Engineering cardiac muscle: new ways to refurbish old hearts?

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Myocardial infarction leads to irreversible cell loss and scar formation, which result in impaired cardiac function, cardiac hypertrophy and, eventually, heart failure. As the intrinsic regenerative capacity of the human heart is very limited, organ transplantation or ventricular assist devices remain the only end-stage therapy options to date.

For at least two decades, alternative therapeutic concepts of heart repair based either on direct cell injection or on the transplantation of in vitro engineered tissue constructs have been considered potential approaches to overcome the shortage of donor organs. However, the multitude of recent experimental and clinical studies applying various adult stem cells have thus far not been able to fulfill the initial high hopes vested in adult stem cell-based therapies. As summarized in a recent meta study, the vast majority of these studies resulted either in no or in only minor functional improvement [1], most likely due to paracrine effects, including accelerated revascularization, enhanced myocyte survival in the infarct border zone and the modulation of scar formation, resulting in improved mechanical properties [2]. Despite sporadic myocyte formation, a significant generation of de novo myocardium could not be demonstrated [3].

Interestingly, very recent studies have demonstrated the possibility of targeted trans-differentiation of cardiac fibroblasts into functional cardiomyocytes (CMs). Inspired by a recent approach utilizing the transcription factor MyoD to drive trans-differentiation of fibroblasts into skeletal muscle [4], a set of cardiac transcription factors was overexpressed for the targeted differentiation of mouse cardiac and tail-tip fibroblasts (Fbs) into cardiomyocytes in vitro [5]. Although trans-differentiation of cardiac Fbs in vivo could be demonstrated [6], the efficiency of such direct conversion experiments is still controversial [7, 8]. At this point, the usefulness of this kind of cell conversion remains uncertain and will critically depend not only on a much higher efficiency, but also on the functional similarity of these artificially converted cells into normal cardiomyocytes.

For many years, embryonic stem cells (ESCs) were considered the only cell source suitable to supply the huge numbers of cardiomyocytes lost after myocardial infarction. However, a potential clinical use of ESCs is highly controversial, since their generation requires the destruction of human embryos and, if not produced through therapeutic cloning, no autologous ESCs are available for cellular therapies.

In 2006, these limitations were overcome by the ground-breaking development of induced pluripotent stem cells (iPSCs) by S. Yamanaka [9], who was awarded the Nobel Prize in Medicine in 2012. In the meantime, the generation of human iPSCs has become a standard procedure in many laboratories and it is now clear that these cells are almost indistinguishable from ESCs with respect to their phenotype, culture characteristics and potential for proliferation and differentiation.

Unfortunately, the availability of human iPSCs and CMs derived from these cells (Figure 1) has solved only one of the most severe limitations for current concepts for myocardial repair. Further critical hurdles are low cell survival and the lack of functional integration of the cellular transplants. In particular, the functional coupling and formation of well-organized myocardium appears to be difficult to achieve. The direct injection of cells to improve heart function was investigated in different animal models with a variety of mouse and human cell sources, including undifferentiated cells as well as cells that had been differentiated to cardiac progenitors or cardiomyocytes. Regardless of the cell type or application method (intramyocardial or intracoronary injection), only up to 5% of the cells remained in the heart, and neither significant integration nor long-term survival could be shown ([10], reviewed by ref. [11]). Although survival and cardiovascular in vitro differentiation was demonstrated, the injection of iPSC-derived cardiovascular progenitors did not result in the formation of structured myocardium [12].

In contrast, a very recent study was the first to report the formation of relatively large electrically coupled and well-structured muscle islands from injected human ESC-derived myocytes in a guinea pig model of myocardial infarction [13]. These data are considered to be very promising, although it was necessary to inject extremely high numbers of myocytes, (i.e. $10^8$ cells, which correlates to $\sim 2 \times 10^{10}$ or approximately four times the total number of myocytes in the adult human left ventricle, when projected from the guinea pig to the human heart, which is ca. two hundred times larger). Even more remarkable is the fact that the formation of large contractile human ESC-derived muscle islands have evidently been confirmed by the same group in a

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non-human primate model of myocardial infarction (preliminary data presented by C. Murry, ISSCR annual meeting in Boston 2013).

The transplantation of in vitro engineered heart tissue may be an alternative approach to overcome the above limitations. Based on natural or synthetic biocompatible or biodegradable materials, tissue constructs can be engineered in vitro either by seeding cells on matrices or by mixing soluble matrix components and cells.

Recently, Ott et al. [14] demonstrated that seeding neonatal rat cardiomyocytes onto a completely acellularized rat heart can lead to a contractile tissue construct. Notably, however, no follow-up studies demonstrating the further development of this concept have been published so far. Whereas reseeding the acellular vascular structures of natural matrices with endothelial cells appears possible, as has been shown for other matrices such as small intestinal submucosa [15], it remains to be demonstrated that larger tissue structures, such as the human heart, can be efficiently reseeded with the necessary cell types. Given the rather low migratory potential of terminally differentiated cardiomyocytes, it is especially questionable as to whether the required high cell density of functional myocardium can be achieved in this way.

Most other myocardial tissue-engineering approaches are based on biocompatible porous scaffolds, in particular animal-derived or synthetic hydrogels, as initially introduced by the group of Eschenhagen et al. [16]. For many years, such approaches were limited by the lack of a suitable human cell source and were based either on neonatal rat cardiomyocytes [17] or on murine ESC-derived cardiomyocytes [18].

More recently, the availability of novel differentiation protocols based on the sequential inhibition and activation of molecular differentiation pathways has for the first time allowed a targeted and efficient differentiation of human ESCs [19]. In the meantime, it has been possible to replace the expensive recombinant proteins used in such protocols by small molecules. This has facilitated the development of highly efficient, scalable protocols that are relatively inexpensive, more robust and result in dramatically improved differentiation efficiencies of up to 90% [20], enabling the production of the vast numbers of cardiomyocytes required for engineering large contractile human muscle patches. In addition, the risk of teratoma formation was addressed through additional strategies for the enrichment of human ESC/iPSC-derived cardiomyocytes. It has been shown that genetic selection approaches in particular result in a pure cardiomyocyte preparation of >99.9% purity [21], and novel techniques of genome engineering allow the efficient targeted integration of genetic selection cassettes into safe harbour loci, thereby dramatically reducing the tumour risk based on insertional mutagenesis [22].

Based on such developments, several groups have reported bioengineered human myocardium based on human pluripotent stem cells (Figure 2). Miniaturized fibrin-based constructs were engineered from human ES-cell derived cardiomyocytes as a novel means for drug screening and safety pharmacology [23]. Tulloch et al. [24] demonstrated that engineered human tissue constructs based on CMs derived from human ESCs and iPSCs became connected to the host vasculature one week after transplantation onto rat myocardium. In another study, it was shown that engineered cell sheets consisting of human iPSC-derived CMs can improve cardiac function in a porcine model of ischaemic cardiomyopathy [25]. Notably, our group has recently shown that human iPSCs with their ESC-like capacity for proliferation and differentiation enable the generation of functional bioartificial cardiac tissue (BCT) that develops contractile forces almost similar...
Figure 2: Engineering bioartificial cardiac tissue based on pluripotent stem cells. CMs: cardiomyocytes; SMCs: smooth muscle cells; ECs: endothelial cells; Fbs: fibroblasts.
to those of native myocardium [21]. Further developments have led to comparable constructs based on defined human and partially synthetic matrix components, which may facilitate clinical applications in the future [26].

Of course, there are still many hurdles to be overcome prior to clinical application. These include the need for proper vascularization, the elucidation and reduction of risks associated with the recently observed chromosomal abnormalities that become apparent after reprogramming and iPSC expansion [27] and the appearance of cardiac arrhythmias.

However, the extremely low incidence of CM-derived tumours in general suggests a low risk factor for a malignant transformation of terminally differentiated iPSC-derived CMs, and potential implantation, the elucidation and reduction of risks associated with the recent iPSC-based transplants to be applied in the clinic and could eventually be broadly applied in the field of cellular heart repair.

Conflict of interest: none declared.

REFERENCES