Magnetic resonance imaging studies on gadonanotube-reinforced biodegradable polymer nanocomposites

Balaji Sitharaman,1,2,3* Meike Van Der Zande,4* Jeyarama S. Ananta,2 Xinfeng Shi,1 Andor Veltien,5 X. Frank Walboomers,4 Lon J. Wilson,2,3 Antonios G. Mikos,1,3 Arend Heerschap,5 John A. Jansen4

1Department of Bioengineering, Rice University, P.O. Box 1892, MS-142, Houston, Texas 77251-1892
2Department of Chemistry, Rice University, P.O. Box 1892, MS-60, Houston, Texas 77251-1892
3The Richard E. Smalley Institute for Nanoscale Science and Technology, Rice University, P.O. Box 1892, MS-100, Houston, Texas 77251-1892
4Department of Periodontology and Biomaterials, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands
5Department of Radiology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

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Abstract: We report about the in vitro cytotoxicity and MRI studies of Gd³⁺ ions-doped ultra-short single-walled carbon nanotube (gadonanotubes), gadonanotubes-reinforced poly(lactic-co-glycolic acid) (PLGA) polymer nanocomposites and in vivo small animal MRI studies using the gadonanotubes. These studies were performed to explore the suitability of gadonanotubes-reinforced PLGA polymer nanocomposite as a model scaffold for noninvasive magnetic resonance imaging (MRI) to evaluate nanotube release during the degradation process of the scaffold and their biodistribution upon release from the polymer matrix in vivo. The gadonanotubes at 1–100 ppm and the gadonanotubes/PLGA nanocomposites (2 wt % gadonanotubes) did not show any cytotoxicity in vitro as demonstrated using the LIVE/DEAD viability assay. For the first time, r2 relaxivity measurements were obtained for the superparamagnetic gadonanotubes in vitro 7T MRI of the superparamagnetic gadonanotubes ([Gd] = 0.15 mM) suspended in a biocompatible 1% Pluronic F127 solution, gave a r2 value of 578 mM⁻¹ s⁻¹. Upon subcutaneous injection of the gadonanotubes suspension into the dorsal region of rats, the high r2 value translated into excellent and prolonged negative contrast enhancement of in vivo T2 weighted proton MRI images. The in vitro characterization of the nanocomposite discs and their degradation process by MRI, showed strong influence of the gadonanotube on water proton relaxations. These results indicate that the gadonanotubes/PLGA nanocomposites are suitable for further in vivo studies to track by MRI the biodegradation release and biodistribution of gadonanotubes. © 2009 Wiley Periodicals, Inc. J Biomed Mater Res 93A: 1454–1462, 2010

Key words: cytotoxicity; gadonanotubes; biodegradable polymer; nanocomposites; contrast agent; magnetic resonance imaging

INTRODUCTION

Single-walled carbon nanotubes (SWNTs) possess a number of unique characteristics that make them desirable for biomedical application. One of their proposed applications is as reinforcing agents in synthetic polymers due to their excellent mechanical properties (~640 GPa in modulus and ~40 GPa in...
tensile strength) and high aspect ratio. Our laboratory has developed SWNT-based nanocomposites for bone tissue engineering. The SWNTs, especially ultra-short SWNTs (US-tubes) induce a significant reinforcement of the polymeric scaffold and show good biocompatibility in vitro and in vivo. Therefore, US-tube nanocomposites may hold exceptional potential for bone-tissue engineering.

Although these carbon nanotubes-based nanocomposites offer promising properties for bone tissue engineering scaffolds, little is known about the biocompatibility and long-term biodistribution of the carbon nanotube materials upon their release from the polymer upon degradation in vivo. It has been shown that debris released from orthopedic implants can accumulate to cause pathologies in vital organs. Recently, microparticle and nanoparticle aggregates of exogenous origin were discovered in the kidneys, liver, and colon of humans suffering from various illnesses. The accumulated particles damaged these organs in a few cases. The origin of these substances is still to be determined. Nevertheless, these studies raise concern about any new nanomaterials-based implants, such as our SWNT and US-tube nanocomposites.

Recently, we reported that Gd³⁺-encapsulated carbon nanotube structures (gadonanotubes) possess unique nano scalar properties exhibiting superior performance as MRI contrast agent (CA) and thus, show promise for molecular imaging and other advanced applications. The high contrast agent efficacy of the gadonanotubes should allow noninvasive monitoring of their release from a polymeric scaffold upon degradation and give better insight about their biodistribution characteristics in vivo. However, before one can perform such extensive in vivo studies, in vitro cytotoxicity testing needs to be performed as they often provide valuable information for further evaluation of biocompatibility. In vitro MRI studies are also essential to optimize the gadonanotube/polymer nanocomposites for the in vivo studies and to optimize the imaging parameters. Towards this end, in this report we present (a) the in vitro cytotoxicity of the gadonanotubes and their nanocomposites with PLGA, (b) the in vitro phantom MRI of the gadonanotubes and gadonanotubes/PLGA nanocomposites and (c) the in vivo small animal MRI of the gadonanotubes.

**EXPERIMENTAL METHODS**

**Generation of US-tubes**

SWNTs were cut into US-tubes by a previously developed method by Gu et al. Briefly, this method involves fluorination of solid SWNT sample for 2 h at 50°C under 1% gaseous fluorine diluted in helium followed by pyrolysis at 1000°C for 1 h under an inert atmosphere that leads to spontaneous cutting of the micron-long SWNT into ultra-short SWNTs (diameter = 1.4 nm and length = 20–80 nm). This procedure also purifies US-tubes from the catalyst impurity. Because of their great flexibility and high surface energy, SWNT tend to aggregate into large bundles and the US-tubes are present as bundles that contain hundreds of individual US-tubes. Although US-tubes can be debundled into individual US-tubes, bundled US-tubes were used in our study.

**Synthesis of gadonanotubes**

Solution-phase filling of the Gd³⁺ inside the US-tubes was achieved by a protocol previously developed in our lab. Briefly, this method involved stirring US-tubes and anhydrous GdCl₃ (1:1 ratio by weight) in HPLC grade water at pH = 3, followed by sonication for 1 h. The solution was then left undisturbed overnight, whereupon the Gd³⁺-loaded US-tubes flocculated from the solution. The supernatant solution was then decanted off and the sample was washed with fresh water followed by sonication to remove any unabsorbed GdCl₃. This procedure of flocculation and washing with fresh deionized (DI) water was repeated several times. The sample was then air dried and an inductively-coupled plasma optical emission spectrometry (ICP-OES) analysis showed that the Gd content of solid gadonanotubes was 3.06% (m/m).

For the relaxivity measurements, a saturated solution of 10 mg of the gadonanotubes in 10 mL of a 1% biologically-compatible 1% Pluronic F127 surfactant solution was prepared. Approximately 10% of the gadonanotubes dispersed and formed a stable suspension. The [Gd] concentration of this supernatant (suspension) solution was then confirmed by ICP. This suspension was used as the stock solution and diluted with 1% Pluronic F127 surfactant solution. The [Gd] concentration of this supernatant (suspension) solution was then confirmed by ICP. This suspension was used as the stock solution and diluted with 1% Pluronic F127 to obtain the [Gd] concentrations used for the cytotoxicity, MRI (in vitro and in vivo) experiments with the gadonanotubes.

**Preparation of the nanocomposites**

The nanocomposites were prepared by dispersing the hydrophobic gadonanotubes at 2 wt % concentration into PLGA polymer (average molecular mass of 5 kDa) using an established procedure of high shearing mixing, sonication and drying. PLGA of 50:50 lactic to glycolic acid copolymer ratio was used for the fabrication of these scaffolds. The nanocomposite material was finally cast into molds that produced discs of 6-mm diameter and 1-mm height.

**Cell culture**

A Fischer rat fibroblast 3T3 like cell line (ATCC, CRL-1764, Manassas, VA) was grown in T-75 culture flasks (Sigma-Aldrich, St. Louis), using Dulbecco’s modified Eagle medium (DMEM, Gibco Life, Grand Island, NY) supplemented with 10 vol % fetal bovine serum (FBS, Journal of Biomedical Materials Research Part A
Gemini Bio-Products, Calabasas, CA) and antibiotics (100 μg/mL penicillin, 100 U/mL streptomycin, and 0.5 μg/mL amphoterricin B, all from Gibco Life). Cell cultures were incubated at 37°C, 95% room air, and 5% CO₂. Cells of passage numbers 4–8 were used in this study.

Preparations of the samples for the in vitro cytotoxicity studies

To evaluate the in vitro cytotoxicity of the gadonanotubes, PLGA polymer and the nanocomposites, the extracts of these materials were first tested in accordance with the International Standard ISO 10993-5 because neither PLGA nor the gadonanotubes are soluble in water. US-tubes were used as controls. All specimens were first sterilized by exposure to UV light for 3 h. Subsequently, PLGA and nanocomposite scaffolds were immersed in culture media at 1 mL media per 3 cm² scaffold contact area, while the gadonanotubes and US-tubes were mixed with culture media at a concentration of 1 mg/10 mL or 100 ppm. Following 24-h extraction at 37°C, the supernatant medium above PLGA and nanocomposites was withdrawn and filtered with a 0.2-μm filter (Nalgen, Rochester, NY). The extracted medium (1× dilution) and their 10-time and 100-time diluted solutions were added to the 96-well plates containing 3T3 cells. To investigate the direct interaction between cells and gadonanotubes, medium containing gadonanotubes at a concentration of 1, 10, 100 ppm was also prepared.

In vitro cytotoxicity tests

For the cytotoxicity studies, the 3T3 fibroblasts were lifted from the T-75 culture flasks at 80–90% confluency, trypsinized, and resuspended at a concentration of 1.5 × 10⁵ cells/mL. The cells were then seeded in 96-well plates at 100 μL cell suspension/well with a seeding density of 4 × 10⁴ cells/cm², and allowed to attach for 24 h. The culture media was then replaced with the experimental solutions (described earlier) and cell was determined after 24 h of incubation (n = 6 wells/treatment).

Thereafter, the cells were washed with phosphate buffered saline (PBS, Gibco Life) and then 100 μL LIVE/DEAD reagent (2 μM calcein AM and 4 μM ethidium homodimer-I (EthD-1; Molecular Probes, Eugene, OR) was added. Following 30 min incubation at room temperature, the fluorescence of each well was measured using a BIO-TEK Instruments FLx800 plate reader (Winooski, VT) equipped with filter sets of 485/528 nm (excitation/emission) for calcein AM (live cells) and 528/620 nm (excitation/emission) for EthD-1 (dead cells). For this test, cells which were only exposed to culture media served as the live control and cells exposed to 70 vol % ethanol solution for 30 min before addition of the LIVE/DEAD reagent served as the dead control. The fluorescence of each sample well was normalized by that obtained from the live and dead control groups, which was defined as the fractions of live and dead cells, respectively. After the fluorescence measurements, the live/dead cell populations of the representative wells were imaged using a Zeiss LSM 510 Axiovert microscope (Carl Zeiss, Jena, Germany).

Magnetic resonance imaging

Magnetic Resonance Imaging experiments were performed on a 7-T/200mm horizontal-bore magnet interfaced to an Surrey Medical Imaging System (SMIS) spectrometer and equipped with a gradient insert (gradient strength = 150 mT/m; rise time = 150 μs).

For the phantom studies, vials containing gadonanotubes dispersed in biologically-compatible 1% Pluronic F127 at a [Gd] of 0.15 mM were prepared. As a reference, a vial containing plain 1% Pluronic F127 solution was also included within the imaging field-of-view (FOV). All phantom studies were done at room temperature (T = 25°C).

For the in vitro MRI of the nanocomposites, the discs were embedded into biological grade agarose and imaged after 2 h, 24 h and subsequently on days 3, 5, and 7. PLGA scaffolds were used as controls.

The phantom and nanocomposite discs were imaged with a homogenous RF birdcage coil (60-mm diameter) using a spin-echo pulse sequence. Imaging parameters were: image matrix size of 128 × 128, field of view (FOV) of 60 × 60 mm², slice thickness (SLT) of 2 mm, 6 slices, one signal average per phase-encoding step and a scan repetition time (TR) of 1000 ms. The best slice containing all samples in 1 slice was then chosen and used for T₂ calculations. The echo time (TE) was varied to calculate T₂ values of the gadonanotube phantom samples (TE = 10, 12, 14, 16, 18, and 20 ms). The T₂ values were calculated by exponential fitting of the datapoints. The T₂ was then calculated as (1/T₂ (gadonanotubes) – 1/T₂ (1% Pluronic F127 solution))/[Gd].

Two healthy male Wistar rats weighing ~200 g were used for the in vivo MRI study. Prior to the in vivo experiments, approval was obtained from the Radboud University Nijmegen animal ethics committee (RU-DEC 2007-096). The rats were immobilized in a ventral position in the MRI under general inhalation anesthesia (isoflurane in an O₂/N₂O mixture). Gadonanotubes were dispersed in 1% Pluronic F127 (100 μL gadonanotubes, [Gd] = 0.15 mM) and were then injected subcutaneously in the back of the rats. The rats were subsequently imaged 2 h and 1 day after administration of the gadonanotube suspension.

For the in vivo MR imaging, a homebuilt dedicated RF microstrip coil and mechanical setup was used. The rat was placed in supine position to get the best signal-to-noise-ratio (SNR) in the disc area. Temperature of the rat was monitored using a fluoroptic rectal thermometer and kept at 37 ± 1°C with a warm water bed. Breathing was monitored with a movement sensor and breathing was kept at 40 ± 5 times/sec by adjusting the anesthetics, which consisted of 1.5-2.5% iso-2-flurane mixed with a gas mixture of 50% O₂ and 50% N₂O.

Images were obtained with a T₂ weighted coronal (i.e. parallel to the coil) multi-slice spin-echo (SE) sequence. Imaging parameters were: image matrix size of 256 × 256, field of view (FOV) of 120 × 120 mm², slice thickness (SLT) of 1 mm, 16 slices, one signal average per phase-encoding step and an echo time (TE) of 15 ms. To reduce breathing artifacts, respiratory gating was used, resulting in repetition times between 1.3 and 1.7 s, depending on the breathing cycle, which was kept between 35 and 45 times/min throughout the complete experiment.
Statistical analysis

The nonnegative least square (NNLS) technique and the Levenberg–Marquardt (LM) nonlinear least squares algorithm were used to analyze the $T_2$ relaxation data. All statistical comparisons were conducted with a 95% confidence interval ($p < 0.05$). Single-factor analysis of variance (ANOVA) was conducted to identify significant differences among treatment groups. The experimental data are expressed as means ± standard deviation.

RESULTS

In vitro cytotoxicity

The depictions of PLGA and the gadonanotubes are shown in Figure 1. Figure 2 shows the viability data for the cells directly contacting 100 ppm gadoxanotubes, and US-tubes as well as cells exposed to 1× dilution extracts from gadoxanotubes, US-tubes, PLGA and the gadoxanotube/PLGA nanocomposites. The fibroblasts directly contacting the gadoxanotubes and US-tubes at 100 ppm concentrations for 24 h showed 100% viability. The fluorescent microscopy image of gadoxanotube [Fig. 3(a)] and US-tube media [Fig. 3(b)] also demonstrated high density of live cells (green color) and very few dead cells (red color) comparable to that of the live control. Because of the hydrophobicity of the carbon nanotubes, they formed aggregates [arrows in Fig. 3(a,b)] in the sizes of submicrometers to tens of micrometers within the confluent cell monolayer. The cells surrounding or attached to these aggregates did not show any signs of damage. Similar results were observed for cells directly in contact with gadoxanotubes and US-tubes at 1 ppm and 10 ppm concentrations respectively for 24 h (Table I). The extracted solutions from the gadoxanotubes and US-tubes at 10× and 100× dilutions also showed ~100% cell viability after 24-h exposure (Table I).

Nearly 100% cell viability was also detected for the cells exposed to 1× dilution of the extracts from PLGA or the gadoxanotube/PLGA nanocomposites (Fig. 2). Similar results were observed at 10× and 100× extracts (Table I). The extraction media from gadoxanotubes nanocomposite did not change the density and morphology of the fibroblasts over 24-h incubation [Fig. 3(c)].

MRI

Representative $T_2$ weighted images at 25°C of samples containing gadoxanotube suspension at 0.15 mM [Gd] and plain 1% Pluronic F127 surfactant solution clearly show the effect of the nanotubes on $T_2$ relaxation (Fig. 4). The multi-echo experiments on the gadoxanotube suspension revealed an apparent $T_2$ value of 11.5 ± 0.5 ms and a $r_2$ value of 578 ± 20 mM$^{-1}$ s$^{-1}$.

Figure 1. Depiction of PLGA and gadonanotubes. The gadonanotubes is drawn not to scale and the dangling carbon atoms at the defect sites as well the Gd$^{3+}$ counterions are not shown. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Figure 2. Cell viability after 24-h incubation with the different groups. Error bars represent standard deviations; $n = 6$. [Graph showing cell viability for different groups with error bars indicating standard deviation.]

Figure 4. Representative $T_2$ weighted images at 25°C of samples containing gadoxanotube suspension at 0.15 mM [Gd] and plain 1% Pluronic F127 surfactant solution clearly show the effect of the nanotubes on $T_2$ relaxation. The multi-echo experiments on the gadoxanotube suspension revealed an apparent $T_2$ value of 11.5 ± 0.5 ms and a $r_2$ value of 578 ± 20 mM$^{-1}$ s$^{-1}$. [MRI images showing relaxation effects.]
The *in vitro* characterization of the nanocomposite discs and the degradation process by MRI is shown in Figure 5. Figure 5(a) shows a schematic picture of the nanocomposite disc configuration within the MR scanner and the orientation of one of the image slices. The corresponding MR images are shown in Figure 5(b–f). Since the MR images are due to the signal from the water protons, no signal was obtained from the solid discs, while the area surrounding the discs was bright, due to the water in the agarose gel. Bright areas (as indicated by the red arrows in Fig. 5) are caused by susceptibility artifacts. The MRI showed enhanced water penetration, which increased with time. Beyond day 1, the discs swelled considerably, the dark parts on the MRI slowly disappeared, and by day 5, there was an increase in image brightness throughout the disc indicating that the water was homogeneously distributed throughout the sample. Enhanced contrast (yellow arrows) was also noticed at the initial time point [Fig. 5(c–d)] at the edges of the water front and at the later time points [days 5 and 7; Fig. 5(e–f)] throughout the disc.

The subcutaneous injection of the gadonanotubes 1% Pluronic F127 solution in rats showed excellent negative contrast. Figure 6 shows a representative MR image of the dorsal region of the rat 1 day after injecting 100 μL of the gadonanotubes ([Gd] = 0.15 mM). The dark spot seen in the right dorsal region is negative contrast generated due to the high $r_2$ relaxivity of the gadonanotubes. There was no observable change in the intensity of the dark spot.

### Table I

<table>
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<tr>
<th>Test Media</th>
<th>Concentration</th>
<th>Fraction of Live Cells (%)</th>
<th>Fraction of Dead Cells (%)</th>
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<td>Live Control</td>
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<td>13 ± 6</td>
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<tr>
<td>Dead Control</td>
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<td>Gadonanotubes in direct contact with Cells</td>
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<td>96 ± 7</td>
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<td>1 ppm</td>
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<td></td>
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<td></td>
<td>100× dilution</td>
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over day long observation indicating that most of
the injected gadonanotubes remained in the vicinity
of the injection site.

**DISCUSSION**

The objective of this study was to assess the
*in vitro* cytotoxicity and MR imaging of gadonanotubes and nanocomposites, as well as *in vivo* small animal MRI studies using the gadonanotubes. This study was performed towards our final goal of evaluating the suitability of gadonanotubes-reinforced PLGA polymer nanocomposites as a model scaffold system for noninvasive MRI monitoring of the gadonanotube release during the degradation process of the scaffold and its eventual biodistribution upon release from the polymer matrix *in vivo*. PLGA was chosen as the polymer matrix because it is not only a biocompatible and FDA approved polymer for human clinical application, but also shows biodegradable characteristics suitable for the time scale for future *in vivo* MRI experiments. Furthermore, previous MRI-based controlled release studies using PLGA served as a point of reference for our current and future MRI experiments.

**In vitro** cell viability

*In vitro* cytotoxicity testing is a standard method routinely used as an initial screening to examine cell response to biomaterials. It often provides useful information before starting more complex and expensive *in vivo* animal experiments. The 3T3 fibroblast-like cell line is a commonly accepted cell line for these evaluations. The LIVE/DEAD viability/cytotoxicity assay (Molecular probes, Eugene, OR) was preferred over the MTT assay to determine cytotoxicity levels due to its suitability for assaying directly carbon nanotube treated cells.

In case of the gadonanotubes-reinforced PLGA nanocomposites, the nanocomposites and the gadonanotubes upon release from the nanocomposites are the two main components that will interact with the surrounding tissues post implantation. Therefore,
we assessed their cytotoxicity to predict a possible tissue response to these components when implanted in vivo.

No cytotoxicity was observed for either the US-tubes or the gadonanotubes at 1–100 ppm concentrations in both extract dilution and direct contact assays (Table I). The cells maintained their morphology (images not shown) and formed agglomerates around nanotube aggregates [arrows in Fig. 3(a)]. These results corroborate with other data on carbon nanotube toxicity as reported by our group and by others.\(^8,27\) The lack of toxicity for the gadonanotubes is encouraging and represents to the best of our knowledge the first study profiling it’s in vitro cytotoxicity. This observation suggests robust sequestering of the \(\text{Gd}^{3+}\) metal ion within the carbon sheath of US-tube for at least one day after their exposure to cells and biological media. However, longer time points exposure studies are clearly needed to ascertain if the \(\text{Gd}^{3+}\) metal ions leak out on prolonged exposure to biological cells and fluids.

The extract dilution assays on the gadonanotubes/PLGA nanocomposites also did not display any toxic effects on the cells and their response was similar with PLGA composite, which was used as control (Table I). Consequently, the favorable cytocompatibility of the nanocomposites allowed their further consideration for in vitro and in vivo MRI studies.

MRI

The \(r_2\) (578 mM\(^{-1}\) s\(^{-1}\)) relaxivity exhibited by the gadonanotubes is among the highest reported value for contrast agents at 7 T\(^{21}\) and should afford better \(T_2\) weighted contrast enhancement for MRI. This large \(T_2\) relaxation effect is possibly due to the gadonanotubes’ superparamagnetism\(^{14}\) and magnetic susceptibility of the carbon nanotubes.\(^{28}\) Nanotechnology-based approaches in the design of MRI CAs have demonstrated great potential for the development of the next generation high-performance CAs. Currently, superparamagnetic CAs are mainly based on iron oxide crystals and can be broadly categorized as ultra-small superparamagnetic iron oxide particles (USPIO) (mean diameter less than \(\sim50\) nm) and superparamagnetic iron oxide particles (SPIO) (mean particle diameter greater than \(\sim50\) nm).\(^{29}\) These CAs exhibit substantially larger \(T_2\) relaxivity compared with Gd chelates in current clinical use, typically by an order of magnitude or more.\(^{29}\) The superparamagnetic gadonanotubes have substantially larger \(T_1\) relaxivity compared with paramagnetic Gd chelates in current clinical use (170 mM\(^{-1}\) s\(^{-1}\) vs. 4.0 mM\(^{-1}\) s\(^{-1}\)).\(^{14}\) The \(r_2\) values for the gadonanotubes reported in this article are the first attempt to characterize their efficacy as \(T_2\) contrast agents. These results suggest that gadonanotubes are useful \(T_1\) positive CAs at lower 1.5 T fields and \(T_2\) negative CAs at higher 7T fields.

The high \(T_2\) relaxivity values shown by the gadonanotubes 1\% Pluronic F127 suspensions were further corroborated by the preliminary in vivo MRI studies (Fig. 6). It has to be noticed that the excellent negative contrast was obtained at a low [Gd] concentrations ([Gd] = 0.15 mM). There was no noticeable change in the intensity of the dark spot at the different times points indicating that most of the injected gadonanotubes are still present in the vicinity of the injection site.

The in vitro MRI studies on the gadonanotubes/PLGA nanocomposites were performed to optimize the final scaffolds and imaging parameters for the eventual in vivo studies. Enhanced contrast [yellow arrows in Fig. 5(b–f)] was noticed at the initial time points at the edges of the water front and at the later time points (days 5 and 7) throughout the disc. This effect may be due to the water molecules being in close proximity to the gadonanotubes getting released upon degradation of PLGA polymer. The bright areas indicated by the red arrows [Fig. 5(b–f)] are probably caused by either susceptibility artifacts due to the water to solid disc transition or by artifacts induced by the high concentration of gadolinium in this area, which induces local susceptibility artifacts. The release of gadonanotubes from the PLGA surface involves a series of degradation stages involving hydration, depolymerization, mass loss, and absorption of the polymer products. Future MRI studies should provide more insight into the kinetics of these processes, along with the accompanying morphological changes.

Although MRI as a noninvasive imaging technique is widely used in medicine and is an established method for imaging food structure and manufacturing process, very few studies have explored their efficacy in the field of pharmaceuticals.\(^{30,31}\) Most of these studies have used MRI to elucidate the internal structure and controlled release of pharmaceutical dosage forms,\(^{32–34}\) to follow the time course of pharmaceutical processes such as mixing and extrusion,\(^{35–37}\) to assess in vivo behavior of dosage forms,\(^{38–40}\) and to map the biodistribution of gadolinium marked-control delivery systems from hydrogels and biopolymer implants.\(^{40–42}\) These investigations have illustrated the tremendous potential of MRI to map the transport, behavior, and the physiological response to delivery systems ranging from oral and parenteral matrix device to colloidal carriers for drug targeting.

Till now, no studies have been done dealing with mapping the biodegradation release and in vivo fate of reinforcing agents upon release from biodegradable polymers by MRI. Ideally, such a study should
provide information about individual agents or a small cluster of agents upon their release from the polymer matrix. However, the agents by themselves will show very poor contrast unless tagged with contrast agents to enhance their imaging in vivo. It is quite possible that distribution of the agents will be in the nanomolar or picomolar range. At such a low concentration, gadolinium CAs in current clinical use do not possess sufficiently large relaxivities to generate sufficient contrast. Such molecular imaging applications require that each Gd\(^{3+}\) center possess an extremely large relaxivity in order to induce sufficient signal intensity. The exceptionally large \(T_2\) relaxivities of the gadonanotubes should provide sufficient signal/noise, even if they are present in the nanomolar concentration range and permit detection of small cluster of gadonanotubes by MRI. Currently, studies are underway in our lab to monitor by MRI the in vivo biodegradation release and distribution of the gadonanotubes from gadonanotubes/PLGA nanocomposites and histologically correlate this distribution.

**CONCLUSIONS**

This study demonstrated by using a LIVE/DEAD cytotoxicity assay that gadonanotubes at concentrations between 1 and 100 ppm and gadonanotubes/PLGA nanocomposites upto 2 wt % are cytocompatible in vitro. For the first time \(r_2\) relaxivity measurements were obtained for the superparamagnetic gadonanotubes. In vitro 7T MRI of the superparamagnetic gadonanotubes [0.15 mM] suspended in a biocompatible 1% Pluronic F127 solution gave a \(r_2\) value of 578 mM\(^{-1}\) s\(^{-1}\); and showed excellent \(T_2\)-weighted contrast enhancement for the MRI phantom imaging. Subcutaneous injection of this gadonanotube suspension into the dorsal region of rats also resulted in an excellent and prolonged negative contrast enhancement. The in vitro MRI of nanocomposite and their degradation process also showed strong gadonanotube water interactions. The study indicates that the gadonanotubes-reinforced PLGA nanocomposites maybe suitable as tissue engineering scaffolds to simultaneously achieve excellent mechanical reinforcement as well as monitoring by MRI of the biodegradation release and biodistribution of gadonanotubes in vivo.

**References**