Magnetic core–shell nanowires as MRI contrast agents for cell tracking

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Abstract
Background: Identifying the precise location of cells and their migration dynamics is of utmost importance for achieving the therapeutic potential of cells after implantation into a host. Magnetic resonance imaging is a suitable, non-invasive technique for cell monitoring when used in combination with contrast agents.

Results: This work shows that nanowires with an iron core and an iron oxide shell are excellent materials for this application, due to their customizable magnetic properties and biocompatibility. The longitudinal and transverse magnetic relaxivities of the core–shell nanowires were evaluated at 1.5 T, revealing a high performance as T2 contrast agents. Different levels of oxidation and various surface coatings were tested at 7 T. Their effects on the T2 contrast were reflected in the tailored transverse relaxivities. Finally, the detection of nanowire-labeled breast cancer cells was demonstrated in T2-weighted images of cells implanted in both, in vitro in tissue-mimicking phantoms and in vivo in mouse brain. Labeling the cells with a nanowire concentration of 0.8 μg of Fe/mL allowed the detection of 25 cells/μL in vitro, diminishing the possibility of side effects. This performance enabled an efficient labelling for high-resolution cell detection after in vivo implantation (~10 nanowire-labeled cells) over a minimum of 40 days.

Conclusions: Iron-iron oxide core–shell nanowires enabled the efficient and longitudinal cellular detection through magnetic resonance imaging acting as T2 contrast agents. Combined with the possibility of magnetic guidance as well as triggering of cellular responses, for instance by the recently discovered strong photothermal response, opens the door to new horizons in cell therapy and make iron-iron oxide core–shell nanowires a promising theranostic platform.

Keywords: Cell tracking, Magnetic resonance imaging, Iron-iron oxide, Core–shell, Nanowires, Cell labeling, T2 contrast

Background
Over the last years, nanomaterials have been widely investigated for improving the diagnosis of diseases and their treatments [1]. Thanks to their structural, chemical, optical or mechanical properties, nanomaterials provide the ability to interact with cells in different ways and with remote control mechanisms [2–4]. Due to the importance of a very early-stage detection of disease, biomedical imaging has become an invaluable tool for both scientific and clinical applications [5]. In particular, magnetic resonance imaging (MRI) is a powerful tool, allowing the non-invasive and real-time monitoring of
dynamic processes in living cells and organisms. Contrast agents (CAs) are often utilized in MRI, to better discern tissues of similar magnetic properties, by shortening the transversal \( T_2 \) and/or the longitudinal \( T_1 \) relaxation times of the nearby water protons at the region of interest [6, 7]. Commercially available \( T_1 \) CAs are paramagnetic complexes, usually gadolinium (Gd\(^{3+}\)) chelates [8], while \( T_2 \) CAs are mostly based on iron oxide (Fe\(_{3}O_{4}\)) nanoparticles (NPs) [9, 10]. Fe\(_{2}O_{3}\) NPs are superparamagnetic and exhibit a magnetization proportional to an external magnetic field, which generates local magnetic field inhomogeneities and accelerates the dephasing of the surrounding protons’ spins [7]. Limitations such as the passive accumulation of Fe\(_{3}O_{4}\) NPs in the liver and spleen [11, 12] together with signal attenuation (low signal-to-noise ratio) [13, 14], strong blooming effect [15], and high background interference (low specificity) [16] have restricted their clinical application [17–19]. Nevertheless, \( T_2 \) CAs are highly appreciated for research purposes in relevant applications such as cell tracking studies [20]. Besides, a new generation of particles is being developed to tackle the previously encountered issues [21, 22] and functionalities are dramatically expanded by imaging and guiding the particles, i.e. magnetic resonance navigation [23]. In order to be an efficient \( T_2 \) CA, a nanomaterial should possess a large transversal magnetic relaxivity constant, \( r_2 \), which is proportional to the effective magnetic moment of the material [24]. This requirement places nanowires (NWs) composed of iron (Fe) in a prime position, due to their high saturation and remanent magnetization values, the latter originating from the strong shape anisotropy [25, 26]. NWs can be grown in nanoporous templates by electrochemical deposition, which is a simple and efficient method that provides accurate control of the length and diameter [27–29].

Previously, the performance of Fe-based NWs and multisegmented Fe/gold NWs was assessed, where both types of NWs appeared to be promising \( T_2 \) CAs [30]. Similarly, an aqueous suspension of coated nickel NWs exhibited good performance for \( T_2 \) contrast with \( r_2 \) values similar to those of commercial Fe\(_{3}O_{4}\) NPs [31]. However, genotoxicity and cytotoxicity effects have been reported in nickel-containing particles [32].

In this paper, we investigate the performance of Fe–Fe\(_{3}O_{4}\) core–shell NWs as tunable \( T_2 \) CAs. We utilize them for MRI cell tracking, which is a rapidly advancing medical method since the earliest studies of stem cell detection [33–35]. In order to employ the therapeutic potential of cells, their precise location, and the dynamics of their migration and differentiation after implantation into the host must be monitored and understood. Fe\(_{3}O_{4}\)-based nanomaterials are among the most used systems for labeling of cells for in vivo tracking experiments [20, 34, 36]. One of the key aspects of cell tracking studies is the sensitivity to detect a small number of cells after implantation. For this task, the strong magnetization [37] and biocompatibility [38–40] of Fe–Fe\(_{3}O_{4}\) core–shell NWs are foreseen as advantageous characteristics.

In this work, we studied the actuation of Fe–Fe\(_{3}O_{4}\) core–shell NWs for \( T_2 \) contrast at representative clinical (1.5 T) and preclinical (7 T) fields, and at a different level of oxidation, and surface coatings. The amount of NWs interacting with the cells was spectrometricaly quantified and the labeling sensitivity was assessed by in vitro \( T_2 \)-weighted images of the cells embedded in an agar gel. In vivo MRI cell tracking studies were carried out at 11.7 T, implanting the labeled cells into mouse brains.

**Results and discussion**

**Nanowire characterization**

NWs composed by Fe were produced with an average diameter of 30 to 40 nm and an average length of 0.7 ± 0.16 μm (n = 100), as illustrated in Fig. 1a, b. Since NWs are exposed to air, water, sodium hydroxide, ethanol, etc., oxidation of their surface is unavoidable. The oxidation creates core–shell NWs with an oxide layer that is typically 4–10 nm thick [39] composed of mainly Fe\(_{2}O_{3}\) and Fe\(_{3}O_{4}\) [37, 40], and has a significant contribution to the biocompatibility [37], functionalizability, and magnetic properties of the NWs [39–43]. The electron energy loss spectroscopy (EELS) composition map in Fig. 1c confirms a core–shell structure with an Fe core (red color) surrounded by an Fe\(_{3}O_{4}\) shell (blue color).

In order to increase the thickness of the oxidation shell and thereby reduce the Fe core, a sample of NWs was subjected to oxidizing conditions (NWs oxidized). The level of oxidation was evaluated by scanning transmission electron microscopy (STEM) and EELS in NWs that were placed in oxidizing conditions, and NWs that remained in ethanol all the time at room temperature. In both cases, the average relative concentrations of oxygen and Fe were measured in a specific region of different single NWs (Additional file 1: Figure S1) showing a mix between both elements in the oxidized shell and Fe in the core, (Additional file 1: Table S1, n = 3 for each condition). The oxygen/Fe ratio indicates the change in the thickness of the oxidation layer, which was then expressed as the percentage of oxygen and Fe in each NW analyzed. The average percentage of oxygen was found to be 33.7 ± 7% for the treated NWs and 17.7 ± 3% for the control NWs with a native oxide layer.

The magnetization curves of both samples of NWs with different oxidation level are shown in Fig. 1d. From these, the values of the saturation magnetization (\( M_s \)) are 108 Am\(^2\)/kg of Fe for the NWs with a native oxide layer and 65 Am\(^2\)/kg of Fe for the oxidized sample. The \( M_s \) of the
The magnetization curves of Fe-based NWs with native oxide layer (black curve) and oxidized NWs (red curve) (Magnetization M is presented as Am²/kg of Fe,  there is the saturation magnetization, and  Hc the coercive field, n = 3).

The NWs oxidized showed approximately 40% reduction in the Ms compared to NWs with native oxide layer, which is directly related to the increase in the thickness of the Fe₃O₄ shell in the oxidized sample [37]. It could also be attributed to corrosion (Fe ions lost in the hydrogen peroxide oxidation step) [49]. If assuming a linear relationship between the oxide shell volume and the Ms reduction, it is easy to show that this 40% reduction in Ms corresponds to an increase of shell thickness of around 4 nm in each NW. The coercive field was ~ 125 kA/m for both formulations since the NWs take random orientations, compared to NWs inside a template.

In this study, NWs with a native oxide layer and NWs oxidized were coated with (3-aminopropyl) triethoxysilane, APTES [50], (APTES-NWs and APTES-NWs oxidized, respectively) and NWs with native oxide layer were coated with bovine serum albumin, BSA, (BSA-NWs) as previously reported [51, 52]. The presence of both coating agents around the Fe-based NWs has been previously confirmed [38, 40, 52], and has been shown to improve the biocompatibility and colloidal properties of the NWs with a reduction of agglomeration and enhancement of dispersion [40, 43]. Such improvement can be attributed to electrostatic interactions supported by a zeta potential of −58 mV [43] and −17.4 mV [52] for the BSA-NWs and APTES-NWs, respectively. Furthermore, the presence of free chemical groups of both, BSA and APTES enables further functionalization of the NWs [40, 43, 51, 53].

**Relaxivity measurements**

The longitudinal (r₁) and transversal (r₂) relaxivities, as well as r₂/r₁ ratios, for different formulations of NWs were calculated from the relaxation times measured on an MR relaxometer at 1.5 T (Additional file 1: Figure S2, Table S2) in a preliminary characterization. Linear fitting of the relaxation rates (R₁ = 1/T₁, Additional file 1: Figure S2A and R₂ = 1/T₂, Additional file 1: Figure S2B), as a function of Fe concentration, was performed using:

\[
R_{1,2}(s^{-1}) = R_{10,20} + r_{1,2}[CA],
\]

where R₁₀₂₀ are the relaxation rates of the solvent, R₁₂ the relaxation rates of NW solutions and [CA] the concentration of CA. Considering the elevated r₂ values and r₂/r₁ ratios obtained for all Fe-based NW formulations, and their practically negligible effect on T₁, these materials can be considered as competitive T₂ CAs (Additional file 1: Table S2) [7]. Moreover, the performance of Fe-based NWs for T₂ contrast (r₂ ranging from 35 to 70 s⁻¹ mM⁻¹) is close to commercial Fe₂O₃-based CAs such as Endorem™ (r₂ ~ 41 s⁻¹ mM⁻¹) and Resovist (r₂ ~ 61 s⁻¹ mM⁻¹) at 1.5 T [54].

Initial exploration of r₂ relaxivities of selected formulations of Fe-based NWs at preclinically (7 T) relevant magnetic field (Table 1), showed that r₂ values varied with time. Therefore, a set of experiments was performed measuring r₂ for 160 min, with a lapse of 20 min between
each time point. The first experimental $r_2$ value was obtained at $t=20$ min (time required for securing the samples inside the scanner, tuning the system, and acquire the required MR images). An exponential decay of $r_2$ with time was observed, and the estimated $r_2$ values (Table 1) at time zero (beginning of the magnetic field exposition) for each sample were obtained after fitting the $r_2$ vs. time plots to a mono-exponential decay equation (Additional file 1: Figure S3A). A semilogarithmic representation of the $r_2$ values over time (Additional file 1: Figure S3B), as well as the half time signal decay ($t_{1/2}$ in Table 1) for each of the NW formulations, confirm the mono-exponential decay of the $r_2$. The Fe-based NWs showed an $r_2$ comparable to the one reported for the commercial formulation Feridex® at 7 T ($166.71$ s$^{-1}$ mM$^{-1}$)[55]. A reduction in $r_2$ values is observed, when comparing non-oxidized and oxidized NWs (NWs vs. NWs$_{oxidized}$ (non-coated formulations) and APTES-NWs vs. APTES-NWs$_{oxidized}$ (coated formulations)). This decrease in $r_2$ of ~40% and ~30% for the non-coated and coated formulations, respectively, is related to the 40% decrease in the $M_S$, due to the oxidation of the NWs, which, in turn, reduces NW agglomeration. It has been reported that different factors, such as chemical composition, particle size and geometry, agglomeration, and polydispersity directly interfere with $r_2$ [53, 56–61]. In fact, low levels of aggregation may lead to an increase of relaxivity, as aggregated particles in solution induce larger alterations of the local magnetic field (inhomogeneities). On the other hand, larger levels of aggregation may lead to particle sedimentation; hence, the relaxivity of the bulk solution will be reduced (approaching the one of the solvent) [62]. The NWs $r_2$ was also affected by the addition of coating agents, where a slight decrease in the $r_2$ was found when comparing the NWs coated with APTES with the non-coated NWs (NWs vs. APTES-NWs and NWs$_{oxidized}$ vs. APTES-NWs$_{oxidized}$). This decrease is attributed to an increase in steric stability [64] and indicates that the coating of the NWs produces a reduction in agglomeration, which affects the $r_2$ values [52, 53]. Besides, since the NWs behave as single magnetic domain structures, the magnetic inhomogeneities affecting the relaxation time of the surrounding protons are generated only at the tips of the NWs with no contribution of the NW body [63–65]. The BSA-NWs present the lowest $r_2$ with a value at least three times lower than the other formulations. This reduction in the $r_2$ can be partially attributed to the large volume of BSA that not only prevents aggregation of particles by increasing the distance between them, by steric hindrance [51], but also increases the distance between the NWs and the surrounding protons, reducing the magnetic inhomogeneity [66, 67]. Besides, BSA-NWs presented the largest half time of the $r_2$ signal, which is related to higher colloidal stability.

Although all samples were sonicated before the measurements, the magnetic field of the imaging system at 7 T enhanced the NW aggregation as exemplified in Additional file 1: Figure S4. There is an abrupt decrease (more than 70%) of the $r_2$ value (from 55.5 to 17.1 s$^{-1}$ mM$^{-1}$) in the samples that remained inside the equipment throughout the measurement time (60 min, Additional file 1: Figure S4A) compared to the lower decrease of $r_2$ (from 67.4 to 51.7 s$^{-1}$ mM$^{-1}$, ~23%) observed for the NWs that were placed in the MRI system only at the measurement time points (0 min and 60 min, Additional file 1: Figure S4B). The NW aggregation and/or sedimentation enhancement by the magnetic field of the MRI equipment occurs through the NW alignment and attractive forces [59, 61]. Several Fe$_3$O$_4$-based nanomaterials have been observed to cluster as a function of time in the presence of a magnetic field [68, 69].

The $T_2$ parametric map of APTES-NWs (Fig. 2) shows a NW concentration dependence of the $R_2$. A sudden decrease in the $r_2$ during the first 40 min is observed similar to the decay observed in Additional file 1: Figure

| Formulation            | $r_2$ (s$^{-1}$ mM$^{-1}$) | $t_{1/2}$ (min) |
|------------------------|---------------------------|-----------------|
| NWs                    | 281                       | 23.6            |
| APTES-NWs              | 221                       | 34.3            |
| NWs$_{oxidized}$       | 176.8                     | 31.7            |
| APTES-NWs$_{oxidized}$ | 155                       | 26.6            |
| BSA-NWs                | 50.1                      | 51.4            |

Table 1 Transversal ($r_2$) relaxivity values and relaxivity half time decay of different nanowire formulations at 7 T.
Additionally, after 160 min, the $r_2$ of the most diluted NW solution tended towards the $r_2$ value of HPLC grade water, used as control. As explained above, this decay of $r_2$ values is attributed to aggregation and sedimentation of NWs in suspension, which is more pronounced in presence of high magnetic fields. Variations in the relaxation times, and therefore the colors, are visible in some of the tubes and can be explained by the inhomogeneous distribution of the NWs in suspension given by the aggregation and sedimentation.

Magnetic resonance imaging of nanowires internalized in breast cancer cells

Studies on the cellular uptake and degradation of NWs with similar dimensions as well as the cellular internalization and biocompatibility of Fe-based NWs (identical to the ones used in this study) coated with APTES and BSA in MDA-MB-231 cancer cells have been previously reported [40, 43, 70, 71]. Cellular internalization of NWs is a continuous process that starts upon contact between NWs and cells [52], and takes place through the activation of the integrin-mediated phagocytosis pathway [26, 72]. Here, inductively coupled plasma mass spectrometry (ICP-MS) measurements were performed to quantify the NWs interacting with the cells. A total amount of ~35 pg of Fe/cell was found for cells incubated with APTES-NWs and ~117 pg Fe/cell for the cells incubated with BSA-NWs for 24 h without transfection agent. These values represent an internalization of ~40% and ~76% from the total amount of APTES-NWs and BSA-NWs added to the cells, respectively. It is worth mentioning that these values refer not only to internalized NWs, but also to NWs embedded in or adsorbed onto the extracellular structures surrounding the plasmatic membrane, and which can be present even after several washing steps.

In vitro studies of Fe NWs internalized in breast cancer cells were performed to evaluate their efficiency as T2 cell labeling CAs (Fig. 3). Serial dilutions from a suspension of MDA-MB-231 cells labeled with APTES-NWs (12 μg of Fe/mL) or BSA-NWs (20 μg of Fe/mL) were sandwiched in an agar gel and a cell suspension of non-labeled cells was used as a negative control (n = 3). 3D-Gradient Echo T2* weighted MRI images were generated for each gel 24 h after implantation in a 7 T MRI equipment, from which a single 2D image was constructed by minimal intensity projection of all stacked slices that showed the presence of cells. The integrated T2* signals of each of these 2D projections were measured for each dilution of cells labeled with NWs and control (non-labeled) cells and the signal intensity was normalized to the value observed for agars. Averaged T2* weighted images of different concentrations of labelled cells in agar are presented together with the mean (± SD) signal intensity, for APTES-NWs (Fig. 3a) or BSA-NWs (Fig. 3b). In T2* weighted images, signal intensity ($S$) is exponentially dependent on the relaxation rate $R_2^*$:

$$S = S_0 \exp(-T E R_2^*)$$

where $S_0$ is the value of $S$ extrapolated to a null echo time TE=0, i.e. in absence of $T_2^*$ relaxation. Thus, according to Eqs. 1 and 2, $S$ is directly proportional to the concentration of CA (Fe inside the cells), so the larger the number of labeled cells, the larger the $R_2^*$ value (Eq. 1), and the lower the mean signal intensity (Eq. 2).

Overall, cell labeling with both formulations show a similar trend in the reduction of the mean signal intensity of MDA-MB-231 cells (Fig. 3). Although BSA-NWs showed an $r_2$ ca. 5 times lower than the APTES-NWs, a similar number of cells with similar mean signal...
intensities were observed when comparing wells enclosing cells with the same Fe concentration for both formulations. This can be partially explained by the higher concentration of the BSA-NWs added to the cells and the higher cellular internalization of this formulation. A minimum cell concentration of approx. 25 cells/µL was detected using a NW concentration of 0.8 μg of Fe/mL and 2.5 μg of Fe/mL for the cells labeled with APTES-NWs (Fig. 3a) and BSA-NWs (Fig. 3b), respectively. T2* weighted images show a signal intensity distributed evenly throughout the whole well, indicating that labelled cells are distributed with minor aggregation. Hypointense regions at the edges of the wells given by cell accumulation were unavoidable, due to how the wells were produced in the gel. Micro air bubbles inside of the agar gel are seen as big black spots in T2* weighted images (Fig. 3a, b) and could influence the signal intensity measured in each gel (false positives). This fact is reflected in the SD at each cell concentration. The resolution of MRI cell detection depends on many factors such as the r2 value of the CA, the amount of internalized nanomaterial per cell, the MRI protocol, scanner specifications, and has reached the point of single-cell detection using Fe3O4-based nanoparticle labels [73]. The limit of cell detection with the core–shell Fe3O4 NWs was not elucidated in our experimental setup. Nevertheless, the results show the efficiency and sensitivity of Fe-based NWs as T2 CAs for cellular detection in systems simulating the tissue environment. The BSA-NWs were selected for further studies, due to their higher internalization compared to the APTES-NWs.

Finally, the sensitivity of MRI detection of Fe-based NWs-labeled cells was tested in vivo by injection of the BSA-NWs labeled cells after implantation in mice brains (n = 3, Fig. 4). Circa 10 cells (Fig. 4a left, 3 μl of a 3500 cells/ml solution) and ca. 100 cells (Fig. 4a right, 3 μl of a 35,000 cells/ml solution) that were previously labeled with BSA-NWs were injected in the left and right hemisphere of a mouse brain, respectively, and immediately imaged by MRI at 11.7 T. Cell deposits are visible along the channel opened by the injection needle (backflow of cells along the needle track is difficult to avoid), and some of the cells ended up in the ventricles as indicated by the hollow arrows in Fig. 4a. The presence of large blood vessels in the field of view (dashed arrows in Fig. 4a) highlights one of the main problems of the in vivo use of negative CAs, which is the lack of specificity between exogenous labeled cells and endogenous negative contrast (in this case lack of signal in vessels due to blood flow). Nevertheless, the labeling provided by BSA-NWs was so effective that the sensitivity of detection could be pushed to the limit of enabling the location of small clusters of labeled cells, as observed in Fig. 4a. A surface rendered reconstruction of the 3D set of all T2* weighted images of the mice brain is shown in Fig. 4b, where the signal generated by the cells containing NWs appear in red color.

MR imaging following the implantation of labeled cells in alive mice confirmed that the NWs produce an intense T2* contrast visible for several weeks (at least 6) after implantation (Additional file 1: Figure S5). High-resolution T2* weighted 3D images were acquired immediately after injection and 10, 20 and 40 days after implantation, showing the presence of NWs in a series of 2D image slice projections obtained from the 3D set. A detailed view of the cell location is presented in Fig. 5, where minimal intensity projections of all 2D slices with presence of NWs were constructed.
Panels B-D in Fig. 5 shows that the labeled cells are distributed not only along the injection needle tracks but also traveled between injection sites along the corpus callosum. This could be potentially due to the activity of macrophages that engulfed injected cells, as part of the immune response to the implantation of exogenous cells in immune-competent mice rather than the active migration of the implanted cells. Our proof of principle in vivo studies do not provide information about the viability of the implanted cells and/or the integrity of the NWs throughout the weeks after the implantation. However, it has been reported that NWs with similar dimensions were observed inside of endosomes 24 h post-incubation with cells, and that a minimal fraction (~2%) of the NWs was dissolved intracellularly after 72 h due to the acidic environment of the lysosomal compartments in the cytoplasm [72]. Furthermore, large biodegradation of Fe nanoparticles was only observed after almost 1 month in a tissue environment and was partially determined by a decay on the $M_s$ value [74]. A comparison between the NW-labeled cells detected by MRI (in vivo) and optical microscope images (ex vivo) of mouse brain slices stained with Pearls’ Prussian blue for Fe detection, confirms the presence of Fe at the implantation site after 40 days and the correlation between NW labeling and the MRI $T_2^*$ contrast.
addition of coating agents. T2* weighted images of MDA-
by modifying the thickness of the oxide shell and by the
cored NWs, despite having a smaller r2 value. There-
a similar efficiency for MRI cell detection to the APTES
dispersion and cellular internalization, but also showed
formulations, whereby BSA coating not only enhanced
environment were produced testing two different NW
MB-231 cells labeled with NWs embed in a tissue-like
was found that the r2 value of the NWs can be tailored
of labeled cells. The capability of these Fe-Fe xOy
act as T2 (T2*) MRI CAs, as well as the effects of differ-
core–shell NWs with their high magnetization values to
iron the NWs and surface coatings, were evaluated by
including variables on the T2 contrast such as level of oxida-
tion of labeled cells. The capability of these Fe–FeOy
core–shell NWs were fabricated by chemi-
and recent photothermal therapy, where the NWs showed
an outstanding heating efficiency [43]. Functionalization
with targeting agents allowed the selective interaction
with leukemic cells [38], and these NWs show potential
for hyperthermia treatment and magnetic guiding [75,
by modifying the thickness of the oxide shell and by the
addition of coating agents. T2* weighted images of MDA-
cells, as well as the effects of different variables on the T2 contrast such as level of oxida-
tion of the NWs and surface coatings, were evaluated by
measuring their r2 values at a preclinical relevant field. It
was found that the r2 value of the NWs can be tailored
by modifying the thickness of the oxide shell and by the
addition of coating agents. T2* weighted images of MDA-
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environment were produced testing different NW
formulations, whereby BSA coating not only enhanced
dispersion and cellular internalization, but also showed
a similar efficiency for MRI cell detection to the APTES
coated NWs, despite having a smaller r2 value. There-
fore, a lower concentration of BSA coated NWs can be
employed to efficiently label the cells reducing, in turn,
the possibility of toxic effects. In vitro, labeling of cancer
cells with NWs at a concentration of 0.8 μg of Fe/mL ena-
bled the detection of ~25 cells/μL. In vivo studies showed
that ca.10 NW-labeled cells could be located in the brain
parenchyma of a mouse and were detectable for at least
40 days after implantation. The efficient and longitudi-
nal cellular detection combined with the possibility of
magnetic guidance of the NW labeled cells as well as trig-
gerating cellular responses opens the door to new horizons
in cell therapy. These results together with the capacity of
functionalization, magnetic responses, and the recently
discovered qualities as a photothermal agent make the Fe–FeOy core–shell NWs a promising theranostic

Conclusions
In this work, it is demonstrated the excellent perform-
ance of Fe-based NWs for T2 contrast in MRI detec-
tion of labeled cells. The capability of these Fe–FeOy
core–shell NWs with their high magnetization values to
act as T2 (T2*) MRI CAs, as well as the effects of different
variables on the T2 contrast such as level of oxida-
tion of labeled cells. The capability of these Fe–FeOy
core–shell NWs were fabricated by chemi-
and 5% CO2.

Methods
The main aspects of the methods are mentioned in this
section. For detailed explanations of some of the proce-
dures, see Additional file 1.

Chemical reagents
Bovine serum albumin (BSA), 98% (3-aminopropyl) tri-
ethoxysilane (APTES), and 0.25% trypsin–EDTA solution
were purchased from Sigma-Aldrich. 30% hydrogen per-
oxide was purchased from Electron Microscopy Sciences,
Pennsylvania, USA. HPLC grade water (Sigma Aldrich)
was used in all experiments if not indicated otherwise.

Cell culture
The MDA-MB-231 cell line was purchased from Ameri-

Preparation and characterization of nanowire formulations
Fe–FeOy core–shell NWs were fabricated by chemi-
cal electrodeposition into nanoporous alumina mem-
branes, as previously reported [27–29, 72]. The NWs’
length of ~700 nm was controlled by the deposition time
(16 min). The NWs were released from the alumina tem-
plate and collected with a magnetic rack (DynaMag™-2;
Life Technologies, Carlsbad, CA, USA) and rinsed thor-
oughly 10 to 15 times with ethanol with sonication steps
of 10 s in-between. Finally, the released NW’s were
suspending in 1 mL of absolute ethanol. Thereafter, the
sample of NWs was suspended in an aqueous solution
of hydrogen peroxide 15% v/v and left in a theromixer
(Thermomixer comfort, Eppendorf) at 400 rpm and 40°C
for 3 weeks. The sample was then washed five times with
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for 3 weeks. The sample was then washed five times with
absolute ethanol with sonication periods in between.
of a hundred NWs from different samples. The chemical composition, as well as the level of oxidation of the NWs, were evaluated by STEM and EELS by comparing the treated samples against control NWs that remained all the time in absolute ethanol at room temperature (n=3). To determine the oxidation level, the average relative concentrations of Fe and oxygen were measured in identical regions of different single NWs, and the resultant ratio was compared between the control and treated samples of NWs (n=3). TEM studies in combination with EELS were performed using a Thermo Fisher (former FEI) Titan 80–300 TEM equipped with a Cs probe corrector and a Gatan Imaging Filter Quantum 966. The microscope was operated at 200 kV. The EELS maps were acquired in STEM mode as so-called spectrum imaging. The impact of increasing the oxide layer thickness on the magnetic properties of the NWs was evaluated by measuring the magnetic hysteresis loops with a LakeShore 7404 Vibrating Sample Magnetometer (n=3) and the measurements were carried out on dried samples of dispersed NWs.

NWs were coated with APTES or BSA as described previously [40]. Briefly, NWs with native oxide layer and oxidized NWs were coated separately with APTES by adding 0.0946 g of APTES per each 100–300 mg of Fe to the NWs suspended in ethanol with a final volume of 5 mL and sonicated for 1 h. Since basic catalysis is required for the reaction, 200 μL of miliQ water and 10 μL of sodium hydroxide 1 M were added, followed by a second sonication period of 1 h. Finally, the NWs were washed five times with 1 mL absolute ethanol and stored at room conditions in an Eppendorf tube. For BSA coating, NWs with native oxide layer were suspended in 10 mM phosphate buffer pH 7.4 (PB), to a final volume of 2.5 mL in a glass vial and added with 0.8 mg of BSA/ mg of Fe, followed by 1.5 of sonication. The BSA coated NWs were washed three times with phosphate buffer and stored at 4 °C in sterile conditions. All NW formulations were stored at room temperature and are stable for at least 12 months after the coating. The Fe concentration in each NW-formulation stock solution and in cell suspensions was quantified by triplicate using an ICP-MS (iCAP-Q instrument, Thermo Fisher Scientific).

Transversal relaxivity and magnetic resonance imaging measurements

Transversal relaxivity measurements and in vitro imaging experiments were performed in a 7 T horizontal bore Bruker Biospec 70/30 USR scanner (Bruker Biospin, Ettlingen, Germany) using a BGA12-S imaging gradient set of 440 mT/m and a 40 mm inner diameter transmit/receive volumetric coil. A set of 5 solutions of each NWs formulation with Fe concentrations ranging from 0.01 to 0.03 mM were measured at 37 °C using a heated water blanket for temperature control. A tube with HPLC grade water was used as a control sample in all measurements. All samples were sonicated individually for 20 s before introducing them in the MRI equipment to ensure proper dispersion of the NWs. T2 relaxation time maps were acquired using a Multi-Slice Multi-Echo sequence with the following parameters: repetition time (RT)=1500 ms, echo time (TE)=20 ms, echo train of 24 equally spaced TE (20 to 480 ms, ∆TE=20 ms), field of view (FOV)=25.6 mm×12.8 mm, image matrix=256×128 points, one slice of 5 mm thickness and 2 averages. T2 relaxation times of each sample were measured at fixed intervals of ca. 20 min throughout 160 min. R2 relaxation rates were obtained as the reciprocal of T2 relaxation times (obtained by fitting MR signal decay with echo time to a mono-exponential function) and the corresponding R2 values were obtained using Eq. 1. T2 parametric maps were generated for each of the NW samples and were calculated by fitting pixel intensities of the multi-echo images to a mono-exponential decay on a pixel by pixel basis.

The capacity of NWs to perform as CA for cell tracking was assessed by labeling of breast cancer cells and studying them with MRI when placed in vitro and in vivo. Cell suspensions with a concentration of ~140,000 cells/mL were prepared from MDA-MB-231 cells labeled with APTES-NWs (12 μg of Fe/mL) and BSA-NWs (20 μg of Fe/mL). Five serial dilutions of the cells treated with each NW formulation were made (4200 to 700 cells /mL). Likewise, 3 dilutions of the non-treated cells were made with a concentration of 4200 to 1400 cells/mL). 30 μL of each dilution was applied to the wells of each one of the three agar gels previously prepared, which thereafter was left for 2 h at room temperature, enabling adsorption of the cells on the surface of the bottom of the wells. Subsequently, another agarose solution of 0.9% was prepared, left to reach just above physiological temperature and added to the three gels for filling the wells, therefore trapping the attached cells. Finally, gels were covered with Parafilm and left at 4 °C until imaging. For MRI acquisition, a Fast Low Angle Shot sequence was chosen to image 25 slices of 0.2 mm thickness. The FOV was 36 × 36 mm², acquired with a matrix of 512 × 512 points, resulting in an in-plane resolution of 70 × 70 μm². For imaging, the following parameters were used: TR=500 ms, TE=7.3 ms, and 20 averages. For the quantitative analysis, all the slices containing cells were used to calculate the minimal intensity projection taking into account that transfected cells produce a dark contrast on MR images, which were normalized by subtracting the agarose gel background signal. Finally, the integrated signal intensity was calculated for each sample from the
minimal intensity projection image, and its dependence with the volume of labeled cells was obtained. Signal intensity was normalized to the value observed for agar to correct for factors such as distance and orientation of sample to the MR coil, magnetic field shimming, receiver gain of the system and other parameters that can vary from one experiment to the next.

In vivo imaging experiments were performed in a 11.7 T horizontal bore Bruker Biospec 117/16 USR scanner (Bruker Biospin, Ettlingen, Germany) using a BGA9-S imaging gradient set of 750 mT/m, and a 72 mm inner diameter transmit volumetric coil and actively decoupled mouse head surface coil for detection (both from Bruker Biospin). All animal experiments were approved by the local IACUC and local authorities and under the European Union Directive 2010/63/EU on the protection of animals used for scientific purposes. A total of 3 mice (c57bl/6, male, ~30 g) were used in the study. Briefly, after induction of anesthesia with isoflurane and 16.8 ms, TR, 3D-Multi-Gradient-Echo sequence, TE were acquired using the following imaging parameters:

Briefly, after induction of anesthesia with isoflurane and transferred to the MRI where images were acquired under isoflurane anesthesia. T2*-weighted MRI images and dehydrated with an ascending gradient of ethanol–water mixtures (ethanol 50%, 75%, 96%, 3–4 min in each mixture). Finally, slides were immersed in Fast Red for 2 min, washed with water and dehydrated with an ascending gradient of ethanol–water mixtures (ethanol 50%, 75%, 96%, 3–4 min in each mixture). Finally, slides were immersed into xylene for 3 min and covered with a DPX mount for histology.

**Supplementary information**

Supplementary information accompanies this paper at https://doi.org/10.1186/s12951-020-00597-3.

**Additional file 1.** Supplementary figures describe the schematic of Fe-based NWs oxidation and its evaluation, the variation of the relaxation rates as a function of the Fe-based NW concentration at 1.5 T for different NW formulations, the decay of the r1 values obtained at 7 T across the time for different NW formulations, the influence of the NWs’ magnetization in the r2 decay across time, and four axial consecutive slices of 200 µm of thickness across the brain of a mouse implanted with BSA-NWs labeled cells. Supplementary tables describe the determination of the oxidation level of Fe NWs through elemental quantification, and the longitudinal (r1), transversal (r2) relaxivities and r2/r1 ratio of different nanowire formulations at 1.5 T. Supplementary methods describe the synthesis of nanowires, the magnetic characterization of nanowires, the quantification of iron nanowires, the relaxivity measurements at 1.5 T, the magnetization effect of iron nanowires at the 7 T, the preparation of cell suspensions for in vitro MRI detection and the preparation of agar gels for MRI imaging phantom studies.

**Abbreviations**

NWs: Nanowire(s); BSA: Bovine serum albumin; APTES: (3-aminopropyl)triethoxysilane; CA: Contrast agent; PBS: Phosphate buffer saline; Fe: Iron; Fe3O4: Iron oxide; FOV: Field of view; ICP-MS: Inductively coupled plasma mass spectrometry; MRI: Magnetic resonance imaging; T1: Longitudinal relaxation time; T2: Transversal relaxation time; TE: Time to echo; TEM: Transmission electron microscopy; TR: Time to repetition; SEM: Scanning electron microscopy; EELS: Electron energy loss spectroscopy; STEM: Scanning transmission electron microscopy; Ms: Saturation magnetization; Hc: Coercivity; r1: Longitudinal relaxivity; r2: Transversal relaxivity; r2/r1: Longitudinal relaxation rate; r2: Transversal relaxation rate.

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**Authors’ contributions**

JK, ALC and PR-C conceived work and designed the experiments. JK, ALC, PRC, TR, and JSM supervised the work. JAM and AIM synthesized and characterized the iron nanowires. AIM-B, AA, SP-C, LC and PR-C performed the MRI experiments. All authors discussed the results and contributed to writing the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article and its supplementary information files.

Ethics approval and consent to participate
All animal experiments were approved by the local IACUC and local authorities and under the European Union Directive 2010/63/EU on the protection of animals used for scientific purposes.

Consent for publication
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Competing interests
The authors declare that they have no competing interests.

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