

Cardiomyocyte Regeneration: A Consensus Statement

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Dear colleagues,

In an effort to foster and accelerate the pace of discovery in the field of myocardial regeneration, *Circulation* has commissioned a Consensus Document. Our intent is to gather key thought leaders in the field in order to a) identify areas of consensus, b) specify areas of controversy, and c) delineate key steps required to move the field forward. To that end, we held two gatherings as follows:

- Discussion of concept at the BCVS Meeting in Phoenix: Wednesday, July 20, 2016.
- Finalizing of consensus paper at AHA Scientific Sessions, New Orleans, November 15, 2016

In each case, we conducted a 2-3 hour gathering marked by lively discussion.

Background

Cell therapy is an exciting option for repairing the injured heart, which has attracted considerable interest over the past 15 years. Consensus exists that the injection/infusion or tissue-based implantation of various cell types may exert therapeutic effects^{1, 2}, and there is general agreement that additional molecular, translational and clinical studies are required to define optimal cell source, method of delivery, and underlying mechanism(s) of action.

One of the underlying questions regards cardiomyocyte turnover under normal and diseased conditions and its contribution to the beneficial effects of cell therapy. While results published in the literature have not been consistent, we believe that time is ripe to reach a consensus regarding some of the pertinent questions.

It is important to emphasize that the focus of this consensus statement is on cardiomyocyte renewal; it is not on cell therapy in general. Whereas we touch on some aspects of therapeutic strategies based on delivery of exogenous cells, our intent here is to define areas of agreement, and areas requiring further elucidation, related to the regenerative potential of the myocardium itself.

We have included references to the scientific literature throughout the document. Whereas it is impossible for us to include all publications in this expansive field, representative studies that corroborate statements herein have been cited

Central questions

1. Definition of cardiomyocyte renewal

In this consensus statement, the term “cardiomyocyte renewal” is defined as the ability to replace lost cardiomyocytes by new ones. It is distinct from the turnover of cardiac proteins or the generation of polyploid cardiomyocytes (i.e. more than two sets of

chromosomes), either by nuclear division giving rise to multinucleation, or the duplication of DNA without nuclear division resulting in polyploid nuclei.

2. Naturally occurring cardiomyocyte renewal and proliferation

- During normal mammalian development
 - (i) Growth of the heart during embryonic and fetal development involves an absolute increase in the number of cardiomyocytes and is brought about by differentiation of precursor cells and by division of relatively immature cardiomyocytes.
 - (ii) The mammalian heart continues to grow by means of cardiomyocyte proliferation (hyperplastic growth) in the early postnatal period.³ During a brief postnatal window of 7 days in rodents, myocardial injury induces a regenerative response resulting in replacement of lost cardiomyocytes by new ones.⁴ Fate mapping studies suggest that this type of myocardial regeneration is mediated primarily by cardiomyocyte proliferation.⁴
 - (iii) While cardiomyocytes appear to continue to renew throughout life, the quantitatively dominant mechanism of growth in the mammalian postnatal heart is an increase in cardiomyocyte size (reviewed in Heineke et al⁵).
 - (iv) In the healthy, uninjured adult human and murine heart, the total number of cardiomyocytes remains essentially stable, and cardiomyocyte turnover is currently estimated at 0.5-2% per year in both species.^{3 6-8}

- Following cardiac injury in adult mammals
 - (i) Cardiomyocyte renewal rates may be higher after injury than under normal conditions.⁸
 - (ii) The experimental determination of cardiomyocyte turnover after cardiac injury can be challenging owing to inflammation, proliferation of stromal and vascular cells, and scar formation.

- After heart or bone marrow transplantation (chimerism)
 - (i) Sex-mismatched heart transplantation in patients with end-stage heart failure or sex-mismatched bone marrow transplantation provide opportunities to experimentally determine cardiomyocyte renewal deriving from extra-cardiac sources.
 - (ii) While data are not completely consistent, the majority of studies suggest that the level of cardiomyocyte chimerism after sex-mismatched transplantation is <1%⁹⁻¹¹, and may arise due to fusion events⁶
 - (iii) Insufficient data are available to determine the time course within which such chimerism develops.

3. Mechanisms of endogenous cardiomyocyte renewal

There is no infallible means of tracking cell renewal in any organ system. However, in preclinical models of cardiomyocyte renewal (e.g. mouse and fish), genetic fate mapping studies provide the strongest level of scientific evidence. Critical biological issues such as promoter fidelity (leakiness and sensitivity), inefficient reporter expression (Cre recombinase activity), and cellular fusion or transfer of reporter proteins are relevant and must be considered in the interpretation of the findings. Further, appropriate control studies are essential to assess for deleterious consequences of haploinsufficiency, which could result from genetic manipulation of an endogenous gene locus.

- Cardiomyocyte proliferation

- (i) The majority of studies suggest that cardiomyocyte renewal in the uninjured adult heart derives from a modest level of pre-existing cardiomyocyte mitosis¹²⁻¹⁴. Support for this interpretation comes from experiments in zebrafish^{15 16}, newts¹⁷ and other species¹⁸ in which cardiomyocyte renewal occurs more robustly than in mammals.

- Resident stem/progenitor cells

- (i) Resident stem/progenitor cells contribute to multiple cell types within the ventricle, including cardiomyocytes. However, in terms of adult myocardial homeostasis in mice, current evidence suggests that their contribution under basal conditions is low (estimates in rodents based on genetic fate-mapping experiments suggest a rate of <0.01% per year)^{19, 20}

- Extracardiac stem/progenitor cells

- (i) The contribution of extracardiac stem or progenitor cells to cardiomyocyte renewal has been studied largely with chimeric mice, in which the bone marrow is genetically labeled, and in parabiotic mice, in which the circulation of a genetically labeled mouse is experimentally linked to another unlabeled mouse. Cell fusion and transdifferentiation events have been evaluated using genetic lineage tracing, and the findings are largely concordant. In humans, the role of extracardiac stem/progenitor cells in cardiomyocyte renewal has been studied by sex-mismatched heart and bone marrow transplantation.
- (ii) Homing of extracardiac bone marrow-derived cells to the uninjured heart is a rare event.
- (iii) Extracardiac bone marrow-derived cells enter the injured heart at a higher rate. The majority of these cells are of hematopoietic origin.
- (iv) A small fraction of cardiomyocytes within injured rodent hearts carry the genetically determined label of bone marrow cells (estimates in rodents based on genetic fate-mapping experiments suggest a rate of <0.2%^{21 22}). Most studies suggest that the majority of these cells originate from cell fusion, and <1% may derive from transdifferentiation (estimates in rodents based on genetic fate-mapping experiments suggest a rate of <0.002% in total²²).

4. Therapeutic manipulation of cardiomyocyte renewal

- (i) Most studies suggest that the infusion, injection or tissue-based implantation of cells of various origins confer therapeutic benefits to the injured heart.
- (ii) Cell-based therapies may affect endogenous cardiomyocyte renewal and/or directly generate new cardiomyocytes from the transplanted cells.
- (iii) The degree of new cardiomyocyte formation depends on the cell type, as well as on retention and survival of those cells within the heart. Retention of unselected bone marrow cells in the heart is low (a study in patients determined a rate of <3% for unselected bone marrow cells and approximately 10-fold higher with CD34⁺ cells 1 hour after coronary infusion²³). It may be higher following cell injection into the myocardium.²⁴ Co-injection of scaffolding materials and use of tissue engineering approaches may increase this rate.²⁵
- (iv) The degree of engraftment and differentiation of transplanted cells into cardiomyocytes does not appear to match the extent of functional improvement, suggesting that other mechanisms account for at least part of the beneficial effects of cell therapy.
- (v) Transplantation of cardiomyocytes derived from pluripotent stem cells can generate new myocardium that beats in synchrony with the host myocardium and may contribute to systolic force generation, although the extent of this contribution has not been precisely determined.
- (vi) Mechanisms of benefit remain obscure, but may involve paracrine actions, including exosome-derived effects on pre-existing cardiac tissue.^{26, 27}

- Bone marrow-derived cells

- (i) Prevailing evidence suggests that unfractionated bone marrow-derived cells do not become cardiomyocytes when infused or injected into the heart²⁸⁻³⁰
- (ii) Fractionated bone marrow populations consisting of c-kit⁺ cells or mesenchymal stem cells may confer structural and/or functional benefits primarily by indirect biological activities that may promote cardiomyocyte renewal^{31, 32, 33}
- (iii) Evidence for clinically demonstrated therapeutic benefit with bone marrow-derived cells has emerged with mesenchymal cells³⁴ and possibly c-kit⁺ cells³⁵.
- (iv) Evidence for the fate of mesenchymal cells after infusion or injection into the heart is inconsistent, but some studies report unmanipulated mesenchymal cells can transdifferentiate into cardiomyocytes at low rates.^{36, 37}
- (v) Genetic or ex vivo manipulation of transplanted cells enhances engraftment as well as structural and functional recovery of uninjured myocardium in preclinical animal models^{38, 39}

- Cardiac-derived stem/progenitor cells
 - (i) Most experiments have been performed with c-kit⁺, cardiosphere-derived cells or Sca1⁺ cells isolated from heart biopsies and cultured in vitro.
 - (ii) These cells can emerge as cells expressing cardiomyocyte markers when cultured in vitro under specific conditions, they can also express some cardiomyocyte markers in vivo^{40 41}. Co-culturing cardiac c-kit cells with mesenchymal stem cells enhances their lineage commitment towards a cardiac myocyte fate⁴².
 - (iii) The degree of functional improvement following in vivo delivery of these cells cannot be explained solely by new cardiomyocyte formation from transplanted cells, which is very low.⁴³⁻⁴⁵

- Pluripotent cells
 - (i) Pluripotent stem cells proliferate in an undifferentiated state indefinitely and, upon exposure to specific culture conditions, can differentiate into almost all cell types of the body including cardiomyocytes.
 - (ii) The efficiency of differentiation of pluripotent stem cells into immature cardiomyocytes in vitro can exceed 80%.⁴⁶⁻⁵⁰
 - (iii) Undifferentiated pluripotent stem cells can form teratomas when injected into the heart of immunocompromised organisms.⁵¹
 - (iv) Pluripotent stem cell-derived cardiomyocytes successfully engraft generating new myocardium when injected into the injured or uninjured heart⁵²⁻⁵⁷. Long-term engraftment of these cells has not been studied.
 - (v) Pluripotent stem cell-derived cardiomyocytes can electrically couple with host cardiomyocytes, beating in synchrony but they have also been found to have proarrhythmic effects^{53, 57}.
 - (vi) Although direct force generation deriving from the injected myocytes may explain some of the functional improvement, it is not clear whether the degree of emergence of new myocardium entirely accounts for the degree of contractile improvement; paracrine signalling events may contribute as well.

- Stimulation of the endogenous cardiomyocyte proliferation
 - (i) The normal turnover of cardiomyocytes can be stimulated as a therapeutic strategy to achieve regeneration
 - (ii) Endogenous cardiomyocyte proliferation can be enhanced by manipulation of cell cycle regulators^{58, 59}, redox regulators⁶⁰⁻⁶², growth factors acting through cell surface receptors^{63 64} or through the transfer of nucleic acids acting intracellularly^{16 65 66}

5. Important questions to be answered

- (i) Identify mechanisms of endogenous cardiomyocyte renewal in mammals as a target for therapy, including characterizing populations of proliferative cardiomyocytes
- (ii) Define the relative roles of progenitor cell differentiation versus cardiomyocyte proliferation in regenerating the injured myocardium
- (iii) Unveil mechanism(s) of benefit deriving from cell-based therapy, including the contribution of new cardiomyocytes, angiogenesis, anti-inflammatory actions, anti-fibrotic actions, or other effects
- (iv) Define the paracrine mechanisms that mediate many of the beneficial effects of cell therapy
- (v) Improve the efficiency of cell therapy with regards to modes of delivery, enhancement of engraftment, and differentiation
- (vi) Define the risk/benefit aspects for genetically-modified stem cells pluripotent stem cell-based therapies, and cell combination strategies.

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