

Letter to the Editor

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Luo *et al.* Fabrication of Synthetic Mesenchymal Stem Cells for the Treatment of Acute Myocardial Infarction in Mice. *Circ Res.* 2017; DOI 10.1161/CIRCRESAHA.116.310374.

To the Editor:

We were very interested by the Luo *et al.*¹ article on the production of synthetic mesenchymal stem cells recently published in the *Circulation Research* journal. The authors manufactured microparticles (MPs) consisting of poly(lactic-co-glycolic acid) (PLGA)-incorporated mesenchymal stem cell (MSC)-conditioned medium. They utilised membranes from MSCs to coat the MPs creating synthetic MSCs (synMSCs). Luo *et al.*¹ showed the ability of the synMSCs for sustained release of growth factors and their potential for storage. Moreover, the authors demonstrated that synMSCs can increase the proliferative capacity and contractile function of neonatal rat cardiomyocytes *in vitro*, and used an *in vivo* myocardial infarction model to demonstrate the regenerative potential of the synMSCs.

We believe this article advances the knowledge about storage and usage of MSCs and their products in myocardial disease. However, we would like to express some concerns and discuss a few important aspects of this study.

In their *in vivo* mouse myocardial infarction experiments, Luo *et al.*¹ induced a permanent left anterior descending (LAD) artery ligation in a mouse, followed by an intramyocardial injection of synMSCs or MSCs. We were somewhat concerned by the fact that, following LAD ligation, control group animals received no treatment while other groups received intramyocardial injections. We believe that the control group in such experiments must always include intramyocardial injection of the appropriate vehicle (PBS in this case). Previous reports have clearly shown that intramyocardial saline injection may induce stretch-activated myocardial protection². Indeed, stretch-induced protection appears to share a common mechanism with ischemic preconditioning³. The technique of administration is also important, but in Luo *et al.*¹, apart from the total volume administered (50 μ l), details are lacking. We would be interested in knowing how many sites were injected, and with what volume at each site. Even the gauge of needle used is important, since too small a needle will cause cell shearing, while too large a needle will cause myocardial injury.

A second point that deserves further discussion is the authors' claim that synMSCs are superior to MSCs in terms of immune tolerance. Indeed, they did not observe recruitment of macrophages to the injection site of synMSCs, although macrophage recruitment was marked after MSCs injection. Firstly, since the synMSCs were coated with randomly orientated membranes from sonicated MSCs it is surprising that macrophages did not recognize exposed phosphatidylserine (the "eat-me" signal⁴). Secondly, MSCs are negative for MHC class II molecules⁵ and have low immunogenicity, with reports suggesting they may have anti-immunogenic and immunomodulatory functions^{6,7}. Furthermore, there is evidence that macrophages that are recruited by MSCs may be beneficial in wound healing⁸, and macrophages are important players in post-myocardial infarction wound healing and angiogenesis⁹. In their experiments, Luo. *et al.*¹ appear to have injected the MSCs immediately after thawing without plating and growing them in culture, since they state the preparation contains "dead MSC caused by harsh freezing/thawing". Injection of dead cells seems highly inadvisable as such damage-associated molecular patterns (DAMPs) can activate

inflammatory and pyroptotic death pathways in addition to efficiently recruit macrophages¹⁰. We are interested to know whether the authors observed similar macrophage recruitment after injection of MSCs prepared from growing cultures of MSCs as opposed to freshly thawed cells.

Lastly, the authors mention exosomes and their potential for mediating some of the paracrine cardiovascular benefits of MSCs¹¹, but do not present any results regarding this. We would be interested to know how much of the regenerative potential of the synMSCs might be due to exosomes contained within the conditioned medium.

References

1. Luo L, Tang J, Nishi K, Yan C, Dinh P-U, Cores J, Kudo T, Zhang J, Li T-S, Cheng K. Fabrication of Synthetic Mesenchymal Stem Cells for the Treatment of Acute Myocardial Infarction in Mice. *Circ Res*. 2017; CIRCRESAHA.116.310374.
2. Whittaker P, Kloner RA, Przyklenk K. Intramyocardial injections and protection against myocardial ischemia. An attempt to examine the cardioprotective actions of adenosine. *Circulation*. 1996; **93**: 2043–57.
3. Gysembergh A, Margonari H, Loufoua J, Ovize A, André-Fouët X, Minaire Y, Ovize M. Stretch-induced protection shares a common mechanism with ischemic preconditioning in rabbit heart. *Am J Physiol*. 1998; **274**: H955-64.
4. Segawa K, Nagata S. An Apoptotic “Eat Me” Signal: Phosphatidylserine Exposure. *Trends Cell Biol*. 2015; **25**: 639–650.
5. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F., Krause DS, Deans RJ, Keating A, Prockop DJ, Horwitz EM. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy*. 2006; **8**: 315–317.
6. Tse WT, Pendleton JD, Beyer WM, Egalka MC, Guinan EC. Suppression of allogeneic T-cell proliferation by human marrow stromal cells: implications in transplantation. *Transplantation*. 2003; **75**: 389–97.
7. Krampera M, Glennie S, Dyson J, Scott D, Laylor R, Simpson E, Dazzi F. Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigen-specific T cells to their cognate peptide. *Blood*. 2003; **101**: 3722–9.
8. Chen L, Tredget EE, Wu PYG, Wu Y. Paracrine factors of mesenchymal stem cells recruit macrophages and endothelial lineage cells and enhance wound healing. *PLoS One*. 2008; **3**: e1886.
9. Lambert JM, Lopez EF, Lindsey ML. Macrophage roles following myocardial infarction. *Int J Cardiol*. 2008; **130**: 147–58.
10. Bell RM, Bøtker HE, Carr RD, Davidson SM, Downey JM, Dutka DP, Heusch G, Ibanez B, Macallister R, Stoppe C, Ovize M, Redington A, Walker JM, Yellon DM. 9th Hatter Biannual Meeting: position document on ischaemia/reperfusion injury, conditioning and the ten commandments of cardioprotection. *Basic Res Cardiol*. 2016; **111**: 41.

11. Davidson SM, Takov K, Yellon DM. Exosomes and Cardiovascular Protection. *Cardiovasc drugs Ther.* 2017; **31**: 77–86.