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MPI quantification of magnetically labeled extracellular vesicles in mouse heart following myocardial infarction

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Abstract

This study aims to develop magnetically labeled induced pluripotent stem cell (iPSC)-derived extracellular vesicles (magneto-EVs) for imaging by high resolution MRI and quantitative MPI and myocardial repair in a theranostic manner. Using optimized electroporation and purification protocols, we prepared magneto-EVs with high SPIO loading (1.77 ng Fe/10^6 EVs quantified using the Ferrozine method), allowing detecting ~1×10^9 EVs/mL, and well-preserved therapeutic effects. *In vivo* MPI shows that MPI can detect magneto-iPSC-EVs injected intramyocardially in mouse hearts for up to 7 days following myocardial infarction. MPI quantification showed that 32% of injected EVs remained in the heart after 24 hours, with a further ~50% decrease by day 7. Despite this low retention and rapid clearance, magneto-EVs led to significant improvement in cardiac function and prevent of fibrosis compared to saline controls. This theranostic EV imaging platform enables quantitative MPI monitoring of EV delivery and predicting therapeutic outcomes.

I. Introduction

Stem cell-derived extracellular vesicles (SC-EVs) present a promising avenue for cell-free regenerative therapy of myocardial infarction (MI) [1, 2]. The advancement of EV-based treatments from preclinical to clinical phases could be significantly supported by a non-invasive imaging technique that accurately tracks the dynamics and biodistribution of the administered EVs. Magnetic labeling of EVs is challenging because it requires high nanoparticle loading to achieve sufficient imaging sensitivity while preserving EV integrity and therapeutic

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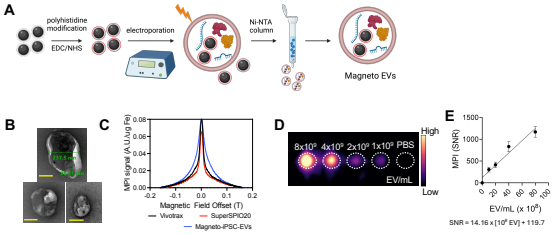

Figure 1: (**A**) Overall work flow. (**B**) TEM images of magneto-iPSC-EVs. Scale bar=100 nm. (**C**) MPI relaxometry of SuperSPIO20 (red), SuperSPIO20-loaded EVs (blue), and Vivotrax® (black). Field sweep range= ±200 mT, drive field=16 mT, excitation frequency= 45 kHz. (**D**) MPI of magneto-iPSC-EVs at different concentrations. (**E**) Linear correlation of MPI signal with EV concentration.

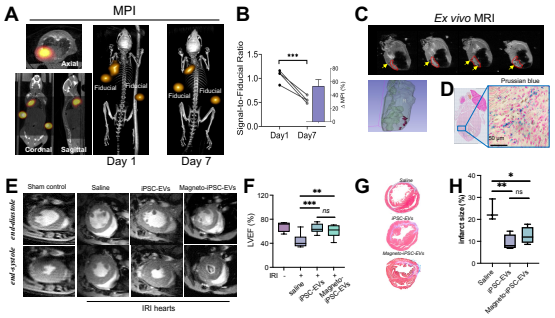

Figure 2: In vivo demonstration of the theranostic capability of magneto-EVs. (**A**) MPI of a representative mouse on day 1 and 7 post-injection. (**B**) Average MPI signal intensity change (quantified by signal-to-fiducial ratio) at day 7. Inset shows the average percentage change in MPI signal after 7 days. (**C**) *Ex vivo* MRI of the mouse heart with arrows indicating magneto-EVs. The 3D reconstructed image (right) clearly shows three injection sites. (**D**) Prussian blue staining. (**E**) Cardiac MRI of two representative mouse hearts. (**F**) Comparison of left ventricular ejection fraction (LVEF, %) among the different groups. (**G**) Representative Masson's trichrome images. (**H**) Infarct size was quantified by the percentage of fibrotic area relative to total myocardial area. (*p<0.05, **p<0.01, ***p<0.001, ns: not significant). In all studies, two fiducial markers (2 µg/mL SuperSPIO20, 100 µL) were included for quantitative analysis and image co-registration.

function. The present study aims to optimize labeling and purification protocols to produce magnetically labeled iPSC-EVs (magneto-EVs) that integrate therapeutic EVs with bimodal MRI/MPI imaging, enabling theranostic treatment of MI. iPSC-EVs were chosen due to their strong cardioprotective effects [3, 4] and higher yields than other SC-EVs [5, 6].

II. Methods and materials

Histidine-tagged SuperSPIO20 (20 nm, SuperBranche) synthesis, magnetic labeling, and purification were performed according to Figure 1A. Electroporation was performed using a Celetrix EX+ electroporator (Celetrix, Manassas, VA) using a modified procedure as described previously[7]. His-tagged SPIO was used because it can be efficiently separated from magneto-EVs using nickel-nitrilotriacetic acid (Ni-NTA) columns[7]. We further optimized the electroporation conditions, including pulse voltage (200-400 V), duration (2- 20 ms), pulse number (1-10) and buffer (PBS or KPBS, with or without 50 mM sucrose or trehalose), and performed comprehensive characterization of the resulting magneto-EVs. The MPI properties of magneto-iPSC-EVs, including sensitivity (maximum signal in the point spread function (PSF)) and resolution (full width at half maximum (FWHM) of the PSF), were characterized using the RELAX module of a Momentum MPI scanner (Magnetic Insight Inc, with key parameters listed in Fig. 1C caption) and compared with those of Vivotrax® (core size∼ 4 nm (70%) and ∼24 nm (30%)). For *in vivo* studies, 2x10^9 magneto-EVs (an effective dose reported previously[8]) were injected intramyocardially into mouse hearts immediately after the reperfusion started after a 35-minute ligation of the left anterior descending (LAD) coronary artery according to previously described [7, 9]. MPI was acquired at 1 and 7 days using a 3D imaging mode (high sensitivity configuration, 21 projections, 20 min). 3D anatomical CT scans were acquired using an IVIS/micro-CT System (Caliper Sciences). *Ex vivo* high-resolution MRI on excised, fixed mouse hearts after the final MPI scan was performed on a vertical bore 11.7 T Bruker scanner using a 3D T1w FLASH sequence with TE/TR=6/150 ms. Therapeutic effects were assessed using cardiac MRI and Masson's Trichrome staining.

III. Results and discussion

Using optimized electroporation conditions (300 V, 2 x 10 ms, in 50 mM trehalose-containing KPBS buffer), we successfully prepared magneto-iPSC-EVs (TEM, Figure 1B) with a high loading efficiency (1.77 ng Fe/10^6 EVs, quantified using a Ferrozine assay[10, 11]), and well-preserved EV cargo (RNA and proteins) and biological activities, despite a modest increase (∼ 46%) in EV size. MPI relaxometry results (Figure 1C) showed that the prepared magneto-iPSC-EVs possess a strong MPI signal (0.055 AU/µg Fe), which is comparable to the commercial MPI tracer Vivotrax® (0.058 AU/µg Fe), one of the most sensitive MPI tracers on the market. The detectability threshold of magneto-EVs was estimated to be 1×10^9 EVs/mL (Figure 1D, E), sufficient for assessing EVs at therapeutic concentration range during a real treatment. *In vivo* MPI quantification (Figure 2A, B, showed that an average of 32% of magneto-EVs remained at the injection site, with approximately 50% of these being cleared after 7 days. The rapid loss of magneto-EVs following

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intramyocardial injection is primarily driven by a combination of mechanical washout from cardiac contractions, rapid drainage through the lymphatic and vascular systems, and phagocytic clearance by macrophages at the site of injury. *Ex vivo* MRI (Figure 2C) can delineate the high-resolution, sub-organ distribution of magneto-EVs. Prussian blue staining confirmed the presence of SPIO-labeled EVs (including possibly released SPIO) in the IRI hearts (Figure 2D). In magneto-EVs-treated IRI mice (n=10), a significant increase in left ventricular ejection fraction (37.3%, P=0.0014) and a decrease in scar size (61.0%, P=0.0182) were observed 30 days after EV administration, compared to saline control (n=10), demonstrating treatment efficacy (Figure 2E-H).

IV. Conclusion

The optimized labeling protocol allows efficient production of therapeutic magneto-EVs, enabling in vivo theranostic applications. This novel theranostic EV platform will be useful to guide future development and clinical translation of EV-based therapy for treating a wide spectrum of cardiovascular diseases. Long-term EV tracking may be challenging due to potential SPIO release in vivo. Future work will be to develop it as a quantitative biomarker for early predicting the treatment outcomes.

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Author's statement

G.C., D. F-F and J.W.M.B, are shareholders and/or employees of SuperBranche. Other authors state no conflict of interest. The JHU IACUC committee has approved all animal protocols (ACUC# MO24K153).

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