

Understanding Stem Cell-Mediated Brain Repair Through Neuroimaging

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Abstract: Transplantation of stem cells into the damaged brain can lead to behavioral recovery. However, at present, the mechanisms by which these cells exert their beneficial effects are still poorly understood. Survival, migration and differentiation are but a few of the factors that are thought to be involved in stem cell-mediated brain repair. It is hoped that neuroimaging, by MRI and PET, will provide serial *in vivo* assessments of transplanted cells that can lead to a greater understanding of the mechanisms involved in brain repair.

Stem cell therapy holds great promise to remedy neurological disorders [1]. The ability to migrate to distinct areas of damage and to integrate into diffuse pathologies, whilst having the ability to differentiate into site-appropriate phenotypes are thought to be crucial for functional repair. The functional significance of the repair and its relevance to clinical translation can only be attested by behavioral studies that probe the cells' ability to recover lost functions. Although behavioral improvement must be the ultimate outcome measure, behavioral testing provides little information about the neural or molecular mechanisms involved in brain repair.

HOW DO NEURAL STEM CELLS PROMOTE BEHAVIORAL RECOVERY?

Until recently, most knowledge about how stem cells recover brain damage has been gained through post-mortem histological investigations. The high specificity and variety of antibodies available to probe different aspects of stem cell therapy provide an invaluable resource to gain an understanding of the differentiation of transplanted cells, cues involved in their migration, trophic factor secretion and even neuroprotection of damaged host cells. In some neurodegenerative diseases, such as Parkinson's disease, a clear pharmacological effect through neurotransmitter replacement is the main target of transplanted cells, whereas in other conditions, such as stroke, a more general replacement of a wide variety of cells is likely to be needed to achieve behavioral improvements [1]. In some cases, such as Parkinson's disease, there appears to be a dose-dependent effect of graft survival on behavior. In other cases though, behavioral recovery can be observed in rats with stroke damage with only a few cells surviving or integrating after transplantation [2, 3]. This low number of cells achieving a functional effect and the limited differentiation of mesenchymal stem cells into neuronal cells question the relationship between repair and the number of surviving/differentiating cells. Paradoxically, the inflammatory and immune response to transplanted cells is also known to lead to behavioral improvements [4] indicating that the relationship between functional repair and graft survival is not as straightforward as it is often assumed. It also remains largely unknown how additional

strategies to improve graft functioning, such as behavioral training [5], exert their anatomical effects.

All in all, the varied influences transplanted cells can exert on host tissue are still poorly characterized, but it is important to determine which elements constitute a significant contribution to functional repair. The wider neurobiological and neurodegenerative context also needs to be accounted for whilst trying to pin down the mechanisms by which transplanted cells work. It is possible that transplanted cells upregulate the proliferation and migration of endogenous stem cells, which could also support various mechanisms of recovery. Some mechanisms of recovery, such as diaschisis [6], are also often disregarded in the explanation of behavioral recovery after cell therapy, as there are no adequate histological markers that would allow a detailed investigation of these functional changes. Understanding the various mechanisms of recovery involved in functional repair will afford the development of more effective cell therapies [7, 8].

Limiting these anatomical analyses to post-mortem excised tissue, nonetheless, confines this approach to a snapshot view of the histological landscape and therefore complicates assertions as to how changes occurred or how these relate to behavioral improvements [9].

THE NECESSITY OF *IN VIVO* IMAGING TO ELUCIDATE STEM CELL-MEDIATED BRAIN REPAIR

The ability to visualize detailed anatomy repeatedly and non-invasively *in vivo* offers the opportunity to investigate how stem cell transplantation affects the damaged brain [9]. Both magnetic resonance imaging (MRI) and positron emission tomography (PET) are excellent *in vivo* imaging techniques to probe cell therapy in both preclinical models and clinical applications (Table 1). Although PET does not provide a high anatomical resolution, the specificity of PET radioligands provides an exquisite tool to investigate molecular targets relevant to transplant-mediated recovery [10]. PET ligands are especially useful to assess metabolism and receptor distribution in the brain.

In contrast, MRI can achieve a high spatial resolution in the dimension of 10 μm , but generally lacks specificity. The development of contrast agents that can increase specificity on MR images, however, promises to overcome this issue. Still, the large particle size of contrast agents (> 600 Da) for

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Table 1. Potentials and Limitations of Imaging Technology in Comparison to Immunohistochemistry

Aspects	MRI	PET	Confocal
Assessment: <i>in vivo</i>	Yes	Yes	No
<i>in vitro</i>	Yes	Yes	Yes
<i>ex vivo</i>	Yes	Yes	Yes
post-mortem	Yes	Yes	Yes
3-dimensional	Yes	Yes	Pseudo 3D
Serial assessment	Yes	Yes	No
Spatial Resolution	10-100 μm	1000 μm	1 μm
Study function	Yes	Limited	Yes
Gene Expression	Limited	Yes	Yes
Specificity	Poor	High	Very high
Changes in extra-cellular matrix	Yes	Limited	Difficult
Changes in water diffusion	Yes	Limited	No

MRI does not generally permit a crossing of the blood-brain barrier (BBB). Providing specific contrast within the brain therefore remains a great challenge. Coating of the contrast agent with BBB- or cell-penetrating peptide fragments [11] is currently actively pursued and potentially will overcome this hurdle [12]. Endogenous contrast based on the paramagnetic properties of deoxygenated blood can also be used to assess the regional activations in the brain non-invasively. This Blood Oxygenation Level Dependent (BOLD) functional MRI (fMRI) can therefore be used to assess the functional anatomy of particular behaviors [13], such as activation of the sensorimotor cortex after sensory stimulations of a hand. The same principle can also be used to determine which areas of the brain are activated by pharmacological agents [14]. This pharmacological MRI (phMRI) can determine non-invasively if a particular cell type (e.g. dopamine cells) has been lost, as no dopamine receptors would be present to produce a change in activity.

In contrast to post-mortem immunohistochemical techniques, neuroimaging can therefore provide an *in vivo* assessment of the dynamic evolution of both functional and anatomical changes, as they relate to behavior and/or the progression of pathology. To understand the dynamic and protracted recovery (over months) involved in stem cell transplantation, it will be necessary to further expand existing assessment approaches and develop novel *in vivo* imaging approaches. Neuroimaging can provide an adequate long-term assessment of the functional anatomy of the whole brain that can be related to behavioral improvements and provide a functional anatomy of graft-mediated brain repair.

CELLULAR AND MOLECULAR MR IMAGING OF TRANSPLANTED CELLS

Nonetheless, to trace transplanted cells *in vivo* poses a major challenge on neuroimaging. Although fetal tissue transplants can be detected without pre-labeling by MRI [15] due to the mass-like structure these grafts form, isolated stem cells migrate widely and integrate seamlessly into the host parenchyma [16]. Stem cell imaging therefore poses a

greater challenge to MRI than detection of other transplants. In addition to the high spatial resolution needed to visualize transplanted cells, it is also necessary to pre-label stem cells *in vitro* with MR contrast agents to allow a distinction from the host brain [17].

The pre-labeling of non-phagocytic cells also comes with its difficulties. To visualize stem cells *in vivo*, MRI contrast agents need to provide enough contrast-to-noise to allow detection of transplanted cells in comparison to host tissue. For this, either a substantial amount of paramagnetic (weakly attracted to the magnetic field) particles need to be incorporated into the cells or a lesser amount of larger particles that are superparamagnetic (strongly attracted to magnetic field). Due to the difference in size of the particles, larger particles (e.g. ferumoxides) need additional help to be chaperoned into non-phagocytic cells, whereas smaller contrast agents can be endocytosed without recourse to additional strategies [17]. The incorporation of iron oxide-based contrast agents into phagocytic macrophages after i.v. injection, however, allows the monitoring of the trafficking of these immune cells to sites of damage or graft rejection [18, 19]. Although embryonic and mesenchymal stem cells can give rise to microglia/macrophages that readily incorporate contrast agents, their precursor populations are not considered phagocytic and generally do not take up contrast agents easily. Two main approaches have emerged for improving cellular uptake into non-phagocytic cells, notably linking of the contrast agent with transfection agents [20, 21] or specific engineering of the outer surface of the particles [22, 23].

At present, superparamagnetic ferumoxides combined with transfection agents to improve cellular uptake are the preferred approach for cellular MR imaging [24]. Especially the high relaxivity and the ability to detect as few as 100 cells *in vivo* [21], convey the attractiveness to this approach. However, the negative contrast effect these particles exert on T2-weighted MR images can also be generated by other aspects, such as small bleeds or air bubbles, that are inherent to the transplantation surgery. Likewise, the often preferred T2* effect of superparamagnetic agents that leads to a

blooming effect attenuating the signal in an area 50 times greater than the particle size can also detect poorly oxygenated blood vessels (similar to the BOLD effect used for fMRI) and hence can potentially lead to difficulties in differentiating the injecting tract from a blood vessel. By dramatically increasing the oxygen content of the inhalation gases used for anesthesia, it is possible to suppress this effect [25]. The large $T2^*$ effect that can be achieved with superparamagnetic particles makes it especially interesting for the detection of small cell clusters or even individual cells [26]. Individual cells can also be visualized by MRI by incorporation of large iron-based particles [27]. Nonetheless, strategies need to be developed to improve the differentiation of contrast agent-loaded cells from bleeds or other potential false positives. The emergence of contrast agents based on the chemical exchange saturation transfer (CEST) could potentially circumvent the issue of small bleeds or the detection of poorly oxygenated blood vessels as they will only produce a signal after excitation at a specific radiofrequency. The agents therefore also hold the promise to differentiate more than one cell population *in vivo* as different CEST agent are responsive at different radiofrequencies [28]. Not only further improvements in contrast agent design will be necessary to circumvent these issues, but also novel pulse sequences [29] that could selectively detect the contrast agent and differentiate it from other sources of contrast will require further developments. It is hoped that this greater specificity will improve the reliable detection of transplanted cells *in vivo*.

The reliance on negative contrast on T2-weighted images also potentially creates another difficulty if the aim is to understand how transplanted cells interact with a pathological condition. Many lesions, such as ischemia, will result in a hyperintense bright signal on T2-weighted images. Although this makes it easy to differentiate the pathology from the contrast agent-labeled stem cells, the averaging of a hypointense (stem cells) and hyperintense (lesion) signal for each voxel could encounter difficulties in interpreting if stem cells were present in this area or if the lesion is still progressing. It could therefore be challenging to continue to use T2-weighted images to reliably monitor the progression of pathology. Nonetheless, this translational aspect has not yet been addressed adequately.

Paramagnetic agents based on gadolinium are generally smaller in size and incorporation into stem cells appears to be more straightforward (i.e. no need for transfection agents). The higher intracellular concentration of particles and their lower relaxivity currently disfavor paramagnetic agents for cellular imaging. Generally, these agents provide a positive contrast in T1-weighted images which can be fairly unequivocally attributed to the presence of paramagnetic particles. Although [30] recently reported an T1-enhancing effect of stem cells transplanted under the kidney capsule, more commonly Gd-based agents appear to lose their T1-effect once they are incorporated into cells. Nonetheless, these agents also induce a T2-effect and it is therefore also possible to detect transplanted cells on T2-weighted images [31]. Occlusion of pathology on T2-weighted images therefore remains a concern for T1-enhancing contrast agents as well. Although the exact mechanisms for this T1 loss are not entirely clear, the loss of a T1 effect is likely to be mediated by the localization of the paramagnetic particles in

endosomes (a result of pinocytosis). In endosomes, paramagnetic particles do not have an unrestricted access to water molecules and therefore cannot affect T1 relaxivity. Changing the localization of the contrast agent by incorporating the particles through electroporation rather than pinocytosis can rescue the T1 effect and produce a dose-dependent signal increase [32].

Not only can the localization of contrast agents within cells produce different relaxivities, but it can also potentially affect cellular function [17]. In general, a good viability has been reported after labeling of cells with contrast agents. However, the time points at which this viability has been assessed mainly focused on the time frame almost immediately after labeling, and it is possible that delayed effects might occur. If a reduced viability is observed, it will therefore be important to determine if this effect is due to the labeling procedure per se, for instance, due to a temporary permeabilization of the cell membrane, or if it is caused by disintegration of the contrast agent exposing cells to the freed metal particles. Reduced viability due to the labeling procedure would probably be less of a concern as it could be expected that viability thereafter will be stable, whereas if freed metal ions are the source of toxicity, it is likely that cells will further undergo cell death and no transplanted cells might survive in the long run. It is often suggested that localization to the lysosomes is favorable to other locations as it is unlikely to affect cellular functions. Still, at present, little evidence exists to support this assumption and [33], for instance, recently reported that iron-oxide labeling of cells could inhibit chondrogenesis in mesenchymal stem cells indicating that a detailed analysis is necessary to ensure that stem cells can fulfill their full potential *in vivo*. The main concern, however, lies with the clearance of metal particles from the cells if the protective coating used to reduce any toxic effect might disintegrate. Iron is thought to be disposed of through the normal cell iron metabolism. In contrast, gadolinium could well be toxic if the protective chelate has been lost. Contrast agents coated with the biologically-inert styrene/divinyl benzene coat [34] are thought to provide the best strategy for long-term persistence. Nonetheless, long-term studies need to be conducted to further address these issues.

Once cells have been transplanted into preclinical models, it is prudent to corroborate *in vivo* imaging detection of transplanted cells by histological means. The use of a bimodal agent is advantageous here as it allows a direct corroboration of the *in vivo* MRI images by, for instance, fluorescent histology [16, 31, 34] or *in vivo* optical imaging [35]. Fluorescence can greatly aid with *in vitro* studies as it can serve as a surrogate marker of contrast agent uptake without recourse to immunohistochemical staining (Fig. 1). Iron oxide particles can be detected based on Prussian Blue staining, but currently, no histological staining method has been described to detect gadolinium particles.

An alternative to MRI tracking of stem cells is to use PET to visualize transplanted cells. PET ligands do not generally interfere with MRI and therefore would not raise the issue of being able to assess pathology by means of T2-weighted MRI. Moreover, the short-half life of radioligands makes it unlikely that a long-term detection can be guaranteed based on *in vitro* pre-labeling, although some

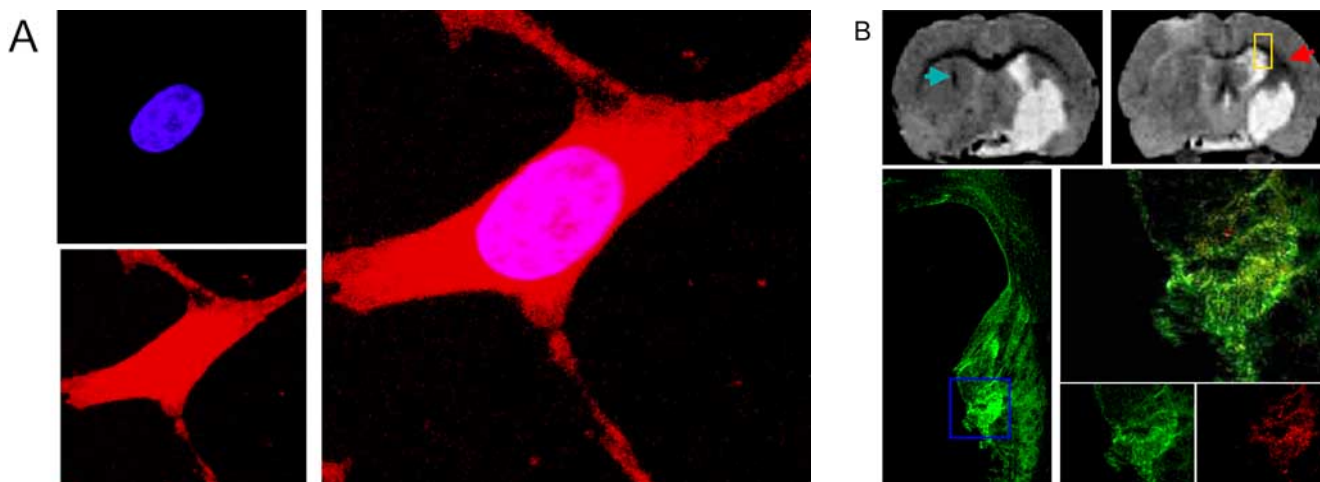


Fig. (1) (A). The use of bimodal contrast agents, such as GRID (16), detectable by both MRI and fluorescent microscopy simplify *in vitro* studies as they allow a direct visualization of contrast agent uptake without recourse to histological staining. (B). This further allows the serial *in vivo* tracking of transplanted cells in rats with stroke damage (31) and corroboration of the cells' arrival around the lesion site (as demonstrated here with a GFAP staining for the glial scar and corroboration of grafted cells based on the red fluorescence of GRID).

studies have demonstrated the proof-of-principle [36]. The small size of PET ligands and their ability to cross the intact blood-brain barriers make it more likely that cells for transplantation will be engineered with a reporter gene that is not normally expressed in host tissue [37]. A PET ligand binding to this reporter gene, so called molecular imaging, could then report on the presence of transplanted cells. However, the issues to overcome consist of convincing regulatory agencies that insertion of a reporter gene will not have deleterious effects. The stable expression of reporter genes over protracted time points (months to years) has also not been thoroughly investigated and it is therefore possible that expression diminishes *in vivo* and hence could compromise detection.

Current developments of MRI contrast agents also promise the visualization of reporter gene products, such as the protein β -galactosidase (β -gal) indicating the expression of the LacZ reporter gene [38, 39]. Although linking of a contrast agent to an antibody can allow specific detection of transgene expression, it is the use of smart contrast agents that has generated most interest. Smart contrast agents refer to a class of MRI probes that will only change relaxivity of the neighboring water molecules if they bind to a given molecule, such as β -gal or Ca^{2+} [39, 40]. More conventional contrast agents will also induce a signal change if they are unbound to the molecules of interest, and it is therefore generally necessary to await a wash-out of the unbound particles before a specific detection can be assumed. Using this reporter construct, it should be possible along similar lines than PET imaging to achieve a high specificity that would allow the serial visualization of transplanted cells long-term without any concern about the particles exerting any undesirable effects inside the cell. Moreover, this approach would preserve lesion detection based on T2-

weighted imaging as it would be expected that the contrast agent would be cleared from the body within a few days. Similar limitations as with PET ligands apply to the expression of reporter genes and will need to form the basis of more chronic molecular biological studies to improve stable and consistent gene expression. Nonetheless, an even more sophisticated use for this molecular or 'reporter gene' imaging can be envisaged in the understanding of stem cell transplantation. Notably, little is known about the molecular cascade involved in *in vivo* stem cell differentiation or if the expression of growth factors in particular regions are relevant to behavioral recovery. It is therefore conceivable to label stem cells with iron oxide particles to allow *in vivo* tracking, but to use smart contrast agents that could induce a T1-enhancement to determine if particular cells will secrete a growth factor in one area, but not in others, which could explain the underlying changes in this area's plasticity as observed with fMRI.

Even more exciting is the combination of bioluminescence imaging (BLI) and MRI to investigate stem cell therapy in preclinical models. The bioluminescent reporter genes used in BLI do not dilute with cell division and therefore can visualize the reconstitution of an entire cell population from single cells over large distances [41, 42]. Linkage of the reporter gene to a gene of interest can also inform about specific gene expression. Nonetheless, the resolution and 3D anatomical information is generally far poorer than with MRI. Combining BLI with cellular MRI imaging, for instance, could therefore provide additional information to study, for instance, cell differentiation in migrating cells or determine the infiltration of particular immune cells involved in graft rejection or graft versus host disease (GVHD) [43]. Moreover, the tagging of neurodevelopmental proteins, such as apolipoprotein E or

presinilin [44], could also inform regarding the relevance of these proteins to plasticity of the host brain and their involvement in stem cell-mediated brain repair. It is currently very unlikely though that this technique will find its translation into larger species or human patients.

Although these technological developments have little to do with stem cell efficacy, they, however, form the basis of our ability to study stem cell repair in living subjects. Once these technologies have reached 'maturity', it will be possible to tackle the important questions regarding stem cell repair. Cellular imaging, for instance, will help to determine if stem cell migration is a necessary condition for behavioral repair. To date, migration and behavior have mainly been linked through histological studies that were conducted after behavioral recovery was achieved. At this protracted time point, it is unlikely that it is still possible to observe the processes/mechanisms that actually led to behavioral recovery. Not all subjects will show recovery, and the use of earlier time points might therefore not necessarily be very informative. Being able to determine that cells are present within a particular brain region at a particular time could help to address the hypothesis that changes in that region, due to stem cell infiltration, are indeed associated with concomitant behavioral improvements. These studies will provide crucial elementary data to formulate novel hypotheses as to how the presence of transplanted stem cells relate to brain repair. However, merely focusing on anatomical aspects might only provide a glimpse at the changes transplanted cells induce.

GOING BEYOND CELLS – THE FUNCTIONAL ANATOMY OF STEM CELL REPAIR

Although being able to visualize graft survival and migration are essential questions that need to be addressed to gain a better understanding of how these cells work, little knowledge can be gained from this approach regarding the functional integration of cells within host neural circuitry. Still, several neuroimaging approaches can be used to investigate the functional anatomy of pathology and repair. At present, the foremost functional neuroimaging technique consists of BOLD fMRI. Both human and animal studies can elucidate the activation of brain regions as a result of behavior or sensory stimulations. However, preclinical animal studies are far more limited in their scope than human studies due to the need of anesthesia to immobilize head movement. Although it is possible to perform fMRI studies on awake animals, most notably primate studies [45], most laboratories currently use anesthesia in small rodents to probe sensory stimulations. In rat models of stroke, for instance, it is possible to stimulate the impaired and intact paw and compare the regions of activation with those commonly activated in normal control animals to demonstrate a functional re-organization [46]. The possibility to gain this data repeatedly from single subjects highlights the potential of this approach to investigate if transplanted cells might further support regional re-organizations through mechanisms such as diaschisis. Metabolic imaging with PET can already elucidate the neurological underpinnings of brain plasticity [47, 48], and it is therefore possible to consider if differences in resting or activated cerebral blood flow maps derived from MRI could

accomplish a similar application. In combination with cellular imaging, however, fMRI would allow us to determine if areas undergoing functional changes contain transplanted stem cells or if grafted cells exert a remote downstream effect. It was, for instance, possible to probe the functional connectivity of fetal tissue grafts by fMRI in Huntington's patients [49].

In preclinical models, it will also be possible to probe if transplanted cells restore functional networks. Based on the directional (i.e. anisotropic) movement of water molecules along fiber tracts (i.e. diffusion tensor imaging), it is possible to generate a 3 dimensional fiber map highlighting connections between different regions of the brain [50]. In neurodegenerative disease, where the connection from the substantia nigra to the striatum is lost, it is hence possible to map the lack of connections and its restoration by transplantation. In order to assess the functional significance of these connections, pre-labeling of cells with $MnCl_2^{2+}$, which enters the cells through calcium channels, could be used to trace axonal pathways [51]. The release of manganese at the synaptic cleft and re-uptake at the postsynaptic terminals provides a mean to map functional circuitry in the brain [52]. Interestingly, only functionally activated cells will incorporate manganese and hence provide a means to assess functional circuitries over time *in vivo* [53]. However, at present it is not very clear if manganese can be applied safely to human subjects.

The integrity of neurotransmitter pathways and the specific loss of neuronal phenotypes can be investigated using phMRI, in clinical and preclinical situations [14]. Again, as with fMRI a considerable confound in preclinical animal studies consists of the use of anesthesia [54]. Nonetheless, careful physiological monitoring and adequate control conditions allow, for instance, the investigation of a loss of dopaminergic cells in the substantia nigra [55] or the differential mapping of D2/D3 receptors [56]. A good correspondence between phMRI, PET, microdialysis and behavior has been demonstrated to provide the foundation to assess transplanted cells and their ability to restore dopamine release in the striatum [57]. To date, the ectopic placement of dopaminergic grafts in the striatum and their pharmacological effects make animal models of Parkinson's an ideal testbed for phMRI in the investigation of cell transplantation. It is possible to foresee how several imaging approaches, such as DTI and phMRI, could be combined to study how nigral transplants might one day be used to probe the restoration of lost connections to the striatum. Still, other diseases that require a phenotypic replacement might benefit from the use of phMRI to assess *in vivo* the receptor status prior to transplantation and its re-emergence from differentiating stem cells.

Along similar lines to phMRI, PET imaging provides highly specific and quantitative data regarding the receptor status in the brain. Although generally PET cannot provide functional activation data that would also afford the investigation of downstream effects of regional activity (i.e. functional circuitry assessment), PET is currently unchallenged by MRI to provide specific and quantitative data for the presence of receptors or molecules in a particular region. Currently, PET is therefore the imaging modality of choice to assess the presence of dopaminergic grafts *in vivo* [58]. The translation from animal to man is also fairly

straightforward and preclinical models can serve to address specific questions regarding dose-response relationships and the specificity of imaging probes. The scarce distribution of PET throughout the world and considerably higher cost implications compared to MRI, nonetheless, limit its wider dissemination, and it is unclear if recourse to this technology would be warranted for routine *in vivo* monitoring if stem cell therapy would become an established treatment strategy.

The integration of PET and MRI into a single device [59] still promises to be an exquisite investigative tool both pre-clinically and clinically to study how stem cells promote brain repair. Unlike with bioluminescence, PET has an excellent deep tissue penetration and quantitation. Experiments investigating gene expression after stem cell transplantation will therefore be feasible with PET detection of a reporter gene and MRI to determine the spatial localization of the expression. It would also be feasible to investigate, for instance, how localization of transplanted stem cells would affect the metabolic turnover in tumors or how downstream effects of amphetamine administration after dopaminergic transplantation are dependent on the number of grafted dopaminergic cells. However, a combination of cellular MRI and phMRI will need to address the issue if a strong negative contrast due to transplanted cells would prohibit the detection of the BOLD response. An integration of cellular MRI, phMRI and PET would really considerably increase our ability to address complex questions *in vivo*.

The ability to visualize molecules *in vivo* would not only provide a greater understanding of how transplanted cells promote behavioral change, but it would also allow a far more detailed study of the pathological processes *per se*. Being able to characterize the lesion environment and study its progression over time can provide indices of the natural progression of the disease that in some cases would be expected to be altered by stem cell transplantation. For instance, in an animal model of Huntington's disease, the natural progression of the lesion indicated that the hyperintense area and the striatum reduce with time, whereas in transplanted animals, the striatal degeneration was stopped and resulted in a preservation of behavioral functions that were normally lost. A correlation between striatal volume over time and behavioral performance confirmed a strong link between the two, suggesting that striatal volume could serve as an imaging index or surrogate marker of behavioral recovery in this model [60]. Moreover, this link strongly implies that the mechanism by which stem cells exert a behavioral effect are linked to preservation of striatal volume. It will therefore now be possible to further probe this effect and dedicate more attention to the mechanisms that reduce striatal degeneration. A similar approach can be used for almost all CNS diseases and provide a heuristic approach to narrow down the areas of interest to help advance effective therapies at a faster pace.

NEUROIMAGING IN PATIENT SELECTION AND MONITORING OF DELIVERY

Imaging of the lesion environment, however, should also play a major role in our understanding of the limitations of stem cell therapy. It is clear, as with all therapeutic interventions, that there will be conditions in which stem

cell therapy will be very efficient and others, where there is no benefit at all. Neuroimaging can help to define these inclusion and exclusion criteria to provide a more rigorous patient selection.

A recent clinical trial of cell therapy in patients with stroke damage revealed that treatment only promoted behavioral change in patients with lesions confined to the basal ganglia in the non-dominant hemisphere [61]. Patients with ischemic lesions in the homologous regions in the dominant hemisphere did not appear to show any recovery. The implication here is that focusing on this subgroup of patients that show recovery, it will be possible to gain a better understanding of how recovery occurred. To gain a more comprehensive view of why the other patients did not recover, a very detailed analysis needs to be performed as it might indicate the limitations of the approach or help to find factors that do not allow the cells to fully fulfill their potential. The group in which no benefit can be observed will be extremely valuable, as no further transplants can be ethically administered to patients with similar lesions until it is clarified how a successful recovery could be achieved. Again, these findings indicate that despite many factors being the same in this trial, not all patients improve and it remains still unclear why only a subgroup of patients recovered. There are currently no reasons to believe that graft survival or integration differed between these different patients, but it might be very specific differences between the different lesion environments that might influence graft success. Along similar lines, [62] demonstrated that parenchymal neural stem cell transplants exerted a different effect in the same lesion compared to intraventricular grafts. These studies highlight the importance of more detailed studies involving behavioral assessment to elucidate the factors necessary to ensure the efficacy of transplant therapy.

The ability of neuroimaging to assess a lesion prior to transplantation offers the opportunity to investigate how lesion location, size, age or physiological status (e.g. presence of penumbral tissue) influences the effectiveness of stem cell therapy. Although some reports suggest that there is little effect of the lesion size on the effectiveness of cell therapy [63], the issue remains poorly addressed in preclinical and clinical studies. It is conceivable, for instance, that for large lesions above a certain threshold where the extracellular matrix has been lost, no viable support for the cells exists and they could therefore not promote behavioral recovery. It is interesting that to date, stem cells on their own have not actually re-grown any tissue, but have only integrated into damaged tissues where an extracellular matrix was still present. The use of 'scaffolds' could be needed to provide support for stem cells to actually rebuild lost tissue [64] and potentially widen the appeal of stem cell therapy. Being able to determine if an extracellular matrix is still present hence could potentially have dramatic implications for the delivery of stem cell therapy.

Not only can neuroimaging determine patient selection, but it is also essential in guiding the therapeutic intervention. Should cells be implanted into the ongoing lesion, the penumbra, or at a more remote site? At present, preclinical studies have failed to address these issues in detail. To deliver cells accurately to their target, it is important to provide a 3-dimensional mapping of the lesion

environment and its location within the brain [65]. These stereotactic co-ordinates will be essential for the neurosurgeon to direct delivery and to plan the surgical intervention. Similar to the resection of brain tumors by neurosurgeons, it will be crucial to determine the presence of blood vessels along the delivery route (by magnetic resonance angiography) and avoid major axonal pathways, as damage to these could affect remote regions and cause novel impairments. At present, magnetic resonance imaging is the most versatile technique in the pre-surgical workup for transplantation. Not only can it provide information regarding the location of the lesion, its age and physiological status, but it can also provide detail regarding arteries and fiber tracts. PET does not have the resolution or versatility to provide this information. Recent work by [66], for instance, indicated that dendritic cell therapy for melanomas was misplaced by experienced clinicians using ultrasound in 50% of cases, whereas cellular MR guided intervention increased the accuracy of delivery. Nonetheless, at present it is unclear to what degree accurate delivery is an essential component of the effectiveness of stem cell therapy. However, there certainly is a concern that misplacement in the brain could be the consequence of poor pre-surgical planning, which could cause an intracerebral bleed due to puncturing of an artery. In the case of stem cell therapy, where the target for the deposit might vary, greater care might need to be taken in the pre-surgical planning. Neuroimaging, and especially MRI, appear to be essential tools to ensure a high success rate of stem cell therapy.

CONCLUSION

The *in vivo* imaging of neural transplants has considerably developed over the past 15 years. The limited success of some clinical studies assessing the efficacy of cell therapy highlights the importance of more detailed translational studies in preclinical models. Pure reliance on stem cell biology will not achieve the full potential of this promising therapeutic strategy. It will be essential to probe therapeutic efficacy in animal models and determine favorable conditions for stem cell therapy. The increasing reliance on neuroimaging as a diagnostic tool in the clinic and its ability to provide baseline assessments in preclinical models necessitate its implication in the translation of stem

cell therapy into an efficient and successful treatment (Table 2).

The development of cellular MR imaging has brought a great advance to neuroimaging and the possibility to study stem cell therapy *in vivo* [17]. Further developments in the labeling of stem cells and its ability to allow the prolonged visualization of cells without any effect on cellular functions are needed to allow implementation of this approach to clinical trials. Especially, strategies relying on reporter genes might in the future provide interesting alternatives to current strategies, if certain limitations, such as a low contrast to noise or crossing of the BBB, can be overcome by novel developments.

Still, the involvement of neuroimaging has to go beyond cellular MRI of stem cell therapy and involve the assessment of the functional substrates of behavioral recovery. As our knowledge of stem cell-mediated brain repair increases, it is becoming more evident that it will also be essential to understand host plasticity in order to maximize behavioral recovery. It is therefore the burden of neuroimaging to further integrate current disparate techniques (e.g. DTI, fMRI) to provide a more holistic assessment of the factors involved in graft-mediated repair.

Nonetheless, neuroimaging studies will only find their significance if they are combined with concomitant behavioral assessment. As mentioned above, the main outcome measure of any therapeutic intervention must be the behavioral recovery experienced by the patient. It is of little help to invest significant sums of money into sophisticated neuroimaging showing, for instance, a reduction in lesion volume, if this does not translate into a significant behavioral benefit. The complexity of studies will therefore dramatically increase, as different expertise from cell biology, neuropsychology, and neuroimaging need to be integrated into a comprehensive assessment. Neuroimaging will hence be the key to the translation of stem cell transplantation from research to therapy.

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Table 2. Examples of How Imaging can Address the Factors Influencing Graft-Mediated Recovery

Influencing factors	The role of <i>in vivo</i> imaging
Site of lesion	Stereotactic coordinates of location can be determined
Severity of lesion	Measurement of lesion volume
Graft survival	Contrast agent-labeling prior to transplantation allows cellular tracking
Number of grafted cells	Change in signal due to contrast agent-labeled cells potentially can determine how many cells are contained within an area
Sites of Implantation	Injection tract visible on T ₂ *-weighted images
Functional Integration	Labeling cells <i>in vitro</i> with manganese and tracing its transport along activated axons to different regions of the brain
Effect on host re-organization	fMRI can probe how functional activity changes as the brain re-organizes
Inflammatory/immunological response	<i>In vivo</i> labeling of macrophages with contrast agent

interdisciplinary research environment dedicated to stem cell research a reality at the Institute of Psychiatry. The generous funding from the Medical Research Council, Department of Trade and Industry, Wolfson Foundation, Research Councils of the UK, Ministère de l'Éducation Nationale du Luxembourg, NATO and ReNeuron that allowed our interdisciplinary studies to flourish are gratefully acknowledged.

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