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Multiwall carbon nanotubes as MRI contrast agents for tracking stem cells

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Abstract

In this study we investigate the potential of multiwall carbon nanotubes (MWCNTs) with low metal impurities (2.57% iron) as magnetic resonance imaging (MRI) contrast agents. Taking into account probable aggregation at high MWCNTs concentration analysis shows that the r_2 relaxivity of MWCNTs in 1% agarose gels at 19 °C is $564 \pm 41 \text{ s}^{-1} \text{ mM}^{-1}$; this is attributed to both the presence of iron oxide impurities and also to the carbon MWCNT structure itself. Stem cells were labelled with MWCNTs to demonstrate the effectiveness of MWCNTs as MRI contrast agents for cellular MRI. The MWCNTs did not impair cell viability or proliferation. These results suggest that the MRI contrast agent properties of the MWCNTs could be used *in vivo* for stem cell tracking/imaging and during MWCNT-mediated targeted electro-chemotherapy of tumours.

1. Introduction

The unique physical and chemical properties of carbon nanotubes (CNTs) have produced considerable interest amongst scientists in the last five years, particularly their potential in the area of biomedical research. Recent developments in the chemical modification and functionalization of multiwall carbon nanotubes (MWCNTs) have opened up new approaches for modification of these nanomaterials, such as conjugation of bioactive species (proteins, carbohydrates, and nucleic acids) (Cui 2007). These studies share a common aim which is to customize these molecular structures at the atomic scale, and hence produce nanomaterials where features such as their optical, chemical and magnetic properties can be tailored. The goal is to produce bespoke multifunctional 'nano-devices'. For example, these MWCNTs can be functionalized and used as magnetic carriers for drug or gene delivery (Foldvari and Bagonluri 2008). Recently we have demonstrated that MWCNTs are able to interact with cells, enter inside the cytoplasm and, when exposed to a magnetic field, induce their migration towards the magnetic source (Pensabene *et al* 2008, Raffa *et al* 2008). Cai *et al* have exploited the magnetic properties of MWCNTs to develop a method of *in vitro* and *ex vivo* gene transfer through 'nanotube spearing', capable of effective cell transfection with

plasmid DNA (Cai *et al* 2005). Carbon nanotubes can also be used as localized heat sources for hyperthermic ablation of tumours, since they strongly absorb near infrared (NIR) light before emitting heat. With this technique, Shi Kam *et al* achieved selective cancer cell destruction *in vitro* with folate-functionalized nanotubes (Kam *et al* 2005). Carbon nanotubes also absorb energy and release heat when exposed to radio frequency (RF) radiation. This approach was used by Gannon *et al* to induce efficient heating of aqueous suspensions of single wall carbon nanotubes (SWCNTs) (Gannon *et al* 2007), enabling the selective thermal destruction of human cancer cells *in vitro*. In this paper we are interested in designing MWCNTs as magnetic resonance imaging (MRI) contrast agents with the potential for *in vivo* cell tracking (Bai *et al* 2008). Carbon nanotubes have potential for multi-modal imaging as they can also be loaded with x-ray contrast agents such as molecular iodine (I_2) (Ashcroft *et al* 2007), and radio-therapeutic agents, for example by doping with $^{211}AtCl$ molecules (Hartman *et al* 2007).

The most commonly used T_2 MRI contrast agents in the clinic are superparamagnetic iron oxide nanoparticles such as Feridex (Qian *et al* 2010). There is considerable on-going research into the development of more sensitive MRI contrast agents with greater selectivity (Modo and Bulte 2007). Several papers have been published on the potential of nanotubes as T_2 MRI contrast agents; typically they use ferromagnetic metals such as Fe, Co, or Ni. Bai *et al* synthesized superparamagnetic iron oxide nanoparticles (SPIONs) directly in the pores of silica nanotubes (SNTs). The integration of SPIONs with SNTs transfers the superparamagnetic characteristics of SPIONs into the SNTs, creating unique magnetic nanoparticles showing promising results (Bai *et al* 2008). Another interesting approach involves the modification of the shape of the magnetic nanoparticles (Park *et al* 2008) to increase the effectiveness of the MRI contrast agent. T_1 MRI contrast agents have also been produced by loading the MWCNT with gadolinium (Gd^{3+}) ions (Sitharaman *et al* 2010). Al Faraj *et al* investigated the potential of super-purified SWCNTs containing only 0.8% iron as an MRI contrast agent, as well as SWCNTs with much higher (10%) iron content. They reported that only the nanotubes with 10% Fe content could be detected *in vivo* by MRI (Al Faraj *et al* 2009). A limitation of the high Fe content materials is cellular toxicity originating from high metal concentrations which can induce oxidative stress (Häfeli and Pauer 1999, Jimenez *et al* 2000). The use of pristine or low Fe content MWCNTs as MRI contrast agents has been reported (Ananta *et al* 2009). One recent publication showed that zigzag SWCNTs are inherently paramagnetic by virtue of their chirality, diameter and length, with shorter length tubes being more magnetic (Chen *et al* 2004). Furthermore, the presence of defect sites in SWCNTs also enhances their magnetic properties (Hod *et al* 2007). This suggests a novel sub-class of T_2 -weighted MRI contrast agents where the performance is dependent on the shape and morphology of the contrast agent.

Recent studies with mesenchymal stem cells (MSCs) have highlighted their potential for tissue regeneration (Bussolati and Camussi 2006). The development of clinical therapies based on the transplantation of the stem cells requires methods for monitoring stem cell delivery and following their bio-distribution over time. In addition, the viability of the stem cells has to be assessed, as well as their effectiveness at targeting the organ of interest and engraftment (Hoshino *et al* 2007, Suzuki *et al* 2007). MRI offers great promise as it is non-invasive and available as pre-clinical and clinical platforms. However, it does require cells to be labelled with MRI contrast agents either by endocytotic internalization or cell surface attachment (Weissleder *et al* 1997, Handgretinger *et al* 1998). There are a variety of superparamagnetic T_2 contrast agents that have been developed for tracking stem cells. An ongoing concern is that the metal ion in the nanoparticles has toxicological effects on the cells (Bulte *et al* 2001). Therefore, it is necessary to develop nanoparticles that are non-toxic, biocompatible, efficient at intracellular labelling, and highly sensitive for MRI detection.

In this paper, we determine the effectiveness of low Fe containing MWCNTs as MRI contrast agents. Specifically, we investigated their ability to modify aqueous longitudinal (T_1) and transverse (T_2) relaxation times, and measure the transverse relaxivity (r_2) of these low Fe containing MWCNTs in 1% agarose gels. Mesenchymal stem cells were labelled with MWCNTs and the effect on cells' viability investigated. We obtain three-dimensional (3D) MRI images of mesenchymal stem cells labelled with low Fe containing MWCNTs and thus demonstrate their potential for use as contrast agents for *in vivo* MRI cell tracking studies.

2. Materials and methods

2.1. MWCNT samples

MWCNTs (provided by Nanothinx SA, Greece) were produced by catalytic chemical vapour deposition (CCVD) of hydrocarbon sources on substrates of metal oxides (Al_2O_3) impregnated with metal catalysts (Fe). The MWCNTs produced in this way have been characterized (Vittorio *et al* 2009) by scanning electron microscopy (SEM), Raman spectroscopy, transmission electron microscopy (TEM), thermogravimetric analysis (TGA), and focused ion beam (FIB) microscopy. The purity of CNTs was assessed by a postdeposition TGA treatment, which also provided a measure of the residual metal catalysts percentage. SEM and TEM were used for the morphology and structural examination of CNTs. Raman spectroscopy was used for the determination of the presence of defects and amorphous carbon content. The measurement of the MWCNTs length was performed by a FIB system FEI 200 (FIB localized milling and deposition) delivering a 30 keV beam of gallium ions (Ga^+). The carbon content of the sample estimated by inductive coupled plasma mass spectrometry, was 97.06% with less than 1% amorphous carbon and the metal particle content was 2.94% (Al and Fe in a ratio Fe/Al of 7 ± 1 (w/w)). Thus the MWCNTs have iron content of 2.57%. The average length of the MWCNT was 2 μm and the diameter ranged from 20 to 40 nm. The magnetic properties of the CNTs utilized in these experiments were previously analysed by the use of a SQUID magnetometer (MPMSXL-7, Quantum Design). The magnetization curve was recorded for solid samples of 5 mg of CNTs. Measurements were performed at 37 °C with an applied magnetic field up to 20 000 Oe. The analysis showed a magnetization curve typical of paramagnetic materials, with a coercivity of about -400 Oe and a remanence of about 0.44 emu g^{-1} . Saturation magnetization (m_s) was about 1.6 emu g^{-1} for a field of about 5000 Oe (Raffa *et al* 2010a, 2010b).

Pluronic F127 (PF127), polyoxyethylene-polyoxypropylene block copolymer (Sigma-Aldrich, St. Louis, MO) surfactant was dispersed in PBS 1X (Sigma) at 0.1% concentration. MWCNTs were added (0.4 mg ml^{-1}) and heated over a hot plate (70 °C) with magnetic stirring for 2 h. The resulting mixture was sonicated with a Branson sonicator 2510 (Branson, CT, USA) at 20 W. The mixture was then centrifuged at 900g for 10 min to remove residual non-suspended nanotubes and any impurities. The concentration of the MWCNT solution, measured by spectrophotometric analysis (Li *et al* 2006) was 100 $\mu\text{g ml}^{-1}$. These dispersed MWCNTs had an average length of 2 μm with a diameter of 40 nm (Vittorio *et al* 2009).

2.2. Mesenchymal stem cell isolation and culture

Rat bone marrow stem cells were collected from the tibia and femur of Wistar Furth animals following the Dobson procedure (Dobson *et al* 1999). The MSCs were cultured in Dulbecco modified culture medium (Sigma-Aldrich, Italy) supplemented with 10% foetal bovine serum (FBS) (Eurobio, Italy), 1% L-glutamine (Sigma-Aldrich, Italy), penicillin (Eurobio, 50 U ml^{-1}), streptomycin (Eurobio, 50 $\mu\text{g ml}^{-1}$), amphotericin B (Sigma-Aldrich 0.2 $\mu\text{g ml}^{-1}$) and incubated at 37 °C in a fully humidified atmosphere containing 95% air and 5% CO_2 .

After seven days, half of the culture medium was changed. On reaching confluence, the adherent cells were detached by 0.05% trypsin and 0.02% EDTA for 5–10 min at 37 °C, harvested and washed with HBSS (Hank's buffered salt solution) and 10% FBS and finally re-suspended in complete medium (primary culture, P0). Cells were re-seeded at 10^4 cells cm^{-2} in 100 mm dishes (P1) for cellular expansion. This was achieved by successive cycles of trypsinization and re-seeding. The frequency of colony forming units-fibroblasts (CFU-F) was measured using the method of Castro-Malaspina. Visible colonies with 50 or more cells were counted and transferred to 10^6 plated cells (number of CFU-F/ 10^6 total nucleated cells). The experimental procedures were carried out with the approval of the Ethical Committee for Animal Experimentation of the University of Pisa.

2.3. MSC–MWCNT samples preparation

Mesenchymal stem cells were seeded in a T 25 flask in a concentration of 2×10^5 cells ml^{-1} with DMEM culture medium. After 6 h incubation, the medium was changed to a modified medium containing MWCNTs dispersed in pluronic solution (PF127-MWCNTs). The modified medium was prepared by mixing PF127-MWCNTs solution and cell culture medium in a ratio of 1:10 (v/v), containing $10 \mu\text{g ml}^{-1}$ of MWCNTs and 0.01% of PF127. This modified medium was stable for over a month from preparation with no evidence of nanotube bundling or any precipitation. In order to study the effect of the surfactant, we prepared PF127 modified cell culture medium at the same concentration previously utilized for the nanotubes (0.01% w/v). After three days, the modified medium was changed and on the fifth day the cells were ready for the *in vitro* assays.

2.4. Cell proliferation assay

The WST-1 (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disul fophenyl)-2H-tetrazolium) cell proliferation assay (BioVision, Mountain View, California) was used to study the effect of MWCNTs on stem cell metabolism. 10×10^3 cells were seeded into each well of a 96-well plate and then incubated with the culture medium. After ten days incubation with MWCNTs solution, $10 \mu\text{l}$ of WST-1 (in accordance with the instructions of the quick cell proliferation assay kit) were added to the culture medium and the plate was incubated for 2 h under standard conditions. The absorbance was measured on a Versamax microplate reader (Molecular Devices, Sunnyvale, California) at a wavelength of 450 nm with background subtracted at 650 nm. The results are expressed relative to the untreated control. The four assay experiments were each carried out six times and expressed as a percentage of the viability of the untreated controls. Values are reported as mean $\pm$ standard error of the mean (s.e.m.).

2.5. Statistical analysis

The results of the cell viability studies were assessed by one way analysis of variance (ANOVA) followed by a post-hoc comparison test (Tukey). Significance was set at 5%.

2.6. Preparation of samples for MRI

The MRI samples were dispersed in low melting agarose (Sigma-Aldrich) gels to avoid settling of the MWCNTs. The 1% agarose (w/v) gels were prepared by dissolving agarose powder in deionized water. The MRI relaxation times were measured for gels containing 97% MWCNTs with concentrations of 10, 18, 50, and $180 \mu\text{g ml}^{-1}$. A control sample without MWCNTs was measured. The mesenchymal stem cells were re-suspended in 1×10^6 cells ml^{-1} in agarose solution and three samples were prepared (one test and two controls); (a) *test*: MSC labelled with surfactant coated 97% MWCNTs and held in agarose gels; (b) *control i*: MSC treated with surfactant and held in agarose gels; (c) *control ii*: cells incubated in normal medium, held in agarose gels.

2.7. Micro-MRI

Micro-MRI data were acquired on a Bruker Avance FT NMR spectrometer with a wide bore 7.1 T magnet resonating at 300.15 MHz for ^1H . A birdcage RF resonator with an internal diameter of 30 mm was used. All acquisitions were made at 19 °C. Two acquisition sequences were collected and averaged to improve the signal-to-noise ratio and reduce artefacts. Relaxation measurements were determined from 128×128 axial planes across the samples with slice thickness of 1 mm. Up to nine samples were studied simultaneously, gels with no contrast agent acted as reference. A recycle time (T_R) of 15 s was used to avoid saturation. The longitudinal (T_1) relaxation times were measured using inversion recovery ($180^\circ - T_1 - 90^\circ$) imaging pulse sequence; eight different inversion times (T_i) that ranged from 100 to 15 000 ms were applied and the echo time (T_E) was 4 ms. The transverse (T_2) relaxation times were measured using a Carr–Purcell–Meiboom–Gill (CPMG) spin echo imaging pulse sequence (Freeman 2003); a train of 16 echoes was acquired with a delay (τ) between 180° pulses of 12 ms. Single exponential relaxation times were calculated from relaxation data. Each sample was studied at least three times from different locations in the sample so as to minimize the problem of uneven distribution of the MWCNTs, and average T_1 and T_2 relaxation times were calculated. Transverse relaxivity (r_2) was calculated using the following equation:

$$r_2 [\text{CA}] = \frac{1}{T_2} - \frac{1}{T_{2o}} \quad (1)$$

where [CA] is the concentration of contrast agent, in this case Fe ions, $1/T_2$ is the transverse relaxation rate in the presence of a known concentration of contrast agent, $1/T_{2o}$ is the transverse relaxation rate of the gel in the absence of contrast agent. The $128 \times 128 \times 128$ three-dimensional (3D) RARE-4 (rapid acquisition with relaxation enhancement) experiments were acquired with a recycle time (T_R) of 250 ms and effective echo time (T_E) of 40 ms. Scanning times were 34 min. The field of view was 30 mm and in-plane resolution was 0.234 mm/pixel. 3D surface representations of the MRI image data sets were produced using Amira® software (Visage Imaging Inc., San Diego, CA USA).

3. Results

3.1. MRI of MWCNTs

The dependency of the ^1H longitudinal (T_1) and transverse (T_2) relaxation rates of 1% agarose gels upon the concentration of 97% MWCNTs is illustrated in figure 1 and tabulated in table 1 A. As commonly observed with iron-based MRI contrast agents (Modo and Bulte 2007), the presence of Fe in the MWCNTs has no significant effect on the longitudinal relaxation rates ($1/T_1$) of the gels. In contrast, the presence of 97% MWCNTs has a significant effect upon the ^1H transverse relaxation rates ($1/T_2$). Relaxivity r_2 ($\text{s}^{-1} \text{mM}^{-1}$) is a measure of the effectiveness of an MRI contrast agent in reducing the aqueous T_2 relaxation times (equation (1)). There is a noticeable scatter in the data at high MWCNT concentration which is probably due to an uneven distribution of CNTs in the agarose gel, despite careful sample preparation and measuring the relaxation rate from at least five different locations. Figure 1(B) presents the relaxation rates ($1/T_2$) of the five different 1% agarose gel samples. A straight line fit of the five points (dashed black line) calculates the r_2 relaxivity as $272 \text{ s}^{-1} \text{mM}^{-1}$, but is a very poor fit with a high χ^2 value of 65. It would appear that the $180 \mu\text{g ml}^{-1}$ MWCNT sample which has an Fe concentration of 0.0828 mM is an outlier (circled). It is known that MWCNTs have a tendency to aggregate and this is more prevalent at higher CNT concentrations. If the data from this sample, with its Fe concentration of 0.0828 mM, are ignored assuming excessive aggregation, then there is a considerable improvement in the straight line fit (solid grey line in figure 1(B)) using the

remaining four data points. The relaxivity calculated using the four data points is $564 \pm 41 \text{ s}^{-1} \text{ mM}^{-1}$, and the 95% confidence intervals are (523, 605) from the χ^2 straight line fit, with a χ^2 value of 0.28. Thus, the relaxivity r_2 of these MWCNTs is greater than that of the commercial iron oxide contrast agent Feridex ($r_2 = 148 \text{ s}^{-1} \text{ mM}^{-1}$) measured under similar conditions (Chen *et al* 2010). This result indicates that the shape and size of the nanoparticles, as well as their iron content significantly affects the r_2 relaxivity and the nanoparticles' effectiveness as an MRI contrast agent.

3.2. Stem cells tracking

The effectiveness of cellular imaging using 97% MWCNTs as an MRI contrast agent was investigated by labelling MSCs. Three vials were prepared: the first (*test*) containing 97% MWCNTs in MSCs treated with surfactants held in agarose gels (CNT), the second (*control i*) containing MSCs treated with surfactants held in agarose gels (PF127), and the third (*control ii*) with MSCs held in agarose gels (C). To simulate a 3D heterogeneous biological sample, all three vials were placed together in a plastic holder and embedded in 1% agarose gel (figure 2(A)). The ^1H longitudinal (T_1) and transverse (T_2) relaxation times of each sample were measured at least three times and an average calculated (table 1(B)). The ^1H T_1 relaxation times of the three vials and the 1% agarose gel did not vary significantly, ranging from 2.81 to 2.99 s. However, differences were observed in their T_2 relaxation times. The T_2 relaxation times of cells containing MWCNTs plus surfactant in agarose ($71.6 \pm 3.5 \text{ ms}$) was lower compared to (*control i*) cells with surfactant in agarose ($92.6 \pm 3.5 \text{ ms}$) and (*control ii*) cells in agarose but without surfactant ($80.8 \pm 6.8 \text{ ms}$). The 1% agarose gel had the highest ^1H T_2 relaxation time ($98 \pm 7 \text{ ms}$).

To determine the effect on image contrast, 3D RARE-4 ($T_R/T_E = 250/40 \text{ ms}$) MRI images of the samples were acquired, this took 34 min which is suitable for *in vivo* imaging. An axial image through the 3D RARE-4 data set is shown in figure 2(B). Regions of hypo-intensity can be seen in the T_2 -weighted RARE image of the MSCs cells containing MWCNTs. This is due to the localized magnetic inhomogeneities produced by the MWCNTs which cause the MRI magnetization in these regions to dephase. A 3D surface representation of the three vials and the agarose gel was obtained by digitally segmenting the MRI data set using Amira® software (figure 2(C)). This study confirms that MWCNTs are effective T_2 MRI contrast agents and their presence inside the cells can be detected in T_2 -weighted images.

We investigated the possible toxicological effect of low Fe containing MWCNTs coated with PF127 on mesenchymal stem cells using the WST-1 proliferation assay. These assays confirmed that cells incubated with MWCNTs ($10 \mu\text{g ml}^{-1}$) for ten days maintained high proliferation rates similar to the control cells (Wörle-Knirsch *et al* 2006) (figure 3). In particular, cells incubated with nanoparticles showed a 97% proliferation rate which is not statistically significant when compared with the control sample, while we observed 25% loss of viability when cells were treated with PF127 surfactant solution. The lack of toxicity to MSCs is attributed to the action of the surfactant which wraps the nanotube's surface and reduces the amount of free PF127 in the medium (Bardi *et al* 2009).

4. Discussion and concluding remarks

There is great interest in the potential applications of CNTs in medicine. Several researchers have proposed novel therapies which exploit particular physical and chemical properties of CNTs, such as the targeted delivery of drugs, the functionalization of scaffold for tissue engineering and also the thermo-ablation of tumours (Richard *et al* 2008, Raffa *et al* 2010a, 2010b). One of the future challenges facing nanotechnology is the design and development of nano-multifunctional 'devices' that are able to simultaneously perform a variety of biomedical tasks. These novel nanotube therapies will require *in vivo* imaging to assess their

efficacy and monitor the bio-distribution of nanotubes in tissues. Consequently we were interested to determine whether the magnetic properties of these low Fe content CNTs were sufficient for them to be effective as MRI contrast agents. Undoubtedly, the recent advances in cell therapy and transplantation urgently require effective imaging contrast agents and protocols for stem cell tracking. There is an interest in the ability of these cells to grow and differentiate on CNT scaffolds (Park *et al* 2007, Kalbacova *et al* 2006). Mooney and colleagues were one of the first groups studying the interaction of dispersed CNTs and mesenchymal stem cells (Mooney *et al* 2008). The authors demonstrated that COOH-functionalized SWCNTs, used at concentration up to $32 \mu\text{g ml}^{-1}$, did not affect a cell's viability, proliferation, differentiation ability, and metabolic activity. However, at higher concentrations, the SWCNTs exerted detrimental effects on the cells. In contrast, OH-functionalized MWCNTs were found to be toxic at all concentrations. The safety and biocompatibility of CNTs appears to be dependent on the size, purity, and functionalization of the nanotubes (Vittorio *et al* 2009). In the present study, the MWCNTs were not functionalized but simply dispersed in an anionic surfactant, at concentrations of $10 \mu\text{g ml}^{-1}$. These well characterized MWCNT had low Fe content; *in vitro* and *in vivo* toxicological studies of these MWCNTs 97% demonstrated that at $10 \mu\text{g ml}^{-1}$ they showed no adverse effects on cells differentiating in osteocytes and adipocytes as well as in untreated control stem cells (Vittorio *et al* 2011).

The results from this study are very encouraging. Taking into account aggregation of the nanoparticles at high concentration resulted in the relaxation rate of $180 \mu\text{g ml}^{-1}$ for the MWCNT sample acting as an outlier. Analysis shows that low Fe content MWCNTs are effective T_2 MRI contrast agents with r_2 relaxivity of about $564 \pm 41 \text{ s}^{-1} \text{ mM}^{-1}$. Furthermore, the presence of MWCNTs can be detected in cells by T_2 -weighted MRI, opening the way for *in vivo* visualization of the cell movement/trafficking with labelled cells. The r_2 relaxivity of CNTs is usually attributed to the presence of magnetic metal impurities such as Fe and Ni. Ananta *et al* suggested that in addition to the metals, the CNT itself has magnetic properties that contribute to relaxivity (Ananta *et al* 2009). This means that measurement of relaxivity relative to iron concentration alone (equation (1)), although conventionally used, is slightly misrepresentative. Even if MWCNTs represent a good candidate for stem cell tracking, more studies are needed to fully understand the internalization mechanisms and the kinetics of endocytosis and exocytosis of these nanoparticles in the stem cells. A recent review on cellular uptake of CNTs (Raffa *et al* 2010a, 2010b) summarized the internalization process of nanotubes by phagocytosis, endocytosis, or diffusion. The phagocytosis is the internalization pathway for nanotube aggregates, bundles, clusters, or single dispersed nanotubes that are $1 \mu\text{m}$ or more in length. Endocytosis is the internalization mechanism for nanotubes that form supramolecular structures. Otherwise the internalization mechanism is by diffusion. However, there are conflicting data about the mechanism as well as the kinetics of the internalization of CNTs in the cells. Therefore, further research is still required. In addition, *in vivo* studies over time in animal models are required to follow the accumulation and elimination of nanoparticles.

In conclusion, we have demonstrated that MWCNTs with low metal impurities (2.57% iron) can be used as MRI contrast agents even at concentrations of tens of microgram per millilitre, as the MWCNTs have a significant effect on the observed 1H transverse ($1/T_2$) relaxation rate of water. Consequently, MSCs labelled with MWCNTs exhibit a reduced image intensity in T_2 -weighted MR images compared to cells without internalized MWCNTs. The 3D MRI cellular study suggests that it should be possible to track stem cells *in vivo* by labelling cells with these low Fe MWCNTs. Future studies will focus on using MRI to map the bio-distribution of MWCNTs and measure the kinetics of accumulation of MWCNTs in animal model systems.

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Figure 1.

MRI relaxation study of 97% MWCNTs in 1% agarose gels at MWCNTs concentration up to $180 \mu\text{g ml}^{-1}$, illustrating the dependency of (A) longitudinal ($1/T_1$) and (B) transverse ($1/T_2$) ^1H relaxation rates of the gels upon iron concentration of the 97% MWCNTs. The $180 \mu\text{g ml}^{-1}$ MWCNTs data point with an Fe concentration of 0.0828 mM is circled. (C) 128 by 128 ^1H T_2 -encoded CPMG axial image of the gels with echo time of 48 ms and slice thickness of 1 mm. MRI measurements were completed at 7.1 T, at 19 °C, with a field of view of 30 mm, and pixel spatial resolution of 0.234 mm/pixel.

Figure 2.

MWCNTs MRI study of MSCs. Three vials were embedded in a container of 1% agarose: vial 1 (*test*) contains 97% MWCNTs in MSCs treated with surfactants held in agarose gels (cnt); vial 2 (*control i*) contains MSCs treated with surfactants held in agarose gels (pf), and vial 3 (*control ii*) contains MSCs cells held in agarose gels (c). (A) Photograph of the dorsal view of the sample; (B) T_2 -weighted RARE-4 axial image of the sample from the $128 \times 128 \times 128$ RARE-4 ($T_R/T_E = 250/40$ ms) data set; (C) 3D surface reconstruction of the sample from the same MRI data set as (B). MRI measurements were completed at 7.1 T, at 19 °C, with a field of view of 30 mm, and voxel spatial resolution of 0.234 mm/pixel.

Figure 3.

MSCs incubated with MWCNTs for five days: (A) black MWCNTs inside the cells after five days of incubation in modified MWCNT-medium. (B) WST-1 assay for MSCs cultured with the control medium (MSCs), the MWCNT-modified medium and PF127 modified medium for ten days. Results of four experiments each carried out in six-plicate (mean $\pm$ s.e.m. * $p < 0.05$) are given as a percentage of the untreated controls.

Table 1

(A) Longitudinal and transverse relaxation rates of 1% agarose gels containing different concentrations of MWCNTs.
 (B) Longitudinal and transverse relaxation times of different preparations of 1×10^6 cells/ml⁻¹ mesenchymal stem cells re-suspended in agarose gel, including cells labelled with MWCNTs and agarose as a control.

(A) Conc. of MWCNTS ($\mu\text{g ml}^{-1}$) in 1% agarose gels	$1/T_2$ (mean) (s^{-1})		$1/T_1$ (mean) (s^{-1})		s.d.
180	30.3	2.9	0.367		0.013
50	19.6	1.8	0.343		0.008
18	11.4	1.1	0.356		0.020
10	9.1	0.3	0.355		0.017
0	6.6	0.5	0.357		0.014

(B) Mesenchymal stem cell samples	T_2 (mean) (ms)		T_1 (mean) (s)		s.d.
Agarose	98.0	6.95	2.936		0.162
Cells	80.8	6.78	2.830		0.154
Cells + surfactant	92.6	3.51	2.811		0.145
MWCNTs + cells + surfactant	71.6	3.45	2.993		0.036