Gadonanotube materials as new intracellular MRI contrast agents for stem cell labeling

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Stem cells possess great potential for different medical applications and every year more investigators join this field of study. As interest in stem cells has increased, it has become essential to track the cells in vivo in order to study their biodistribution and possible tissue accumulation. Here, we review the use of two new carbon nanotube (CNT)-based contrast agents (CAs) for magnetic resonance imaging (MRI) called Gadonanotube (GNT) materials, which contain Gd$^{3+}$-ion clusters or Gd$^{3+}$ chelates within the sidewall cavities of 20-80 nm long carbon nanotube capsules. These ultra-high-performance $T_1$-weighted CAs have been used to label a number of mammalian cells, including porcine bone marrow-derived mesenchymal stem cells without any observed cytotoxicity. Furthermore, various in vitro and in vivo preclinical studies have demonstrated the safety and potential of these new CNT-based materials as intracellular CA labels for stem cell tracking by MRI.

Keywords: mesenchymal stem cells; gadonanotube; carbon nanotube; contrast agent; MRI


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During the past two decades, there has been a dramatic surge in studies and clinical trials involving stem cell therapies, and such findings are forming the foundation for an emerging medical field centered around the prevention and treatment of various diseases. Current research focuses on the use of patient-derived stem cells to replace damaged tissue and to provide extracellular support due to the release of growth factors or cytokines to promote healing and improvement of tissue function [1]. Despite the numerous advances and promising findings that have been achieved, many remaining issues need to be addressed to fully understand the behavior of stem cells and to develop new strategies to optimize their potential. There are three main challenges that still present fundamental problems for stem cell therapy: 1) the delivery method of the cells and its safety, 2) the retention of the cells at the site of interest and the long-term engraftment of the cells, and finally 3) the spatial distribution of the cells [2]. These problems, although present for all stem cell therapies, are particularly critical for cardiac applications due to constant heart contractions and the abundant blood flow in the heart [2, 3].

To address the last two of these problems: retention and biodistribution of the cells, a powerful, new $T_1$-weighted MRI contrast agent (CA) known as the Gadonanotubes (GNTs) was first synthetized by Wilson and co-workers and its performance evaluated in 2005 [4, 5]. GNTs are ultra-short (US-tubes, 20-80 nm in length) single-walled carbon
nanotube (SWCNT) materials encapsulating clusters of Gd\textsuperscript{3+} ions (Figures 1A, 1B) \cite{4}. GNTs exhibit the greatest $T_1$ relaxivity ($r_1$) known, with values of up to 170 mM\(^{-1}\text{ s}^{-1}\) per Gd\textsuperscript{3+} ion at 40 °C and 1.5 T \cite{4}. To function in biological media, GNTs are usually suspended in a 0.17% Pluronic\textsuperscript{®} F-108 solution via sonication. Pluronic\textsuperscript{®} is a non-ionic surfactant that wraps the GNTs non-covalently allowing the material to be suspended in aqueous media while maintaining its inherent hydrophobicity. Due to the distinctive properties of the GNTs, such as their amphiphilic character (when suspended in surfactants) and biocompatibility, these carbon-based nanostructures are able to translocate across cell membranes without the need of a transfection agent.

Hence, the GNTs have been used to intracellularly label a variety of cells \cite{6-8}, including porcine bone marrow-derived mesenchymal stem cells (MSCs) \cite{9, 10}. MSCs were chosen among the several adult progenitor cells that are present in mammals because they can be easily isolated, easily expanded in cell culture, and they have shown promising outcomes for cardiac tissue repair \cite{2, 11, 12}. Upon cell internalization of GNTs in MSCs, it was found that $\sim 10^9$ Gd\textsuperscript{3+} ions per cell were delivered without affecting cell viability, differentiation potential, or other important properties of MSCs \cite{9}. A nearly two-fold decrease in the $T_1$ relaxation time of GNT-labeled MSCs was obtained when compared to control cells (Figure 1D). Transmission electron

**Figure 1.** Gadonanotube as an intracellular MRI contrast agent for stem cells. (A) Schematic of a single Gadonanotube (GNT): ultra-short single-walled carbon nanotube (US-tube) loaded with hydrated Gd\textsuperscript{3+}-ion clusters. Gd\textsuperscript{3+} loading is possible through side-wall defects created by chemically cutting full-length SWCNTs into US-tubes. (B) TEM image of bundled GNTs showing the Gd\textsuperscript{3+}-ion clusters (black arrows) formed within US-tubes as confirmed by energy-dispersive spectroscopy (EDS) measurements. (C) TEM images of GNT-labeled MSC. Red arrows indicate GNT aggregates in the cytoplasm, and yellow arrows outline the nucleus of the MSC. Scale bar = 1µm. (D) $T_1$-weighted MRI phantom images at 1.5 T and 25 °C of cell pellets; unlabeled MSCs (control cells, left) and GNT-labeled MSCs at TI = 500 ms Figures (A) and (B) adapted from [4], Copyright (2005), with permission from Royal Society of Chemistry. Figures (C) and (D) adapted from [9], Copyright (2010), with permission from Elsevier.

**Figure 2.** Photographs of the in vivo retention of the GNT-labeled MSCs for a porcine heart. (A) A butterfly needle was used to inject the MSCs around a 1.3 T NdFeB ring magnet sutured onto the left ventricular anterior wall of the heart. (B) The white arrows indicate the presence of the GNT-labeled MSCs near the injection sites post-organ removal. Figure adapted from [10], Copyright (2014), with permission from Elsevier.
microscopy (TEM) images showed that GNTs do not translocate into the nucleus but instead accumulate in the cytoplasm in aggregated form with no visible vesicles enclosing the GNTs, suggesting that a passive form of cell transport takes place (Figure 1C) [9].

Besides being MRI-active, GNTs are magnetic, and hence, they respond to an external magnetic field. This behavior is due to paramagnetic/superparamagnetic metal impurities present in the US-tubes, such as nickel (as nickel oxide), which is one of the metal catalysts used in the synthetic process to prepare the SWCNTs from which the US-tubes are derived (AS-SWCNTs, Carbon Solutions Inc.) [13]. As a result, GNTs can render MSCs highly magnetic once internalized. The magnetic properties of the GNTs have been studied in vivo for the purpose of using GNTs as magnetic facilitators to increase the retention of MSCs at the site of injection. In a previous study, GNT-labeled MSCs were injected transepicardially into the left ventricle of female juvenile domestic pigs [10]. Prior to injection, a 1.3 T NdFeB ring magnet was sutured onto the anterior wall of the left ventricle, as shown in Figure 2A, and the GNT-labeled MSCs were then transplanted around the inner and outer perimeters of the ring magnet. In the study, it was found that GNTs can be used as a magnetic facilitator to effectively increase cell retention at target cardiac sites in the presence of a strong external magnetic field. GNTs improved the retention of transplanted cells by three times over unlabeled control cells, while the implantation of a strong magnet onto the heart was well tolerated with no inflammation of tissue for up to 168 h. A group of animals was kept alive for up to 30 d, indicating the safety of using MSC-labeled GNTs for cardiac therapy in a large animal model [10, 14]. The amount of GNT-labeled MSCs retained in the tissue was obtained quantitatively by inductively-coupled plasma mass spectrometry (ICP-MS). From this study, it was concluded that GNTs can be used to address two of the main challenges associated with stem cell therapy: tracking and retention of
In a subsequent study, water-suspendible GNTs were synthetized by covalently functionalizing the surface of the US-tubes (Figure 3A)\[^{15}\]. Acidic conditions are used for the functionalization, and it was found that when GdCl\(_3\) is used as the Gd\(^{3+}\)-ion source, 90% of the Gd\(^{3+}\) ions leak out of the carbon nanotubes during the acidic functionalization process\[^{15}\]. Therefore, two alternative Gd-based agents were explored in order to load Gd\(^{3+}\) ions within the cavities of US-tubes: 1) Gd(acetylacetone)\(_3\)•2H\(_2\)O chelates (Gd(acac)\(_3\)•2H\(_2\)O) and 2) Gd(hexafluoroacetylacetone)\(_3\)•2H\(_2\)O chelates (Gd(hfac)\(_3\)•2H\(_2\)O). After encapsulation of the gadolinium chelates within the US-tubes as previously reported\[^{16}\], the surface of the US-tubes was repeatedly functionalized (4×) with \(p\)-carboxyphenyldiazomium (PCP) tetrafluoroborate salt which covalently attached benzoic acid moieties to the outer surface of the US-tubes, producing a material that was highly water suspendible (35 mg/mL). The hydrophilic PCP-Gd(acac)\(_3\)•2H\(_2\)O@US-tubes, containing 3.8% of Gd\(^{3+}\) by weight, were shown to be the most suitable CA, which displayed the highest performing \(T_1\)-weighted MRI phantom images using a 1.5 T scanner at 25 °C (Figure 3C).

The water-soluble PCP-Gd(acac)\(_3\)•2H\(_2\)O@US-tubes were then used to intracellularly label porcine bone marrow-derived MSCs, delivering approximately \(10^9\) Gd\(^{3+}\) ions per cell, which is the same Gd\(^{3+}\)-ion concentration per cell that is delivered by Pluronic\(^\circledR\)-wrapped GNTs. Viability and cytotoxicity studies of the labeled cells showed more than 98% cell viability when compared to control cells. TEM images of the labeled cells demonstrated accumulation of the material in the cytoplasm, with no evidence of the material translocating into the nucleus (Figure 3B).

TEM analysis showed that both the Pluronic\(^\circledR\)-wrapped GNTs and water-soluble PCP-Gd(acac)\(_3\)•2H\(_2\)O@US-tubes formed agglomerates in the cytoplasm of the MSCs. Despite the fact that the GNTs form more aggregates within the cells due to their higher hydrophobicity which make them accumulate in bundles, both CAs delivered the same concentration of Gd\(^{3+}\) ions intracellularly.

In summary, the two new CNT-based MRI CAs described in this article are easily taken up by MSCs without the need of transfection agents, which tend to cause some cytotoxicity\[^{17, 18}\]. Both CAs delivered \(\sim 10^9\) Gd\(^{3+}\) ions per cell, thus rendering the cells MR active. No adverse effects on the MSCs were observed with either agent. Although labeled MSCs have been thoroughly studied for the case of Pluronic\(^\circledR\)-wrapped GNTs, in order to make a complete comparison between the two CA materials, more studies are needed to evaluate the phenotypic and genotypic behavior of labeled MSCs for the case of the water-suspendible PCP-Gd(acac)\(_3\)•2H\(_2\)O CA. Nevertheless, by eliminating the use of surfactant, the PCP-Gd(acac)\(_3\)•2H\(_2\)O@US-tubes should have greater potential of also becoming an extracellular MRI CA \(in vivo\) via intravenous injection, thus further expanding their clinical potential in the future\[^{19}\].

**Conflicting interests**

The authors have declared that no conflict of interests exist.

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**Abbreviations**

MSC: mesenchymal stem cells; CA: contrast agent; CNT: carbon nanotube; SWCNT: single-walled carbon nanotube; GNT: gadonanotube; Gd(acac)\(_3\)•2H\(_2\)O: Gd(acetylacetone)\(_3\)•2H\(_2\)O chelates; Gd(hfac)\(_3\)•2H\(_2\)O: Gd(hexafluoroacetylacetone)\(_3\)•2H\(_2\)O chelates; PCP: \(p\)-carboxyphenyldiazomium.

**References**


