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The interstital matrix creates a barrier to therapeutic agent delivery. To overcome this barrier we must study the matrix characteristics and the effect of manipulations and therapy. We proposed to establish the use of magnetic resonance (MR) methods for studying tumor interstitium. We wished to 1) determine by MR the collagen and glycosaminoglycan (GAG) concentration and diffusivity of small solutes in the interstitial matrix in breast cancer. 2) investigate the effect of antiestrogen therapy on the interstitial matrix composition in estrogen dependent breast cancer. 3) study the effect of enzymatic modulation and biological response modifiers on the interstitial matrix. In pilot studies, Sodium MR was found to have insufficient resolution for determining GAG in tumor tissue. We developed a proton MR technique involving the contrast agent Gd-DTPA^{2-} and showed that compared with sodium methods it was equally sensitive, and provided far higher spatial resolution. The latter allowed us to observe matrix accumulation in polymer scaffolds seeded with cells. We observed that the high cellularity of tumors affected the estimate of GAG content from the MR measurement. Subsequent theoretical analyses established a framework accounting for cellularity. We began to characterize diffusion imaging and its dependence on hydration in extracellular matrices.

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INTRODUCTION:

Chemotherapeutic treatment of metastatic breast cancer has been disappointing. One reason for treatment failure is the less than optimal delivery of the therapeutic agents through the interstitial matrix of the tumor (Jain, 1994). A quantitative understanding of the structure and function of the tumor interstitial compartments is a prerequisite for overcoming this barrier. To this end, we have been investigating various methods of studying the interstitial matrix and interstitial transport in tumors (Jain, 1994, Berk et al., 1997). In the past we have utilized magnetic resonance imaging (MRI) to study blood flow and metabolism in tumors (Eskey et al., 1992, 1993). In the current project our goal is to develop the use of MRI for measuring components of the interstitial matrix such as collagen, and glycosaminoglycan (GAG), as well as for measuring diffusion through the tumor interstitium. We have developed these methodologies in cartilage (Lesperance et al., 1992, Gray et al., 1995, and Burstein, et al., 1993) and are using this system to better understand technical difficulties encountered when imaging the tumor interstitial matrix and diffusivity. The hypothesis addressed by the proposed research is that the nondestructive and noninvasive measurements of tumor interstitium, as provided by MRI, will significantly enhance our ability to design and evaluate therapeutic interventions. The purpose of the proposed research was to initiate studies of the tumor interstitium by MRI, and to follow changes in the interstitium induced by biological response modifiers and the enzymatic degradation of interstitial matrix components. These interventions are designed to increase drug delivery to tumor cells. The technical objectives were:

1. To determine by MRI the collagen and glycosaminoglycan concentration and diffusivity of small solutes in the interstitial matrix of breast cancer.
2. To investigate the effect of antiestrogen therapy on the interstitial matrix composition and pathophysiology of estrogen dependent breast cancer xenografts.
3. To study the effect on the interstitial matrix of enzymatic modulation with trypsin, hyaluronidase, and collagenase, as well as with biological response modifiers.

In pilot experiments to prove the principle of MR imaging of the tumor interstitial matrix we used LS174T tumor xenografts after irradiation to optimize fibrosis. We had a good understanding of the interstitial matrix and physiological properties of this tumor from previous studies (Berk et al., 1997, Boucher et al., 1997). In these initial studies it was clear that the exact same methodologies used to image cartilage could not be used with good resolution and sensitivity in the tumor due to the high cellularity and relatively sparse interstitial matrix. Thus, further modifications of the imaging methodologies were explored in cartilage, a more homogeneous tissue that we could easily, and uniformly modulate in a predictable fashion. In addition, we developed a model in which chondrocytes are seeded on polymer scaffolds allowing us to image the accumulation of extracellular matricies in a highly cellular model. This model more closely resembles the characteristics of the tumor tissue and is easily modified to examine the effect of increasing cellularity on the imaging resolution and sensitivity. We first developed the use of Gd-DTPA^{2-} based imaging of GAG in lieu of the proposed sodium based imaging. High resolution and sensitivity was then possible. We then set out to determine how high cellularity effects the accuracy of GAG imaging, magnetization transfer (MT) based imaging of collagen, and MR measurements of diffusion in the cartilage systems so we could optimize tumor imaging of these parameters.

BODY:

Experimental Methods and Procedures:

Tumor model:
LS174T human colon adenocarcinoma xenografts were established in the hindleg of three male nude (immuno-deficient) mice (n=3). After two weeks the tumor had grown to approximately 180 mm^3 in size. At this time the three mice received a single radiation dose of 20 Gy, using a Cesium irradiator. One week after irradiation the mice were anesthetized for MRI imaging. Following imaging the tumors were excised and analyzed by MR spectroscopy (sodium MR to determine GAG, diffusion MR to evaluate extracellular matrix density, and magnetization transfer (MT) MR to evaluate collagen concentration and structure). Mammary carcinoma xenografts, estrogen withdrawal experiments, enzymatic degradation of the matrix, and the use of biological modifiers will be performed as describe in the original grant application.

Chondrocyte polymer scaffold co-culture:
Chondrocytes were seeded on disk-shaped fibrous polyglycolic acid scaffolds, and cultured for 1-6 weeks. After each week in culture, a disk was removed from culture and analyzed for GAG and diffusivity using the MRI methods described below.

Fluorescence recovery after photobleaching (FRAP):
FRAP was performed as described by Berk et al. (Berk et al., 1997). Briefly, HGL21 human glioblastoma, or HSTS26 human sarcoma xenografts are grown in a dorsal chamber of SCID (severe combined immunodeficient mice) for two weeks when the tumors measured 180 mm^3 in size. The glass coverslip was removed and the tumors were topically treated with either saline (n=3) or type 1 collagenase (n=4) before the IV infusion of rhodamine labeled IgG for FRAP analysis. FRAP measurements of interstitial mobility are performed 24 h after infusion to allow sufficient accumulation and quasi-equilibrium. An argon ion laser (model 2020; Spectra-Physics), tuned to a wavelength of 488 nm, is focused onto the tissue through the microscope (X20, NA 0.4) to form a circular spot with nominal diameter of 40 μm. After a 100 ms exposure to laser illumination, wide-field epifluoresence images are projected onto an ICCD (model 2400; Hamamatsu photonics, Hamamatsu City, Japan), digitized and stored at 5 images/second over 100 seconds. Photobleaching recoveries are quantified by spatial Fourier analysis.

MRI methods:
MRI experiments are performed on an 8.45 Tesla Bruker spectrometer (Bruker Instruments, Inc, Billerica, MA) equipped with a microimaging accessory. Sodium, magnetization transfer, and proton diffusion images were obtained in tumor tissue and in the cartilage models. Several standard solutions of known volume and concentration were utilized to ensure day-to-day consistency of the system and as standards with which to calibrate the system. These include 150 mM saline for the diffusion and sodium measurements, and a 2% agarose gel for the MT measurements (Gray et al., 1995).

Sodium-based GAG Imaging: Intracellular and extracellular sodium images were obtained with a standard spin echo sequence and projection reconstruction imaging sequences (Gewalt et al., 1993). A repetition time (TR) of 60 or 100 ms was used, along with a TE of 1 ms for spin echo imaging or 0.5 ms for projection reconstruction. The sodium image was scaled to intracellular or extracellular water content by dividing each voxel by the corresponding intracellular and extracellular voxel in a proton density. Finally, the signal was calibrated to a mM scale with the use of a 150 mM NaCl standard which was imaged along with the samples. Sodium concentrations were related to tissue

fixed charge density (FCD) through the use of Donnan theory, and FCD was used to estimate GAG concentration by assuming the net charge is all due to GAG, each disaccharide has 2 negative charges, and the molecular weight of the disaccharide is 502.5 g/mole (Gray et al, 1995).

Gd-DTPA^{2-} Based GAG Imaging: Gd-DTPA was used as an alternative to sodium for estimating GAG. Experiments involving Gd-DTPA involved equilibrating the samples in a saline solution containing 1 mM GdDTPA. For spectroscopy experiments, T1 was measured with an inversion recovery pulse sequence with 12 inversion delays ranging from 2 ms to 2.5 s. A reference value for T1 was obtained following equilibration in saline (without Gd-DTPA), using 12 inversion delays ranging from 0.02s to 10s. In imaging experiments, T1 was determined on a pixel by pixel basis using an inversion recovery spin echo pulse sequence, with a TR/TE of 200/23 ms. For both imaging and spectroscopy studies, Gd-DTPA concentraion was computed as $R^{-1}(T1_{Gd}^{-1} - T1^{-1})$, where the relaxivity $R = 4.5$ (mM-sec)$^{-1}$, $T1_{Gd}$ is the T1 in the presense of Gd-DTPA and T1 is T1 in the absence of Gd-DTPA. FCD was computed from the concentration of Gd-DTPA as previously described (Bashir et al., 1996).

Magnetization Transfer Imaging: MT images were obtained with a MT preparation pulse before ultrafast (turboFLASH) image sequence readout (Atkinson et al. 1990). An image (Ms) was obtained with a saturation pulse of 6 seconds, 6kHz off of the water resonance; at a power of 1.2 X 10^{-5} Tesla. A control (Mo) image was obtained by transmitting the saturation pulse far off resonance (1MHz) such that even the macromolecular protons are not saturated. An image of Ms/Mo was calculated by dividing the two images pixel by pixel.

Diffusion Weighted Images: The imaging version of the Stejskal Tanner pulsed gradient diffusion experiment is used as a model (Tanner, 1970). Diffusion gradient durations of 3 ms and diffusion times of 100 ms are used. Eight images are acquired with different diffusion gradient strengths of 0 to 20 G/cm. A calculated diffusion image is acquired, taking image gradients into account (Neeman, et al., 1991).

Results and Discussion:

1. In vivo imaging of a subcutaneous tumor

The first experimental goal was to use the MR techniques as developed for cartilage (a relatively pure interstitial matrix) to see how a tumor might appear by MR before and after irradiation. We felt that irradiation represents a treatment modality applicable to mammary carcinoma and that irradiation would lead to optimal fibrosis allowing us to determine if we could image changes in the interstitial matrix occurring with fibrosis. We were most familiar with the interstitial matrix of the LS174T human colon adenocarcinoma xenograft as well as the extent of fibrosis occurring in this tumor line after irradiation (Zaneti et al., 1997). We used this tumor model for the initial proof of principle. The tumor was visible but the resolution was poor and we could not distinguish cellular tissue from the matrix in these tumors. From this pilot data it became clear that the extracellular matrix density (especially the proteoglycan concentration) was relatively low and the cell density very high compared with cartilage, and that these differences would significantly affect the interpretation of the MR data. Therefore, the remainder of the year has been spent developing the groundwork for these techniques in a cellular model in which the exctracellular matrix composition and cellular composition could be varied over a wide range.

2. Measuring GAG content in tumor interstitial matrix

The MR measurement of GAG content is based on the knowledge that ions in the interstitial fluid will distribute in a manner commensurate with the amount of charge in the extracellular matrix. The charge on the extracellular matrix is due mainly to glycosaminoglycans. Therefore, by measuring the concentration of charged solute we can determine the GAG concentration. The method described in the original proposal involved sodium MR: i.e. measuring the interstitial sodium concentration and inferring the GAG concentration. The pilot data illustrated two important problems: One - the resolution necessary for sodium imaging demanded imaging times of at least 8 hours; Two - the effect of cells on the calculated GAG concentration needed to be theoretically and experimentally evaluated. These two problems were addressed as outlined below.

New MR method for measuring GAG

Sodium is a convenient charged solute because it is naturally present and can be measured by MR. Compared with proton (water) imaging, the signal is about two orders of magnitude smaller, with commensurate implications for imaging time and/or resolution. We therefore investigated a charged solute whose concentration could be measured by proton MR techniques. Specifically, we evaluated the use of the contrast agent gadopentetate dimeglumine (which upon dissociation yields the divalent anion, gadopentate (Gd-DTPA^{2-})). This FDA approved contrast agent is routinely used for MRI studies and does not appear to be cytotoxic. The T1 relaxation of protons is affected by and can be quantitatively related to the concentration of Gd-DTPA^{2-}. We therefore tested the conjecture that measurement of [Gd-DTPA^{2-}] could yield a measurement of [GAG] which was equivalent to the estimate of [GAG] obtained by MR measurements of [Na^+]. By manipulating a tissue with very few cells (cartilage) to achieve a wide range of GAG concentration, we found that [GAG] as estimated from Na MR was very highly correlated to that measured by Gd-DTPA-MR (Figure 1).

We next explored whether the Gd-DTPA-based approach to measuring GAG was likely to be sensitive to changes in GAG content which might occur in a developing tumor. Specifically, we seeded chondrocytes on disk-shaped fibrous polyglycolic acid scaffolds, and cultured them for 1-6 weeks. Chondrocytes were selected because of their ability to rapidly synthesize an extracellular matrix. (Tumor cells can also be used, but the experiment was estimated to require 12 -18 weeks. In view of the objective of the particular study to examine whether we could use the Gd-DTPA-based method to observe the formation of a matrix, we elected to use a system which provided the most rapid accumulation of matrix.) After each week in culture, a disk was removed from culture and analyzed for GAG and diffusivity. The accumulation of matrix was clearly observed to progress with time, with accumulation being more rapid at the edges of the disk (e.g. Figure 2). The diffusion data, not shown, had very similar profiles, with the apparent diffusion coefficient of water decreasing with time, and with the edges decreasing more rapidly than the center of the disk.

The significance of these results is that we now have a proton-MR method for measuring tissue GAG concentration, a method provides resolution commensurate with standard proton imaging and which is able to resolve GAG differences expected to occur in tumor interstitium. Furthermore, we were able to observe consistent changes in the diffusivity of water as the extracellular matrix "matured."

Consequences of having a cellular tissue for measuring GAG content of extracellular matrix

The calculation of GAG concentration from MR measurements of either Na^+ or Gd-DTPA^{2-} uses a one compartment Donnan equilibrium model and thereby essentially assumes that the charge on the GAG is uniformly distributed throughout the tissue. Cells comprise a compartment in tissue which excludes the GAG. In the case of Na^+, the

intracellular concentration is relatively constant at 10 mM, and independent of [GAG]; for Gd-DTPA^{2-} the intracellular concentration is expected to be zero. From a theoretical perspective, we assume the content of Na^+ or Gd-DTPA^{2-} in the tissue is determined by the weighted average of their concentration in the intracellular and extracellular compartments. The concentration in the extracellular compartment is presumed to vary with GAG content in accordance with a Donnan distribution. The GAG content predicted by MR (in the simplest analysis) ignores the presence of two compartments and assumes that the content is uniformly distributed throughout the tissue. The consequences of this analysis are that the prediction of GAG concentration become very imprecise as the cell density increases (Figure 3). However, when comparing samples of similar cell density, the method should be sensitive to differences in GAG content (Figure 3). One way to correct for the precision problem is to measure cell volume. We have started to work in that direction. Studies are ongoing to experimentally verify the predictions of the theoretical modeling. If validated, then we can use this technique in vivo to evaluate changes in GAG concentration in tumors as originally planned for the sodium technique.

3. Measuring Diffusion and MT (Magnetization Transfer) in tumor interstitial matrix

To determine the sensitivity of diffusion and MT to changes in extracellular matrix density, we investigated a pure extracellular matrix by depleting cartilage of GAG, and then controlling the hydration. The sensitivity of these measures to matrix hydration is illustrated in figure 4. In terms of their utility in evaluating tumors, it appears that MT may be sensitive to changes in collagen content. The signal to noise in the diffusion measurement limits its sensitivity. The next step for each is to evaluate whether the presence of a large number of cells confounds the measurement so as to make it uninterpretable.

4. In vivo measurement of interstitial diffusion with fluorescence recovery after photobleaching (FRAP).

To prove the principle that the enzymatic degradation of the interstitial matrix effects diffusivity and that there is the potential to image this change using MRI we used the fluorescence photobleaching (FRAP) method to measure the interstitial diffusion rates of IgG in U87, human glioblastoma, and HSTS26, human sarcoma, xenografted tumors. Diffusion coefficients were calculated by comparing the mobility of the monoclonal antibody in collagenase and saline treated tumors. An important finding of this study was that the interstitial diffusion coefficient of nonspecific IgG (MW 150 kDa) is 2-fold higher in both tumor types when treated with collagenase (Figure 5).

Recommendations in Relation to the Statement of Work:

As expressed in the statement of work, tumors were grown for the initial baseline measurements of diffusivity, collagen, and GAG content. To demonstrate the initial proof of principle we obtained the first MR images on the LS174T colon adenocarcinoma after irradiation. We felt that irradiation represents a treatment modality applicable to mammary carcinoma and that irradiation would lead to optimal fibrosis allowing us to determine if we could image changes in the interstitial matrix occurring with fibrosis. We were most familiar with the interstitial matrix of the LS174T human colon adenocarcinoma xenograft as well as the extent of fibrosis occurring in this tumor line after radiation. It was clear from this pilot study that because of low levels of extracellular components in this highly cellular tissue the resolution would be quite low using sodium imaging MR. To solve this problem Gd-DTPA^{2-} based imaging was developed in cartilage, a relatively acellular tissue in which the exctracellular matrix composition could be enzymatically modulated over a wide range. The signal to noise ratio for MT, which is sensitive to changes in collagen

content, and diffusion measurements were also to high in the tumor tissue limiting the sensitivity. Subsequently, cartilage cells were seeded in a polymer matrix and cultured so that the cells could synthesize an extracellular matrix. Both the Gd-DTPA-based technique and diffusion MR were sensitive to changes in extracellular matrix accumulation. Theoretical analyses revealed approaches for accounting for the high cellularity typically seen in tumors. Thus, the sensitivity of these measurements as assessed theoretically and experimentally in extracellular matrices (derived from cartilage or from polymers) with varying hydration and cellularity appears to now be adequate to evaluate tumors in vitro. As stated, we have established tumors for the enzymatic modulation of the interstitial matrix, using type I collagenase, and have demonstrated an increase in the diffusion of antibodies, using FRAP. This data proves the principle that we can use enzymes to modulate the diffusion of macromolecules and we should be able to use MR to image these changes. A manuscript has been submitted describing this baseline data in cartilage. Thus, in the first year of this work we have addressed Task 2, Task 3, Task 4, Task 5, and Task 6. We have done initial work in tumors and required a more defined, system that could be easily modulated. To this end, we have completed much of the ground work for this project using extracellular matrices derived from cartilage. As such, we have begun to address objectives 1, 2, and 3. The proposed estrogen dependent tumor models are routinely used in our laboratory and available for the MR studies in the upcoming year, once the system has been fully developed using the cartilage model. In light of the initial difficulties in establishing the use of MR to measure collagen, GAG, and diffusivity in tumor tissue we propose the following statement of work for the upcoming year:

Task 1: Month 1-2: Grow tumors (6 of each tumor line, MCF-7, Zr-75, and MDA-MB-231, for each treatment group) for Gd-DTPA^{2-} based MR imaging of GAG.

Task 2: Month 2-3: MRI of GAG using Gd-DTPA^{2} in the breast carcinoma xenografts as well as in polymer scafolds in which varrying densities of tumor cells are grown.

Task 3: Month 2-4: MRI of GAG before and after estrogen withdrawal and irradiation (allowing optimal fibrosis) in tumor xenografts.

Task 4: Month 1-4: Understand how cell volume effects the precision of GAG imaging and validate the systems accordingly.

Task 5: Month 6: Write manuscript on MRI imaging of GAG and matrix in mammary carcinoma and the effect of treatment.

Task 6: Month 4-9: Define the use of MT for measuring collagen content and MR for diffusion measurements in cartilage, cell polymer scafold co-cultures and tumor tissue.

Task 7: Month 9-12: Measure MT, Diffusion, and GAG in tumors treated with intratumoral enzymatic treatment, as well as in tumors treated with IV biological response modifiers .

Task 8: Month 12: Write manuscript about the enzymatic and biological modifications of tumor interstitial matrix constituents.

FIGURES

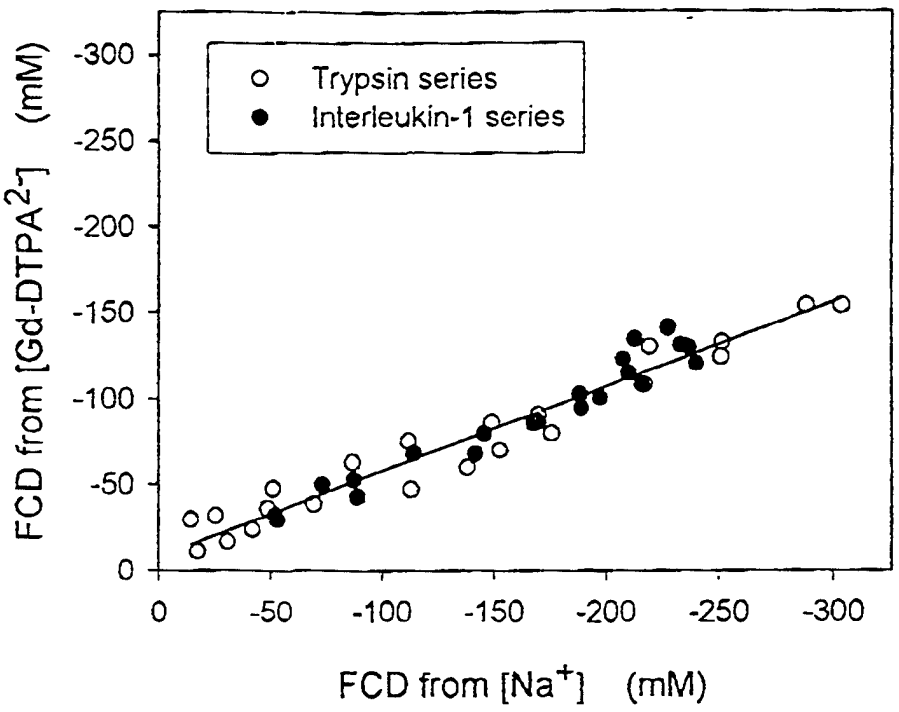


Figure 1: The density of fixed charge (FCD) extracellular matrix containing less than 10% cells by volume was determined using the previously validated sodium MR method, and compared with the new Gd-DTPA-based method. The FCD can be directly related to tissue GAG concentration. Data for a variety of extracellular matrix model systems (solutions of GAG, solutions of GAG and collagen, harvested extracellular matrix with natural or enzyme-induced alterations in tissue GAG content) from human and bovine sources appears very similar. The correlation coefficient between the two methods is consistently greater than 0.95. These data suggest that, at least for extracellular matrices containing relatively few cells, the Gd-DTPA-based method provides results comparable to the sodium-based method.

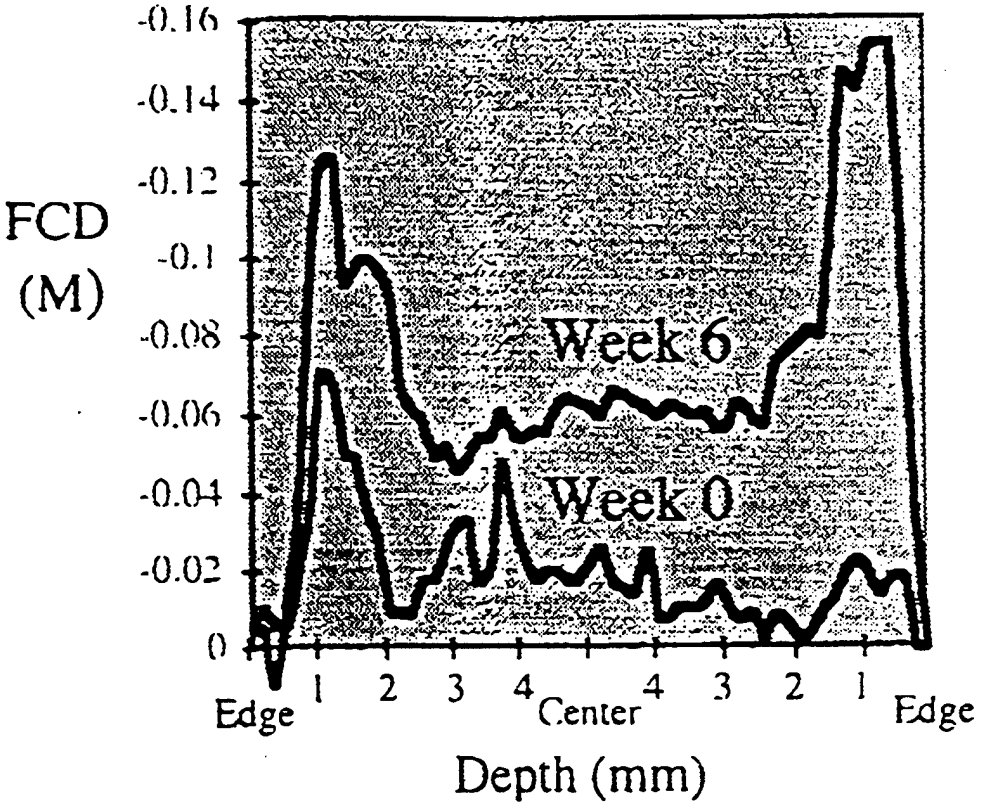


Figure 2: Profiles across the diameter of cell-laden polygalactic acid scaffolds showing the accumulation of GAG. These tissue constructs have a range of GAG expected to be found in tumors (though accumulating more rapidly than expected for tumors). The resolution of the MR measurement was approximately 100 μm.

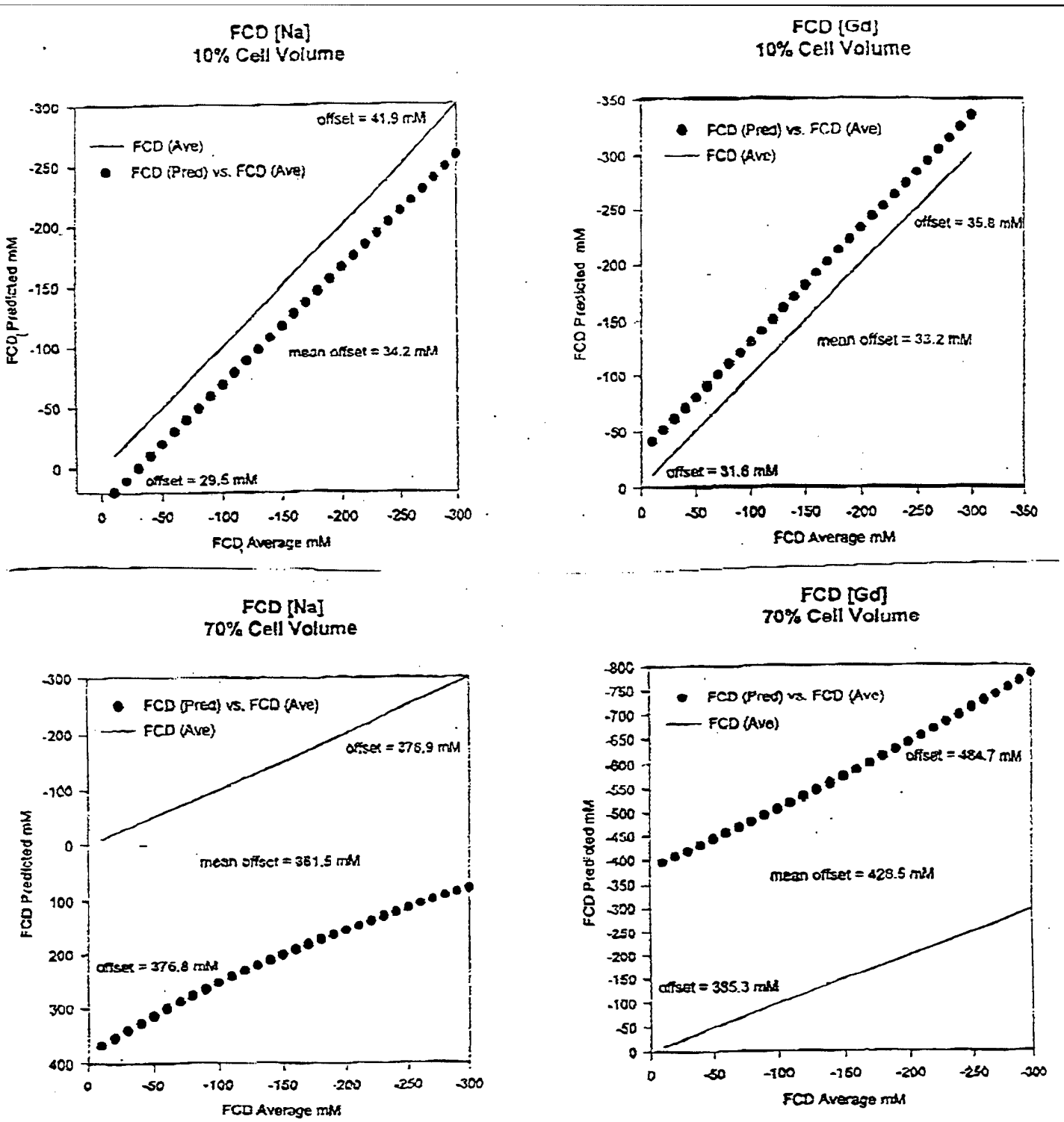
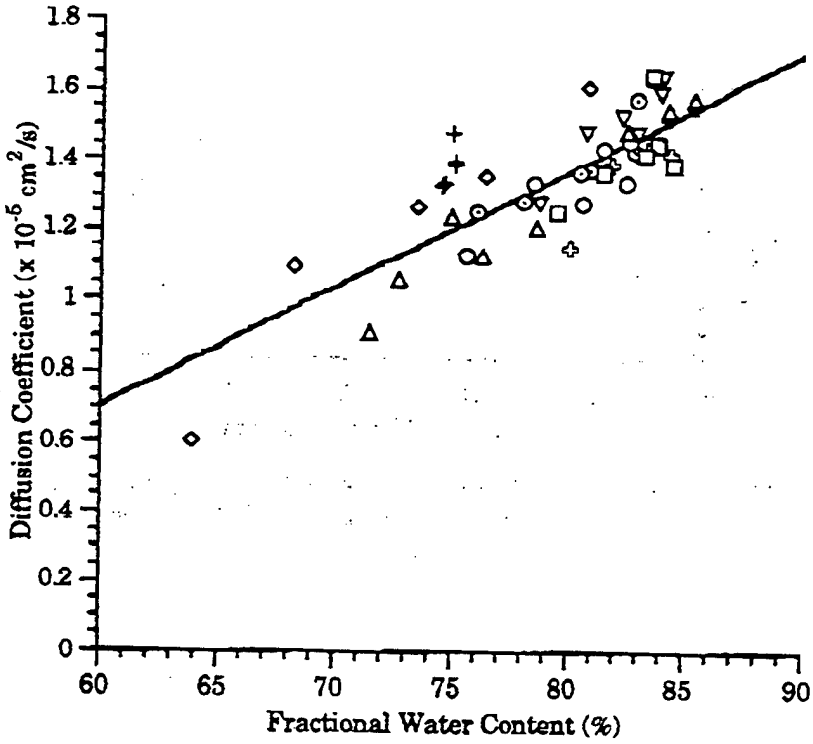


Figure 3: Four panels illustrating the theoretically-determined impact of cells on the sodium and Gd-DTPA-based approaches to measuring tissue GAG content (FCD). The left and right hand sides show the results for the sodium-based and Gd-DTPA-based methods, respectively. The top and bottom panels show the results for 10% and 70% cell volumes, respectively. In each plot the actual FCD (averaged across cells and matrix) is plotted on the x-axis, and the FCD which would be predicted by the MR method is plotted on the y-axis. The solid line shows the unity line; the dots show the results of the calculation. In each case, it is clear that the prediction does not precisely match the actual FCD, and the difference between the prediction and actual FCD is greater for higher cell densities. At any given cell density the slop of the relationship between actual FCD and predicted FCD is nearly one, suggesting that while the prediction may not be accurate for determining the absolute FCD, it is likely to be accurate in determining changes in FCD.



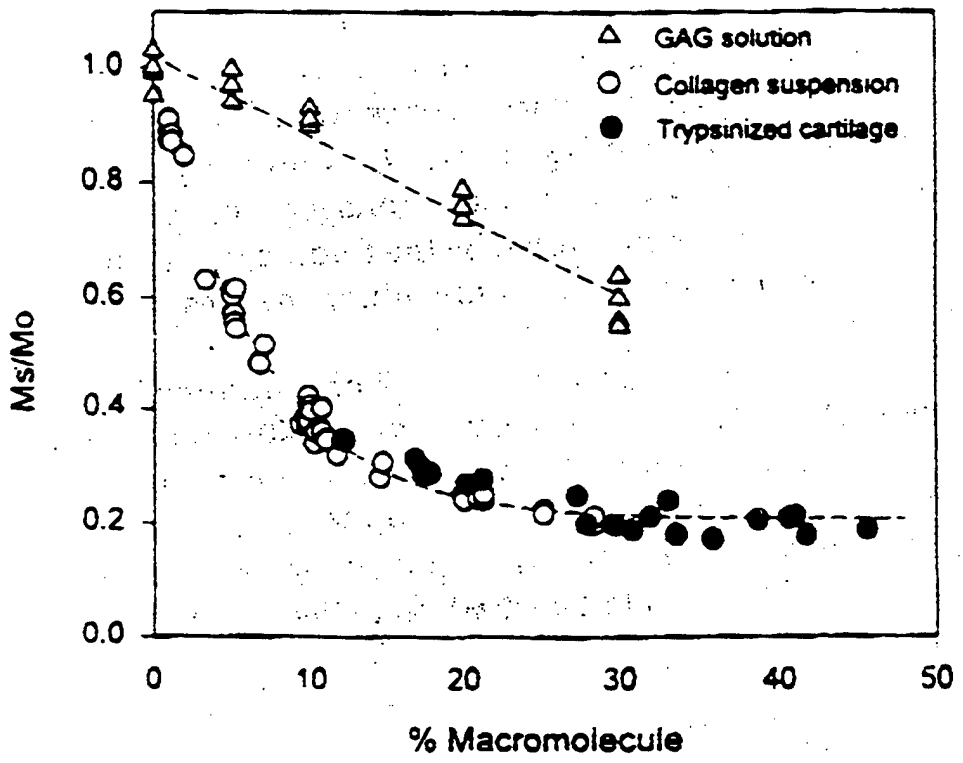


Figure 4: The sensitivity of MR measurements of diffusion and magnetization transfer (MT) to differences in water content were examined in a pure collagen matrix subjected to varying degrees of compression. Both parameters depend on water content, but are relatively insensitive to subtle changes in water content at water contents of 70-80%. They are, however, more sensitive to changes between 85-95% water content, a fact that might be important if that situation should occur in a region of a growing tumor.

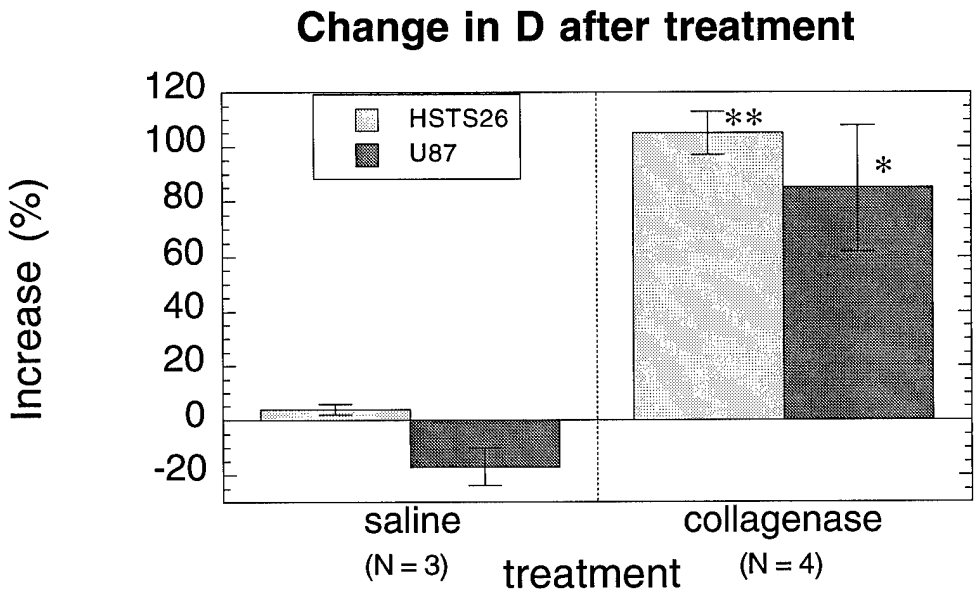


Figure 5. Relative interstitial diffusion coefficients (D) of proteins in two human tumor xenografts. Fluorescence recovery after photobleaching (FRAP) was used to measure D for IgG. Treatment of human sarcoma HSTS 26 and the human glioblastoma U87 with collagenase increased the mobility of IgG 2-fold. Paired-t-test **$p<0.0001$, *$p<0.01$.

CONCLUSIONS:

The purpose of the proposed research is to initiate studies of tumor interstitium by MRI, and to follow changes in the interstitium induced by interventions designed to increase drug delivery to tumor cells. The technical objectives are:

1. To determine by MRI the collagen and glycosaminoglycan concentration and diffusivity of small solutes in the interstitial matrix of breast cancer.

In initial pilot studies, designed to optimize fibrosis and interstitial matrix components, we were unable to image GAG using sodium based imaging. The resolution and sensitivity were to low to obtain meaningful data due to the high cellular density and relatively low expression of interstitial matrix components. Similar difficulties were encountered when using MT imaging and diffusion imaging.

Here we developed a new method for investigating GAG content that should offer a resolution two orders of magnitude better than that provided by the sodium method originally proposed. Moreover, we demonstrated that the new GdDTPA-based method could be used to track changes in GAG in a developing interstitium. While a high cell density, as would be expected in tumors, will significantly affect the accuracy of the method, there is a high sensitivity to changes in GAG content (and there are approaches we haven't yet investigated which could improve the accuracy). Having laid this groundwork, we are now in a much better position to reexamine tumors in vivo. Conditions are being perfected in our established cartilage model so we could optimize tumor imaging. This model allows us to predictably modulate relative cellular content, matrix content, and hydration to define technological paramters useful for tumor imaging. We are investigating modulations in the GdDTPA-based GAG imaging to account for high cellularity based on cell volume. We are also investigating problems encountered while imaging tumor interstitial diffusion as well as collagen with MT based imaging. We have demonstrated as proof of principle that we can modulate the diffusion of IgG through the interstitium with type I collagenase, using FRAP, and we should be able to image these changes with MRI methodologies.

We have gained an understanding of the system modulations needed to image highly cellular tissues to determine GAG content, collagen, and diffusion and will begin to re-image tumors as proposed. Once the baseline imaging of tumor structure and physiology are complete we will pursue the following technical objectives as originally stated:

1. To investigate the effect of antiestrogen therapy on the interstitial matrix composition and pathophysiology of estrogen dependent breast cancer xenografts.
2. To study the effect on the interstitial matrix of enzymatic modulation with trypsin, hyaluronidase, and collagenase, as well as biological response modifiers.

REFERENCES

Atkinson, D.J., Burstein, D., Edelman, R.R. First-pass cardiac perfusion: Evaluation with Ultra-fast MR imaging. Radiology 174:757-762, 1990.

Bashir, A., Gray, M., Burstein, D. Gd-DTPA as a measure of cartilage degradation. Mag. Reson. Med. 36:665-673, 1996.

Berk, D., Yuan, F., Leunig, M., Jain R.K. Direct in vivo measurements of targeted binding in tumor xenograft. PNAS. 94:1785-1790, 1997.

Boucher, Y., Brekken, C., Netti, P., Baxter, L., Jain, R.K. Intratumoral infusion of fluid, estimation of hydraulic conductivity and compliance: Implications for delivery of therapeutic agents, submitted.

Burstein, D., Gray, M.L., Hartman, A.L., Gipe, R., Foy, B.D. Diffusion of small solutes in cartilage as measured by nuclear magnetic resonance (NMR) spectroscopy and imaging. J. Orthopaed. Res. 11:465-478, 1993.

Eskey, C.J., Koretsky, A.P. Domach, M.M., Jain, R.K., 2H-nuclear magnetic resonance imaging of tumor blood flow: spatial and temporal heterogeneity in a tissue-isolated mammary adenocarcinoma. Cancer Res. 52:6010-6019, 1992.

Eskey, C.J., Koretsky, A.P., Domach, M.M., Jain, R.K. Role of oxygen vs. glucose in energy metabolism in a mammary carcinoma perfused ex vivo: direct measurement by 31P NMR. PNAS USA 90:2646-2650, 1993.

Gewalt, S.L., Glover, G.H., Hedlund, L.W., Cofer, G.P., MacFall, J.R., Johnson, G.A. MR microscopy of the rat lung using projection reconstruction. Magn. Reson. Med. 29:99-106, 1993.

Gray, M.L., Burstein, D., Lesperance, L.M., Gehrke, L. Magnetization transfer in cartilage and its constituent macromolecules. Magn. Reson. Med., 34:319-325, 1995.

Jain, R.K. Barriers to drug delivery in solid tumors. Scientific American 271:58-65, 1994.

Lesperance, L.M., Gray, M.L., Busrstein, D. Determination of fixed charge density in cartliage using nuclear magnetic resonance. J. Orthopaed. Res. 10:1-13. 1992.
Tanner, J.E. Use of stimulated echo in NMR diffusion studies. J. Chem. Phys. 52:2523-2526.

Neeman M., Freyer, J.P., Sillerud, L.O., A simple method for obtaining cross-term-free images for diffusion anisotropy studies in NMR microimaging. Magn. Reson. Med. 21:138-143, 1991.

Zaneti, C. A., Boucher, Y., Rosenstein, M., Turner, D., Bloomer, W.D., Watkins, S., Jain, R. K. Change in the fluid transport and interstitial matrix of tumors after irradiation, submitted.

ANIMAL USE REPORTING

Activity Name & Address: Radiation Oncology, MGH, 100 Blossom St
Boston, MA 02114

Contract number: DAMD17-96-1-6282

Animal Type Genus/Species	Animals Purchased or Bred	Animals Used	USDA Pain Column C	USDA Pain Column D	USDA Pain Column E	AAALAC Accreditation
nu/nu/sed nude mice	3	3				yes
scid/sed scid mice	7	7				yes

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