COMMENT

# Hallmarks of cardiac regeneration

*Alessandro Bertero*[1] *and Charles E. Murry*[1,2]*

Despite substantial advances, bona fide regeneration of the damaged human heart is still an unmet ambition. By extracting our current knowledge from developmental biology, animal models of heart regeneration, and clinical observations, we propose five hallmarks of cardiac regeneration and suggest a holistic approach to reconstituting human heart function.

Regeneration of the cardiac muscle in patients with heart disease has been a holy grail, but despite decades of efforts and several successes in preclinical models, such outcomes have not yet positively translated into human clinical trials. Therefore, we must go back to the drawing board and rethink our approach. This Focus Issue of *Nature Reviews Cardiology* aims to catalyse this process by providing a broad overview of the most important recent advances in cardiac development and regeneration.

Over the past decade, the rise of revolutionary technologies such as single-cell genomics, CRISPR–Cas9 gene editing, and pluripotent stem cells has dramatically shifted our understanding of cardiac biology. Our knowledge of cardiogenesis and the underlying gene regulatory networks has been highly refined, and the heterogeneity and plasticity of the resulting cell states is becoming increasingly clear (reviewed in this Focus Issue by Meilhac and Buckingham, and by Christoffels and colleagues). We have not only identified and characterized the spatiotemporal function of crucial pathways involved in cardiomyocyte specification, proliferation, and survival (reviewed in this Focus Issue by Martin and colleagues, and by MacGrogan, Münch, and de la Pompa), but we have moved well beyond the cardiomyocyte to increasingly appreciate the physiological importance and therapeutic potential of the epicardium, stroma, vasculature, and immune system (reviewed in this Focus Issue by Cao and Poss, and by Forte, Furtado, and Rosenthal). Thus, today we face the unprecedented opportunity to integrate all this new knowledge with the lessons learned from existing preclinical and clinical experience in order to devise the next generation of cardiac regeneration therapies (reviewed in this Focus Issue by Menasché, and by Hashimoto, Olson, and Bassel-Duby).

In 2000, the classic paper from Hanahan and Weinberg propelled a dramatic shift in cancer biology from a reductionist view focused on the neoplastic cell to one regarding the tumour as a complex, heterotypic tissue[1]. A number of 'hallmarks of cancer' were proposed to explain the variation of cancer phenotypes down to a core of acquired properties. By analogy, we propose a minimal set of five 'hallmarks of cardiac regeneration' that are consistently observed in organisms with an innate cardiac regeneration capacity such as zebrafish or in the neonatal mouse heart[2] (FIG. 1). We suggest that all these properties should be implemented in a new generation of holistic approaches to achieve bona fide reconstitution of the heart muscle.

## Remuscularization

Regeneration of the cardiac wall must by necessity involve its primary functional component, the cardiac myocyte. Several approaches have been devised and can be broadly classified in three groups: stimulation of endogenous cardiomyocyte proliferation, for example, through modulation of developmental signalling pathways or direct regulation of cell cycle regulators[3]; reprogramming of resident stromal cells into cardiomyocytes by forced expression of cardiogenic factors[4]; or supplementation with exogenous stem or progenitor cells with putative, but still controversial, cardiogenic potential, or with bona fide cardiomyocytes derived from human pluripotent stem cells (hPSCs)[5,6]. All these strategies have shown moderate to substantial promise, but care must be taken when assessing the efficacy of true remuscularization. For instance, current clinical strategies based on stem or progenitor cells are now believed mainly to lead to paracrine effects that, while possibly beneficial, do not directly realize this central hallmark[7]. By contrast, transplanting cardiomyocytes derived from hPSCs has so far created the highest volume of new myocardium[5,6], but has not yet been attempted in humans. We anticipate that our improved understanding of cardiac development will inform improved approaches both to drive cellular proliferation and reprogramming, and to obtain hPSC-derived cardiomyocyte subtypes most suited for remuscularization.

## Electromechanical stability

New cardiomyocytes will not only be mechanically feeble, but could lead to adverse effects if they are not properly connected within the innate cardiac electrical topology. While these concerns were once purely theoretical, they are now corroborated by preclinical data showing that transplantation of human or monkey PSC-derived cardiomyocytes into non-human primates can indeed induce sustained ventricular arrhythmias[5,6]. Other approaches

[1]*Institute for Stem Cell and Regenerative Medicine, Center for Cardiovascular Biology, and Department of Pathology, University of Washington, Seattle, WA, USA.*

[2]*Departments of Medicine, Cardiology, and Bioengineering, University of Washington, Seattle, WA, USA.*

**e-mail: murry@uw.edu*

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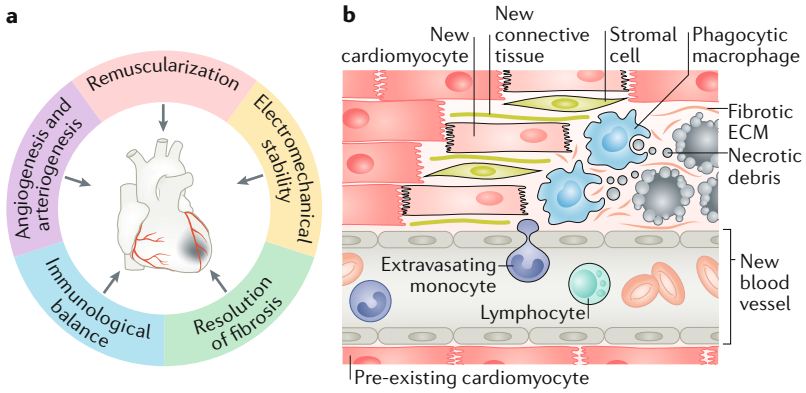


Fig. 1 | **Properties of the regenerating heart. a** | We propose that five hallmarks should be realized to truly reconstitute the human heart muscle. **b** | Optimally regenerating myocardium, in which new cardiomyocytes electromechanically couple with the surviving host myocardium, while stromal cells, new blood vessels, and immune cells create the necessary favourable microenvironment. ECM, extracellular matrix.

might have similar drawbacks when used for large-scale remuscularization. To overcome these limitations, new cardiomyocytes will have to rapidly achieve adult-like functionality of crucial components of the excitation–contraction coupling machinery (including ion channels, gap junctions, transverse tubules, and myofibrils)[8]. Repressing the automaticity of new cardiomyocytes will be particularly important, and to achieve this aim, we can leverage our emerging understanding of the gene regulatory networks underpinning the development of the cardiac pacemaker and conduction system. Finally, efficient and specific delivery methods for cells, gene vectors, and molecular therapies will be needed to ensure spatially precise and homogeneous remuscularization of the damaged region, so as not to interfere with healthy muscle.

## Angiogenesis and arteriogenesis

An extensive and hierarchical vascular network is required to robustly supply the regenerating muscle with oxygen and nutrient exchange. While pre-existing vessels and vascular progenitor cells can innately contribute to angiogenesis[5], this process can be markedly boosted by a number of paracrine factors that can be supplemented exogenously (such as growth factors, microRNAs, and exosomes). Endogenous pro-angiogenic signals are also secreted by the stroma, immune system, and epicardium. Regeneration could be further boosted by providing new vasculogenic cells either as progenitors or as differentiated progenies. Importantly, angiogenesis creates a fairly disordered plexus that is not robustly perfused. Complete myocardial regeneration will also require arteriogenesis. This can either result from biological remodelling, for example through enlargement of capillary-sized vessels into muscularized conduction vessels, or be designed though emerging bioengineering solutions for the generation of engineered heart tissues[9].

## Resolution of fibrosis

While production of extracellular matrix (ECM) in the heart was once considered primarily a pathological mechanism, the ECM is now known to be pivotal in preventing early rupture of the damaged myocardium, and also to facilitate cardiac regeneration in the neonatal heart and in regenerative organisms. Overall, cells of the stroma, primarily fibroblasts and myofibroblasts, represent a double-edged sword that must be carefully balanced to sculpt an environment appropriate to efficient remuscularization and vascularization. A number of approaches have been explored to modulate the plasticity of these populations and control the composition and stability of the ECM, including administration of antifibrotic paracrine factors either directly or via stem or progenitor cells. Excessive fibrosis is a particular challenge when surgically applying epicardial cardiac 'patches', because the resulting scar strongly limits electromechanical coupling and angiogenesis of the graft, limiting its functional benefit and long-term survival[9].

## Immunological balance

The immune system has multiple essential roles in both the early and chronic stages after cardiac injury. In particular, balanced activation of the innate immune system is necessary to clear necrotic cells, initiate angiogenesis, and promote fibroblast ingrowth, whereas rapid resolution of inflammation is required for regeneration[10]. In this context, the interplay between the stroma and immune cells has a crucial role, offering promising therapeutic avenues. The adaptive immune system is important in cell transplantation[5,6], because rejection must be prevented either by inducing immunotolerance through administration of immunosuppressive drugs or biologic agents, or by using cells that are autologous or engineered to avoid immune rejection.

The first generation of regenerative therapies was focused on individual agents or approaches, because of both practical and regulatory limitations. Looking ahead, we envision holistic approaches encompassing bioactive molecules, stem cells, bioengineering, and gene editing. By embracing complexity, we can target these five cardiac regeneration hallmarks and, we think, achieve clinically meaningful heart repair in the next decade.

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**Competing interests**
C.E.M is a scientific founder and equity holder in Cytocardia. A.B. declares no competing interests.