Cardiovascular diseases represent a significant cause of death and disability worldwide.1 The decline of cardiovascular mortality as a result of modern medicine and surgery has in turn led to a rapid increase of patients having heart failure, with the only definite cure being heart transplantation. However, many patients are unable to undergo transplantation surgery because of complications from existing comorbidities, and among suitable patients, the procedure is plagued by limited donor supply, high costs, and the need for chronic immunosuppressant therapy. Hence, recent advances in cardiac regenerative therapy have emerged as an attractive alternative.

Currently, there are several methods to achieve cardiac regeneration. Endogenous cardiac repair that involves generation of new cardiomyocytes from differentiation of cardiac progenitor cells (CPCs) or renewal of pre-existing adult cardiomyocytes is one such approach, albeit a rare and inefficient process to cope with the loss of cardiomyocytes after myocardial infarction or other cardiac diseases.2 Alternatively, functional myocardium may be salvaged or replenished through transplantation of exogenous stem cells.3,4 However, the poor long-term engraftment and survival in current transplantation have largely precluded substantial cell replacement, and instead supports the paracrine hypothesis that the release of external factors contributes to myocardial salvage or repair.

Historically, the paracrine hypothesis is thought to be mediated primarily by chemical and physical signals, such as soluble proteins, gene products, lipids, and gases. Indeed, various studies have demonstrated that stem cells produce and secrete a broad range of cytokines, chemokines, and growth factors that are involved in cardiac repair.5 Strong support of a paracrine hypothesis came from experimental studies in which the administration of conditioned medium from stem cells was able to confer beneficial effects without the physical presence of stem cells within the infarcted heart.5,6 There is a growing body of evidence showing that stem cells are also able to release membranous vesicles into extracellular space that can contribute to cell-to-cell communication, including microparticles, microvesicles, and exosomes.7-9

Exosomes are phospholipid bilayer microvesicles released from the endocytic compartment of live cells, typically ranging between 30 and 100 nm in size (Figure).10 Early endosomes form by the fusion of small vesicles of different sizes that originate from invaginations of the plasma membrane. As the endosomes mature, exosomes form multivesicular bodies by accumulating intraluminal vesicles through the invagination of the limiting membrane of the endosomes. Multivesicular bodies that are not degraded through fusion with lysosomes subsequently fuse with the plasma membrane and release intraluminal vesicles into the extracellular environment as exosomes.11 A wide range of cargo is transported within exosomes, including mRNA, miRNA, proteins, molecular chaperones, and signaling molecules. The ability of exosomes to mediate the cross talk between different cell types has been increasingly documented since the seminal 2002 study, which demonstrated that dendritic cell-derived exosomes were capable of activating naïve CD4⁺ T cells;12 this was further corroborated by another landmark study that validated exosomes as a natural carrier system capable of transporting miRNA, mRNA, and proteins among cells.13 The growing role of exosomes in stem cell biology has been demonstrated during the past few years, primarily based on their potential utility as cell-free therapeutic candidates that can mediate cardiac regeneration.7,14-16 In addition, exosomes have been implicated in mediating cardioprotection through distinct mechanisms, such as enhancement of angiogenesis,15 less fibrosis,17 and reduced apoptosis,16 highlighting the broad potential of exosomes.

In this issue of Circulation Research, Khan et al18 presented results showing that mouse embryonic stem cells–derived exosomes (mES-Exo) can promote endogenous repair and preserving cardiac function in a mouse model of myocardial infarction, effects that are mediated at least, in part, by the transfer of miR-294. Initial in vitro experiments revealed that mES-Exo–treated H9c2 myoblasts experienced decreased caspase-3 expression on exposure to H₂O₂ when compared with cells treated with mouse embryonic fibroblasts–derived exosomes (mEF-Exo). Consistently, intramyocardial injection of mES-Exo into infarcted mouse myocardium was associated with preserved cardiac function 4 weeks post surgery when compared with mEF-Exo or saline control. Although the transplantation of undifferentiated ES cells is often associated with tumor formation,19 the authors did not observe any tumor formation in mice treated with mES-Exo, indicating that exosomes are an attractive option for avoiding the tumorigenic potential of ES cells while preserving their therapeutic modality. The preserved cardiac function seen in
mES-Exo–treated hearts was associated with increased neo-
vascularization, decreased apoptosis, and enhanced myocyte
proliferation. Moreover, mES-Exo were found to increase the
number of proliferating CPCs in the infarcted heart for up to
4 weeks post infarct.

To complement their in vivo studies, Khan et al18 designed
in vitro experiments to investigate the mechanisms associated
with mES-Exo that could contribute to enhanced survival and
proliferation of endogenous CPCs. Mirroring the results seen
in mice, the authors found that CPCs treated with mES-Exo
had better survival in response to H2O2 challenge when com-
pared with mEF-Exo or nontreated CPCs, which was attributed
to increased proliferation and metabolic activity. The authors
went on to demonstrate that after myocardial infarction, mice
that received transplantation of CPCs pretreated with mES-
Exo had improved LV function compared with those received
cells pretreated with mEF-Exo. CPCs pretreated with mES-
Exo had better survival and proliferation, resulting in increased
angiogenesis and to a certain extent differentiating into new
myocytes, indicating the potential usefulness of mES-Exo as
a therapeutic candidate for enhancing cell survival and func-
tion. Because exosomes are known to harbor a plethora of bi-
ological molecules that can be transferred to target cells leading
to phenotypic modulation, Khan et al18 sought to investigate
miRNAs that are enriched in mES-Exo as potential modulators
of cardiac regenerative mechanisms. The authors showed that
mES-Exo were enriched for ES cell-specific miR-290 family,
and subsequent gain-of-function studies revealed miR-294 as
a primary candidate that accelerated cell cycle in CPCs treated
with miR-294 mimics, suggesting a central role in mediating
the effects of mES-Exo in promoting cardiac regeneration.

The study by Khan et al18 provides important and novel
insights into the potential application of exosomes as cell-free
therapeutic agents in place of autologous or allergenic cell
administration, which is often hampered by issues such as poor
cell survival, electric/mechanical coupling, and immu-
nogenicity. Importantly, this study paves the way for expa-
sion of exosomes beyond ES cells, showing that they could be
harnessed for other cell types such as induced pluripotent
stem cells.20 However, some concerns must be addressed be-
fore the immense potential of exosomes as a biomedical tool
in stem cell–based cardiovascular therapeutics can be fully
capitalized. Although stem cell–derived exosomes have gen-
erally been found to be less immunogenic than parental cells,
mainly because of lesser membrane-bound proteins such as
MHC (major histocompatibility complex),21 there is still an
inherent risk of exosomes triggering an immune response,
especially in the infarcted myocardium. Notably, the authors
emphasized the role of miR-294 as one of the contributing
factors that underlie the beneficial effects of mES-Exo. Given
that the cargo of exosomes is extremely complex, focusing
on miRNAs is likely only part of the equation and it would be
interesting to perform in-depth characterization of mES-
Exo’s full content in future studies using RNA-sequencing
or proteomics. Furthermore, because previous studies have
demonstrated that cells secrete exosomes differentially under
physiological and maladaptive conditions,22 it would be illu-
minating to perform additional characterization of mES-Exo
when ES cells are exposed to hypoxic conditions to mimic
the ischemic heart. Although it is conceivable that the transfer
of ES cell-specific miR-294 from exosomes into the heart can
stimulate pre-existing cardiomyocyte proliferation because
of its inherent role in accelerating G1-S transition, it is some-
what intriguing to contemplate how miR-294 can promote the
switch of CPCs into cardiomyocytes, given that the miR-290
cluster has been reported to actually inhibit differentiation
of cells, which is in line with its role of accelerating the cell cy-
cle.23 24 Along the same line, given that miRNAs are capable
of affecting multiple targets, future efforts should be made
to ensure proper targeting of exosomes to specific tissues to
prevent any undesirable off-target effects.

Taken together, the work of Khan et al18 shows that
exosomes can be harnessed as an extremely useful tool for
cardiac regenerative strategies. Although the molecular
mechanisms of exosomal-mediated cardiac repair are not
fully understood, the fact that exosomes are capable of medi-
sing such effect is extremely encouraging. Future work will
undoubtedly shed more light on the biology of these natural
carriers of biological molecules, such as in-depth systems
biology for characterizing exosomes,26 paving the way for
novel and exciting possibilities for the use of exosomes in
regenerative medicine.

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References


5. Gneccci M, He H, Liang OD, Melo LG, Morello F, Mu H, Nioseux N, Zhang L, Pratt RE, Ingwall JS, Dzau VJ. Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. Nat Med. 2005;11:367–368. doi: 10.1038/nm0405-367.


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