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Dynamic mechanical characteristics of intact and structurally modified bovine pericardial tissues

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Abstract

Bovine pericardium (BP) is a source of natural biomaterials with a wide range of clinical applications. In the present work we studied the dynamic mechanical behavior of BP in native form and under specific enzymatic degradation with chondroitinase ABC extracted a 17% of the total glycosaminoglycans (GAGs). The GAGs content of native BP was composed mainly from hyaluronan, chondroitine sulfate and dermatan sulfate. Dynamic tensile mechanical testing of BP in the frequency range 0.1–20 Hz demonstrated its viscoelastic nature. The storage modulus was equal to 6.5 (native) and 5.5 (degraded) MPa initially, increased in the region nearby 1 Hz by about 15%. This was related with physical resonance mechanisms activated in this frequency region. The high modulus (modulus of the high linear phase of stress–strain) was equal to 14 (native) and 10 (degraded) MPa, dropped at high frequencies to 7 and 5 Mpa, respectively. The damping, expressed by the hysteresis, was equal to 20% of the loading energy, changed exponentially with the frequency to 30% at 20 Hz. It seemed that of the elastic mechanical parameters, the storage modulus and the high modulus were even slightly dropped as a result of degradation. As a final conclusion, there was evident that GAGs may play a non-negligible role in the dynamic mechanical behavior of BP and, probably in other soft tissue biomechanics. It is suggested that the GAGs content may be considered during the design and chemical modification of biomaterials based on BP and other soft tissues.

Keywords: Bovine pericardium; Dynamic mechanical testing; Glycosaminoglycans; Viscoelasticity; Soft tissue biomechanics

1. Introduction

Bovine pericardial (BP) tissues have been widely used, after treatment with different cross-linking methods, for the construction of bioprosthetic heart valves and for repair of several soft tissue deficiencies, such as cardiac patches, staple line reinforcement and vascular stents in cardiovascular and lung surgery. Severe clinical complications have been reported, especially for bioprosthetic heart valves, caused from incompetent mechanical performance and calcification (Bruck, 1983–84; Schoen and Levy, 1999).

BP can be characterized mechanically as a hardly non-linear, anisotropic, multilaminate composite pliable material, which is usually imposed in large deformations during its physiological function as implant material. Histologically, BP is composed from a complicated network of collagenous and elastic fibers. These are embedded in an amorphous matrix, which maintains the structural integrity and functionality of the tissue. The matrix is composed mainly of proteoglycans (PGs) and glycosaminoglycans (GAGs), which engage fibers, cells and interstitial fluid (water and soluble electrolytes). PGs, which are important components of the extracellular matrix of all connective tissues, comprise a class of polyanionic macromolecules consisting of a protein core onto which sulfated GAGs and oligosaccharide chains are covalently bound (Iozzo, 1998). The common GAGs include the galactosaminoglycans—chondroitin sulfate (CS) and dermatan sulfate (DS)— and the glucosaminoglycans—hyaluronan (HA), heparan sulfate (HS), heparin, and keratan sulfate (KS) (Kjellen

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and Lindahl, 1991; Hardingham and Fosang, 1992). Hyaluronan is a high molecular weight non-sulfated GAG, which does not occur as a PG but as a free GAG.

During mechanical loading and deformation a rearrangement of all fibrous components of the tissue is observed, involving gradual straightening, rotation and elongation of the crimped unloaded fibers (Zioupos and Barbenel, 1994). Static tensile experiments have shown that the mechanical performance of soft tissues resulted from that of the fibrous components, which are the main load-bearing elements, (Mavrilas and Missirlis, 1991; Christie, 1992; Sacks and Chuong, 1998). Although recently the mechanical contribution of the matrix attempted to be computed in soft tissue modeling, no mechanical data are available (Sacks, 2000). In creep, stress relaxation and forced vibration experiments the viscoelastic nature of BP has been demonstrated and studied (Naimark et al., 1992; Garcia Sestafe et al., 1994; Courtman et al., 1994; Duncan and Boughner, 1998).

It is obvious that during cyclic loading all or part of the non-fibrous tissue components should potentially contribute to its mechanical characteristics by direct or indirect interaction with the deformed fibers. Studies in articular cartilage mechanics have shown that GAGs contribute to its viscoelastic performance by regulating its internal friction mechanism due to a swelling effect (Lai et al., 1981; Flahiff et al., 2002). Alterations of GAGs content have also been reported in certain connective tissues deficiencies, as in atherosclerosis and aneurysm of blood vessels, scoliosis of nasal cartilage and osteoarthritis of articular cartilage, in parallel with changes of the mechanical performance of those tissues (Silver et al., 2002; Theocharis et al., 2002A, B). In the native heart valve, highly hydrated GAGs present in the middle spogiosa layer allow shearing between the two outer layers, the fibrosa and ventricularis, which are composed predominantly of a dense network of collagen fibers, during valve function (Schoen and Levy, 1999; Vyavahare et al., 1999). Chemically modified porcine heart valves with glutaraldehyde for use as implant material demonstrate collagen denaturation and loss of a great part of GAGs during fatigue loading, accompanied with reduced bending strength (Vyavahare et al., 1999). However, the possible contribution of GAGs components in the mechanical performance of BP has not been elucidated.

In this work we studied the dynamic mechanical characteristics of BP in two modes: fresh intact tissue and after specific enzymatic degradation which selectively decomposed a significant part of GAGs without affecting the rest structure of the tissue. We attempted so to study the dependence of the viscoelastic mechanical parameters of BP, such as the storage and high modulus and the hysteresis, on the frequency of the applied cyclic load and the potential contribution of GAGs in the mechanical performance of soft tissues.

2. Materials and methods

BP tissues from healthy animals were supplied fresh from the slaughterhouse and transported to laboratory in iced saline. The tissues were carefully trimmed for removing the external fatty laminates. Two adjacent portions of the trimmed tissue were cut from each sack. One was used for dynamic mechanical testing as intact BP and the other for biochemical analysis, modification and subsequent mechanical testing.

2.1. Biochemical modification and analysis of BP

2.1.1. Outline of the method

Portions of intact BP tissue samples, after external trimming, were used for various determinations. Uronic acid (UA) and hence the total GAGs were determined after papain digestion of part of the samples followed by chemical analysis. High-performance liquid chromatography (HPLC) and electrophoresis were used for the determination of disaccharides and GAGs of the digests, respectively. A second part of intact BP was imposed to alkaline hydrolysis for the determination of hydroxyproline (Hyp) and hence collagen content of BP. Other portions were imposed to a structural modification by a first enzymatic degradation with chondroitinase ABC for partial removing of GAGs from the extracellular matrix. From the products of this first degradation, the supernatant (SN_1) was used to determine UA_1 while the $residue_1$ was used partly for dynamic mechanical testing of degraded BP, as with the native ones, and the rest for subsequent degradation. After a second degradation with chondroitinase ABC the supernatant SN_2 was used for the determination of UA_2, and the $residue_2$ was digested with papain for the determination of UA_3 remaining after the two degradations with chondroitinase ABC. The rational of determining the GAGs at SN_2 was to secure that the greatest possible amount of GAGs was liberated at SN_1. Fig. 1 presents a brief flow diagram of the experimental procedure.

2.1.2. Details of the procedure

Chemicals: Twice crystallized papain (EC 3.4.22.2), chondroitinase ABC from *Proteus vulgaris* (EC 4.2.2.4), chondroitinase AC II from *Arthrobacter aurescens* (EC 4.2.2.5), as also GAG standards were purchased from Sigma Chemical Co. (Saint Louis, MO, USA). Standard preparations of chondroitin disaccharides (Δdi-4S, Δdi-6S and Δdi-0S) and hyaluronan (Δdi-0S) were purchased from Seikagaku Kogyo (Tokyo, Japan). Toluidine-blue salt was purchased from Serva (Heidelberg, Germany) and 1,9-Dimethyl-methylene Blue (DMB) from Aldrich