

Site-Specific Molecular Diffusion in Articular Cartilage Measured using Fluorescence Recovery after Photobleaching

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Abstract—Diffusive transport of solutes is critical to the normal function of articular cartilage. The diffusion of macromolecules through cartilage may be affected by the local composition and structure, which vary with depth from the tissue surface. We hypothesized that the diffusion coefficient of uncharged molecules also varies with depth and molecular size. We used fluorescence recovery after photobleaching (FRAP) to measure site-specific diffusion coefficients of fluorescent dextran molecules (3, 40, 70, and 500 kDa) in porcine articular cartilage. The diffusion coefficients measured using FRAP exhibited an inverse size dependence and were in general agreement with those measured using other techniques. The diffusion coefficients for all molecules varied significantly with depth in a manner that depended upon the size of the diffusing molecule. The diffusion coefficients for the 3 and 500 kDa dextrans were 1.6 and 2.4 times greater, respectively, in the surface zone as compared to the middle and deep zones, whereas the diffusion coefficients of the 40 and 70 kDa dextrans were 0.3 and 0.2 times lower in the surface zone as compared to the middle and deep zones. These differences may reflect variations in the structure and composition of collagen, proteoglycans, and other macromolecules among the zones. © 2003 Biomedical Engineering Society. [DOI: 10.1114/1.1581879]

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INTRODUCTION

Articular cartilage is a soft, load-bearing tissue that lines the ends of joints and provides a smooth, low friction surface for joint articulation. It is comprised primarily of water (60%–80%) with type II collagen (~75% dry tissue weight) and proteoglycans (~20%–25% dry tissue weight) as the major structural components. The solid matrix of articular cartilage has a complex ultrastructure that varies with depth from the cartilage surface to the subchondral bone.^{9,18–20,28} In this regard, the tissue may be divided into a number of successive “zones” based on extracellular matrix structure and composition, as well as cell shape and cell arrange-

ment. The surface zone, where the cartilage on one joint surface articulates with the cartilage on the opposing joint surface, is characterized by densely packed collagen fibers that run parallel to the surface in a preferred direction termed the “split line” direction.²⁰ The surface zone is also characterized by a high cell density and collagen content and low proteoglycan content relative to the other zones.^{9,20,28} The middle zone is characterized by fewer and more randomly oriented collagen fibers, fewer cells, and high proteoglycan content. The deep zone, where the cartilage attaches to the bone, is characterized by large, bundled collagen fibers that run perpendicular to the surface, lowest cell density, and lower proteoglycan content.^{9,20,28} Water content also varies between the three zones decreasing linearly with depth from 80% in the surface zone to 65% in the deep zone.²⁴

Adult articular cartilage is an avascular tissue, implying that movement of any molecules (e.g., nutrients, wastes, oxygen, signaling molecules, matrix macromolecules) through the tissue occurs primarily by diffusion or by convection of interstitial fluid. While fluid movement due to joint loading and unloading can increase the transport of large molecules in the extracellular matrix,³⁰ diffusion is believed to be the primary mode of molecular transport. Alterations in the rates of molecular diffusion, which may occur with aging or disease, therefore have the potential to influence chondrocyte metabolism by affecting the transport of nutrients, signaling molecules, or pharmacologic agents. Many factors have been shown to influence the rate of molecular diffusion in articular cartilage, with specific effects that often depend on the size and nature of the diffusing molecule. For example, diffusion coefficients in cartilage are generally inversely proportional to molecular size^{28,40} and increase with temperature in accordance with the Stokes–Einstein relationship.³⁹

Importantly, the structure and composition of the cartilage extracellular matrix appear to have significant effects on the rate of diffusion in the tissue. Torzilli *et al.*⁴¹ have shown that removal of 71% of proteoglycans by enzymatic degradation increases the diffusion coefficient

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of 70 kDa dextran molecules, but not that of glucose or insulin. Further digestion to remove 93% of proteoglycans increases the diffusion coefficient of both the dextran and insulin, but not glucose.⁴¹ In addition, removal of the entire surface zone decreased the bulk tissue diffusion coefficients of glucose, insulin, and 10 kDa dextran, but increased the diffusion coefficient of 70 kDa dextran.³⁹ Maroudas²⁶ has shown that the diffusion coefficients of larger molecules (10 and 40 kDa dextran) decrease with increasing fixed charge density, a measure of proteoglycan content, whereas the diffusion coefficient of smaller molecules (urea and glucose) did not vary with fixed charge density. More recently, it has been demonstrated that static compression of cartilage can decrease diffusion coefficients of relatively large molecules (3, 10, and 40 kDa dextrans)³⁵ as well as that of water.⁶ Positively charged sodium ions diffuse more slowly than negatively charged chloride ions, presumably due to interaction with the negative charges on the proteoglycans.²⁷

Diffusion coefficients have been measured in cartilage using magnetic resonance imaging (MRI),^{10,11} nuclear magnetic resonance (NMR),⁶ tracking net movement of radiolabeled solutes,^{39,41,42} and fluorescence³⁵ or radiotracer desorption.^{26–28} To date, MRI and NMR have only been used to track the movement of very small molecules and ions. While tracking radiolabeled solutes and fluorescence desorption allow for the measurement of a variety of molecules, these methods measure spatially averaged diffusion coefficients over relatively large volumes of tissue. Due to the uneven distribution of macromolecules that may impede the movement of a diffusing molecule, it is likely that the diffusion properties of cartilage vary with site in the tissue.

We hypothesize that the inhomogeneous structure and composition of cartilage leads to a variation in diffusion coefficients among the three zones. The goal of this study was to examine the site dependence of diffusion coefficients of a size range of molecules in articular cartilage using fluorescence recovery after photobleaching (FRAP). FRAP allows site-specific measurement of the diffusion coefficient of a fluorescently labeled molecule. After the molecule is allowed to permeate the tissue, an intense laser beam is focused on a small portion of the sample, causing irreversible breakdown of the fluorophores in that region. This creates a dark spot, which gradually recovers its fluorescence due to the diffusion of fluorescent molecules into the bleached region and photobleached molecules out of the bleached region. The rate of the recovery of fluorescence is dependent on the diffusion coefficient of the molecule.² FRAP has been used to measure the apparent diffusion coefficient of fluorescently labeled molecules in a localized region, such as within tumors,^{29,32} bacterial biofilms,⁵ muscle fibers,³¹ cytoplasm,²⁵ nucleus,³⁶ and cell membrane.³⁸ In

this study, we used FRAP to measure the diffusion coefficients of inert dextran molecules, spanning over two orders of magnitude in size, throughout the surface, middle, and deep zones of articular cartilage.

MATERIALS AND METHODS

Preparation of Specimens

Full-depth explants of articular cartilage were harvested from the femoral condyles of ~2–2.5-year-old female pigs. Only cartilage that appeared macroscopically healthy, showing no fibrillation or other signs of degeneration, was used. The samples were approximately 5 mm by 2 mm by the thickness of the cartilage. Fluorescein conjugated dextrans of 3, 40, 70, and 500 kDa molecular weights (Molecular Probes, Eugene, OR) were suspended in Dulbecco's phosphate buffered saline (Gibco, Gaithersburg, MD) at concentrations of 0.38 mg/ml (0.13 mM), 0.93 mg/ml (0.023 mM), 2.27 mg/ml (0.032 mM), and 2.00 mg/ml (0.004 mM), respectively. Samples were incubated in dextran solutions for 2 days at 4 °C to allow the dextrans to fully permeate the tissue. Dextrans are polysaccharides comprised of glucose and are produced by bacteria. The diffusive properties of dextrans were studied because they are uncharged, inert molecules and are available in a range of sizes, which allowed us to examine the passive diffusive characteristics of cartilage. There are many physiologically relevant molecules found in cartilage that are encompassed by the size range of dextrans used (Table 1). It is important to note, however, that factors other than size, such as active cellular uptake or binding of molecules with extracellular matrix proteins, may also influence the effective diffusive properties of these molecules.²⁸

FRAP Experiments

All FRAP experiments were performed on a Zeiss LSM 510 laser scanning confocal microscope (Carl Zeiss, Inc., Thornwood, NY). All samples were allowed 20 min to equilibrate to room temperature before testing, and all tests were performed at room temperature (~25 °C). Previous studies have shown that diffusion in cartilage follows the Stokes–Einstein relationship and diffusion coefficients can be extrapolated to any relevant temperature.³⁹ Each cartilage sample was placed in a custom built chamber that held the tissue block in place against a coverglass bottom. The chamber was filled with Dulbecco's phosphate buffered saline (Gibco, Gaithersburg, MD).

All photobleaching was performed at 488 nm emission at 100% laser power. All imaging was performed with a 15.0 mW argon laser at 1% power with excitation at 488 nm and emission recorded above 505 nm. The

TABLE 1. Size comparison of dextrans studied and physiologically relevant molecules. Numbers taken from Heinegard and Oldberg (see Ref. 17) and Seyedin and Rosin (see Ref. 37).

Molecule	Molecular weight (kDa)
Proteoglycan aggregate	200,000
Proteoglycan	2500
Hyaluronic acid	100–10,000
Fibronectin	550
Cartilage oligomeric matrix protein	500
Dextran-500	500
Single type II procollagen chain	285
Biglycan	76
Decorin	75
Dextran-70	70
Fibromodulin	59
Link Protein	45
Dextran-40	40
Cartilage derived growth factor	31
BMP-1	30
TGF- β	25
BMP-2	18
IL-1	17.5
BMP-3	16
IGF	7.5
EGF	6
Insulin	5
Dextran-3	3
Glucose	0.18

fluorescence intensity in the unbleached portion of the images did not change over the course of the experiment, indicating that there was no significant photobleaching of the samples during the imaging portion of the experiment. For each experiment, an initial background image was taken, the photobleaching was performed, and then a series of images was recorded to track the fluorescence

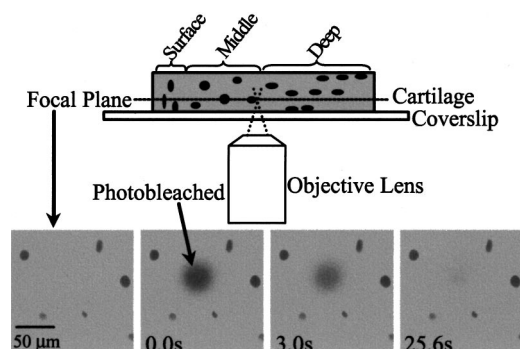


FIGURE 1. The upper panel shows the setup for a FRAP experiment where the cartilage specimen is placed on its side such that surface, middle, and deep zones are visible through the microscope. The confocal microscope allows the focal plane to be at a depth in the tissue away from the cut face. The lower panels show the photobleaching and recovery process on a cartilage sample. The smaller dark spots are viable chondrocytes that exclude the fluorescent dye.

recovery (Fig. 1). All images were recorded with a $20 \times / 0.5$ numerical aperture Plan-Neofluar objective (Zeiss) with a confocal pinhole of $166 \mu\text{m}$, which resulted in an optical slice of less than $7.2 \mu\text{m}$. Images were 512 by 512 pixels, corresponding to a resolution of $0.9 \mu\text{m}/\text{pixel}$.

FRAP experiments were carried out in the surface, middle, and deep zones of each piece of tissue. Zones were discriminated by cellular morphology and tissue architecture.⁹ The surface zone was distinguished by the closely packed, elongated cells; surface zone FRAP experiments were performed within 15% of the cartilage thickness from the surface. The middle zone was distinguished by single rounded cells; middle zone FRAP experiments were performed at approximately one-third of the cartilage thickness from the surface. The deep zone was distinguished by a sparse cell population and radial stacks of rounded cells; deep zone FRAP experiments were performed at approximately four-fifths of the cartilage depth. For each molecule, a minimum of three FRAP experiments were performed in each zone on each piece of tissue. Tissue from at least two different animals was used for each molecule. In addition, another series of FRAP experiments was performed sampling uniformly across all zones to generate a zone-averaged data set that could be compared with previous studies that did not account for zonal variations of the tissue. Four explants each were taken from two different animals, and one explant from each animal was placed in the four different sized dextran solutions. Six FRAP experiments were performed in each explant, distributed uniformly across the zones.

Image Processing

All image processing was performed using custom written code in Matlab (The Mathworks, Inc., Natick, MA). Images were normalized using background division and median filtering.¹³ The program then determined the radius of the bleached circle as the average distance at which the fluorescence intensity falls to e^{-2} of the maximum value in the center of the bleached area in the time zero, immediately after bleaching, image. The mean radius of the bleached area was $37.3 \pm 10.6 \mu\text{m}$. The mean fluorescence intensity within this bleached region was calculated for each image over the recovery period. Because the fluorescence intensity is averaged over this area, the size of this area must be large enough to be considered homogeneous, but small enough to capture the heterogeneity of interest. This size area is significantly larger than the most widely spaced molecular structure (deep zone collagen fibers, which are 200 nm apart), but small enough to fit well within the smallest cartilage zone (surface).

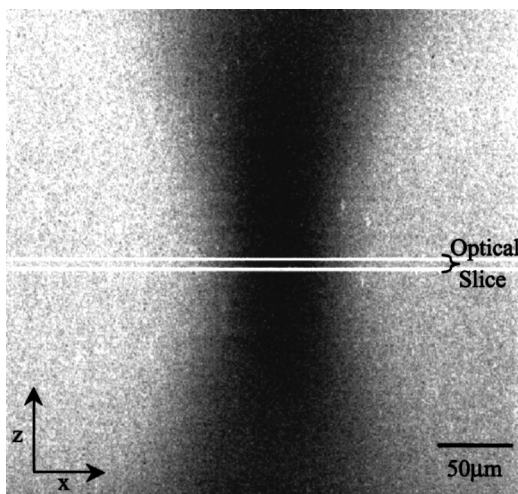


FIGURE 2. Image shows the depth (z dimension) of the area that is photobleached in a FRAP experiment is large relative to the optical slice in which fluorescence recovery is observed. The optical slice is less than $7.5 \mu\text{m}$.

Calculation of Diffusion Coefficients

Diffusion coefficients were calculated from the FRAP experiments using the method described by Axelrod *et al.*² This analysis assumes a two-dimensional, planar configuration. Photobleaching occurs as a slightly flaring column through a very thick section of the tissue, but the images are recorded in a thin section in the center of the bleached section (Fig. 2). Therefore, diffusion in and out of the imaging plane (the third dimension) is neglected in this analysis.⁴

To determine the diffusion coefficient, the mean fluorescence in the bleached region over time was converted to a normalized fractional fluorescence intensity

$$f = \frac{F(t) - F(0)}{F(\infty) - F(0)},$$

where $F(t)$ is the fluorescence at time t , $F(0)$ is the fluorescence immediately after bleaching, and $F(\infty)$ is the fluorescence after complete recovery. The fractional fluorescence intensity was plotted versus time and fitted with a logarithmic curve (Fig. 3). The recovery rate will vary with size of the bleached area, location of the bleached area (i.e., cartilage zone), and the size of the diffusing molecule. The equation for the curve was used to determine the half-recovery time ($f=0.5$). The bleaching parameter, which describes the relationship between the half-recovery time and the characteristic diffusion time, was also calculated according to Axelrod *et al.*² The half-recovery time, $\tau_{1/2}$, the measured initial spot radius ω (as described earlier), and the bleaching parameter, γ_D , were used to determine the diffusion coefficient, D^2 :

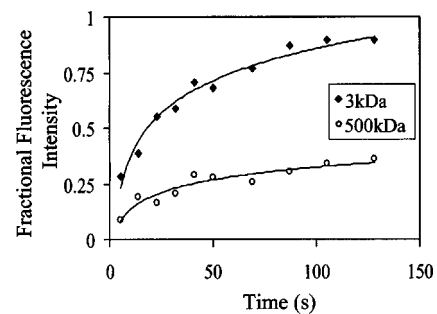


FIGURE 3. Examples of fluorescence intensity recovery in the bleached region vs. time since bleaching ended for 3 kDa dextran (closed diamonds) and 500 kDa dextran (open circles). The data are fit with logarithmic curves 3 kDa: $y = 0.21 \cdot \ln(x) - 0.1034$, $r^2 = 0.97$, and 500 kDa: $y = 0.081 \ln(x) - 0.048$, $r^2 = 0.91$.

$$D = \left(\frac{\omega^2}{4 \tau_{1/2}} \right) \gamma_D.$$

Precision

Experiments were carried out to test the precision (i.e., repeatability) of this method. Multiple FRAP experiments were carried out at the same spot on a piece of cartilage, waiting 20 min between experiments to allow the tissue to completely recover. All experiments showed complete recovery, indicating no fluorophore was immobilized.² A minimum of five precision experiments were carried for each size dextran on tissue from a single animal. All experiments were performed in middle zone cartilage. The coefficient of variation was calculated for each dextran as the standard deviation of the diffusion coefficient divided by the mean diffusion coefficient.

Statistical Analysis

Comparisons of diffusion coefficients between molecules and between zones were made using one factor analysis of variance (ANOVA) using Statistica (Statsoft, Inc., Tulsa, OK). Least significant difference (LSD) post hoc tests were used for *a posteriori* comparisons of groups.

RESULTS

The coefficients of variation for the 3, 40, 70, and 500 kDa dextrans were 0.23, 0.16, 0.16, and 0.29, respectively. The mean coefficient of variation in repeated measurements averaged over all sized dextrans was 0.21.

Diffusion coefficients varied significantly by size when averaged across all zones (ANOVA, $p < 0.00001$, Fig. 4). Diffusion coefficients varied inversely with dextran size; 3 kDa dextran had the highest diffusion coefficient, followed by the 40, 70, and the 500 kDa dextran, which had the lowest diffusion coefficient. The diffusion

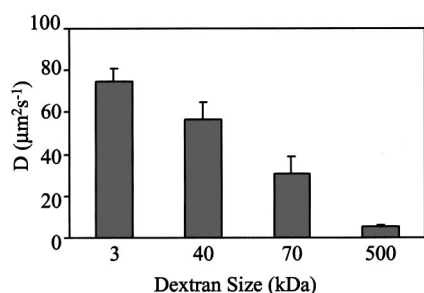


FIGURE 4. Diffusion coefficients in articular cartilage vary significantly with the size of the diffusing molecule. All groups are significantly different from one another (ANOVA $p < 0.00001$, LSD post-hoc $p < 0.05$, $n = 12$ per group).

coefficient was correlated to the inverse of the published hydrodynamic radii^{21,25} of the dextrans as predicted by the Stokes–Einstein relationship ($R^2 = 0.88$).

Diffusion coefficients varied significantly by zone for all sizes (ANOVA, $p < 0.04$); however, the direction of the trend depended upon the size of the dextran. In addition, all significant differences were from the surface zone as compared to the middle and deep zones. For the 3 kDa dextran the diffusion coefficient in the surface zone was significantly greater than that of the middle and deep zones [Fig. 5(a)]. In contrast, the diffusion coefficients of the 40 and 70 kDa dextrans were significantly lower in the surface zone than in the middle and deep

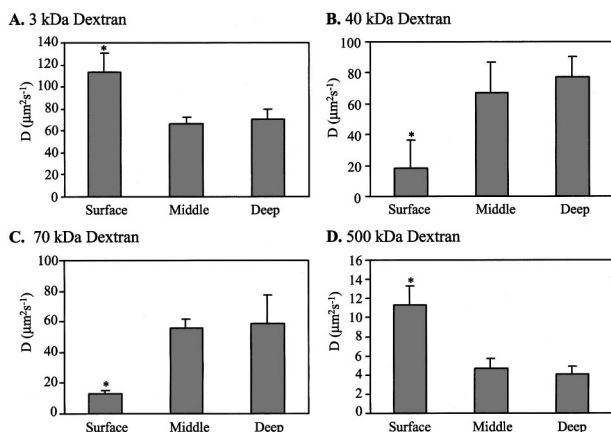


FIGURE 5. Diffusion coefficients of four different sized dextrans in articular cartilage vary significantly with zone. Bars indicate mean plus standard error; asterisks indicate significant difference between marked group and the other two groups (LSD post-hoc test, $p < 0.05$). (a) 3 kDa dextran diffuses more rapidly in the surface zone than in the middle and deep zones ($p = 0.01$ ANOVA, $n > 15$ per group). (b) 40 kDa dextran diffuses more slowly in the surface zone than in the middle and deep zones ($p < 0.05$ ANOVA, $n > 7$ per group). (c) 70 kDa dextran diffuses more slowly in the surface zone than in the middle and deep zones ($p < 0.0005$ ANOVA, $n > 13$ per group). (d) 500 kDa dextran diffuses more rapidly in the surface zone than in the middle and deep zones ($p < 0.001$ ANOVA, $n > 14$ per group).

zones [Figs. 5(b) and 5(c)]. The 500 kDa dextran showed the same trend as the 3 kDa dextran with the diffusion coefficient in the surface zone being significantly greater than the diffusion coefficients in the middle and deep zones [Fig. 5(d)].

DISCUSSION

The findings of this study indicate that the diffusion coefficients of uncharged dextran molecules vary with zone in articular cartilage in a manner that depends on the size of the diffusing molecule. In particular, significant differences were observed between the properties of the surface zone as compared to the middle and deep zones, which had similar diffusion properties. These findings suggest that the zonal differences in the structure and composition of articular cartilage have a significant influence on the diffusive transport of a wide range of molecular sizes.

Our repeatability studies indicate that FRAP provides a precise method for measuring site-specific diffusion within articular cartilage. This technique provides an important advance over standard techniques for measuring diffusion in cartilage such as fluorescence desorption and radiotracer movement^{26–28,35,41} by allowing measurements at high spatial resolution (i.e., micron scale) in a nondestructive manner. The diffusion coefficients measured in the present study using FRAP are in good agreement to those measured previously using fluorescence desorption³⁵ and radiotracer movement.⁴¹ For example, Torzilli *et al.*⁴¹ measured the diffusion coefficient of 70 kDa radiolabeled dextran in cartilage as $40 \mu\text{m}^2 \text{s}^{-1}$, as compared to our measurement of $31 \mu\text{m}^2 \text{s}^{-1}$. Using fluorescence desorption, Quinn *et al.*³⁵ measured the diffusion coefficient of 3 and 40 kDa dextrans in middle zone cartilage as $50\text{--}70$ and $35\text{--}40 \mu\text{m}^2 \text{s}^{-1}$, as compared to our measurements of 75 and $56 \mu\text{m}^2 \text{s}^{-1}$. There is no other data available for diffusion of a molecule as large as the 500 kDa dextran in cartilage. The largest molecule diffusion coefficient measured prior to this study is for IgG, which has a molecular weight of 150 kDa and a diffusion coefficient of about $50 \mu\text{m}^2 \text{s}^{-1}$.²⁸

Our findings corroborate previous findings on molecular diffusion in the surface zone of cartilage.³⁹ In a previous study, it was shown that removal of the surface increases the coefficient of diffusion of 70 kDa dextran for the bulk tissue sample. This finding is consistent with our results of a lower diffusion coefficient in the surface zone as compared to the middle and deep zones.³⁹ Conversely, for the 3 kDa dextran we found that the surface zone diffusion coefficient is higher than that in the other zones, which implies that removal of the surface zone would decrease the tissue averaged diffusion coefficient. Likewise, Torzilli³⁹ found that surface zone removal de-

creased the diffusion coefficients of smaller, similarly sized molecules such as insulin (~5 kDa) and 10 kDa dextran.

The zonal differences in diffusion coefficients presumably arise from interactions between the diffusing molecules and extracellular matrix structures that act as barriers to diffusion. It has been shown both theoretically and experimentally that fibrous structures can slow the progress of a diffusing molecule, thereby decreasing the effective diffusion coefficient.^{7,15,33}

For example, proteoglycans are believed to slow the diffusion of certain molecules in cartilage, and proteoglycan removal increases the diffusion coefficient of 70 kDa dextran and insulin.⁴¹ Torzilli *et al.*,⁴⁰ however, found no correlation between bulk tissue proteoglycan content and diffusion coefficient in cartilage. When the cartilage was divided into slices from different depths, on the other hand, Maroudas²⁶ showed some evidence for a decrease in diffusivity with increasing GAG content. However, our finding that all molecules did not show the same trend in diffusivity with zone suggests that the differences in GAG content among zones cannot be the only factor affecting the rate of diffusion.

Typically, collagen has not been considered a significant influence on the diffusion of molecules in cartilage because the fibers are relatively large and widely spaced compared to the proteoglycans.²⁷ Collagen fibrils in the surface zone are 25–50 nm in diameter, whereas those in the middle and deep zones are 60–160 nm in diameter.¹⁹ The distance between collagen fibers may be as low as 60 nm in the surface zone and up to 200 nm in the deep zone.⁸ The volume fraction of collagen fibers in cartilage, however, is approximately 30%.²² Although the spaces between fibers are larger than the largest molecule we examined, the volume fraction of fibers is sufficiently high that they may still hinder diffusion. Theoretical simulations show that diffusion of a molecule in a solution of fibers of similar diameter to the diffusing molecule (as would be the case for the 500 kDa dextran in the surface zone) can be slowed by 25% when the fibers are present at a volume fraction of 30%.¹⁵ Even for a fiber that is 2.5 times larger than the diffusing molecule, the effective diffusion coefficient can be decreased by 20%. This prediction suggests that rate of diffusion of even the 40 and 70 kDa dextrans could be affected by the collagen fibers in the surface zone.

These previous studies, in combination with our current findings, suggest that both proteoglycans and collagen may play a role in determining the diffusion rate of large molecules in cartilage. The variation in patterns of zonal diffusion for each sized molecule may arise from the increased numbers and distribution of barriers each molecule must face. The 3 kDa dextran may be slowed by proteoglycans only, and its pattern of zonal diffusion inversely tracks the distribution of proteoglycans. The

hindrance of 3 kDa dextran diffusion by proteoglycans is consistent with previous studies demonstrating that removal of proteoglycans from cartilage increases the diffusion coefficient of insulin, a similarly sized molecule.⁴¹

The 40 and 70 kDa dextrans, which are of similar size,²¹ exhibited similar zone-dependent properties, with lowest diffusion coefficients in the surface zone. This finding suggests that their diffusion is hindered by small, dense collagen fibers that are prevalent in the surface zone. Although it has been shown that proteoglycans do slow the diffusion of 70 kDa dextran in cartilage,⁴¹ its relatively low diffusion coefficient in the surface zone suggests that other matrix macromolecules, such as collagen, may hinder its motion. It is also important to note that the structure of aggrecan, the primary proteoglycan of cartilage, may change significantly with depth. A single aggrecan molecule consists of a large protein core and numerous glycosaminoglycan side chains. Chondroitin and keratan sulfates are the dominant side chains for the majority of aggrecan in mature articular cartilage,¹⁶ and the ratio of keratan to chondroitin sulfate increases with depth from the surface.^{3,12} The specific influences of aggrecan size and structure on molecular diffusion in cartilage remain to be determined.

Theoretically, the 500 kDa dextran is large enough relative to the collagen fibers that its diffusion could be significantly hindered by the collagen fibrils in the surface zone;¹⁵ however, its diffusion coefficient was highest in that zone. This observation suggests that other matrix macromolecules may hinder its diffusion in the middle and deep zones. The largest gaps between the side chains of proteoglycan aggregates are approximately 20 nm.⁴¹ Unlike the other molecules tested, the 500 kDa dextran is large enough (hydrodynamic radius of 24.3 nm)²⁵ that it may not fit between or may become entangled within the side chains of the proteoglycan aggregates.

The size range of dextran examined in this study was selected to encompass the sizes of a wide range of physiologically relevant molecules (Table 1). The large 500 kDa dextran is similar in size to large extracellular matrix molecules such as fibronectin or cartilage oligomeric protein. The 70 kDa dextran is similar in size to some of the smaller matrix molecules such as decorin and biglycan, while the 40 kDa dextran and the 3 kDa dextran are similar in size to some important growth factors such as bone morphogenetic protein-1 and insulin-like growth factor (IGF). However, it is important to note these dextrans have a linear structure that may not duplicate the molecular structure of other physiologically relevant molecules in cartilage, which may be more globular in structure. Pluen *et al.*³³ have shown that flexible macromolecules have greater mobility than similarly sized globular molecules in a random fiber matrix. Furthermore, the effects of active cellular uptake or binding of

such molecules with extracellular matrix proteins may influence their diffusive properties.

Diffusion coefficients have been measured for other tissues and cells using the FRAP technique. The diffusivity of tumors may be as high as three orders of magnitude greater than that of cartilage for comparably sized molecules, suggesting that tumor tissue is much less dense than cartilage.^{29,32} Within the cytoplasm of hepatoma cells, the diffusivity of 40 and 70 kDa dextrans is approximately 15 times slower than that of cartilage, and in the nucleus it is 50 times slower than that of cartilage.²¹ If this holds true for chondrocytes as well, diffusion of large signaling molecules within cells may be much slower than between cells.

In summary, we have shown that FRAP provides an effective way to measure site-specific diffusion in cartilage. We have also shown that diffusion of large molecules in cartilage is size dependent, and that diffusion of molecules in cartilage is zone dependent, but the pattern varies with molecular size. This technique may be useful in examining age- or disease-related changes in molecular diffusion in cartilage, which may cause site-specific changes in tissue properties. For example, loss of proteoglycans and disruption of the collagen network in fibrillated areas in the surface zone due to osteoarthritis could lead to altered diffusive properties in those regions.¹⁴ In addition, the matrix immediately surrounding chondrocytes, the pericellular matrix, is structurally distinct from the surrounding extracellular matrix, and may also be altered with osteoarthritis.^{1,23,34} The ability of FRAP to measure site-specific diffusion in cartilage provides a means of examining localized changes in these properties.

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