Cartilage: Biomimetic Study of the Extracellular Matrix

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ABSTRACT

Cartilage is a complex biological tissue that exhibits gel-like behavior. Its primary biological function is providing compressive resistance to external loading and nearly frictionless lubrication of joints. In this study, we model cartilage extracellular matrix using a biomimetic system. We demonstrate that poly(vinyl) alcohol (PVA) hydrogels are robust biomaterials exhibiting mechanical and swelling properties similar to that of cartilage extracellular matrix. A comparison is made between the macroscopic behavior of PVA gels and literature data reported for cartilage.

INTRODUCTION

Cartilage is located at the end of bones. It has multiple biological roles such as load bearing, cushioning, lubrication. Cartilage is aneural and avascular. Since it contains a limited number of cells cartilage regenerates very slowly after damage [1-3].

Cartilage is synthesized by chondrocytes. These cells represent about five percent of the tissue volume. In addition, the matrix is comprised of collagen (10% to 20%), proteoglycans (5% to 15%), and water (70% to 80%) (7).
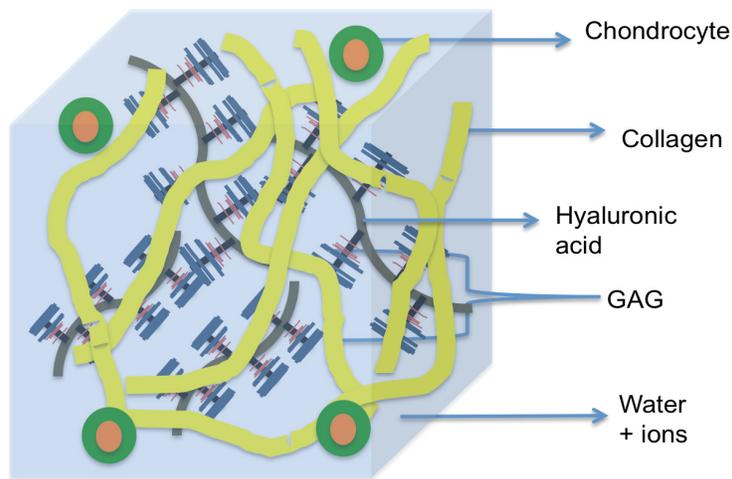

Figure 1: Illustration of the complexity of cartilage tissue.

Within the cartilage matrix, aggrecan is the most prominent proteoglycan. It has a bottlebrush architecture consisting of a protein core and mainly chondroitin sulfate and keratan sulfate side chains. These molecules possess high net negative charges that repel

each other in aqueous solution resulting in an extended molecular configuration. The hydrophilic chains attract water molecules and form a microgel, which assists the matrix to resist external loading. In the cartilage matrix aggrecan bottlebrushes self-assemble along linear hyaluronic acid chains [4-6].

Collagen provides the skeleton of the cartilage matrix; it is a triple helical polypeptide chain. Type II collagen is the most common type of collagen found in cartilage. Collagen serves two major functions: (i) its triple helical structure gives cartilage the ability to sustain tensile stress; (ii) it contains the aggrecan-hyaluronic acid complex, which is essential to maintain structural integrity [7-10].

The objective of our work is to develop a biomimetic model system that possesses cartilage-like mechanical and swelling properties. We characterize well-defined poly(vinyl alcohol) gels and compare these results with those obtained for cartilage tissue.

BIOMIMETIC MODEL

There are several requirements for an appropriate biomimetic model system. These include but are not restricted to
  (i) Tissue-like architecture,
  (ii) Easily controllable chemical composition,
  (iii) Controllable mechanical and swelling properties,
  (iv) High hydration capability and enhanced stability in the physiological
       milieu,
  (v) Fast response to loading and full shape recovery after unloading,
  (vi) Biocompatibility.
Since cartilage exhibits gel-like behavior (it comprises of a swollen network structure which contains over seventy percent water), hydrogels are natural choices for modeling cartilage. Poly (vinyl alcohol) (PVA) is a particularly desirable model system because it meets all the requirements listed above [11-20]. Its low coefficient of friction coupled with its biocompatibility makes it a suitable substitute artificial tissue for cartilage.

The most important mechanical properties relevant to cartilage biomechanical functions are the elastic modulus and the osmotic swelling pressure. The elastic modulus quantifies the load bearing property of the system, while the osmotic swelling pressure defines its compressive resistance. High osmotic pressure is desirable for effective load bearing. The chemical cross-links counteract the swelling and help to maintain structural integrity (in cartilage the collagen network performs this role).

MATERIALS AND METHODS

Gel preparation

Poly(vinyl alcohol) (PVA, M_w = 100 000, degree of hydrolysis > 99 mol%) gel cylinders (1 cm diameter, 1 cm height) were made by cross-linking of solutions with glutaraldehyde at polymer concentrations of 4 and 10% (w/w) at pH ≈ 1.5. The molar ratio of the monomer of the PVA to glutaraldehyde was set at 50, 100 and 200. After gelation the samples were washed in pure water to remove sol fraction (e.g., uncross-linked polymer), and then equilibrated with deionized water. Gel slabs (> 2 mm thick)

were cast for the osmotic swelling pressure measurements and for AFM nanoindentation.

Shear modulus measurements

Shear modulus was determined from uniaxial compression measurements made on gel cylinders using a TA.XT2I HR Texture Analyzer (Stable Micro Systems, UK). Stress–strain isotherms were determined at 25 °C in about 3–5 min with no detectable change in gel composition. The stress–strain isotherms were analyzed by Eq. 1. The shear modulus G was calculated from the nominal stress, f (force per unit undeformed cross section), using the relation

$$G = f/(\Lambda - \Lambda^{-2}) \tag{1}$$

where Λ is the deformation ratio. Measurements were carried out in the range $0.7 < \Lambda \leq 1$.

Atomic Force Microscopy (AFM)

AFM was used to construct elastic modulus maps [21,22]. General-purpose, oxide-sharpened, silicon nitride tips of pyramidal shape were used for the AFM measurements, performed with a commercial AFM (Bioscope SZ with Nanoscope V controller, Veeco). The spring constants of the cantilevers were measured by the thermal tune method. Code written in Matlab was used to analyze each force-indentation data set and extract values of shear modulus. The shear modulus was obtained from the relationship derived by Bilodeau:

$$F = 1.4906 \, G \, \tan\theta \, \delta^2/(1-\mu) \tag{2}$$

where F is the force, δ is the indentation depth for a pyramidal indenter, 2θ is the tip angle and μ is Poisson's ratio of the indented material.

Osmotic swelling pressure measurements

A home-built Tissue Micro-Osmometer (TMO) was used for the measurement of the osmotic swelling pressure [23]. In this apparatus the quartz crystal electrode was coated with PVA solution. Quartz crystals are piezoelectric materials that vibrate in response to an external alternating electric field. If a rigid film is deposited on the surface of the crystal it couples to the oscillation and changes the resonant frequency. Sauerbrey[23] showed that the decrease in the resonant frequency, Δf, is proportional to the mass, Δm, deposited per unit area

$$\Delta f = f_{dry} - f = (2f_o^2/\rho v) \, \Delta m \tag{3}$$

where f_o is the resonant frequency of the quartz crystal and ρ and v are the density and shear velocity of sound waves in the quartz crystal, respectively. The electronics used in the present instrument allow us to measure frequency changes within 1 Hz. The 10-MHz quartz crystal is suitable for detecting very small mass changes $\approx 10 \, ng/cm^2$.

The coated quartz crystals were equilibrated with NaCl solutions of known water

vapor pressure. The vapor sorption measurements were carried out in a temperature-controlled sample chamber placed in a Faraday cage. Changing the concentration of the NaCl solution induced changes in the water vapor pressure. The mass of the polymer (PVA) was determined by drying the sample.

RESULTS AND DISCUSSION

Elastic modulus measurements

In Figure 2a the nominal stress f is plotted as a function of the corresponding deformation for different PVA gels swollen in water. It can be seen that the curves exhibit a non-linear upwards trend indicating that gel stiffness increases with increasing deformation. In Figure 2b the same data are shown according to Eq. 1. In this representation $f/(\Lambda - \Lambda^{-2})$ is practically independent of $1/\Lambda$.
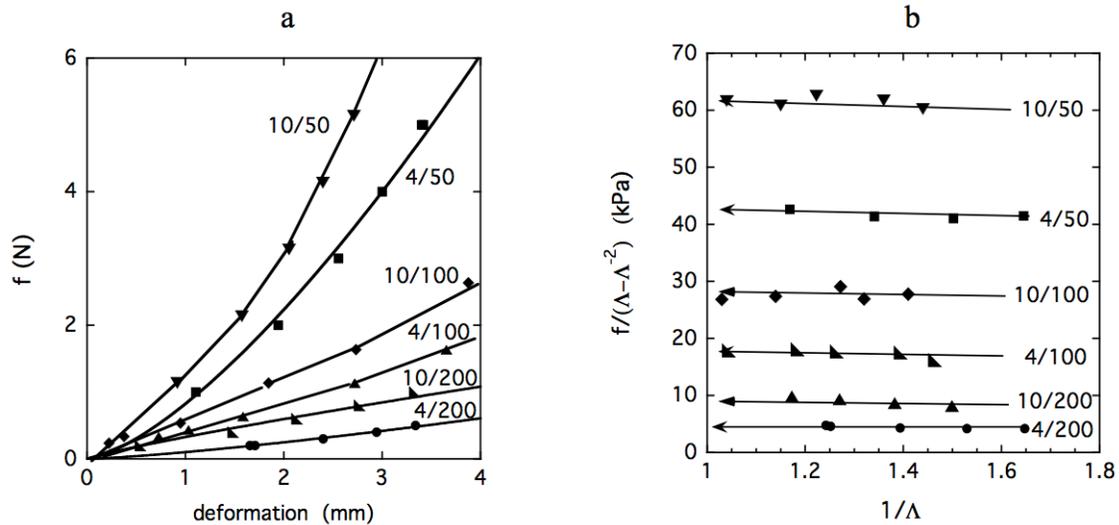

Figure 2. a. Force-deformation curves for PVA hydrogels equilibrated with water. b. Linearized data of Figure 2a yields the elastic (shear) modulus G of PVA gels using equation 1.

In order to determine the elastic modulus Eq. 1 was used (see Figure 2.b). In this representation, extrapolation to the y-axis yields the elastic modulus, which varies between 5 and 65 kPa. The results also show that G increases with
  (i) increasing polymer concentration (at constant cross-linking density), and
  (ii) increasing cross-linking density (at constant polymer concentration).

Figure 3 illustrates the robustness of PVA hydrogels. It can be seen that the gel completely recovers (retains its original size and shape) after unloading.
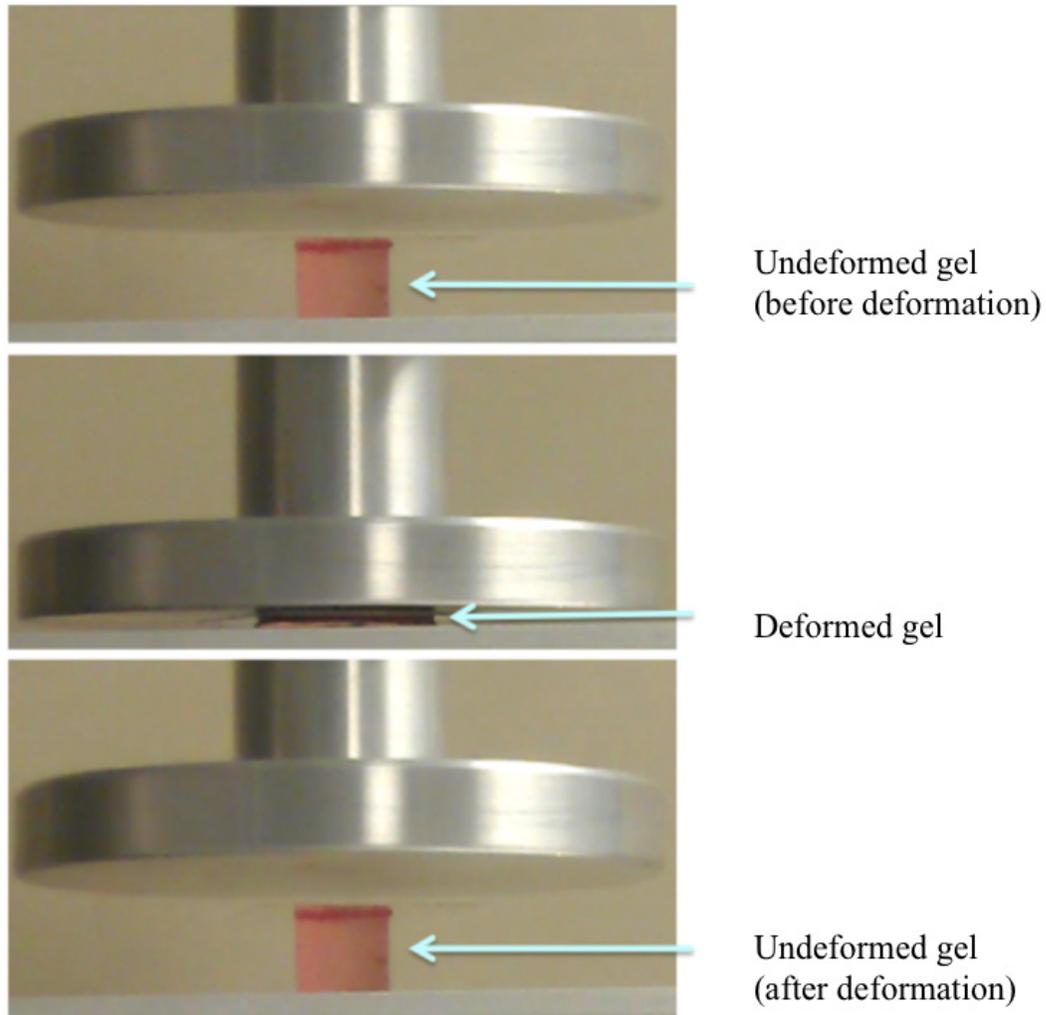
Figure 3. Illustration of size and shape recovery of a PVA gel after uniaxial compression.

Osmotic pressure measurements

The osmotic swelling pressure Π of PVA gels was determined as a function of the swelling degree. **Figure 4a** shows typical curves for gels differing in cross-linking densities. It can be seen that Π, which governs the compressive resistance of the gel, decreases with increasing swelling degree. In Figure 4b are displayed swelling pressure data measured on healthy and osteoarthritic cartilage samples reported in the literature [25]. Osteoarthritis causes the cartilage to become stiff and lose its elasticity. It can be seen that the diseased cartilage exhibits reduced osmotic swelling pressure and swells more than the healthy tissue.
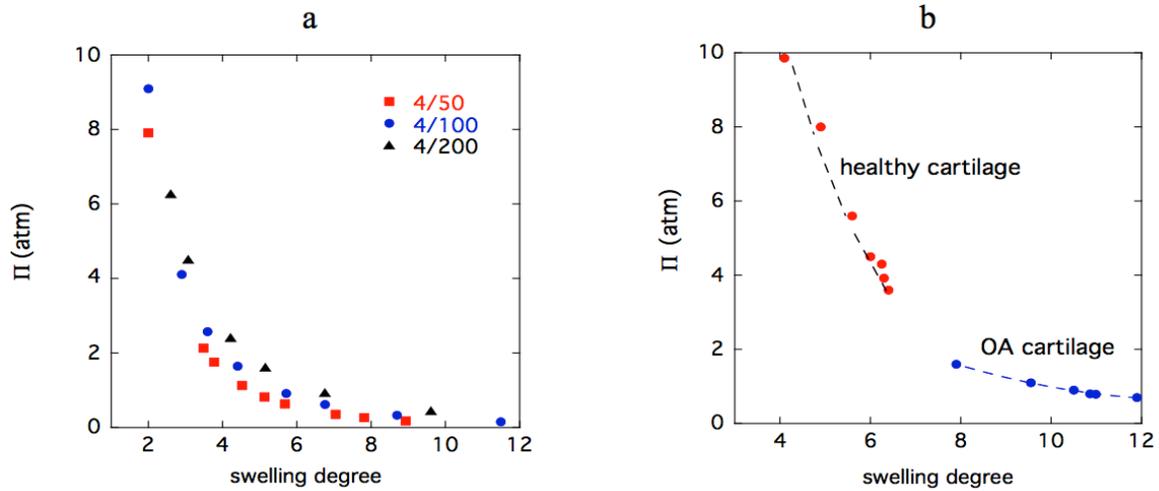
Figure 4. a) Osmotic swelling pressure (Π) of PVA gels as a function of swelling degree. b) Osmotic swelling pressure for healthy and osteoarthritis (OA) cartilage as a function of the swelling degree [24].

Elastic modulus maps

To gain insight into the structure and interactions at high resolution, we constructed elastic modulus maps for PVA and cartilage samples (Figure 5). We investigated the spatial variation of the shear modulus by AFM [25,26]. The maps represent the distribution of the elastic modulus across the surface of the samples. The results reveal that not only the magnitude of the moduli but also the spatial heterogeneity of the two systems is similar.
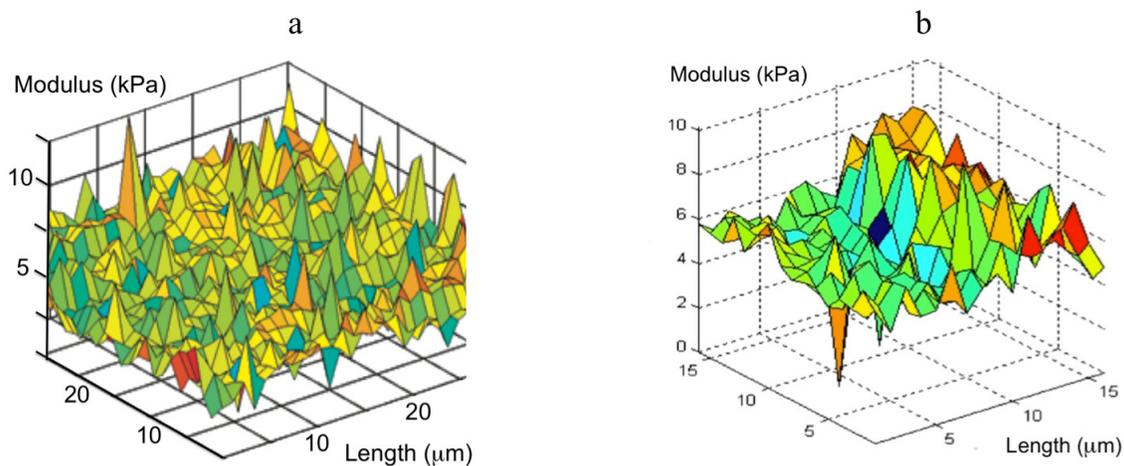

Figure 5: AFM topography images: a) 3D shear modulus map of a PVA hydrogel. b) 3D shear modulus map of mouse cartilage tissue.

CONCLUSIONS

Our results demonstrate that both cartilage and PVA hydrogels exhibit similar mechanical and osmotic properties. In cartilage, the collagen network provides the tensile strength. The chemical cross-links in PVA hydrogels play a similar role. Mechanical measurements made by macroscopic method and AFM nanoindentation indicate that the elastic modulus of PVA gels can be tailored by varying either the polymer concentration or the cross-link density (or both) to mimic the biomechanical properties of cartilage. It has been demonstrated that elastic modulus maps provides insight into the spatial variation of the elastic properties of tissues at high-resolution. This knowledge is particularly important in biomechanical studies, such as tissue characterization and the development of biomimetic tissue analogs.

ACKNOWLEDGEMENTS

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