L-Hydro In-Service Guide
BACKGROUND

TISSUE PRESERVATION

Since the late 1960’s a number of methods have been developed or proposed and used for preservation of tissue products. These include solutions containing alcohol, high ionic strength or high osmolarity. Most of these methods aim at preventing the protein components in tissues from changing their configuration. However, the substances used may inhibit subsequent chemical treatment methods or cause complications in subsequent processes.
GLUTARALDEHYDE
METHODS

What is it?
How it works?

Glutaraldehyde as tissue preservation has been used for over 40 years, and has been successful in treating different biological xenograft tissues used in heart valves.

Glutaraldehyde's main function is to establish crosslink connections in the collagen that can provide structure and resistance to the tissue.
Advantages

1. Glutaraldehyde reduces immunogenicity: it is one of the main reasons for the success of this method of preservation, such as in glutaraldehyde-treated xenograft tissues for bioprosthetic heart valves.³

2. An excellent sterilant: One must remember this when working with a non-glutaraldehyde method of treating implanted tissues.

3. Relatively durable: The durability of glutaraldehyde treated tissue valves demonstrated by in vitro fatigue tester, was partly responsible for the successful development of the clinical glutaraldehyde treated porcine and pericardium valves.²

Disadvantages

1. Cytotoxic: It has been demonstrated that glutaraldehyde treated tissues remain toxic to cells in culture environments after repeated long-term washing.

2. No re-establishment of endothelium: There is no evidence to date demonstrating the re-establishment of an endothelial layer on the leaflets of glutaraldehyde-treated valves.⁴

3. Not supporting cell in-growth: Cells infiltrating glutaraldehyde-treated tissues can not survive.⁴

4. Calcification – Calcification of glutaraldehyde-treated valves is prevalent in young patient.¹
Glutaraldehyde vs. Calcification

Many publications have shown that glutaraldehyde is still a good option for tissue treatment, as it obtains good results for old patients in durability and resistance.

However calcification is a big problem for this technology, for many years methods to reduce calcification have been developed. None of them 100% calcium free, proof that this feature is not well seen in congenital defects repair.
So What is the Goal in Tissue Treatment?

- Establish biological tissue
- Inhibit antigenicity
- Reduce / Suppress the inflammatory response
- Guarantee sterility
- Durability
- Enable cell reconstruction after implant
- No calcification
L-Hydro Principles

1. Present to receptor cells a non viable xenograft matrix (acellular), similar to natural human matrix.

2. Cells grow in same natural function conditions.

3. Reconstruction stimulated by similar natural matrix and physiologic conditions “In Vivo – tissue engineering.”

Based on these principles, Labcor in collaboration with Dr. David Cheung, PhD, Heart Institute of Montana have developed this method of tissue treatment technology, by the name L-Hydro.

Objectives

Preserve xenograft products to be implanted in humans as potentially capable to functionally reconstruct with no rejection and/or calcification. (i.e Grow)
L-Hydro Tissue Treatment  

How It works

1. Salts ETOH  
   Extraction of Antigens
   Fresh tissue is harvested from the slaughterhouse and quickly submerged in a cold ethanol salts solution - Bio burden reduction, Protection of the integrity of the collagen and elastin fibers and antigens extraction.

2. Mild Chemical Oxidation
   Perform mild chemical oxidation reducing potential oxidizable materials that can attract inflammatory reaction from the host.

3. Poly-Glycol
   A well-known poly-glycol which binds tightly to collagen is also added to mask the potential non-extracted antigen.
An anti-inflammatory agent is added to reduce inflammatory response. During the implant procedure a heparin coating reduces cell adhesion.

The resulting material is non-antigenic, yet having the exact properties of the natural tissue.
Post-Implantation

1. Inactivation of Inflammatory Cells

2. Attachment of Endothelial Cells

3. Infiltration of Connective Tissue Cells
With the inactivation of inflammatory cells, endothelial cells are permitted to grow. This way endothelial cells allows infiltration of connective cells becoming the synthesis of a new tissue matrix.
Glutaraldehyde vs. L-Hydro

**Glutaraldehyde**
- Non-Vital

  **Stabilization**
  - Crosslinking
  - Polymerization reaction
  - Coating, Cytotoxic glutaraldehyde
  - Modification of Amino Groups
  - Break down of toxic polymers
  - Persistent residual antigenecity
  - Persistent residual cytotoxicity

  **Sterilization**
  - Conventional - depends on Toxicity of glutaraldehyde
  - Improved - multi-step reduction of bioburden, then final sterilization
  - Aldehyde storage/shipping solution

  **Post-Implantation**
  - Non-vital Implants

**L-Hydro**
- Non-Vital

  **Stabilization**
  - No Crosslinking
  - No Polymerization reaction
  - Coating, non-Cytotoxic poly-glycol
  - No modification of Amino Groups
  - No break down of toxic polymers
  - No persistent residual antigenecity
  - No persistent residual cytotoxicity

  **Sterilization**
  - Multi-step reduction of bioburden
    - L-Hydro process
    - ETOH
  - Final liquid phase peroxide sterilization
  - Storage solution without toxic substance

  **Post-Implantation**
  - Vital-implants
Electron Microscopy Evaluation

Glutaraldehyde
- SEM - Lack of Endothelial Cells
- TEM - Non-viable Cells

L-Hydro
- SEM - Layer of confluent Endothelial Cells
- TEM - Viable Fibroblasts
Advantages

- Non Toxic
- Endothelialization
- Tissue Growth – New Tissue Matrix
- No persistent residual antigenicity
- Very low level of calcification
- Preserve natural structural and mechanical characteristics
**Applications**

*Biological tissue treatments*

- Heart valves
- Vessels
- Pericardium

**Labcor Products**

*Treated with L-Hydro*

- Babygraft / Corograft / Perigraft
  *(Mesenteric artery)*

- Velum
  *(Pericardium conduit, portion closed with porcine pulmonary valve)*

- Vasti
  *(Valved jugular vein)*

- Vivendi
  *(Pericardium repair membrane)*
References


5 - David T. Cheung, Ph.D.: Director of Research, Cardiovascular Tissue Engineering, The International Heart Institute of Montana Foundation, 500 West Broadway, Suite 350, Missoula, Montana 59802 USA