Tracking Cell Fate With Noninvasive Imaging*

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Cell-based cardiac therapy is emerging as a potential strategy (1) to address substantial unmet clinical needs in cardiovascular medicine (2). Unlike all other therapies employed in the treatment of acute and chronic ischemic heart disease, cell-based therapies are conceived to replace lost myocardium (3). There is evidence comprising both basic science (4) and early clinical trials (5) that, together, supports the safety and efficacy of cell therapy for heart disease. The work to date has led to a set of key questions pertaining to dosage, delivery, and timing of cell therapy that must be answered to advance the field. In this context, methodologies to track the fate of injected cells and their therapeutic impact are paramount.

In this issue of the *Journal*, Terrovitis et al. (6) address tracking cell fate by the use of positron emission tomography (PET) to detect cardiosphere-derived stem cells (CDCs) (7) labeled with [^{18}F]-fluoro-deoxy-glucose ^{18}FDG), an agent in clinical use (8). The goal of the authors was to track the fate of cells employing different delivery strategies that can be implemented in clinical practice. In a demonstration of their approach, the authors acutely evaluated different injection efficiencies in a rodent infarct model and confirmed their results with quantitative polymerase chain reaction (PCR). Additionally, the authors attempted to determine the effects of acute retention on long-term engraftment and function with quantitative PCR, immunohistochemistry, and echocardiography.

The rats, after undergoing infarction by left anterior descending coronary artery permanent ligation, received direct intramyocardial injection of CDCs into 2 sites within the infarcted area. Cardiac arrest was found to enhance immediate cell retention to the greatest extent, whereas adenosine-induced bradycardia, fibrin glue, and a combination of the 2 all increased cell retention significantly acutely and in the long term.

Technological advances have been rooted in delivering as many cells as possible to the infarcted myocardium; yet, the quantity of cells required to achieve the highest degree of

repair is unknown (9). For instance, a particularly clinically relevant observation by Terrovitis et al. (6) is the effect of cardiac arrest on cellular retention. This observation could be of clinical relevance for cell delivery at the time of cardiac surgery, where time on cardiac bypass should be limited to a minimum. However, should cell retention prove to be crucial to long-term response, a 5-fold increase might warrant limiting cardiac blood flow during injection. This is also relevant for the choice of cardiovascular support during surgery —on or off cardiopulmonary bypass (10).

The PET-FDG provided an accurate estimate of cell delivery—but is this all that we need to know? Factors other than delivery quantity determine functional outcome. Cells that engraft might have the capacity to proliferate during differentiation; thus the outcome of cell therapy might depend on more than the quantity of cells retained. This concept is supported by the finding of Hamamoto et al. 11), in which a flat dose response to a wide range of concentrations of mesenchymal precursor cells was revealed. Indeed, a recent comprehensive meta-analysis also supports the idea that both higher and lower cell doses can provide meaningful clinical benefits (12).

With these considerations in mind, imaging technology is crucial, because current tracking and imaging modalities are able to not only track and quantify cell fate but also determine their functional effects on the host environment. In this regard, Terrovitis et al. (6) employed computed tomography (CT) in conjunction with PET to anatomically register and track the cells but did not extrapolate any functional data from these images. As previous work has shown, using multiple imaging types can enhance the structural and functional assessment of the post-stem cell transplant heart (13), and importantly CT is emerging as a modality capable of examining both regional and global cardiac function, delineating infarct size, and precisely describing the anatomic ultrastructure associated with a regenerative response.

The choice of PET to track the cells in vivo provides clarity and contrast in differentiating tagged cells from the native heart (14) and allows for precise quantification. Although PET is extremely robust at this, it lacks the spatial and temporal resolution afforded by other imaging types, including the ability to functionally phenotype the heart. Additionally, current PET tagging compounds have short half-lives limiting their use for longitudinal investigations. These deficiencies might be overcome in other imaging modalities, such as CT and magnetic resonance imaging (MRI), both of which enable structural and functional characterization (15).

Magnetic resonance imaging, for instance, provides contrast between materials and can measure function intricately, including morphology, regional contractility, and myocardial perfusion. Cell labeling has been achieved with super paramagnetic iron oxide particles (Molday ION Rhodamine B, Biopal Inc., Worcester, Massachusetts), which are readily taken up by cells. Precise quantification is more complex with MRI (16), but it has the unique ability to track cell spread and movement, and repeated scanning poses minimal risk. An MRI can also localize specific locations in 3 dimensions (17), significant due to the localized nature of cell-based therapies.

All imaging modalities, however, have suffered from lack of robust labeling techniques. Many labeling techniques, requiring large amounts of resources and materials to efficiently label clinically relevant quantities of cells, only function in the short-term (18); and later proliferation, crucial to cell therapy, has been difficult to track (19,20). Recent work aimed at remedying these problems has used transgenic molecular labeling techniques. For example, various detectable proteins such as red fluorescent protein, firefly luciferase, and herpes simplex virus thymidine kinase, can be employed as a multimodality detectable set of genes that can be transduced into a cell's genome. Organization of the genes behind specific promoters for proliferation and differentiation allow for the in vivo cell fate to be determined (21,22). Although these approaches are promising at the experimental level for distinguishing between cell engraftment and differentiation, their clinical application poses additional regulatory challenges.

Imaging technology has the potential to facilitate the development of the cell therapy field by offering cell-tracking coupled to functional imaging of the organ in question. As the study by Terrovitis et al. (6) reveals, existing clinical approaches and reagents can be applied to track cell fate and can aid in clinical trials. It is important to again emphasize that the quantity of cells delivered might not necessarily equate with therapeutic outcome, but it is the application of multimodality imaging that is a likely avenue to provide the answers. Biological considerations indicate that outcomes result from the interplay of cell delivery and the long-term survival, mitosis, and rates of differentiation of the delivered cells. Ultimately, the promise of noninvasive imaging is that each of the features can be measured over time. The ultimate promise of imaging is that cell properties can be linked spatially and temporally to the phenotype of the recovering heart.

Acknowledgments

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