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COMMENTARY

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Current challenges for the targeted delivery and molecular imaging of stem cells in animal models

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ABSTRACT

In contrast to conventional, molecular medicine that focuses on targeting specific pathways, stem cell therapy aims to perturb many related mechanisms in order to derive therapeutic benefit. This emerging modality is inherently complex due to the variety of cell types that can be used, delivery approaches that need to be optimized in order to target the cellular therapeutic to specific sites *in vivo*, and non-invasive imaging methods that are needed to monitor cell fate. This review highlights advancements in the field, with focus on recent publications that use preclinical animal models for cardiovascular stem cell therapy. It highlights studies where cell adhesion engineering (CAE) has been used to functionalize stem cells to home them to sites of therapy, much like peripheral blood neutrophils. It also describes the current state of molecular imaging approaches that aim to non-invasively track the spatio-temporal pattern of stem cell delivery in living subjects.

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Introduction

Stem cell therapy is fundamentally different from the conventional cornerstones of modern medicine that rely upon surgery or pharmaceutical therapy. Surgery treats physical ailments (e.g. inflamed appendix) and accompanying symptoms (abdominal pain) through "physical" tissue level resection (appendectomy) and/or reconstruction. Both the responses (wound-healing, regeneration) and known complications (coagulopathy, infections) can be anticipated based on a priori knowledge. Similarly, pharmaceuticals target specific receptors or pathways, with the goal of improving disease outcome. Here, predictive, pharmacokinetic models enable both the design of the therapeutic and its application regimen. In contrast to these conventional approaches that target specific processes using exogenous means, the field of cellular therapeutics aims to introduce stem cells to function as 'catalysts' that may either replace injured tissue or accentuate the endogenous repair mechanisms already at work in living organisms.[1,2] Here, the molecular target is not one receptor or a localized feature. Instead, it is a series of related molecular processes and associated, heterogenous cell types that aim to increase tissue mass, augment differentiation, stimulate endogenous repair, establish supportive tissue regeneration (e.g., angiogenesis), and/or reduce inflammation. Validating this concept, numerous studies conducted in animals support the promise of this approach, with evidence that stem cells may either undergo differentiation[3] or secrete paracrine factors that enhance tissue repair and functional outcome.[4]

The clinical application of stem cells has reached center stage. In the last 15 years, both adult and pluripotent-derived stem cells have proceeded through preclinical models, paving the way for commercialization, and motivating numerous clinical trials[5]. However, the successes of clinical studies have been scant, with successful clinical transplantation only being reported recently.[6] While much effort has been focused on techniques for stem cells derivation, characterization, and cultivation, future success may hinge upon two less-appreciated areas of investigation:

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targeted delivery of therapeutic stem cell and noninvasive molecular imaging. This commentary reviews these two emerging areas, with focus on recent advances in cell delivery strategies[7] and imaging.[8-10]

Targeted delivery of stem cells

Methods for the delivery of stem cells are broadly classified into: i. local methods where cells are directly injected into the damaged tissue; and ii. systemic methods that use intra-venous (i.v.), intra-arterial (i.a.) or intra-coronary (i.c.) infusion to introduce the stem cells. Both methods result in relatively low levels of engraftment with only 1–2% of the cells being retained.[11] Although direct cell delivery to a focal area of tissue damage could be beneficial, poor vascularization leading to oxygen depletion, high cellular nutrient demand at sites of injury, along with the risk of tissue perforation likely limit the utility of this approach. Thus, there is growing interest in developing targeted, systemic cell injection techniques (Table 1). In this regard, i.v. injection introduces all therapeutic cells to the right-side of the heart, and this can lead to cell trapping, and retention within non-targeted lung alveolar capillaries.[12] Arterial injections may offer more utility, but non-targeted delivery may simply result in passive entrapment within arterial microvasculature without extravasation.

Engineering of the cell delivery pathway should ideally target the well-vascularized, viable, but compromised, tissue that immediately surrounds the area of damage in specific organs. Since these vulnerable regions are often inflamed and susceptible to neutrophil recruitment, a number of laboratories have hypothesized that modifying stem cell surface adhesion molecules to more closely resemble peripheral-blood neutrophils may enable the efficient, systemic delivery of therapeutic cells. Broadly, this approach is termed 'Cell Adhesion Engineering' (CAE). With respect to this, while early studies suggested that mesenchymal stem cells (MSCs) may constitutively express the P-selectin ligands and VLA-4 that are necessary to target these regions,[13] such expression may not be robust since the MSCs are a heterogeneous cell type and their surface markers may change during *in-vitro* propagation.[14] Thus, there is a need to engineer simple but robust cell surface modifications to enable leukocyte-like stem cell capture. Key challenges in the field include:

Glycoengineering selectin-ligands on stem cells: The blood neutrophils are captured from flow when sialofucosylated carbohydrates expressed on their cell-surface bind E- and P-selectin expressed on the inflamed endothelium. These glycans are commonly decorated by α(2,3)sialic acid and α(1,3)fucose on Type-II lactosamine chains, with sialyl Lewis-X (sLe^X) representing a prototypic selectin-ligand. In mammals, such structures can be synthesized by various glycosyltransferases including the α(1,3)fucosyltransferases FUT3-FUT7 and FUT9, and the α(2,3)sialyltransferase ST3Gal-4 and ST3Gal-6.[15] Among these, the enzymes responsible for selectin-ligand biosynthesis in human neutrophils are FUT4, 7 and 9,[16] and ST3Gal-4.[17]

Stem cells often lack the robust expression of α(1,3) fucosylated glycans. Thus, Xia et al.[18] enforced fucosylation and sLe^X expression on these cells, more specifically the human umbilical cord blood $CD34^+$ cells, using the exogenous FUT6 along with the donor GDP-fucose (guanosine diphosphate-fucose). This modification enabled $CD34^+$ cell rolling on P- and E-selectin at a shear stress of 0.5 dyn/cm^2 *in-vitro*. Transplantation of these modified cells into immunodeficient mice also enhanced cell engraftment. Sackstein et al.[14] extended this approach to MSCs and demonstrated that such α(1,3)fucosylation can enhance targeting of MSCs to the bone, since marrow vessels constitutively express E-selectin.

The high on- and off-rates of the physiological selectin-ligand bond are a key for the capture of cells from free flowing blood.[19] Here, the expression of the sLe^X glycan alone in the absence of the physological glycoprotein scaffold(s) results in low affinity cellular interactions and only rapid rolling.[20] Based on this, Lo et al.[21] engineered HEK (human embryonic kidney) cells for the high-level expression of a fusion protein 19Fc[FUT7+] (originally described in[22]), where the functional end of the P-selectin glycoprotein ligand-1 (PSGL-1, CD162) was fused to a human IgG1 tail. The protein was non-covalently and transiently coupled to MSC surface using palmitated-protein G chemistry.[23] Such cell-surface PSGL-1 modification resulted in robust cell recruitment on substrates bearing P-selectin. Extending this approach, more recently, these authors showed that α(1,3)fucosylation of MSCs more prominently enhanced E-selectin recognition rather than P-selectin binding.[7] Combining the PSGL-1 and fucosylation strategies thus resulted in robust binding on both selectin-types in *in vitro* flow

Table 1. Effect of stem cell modifications on *in vivo* targeted delivery.

Experiment	Modification	Key finding	Citation
Rat BM-MSC transfused into the left ventricular cavity of MI rats	No modification	1% of cells migrate to the infarcted myocardium at 4 h with significant retention in lung	12
Murine MSC-like cells were injected into the tail vein of 4-mo-old mice	Overexpression of CXCR4 on MSCs through adenovirus infection	~8 fold increase in retention to bone marrow	35
Murine MSCs were intramyocardially injected in mice with myocardial infarction	Overexpression of CCR-1 chemokine receptor on MSCs	Increase in MSC survival, migration, and engraftment in ischemic myocardium	36
Rat MSCs were intravenously infused into tail vain of myocardial infarcted rat	Overexpression of CXCR4 on MSCs	2.5-fold increase engraftment to the infarcted myocardium, leading to reduced LV remodeling and enhanced recovery of function	37
Human and rat GRPs and MSCs were transplanted into the internal carotid artery of rats	Altering cell size, cell dose, and cell infusion velocity	Stroke at infusion velocity over 1 ml/min, profound decrease in cerebral blood flow for large cells infusion, stroke lesions for dosage injection more than 1×10^6	38
Primary human MSCs were injected into the tail vein of an inflamed model of mice.	Immobilization of SLe^x on MSC surface using prior surface immobilization of biotin and streptavidin	56% efficiency increase in cell localization to the inflamed ear	20
Human umbilical cord blood cells were injected intravenously into sublethally irradiated immunodeficient (NOD/SCID) mice	Enforced α(1,3)fucosylation and SLe^x expression on CB cells surface	Enhanced selectin binding and bone marrow engraftment of CB cells in irradiated NOD/SCID mice	18
Human MSCs were intravenously infused into the tail veins of immunodeficient (NOD/SCID) mice	Enforced α(1,3)fucosylation and SLe^x expression on MSCs surface	Robust tethering and rolling interactions and firm adherence of cells on sinusoidal vessels and rapid infiltration to the marrow parenchyma	14
Murine MSCs were injected into the mice with inflammatory bowel disease	Coating MSCs with VCAM-1 antibody using protein G	Highest delivery efficiency to inflamed mesenteric lymph node	39
Lin- Sca+ murine stem cells were intravenously injected into mice with infarcts created by ligation of LAD	Cells modified with bispecific antibodies against murine stem cell c-kit and VCAM-1 up-regulated on injured myocardial cells	Increased retention to injured myocardium	40
Human HSC intravenously injected into the xenogeneic rat model with ischemic injury induced by transient ligation LAD	Decorating HSCs with Bispecific antibodies that binds human CD45 and myosin light chain, an organ-specific injury antigen expressed by infarcted myocardium	Enhanced cell homing to myocardial infarcted tissue	41
Human MSCs intra-ventricularly injected through the left ventricle of mice with myocardial infarction	Coating MSCs with palmitated derivatives of phage-peptides (CRPPR, CRKDKC, KSTRKS, and CARSKNKDC)	Increased binding to infarcted regions	42
Swine CDC and MSC intracoronary infused into the brief cardiac IR injury swine model	Coupling CDCs and MSCs with 19Fc[FUT7+] plus FUT7 over-expression in the cells	28% of cells localized in LAD proximal to IR site	7

Abbreviations: BM-MSC: Bone Marrow-derived Mesenchymal Stem cells; MI: Myocardial Infarction; MSC: Mesenchymal stem cells; LV: Left ventricle; GRP: Glial restricted precursors; NOD/SCID: Nonobese diabetic/ sever combined immunodeficient; VCAM: vascular cell adhesion molecule; AD: Left anterior descending; HSC: haematopoietic stem cells; CDC: Cardiosphere derived cells; IR: Ischemia reperfusion.

chamber studies (Fig. 1). Glycoengineering of stem cells using both strategies also enabled short-term retention of 28% of the cells in the left anterior descending artery of the pig heart in a brief ischemia-reperfusion model.[7] In addition to the demonstration of stem cell delivery, the study provides promising data suggesting that engineered stem cells may be safe for cardiovascular applications.

Optimizing conditions for the capture of stem cells from free flowing blood: Mesenchymal stem cells are often larger (20–25μm) than peripheral blood neutrophils (10–15μm). The fluid drag force that must be overcome in order to capture these cells from flow varies approximately as a square of the cell radius. Thus, the drag force applied on stem cells is ~3.2 (=[22.5/12.5]^2) fold higher than that applied on neutrophils. Additionally, while neutrophils are captured in the post-capillary venules where the wall shear stresses are low at 0–3 dyn/cm^3, the engineered adhesion molecules on stem cells may have to be designed to overcome higher stresses at sites of inflammation in arteries. For these reasons, in addition to examining the simple rolling of stem cells on the endothelium, studies that aim to deliver stem cells following systemic injection must also emphasize cell adhesion engineering (CAE) methods that enhance cell recruitment or capture onto the endothelium. Beyond mimicking neutrophils, in the long run, it may also be

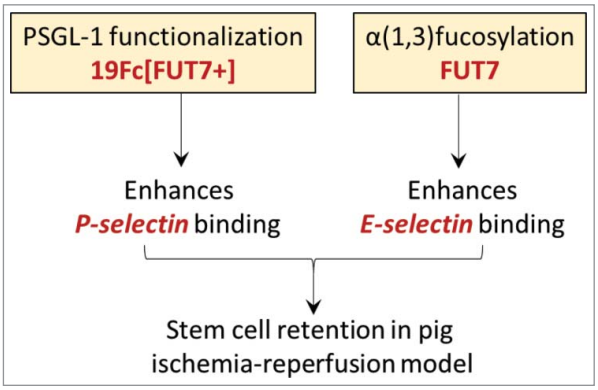

Figure 1. Complementary glycoengineering methods to enhance stem cell delivery. Coupling the recombinant PSGL-1 protein (19Fc[FUT7+]) to stem cell surface enhances cell binding to P-selectin. Overexpression of the α(1,3)fucosyltransferase FUT7, on the other hand, enhances cell binding to E-selectin. CDCs functionalized with both modifications were retained in the pig heart in a brief ischemia-reperfusion model (ref. [7]).

necessary to identify additional stem-cell specific sialofucosylated glycoconjugates that can enhance cell capture. While CD44 has been implicated to be one of the stem cell glycoproteins that is prominently sialofucosylated to display the HCELL epitope,[14] it is necessary to also identify other players that may be similarly modified.

Optimizing conditions for stem cell transmigration across the endothelium: Besides selectins, stem cells express a variety of endogenous chemokine and growth factor receptors that aid the activation of cell surface integrins and enhance cell homing to sites of inflammation and injury.[11] Depending on the source of the cells, this includes chemokine receptors that bind SDF-1 (stromal cell-derived factor-1, CXCL12), MIP (Macrophage inflammatory protein, CCL3/4) and RANTES (Regulated on activation, normal T cell expressed and secreted, CCL5), and growth factor receptors that bind FGF (fibroblast growth factor), PDGF (platelet-derived growth factor) and VEGF (vascular endothelial growth factor). Expansion of stem cells can down-regulate many of these homing molecules in culture and thus methods to over-express these using non-viral techniques is of current interest. Among these, a large emphasis is on the receptor CXCR4 as it binds SDF-1 since numerous publications illustrate the importance of this binding on stem cell homing.[24] Besides these, metalloproteinases (MMP-2 and MT1-MMP) may also play a role in degrading extracellular matrix components to enhance cell migration to sites of injury.

Molecular imaging of stem cells

Stem cell imaging is an emerging sub-topic within molecular imaging, a field where biological processes are visualized and quantified in living subjects.[25] Current approaches for monitoring stem cells *in vivo* are commonly destructive and not quantitative, and they involve the use of methods like RT-PCR, immunohistochemistry, and fluorescence in situ hybridization.[26] The inability to perform longitudinal studies that track cell number, location, and differentiation state *in vivo*, repeatedly, reduces the ability to relate cell *in vivo* fate to tissue regeneration, and our understanding of inter-individual variability. To address this major limitation, more recent investigations have attempted serial non-invasive cell imaging in living subjects. A comparison of these approaches follows.

Cell prelabeling: Cell prelabeling, consists of intracellular loading of imaging agent (molecules or nanoparticles) which enhance imaging sensitivity. This technique, which can be used in conjunction with SPECT (single photon emission computed tomography), PET (positron emission tomography) and MRI (magnetic resonance imaging), boosts imaging sensitivity because large amounts (μg to mg) of imaging agents can be specifically loaded into cells *ex vivo* prior to introduction into animals. The main issue here is cellular toxicity and dilution of imaging-probe mass over time due to cell proliferation *in vivo*. Cell prelabeling during SPECT can be accomplished with ^{111}In oxine or [^{111}In] Indium oxinate$_3$, in which the ^{111}In isotope of Indium complexes with the chelator 8-hydroxy quinolone.[27] This chelator is subsequently released after intracellular entry. This technique is used in patients for imaging whole body leukocyte migration for fever of unknown origin. A minimum of 2×10^8 cells are labeled for adequate visualization, probe half-life is 2.8 d, and spatial resolution is ~10 mm (Table 2, [27]).

During PET, positron-emitting ^{18}F-fluorodeoxyglucose (^{18}F-FDG) is commonly used to prelabel cells. PET has been used to image stem cells in mice.[28,18]^{18}F has a half live of 110 minutes, shorter than [^{111}In], but PET is an order of magnitude more sensitive than SPECT (10^{-12} M Molar (M) vs. 10^{-11} M), and thus can detect fewer numbers of cells with the same mass of prelabel (Table 2).

Nanoparticle (30–100nm) (NP) based cell prelabeling use quantum dots, silica, polymer-based, gold or

Table 2. Comparison of cell prelabeling versus reporter gene for all major imaging modalities.

Label	Modality	Cell sensitivity (Small animal)	Cell sensitivity (Large animal)	Advantages	Disadvantages
Prelabeling					
Near Infrared dye	IVM, FMT	1×10^{0} [31]	n/a	high sensitivity(10^{-17}M) high spatial resolution (<1 μm) (IVM) inexpensive multiplex capability improved depth penetration (FMT)	low depth penetration (IVM) limited clinical use invasive procedure (IVM) loss of signal with depth
Indium oxine	SPECT	6×10^{5} [43]	1×10^{7} [44]	high sensitivity (10^{-11}M) ease of use multiplex capability	label dilution radioactive dose signal decay planar (not tomographic) low spatial resolution (2–10mm)
FDG	PET	5×10^{4} [28]	3×10^{7} [45]	high sensitivity (10^{-12}M) tomographic natural molecule labeling quantitative	label dilution radioactive dose signal decay cyclotron required expensive low spatial resolution (2–10mm)
SPIO NP	MRI	2×10^{2} [46]	1.5×10^{7} [10]	high spatial resolution improved sensitivity no radioactivity	label dilution toxicity low sensitivity (10^{-12}M, MR) highly sequence dependent many imaging artifacts negative contrast method semiquantitative
Reporter Genes					
GFP	IVM	1×10^{0} [31]	n/a	high sensitivity (10^{-17}M) high spatial resolution (<1 μm) inexpensive multiplex capability serial imaging	low depth penetration limited clinical use invasive procedure loss of signal with depth
Firefly Luciferase	BLI	1×10^{3} [9]	n/a	high sensitivity (10^{-17}M) inexpensive multiplex capability (Rluc) serial imaging	low spatial resolution loss of signal with depth low light cooled CCD required
HSV1TK/SR39TK 18F-FHBG	PET	1×10^{7} [47]	2.5×10^{8} [10]	high sensitivity (10^{-12}M) tomographic serial imaging clinically approved	radioactive dose signal decay cyclotron required expensive

Abbreviations: IVM: intravital microsopy; FMT: fluorescence molecular tomography; SPECT Single photon CT; PET: Positron emission tomography; MRI: Magnetic resonance imaging; BLI: Bioluminescence; SPIO: Superparamagnetic Iron Oxide Particles; NP: nanoparticles; HSV1-TK: Herpes Simplex Virus Type I truncated thymidine kinase; SR39TK: Mutant Herpes Simplex Virus Type I truncated mutated thymidine kinase; 18F-FHBG: 18F-radiolabelled 9-[4-fluoro-3-(hydroxyl methyl) butyl] guanine

superparamagnetic (SPIO) particles for imaging with fluorescence, PET, SPECT, photoacoustics and magnetic resonance imaging (MRI). Among these, prelabeling with SPIO NP (MRI) is an established, clinically approved technique that has been used to monitor stem cell delivery.[29] Here, SPIO NP mass, strength of magnetic field, signal to noise ratio, pulse sequence, and acquisition parameters all affect imaging quality and sensitivity.[30] Using SPIO NP and a 3T MRI for cardiac imaging, Parashurama et al.[9] demonstrate that a minimum of 1.5×10^{7} MSCs are needed for large animal imaging. Here, only a fraction (~20–30%) of the infused MSCs, rather than all MSCs, were SPIO NP loaded and this reduced overall toxicity. With this technique, the authors analyzed MRI data across the cardiac cycle, rather than in a single frame, leading to a linear relationship between signal and cell number. Because of the dilution of the prelabel with cell division, the above techniques are valuable for obtaining sensitive images for initial stem cell localization studies, for a duration of hours to days.

Optical Reporter gene (RG) imaging: Here, a reporter gene (RG) is stably expressed in cells. An imaging probe is then introduced which interacts with the RG to produce signal. Due to this, RG imaging signal sensitivity depends on reporter levels in cells, the number of cells expressing the RG, probe transport to the reporter, the strength of the signal generated, probe toxicity and safety, and the physics of the imaging device. In this case, as the RG is stably expressed, the imaging signal is constant rather than being diluted with cell division and can be repeated indefinitely. Promoter silencing is one process that can prevent long term, serial imaging. A short-half RG half-life (e.g. hours) is requisite so that the measured imaging signal reflects changes in reporter gene promoter levels. Here, in a prototypic example, green fluorescent protein (GFP) has been used for serial, intravital, stem cell imaging.[31,32] However, the tissue depth is limited (~150 microns) and this is not useful for whole body imaging due to visible-light absorption, scattering, and high background. Bioluminescent RG imaging using firefly luciferase (Fluc), on the other hand, enables highly sensitive, whole body imaging although the resulting optical signal also varies linearly with depth. Here, as little as 1000 MSCs can be detected in small animals (mouse) following localized subcutaneous injection, with the measured signal varying linearly with cell number.[9] Interestingly, the measured bioluminescence signal persisted longer in the injured

compared to the normal myocardium, suggesting fundamentally different MSC cell fates in these two environments.[9] In addition to monitoring a single parameter, the use of two luciferase RGs (Fluc and Renilla(Rluc)) can enable multiplexing *in vivo* by usage of substrates specific to each of the luciferases. Using this approach, Ahn et al. engineered pluripotent stem cells co-expressing constitutively-active Fluc along with Rluc driven by Oct4/differentiation promoter.[8] Stem cell proliferation and differentiation fates could then be independently monitored following local subcutaneous injection. In contrast to *in vitro* imaging data, the initial decrease in Rluc/Fluc signal was followed by an increase indicating complex stem cell regulatory mechanisms *in vivo*. Unfortunately, neither GFP nor bioluminescence studies are feasible in large animals due to lack of signal strength at greater tissue depths.

PET RG Imaging: PET RG imaging involves expressing a genetically encoded PET reporter gene (HSV1TK or its mutant SR39TK) in the transplanted stem cells. These RG expressing stem cells are then detected using the PET reporter probe (18F-FHBG (18F-radiolabelled 9-[4-fluoro-3-(hydroxyl methyl) butyl] guanine).[33] Here, the PET RG in the stem cells selectively phosphorylates the PET reporter probe and traps it intracellularly, leading to a detectable signal. Only trace doses of the PET reporter probe are infused in order to limit patient exposure to radioactivity. Because the probe distributes throughout the body, less than 1% of the injected probe actually accumulates in the cells of interest. Due to this, in the first limit of detection study in larger animals, when different concentrations of MSCs expressing the PET-RG SR39TK were locally injected into the porcine left ventricle, a minimum of $\sim 2.5 \times 10^8$ cells were required for 18F-FHBG PET signal detection.[10] As in previous studies, a number of parameters affect the imaging signal including PET RG expression levels, vascularity of target organ, cardiac motion, and animal fluid status. Thus, while PET is a powerful clinical imaging modality, additional improvements are necessary before the routine use of PET RG in stem cell based studies.

Multimodality molecular imaging: Since each imaging modality has its pros and cons (Table 2), it would be beneficial to combine complementary approaches by developing multimodal methods. For example, combining PET RG imaging (high sensitivity) with MRI (high spatial resolution) is a simple approach to improving visualization of stem cell therapies. In this case, the stem cell signal, derived from PET RG imaging, may be visualized in relationship to relevant, <10 μm, anatomical structures highlighted by MRI. Thus, imaging of the vascular endothelium with MRI can improve the ability to engineer and visualize cell delivery, and MR imaging of border tissue near myocardial infarction can help visualize stem cell migration and subsequent tissue repair. If the PET RG-expressing stem cells are also prelabeled with SPIO NP, then further benefit is gained from a multimodality perspective.[10] Here, SPIO prelabeling can enable initial localization and validation of PET signals, and the PET signal can also validate the MR images of prelabeled cells. Further, because injections can be validated with two modalities, this approach strengthens the ability to track two injections in two locations in the same animal, potentially with two different cell numbers or two different RGs, independently. Other modalities can also be combined within a single study for benefit.[10]

Conclusion

Stem cell therapies represent a new and exciting approach for treating major health problems like ischemic heart disease. However, the results of clinical studies have been mixed. This highlights the need to better understand tissue specific stem cell differentiation mechanisms, delivery methods and imaging techniques.

In the case of cardiac cell therapy, preclinical data demonstrates that several cell types, including unfractionated bone marrow cells, bone marrow stromal cells (MSC), cardiac stem/progenitor cells (C-kit positive or Sca-1 positive), pluripotent stem cell-derived progenitors are all potential therapeutic candidates.[34] The question naturally arises, which one cell type should be used therapeutically and/or how can they be used synergistically?

A vast literature has appeared on the mechanisms by which stem cells naturally home to sites of injury. These studies highlight caveats in the culture procedure of these cells that may alter the natural repertoire of homing receptors. To address this shortcoming, recent studies have begun to use 'cell adhesion engineering' approaches to accentuate the natural homing properties of the stem cells by glycoengineering selectin-ligands on their surface and also decorating chemokine

receptors that aim to augment the natural tropism of these cells. Further studies may enable the improvement of such delivery strategies, and related simplifications that are necessary for clinical applications.

Both prelabeling and RG imaging enable noninvasive monitoring of stem cell fate. While prelabeling is sensitive, it is not ideal for long-term studies that last weeks to months. Reporter genes are more suitable for long term studies though improvements in sensitivity and spatial resolution are necessary. Further advancements in nanoparticle based prelabeling methods, RG design for PET and multimodal imaging may facilitate longitudinal stem cells studies, particularly in large animals. In the future, a combination of these advanced imaging modalities with the targeted cell adhesion engineering approach may pave the way for more robust validation of basic science concepts in large animals and their successful translation to humans.

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