RELATIONSHIP BETWEEN FIBRE ORIENTATION AND TENSILE STRENGTH OF NATURAL COLLAGEN MEMBRANES FOR HEART VALVE LEAFLETS

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ABSTRACT

Heart valve prostheses are used to replace native heart valves which that are damaged because of congenital diseases or due to ageing. Biological prostheses made of bovine pericardium are similar to native valves and do not require any anticoagulation treatment, but are less durable than mechanical prostheses and usually fail by tearing. Researches are oriented in improving the resistance and durability of biological heart valve prostheses in order to increase their life expectancy. To understand the mechanical behaviour of bovine pericardium and relate it to its microstructure (mainly collagen fibres concentration and orientation) uniaxial tensile tests have been performed on a model material made of collagen fibres. Small Angle Light Scattering (SALS) has been also used to characterize the microstructure without damaging the material. Results with the model material allowed us to obtain the orientation of the fibres, relating the microstructure to mechanical performance.

RESUMEN

Las prótesis cardiacas se emplean para substituir las válvulas nativas que han dejado de cumplir su función por enfermedades congénitas o por envejecimiento. Las prótesis biológicas hechas con pericardio bovino son similares a las naturales y no requieren de tratamientos anti-coagulantes, pero tienen una menor durabilidad que las prótesis mecánicas y suelen fallar por desgarro. Las investigaciones se orientan en mejorar la resistencia y durabilidad de las prótesis cardiacas para aumentar su esperanza de vida. Para entender el comportamiento mecánico del pericardio bovino y relacionarlo con su microestructura (contenido de fibras de colágeno y su orientación, principalmente), se han realizado ensayos de tracción uniaxial en un material modelo fabricado con fibras de colágeno. Asimismo se ha empleado la Difracción de Bajo Ángulo (SALS) para caracterizar la microestructura sin destruir el material. Los resultados con el material artificial nos permiten obtener la orientación de las fibras, relacionando la microestructura con sus propiedades mecánicas.

KEYWORDS: Bovine pericardium, Tensile strength, Light Scattering, Collagen fibre

1. INTRODUCTION

Pericardium is a collagenous membrane. It is composed of collagen and elastin fibres embedded in an extracellular matrix. Pericardium has been of interest for the last half century for its ability to be used, after specific treatment, as a biological suitable material for heart valve leaflets replacement. Biological prostheses, also known as bioprostheses, are more interesting than the mechanical prostheses on many aspects. They are non-thrombogenic, they show a good hemodynamic, similar to the one of the natural material, and they do not necessitate any anticoagulation medication for the patient [1]. However, they are not perfect, and they show durability problems. The main reasons are calcification and tearing of the material.

Calcification is the accumulation of calcium and phosphate in the implanted material due in part to the dead state of the implant, and partly to the pre-treatment they are subjected to. Tearing occurs either at sutures or in the material itself, showing that the implanted part is not always able to show enough wear resistance to withstand the stresses it is subjected to. Suture techniques and materials have been studied and compared [2, 3]. Tissue selection has to be improved to reduce tearing risks in the implanted cusp.

Solutions to calcification have been intended to be defined by the use of decellularization and chemical treatments. Decellularization is used for calcification prevention in porcine valve leaflets, and do not induce changes in the mechanical properties [4]. Pericardium has as well to be decellularized to suppress antigen. It has been shown that non ionic agents were safe for the receiver, and that decellularization did not affect the material strength, tensile modulus nor collagen structure network [5].

With respect to chemical treatments, glutaraldehyde treated pericardium has been used since more than 40 years [1]. Other chemical treatments have been tested [6] but glutaraldehyde is admitted to be the most suitable. Question of glutaraldehyde inducing calcification has been raised, and it has been shown that it did not induce it [7]. On the contrary Suh et al. showed a few years later, that glutaraldehyde cross-linked materials were showing a high and rapid accumulation of calcium in the early stage of the study (3 days) when compared to UV irradiation cross-linking [8].

Some alternative agents or techniques have been tested as a solution to substitute glutaraldehyde. UV irradiation [8] and No-react [9] are showing better reaction to calcification than glutaraldehyde, but long term studies have to be done for attesting the No-react efficiency. The calcification process is not well understood yet and some tests are made in the way to fill the lack of knowledge in this field. Physiological fluids with various concentrations of pyrophosphate, etidronate or phytate have been used as fluids for calcification inducing reagents on pericardium [10]. Results are showing that phytate was the most suitable to reduce calcification in vitro by measuring the calcium and phosphorous levels. It has to be noted that none was preventing calcification.

Tearing in the implanted material away from sutures implies a better tissue selection [11]. For this purpose, the aim is to find a way to define which part would be the most suitable for being a bioprosthetic leaflet. This has to be done via a non-destructive technique. Small Angle X-ray scattering (SAXS) have been widely used upon various materials. Soft matter [12], coal [13] compact powders [14] have been characterized by SAXS at the interaction and nanoscale structure level. The relation between crystallite thickness and organic preservation has been used for absolute and relative dating of bones based on the X-ray diffraction [15]]. Arterial wall has been tested in order to visualize the nanoscopic structural changes while applying a load [16]. Pericardium as well has been under X-ray observation via SAXS for collagen ultrastructure characterization (molecule packing, fibril spacing and orientation) [17]. The field of view however is in the order of magnitude of the nanometer, hence another technique, more suitable for microscale, should be used.

Visible light presenting a wavelength longer than X-ray's, seems to be of use for this purpose. Small angle light scattering (SALS) has shown good results with polymeric materials as a complement to rheometer for spinodal decomposition [18] and for time dependent phase change in polymer dispersed liquid crystal composites [19]. Sacks et al. set up a system for soft tissue microstructure analysis [20]. They have shown the ability of their system to define the main orientation of a fibre population and define some variables such as the orientation index as a quality of the angle definition. They also showed the capacity of the technique to separate two populations having an angular difference higher than 5°. The set up has been used for valve leaflet analysis [20], tendons [21], and pericardium [22].

It is admitted that collagen fibres concentration, distribution and orientation greatly influence the mechanical response of the tissue. Hence, the importance of knowing the microstructure of pericardium will lead to a better tissue selection for bioprostheses. The aim of this paper is to explore a methodology to link the microstructure of pericardium with its mechanical properties. For this purpose, an artificial collagen-based material has been used. The material is much thinner and transparent than pericardium. The advantage it presents is the more regular alignment of its collagen fibres and its higher homogeneity. A home-made SALS device was used to define the fibre orientation, and uniaxial tensile tests were performed in order to relate the fibre orientation with the tensile strength, stiffness, and elongation at rupture.

2. MATERIAL AND METHODS

2.1 Material

The artificial collagen-based material used is made of porcine skin smashed, pressed and then extruded. The manufacturing process aligns the fibres mainly in one direction. Globally, the composition of the material is collagen fibres of reduced length and a diameter of the order of magnitude of 1μm in a matrix. The material presents a good transparency and it is possible, by looking through it, to determine what the collagen fibres main orientation is. That is of importance for the first step of the methodology described here. As stated above, the material is of relative homogeneity while compared with pericardial tissue. This is also an important characteristic for the purpose of the tests presented below.

The material, like pericardium used in bioprostheses, has been treated with glutaraldehyde for cross-linking fibres (standard treatments).

According to those specificities of the material and the tests performed, we can expect that using SALS, the material may be assumed as being a diffraction grid. Hence, with optical laws, the pattern given by diffraction should be rotated by 90° with respect to the fibres direction. Moreover, when keeping the material orientated in one direction, the pattern should give the same orientation, with very few variations whichever location of it is chosen. Besides, and with respect to mechanical tests, the material should be stronger in the direction of the collagen fibres than in the perpendicular one. It should also show a greater elongation in the perpendicular direction compared to the fibres' one.

2.2 Small Angle Light Scattering

The small angle light scattering device is based on [20]. It consists of a 5mW laser (λ=632.8nm) mounted on an optical bench. The sample is then manually placed on the light path, and a screen is placed to receive the image (Figure 1). Pictures are taken by a digital camera placed behind the screen. Since the material can be compared to a set of diffraction grids, the expected result is an elliptical shaped spot on the screen having its mayor axis perpendicular to the main fibre orientation. Analyzing the picture will allow us to give the angle of the main fibre direction with the horizontal.

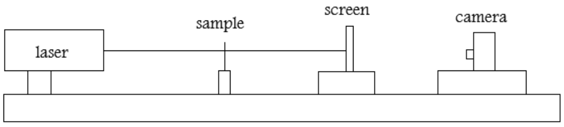

Figure 1: Scheme of the SALS device

2.3 SALS analysis

Using software for imaging data treatment a grey level scale is deduced from the picture. The analysis is based on analyzing the grey level (intensity) alongside a circle concentric with the ellipse and which diameter is the mayor axis of the ellipse shaped spot. The analysis will give a curve showing the grey level with respect to its angular position. Like shown by Sacks et al. [20], for a single population, the curve is showing 2 maxima and 2 minima. Distance between maxima is 180° and distance from a maximum to a minimum is 90°.

2.4 Tensile tests

Tensile uniaxial tests were performed under the flowing physiological serum at 37°C and at a constant crosshead speed of 0.03mm/s. Specimens were cut in a rectangular shape (5x30mm) and the thickness was measured. They were then fixed to the clamps mechanically and chemically. The free length between the clamps is of about 20mm. The tests are started with the material being free from any preloading, and are run until rupture. Data are recorded for analysis.

2.5 Data analysis

Applied force and distance between the clamps were recorded. Distance is set to be zero at a known distance between clamps. Therefore, specimen length and force at each time t is known. Calculations are done in order to obtain stress-elongation curves. L_0 the initial length of the specimen is manually found and the elongation is calculated as $\lambda=L/L_0$. The corresponding true stress is $\sigma=\lambda*(F-F_0)/A_0$ where L is the current length, F the current force, F_0 the force at initial length and A_0 is the initial cross sectional area. Stress-elongation curves are then compared for the same testing direction as well as compared to the perpendicular one.

The stiffness of the material is measured as being the slope of the linear part of the stress-elongation curve. A simple linear regression function is used for this purpose.

3. RESULTS AND DISCUSSION

3.1 SALS

Since the material is composed of well aligned fibres and its transparency allows the direct determination of their direction, it is quick to verify that data given by the device match the theory of light diffraction, and that the diffracting component is collagen and not part of the matrix. Then, the homogeneity of the material has, as well, to be assessed. For this purpose, a large piece of material has been placed on the rail. The material has been scanned in different places, and the resulting grey level-angular position curves are shown in Figure 2. Curves show a maximum intensity at a constant angular position. The homogeneity of the material's microstructure is then verified. As stated above, only the fibre main orientation is obtained.

Main fibre orientation indicated by the analysis makes an angle of about 95° with the horizontal. This is consistent with the sample positioned horizontally, indicating an actual angle of the fibres close to 5°. The pattern showed two maxima separated by an angular position of about 180°. Due to the symmetry of the plot, only the grey levels from 0º to 180º are shown in Figure 2.

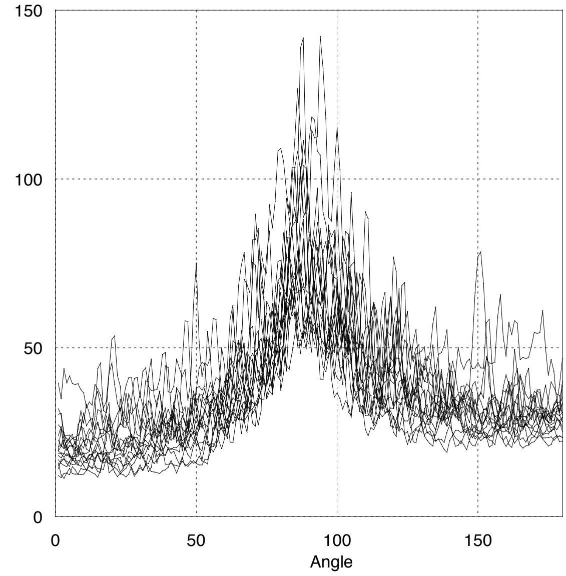

Figure 2: SALS data analysis of the patterns in 20 spots along a large sample.

3.2 Tensile tests

Uniaxial tensile tests have been performed along two perpendicular directions, one being the main direction of the fibres. Stress-elongation curves have been compared with respect to the testing direction in figure 3. The dashed lines are representing the perpendicular direction, while the solid ones, that are representing the parallel direction, are less extensible. The stiffness also differs significantly with respect to the direction.

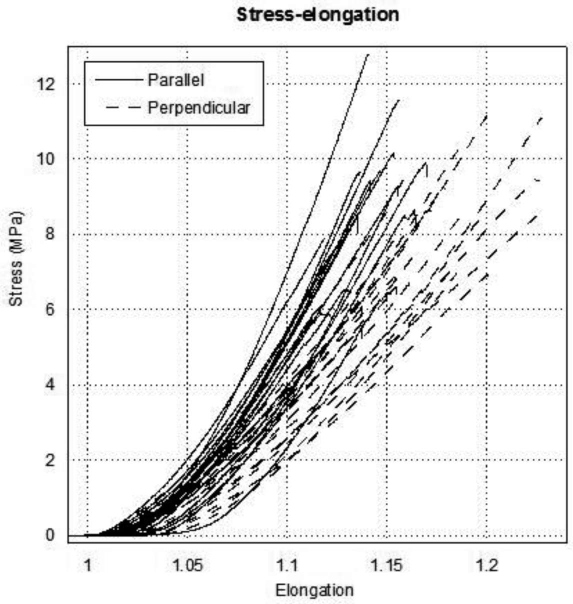

Figure 3: Stress-elongation curves of collagen based material in the parallel direction (solid lines) and the perpendicular direction (dashed lines)

In order to quantitatively compare the mechanical performance and link the fibre orientation to the mechanical properties, some specific parameters have been obtained: stiffness, ultimate stress and the maximum elongation. Table 1 shows a significantly different stiffness and elongation ($p<0.0001$). This is consistent with the material structure. The matrix is supposed to allow a greater elongation in the perpendicular direction, and the collagen fibres increase the stiffness in the longitudinal direction. The ultimate stress however is not significantly different. This is probably due to the cross-linking treatment reinforcing the perpendicular direction.

Table 1: *Mean values and standard errors of stiffness, elongation and ultimate stress in the parallel and perpendicular directions*

	Parallel n=17	Perpendicular n=19
Stiffness (MPa)	84±14	58±7
Elongation	1.14±0.016	1.18±0.02
Stress (MPa)	8±4	8±1.5

4. CONCLUSIONS

We have obtained microstructural information by using light diffraction on a model collagen-based material. This non destructive method has shown its ability to give the main direction of collagen fibres.

Uniaxial tensile tests have been performed and compared. Comparison has shown a clear difference in the mechanical performance with respect to the fibre orientation.

The link between the fibre orientation and the mechanical performance has been shown. The methodology is suitable to determine collagen fibres orientation and hence for tissue selection. Having the direction of the collagen fibres by SALS analysis will allow one to have a global idea on how to orientate the material in order to get the best mechanical properties for a specific application.

Further work will have to be done on the artificial material in order to increase the quality of the data and allow a better determination of precise mechanical features. This implies definition of parameters on SALS analysis for quantification of the data quality and the fibre organization. More, pericardium is a much more complex material than the one used in this work, and SALS device characteristics will have to be determined or improved, such as the ability of the device to separate two or more overlapping fibre populations. Mathematical models for the simple artificial material will have to be expressed, to show the influence of the fibre orientation on mechanical behaviour.

5. ACKNOWLEDGES

The authors gratefully acknowledge the financial support from the Fundación Marcelino Botín through the project “Diseño y desarrollo de nuevos biomateriales para medicina regenerativa” and to the Ministerio de Ciencia e Innovación through the project MAT2009-10258.

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