BASIC SCIENCE

Nanomedicine: Nanotechnology, Biology, and Medicine
11 (2015) 19–29

nanomedicine
Nanotechnology, Biology, and Medicine

Original Article

nanomedjournal.com

Increasing magnetite contents of polymeric magnetic particles dramatically improves labeling of neural stem cell transplant populations

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Received 21 March 2014; accepted 10 July 2014

Abstract

Safe and efficient delivery of therapeutic cells to sites of injury/disease in the central nervous system is a key goal for the translation of clinical cell transplantation therapies. Recently, '*magnetic cell localization strategies*' have emerged as a promising and safe approach for targeted delivery of magnetic particle (MP) labeled stem cells to pathology sites. For neuroregenerative applications, this approach is limited by the lack of available neurocompatible MPs, and low cell labeling achieved in neural stem/precursor populations. We demonstrate that high magnetite content, self-sedimenting polymeric MPs [unfunctionalized poly(lactic acid) coated, without a transfecting component] achieve efficient labeling (≥90%) of primary neural stem cells (NSCs)—a '*hard-to-label*' transplant population of major clinical relevance. Our protocols showed high safety with respect to key stem cell regenerative parameters. Critically, labeled cells were effectively localized in an *in vitro* flow system by magnetic force highlighting the translational potential of the methods used.

From the Clinical Editor: Utilizing self-sedimenting polymeric magnetic particles, the authors demonstrate an efficient method for magnetically labeling primary neural stem cells for magnetic localization in the central nervous system.

Key words: Polymeric magnetic particles; Neural stem cells; Labeling; Transplant cells; Magnetic cell targeting

Advances in stem cell technology have had a major impact in the field of regenerative neurology. Several transplant cell populations show improved neurological outcomes in pre-clinical models of injury and disease, including spinal cord injury (SCI), stroke, Parkinson's disease, Huntington's disease and birth defects.[1] A major obstacle in the translation of cell transplantation is the safe and efficient delivery of cells to sites of disease/injury. The two main methods for cell delivery (systemic and direct local injection) have associated problems in this regard. Injecting cells systemically can lead to their eventual clearance in the spleen, liver or lung resulting in low cell accumulation at the desired site.[2,3] Multiple direct injections can result in secondary pathology due to blood brain barrier damage, bleeding or embolization.[4] To overcome these issues, several reports have shown that transplant cells labeled with magnetic particles (MPs) can be efficiently "trapped" at foci of injury by application of a magnetic field gradient, as part of so called '*magnetic cell localization*' strategies.[5–9] Application of fields

This work is supported by a research grant from the Biotechnology and Biological Sciences Research Council, UK (DMC), the Engineering and Physical Sciences Research Council funded Doctoral Training Centre in Regenerative Medicine (CFA) and by the USA Award Number 5R01HL107771 from the National Heart, Lung and Blood Institute (BP). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Heart, Lung and Blood Institute or the National Institutes of Health.

This work has been presented as a poster at the Cell Tracking Symposium, Liverpool University, July 2013, Doctoral Training Centre in Regenerative Medicine's Joint Conference, Sheffield University, July 2013 and the FIRM symposium, Girona, September 2013.

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http://dx.doi.org/10.1016/j.nano.2014.07.001
1549-9634/

over sites of pathology can trap systemically injected cells as they pass through the vasculature.[6,8] This would offer considerable benefits if used in conjunction with the 'homing' capability of some stem cell types to sites of injury (after intravenous administration).[10] Field application has also been suggested to localize cells near injury sites after intrathecal injection[7] and can thereby remove the need for multiple injections over time. Enhanced cell accumulation using this method has been shown in the heart,[9] on the surface of intraarterial steel stents[8] and also for some neurological applications.[5–7]

Despite being a promising method to safely enhance cell accumulation at injury sites, a major limiting factor for neurological applications is the relatively low MP labeling efficiency achieved in stem cell transplant populations; few neurocompatible particles have been developed for neurological use. Diverse chemical/biological strategies have been adopted to promote intracellular MP accumulation. These include chemical transfection coating agents (such as chitosan or polylysine)[6,11,12] or cell uptake enhancing molecules (such as the RGD/TAT peptides).[13] While effective from a research perspective, such strategies are not optimal in the clinical context as the related methods can be time consuming, involve significant technical challenges in generating nanoparticle constructs, and are limited by non-specificity of cellular targets. Moreover, these can be associated with cellular toxicity[14,15] and the effects of targeting molecules on neural cell physiology are poorly understood.

As an alternative *physical* delivery approach, MPs have been deployed with external magnetic fields to enhance cellular uptake.[16,17] From a translational perspective, these magnetic assistive methods rely on intrinsic endocytotic uptake mechanisms of cells and have high associated safety.[16] As magnetic force is proportional to the particle magnetic moment, magnetite entrapment within MPs is a major parameter that can influence cell–particle interactions and cellular uptake. Despite this, the relationship between magnetite concentration, applied magnetic fields and cellular labeling in '*hard-to-label*' stem cell transplant populations has never been investigated. It should be noted that studies investigating the relationship between magnetic force and cell loading in neural cells, using applied magnetic fields, have primarily used transfection (gene delivery) grade MP reagents, many of which have low iron content, and where particle uptake is strongly influenced by properties specific to the transfecting component.[18] As such, these cannot provide insights into the relationship between MP magnetite content and '*magnetolabeling*' of stem cells.

To address these issues, the goal of this study is to investigate the effects of systematically modulating MP magnetite concentration on labeling of multipotent, primary neural stem cell (NSC) transplant populations, in conjunction with applied magnetic fields (static and oscillating). The translational potential of the labeling methods has been evaluated by assessing the magnetic cell localization potential of the labeled NSCs in a flow system, using applied magnetic force. NSCs were selected as the target stem cell population given their high clinical relevance for the repair of neurological injury and their capacity to migrate towards sites of pathology, a phenomenon termed '*pathotropism*'. Available MP labeling studies indicate that NSCs have intrinsically low MP labeling efficiencies in the absence of delivery enhancing strategies,[19–21] making these an ideal test population for the current study.

Methods

Magnetic particle formulation and characterization

Fluorescent poly(lactic acid) (PLA) coated non-magnetic (termed Non-mag) and MP samples with varied magnetite content (MP-1X, MP-3X and MP-5X, indicating their relative magnetite content) were prepared using published methods.[17,22] Expanded methods including reagent information/particle formulation are in Supplementary Information. Particles were fully characterized using dynamic light scattering (DLS), transmission electron microscopy (TEM), magnetometry, zeta potential measurement, Fourier-transform infrared (FTIR) spectroscopy, elemental analysis, and powder x-ray diffraction (XRD). Full experimental details are also in the Supplementary Information. Figure 1 illustrates a schematic procedure for preparing the PLA MPs.

Preparation of NSC cultures and MP labeling procedures

NSCs derived from the subventricular zone of CD1 mouse pups (postnatal days 1-3)[23] were maintained as neurospheres in complete medium (defined in Supplementary Information). Neurospheres (passages 1-3) were dissociated to a single cell suspension and maintained as monolayers by plating 1.2×10^5 cells in 600 μL of monolayer medium (defined in Supplementary Information) onto glass coverslips (or aclar for TEM) coated with poly-ornithine and laminin in 24 well plates. Cells were allowed to adhere for 24 hours before changing to fresh monolayer medium with or without particles.

To prepare particle suspensions, lyophilized aliquots (containing the same number of particles for each particle type) were re-suspended in water and added to monolayer medium so that final suspensions contained a 1:1000 ratio of particle solution to medium (approximately 15 μg/mL of dry weight for MP-1X, 19 μg/mL for MP-3X and 26.5 μg/mL for MP-5X). Cells were incubated for 24 hours under no field, static (oscillation frequency: $F = 0$ Hz, 200 μm amplitude) or oscillating ($F = 4$ Hz, 200 μm amplitude) magnetic fields for the first 30 minutes. Field application was restricted to 30 minutes as heating effects were observed in pilot experiments using oscillating fields for longer time periods, and static fields applied for 24 hours resulted in significant particle aggregation. After incubation, cells were washed 3-5 times with phosphate buffered saline (PBS) to remove particles not internalized. Cells were fixed using 4% paraformaldehyde (15 minutes, room temperature) for immunocytochemistry or 2.5% glutaraldehyde for TEM analysis or switched to differentiation medium (complete medium minus growth factors, supplemented with 1% FCS). Cells in differentiation medium were cultured for a further 7 days with medium changes every 2-3 days. TEM samples were processed as previously described.[24]

Assessment of proliferation, stemness and differentiation of labeled NSCs

To assess safety of the procedures, cells fixed at 24 hours post particle addition were stained for the NSC specific markers