JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 6, NO. 3, 2021

EDITORIAL COMMENT

Balls to the Wall

Human Pluripotent Cell-Derived Cardiac Muscle Spheres Enhance Preclinical Heart Repair*

Michael D. Schneider, MD

"Balls to the wall" (U.S., idiomatic, slang): Full throttle; (at) maximum speed.
—Wiktionary (1)

How to make new heart muscle bloom in the desert? The scope and irreversibility of cardiac muscle death in myocardial infarction, together with the lack of sufficient donor hearts to enable widespread transplantation, have inspired a long quest for cardiac regeneration, in basic and translational science alike. Some paradigms have arisen, such as heart muscle formation by grafted bone marrow cells, only to be toppled as more evidence appears, prompting a search for cells with less questionable potential to create new myocardium. A second paradigm shift resulted from the discovery that grafted cells—regardless of type—often persist just briefly in recipient hearts. Consequently, under the routine conditions for delivery (typically, injecting a naked suspension of single myocytes into the ventricular wall or coronary arteries), any observed benefits are likelier due to evanescent cell-to-cell signals, than to durable, donor-derived, new myocardium (2). This daunting limitation characterizes the current state of the art, and holds true even for grafts of pre-formed, differentiated, beating cardiac muscle cells, which now can be produced using pluripotent stem cells (PSCs) as the primitive, undifferentiated, easily grown starting point. Experimentally validated signals include secreted proteins, microRNAs, or vesicles containing both, improving mechanical performance of the injured heart through indirect mechanisms: effects on angiogenesis, the inflammatory milieu, cardiomyocyte survival, and perhaps restarting heart muscle cell proliferation. Although there is clear potential to exploit one or more of these secreted signals for therapeutic benefit, a more direct solution has remained attractive but elusive.

In this issue of *JACC: Basic to Translational Science,* Kawaguchi et al. (3) report an encouraging improvement in experimental heart repair. Their approach combined the use of human induced PSCs (hiPSCs) as the donor cell source, differentiation and purification of cardiac myocytes on a large scale, myocytes' aggregation into cardiac spheroids to improve cell retention, and intramyocardial transplantation of the spheroids together with a gelatin hydrogel into rat and porcine models of heart failure (cryoinjury in immunodeficient rats and immunosuppressed "microminipigs," transplanted 1 and 4 weeks later, respectively). Compared 8 weeks after transplantation to an equivalent number of single myocytes or the hydrogel alone, spheroid-transplanted rats showed an improved left ventricular (LV) ejection fraction (36% and 32%, respectively) and systolic function ($+dp/dt_{max}$; nearly 70% for each). Likewise, long-term retention of the human cardiomyocytes was improved, assessed by costaining for cardiac troponin I plus human nuclear antigen as well as by bioluminescent imaging for a genetically incorporated label, luciferase. Even 2 months after grafting, roughly 20% of the luciferase remained.

Taking this forward into the more relevant porcine model, a 6-needle device for epicardial injection was used, previously developed for better cell retention, featuring multiple side holes and a closed, domed tip, then polyglycolic acid felt glued to the injection site

*Editorials published in *JACC: Basic to Translational Science* reflect the views of the authors and do not necessarily represent the views of *JACC: Basic to Translational Science* or the American College of Cardiology.

From the National Heart and Lung Institute, Imperial College London, London, United Kingdom.
The author attests he is compliance with human studies committees and animal welfare regulations of the author's institution and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

ISSN 2452-302X https://doi.org/10.1016/j.jacbts.2020.12.011

with factor XIII and fibrinogen. In spheroid-injected pigs 8 weeks after transplantation, LV ejection fraction improved by 29% and 17%, compared with hydrogel-treated and untreated infarcts, and LV end-systolic volume decreased by 70%. The scar area decreased nearly 50% by both late gadolinium enhancement magnetic resonance imaging and histochemical staining. Myocyte retention was readily detected at 2 weeks, but not later, using far-red-stained donor cells. Endothelial cell density was increased in the border zone and remote myocardium; using cytokine arrays, vascular endothelial growth factor (VEGF) was the principal angiogenic factor detected. As in related large-mammal investigations (4), arrhythmias including ventricular tachycardia were noted, a challenge to future clinical implementation.

Some limitations of this study should be mentioned, though intriguing and highly commendable overall (3):

1. Cryoinjury is reproducible and readily implemented, but does not model all relevant aspects of myocardial infarction in humans, including inflammation. Immunodeficient or immunosuppressed animals were mandated, given the inherent requirement for transplantation across species, confounding a normal inflammatory response even if ischemia-reperfusion had been used; a lesser degree of immunosuppression might suffice clinically, for allogeneic human cells.
2. The absence of humans' relevant comorbidities (obesity, diabetes, hyperlipidemia, hypertension, and mere aging) is another feature, which historically impairs the predictive power of animal models to inform cardiovascular therapeutics.
3. Ideally, one might wish for an assessment of spheroids versus single myocytes in both species, not just rats; scar size in both, not just pigs; and additional details of myocyte attrition in the large-mammal model. Was the eventual loss of grafted myocytes just incomplete immunosuppression, as the investigators suggest?
4. The present report's mode of cell delivery competes with alternative tissue engineering solutions such as cardiac muscle patches, which likewise have been taken forward into large mammal testing, and which possibly have less arrhythmogenicity (5); long-term efficacy and safety will need to be compared.
5. Lastly, the relative immaturity of stem cell-derived cardiomyocytes is a recognized issue that influences these cells' utility both in vitro, as a human platform to improve cardiac drug discovery, and in vivo, as cell therapy (6). Though not profiled systematically in the present study, features of immaturity were noted by immunostaining. Graft-related arrhythmias seem to involve focal automaticity, rather than re-entry (4). Hence, it could be important to bridge the present investigation's advances in cardiomyocyte delivery with recent approaches that confer more complete metabolic, transcriptomic, mechanical, and electrophysiological maturity (6).

FUNDING SUPPORT AND AUTHOR DISCLOSURES

Dr. Schneider was partially supported by the Wellcome Trust grants WT10638/Z/14/Z and WT205256/Z/16/Z, the British Heart Foundation grants CH/08/002/29257, CH/08/002/29257, RG/15/1/31165, and RM/13/1/30157, and Apollo Therapeutics grant AP22. Dr. Schneider has reported that he has no relationships relevant to the contents of this paper to disclose.

ADDRESS FOR CORRESPONDENCE: Prof. Michael D. Schneider, National Heart and Lung Institute, Imperial Centre for Translational and Experimental Medicine, Du Cane Road, Imperial College London, London W12 0NN, United Kingdom. E-mail: m.d.schneider@imperial.ac.uk.

REFERENCES

1. Wiktionary. Balls to the wall. Available at: https://en.wiktionary.org/wiki/balls_to_the_wall. Accessed December 10, 2020.

2. Sun X, Wu J, Qiang B, et al. Transplanted microvessels improve pluripotent stem cell-derived cardiomyocyte engraftment and cardiac function after infarction in rats. Sci Transl Med 2020;12:eaax2992.

3. Kawaguchi S, Soma Y, Nakajima K, et al. Intramyocardial transplantation of human iPS cell-derived cardiac spheroids improves cardiac function in heart failure animals. J Am Coll Cardiol Basic Trans Science 2021;6:239-54.

4. Romagnuolo R, Masoudpour H, Porta-Sanchez A, et al. Human embryonic stem cell-derived cardiomyocytes regenerate the infarcted pig heart but induce ventricular tachyarrhythmias. Stem Cell Reports 2019;12:967-81.

5. Gao L, Gregorich ZR, Zhu W, et al. Large cardiac muscle patches engineered from human induced-pluripotent stem cell-derived cardiac cells improve recovery from myocardial infarction in swine. Circulation 2018;137:1712-30.

6. Feyen DAM, McKeithan WL, Bruyneel AAN, et al. Metabolic maturation media improve physiological function of human iPSC-derived cardiomyocytes. Cell Rep 2020;32:107925.

KEY WORDS cardiac regeneration, cell therapy, heart failure, human induced pluripotent stem cells