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Ultrahigh-Field DCE-MRI of Angiogenesis in a Novel Angiogenesis Mouse Model

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Purpose: To be able to screen and identify potential candidate agents for noninvasive imaging of diseases involving angiogenesis, a standardized in vivo angiogenesis model is needed. Angiogenesis is a common feature of many pathological conditions and has become an important target for diagnosis and treatment, with many noninvasive imaging agents emerging.

Materials and Methods: Uniform scaffolds consisting of porous and flexible polycaprolactone were implanted subcutaneously in mice and studied after 1 to 6 weeks to describe the time course of angiogenesis. The model was characterized by histology and dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI).

Results: Microscopic examination revealed progressive ingrowth of new vessels from the periphery, leading to a fully vascularized scaffold within 6 weeks. Blood flow through the new vessels, assessed by DCE-MRI, revealed peripheral vascularization corresponding to 12.3% (SD 6.1%) of scaffold area at week 1 and a more uniform and complete distribution of vessels corresponding to 84.1% (SD 16.2%) of scaffold area at week 4.

Conclusion: In agreement with microscopic examination, noninvasive DCE-MRI visualized progressive development of new vessels in a novel and standardized murine angiogenesis model, making this model suitable for screening angiogenesis-related drugs and contrast agents.

Key Words: angiogenesis; dynamic contrast-enhanced magnetic resonance imaging; scaffold; immunohistochemistry

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and allow discrimination between preexisting and newly formed vessels to provide conclusions about angiogenesis. Preferably, the site of angiogenesis should be readily available for imaging and harvesting of tissue for histological examination.

In this study we aimed at developing a model meeting these demands by implanting porous polycapro-lactone (PCL) scaffolds in the subcutaneous space in mice. We followed angiogenesis in the model with dynamic contrast-enhanced (DCE)-MRI at ultrahigh magnetic fields along with histology and found a time-dependent angiogenesis response well suited for studies of therapeutic and diagnostic agents aimed to target angiogenesis.

MATERIALS AND METHODS

PCL Synthesis and Pretreatment

Porous PCL scaffolds were prepared as described by Andersen et al (12), but with several important modifications. In brief, scaffolds were synthesized by thermally induced phase separation of 41.8 mg PCL in 1 g 1.4-dioxane and 100 ng H2O. After lyophilization, scaffolds were frozen in liquid N2 and discs (Ø = 8 mm, h = 2 mm) were cut. Newly synthesized PCL scaffolds were very hydrophobic, which was unwanted in the hydrophilic environment they were to be implanted in. To better fit the needs of the in vivo environment, scaffolds were treated by adding carboxyl-groups to the scaffold surface. These COOH groups imparted a natural hydrophilicity to the scaffolds, which after a thorough rinsing procedure were ready for use. The discs were made water-soluble by incubating in decreasing concentrations of EtOH (99%, 96%, 70%, and 50% each for 30 minutes) and finally in double-distilled H2O (5 × 15 minutes). The scaffolds were etched in 1.25 mol/L NaOH for 16 hours and 1.0 mol/L HCl for 1 hour. Scaffolds were washed in phosphate-buffered saline (5 × 30 minutes) then in saline (5 × 15 minutes) and stored in saline at 4°C.

3D Microstructure of PCL Scaffolds

PCL scaffolds were examined with a high-resolution scanning electron microscopy (HR-SEM) to characterize the 3D structure. Scaffolds were placed on aluminum stubs and scanned using a Nova electron microscopy system (low vacuum detector, spot size: 3, voltage: 3 kV, working distance <5 mm).

Insertion of PCL Scaffolds

All procedures involving experimental animals were approved by the National Animal Experiments Inspectorate. Sixty-six mice were used: 36 were assigned to the DCE-MRI study (six groups with six mice in each) and 30 mice were used for histologic evaluation (six groups with five mice in each) to support the imaging data. Male CDF1 mice (12–20 weeks old) were anesthetized intraperitoneally (i.p.) with 10 µL/g of a mixture of 2.5 mg/mL fentanyl citrate + 1.25 mg/mL diazepam. Then a 1–1.5 cm incision was made in the skin at the midline of the back, starting 0.5–1 cm cranial to the hind legs and toward the head. From the incision, two subcutaneous pouches were created by blunt dissection, one in front of each hind leg of the mice. PCL scaffolds soaked in saline were inserted into the pouches, with one scaffold in each pouch. Adding 0.5 mL saline into the pouches eased the insertion of the fragile scaffolds. Implantation of PCL scaffolds did not interfere with the mobility of the mice. The incision was sutured using a 5.0 thread, and after surgery mice were caged individually to prevent other mice from removing the implants. Mice were sacrificed at weekly intervals from 1 to 6 weeks after implantation.

In Vivo DCE-MRI

A 16.4 T Bruker Avance II wide-bore NMR spectrometer (Bruker BioSpin, Rheinstetten, Germany) equipped with a GREAT 60 gradient system with maximum gradient strength 1.5 T/m and a Micro 2.5 probe (25 mm, inner diameter, coil) was used for DCE-MRI. The mice were anesthetized i.p. with 10 µL/g of a mixture of 10 mg/mL ketamine + 1 mg/mL xylazine and restrained in the 25 mm coil, with the implants aligned to the approximate middle of the coil to visualize both implants, if possible. An intravenous (i.v.) line was applied in a tail vein for administration of the contrast agent gadolinium-diethylenetriamine penta-acetic acid (Gd-DTPA, Magnevist, Schering, Berlin, Germany), and an i.p. line was inserted for administration of top-up anesthesia.

When acquiring the DCE-MRI images, a single slice through the center of the implant(s) was chosen to facilitate comparison with histology. The imaging protocol included a variable repetition time (VTR) sequence for T1 mapping with field of view (FOV) = 25 × 25 mm, slice thickness = 1 mm, matrix size = 128 × 128, number of averages = 1, two dummy scans, spectral width = 75 kHz. 6 T R values within the interval 22.2 msec to 2430 msec, and T E = 12.6 msec, followed by dynamic acquisition of 100 images using a fast spoiled gradient echo sequence with FOV = 25 × 25 mm, slice thickness = 1 mm, matrix size = 128 × 128, number of averages = 4, 10 dummy scans, spectral width = 50 kHz, flip angle = 15°, T R = 11.7 or 12.5 msec, and T E = 3.1 msec. T R and flip angle in the dynamic sequence was chosen to give good signal at the upslope of the signal-time curves and four averages were possible within the desired dynamic image time resolution of ~6 seconds. The slice thickness of 1 mm was larger than the in-plane resolution of 0.2 mm in order to obtain a high signal-to-noise ratio (SNR) for the data analysis and the vascular variation was expected in the image plane between the scaffold’s periphery and the center. During the initial 4 seconds of the sixth image acquisition, Gd-DTPA was administered i.v. at a dose of 0.1 mmol/kg and concentration of 0.02 mmol/mL.

Both the VTR and dynamic images were smoothed using a 5 × 5 matrix Gaussian filter with a standard deviation (SD) of 1. T1 maps were calculated from the VTR images. We assumed a linear relationship
Images 2–5, and SI0 was calculated from Eq. 3. For baseline precontrast SI was averaged from dynamic late maps of vascular parameters. The semiquantitative parameter initial area under the curve (IAUC) was estimated by optimization of Eq. 3 using the Nelder-Mead simplex direct search method in MatLab 7.11 (MathWorks, Natick, MA). The contrast agent concentration causing the difference between $T_1,\text{post}$ and $T_1,\text{pre}$ was then calculated from Eq. 2.

where $T_1,\text{post}$ is $T_1$ after contrast agent administration, $T_1,\text{pre}$ is $T_1$ before contrast agent administration, and $r_1$ is the specific longitudinal relaxivity for the contrast agent. The relaxation $r_1 = 3300 \text{ L/(s mol)}$ was used (13). Signal intensity in the dynamic images was then converted to Gd-DTPA concentration using the signal equation for the dynamic sequence and the $T_1$ value estimated before the dynamic images in order to produce Gd-DTPA concentration-time curves for each voxel. The signal equation for the dynamic sequence assuming negligible $T_2^*$-weighting is:

$$SI = SI_0 \cdot \frac{1}{1 - e^{-\frac{T_R}{T_1}}} \frac{1}{1 - e^{-\frac{T_R}{T_1}} \cdot \cos(\Theta)}$$

where $SI_0$ is the signal with full longitudinal relaxation, $T_R$ is repetition time, and $\Theta$ is flip angle. The baseline precontrast SI was averaged from dynamic images 2–5, and $SI_0$ was calculated from Eq. 3. For each timepoint after contrast agent injection, $T_1$ was estimated by optimization of Eq. 3 using the Nelder-Mead simplex direct search method in MatLab 7.11 (MathWorks, Natick, MA). The contrast agent concentration causing the difference between $T_1,\text{post}$ and $T_1,\text{pre}$ was then calculated from Eq. 2.

Voxel concentration–time curves were used to calculate maps of vascular parameters. The semiquantitative parameter initial area under the curve (IAUC) was calculated by trapezoidal integration of the Gd-DTPA concentration over the first 90 seconds postadministration. Scaffold voxels with a low initial contrast agent uptake cannot be used for model analysis and are assumed not vascularized. Therefore, voxels with initial IAUC values less than 0.0005 Ms were excluded from IAUC regions of interest (ROIs). The following quantitative analysis for supporting consistency between IAUC and model ROIs. The threshold was chosen empirically such that the largest portion of the remaining voxels provide acceptable curve fits.

The standard DCE-MRI model including a vascular term (three-parameter model) was fitted to the curves for quantitative estimation of the transfer constant, $K_{\text{trans}}$, the rate constant, $k_{\text{ep}}$, the extravascular extracellular space, $v_e = K_{\text{trans}}/k_{\text{ep}}$, and the plasma volume fraction, $v_p$ (14). This is a model standardized by researchers in the field, and an expansion is made such that $v_p$ is estimated. However, other relevant DCE-MRI models exist (15,16). The same model without the vascular term (two-parameter model) was also applied; this model is often used in preclinical and clinical studies. Tracer plasma concentration was based on measurements by Furman-Haran et al (17) and adapted in this experiment with the current dose assuming a blood volume of 65 mL/kg and a hematocrit of 0.49 (18).

Fitting was performed using nonlinear least squares minimization in MatLab. Both models were fitted for each voxel in the IAUC ROI in which voxels with low contrast uptake were excluded. The criteria for an acceptable fit were positive parameter values and a mean fit point distance to the measured points where the concentration was larger than or equal to 0.5 M. Voxels not satisfying these criteria were excluded from the model ROIs. In voxels where a two-parameter fit was superior to a three-parameter fit, the values of the two-parameter model were adopted into the three-parameter model with $v_p$ set to zero. Despite trying a range of different initial values, the 3-parameter fit may fail with, eg, negative parameter values as the optimization is unconstrained, or be inferior to the 2-parameter fit. This happened for some voxels because curve fitting with three parameters was more dependent on initial values and local minima of the optimization function, often when blood volume was low.

ROIs were drawn manually around the implanted scaffolds on the MR images. $v_p$ estimates within the ROIs were used to calculate vascularization percentage within the implants (voxels with a value above zero) and blood volume in percentage of the implant voxels (estimated from average voxel $v_p$ and a previously measured hematocrit of 0.49 (18)).

**Histology**

The entire animal was immersion fixed in phosphate-buffered 4% formaldehyde solution (pH 7.2) for 24–48 hours. Noting the orientation of the mice, scaffolds were excised, cut in half at the middle, orientation kept exactly as on image slices from DCE-MRI, paraaffin-embedded, sectioned, and stained for microscopic examination. Cellularity and overall structure were assessed in hematoxylin and eosin (H&E)-stained sections, and vascularization was evaluated on collagen IV immunohistochemical stains (endothelial basement membranes; Cat. no. ab19808, Abcam, Cambridge, MA). Vessel density (number of vessels/mm²), average vessel size ($\mu$m²), and area of vessels within the implants (vessel area/implant area) were quantified. Briefly, on images of collagen IV-stained sections, vessels were traced in Adobe Photoshop (San Jose, CA) and tracings were exported to ImageJ (National Institutes of Health, Bethesda, MD, http://imagej.nih.gov/ij/; 1997-2011) for analysis. Vessels were identified using the following criteria: 1) collagen IV-staining should encircle a definable lumen; 2) at least one endothelial cell-nucleus should be identified around the lumen; and 3) lumen should not contain cell structures besides red blood cells or leukocytes (no implant structures or other tissue-related cells).

**Statistical Analysis**

Data are presented as mean ($\pm$SD). Data distribution of all variables in all groups was considered normal.
on the basis of Q-Q plots. Comparison between groups in the same graph was made by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test. Graph curves were analyzed for linear trend by performing one-way ANOVA followed by a post-test for linear trend. $P < 0.05$ was considered significant.

RESULTS

3D Microstructure of PCL Scaffolds

In Figure 1a the intricate but uniform porous structure of the scaffold is shown using high-resolution scanning electron microscopy. Major pores with a diameter of $\approx 80-120 \, \mu m$ are separated by walls with a thickness of 10–40 $\mu m$. The walls contain smaller cavities with diameters up to 10 $\mu m$, which greatly increase the surface area of the scaffold (Fig. 1b).

Histology: Cell Infiltration in PCL Scaffolds

H&E staining of scaffolds revealed cellular invasion starting from the periphery of the implant and moving toward the middle over the time-course of the study (Fig. 2). The cell infiltration begins with a few cells at week 1 over a more dense infiltration at weeks 2–5 to a fully infiltrated implant at week 6. As the mice (and hence the scaffolds) were not perfusion-fixed, we were able to observe red blood cells within very defined compartments of the implants, indicating the presence of new vessels (enlargement of the H&E stains in Fig. 2g–i). We did not observe obvious intra-implant hemorrhage, indicating that the new vessels were stable and not leaking erythrocytes into the extra-vascular matrix. Cellular infiltration was not always uniform throughout the implant, resulting in some implants being more cell-rich on one side compared to the other.

Histology: Vessel Analysis

In Fig. 3 the results from the vessel analysis are depicted and all three time-trend curves show the expected increase in vascularization from week 1 toward week 6.

Error bars at week 5 indicate that there is some variation in this group, and a single outlier is responsible for most of the observed variation seen in the graphs for both vessel-area/scaffold-area and for the vessel density. This outlier could be due to the localization of the scaffold in the mouse, as it was placed in a highly vascularized area. Furthermore, the interface of scaffold and host-tissue seemed more closely attached compared to other implants, which could contribute to better compatibility and higher vascularization. The mean values at week 5 are lowered to some extent when the outlier is removed, which in some cases resulted in changes of the $P$-value between groups to a significant level. However, the outlier did not change the time-trend curves for any of the parameters and hence did not change the overall conclusion of the study.

The average vessel size rapidly increases to around 200 $\mu m^2$ at week 2 and reached $\approx 350 \, \mu m^2$ at week 5. The average vessel diameter, calculated from the formula: average vessel diameter $= 2*\sqrt{\text{average vessel size}/\pi}$, assuming all vessels are circles, was found to be 16 $\mu m$ and 21 $\mu m$ at weeks 2 and 5, respectively.

DCE-MRI: Vascularization and Blood Volume in Implants

The presence of functional blood vessels and blood volume in implanted scaffolds was assessed by MR data analysis estimating the plasma volume fraction ($v_p$). In Fig. 4 a $v_p$ signal was observed in the periphery of implants at weeks 1 to 3, indicating new vessels
in the rim of the scaffolds. A more uniform distribution of signal was observed at weeks 4 to 6, indicating the existence of vessels within the center of the implants as well. The percentage of vascularized area within the implants is shown in Fig. 5 and blood volume as a percentage of voxels within the scaffolds are depicted in Fig. 6. Both time-trend curves showed a significant increase over the 6-week time period. All these findings were in agreement with the angiogenic response observed by histology (Figs. 2, 3), indicating that the functional vessels followed the advancing front of ingrowing new vessels.

**DISCUSSION**

The aim of this study was to find a suitable animal model for the noninvasive MRI evaluation of angiogenesis, which in turn can be used to screen new drugs and contrast agents for their effectiveness to target angiogenesis. Many existing angiogenesis models fell short in this regard as their site of angiogenesis was difficult to distinguish from already existing vessels on noninvasive imaging modalities (19–21). Harvesting of tissue for histology was also difficult in these models, making them less favorable as animal models for our purpose.

Other angiogenesis assays rely on tumor-associated angiogenesis either within a Matrigel plug (22) or a window chamber (23). These angiogenesis assays qualify for both of the above-mentioned criteria, but introduce an unknown quantity of growth factors and chemokines. Furthermore, tumor tissue tends to be very heterogeneous, having an irregular blood flow and an uneven perfusion within the same tumor (3,24). To get a “pure” angiogenesis model, we wanted no growth factors, except the stimulus coming from the animal itself, and no tumor cells influencing the angiogenesis process. An advantage of having a “pure” angiogenesis model is the ability to either induce or inhibit neovessel formation knowing that there will be no interference from tumor-associated pro-/antiangiogenic chemokines. Finally, promising diagnostics and drugs identified in a “pure” angiogenesis model most likely have a greater universal potential than agents identified in a disease-specific model.

The model that came closest to these criteria was the subcutaneous sponge implant model introduced by Andrade et al in 1987 (25). This model provides a scaffold for dividing and migrating endothelial cells, and the size and shape of the implanted scaffolds are consistent throughout the experiment (26–28). The angiogenesis process is propelled by a mild foreign-body response making use of the animals own growth factors, and the neovasculature is created within the scaffold making it easily identifiable. However, in our hands the polyether-polyurethane sponge model failed...
to give a robust angiogenic response, as we only observed a peripheral vascularization of the scaffolds. The lack of vascularization in the center was a major drawback for the noninvasive MRI we required, and hence we explored different scaffold materials and different treatment remedies of these materials.

PCL is a material that is biologically compatible, nontoxic, and slowly degradable (29). Introducing cavities in the walls of the PCL scaffolds hugely increased the surface area, and etching the surface to contain massive amounts of hydroxy groups enhanced hydrophilicity, both creating a better environment for cell adhesion and migration. Using these hyperporous and etched PCL scaffolds instead of polyether-polyurethane enabled us to get a fully vascularized scaffold within 6 weeks. Hence, we chose this material to support the neovessel formation in our experiments. Although the mice were genetically similar, we did observe differences in the rate and location of cell infiltration and in the amount of vessel formation within the different groups. Some scaffolds contained more cells and vessels on one side as compared to the other side of the implant, and we observed minor differences between implants coming from the same mouse. This indicates that the location and the surrounding tissue of the scaffold have some importance on the resulting vascularization. This was also observed by Mendes et al (28), who reported major differences when placing implants at different anatomical sites.

Initially, the implants were infiltrated by small vessels of capillary size, which grew larger in diameter as they matured and underwent remodeling. This process is very similar to the proliferative phase of wound healing (30) and the foreign body response, and can explain the initial increase in vessel density (Fig. 3c), followed closely by the increase in average vessel size (Fig. 3b). The increase in vessel density and vessel size meant that more space within the scaffold was occupied by vessels (Fig. 3a). When implants became fully infiltrated and vascularized, the vessel density ceased to increase around weeks 4–5 (Fig. 3c). The cessation of vessel density was followed shortly by the average vessel size as all the newly formed vessels had matured around week 5 (Fig. 3b). As the vessel-area/scaffold-area percentage illustrates a combination of vessel density and average vessel size, this parameter peaked around week 5 as well (Fig. 3a).

The DCE-MRI data provide similar information when it comes to characterization of the angiogenesis model. Like histology, where we observe an increase in vessel density, we observe an increase in the vascularization percentage of the scaffolds (Fig. 5 compared with Fig. 3c). The vascularization percentage increases from week 1 and continues to rise until week 4, whereas the vessel density increases from week 1 and continues to rise until weeks 4–5.

Comparing the graph in Fig. 6 to the vessel-area/scaffold-area graph seen in Fig. 3a, we find that both time trends show a significant increase. The values at each week are much higher in Fig. 6 than the values in Fig. 3a (eg, at week 5 we have 2.6% in Fig. 6 compared to 2.2% and 1.5% in Fig. 3a). This difference in absolute value may be ascribed to one or more of the following: 1) The DCE-MRI data analysis could have overestimated the v_p-value because of the following assumptions and uncertainties. The relaxivity value used for the contrast agent was adapted from a study at the field strength 3 T because we did not have an estimate at the current high field strength. Also, the individual plasma concentration–time curves may vary from the common curve adapted in this study. 2) The DCE-MRI values are created from 3D voxels measuring 1 mm in thickness as compared to the 3D histology with a thickness of 4–5 μm. The histology is taken from the very center of the implant, which is the least vascularized area of the scaffold. The same

Figure 3. Vessel analysis of implants excised after 1 to 6 weeks and stained with an anti-collagen IV antibody. a: Vessel-area/scaffold-area (%); b: average vessel size (μm²); and c: vessel density (vessel/mm² scaffold). Solid line represents data including the single outlier at week 5 and the dotted line represents data excluding the outlier. Results are depicted as mean values with error bars representing SD. Asterisks (*) represent significant differences between groups of data including the outlier (solid line), and daggers (†) represent significant differences between groups of data excluding the outlier (dotted line). P-value below 0.05 was considered significant.
applies to the MR images, but because the thickness is much larger in the MR images they average information from a greater area, resulting in a higher mean value. 3) The drawing of the ROIs could have included host-tissue in calculations of the $v_p$ value. The surrounding host-tissue will be vascularized in order to support the angiogenesis occurring within the scaffold, and might elevate the $v_p$ value if included in the calculations. Despite the high resolution of the MR images, it sometimes proved difficult to trace the ROIs at the exact host-scaffold interface.

When interpreting the MR data, we must remember the several assumptions made with regard to the MR images obtained as stated above. Although these are not expected to give large differences in the results, they should be mentioned since the MR scanner used in this study had a high magnetic field (16.4 T). The

![Figure 4](image1.png)

**Figure 4.** Functional vessels within implants. Intravenous injection of contrast agent gives rise to signal increase in blood vessels on DCE-MRI. Analysis of images before, during, and after the injection gives an estimate of the plasma volume fraction ($v_p$) in each voxel creating a heat-map over functional vessels. Regions of interest representing the implants within the animal are cut out of the $v_p$ heat-map and overlaid on a grayscale MR image of the mouse. This gives an overview of new vessels formed within the implants, which is depicted for one representative animal from each of the six groups. a–f: 1–6 week implants showing the increase in plasma volume from week 1 (a) to week 6 (f). Voxel resolution is $200 \mu m \times 200 \mu m \times 1000 \mu m$ before smoothing.

![Figure 5](image2.png)

**Figure 5.** Vascularization of the implants estimated by DCE-MRI. Voxels from each implant were analyzed for values above zero. Voxels having a value above zero were counted as vascularized voxels and these voxels were divided by the total number of voxels within the implants. Results are depicted as mean values with error bars representing SD. Asterisks (*) represent significant differences between groups ($P < 0.05$).

![Figure 6](image3.png)

**Figure 6.** DCE-MRI evaluation of blood volume in percent of implant voxels. Results are depicted as mean values with error bars representing SD. Asterisks (*) represent significant differences between groups ($P < 0.05$).
drawing of the ROIs could give some variation at the very edges of the implants where the host–scaffold interface might not be 100% correctly identified. This could give rise to small variations such as some of the vascularization located at higher levels of the implants. This vascularization could actually come from the host-tissue and been incorrectly included. However, the opposite scenario could also apply (ie, some of the scaffold has been left out, decreasing the value of \( v_p \)). Hence, the definition between host and scaffold may vary on DCE-MRI, whereas histology paints a more valid picture of the vascularization. Altogether, the MR data resembles the gold standard of histology, and the model can be used for monitoring/evaluating the angiogenesis process.

In conclusion, the novel murine angiogenesis model described in this article displayed a robust neovascularization response in subcutaneously implanted PCL scaffolds. This model meets all of the listed criteria for a suitable angiogenesis model for noninvasive evaluation of developmental diagnostics or drugs that target angiogenesis. The MR data, supported by histologic findings, indicate that 3–4 weeks after implantation might be an optimal timepoint for testing agents. At this point new vessels are still emerging, and newly formed vessels have not yet completely matured. Further, this study shows that MRI is an excellent tool for monitoring angiogenesis in vivo.

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REFERENCES

13. Pintaske J, Martiorean S, Graf H, et al. Relaxivity of gadopentate dimeglumine (Magnevist), gadobutrol (Gadovist), and gadobenate dimeglumine (Multihance) in human blood plasma at 0.2, 1.5, and 3 Tesla. Invest Radiol 2006;41:213–221.