Nanotheranostic approach by magnetic nanoparticles labeled stem cells for cancer therapy

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ABSTRACT SUMMARY
Mesenchymal stem cells (MSCs) are of great interest for their self-renewal, multilineage differentiating capabilities and tumor-tropic migratory behavior. In the present study, we have demonstrated that the formulated aqueous dispersible glyceryl monooleate (GMO) coated magnetic nanoparticles (MNPs) can act as a better labeling and efficient tracking agent in vitro due to their long-term retention inside the MSCs even up to one month. We have shown the retention of different properties of stem cells (phenotypic expression and functionality showing multilineage differentiation) after labeling with MNPs even after a long time period. To prove the above system to be used as a targeted delivery system, we have shown that external magnet mediated movement of labeled MSCs towards a desired location in vitro condition. The high T₂ relaxivity of MNPs labeled MSCs facilitated the stem cell tracking by magnetic resonance imaging (MRI) which in turn potentiates the use of MNPs labeled MSCs as a prospective theranostic tool. Most importantly, the labeled MSCs showed tumor tropism towards the tumor spheroid (in vitro 3D model for tumor). Thus, combination of the magnet mediated migration and the tumor tropism of MNPs labeled stem cells will help to develop a better theranostic vehicle for targeted delivery of therapeutics to the tumor site in near future.

INTRODUCTION
Stem cell-based therapies have shown great potential in a wide variety of degenerative, malignant, and genetic diseases owing to their capacity to differentiate into a wide range of specialized cells. MSCs have tendency to migrate to the site of pathogenesis like wounds or ischemia. Recently, Loebinger et al have shown that MSCs have the tendency to migrate towards tumor vasculature like pulmonary metastases (1). With this unique property of MSCs, we are hypothesizing that MSCs can also be an effective approach to deliver as nanovehicles at the tumor site for the treatment of different cancer tissues and also be a good platform for early detection. Different chemokine-receptor pairs including stromal derived growth factor SDF-1/CXCR4 together with ECM proteins have been implicated for the migration of stem cells towards the inflammation site. The clinical development of stem cell therapies requires a suitable method that can follow the fate of delivered cells in vivo non-invasively at high resolution. Theranostic nanoparticles like superparamagnetic nanoparticles hold the promise by enabling the clinicians for real-time, non-invasive monitoring of nanoparticles by MRI (2).

Recently, our group has established a novel magnetic nanoparticle formulation coated with glyceryl monooleate (GMO) that is dispersible in water without the use of any surfactant (3). We have established the biocompatibility of our formulated magnetic nanoparticles in vitro system. The theranostic ability of the MNPs has been tested in leukemia cell line. Herein, we have established the high labeling efficiency of our formulated MNPs and report to construct MNP labeled mesenchymal stem cells that have the ability to be monitored by the MRI due to its enhanced MRI contrast property. In addition, these labeled cells have the inherent capacity to migrate towards the tumor site as well as the movement of these labeled MSCs can be modulated by an external magnetic field. Thus, the MNP labeled MSCs may result in developing a future targeted theranostic agent.

EXPERIMENTAL METHODS
GMO coated MNPs were synthesized by co-precipitation method. The mesenchymal stem cells were isolated from the bone marrow of tibia and fibula of mice. The labeling of MSCs by MNPs and retention of MNPs inside the MSCs were evaluated by confocal microscopy, flow cytometry and Prussian blue assay. To test the effect of labeling using MNPs, some of the phenotypic markers (CD 106, CD 29 and Sca-1) were studied by immunostaining by confocal microscopy and by flow cytometry and the differentiating capability was checked by inducing adipogenic and osteogenic differentiation by culturing MSCs cultured in adipogenic and osteogenic specific media. The effect of external magnet on the MNPs labeled MSCs was determined at different time interval using confocal microscopy. To study the ability of labeled MSCs to be tracked by MRI, T₂ MRI studies were performed both in vitro and in vivo. MSCs labeled with the MNPs were observed both in vitro and in vivo using a 3.0 T MRI system. The tumor tropic migration of MNPs labeled MSCs was studied in a MCF-7 tumor spheroid model.

RESULTS AND DISCUSSION
Labeling the mesenchymal stem cells with magnetic nanoparticles is a new avenue in the field of regenerative medicine and theranostics related to cancer therapy and diagnosis. The MNPs possess a hydrodynamic size of ~ 158 nm and a surface charge of ~ 26 mV (3). In the present study, we have analyzed the labeling efficiency of our MNPs as labeling agent. The confocal microscopy results showed that with an increasing concentration of 6-coumarin-MNPs, the fluorescence intensity gradually increased and gradual saturation in uptake was seen after
a concentration 250 µg/ml. Liu et al have shown that cells labeled with 0.5 µg/ml Fe for 24 h have similar iron content as 7 µg/ml Fe for only 1 h (4). In another set experiments, Lu et al have shown a saturation of labeling after a concentration of 100 µg/ml of iron oxide (5). But, our formulated MNPs showed a higher uptake (> 250 µg/ml) by the MSCs and thereby the labeling by MNPs may open up an area to use them as a theranostic agent in cancer therapy.

Figure 1 (a) Labeling of MSCs by 6-coumarin loaded MNPs. Confocal microscopy pictures of MNP labeled MSCs were taken at 72 h. (b) TEM picture showing retention of MNPs in the cytosol after 30 days of labeling.

The retention of the MNPs inside the MSCs were checked up to 30 days of labeling. For a detailed analysis of localization of MNPs inside the MSCs, transmission electron microscopy images were analyzed. The TEM results confirmed that MNPs are confined to the intracellular space, but not found in cell nuclei. As MSCs express specific surface receptors or markers, so to check the effect of MNP labeling on the phenotypic expression of surface markers, the MSC specific markers (CD 106, CD29 and Sca-1) were analyzed in both control and MNPs labeled MSCs. The confocal microscopy results showed that both the control and labeled cells were expressing the surface markers.

Figure 2 (a) Phenotypic expression of labeled MSCs (b) Osteogenic differentiation and (c) Adipogenic differentiation of labeled MSCs

The retention of functionality of MSCs was confirmed, following osteogenic and adipogenic differentiation of the control and MNPs labeled MSCs. Alizarin stain stains the mineralization during osteogenesis and the adipogenic differentiation was observed by presence of oil lobules. To ascertain the ability of our formulated MNPs to induce movement of MNPs labeled MSCs in presence of an external magnet, labeled MSCs culture discs were exposed to an external magnet and observed up to 5 days. The results suggested that gradually the cells are congregating at one place where the magnet was placed.