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Large-scale production of human pluripotent stem cell derived cardiomyocytes☆

Henning Kempf, Birgit Andree, Robert Zweigerdt *

Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), Department of Cardiac, Thoracic-, Transplantation and Vascular Surgery, Hannover Medical School, Germany
REBIRTH-Cluster of Excellence, Hannover Medical School, Carl-Neuberg-Str. 1, 30625 Hannover, Germany

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ABSTRACT

Regenerative medicine, including preclinical studies in large animal models and tissue engineering approaches as well as innovative assays for drug discovery, will require the constant supply of hPSC-derived cardiomyocytes and other functional progenies. Respective cell production processes must be robust, economically viable and ultimately GMP-compliant. Recent research has enabled transition of lab scale protocols for hPSC expansion and cardiomyogenic differentiation towards more controlled processing in industry-compatible culture platforms. Here, advanced strategies for the cultivation and differentiation of hPSCs will be reviewed by focusing on stirred bioreactor-based techniques for process upscaling. We will discuss how cardiomyocyte mass production might benefit from recent findings such as cell expansion at the cardiovascular progenitor state. Finally, remaining challenges will be highlighted, specifically regarding three dimensional (3D) hPSC suspension culture and critical safety issues ahead of clinical translation.

Contents

Abbreviations: ATMP, advanced therapy medicinal product; CM, cardiomyocyte; cTNT, cardiac Troponin T; CVP, cardiovascular progenitor; EB, embryoid body; GMP, good manufacturing practice; hPSC, human pluripotent stem cell; MHC, myosin heavy chain; RI, ROCK inhibitor; SA, sarcomeric actinin.

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Tissue engineering of the heart: from in vitro models to regenerative solutions".

* Corresponding author at: Leibniz Research Laboratories for Biotechnology and Artificial Organs, Carl-Neuberg-Strasse 1, 30625 Hannover, Germany.
E-mail addresses: Kempf.Henning@mh-hannover.de (H. Kempf), Andree.Birgit@mh-hannover.de (B. Andree), Zweigerdt.Robert@mh-hannover.de (R. Zweigerdt).

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1. Introduction: the need for mass production of cardiomyocytes from hPSCs

Restricted lumen and flexibility of coronary arteries by atherosclerotic plaques often precede myocardial infarction (MI). Ultimate vessel occlusion triggers MI by interrupting oxygenation and nutrition to downstream areas. Such tissue ischemia, followed by reperfusion in the clinic, typically results in irreversible loss of billions of cardiomyocytes (CMs) in each affected heart [1]. Since mature CMs have an arrested cell cycle [2] they exhibit very limited proliferation, which is further progressively lost through aging and heart diseases [3]. Recent evidence has also challenged the presence of a stem cell population with relevant regenerative potential in the heart [4]. Consequently, rather than tissue regeneration, an akinetic fibrotic scar is formed post-MI and typically leads to reduced heart function which might ultimately result in organ failure. The only curative option, heart transplantation, is limited by the lack of donor organs and the requirement of lifelong immunosuppression. Despite advances in the engineering of left ventricular assist devices (LVAD) [5] the technique is still hampered by severe limitations typical for artificial prostheses. These include infections and thrombosis that might occur despite the necessity to take anti-coagulation therapy which, in itself, can provoke undesirable side effects such as uncontrolled bleeding.

Cell therapy has been envisioned as a promising alternative strategy for heart repair. Despite numerous clinical trials focused on the transplantation of patients' own tissue-derived cells, for example from the skeletal muscle, bone marrow, or peripheral blood, very limited recovery of heart function was achieved [6,7]. Furthermore, the hypothesized formation of CMs from patients' adult (stem- or progenitor-) cells after delivery to the heart has been unsuccessful [2].

An alternative concept aims to transplant in vitro differentiated human pluripotent stem cell (hPSC)-derived cardiomyocytes (CMs) [1] alone or in combination with other cell lineages. This could be achieved either by direct cell transplantation, matrix embedding approaches or by more advanced in vitro tissue engineering prior to transplantation (Fig. 1), addressed in detail in other articles in this issue [8–11].

Cell types that might foster cell retention, survival and repair in the heart [12,13] have recently been derived from hPSC (including human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs)) with increasing efficiencies, as demonstrated for endothelial cells [14,15], pericytes [16], smooth muscle cells [17], mesenchymal stem cells [18] as well as bona fide CMs [19]. In addition to their potential to differentiate into essentially any somatic cell type, PSCs have the property of unlimited proliferation at the pluripotent state when cultured under appropriate culture conditions, thus providing an ideal "raw material" for regenerative medicine [20].

Notably, iPSC technology [21–23] is not only overcoming ethical issues related to hESCs, but enables the derivation of patient-specific iPSC lines, which in principle should preclude rejection of iPSC-derived progenies when transplanted back to the patient of origin. Moreover, this technology has led to the exciting possibility of establishing patient- and tissue-specific disease models in vitro, which has the potential to revolutionize drug screening and safety pharmacology as described in recent papers and reviews elsewhere in this issue [10, 24–26].

However, regenerative medicine, preclinical studies in (large) animal models, tissue engineering and high throughput assays for drug screening will all require the constant supply of billions of hPSC-derived CMs and other functional progenies, which will require robust,

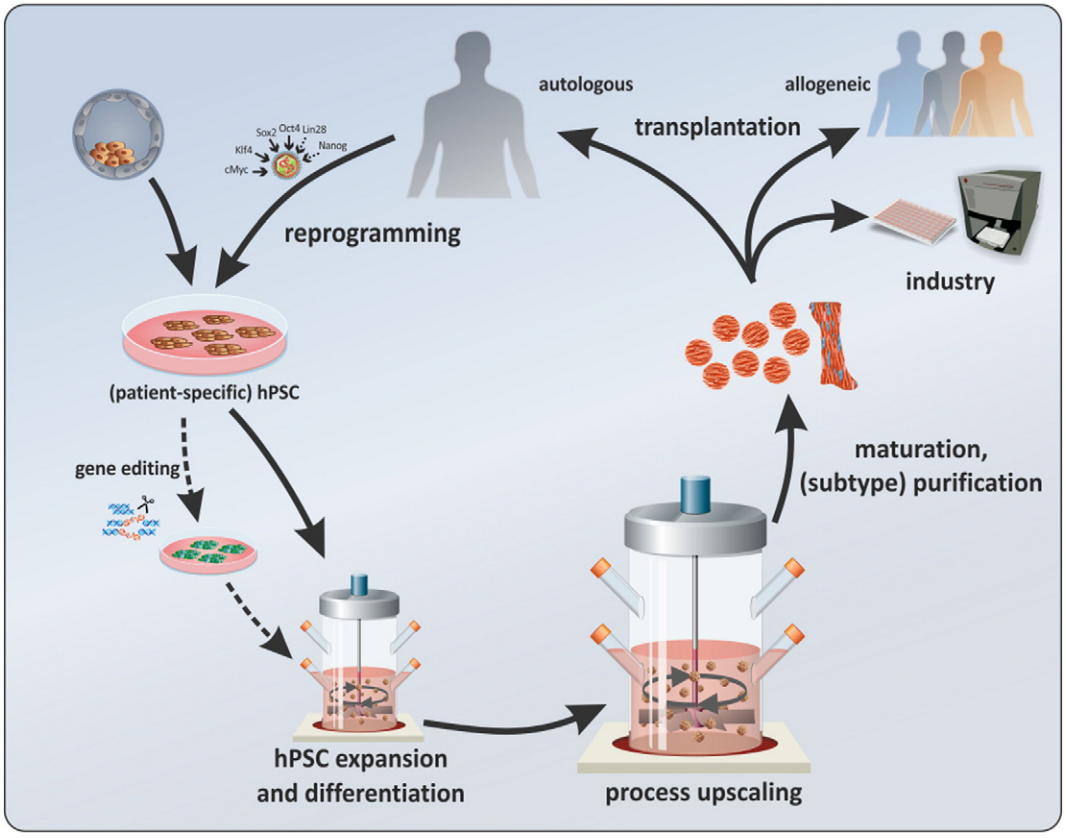

Fig. 1. Strategies for PSC-derived mass production of cardiomyocytes. hPSCs are derived from the inner cell mass of the blastocyst (hESC) and reprogrammed from somatic cells (hiPSC), respectively. Application of designer nucleases additionally enables the tailored genetic modification of hPSCs, i.e. to correct patient-specific mutations. Expansion of acquired hPSCs and the subsequent cardiac differentiation in scalable culture platforms will enable the mass generation of required numbers of cardiomyocytes for transplantation purposes and industrial application such as high content screening and toxicity testing.