AD_____

GRANT NUMBER DAMD17-96-1-6282

TITLE: MRS and MRI Studies of the Structure and Function of Tumor Interstitial Matrix

PRINCIPAL INVESTIGATOR: Rakesh K. Jain, Ph.D.

CONTRACTING ORGANIZATION: Massachusetts General Hospital
Boston, Massachusetts 02114

REPORT DATE: October 1998

TYPE OF REPORT: Final

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 1998	Final (3 Sep 96 - 2 Sep 98)

4. TITLE AND SUBTITLE
MRS and MRI Studies of the Structure and Function of Tumor Interstitial Matrix

5. FUNDING NUMBERS
DAMD17-96-1-6282

6. AUTHOR(S)
Rakesh K. Jain, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Massachusetts General Hospital
Boston, MA 02114

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200)*

The interstitial 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. Our goals were to determine 1) by MR the collagen and glycosaminoglycan (GAG) concentration and diffusivity of small solutes in the interstitial matrix of mammary tumors, 2) the effect of antioestrogen therapy on the interstitial matrix in estrogen dependent mammary tumors and 3) the effect of enzymatic modulation and biological response modifiers on the interstitial matrix. In our initial studies the resolution of sodium MR was insufficient for estimating GAGs in tumor tissue because of high cellularity. We developed a proton MR technique involving the contrast agent Gd-DTPA^{2-} and found that compared with sodium methods it was equally sensitive with a significantly improved resolution. However, in *in vitro* matrices with high cell numbers the GAG estimates were not precise. Similarly, in tumors the lack of sensitivity of MR imaging prevented accurate measurements of GAG and collagen levels.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Breast Cancer	19
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRA
Unclassified	Unclassified	Unclassified	Unlimited

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

____ Where copyrighted material is quoted, permission has been obtained to use such material.

____ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

RKJ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

RKJ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

____ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

____ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

____ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

____ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Robert K. Jan 02/09/99

PI - Signature Date

TABLE OF CONTENTS:

INTRODUCTION:

Chemotherapeutic treatment of metastatic breast cancer has been disappointing. One reason for treatment failure is the less than optimal delivery of therapeutic agents through the interstitial matrix of tumors (Jain et al., 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; Boucher et al., 1998; Netti et al., 1999). In the past we have utilized magnetic resonance imaging (MRI) to study blood flow and metabolism in tumors (Eskey, 1992, 1993). In the current project our goal was to develop the use of MRI for measuring interstitial matrix constituents 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 in the 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 degrading enzymes. These interventions were 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.

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 was performed as described 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, a human glioblastoma (U87), a human sarcoma (HSTS-26T), a human colon adenocarcinoma (LS174T) and a murine mammary carcinoma (MCa-IV) were grown in the transparent dorsal skin fold chamber of SCID (severe combined immunodeficient) mice. FRAP measurements of interstitial mobility were performed 24 h after the intravenous injection of non-specific IgGs or albumin to allow sufficient accumulation and quasi-equilibrium of the proteins in the interstitial space. An argon ion laser (model 2020; Spectra-Physics), tuned to a wavelength of 488 nm, was focused on the tissue through the microscope (X20, NA 0.4) to form a circular spot with a nominal diameter of 40 μm. After a 100 ms exposure to laser illumination, wide-field epifluoresence images were projected onto an ICCD (model 2400; Hamamatsu photonics, Hamamatsu City, Japan), digitized and stored at 5 images/second over 100 seconds. Photobleaching recoveries were quantified by spatial fourier analysis. In other experiments, the glass coverslip covering the tumor was removed and saline or collagenase was topically applied on the tumor surface before the intravenous infusion of rhodamine labeled IgG for FRAP analysis.

MRI methods:
MRI experiments were 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 cartilage. 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 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 $(mM\text{-}sec)^{-1}$, $T1_{Gd}$ 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 of 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 were 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 was used as a model (Tanner, 1970). Diffusion gradient durations of 3 ms and diffusion times of 100 ms were used. Eight images were acquired with different diffusion gradient strengths of 0 to 20 G/cm. A calculated diffusion image was 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 sodium MR to determine GAG levels in tumor tissue. We felt that irradiation represented 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 (Znati et al., 1996). 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, we developed another MR approach using the contrast agent Gd-DTPA^2. (see section # 2, *New MR method for measuring GAG*) In comparison to the sodium method Gd-DTPA^2 was equally sensitive with an increased spatial resolution. To test the Gd-DTPA^2 approach in tumors we used the mammary carcinoma Zr-75. Zr-75 is a cellular tumor which under natural conditions has a greater amount of extracellular matrix has compared to LS174T tumors. The cellular volume in Zr75 was also too high, and prevented the precise imaging of interstitial matrix constituents. Thus it appears that MR imaging is not sensitive enough for quantitative measurements of extracellular matrix constituents.

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 of the extracellular matrix is due predominanatly to GAGs. Therefore, by measuring the concentration of charged solute, GAG concentration can be estimated. 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, the method provides resolution commensurate with standard proton imaging and 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; panel a). However, when comparing samples of similar cell density, the method should be sensitive to

differences in GAG content (Figure 3; panel b). One way to correct for the precision problem is to measure cell volume.

3. 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.

4. Fibrillar collagen limits diffusiove transport and bulk flow in tumors

In complimentary studies we have characterized with other technologies how the composition of the tumor interstitial matrix influences the diffusion and bulk flow of large molecules (Boucher et al., 1998; Netti et al., 1999). We speculated that fibrillar collagen limits the penetration of large molecules in tumors. The hypothesis was tested by measuring with FRAP the diffusion coefficient of bovine serum albumin (BSA) and non-specific IgGs in tumors with different collagen contents. In comparison to diffusion in solution, the diffusion of BSA and IgG in tumors was restricted (Fig. 5). The mobility of BSA was similar for the the 4 tumor types, however the diffusion coefficient of the larger IgG molecule was significantly restricted in HSTS-26T and U87 as compared to MCaIV and LS174T. These findings indicate that the pore size (available space) is larger than the size of albumin in the 4 tumors studied, and that in HSTS-26T and U87 the pore size is comparable to the IgG dimensions. The diffusion coefficient measurements were correlated with the total collagen content and glycosaminoglycan content of the tumor interstitial matrix. There was no significant difference in total GAG content, hyaluronic acid or sulfated GAG contents between the 4 tumor types (Fig. 6). The low diffusion of IgG in HSTS-26T and U87 was associated with high collagen levels, whereas MCaIV and LS174T with faster diffusion coefficients had lower collagen levels.

To further characterize the significance of fibrillar collagen in impeding interstitial transport, collagen was degraded with the enzyme collagenase. The interstitial diffusion coefficient of IgG was measured before and after collagenase treatment (Fig. 7). Collagenase treatment caused a significant increase in IgG mobility (approximately twofold) in HSTS-26T and U87 tumors, whereas saline treatment had no effect.

Intratumoral injections are used to deliver therapeutically relevant molecules (e.g. gene products) in tumors. We have developed a new in vivo technique to measure hydraulic conductivity (e.g. measurement of ease of fluid movement) a parameter which influences the distribution of injected molecules (Boucher et al., 1998). The direct infusion at low flow rates (0.5, 1.0 or 1.4 μl/min) revealed an important qualitative difference in the spatial pattern of Evans-blue albumin deposited in the tumors. In LS174T, labeled albumin spread radially and uniformly outward from the tip of the infusion needle. In HSTS-26T however, the protein solution flowed along one or more preferential pathways, often pooling in a distant necrotic region as shown in Fig. 8. The heterogeneous distribution of albumin in HSTS-26T is also consistent with the relatively low hydraulic conductivity and high collagen content in that tumor.

FIGURES

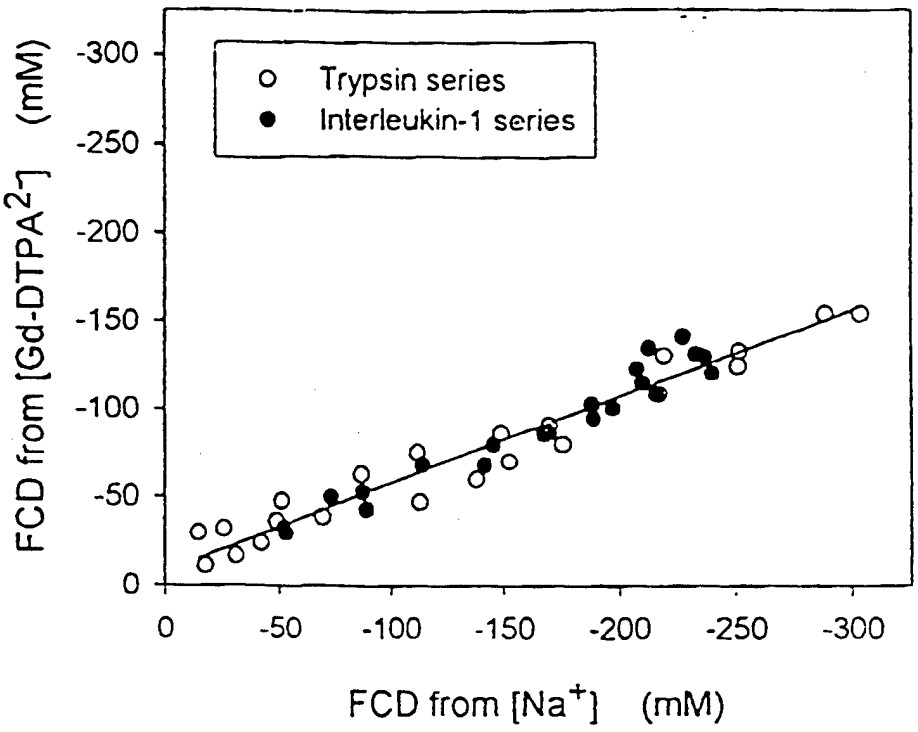


Figure 1: The density of fixed charge (FCD) extracellular matrix containing less than 10% cells by volume was determined using the previously validated sodium MR method, and compared with the new Gd-DTPA-based method. The FCD can be directly related to tissue GAG concentration. Data for a variety of 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.

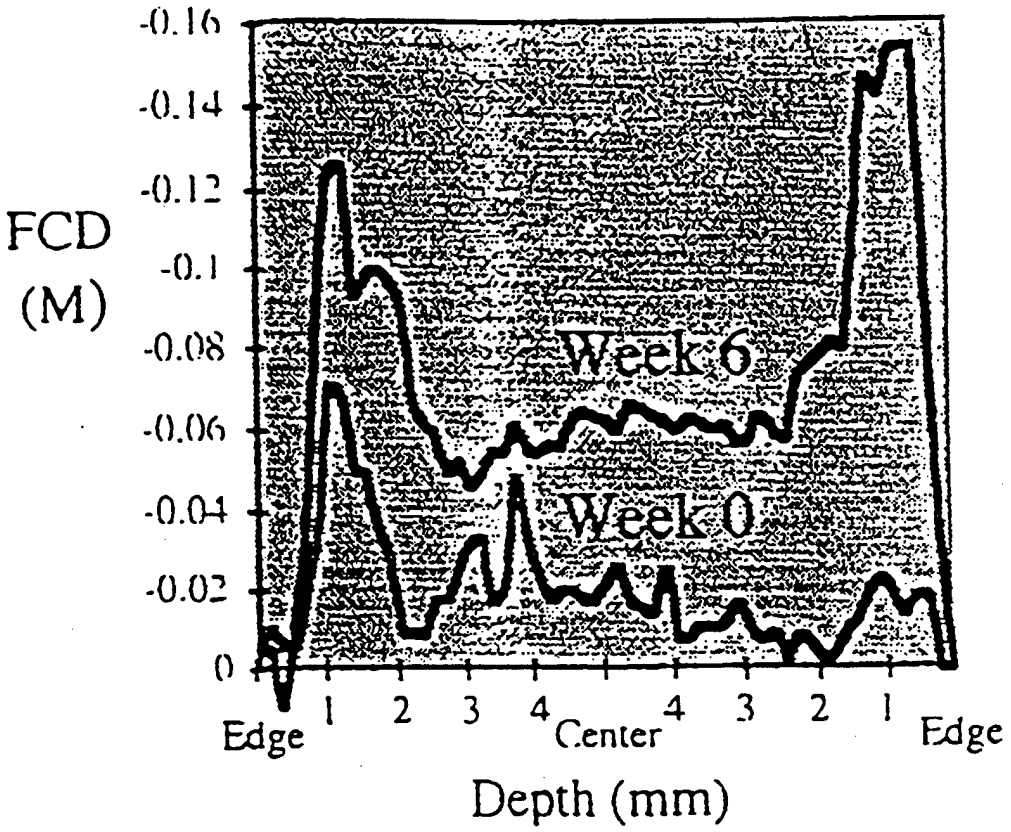


Figure 2: Profiles across the diameter of cell-laden polygalactic acid scaffolds showing the accumulation of GAG. These tissue constructs have a range of GAG expected to be found in tumors (though accumulating more rapidly than expected for tumors). The resolution of the MR measurement was approximately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

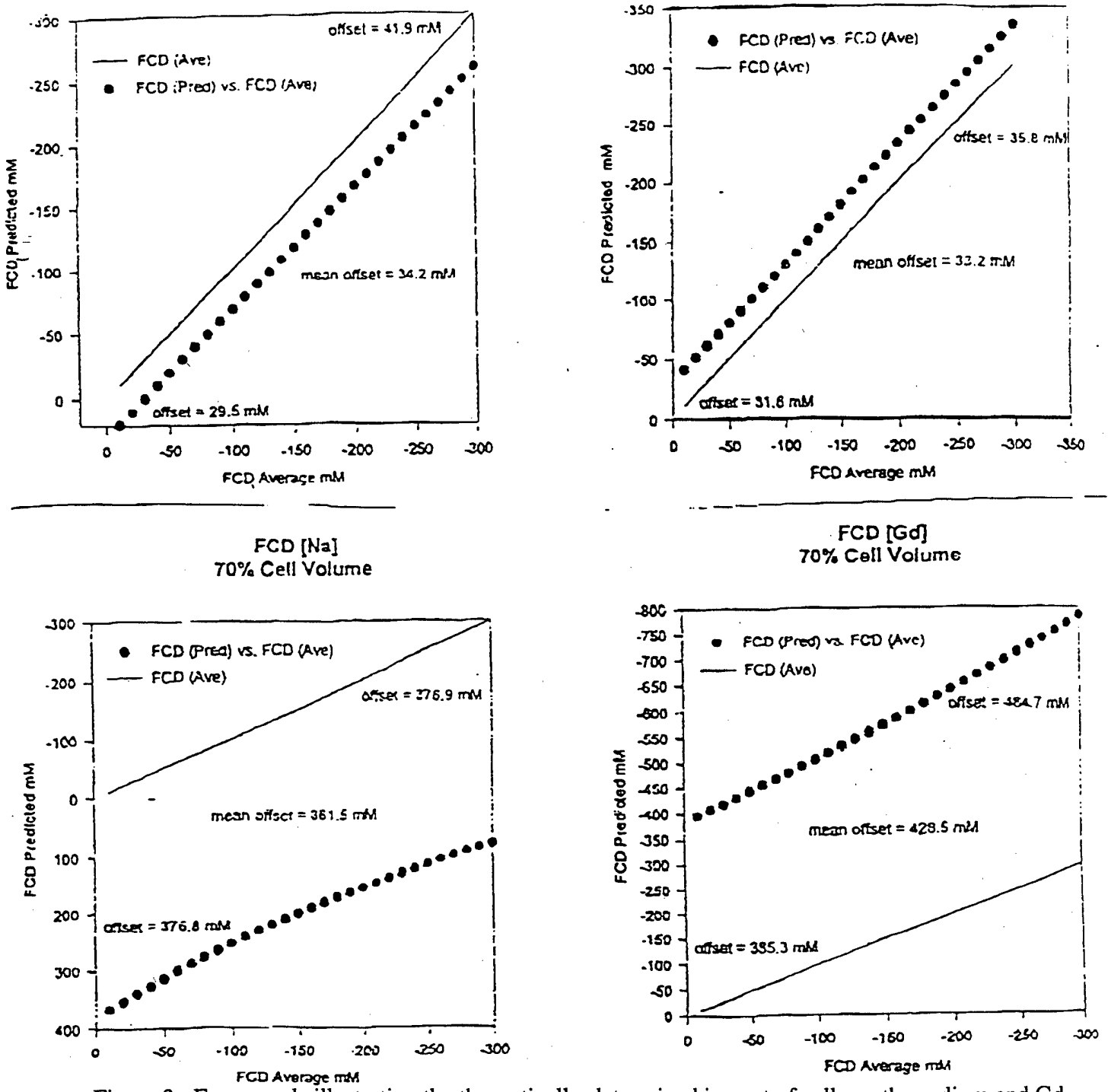
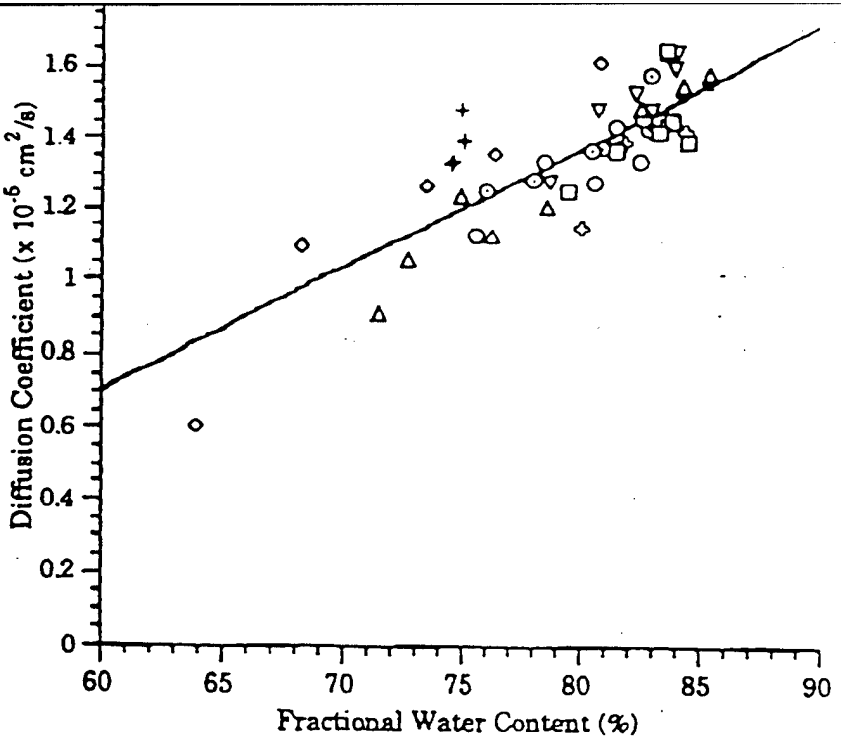


Figure 3: Four panels illustrating the theoretically-determined impact of cells on the sodium and Gd-DTPA-based approaches to measuring tissue GAG content (FCD). The left and right hand sides show the results for the sodium-based and Gd-DTPA-based methods, respectively. The top and bottom panels show 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.



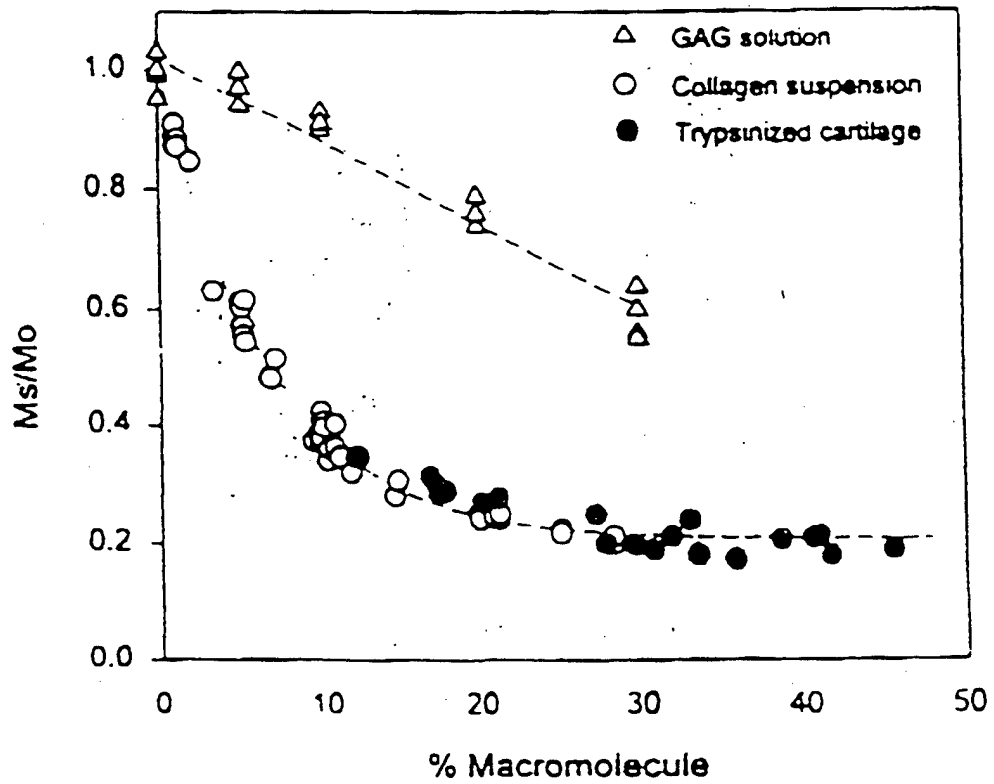
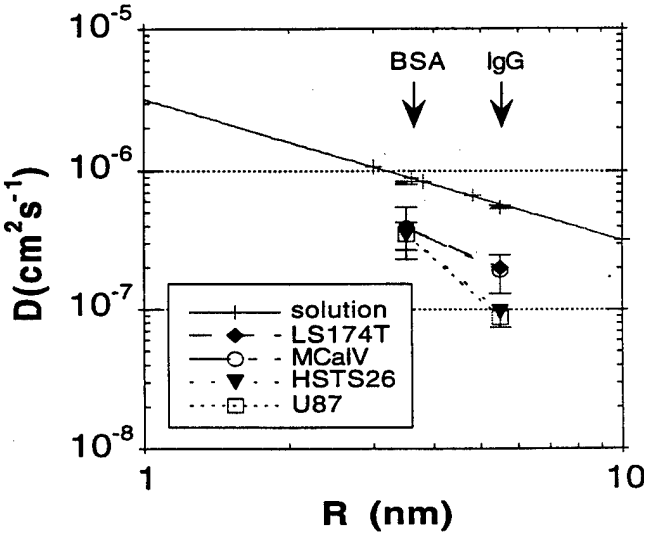


Figure 4: The sensitivity of MR measurements of diffusion and magnetization transfer (MT) to differences in water content were examined in a pure collagen matrix subjected to varying degrees of compression. Both parameters depend on water content, but are relatively insensitive to subtle changes in water 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. **Interstitial protein diffusion coefficients in three human tumor xenografts and a mouse allograft.** The diffusion coefficient was measured by FRAP. Diffusion coefficients in saline solution (+) are shown for lactalbumin (14 kDa) ovalbumin (45 kDa), albumin (70 kDa) and IgG (150 kDa). Measurements were performed in N≥5 tumors with n≥50 measurements for each molecule and tumor type. No difference in BSA diffusion was found between the 4 tumor types. Note the significantly lower diffusion coefficient of IgG in U87 and HST-S26T human sarcoma as compared to LS174T and MCaIV ($p<0.001$).



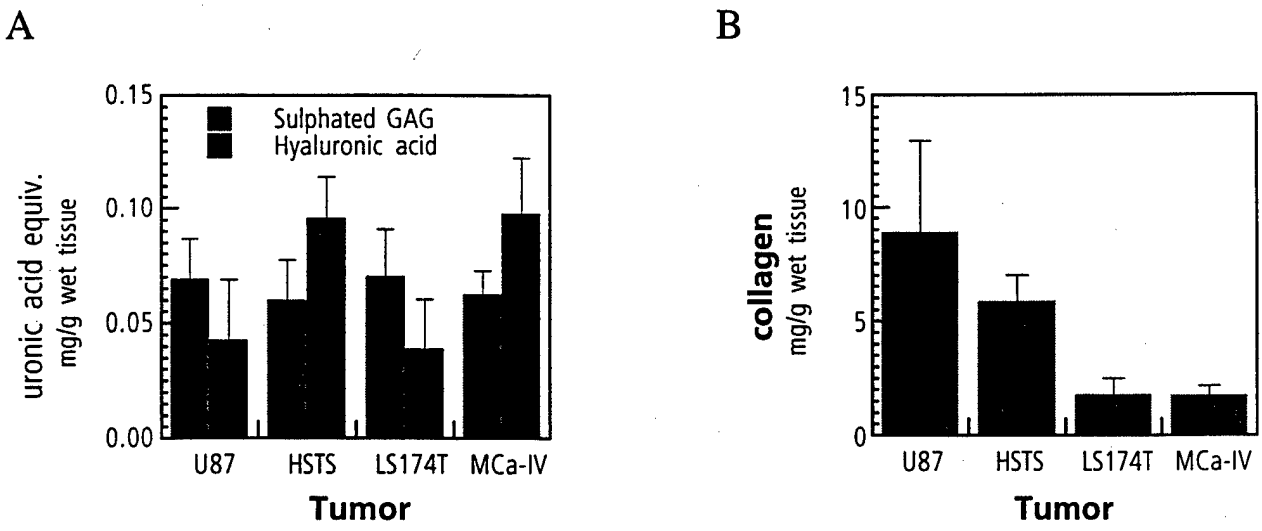
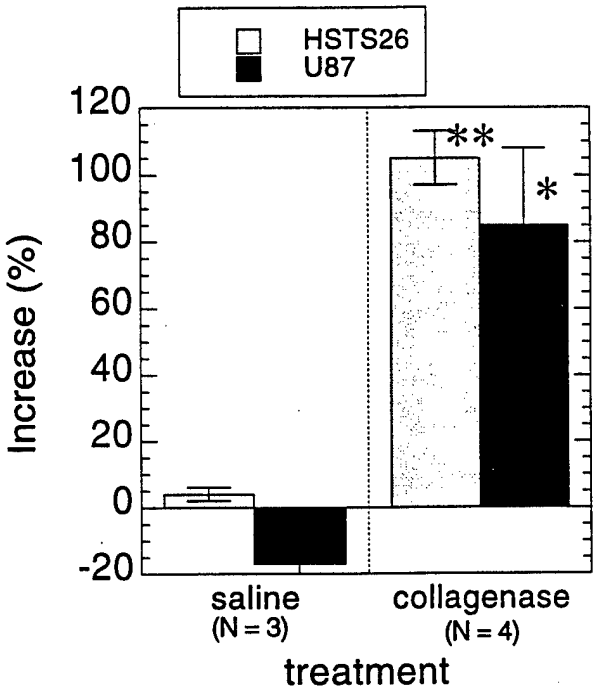
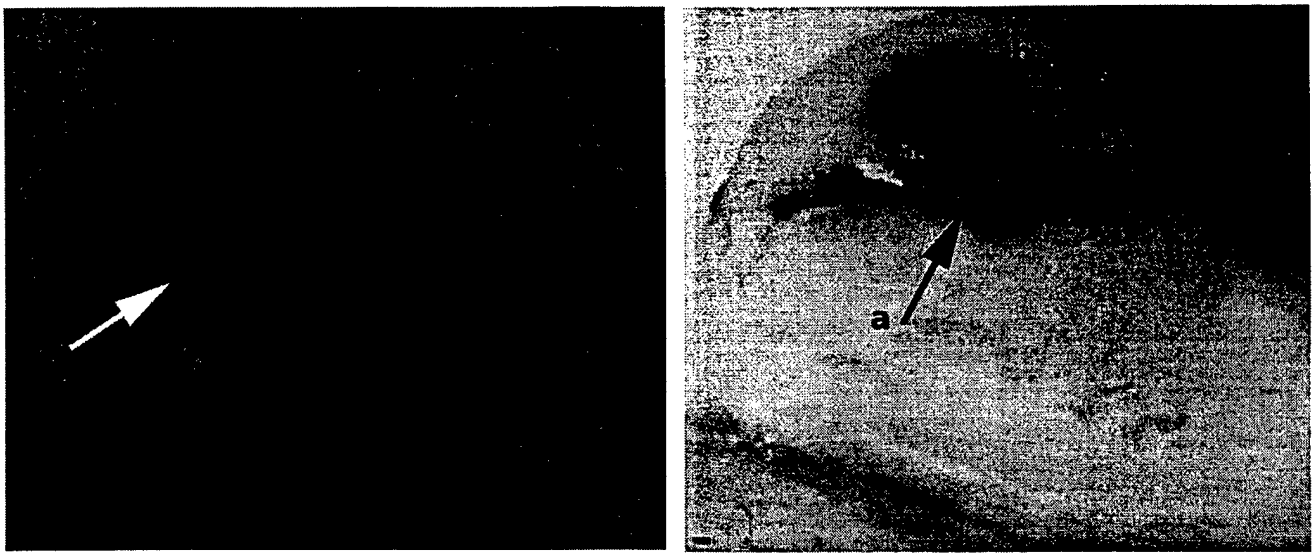


Figure 6. Biochemical analysis of the interstitial matrix of U87 tumors . (A) Glycosaminoglycan (GAG) content measured as equivalent uronic acid. (B) Total collagen content determined by colorimetric assay for hydroxyproline and compared to a collagen standard. The levels of sulfated GAGs are similar between the 4 tumors, significantly higher levels of hyaluronic acid were found in HSTS-26T and MCa-IV. The higher levels of collagen in U87 and HSTS-26T are most likely responsible for the slower diffusion coefficients of IgG in these 2 tumors.

Figure 7. Effect of collagenase treatment on the interstitial diffusion coefficient of IgG in HSTS-26T and U87. The tumors were superfused with collagenase and diffusion was measured one day later. The effect of saline treatment was not significant (paired t-test). Collagenase treatment produced a significant increase in IgG mobility in HSTS-26T ($p<0.0001$) and U87 ($p<0.05$).





A B

Figure 8. Spatial distribution of infused albumin in two tumor types. Evans-blue-labeled albumin was infused at the center of each tumor. The grayscale images shown here are the red color component of digitized photographs. (A) In LS174T, the material spread radially outward from the needle tip (arrow) through the tissue and filled a spheroidal volume of tissue. (B) In HSTS-26T, the material initially spread asymmetrically from the needle tip (larger arrow marked a), then traveled through a channel (smaller arrow b) to accumulate predominantly in a necrotic region of the tumor. Backflow along the needle track is also evident in this photograph.

CONCLUSIONS:

The purpose of the proposed research was to determine if MRI could be used to quantify interstitial matrix constituents in tumors, and to follow changes in the interstitium induced by interventions designed to increase drug delivery to tumor cells. The technical objectives were to determine by MRI the collagen and glycosaminoglycan concentration, and diffusivity of small solutes in the interstitial matrix of breast cancer. In initial studies, in irradiated tumors, 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.

We thus developed a new method for investigating GAG content that offered a resolution two orders of magnitude better than that provided by the sodium method originally proposed. Moreover, we demonstrated that the new $GdDTPA^{2-}$-based method could be used to track changes in GAG in a developing *in vitro* matrix. Conditions were perfected in our established cartilage model so we could optimize tumor imaging. The model was used to modulate relative cellular content, matrix content, and hydration. We investigated modulations in the GdDTPA-based GAG imaging to account for high cellularity. 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. The $GdDTPA^{2-}$ method was applied to mammary tumors, however the high cellurarity of the tumors limited significantly the sensitivity of the approach, so it was impossible to quantify GAG or collagen contents in tumors. In parralel studies we have shown that fibrillar collagen impedes the transport of large molecules like IgG and that the degradation of collagen by collagenase increases significantly the diffusion of IgG.

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 implications for the delivery of therapeutic agents. Brit. J. Cancer 78:1442-1448, 1998.

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.

Netti, P. A., Berk, D., Swartz. M.A., Grodzinsky, A.J., Jain R.K. Role of extracellular matrix assembly in interstitial transport in solid tumors. Submitted for publication.

Znati, 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 for publication.

APPENDIX A:

Personnel receiving pay:
Dr. Deborah Burstein
Dr. Yves Boucher
Ms. Julia Kahn