third step, a rapid cooling rate was obtained by transferring the bag to the gaseous phase of the liquid nitrogen compartment (-142 ºC). After the storage period (30 days) at –142 ºC (Mark III, Temperature and Liquid Level Controller, Taylor, Wharton, Theodore, Alabama U.S.A.), the veins were thawed. A two stage warming protocol was selected [Bia, 2007]. First, the bag was transferred from the nitrogen gaseous phase to room temperature (20 ºC) during 30 minutes. Then, the bag was immersed in a 40 ºC water bath, until the segments were completely thawed. After thawing, to prevent osmolar stress, the cryoprotectant solution was removed in four 10 min-steps by immersion in tapered concentrations (10, 5, 2.5, and 0% of DMSO). Finally, the veins were sent, immersed in saline solution to the biomechanics’ laboratory.

**In vitro biomechanical tests:** The vessels were mounted (at in vivo length) in a circulation mock and remained immersed and perfused with Tyrode’s solution (at 37 ºC, oxygenated, pH=7.4) [Bia, 2005]. The perfusion line consisted in polyethylene tubing powered by a pneumatic pump (Jarvik Model 5, Kolff Medical Inc., Salt Lake City, Utah, USA) regulated by an air supply machine [Bia, 2005]. The circulation mock controls allowed adjusting pressure values and waveforms. Pressure was measured with a solid-state transducer (1200 Hz frequency response, Königsberg Instruments, Inc., Pasadena, CA, USA). To measure the vascular external diameter ultrasonic crystals (5 MHz, 2 mm diameter) were sutured to the segments. 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 instrumented, the segments were allowed to equilibrate under a steady state of flow and pressure. In addition, 6 segments (6 cm in length) of ePTFE (Gore-Tex Vascular graft, W.L.Gore & Associates, Inc.,Flagstaff, Arizona, USA) were instrumented and mechanically tested, following similar procedures to those followed for the venous segments.

The segments were evaluated during haemodynamic conditions mimicking the patients’ hemodynamic states. Pressure and diameter signals were measured under dynamic conditions, displayed in real time, digitized every 5 ms and stored for off-line analysis. Twenty consecutive beats were sampled. For each segment, the PWV was calculated using the Moens-Korteweg equation:

\[
PWV = \sqrt{\frac{1333 \cdot E_{pd} \cdot h}{2 \cdot R \cdot \rho}}
\]

where \(E_{pd}\) is the pressure-diameter elastic index, \(h\) is the wall thickness, \(R\) is the midwall radius, and \(\rho\) is the arterial wall density (assumed as 1.06 g/cm³) [Armentano, 2006; Bia, 2005].

### 3. Biomechanical mismatch

The BM was calculated as:

\[
BM = \frac{PWV_{Non-invasive} - PWV_{in vitro}}{PWV_{Non-invasive} + PWV_{in vitro}}
\]

[Bia, 2007]. The BM ranges between 1 and –1. A BM=0 represents an optimal matching (vascular substitute and the native artery with identical biomechanical behavior), while values far from 0 indicate an increasing mismatch [Bia, 2007].

Statistical analysis: PWV values were expressed as mean ± standard deviation (MV±SD). Comparisons among the groups were performed using ANOVA + Bonferroni. A p<0.05 was considered
Results
Table shows the PWV and BM mean values obtained from each group (Table 1).

Table. Pulse wave velocity and biomechanical mismatch between patients’ vessels and grafts

<table>
<thead>
<tr>
<th></th>
<th>H</th>
<th>CHDP</th>
<th>FHV</th>
<th>CHV</th>
<th>ePTFE</th>
</tr>
</thead>
<tbody>
<tr>
<td>PWV [m/s]</td>
<td>8.2±1.3</td>
<td>10.3±0.9†</td>
<td>14.8±1.5 †</td>
<td>14.5±1.5 †</td>
<td>86.1±0.6 ‡</td>
</tr>
<tr>
<td>BM with respect to CHDP</td>
<td>-0.29</td>
<td>-0.26</td>
<td>-0.35</td>
<td>-0.83</td>
<td></td>
</tr>
<tr>
<td>BM with respect to CHV</td>
<td>-0.15</td>
<td>-0.15</td>
<td>-0.78</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

M±SD. Groups: H: healthy subjects; CHDP: chronically hemodialyzed patients; FHV and CHV: fresh and cryo-preserved human veins, respectively; ePTFE: expanded polytetrafluoroethylene. Statistics † and ‡: p<0.05 with respect to PWV of H and CHDP, respectively. * and ‡: p<0.05 with respect to PWV of FHV and CHV, respectively.

As was expected, CHDP showed higher PWV (stiffness) respect to healthy individuals (p<0.05), and the cryopreservation procedures did not modify the biomechanical properties of human veins.

The higher BM was found when ePTFE was studied (p<0.05). No differences in BM were evidenced when FHV and CHV were considered.

Discussion
This work contributes to the knowledge of the potential alternatives in the VA confection, due to its main finding:

Cryopreserved human veins could reduce the biomechanical mismatch existent between synthetic prosthesis and native vessels of patients with chronic renal failure under treatment with chronic hemodialysis.

The research methodology employed in this study has been largely used by our group, both in clinical studies and in in vitro analysis of biomechanical properties of arteries, veins and prosthetic conduits [Bia, 2005; Cabrera Fischer, 2005; Armentano, 2006]. It includes the development and use of biomechanical indexes and the utilization of the well known pulse wave velocity that was employed since the earliest 1890’s.

As was mentioned above, intimal hyperplasia decreases the intra vascular lumen causing severe access dysfunction that determines the failure of renal replacement therapy in uremic patients [Haruguchi, 2003; Hofstra, 1995]. Both, VA performed with native vessels or using ePTFE conduits are affected by obstructive processes, the origin of which is associated with the mechanical mismatch around the anastomoses [Hofstra, 1994]. With the employment of ePTFE conduits in the VA confection the vascular occlusive thickening due to intimal hyperplasia could occur in a brief period of time.

Since renal replacement therapy in chronic patients is highly limited by VA dysfunction several alternatives have been investigated including the use of animal conduits in the arterio-venous fistulae confection. One of them reported the reduction of the elastic mismatch among the conduits used to perform the VA [Galli, 2007]. During the last years, cryopreserved vessels have been used in humans providing a very interesting alternative that has a good performance in infected vascular territories. Besides, the cryopreservation procedure has the advantage that the vessels can be stored and used at discretion [Armentano, 2006].

In this study not only in healthy individuals but also in CHDP, FHV and CHV could reduce the BM observed between NV and ePTFE. Consequently, from the biomechanical point of view CHV could be considered as an alternative to perform VA because their BM is lower than that of the ePTFE and similar to that observed with FHV.

Conclusion
Cryopreservation did not modify PWV of human vein grafts. Since it allows reducing the BM, cryopreserved human veins could be considered alternatives to construct vascular access in patients
with chronic severe renal failure submitted to chronic hemodialysis.

Acknowledgments
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References


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