Research attempts to address developing world needs

Prof Francis E Smit
MD PhD FACC
Fundamentals to Innovation
SA Heart Association Congress
Johannesburg
November 2017
Prosthetic heart valves: Catering for the few

Figure Typical age-distribution of patients undergoing heart valve replacement in the First World and in a Developing Country. While prosthetic valve recipients in a First World population are predominantly in the age group of 60–69 years (red line) they are broadly disseminated over an age spectrum from 20 to 70 years in a Developing Country such as South Africa (blue line). As the age distribution of 2000 consecutive heart valve recipients at the Groote Schuur Hospital (University of Cape Town) shows, a significant proportion of patients is even younger than 20 years. Zilla P, Brink J, Human P, Bezuidenhout D. 2008. Prosthetic heart valves: Catering for the few. Biomaterials 29: 385–406.
<table>
<thead>
<tr>
<th></th>
<th>USD per capita per annum</th>
<th>Physicians per 1000 population</th>
<th>Hospital beds per 1000 population</th>
</tr>
</thead>
<tbody>
<tr>
<td>World</td>
<td>1030</td>
<td>1,5</td>
<td>..</td>
</tr>
<tr>
<td>Euro zone</td>
<td>3884</td>
<td>3,7</td>
<td>5,6</td>
</tr>
<tr>
<td>High Income</td>
<td>4635</td>
<td>3,0</td>
<td>5,7</td>
</tr>
<tr>
<td>Low income</td>
<td>31</td>
<td>0,2</td>
<td>..</td>
</tr>
<tr>
<td>Middle Income</td>
<td>262</td>
<td>1,3</td>
<td>2,3</td>
</tr>
<tr>
<td>Sub Saharan Africa</td>
<td>95</td>
<td>0,2</td>
<td>..</td>
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</tbody>
</table>
Survival after heart valve replacement reduced vs. expected survival in the general population

Figure 2. Observed survival (O[s]) among 2,805 patients undergoing aortic or mitral valve replacement, or both. The expected survival rate (E[s]) line represents the experienced survival in a gender- age- and year of operation-matched control group. The vertical bar indicates the 95% confidence limits (95CL). PAT = patient; POST-OP = postoperatively.
Pre-operative heart failure reduces long-term survival

Fig. 8. Relative survival after primary mitral valve replacement by preoperative NYHA functional class in patients who survived the first postoperative month. NYHA II (— □ —), IIIA (— — —) ≥ IIIB (— ○ —). 95% confidence intervals at 5 and 10 years and the number (N) of patients at risk after one month, 2, 4, 6, 8 and 10 years are given.

Valve requirements in the Young (< 65 Y/age)

- Durability limitations of bio prostheses versus life long anti-coagulation mechanical valves in the young is a real problem in Africa
- Limited choices with compromised outcomes
- Repair versus replace debate for advanced valvular pathology and a once-off procedure

Aorta valve:

- Ross procedure limitations in a RHD population
- Availability of homografts
Research Approach to Heart Valve Substitutes

- Expand the homograft pool

- Development of Biological valves which are durable and do not calcify

- Ideally valved conduits should grow in children with CHD and maintain its own tissue integrity

- Development of Mechanical valves requiring no or very low dose anti-coagulation

- Alternative materials

- TAVI
Expanding the homograft pool

Does prolonged post-mortem cold ischemic harvesting time influence cryopreserved pulmonary homograft tissue integrity?

Francis Edwin Smit · Dreyer Bester · Johannes Jacobus van den Heever · Franziska Schlegel · Lezelle Botes · Pascal Maria Dohmen

Received: 28 October 2014 / Accepted: 2 February 2015 © Springer Science+Business Media Dordrecht 2015

Abstract This study investigated cryopreserved pulmonary homograft (CPA) structural integrity after prolonged cold ischemic harvesting times in a juvenile sheep model. Three groups with different post-mortem cold ischemic harvesting times were studied, i.e. Group 1 (24 h, n = 10); group 2 (48 h, n = 10); group 3 (72 h, n = 10). In each group, 5 CPAs were studied in vitro after cryopreservation and thawing. The other 5 CPAs were implanted in juvenile sheep for a minimum of 180 days. Serology samples were obtained and echocardiography was performed before euthanasia. Hematoxylin and eosin (H&E), scanning electron microscopy (SEM), von Kossa, Picrosirius red, α-actin, immunohistochemistry [von Willebrand factor (vWF), CD4, CD31 and CD34] and calcium content analyses were performed on explanted CPAs. The in vitro and in vivo studies failed to demonstrate any change in tensile strength, Young’s Modulus and thermal denaturation ($T_d$) results between the groups. SEM demonstrated a reduction in endothelial cells (50 % at 24 h, 60.9 % at 48 h and 40.9 % at 72 h), but H&E could not demonstrate autolysis in any CPA in vitro. All cultures were negative. In the explanted groups, IgE, IgM and IgG results were inconclusive. Echocardiography demonstrated normal valve function in all groups. H&E and Picrosirius red staining confirmed tissue integrity. vWF, CD31 and CD34 staining confirmed a monolayer of endothelial cells in all explanted valves. Calcium content of explanted CPA leaflets was similar. This experimental study supports the concept of prolonging the cold ischemic harvesting time of cryopreserved homografts to reduce homograft shortage.
**Pulmonary Homografts In Children – Expanding The Donor Pool By Extending Post Mortem Harvest Times**

Francis E. Smit1, Johannes J. van den Heever2, Dreyer Bester3, Lezelle Botes2, Susan Vocloso1, Pascal M. Dohmen2,3

1Dept of Cardiothoracic Surgery, University of the Free State, Bloemfontein, South Africa, 2Dept of Health Sciences, Central University of Technology, Bloemfontein, South Africa, 3CCM Med Klinik für Kardiovaskuläre Chirurgie, Charité Universitätmedizin Berlin, Berlin, Germany

**OBJECTIVE**

Pulmonary homografts remain the golden standard for Right Ventricular Outflow Tract (RVOT) reconstruction in children with congenital heart defects requiring a valved conduit. Availability is restricted by an international donor shortage.

Currently donors are restricted to beating heart donors, <6 hours (h) or limited to <24 post mortem (PM) worldwide and most cadaver programs have closed down.

Between 1988 and 2014 the Bloemfontein Homograft Bank have harvested 3182 cadaver homografts with 1710 homografts issued for implantation. The mean PM harvest time was 30h (range 1-82h).

This study evaluates the clinical outcomes of pulmonary homografts implanted in the RVOT of children <14 years of age. Freedom from valve failure requiring re-intervention of pulmonary homografts harvested from cadavers <24h PM and then cryopreserved versus those harvested >24h before cryopreservation were compared.

A retrospective analytical cohort study was conducted in 253 children younger than 14 years operated between 1990 and 2013. The impact of extended PM harvest time (>24h PM before processing and cryopreservation) on freedom of re-operation was compared to those homografts harvested ≤24h PM.

Follow-up was complete for 214 homograft implants. Group 1 (107 implants in 102 patients) received homografts harvested <24 h PM and Group 2 (107 implants in 99 patients) homografts harvested ≥24h PM. Valve failure requiring re-intervention was defined as a systolic gradient in excess of 40-50 mmHg or valve regurgitation more than moderate associated with corresponding right ventricular morphological changes.

**METHODS**

Age at first operation, gender, follow-up and freedom from homograft failure was recorded and analysed (Table 1 and Table 2).

Modes of degeneration was noted and compared (Table 3).

95% of homografts in Group 1 and 93% of homografts in Group 2 were functioning normally and did not require re-intervention at a mean age of 11.1 years (Group 1) and 9.7 years (Group 2). 94% of all pulmonary homograft implants did not require re-intervention at 10.4 years.

Statistical analyses included Log-Rank, Wilcoxon and -2log (LR) tests and could not demonstrate any difference in the failure rate between Group 1 and Group 2.

Table 1: Age at first operation, gender, follow-up and freedom from valve failure (1988 – 2014)

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>GROUP 1 &lt;24h PM</th>
<th>GROUP 2 &gt;24h PM</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of grafts</td>
<td></td>
<td></td>
<td>253</td>
</tr>
<tr>
<td>Number of implants available for analysis</td>
<td>107 in 102 patients</td>
<td>107 in 99 patients</td>
<td>214</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>63 (60.8%)</td>
<td>64 (64.6%)</td>
<td>127</td>
</tr>
<tr>
<td>Male</td>
<td>39 (39.2%)</td>
<td>35 (35.4%)</td>
<td>74</td>
</tr>
<tr>
<td>Age at implantation (years)</td>
<td>10.9 (11.6)</td>
<td>13.7 (11.9)</td>
<td>12.3</td>
</tr>
<tr>
<td>Follow-up time (days)</td>
<td>3.5 ± 4.2</td>
<td>4.5 ± 4.8</td>
<td>4.0</td>
</tr>
<tr>
<td>Freedom from valve failure</td>
<td>4043.7</td>
<td>3550.6</td>
<td>3797.2</td>
</tr>
<tr>
<td>Years</td>
<td>11.1</td>
<td>9.7</td>
<td>10.4</td>
</tr>
</tbody>
</table>

**RESULTS**

5 patients in Group 1 and 8 patients in Group 2 received a second homograft in the study period.

95% of homografts in Group 1 and 93% of homografts in Group 2 were functioning normally and did not require re-intervention at a mean age of 11.1 years (Group 1) and 9.7 years (Group 2). 94% of all pulmonary homograft implants did not require re-intervention at 10.4 years.

Statistical analyses included Log-Rank, Wilcoxon and -2log (LR) tests and could not demonstrate any difference in the failure rate between Group 1 and Group 2.

**CONCLUSION**

No difference could be demonstrated between the freedom of re-operation between processed and cryopreserved pulmonary homografts harvested ≤24h PM and those harvested >24h PM implanted in the RVOT of young children.

Valve degeneration occurred mainly as a result of regurgitation in Group 1 (<24h harvested homografts) and as a result of calcification in Group 2 (>24h harvested homografts). The number of failed homografts are too small for any conclusions.

As a whole, pulmonary homografts implanted in the RVOT from the Bloemfontein Homograft Bank in children <14 years, demonstrated freedom from homograft failure requiring re-intervention for a mean of 10.4 years. This is a very respectable outcome in this age group.

This study failed to demonstrate that extending PM harvest times beyond 24h was associated with an increase in the homograft failure rate. Therefore the re-evaluation of cadaver based donor programs in an attempt to address the world wide homograft donor shortage is justified.

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**Figure 1: Pulmonary homograft**
Tissue Engineering

- Artificial scaffolds – too far, too expensive
- Aorta homografts
- Pulmonary homografts
- Mitral homografts
- Pericardium - bovine, other
- Porcine valve modification
Decellularized pericardium and tissue engineered mitral valves

Comparison between the decellularized bovine pericardium and the conventional bovine pericardium used in the manufacture of cardiac bioprostheses

Decellularization as an anticalcification method in stentless bovine pericardium valve prosthesis: a study in sheep

Tissue-engineered mitral valve: morphology and biomechanics

Claudinei Collatusso¹, João Gabriel Roderjan², Eduardo Discher Vieira³, Nelson Itiro Myague⁴, Lúcia de Noronha⁵, Francisco Diniz Affonso da Costa⁶

Pavel lablonskii⁷, Serghei Cebotari, Igor Tudorache, Marisa Granados, Lucrezia Morticelli, Tobias Goecke, Norman Klein, Sotirios Korossis, Andres Hilfiker and Axel Haverich

Jean Newton Lima COSTA, Pablo Maria Alberto POMERANTZEF, Domingo Marcolino BRAILE, Vladimir Aparecido RAMIREZ, Gilberto GOISSIS, Noedir Antônio Groppo STOLF
Pericardium
**Pericardium**

### Strength

<table>
<thead>
<tr>
<th></th>
<th>Fresh</th>
<th>Decellularized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tensile Strength (MPa)</td>
<td>11.25 ± 3.55</td>
<td>7.96 ± 3.88</td>
</tr>
<tr>
<td>Young's Modulus (MPa)</td>
<td>82.30 ± 23.72</td>
<td>70.30 ± 46.00</td>
</tr>
<tr>
<td>Thickness (mm)</td>
<td>0.500 ± 0.103</td>
<td>0.431 ± 0.117</td>
</tr>
</tbody>
</table>

### Protein (µg/mg wet weight)

<table>
<thead>
<tr>
<th></th>
<th>Fresh</th>
<th>Decellularized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen</td>
<td>190.42 ± 34.03</td>
<td>165.60 ± 15.28</td>
</tr>
<tr>
<td>Denatured Collagen</td>
<td>0.754 ± 0.113</td>
<td>0.578 ± 0.326</td>
</tr>
<tr>
<td>Elastin</td>
<td>27.95 ± 13.34</td>
<td>19.54 ± 8.59</td>
</tr>
<tr>
<td>Gags</td>
<td>1.469 ± 0.46</td>
<td>0.177 ± 0.03</td>
</tr>
</tbody>
</table>

### Glycaride quantification

- **Calcium Content**
  - Fresh
  - H&H
  - D
  - Decellularized
  - Series 1: 0.98243243 0.218402848 0.504463442 0.071896483

- **Alpha gal concentration/mg tissue**
  - Fresh: 15.52
  - Decellularized: 11.86
Engineering - Valve design and construction

A - Eligiloy wireform stent
   Acetyl homopolymer stent
   Stellite ring
   Haynes alloy eyelets

B - Eligiloy/polyester ring and stent posts
   Acetyl stent
   Silicone base ring

C - Polyester covered stent/base, and outer layer of pericardium
   Sorin Mitroflow

D - Stainless steel frame
   Bovine pericardial tissue
   Nitinol frame
   Porcine pericardial tissue

Aortic valve parts:
- Atrial annulus
- Aortic annulus
- Medtronic device
- Edwards FORTIS
Decellularized fresh homografts for pulmonary valve replacement: a decade of clinical experience†

Samir Sarikoucha,‡*, Alexander Horkea,‡, Igor Tudorachea, Philipp Beerbaumb, Mechthild Westhoff-Bleckc, Dietmar Boethiga,b, Oleg Repind, Liviu Maniucd, Anatol Ciubotarud, Axel Havericha and Serghei Cebotaria
TEM - Decellularized PuH Leaflet

TEM- 14 day explants

TEM-Transmission Electron Microscopy, PuH-Pulmonary
OBJECTIVES
Explantation of pristine poppet valves decades after implantation lead to the redesign of the 1960’s version of the University of Cape Town poppet valve as the hemodynamically improved Glycar poppet valve. The valve was re-engineered using a stainless steel frame and a silicone poppet shown in Figure 1.

METHOD
This study compared the hemodynamic performance of the Glycar valve to a Sorin bi-leaflet mechanical valve, using a combination of steady-state computational fluid dynamics (CFD), pulse duplication and particle image velocimetry (PIV). The numerical CFD analysis for the Glycar valve was performed with FloEFD CFD software. Pulse duplication (Vivotrabs pulse duplicator) and PIV (Dantec Dynamics combined with a custom pulse duplicator) evaluations were conducted on the 21mm Sorin bi-leaflet aortic valve and the re-engineered 21mm Glycar poppet valve. The tests were performed at a variety of cardiac outputs (CO), ranging from 3.6 L/min to 9.6 L/min. The velocity vector field and viscous shear stress (VSS) of the CFD and PIV were then compared for the Glycar valve.

RESULTS
Computational Fluid Dynamics:
The surface VSS obtained for the Glycar valve from the CFD simulations is shown in Figure 2. A maximum VSS of 20 Pa occurs at the poppet edge before the flow separates, with reattachment of the flow near the poppet tail, resulting in a maximum VSS of 15 Pa at the poppet tail.

Pulse Duplication:
The results of the pulse duplication tests performed at a CO of 6.4 L/min are summarised in Table 1. The pressure drop of the Glycar valve is lower than that of the Sorin bi-leaflet valve. The EOA of both the Glycar and bi-leaflet are above required industry standards. However, the Glycar valve exhibits higher closing volumes than the bi-leaflet (3.2% vs 1.36%) with increased regurgitation above a cardiac output of 8 L/min (data not shown). Leakage volumes of the Glycar valve was lower than in the bi-leaflet valve.

All pulse duplication performance data requirements (ISO 5840) were exceeded by the Glycar valve.

CONCLUSION
Effective orifice area (EOA), pressure drop, closing volume and leakage volume of the Glycar valve exceeded ISO 5840 standards and was comparable to a bi-leaflet valve of similar size. Recirculation and stagnant flow areas were identified for future research using CFD and PIV. VSS values were consistently below platelet activation values on both valves. Further design changes to the poppet valve are envisaged to reduce recirculation and regurgitation at high flows.
Tri leaflet mechanical valves

FIGURE 2. A trileaflet mechanical heart valve provides flow characteristics that more closely mimic native aortic valves. The valve contains a pivot mechanism that allows airfoil-like leaflets to close before flow reverses, a phenomenon that also occurs in the body. (a) Valve from above in fully open position. (b) Elevated isometric view of the valve in a fully open position. (c) Disassembled valve with three pyrolytic carbon leaflets and ring. Images are courtesy of Ulrich Steinseifer, Helmholtz Institute, Aachen, Germany.
Polyurethane valves

- Design modifications
- Mounted on a titanium or PEEK stent

TAVI

- Improvement of material - SIBS (styrene isobutylene styrene)
- Processes - dip molding, spray, electro spinning
Surgery vs Cardiac Interventionist
Rheumatic Valvular Disease in Africa

Common sense:
• Prevent RHD
• Diagnose valvular disease before advanced heart failure
• Early surgery
• Repair if possible

Where?
• Valvular surgery (OR)
• TAVI
In Africa for Africa

- Increase homograft or modified homograft use
- Tissue engineered biological alternatives
- Mechanical valves – tri-leaflet/poppet valve versus bi-leaflet valve
- Polyurethane or other material
- TAVI feasibility in RHD

Question:

- Is disease modification after valvular surgery required/possible, especially in patients who presented pre-operatively with advanced failure - monitoring, medication, genetic pathways
Recent process example


• “The novel tissue preservation technology introduces several new approaches to preservation of tissue integrity: functional group capping, glycerolization, and a new terminal ethylene oxide (EO) sterilization process.

Capping is used to reduce functional groups (eg, aldehydes) present in the gluteraldehyde-fixed tissue to prevent oxidation of the tissue and to mitigate tissue calcification. There are several ethanol rinse steps between capping and glycerolization that lower the amount of residual chemicals to acceptable levels.

The other novel aspect of the technology is that the valves undergo treatment with a glycerol and ethanol mix, which displaces most of the water present in the pericardial tissue and replaces it with glycerol. As a result of glycerolization, the valves can be packaged and stored dry, without the need for any liquid-based storage solution such as gluteraldehyde. The valves are sterilized via 100% EO. Rinsing is not required before use. “

• Of course, none of the valve companies ever reveal the ultimate production details of their anti-mineralization technologies. This is something we have to live with. No-body knows how to make Coca-Cola except the company. - J Schmoker ( editorial)