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Diffusion and partition of solutes in cartilage under static load

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Abstract

We describe experimental apparatus, methodology and mathematical algorithms to measure diffusion and partition for typical small ionic solutes and inulin (a medium size solute) in statically loaded cartilage. The partition coefficient based on tissue water (K_{H_2O}) of Na^+ increased from 1.8 to 4.5 and for SO_4^{-2} decreased from 0.5 to 0.1, when the applied presure was raised from zero to 22 atm K_{H_2O} of inulin decreased from 0.3 to 0.05, for an increase in pressure from zero to 11 atm. Our theoretical interpretation of the results is that the partition coefficient can be expressed as a function of fixed charge density (FCD) for both loaded and unloaded cartilage. The partition coefficient shows good agreement with the ideal Gibbs–Donnan equilibrium, particularly when FCD is based on extrafibrillar water (EFW). The diffusion coefficients, $\bar{D}$ also decreased with an increase in applied pressure; raising the pressure from 0 to 22 atm resulted in the following changes in the values of $\bar{D}$: for Na^+ from 2.86×10^{-6} to 1.51×10^{-6} cm^2/s, for SO_4^{-2} from 1.58×10^{-6} to 7.5×10^{-7} cm^2/s, for leucine from 1.69×10^{-6} to 8.30×10^{-7} cm^2/s and for inulin from 1.80×10^{-7} to 3.30×10^{-8} cm^2/s. For the three small solutes (two charged and one neutral) the diffusion coefficient $\bar{D}$ is highly correlated with the fraction of fluid volume in the tissue. These experimental results show good agreement with the simple model of Mackie and Meares: hence solute charge does not affect the diffusion of small solutes under load. For inulin $\bar{D}$ & K show some agreement with a modified Ogston model based on two major components, viz., glycosaminoglycans (GAG) and core protein. We conclude that the changes in the partition and diffusion coefficients of small and medium size solutes in statically loaded cartilage can be interpreted as being due to the reduction in hydration and increase in FCD. The change in the latter affects the partition of small ionic solutes and the partition and diffusion of larger molecules. Our results throw light on the ionic environment of chondrocytes in loaded cartilage as well as on the transport of solutes through the matrix.

Keywords: Compressed cartilage; Hydration; Two-compartment model; Fixed charge density; Extrafibrillar water; Exclusion volume

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1. Introduction

Articular cartilage functions as a weight bearing, low friction, wear-resistant tissue which enables synovial joints to withstand high pressure without developing peak stresses. A wide range of mechanical loading forces in vivo produce time varying and spatially non-uniform strains (compressive, tensile and shear deformations) within the tissue. The ability of cartilage to perform its physiological function under load depends critically on the structure, composition and integrity of its extracellular matrix, which is composed of three major components: collagen, proteoglycans (PG) and water. The compressive properties of cartilage are largely dependent on a high density of negatively charged fixed groups (FCD) residing on the PG [1,2]. These ionized carboxy and sulfate groups of the glycosaminoglycan (GAG) side chains of the PG attract counter ions, which tend to imbibe water and thus endow cartilage with a large osmotic pressure (Π_{osm}); this tendency to expand is balanced by the tensile stiffness of the collagen framework [3].

Although cell density in human cartilage is very low (approx. 1% by volume), cells perform the essential function of maintaining the composition of cartilage at its optimum throughout life. Biosynthetic processes of the cells require transport of common nutrients and regulatory substances such as growh factors and cytokines. Metabolic waste products must also be transported out of the matrix. Since cartilage is an avascular tissue, solute transport occurs by diffusion and convection (the contribution of the latter is only significant in the case of large solutes. We shall not deal with it here).

Since solute transport takes place through the aqueous phase, it is dependent on the water content of the tissue. The latter in turn depends on the pressure which is applied during load-bearing. In the present work, we shall examine the effects of static compression on solute transport in cartilage, a subject, which has received relatively little attention in the past. In particular, we shall deal with the partition coefficient, K, and the diffusion coefficient, D, of small solutes (<200 Da) and inulin (5600 Da). The latter lies within the size range of molecules, which are partially excluded from cartilage and is close in size to the physiologically important insulin-like growth factor, IGF.

It should be noted that we treat the aqueous phase in the cartilage matrix as a two-compartment system: extrafibrillar water (EFW), lying outside the collagen fibrils and intrafibrillar water (IFW), lying inside the fibrils. Intrafibrillar pores in collagen are smaller than extrafibrillar pores, and also much smaller than the PG molecules. Hence, PG cannot penetrate into the IFW compartment [4,5]. The size of the intra-fibrillar compartment is determined from low-angle X-ray diffraction measurement of the spacing between collagen molecules. The spacing depends on a balance between the tendency of collagen molecules to repel each other, and the tendency of extra-fibrillar osmotic pressure to extract the water from in between the collagen molecules and thus push them together [6]. Small neutral solutes distribute themselves equally between the EFW and IFW compartments [4]. In contrast, small ions have an unequal distribution depending on their charge, because the EFW compartment is strongly negative due to its PG content.

Several methods have been described in the literature for measuring the diffusion coefficient in tissues. Maroudas et al. [7] used a cartilage slice clamped between two chambers of solution; the diffusion coefficient was determined by measuring the flux of tracer across the cartilage slice. This method is useful for small solutes, but for a large solute the flux is very small, hence the measurement is not accurate. Later Maroudas et al. [8,9] used the desorption method to measure $\bar{D}$ for large solutes. This method is not accurate for small solutes since their desorption is very fast; but for large solutes it is accurate and practical. Winlove and Parker [10] used concentration/distance curves to measure the diffusion in GAG solutions. This method is suitable for tissues like the intervertebral disk or fibrillated cartilage, which would swell if placed in a permeation cell [2].

All the above methods are limited to unloaded tissue: the first two because load will stop the flux from cartilage surfaces. In the third method, static pressure cannot be applied uniformly along the strip, since the end of the strip (which is pulsed with the tracer) cannot be confined.

Fluorescence recovery after photobleaching with a confocal laser scanning microscope was used by Gribbon et al. [11,12] to determine the diffusion of large solutes, such as aggrecan and hyaluronan in gels.

Recently, Quinn et al. [13] have measured the partition and diffusion of 430-Da tetramethylrhodamine and of 3-, 10- and 40-kD dextran conjugated with this reagent in statically compressed bovine articular cartilage: the desorption took place from the periphery of the tissue and was continuously monitored in cartilage. Their work showed that partition and diffusion decreased with increasing solute molecular weight and that diffusivity decreased significantly with strain. The authors did not find a correlation of K with glycosaminoglycan content, which is surprising in view of the known excluded volume effect of the latter on the partition of larger solutes [9]. Fluorescence methods are restricted to large solutes, where the size of the conjugated group is negligible compared to solute size.

Burstein et al. [14] used nuclear magnetic resonance spectroscopy (NMR) and magnetic resonance imaging (MRI) to measure D of small solutes in loaded as well as unloaded samples of cartilage. Their results [14] on compressed cartilage were only preliminary, but these workers pointed out the necessity for correlation of K and D not with load, but with the effect of load on matrix composition and structure. In the present treatment we shall develop this line of reasoning, with a method for correlation of D as a function of hydration or fixed charge density (FCD), which are themselves functions of the applied load. We have also developed an experimental technique for measuring concentration/distance profiles in cartilage under load.

In unloaded tissue, small non-ionic solutes (urea, amino acids, oxygen and glucose) distribute themselves equally between the extracellular water in cartilage and external solution (partition coefficient close to unity), and their distribution is not affected by FCD. Fixed negatively charged groups attract small cationic solutes; hence the partition coefficients of the latter in EFW are higher than unity [9] (the converse applies to anionic solutes). In absence of binding, the larger the solute the higher the exclusion and the slower the diffusion. Conversely, the more hydrated the matrix, the higher are partition and diffusivity. With increased proteoglycan and collagen, solute movement and distribution are restricted. Diffusion coefficients of large solutes decrease exponentially with Stokes radius [8]. The measured diffusion coefficient of small solutes in unloaded cartilage is roughly 40% of the diffusivity in free solution [14].

2. Materials

Rhodamine B, ethylenediaminetetraacetic acid (EDTA), C-amino-*n*-caproic acid, *N*-ethylmaleimide, benzamidine, 2,5-diphenyl-oxazole and Rhodamine B were obtained from Sigma Int. (USA), [^{3}H]inulin and [^{3}H]leucine from Dupont NEN, [^{22}Na] and [$^{35}SO_4$] from Amersham Int. England, toluene from Frutarom LTD, Triton X-100 from Riedel-deHaen.

2.1. Cartilage samples

Human femoral heads age range 40–90 years, were obtained at post-mortem and at operations for femoral neck fractures. Only cartilage with completely intact and smooth surface (as shown by Indian ink staining) was tested. FCD was measured using the tracer cation method of Maroudas and Thomas [15], either on control samples adjacent to test specimens or on test specimens at the end of the experiment.

3. Methods

3.1. Inulin purification

In preliminary experiments, approximately 1.5% breakdown products of a radioactive inulin were produced during 1 week. Since the diffusion coefficient of the small fragments is significantly larger than that of inulin, they were separated by dialysis through 3000 Da tubing (Isramex cat No. 42403). This applied both to the initial inulin ('hot solution') and to inulin in the cartilage desorbates.

3.2. Preparation of samples for diffusion measurements

For each solute six thin strips were cut. To cut these strips, we first delaminated an area of full depth cartilage from the bone (approx. 6×3 cm). The rectangular lamina was laid flat and frozen onto a microtome stage. Full depth rectangular strips (approx. 3×0.5 cm) were cut, using two accurately set parallel blades. The end of each strip was squared off by mean of a cutter, which consisted of a scalpel blade mounted on a micrometer axis. The superficial and deep zone slices were shaved off each strip (unlike in partition experiments where we wished to examine both the superficial and the middle slices) using a freezing microtome, to obtain a middle zone sample strip, of uniform depth (approx. 1 mm) and therefore, as far as possible, uniform properties. Each solute had its own set of six strips, three on either of the apex of a femoral head. We shall refer to them as $S_{1,1}$, $S_{1,2}$ and $S_{1,3}$ and $S_{2,1}$, $S_{2,2}$ and $S_{2,3}$, respectively. The middle strip of each set (i.e. $S_{1,2}$ and $S_{2,2}$) was used to measure D for unloaded cartilage, while the remaining strips were used to measure D under applied pressure.

It should be noted that the length of the strip (~3 cm) is a compromise between two demands: one (which calls for a short length) is diffusion in a reasonably short time (2–3 weeks incubation), and the other (calling for a long strip) is accuracy, since the length of each cut segment should be of sufficient weight for accurate measurement of properties such as hydration, FCD and tracer activity.

3.3. Diffusion measurement

The diffusion apparatus is shown in Fig. 1. The design, with an air gap between the paper strip and the platen, solves an awkward technical problem: namely, how to prevent tracer solution from 'short circuiting' its cartilage path by creeping around the edges of the strip and into the free space under the loaded piston. In our design, there is only one possible contact between the solution and the tip of the cartilage, viz., via the paper strip. In order to make sure that there was no capillary seepage of solutes around the cartilage specimen, the movement of Rhodamine B was visually observed and photographed, to confirm that no short-circuiting occurred, i.e. penetration was purely by diffusion through the body of the strips.

For each strip, 3 ml of non-radioactive ('cold') solution and 3 ml of radioactive solution were prepared. For sulfate and leucine the cold solution contained 2 mmol per liter of 0.15 M NaCl solution; for inulin, we used 1 g/l. In all cases we added protease inhibitors, viz., 25 mM EDTA; 10 mM *N*-ethylmaleimide; 25 mM C-amino-*n*-caproic acid and 5 mM benzamidine (in preliminary experiments we had measured the FCD of control samples adjacent to the test strip and found that in the presence of protease inhibitors FCD remains practically constant up to 3 weeks of incubation at 4 °C). 'Hot' solution was prepared by adding tracer to the 'cold' solution, with sufficient radioactivity (at least 10^7 DPM per ml for a small solute and 10^8 DPM per ml for inulin) to achieve a detectable tracer activity profile within a reasonable incubation time.

The cartilage strips were immersed in 'cold' solution for 1 day prior to the experiment proper in order to take up the enzyme inhibitors. A paper strip was connected to each of the two reservoirs, one containing 'cold' and one 'hot' solution. The paper strips were left to achieve equilibrium (at most 4 h, as found by initial experiments). The next step was to make the cartilage specimen reach equilibrium water content under load while touching the 'cold' paper strip. The cartilage specimen was placed on the base platen by using a special holder that prevented it from slipping under load. The equilibrium took 1–2 days. After removing the preloaded cartilage strip from the apparatus and wiping the tip, we quickly put it back in its holder and placed it in contact with the 'hot' paper strip. The diffusion of the tracer was timed from this point. The same load was applied as before and equilibrium hydration was achieved practically instantaneously since the pre-loaded strip had been unloaded only for a very short time (<2 min). During both incubations the incubator remained tightly closed. Air had been flushed from the incubator using 2–3 l/min flow of N_2 saturated

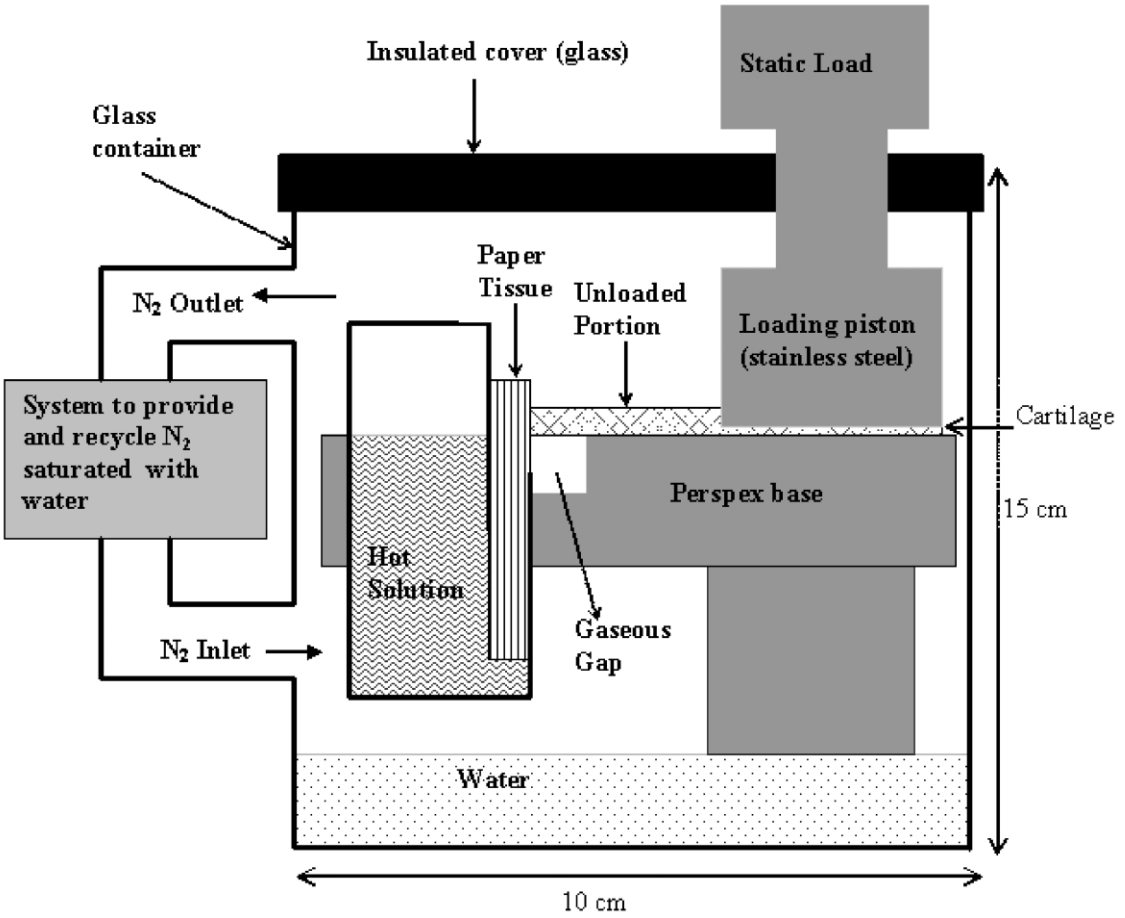

Fig. 1. The system consists of two units. The first unit is used to prepare water-saturated N_2 to achieve 100% humidity in the diffusion cell (to prevent drying of the cartilage strip) and an anaerobic environment to suppress fungal growth. The second unit is an insulated incubator (gas-proof) containing the diffusion cell, and maintained at 4 °C. A radioactive tracer is drawn from the reservoir containing the radioactive ('hot') solution through the paper strip (3×0.5 cm) by capillary action, it then diffuses into the cartilage strip, with one end in contact with the paper strip. The first portion of the cartilage strip is not loaded, the remainder of its length is loaded by the piston.

with water until 100% humidity was reached. The temperature was kept constant at 4 °C.

At the end of the tracer diffusion, the hot cartilage specimen was taken out, and its tip wiped to remove surplus hot solution (if any) left by the 'hot' paper strip. The cartilage specimen was frozen rapidly by plunging directly into liquid N_2 to prevent water and solute redistribution.

While frozen, the specimen was cut into short sequential segments (0.1 cm for inulin and 0.2 cm for the small solutes) using a scalpel blade mounted on a micrometer. For each cut segment hydration, radioactivity due to the solute in question and FCD were measured. Gamma radiation of $^{22}Na^+$ was counted directly by placing the cartilage segments in a Packard γ counter. For β-emitting tracers, the cartilage specimens were desorbed in two successive aliquots of 1 ml 'cold' solution. The equilibration times were 4 h for sulfate, sodium and leucine and 2 days for inulin. The radioactivity in the solutions was counted in a Packard β-counter, after addition of 5 ml of

scintillation fluid (consisting of 740 ml of toluene, 260 ml of triton X-100 and 3.4 g of 2,5-diphenyloxazole) to 0.5 ml of desorbate. (Note: tritium from [^{3}H]leucine might exchange with hydrogen in water. Hence, water was removed by freeze-drying before desorption at two stages of the experiments: (i) at the beginnig water was removed from the fresh 'hot' solution; (ii) at the end of diffusion water was removed from the cut segments. The first separation is critical since during storage (months) a significant amount of exchanged tritum might have accumulated. The second separation was performed to remove exchanged tritum produced during the experiments. These quantaties were found to be less than 2%.)

For the purpose of subsequent correlations the extrafibrillar water (EFW) and the fixed charged density based on EFW (FCD_{EF}), were estimated from the measured values of FCD and hydration as described in Basser et al. [1] for isotropic (osmotically applied) compression, using data of cartilage composition and empirical relationships (i) between intrafibrillar water and extrafibrillar osmotic pressure due to PG and (ii) between osmotic pressure and the FCD of PG solution.

$$FCD_{EF} = FCD_{H_2O}\frac{H_2O_{TOTAL}}{H_2O_{EF}} \quad (1)$$

Where FCD_{H_2O} is the FCD based on total water content. H_2O_{TOTAL} and H_2O_{EF} are total and extrafibrillar water contents, respectively.

3.4. Corrected concentration profiles for non-uniform tissue

The cartilage specimens were not always uniform in FCD along their length, hence their partition coefficients must also have varied. To correct this, the measured tracer concentration in each segment of the strip was normalized with respect to the equilibrium concentration, i.e. divided by the value of the estimated local partition coefficient and multiplied by the mean partition coefficient of the strip. The partition coefficients corresponding to the local FCD were taken from experimental graphs (Figs. 5 and 8).

3.5. Measurement of partition

Partition coefficients of unloaded cartilage (K) have been measured in the past by the use of radioactive tracer techniques [9]. We extended this method to loaded cartilage. A cartilage plug of uniform thickness (some of the deep zone cartilage having been trimmed off by microtome) was placed between two sintered Pyrex discs. The upper disc was under the piston and the lower one was placed on the base platen, using a centering ring. The 'sandwich' of cartilage-Pyrex discs was immersed in 'cold' solution until equilibrium hydration was reached (2 days at 4 °C). Exuded 'cold' solution and any adherent droplets were then sucked up with a syringe and paper blotter, while the specimen remained under load. The 'cold' solution was replaced by one containing the radioactive solute of interest. It should be noted that the original sintered discs were also replaced by a pair, which had been pre-soaked in the radioactive solution to be used in the actual experiment. The sample was left under compression. After equilibration (1–2 days, depending on solute size), all 'hot' liquid was removed and aliquots counted. The 'hot' cartilage plug was weighed to obtain its weight under load (W), and was then cut into two slices: superficial ($\sim$300 μm) and the remaining middle and deep zones (note that unlike in the diffusion experiments, separate data were obtained for the superficial zone and the remainder of the cartilage sample). Both slices were weighed (to obtain their weight when under load), then desorbed in 'cold' solution, and the two desorbates were counted. After relaxation during desorption, both slices were again wiped and weighed (this time to obtain their unloaded weight). FCD was measured. Finally, the slices were freeze-dried.

The partition coefficient was calculated, based on total water weight under load.

$$K_{H_2O} = \frac{\bar{C}}{C} = \frac{CPM_{DES}}{CPM_{HOT}\cdot W_{SLICE}\cdot[H_2O_{TOTAL}]} \quad (2)$$

Where $\bar{C}$ and C are tracer concentration in the cartilage and in the hot solution, respectively. CPM_{DES} are counts per minute of tracer in the

desorbate. CPM_{HOT} are counts per minute per ml of 'hot' solution. $[H_2O_{TOTAL}]$ is the fractional water content in the loaded cartilage. W_{SLICE} is the weight of the cartilage under load.

3.6. Calculations of K and $\bar{D}$ based on theoretical considerations

For a one-compartment model, the partition coefficients for Na^+ and SO_4^{-2} ($K_{H_2O}^{Na^+}$ and $K_{H_2O}^{SO_4^{-2}}$) based on total water contents, were predicted, using the ideal Gibbs–Donnan [16] equilibrium equations.

$$K_{H_2O}^{Na^+} = \frac{FCD_{H_2O} + \sqrt{FCD_{H_2O}^2 + 4\cdot[Na^+]\cdot[Cl^-]}}{2\cdot[Na^+]} \quad (3)$$

$$K_{H_2O}^{SO_4^{-2}} = \frac{4\cdot[Na^+]^2}{\left[FCD_{H_2O} + \sqrt{FCD_{H_2O}^2 + 4\cdot[Na^+]\cdot[Cl^-]}\right]^2} \quad (4)$$

Where FCD_{H_2O} is the fixed charge density based on total water. $[Na^+]$ and $[Cl^-]$ are respective concentrations of Na^+ and Cl^- in external solution (0.15 M).

An effective total partition coefficient, K' was also calculated, based on the two-compartment model [4,5].

$$K' = K_{EF}\cdot[H_2O_{EF}] + K_{IF}\cdot[H_2O_{IF}] \quad (5)$$

Where $[H_2O_{EF}]$ and $[H_2O_{IF}]$are fractions of extra and intrafibrillar water at equilibrium. K_{EF} and K_{IF} are partition coefficients at equilibrium, based on extra and intrafibrillar water content, respectively. Note that K_{IFW} is zero for large solutes and unity for small solutes [4]. For Na^+ and SO_4^{-2}, K_{EF} was calculated by means of equations analogous to Eqs. (3) and (4), where FCD_{H_2O} is replaced by FCD_{EF}.

The reduction of the diffusion coefficient in the tissue as compared with free solution ($\bar{D}/D$) was calculated for small solutes by means of the Mackie and Meares equation [17,18] which correlates the decrease of the diffusion coefficients in porous media with the increase in tortuosity. Thus:

$$\bar{D}/D = \left[\varepsilon/(2-\varepsilon)\right]^2 \quad (6)$$

Where ε is the volumetric fraction of fluid in cartilage.

For inulin Ogston's model [19,20] was used in addition to Mackie and Meares' equation. The following assumptions were made:

1. Inulin is completely excluded from the intrafibrillar compartment (this assumption is based on experimental results, see Schneiderman et al. [21]).
2. Partial exclusion of inulin from the extrafibrillar space is treated in two ways: (i) considering exclusion by GAG chains alone, i.e. chondroitin sulfate, CS and keratan sulphate, KS, (Eq. (7a)); (ii) exclusion by GAG and core protein (Eq. (7b)). This is the first time that core protein is considered as an excluding species, alongside CS and KS. The exclusion by the relatively thick collagen fibrils is negligible in the extrafibrillar space.
3. The mass fraction of CS and KS were determined from the experimentally obtained values of FCD and the ratios of CS/KS known from the data of Maroudas et al. for both the superficial zone and the remainder of cartilage [22].
4. The mass of total PG protein in relation to GAG was also taken from the data of Maroudas et al. [22]. The proportion of linear core protein was assumed to be 2/3 of total PG protein. The concentration of core protein in each specimen was taken to be proportional to the GAG content and, hence, like the latter, to the FCD.
5. The exclusion by the globular portions of the core protein (i.e. G1, G2, G3 and the link) was calculated to be negligible compared with the exclusion by the GAG and the linear portion of the core.
6. The GAG and core protein constitute a single network of rods. Hence, a mean value of the rod diameter was used, account being taken of the relative proportions of the two constituents.
7. The 'dead volume' of the collagen fibrils led to an increase in the tortuosity of the path. We have estimated the resulting reduction in the diffusion coefficient by the Mackie and Meares equation.

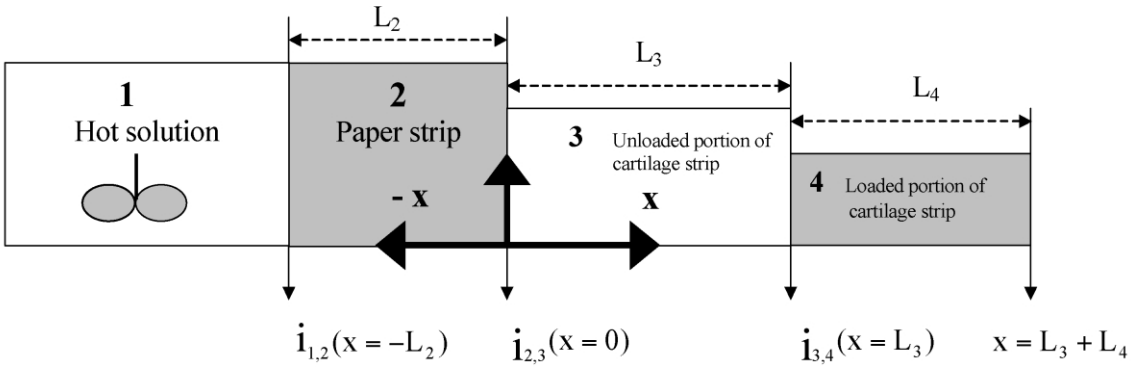

Fig. 2. This figure illustrates the system of coordinates in our set up. The coordinate origin ($x=0$) is located at the interface between the paper strip and the unloaded portion of cartilage.

Using the above assumptions, we calculated K and $\bar{D}/D$ as follows.

K_{EF} was calculated in two ways: (i) considering GAG as the only excluding species. In this case,

$$K_{EF}=\exp-\left[\varphi^{EF}_{GAG}\cdot(1+r_s/r_{GAG})^2\right] \quad (7a)$$

Where is the volumetric fraction of GAG in EF space; r_s is solute radius. For inulin: $r_s=1.2$–1.3 nm [21].

(ii) Taking into account core protein as an additional excluding species.

$$K_{EF}=\exp-\left[(\varphi^{EF}_{GAG}+\varphi^{EF}_{\text{core protein}})(1+r_s/r_{mean})^2\right] \quad (7b)$$

Where and $\varphi^{EF}_{\text{core protein}}$ are the volumetric fractions of GAG and core protein in EF space; r_{mean} is the mean radius of excluding molecules, i.e. $r_{mean}=\varphi^{EF}_{GAG}\cdot r_{GAG}+\varphi^{EF}_{\text{core protein}}\cdot r_{\text{core protein}}$. We took $r_{GAG}=0.5$ nm [23] and $r_{\text{core protein}}=0.23$ nm.[1]

The mass ratios of PG protein to GAG were taken as 0.850 for surface zone and 0.806 for the remainder of the cartilage [22]. The proportion of G1, G2, G3 and link protein were taken as negligible in relation to the linear portion of the core protein. Specific volumes of GAG and core protein are 0.54 and 0.74 cm^3/g, respectively [25].

The diffusion coefficient of inulin is calculated by Eq. (8):

$$\bar{D}/D=f(\varphi^{\text{tissue}}_{\text{collagen fibril}})\cdot\exp-\left[(1+r_s/r_{mean})\sqrt{\varphi^{\text{tissue}}_{GAG}+\varphi^{\text{tissue}}_{\text{core protein}}}\right] \quad (8)$$

Where $\varphi^{\text{tissue}}_{GAG}$, $\varphi^{\text{tissue}}_{\text{core protein}}$ and $\varphi^{\text{tissue}}_{\text{collagen fiber}}$ are the volumetric fractions of GAG, core protein and collagen fibrils (including intra-fibrillar water) based on total tissue volume. According to Mackie and Meares, $f(\varphi^{\text{tissue}}_{\text{collagen fibril}})=(1-\varphi^{\text{tissue}}_{\text{collagen fibril}})/(1+\varphi^{\text{tissue}}_{\text{collagen fibril}})$.

3.7. Mathematical model for evaluation of diffusion coefficient from experimental data

In our system the solute concentration at the interface between the paper strip and cartilage may vary with time. Since interfacial concentration could not be measured during incubation, we opted for an approximate solution of the diffusion equation.

3.7.1. Approximate solution

We solve for a composite medium for our system using a semi-infinite approximation for each element (#3.4 Crank [26]). In our experimental set up (Fig. 2), this approximation holds

[1] The diameter of core protein is inferred from packing distances between extended chains and between beta sheets. These values have been determined by X-ray diffraction to vary between approximately 4.75 Å and 5.7 Å [24]. We took the first value (4.7 Å).

since the length of each element is sufficient to fulfill the condition: $(D_i \cdot t)/L_i^2 < 0.1$.

We calculated D from the profiles of radioactivity, water content and the partition coefficient corresponding to the measured values of FCD.

3.7.2. Basis of calculation

Each of the three elements (paper strip, unloaded portion and loaded portion of cartilage specimen) can be approximately treated as semi-infinite as follows, i.e.:

$$C(x,t) = B \times \mathrm{erfc}\left(\frac{x}{\sqrt{4 \cdot \bar{D} \cdot t}}\right) \quad (9)$$

Whence we expect a linear plot of the form $y = \alpha x$ where y is $\mathrm{erfc}^{-1}[C(x,t)/B]$ and α is $1/\sqrt{4 \cdot \bar{D} \cdot t}$ (erfc is the error function complement and erfc^{-1} is the inverse of erfc, B is a constant which is determined for each element from the appropriate boundary condition). We extract $\bar{D}$ for each element from a linear plot of $\mathrm{erfc}^{-1}[C(x,t)/B]$ vs. x. What we measure is concentration C at distance x at time t (t being the time at which we stop the experiment and cut each element into segments to get values of x and C).

It can be shown that Eq. (9) holds for each strip given the following assumptions and boundary conditions.

3.7.3. Assumptions

1. The interface (between each two elements of Fig. 2) is a two-dimensional plane perpendicular to the diffusion direction.
2. We neglect the transition zone, i.e. we assume a step decrease in height at the interface between the unloaded and loaded elements.
3. The instantaneous deformation in the loaded portion is neglected as we were not able to measure it in the existing apparatus. The values given in the literature [27] vary from 5 to 15% strain for cylindrical specimens and are strongly dependent on the orientation with respect to predominant collagen fibril direction. The latter was not identified in the present work.
4. The diffusion coefficient is constant in each element.
5. As with previous methods [8,10–12,14], binding kinetics are not considered.

3.7.4. Initial condition

$$t = 0 \quad C_1(x,0) = C_{\text{reservoir}}; \quad C_2(x,0) = C_{0,2}; \quad C_3(x,0) = C_4(x,0) = 0 \quad (10)$$

Where $C_{\text{reservoir}}$ is the concentration of tracer in the hot solution reservoir (constant), $C_{0,2}$ is initial tracer concentration in the paper strip. Note that: $C_{0,2}/C_{\text{reservoir}}$ is equal to the partition coefficient between the paper strip and the hot solution (not measured).

3.7.5. Boundary conditions

1. Equilibrium at the interface (paper strip/cartilage strip).

$$x = 0 \quad \frac{C_3(0,t)}{C_2(0,t)} = K_{\text{paper/cartilage}} \quad (11)$$

Where $K_{\text{paper/cartilage}}$ is the partition coefficient between the tip of the cartilage specimen and the paper strip.

2. Continuity of flux through the interface paper/cartilage ($x = 0$):

$$D_2 \cdot \frac{\partial C_2(0,t)}{\partial x} = D_3 \cdot \frac{\partial C_3(0,t)}{\partial x} \quad (12)$$

Where D_2 and D_3 are the diffusion coefficients in the paper and the unloaded portion of the cartilage strip, respectively.

3. Continuity of flux at the second interface (unloaded/loaded portions of cartilage $i_{3,4}$).

$$D_3 \cdot \frac{\partial C_3(L_3,t)}{\partial x} = D_4 \cdot \frac{\partial C_4(L_3,t)}{\partial x} \quad (13)$$

Where D_4 is the diffusion coefficient in the loaded portion of the cartilage strip. L_3 is the length of the unloaded portion of the cartilage strip.

From these assumptions and boundary conditions the concentration profiles in the unloaded

specimens (or in the unloaded portions of the loaded specimens) are given by Eq. (9) as: $\mathrm{erfc}^{-1}[C_3(x,t)/C_3(0,t)] = \alpha_3 x$, where $C_3(0,t)$ is the tracer concentration in the unloaded cartilage strip at $x=0$. Its value is obtained by extrapolation of the measured profile, using the Taylor series expansion of second order (first and second derivatives are calculated using second order forward difference).

The diffusion coefficient, D_3 is calculated from the slope α_3, as in Eq. (9).

$$\text{where} \quad \alpha_3 = 1/\sqrt{4 \cdot D_3 \cdot t} \tag{14}$$

In the loaded portion the concentration profile $C_4(x,t)$ is given by a slightly different formula:

$$C_4(x,t) = C_3(0,t) \times \Psi(D_3,D_4,L_3) \times \mathrm{erfc}\frac{x}{\sqrt{4 \cdot D_4 \cdot t}} \tag{15}$$

where

$$\Psi(D_3,D_4,L_3) = \sqrt{\frac{D_3}{D_4}} \times \exp\left(\frac{L_3^2}{4D_4 \cdot t} - \frac{L_3^2}{4D_3 \cdot t}\right)$$

Function Ψ takes into account the step change in D at the interface 3/4 and is solved by iteration. First we assume $D_4 = D_3$, then we obtain from α_4 a new value of D_4. The process is repeated until convergence of D_4. The diffusion coefficient of the loaded portions, D_4 is calculated from the slope α_4 of the plot $\mathrm{erfc}^{-1}\ C_4(x,t)/[C_3(0,t) \times \psi(D_3, D_4,L_3)]$ vs. x. From Eq. (15), α_4 is given, as before, by:

$$\alpha_4 = 1/\sqrt{4 \cdot D_4 \cdot t} \tag{16}$$

3.8. Numerical example

As an example we will illustrate the calculations of the diffusion coefficients for Na^+ (D_3 and D_4) for two typical strips: loaded strip, $S_{1,1}$ and unloaded strip, $S_{1,2}$ (Table 1, Scheme 1). The measured concentration profiles, $C_3(x,t)$ and $C_4(x,t)$ were corrected to take account of the equilibrium partitioning where necessary. The corrected profiles are obtained by multiplying the measured concentrations profile by the expression:

$$\frac{K_{\mathrm{mean}}}{K(x)} \tag{17}$$

where $K(x)$ is the partition coefficient for the given cut segment; K_{mean} is the average value for the element in question (for calculation of D_3 the average is taken along the unloaded portion without the transition zone and for the calculation of D_4 the average is taken along the loaded portion).

i. *Calculation of D_3 for unloaded strip $S_{1,2}$*: is given in Table 1 (first three columns) and plotted vs. x in Fig. 3a. Substituting $\alpha_3 = 0.645 \pm 0.021$, and time $t = 202752$ s in Eq. (14) yields, $D_3 = 2.961 \pm 0.203 \times 10^{-6}$ cm^2/s.
ii. *Calculation of D_3 for unloaded portion of loaded strip $S_{1,1}$:* is tabulated in the last two columns of Table 1 and plotted vs. x in Fig. 3b, unloaded portion. Substituting slope $\alpha_3 = 0.312 \pm 0.028$, and time $t = 815544$ s in Eq. (14) yields, $D_3 = 3.15 \pm 0.66 \times 10^{-6}$ cm^2/s (error 21%).
iii. *Calculation of D_4 from loaded portion of $S_{1,1}$*: for first iteration D_4 is set equal to the unloaded value, $D_3 = 3.15 \times 10^{-6}$ cm^2/s, and used in Eq. (15) (Table 1 'loaded portion', and Fig. 3b) to obtain, as first approximation, $D_4 = 1.444 \pm 0.0.280 \times 10^{-6}$ cm^2/s (slope $\alpha_4 = 0.461 \pm 0.051$, $t = 815544$). The process is repeated until convergence of D_4 (differences of two successive iterations less than 1×10^{-9}). The final value of (iteration 10, last column of Table 1) is plotted in Fig. 3b to give: $\alpha_4 = 0.412 \pm 0.050$ and $D_4 = 1.813 \pm 0.455 \times 10^{-6}$ cm^2/s.

The effect of errors arising from the uncertainty at the transition zone and the concentration, $C_3(0,t)$ at the interface cartilage/paper was estimated as follows:

i. We estimate (from the measured 35% water loss in the loaded zone) that there would be approximately 9% loss in the transition zone. If we had added this segment to our calculation, the value of D_3 would have changed by only 6.6%.

Table 1
Typical data for calculation of diffusion coefficient of Na^+ for unloaded ($S_{1,2}$) and loaded strip ($S_{1,1}$)

$x_{S_{1,2}}$ (cm)	$C_{S_{1,2}}(x,t)$ (cpm/g tissue)	$y_{S_{1,2}}(x,t)$	$x_{S_{1,1}}$ (cm)	$C_{S_{1,1}}(x,t)$ (cpm/g tissue)	$y_{S_{1,1}}(x,t)$ Iteration = 1	Iteration = 10	
0	• 4,275 500	0	0	• 2,780 100	0	0	▲
0.0945	3,973 800	0.0626	0.0935	2,699 000	0.0259	0.0259	
0.2945	3,460 600	0.1705	0.2935	2,534 600	0.0784	0.0784	
0.4945	3,076 500	0.2539	0.4935	2,346 500	0.1391	0.1391	Unloaded portion
0.6945	2,478 600	0.3916	0.6935	2,134 100	0.2089	0.2089	
0.8945	1,899 700	0.5409	0.8935	1,955 700	0.2692	0.2692	
1.0945	1,446 500	0.6770	1.0935	1,687 100	0.3639	0.3639	▼
1.2945	1,015 800	0.8351	1.2935	1,600 000	Transition zone		
1.4945	738 900	0.9639	1.4935	1,422 000	0.4642	0.6710	▲
1.6945	505 600	1.1046	1.6935	1,253 900	0.5330	0.7296	
1.8945	356 900	1.2239	1.8935	1,164 500	0.5716	0.7630	
2.0945	220 700	1.3762	2.0935	957 300	0.6686	0.8475	
2.2945	151 600	1.4871	2.2935	775 000	0.7659	0.9336	Loaded portion
2.4945	90 800	1.6289	2.4935	653 600	0.8396	0.9995	
–	–	–	2.6935	511 700	0.9393	1.0897	
–	–	–	2.8935	337 600	1.0951	1.2324	
–	–	–	3.0935	226 300	1.2323	1.3596	▼

Legend: $C_{S_{1,2}}(x,t)$and $C_{S_{1,1}}(x,t)$ are the corrected concentration profiles of Na^+ for loaded ($S_{1,1}$) and unloaded strip ($S_{1,2}$). Each point is an average concentration of a cut segment localized at the centre of the segment. $x_{S_{1,1}}$and $x_{S_{1,2}}$are distances of the center of the cut segments from the interface cartilage/paper.

$$y_{S_{1,1}}(x,t) = erfc^{-1}[C_{S_{1,2}}(x,t)/C_{S_{1,2}}(0,t)]$$

$$y_{S_{1,1}}(x,t) = \begin{cases} x \le L_3 & erfc^{-1}[C_{S_{1,1}}(x,t)/C_{S_{1,1}}(0,t)] \\ x > L_3 & erfc^{-1}[\Psi(D_3,D_4,L_3)\times C_{S_{1,1}}(x,t)/C_{S_{1,1}}(0,t)] \end{cases}$$

The length of the unloaded portion of $S_{1,1}$, $L_3 = 1.2935 + 0.1 = 1.3935$ cm. The diffusion time of $S_{1,1}$ and $S_{1,2}$ are $t = 815\,544$ and $t = 202\,752$ s, respectively.

Concentration at $x = 0$, extrapolated from the measured profile.

ii. $C_3(0,t)$ was not known but had to be estimated by extrapolation from the measured profile, leading to uncertainties in the origin of the $erfc^{-1}$ function. However, because the slope of the concentration profile is relatively flat, and the transition zone relatively narrow, we estimate this particular error in D_3 to be less than 5%.

3.9. Statistics

As a measure of correlation between two variables we compared Spearman's rank coefficient, r_{sp}^{calc}, calculated from the results, with the theoretical coefficient, r_{sp}^{crit} (the latter depends on the number of samples and selected risk [28]).

4. Results

4.1. Partition coefficients

The ranges of applied pressures, initial and final hydrations and FCDs are summarised in Table 2. It should be noted that our specimens consisted of sections from superficial and middle zones (though the latter contained portions of the deep zone as well).

4.2. Partition of Na^+ and SO_4^{-2}

Since, under the effect of static compression, there is a decrease in the water content and hence an increase in FCD, we wished to verify whether the measured partition coefficients of Na^+ and

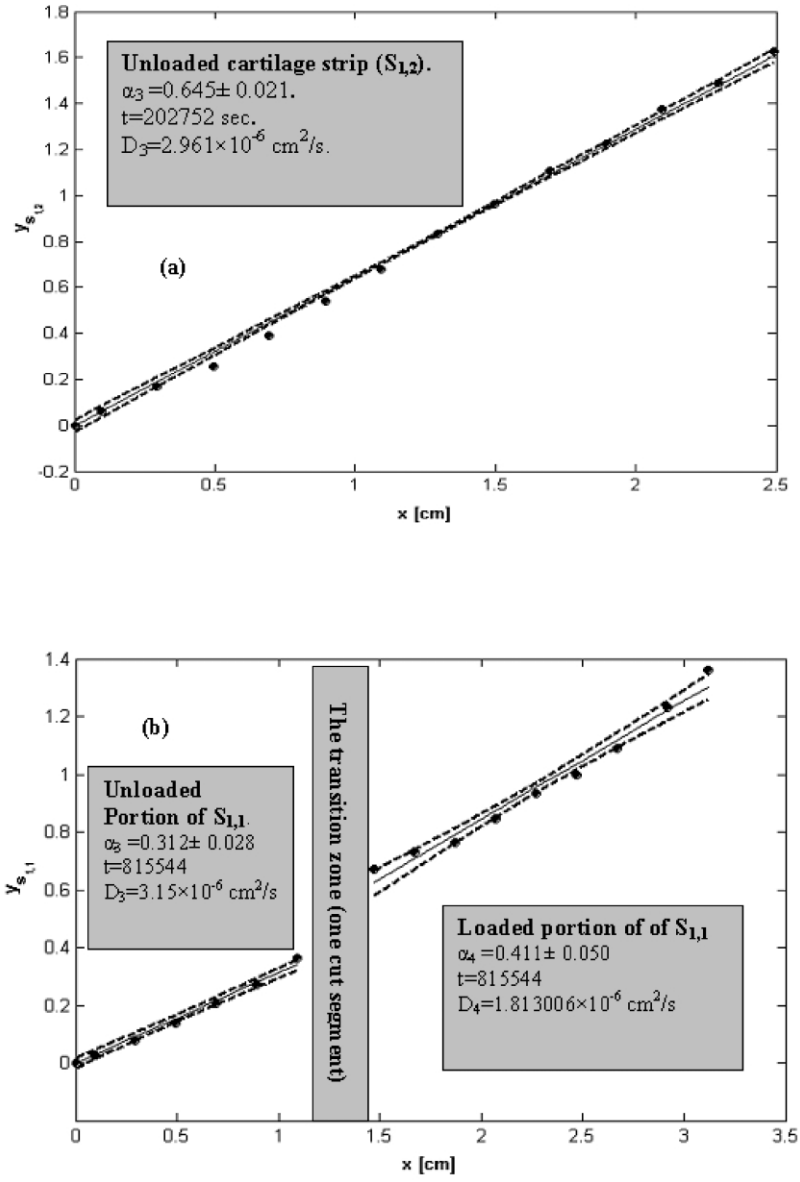

Fig. 3. In this graph the experimental data of Table 1 were ploted to calculate the diffusion coefficient D_3 and D_4 (a): $y_{S_{1,2}}$ vs. x for the unloaded strip $S_{1,2}$. (b) $y_{S_{1,1}}$ vs. x for loaded strip $S_{1,1}$. Dotted lines are confidence limits, based on a t-distribution with confidence of 95%. The middle line is a linear regression plot. The transition zone is not considered in the evaluation.

SO_4^{-2} in compressed cartilage depend on FCD in the same way as previously found for uncompressed tissue [9,23,29].

Fig. 4a,b shows graphs of $K_{H_2O}^{Na^+}$ and $K_{H_2O}^{SO_4^{-2}}$ vs. applied pressure. At $P=0$ there is considerable scatter in the value of $K_{H_2O}^{Na^+}$ and $K_{H_2O}^{SO_4^{-2}}$, which is

Table 2
Range of initial and final measurments in the partition experiments

Solute	Applied pressure (atm/)	Initial FCD $\left(\frac{\text{meq}}{\text{g } H_2O}\right)$	Final FCD $\left(\frac{\text{meq}}{\text{g } H_2O}\right)$	Initial hydration $\left(\frac{\text{g water}}{\text{g dry tissue}}\right)$	Final hydration $\left(\frac{\text{g water}}{\text{g dry tissue}}\right)$
SO_4^{-2}	5.5–21.3	0.193–0.269	0.308–0.530	1.992–2.533	0.954–1.767
Na^+	2.5–22.0	0.124–0.280	0.282–0.452	1.879–2.750	0.886–2.073
Inulin	4.1–11.0	0.133–0.298	0.194–0.457	1.762–2.764	1.015–2.131

due to the fact that the test specimens exhibited initially a wide range of FCDs (see Table 2) and that partition coefficients of ions are strongly dependent on FCD (e.g. [9]). When external pressure is applied, fluid is expressed until an equilibrium hydration and FCD are finally reached. At this point the applied pressure becomes equal to the osmotic pressure, π_{PG}, of the specimen's PG less the residual tension in the collagen network [25]. Since π_{PG} and the partition coefficients of ionic solutes both depend on the equilibrium value of FCD, a correlation between π_{PG} and K_{ionic} is to be expected, hence also between the applied pressure and K_{ionic}.

However, when K values are plotted against final water content (Fig. 4c,d) much scatter is observed throughout the entire range. Since the equilibrium hydration depends not only on the applied pressure but also on the initial FCD, the scatter is not surprising. Better correlations are obtained as follows.

For both unloaded and loaded specimens $K_{H_2O}^{Na^+}$ experimental (Fig. 5a) correlated well with the experimentaly determined FCD_{H_2O}, increasing with an increase in the latter. The curve of $K_{H_2O}^{Na^+}$ vs. FCD_{H_2O} lay close to the calculated Donnan equilibrium curve, though some deviation was observed. However, for sulfate there is considerable divergence between experimental results and one compartment Donnan theory. When the two-compartment model is used for calculating K_{Total} (Eqs. (5) and (3), with FCD_{EF} instead of FCD_{H_2O}) a much better agreement is observed (Fig. 5b). We do not know why the experimental partition coefficients diverge from those calculated at low values of FCD, no matter which of the two models is used, i.e. whether FCD is expressed on total water or on extrafibrillar water.

For the sake of comparison we also used our experimental data to calculate the partition coefficients of Na^+ and SO_4^{-2} based on EF water alone (Fig. 6a,b), obtained as detailed in Basser et al. [25] and plotted them vs. FCD_{H_2O} (Fig. 6a,b) since it is the extrafibrillar space that provides the actual environment for the chondrocytes. It can be seen that as FCD increases, the increase in $K_{EF}^{Na^+}$ is a little more pronounced and decrease in $K_{EF}^{SO_4^{-2}}$ much more pronounced than when the partition coefficients are based on total water (Fig. 5a,b).

4.3. Diffusion coefficient of small solutes

Fig. 7a shows a plot of diffusion coefficients (normalised to those in water) vs. applied pressure for two charged solutes (Na^+ and SO_4^{-2}) and one neutral solute (leucine). For all three solutes, the values of D/D decrease with increase in pressure. However, considerable scatter and a weak correlation is observed ($r_{sp}^{calc} < r_{sp}^{crit}$).

In studies of diffusion in CS solutions [23,29] and in uncompressed cartilage [30], it was found that D/D depends on the fraction of fluid volume in the tissue. Since, at any given applied pressure, fluid content varies with initial FCD, the scatter in Fig. 7a is understandable. Hence in Fig. 7b we plotted D/D vs. fractional water volume ε. This time, a good correlation was obtained ($r_{sp}^{calc} > r_{sp}^{crit}$) exept for leucine (probably because specific activity was relatively low). The dotted line represents the ratio D/D calculated by the Mackie and Meares Eq. (6), which lies close to (though a little below) the experimental points.

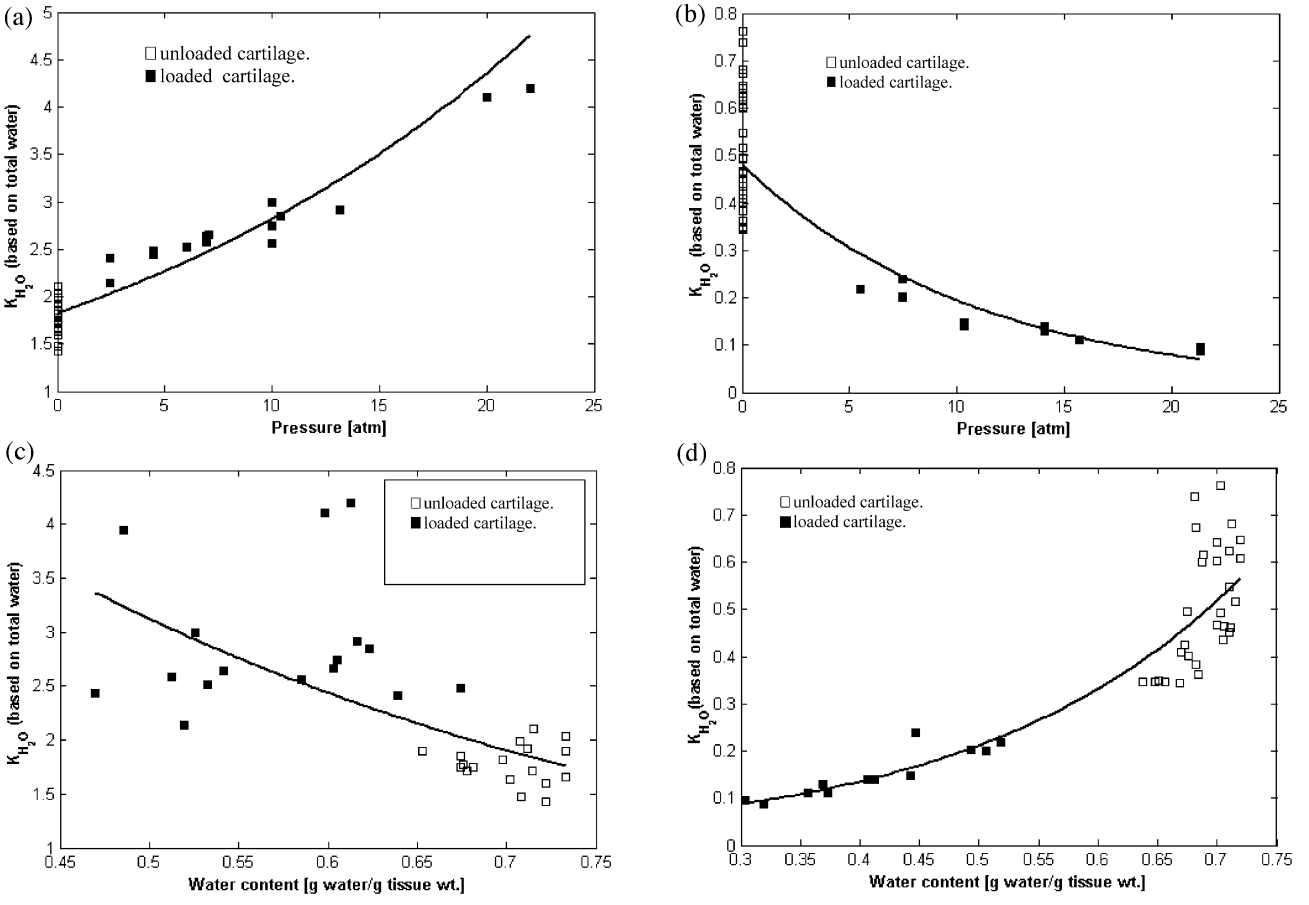

Fig. 4. (a) $K^{Na}_{H_2O}$ vs. applied pressure (P). Correlation: $y=1.8258\exp(0.0435x)$; $R^2=0.9747$; $r^{calc}_{sp}=0.7254$ ($n=34$). If $P=0$ is excluded, $=0.9412$ (16 loaded samples). (b) $K^{SO_4}_{H_2O}$ vs. P. $Y=0.48\exp(-0.0902x)$; $R^2=0.9944$; $r^{calc}_{sp}=0.3056$ ($n=42$). If $P=0$ is excluded, $r^{calc}_{sp}=0.9476$ (12 loaded samples). (c) $K^{Na}_{H_2O}$ vs. water content. $Y=10.7547\exp(-2.4721x)$; $R^2=0.8874$; $r^{calc}_{sp}=0.7371$. (d) $K^{SO_4}_{H_2O}$ vs. water content. $Y=0.0227\exp(4.4673x)$; $R^2=0.9962$; $r^{calc}_{sp}=0.8543$. $r^{crit}_{sp}=0.547$ For Na^+ (risk of 0.001, 42 samples) and for SO_4^{-2} $r^{crit}_{sp}=0.495$ (risk of 0.001, 42samples) in all above cases. □, ■ Represent unloaded and loaded samples, respectively.

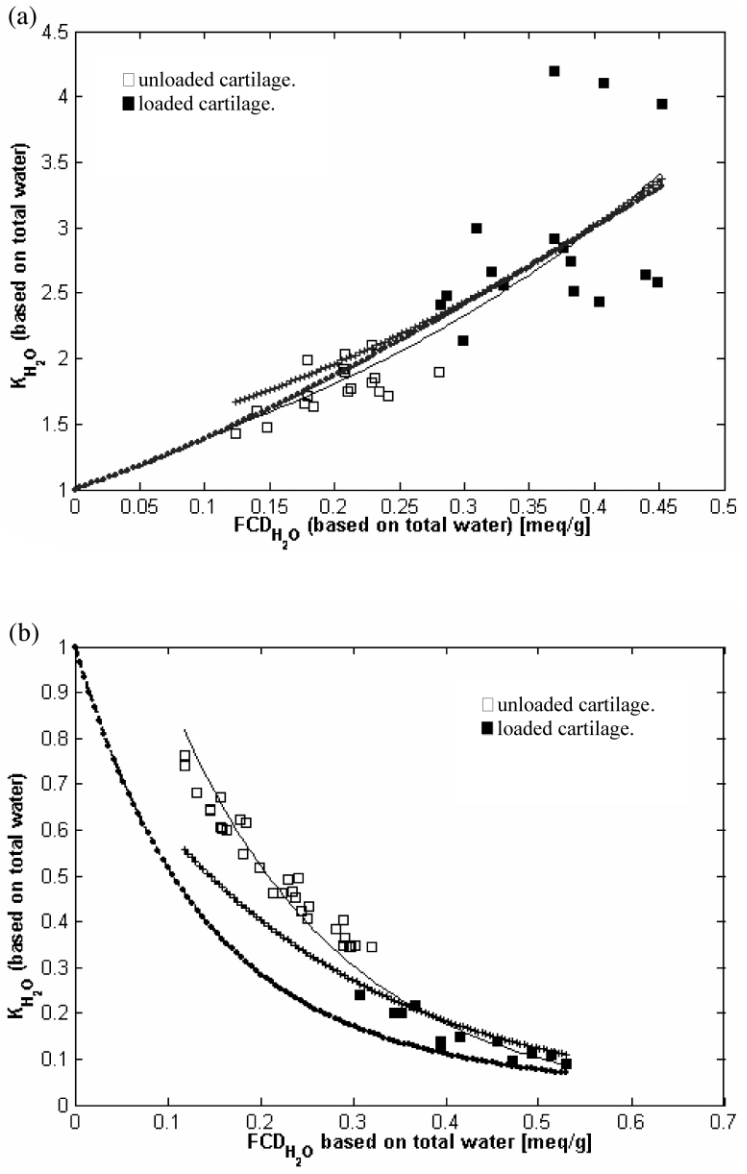

Fig. 5. (a) $K_{H_2O}^{Na^+}$ vs. FCD based on total water (FCD_{H_2O}). $Y=1.0886\exp(2.5349x)$; $R^2=0.9359$; $r_{sp}^{calc}=0.8671$ ($n=34$); $r_{sp}^{crit}=0.547$ (risk of 0.001, 34 samples). (b) $K_{H_2O}^{SO_4^{-2}}$ plotted vs. FCD based on total water (FCD_{H_2O}). $Y=1.5505\exp(-5.4272x)$; $R^2=0.9992$; $r_{sp}^{calc}=0.9885$; $r_{sp}^{crit}=0.495$ (risk of 0.001, 42 samples). ● Denotes the ideal Donnan curve based on one-compartment, calculated by Eq. (3). + Denotes the ideal Donnan curve based on a two-compartment model, calculated using Eq. (3), with FCD_{EF} instead of FCD_{H_2O} and Eq. (5). □, ■ Represent unloaded and loaded samples, respectively.

4.4. Partition and diffusion coefficient of inulin

The log of the partition coefficient of inulin calculated: (i) on the basis of total water; and (ii) on the basis of total EF water is plotted vs. FCD based on total water (Fig. 8a) and vs. FCD_{EF} (Fig. 8b), for loaded and unloaded cartilage. A strong linear correlation is observed ($r_{sp}^{calc}>r_{sp}^{crit}$). Howev-

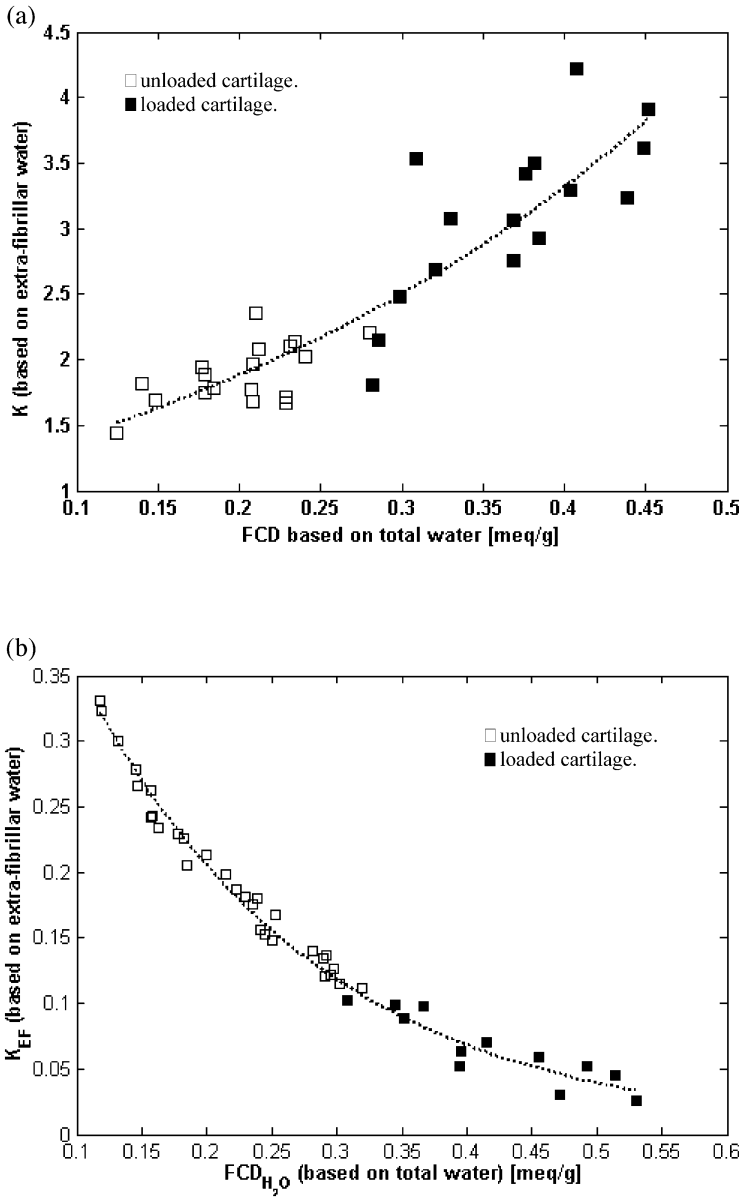

Fig. 6. (a) $K_{H_2O}^{Na^+}$ vs. FCD_{H_2O}. (b) $K_{EF}^{SO_4^{-2}}$ vs. FCD_{H_2O}. (a) And (b) partition coefficients of Na^+ and SO_4^{-2} based on extrafibrillar water and $K_{EF}^{SO_4^{-2}}$, were calculated by equation analogous to Eq. (3), (FCD_{H_2O} being replaced by FCD_{EF}) and plotted vs. FCD based on total water. □, ■ Represent unloaded and loaded samples, respectively.

er, due to exclusion of inulin from EF space a better correlation is obtained in case (ii). In both graphs, the experimental points at higher values of FCD lie well below the line calculated on the basis of the Ogston equation (Fig. 8b) when GAGs alone are considered as the excluding species. However, when core protein is also taken into consideration, a much better agreement is obtained.

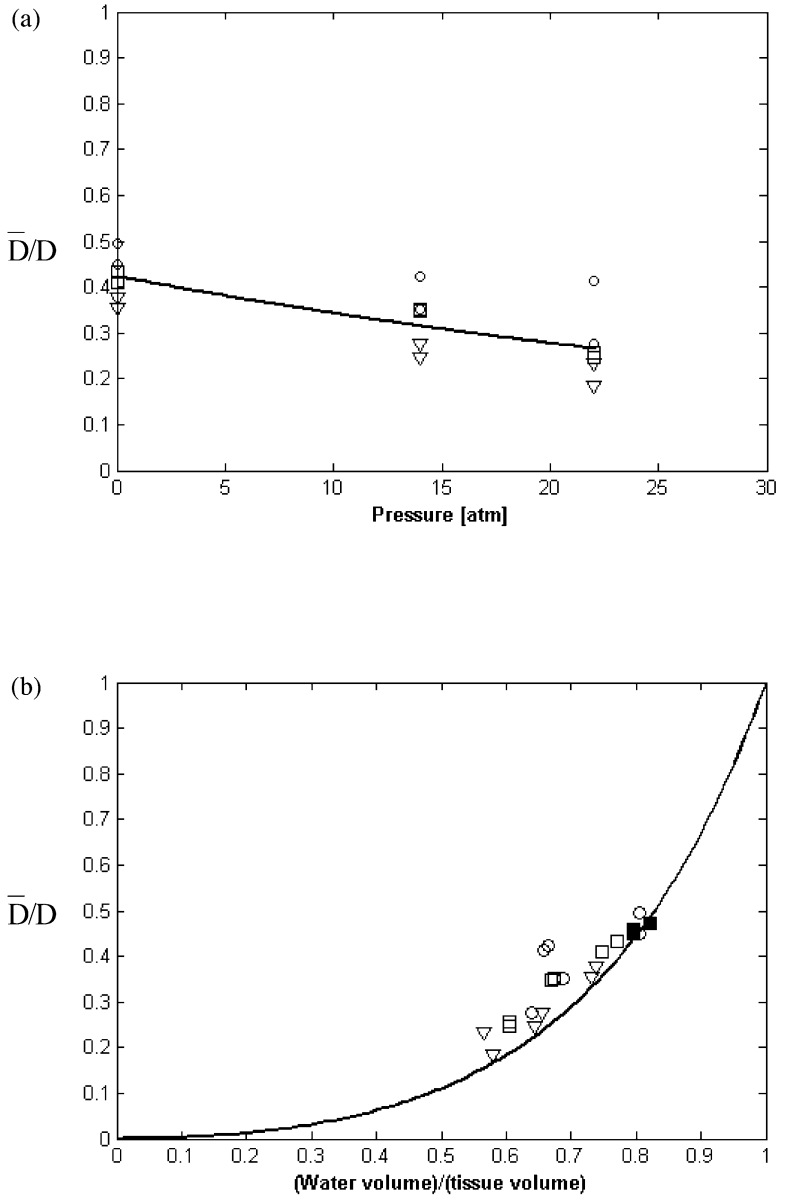

Fig. 7. (a) $\bar{D}/D$ vs. pressure. $Y=0.4234\exp(-0.0211x)$; $R^2=0.9983$; $r_{sp}^{calc}=0.6316$ ($n=18$). (b) $\bar{D}/D$ vs. volumetric fraction of water (ε). $r_{sp}^{calc}=0.8163$ ($N=18$). $r_{sp}^{crit}=0.728$ (risk of 0.001, 18 samples). Diffusion coefficients in water for Na^+ and SO_4^{-2} were obtained from [31] at 25 °C. The latter values were corrected by means of the Stokes–Einstein equation to 4 °C, giving 7.0×10^{-6} cm^2/s and 4.77×10^{-6} cm^2/s, respectively. D for leucine was estimated from D for alanine [32] using the formula: $D_{W\ \text{alanine}}/D_{W\ \text{leucine}}=(M_{W\ \text{leucine}}/M_{W\ \text{alanine}})^{2/3}$. From the above equation $D_{\text{leucine}}=3.72\times10^{-6}$ cm^2/s at 4 °C. Dashed curve represents Mackie and Meares equation (Eq. (6)). □, ▽, ○ Represent Na^+, SO_4^{-2} and leucine diffusion. ■ Na^+ diffusion coefficients for the unloaded portions of loaded specimens.

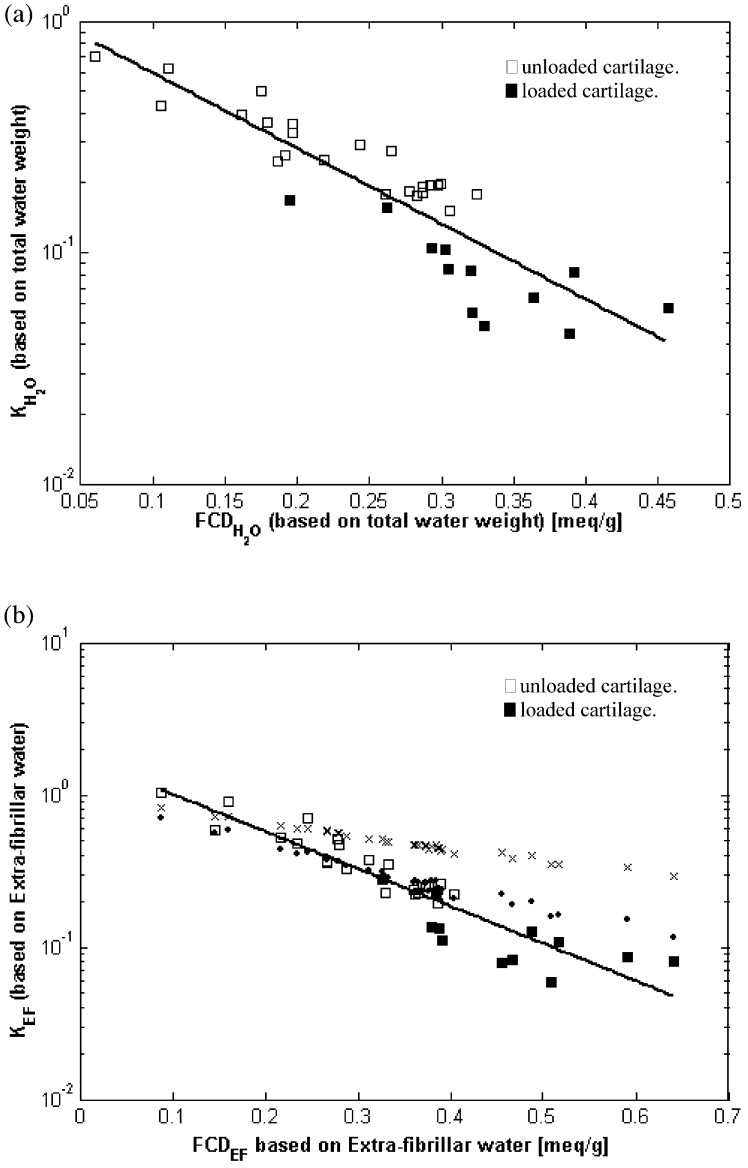

Fig. 8. (a) K_{H_2O} vs. FCD_{H_2O}. $Y=1.268\exp(-7.5104x)$; $R^2=0.9989$; $r_{sp}^{calc}=0.8863$ ($n=35$). (b) K_{EF} vs. FCD_{EF}. $Y=1.7881\exp(-5.645x)$; $R^2=0.9979$; $r_{sp}^{calc}=0.9370$ ($N=35$). $r_{sp}^{crit}=0.539$ (risk of 0.001, 35 samples). □, ■ Represent unloaded and loaded samples, respectively. × Were calculated from Ogston's equation, based on GAGs alone. ● Were calculated from Ogston's equation based on GAGs and core protein Eqs. (7a) and (7b).

It should be noted that in aged human articular cartilage, the ratio of protein to GAG is by no means negligible [22].

Inulin diffusivities are correlated with pressure (Fig. 9a). However, a better correlation was obtained with FCD_{EF} (Fig. 9b). The calculated

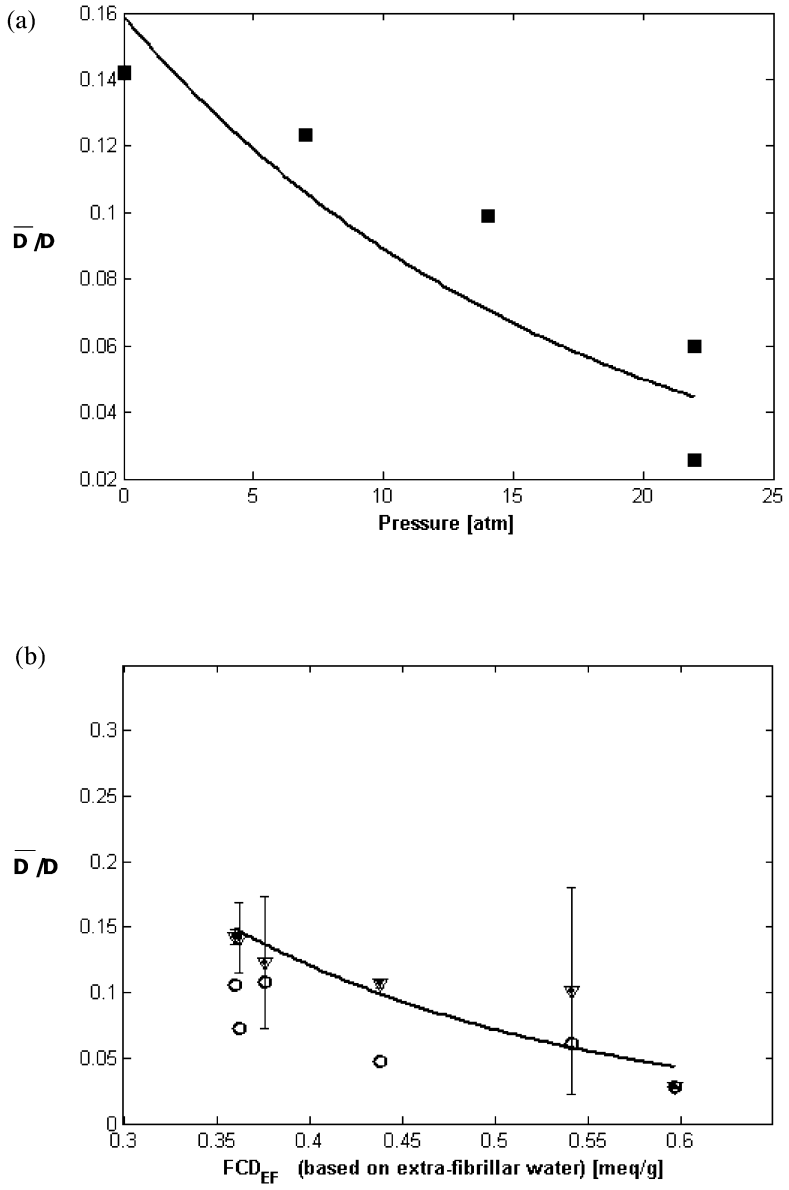

Fig. 9. (a) $\bar{D}/D$ vs. P. $Y=0.159\ \exp(-0.058x)$; $R^2=1.000$; $r_{sp}^{calc}=0.943$ ($N=6$). (b) $\bar{D}/D$ vs. FCD_{EF}. $Y=1.4872\ \exp(-6.4139x)$; $R^2=1.0$; $r_{sp}^{calc}=1.0$ ($n=6$). $r_{sp}^{crit}=0.943$ (Risk of 0.02, six samples). ▽ Represents experimental data. ○ Points calculated by Eq. (8). Bars represent confidence limits, based on a t-distribution with confidence of 95%. D for inulin was quoted by Lanman et al. [33] as 2.96×10^{-6} cm^2/s at 37 °C, which, when corrected by means of the Stokes–Einstein equation to 4 °C, gives a value of 1.27×10^{-6} cm^2/s.

points (Eq. (8)) lie below the experimental results, especially at low FCD values. We have no explanation for this.

5. Discussion and conclusions

5.1. Partition

The strong correlation which we obtain between the partition coefficient of small ionic solutes and fixed charged density (calculated on the basis of water content in both unloaded and loaded tissue) demonstrates that the effect of static mechanical compression on ionic concentrations is mainly through fluid loss and the consequent increase in the charged GAG content. This finding is of considerable importance for cartilage physiology. For instance, it has been shown that static loading decreases the PG synthesis rate. From several pieces of experimental evidence, it can be argued that a major factor causing the fall in synthesis rate under static load is the decrease in the water content, resulting in an increase in pericellular FCD, acompanied by an increase in osmotic pressure and a decrease in pH.

The contribution of the present work is to assign quantitative values to the ionic concentrations in the extrafibrillar compartment, since it is that compartment which provides the environment of cells in cartilage, both in the absence and presence of static load.

5.2. Diffusion

Our method of determining diffusion coefficients is based on the assumption that we can solve the one-dimensional problem of diffusion in a layered medium consisting of three elements: paper strip/unloaded cartilage strip/loaded strip. Each layer is considered semi-infinite, in spite of the fact that the tracer has to penetrate through the first two elements in order to reach the loaded cartilage. This assumption required justification. Firstly, on theoretical grounds, we found convergence to an exact solution when the length L of cartilage was more than 2 cm, the time t less than 1 week and the average D less than 4.0×10^{-6} cm^2/s (hence, $D{\cdot}t/L^2<0.1$). Thus, theoretically we appeared to be justified in expecting a linear correlation. Experimental results also support our assumptions: (i) $y_{S_{1,1}}(x,t)$ and $y_{S_{1,2}}(x,t)$ vs. x were indeed linear (Fig. 3); (ii) the diffusion coefficient of the unloaded strips is close to that of the unloaded portion of the loaded strips (as shown by the example for $S_{1,1}$ and $S_{1,2}$); (iii) the diffusion coefficient was independent of the time of diffusion. Hence our method for solving the diffusion equation appears to be reasonable.

As shown in Fig. 7b, solute charge does not affect diffusion of small solutes and the simple model of Mackie and Meares (based on tortuosity, hence water content) shows reasonable agreement with the experimental results. The results on loaded tissue are consistent with previous tests on unloaded tissue [9]. Hence for small solutes we can conclude that the reduction of diffusion under load is basically due to the reduction of fluid volume in the compressed tissue. This provides a means of estimating the diffusion coefficients of small solutes in compressed cartilage.

The reduction in diffusion coefficients of small solutes is obviously relevant to the rate of supply of such solutes to the chondrocytes and hence to the balance between the rate of diffusion on one hand, and the rate of consumption/production of metabolites by the chondrocytes on the other hand.

Regarding inulin, both experimental and calculated values of the partition and diffusion coefficients show an exponential relationship to FCD_{EF}. However, there is considerable discrepancy between the experimental and the calculated values when we use Ogston's equation and consider exclusion by GAGs alone. Clearly the protein core, of which there is a cosiderable proportion in adult human cartilage, should also be taken into account. When this is done, there is indeed a much better agreement between experimental data and Mackie and Meares' equation combined with Ogston's theory. (However, we cannot rule out other possible reasons, since many assumptions were made in our calculations.) From the physiological point of view the considerable exclusion of inulin from the extrafibrillar space, particularly at high FCD (i.e. low hydration levels), implies a decreased transport under static load of biologically active molecules

such as cytokines and growth factors of similar size to inulin.

Our results are different from those shown by Quinn et al. [13] who found that the partition coefficients (of dextrans 3 and 10 kDa) are not affected by GAG concentration. By contrast, our results show that partition (of inulin) decreased markedly with GAG concentration, as expected from Ogston's theory. As far as the diffusion coefficients are concerned, Quinn observed a sligtly lower decrease with strain for dextran 3 kDa than we have for inulin (5.6 kDa) so that the two sets of results show qualitative agreement.

The significance of our results in general is that by combining the concepts of hydration, fixed charge density and tortuosity we now seem to be in a position to predict both diffusion and partition of small solutes under static load. At present we cannot predict quantitatively the behavior of large solutes because of inadequate data of excluding species in cartilage.

We have not considered the effect of fluid flow, such as occurs in cyclic compression. However, since cyclic compression also causes some fluid loss (hence some decrease in both EFW and IFW) we would expect static load results to be also applicable to this aspect of solute transport under dynamic loading of cartilage. Our ability to predict partitions and diffusion coefficients is of significance in considering transport to and from chondrocytes as well as the ionic environment around the chondrocytes.

Acknowledgments

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