Particle embedded culture dishes for magnetic cell labeling

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INTRODUCTION: Magnetic cell labeling for the purpose of MRI based cell tracking has been accomplished using a myriad of different labeling methods and probes. A common procedure involves preparing a nanoparticle complex with a positively charged agent such as poly-lysine or protamine sulfate, then incubating with cells, which endocytose the complex (1). Another procedure involves the targeting of nano- or microparticles to cells by way of affinity ligands, either directly attached to the particles or prelabeled to cells (2). A third procedure involves the simple incubation of microparticles with adherent cells which endocytose particles efficiently (3). Lastly, electroporation in the presence of magnetic nanoparticles has also been shown to efficiently label cells for MRI (4). Moving forward towards translating promising experiments into large animals and humans will require standardization of labeling protocols as well as sterile methods for achieving cell labeling. Here we demonstrate a potentially standardized, sterile, reproducible and stand alone new method for magnetic cell labeling. The basic idea is a plastic cell culture dish coated with various biopolymers commonly used in cell culture in which magnetic nano- or microparticles are embedded. Adherent cells are plated on the dish, and upon attachment endocytose particles directly off the dish. As many cell types require culture either as a step in their purification or, as in the case of stem cells, to allow for proper differentiation into cell types, a culture vessel which includes the magnetic cell labeling step could prove useful in accelerating cell labeling times and ease, particularly in clinical environments.

MATERIALS AND METHODS: 6 well plastic flat culture plates were coated with a mixture of various particle types and different biological polymers or matrices in sterile doubly deionized water (DDW). The particles that were used and their characteristics are listed in Table 1. 2.0 X 10^7 Bangs, JSR or Chemicell particles or Feridex (1 mM iron) were mixed with 0.5 ml of 0.1 mg/ml or 1 mg/ml of hyaluronan, gelatin or poly-L-lysine (PLL) and vortexed. After overnight incubation of mixtures on plates, the mixtures were aspirated and plates were extensively washed with DDW. Next, 5.0 X 10^5 mouse embryonic fibroblasts were plated for overnight incubation. After 16 hours, plates were washed with PBS and cells were trypsinized to remove them from the dish. Cells were washed 3 additional times to remove free particles, and were re-plated in fresh, uncoated dishes. Cells were allowed to attach at least 4 hours before formalin fixation. Assessment of particle embedding onto the plates as well as the degree of cell labeling was performed using a Leica fluorescent stereomicroscope.

particle	Coat/ charge	size
JSR	Hydrophobic polymer, Carboxyl (COOH), negative	2.0 µm
Bangs	Styrene/Divinyl benzene, Carboxyl (COOH), negative	1.63 µm
Chemicell	Silica, Amine (NH_2), positive	1.0 µm
Feridex	Dextran, hydroxyl (OH), negative	150 nm

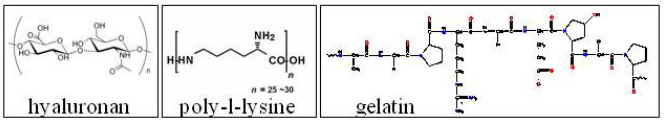

Table 1: Particle and polymer characteristics and structures.

RESULTS: *Particle embedding:* Embedding plates with the three largest particles (Bangs, JSR and Chemicell) achieved generally homogeneous coverage of the entire dish, regardless of the biopolymer used for embedding, as shown in Fig 2A and D. The use of the nanoparticle however, Feridex, resulted in clumping, especially with hyaluronan and higher concentrations of PLL.

Cell labeling: Cells that were plated with different particle compositions exhibited varied labeling efficiencies (Figure 1 B-C, E-F). In general, PLL and gelatin were found to be the most efficient polymers for the two larger micron sized particles tested, that is, cells were labeled with the most beads while retaining the ability to be replated. Replating of labeled cells is an indication of viability as dead or damaged cells will not re-adhere to culture vessels. For hyaluronan coating, only the largest particle was effective for cell labeling.

	HA		gelatin		Poly-L-Lysine	
	0.1mg/ml	1mg/ml	0.1mg/ml	1mg/ml	0.1mg/ml	1mg/ml
JSR	√√	√√	√√√	√√√	√√√	√√√
Bangs	some	√	√	√√	√√	√√
Chemicell	some	some	√	√	√√	√√
Feridex	√	√	√	√√	√√	Clumping

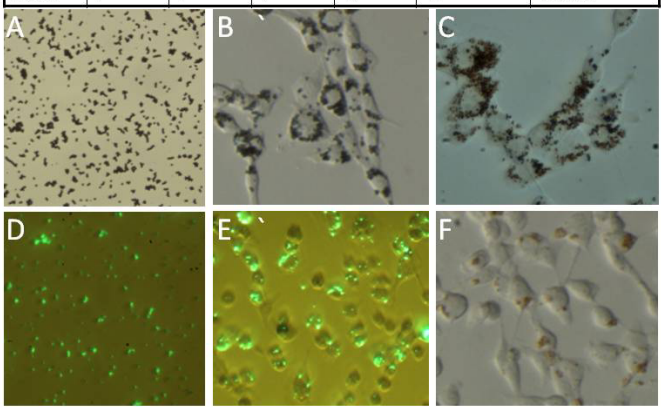

Figure 1: Top – table summarizing results of degree of cell labeling under specified conditions. Panel A) JSR particles embedded on a dish in 0.1 mg/ml PLL; B) Cells labeled with JSR beads from A; C) Cells labeled with Chemicell beads from 0.1 mg/ml PLL; D) Bangs beads embedded on a dish in 0.1 mg/ml PLL; E) Cells labeled with Bangs beads from D; F) Cells labeled with Feridex from 0.1 mg/ml PLL.

DISCUSSION: Standardized cell labeling protocols for MRI-based cell tracking will facilitate translation of experimental models to larger animals and perhaps even humans. Here we demonstrate a new method that has the potential for standardization of magnetic cell labeling protocols for adherent cells. We chose to use biodegradable and biocompatible polymers that would not interfere with cellular properties and functions. In fact, gelatin is used in the food industry, hyaluronan is used in cosmetics and PLL is well documented both as a transfection agent as well as in magnetic cell labeling protocols. We also chose these polymers to sample charge differences as PLL is positively charged, hyluronan is negatively charged and gelatin is near neutral. For magnetic beads, we chose to sample a variety of sizes with different surface charge groups, the details of which are in Table 1. Three major observations were recorded. 1) Hyaluronan is not a good biopolymer for embedding magnetic beads to plates. This may be due to the fibrous nature and low solubility of the biopolymer. 2) The three micron sized beads homogeneously coated plates independent of the biopolymer matrix. This is likely due to the fact that these beads will settle due to gravity as opposed to Feridex which remains colloidal in solution. 3) Only the two large negatively charged micron sized beads were effective for cell labeling in this labeling protocol, independent of biopolymer. The positively charged micron sized bead was effective only when embedded in PLL. This may be due to the very high positive charge of the particles, 35 times more charged than the negatively charged JSR beads, which may have prevented release from the negatively charged hyaluronan. Future experiments will aim to optimize both polymer composition and also embedding procedure.

References: 1) Arbab AS, et al, Transplantation (76) 2003; 2) Shapiro EM, et al, Contrast Media Mol Imaging (2) 2007; 3) Shapiro EM, et al, MRM (53) 2005; 4) Walczak P, et al, MRM (54) 2005.