Eur Biophys J (2007) 36:529–538
DOI 10.1007/s00249-006-0098-y

ARTICLE

Comparison of the effects of mechanical and osmotic pressures on the collagen fiber architecture of intact and proteoglycan-depleted articular cartilage

Galit Saar · Hadassah Shinar · Gil Navon

Received: 13 July 2006 / Revised: 20 August 2006 / Accepted: 30 August 2006 / Published online: 22 September 2006

Abstract One of the functions of articular cartilage is to withstand recurrent pressure applied in everyday life. In previous studies, osmotic pressure has been used to mimic the effects of mechanical pressure. In the present study, the response of the collagen network of intact and proteoglycans (PG)-depleted cartilage to mechanical and osmotic pressures is compared. The technique used is one-dimensional ^{2}H double quantum filtered spectroscopic MRI, which gives information about the degree of order and the density of the collagen fibers at the different locations throughout the intact tissue. For the nonpressurized plugs, the depletion had no effect on these parameters. Major differences were found in the zones near the bone between the effects of the two types of application of pressure for both intact and depleted plugs. While the order is lost in these zones as a result of mechanical load, it is preserved under osmotic pressure. For both intact and PG-depleted plugs under osmotic stress most of the collagen fibers become disordered. Our results indicate that different modes of strain are produced by unidirectional mechanical load and the isotropic osmotic stress. Thus, osmotic stress cannot serve as a model for the effect of load on cartilage in vivo.

This paper is presented in part in ISMRM 11th Scientific Meeting, p. 55, 2003 and ISMRM 14th Scientific Meeting, p. 59, 2006.

Dedicated to Prof. K. Arnold on the occasion of his 65th birthday.

G. Saar · H. Shinar · G. Navon (✉)
School of Chemistry, Tel Aviv University,
Ramat Aviv, Tel Aviv 69978, Israel
e-mail: navon@post.tau.ac.il

Abbreviations

PG	Proteoglycans
GAG	Glucoseaminoglycan
DQF	Double quantum filtered
IP-DQF	In-phase DQF
PEG	Polyethyleneglycol
SEM	Scanning electron microscopy
PLM	Polarized light microscopy
STIM	Scanning transmission ion microscopy

Introduction

The collagen fibers in articular cartilage define the shape and the tensile strength of the tissue. The cartilage also contains proteoglycans (PG) that consist of an inner protein core with glucosaminoglycan (GAG) molecules that have a high content of negatively charged groups. Those negatively charged groups are responsible for the strong water binding ability of the PG that generates a swelling pressure (Maroudas and Bannon 1981; Urban et al. 1979). Both the swelling power of the PG and the tensile strength of the oriented collagen fibers provide the unique characteristics of the articular cartilage.

Articular cartilage can be divided into four zones (Kääb et al. 1998; Nötzli and Clark 1997). At the superficial zone the collagen fibrils are parallel to the surface, rather randomly oriented at the transitional zone, and perpendicular to the surface at the radial and calcified zones. The relative size of the different zones varies from one sample to another.

The main biological function of articular cartilage is to withstand pressure and thus a large amount of

research is devoted to the study of cartilage under load (Gründer et al. 2000; Kääb et al. 1998; Kaufman et al. 1999; Mow and Guo 2002; Nötzli and Clark 1997; Regatte et al. 1999; Rubenstein et al. 1996). When pressure is applied to cartilage, water is forced out of the extracellular matrix. Thus the concentration of the PG increases, resulting in an increase of the swelling pressure within the extracellular matrix, and rearrangement of the macromolecules. We have recently studied, by ^{2}H double quantum filtering (DQF) techniques, the effect of unidirectional mechanical load on the fiber architecture in intact cartilage bone plugs as well as on cartilage detached from its underlying bone. We have found that there is an increase of the width of the surface zone with fibers parallel to the surface and a gradual loss of the orientation of the collagen fibers in the deep radial zone (Shinar et al. 2002). The stabilizing effect of the attachment to the bone was shown in a load experiment on the detached cartilage (Keinan-Adamsky et al. 2005). In several studies the effect of osmotic stress, on the relaxation rates (Lüsse et al. 1995), on the hydration (Maroudas et al. 1985), and on the tensile stress of collagen network (Basser et al. 1998) in articular cartilage, were investigated.

Since, technically, it is much more convenient to apply osmotic pressure, osmotically loaded cartilage has been used as a model for the in vivo loaded tissue (Maroudas and Bannon 1981). It has been shown that the same levels of hydration can be achieved by applying the osmotically active solution of polyethyleneglycol (PEG) and an equivalent mechanical load (Maroudas and Bannon 1981; Schneiderman et al. 1986). Lai et al. (1998) also dealt with the question of equivalence of osmotic and mechanical loading on the articular cartilage and came to the conclusion that an equivalence between the two methods can be reached only if the mechanical load is applied in isotropic manner. One of the aims of this study was to compare the effect of unidirectional mechanical load to the effect of osmotic stress on the collagen fibers architecture. Results obtained for intact cartilage bone plugs as well as for PG-depleted plugs, which serve as a model for the study of osteoarthritis, are presented.

Theoretical background

The ordered collagen fibers in articular cartilage induce anisotropic motion to water molecules interacting with them. This anisotropic motion results in residual proton–proton dipolar interaction. However, this interaction is partially averaged by proton exchange between water molecules and thus in biological tissues at body temperature the splitting is not resolved (Dehl and Hoeve 1969; Eliav and Navon 1999). Still, the residual broadening is responsible for the short ^{1}H T_2 of water in cartilage and tendon.

For deuterated water, the anisotropic motion is manifested in nonzero ^{2}H residual quadrupolar interaction. As this interaction is not averaged by the exchange of deuterium atoms, the splitting is resolved and is equal to twice the quadrupolar interaction. In most biological tissues, the ^{2}H splitting is small due to the small fraction and the small order parameter of the water bound to oriented macromolecules and thus, is masked by the strong signal from the isotropic media in the single quantum (SQ) spectra. This can be overcome by using DQF (Eliav and Navon 1999; Shinar et al. 2002) where only the signals of molecules with anisotropic motion and those in fast exchange with them are detected and the large isotropic signal is filtered out.

The one-dimensional (1D) ^{2}H in-phase DQF (IP-DQF) spectroscopic imaging pulse sequence is given in Fig. 1 (Eliav and Navon 1999) where τ is the creation time of the second rank tensors, t_{DQ} is the DQ evolution time, and t_{ZQ} is the evolution time for the zero quantum coherence. The line shape and the intensity of the IP-DQF spectra vary as a function of the creation time τ. The signal intensity is given by signal $\propto \sin(\omega_Q\tau)e^{(-\tau/T_2)}$, where ω_Q is the quadrupolar interaction. By applying the proper phase cycling only the DQ coherences are allowed through the filter. The imaging gradients are applied during the DQ evolution time, which is relatively long for ^{2}H water signals in connective tissues (Sharf et al. 1995; Shinar et al. 1997). Moreover, the effect of the gradients is doubled during the DQ evolution time, partially compensating for the low γ of ^{2}H. The gradients are applied in parallel to the

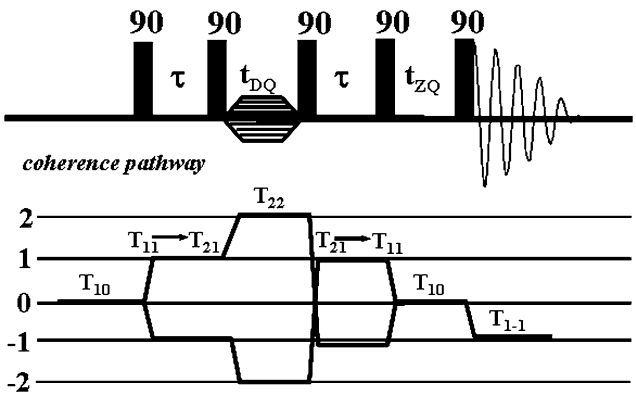

Fig. 1 One-dimensional ^{2}H in-phase DQF (IP-DQF) spectroscopic imaging pulse sequence and coherence transfer pathway diagram. τ is the creation time of the second rank tensor, t_{DQ} is the double quantum evolution time, and t_{ZQ} is the zero quantum evolution time

symmetry axis of the plugs to give the spatial dimension of the image, and thus the variation of the quadrupolar splitting in the different layers of the cartilage can be observed.

In the presence of macroscopic order as in articular cartilage and tendon, the water ^{2}H quadrupolar splitting depends on the orientation of the tissue in respect to the external magnetic field, and on the fraction of water molecules interacting with the collagen fibers according to the equation: $\upsilon_{(\exp)} = p_b \upsilon_b \frac{(3\cos^2\theta - 1)}{2}$ where υ_b is the quadrupolar splitting of the bound water molecules, p_b is the fraction of bound water molecules, and θ is the angle between the director of the interaction and the magnetic field. For water and collagen it has been shown that the director is parallel to the collagen fibers (Berendsen 1962; Migchelsen and Berendsen 1973; Navon et al. 2001). When the symmetry axis of the plug is parallel to the magnetic field, $\theta = 0°$, a maximum splitting is obtained, half maximum at $\theta = 90°$, and no splitting is expected at the ''magic angle'', $\theta = 54.7°$.

Materials and methods

Cartilage-bone plugs, 7 and 8 mm in diameter, were obtained from bovine femoral lateral and medial condyles. All plugs were equilibrated for approximately 3 h in deuterated saline, wiped dry, and immersed in fluorinated oil (Fluorinert, FC-77, 3M, St. Paul, MN, USA), which has a low water solubility and susceptibility similar to that of water.

Spectroscopic images were recorded on a Bruker DMX-360WB NMR spectrometer using a 10 mm imaging probe (200 G/cm) tuned for deuterium (55.3 MHz).

The MRI parameters used for the 1D IP-DQF spectroscopic images were τ = 1,200 μs, field of view 1.0 cm × 20 kHz, and data matrix 64 × 2048. The 2D FT was performed after zero filling of data to 128 × 4,096.

For mechanical compression a home built pressure device with a series of weights was used (Keinan-Adamsky et al. 2005). Two plugs separated by a Teflon spacer were placed in a heavy walled NMR tube (513-7TRM-7, Wilmad, Buena, NJ, USA). In this way the physiological situation in the body is mimicked. After each application of pressure, a 30-min interval was allowed in order to reach a new equilibrium.

Solutions of PEG with molar mass of 20,000 Da were used to obtain the osmotic stress. The osmotic pressure of the different PEG solutions in 150 mM NaCl in D_2O was calculated using the following relation taken from Parsegian et al. (1986) after interpolation for 25°C: $\log[\Pi_{20}(w; 25°)] = 1.61 + 2.74w^{0.21}$, where Π_{20} (dyne/cm^2) is the osmotic pressure of PEG solution at PEG concentration w (%).

In order to prevent penetration of the polymer molecules into the cartilage matrix, the plugs were separated from the PEG 20,000 solution using a dialysis bag. The samples were incubated for at least 20 h in order to reach equilibrium state, and then were removed from the dialysis bag, immersed in fluorinated oil, and placed in the NMR tube.

For the PG depletion, cartilage bone plugs were incubated for 12 h in 1 mg/ml trypsin (Sigma, St. Louis, MO, USA) in PBS for 12 h, at 25°C with constant stirring. In this way, a full depletion of the cartilage PGs is achieved. The samples were then re-equilibrated in deuterated saline and measured.

The level of PG depletion was measured by following the increase of the ^{23}Na quadrupolar splitting (Danziger et al. 1999; Shinar and Navon 2006) using the modified CPS pulse sequence (Eliav et al. 2003; Shinar and Navon 2006).

Results

The 1D IP-DQF spectroscopic image of two cartilage bone plugs, excised from bovine femoral condyles, is given in Fig. 2. The x-axis is the frequency dimension and the y-axis is the spatial dimension. Some spectra extracted from the upper plug at different locations from bone to surface are given as well. Near the bone and in the radial zone two pairs of satellite transitions are observed. The quadrupolar splittings decrease in the upper radial zone and only one pair of satellites is observed in the transitional and surface zones. The presence of two pairs of satellites indicates two types of collagen fibers, with different densities and degrees of hydration, near the bone and at the deep radial zone. This has recently been observed by Shinar et al. (2002) and Keinan-Adamsky et al. (2005).

In order to compare the effect of mechanical and osmotic loads, the same level of pressure has to be achieved by the two methods. Results of such an experiment are given in Fig. 3 where the 1D ^{2}H IP-DQF spectroscopic images of an intact plug at rest and under mechanical and osmotic stress of 0.5 MPa are shown. The width of the plug decreased from 2.2 mm at rest to 1.5 mm under both forms of load, indicating that the same amount of compression of the cartilage is achieved. Similar results were obtained for a few

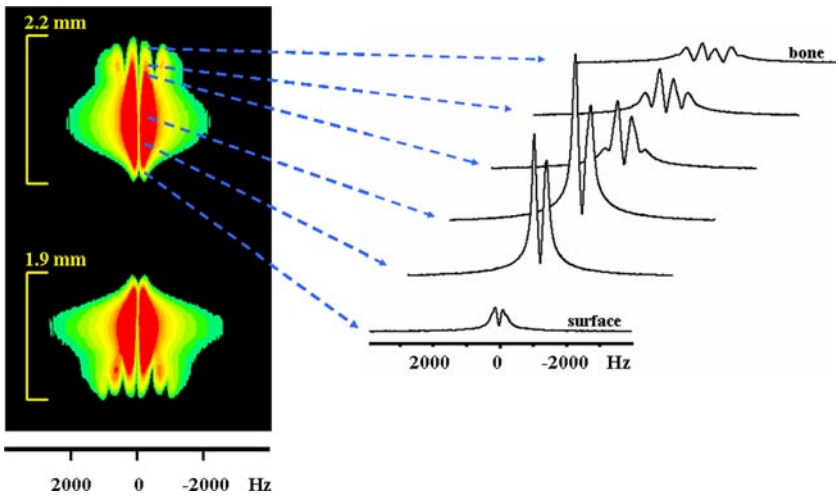

Fig. 2 1D ^{2}H IP-DQF spectroscopic image of two bovine articular cartilage bone plugs equilibrated in deuterated saline. The cartilage bone plugs were placed surface to surface in the NMR tube. The *x*-axis represents the frequency dimension and the *y*-axis represents the spatial dimension. On the *right* ^{2}H IP-DQF spectra extracted from the upper plug at different locations from bone to surface

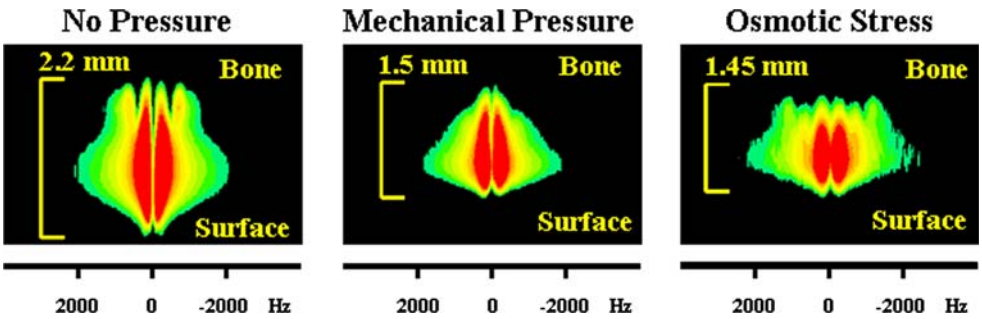

Fig. 3 ^{2}H IP-DQF spectroscopic images of bovine articular cartilage bone plug equilibrated in deuterated saline, at rest (0 MPa) and under 0.5 MPa mechanical and osmotic stress

cartilage bone plugs at 0.5 MPa with an average compression of 77.3 ± 3.7% (n = 13) and 78.0 ± 3.7% (n = 8) for mechanical and osmotic stress, respectively. At 1.0 MPa the average compression was 67.5 ± 4% (n = 10) and 64.3 ± 3.1% (n = 3) for mechanical and osmotic stress, respectively. Some spectra extracted from the intact, mechanically and osmotically compressed cartilage at 0.5 MPa are given in Fig. 4. For intact cartilage, without applied pressure, two pairs of quadrupolar split satellites of 1,300 and 400 Hz are observed near the bone. The mechanical load resulted in the disappearance of the large splitting in agreement with previous reports (Keinan-Adamsky et al. 2005; Shinar et al. 2002). For osmotic stress at the same load the quadrupolar splitting of 1,300 Hz is clearly detected and a larger splitting of 2,250 Hz appeared. At locations between 50% from bone and the surface, the line shapes of the cartilage under mechanical and osmotic loads were similar.

The developments of the spectral changes near the bone as the mechanical and osmotic pressures increased from 0 to 1.0 MPa are given in Fig. 5. The same sample was used for each of the mechanical and the osmotic pressures. As can be seen from the figure the main spectral features are fairly reproducible among the different samples before the application of pressure. For mechanical compression, the large quadrupolar splitting is already lost at 0.5 MPa while for osmotic loads it is clearly resolved even at 0.7 MPa.

The effect of mechanical and osmotic loads was also studied on PG-depleted cartilage (Figs. 6, 7). Measurements of the effects of mechanical load were performed first on intact cartilage-bone plugs and were repeated on the same plugs after the PG depletion. After the release of pressure, the samples were re-equilibrated in deuterated saline and re-measured. The images obtained at this stage were identical to the images obtained before the application of pressure. An example of such an experiment is given in Fig. 6. Without load, the quadrupolar splittings obtained for the intact and PG-depleted plugs are almost identical. This result indicates that the orientation of the collagen fibers is not effected by the depletion of the PG in agreement with previous results (Keinan-Adamsky et al. 2005). The same degree of compression of the intact and depleted cartilage was achieved by applying loads of 0.5 and 1.0 MPa to the intact cartilage and loads of 0.12 and 0.2 MPa to the depleted cartilage, respectively. Near the bone, for both intact and PG-depleted cartilage, the large splitting disappeared

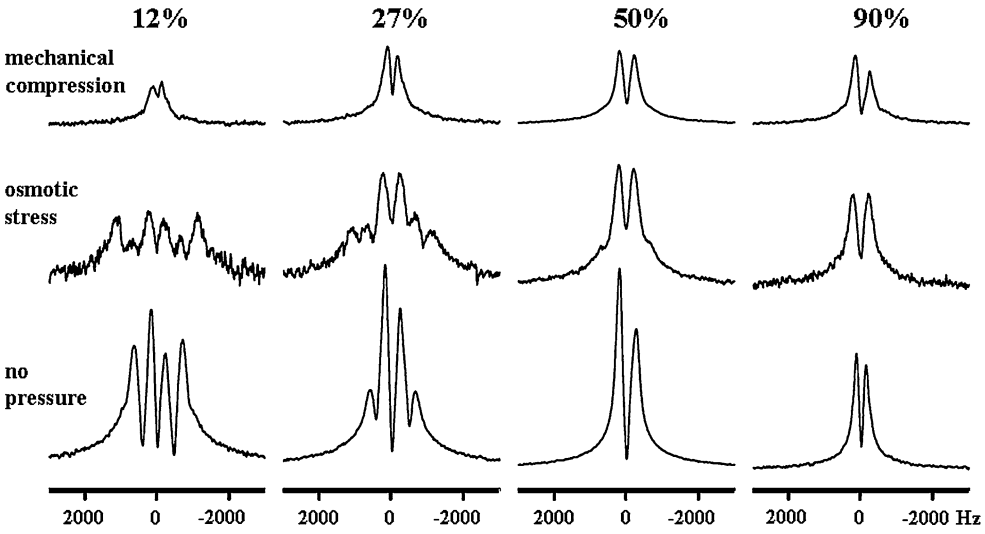

Fig. 4 ^{2}H IP-DQF spectra extracted from the spectroscopic images of bovine articular cartilage bone plugs given in Fig. 3, at rest and under 0.5 MPa mechanical and osmotic stress. Spectra are given for different locations from bone (0%) to surface (100%)

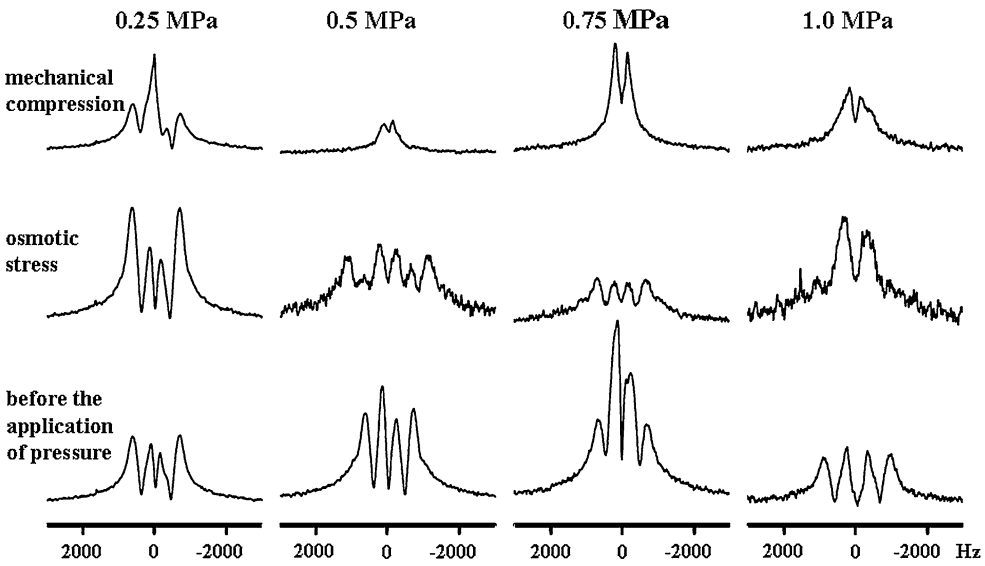

Fig. 5 ^{2}H IP-DQF spectra extracted from spectroscopic image of bovine articular cartilage bone plug, at rest and under 0.25, 0.5, 0.7, and 1.0 MPa mechanical and osmotic stress at 12% from the bone. The same sample was used for each of the mechanical and osmotic stress

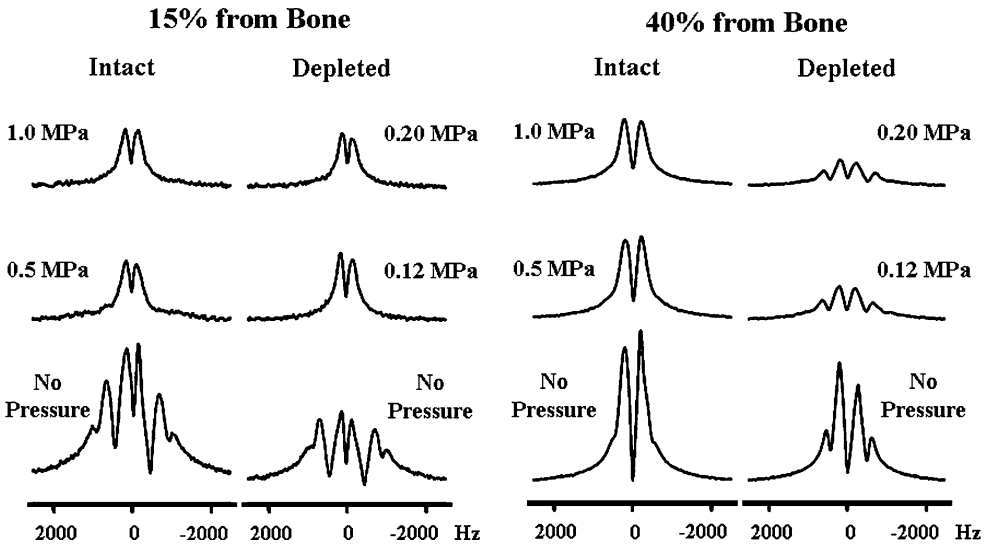

Fig. 6 ^{2}H IP-DQF spectra extracted from spectroscopic images of intact and PG depleted bovine articular cartilage bone plug, at rest and under mechanical pressure. Spectra are given for locations of 15 and 40% from bone. One plug was used for the whole set of measurements

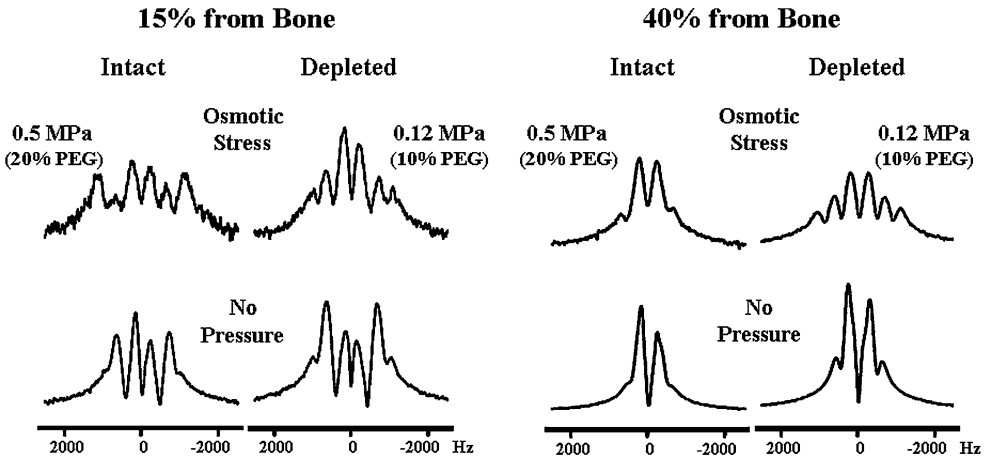

Fig. 7 ^{2}H IP-DQF spectra extracted from spectroscopic images of intact and PG depleted bovine articular cartilage bone plugs, at rest and under osmotic stress. Spectra are given for locations of 15 and 40% from bone

under the applied mechanical loads. A striking difference between intact and depleted cartilage is observed from depths of 40% from the bone to the surface. For the intact cartilage only one pair of quadrupolar split satellite is observed, while two pairs are clearly resolved for the depleted cartilage. For the depleted cartilage as the pressure increases the intensity of the small splitting, relative to the intensity of the large splitting decreased. This may indicate a preferential decrease in the amount of water which resides near those collagen fibers that are responsible for the smaller splitting in response to the application of load.

Unlike the case of mechanical compression, the large quadrupolar splitting of approximately 1,500 Hz at 15% from the bone did not disappear following the application of osmotic stress for both intact and depleted cartilage (Fig. 7). A quadrupolar splitting of about 2,300 Hz that was seen for the intact plug (see Fig. 4) is evident also in the depleted plug. For the depleted plug at osmotic load of 0.12 MPa, all the three pairs of satellites are also resolved at 40% from the bone.

As mentioned earlier, the ^{2}H quadrupolar splitting of deuterated water interacting with oriented collagen fibers depends on the fraction of bound water as well as on the geometric factor $(3\cos^2\theta–1)$, where θ is the angle between the direction of the quadrupolar interaction and the external magnetic field. Thus, by measuring the quadrupolar splitting at different orientations, with respect to the external magnetic field, the ordering of the collagen fibers can be deduced. Due to technical reasons we were only able to perform these measurements for cartilage bone plugs without load and those under osmotic stress.

For the intact and depleted plugs, before the application of osmotic stress, the results are similar. In the radial zone, a maximal splitting is obtained when the symmetry axis of the plug is parallel to the external magnetic field and approximately half of the splitting after a rotation of the plug by 90° (Fig. 8a). This result indicates that the collagen fibers in this zone orient perpendicular to the surface. At the ''magic angle'' ($\theta = 55°$), the intensity of the DQF signal is significantly reduced, yet the splitting does not completely vanish, probably indicating a distribution in the alignment of the collagen fibers resulting in a powder-like spectrum. Similar results are obtained near the bone (Fig. 8b): without pressure when the symmetry axis of the plug is parallel to the external magnetic field ($\theta = 0°$) two quadrupolar splittings are observed both for the intact and the depleted plugs. When $\theta = 90°$ and 55°, the two pairs are not resolved.

The orientation dependence of the quadrupolar splittings was measured for intact plugs subjected to 0.5 MPa osmotic stress and for depleted plugs subjected to 0.12 MPa stress. In the radial zone, as can be seen from Fig. 8a, there is only minimal angular dependence of the quadrupolar splitting in the intact and depleted plugs under osmotic stress. Near the bone (Fig. 8b) three pairs of satellites are observed. For the intact plug under an osmotic load of 0.5 MPa, the large quadrupolar splitting of 2,300 Hz is not evident at 90° and 55° and the value of the other two splittings is invariant to rotation. This result indicates that following osmotic stress most of the collagen fibers become disordered and the residual dipolar interaction that we are detecting stems from local order—similar to the case of nasal cartilage (Sharf et al. 1995) and not macroscopic order that is shown here for the intact plug.

The quadrupolar splittings as a function of the distance from the surface, at three orientations of the plug with respect to the magnetic field, for an intact plug at 0 and 0.5 MPa osmotic loads and for a depleted plug at 0 and 0.12 MPa osmotic loads are given in Fig. 9.

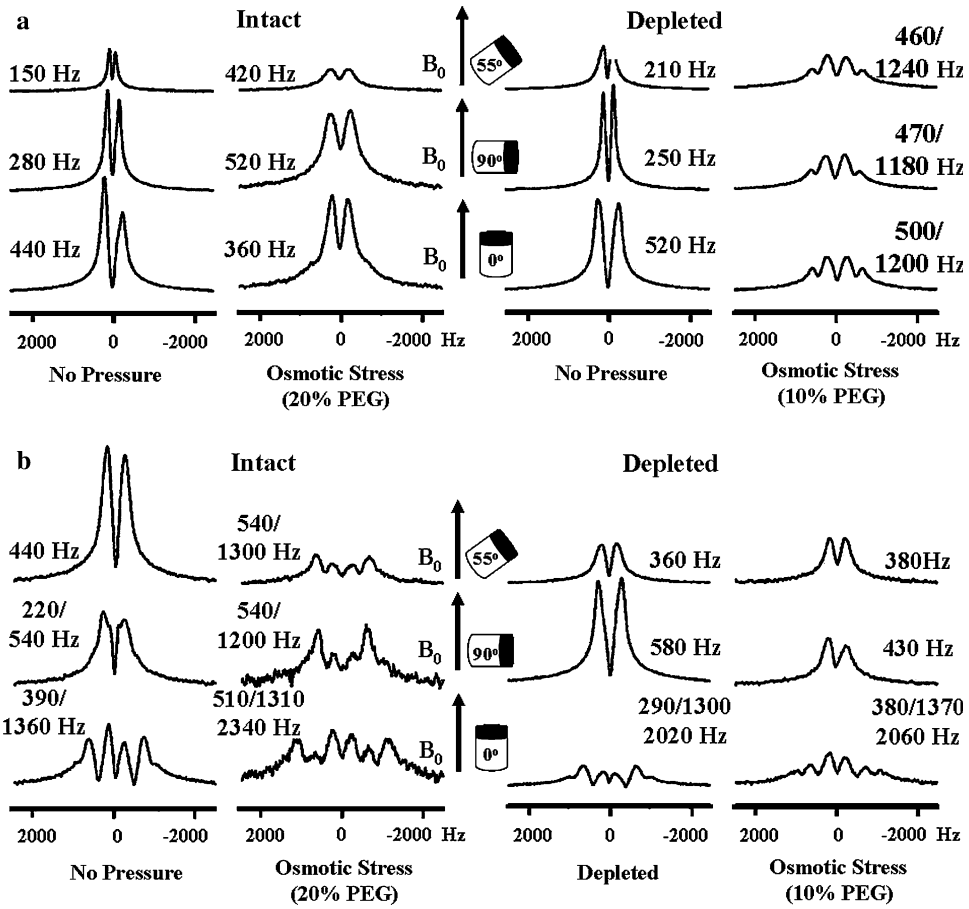

Fig. 8 ^{2}H IP-DQF spectra extracted from spectroscopic images of intact and PG depleted bovine articular cartilage bone plugs, before and after the application of osmotic stress, at three orientations of the plugs relative to the external magnetic field. Spectra are given for two locations: **a** 60% from bone and **b** 15% from bone. The splittings are given in the figures

When the symmetry axis of the plugs is parallel to the external magnetic field at least two pairs of quadrupolar splittings are observed in the calcified and radial zones. These splittings decrease toward the transitional zone to a point in which only one splitting is observed (Fig. 9a, c). A rotation of the intact and PG-depleted plugs by 90° resulted in the decrease of the quadrupolar splitting by a factor of approximately 2 in the calcified and radial zone. These results show that the orientation dependence of the collagen fibers did not change as a result of the PG depletion.

Under osmotic stress, we found that the orientation dependence for both intact and PG depleted cartilage is lost. The two smaller quadrupolar splittings at different locations of the plugs at all three orientations (Fig. 9b, d) are similar. Moreover, unlike the non-pressurized plugs in which the quadrupolar splittings are largest near the bone and decrease toward the surface, for the plugs under osmotic stress the splittings are rather uniform throughout the sample.

For both intact and depleted cartilage, the largest quadrupolar splitting observed in the calcified and deep radial zone (approximately 2,400 Hz) when the symmetry axis of the plug is parallel to the magnetic field was not detected at the other two orientations. The fibers responsible for that splitting may be oriented parallel to the plug symmetry axis. In that case, at the perpendicular orientation, the 2,400-Hz splitting is not observed and is probably reduced to 1,200 Hz and coincides with the 1,300 Hz intermediate splitting.

Discussion

In contrast to other techniques, like SEM and PLM where fixation, dehydration, sectioning, and decalcification are performed before the measurements, or STIM that required cryosection and dehydration (Reinert et al. 2001), for ^{2}H NMR only equilibration of the cartilage in deuterated saline is required. Moreover, while in the other techniques a different sample is used for each measurement, a full load experiment can be done on one cartilage bone plug in the case of NMR measurements.

^{2}H DQF spectroscopic imaging was used in order to deduce the collagen fiber orientation in articular cartilage. Water molecules in the vicinity of the oriented collagen fibers tend to align along the fibers and their

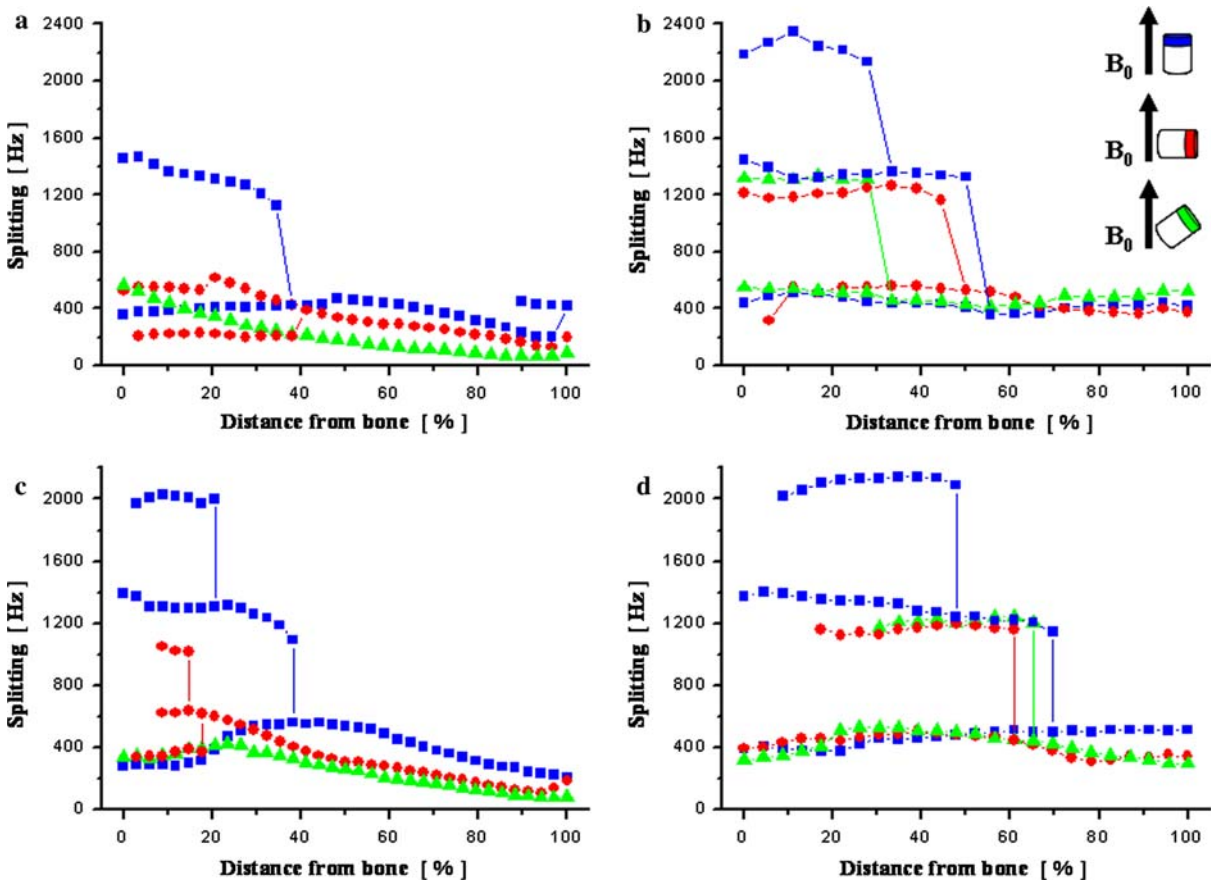

Fig. 9 ^{2}H quadrupolar splitting of bovine articular cartilage bone plug, equilibrated in deuterated saline, as a function of the distance from bone (0%) to surface (100%), at three orientations of the plug relative to the magnetic field. The ^{2}H IP-DQF spectroscopic images were obtained with the symmetry axis of the plug parallel (*blue*), perpendicular (*red*), and at 55° (*green*) relative to the external magnetic field. Results are given for intact plug under **a** 0 MPa and **b** 0.5 MPa osmotic stress and for PG-depleted cartilage under **c** 0 MPa and **d** 0.12 MPa osmotic stress

motion is anisotropic. For cartilage equilibrated in deuterated saline, the ^{2}H quadrupolar interaction does not vanish and quadrupolar split satellites are detected. From the orientation dependence of the quadrupolar splitting the alignment of the collagen fibers is obtained.

It has been shown that the same levels of hydration can be achieved by applying either osmotically active solutes (e.g. polyethylene glycol), which produce an isotropic load to the cartilage, or an equivalent unidirectional mechanical pressure (Maroudas and Bannon 1981; Schneiderman et al. 1986). We have found that under matched conditions the collagen fibers near the bone and in the deep radial zone react in a different fashion to the applied mechanical load, and to the equivalent osmotic stress. At 12% from the bone, the quadrupolar splitting of the order of 1.3 kHz is no longer evident under the mechanical load, while under osmotic pressure, three pairs of satellites are resolved. This phenomenon persists to a lesser extent at 27% from the bone. At the surface (90% from the bone) both forms of load produce similar effect on the ^{2}H DQF spectra. Lai et al. (1998) have dealt with the question of the equivalence of osmotic and unidirectional mechanical loads. From their calculations they have concluded that the two types of loads may be equivalent in terms of matrix deformation only when the tissue is mechanically loaded in isotopic manner. Indeed our ^{2}H DQF results indicate that different modes of strain are produced by unidirectional mechanical load and osmotic pressure. The equivalence of osmotic and isotropic mechanical stress is still pending for experimental testing. In any case osmotic stress cannot serve as a suitable model for the physiologic condition, which is the unidirectional mechanical stress.

The effect of PG depletion on the ^{2}H quadrupolar splitting was found to be minimal. Studies of the orientation dependence of the splittings in PG-depleted cartilage have shown that the loss of GAG did not

affect the zonal architecture and the alignment of the collagen fibers. This is in full agreement with our previous conclusion that the anisotropic motion of the water molecules stems from their interaction with the oriented collagen fibers (Keinan-Adamsky et al. 2005) and not as a result of interaction with the negatively charged groups of the PG (Gründer et al. 1998). This is in line with the fact that also T_2 values in intact and depleted plugs are very similar (Kaufman et al. 1999; Regatte et al. 1999).

Relative to intact cartilage, significantly lower loads are needed in order to achieve the same degree of compression for the PG-depleted cartilage. Thus, we have compared the effect of 0.5 and 1.0 MPa on the intact plug to 0.12 and 0.2 MPa on the depleted plug, respectively. A significant difference was observed between the orientation dependence of the quadrupolar splittings for both intact and PG-depleted plugs and the same plugs subjected to osmotic stress. For the nonpressurized plugs, the orientation dependence experiments verify the fact that the collagen fibers in the calcified and radial zones are oriented parallel to the main axis of the plug.

Under osmotic stress, the value of the small (400 Hz) and intermediate (1,300 Hz) quadrupolar splittings for the intact and depleted plugs at 0.5 and 0.12 MPa, respectively, did not change with the rotation of the plugs with respect to the field. This result indicates that most of the collagen fibers are disordered after the application of osmotic stress. The residual dipolar interaction in this case, like in the case of nasal cartilage (Sharf et al. 1995) is a result of local order. These results constitute further support for the claim that osmotic stress cannot serve as a model for mechanical compression.

For the nonpressurized plugs, the quadrupolar splittings are largest near the bone and decrease toward the surface. For the plugs under osmotic stress, the splittings are rather uniform throughout. Since the size of the splitting is proportional to P_b, the fraction of bound water molecules. This indicates that larger amount of water is extruded from the surface and intermediate zones and a smaller amount from the deep radial zone.

Acknowledgments Support by the Israel Science Foundation of the Israel Academy of Sciences and Humanities is gratefully acknowledged.

References

Basser PJ, Schneiderman R, Bank RA, Wachtel E, Maroudas A (1998) Mechanical properties of the collagen network in human articular cartilage as measured by osmotic stress technique. Arch Biochem Biophys 351:207–219

Berendsen HJC (1962) Nuclear magnetic resonance study of collagen hydration. J Chem Phys 36:3297–3305

Danziger O, Shinar H, Eliav U, Navon G (1999) Differentiation between the action of different enzymes on the structure of articular cartilage using multiple quantum filtered ^{23}Na NMR. In: Proceedings of the 7th annual meeting ISMRM, Philadelphia, p 1522

Dehl RE, Hoeve CAJ (1969) Broad-line NMR study of H_2O and D_2O in collagen fibers. J Chem Phys 50:3245–3251

Eliav U, Navon G (1999) A study of dipolar interactions and dynamic processes of water molecules in tendon by ^{1}H and ^{2}H homonuclear and heteronuclear multiple-quantum-filtered NMR spectroscopy. J Magn Reson 137:295–310

Eliav U, Keinan-Adamsky K, Navon G (2003) A new method for suppressing the central transition in I = 3/2 NMR spectra with a demonstration for ^{23}Na in bovine articular cartilage. J Magn Reson 165:276–281

Gründer W, Wagner M, Werner A (1998) MR-microscopic visualization of anisotropic internal cartilage structures using the magic angle technique. Magn Reson Med 39:376–382

Gründer W, Kanowski M, Wagner M, Werner A (2000) Visualization of pressure distribution within loaded joint cartilage by application of angle-sensitive NMR microscopy. Magn Reson Med 43:884–891

Kääb MJ, Ito K, Clark JM, Nötzli HP (1998) Deformation of articular cartilage collagen structure under static and cyclic loading. J Orthop Res 16:743–751

Kaufman JH, Regatte RR, Bolinger L, Kneeland JB, Reddy R, Leigh JS (1999) A novel approach to observing articular cartilage deformation in vitro via magnetic resonance imaging. J Magn Reson Imaging 9:653–662

Keinan-Adamsky K, Shinar H, Navon G (2005) The effect of detachment of the articular cartilage from its calcified zone on the cartilage microstructure, assessed by ^{2}H-spectroscopic double quantum filtered MRI. J Orthop Res 23:109–117

Lai WM, Gu WY, Mow VC (1998) On the conditional equivalence of chemical loading and mechanical loading on articular cartilage. J Biomech 31:1181–1185

Lüsse S, Knauss R, Werner A, Grunder W, Arnold K (1995) Action of compression and cations on the proton and deuterium relaxation in cartilage. Magn Reson Med 33:483–489

Maroudas A, Bannon C (1981) Measurement of swelling pressure in cartilage and comparison with the osmotic pressure of constituent proteoglycans. Biorheology 18:619–632

Maroudas A, Ziv I, Weisman N, Venn M (1985) Studies of hydration and swelling pressure in normal and osteoarthritic cartilage. Biorheology 22:159–169

Migchelsen C, Berendsen HJC (1973) Proton exchange and molecular orientation of water in hydrated collagen fibers. An NMR study of H_2O and D_2O. J Chem Phys 59:296–305

Mow VC, Guo XE (2002) Mechano-electrochemical properties of articular cartilage: their inhomogeneities and anisotropies. Annu Rev Biomed Eng 4:175–209

Navon G, Shinar H, Eliav U, Seo Y (2001) Multiquantum filters and order in tissues. NMR Biomed 14:112–132

Nötzli H, Clark J (1997) Deformation of loaded articular cartilage prepared for scanning electron microscopy with rapid freezing and freeze-substitution fixation. J Orthop Res 15:76–86

Parsegian VA, Rand RP, Fuller NL, Rau DC (1986) Osmotic stress for the direct measurement of intermolecular forces. Methods Enzymol 127:400–416

Regatte RR, Kaufman JH, Noyszewski EA, Reddy R (1999) Sodium and proton MR properties of cartilage during compression. J Magn Reson Imaging 10:961–967

Reinert T, Reibetanz U, Vogt J, Butz T, Werner A, Gründer W (2001) Visualisation of collagen fibrils in joint cartilage using STIM. Nuclear Instrum Methods Phys Res Sect B: Beam Interact Mater Atoms 181:511–515

Rubenstein JD, Kim JK, Henkelman RM (1996) Effects of compression and recovery on bovine articular cartilage: appearance on MR images. Radiology 201:843–850

Schneiderman R, Keret D, Maroudas A (1986) Effects of mechanical and osmotic pressure on the rate of glycosaminoglycan synthesis in the human adult femoral head cartilage: an in vitro study. J Orthop Res 4:393–408

Sharf Y, Eliav U, Shinar H, Navon G (1995) Detection of anisotropy in cartilage using ^{2}H double-quantum-filtered NMR-spectroscopy. J Magn Reson Ser B 107:60–67

Shinar H, Navon G (2006) Multinuclear NMR and microscopic MRI studies of the articular cartilage nanostructure. NMR Biomed 19(7) (in press)

Shinar H, Seo Y, Navon G (1997) Discrimination between the different compartments in sciatic nerve by ^{2}H double-quantum-filtered NMR. J Magn Reson 129:98–104

Shinar H, Seo Y, Ikoma K, Kusaka Y, Eliav U, Navon G (2002) Mapping the fiber orientation in articular cartilage at rest and under pressure studied by ^{2}H double quantum filtered MRI. Magn Reson Med 48:322–330

Urban JP, Maroudas A, Bayliss MT, Dillon J (1979) Swelling pressures of proteoglycans at the concentrations found in cartilaginous tissues. Biorheology 16:447–464