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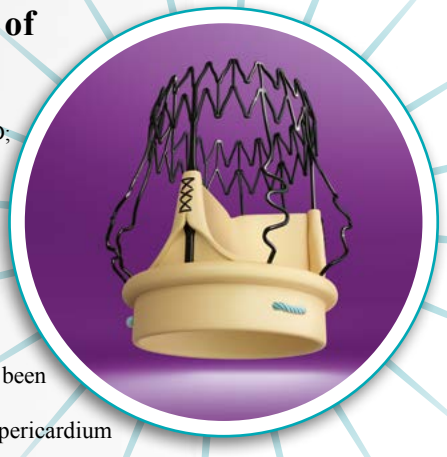
A Novel Tissue Treatment to Reduce Mineralization of Bovine Pericardial Heart Valves

THE JOURNAL OF
THORACIC AND
CARDIOVASCULAR SURGERY

JTCVS-17-1107

A Novel Tissue Treatment to Reduce Mineralization of Bovine Pericardial Heart Valves.



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ABSTRACT.

Objective. With the increasing use of bioprostheses worldwide, continuous efforts have been made to improve tissue durability. We introduce a new treatment for bovine pericardium combining octanediol-ethanol based phospholipid removal with taurine-based glutaraldehyde neutralization and storage in an aldehyde-free solution (FREE treatment).

Methods. Treated tissues were evaluated by mechanical and biochemical characterization, phospholipid content, aldehyde levels, cell cultures on pericardial samples (L929 fibroblasts and HUVEC cells), rat subcutaneous implantations and long-term juvenile sheep mitral valve implantations (n=3). Comparisons were made to glutaraldehyde fixed bovine pericardium or to samples from commercially available biological valves (Trifecta™ and Perimount Magna Ease™).

BACKGROUND

FREE TISSUE TREATMENT: the latest evolution in the treatment of bovine pericardial valves designed to reduce the sources of calcification and improve tissue durability.

- All tissue valves are subject to structural valve deterioration (SVD), mainly due to calcification.
- Phospholipids and aldehydes are major sources of calcification
- Phospholipids are intrinsically present in biological tissue, while aldehydes are a consequence of the fixation process. Both contribute to calcification: phospholipids directly as calcium binding sites[7-11]; aldehydes through their high chemical reactivity eliciting toxicity.[1-6]
- Valves are usually stored in liquids containing aldehydes. This, however, exposes once again the tissue to the detrimental effects of remaining free aldehydes. Even pre-implant rinsing, as per IFU indications, does not guarantee complete removal of toxic aldehydes.

AN INNOVATIVE TISSUE TREATMENT AND A READY-TO-USE VALVE

The FREE tissue treatment addresses both phospolipid and aldehyde and it's ready to use, straight from the jar, thanks to an aldehyde-free storage. This combination enhances anticalcification properties and may improve tissue durability.

METHODS

- The FREE treatment was investigated both in vitro and in vivo for safety and efficacy.
- FREE treated tissues were evaluated in rat subcutaneous implantations and long-term juvenile sheep mitral valve implantations
- Comparisons were made to glutaraldehyde fixed bovine pericardium (control group) or to samples from commercially available biological valves (Trifecta™ treated with LinX™ and Perimount Magna Ease™ treated with ThermaFix™).

RESULTS: IN VITRO

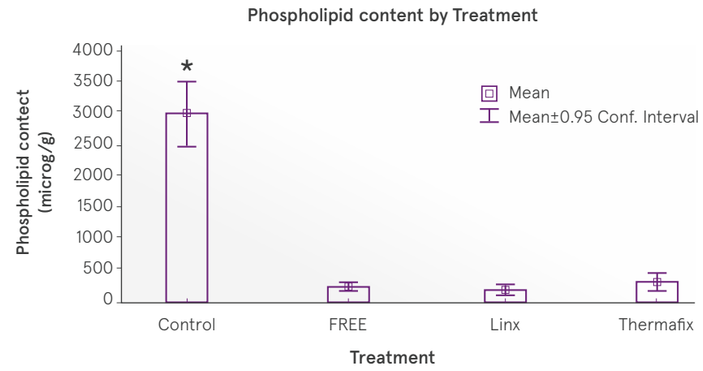
Mechanical and Biochemical Characterization

The resistance to mechanical stress was assessed and compared to clinically approved references.

- **FREE treated tissue guarantees the same mechanical and biochemical performance and stability as the tissue in commercially available valves.**

Phospholipid Content

- **Phospholipid content in FREE treated tissue is reduced up to 96% vs control group**
- **Similar low levels of phospholipids compared to samples from Linx™ and ThermaFix™ treated valves.**



Phospholipid content for GA-only fixed tissue, FREE treated tissue, Linx- treated and ThermaFix-treated tissue. Columns represent the mean values, error bars are the 95% confidence interval.

Aldehyde Content

3 analyses were performed to test the aldehyde level:

1. **Qualitative: colorimetric assay**

   Tissue samples from different valve models are exposed to a staining reagent that is sensitive to aldehydes. The purpose is to visually determine the level of aldehydes that remain in the valve tissue. The darker the blue, the more the aldehydes are present.

   FREE outperforms other treatments, confirming a very low aldehyde presence.

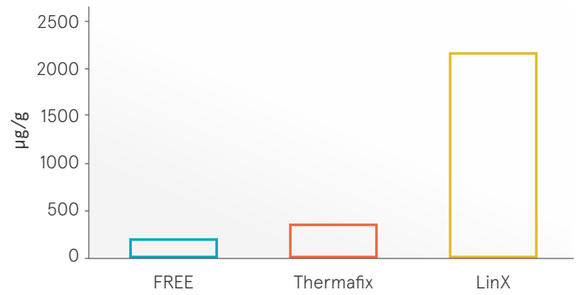
Bovine Pericardium Samples

* The link between aldehydes concentration and toxicity is mentioned in the paper.

2. **Quantitative: biochemical assay**

   To quantify the amount of aldehydes that are extracted from the valve samples. This amount of aldehydes is the one that potentially will be released by the valve after implantation.

Cumulative aldehydes content extracted by the valves



3. **Tissue toxicity level**

A cell adhesion and proliferation test on pericardial samples was performed in order to confirm the neutralization of aldehydes in the FREE treated valves. It measures the tissue toxicity through the ability of cells to adhere and proliferate. In the presence of residual aldehydes cells do not adhere and die. FREE treated tissue showed good cell viability and proliferation after 48 and 72 hours from cells seeding, while cells on GA treated samples were not able to adhere and proliferate.

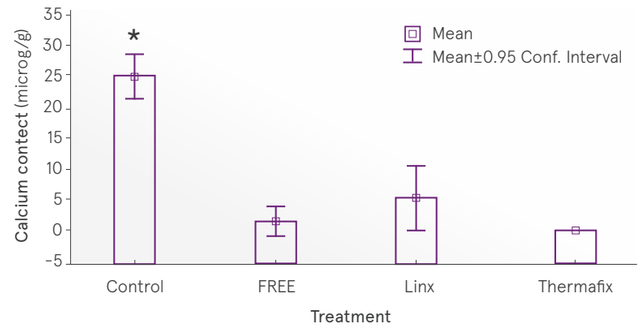
Thanks to the combination of highly effective neutralization of aldehydes and a completely aldehyde-free storage, the FREE treatment delivers a final product that has a very low level of aldehydes.

RESULTS: IN VIVO (PRE-CLINICAL ANIMAL STUDY)

Rat Subcutaneous Implantations

The subcutaneous tissue implantation in rat model is the gold standard to assess tissue propensity to mineralize in vivo as it resembles the dystrophic mineralization occurring in human patients after years of implantation.

Calcium content vs. Treatment



* Outlier data

Calcium content within bovine pericardial tissues after 60 days subcutaneous implantation in juvenile rats.

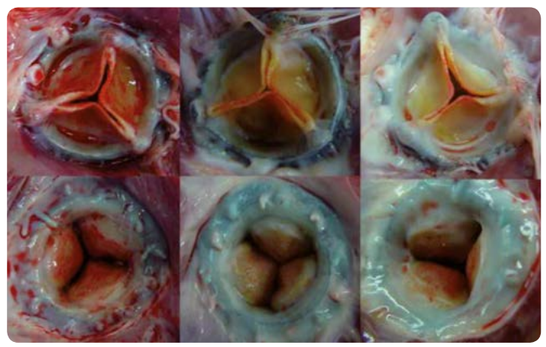
- **Extremely low calcium contents were observed in FREE treated tissues after implantation; similar to ThermaFix™ tissue and slightly lower than Linx™ tissue.***

* Among the treated samples, there were no significant differences.

Mitral Valve Implantations in Juvenile Sheep

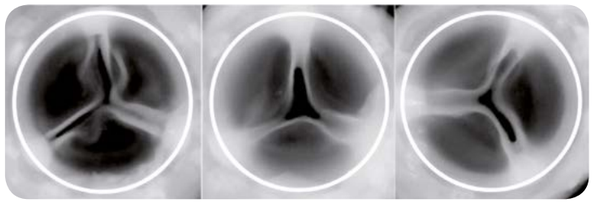
Valve replacement in juvenile sheep is the standard model to assess valve safety and performance, as it most closely resembles clinical conditions and represents an accelerated model for SVD (high pressures, stress of the mitral position and accelerated calcium metabolism of growing animals).

FREE treated valves showed no major abnormalities on both the inflow and outflow sides with pliable leaflets and no visible signs of dystrophic calcification.



Outflow (upper panels) and inflow (lower panels) sides of mitral valve prostheses made of bovine pericardium subjected to the FREE treatment after 20 weeks of implantation in juvenile sheep.

X-ray imaging of the explanted valves revealed no signs of dystrophic mineralization at 20 weeks after implantation.



X-ray images of the FREE treated bioprostheses after 20 weeks implantation in mitral position of juvenile sheep.

The FREE treatment showed no signs of valve mineralization and degeneration, even in the challenging model of chronic mitral valve implantation in juvenile sheep.

FREE TISSUE TREATMENT: the latest evolution in the treatment of bovine pericardial valves designed to reduce the sources of calcification and improve tissue durability.

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INDICATIONS: The PERCEVAL prosthesis is indicated for the replacement of diseased native or a malfunctioning prosthetic aortic valve via open heart surgery. The prosthesis is indicated for use in adult patients who are diagnosed to have aortic valve stenosis or steno-insufficiency.
TOP POTENTIAL SIDE EFFECTS: The risks or potential adverse events associated with cardiac valve replacement with a bioprosthesis include, but may not be limited to: cardiac arrhythmias, death, endocarditis, heart failure, hemorrhage, intravalvular and/or paravalvular leak, stroke or any related neurologic disorders, structural valve deterioration, reoperation and explant. Beyond the previously mentioned adverse events, specific events related to the implant of the PERCEVAL prosthesis may include, but not be limited to dislodgment and/or migration of the prosthesis.
MRI conditional.

12/2018 IM-01944 C RE0210252

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