

## Prometheus's heart: what lies beneath

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### Abstract

A heart attack kills off many cells in the heart. Parts of the heart become thin and fail to contract properly following the replacement of lost cells by scar tissue. However, the notion that the same adult cardiomyocytes beat throughout the lifespan of the organ and organism, without the need for a minimum turnover, gives way to a fascinating investigations. Since the late 1800s, scientists and cardiologists wanted to demonstrate that the cardiomyocytes cannot be generated after the perinatal period in human beings. This curiosity has been passed down in subsequent years and has motivated more and more accurate studies in an attempt to exclude the presence of renewed cardiomyocytes in the tissue bordering the ischaemic area, and then to confirm the dogma of the heart as terminally differentiated organ. Conversely, peri-lesional mitosis of cardiomyocytes were discovered initially by light microscopy and subsequently confirmed by more sophisticated technologies. Controversial evidence of mechanisms underlying myocardial regeneration has shown that adult cardiomyocytes are renewed through a slow turnover, even in the absence of damage. This turnover is ensured by the activation of rare clusters of progenitor cells interspersed among the cardiac cells functionally mature. Cardiac progenitor cells continuously interact with each other, with the cells circulating in the vessels of the coronary microcirculation and myocardial cells in auto-/paracrine manner. Much remains to be understood; however, the limited functional recovery in human beings after myocardial injury clearly demonstrates weak regenerative potential of cardiomyocytes and encourages the development of new approaches to stimulate this process.

**Keywords:** cardiac regeneration • cardiomyocyte turnover • tissue plasticity • heart

### Introduction

The adult human heart has been viewed as a static terminally differentiated organ, formed of oriented mature cardiomyocytes that allow for the conduction of electrical signals and the synchronous contractile function aimed at maintaining the physiological systemic perfusion. Adult human myocardium has been considered a tissue capable of increasing its mass by hypertrophy of existing myocytes in response to increased after- and/or pre-load. Indeed, historical evidence highlighted that, in physiological or pathological conditions, a post-natal mammalian cardiomyocyte could not re-enter the cell cycle and replace itself. However, no clear evi-

dence in favour of the above possibility was provided prompting the international scientific community to reveal the hidden truth in definitive way. Since the late 1800s, the question of regeneration of cardiac muscle fibres has often been discussed. Goldenberg [1] reviewed the previous reports and concluded that longitudinal splitting of cardiomyocytes could occur, yet the increase of cardiac tissue was largely by hypertrophy of muscle fibres. Conversely, other investigators demonstrated that the increase of the heart mass was mainly due to hypertrophy rather than hyperplasia of cardiomyocytes, on the basis of the lack of evidence of myocardial

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mitotic figures [2–5]. In the early 1920s, counts and measurements of muscle fibres made by Collier [6] and Karsner *et al.* [7] confirmed that myocardial hypertrophy and hyperplasia are occurred. Thereafter, Macmahon [8] indicated that the principal difficulty was failure to demonstrate mitotic figures in adult cardiomyocyte nuclei, but not in myocardium of infants and children. Thus opinions on cardiomyocyte turnover in adult heart was divided for many years, but in the late 1960s and early 1970s the evaluation of DNA synthesis in cardiomyocyte nuclei was shown to be negligible, strengthening the notion that cardiomyocytes can increase in volume but not in number [9, 10].

The steps forward of the molecular cardiology and the development of advanced microscopy techniques have modified the scale of analysis and allowed conceivable that the early cardiomyocyte response to homeostatic and pathophysiological environments is a dynamic consequence of alterations in the effector pathways, which regulate myocyte growth and proliferation [11–14]. Intercellular signals are generated from resident cells functionally mature or immature, and tend to maintain a homeostatic code in order to preserve the myocardial architecture. The dissection of the mechanisms is still in progress and this is changing dramatically our understanding of cardiac biology by witnessing the principles dictating initial cardiac genesis during development so as to exclude any artefact.

## Adult cardiomyocyte turnover

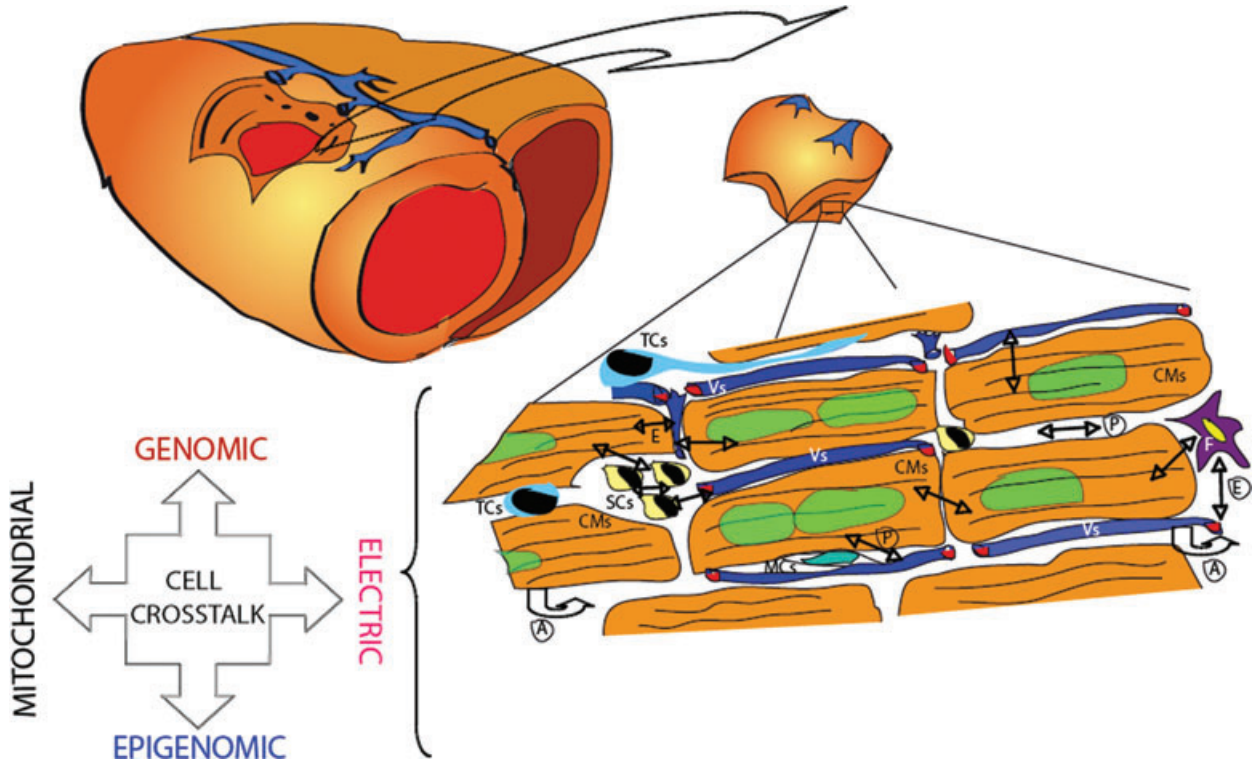
At birth the number of cardiomyocytes in the heart of mammals is largely determined. The hyperplastic growth occurring during embryonic life is quickly replaced by a hypertrophic process soon after birth. Studies based on the method of incorporation of thymidine showed that DNA synthesis accompanied by cytokinesis in mice decreases from 70% at E8 to 45% at E11 and almost disappears 1–2 days after birth [15]. In the second phase, starting just after the birth, karyokinesis occurs in the absence of cytokinesis thus determines an increasing percentage of binucleated cardiomyocytes from 2.5% at day 2, to 80% at day 14 [16]. These two distinct phases in the heart development are reflected in the expression levels of regulators of cell cycle such as cyclin-dependent kinases (Cdks) and their inhibitors. The expression patterns of cell cycle regulatory proteins are well reviewed by Pasumarthi K.B. & Field L.J. [11]. In general, Cdks exert their positive effects in terms of promoting mitosis of cardiac cells during the embryonic life while they are down-regulated in the adult heart. Therefore, the replicative capacity of the heart suffers a sharp slowdown after birth and starting from this certainty, several scientists have wondered if the adult heart was still able to retain a regenerative capacity in adult life. Recently, two independent studies have addressed the issue following a similar approach but in two different species [11, 17]. Although it was known that many invertebrates such as the zebrafish can regenerate the heart after amputation up to 20% [18]. Jopling *et al.* first established that the contribution of existing cardiomyocytes to regenerate tissue is of paramount impor-

tance. Using a CRE/lox approach they demonstrated that after partial amputation of the ventricle, survived cardiomyocytes adjacent to the damaged region express regulators of cell-cycle progression, such as *plk1* and *mps1*, de-differentiate detaching from one another. They lose their sarcomeric structure and re-enter the cell cycle [19]. A more recent paper by Porrello *et al.* showed that similar events occur in 1-day-old mouse when the ventricular apex is resected. The region of resection is progressively interested by a robust inflammatory response followed by the formation of blood clot that is subsequently replaced by new functional heart, which derives mainly from the pre-existing cardiomyocytes. At 21 days the majority of the new formed cardiomyocytes were positive for the mitotic index, phosphohistone H3 and the aurora B kinase. The mouse heart loses its regenerative potential within the first week of postnatal life [17]. While demonstrating the absolute importance of the existing cardiomyocytes in regenerating new cardiac tissue, none of the above-cited papers can exclude the contribution of resident progenitor/stem cells.

## Cardiac tissue generation: contribution of resident interstitial cells

The adult myocardium is a well-organized tissue composed by different resident cell types forming a morpho-functional unit [20]: cardiomyocytes, endothelial cells, smooth muscle cells and a variety of interstitial cells including cardiac resident stem/progenitor cells (Fig. 1). Among the interstitial cells, cardiac fibroblasts, which account for two-thirds of total myocardial cells, play important physiological role in ensuring a spatial orientation, a structural organization and mutual interplay of resident cardiac cells, which work interdependently in order to promote an efficient global and regional function of the heart [21]. The intrinsic characteristics of tension-generating fibroblasts also have a continuous mechanical interplay with their extrinsic environment [22]. Moreover, the cardiac fibroblasts are the main myocardial source of collagen, which composes the post-ischaemic infarct scar, and release several humoral mediators involved in myocardial remodelling: proinflammatory cytokines, including tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ), and interleukin-6 (IL-6) [23, 24], growth factors involved in myocardial remodelling, such as platelet-derived growth factor [25], high molecular weight fibroblast growth factor-2 (FGF-2) [26], insulin-like growth factor-1 [27] and vascular endothelial growth factor (VEGF) [28]. Cardiac fibroblasts are able to interact with other interstitial cells resident into the myocardium, such as mast cells (MCs) [29] and telocytes (TCs) [30].

Mast cells are multifunctional cells releasing different humoral mediators, such as cytokines, and histamine, and they have been identified in injured myocardium [31]. MCs have been suggested also to play a role in adverse remodelling in human failing heart [32]. Chymase, which is an enzyme stored in the cardiac MCs, is insensitive to ACE inhibitors and promotes interstitial fibrosis by affecting collagen metabolism *via* transforming growth factor- $\beta$ .



**Fig. 1** Cross-talk of resident cardiac cells. Schematic representation of resident cardiac cells potentially involved in myocardial regeneration and modalities of intercellular cross-talk. Vs: vessels; CMs: cardiomyocytes; SCs: stem cells; F: fibroblast; TCs: telocytes; MCs: mast cells; P: paracrine action; A: autocrine action; E: endocrine action.

Chronic chymase inhibition decreases the cardiac angiotensin II levels, myocardial fibrosis and contractile failure [33]. Conversely, Kwon *et al.* [34] recently showed that the intramyocardial injection of MC granules prolonged survival of cardiomyocytes and promoted angiogenesis in rodent infarcted hearts. However, the role of cardiac MCs during remodelling, which are also CD117(+), remains controversial and some investigators even suggested them as cardiac progenitor cells (CPCs) [35].

Telocytes have been recently described as a novel different types of interstitial cell type in adult mammalian heart [36, 37]. They are characterized to shed exceptionally long cellular processes – telopodes – which form an interstitial network, connecting different types of cell from myocardium, epicardium and endocardium. TCs seem to be involved in a long distance intercellular signalling, due to both membrane-to-membrane junction and release of vesicles [30, 36]. TCs presumably contribute to neo-angiogenesis *via* paracrine secretion of VEGF and microRNAs (*e.g.* let-7e, 10a, 21, 27b, 100, 126–3p, 130a, 143, 155) [38]. Particularly important is the role of TCs in supporting the renewal and survival of CPCs [30].

Cardiac progenitor cells reside in the heart as well as in other solid organs, within niches that are composed of extracellular matrix, stem/supporting cells and blood vessels [39, 40]. In the last decade, several independent groups have tried to isolate pro-

genitor cells from adult heart tissue with a view to reuse them as cell source for autologous cell transplantation in injured heart [41–46]. With the heterogeneity of markers that have been chosen to define cardiac progenitor populations, few scientists have bothered to study the role of these progenitors in the normal cardiac physiology. So far it is not clear if these populations are related and which is their origin. The *in vitro* condition that better recapitulates the features of CPC niche is the three-dimensional culture of CPCs as cardiospheres, in which the cell–cell and cell–matrix interactions are conserved and the cell survival and functional benefits are ensured [47, 48].

An indirect evidence that precursors may contribute to generate new cardiomyocytes in the area of the injury derives from paper by Hsieh *et al.* [49]. They used an elegant ‘fate-mapping’ approach establishing a double transgenic mouse in which all the cells positive for the cardiac specific promoter Mhy6 were constitutively expressing the  $\beta$ -galactosidase reporter gene ( $\beta$ -gal+). The latest is replaced by the GFP when mice are pulsed with 4-OH tamoxifen. Theoretically, all the cardiomyocytes that were  $\beta$ -gal+ become GFP+ after pulse. The findings consist in the fact that following the induction of the infarct in the transgenic mice, the number of GFP+ myocytes were significantly decreased with respect to the  $\beta$ -gal+ cells indicating the presence of new cardiomyocytes deriving from the differentiation of progenitor cells. Although

authors cannot exclude that some progenitors were labelled by the 4-OH-tamoxifen, the percentage of new cardiac cells in myocardial infarction border areas that were not derived from the mitosis of pre-existing cardiomyocytes, reached 34%. Thus cardiomyocytes can be generated by activation of resident [50] or in part by migration of progenitor cells from distant organs [51]. Similar genetic mapping approach to track cell fate in injured heart has been followed by Loffredo *et al.* [52]. In this article, authors establish that the dilution in GFP<sup>+</sup> and complimentary increase in  $\beta$ -gal + cardiomyocytes in infarcted transgenic mice is associated with an increase in the number of nkx2.5- and GATA4-positive progenitor cells. Moreover, in the same set of experiments they were able to assess that the delivery of exogenous bone marrow-derived c-kit<sup>+</sup> cells exert effects by releasing a paracrine factors able to stimulate regenerative capacity of progenitors rather than proliferation of pre-existing cardiomyocytes [52]. However, recent findings showed that the function and potential plasticity of stem cells, potentially activated or recruited after injury into the myocardium, is impaired by aging [53] and severity of cardiac disease [54].

The consistency of cell turnover due to CPC differentiation was analysed in mouse by the *in vivo* incorporation of the BrdU [40]. BrdU retaining myocyte was 19%, 15% and 10% in the apex, atria and base-midregion, respectively [40].

In human beings who live much longer than rodents, it is reasonable to expect a slow cell turnover that implies cardiomyocytes replacement. Clear evidence that cell division occurs in human heart is shown by Bergmann *et al.* [55]. During the Cold War the concentration of <sup>14</sup>C in the atmosphere had increased sharply following an exponential fluctuation with a peak around 1963. By relating this information with the levels of <sup>14</sup>C in the DNA of cardiomyocytes of individuals born before and after the nuclear bomb tests, authors reached a conclusion that the amount of <sup>14</sup>C present in heart cells of individuals correspond to atmospheric levels several years after the persons' birth. It follows that the DNA of cardiomyocytes has been synthesized many years after the birth thus implicating cell renewal. Using elaborate mathematical models and excluding contamination of other cell types by cell-sorting, authors came to the following conclusions: at the age of 50, 55% of the cardiomyocytes remain from the time birth and 45% have been generated later; cardiomyocytes are renewed at a rate of ~1% per year at the age of 25, 0.45% at the age of 75; the age of cardiomyocytes is on average 6 years younger than the individual.

This article was recently criticized in numbers and methodology by Kajstura J. *et al.* [56]. They use a different approach based on the detection of the iododeoxyuridine, analogue of thymidine, into the cardiac cells of patients undergoing infusion of this radiosensitizer. The results were 'dramatically' different with an average of myocytes renewal of about 22% per year suggesting that the heart is capable to renew itself several times during the course of life [56]. Moreover, the myocyte turnover increases further with age reaching the 40% in a heart of female of 100 years of age.

The above experimental evidence, obtained by the use of more accurate technologies than optical microscopy, seemed to have

answered the question that tormented too long cardiovascular pathologists. Over the next two decades, the significant lack of further technological progress had mitigated the interest in the field of regenerative cardiology maturing awareness that a deep understanding of the mechanisms should be guaranteed only by a change of point of view of the biological phenomena.

## Signalling of miocardiogenesis: a developmental perspective

Unravelling how different signalling pathways dictate cardiac precursor specification and subsequent behaviour is critical for predicting how damaged myocardium will self-renew (Fig. 1). To date, studies on cardiogenesis offered new hypothesis to highlight the mechanisms underlying the regeneration of adult myocardium. Several signalling pathways integrate and cross-talk during cardiogenesis and it is conceivable that multiple molecular inputs regulate the balance in the earliest steps of cardiogenesis through mesoderm specification and allocation.

TNF-related weak inducer of apoptosis (TWEAK), a member of the TNF- $\alpha$  family, regulates proliferation in multiple cell types and its effects are mediated through the activation of fibroblast growth factor-inducible molecule 14 (FN14) receptor [57]. TWEAK caused a dose-dependent increase in DNA synthesis of cardiomyocytes and down-regulates the cell cycle inhibitor p27KIP1. TWEAK/FN14-mediated patterning involves the activation of ERK, and PI3K, as well as inhibition of GSK-3 $\beta$  which in turn led to stabilization and accumulation of total  $\beta$ -catenin and accumulation of dephosphorylated  $\beta$ -catenin in the nucleus. However, TWEAK does not affect proliferation of adult rat cardiomyocytes due to progressive down-regulation of FN14 gene and protein expression after birth. Therefore, a guided post-natal overexpression of FN14 receptor is able to induce an efficient cell cycle reentry in adult cardiomyocytes on the basis of the presence of endogenous TWEAK protein in these cells [14]. Terminal differentiation of organ involves the retinoblastoma family of tumour-suppressor pocket proteins and Cdks that modulate their function [58]. However, the role of these proteins has been substantiated in cardiac cell cycle control [59] and needs the activity of telomerase reverse transcriptase (TERT). Continuous cell division erodes the telomeric repeat because of incomplete replication of the distal 39 strand during active replicative growth [60]. In adult human beings, telomerase activity is found predominantly in germ cells, tumour cells and stem cells but not somatic cells that ultimately senesce. Failure to express TERT is the principal mechanism for low levels of telomerase activity seen with replicative senescence. Telomerase activity and TERT expression are lacking or are markedly decreased in the adult heart, but not during cardiogenesis [61]. On the basis of this notion, Oh *et al.* [62] showed that the replacement of ancestral telomerase activity in adult cardiomyocytes delays cell cycle exit, induces hypertrophy and promotes cardiac myocyte survival.

Other investigations showed that Nodal/Activin/TGF- $\beta$ , bone morphogenetic proteins (BMPs) and FGFs drive cardiogenesis [63] and suggested how their re-activation might improve the self-regenerative capacity of the heart.

BMPs promote mesoderm formation from embryonic stem cells through a Wnt/Activin/Nodal-dependent mechanism [64]. Thereafter, Notch regulates CPC proliferation and fate through an interaction with Wnt/b-catenin signalling [64]. Previous study showed that cardiomyogenesis in the adult heart depends on the activation and differentiation of cardiac stem cells [65]. In accordance with the traditional model of development of self-renewing organs, stem cells give rise to progenitor precursor cells, which then proliferate and eventually reach terminal differentiation and growth arrest. Newly generated cardiomyocytes might proliferate and concurrently differentiate, mimicking cellular hyperplasia and hypertrophy [66]. Activation of the Notch1 cascade is crucial for cell-to-cell interaction during cardiovascular development and may influence differentiation, proliferation and apoptotic events of CPC [67, 68]. Alteration of Notch1 pathway hampers coronary vessel wall maturation during myocardium growth and development [69] and even predisposes to the onset of lethal cardiomyopathy [70]. Notch1 signalling is limited by the abundance of Notch1, which is transcriptionally controlled by Nrf2 (nuclear factor erythroid-derived 2-related factor 2), which is a prosurvival transcription factor that plays a pivotal role in maintaining cellular homeostasis following oxidative stress [71–73] and pro-inflammatory state [74] in adult heart. Nrf2-mediated regulation of Notch1 expression may be most critical in postnatal stages, rather than during development, because Nrf2 disruption does not produce altered phenotypes during early development stages. Nrf2 is abundant in tissues containing stem cells, such as liver, kidney and gastrointestinal-tract. Wakabayashi *et al.* [75] showed original evidence that Nrf2 facilitates tissue regeneration in the liver by regulating Notch1 expression. Moreover, recent experimental evidence showed that an increased expression of Nrf2 improves the effectiveness of cell therapy delivered in infarcted rodent heart [76].

Wnt signalling has been shown to influence the development of the heart. Recent data suggested canonical Wnts promote the emergence and expansion of cardiac progenitors in the gastrula embryo. Martin *et al.* showed that canonical Wnt signalling up-regulates expression of the cardiac stem cell marker c-kit and pluripotency genes Oct25 and Oct60 without a corresponding inhibition of cardiogenic differentiation [77].

During cardiomyogenesis, Notch and TGF- $\beta$  signalling pathways are involved in endothelial-to-mesenchymal transition of endothelial cells. Notch activation modulates TGF- $\beta$  signalling pathways in a receptor-activated Smad-specific manner [78]. In particular, Notch increases SMAD3 mRNA expression and cooperatively induces histone H4 acetylation in cooperation with TGF- $\beta$ . Recently, Maioli *et al.* [79] showed that the transcription of the cardiogenic gene Nkx-2.5 in embryonic stem cells is enhanced through Smad4 binding to its own consensus Smad site. The above signalling pathway driving cardiogenesis is modulated by novel molecules, which maintains acetylation of histone H4 in stem cells and adult cardiomyocytes, such as hyaluronan mixed esters of butyric and retinoic acids [80].

## Imaging of self-renewed myocardium

The phenomenon of myocardial self-regeneration needs more detectable and quantifiable evidence in response to historical controversies [81]. In the past, it was very difficult to determine the sequence of the process of myocardial remodelling and/or repair after an injury as well as the plausible interference of a therapy during the healing's phase without the use of histological analysis and, therefore, the sacrifice of a large number of animals. In addition, the tools available were not advanced enough to promote accurate morphological and functional study at regional and molecular level. So, it was common opinion that the *no detection* of a global cardiac effect, such as following a potential healing treatment, reflects the absence of an adaptive response post-injury (repair/regeneration), which was rather active regionally to a different scale. To date, the technological progress in non-invasive diagnosis allowed the development of more accurate tools for the ongoing detection of new forming coronary vessels or myocardium as well as for the real-time assessment at higher magnification of contractile function and structure *in vivo* [82–84]. The process of myocardial remodelling is initiated with a number of dynamic adverse effects in injured heart. Firstly, the non-contracting tissue expands and becomes thinner. The extreme thinning of the infarcted cardiac wall is easily detectable in both animal models and human beings. Secondly, the reactive growth of the cardiac tissue causes an increased volume load, after which compensatory mechanisms also lead to an increased pressure load on the heart. The mechanical overload results in LV hypertrophy and then dilation with increasing of LV cavity volume.

Finally, the occurrence of myoarchitectural disarray in the ventricular wall, *i.e.* the presence of abnormally organized cardiomyocytes, characterizes the remodelled myocardium in cardiovascular disease, such as hypertrophic cardiomyopathy, hypertension and myocardial infarction [85]. The cardiac muscle architecture lies at the basis of the mechanical and electrical properties of the heart, and dynamic alterations in fibre structure are known to be of prime importance in healing and remodelling after myocardial infarction. The amount of disarray present in the hearts after myocardial infarction, in both the border areas and the scar tissue formed by the infarction, is quantifiable by magnetic resonance imaging (MRI). In fact, diffusion tensor imaging offers a non-destructive tool for the study of myocardial fibre orientations [86, 87] as well as for the evaluation of structural tissue changes caused by ischaemia [88].

Myocardial wall anatomy and function of normal/injured cardiac regions are also accurately assessed by MRI using velocity encoding, tagging or DENSE (displacement encoding with stimulated echo) imaging [89–92]. The delayed enhancement-MRI of the infarcted myocardial wall after injection of a gadolinium-based contrast agent has been demonstrated to correlate with infarct size [93].

Regional myocardial wall perfusion in infarcted heart is quantified by positron emission tomography (PET) after intravenous injection of specific radiotracers, such as  $^{13}\text{NH}_3$  (ammonia) [91, 94],  $^{11}\text{C}$ -acetate [95] or  $(^{15}\text{O})$ -labelled water [96]. Recent

investigations also demonstrated that it is feasible to quantify regional myocardial perfusion in ischaemic heart using gated single photon emission computed tomography (SPECT) [97] or hybrid SPECT/coronary computed tomography angiography technology [98]. The nuclear medicine has made possible an accurate assessment of the myocardial viability through a monitoring of its metabolism. Radionuclide modalities currently used in the assessment of viability include (201)Tl SPECT, (99m)Tc-based SPECT imaging and (18)F-fluorodeoxyglucose ((18)F-FDG)-PET imaging. The initial myocardial uptake early after systemic delivery of thallium reflects the magnitude of the regional blood flow. However, redistribution of <sup>201</sup>Tl is related to the rate of <sup>201</sup>Tl 'washout' from the myocardium, dependent on the thallium concentration gradient between myocytes, blood pool and the integrity of the membrane-based Na<sup>+</sup>/K<sup>+</sup> ATPase pump [99].

Despite <sup>201</sup>Tl, <sup>99m</sup>Tc-based radiopharmaceuticals have a worse relationship to flow and minimal redistributing properties, and the mitochondrial uptake of these compounds requires an intact mitochondrial membrane and oxidative metabolism, thus the basis for sensitive viability detection [100]. Recent study compared the detection of viable myocardium by <sup>18</sup>F-FDG, MRI and <sup>99m</sup>Tc-sestamibi (MIBI) and demonstrated that a significantly higher number of segments were identified as scar by MIBI than by either <sup>18</sup>F-FDG or CMR [101]. However, the greatest discrepancy between MIBI and <sup>18</sup>F-FDG and MRI was in detection of scar of the inferior and lateral wall. Cardiac PET utilizing <sup>18</sup>F-FDG is considered the most sensitive modality for detecting scar and viable myocardium. To date, <sup>18</sup>F-FDG PET is considered the most sensitive means of assessing viable myocardium and hence predicting LV functional recovery post-coronary revascularization [102].

However, no single imaging modality can provide all the information required to monitor the repair or regeneration of injured myocardium; hence, there is a necessity for combining complementary imaging methodologies. Indeed, the combination of MRI and PET modalities allows the acquisition of anatomical, physiological and metabolic information from the same subject. Recent study showed that multimodal imaging approach allowed an accurate and regional assessment of the new myocardium regenerated after cardiac transplantation of stem cells [103–106] or administration of new drugs, which elicit self-repair of the myocardium, such as hyaluronan mixed esters of butyric and retinoic acid [80]. These studies showed that the myocardial structure, function and viability of infarcted heart following regenerative treatment is equal to uninjured myocardium and the infarct scar size is significantly reduced.

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## Conclusions

Since ancient times, the heart's ability to regenerate itself has always intrigued the human curiosity. Hyginus has narrated the myth of Prometheus, the Titan punished by the wrath of Zeus for stealing the fire from the gods. The Prometheus's heart grows back during the night after being eaten by eagle on the day. The mythology seems to have foreseen that the human heart could not beat for the whole life with the same cardiomyocytes. The heart is an organ exposed to constant and variable mechanical forces and so it is potentially exposed to wear. The heart, similar to all adult tissues, has a plasticity that provides a partial renewal, yet it is weak, slow and limited. The plasticity of the heart depends both on rare cardiac progenitors resident into orderly architecture of the myocardium as well as on continuous cross-talk with other cells that shape the adult myocardium (cardiomyocytes, endothelial cells, fibroblasts), and it is also due to own ability of the myocardium to recruit and activate circulating inflammatory and progenitor cells. Some pathways of intercellular communication have been revealed ensuring the development of new pharmacological modulators. The future perspective will be to identify the complete code by which heart cells communicate with each other to promote a slow and continuous regeneration of the cells worn by mechanical forces and vascular insults. This new frontier of research in the cardiovascular field will lead to the affirmation of the pro-regenerative drugs for the treatment of cardiac dysfunction.

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## Conflict of interest

Authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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