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Human myocardial grafts: do they meet all the criteria for true heart regeneration?

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Ischemic heart disease has long been the number one cause of death in the USA and has recently become the number one cause of death worldwide [1]. While remarkable progress in understanding the pathophysiology of the disease has been made in the last several decades, there are clearly many challenges ahead. Current therapy using various surgical, catheter-based and pharmacological approaches can reduce symptoms or delay disease progress; however, these do not cure the underlying damage to the heart. One of the key issues to overcome is bringing back functional cardiomyocytes (CMs), of which approximately a billion are lost during a typical myocardial infarction. While cardiac transplantation is an effective therapy, patients suffer from lifelong immunosuppression and it will never be available to all the patients in need because of the limited number of donors. Since the heart contains little intrinsic regenerative capacity, finding other sources for new heart muscle is critical. Stem cell therapy is the emerging attempt to regenerate cardiac tissue that could lead to the functional recovery of the whole heart [2]. This editorial focuses on current research on stem cell-derived CMs and their application as transplantable myocardial grafts.

There are several types of stem cell that have been proposed for cardiac repair, beginning with skeletal muscle and moving to bone marrow cells, mesenchymal stem cells, resident cardiac progenitor cells and pluripotent stem cells, such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) [3]. When considering them as the therapeutic tool, there are some critical features those cells need to possess. First of all, they have to show the electrophysiological, structural and contractile properties of genuine CMs. Second, they need to survive, integrate structurally and functionally couple with host tissue, which are often ischemic and harsh environments for cells. Third, they should have a proliferative potential, which would provide sufficient CMs for transplantation. Finally, it is preferable

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for them to be autologous since allogeneic transplantation requires long-term immunosuppression. We should say at the outset that, at present, none of the existing cell sources are able to meet all the above criteria. Interestingly, while bone marrow cells, mesenchymal stem cells and resident cardiac progenitor cells all have some efficacy in improving cardiac function in preclinical animal models, they do not appear to differentiate into new CMs. We now know that most of these cells die within a few weeks of transplantation and their benefits are mediated principally through production of paracrine factors that promote endogenous repair mechanisms [4]. By contrast, human ESCs and iPSCs (hESCs and hiPSCs, respectively) form definitive beating CMs *in vitro* and readily identified grafts of human myocardium after transplantation. Given these attributes, hESCs and hiPSCs have become very promising cells for clinical heart regeneration and the remainder of this editorial focuses on their preclinical applications.

In order to achieve true heart regeneration, we have to generate cardiac tissue that would meet both structural and physiological criteria upon transplantation. The structural criteria include presence of *de novo* CMs together with appropriate vascular and connective tissue. This new cardiac muscle should be well incorporated into of human myocardium after host myocardium, with an organized cell alignment. For the physiological criteria, improvement of the mechanical function is definitely required. Since scar tissue, which is formed after the infarction, causes ventricular remodeling and consequently abnormal electromechanical coupling, regenerated CMs need to be able to normalize this phenomenon by providing stable and synchronized contraction with host tissue.

Since the initial report of hESC derivation in 1998, various studies have established *in vitro* differentiation systems to CMs [5–7]. Those hESC-derived CMs not only show spontaneous beating, but also make electromechanically coupled networks of synchronously beating CMs [8]. Several studies have shown that hESC–CM grafts can survive, proliferate and mature when transplanted into the surgically infarcted hearts of rodents [9–11]. Engraftment could be observed even 3 months after the transplantation and grafts are able to structurally integrate with host tissue, suggested by the presence of microvessels connected to the host coronary vasculature and, less frequently, formation of intercalated disks between graft and host CMs [11]. Importantly, some functional improvements, including reduced ventricular dilation, enhanced contractile property and reduction of scar size have been reported, although in some cases, the improvements did not last long term [9].

Until recently, it was not evident that the beneficial effects of CM transplantation were directly attributable to the contractile property of transplanted CMs. One of the key questions was whether hESC–CM grafts could contract synchronously with infarcted host hearts through electrical coupling by gap junction. Recent work from our group shed light on this by creating a line of hESC–CMs expressing a calcium indicator protein, GCaMP3 [12]. These CMs flash green with permitting us to identify when the grafts beat in the hearts of recipient guinea pigs. We found that, in most cases, hESC–CM grafts electromechanically couple and contract synchronously with host myocardium. In some instances, however, grafts were isolated by scar tissue and beat autonomously. Interestingly, the presence of the human grafts markedly reduced the susceptibility of these hearts to pacing-induced arrhythmias, indicating human grafts enhance both mechanical and electrical function of the heart. The finding that human CMs have the capacity for electromechanical integration has given a boost to developing stem cell-based therapy as a new clinical strategy.

The generation of hiPSCs can overcome several of the most challenging limitations of hESCs, since they can be generated without disruption of human embryos [13]. Many studies show that CMs can be derived from hiPSCs using similar differentiation protocols to hESCs [14]. While there are several reports stating that cardiogenic efficiency is much lower

than hESCs, in our experience the two cell types are comparable. Both show variable line-to-line efficiency in cardiogenesis with a given protocol and if one takes care to understand the signaling requirements for a given line, it should be possible to derive >50% CMs from most pluripotent lines. hiPSC-derived CMs show contractility, defined sarcomeres and electromechanical coupling similar to hESC-CMs [15]. Since research on hiPSC-CMs is still in early stages, not many experiments have been performed to determine the functional effects of hiPSC-CMs in the infarcted hearts of animals. Recently, Miki *et al.* [16] and Kawamura *et al.* [17] published papers showing that bioengineered hiPSC-CMs were able to survive and integrate in the hearts of rat and porcine chronic myocardial infarction models, respectively. In both cases, transplantation significantly improved cardiac function as demonstrated by systolic performance and attenuated left ventricular dilation.

Thus, while some promising results have been reported in preclinical studies with hESC-CMs and hiPSC-CMs, several factors need to be improved before beginning clinical application. Although the differentiation protocols have improved dramatically, these are still small scale calcium transient, (10–100 million CMs). It will be important to develop large-scale differentiation protocols (e.g., yielding 10–100 billion CMs) with consistently high purity. This goal will be helped by the development of effective high-throughput purification methods to enrich CMs without genetic modification. In addition, it is often pointed out that hESC-CMs demonstrate a relatively immature phenotype compared with adult CMs. At present, we have no evidence that immature CMs will be disadvantageous to be implanted and we also know that fully mature CMs will not survive the rigors of transplantation [18]. Further analysis comparing immature and mature CMs, and their functional contribution to the infarcted heart will be necessary.

There are several additional issues to be considered before hiPSC-CMs go into clinical use. The initial protocols for iPSC generation involved virus-mediated expression of reprogramming factors, which give rise to the concern of tumor formation when applied in the clinical settings. Now nonviral approaches have significantly improved and are becoming the standard method for generating hiPSCs [19]. There has been surprisingly little transplantation immunology work carried out with iPSCs. The one study of which we are aware showed that, contrary to expectation, undifferentiated mouse iPSCs were rejected when transplanted into syngeneic hosts [20]. The basis for this rejection is not yet clear, but will need to be worked out before clinical trials with these cells can begin. The biggest hurdle to the autologous use of hiPSCs, however, is cost. Rather than making a large batch of cells where a single round of quality control studies serves many patients, for autologous cells, each patient requires a single batch. It is not clear that there is a business model that makes such an approach commercially viable.

Over the last decade, stem cell derived-CMs and their application in cardiac repair have been extensively studied. On the way to clinical trials, researchers will need to address practical questions including what kind of transplantation methods or immunosuppressive agents should be used. In order to tackle these problems, studies using larger animals, such as pigs, dogs or nonhuman primates, are necessary. As many of the efforts are now concentrated on solving these problems, it is likely that standardized protocols for clinical application will be established in the near future. As we proceed further with more innovation and new findings, the goal for real human heart regeneration is certainly approaching.

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