ESC European Society of Cardiology
European Heart Journal (2018) **39**, 1848–1850
doi:10.1093/eurheartj/ehy097

EDITORIAL

# Induced pluripotent stem cells as a biopharmaceutical factory for extracellular vesicles

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*Online publish-ahead-of-print 13 March 2018*

**This editorial refers to 'Acellular therapeutic approach for heart failure: *in vitro* production of extracellular vesicles from human cardiovascular progenitors'†, by N. El Harane *et al.*, on page 1835.**

Over the past two decades, many pre-clinical and clinical studies have been conducted to establish stem cell therapy as a potential treatment for heart failure.[1] Although the original purpose was to replace the scar lesion with new cardiomyocytes generated from stem cells, there is growing evidence that therapeutic benefits may be derived mostly from cardioprotective paracrine factors released by stem cells.[2] Paracrine factors from several cell sources, including bone marrow-derived cells, endothelial cells, and mesenchymal stromal cells, have been shown to stimulate growth of new blood vessels and activate the endogenous repair pathway in the myocardium. Several studies hypothesized that these paracrine effects work through extracellular vesicles (EVs) secreted from stem cells.[3–6] EVs, including exosomes and microvesicles, are released by different types of cells and involved in both physiological and pathophysiological processes.[3–7] EVs are believed to mediate intercellular communication by transmitting information from the cells of origin to their target cells. Thus, attempts are being made to utilize EVs as novel tools for various therapeutic approaches such as antitumour and regenerative therapies, and some antitumour EVs have already entered phase II human clinical trials.[3,7]

Since the discovery of human induced pluripotent stem cells (iPSCs) by Shinya Yamanaka *et al.*,[8] they are increasingly being used in cardiovascular research for disease modelling, drug screening, personalized medicine, and regenerative medicine.[9,10] Although previous studies have shown that transplantation of either human embryonic stem cell-derived cardiomyocytes (hESC-CMs)[11] or iPSC-derived cardiomyocytes (iPSC-CMs)[12] to non-human primates can engraft or improve cardiac function through direct cardiomyocyte replacement, there are still several hurdles to overcome, including short-term ventricular arrythmias and long-term sustainable engraftment.[12–14]

In this issue of the journal, El Harane *et al.* sought to test whether EVs secreted by iPSC-derived cardiovascular progenitors (iPSC-PGs) can recapitulate the therapeutic effects of direct transplantation of iPSC-PGs.[15] They successfully isolated EVs from iPSC-PGs, and found that iPSC-CMs did not produce EVs althogh the mechanism is unclear. EVs were internalized *in vitro* to target cells, which improved cell survival and proliferation of cultured H9C2 cardiomyocytes, and promoted angiogenesis including scratch wound healing and tube formation. Similarly, *in vivo* EV injection improved cardiac function in a murine myocardial infarction model. Surprisingly, the injection of EVs outperformed transplantation of their parent progenitor cells. The EVs were enriched with 16 highly conserved microRNAs (miRNAs), which are associated with biological functions expected to ameliorate heart failure. In terms of target genes of the miRNAs, they showed that the hearts from the three groups treated with iPSC-CMs, iPSC-PGs, and EVs showed distict gene expression patterns.

The work by El Harane *et al.* highlights the effect of EVs secreted by iPSC-PGs on chronic heart failure following myocardial infarction. In terms of clinical application, these results have three important implications. First, the source of EVs is progenitor cells differentiated from iPSCs, which are more available than hESCs from the ethical point of view. Second, the EV therapy is considered to be relatively safe because it is a cell-free therapy. Third, EV therapy was more effective in improving cardiac function *in vivo* than the direct transplantation of parent stem cells.

In their previous studies, Kervadec *et al.* showed that the injection of EVs secreted from human embryonic stem cell-derived cardiovascular progenitor cells (hESC-PGs) could provide beneficial effects equivalent to those of cell transplantation therapy of parent hESC-PGs in the treatment of chronic heart failure in mice.[16]. While EV

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† doi:10.1093/eurheartj/ehy012.

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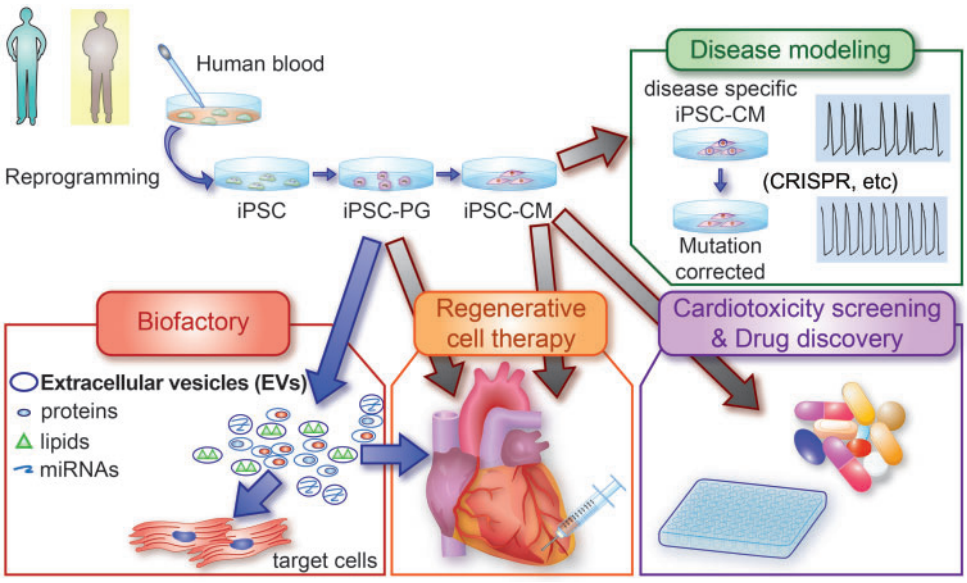


**Take home figure** New application of iPSCs as a 'biofactory'. The blood cells from patients or healthy individuals can be reprogrammed into iPSCs and differentiated to cardiovascular progenitor cells (iPSC-PGs) and cardiomyocytes (iPSC-CMs). These cells have been used for cardiac disease modelling, high-throughput drug screening, and regenerative medicine. Notably, this study also presented a new way to use iPSCs as a 'biofactory' of extracellular vesicles (EVs). EVs released from iPSC-PGs were shown to be potentially effective for treating chronic heart failure in a murine model.

therapy for heart failure had potential for being a new cell-free therapy, there were several hurdles to put it into practice because of the limited availability and ethical issues of hESCs. To overcome this problem, they switched the cell source from hESCs to iPSCs in the current study and successfully isolated EVs from iPSC-PGs to show the therapeutic effects of the iPSC-PG-EVs. The switch is an important step for clinical application of the EV therapy, because iPSCs are more available than hESCs in terms of regulatory restrictions and ethical issues.

Although the exact mechanism of therapeutic benefit of EV therapy is still unclear, it is notable that the EV therapy here is cell free and is shown to be equally if not more effective than direct cell transplantation. If true, this means that EV therapy may have significant safety advantages because it is probably less likely to induce malignancy, immune reactions, and arrhythmias compared with direct transplantation of hESC-CMs or iPSC-CMs.

Surprisingly, El Harane *et al*. found that EV therapy not only could mimic paracrine effect of cell transplantation, but also was more effective than parent cells in improving EF *in vivo*. This is probably due to the concentration of cardioprotective factors such as miRNAs in the EVs, because EVs injected into mice hearts should be highly concentrated during the *in vitro* culture process prior to injection. This may also confer an advantage for EV therapy because the concentration of EVs can be controlled and optimized by adjusting culturing conditions, whereas, in comparison, it is much more difficult to control the behaviour of cells already transplanted into the hearts. Thus, this EV therapy potentially can even maximize the cardioprotective paracrine effects of the parent stem cells.

To translate EV therapy into an effective clinical treatment option for heart failure, it will be important to assess its possible serious side effects given that its precise mechanism is unclear at present. To that end, it will be essential to perform optimization to increase the benefits and decrease the side effects, such as by optimizing the amounts of EVs to be injected and their delivery to the heart, as well as how their parent stem cells are to be cultured. In addition, the selection of parent cell types for EVs may be important. Here, El Harane *et al*. used iPSC-PGs and iPSC-CMs as the source of EVs. However, other cell types or combinations of cell types may be even better at generating cardioprotective EVs, including endothelial progenitors that may be able to produce EVs with greater angiogenesis or anti-apoptotic effects. Furthermore, there are still several important unanswered points that should be investigated: (i) how consistently can iPSC-PGs produce effective EVs that contain the same set of miRNAs within the same iPSC line and also over different passage numbers; (ii) how robustly can other iPSC lines produce effective EVs because iPSCs have significant variability depending on the donors; (iii) how long is the biological half-life of EVs and how does that translate to sustained improvement in heart failure; and (iv) how can we standardize the process to make EVs with consistent quality?

Lastly, this study also shows that EVs not only could mimic the paracrine effect of regenerative cell therapy, but also may be used as a drug delivery system.[3,7] El Harane *et al*. notably used iPSCs as a biopharmaceutical 'factory' to produce EVs. Because EVs can deliver diverse substances such as proteins and nucleic acids to target tissues, and iPSCs can be differentiated into any types of cells, iPSC-derived EVs have a potential as next-generation biopharmaceutical drugs capable of targeting tissues beyond the reach of current recombinant proteins. However, additional research is necessary for these diverse applications, including ways to optimize the beneficial paracrine factors produced by iPSC-derived cells (*Take home figure*).[17]

## Acknowledgement

This publication was supported in part by research grants from the National Institutes of Health (NIH) R01 HL113006 and NIH R01 HL133272, American Heart Association (AHA) 17MERIT3361009, and the California Institute of Regenerative Medicine (CIRM) DR2A-05394 and RT3-07798 (J.C.W.).

**Conflict of interest:** none declared.

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