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EDITORIAL

Cardiac cell-derived exosomes: changing face of regenerative biology

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This editorial refers to 'Exosomes secreted by cardiosphere-derived cells reduce scarring, attenuate adverse remodelling, and improve function in acute and chronic porcine myocardial infarction'†, by R. Gallet *et al.*, on page 201.

> . . . The difference between past, present and future is only a stubbornly persistent illusion
>
> Albert Einstein

Repair of the damaged cardiac tissue has been considered the holy grail of cardiac medicine driving researchers for years. Myocardial damage in an adult heart leads to widespread cardiomyocyte loss without active replacement of the dead cardiac tissue, ultimately resulting in formation of scar at the site of injury. Current therapies are designed only to limit the extent of scar formation and are unable to offer restoration of lost cardiac tissue, leading to a progressive decline in cardiac function. As a consequence, cardiac diseases continue to rise and are a significant cause of morbidity and mortality around the world. The last 10 years have witnessed the dawn of stem cell therapy for cardiac repair with promising true cellular replacement and restoration of cardiac tissue damaged due to pathological injury. Multiple stem cell types derived from bone marrow, heart, adipose tissue, and cortical bone have been tested in small and large animal models of myocardial damage. Among these, stem cells derived from the heart hold particular interest and represent the most suitable cell type for cardiac repair.[1] Different populations of cardiac stem cells have been reported to date and, importantly, all of them have been shown to differentiate into three cardiac cell types, i.e. myocytes, endothelial cells, and smooth muscle cells, contribute towards neovascularization, and significantly improve function after myocardial damage.[1,2] Promising pre-clinical results have laid down the basis for multiple phase I and II trials conducted to test the safety and effectiveness of cardiac-derived stem cells for patients with cardiovascular diseases.[3,4] The outcome largely shows that cardiac-derived stem cell therapy is safe for use in patients, yet the improvement in cardiac function is incremental and modest. Moreover, controversy exists regarding the exact mechanism behind the beneficial effect of stem cell therapy. Data from animal studies convincingly demonstrated transdifferentiation ability of cardiac stem cells[1] in the heart, although investigators found very few adoptively transferred cells in the heart beyond the first few days of transplantation.[5] Moreover, lack of available methodologies to trace transplanted stem cells effectively in patients with cardiovascular disorders certainly represents a limitation complicating evidence for transdifferentiation. As a result, development of strategies aimed at augmenting stem cell survival and their transdifferentiation ability have become the focus of current research. Alternatively, a large body of evidence implicates the ability of transplanted stem cells to secrete growth factors, cytokines, and chemokines at the site of injury as explaining their cardioprotective effects.[6,7] Consequently, cardiac stem cells are not thought to stick around for a long time, but rather release paracrine factors that stimulate endogenous myocardial repair processes consisting of enhanced cardiomyocyte cell cycle activity, neovascularization, and increased stem cell participation in myocardial repair.[8,9] Emerging data recently have linked exosomes as one of the main components of the paracrine signalling regulating the salutary effects of stem cells in a cell-free system independent of the burdens associated with whole-cell transplantation.[10,11] A number of recent studies have effectively shown that exosomes derived from different stem cells including cardiac-derived stem cells are a viable therapy for augmentation of cardiac function recapitulating in large part the benefits of adoptive cell transfer. Nevertheless, there still remains a need for translation of small animal findings into a clinically relevant large animal model for myocardial damage.

This issue of the journal covers the study by Gallet *et al.* that provides one of the first reports on the safety and efficacy of cardiosphere-derived cell (CDC) exosomes in large animals after acute and chronic myocardial injury.[12] CDCs have been described for well over a decade now, extensively validated in small and large animal models of cardiac myocardial injury. Two recently concluded phase I clinical trials have demonstrated the ability of CDCs in augmenting cardiac function by attenuation of ventricular remodelling and scar reduction in patients with heart failure.[4] Patients receiving CDCs demonstrated persistent improvement in cardiac function at

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1-year follow-up yet received only a single injection at the start of the study. This finding subsequently led to the hypothesis that transplanted CDCs in large part extend their benefits by secreting paracrine factors including exosomes at the site of injury, leading to acute cardioprotection together with long-term activation of endogenous myocardial repair (*Figure 1*). One of the earlier studies by the same group validated the paracrine hypothesis and showed that human CDC-derived exosomes are able to recapitulate in large part the benefits of CDC therapy and augment cardiac function after myocardial infarction. Mechanistically, CDC exosomes were found to be enriched in cardioprotective microRNAs that are delivered to the injured cardiac tissue, promoting endogenous repair processes. The next logical question was to determine the therapeutic efficacy of CDC exosomes in a clinically relevant large animal model. In order to test this hypothesis, the authors make use of an ischaemia–reperfusion injury model in Yucatan mini-pigs as previously reported. CDCs have been shown previously to be mildly immunogenic with no signs of systemic immune response or toxicity; therefore, the authors set out to determine whether human CDC exosomes exhibit similar properties when tested in swine with myocardial injury. Human CDC exosomes were isolated using previously described methods, characterized, and then injected into animals with acute and chronic cardiac injury. Moreover, two different routes for exosome delivery, i.e. intracoronary and intramyocardial, were also compared for their effectiveness in improving cardiac function. The results demonstrated higher exosome retention and efficacy after i.m. delivery compared with i.c., subsequently leading to marked reduction in scar size and increased ejection fraction. Based on these results, the authors designed a pre-clinical randomized study using a NOGA-guided intramyocardial exosome injection. NOGA-guided delivery systems have been recently used as an effective way to transplant stem cells into the myocardium, at the same time providing the ability to map and visualize the ischaemic regions following injury.[13–15] As a result, there is enhanced probability that stem cells are delivered in the border zone rather than the ischaemic region, promoting the effectiveness of cell therapy approaches. Animals receiving human CDC exosomes showed preservation of ventricular volumes and function parallel with scar reduction. Histological analysis revealed decreased collagen content in the infarct and border zone but also increased neovascularization and viable mass evidenced by $Ki67^{+}$ cardiomyocytes. Importantly, human CDC exosomes did not increase inflammatory infiltrates or cardiomyocyte necrosis, suggesting lack of an immune reaction in pigs. These are critically important observations and support the notion that CDC exosomes might be ripe to be tested in clinical trials.

The discovery of tiny shuttles called ‘exosomes’ secreted by cells in response to changes in their physiological environment represents a paradigm shift in the way we look at biological processes. First described 30 years back, exosomes were long regarded to be nothing more than cellular garbage, a way for the cells to remove unwanted debris. Since then, exosomes have exploded onto the field, regulating diverse molecular processes, proposed as biomarkers, and play vital role in progression of disease including cardiovascular disorders. In particular, exosomes for cardiac repair and regeneration have generated significant interest as an alternative to cell therapy (*Figure 2*).[16] Adoptive transfer of cardiac stem cells improves cardiac function yet the transplanted cells are unable to persist in the heart beyond the first few days. In contrast, exosomes are more stable than cells under various physiological conditions and are to a certain degree immune privileged. Moreover, exosomes can be stored long term, making them ideally suited for therapeutic interventions. Nevertheless, this optimism and excitement must be channelled into rigorous assessment of exosome characteristics

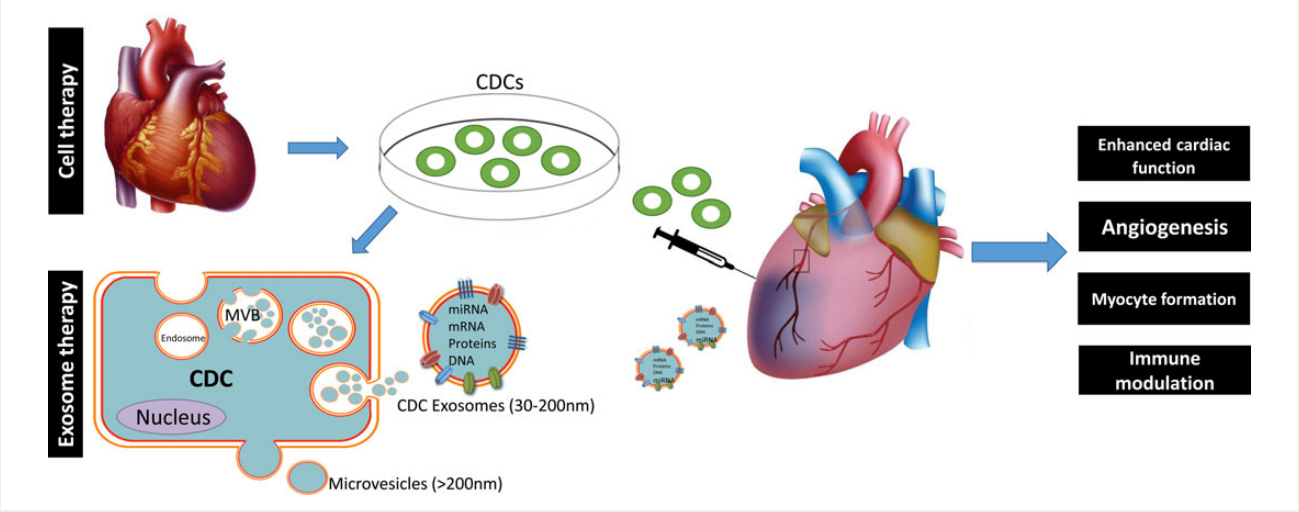

Figure 1 Exosome therapy recapitulates the benefits of cell therapy. CDC derived exosomes are generated by inward budding of endosomal membranes and accumulate in multivesicular bodies. They are released into extracellular space following the fusion of the endosomal compartment with the plasma membrane. Their cargo consists mainly of proteins and nucleic acids, with the greatest abundance and variety presented by miRNA and mRNA. Delivery of CDC exosomes to heart after injury recapitulates cardiac structural and functional effects, angiogenesis and immune modulation as seen by cell therapy.

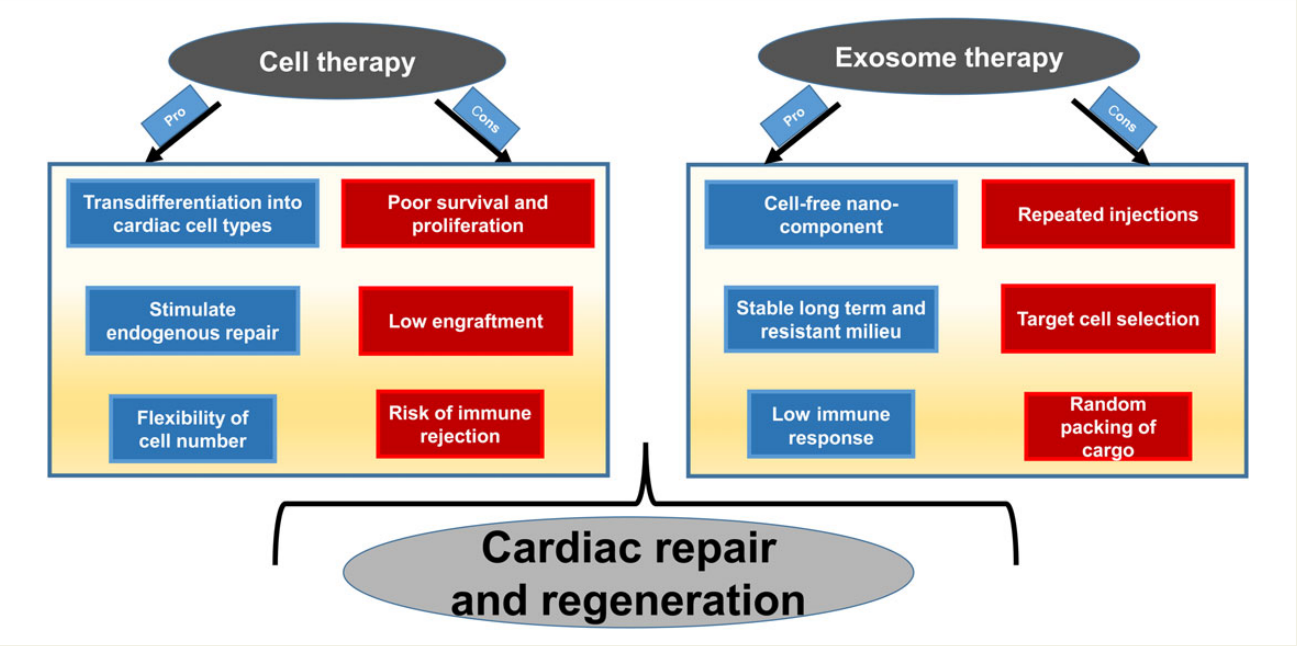

Figure 2 Comparative assessment of associated pros and cons of cell therapy vs. exosome therapy. Cell therapy offers direct cell injections; the surviving cells are capable of developing into cardiac tissue and stimulate endogenous myocardial repair processes (blue boxes) but carry a number of well-documented problems (red boxes). Exosome therapy relies on exosomes derived from the cells themselves and presents a cell-free system for myocardial repair without the burdens associated with cell transplantation (blue boxes). Nevertheless, there are some limitations (red boxes) associated with exosome therapy as highlighted.

including their ability to alter cardiac repair processes. An important area for consideration is how exosomes pack their cargo and whether each and every exosome from the parent cell type cultured under the same conditions has a similar content. Stem cells derived from heart failure patients are known to be functionally impaired, and exosomes derived from such cells may already have compromised ability. Therefore, careful examination of exosome biology is required, including the effect of changes in the physiological microenvironment of parent cells on exosome content. Exosomes have a very short half-life and are quickly taken up by the target cells, limiting their effect to persist for a certain amount of time. Repeated injections may be something that needs to be developed in the future to enhance the effects of exosome therapy. Similarly, exosome target selection is another potential area of consideration for successful implementation of exosome therapy. The myocardium consists of cardiomyocytes, endothelial cells, stem cells, fibroblasts, and other interstitial cells. Exosomes may instigate opposite or similar responses in different target cells, influencing the outcome of the therapy, and require careful assessment and validation.

In conclusion, exosomes may significantly alter cardiac regenerative medicine, and this study provides the foundation for future research for development of a viable therapy based on exosomes for the repair of damaged cardiac tissue in heart failure patients.

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