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Biochemical and mechanical behavior of ostrich pericardium as a new biomaterial

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Abstract

We have performed a comparative analysis of glutaraldehyde-preserved ostrich pericardium, as a novel biomaterial, with bovine pericardium. The biochemical characteristics (histology, water content, amino acid composition, and collagen and elastin contents), mechanical properties, and in vivo calcification in a subcutaneous rat model were examined. Ostrich pericardium is slightly thinner and shows a higher water content ($70 \pm 2\%$ vs. $62 \pm 2\%$) than bovine pericardium. Additionally, ostrich pericardium presents 1.6-fold lower elastin content and a lower percentage of collagen in reference to the total protein content ($68 \pm 2\%$ vs. $76 \pm 2\%$). However, ostrich pericardium shows better mechanical properties, with higher tensile stress at rupture (32.4 ± 7.5 vs. 11.5 ± 4.6) than calf pericardium. In vivo calcification studies in a rat subcutaneous model show that ostrich pericardium is significantly less calcified than bovine pericardium (23.95 ± 13.30 vs. 100.10 ± 37.36 mg/g tissue) after 60 days of implantation. In conclusion, glutaraldehyde-stabilized ostrich pericardium tissue shows better mechanical properties than calf tissue. However, calcium accumulation in implanted ostrich tissue is still too high to consider it a much better alternative to bovine pericardium, and anticalcification treatments should be considered.

Keywords: Calcification; Collagen; Cross-linking; Elastin; Mechanical properties

1. Introduction

The impact of biomaterial technologies is great; worldwide, the use of biomaterials (e.g., in wound dressings or artificial heart valves) and the number of different applications for them is increasing. Due to better medical care, the population is aging and requires long-lasting devices such as heart valves. Attempts to replace diseased human cardiac valves with prostheses began more than three decades ago. By comparison with other fields of medicine that have evolved quite fast, the development of artificial heart valves has progressed rather slowly since its beginning in 1960. Bovine pericardial tissue and aortic porcine valves (stented or unstented) fixed with glutaraldehyde (GA) are used to construct heart valves in which the design characteristics are the main differences between heart valve manufacturers [1]. An ideal bioprosthesis has not yet been achieved, as a result of major drawbacks like their limited durability due to tissue failure and/or calcification, which lead to clinical failure [2]. Alternatives to stented heart valves bioprostheses are being developed. Thus, stentless bioprostheses are becoming an actual alternative [3,4], and tissue-engineered valve leaflets are currently under research [5–8]. Additionally, alternatives to porcine and bovine tissues are being analyzed, such as kangaroo pericardium or aortic wall [9–11]. Similarly, the present report examines ostrich

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pericardium, a new tissue that may prove to be an alternative to the porcine and bovine tissues currently employed in the manufacture of cardiac valve prostheses. We have first performed a biochemical characterization of the tissue, with a quantitative assessment of the collagen and elastin content, and analyzed the effect of GA-fixation on the stability of the collagen cross-linking. The histological and mechanical properties of GA-fixed specimens have also been studied as well as the influence of this treatment on the calcification potential of ostrich pericardium after subcutaneous implantation in a rat model.

2. Material and methods

2.1. Materials and chemical treatments

Ostrich pericardium was obtained directly from a local slaughterhouse and transported to our laboratory in sterile saline solution (0.9% NaCl, w/v). Afterwards, the tissue was cleaned to remove fat, and portions were selected for different biochemical studies and for subcutaneous implantation by visual inspection and thickness measurements to ensure similar characteristics in all the samples. All reagents were purchased from Sigma (St. Louis, MO, USA). Unless otherwise stated, ostrich pericardium specimens were treated for 24 h at room temperature with 0.625% (v/v) GA in 0.1 M sodium phosphate buffer, pH 7.4. Bovine pericardium was obtained directly from a local slaughterhouse from young calves (6–9 months-old) and it was processed similarly to the ostrich pericardium.

2.2. Water content

Small pieces of GA-treated ostrich or calf pericardium (1×1 cm; $n = 12$) were blotted on filter paper to remove surface saline solution and weighed to determine their wet weight. They were then put in an oven at 37 °C until constant weight was reached (dry weight). Water content was calculated from the mean values of the differences between wet and dry weights and expressed as a percentage.

2.3. Thickness measurement

The thickness of each tissue fragment was determined measuring a series of three points in 12 random samples using a Mitutoyo digital micrometer (Elecount, series E:A33/8 Digital) with a precision at 20 °C of ± 3 μm.

2.4. Amino acid analysis and cross-linking stability assessment

To analyze the amino acid composition, native or GA-modified pericardium samples of known weight (1–2 mg of wet tissue) were hydrolyzed in vacuum-sealed Pyrex tubes at 105 °C for 24 h in the presence of 0.5 ml of 5.7 N constant boiling HCl, containing 0.1% phenol and norleucine as internal standard. The samples were then dried by vacuum evaporation, dissolved in the application buffer and assessed with a Beckman 6300 amino acid analyzer (Beckman Instruments Inc., Palo Alto, CA). Lysine (Lys) and hydroxylysine (Hyl) residues in pericardium may be found either in a free form or forming endogenous cross-links. These cross-links are almost quantitatively broken by acid hydrolysis yielding free Lys and Hyl residues. However, only the free Lys and Hyl residues are susceptible to modification by exhaustive chemical treatments like exposure to 0.625% GA for 24 h. GA-modified residues are not restored to their free form after hydrolysis. Variations in Lys and Hyl residues after GA treatment were always determined after normalization in terms of the content in Val and Leu residues, which remained unchanged after this treatment, as described elsewhere [12].

The collagen percentage over the global protein content in the pericardium was determined from the amino acid composition according to the following formula: percentage of collagen = 2Hyp/(2Hyp + 5(Pro-Hyp)), taking into account that interstitial collagen presents approximately equal amounts of hydroxyproline (Hyp) and Pro, and that Pro content in non-collagenous proteins is around 5 times lower than in collagen [13]. Collagen content was also evaluated on a weight basis considering that interstitial collagen contains 13% (w/w) Hyp.

2.5. Elastin content

Insoluble elastin was quantified according to its resistance to hydrolysis during treatment with diluted hot alkali according to a modification of the method described by Wolinsky [14]. Briefly, tissue specimens of around 2 cm^2 ($n = 12$) were first subjected to delipidation in chloroform/methanol (1:1 v/v) and dried. Cell proteins were extracted after cell lysis in 0.3% sodium dodecyl sulphate for 12 h with mechanical agitation [11]. The remaining tissue was minced and extracellular proteins (but not elastin) were then solubilized by four extractions in nine volumes of 0.1 M NaOH for 15 min in a boiling-water bath. The insoluble elastin residue was then lyophilized and the dry weight was quantified and expressed as micrograms per milligram of tissue (dry weight).

2.6. Histological examination

Native and glutaraldehyde tissue samples were fixed in 4% formaldehyde in PBS, processed and stained with hematoxylin–eosin to assess general morphology and with the orcein van Gieson technique to visualize elastin, following standard histological techniques.

2.7. Stress–strain characteristics of the ostrich pericardium

Samples measuring 12×2 cm^2 were cut from each pericardium membrane, in the root-to-apex direction. A total of 24 specimens were prepared for the assay: 12 from the bovine pericardium and 12 from the ostrich pericardium.

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