

Hearty slices to plan for future health

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Clinical trials have shown that heart function can only be moderately improved by injection of stem cells.^{1,2} Gains to date are most likely the result of increased wall stiffness and angiogenesis rather than generation of new, beating myocytes.^{3,4,5} Poor results could be due to the number and kind of cells used, the timing and method of delivery, lack of engraftment in an inhospitable local environment, general immune responses and many more variables. Thus, although the current approach has been responsive to public pressure to rush to translate new ideas to a cure, the lack of a sound foundation in the basics makes the failure of stem cell therapy to date unsurprising. Working with whole animals is a most difficult way to learn because so much is beyond the control of the experimentalist and assays are limited. It is clear is that experimental platforms are needed to provide the rational groundwork before future cell therapy trials for cardiovascular disease are carried out.

It is a pleasure to find a simple new platform of an *in vitro* beating heart model for long-term assessment of experimental therapeutics in this issue by Monville's group.⁶ Ironically, some of the earliest *ex vivo* methods used explanted slices of organs in culture which persisted with great success for the brain but were abandoned for the heart because physiologic function lasted only a few hours. Surprisingly, 800 micron-thick heart slices grown on semiporous membranes with an air-medium interface survive three months, which is far better than previous immersion methods.⁶ The thickness is puzzling since beating heart cells

consume energy at a very high rate and are exquisitely sensitive to the lack of oxygen, relying heavily on oxidative metabolism to produce the ATP for the motor proteins and ion pumps. Isolated bundles and tissue-engineered constructs show cell death 150-200 microns below the surface, thought to be due to poor diffusion.⁷ Since oxygen at 20% of air dissolves very poorly in water, it is hard to imagine how cells live deep in the heart slice because the vessels are useless without circulating blood. However, proof is provided by live nuclei seen in many figures and by the quantitative analysis of live human stem cells as a function of depth (Fig. 3 supplement)⁶. Anatomy is science, too. Unfortunately, the field of heart stem cell science has been populated by sloppy numerical analyses of how many of which kind of cells are really present. Results have not been reproduced from one lab to another (see Murry et. al^{5,8} for a discussion of such issues).

The first application of this heart slice platform is the rat ventricular tissue for analysis of human embryonic stem cell engraftment into the myocardium *in vitro*. Both pharmacologic interventions and cell therapeutics were characterized at the tissue organization level. Quantitative immunohistochemistry, gene expression and mechanical function fully documented the conclusions. Assessment of various cell populations along with their respective engraftment capabilities is now possible. The novelty of this system is that the complex natural base provides a scaffold with live cell cues, scaffold matrix and microstructure, so this

might be better than other models to date.

There are several *in vitro* models with progressive complexities that have been used for stem cell research in addition to blind injection into the living animal. The simplest is the growing of stem cells on microwells, which can control both topography and growth factors but not physiologic functions.⁹ Next, cardiac differentiation markers have been expressed in endothelial progenitor cells,¹⁰ human amniotic mesenchymal cells¹¹ and bone marrow-derived mesenchymal stem cells¹² after co-culture with a feeder layer of primary cardiomyocytes with spontaneous beating but unknown chemical milieu. A key point here is that although some cardiac differentiation markers were present in the stem cells, contraction was not. Tissue-engineered models in three dimensions are advantageous in the application of external forces to mimic the mechanically dynamic heart.⁷ However, integration of stem cells might be hampered because the constructs are covered with a sheath that might be difficult to penetrate. Lastly, an animal heart was completely decellularized, leaving the extracellular matrix and vascular architecture for repopulation by endothelial stem cells to make vessels and rat neonatal cardiac myocytes.¹³ Electrical stimulation, paced contraction and pumping for several weeks provides the proof of principle for future studies with numerous potential cardiac progenitor populations. However, despite the merits of a natural scaffold, good circulation and beating, this is not similar to the situation in the infarcted human heart, where many live cells are still

present.

The model established by Habeler et al.⁶ adds to this base of experimental models for assessment of cell therapeutics for heart disease. Hopefully, we can now move forward in a rigorous scientific manner to explore which cells can generate cardiac myocytes. Will these be from the bone marrow or the blastocyst of the embryo? What cell population determined by the infinite combinations of all cell-surface markers will be optimal? What are the advantages and pitfalls of each cell type for clinical therapy? How does mechanical and electrical function affect engraftment? Once these questions are answered, whole-animal studies will still be essential. Newly generated myocytes must survive, engraft, connect mechanically with the host myocardium and beat synchronously when they do. The ultimate test is improved cardiac contractile function after infarction in the complex systemic environment and in the presence of immune responses. These new heart slices now allow us to plan for future health.

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