Collagen-Based Matrices Improve the Delivery of Transplanted Circulating Progenitor Cells

Development and Demonstration by Ex Vivo Radionuclide Cell Labeling and In Vivo Tracking With Positron-Emission Tomography

Yan Zhang, MD, MSc; Stephanie Thorn, MSc; Jean N. DaSilva, PhD; Marc Lamoureux, BSc; Robert A. deKemp, PhD; Rob S. Beanlands, MD; Marc Ruel, MD, MPH; Erik J. Suuronen, PhD

Background—Collagen delivery matrices have been reported to improve the results of cell therapy, but knowledge of their mechanisms of action is limited. To evaluate whether a collagen matrix improves early engraftment posttransplantation, 2-[18F]fluoro-2-deoxy-D-glucose ([18F-FDG]) was used to label transplanted circulating progenitor cells (CPCs) and track them in vivo with positron-emission tomography.

Methods and Results—Efficiency of [18F-FDG] cell labeling was CPC-concentration dependent (r=0.61, P<0.001) but not [18F-FDG]-dose dependent. Labeled human CPCs (2×10^6) were injected with or without a collagen-based matrix in the ischemic hind limb of rats (n=12 per group) 2 weeks after femoral artery ligation. Imaging of labeled cells, acquired by small animal positron-emission tomography at 150 minutes postinjection, revealed greater CPC retention in the ischemic hind limb and less nonspecific leakage to other tissues (retention ratio, 0.44±0.08) when CPCs were delivered within the matrix, compared with cells injected alone (0.22±0.13, P=0.040) and with [18F-FDG] injected with or without the matrix (0.10±0.05 and 0.11±0.05, respectively, P<0.005). Tissue radionuclide biodistribution was performed after completion of positron-emission tomography imaging. When [18F-FDG]-labeled cells were injected with the collagen matrix, accumulation was significantly increased (by 69.6%, P=0.021) in the target ischemic hind limb muscle and significantly reduced (by 14.8% to 31.4%, P<0.05) in nonspecific tissues, compared with cells injected alone. Histology confirmed the increased retention in target tissue associated with the matrix.

Conclusions—Early posttransplantation, a collagen matrix enhances progenitor cell retention and limits distribution to nonspecific tissues, as measured by the use of [18F-FDG] labeled cells and positron-emission tomography imaging and confirmed by biodistribution and histology. (Circ Cardiovasc Imaging. 2008;1:197-204.)

Key Words: stem cells ■ tissue engineering ■ transplantation ■ ischemia ■ imaging

Growing evidence from experimental studies1-6 and clinical trials3-6 suggest that cell therapy can restore perfusion and improve function in ischemic and/or infarcted myocardium. Circulating progenitor cells (CPCs) have been shown to contribute to neovascularization in ischemic tissues, where they may differentiate into endothelial cells in situ and result in “vasculogenesis.”7-9 CPCs can be obtained by noninvasive means, thus providing the potential for autologous clinical use.

Clinical Perspective see p 204

However, stem/progenitor cell therapy is hindered by a low rate of engraftment and low persistence of cells in the target tissue. Depending on the method of delivery and the cell fraction used, transplanted cells can quickly fade from the target tissue in a matter of hours to a few days.10,11 Therefore, insufficient cell numbers and inadequate cellular interactions may not allow for an optimal therapeutic effect. Furthermore, delivery of cells to other nonspecific body sites constitutes an unwanted potential side effect. Considering the rapid loss of the delivered cells and the modest benefits of cardiac cell therapy using bone-marrow derived cells observed in clinical studies,5,6 it is likely that improvements in stem/progenitor cell delivery, engraftment and survival will be needed for more effective restoration of myocardial function.

Previous work, including our own, has shown that tissue engineered collagen-based matrices can support multiple tissues and cell types,12,13 and constitute suitable cell delivery vehicles.13-15 It has been hypothesized that the collagen, which mimics the extracellular matrix, may provide local

Received March 20, 2008; accepted September 19, 2008.

From the Division of Cardiac Surgery (Y.Z., M.R., E.J.S.); Cardiac PET Centre, Division of Cardiology (Y.Z., S.T., J.N.D., M.L., R.A.d., R.S.B.); the Department of Cellular and Molecular Medicine (Y.Z., S.T., J.N.D., R.S.B., M.R., E.J.S.); and the Molecular Function and Imaging Program (all authors), University of Ottawa, Ottawa, Canada.

Correspondence to Erik J. Suuronen, PhD, Division of Cardiac Surgery, University of Ottawa Heart Institute, 40 Ruskin Street, Ottawa, Canada K1Y 4W7. E-mail esuuronen@ottawaheart.ca or mruel@ottawaheart.ca

© 2008 American Heart Association, Inc.

Circ Cardiovasc Imaging is available at http://circimaging.ahajournals.org

DOI: 10.1161/CIRCIMAGING.108.781120
physical retention and a good platform for cell seeding during direct cell transplantation. However, the evaluation of the ability of matrices to improve cell viability and engraftment has so far been limited primarily to histological assessment of tissue at a single time point. This study was therefore designed to use in vivo imaging to examine one mechanism by which a collagen matrix may enhance the short-term effects of CPC transplantation through improved early retention of transplanted cells.

To this end, we used small animal positron-emission tomography (PET), which is an advanced nuclear imaging technology with high sensitivity and high spatial resolution. PET not only provides the potential for determining the nature of transplanted cells and of their progeny in vivo, but also offers serial monitoring capabilities that add to the clinical relevance of this modality. PET imaging with $^{18}$F-FDG, which is an advanced nuclear imaging technology with high sensitivity and high spatial resolution, has been used to track a few types of cells, such as monocytes, bone marrow-derived cells, hematopoietic stem cells, in animal and human studies. However, use of $^{18}$F-FDG to label CPCs for the assessment of delivery matrix effects on transplanted cells has not been reported.

In the present study, we evaluated the feasibility of using $^{18}$F-FDG to label CPCs for tracking by small animal PET and examined the effect of collagen-based matrices on the early retention of transplanted CPCs and their distribution to nonspecific tissues in a rat model of hind limb ischemia.

**Methods**

**Cell Isolation and Culture**

Human CPC procurement procedures were approved by the Human Research Ethics Board of the University of Ottawa Heart Institute. Total peripheral blood mononuclear cells were freshly isolated (with informed consent) from the blood of volunteer, healthy human donors by Histopaque 1077 (Sigma-Aldrich, Oakville, Canada) density-gradient centrifugation ofuffy coats, as described previously. Briefly, cells were cultured on fibronectin-coated plates in endothelial basal medium-2 (Clonetics, Guelph, Canada) supplemented with EGM-2-MV-SingleQuots (Clonetics). After 4 days, the adherent population (CPCs) was collected.

**Collagen Matrix Preparation**

Similar to methods described previously, collagen-based matrices (pH 7.5) were prepared on ice. Briefly, matrices consisted of a mixture of blended neutralized type I rat tail tendon collagen (0.4%, wt/vol; Becton Dickinson, Mississauga, Canada) and chondroitin 6-sulfate (1:6, wt/wt; Sigma), cross-linked with 0.02% (vol/vol) glutaraldehyde and followed by glycine treatment of unreacted aldehyde groups. This formulation allowed the matrix to thermogel at 37°C, following injection into animal tissue.

**$^{18}$F-FDG Cell Labeling**

CPCs were incubated with $^{18}$F-FDG at 37°C for 30 minutes in a 15-mL centrifuge tube (Fisher Scientific, Ottawa, Canada), under sterile conditions. Various dose ranges of $^{18}$F-FDG (0.5 to 4.0 mCi [18.5 to 148 MBq], 4.1 to 6.0 mCi [151.7 to 222 MBq], and 6.1 to 8.0 mCi [225.7 to 296 MBq]) and different concentrations of CPCs (2×10^6 cells, in 1, 3, or 5 mL of media) were tested to optimize the efficiency of the labeling procedure. In select experiments, insulin (0.1 U/mL) and heparin (10 U/mL) were added to the incubation in an attempt to improve labeling efficiency. At the end of incubation, cells were washed in PBS to remove unbound radioactivity. The radioactivity was measured both in cells and in the supernatant by using a dose calibrator (Capintec, Ramsey, NJ). With correction for radiolabel decay, cell-labeling efficiency was calculated as the activity in cells over the total activity used in the incubation.

**Stability and Viability of Labeled Cells**

To assess the stability of the labeling procedure, labeled cells were rinsed and centrifuged, and the cell pellet was resuspended in 2 mL PBS and incubated at 37°C for 2 hours. The retention of $^{18}$F-FDG within CPCs was calculated as above. To determine the effects of labeling on cell viability, labeled or nonlabeled cells (1×10^6) suspended in 1.0 mL endothelial basal medium were plated and incubated for an additional 0.5 hours, 24 hours, or 5 days. Cell viability was assessed using the Vi-CELL analyzer (Beckman Coulter, Mississauga, Canada) with a Trypan Blue Dye Exclusion Method.

**Retention of $^{18}$F-FDG in Matrices**

$^{18}$F-FDG-labeled CPCs (1×10^6) in 50 μL of PBS or $^{18}$F-FDG alone (using the same radioactivity and volume as the labeled CPCs) were added to the matrix solution (450 μL) on ice. Gels were plated at 500 μL per well in 24-well flat-bottom plates (VWR, Mississauga, Canada) and incubated at 37°C for 60 minutes to allow complete gelation of the matrix. Matrix gels were transferred into 2 mL PBS and incubated at 37°C for 2 hours. After incubation, the radioactivity in matrices was counted to determine the retention of $^{18}$F-FDG in matrices. Controls consisted of 1×10^6 $^{18}$F-FDG-labeled cells in 500 μL of PBS.

**Hind Limb Ischemia Animal Model**

Procedures were performed with the approval of the University of Ottawa Animal Care Committee, in accordance with the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals. The left proximal femoral artery of anesthetized (2% isoflurane) 8- to 9-week-old Sprague Dawley rats (Charles River, Wilmington, Mass) was ligated to induce ischemia, as previously described. Survival for all treatment groups was 100%. Two weeks after ligation, anesthetized rats randomly received one of the following treatments, administered by intramuscular injection into the ischemic thigh muscle, using a 28-gauge needle: (1) 2×10^6 $^{18}$F-FDG-labeled CPCs (34.7 to 56.9 μCi) in 400 μL of matrix (n=15); (2) 2×10^6 $^{18}$F-FDG-labeled CPCs (32.0 to 61.0 μCi) in 400 μL PBS (n=15); (3) $^{18}$F-FDG (54.4 to 130.2 μCi) in 400 μL of matrix (n=12); or (4) $^{18}$F-FDG (51.92 to 148.4 μCi) in 400 μL PBS (n=12).

**Small Animal PET Imaging for Localization of $^{18}$F-FDG-Labeled CPCs**

For some animals (n=3 to 4 per treatment group), whole body $^{18}$F-FDG images (150 minutes postinjection) were acquired for 15 minutes using the Inveon small animal PET scanner (Siemens, Knoxville, Tenn). Images were reconstructed using OSEM2D, resulting in a reconstructed image resolution of ~1.3 mm. To determine relative retention in the injected hind limb, Inveon Research Workplace (Siemens, Knoxville, Tenn) was used to draw 1 cuboid volume that completely encompassed the hind limb, and a second cuboid volume that contained the whole body. After correcting for injected activity, total counts in both volumes were expressed as a retention ratio of hind limb/whole-body counts.

**Biodistribution of $^{18}$F-FDG Labeled CPCs**

Although under anesthetic, rats were euthanized by cervical dislocation (at 180 minutes postinjection) and their tissues were dissected. Biodistribution of the specific radioactivity accumulation in different tissues was determined by a γ counter (PerkinElmer Life and Analytic Sciences, Waltham, Mass), and the tissues were weighed. Data are expressed as percentage of the injected dose per gram of wet tissue for all tissues other than the ischemic hind limb, and as percentage of the injected dose per...
organ for the ischemic hind limb, to account for differences in harvested and injected hind limb tissue locations.21

**Immunofluorescence Assessment**

Hind limb muscles were dissected from 0.5 cm above to 0.5 cm below the marked injection site, fixed with 4% paraformaldehyde, and sectioned at 2.5 mm thickness to ensure equivalent sampling. Tissue samples were stored in 10% neutral buffered formalin, paraffin embedded, and slides were prepared using 5-μm serial sections. According to the manufacturer’s protocol, transplanted human CPCs were localized by immunofluorescence staining using antihuman mitochondria antibodies (1:40; Chemicon, Temecula, Calif). The sections were mounted with mounting medium containing 4',6-diamidino-2-phenylindole (Vector Laboratories, Burlingame, Calif) to label cell nuclei. The percentage of transplanted cells per field of view was calculated from 4 random sections at different levels as:

\[
\frac{\text{number of human mitochondria}^+ \text{DAPI}^+ \text{cells}}{\text{number of DAPI}^+ \text{cells}} \times 100\%
\]

**Statistical Analysis**

Data are expressed as mean±SD. Statistical analyses between groups were performed with a one-way analysis of variance. For multiple comparisons of 18F-FDG PET imaging, a Bonferroni correction was applied to each test. Correlation analyses were performed by linear regression. Differences with \( P<0.05 \) were considered statistically significant.

**Statement of Responsibility**

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

**Results**

**18F-FDG Cell-Labeling Efficiency Depends Primarily on the Concentration of CPCs**

Using \(1\times10^6\) CPCs, there was no correlation between the retention and the amount of 18F-FDG in the incubation (Table). Therefore, within the 18F-FDG dose range used, CPC labeling efficiency was not 18F-FDG-dose dependent (\(P=0.18\), \(n=5\)/group).

A total of \(2\times10^6\) CPCs and 2 mCi 18F-FDG were incubated in 3 different volumes (Figure 1A). The highest 18F-FDG retention in CPCs (7.6±4.4%, \(n=12\)) was obtained in the most concentrated group (ie, 1 mL total incubation volume). 18F-FDG accumulation decreased as incubation volumes were increased, indicating that the labeling efficiency of 18F-FDG was dependent on the concentration of CPCs (\(r=0.61, \ P<0.001, \ n=9\), with an overall labeling efficiency range of 1.5% to 12% (Figure 1B). Additional experiments revealed that insulin and heparin did not increase the retention of 18F-FDG in CPCs (data not shown).

**Table. Retention of 18F-FDG in CPCs**

<table>
<thead>
<tr>
<th>Dose (mCi)</th>
<th>CPC (×10^6)</th>
<th>FDG Accumulation in Cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5–4.0</td>
<td>1.0</td>
<td>1.5±0.6</td>
</tr>
<tr>
<td>4.1–6.0</td>
<td>1.0</td>
<td>1.6±0.5</td>
</tr>
<tr>
<td>6.1–8.0</td>
<td>1.0</td>
<td>1.4±0.5</td>
</tr>
</tbody>
</table>

\(n=5\) per group.

**Figure 1.** The effect of CPC concentration on cell-labeling efficiency. A, The maximum labeling efficiency (7.6±4.4%) was obtained in the group with \(2\times10^6\) CPCs in 1 mL total incubation volume (\(n=12\) per group). B, The labeling efficiency was proportional to CPC concentration (\(r=0.61, \ P<0.001, \ n=9\) per group).
Labeled CPCs Exhibit Good Short-Term Stability and Viability

The retention of 18F-FDG in CPCs was 42.1±3.1% (n=6) after a 2-hour incubation, indicating that labeled cells showed efflux of radioactivity into media.

To investigate the effect of 18F-FDG labeling on cells, labeled cell viability was measured at 0.5 hours, 24 hours, or 5 days after labeling. As shown in Figure 2, radiolabeling had no effect on CPC viability (P>0.21) up to 24 hours postlabeling. However, there was a slight reduction in viability (15.9±6.6%) after 5 days (P<0.05).

18F-FDG Radioactivity Postlabeling Indicates Persistence in Cells Rather Than Nonspecific Retention in the Matrix

To evaluate whether matrices retain 18F-FDG and may interfere with PET imaging of labeled CPCs, the retention of 18F-FDG in matrices was measured in vitro under nonflow conditions. After 2-hour incubation, the retained 18F-FDG radioactivity of labeled cells within the matrix was 32.3±6.9%, whereas the 18F-FDG radioactivity of labeled cells alone was 26.8±3.0% (Figure 3), with no significant difference between the 2 groups (P=0.11). Retained 18F-FDG radioactivity in the collagen matrix alone (without cells) was only 4.1±1.0%, and significantly less than the other 2 groups (P<0.005).

Collagen Matrices Maintain Labeled Cells Within the Target Tissue and Minimize Delivery to Nonspecific Tissues and Organs

Determination by PET Imaging of 18F-FDG-Labeled Cells

Small animal PET whole-body imaging was performed in rats after treatment with 18F-FDG-labeled cells with or without the matrix, to assess the ability of matrices to improve retention of the transplanted CPCs in the hind limb. At 150-minute postinjection (Figure 4), greater signal intensities in the ischemic hind limb relative to the whole body were observed when CPCs were delivered with the matrix. The retention ratio of 18F-FDG radioactivity in the ischemic hind limb to whole-body 18F-FDG radioactivity for hind limbs receiving CPCs in the matrix (0.44±0.08) was significantly greater than that observed for labeled CPCs in PBS (0.22±0.13; P=0.040), and to 18F-FDG injected in the matrix or in PBS (0.10±0.05 and 0.11±0.05, respectively; P<0.005). PET imaging results also indicate that the accumulation of 18F-FDG in heart, bladder, and other nonspecific organs was lower when CPCs were delivered with matrix versus the other treatment groups (Figure 4).

Determination by Biodistribution of 18F-FDG-Labeled Cells

For confirmation, biodistribution of the specific radioactivity accumulation in different organs was determined after completion of PET scans. As shown in Figure 5, with use of the matrix there was a significant increase in retention (69.6%, P=0.021) of 18F-FDG-labeled CPCs within the ischemic hind limb muscle, and an overall reduction (by 14.8% to 31.4%, P<0.05) of labeled cell distribution to nontarget tissues, including the heart, lung, kidney, spleen, liver, and brain.

Determination by Immunofluorescence Analysis of Transplanted Cells

The retention of transplanted human CPCs in the ischemic hind limb was also confirmed by immunofluorescence analysis, using antihuman mitochondria antibody and 4',6-diamidino-2'-phenylindole (Figure 6). The percentage of
cells per field of view that were transplanted cells was greater in the ischemic hind limb of rats injected with cells + matrix (3.0±2.1%) compared with injection of cells alone (1.9±0.8%, *P*=0.048). The retention ratio calculated from PET images correlated with the number of transplanted cells in the hind limb as determined by immunofluorescence (*r*=0.845, *P*=0.017).

**Discussion**

The main findings of this study are (1) that use of a collagen-based matrix increased the early retention of CPCs delivered in vivo (observed only 2 to 3 hours posttransplantation); (2) that a collagen-based matrix minimized the distribution of CPCs to nonspecific tissues; (3) that 18F-FDG is a usable tracer for monitoring transplanted CPCs, and

**Figure 4.** Small animal PET whole-body images at 150 minutes postinjection. Images of transplanted, 18F-FDG-labeled CPCs delivered with the matrix showed significantly higher hind limb/whole-body retention ratios, indicating minimized signals in the nonspecific organs and less clearance through the bladder compared with the other groups. Images are normalized based on the injected activity of 18F-FDG to be visualized on a common scale.

**Figure 5.** Biodistribution of 18F-FDG accumulation in different organs 180 minutes after injection. A, Radioactivity in the ischemic hind limb muscle. Distribution of 18F-FDG-labeled CPCs in the group delivered in matrix (n=15) was significantly greater in the target tissue (ischemic hind limb). B, Radioactivity in the nonspecific tissues. There was an overall reduction of labeled-cell distribution in kidney, spleen, and other nonspecific tissues. #*P*<0.05 versus FDG (n=12); Δ*P*<0.05 versus FDG with the matrix (n=12); **P*<0.05 versus FDG-labeled CPCs (n=15).
provides a promising platform for the development of noninvasive PET imaging approaches for trafficking of CPC delivery within matrices in real time; and (4) that the success of the PET imaging methods in evaluating the effect of the matrix on CPC transplantation was validated by 2 conventional methodologies (biodistribution and immunofluorescence). Our study therefore introduces a novel concept that in addition to improving the long-term engraftment of transplanted cells, a collagen delivery matrix may also enhance the short-term effects of cell therapy because differences in cell retention were observed at an early stage posttransplantation.

Stem cells and/or progenitor cells are being widely investigated as a potential therapy for ischemic heart disease. Satisfactory cell deposition and engraftment in the target area are considered likely therapeutic prerequisites. The use of CPCs for cardiac angiogenic activity has previously resulted in some benefits, but the retention and survival of implanted cells in the myocardium is only between 1% and 10%. The development of tissue engineered matrices for the delivery and support of transplanted cells has recently attracted interest in the cardiac field. Collagen-based biomaterials have been developed to support cell growth and for the restoration of myocardial infarction. The advantages of collagen matrices are their large surface area for cell seeding, porosity for capillary in-growth, stability for mechanical support, biodegradability, and minimal immunogenicity. Previous work from our group indicated that in vitro adhesion of the CPC derived CD133\(^+\) cells was greatest on a collagen type I substrate; and use of a collagen-based matrix for the delivery of CPCs into ischemic hind limbs of rats improved the retention of the transplanted cells and increased tissue vascularization, as determined by immunohistochemical analysis performed 2 weeks after transplantation.

In this study, noninvasive in vivo PET imaging and subsequent biodistribution and immunofluorescence analysis demonstrated related observations at earlier time points after transplantation than previously investigated. CPC retention in the ischemic hind limb muscle was \(\approx 11\%\) of injected dose at 180 minutes after injection without the matrix. With use of the matrix, retention was enhanced by \(\approx 70\%\) in the target tissue.

To date, cardiac cell therapy has resulted only in modest clinical benefits, perhaps limited by the low retention and survival of implanted cells. It has been demonstrated that the loss of injected cells from the target tissue occurs at an early stage posttransplantation, but their effects can be sustained by host tissues that are induced by the transplanted cells to express humoral factors involved in angiogenesis, antiapoptosis, and chemotraction of bone marrow cells. Therefore, the observation that our collagen matrix improved the very early retention of cells within the target tissue suggests that its use may confer enhanced short-term therapeutic cell effects in addition to its benefits to long-term cell engraftment. It is conceivable that by minimizing the rapid loss of transplanted cells, the collagen matrix may augment and/or prolong the cell transplant-mediated host humoral response.

The ability to assess the engraftment and survival of transplanted cells is also of importance for the study of cell-based therapeutic strategies. Traditionally, the fate of transplanted cells is assessed by postmortem histological examination at a single time point in animal studies. For visualization, ex vivo cell labeling methods are used before transplantation using a vital dye (eg, 4',6-diamidino-2'-phenylindole), a thymidine analog (eg, 5-bromodeoxyuridine) or a conventional reporter gene (eg, green fluorescent protein). Alternatively, established radiotrace methods used in clinical nuclear medicine offer an attractive option for cell imaging, with the advantage of noninvasive cell tracking at several time points. The present study demonstrated that PET imaging with \(^{18}\)F-FDG cell labeling was feasible for the assessment of CPC retention and distribution in the early stages of transplantation with and without a matrix. To our knowledge, this is the first study using PET imaging to investigate and demonstrate the effect of collagen matrices on cell transplantation in vivo.

\(^{18}\)F-FDG is an attractive radiotracer for labeling stem cells because it enables PET imaging of cell tracking in vivo, and exploits normal metabolic activity of target cells, thereby reducing any risk of functional alteration. In this study, we successfully labeled human CPCs with \(^{18}\)F-FDG with minimal alteration of viability. The viability of labeled cells was preserved up to 24 hours, with a slight subsequent reduction observed at 5 days. Our results also demonstrated that cell-labeling efficiency of \(^{18}\)F-FDG was CPC-concentration dependent but not FDG-dose dependent. Similar observations have been reported in studies using \(^{111}\)In or \(^{99m}\)Tc.

Because only viable cells will retain \(^{18}\)F-FDG in the cytoplasm, an added benefit to its use is that retention of \(^{18}\)F-FDG might reflect the viability state of the cells. However, a possible limitation of labeling cells with \(^{18}\)F-FDG is that significant leakage of the radiotracer after initial cellular accumulation may occur. Several studies using \(^{18}\)F-FDG...
FDG have reported a labeling efficiency of <10% for some types of stem cells because of a high efflux rate in the first hour post-labeling. Ma et al29 showed that rat mesenchymal stem cells can be labeled with $^{18}$F-FDG but 98% release of the radiotracer occurs in the washing process. Our results also showed a significant rate of radiolabel release from CPCs during the washing process. Stability studies on labeled cells showed a ~50% label loss after 2-hour incubation. Although insulin has been shown to improve $^{18}$F-FDG retention in cells, it was not effective in improving the labeling efficiency in CPCs.31 It is possible that CPCs may have an insufficient level of G-6-phosphatase activity, or perhaps insulin does not have an inhibitory effect on G-6-phosphatase activity in CPCs.30 Importantly, despite the dosage loss, the radioactivity could still be monitored by PET in vivo.

Another limitation of $^{18}$F-FDG cell labeling is the rather short half-life of $^{18}$F (110 minutes) that only permits the monitoring of cell fate for several hours. Advances in nanotechnology are enabling the development of new PET agents with improved sensitivity for the tracking of cells using long-lived radionuclides. For example, $^{64}$Cu-labeled nanoparticles have been used to image the macrophages in inflammatory atherosclerosis,31 and although not yet widely available, agents such as these may provide an appealing alternative for long-term or repeated imaging.

In summary, $^{18}$F-FDG is a usable tracer for monitoring transplanted CPCs, and provides a promising platform for the development of noninvasive PET imaging approaches for tracking of CPC delivery within matrices in real time. This study used PET imaging and traditional confirmatory techniques to demonstrate that collagen-based matrices significantly improved the very early retention of transplanted CPCs in the ischemic tissue and limited their nonspecific distribution. This mechanism conferred by the matrix may have implications on the effects of cell therapy at the early stages after transplantation in addition to the long-term benefits of improved cell engraftment.

Sources of Funding

This work was supported by the Heart and Stroke Foundation of Ontario (program grant PRG 6242; grant NA5905 to Drs Ruel and Beanlands; and grant NA6121 to Dr Suuronen), the Canadian Institutes of Health Research (grant MOP-77536 to Drs Ruel and Suuronen), and the Canadian Foundation for Innovation (award 7346 to Dr Ruel). Dr Beanlands is a Career Investigator of the Heart and Stroke Foundation of Ontario and Dr Zhang is the recipient of an Ontario Graduate Scholarship in Science and Technology.

Disclosures

None.

References

Regenerative medicine for the treatment of cardiac disease is rapidly developing. However, our understanding of the efficacy of cell therapies and of the adjuvant role of tissue engineered materials, such as collagen matrices, is largely limited to postmortem histological assessment, which is unsuitable for clinical use. As a noninvasive molecular imaging modality, PET imaging can be applied longitudinally and has the capability to assess biological processes at the molecular and cellular levels. The current study used 18F-FDG cell labeling and PET imaging to monitor the distribution of transplanted endothelial progenitor cells in real time and to better understand the role of delivery matrices in cell therapy. By using this imaging technique, we found that a collagen-based matrix can improve the early retention of transplanted cell in the target tissue. We believe with further refinement, molecular imaging techniques of transplanted cells will contribute to the elucidation of the optimal stem cell type(s) and dose, the evaluation of adequate administration methods, the assessment of delivery of the cells and biopolymers, and the development of novel tissue engineering strategies. Therefore, tissue engineering and PET imaging could help expand the clinical applications of cell therapies in years to come.

**CLINICAL PERSPECTIVE**

Regenerative medicine for the treatment of cardiac disease is rapidly developing. However, our understanding of the efficacy of cell therapies and of the adjuvant role of tissue engineered materials, such as collagen matrices, is largely limited to postmortem histological assessment, which is unsuitable for clinical use. As a noninvasive molecular imaging modality, PET imaging can be applied longitudinally and has the capability to assess biological processes at the molecular and cellular levels. The current study used 18F-FDG cell labeling and PET imaging to monitor the distribution of transplanted endothelial progenitor cells in real time and to better understand the role of delivery matrices in cell therapy. By using this imaging technique, we found that a collagen-based matrix can improve the early retention of transplanted cell in the target tissue. We believe with further refinement, molecular imaging techniques of transplanted cells will contribute to the elucidation of the optimal stem cell type(s) and dose, the evaluation of adequate administration methods, the assessment of delivery of the cells and biopolymers, and the development of novel tissue engineering strategies. Therefore, tissue engineering and PET imaging could help expand the clinical applications of cell therapies in years to come.
Collagen-Based Matrices Improve the Delivery of Transplanted Circulating Progenitor Cells: Development and Demonstration by Ex Vivo Radionuclide Cell Labeling and In Vivo Tracking With Positron-Emission Tomography

Yan Zhang, Stephanie Thorn, Jean N. DaSilva, Marc Lamoureux, Robert A. deKemp, Rob S. Beanlands, Marc Ruel and Erik J. Suuronen

Circ Cardiovasc Imaging. 2008;1:197-204
doi: 10.1161/CIRCIMAGING.108.781120

Circulation: Cardiovascular Imaging is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2008 American Heart Association, Inc. All rights reserved.
Print ISSN: 1941-9651. Online ISSN: 1942-0080

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circimaging.ahajournals.org/content/1/3/197