EVALUATION OF STORAGE TREATMENTS OF DURA MATER AND PERICARDIUM AS VALVE MATERIALS - PRELIMINARY STUDY OF MECHANICAL PROPERTIES -

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1. INTRODUCTION

The application of various treatments for tissue preservation has been evaluated with regard to sterilization, preservation of mechanical properties, long-term storage, the suppression of antigenecity and the prevention of the denaturation of collagen. Of many storage techniques, formaldehyde and glutaraldehyde solutions are used rather commonly.

Because of the strong sterilization and the ease of handling and application, a 4% formaldehyde treatment was used extensively for the cardiac prostheses in the past in Cleveland Clinic (1). It has been pointed out, however, that the cross-linkages made with formaldehyde are weak, unstable and reversible in nature. It has been believed that this is not the case with the glutaraldehyde treatment. In contrast to formaldehyde which is a simple aldehyde, glutaraldehyde has two active carbonyl groups, which react with protein more effectively under proper conditions, resulting in a stronger cross-linking reaction.

Based upon our studies of aldehyde treatment, a 0.45% glutaraldehyde solution has been used recently for the biolization and storage of the components for the cardiac prostheses (2). The aldehyde treatments have also been applied to stabilize the bovine pericardial tri-leaflet valves (3) and porcine xenografts.

These successful applications of these treatments for natural tissues, particularly for valve materials, imply that the 0.45% glutaraldehyde treatment is very effective for the preservation of mechanical properties and the antithrombogenecity.

On the other hand, tri-leaflet valves fabricated from human dura mater have been used for the valves of cardiac prostheses in Cleveland Clinic (2). The dura mater is not chemically treated, but is sterilized by dehydration in glycerol for over 2 weeks according to the experimental studies of Zerbini's group in Brazil, who have been successfully using these valves for the past several years in over 1,000 clinical cases of cardiac valve replacement.

However, we have recently detected microbial infection in a few cases of the calves implanted with cardiac prostheses. The aldehyde treatment is expected to have a possibility to improve the sterilization effect for the dura mater as for the case of pericardium.

In spite of the fact that the aldehyde treatments have been employed rather commonly as stated above, their effects on the quality and durability of natural tissues have not been well documented. Sterilization and storage treatments should affect the molecular structure of natural tissues and therefore, their subsequent quality and durability. Changes in molecular structure like the cross-linking of collagen molecules and ground substance could also influence the response of the tissue to external force. Mechanical properties such as flexibility, strength and durability are very important factors partic-
ularly to obtain a good performance of valves. It appears that tensile testing, which is the standard engineering method for determining the stress-strain behavior of materials, might prove a valuable means for quantitating the structural changes brought about by sterilization and storage treatments.

In this study, effects of the aldehyde treatments on the mechanical properties of human dura mater and bovine pericardium were observed experimentally by the tensile test. The results obtained were used to evaluate the performance of valves which were fabricated with these chemically treated materials.

2. EXPERIMENTAL PROCEDURES

Almost all human dura mater tissues were obtained from Brazil and shipped to our laboratory, being kept in 98% glycerol. The glycerol treatment results in dehydration of the tissue and is believed to have the bacteriostatic effect. For the study of mechanical properties of dura mater tissues, specimens taken from glycerol were rinsed in saline solution for over 30 minutes and then preserved in aldehyde solution for a certain period of time. All samples of dura mater tissues were divided into 4 groups. In the first control group, the specimens were tested after soaking in only saline solution for 1 hour, 1 day, 4 days and 7 days, respectively. The second group of dura mater were kept in 0.45% glutaraldehyde solution for 1 hour, 4 days and 7 days, respectively. The third group of dura mater were stored in 2% glutaraldehyde solution for 4 days and 7 days, respectively. A 4% formaldehyde solution was used for the fourth group.

On the other hand, bovine pericardium were rinsed in saline solution for 3 hours after receiving fresh from the slaughterhouse. After removing the extra tissues and fat, these pericardium were divided into 3 groups. These three groups of pericardium were separately preserved in saline solution, 0.45% glutaraldehyde solution and 4% formaldehyde solution, respectively. The storage periods were 1 day, 4 days and 7 days for each treatment.

In these treatments, all aldehyde solution were buffered to pH 7.4 with phosphate, while the pH of saline solution used was 5.0. After these treatments, all specimens were rinsed in saline solution for 30 minutes and cut by a special punch to the dumbbell shape which is standardized by the ASTM D1708-66 (gage length: 22.25 mm, width: 5.0 mm). All specimens were again rinsed in saline solution for 30 minutes and then subjected to the tensile testing under the cross-head speed of 12.7 mm/min.

3. EXPERIMENTAL RESULTS

Since the stress developed in a valve leaflet is estimated below 100 Gm/mm², all data analyses were performed for the stress range below this value. The stress-strain relations of dura mater below the stress of 100 Gm/mm² were described by:

\[
\ln (\sigma + 1) = D (\lambda^2 - 1),
\]

where \(\sigma\) is the stress and \(\lambda\) is the extension ratio which is defined as the ratio of the length at the stress \(\sigma\) to that at no stress. The coefficient D represents the stiffness of material and called as the stiffness parameter. As shown in Table 1, significant stiffening was observed on the specimens preserved in the glutaraldehyde solution. This stiffening is ascribed to the cross-linking of collagen and ground substance caused by the glutaraldehyde treatment. There was almost no effect of the concentration on the stiffness between 0.45%
and 2% of glutaraldehyde. On the other hand, the stiffening of tissues due to 4% formaldehyde treatment is considerably less than those treated with glutaraldehyde.

The stress-strain relations of pericardium have two phases depending upon the stress level. Below 10 Gm/mm\(^2\), they were expressed by the linear relation:

\[ \sigma = K (\lambda - 1). \] \hspace{1cm} (2)

Between 10 and 200 Gm/mm\(^2\), on the other hand, they were described by the same equation as shown in Eq. (1). Since the stress induced in a valve leaflet is estimated between 0 and 100 Gm/mm\(^2\), both of these two parameters, K and D, should be considered to evaluate the material stiffness.

As shown in Table 2, the K-value of pericardium increased and, on the contrary, the D-value decreased significantly by the 0.45% glutaraldehyde treatment. The 4% formaldehyde treatment gave the data between those obtained by the 0.45% glutaraldehyde treatment and the soaking in saline solution.

### Table 2 Stiffness (K and D in Gm/mm\(^2\)) and thickness (t in mm) of bovine pericardium.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>K</th>
<th>D</th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (1 d)</td>
<td>58.5</td>
<td>17.5</td>
<td>0.17</td>
</tr>
<tr>
<td>0.45%G (4 d)</td>
<td>173.4</td>
<td>5.2</td>
<td>0.26</td>
</tr>
<tr>
<td>4%F (4 d)</td>
<td>122.8</td>
<td>7.4</td>
<td>0.28</td>
</tr>
</tbody>
</table>

As for the tensile strength which is considered to be closely concerned with the durability of material, these aldehyde treatments did not improve it so significantly compared with the storage in saline solution. A large amount of thickness change was observed after these glutaraldehyde and formaldehyde treatments as shown in Tables 1 and 2. This change of thickness is very important to analyze the valve performance.

On the bases of these experimental results on the material stiffness and thickness change, the stress developed in a valve leaflet and the critical valve opening pressure were calculated theoretically using the valve design criteria proposed by Ghista (4). The analytical results showed that non-treated dura mater valve which is currently being used in Cleveland Clinic gives the lowest value of critical valve opening pressure of all the other chemically treated dura mater and pericardial valves. However, the stress in a leaflet and the critical opening pressure are fairly higher even for non-treated dura mater valve compared with that in the natural human aortic valve.

REFERENCES