Architecture and function of cartilage matrix: lessons from nature

Ferenc Horkay

Section on Tissue Biophysics and Biomimetics; Program in Pediatric Imaging and Tissue Sciences, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, 13 South Drive, Bethesda, MD 20892, USA, horkay@helix.nih.gov

Understanding of the interactions between the macromolecular components of cartilage extracellular matrix is essential to develop successful tissue engineering strategies for cartilage repair. In this study the contributions of the major macromolecular components to the compressive strength of cartilage is determined. In cartilage the bottlebrush shaped aggrecan molecules form complexes with linear hyaluronic acid (HA) chains. We found that in the physiological concentration range the osmotic modulus of the aggrecan-HA system exceeds that of the pure aggrecan solution. It is also demonstrated that the osmotic modulus of the collagen solution is more than an order of magnitude smaller than that of the proteoglycan systems indicating that the load-bearing capability of cartilage is primarily sustained by the proteoglycan assemblies.

Introduction

Articular cartilage is a hierarchically organized tissue exhibiting high stiffness, low friction, excellent lubrication and wears characteristics [1-3]. It consists of a fibrous collagen network (mainly type II collagen), which is pre-stressed by the osmotic swelling pressure exerted by negatively charged glycosaminoglycan aggregates (GAG's) embedded in the collagen matrix. Besides collagen and GAG's, cartilage also contains other components such as link protein, which has an important role in the stability of the extracellular matrix.

Cartilage has a very limited capacity for repair after damage [4,5]. Tissue engineering is used to study the process of cartilage formation in a controlled environment and can potentially provide transplant material for cartilage reconstruction. It has been found that the mechanical properties of engineered cartilage strongly depend on the culturing conditions, such as scaffold material, cell seeding density, mechanical loading. The effect of extracellular matrix constituents on the mechanical properties was investigated, and a strong dependence of the elastic modulus on both collagen and GAG contents was observed [6]. Better control of the biomechanical properties of engineered cartilage is hampered by a lack of understanding of the physical chemical interactions among the macromolecular constituents of cartilage extracellular matrix.

The aim of the present work is to quantify the mechanical and thermodynamic properties of cartilage polymers. We determine the interactions between the major macromolecular components of cartilage extracellular matrix from osmotic pressure measurements and scattering measurements. The load-bearing resistance is evaluated from the concentration dependence of the osmotic pressure from which the osmotic modulus is derived.

Organization of Cartilage Extracellular Matrix

From the viewpoint of polymer science cartilage is a fiber-reinforced, highly permeable composite gel filled with physiological salt solution. The major fibrous component is type II collagen, which provides the tensile strength of cartilage. Cartilage extracellular matrix exhibits a zonal organization differing in composition, fiber structure and orientation. The pores of the collagen matrix are filled with proteoglycan aggregates. The major cartilage proteoglycan is the bottlebrush shaped aggrecan molecule. In cartilage extracellular matrix

aggrecan molecules interact with hyaluronic acid and form a secondary bottlebrush structure. The swelling of proteoglycan assemblies pre-stresses the collagen fibers. The mechanical properties of cartilage are governed by the balance of the swelling pressure of the aggrecan-hyaluronic acid aggregates and the elastic pre-stress developed in the collagen network.

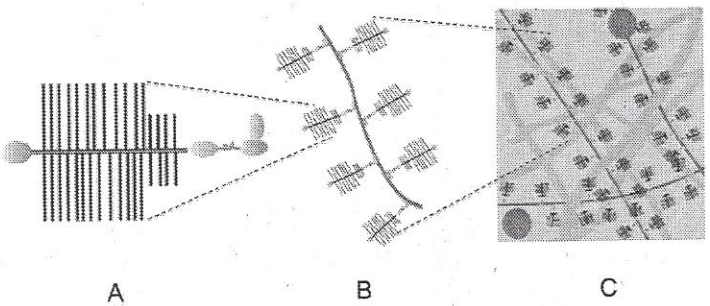

Figure 1: Hierarchy of cartilage matrix. (A) Aggrecan bottlebrush. (B) Aggrecan-hyaluronic acid aggregate. (C) Aggrecan-hyaluronic acid aggregates in the collagen matrix.

Figure 1 illustrates the hierarchical organization of the main macromolecular components in the cartilage matrix. The aggrecan-hyaluronic acid complexes are enmeshed in the fibers of the collagen network.

Materials and Methods

Sample Preparation

Aggrecan (bovine articular cartilage, Sigma) and aggrecan-hyaluronic acid solutions were prepared in 100 mM NaCl. In the latter case the ratio of aggrecan to hyaluronic acid was set at 100 to1.

Collagen (from chicken sternal cartilage, Sigma-Aldrich) was first dissolved in acetic acid then neutralized. The solutions were allowed to homogenize for 2-3 days.

Osmotic Pressure Measurements

The osmotic pressure of the solutions was determined as a function of concentration by bringing them to equilibrium with polyvinyl alcohol (PVA) gels of known swelling pressure [7,8]. The size of the PVA gel filaments was measured by optical microscopy after equilibration in the solution (ca. 24 h). All measurements were made at 25 ± 0.1 °C.

Atomic Force Microscopy (AFM)

AFM images were made using a commercial AFM (Bioscope I with Nanoscope V controller, Veeco, Santa Barbara, CA). Silicon nitride cantilevers were used (MSCT, Veeco Metrology, Santa Barbara, CA), which have a nominal spring constants of 0.01-0.12 nN/nm, resonant frequency in fluid of ~10 kHz, and nominal tip radii of 10 nm. Data analysis was made using procedures reported previously [9,10].

Dynamic Light Scattering Measurements

Dynamic Light Scattering (DLS) measurements were performed on aggrecan and aggrecan-HA solutions with a Precision Detector - Expert Laser Light Scattering DLS Workstation equipped with a He-Ne laser (wavelength: 698 nm). The solutions were not filtered to avoid shear degradation.

Results and Discussion

Figure 2 shows the concentration dependence of the osmotic pressure Π both for the aggrecan solution and for that containing aggrecan-HA complex. At low concentration the osmotic pressure is smaller when HA is present. This reduction is evidence for complex formation between aggrecan and HA. Complexation removes free aggrecan molecules from the solution and the osmotic pressure decreases. The difference between the two systems

progressively decreases with increasing aggrecan concentration, and at higher concentration the osmotic pressure of the aggrecan-HA system exceeds that of the aggrecan solution [11].

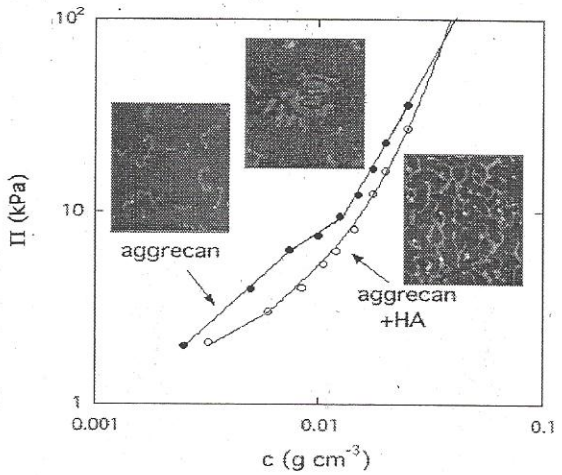

Figure 2. Concentration dependence of the osmotic pressure Π of aggrecan (filled circle) and aggrecan/hyaluronic acid (open circle) solutions in 100 mM NaCl. The AFM images show aggrecan adsorption patterns with increasing surface concentrations. Scan area: 1.5 x 1.5 μm^2; scan frequency: 1.5 Hz.

The load-bearing properties of proteoglycan assemblies can be estimated from the concentration dependence of the osmotic pressure from which the osmotic modulus $K_{os} = c\partial\Pi/\partial c$ can be derived. Figure 3 shows the variation of K_{os} for the two proteoglycan systems, as well as for the collagen solution. In the physiological concentration range ($c > 0.04$ g/cm^3) the osmotic modulus is greater in the aggrecan-HA system than in the pure aggrecan solution. It can also be seen that K_{os} increases faster in the aggrecan-HA system indicating that complex formation enhances the mechanical stability of the assemblies [11].

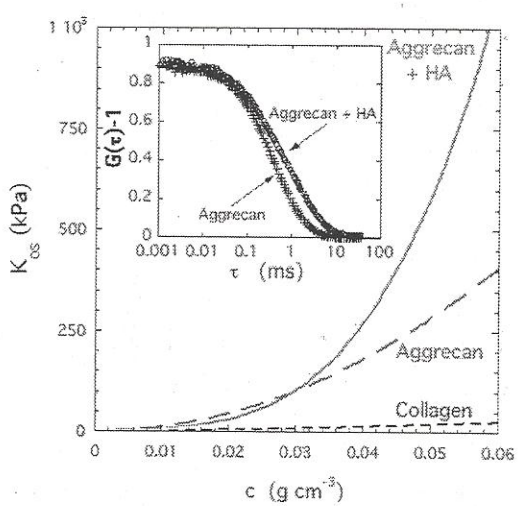

Figure 3. Concentration dependence of the osmotic modulus K_{os} of solutions of cartilage polymers. Inset: DLS intensity correlation functions $G(\tau)$ for solutions of aggrecan and aggrecan-hyaluronic acid complex. τ is the delay time.

In the collagen solution the value of K_{os} is small and its variation with concentration is significantly weaker than in the two proteoglycan systems. This finding is consistent with the limited contribution of collagen to the load-bearing function of cartilage. However, the fibrillar collagen network provides tensile strength and complete shape retention.

The inset in Figure 3 shows the intensity correlation functions measured by dynamic light scattering in aggrecan and in aggrecan-HA solutions at 90°. The dynamic behavior of proteoglycan assemblies has important consequences for the functional properties of cartilage. The rate of volume change, due either to external loading or to changes in the ionic composition, defines the recovery rate of cartilage. It can be seen that the relaxation

properties of the latter system are slightly slower than that of the pure aggrecan solution but the difference between these curves is small, indicating that the dynamics of the aggrecan-HA complex is hardly affected by its connectivity. The relaxation curves cannot be described by a simple exponential relationship as the correlation function of aggrecan exhibits a wide range of relaxation times, extending from about 10^{-2} to 10 ms. The relative rigidity of the aggrecan bottlebrush, arising from the highly charged polysaccharide bristles, prevents mutual interpenetration and chain overlap [12].

Conclusions

Osmotic pressure measurements made on aggrecan and aggrecan-HA solutions reveal the formation of aggrecan-HA complexes. Complexation reduces the osmotic pressure by approximately 30%. The osmotic pressure increases faster with the polymer concentration in the aggrecan-HA solution. In the physiological concentration range the osmotic modulus of the aggrecan-HA system exceeds that of the solution of random assemblies of aggrecan molecules. Dynamic light scattering measurements indicate that complex formation only weakly affects the dynamic response. The osmotic modulus of the collagen solution is much smaller, which is consistent with the limited role of collagen in the load-bearing function of cartilage.

Acknowledgements

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