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Magnetic Tagging Increases Delivery of Circulating Progenitors in Vascular Injury

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Objectives We sought to magnetically tag endothelial progenitor cells (EPCs) with a clinical agent and target them to a site of arterial injury using a magnetic device positioned outside the body.

Background Circulating EPCs are involved in physiological processes such as vascular re-endothelialization and post-ischemic neovascularization. However, the success of cell therapies depends on the ability to deliver the cells to the site of injury.

Methods Human EPCs were labeled with iron oxide superparamagnetic nanoparticles. Cell viability and differentiation were tested using flow cytometry. Following finite element modeling computer simulations and flow testing in vitro, angioplasty was performed on rat common carotid arteries to denude the endothelium and EPCs were administered with and without the presence of an external magnetic device for 12 min.

Results Computer simulations indicated successful external magnetic cell targeting from a vessel with flow rate similar to a rat common carotid artery; correspondingly there was a 6-fold increase in cell capture in an in vitro flow system. Targeting enhanced cell retention at the site of injury by 5-fold at 24 h after implantation in vivo.

Conclusions Using an externally applied magnetic device, we have been able to enhance EPC localization at a site of common carotid artery injury. This technology could be more widely adapted to localize cells in other organs and may provide a useful tool for the systemic injection of cell therapies. (J Am Coll Cardiol Intv 2009;2:794–802) © 2009 by the American College of Cardiology Foundation

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One of the current challenges in the biomedical sciences is the localization of stem cells to sites of interest for the repair of tissue damage. Cellular therapies are increasingly applied in clinical trials, and in recent years the use of hematopoietic progenitors as repairing modules in the compromised cardiovascular system has been the focus of considerable attention (1–3). In the context of ischemic heart disease, these efforts have led to modest success (4,5). However, delivery to specific targets within the body generally remains a difficult task, in part due to low uptake at the site of injury. As such, cell therapies would greatly benefit from methodologies aimed at targeting and monitoring cell trafficking.

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Superparamagnetic iron oxide nanoparticles (SPIO) offer attractive possibilities in biomedicine as they can be incorporated into cells affording a controllable means of "tagging" (6). These particles lead to a marked decrease in the magnetic resonance imaging (MRI) parameter T2* and the possibility of visualizing their localization noninvasively on T2*-weighted MRI (7–9). Furthermore, the magnetic properties of SPIOs allow them to be manipulated mechanically by a magnetic field gradient. This "action at a distance," combined with the intrinsic penetrability of magnetic fields into human tissue, opens up potential applications involving the transport of magnetically tagged biological entities. Such tagging has to date mainly been the focus of drug delivery systems (10–12), including drug targeting in humans (13). Recent efforts on cell targeting in the arterial circulation have been limited to animal models; to achieve cell capture, these have necessitated the use of large nonbiodegradable micron-sized beads (14–16) or nanoparticle composites fabricated in-house (17), and in most cases, the additional introduction of permanent intravascular metallic devices not currently approved for human use (15–17). It may also be possible to attract gene-carrying cells to post-capillary venules of tumors (18). However, the targeted delivery of progenitor cells using external magnetic devices and clinically approved iron-bearing agents has not yet been accomplished.

Early endothelial progenitor cells (EPCs) derived from $CD34^+/CD133^+$ cells (19,20) have been shown to be involved in post-ischemic neovascularization (21) and re-endothelialization, reducing neointima formation following arterial injury (22). We chose to target EPCs to a site of rat common carotid artery (CCA) injury, as localization of such progenitors to sites of vascular catheterization may help prevent post-angioplasty restenosis (23,24). Although various SPIO formulations have been used in the past, their future use as clinical agents is not straightforward due to safety issues raised by cell labeling and the use of transfection agents. To obviate these concerns, we have chosen to label human EPCs with the U.S. Food and Drug Administration (FDA)-approved nano-sized SPIO compound Endorem (Guerbet, Paris, France), the only agent to date that has been used to monitor cells in humans using MRI (8).

Methods

Cells. Human mononuclear cells (MNCs) were collected by leukapheresis from peripheral blood of donors stimulated with granulocyte colony-stimulating factor (25). We magnetically isolated the $CD133^+$ progenitor cells using anti-human CD133 (epitope 1) microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany). These beads are biodegradable and typically disintegrate after a few days in culture. Then, EPCs were derived by culturing $CD133^+$ cells for up to 21 days on fibronectin-coated plates (BD, Franklin Lakes, New Jersey) in growth medium consisting of 20% fetal bovine serum/endothelial basal medium 2 (Lonza, Basel, Switzerland) and supplemented with fibroblast, insulin-like, and endothelial growth factors; ascorbic acid; heparin; and recombinant human vascular endothelial growth factor 165 (25 ng/ml) (R&D Systems, Minneapolis, Minnesota) (26). Growth media were supplemented with recombinant human stem cell factor (100 ng/ml), rhFlt-3/Flk-2 ligand (50 ng/ml), and recombinant human interleukin 3 (10 ng/ml) (R&D Systems) for the first 72 h. MNCs were grown for 2 h in serum-free Dulbecco's modified eagle medium (Gibco, Invitrogen, Carlsbad, California) followed by removal of nonadherent cells and culture of the adherent fraction in 10% fetal bovine serum/Dulbecco's modified eagle medium. All blood samples were obtained with written consent and were approved by local ethics committees.

Abbreviations and Acronyms

CCA = common carotid artery
EPC = endothelial progenitor cell
FDA = Food and Drug Administration
MNC = mononuclear cell
MRI = magnetic resonance imaging/images
SPIO = superparamagnetic iron oxide

Labeling of cells with SPIO. At day 9 of culturing, the $CD133^+$ cells were labeled with the FDA-approved SPIO Endorem (also known as Feridex in the U.S.) (Guerbet) using 0.5 mg/ml SPIO in growth medium. Suspension $CD133^+$ cells were labeled for 24 h and adherent cells for 1 h. Additionally, adherent cells were labeled for 2 or 24 h to increase the amount of iron in the cells. Mononuclear cells were labeled with SPIO by overnight incubation. Labeled cells were washed twice in 15 ml phosphate-buffered saline.

Quantification of cellular SPIO uptake. We used a superconducting quantum interference device (27) to measure the amount of Fe_3O_4 in the cells. To separate SPIO signal from background signal coming from the cells and media, measurements were performed at −263°C. The samples were saturated in an applied field of 2-T, which was subsequently