A Gold Nanoparticle-Based System to Monitor Mesenchymal Stem Cells Delivered via a PEGylated Fibrin Matrix for Ischemic Repair

Laura M. Ricles[1], Seung Yun Nam[2], Stanislav Y. Emelianov[1], Laura J. Suggs[1]
[1]Department of Biomedical Engineering and [2]Department of Electrical and Computer Engineering, The University of Texas at Austin, Austin, TX, U.S.A., 78712-0238

Statement of Purpose: Cardiovascular diseases (CVDs) are the number one cause of death globally [1]. The currently available therapies for CVDs are not very effective, and thus many are investigating the use of stem cell therapy. Mesenchymal stem cells (MSCs) possess angiogenic properties and are easily obtained and expandable in culture [1]. We have previously demonstrated that an insoluble PEGylated fibrin biomatrix can promote MSC tubulogenesis without the addition of growth factors, and potentially lead to increased neovascularization *in vivo* [2]. However, clinical trials using stem cell therapies have been limited by the inability to track administered cells. The current study aims to design a nanoparticle (NP)-based system to allow simultaneous tracking of NP loaded MSCs following implantation via a PEGylated fibrin matrix and determining the transfer of contrast agents to non-stem cells (Figure 1). The results of this study would provide information about the potential to monitor NP labeled MSCs with combined ultrasound/photoacoustic (US/PA) imaging and to distinguish MSCs from other cell types infiltrating the wound area. *In vivo* applications of this NP-based system would contribute to increased understanding of the role of MSCs in vascularization and the angiogenic benefits of PEGylated fibrin.

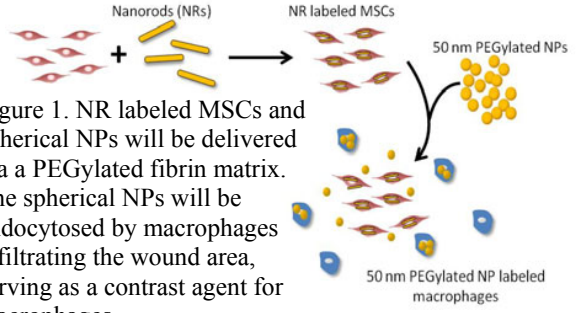

Figure 1. NR labeled MSCs and spherical NPs will be delivered via a PEGylated fibrin matrix. The spherical NPs will be endocytosed by macrophages infiltrating the wound area, serving as a contrast agent for macrophages.

Methods: MSCs were isolated from the bone marrow of Lewis rats as described previously [2]. For NP labeling, MSCs were incubated with gold nanorods (NRs) for 24 hours. The NRs were synthesized and coated with a layer of silica (20 nm thickness) as previously described [3]. A layer of poly-L-lysine (Sigma, 1-5 kD, 0.11 mM) was deposited on the surface to promote cellular uptake. For NP labeling of macrophages (J774A.1; ATCC), cells were incubated with gold spherical NPs for 24 hours. The nanoparticles (50 nm) were synthesized as described previously [4] and coated with a layer of PEG (5 kD; $1x10^{-3}$ mM). NP labeling for both cells was confirmed using microscopy and ultraviolet-visible light (UV-vis) spectrophotometry. The cells were also co-cultured, incubated with NPs (NRs and spherical NPs), and analyzed using microscopy. Cell viability following NP labeling was analyzed using live/dead staining and MTS. The feasibility of imaging the NP labeled cells and distinguishing the cell types was demonstrated using *in vitro* US/PA imaging. Briefly, the cells were mixed in various ratios in a gelatin solution, and imaged using spectroscopic multiwavelength imaging.

Results: Nanoparticle labeling of stem cells: NP labeling of MSCs and macrophages was analyzed using dark field microscopy and UV-vis and compared to control cells. MSCs endocytosed NRs as demonstrated by the yellow-green color of the cells compared to control cells (Figure 2A-B). In addition, macrophages endocytosed spherical gold NPs as demonstrated by the pink color of the cells compared to control cells (Figure 2C-D). UV-vis also confirmed the presence of NPs within the cells.

Co-culture system: Microscopic analysis of MSCs co-cultured with macrophages confirmed that MSCs preferentially endocytose NRs and macrophages are capable of endocytosing spherical gold NPs in the presence of MSCs.

Maintenance of cell function: Cell viability and proliferation were analyzed using live/dead staining and MTS, respectively. Both cell types maintained cell viability and continued to proliferate over one week.

Combined ultrasound/photoacoustic imaging: *In vitro* US/PA imaging (Figure 2E) demonstrated MSCs and macrophages can be detected using US/PA and the cell types can be distinguished using photoacoustic imaging.

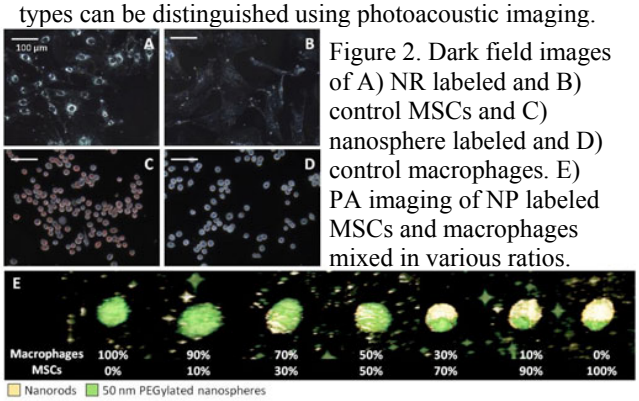

Figure 2. Dark field images of A) NR labeled and B) control MSCs and C) nanosphere labeled and D) control macrophages. E) PA imaging of NP labeled MSCs and macrophages mixed in various ratios.

Conclusions: This study presents a NP-based system which is capable of tracking MSCs *in vivo* and detecting the presence of macrophages. Macrophages which infiltrate the wound area will endocytose the delivered spherical NPs, allowing for photoacoustic detection of the cells. Future work includes *in vivo* studies to evaluate the benefits of delivering MSCs via a PEGylated fibrin gel, including vascular network formation and regeneration of ischemic tissue. In addition, *in vivo* noninvasive longitudinal US/PA imaging will be performed in order to track MSCs and monitor their role in vascular repair.

References:

1. Copland IB. J Cardiovasc Dis Res. 2011;2:3-13. **2.** Zhang G. Act Biomater. 2010;6:3395-3403. **3.** Chen YS. Opt Express. 2010;18:8867-8878. **4.** Ricles LM. Int J Nanomedicine. 2011;6:407-416.