Statement of Purpose: Stem cell-derived exosomes are nanoscale extracellular vesicles (EVs) that contain biologically active components (mRNA, miRNAs, proteins factors) from stem cells and have been shown to have beneficial effects on cardiac repair after injury. Since the half-life of exosomes in vivo is short, repeated dosing is necessary for efficient exosome therapies. Though inhalation has been widely used in the treatment of respiratory diseases for over 50 years, very limited studies have adopted an inhalation approach for cardiovascular diseases. Blood flow from the pulmonary circulation first flows to the heart via the pulmonary vein. Plus, our preliminary data proved that exosomes share an average size of 30-100nm and could potentially pass the air-blood barrier (ABB) to reach the injured heart. Thus, we develop a noninvasive and repeatable method for exosome delivery via a nebulizer after myocardial infarction (MI), named Stem Cell-Derived Exosome Nebulization Therapy (SCENT) (Fig. 1a).

Methods: First, lung spheroid cell-derived-exosomes (LSC-Exos) were isolated from secretome by ultrafiltration and characterized by zetasizer and transmission electron microscopy (TEM). We labelled them with a NIR-680 dye, before delivering them respectively to C57BL/6 mice with MI model via a nebulizer for 7 consecutive days. IVIS live imaging was used to detect the biodistribution of exosomes in vivo and fluorescence microscopy was used to confirm that ex vivo. Echocardiographies were performed to monitor the cardiac function after SCENT in MI mouse and histological analysis helped investigating the myocardial repair, in comparison with the controls. Single cell RNA sequencing of the whole heart was performed to explore the mechanism of action after SCENT. Finally, a porcine model of ischemia/reperfusion (I/R) was created and SCENT was examined in pigs for therapeutic efficacy.

Results: Exosomes were examined by TEM for Morphology of exosomes was examined by TEM to be bilayer membrane vesicles (Fig. 1a). After delivery, they were first detected in the nostril and airway, and then distributed to the mouse lung in 1 hour post-treatment. Notably, when exosomes started to accumulate in the heart within 24 hours, it was paralleled by a reduction of signals in the lungs, which indicated a continuous passage of exosomes across the ABB. It was further confirmed by immunofluorescence microscopy (Fig. 1b). SCENT led to significantly improved left ventricular function to our mouse MI model at 2 weeks (EF: 66.97 ± 2.49%), compared to that of MI control (EF: 36.13 ± 3.57%) (Fig. 1c), along with decreased infarct size (Fig. 1d). Remarkably, Ki67+ proliferating cardiomyocytes were increased after SCENT compared to the control (Fig. 1e). To illustrate the mechanism, we also performed single cell RNA sequencing which revealed that the functional recovery was attributed to a shift in endothelial cell (EC) phenotypes in the heart. Finally, we translated SCENT into a pig model of I/R.

Figure 1. SCENT promotes heart repair in a mouse model of MI. a. Schematic image showing the inhalation delivery of exosomes, including a TEM image. Scale bar, 100 nm. b. Fluorescence images showing the inhaled exosomes (red) distributed in heart including cardiomyocytes (green). Scale bar, 5 μm. c. LVEF were determined at baseline, day 2, day 7, day 14 post-MI. d. Measurement of infarct size of hearts after SCENT and control. e. Quantification of Ki67+ cardiac cells including cardiomyocytes after SCENT and control.

Conclusions: In this study, we delivered stem cell-derived exosomes via nebulization to the infarcted heart and revealed the mechanism behind therapeutic effects by single cell RNA sequencing. SCENT distributed nanoscale exosomes to infarcted heart through the ABB, which promoted cardiac function recovery and heart repair in a mouse MI model. Single cell RNA sequencing illustrated the important role of endothelial cells in MI and post-MI repair. Large animal model confirmed that SCENT is a promising solution for the repeatable and noninvasive treatment for heart repair post-MI.