Minimally Invasive Delivery of Mesenchymal Stem Cells by Intrapericardial Hydrogel Injection for Cardiac Repair

Junlang Li [1,2], Ke Cheng[1,2]

[1]Department of Molecular Biomedical Sciences, North Carolina State University, Raleigh, North Carolina, 27607, USA

[2]Joint Department of Biomedical Engineering, North Carolina State University & University of North Carolina at Chapel Hill, Raleigh, North Carolina, 27607, USA

Statement of Purpose: To date, even though there are many strategies that can be used to inject cells into the heart, none of them have been globally accepted by researchers or surgeons. All of the injection methods have their pros and cons. For example, intracoronary (IC) injections and intravenous (IV) injections using catheters are minimally invasive but have the risk of blood vessel obstruction, which could result in a subsequent infarction. In addition, fast blood flow washes out the injected cells in a short time, reducing the treatment efficacy. In contrast, the intramyocardial (IM) delivery route appears to yield higher cell retention rates, but require open-chest surgery, which makes it riskier. Moreover, the efficiency of the IM delivery option is uncertain, according to an overview of past preclinical and clinical studies. Therefore, the field of cell-based heart regeneration has shifted to the development of alternative, more effective cell delivery routes, such as intrapericardial cavity (IPC) injections.

Methods: Mouse green fluorescent protein (GFP)-labeled bone marrow mesenchymal stem cells (MSCs) were combined in extracellular matrix (ECM) hydrogel and injected into the pericardial cavity or the myocardium of the heart of C57BL/6 mice that had been subjected to a myocardial infarction. Echocardiographies were performed to monitor the cardiac function and an ELISA assay was used to assess cellular retention *ex vivo*, cooperated by IVIS live imaging *in vivo*. CD63-RFP exosome labeling system was established through lentiviral transduction and confirmed *in vitro*. Exo-RFP-MSCs were injected into the mouse MI hearts via IPC route in comparison with IM, to evaluate the paracrine activity of MSCs injected. Finally, video-assisted thoracoscopic surgery was performed for IPC injections in Yorkshire male pigs.

Results: The IPC injection, as an alternative cell delivery route, led to better cardiac function in our mouse model with myocardial infarction, which was showed by echocardiographies in the short term (2 weeks) and the long term (6 weeks). This result was attributed to 10-fold higher engraftment of MSCs injected via IPC route (42.5 ± 7.4%) than that of MSCs injected intramyocardially (4.4 ± 1.3%). Immunohistochemistry data revealed better cellular proliferation, less apoptosis, and better vascular regeneration in the myocardium after IPC delivery of MSCs. CD63-RFP exosome labeling system showed that heart cells including cardiomyocytes absorbed MSC-exosomes at higher rates when MSCs were injected via IPC route, compared to the results from IM injections, indicating more extensive paracrine activity of MSCs after IPC injections. What is more, the feasibility and safety of IPC injection were demonstrated in a porcine model with minimally invasive procedure.

Conclusions: Notably, when we conducted a systematic literature review to study cellular retention rates in the heart, differences in animal models, cell types/doses, the timing of the delivery, cardiac injury (e.g. arterial ligation vs. ischemic reperfusion), and the quantification methods used made the results difficult to compare. In addition, every delivery route is susceptible to operator error in practice. Therefore, in this study, we made a head-to-head comparison between the IPC injection and the general IM injection, instead of using data from previously published studies. The primary objective of our study is to provide an alternative delivery route for cell-based cardiac therapy which can resolve the low retention issue in a significant way. The retention rate that we found was significantly high when compared to all other reported retention rates in the literature. It is important to note that we successfully explored the feasibility and safety of IPC injection in large animal model with minimally invasive procedure, making the IPC delivery route become more translational clinically.

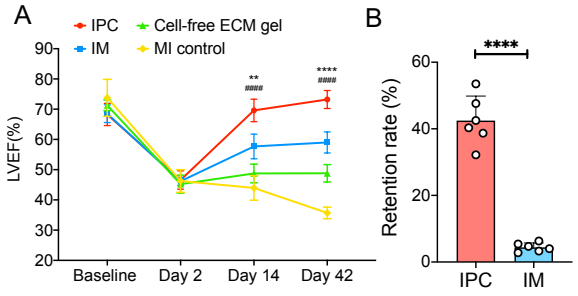

Figure 1. IPC delivery of MSCs generated higher cell retention and greater therapeutic effects than IM delivery. A. LVEF were determined at 2 days, 14 days and 42 days after the MI (n=6). **B.** Quantification of retention rate based on the standard curve and ELISA from IPC group and IM group. (n=6). IPC group, MI mice with intrapericardial injection of MSCs in ECM gel; IM group, MI mice with intramyocardial injection of MSCs in ECM gel. All data are means ± SD.

References: Li, J., Hu, S., Zhu, D., Huang, K., Mei, X., López de Juan Abad, B., & Cheng, K. (2021). All Roads Lead to Rome (the Heart): Cell Retention and Outcomes From Various Delivery Routes of Cell Therapy Products to the Heart. *Journal of the American Heart Association*, *10*(8), e020402.