Unambiguous Identification of Implanted Cells After Cellular Cardiomyoplasty: A Critical Issue

To the Editor:

In their article describing cell implantation in the myocardium of rats, Davani et al. stress the attention on integration of the transplanted syngenic mesenchymal progenitor cells within myocardial architecture and on their differentiation into smooth muscle and endothelial cells. Their conclusions are based on experimental work using 4′,6-diamidino-2-phenylindole (DAPI) as a cell marker. They raise the legitimate concern of false-negative results resulting from the dilution of the marker on cell proliferation. We think, however, that the occurrence of false-positive is also a concern with this type of cell labeling.

Our group has conducted research on an autologous muscle cell grafting procedure in canine urethra as well as in ovine myocardium. To validate the best tools for non-genetic cell cell grafting procedure in canine urethra as well as in ovine myocardium, we performed in vitro and in vivo assessment of chemical fluorescent markers, one of which was DAPI (Sigma). Cells were marked with 50 μg/mL of DAPI during 30 minutes and then washed off 3 times to remove unbound DAPI. Those cells were suspended in Dulbecco’s Modified Eagle Medium and destroyed through 3 rounds of temperature variations (from −160°C to 80°C). One hundred percent cellular mortality was obtained. The dead cells (30 million cells) were then poured onto one Petri dish containing adherent muscle cells (1 million cells per dish) that had never been exposed to DAPI. Cultures were observed after 2, 12, and 24 hours under fluorescent microscopy. Nuclei of adherent muscle cells were all marked with DAPI starting as early as 2 hours after the experiment.

In addition to these in vitro assays, we carried out skeletal muscle cell implantation in the urethra of a dog and harvested the grafted area 24 hours post-surgery. We found a pocket of DAPI-labeled cells (presumably the grafted muscle cells) within the urethral wall. Many of these cells looked necrotic. The outer muscle layer was intensely marked with DAPI. Because implanted cells could not have possibly had time to colonize the whole urethra and change their phenotype to smooth muscle cells, this seriously raises the issue of resident cell labeling with DAPI released by necrotic donor cells. This issue has also been raised when grafting 1,1'-dioctadecyl-3,3,3'-tetramethylindocarbocyanine perchlorate (DiI) labeled cells in the central nervous system. This is not unlikely when considering the massive cell death reported after cell transplantation.1

We also performed in vivo implantation of skeletal muscle derived cells as follows: We implanted either live DAPI-labeled cells, destroyed DAPI-labeled cells, or free DAPI in sheep myocardium. The grafted areas were harvested 1 hour after implantation. We did observe the live grafted cells and intensely DAPI-labeled neighboring cardiomyocytes. We also found numerous marked resident cardiomyocytes in the destroyed DAPI-labeled cell or free DAPI assays. Small DAPI-labeled capillaries were observed as well.

We conclude that re-uptake of DAPI disqualifies the use of this reagent for accurate tracking of implanted cells. We think that only genetic labeling will permit unambiguous identification of the implanted cells and analysis of their fate.

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Response
We have recently demonstrated the plasticity of 4′,6-diamidino-2-phenylindole (DAPI)-labeled mesenchymal stem cells (MSCs) in rats.1 Similar results have been documented in a murine model by Eliopoulos et al.2 However, our study is to our knowledge the first report for this species. The observations reported by Borenstein et al are not entirely new and have been previously discussed by Dorfman et al.3 We feel the following points are pertinent to this discussion:

1. The in vitro study by Borenstein et al showed that the release of DAPI by dead cells and its re-uptake by live cells requires both a high concentration of DAPI and direct contact between cells. A high number of dead cells (30×10^6) were also required to mark only 1×10^6 live cells after 2 hours. Therefore, this would mean that if 1×10^6 DAPI-labeled cells (as used in our study) have released their DAPI, only 0.03 cells in the host myocardium would be stained.
2. In the first in vivo study by Borenstein et al, the authors did not provide the phenotype of marked cells in the outer muscle layer. Importantly, Skuk et al have shown a high infiltration of immune cells (neutrophils, macrophages, and lymphocytes) in the donor-cell implanted area. The staining of these cells by DAPI can produce false-positive results.
3. In the second in vivo study by Borenstein et al, their observations raise some questions: (A) Why did they analyze DAPI-labeled cells 1 hour after implantation and (B) why did they not provide any information about the fate of these cells in the long term? In the their experiments, Dorfman et al showed that the injection of free DAPI did not mark cardiomyocytes 7 days after injection. In addition, the authors did not provide information for either the number of viable cells injected or any quantification of the cardiomyocytes and capillaries labeled. In our study,1 we have observed that 1 month after the administration of 1×10^6 DAPI-labeled MSCs, 22% of capillaries and vessels contained individual DAPI-labeled cells in the whole of the infarct area. This distribution of DAPI-labeled cells supports the idea of colonization of vessels by MSCs and their subsequent division at the luminal face. In addition, because of size, more cells must be injected in sheep than in rats. Therefore, statistically there are more dead cells in the injected area and consequently a higher risk of false-positive labeling.
The data reported here do not allow disqualification of DAPI for cell labeling. They do show, however, that using high amounts of dead cells labeled with DAPI can induce false-positive results mainly in the first hours after implantation. For us, the release of DAPI by dead cells and its re-uptake by live cells is a minor phenomenon and probably could not be observed when a low quantity of cells is used, as in our study. Further long-term studies are necessary to disqualify DAPI for cell labeling. Meanwhile, DAPI labeling is actually used for tracking grafted cells and can provide useful information of the fate of grafted cells when its limitations are known.5

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