In 2003, C. Zandonella reviewed in *Nature* how much was achieved of the 1999 promise to grow a functioning heart in the dish in a decade and what the perspectives were at that time. Today, more than a decade after its public prediction, the heart in the dish is still an unfulfilled dream, but repairing injured hearts with engineered myocardial tissue patches is a viable and increasingly realistic perspective in regenerative cardiology.

This is not so much because of the progress in tissue engineering techniques but rather because of the dramatic advances in stem cell biology. In 1998, the first human embryonic stem cells (hESC) were described, and 3 years later the generation of cardiac myocytes from hESC. Despite widespread ethical concerns and strict legal restrictions in many countries of the world including the US and Germany, hESC proved to be enormously important for several reasons. First, they helped to better understand the earliest steps of human development and human stem cell biology, which differ in critical aspects from those in the mouse. Second, human ESC provided, for the first time, an unlimited cell source with an undisputed capacity to differentiate into essentially all types of cells of the body, including cardiac myocytes. Their discovery boosted cardiac tissue engineering principally by answering the question as to where the several hundred millions of human cardiac myocytes may come from that are necessary to make large myocardial patches. Third, ironically, the ethical concerns against hESC work stimulated and in some cases initiated the search for pluripotent alternatives that would be devoid of the regulatory and ethical problems of human ESC. This motivation, combined with the technical experience acquired with mouse and human ESC cultures, was an important driver for the progress in stem cell technology, including the nondestructive derivatization of pluripotent stem cells from single mouse blastomeres or human IVF embryos, and human spermatogonial stem cells, or human parthenogenic blastocysts. Finally, the seminal discovery of methods to induce pluripotency in mouse and human somatic cells by introducing a combination of pluripotency factors would not have been possible without the longstanding experience both groups previously had with ESC.

Human ESC, at least in principle, solved the issue of a renewable human cardiac myocyte source for cardiac regeneration and human-induced pluripotent stem (hiPS) cells that of a patient-derived autologous approach. It should be mentioned, however, that even autologous iPSC may induce an immune response, likely due to abnormal gene expression. Another important bottleneck remained for years: the low efficiency of cardiac myocyte differentiation from pluripotent stem cells, which precluded the generation of real human heart muscles as well as efficient stem cell therapy. This problem was solved by studies that carefully deciphered the factors guiding the earliest steps of cardiac development under normal conditions and translated this knowledge into cell culture protocols. Thus, by using a multistep protocol with growth factors that subsequently drive mesodermal and then cardiac specification, cardiac myocyte differentiation rates went up from approximately 1% to 50% and more. By optimizing growth factor combinations and timing, cardiac myocyte differentiation rate can be increased even further and, importantly, the new protocols can be applied to essentially all pluripotent stem cell lines including hiPS.

In light of these findings, one may ask why one should choose the cumbersome strategy of engineering a tissue patch in the dish instead of simply injecting stell cells or their derivatives into the heart. The arguments are severalfold. (i) The retention and survival rate of cells is consistently low when injected into the myocardium and even lower when cells are infused. When using potentially tumorigenic cells, such as ESC, the high wash-out rate after cell injection leads to cell deposition in the lung, liver, kidney, and spleen and constitutes a clear risk of systemic tumor formation as demonstrated in mice. (ii) There is not much evidence to support the intuitively attractive idea that the adult mammalian heart provides a "cardiogenic milieu" that will drive maturation and orientation of cardiac myocytes from pluripotent stem cells. Thus, it is unclear how massive numbers of immature cells that are required for cardiac repair shall form a well-organized tissue that functions in synchrony with the host myocardium and support its contractile function. Indeed, although survival of injected embryonic stem cell–derived cardiac myocytes has been reported, the efficiency of the formation of new, electrically coupled, and well-differentiated myocardium appears rather low. (iii) Injecting hundreds of millions of cells, even if they would be retained at the site of injection, carries the potential risk of inflammation. Such effect has been shown in studies with bone marrow–derived cells, but not in several others that reported instead anti-inflammatory effects after injection of mesenchymal or cardiosphere-derived cells. (iv) Importantly, propagating iPSC and maybe any other pluripotent cell to very large numbers in the dish carries a substantial risk of mutations.
and chromosomal aberrations, likely because endogenous control mechanisms are lacking. The consequences of this observation are difficult to predict at present, but the risks may be more difficult to control in approaches that use injections of a mixture of more-or-less defined cells than in one in which a beating cardiac patch is being grown in the dish prior to implantation. However, because no head-to-head comparison of a tissue engineering and cell injection approach with pluripotent cells or their derivatives has been performed, the advantages of the patch remain theoretical.

In a study published in this issue of Circulation Research, Tulloch and colleagues generated engineered heart tissues (EHT) from hESC and hiPS by using a collagen I–based method described earlier, and a commercially available system from FlexCell. The experiments reproduced in a human context the main conclusions made earlier in EHTs from neonatal rat and chick embryonic heart cells, namely, that mechanical load and the presence of nonmyocytes are important to generate well-organized, functional heart tissue–like structures. As acknowledged by the authors, the study is not the first to show that 3-dimensional (3D) structures can be made from hESC. However, it goes significantly beyond the state of the art in various respects.

First, and most important, the human cardiac tissue structure achieved in this study is far better than what had been published previously in terms of myocyte structure and alignment and homogenous tissue orientation as well as overall fraction of myocytes in comparison with nonmyocytes. Good structure was reflected in relatively good contractile function and a qualitatively normal force response to stepwise increases in muscle length. This result was achieved by combining, for the first time, a high-efficiency cardiac myocyte differentiation protocol for human pluripotent cells with a hydrogel-based tissue engineering method that subjects the growing heart tissue to continuous mechanical load.

Second, the methods used to achieve the high fraction of myocytes obviated the need for manual dissection of beating clusters or genetic selection, with their inherent limitations. Importantly as well, serum-free protocols with defined media were used throughout the study.

Third, the study found that mechanical stress, irrespective of whether it is static or cyclic, improves not only myocyte strand orientation (as known previously), but also collagen fiber orientation, another important aspect of heart structure.

Finally, the addition of endothelial cells alone, particularly in combination with mesenchymal stem cells or mouse embryonic fibroblasts, induced the formation of blood vessel–like structures that slightly stimulated cardiac myocyte proliferation in EHT without disturbing their orientation. Similar findings have been reported previously. Importantly, the vascular structures that were formed in vitro participated in the formation of blood-perfused vasculature after implantation. This indicates that, as previously suggested in nonhuman tissue engineering approaches, preformed primitive blood vessels connect to ingrowing host vasculature. Taken together, the results of this study are an important step forward in the engineering of surrogate human heart muscle.

Where do we go from here? Obviously, there is room for improvement. (i) The cardiac myocyte differentiation efficiency from human pluripotent stem cells can be further increased, alleviating the need for any purification steps to make high-quality EHTs. (ii) The degree of cardiac maturation, though better than previously reported and observed in standard 2D cultures, is still limited and needs to be further improved. As an example, the force generated by the constructs in this study amounted to 0.08 mN/mm² (at a passive force of 0.4 mm²; Tulloch et al’s, 2011, Fig. 7). This is 600-fold less than a normal heart muscle and 25-fold less than what has been reported for rat EHT. The widely held belief that the 3D environment and load alone will induce terminal maturation is not well supported by the data. More likely, factors will be identified that, similar to those that drive mesodermal and cardiac specification, induce a mature, ventricular cardiac myocyte phenotype. (iii) Though potentially important in helping fast connection to the host vasculature, the primitive blood vessels in human EHTs are unlikely to be functional in terms of supplying oxygen or nutrients. Thus, in vitro perfusion is probably necessary to generate grafts with a compact muscle thickness of 1 mm or more. (iv) The electric coupling of the transplanted human EHT to the rat myocardium was not studied. Previous work with similar approaches has provided evidence that coupling occurs, but the exact mechanisms remain elusive. (v) Although the authors did not find evidence for teratoma formation after implanting the human constructs onto hearts of athymic rats, the study was not designed to evaluate this question. Certainly, this risk needs further attention and evaluation.

In conclusion, the present study by the Murry group is a nice piece of work that advances the field of cardiac tissue engineering and regeneration by establishing a method to generate functional human heart muscles from pluripotent stem cells. Many questions including those formulated above need to be answered, but the research direction is increasingly clear. Given the fast progress in stem cell technology and tissue engineering, it is likely that the approach described in this study or the like will reach the level of an efficacy study in a large animal model in the next 5 years.

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Disclosures

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