A NEW DEVICE FOR DIRECT MEASUREMENT OF OSMOTIC PRESSURES OF PROTEOGLYCAN SOLUTIONS AT HIGH CONCENTRATIONS

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Introduction: It is known that proteoglycan (PG) swelling pressure plays an important role in maintaining articular cartilage hydration, and shares in load support when the tissue is compressed. Estimates of the contribution of this swelling pressure towards the tissue’s compressive stiffness vary from 100% [1-3] to approximately 50% [4,5]. The tissue’s osmotic pressure has been previously inferred [1-3] using an indirect method, by determining the water content of cartilage or intervertebral disc specimens when equilibrated against polyethylene glycol (PEG) solutions of known osmotic pressure [6].

The osmotic pressure of PGs reported in these studies ranged from 0.03 to 0.3 MPa, which has led to the conclusion that this Donnan osmotic pressure, associated with the charges on PGs, is the sole source of the tissue’s compressive stiffness. However, in later studies [7,8], this indirect method was challenged on a number of technical points that suggested that inaccuracies may exist in the osmotic pressures reported earlier [1-3], arising from: i) extrapolating the osmotic pressure data from 25°C [6] to 4°C; and ii) extrapolating from the absence of NaCl to 0.15M and 1.5M NaCl. Thus, the fundamental question regarding the contribution of Donnan osmotic pressure to the compressive modulus of cartilage remains unanswered.

The support load mechanism in cartilage derives from three distinct sources: 1) the hydraulic pressure generated in the interstitial fluid caused by the resistance against flow in the porous-permeable extracellular matrix; 2) the Donnan osmotic pressure in the interstitium associated with the charges on PGs; and 3) the intrinsic equilibrium stiffness of the collagen matrix. Under equilibrium loading conditions when the hydraulic pressure has subsided, the measured compressive aggregate modulus of the tissue is given by $H_o = H_r - \Delta p / \varepsilon$, where $H_r$ is the intrinsic equilibrium modulus of the collagen matrix and $\Delta p$ is the change in Donnan osmotic pressure resulting from the application of a strain $\varepsilon$ [9]. By measuring the osmotic pressure of PGs or glycosaminoglycan solutions of known concentrations, the relative contribution of $H_r$ and $-\Delta p / \varepsilon$ can be determined.

To achieve these measurements while avoiding potential inaccuracies due to non-validated extrapolations, a new direct method for measuring PG osmotic pressure is employed in this study, using a custom-built osmometer capable of directly measuring osmotic pressure of a macromolecular solution up to about 1.0MPa. Using this new instrument we report experimental data confirming previous findings, the results of this study suggest that Donnan osmotic pressure accounts for significantly less than 100% of its compressive aggregate modulus, in support of our hypothesis.

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Materials and Methods: The osmotic pressure device designed and constructed for this study is based on the same principle used in [8] (Figure 1). The device has two chambers, the lower chamber for a reference saline solution (eg, 0.15M NaCl) and the upper one for the sample to be measured. An ultrafiltration membrane (Millipore, MA) separates the two chambers with water-tight sealing. The design of the membrane holder is adopted from current commercial filter devices which provide water-tight sealing and easy usage. A miniature stainless steel diaphragm-type pressure transducer (SENSOTEC, OH) is used to monitor the pressure changes inside the lower chamber, and a precision pressure gauge for the upper chamber. Because the membrane is selectively permeable only to the solvents in the reference solution, it will generate a sub-ambient pressure inside the lower chamber. The sub-ambient pressure is counter-balanced by the applied pressure in the upper chamber. At equilibrium, this applied pressure is the osmotic pressure exerted by the specimen. During the experiments, the pressure at the lower chamber was not allowed to exceed 0.33kPa to minimize membrane deflection which can affect the pressures inside each chamber due to volume change. Equilibrium is reached when a zero-pressure reading in the lower chamber persists for 120 seconds. The temperature in the chamber is maintained at 21°C.

The standardized CS as used in [1-3] was purchased from SIGMA. The CS was dissolved in distilled water with concentrations of 50, 100, 150, 200 mg/ml. The reference solution was 0.15M NaCl. Samples were dried for 48 hours to determine the ratio of true dry weight to wet weight. The relationship between CS concentration and fixed charged density (FCD) can be estimated using the conversion factor of 2.73 meq/g of CS dry weight.

Results and Discussion: Table 1 shows the measured osmotic pressure for CS at four physiological concentrations. These concentrations are corrected using the dry weight to wet weight ratio, and given in the second column of the table. Using CS FCD in terms of dry weight, 2.73 meq/g, the FCDs in terms of volume were converted and given in the third column, and the relationship between FCD and the directly measured osmotic pressure is plotted in Figure 2. Our results show that the osmotic pressure varies from 0.03 to 0.31MPa for the range of FCD from 0.10 to 0.41 meq/ml. These results agree with those given in [2] using the indirect method of equilibrium dialysis against solutions of PEG in 0.15M NaCl. However, compared with the equilibrium dialysis method, the present method is suitable for any temperature and solution ionic environment; it also requires much less sample volume (0.3 ml) and provides a quick and efficient way to determine the osmotic pressure up to 1.0MPa. Finally, with the data and the measured FCD and $H_o$ of a cartilage sample, one can calculate the specific contribution of Donnan osmotic pressure to its compressive stiffness. Since for most normal articular cartilage the measured aggregate modulus ranges from 0.5 to 1.0 MPa, the results of this study suggest that Donnan osmotic pressure accounts for approximately 50% of CS compressive modulus.

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