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FULL ARTICLE

The potential of biophotonic techniques in stem cell tracking and monitoring of tissue regeneration applied to cardiac stem cell therapy

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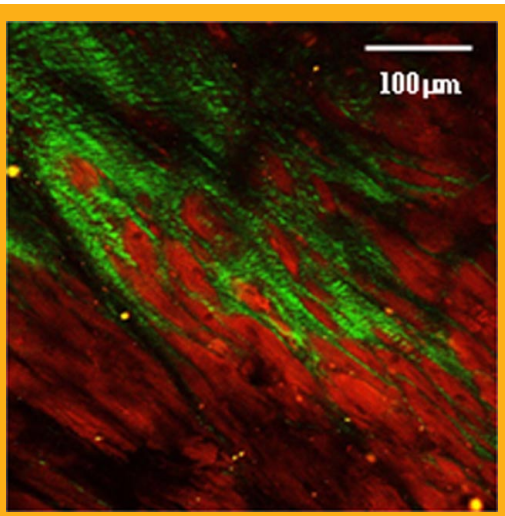
The use of injected stem cells, leading to regeneration of ischemic heart tissue, for example, following coronary artery occlusion, has emerged as a major new option for managing 'heart attack' patients. While some clinical trials have been encouraging, there have also been failures and there is little understanding of the multiplicity of factors that lead to the outcome. In this overview paper, the opportunities and challenges in applying biophotonic techniques to regenerative medicine, exemplified by the challenge of stem cell therapy of ischemic heart disease, are considered. The focus is on optical imaging to track stem cell distribution and fate, and optical spectroscopies and/or imaging to monitor the structural remodeling of the tissue and the resulting functional changes. The scientific, technological, and logistics issues involved in moving some of these techniques from pre-clinical research mode ultimately into the clinic are also highlighted.



Composite image of second harmonic generation (green) and 2-photon excitation fluorescence (red) in ischemic rat myocardium following stem cell treatment

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1. Introduction

The promise of regenerative medicine has led to a great deal of activity in recent years to study stem cells and apply them to the organ/tissue regeneration, including in the heart: see representative reviews [1–4]. Suffice it to say that the prospects of and progress towards eventual organ replacement or rejuvenation have been excellent and with continually improving results. Important breakthroughs include: 1) in the general regenerative medicine case, the ability to induce pluripotent human embryonic stem cells without having to extract them from embryos or cord blood [5] but rather by reprogramming an individual's own skin fibroblasts or somatic cells, thus also circumventing the need for immunosuppression, and 2) in the specific case of cardiac stem cells, the capability now to purify cardiac stem cells from human embryonic stem cells with up to 50% yield [6].

Stem cell research and the eventual clinical translation to regenerative medicine are replete with scientific and technological challenges, many of which are already being addressed or could potentially be addressed using biophotonic techniques. These can be grouped as shown in Table 1, which also identifies some of the major needs in each challenge. This list was developed during a 2008 workshop at the NSF Center for Biophotonics that involved stem cell and biophotonics experts, working together to describe needs and possible biophotonics-based solutions. These needs run the gamut from stem cell identification, to characterization of the type and stage of differentiation of stem cells, to means of enriching or cultivation of specific stem cell types, to means of implanting them and monitoring their location and potential therapeutic effects on target tissue. In this paper we will focus of the challenges of stem cell tracking after implantation and monitoring of the tissue response to treatment. We will further narrow the focus to the specific example of stem cell therapy of ischemic heart disease, and use this to illustrate many of the key issues in the use of biophotonics to meet the above challenges

Cardiovascular disease is a major killer worldwide and a significant part of this mortality is due to ischemic damage to the myocardium following coronary artery occlusion that is the consequence of atherosclerotic plaque, as illustrated in Figure 1. The damaged (ischemic) heart muscle is then unable to perform its normal function. There are over 1 million cases of myocardial infarct per year in the USA and this disease is predicted by the World Health Organization to become the largest cause of death worldwide [7].

The concept of cardiac stem cell therapy is to remodel the ischemic myocardial tissues; a wide range of different stem cell types have been investigated for this purpose, both embryonic and adult, including skeletal myoblasts, mesenchymal stem cells and stem cells derived from bone marrow, as well as cells originating in the heart tissue itself, i.e. adult cardiac stem cells [8]. These various cell types have been tested in a variety of animal models, with typically $\sim 10^4$–10^5 cells injected locally into or surrounding the region of damaged myocardium following ischemia induced by coronary blood vessel ligation. Outcome endpoints include both architectural and functional measures of tissue remodeling. There have also been over a dozen human clinical trials reported [8, 9]. These have targeted different patient groups (type and stage of heart disease), with varying stem cell type and number, route and timing of administration and outcome measures. The responses have also been highly variable, with some trials reporting clinically-significant improvements, others showing no effect, and some showing negative side effects. There is no clear pattern in these studies nor correlation between outcome and the particular protocol used. Hence, as is not uncommon in medicine, the clinical exploration has outrun the underlying basic science, and there is growing concern about clinical studies

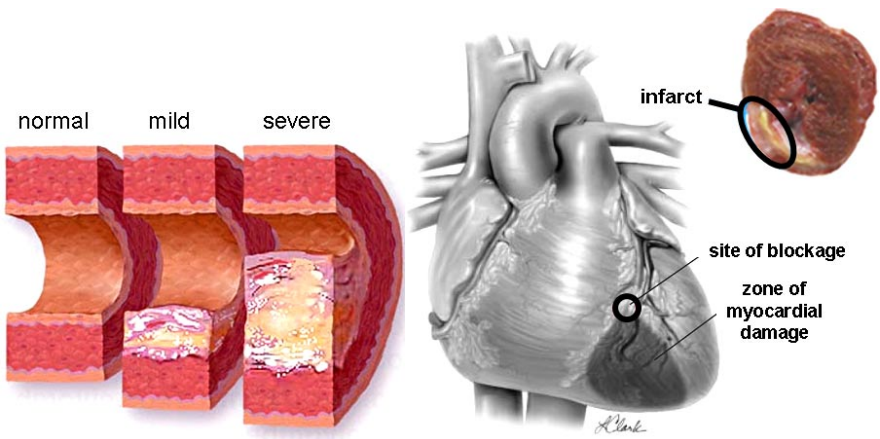

Figure 1 (online color at: www.biophotonics-journal.org) Development of atherosclerotic plaque (left) leading to myocardial ischemia, with a histological section showing a region of infarct.

Table 1 Challenges in stem cell therapy and examples of significant unmet needs.

	Challenge	Unmet needs
Stem Cell	Identification	Specific biomarkers of 'stemness' prior to implantation
	Characterization	Degree of differentiation
	Enrichment	Selection of specific lineages
	Implantation	Minimally-invasive means to implant near identified ischemic zone
	Tracking	Localization, number, function and fate of stem cells following implantation
Target tissue monitoring	Angiogenesis and reperfusion	Spatial extent of angiogenesis and blood flow recovery and level of tissue re-oxygenation
	Structural remodeling	Quality and extent of normal tissue structure replacing damaged zone
	Recovery of mechanical and electrical function	Recovery of biomechanical properties of tissue and proper electrical functioning of the cardiomyocytes

without better understanding the biological mechanisms involved [9]. Herein lies the opportunity to apply biophotonic techniques and technologies at the pre-clinical level, some of which may also be translatable to the clinic.

In this paper, we will focus on two of the main challenges outlined in Table 1, namely:

- methods to assess the fate and survival of the administered stem cells *in vivo* [10] and
- methods to monitor the resulting myocardial repair over time [11].

The first may be further split into i) tracking the macro and micro distribution of the stem cells after administration (when do they go?, are they viable?, do they proliferate?, what molecular/functional changes do they undergo?) and ii) determining their interactions with the tissues. The second challenge comprises iii) monitoring the initial changes triggered by the presence of the stem cells, which includes in particular induction of angiogenesis and blood reperfusion that are prerequisites for repair, iv) monitoring the actual tissue remodeling process, which includes both the cellular and structural (e.g. collagen) elements of the tissue, and v) assessing, again over time, the functional recovery of the tissue, both electrical and biomechanical.

For both stem cell tracking and tissue regeneration monitoring, there are a variety of non-optical techniques under investigation [12–14]. However, the focus here will be on some of the potential biophotonic techniques for these purposes: as will become apparent, these are at very different stages, both in the development of the technologies themselves and in their investigation for these specific applications. It is not the intent here to be comprehensive, but rather to illustrate some of the opportunities and challenges.

2. Stem cell tracking

Non-optical imaging techniques for tracking of stem cells *in vivo*, either at a micro and macro scale [12, 13], include magnetic resonance imaging with superparamagnetic nanoparticle labeling of the stem cells, radionuclide imaging (including positron emission tomography) of stem cells that have been incubated with radioisotopes, and ultrasonography using cells labeled with microbubbles, liposomes or nanoparticles. Each has advantages and limitations, although they all have the substantive advantage over the optical techniques discussed below that the imaging can be carried out *in vivo* non invasively (apart from the labeling of the administered stem cells) in patients, either across the chest wall or throughout the whole body. However, the optical methods have the advantages of offering very high spatial resolution and providing information on specific biological functions of the stem cells, albeit primarily in preclinical small animal models.

The ideal biophotonic technique for stem cell monitoring would allow minimally-invasive tracking of individual stem cells or stem cell populations at specific locations or throughout the whole body, enable determination of their viability, provide information on the specific tissue niches to which they localize and on particular molecular changes as they interact with the host tissues, enable survival to be distinguished from proliferation, and provide this information over time in a minimally-invasive way that does not interfere with the processes involved. Clearly, there is no single technique or even combination of techniques that meets all these needs, but significant progress has been reported on some aspects of the problem. To date, studies have been based on two main approaches: intravital fluores-

cence microscopy/microendoscopy and *in vivo* bioluminescence imaging, and we will illustrate each of these.

Fluorescence tracking can be done by labeling the stem cells either with reporter genes (e.g. β-galactosidase or green fluorescent protein), or with fluorescent dyes. Intravital microscopy for tracking stem cell migration at the microscopic level has been reported by several groups. As presented elsewhere in this issue [15], the work of Lin and colleagues is among the most sophisticated, using high-resolution confocal imaging to track individual fluorescent stem cells (Figure 2a). This work has mainly focused on stem cell niches in bone, and so is not specifically related to cardiac stem cell therapy, but nevertheless demonstrates the power of the technique. As shown by Rhuparwar et al. [16], the challenge with the heart, as for many of the techniques that we will discuss, is that the tissue is moving rapidly and over distances that are large compared with the imaging depth. Nevertheless, *in vivo* stem cell tracking in dog heart (at open surgery) has been achieved: as shown in Figure 2b, this results in significantly poorer resolution because of heart wall movement, even after image processing, but does reveal the overall distribution of the fluorescent cells. Another potential solution is to use a small-diameter fiberoptic microendoscope, such as shown in Figure 2c, which could be placed in contact with the heart, at least using an intraoperative or externalized-heart model approach. The use of cellular autofluorescence to follow changes in stem cells as they proliferate and differentiate may also be a possibility, as suggested by the work of Georgakoudi and colleagues [17], who have used 2-photon fluorescence microscopy to track the Redox ratio as a functional index in stem cells as they undergo differentiation. Whether or not there would be enough signal to follow individual stem cells in this way *in vivo* is not clear.

Bioluminescence imaging, using stem cells that have been transfected with the luciferase gene, is a powerful technique for tracking the population distribution of stem cells in animal models [12, 13, 18]. An example is shown in Figure 2d, where bone marrow stromal cells were injected into rats in which myocardial ischemia had been induced by coronary vessel ligation. The question asked was, what is the optimal route of administration of the stem cells – intravenously (IV), injection through a coronary artery (IA) or injection directly into the myocardium (IM) adjacent to the infarct? As seen, the differences were large, with IV injection resulting in most of the stem cells being sequestered in the lungs, some targeting being achieved using IA injection, but the best result (maximum number of stem cells remaining localized in the heart) being by direct IM injection.

This type of study, which is relatively straightforward in that it used well-established biophotonic technologies, addressed an issue with important clinical implications. In the clinical trials reported to

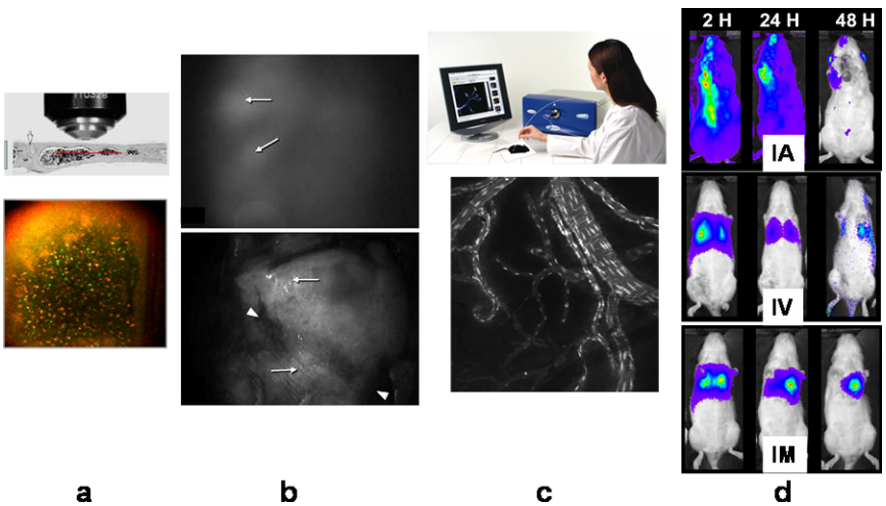

Figure 2 (online color at: www.biophotonics-journal.org) Examples of optical imaging approaches for individual stem cells or stem cell populations. **a**) intravital confocal microscopy of fluorescently-labeled stem cells in a bone model *(courtesy C. Lin, Mass. Gen. Hosp, USA)*. **b**) intraoperative imaging of fluorescently-labeled stem cells at 2 injection sites (arrows) in dog heart, before (top) and after (bottom) image processing to reduce the motion blur *(from ref. [16], with permission)*. **c**) fiberoptic microendoscopy showing (top) the Cellvizio system *(courtesy Mauna Kea technologies, www.maunakeatech.com/)* and (bottom) an example of stem cell tracking in the microvasculature using a confocal microendoscope *(courtesy Optiscan Pty Ltd, www.optiscan.com)*. **d**) whole-mouse *in vivo* bioluminescence imaging to track the spread of stem cells after administration by intra-arterial, intra-venous or intra-myocardial injection (*adapted from Li et al. [18] with permission*).

Table 2 Potential advantages and current limitations of fluorescence and bioluminescence imaging of stem cells for *in vivo* tracking in cardiac stem cell therapy.

	Advantages	Limitations
Fluorescence	Can track single stem cells Multiplexing Labeling or gene transfection	Small depth of high-resolution confocal imaging Tissue autofluorescence background With dye labeling, signal is attenuated with cell division
Bioluminescence	Non-invasive, allowing longitudinal studies Low background No excitation light needed	Limited spatial resolution Not translatable into patients
Both	Whole-body small animal imaging	Need to label stem cells

date, there was corresponding disparity in route of administration. Clearly, IV injection would be the simplest and IM the most challenging, but this study showed that the IM route, or at least the IA route, is preferable. It also demonstrated another major problem in stem cell therapy, in that the survival of the cells was probably very low (as assessed by loss of the bioluminescence signal). In addition, it showed that the timing of stem cell administration following infarct is important, since there were significant differences when the stem cells were given 3 days after ligation versus 28 days.

While bioluminescence imaging has thus shown its value in preclinical studies, there are clearly limitations, some of which are fundamental and some of which could be overcome with further development. The former include the non-translatability of bioluminescence imaging into patients, since administering luciferase (*luc)* gene-transfected stem cells raises serious safety and ethical concerns. The more tractable problems even in the preclinical setting are the difficulty in making these measurements truly quantitative and distinguishing loss of metabolic activity from cell death. The latter could be addressed by double transfection, combining bioluminescence (metabolic activity) and fluorescence protein transfection (cell death). An attractive feature of bioluminescence is that the luciferase gene can be placed downstream of another gene of functional interest, so that the changes its expression *in vivo* could be tracked. Table 2 summarizes the strengths and limitations of fluorescence and bioluminescence labeling of stem cells for cardiac applications.

3. Monitoring angiogenesis/tissue reperfusion

In order for the ischemic tissue to become functional again, it must be reperfused with oxygen. Hence, angiogenesis must take place to regenerate the microvascular network of the tissue. There are several optical techniques that, in principle, could be used to monitor the changes in microvasculature, details of blood-flow dynamics and/or changes in blood perfusion and tissue oxygenation, either directly or indirectly: intravital microscopy, optical coherence tomography (OCT), photoacoustic imaging and near infrared spectral imaging.

As in the case of stem cell tracking, microimaging of new blood vessel formation is most easily performed using (confocal) fluorescence microscopy [19], in which a fluorophore that remains within the blood stream allows the vessels to be distinguished from the other tissue structures. As shown in Figure 3a, FITC-labeled high-molecular weight dextran is commonly used for this purpose. As with stem cell tracking, the heart poses the additional problem of movement and, to our knowledge, monitoring of cardiac regeneration/reperfusion *in-vivo* has not been reported: certainly confocal microendoscopy, such as illustrated in Figure 2c, where the fiber probe could be placed onto the heart wall to give high-resolution images of the microvascular architecture, may present a valuable approach. This would be analogous to applications such as monitoring angiogenesis in the gastrointestinal tract [20], which has been demonstrated in patients. One of the advantages of 3D confocal imaging of the neovascular network is that there are a number of quantitative metrics that may be derived from the images that are biologically relevant [19], such as vessel connectivity and the density of branching points on the vascular network. It is also possible, using a second fluorescent marker, to image simultaneously with the neovasculature the expression of, for example, vascular endothelial growth factor (VEGF) expression that is a molecular promoter of new vessel formation. Thereby, additional information is gained on (the dynamics of) the neovascularization process.

Vessel micro-architecture and flow dynamics may also be directly assessed by OCT, specifically *via* its Doppler and speckle-variance extensions [21]. In the former, interferometric phase-sensitive methods are employed to quantify the flow of red-blood cells in the microcirculation [22], enabling quantifiable de-

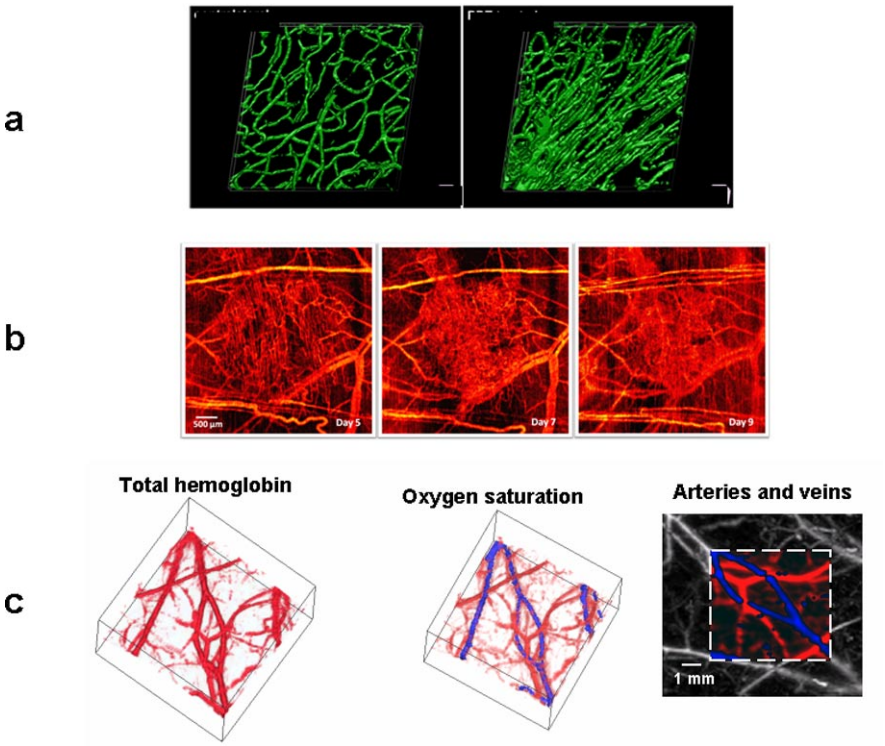

Figure 3 (online color at: www.biophotonics-journal.org) Examples of monitoring of angiogenesis, using three biophotonic techniques for high-resolution qualitative imaging of tissue microvascular networks. **a**) confocal microscopy (brain) showing increased microvessel architecture by the green fluorescence from circulating FITC-dextran before (left) and after (right) pro-angiogenic treatment (*after ref. [19], with permission*). **b**) longitudinal imaging of angiogenesis (in a renal cell carcinoma line/mouse window chamber preparation) using speckle-variance OCT, showing the increasing microvascular network density *(after ref. [25], with permission)*. **c**) spectral photoacoustic imaging of single subcutaneous blood vessels in a rat model *in vivo*: Hb_{tot} was acquired at the isosbestic 584 nm wavelength; SO_2 shows arteries and veins in red and blue, respectively; overlay is based on the maximum amplitude projection towards the skin surface. *(courtesy Dr. L. Wang and after ref. [27], with permission*]). Note that the images in (b) and (c) were taken without an exogenous contrast agent.

tection of the microvasculature. Endoscopic, intraoperative, intravascular and interstitial implementations of OCT systems are available [22–24], although OCT assessment of cardiac regeneration following stem cell therapy *in vivo* in a beating heart has yet to be reported. Recent advances in Fourier-domain OCT have made high-speed OCT imaging with good signal-to-noise a reality [23], and this could be particularly important in cardiac tissue assessment. Figure 3b shows an example of speckle-variance OCT longitudinal imaging of angiogenesis, albeit not in cardiac tissue [25]: note that this technique has the advantage that it does not require use of an exogenous contrast agent. For both Doppler and speckle-variance OCT, a variety of quantifiable metrics of the microvascular architecture are currently being developed [26]. In the cardiovascular context, the degree of heart muscle ischemia and the change in microvasculature following stem cell therapy are both attractive targets for OCT microvascular imaging.

A third microvascular imaging technique that could be useful, but to our knowledge has yet to be reported for this application, is photoacoustic imaging, as exemplified by the work of Wang and colleagues [27]. This technique is based on imaging the ultrasound field that is generated in tissue following localized optical absorption (by endogenous or exogenous chromophores) of a short laser pulse that causes transient local thermal expansion of the tissue. This has been developed for imaging at both the macro and micro scales. Using different incident wavelengths can provide spectral information on the absorbers, and this can be used, for example, for microvascular imaging, distinguishing between the oxygenated and deoxygenated states of hemoglobin in the vessels, as illustrated in Figure 3c.

Each of these various microvascular imaging techniques has strengths and limitation, that together represent a powerful platform of tools for monitoring angiogenesis in general, and that induced by stem cell therapy in particular, at the microvascular level with high resolution.

Tissue reperfusion on a macroscopic scale, resulting from angiogenesis, may also be assessed by spec-

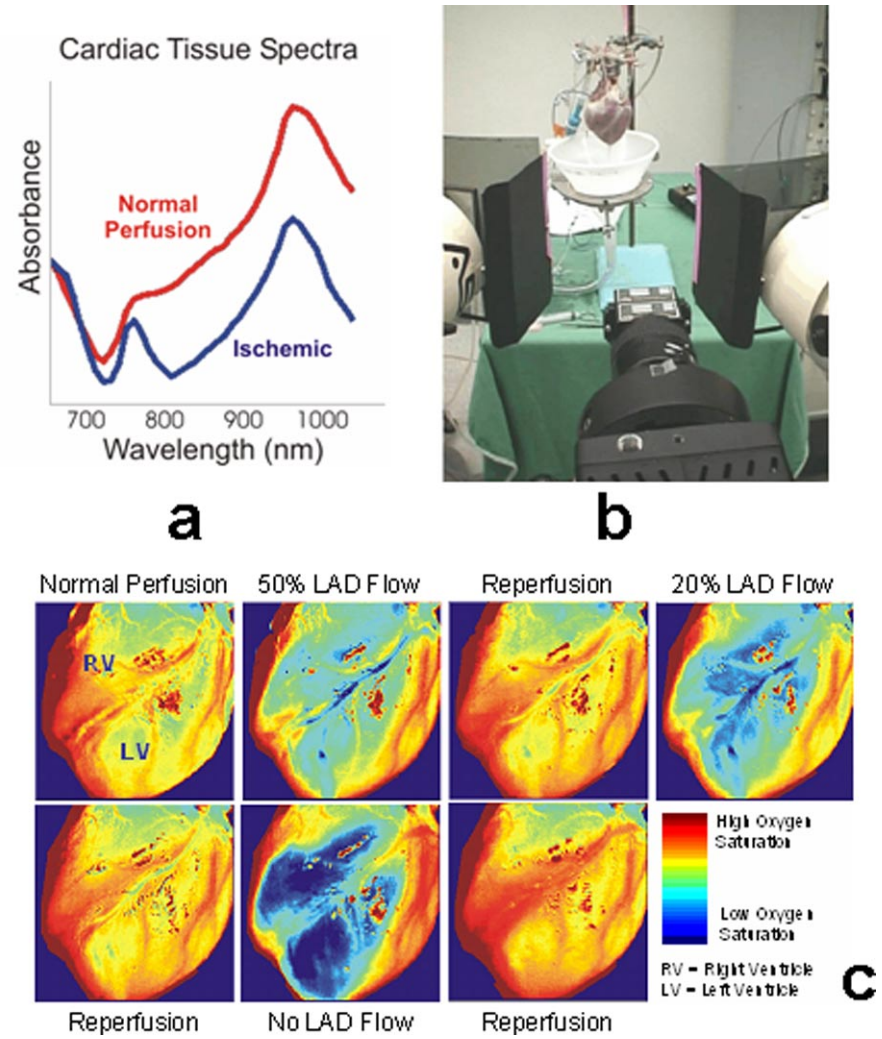

Figure 4 (online color at: www.biophotonics-journal.org) NIR spectral imaging of the heart. **a**) optical absorption spectra of normally-perfused and ischemic myocardium. **b**) set-up for NIR imaging of externalized heart. **c**) images of a heart subject to intermittent episodes of partial and complete restriction of blood flow through the left anterior descending artery (LAD) artery, with reperfusion between those episodes. The images are color coded according to the tissue oxygen saturation derived in each pixel by fitting the HB and HbO_2 spectra to the tissue spectra (a), and show clear patterns of oxygen tension modulation under different ischemic conditions. (*courtesy Dr. A. Shaw, NRC-IBD, Canada, and adapted from ref. [28], with permission*).

tral photoacoustic imaging applied to a large tissue volume (with resulting reduced spatial resolution), or by direct near infrared (NIR) diffuse reflectance imaging [28]. The latter is illustrated in Figure 4. As can be seen, this technique can provide large-scale information on functional recovery of regional blood flow in the heart wall, which is a clinically-relevant endpoint that is analogous to, for example, radionuclide perfusion imaging, but with the added advantage that it shows not only blood perfusion but also the oxygenation status (oxygen saturation) of the tissue.

4. Monitoring tissue remodeling

Following administration of stem cells, there are many and complex biological responses of the myocardial tissue, leading to structural and functional regeneration. There are also confounding responses, such as fibrosis leading to scar formation [11] that may impede the response, and there is interest in interventions to reduce these effects. There are, in principle, several linear and non-linear optical techniques that could be used to monitor the status of damaged and regenerating myocardium in response to stem cell therapy, either in point spectroscopic and/or imaging mode, reporting molecular, cellular, microstructural and physiological/metabolic metrics. These include: near infrared Raman spectroscopy, coherent anti-Stokes Raman spectroscopy (CARS), second harmonic generation (SGH) spectroscopy/imaging, 2-photon excitation of fluorescence (TPEF) and tissue polarimetry. A key advantage of these methods is that they are label-free, so that they report the intrinsic (endogenous) signatures of the tissues, without the need for an exogenous contrast agent. Raman, i.e. inelastic scattering spectroscopy, which is sensitive to common vibrational bonds in cells and tissues, could provide useful information on the biochemical status of the infarct and its remodel-

ing response. (Raman spectroscopy has also been exploited by Chan et al. [29] to separate human embryonic stem cell cardiomyocytes that do not have a known cell-surface marker.) For cardiac tissue, Ogawa et al. [30] have recently shown in an externalized heart model that the near-IR Raman spectra of cardiomyocytes have major contributions from collagen type-1 chromophores. Using spectral principle component analysis, fibrosis, which is known to replace normal myocardium in infarcts, could thereby be distinguished from normal cardiomyocytes in intact tissue, as shown in Figure 5a.

The main limitation of Raman spectroscopy is that the signal is very weak, so image generation pixel-by-pixel is extremely slow. CARS, which uses fs pulsed lasers in a pump-probe configuration, gives orders-of-magnitude resonant enhancement of the Raman signal. CARS imaging has been implemented in confocal microscopy [31], where it is particularly good at imaging lipid-rich structures. Its role in myocardial tissue assessment is less clear and to our knowledge this has not been investigated to date. By contrast, in SHG intense light interacting with non-centrosymmetric molecular structures generates a frequency-doubled optical signal. If a second detection channel is also used, then 2-photon excitation fluorescence (TPEF) and SHG images can be obtained simultaneously and

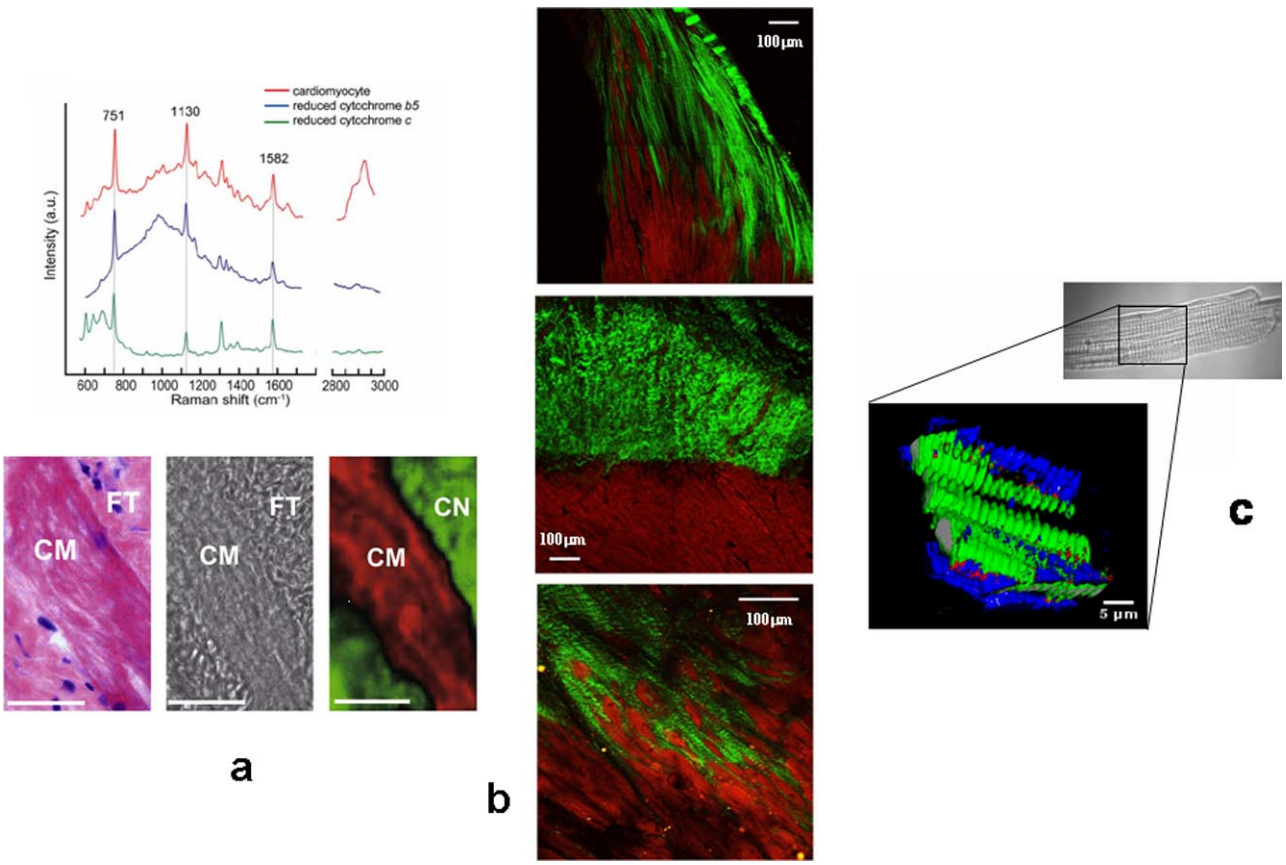

Figure 5 (online color at: www.biophotonics-journal.org) Microstructural and compositional label-free spectral imaging of cardiac tissues and cells. **a**) near-infrared Raman in excised whole rat heart at the myocardial-ischemia border, showing (top) the spectra of single cardiomyocytes (red) and heme proteins (blue, green) indicating common vibrational bands, and (bottom) H&E section (left), unstained white light image (center) and Raman Principle Component false-color image (right) of myocardial tissue, showing differentiation between a cardiomyocyte (CM: red) and a fibrotic region (FT: collagen (CN) in green) (*adapted from ref. [30], with permission*). **b**) simultaneous SHG (green) and TPEF (red) images in rat myocardium *ex vivo*, showing (top) temporary myocardial ischemia (60 min LAD ligation followed by 9 weeks reperfusion), (middle) chronic myocardial ischemia (9 weeks ligation) and (bottom) 4 weeks after mesenchymal stem-cell therapy of chronic ischemia (2 weeks post ligation). Note the diffuse scar/healthy tissue demarcation in the top image, the sharp boundary in the middle image, and the reduction/integration of the scar tissue compartment in the bottom image. (*courtesy Marika Wallenburg, University Health Network, Toronto*). **c**) SGH (green) and THG (blue) images of an isolated cardiomyocyte and their overlap (red). The grey-scale image shows the whole cell under white light (~ 100 µm long). *(adapted from ref. [32], with permission).*

intrinsically co-registered. The two signals are spectrally separated by measuring the fluorescence beyond the frequency-doubled waveband: e.g. for 820 nm excitation, a detector centered at 410 nm (+/− ~15 nm) picks up predominantly the SHG signal, with only a small admixture of TPEF; conversely, longer-wavelength detection isolates the TPEF signal. In the heart, the non-centrosymmetric nature of collagen gives a strong SHG signal, while the cellular component (cardiomyocytes) can be readily distinguished by their strong TPEF signature. Thus, areas of localized infarct containing scar tissue that is richer in collagen than normal myocardium should show larger SHG signals than the surrounding tissue. This is borne out by the images in Figure 5b, in which the nature of localized versus diffuse infarct, and the resultant recovery following stem cell treatment, are readily apparent.

As illustrated in Figure 5c, the application of non-linear microscopies has been taken one step further by Barzda and colleagues [32], who used simultaneous SGH and 3rd harmonic generation (THG) imaging to probe the structure of single isolated cardiomyocytes. The SHG signals were attributed to anisotropic bands of myofibrils, while the THG signal, which is sensitive to interfaces between intracellular structures, was attributed to the multilamelar structure of the densely folded cristae and the outer and inner mitochondrial membranes. Note that the effective depth of tissue sampling is small in all these non-linear techniques, so that *in vivo* they would essentially be able to probe only accessible tissue surfaces.

The use of polarized light to probe monitor myocardial remodeling is a further option that can, in principle, furnish a wealth of useful biophysical information. For example, depolarization rates report on tissue scattering and organization, while birefringence reflects the inherent structural anisotropy of interrogated samples. There are many challenges in applying polarimetric technique to tissue, including the marked depolarization due to multiple light scattering and the simultaneous occurrence of the several polarization processes. However, these are being successfully mitigated, applying rigorous models of polarized light propagation in optically-turbid media [33, 34]. In the present context, it is known that healthy myocardium is a highly organized anisotropic tissue consisting of aligned collagen fibrils and cardiomyocytes; the organization of compromised myocardium is less well known, but is suspected to be more chaotic and so more isotropic. Thus, a polarimetric measurement of, for example, tissue linear birefringence may prove useful in assessing the extent/severity of the scar and in following the remodeling changes associated with stem cell therapy [34]. This is illustrated in Figure 6, which shows how the polarization retardance (a measure of the tissue birefringence and anisotropy) is altered in the infarct

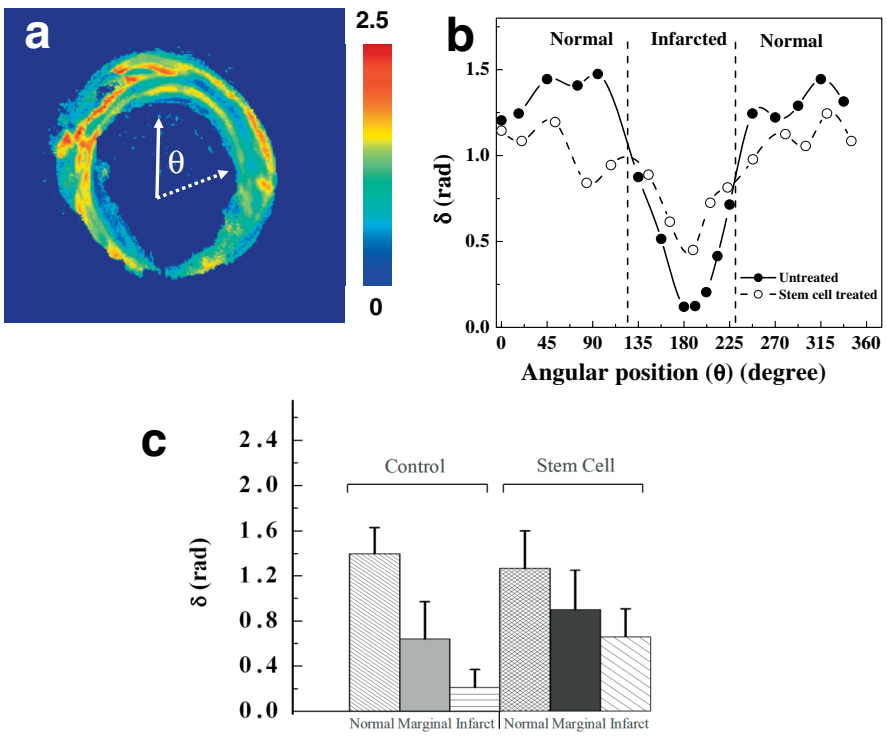

Figure 6 (online color at: www.biophotonics-journal.org) Polarimetry *ex vivo* in heart tissue section of injured and stem-cell treated rat myocardium. **a**) pretreatment image of retardance, δ (rads), showing reduced anisotropy in the scar region (~180°). **b**) corresponding profiles around the myocardial section, showing an increase in scar organization/anisotropy following stem cell therapy. **c**) summary of results for stem cell treatment in this pre-clinical model, showing statistically-significant changes in the infracted scar regions. (*after ref. [34], with permission*).

and how remodeling after stem-cell treatment can be followed. *In vivo* implementation of polarimetric measurements may be facilitated by fiberoptic polarization-sensitive OCT [35].

5. Monitoring tissue biomechanics and electrophysiology

We will mention three optical techniques that are potentially applicable to the challenge of assessing the recovery of biomechanical or electrical functioning of the myocardium following ischemic injury and subsequent stem cell therapy: laser speckle imaging, optical elastography and laminar optical tomography. The first two can potentially report the elastic properties of the tissue, while the third provides functional electrical mapping.

Laser speckle occurs when coherent light propagates through and is reflected from random scatterers, from which the Brownian motion of endogenous light-scattering particles can be detected. The demodulation of the speckle over time depends on the motion of the particles, which is influenced by the tissue viscoelasticity. Laser speckle imaging has been applied by Nadkarni and colleagues [36] to investigate the viscoelastic properties of atherosclerotic plaque, using a fiberoptic bundle, and one could envisage that the technique could be used for monitoring myocardial viscoelastic properties pre and post stem cell treatment. Since the depth of sampling is limited, this would likely be useful only for probing the surface tissue properties, for example, in an externalized heart model.

Ultrasound imaging of the strain distribution in the myocardium has been reported to show significant differences between normal and post-infarct patients and the physical basis of the technique has recently been discussed by Chen et al. [37]. Tissue elastography has also been performed using MRI [38]. It is possible, in principle, that more direct measurement of the tissue elastic properties, specifically the microscale elastic moduli, could be obtained by optical techniques, in particular by monitoring changes in the structural OCT images following mechanical deformation of the tissue at different applied frequency (optical coherence elastography) [39]. To date this has been applied mainly to look at differences between tumor and non-tumor tissue, but could be applied to myocardial tissue, using fiberoptic OCT instrumentation.

For the third technique, Hillman and colleagues have pioneered laminar optical tomography (LOT) to track the propagation of electrical waves across the surface of the perfused heart on a macroscopic scale [40]. Clearly, this can be markedly disrupted by the presence of an ischemic zone of myocardial tissue, and the recovery of the electrical function is a prerequisite to improved cardiac function. This technique was developed originally for functional brain imaging, and is based on the use of near infrared voltage-sensitive dyes, the optical absorption or fluorescence of which is altered by changes in (cardiomyocyte) trans-membrane potential: traditionally, these are used in high-resolution micro-imaging mode. The use of near-IR dyes gives an increased imaging depth, so that the signal is generated from a few mm of sub-surface tissue. The principle of LOT is that, by measuring the surface spatial distribution of diffusely reflected or fluorescent light from the tissue under focused beam illumination, it is possible to reconstruct the 3D distribution of the signal from the tissue, by scanning the excitation beam. The LOT data can then be co-registered with electrocardiogram measurements, under different electrical stimulation conditions, as illustrated in Figure 7. Voltage-sensitive dyes could also be of value for identifying embryonic stem cells that differentiate into adult 'pacemaker' cardiomyocytes that create action potentials and are essential in recovery of the electrical functioning of the tissue.

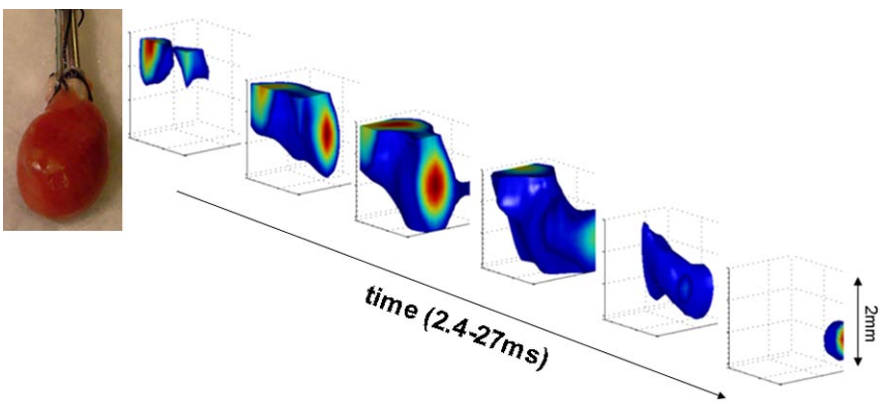

Figure 7 (online color at: www.biophotonics-journal.org) Optical assessment of myocardial electrical function by laminar optical tomography using a voltage sensitive dye in an externalized perfused rat heart under epicardial pacing, showing the wave of electrical activity arising, spreading across a $2 \times 2 \times 2$ mm region of the heart surface and dying out as a function of time after stimulation: *(adapted from ref. [40], with permission)*

6. Discussion

The motivation for cardiac stem cell research is to create a therapeutic means to restore heart function in patients who have suffered myocardial infarct, have chronic arrhythmias or have experienced cardiac arrest due to catastrophic electrophysiological malfunction. The primary methods used to date for clinical therapy of cardiac disorders has been to either implant/inject cardiac stem cells or to graft an ensemble of cardiac stem cells onto a region of the heart that has been damaged by infarct. At this time there is no solid evidence of heart muscle regeneration, but there have been observations of cardiomyocytes derived from human embryonic stem cells (HESC) being integrated into the heart tissue. The major benefit so far has been to make the heart muscle more compliant and its cellular structure less susceptible to rupture, something which modestly improves cardiac output, but is far from full regeneration of heart function.

Imaging, both in the laboratory and in the clinic, has important roles to play at all size and time scales in understanding the sequence of biological events that happen following stem cell administration. This is critical to further optimize the treatment, since, as mentioned in the Introduction, cardiac stem cell therapy has advanced through largely empirical clinical studies that have outstripped understanding of what happens to the stem cells following administration, or of the mechanisms and dynamics of tissue repair. For pre-clinical cell/tissue/organ/whole animal studies there are particular advantages to optical imaging techniques, through the high resolution (spatial and temporal) and rich biomolecular information that can be obtained. To date the techniques illustrated above have been used only *in vitro*, on *ex vivo* tissues, in externalized heart models, or with *in vivo* pre-clinical animal-models. Some have not yet been applied specifically to cardiac stem cell therapy but, from their other applications, one can appreciate the type of information that they yield and extrapolate to this specific application.

At the same time, it is worth briefly considering the question of whether and, if so, how some of these techniques could be moved into clinical use, either in the clinical trial setting or even eventually as part of routine treatment optimization and patient monitoring. Some of the challenges in applying optical techniques in patients undergoing cardiac stem cell treatment should be apparent from the above discussion, and include:

1) the risk involved in using stem cells that have been labeled by gene transfection to make them optically active (e.g. by bioluminescence or even by fluorescence),
2) the loss of optical signal from stem cells upon division or differentiation,
3) the limited penetration of light, including near-infrared, through the chest wall, in order to probe the heart tissue
4) the large and rapid movement of the heart wall, which makes optical imaging at useful spatial resolution extremely difficult, and
5) the changing information required at different time points following treatment- stem cell tracking (location, number, survival and differentiation), angiogenesis, structural remodeling, and recovery of function- which means that different optical 'reporters' are needed throughout the course of treatment and follow-up.

Issue #1 needs to be addressed through extensive safety and toxicity studies and it may be more acceptable to use fluorescent dye labeling of the cells. Issue #2 represents a challenge to the molecular biology community. Addressing #3 will require the use of either fiber-optic based probes or implanted optical microbiosensors (e.g. such as those under development based on integrated micro-lasers/optics/photodetectors, with telemetric read-out [41]). These approaches carry a degree of invasiveness. Also, for the former there will be limited longitudinal sampling of myocardial tissue response, while the amount of information that can be obtained by the latter may be quite limited. The 4th issue of the heart movement represents an engineering challenge: this may involve hardware development and/or, as seen in the example of Figure 2b, spectral/image data processing to compensate or correct for motion blurring. Finally, point #5 highlights a major strength of biophotonic-based approaches, namely the ability to use multiple simultaneous or sequential techniques that report different information at different times in the course of treatment and follow-up. However, the cost and complexity of implementing multiple modalities in the clinic are substantial concerns. Nevertheless, if the ongoing pre-clinical studies demonstrate the potential clinical value in the information that any of the biophotonic techniques can provide, then the pay-off may merit the effort of overcoming some of these major challenges in transitioning to patients.

In conclusion, the main focus of this paper has been the potential of optical techniques for tracking stem cells and monitoring tissue responses following stem cell administration. Although we have used cardiac stem cell therapy as the exemplar of this approach, the various biophotonic techniques are clearly more widely applicable to the whole field of regenerative medicine: indeed, the heart may be one of the most difficult sites, because of the limited access and severe motion, as discussed in the last section. Referring back to the Introduction, there are several steps prior to cell implantation where

there are major shortcomings and where biophotonics may contribute, namely in the identification, characterization and enrichment of the stem cells. Thus, to end on a speculative note, in stem cell identification one could foresee the use of an endogenous or exogenous optical signature that would be a more sensitive and specific biomarker than current cell-surface biomarkers, exploiting perhaps fluorescence, Raman or nanoparticle-based surface-enhanced Raman (SERS) signatures or a combination of several spectroscopic techniques. In stem cell characterization, optical signatures may also aid in, for example, assessing degree of cell differentiation and specific gene expression. From a practical perspective, it would be useful to do this in a massively parallel format, to which optical technologies are well suited. For enrichment of the stem cell population from a mixed-cell sample, some form of modified cell sorting would be invaluable, such as, for example, the use of an array of laser traps that again allows massively-parallel separation [42]. In this case, integration of (multiplexed) optical identification into the sorting platform will be needed. Finally, in terms of the actual implantation process, optical guidance could be of value in localizing the boundary of the ischemic zone so that the cells could be implanted at optimal locations.

From the perspective of biophotonics research and development, it should be clear that there is no shortage of significant challenges in the field of regenerative medicine and that this will become a substantial focus of ongoing effort.

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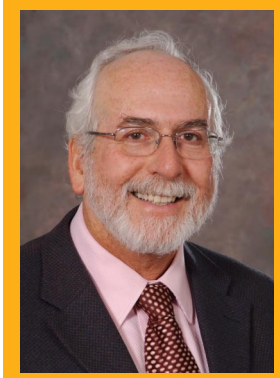

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