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Response to uniaxial load of chemically modified bovine pericardium at different temperatures

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Non-destructive uniaxial load tests were performed on glutaraldehyde-fixed bovine pericardium at two different temperatures. Samples were tested first in isotonic saline at room temperature and then stored overnight in buffered glutaraldehyde. This allowed the tissue to recover from the test. The following day after all memory of the previous test had faded, the procedure was repeated on the same samples and continued for five consecutive days. Mean response curves to load were constructed. The temperature of the saline test medium was raised to body temperature at 37° C. An identical series of tests over five consecutive days on the same samples was repeated at this temperature. Mean response curves were computed. Analysis of variance demonstrated no statistically significant difference in the response to load of the tissue at the two temperatures. Hence, it appears that the temperature is not an important parameter in the mechanical and accelerated fatigue tests of pericardial heart valve substitutes.

1. Introduction

Prosthetic replacement of natural organs is now a routine procedure. The design of prostheses which are both safe and efficient relies on a detailed knowledge of the materials used in their construction. Heart valve substitutes have been used clinically since the late 1950s [1]. Mechanical valves have a long fatigue life and relieve the clinical symptoms associated with diseased natural valves. However, the presence of flow occluders in these prostheses causes acute blood trauma. This design defect is compensated for by chronic anticoagulation [1]. In contrast, valve substitutes constructed from natural tissues provide a central flow orifice which causes minimal cell damage [2]. Unfortunately, the durability of these bioprostheses is suspect [3, 4].

Bovine pericardium, chemically modified by glutaraldehyde fixation, has been used in the construction of valve substitutes for over a decade [5]. Considerable time and effort has been expended on investigating the biochemical consequences of modifying this tissue by glutaraldehyde fixation [6]. Until recently scant attention has been paid to the influence of fixation on the tissue's mechanical properties, even though it is these characteristics which may ultimately determine the fatigue life of the valve substitute [4]. Unlike man-made material, produced in a controlled environment, significant anatomical variations have been identified in the mechanical and ultrastructural properties of the bovine pericardial sac [7]. These results were obtained from tests performed at room temperature. However, heart valve substitutes operate at a body temperature of 37° C. The present study investigates the effect of these temperature changes on the response to uniaxial load of glutaraldehyde-fixed bovine pericardium.

2. Materials and methods

Pericardial sacs obtained from 16 to 20 week old calves were transported from the abattoir in ice-cold isotonic saline (0.9% NaCl). After stripping all visible fat by hand from the tissue, the sacs were mounted loosely on 150 mm diameter embroidery frames and chemically modified by immersion in a 0.2% solution of glutaraldehyde (BDH Chemicals Limited) buffered to pH 7.4 in 0.15 M phosphate buffer (Sørensen) for 7 days. A single sac was used in this study. A template was positioned over the diaphragmatic attachment and sternopericardial ligaments which were still attached to the pericardium. This enabled five equally distributed and randomly orientated strips to be generated from the sac [8] (Fig. 1). The specimens were excised and prepared as described before [9]. The thickness and width of each sample was measured at five equally spaced positions along the longitudinal axis and mean values obtained. The mean cross-sectional area was computed. The uniaxial test apparatus and procedure was similar to that outlined in a previous study [9]. The system was set to operate up to a maximum load which corresponded to a maximum stress level of $0.6\,N\,mm^{-2}$, depending on the initial cross-sectional area of the test specimen. The undeformed length of the sample was measured and eight cyclic tests performed at an extension rate of $60\,mm\,min^{-1}$. The length of the specimen was recorded after the test. The strips were then returned

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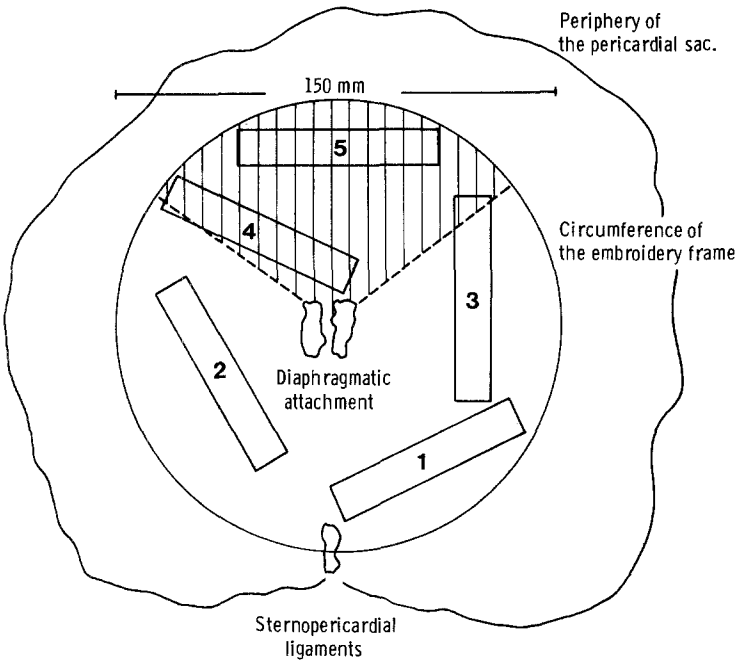

Figure 1 A schematic representation of the pericardium with the five different test sites shown.

to their standard buffered glutaraldehyde storage medium where they remained for 24 h at room temperature to allow the tissue to return to its original gauge length [8]. The following day, confirmation of the return to the original specimen length was obtained by measuring the undeformed length. The mean sample thickness and cross-sectional width were determined and the cross-sectional area of each specimen was computed. All five strips were tested consecutively in the same order as on the previous day. After the test, the length of the sample was again recorded and the specimens returned to their standard storage medium for 24 h.

This procedure was continued for ten consecutive days. During the first five days, all the tests were performed in isotonic saline at room temperature. For the subsequent tests, the temperature of the saline was raised to 37° C. The specimens were placed in the water bath for 40 min until the saline, and presumably the specimens, reached this temperature. From the tests a mean response curve to load for each site was obtained at room temperature and 37° C. The time taken for each mechanical test was recorded.

2.1. Experimental determination of the undeformed length of each specimen

To ensure that the undeformed length corresponded to zero load (and hence zero stress) the specimen was stretched up to the minimum recordable load (0.01 N) and then unloaded until zero force was again recorded. The undeformed length between the perspex grips was measured with vernier calipers to an accuracy of 0.01 mm [10].

2.2. Statistics

Three way analysis of variance, using the F statistic, and a paired t-test were used as tests of significance throughout this study as indicated.

Data are presented as mean $\pm$ one standard error about the mean.

3. Results

Fig. 2 shows the first cycle mean loading curves computed from the five tests at the two different temperatures for each of the five positions. Three-way analysis of variance showed that there was no significant difference in extension at the different temperatures at any of the stress levels in the loading curve. In addition, there was no significant difference (paired t-test, $n = 23$) between the mean percentage extensions of the samples after the first cycle, that is at zero stress, at the different temperatures (room temperature 5.23 $\pm$ 0.44%, body temperature 4.69 $\pm$ 0.27%). This trend continued through to the extensions measured in the unstressed states after the 8th cycle of loading/unloading. There was no significant difference (paired t-test, $n = 22$) between the mean percentage extensions at zero stress of the specimens at the different temperatures. There was also no significant difference (paired t-test, $n = 21$) between the mean maximum extensibility observed during the 8th cyclic test at room temperature (25.12 $\pm$ 1.07%, $n = 22$) and body temperature (24.72 $\pm$ 0.90%, $n = 24$). These data indicate that the response to load of all five specimens is independent of the temperature of the test medium.

Three-way analysis of variance demonstrated no significant difference in thickness, width or gauge length from test to test or temperature to temperature. Each specimen had returned to its original gauge length by 24 h after every mechanical test.

The mean temperature for the first five tests for the five positions was (17.72 $\pm$ 0.23° C, $n = 25$) and for the subsequent tests was (36.96 $\pm$ 0.03° C, $n = 25$).

There was no significant difference between the time taken for the tests performed at room temperature (7.96 $\pm$ 0.92 min, $n = 25$) and body temperature (7.08 $\pm$ 0.57 min, $n = 25$).

4. Discussion

This study indicates that the response to uniaxial load

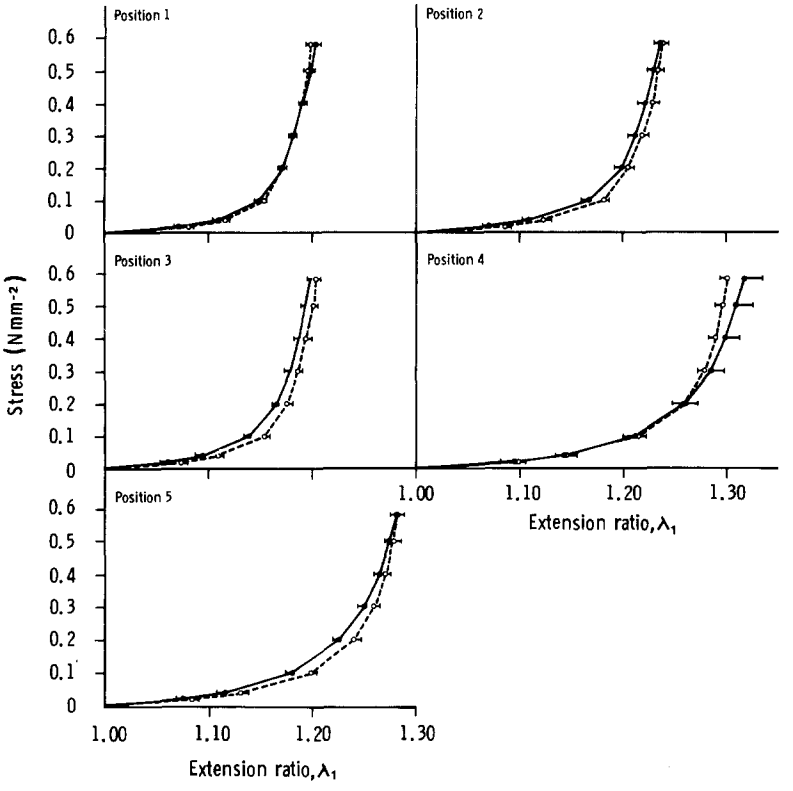

Figure 2 First cycle mean loading curves computed from the five tests at the two different temperatures for each of the five positions in a sac of glutaraldehyde-fixed pericardium. (——) Load curve, 1st cycle, tests performed at room temperature ($\bar{x} \pm$ S.E.M., $n = 5$). (– – –) Load curve, 1st cycle, tests performed at body temperature ($\bar{x} \pm$ S.E.M., $n = 5$).

of bovine pericardium, chemically modified by glutaraldehyde fixation, was unaltered when the temperature of the test medium was raised from room temperature ($17.7 \pm 0.23°$ C) to body temperature ($37.0 \pm 0.03°$ C).

Bovine pericardium in both its natural state and after chemical modification has a memory of previous strain histories that fades with time [8, 11]. This characteristic was used to obtain mean curves for the response to uniaxial load at the two temperatures for five different sites in the pericardial sac. By using an analysis of variance procedure the hypothesis of a temperature-independent response to uniaxial load was tested statistically. There are three possible sources of variation in the experimental design. The first arises from the two different temperatures used for the mechanical tests. The second stems from the inherent anatomical variation in tissue mechanical properties found in the pericardial sac. The third source is related to the five consecutive tests performed. As before [7, 11], significant differences in the response curves were observed at the different sites. However, at a given site, there was no significant difference between the tests or the two temperatures at any stress level.

In its natural state bovine pericardium is a pliant composite material. It consists of a fibrous weave of collagen and elastin in a viscous mixture of ground substance and water. Chemical modification with glutaraldehyde introduces an increased number of both inter- and intramolecular cross-links into the crystalline proteins [6]. These cross-linked structures, as well as introducing biochemical stability, are thought to enhance the resistance to proteolytic degradation. Hence the antigenicity of the tissue is reduced. The cross-linking procedure also increases the extensibility of the material by removing the abrupt stiffening under load associated with the natural tissue [7]. Furthermore, fixation with buffered glutaraldehyde raises the hydrothermal shrinkage temperature of natural bovine pericardium from 50° C to around 84.5° C [12].

Lee and Boughner [13] have suggested that each component of natural pericardium plays several roles in determining tissue behaviour. They proposed that:

1. the collagen network determines the relative isotropy of the structure, its mechanical strength and the different members of the family of stress/extension ratio curves observed during mechanical conditioning,

2. the complementary elastin network modulates the rearrangement of the collagen network affecting the stress/extension ratio response and the amount of stress relaxation. The correlation between elastin ultrastructure and tissue extensibility at different anatomical sites in the pericardial sac [7] adds weight to this proposition,

3. the ground substance matrix provides the viscous nature of the mechanical responses.

In isolation, each of these components is affected by changes in temperature [14]. As a composite in its natural state, samples of canine pericardium taken from a single site demonstrated decreased ultimate tensile strength and tissue modulus and increased resilience, at body temperature compared to room temperature [13]. Furthermore, stress relaxation and creep were both increased at the higher temperature of 37° C [13]. However, the strain at fracture was unaffected by temperature [13]. No results were presented for chemically modified tissue.

With the evidence above, the results of the present study came as a surprise. It is possible that the increase in network stability created by the introduction of more numerous molecular cross-links reduces the potential for different molecular rearrangements

under load at the temperatures used here. The body temperature of 37° C is much closer to the hydrothermal shrinkage temperature of the natural tissue (50° C) than that associated with the tissue after fixation (85° C).

Further studies are required before the temperatures at which changes in response to load can be determined. Nevertheless, this study does show that as far as implantation of heart valve substitutes is concerned, raising the tissue temperature to that of the body is unlikely to alter its mechanical properties. Hence, conclusions drawn from mechanical testing of tissue [7, 8, 11] and accelerated fatigue testing of pericardial heterografts are unlikely to be altered significantly by temperature changes in the physiological range.

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