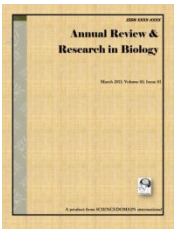

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Image analysis in *In Vivo* Stem Cell Tracking

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Review Article

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ABSTRACT

Regenerative medicine has begun to define a new perspective of future clinical practice. The lack of basic data regarding to basic stem cell biology-survival, migration, differentiation, integration in a real time manner when transplanted into damaged tissue remains a major challenge for design stem cell therapies. So, visualization of injected stem cells provides additional insight into the future therapeutic benefits. Although current imaging modalities including magnetic resonance imaging, positron emission tomography, single photon emission computed tomography, bioluminescence imaging, and fluorescence imaging offer some morphological as well as functional information, they lack the ability to assess and track in vivo biological phenomenon, a pivotal link for greater mechanistic understanding following cell-based intervention. This review will therefore discuss currently available in vivo imaging modalities and image processing techniques which may potentiate this field of research.

Keywords: Stem cell; tracking; In-vivo; image analysis;

ABBREVIATIONS

BLI: *Bioluminescence Imaging;* ***FDA:*** *Food and Drug Administration;* ***FLI:*** *Fluorescence Imaging;* ***MRI:*** *Magnetic Resonance Imaging****;*** ***MSC:*** *Mesenchymal Stem Cells;* ***MPIOs:*** *Micron Sized Particles of Iron Oxide;* ***MRS:*** *Magnetic Resonance Spectroscopy;* ***NMR:*** *Nuclear Magnetic Resonance;* ***PET:*** *Positron Emission Tomography****;*** ***ROI:*** *Regions of Interest;* ***SPECT:*** *Single Positron Emission Computed Tomography****;*** ***SPIO:*** *Super Paramagnetic Iron Oxides****;*** ***USPIO:*** *Ultra Small Super Paramagnetic Iron Oxides****.***

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1. INTRODUCTION

Making alive medication that can patrol our bodies, find the injured tissue and autonomously augment endogenous repair mechanisms is highly desirable. Cell therapy has raised the hope for renovation of failing organ with new cells (Terizc and Nelson, 2010; Javanmard et al., 2010).

Although cell therapy is a fairly new discipline, overflowing new investigations during the last decade develop great potential to provide future effective therapeutic approaches. In contrast to traditional pharmaceuticals, regenerative cytotherapy products contain viable cells as the active building blocks. Stem cell therapy has been considered as a most promising core component of modern medical practice (Terizc and Nelson, 2010; Lau et al., 2010). Tissue repair might provide restorative advantages in a spectrum of pathologies ranging from congenital diseases to acquired, age-related degenerative diseases. Applied in the treatment of cardiovascular diseases, the quickly developing stem cell therapy promises prodigious outcomes for increased quality of life and improved patient care, has been built on advances in stem cell biology. However, it seems that successful development of cell therapy needs translational studies and a close interaction between physician-scientists and basic researchers.

The first crucial problem in developing cell-based therapies has been identifying the ideal stem cell types. For example, the conflicting results of cardiac stem cell clinical trials highlight the need for suitable techniques that can monitor cell-based treatment trials (Lau et al., 2010; Lunde et al., 2006; Wollert et al., 2004; Schächinger et al., 2006). The other important questions are the most suitable timing of stem cell delivery, dose, and delivery route (Lau et al., 2010).

To answer these questions almost all cardiovascular stem cell trials of ischemic heart disease have used at least one imaging modality (Wollert et al., 2004; Schächinger et al., 2006; Bartunek et al., 2005; Britten et al., 2003; Fernandez-Aviles et al., 2004; Gyöngyösi et al., 2008; Herbots et al., 2009; Janssens et al., 2006; Lunde et al., 2006). Cellular imaging techniques are needed for their ability to identify, localize, and monitor stem cells fate *in vivo* longitudinally after implantation. These imaging modalities are likely to provide greater insight into the fundamental mechanisms underlying stem cell migration, homing and survival.

2. IMAGING MODALITIES

The perfect imaging modality should provide data on cell survival, proliferation and differentiation as well as its effects on cardiac function. Furthermore, the modality should have the sensitivity to track low numbers of cells, the exact quantification and location of the cells to be determined. Certainty, no modality is optimal, and it is necessary to consider the advantages and shortcoming of each technique for the unique application in question (Pearl and Wu, 2008; Terrovitis et al., 2010).

Many studies on stem cell fate have used ex vivo analysis such as histologic staining for green fluorescent protein or β-galactosidase (Wu, 2008). However to realize stem cell fate in vivo, noninvasive techniques must be developed. Common non-invasive imaging modalities available for in vivo tracking of the biological fate of stem cells consist of direct Radionuclide

Labeling**,** Magnetic Resonance Imaging (MRI), and indirect reporter gene labeling based methods such as Bioluminescence Imaging (BLI), and Fluorescence Imaging (FLI).

2.1 Radionuclide Labeling Based Imaging

This imaging modality includes positron emission tomography (PET) and single positron emission computed tomography (SPECT). In these techniques the cells should be directly labeled with a radioisotope prior to transplantation (Acton and Zhon, 2005). The advantages of this modality are high sensitivity and instantaneous translation to clinical application, because indium (In)-111-oxine and F18-fluorodeoxyglucose ([18F]-FDG) are approved by the Food and Drug Administration (FDA) for clinical practice (Villa et al., 2010). The technique has demonstrated utility for tracking cell homing in infarcted myocardium and distribution following injection. It has been shown that [^{111}In]-oxine labeled endothelial and hematopoietic progenitor cells home to infarcted rat myocardium after intravenous injection (Higuchi et al., 2009). However, by 96 hours post injection, only ~5% of the injected dose of radioactivity was found in the heart (Janssens et al., 2006; Messina et al., 2004). Love et al. have demonstrated PET and CT fused images, were able to locate and detect as few as 10,000 mesenchymal stem cells (MSC) for over a two-month period in mice (Love et al., 2007). These data presents the sensitivity of the method. An inherent limitation of radionuclide labeling is the short half-life of the radionuclides, which prevent long-term imaging. For radiotracers with a shorter half life ([^{18}F]-FDG is 110 minutes), one can follow those cells at most out to 1 day. Even for radiotracers that have longer half-lives (e.g., [^{111}In]-oxine, 67.3 h, [^{201}Tl]-thallium, 74 h, [^{124}I-iodine, 96 h), the longest time period of imaging would still be limited to within the first 1 to 2 weeks (Pearl and Wu, 2008). Furthermore, the radiotracer itself has other concerns, specifically potential adverse effects on stem cell viability and the genetic properties, differentiation and the leakage of the tracer from the labeled cells, which can provide a false signal (Hoehn et al., 2002).

2.2 Magnetic Resonance Imaging

MRI is the most readily accessible modality for in vivo imaging of transplanted cells and can detect the nuclear spin of molecules (Cassidy and Radda, 2005). There are two general MRI modalities, T1 and T2. Lanthanide gadolinium (Gd^{3+}) is applied for T1-weighted contrast enhanced images and T2 contrast agents commonly used in stem cell tracking methods include super paramagnetic iron oxides (SPIO) (Wang et al., 2007), ultra small super paramagnetic iron oxides (USPIO), and micron sized particles of iron oxide (MPIOs) (Modo et al., 2005). MRI can be used to track cells labeled with iron particles for at least 16 weeks after injection. The magnetic properties of iron-labeled donor cells can be used for their subsequent isolation from host tissue to enable further characterization (Stuckey et al., 2006). MRI techniques have been utilized to detect the presence and/or migration of transplanted stem cells in various animal models (Kim et al., 2007).

Compared with radionuclide imaging, the main advantage of MRI is its capacity for high anatomic resolution. The ability of MRI is limited to detect $>10^5$ cells in the heart, so, it has lower sensitivity than radionuclide based labeling methods (Pearl and Wu, 2008).

Furthermore, the ferromagnetic probe dilution with cell division, making it difficult to enumerate proliferating cells. Finally, as the iron particles can remain in nonviable cells and tissue macrophages the detected signal does not necessarily indicate the cells viability (Pearl and Wu, 2008).

A specialized technique associated to MRI is magnetic resonance spectroscopy (MRS). MRS, also known as nuclear magnetic resonance (NMR) spectroscopy, is a non-invasive analytical technique that has been used to study metabolic changes *in vivo*. So, despite of MRI which gives information about the structure of the body, MRS allows obtaining biochemical information about the tissues in a non-invasive way (Weber et al., 2006). MRS can be used for tracking of the metabolically related events in vivo such as proliferation and differentiation (Couillard-Despres and Aigner, 2011).

2.3 Reporter Gene Based Imaging

Specific labeling of targeted stem cells could be achieved through genetically labeling by a reporter gene. This method can be used to illustrate both cellular and molecular processes in vivo. Genetically modification of stem cells to express reporter gene(s) before transplantation led to express reporter protein(s) that can act together with a corresponding reporter probe to produce an imaging signal detectable by different imaging modalities. This signal can be detected by charged-coupled device (CCD), PET, SPECT, or MRI (Rodriguez-Porcel M., 2010).

The reporter gene DNA can be transferred into cells by transfection (via a viral vector such as lentivirus, retrovirus, adenovirus, adeno-associated virus) or transduction (via a nonviral vector e.g., plasmid). It should be mentioned that introducing DNA into cells by non-viral methods i.e. transfection only impacts the cells that directly receive the transfected DNA, so, the reporter gene is not passed from generation to generation during cell division and therefore the labeling is not permanent. Despite the transfection, transduction stably introduces a target gene into a host cell's genome and allows the transfected DNA to be carried stably from generation to generation. However, the advantages of using non-viral delivery compared to viral vector delivery are decreased immunogenicity and mutagenesis. For more information please see (Denèfle, 2011)

These reporter genes enable us to investigate consequent gene expression associated with key cellular events (differentiation, proliferation and cell viability) (Zhong et al., 2010; Denayer et al., 2008; Wang et al., 2007). An advantage of reporter gene imaging is that the construct can be designed and linked to the suitable promoter sequence according to research question. For example, linking a cardiac specific promoter (α-cardiac myosin heavy-chain promoter) to a reporter gene enables us to detect the stem cells differentiation into cardiac phenotype. Promoters can be categorized as constitutive (continuous expression), inducible (controlled expression), or cell/tissue specific (Pearl and Wu, 2008).

One of the frequently used reporter gene based imaging modality is firefly luciferase (*Fluc*)-based optical BLI. The main reporter gene is *Fluc*, which oxidizes the D-Luciferin to emit light at about 560 nm (Wang et al., 2003), similar to the light is generated within the firefly insect. The emitted photons can be detected and quantified by CCD cameras. Although the bioluminescence imaging can be distorted by the absorbance and scattering the light given off by the luciferase marker by surrounding living tissue (Frangioni and Hajjar, 2004). Bioluminescence imaging is safe, providing the repeated tracking of small numbers of labeled cells. However it has lower sensitivity to detect small number of cells than MRI and PET. In addition, this technique is restricted to small animal use because the emitted photons become attenuated within deep tissues and thus are currently not applicable to large animal or human studies. However, BLI has been used successfully for in vitro tracking imaging in a few large animal studies (Gyöngyösi et al., 2011).

Another widely used reporter gene is herpes simplex virus thymidine kinase (*HSV-tk*) utilized in PET imaging. Compare to *Fluc*, the HSV-tk reporter gene constructs have been applied to both animal and human trials (Pearl and Wu, 2008). The HSV-tk phosphorylates the injected radiolabeled thymidine analog (e.g., [18F]fluoro-3-hydroxymethylbutyl) guanine or [^{18}F]-FHBG) and consequently entrapped inside the cell and then can be detected by PET (Pearl and Wu, 2008).

In both *Fluc* and *HSV-tk* the stem cells must be genetically modified before transplantation and it may be potentially immunogenic and mutagen. Though, Fluc is usually used as a single transient transfection marker and used in combination with other reporter as a bi- or trifusion reporter gene, then used for stable transfection. Other benefits of transduction consist of: 1) only viable cells can generate signal, 2) proliferation of stem cells expressing the reporter gene can be detected by continuing rise of imaging signals over time, 3) the reporter genes will be missing if the transplanted cells are died , so minimizing false signals (Pearl and Wu, 2008).

Cao et al. (Cao et al., 2006) injected mouse embryonic stem cells stably transfected with a trifusion reporter gene (firefly luciferase, red fluorescent protein, and HSVtk) in the rodent heart to demonstrate multimodality imaging of cell survival, cell proliferation, and ablation of cell misbehavior. Similarly, Gyongyosi et al. (Gyöngyösi et al., 2008) injected porcine mesenchymal stem cells stably transfected with a variant of the trifusion reporter gene into the pig heart and performed clinical PET imaging to document cellular persistence at 10 days post-delivery. Rodriguez-Porcel et al. (Rodriguez-Porcel et al., 2008) showed the feasibility of quantifying regional myocardial transgene expression with clinical PET/computed tomographic imaging by using endomyocardial catheter delivery of adenovirus carrying the HSVtk reporter gene in healthy pig myocardium. Using a transgenic mouse model that stably expresses firefly luciferase and green fluorescent protein, Sheikh et al. (Sheikh et al., 2007) provided insights into the “spatiotemporal kinetics” of how bone marrow cells can home in on ischemic hearts after intravenous delivery. In a follow-up study, van der Bogt et al. (Bogt et al., 2008) performed head-to-head comparisons of the post-intramyocardial transplantation survival and efficacy of bone marrow cells, skeletal myoblasts, and mesenchymal stem cells derived from these colored transgenic mice. Finally, Hung et al. (Hung et al., 2008) showed that mouse embryonic stem cells delivered into the normal (remote) zone have better cell viability compared with that seen with injections in the intrainfarction and peri-infarction zones.

Some candidate reporter genes have been used for detection of gene expression by MRI. Recent studies have suggested the use of genes associated with intracellular metalloproteins including the transferring, ferritin, tyrosinase. However, accumulation of iron inside the cells may be cytotoxic and iron accumulation does not inevitably correlate with cell viability (as in direct labeling) (Gilad et al., 2007).

Since each imaging modality possesses unique limitations and advantages, multimodality imaging constructs have been developed that combine the best features of each technology in an effort to fulfill all the needs of stem cell imaging. Additionally, it seems that image processing will empower us to detect more precise information from stem cell imaging method.

3. SIGNAL/IMAGE PROCESSING AND CELL TRACKING

The main purpose of "image processing" (Sonka et al., 1999) is improvement of pictorial information for human interpretation. For this reason we must at first prepare the image by "preprocessing" (e.g., image enhancement (Wang et al., 1983) and image restoration (Bates et al., 1986; Andrews et al., 1977) to enable object detection, classification, and tracking. In many advanced imaging modalities including "cell imaging devices" (Dmitri et al., 2009; Wu and Castleman, 2008), images can be stored in digital format. A "digital image" is a two dimensional spatial array of data, representing some sensed quantity of interest. Using digital image gives us this ability to use a lot of computer softwares that help us to extract the useful information from the images. Fig. 1 shows an example of analog to digital conversion. This process includes two steps, 1) "Sampling", Dividing a continuous region into small squares (pixels), and taking average value of each square, 2) Quantization, Mapping each value into one in a set of discrete values.

In "high content screening" (Wu and Castleman, 2008; Carpenter and Sabatini, 2004; (Abraham et al., 2004), we want to know about cells, what is in them, around them, and between them, their growth and dying, their signaling, influencing, stimulating, inhibiting, and about cell division and proliferation, differentiation, and migration. The difficulty of interpreting large-scale image datasets by human visual inspection, leads us to use image processing tools. For this reason, according to Fig. 2, after image acquisition, the main steps are image preprocessing, segmenting individual cells, linking cells over time, and feature extraction.

3.1 Preprocessing

Preprocessing is one of the main stages of any image processing system (Sonka et al., 1999; Wang et al., 1983; Bates et al., 1986; Andrews et al., 1977). The main purpose of using this step is preparing the image for main processing such as feature extraction and pattern recognition (Micheli-Tzanakou, 2000). Usually images are corrupted during acquisition and transmission. This corruption depends on the imaging modality and the communication media (Wang et al., 1983; Bates et al., 1986; Andrews et al., 1977). For example, it has been shown that the dominant noise in CT, MR and PET images can be statistically modeled by Gaussian (Rabbani et al., 2009), Rician (Rabbani et al., 2009; Nowak, 1999) and Poisson (Snyder et al., 1987) distributions respectively. Fig. 3.B illustrates a noisy and denoised image.

In addition to (statistical) noise, degradation may come in many other forms such as motion blur, and camera mis focus (Campisi and Egiazarian, 2007). Thus, by considering the effect of noise, a popular and simple model for captured image can be defined using convolution operator and the additive noise (Campisi and Egiazarian, 2007). Here, "Image restoration" (Bates et al., 1986; Andrews et al., 1977) (i.e., the process of recovering image from degraded observation called "deblurring" (Andrews et al., 1977; Hansen, 2006) for known blur function, and "blind deconvolution" (Stockham et al., 1975; Dmitri et al., 2009) for unknown blur function) is the main purpose of preprocessing. Figure 3.C shows an example of for deblurring as a preprocessing technique for solving this problem.

There are other issues for preprocessing such as intensity adjustment (Wu and Castleman, 2008), contrast enhancement (Armitage et al., 1965), interference removal (Wu and Castleman, 2008), registration (prior motion correction for alignment of sequences) (Brown,

1992), etc. For example, due to uneven illumination over the field of view, there is a large variation in image intensity that must be removed using contrast enhancement methods. Fig. 3.D shows the application of image equalization for contrast enhancement using *Image Histogram and Intensity Adjustment Demo* in *MATLAB* (MATrix LABratory) environment (http://www.mathworks.com).

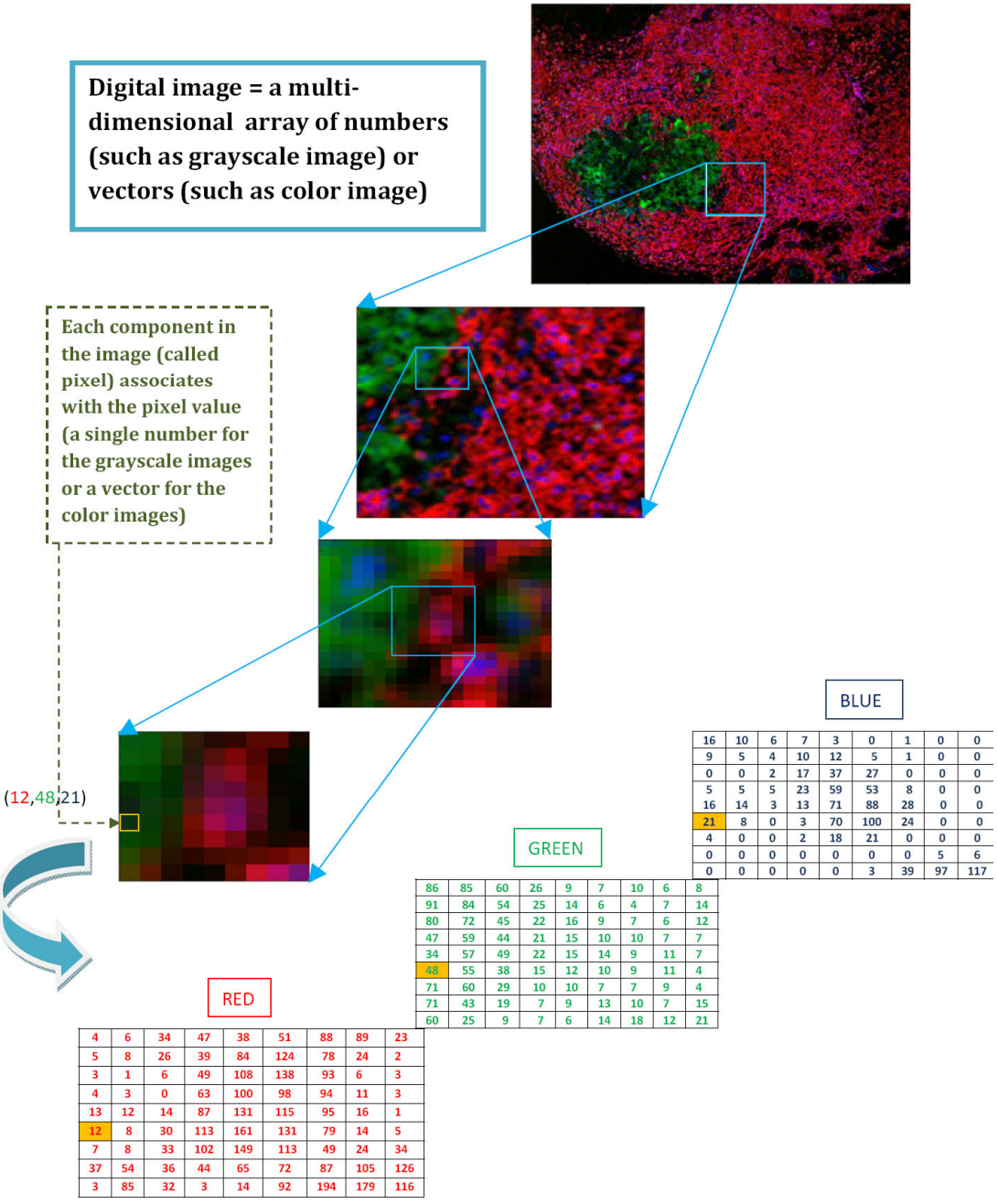

Fig. 1: An example of analog to digital conversion

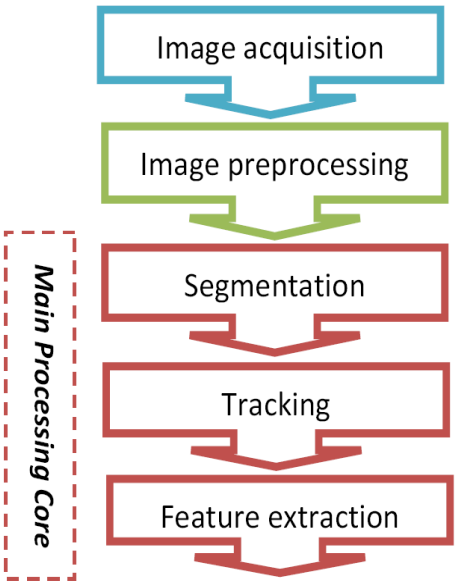

Fig. 2: The main steps of signal/image processing tools for cell tracking

3.2 Segmentation and Object Detection

After employing a series of image-processing methods for improving the quality of raw in preprocessing step, we can go toward the main image processing goals in cell tracking, i.e., segmentations, tracking and feature extraction. Segmentation refers to the process of partitioning an image into meaningful segments. In this base, a new image is obtained that for each pixel a label indicating to which segment it belongs is extracted (Pham et al., 2000; Shapiro and Stockman, 2001). Fig.4. shows a simple example for segmentation. In this example that is from "Image Processing Toolbox of MATLAB" the main goal is detection of prostate cancer cell. For this reason an appropriate edge detection algorithm (Lindeberg, 2001) and basic morphology tools (Soille, 2003) are used. To detect the entire cell in this image (Fig. 4.A) we benefit from the great difference between the contrast of the object and the background. In this way, the gradient of the image is calculated and an appropriate threshold is applied to create a binary image (Fig. 4.B). Since the obtained lines in this binary image do not quite determine the outline of the object of interest, this image is dilated by linear structuring elements (morphology operators) (Fig. 4.C). In the next step, the interior gaps are filled using another morphology operator (Fig. 4.D). Finally the additional connected objects in the border are removed (Fig. 4.E) and the object is smoothed by eroding the image (Fig. 4.F).

In cell tracking, we must separate cells from the background. As we explained in Fig. 4, we can choose a single threshold, compare the intensity with it, and label the value under the threshold as background (Meijering et al., 2009). However, "intensity thresholding" methods fail when cells are not well separated and their intensities don't differ adequately and consistently from the background (Meijering et al., 2008). Up to now many methods have been reported to overcome to this limitation (Meijering et al., 2009; Meijering et al., 2008; Kachouie et al., 2006; Wählby et al., 2004; Zimmer et al., 2004; Padfield et al., 2009).

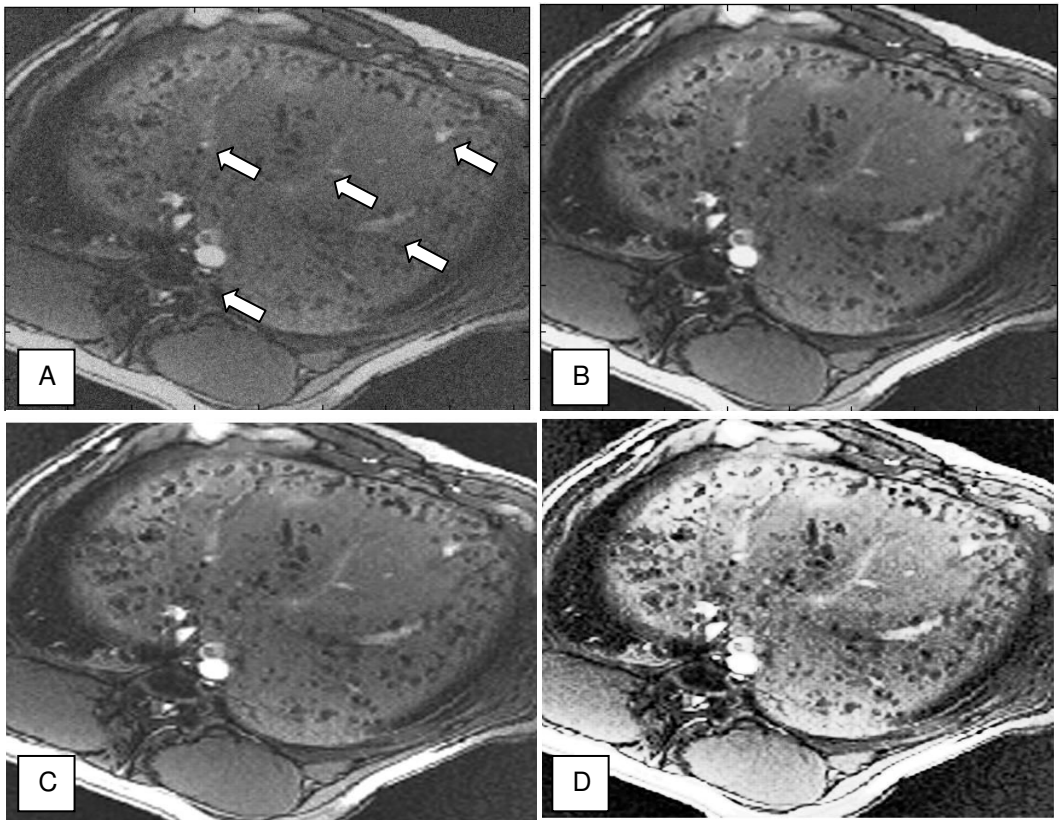

Fig. 3: Examples of image preprocessing which have been applied in *in vivo* cell tracking.
*In vivo MR images obtained 5 minutes after intraportal infusion of human pancreatic islets cells encapsulated into ferumoxides-labeled semipermeable alginate capsules shows them as hypointense cavities throughout liver (**A**). The same picture after using proposed denoising method in (Rabbani, 2009) (**B**). Image restoration and deblurring (Bates et al., 1986; Andrews et al., 1977) of the same image (**C**). Application of image equalization for contrast enhancement using Image Histogram and Intensity Adjustment Demo in MATLAB (MATrix LABratory) environment (http://www.mathworks.com) (**D**). The original image has been reprinted from (Bulte, 2009; Barnett et al., 2007).*

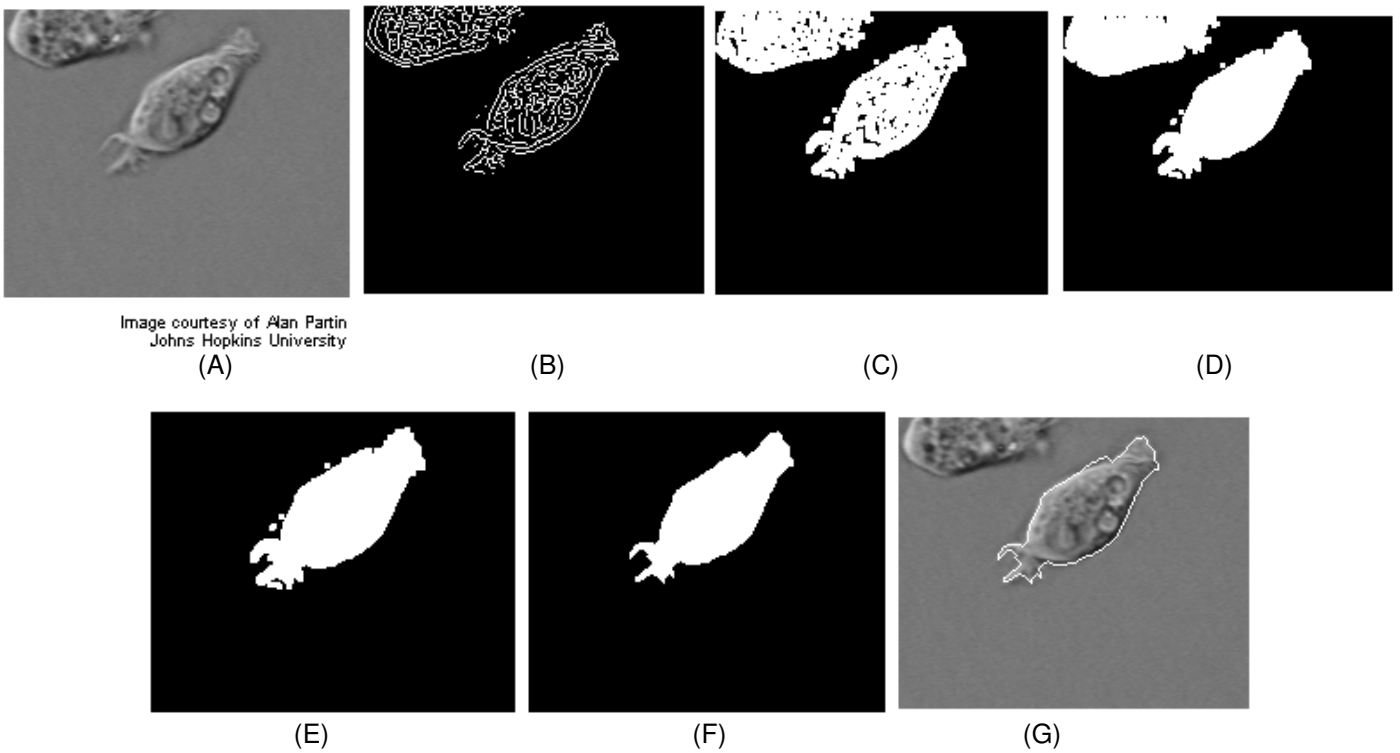

Fig. 4: An example for image segmentation from Image Processing Toolbox of MATLAB.
*The original image **(A).** Binary gradient mask image **(B).** Dilated image **(C).** Filled holes image **(D).** Cleared border image **(E).** Segmented image **(F).** Displaying the segmented object by placing an outline around the segmented cell **(G)** (http://www.mathworks.com).*

For example, in “template matching” technique (Kachouie et al., 2006), a predefined cell intensity profile is chosen as a template and the cells are extracted by comparing and matching this template against the image. However, the shape of many cells changes in successive sequences and different templates are needed for this technique (Wählby et al., 2004). “Deformable models” (Zimmer et al., 2004; Padfield et al., 2009) give us the power to capture topological deformations, such as cell division. Deformable models are based on minimization of an energy function that iteratively improves the segmentation process in each step (Zimmer et al., 2004; Padfield et al., 2009). Fig. 5 compares the results of segmentation by thresholding and deformable models (Meijering et al., 2009).

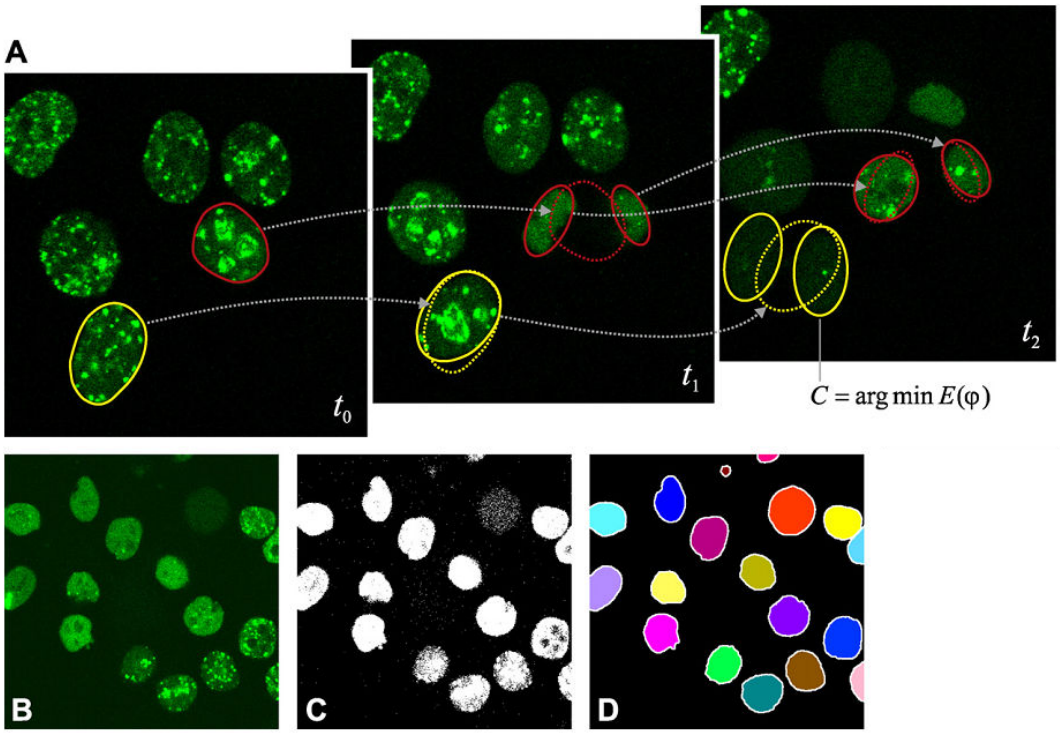

Fig. 5: A comparison between the results of segmentation by thresholding and deformable models.

*Three frames of time-lapse fluorescence microscopy image (**A**). The appearance of nuclei in one frame (**B**). The results of thresholding method (choosing at low thresholds results in very noisy results and choosing or high thresholds results in fragmented segmentation) (**C**). Results of model-based segmentation (**D**) (Meijering et al., 2009).*

3.3 Cell Tracking and Feature Extraction

After segmentation, we must link the segmented cells from frame to frame in the image sequence to obtain cell trajectories. For this reason, we must associate each cell in any frame to the “nearest” cell in the next frame. Note that “nearest” include similarity in any appropriate “feature” such as intensity, perimeter or surface, orientation, boundary curvature, estimated displacement, or a combination of these features (Debeir et al., 2005; Chen et al., 2009; Wilson and Theriot, 2006; Matula et al., 2006; Yang et al., 2008; Ray and Acton, 2004; Xie et al., 2009; Cui et al., 2006).

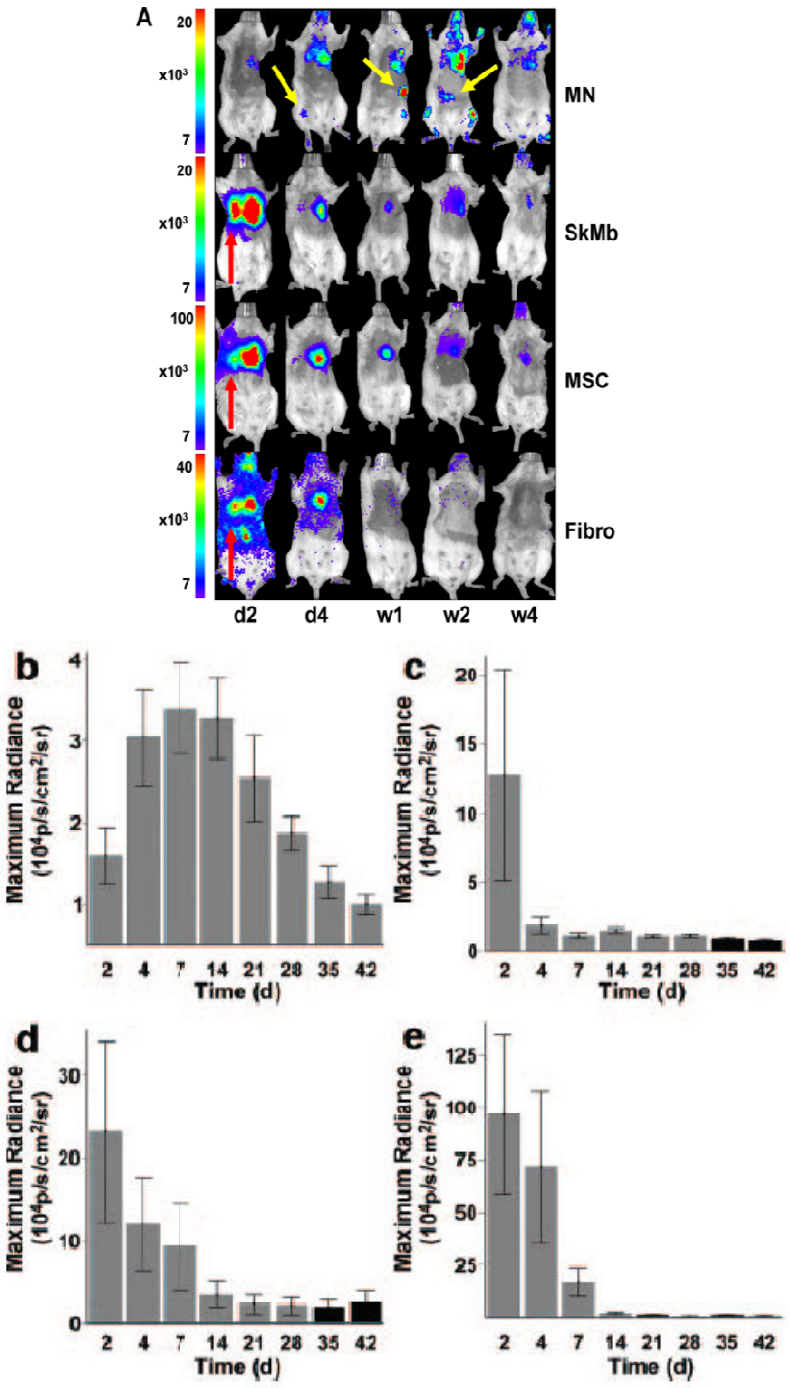

Fig. 6: Longitudinal in vivo optical BLI of several cell type injected to regenerate ischemic myocard (A). Quantification of signals (p/s) over fixed regions of interest (ROI) of each images from the same representative animal from each group reveal cell proliferation, death, and migration (b-e). The original images have been reprinted from (van der Bogt, 2008).

Usually proposed image processing methods for cell tracking are more complicated comparing to conventional tracking methods. For example, as we can see in Fig. 5, the proposed cell tracking method must be able to perform properly in the presence of cell deformation and cell division. Note that although tracking has a specific definition in the field of electrical and computer engineering, however the cell tracking can be refer to extraction of any information (useful features) about cell living including what is in them, around them, and between them, their viability and proliferation, their signaling, influencing, stimulating, inhibiting, and about cell differentiation, and migration.

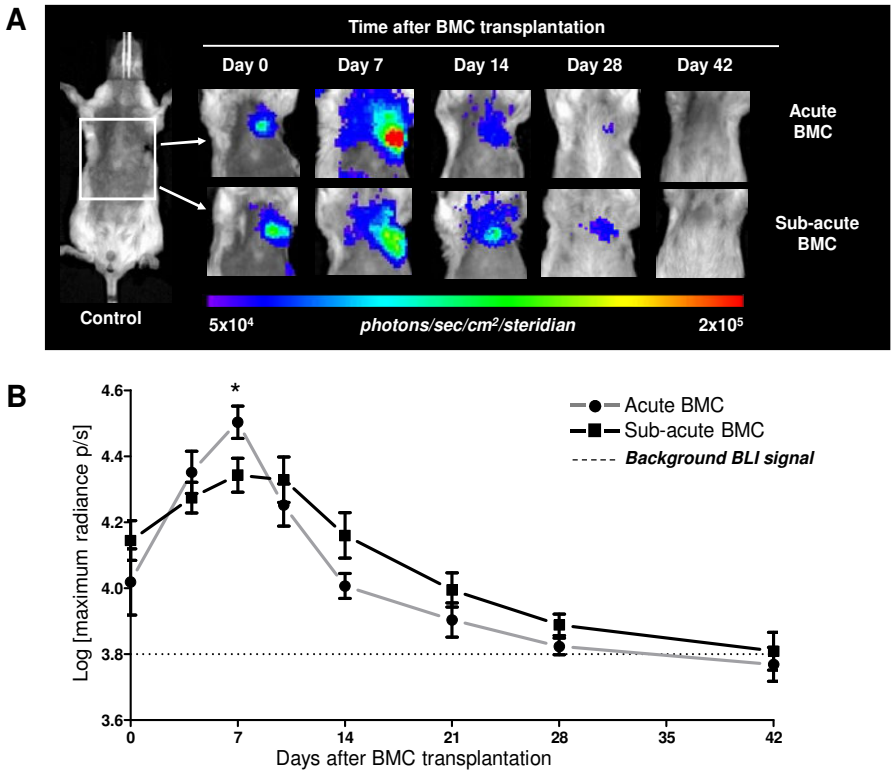

Fig. 7: Use of BLI imaging modality for longitudinal in vivo tracking of transplanted BMCs.

*BMC transplanted animals either acutely (upper panels) or 7 days after MI (lower panels) (**A**). In both groups the BLI signal increased till 10 days showing proliferation of the cells early after Transplantation and then, the BLI signal decreases progressively over time to reach background levels at day 42. Color scale bar values are in photons/s/cm2/sr (**B**). The original images have been reprinted from (Swijnenburg et al., 2010).*

However most of the above mentioned techniques have been used in *in vitro* studies and these features are hard to measure in *in vivo* settings, so, the most common used feature is cell labeling with some contrast generating agent relevant to imaging modality. One of the most common of such methods is cell labeling with Luciferin and imaging by bioluminescence technique. In this imaging modality the main tracking method is color-based image processing techniques. There are several studies which have used bioluminescence signal intensity as a feature to probe the injected stem cell viability,

migration and survival and several other questions. For example, van der Bogt et al (van der Bogt, 2008) have used this method to find the best stem cell type and best root of delivery for the treatment of myocardial infarction (Fig. 6). In another study Swijnenburg et al (Swijnenburg et al., 2010) also used this feature to find the best timing of bone marrow stem cell delivery for the treatment of myocardial infarction (Fig. 7). Table 1 has illustrated a collection of in vivo stem cell tracking studies. In addition to several small-animal imaging studies have been done to develop and test new molecular imaging methods there are few studies that stem cell labeling have been used in human subjects, such as Schächinger et al study [5]. They infused ^{111}In-oxine–labeled proangiogenic cells to patients after myocardial infarction to determine the amount of proangiogenic progenitor cells retained in the heart and homing of progenitor cells to the myocardium.

4. CONCLUSION AND PERSPECTIVES

In conclusion, imaging can be used as a noninvasive screening tool for stem cells based therapies. Outcome measures of therapeutic efficacy, including adverse effects can be determined noninvasively as well. Because of the tumorigenic potential of stem cells, tracking them in vivo is of high importance for future clinical application (Amjadi et al., 2011). Whole-body imaging with several imaging modalities allows the testing of new therapies in relevant disease models to study the safety and efficacy with outcome measures of stem cells in future clinical trials. To make stem cells based therapies safe, a good solution can be to use a reporter–suicide gene mechanism that would allow for the in vivo tracking of the transplanted pluripotent cells and would target these cells for removal in the case of tumor formation.

Table 1: A collection of *in vivo* stem cell tracking studies

Study	Imaging modalities	Research question
Swijnenburg et al. (Swijnenburg et al., 2010)	Bioluminescence imaging	Viability, timing of SC delivery
Gyöngyösi et al. (Gyöngyösi et al., 2008)	PET–reporter gene PET-CT	the engraftment and spatial migration of SCs in vivo following intracoronary or intramyocardial injection
Blackwood et al. (Blackwood et al., 2009)	SPECT	cell viability
Li et al (Li et al., 2009)	luciferase reporter gene Bioluminescence imaging	Route of delivery biodistribution and myocardial retention of SCs
Sheikh et al. (Sheikh et al., 2007)	Bioluminescence imaging	cell migration and survival in vivo for 4 weeks
Wisenberg et al. (Wisenberg et al., 2009)	SPECT (^{111}Indium) and late gadolinium enhancement cardiovascular magnetic resonance	cell engraftment and biological clearance of cells from the injection site, concurrent measurement of infarct evolution and remodeling

It is unclear whether the different stem cell labeling techniques are safe and can be applied straightforwardly to clinic. There are several opportunities of genetic modification in animals which enable us to examine the response to specific interventions in stem cell research. However, there are serious challenges for translation of the results of animal studies to the clinical arena. It seems that development of new image processing strategies provides a creative platform for finding more rigorous and reliable answers for stem cell research field open questions.

Currently, although advanced image acquisition devices contain interfaces for users performing mentioned image processing steps (preprocessing, segmenting individual cells, linking cells over time, and feature extraction), development of new protocols of image processing will shed more light on stem cell clinical research questions and may solve some safety concerns for using these potent therapeutic intervention (For example instead of only using colour as the main feature for tracking, other features such as explained features in section 3.3 can be used).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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