Magnetic Resonance Imaging-Guided Transplantation of Neural Stem Cells into the Porcine Spinal Cord

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Keywords

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Abstract

Background: Cell-based therapies are a promising treatment option for traumatic, tumorigenic and degenerative diseases of the spinal cord. Transplantation into the spinal cord is achieved with intravascular, intrathecal, or direct intraparenchymal injection. The current standard for direct injection is limited by surgical invasiveness, difficulty in re-injection, and the inability to directly target anatomical or pathological landmarks. The objective of this study was to present the proof of principle for minimally invasive, percutaneous transplantation of stem cells into the spinal cord parenchyma of live minipigs under MR guidance. **Methods:** An MR-compatible spine injection platform was developed to work with the ClearPoint SmartFrame system (MRI Interventions Inc.). The system was attached to the spine of 2 live

minipigs, a percutaneous injection cannula was advanced into the spinal cord under MR guidance, and cells were delivered to the cord. **Results:** A graft of 2.5×10^6 human ($n = 1$) or porcine ($n = 1$) neural stem cells labeled with ferumoxytol nanoparticles was transplanted into the ventral horn of the spinal cord with MR guidance in 2 animals. Graft delivery was visualized with postprocedure MRI, and characteristic iron precipitates were identified in the spinal cord by Prussian blue histochemistry. Grafted stem cells were observed in the spinal cord of the pig injected with porcine neural stem cells. No postoperative morbidity was observed in either animal. **Conclusion:** This report supports the proof of principle for transplantation and visualization of pharmacological or biological agents into the spinal cord of a large animal under the guidance of MRI.

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Introduction

Stem cell-based therapies are under clinical evaluation for the treatment of a range of tumorigenic, degenerative and traumatic diseases of the spinal cord, including

amyotrophic lateral sclerosis, spinal cord injury, and multiple sclerosis. Intravascular, intrathecal, and intraparenchymal delivery methods have been employed in these trials [1]. Direct intraparenchymal injection is currently the most straightforward, reliable method for transplanting cell therapies directly into the spinal cord. Furthermore, the safety profile of spine-mounted stereotactic platforms capable of performing multiple direct injections into the spinal cord parenchyma following laminectomy is established in preclinical and clinical studies [2–14].

Image-guided approaches have been employed in the brain to replace procedures traditionally done with stereotactic systems. The scope and number of MRI-guided interventions has advanced in recent years due to hardware and software developments that exploit the excellent soft tissue contrast, high spatial resolution, and multiplanar imaging capabilities of MRI. Interventional MRI is the method of choice in many centers for guiding implantation of deep brain stimulation electrodes [15–17].

Moreover, image-guided approaches have been used to access the spine and spinal cord. Computed tomography and MRI are used in the clinic to guide percutaneous cordotomy [18–20] and other procedures in the spine and spinal cord [21–24]. Preclinical studies in canines employed fluoroscopic guidance to percutaneously transplant stem cells into the spinal cord [25, 26]. Recently, MRI guidance was used to deliver stem cells to the porcine intervertebral disk [27] and guide transpedicular biopsy in patients [28]. However, these previous studies have not introduced a cannula into the spinal cord through the interlaminar space under MRI guidance and have not delivered cells to specific locations identified with MRI.

The purpose of this study was therefore to establish the proof of principle and feasibility of MR-guided percutaneous injection of cellular therapeutics into the spinal cord. The benefits of MR-guided injection into the spinal cord would be transplantation without surgical laminectomy, direct targeting of anatomic locations such as the ventral gray matter horn of the spinal cord or pathologic landmarks in the spinal cord such as multiple sclerosis lesion or malignancy, and immediate confirmation of targeting accuracy. Many of these soft tissue anatomical or pathological variations cannot be seen with modalities other than MRI. The reduced invasiveness could improve procedural recovery time and allow for reinjection into the same spinal cord segments. The use of MRI and this targeting system also allows for real-time adjustment of the cannula trajectory to improve access to the designated target site and reduce procedure time.

In this report, we describe a methodology for minimally invasive delivery of labeled cellular therapeutics into the pig spinal cord under the guidance of MRI using a custom-built spine mounted platform and the ClearPoint system (MRI Interventions Inc.).

Methods

Ethics Statement

All procedures were conducted at the Division of Animal Resources in accordance with a protocol approved by the Institutional Animal Care and Use Committee at our University.

Cell Preparation

Human neural progenitor cells (hNPCs) isolated from the fetal cortex were provided as frozen stocks (Clive Svendsen at Cedars-Sinai Medical Center [30, 31]). The hNPCs were expanded in culture as neurospheres [32] and labeled with ferumoxytol (Feraheme, AMAG Pharmaceuticals), an ultrasmall superparamagnetic iron oxide nanoparticle (SPIO), previously used for cellular tracking with MRI [33, 34]. Immediately following mechanical passage, the reforming neurospheres were incubated with 400 µg/mL of ferumoxytol for 7 days. Immediately prior to surgery, the neurospheres were washed and chemically dissociated to single cells. Green fluorescent protein-tagged pig neural progenitor cells (GFP-pNPCs) were provided as dissociated cells prepared for transplantation (Neuralstem Inc.) and were labeled with ferumoxytol nanoparticles for 24 h. Cell viability and concentration were assessed with a trypan blue exclusion assay (>80% viable required for transplantation). The hNPCs and pNPCs were maintained on ice in hibernation medium until transplantation.

MR-Compatible Injection System

The prototype injection platform was MRI-compatible and constructed of polyoxymethylene resin. The device was designed to attach to spine laminae rostral and caudal to the region of interest with custom-built percutaneous aluminum posts and titanium lamina screws (4 mm anchor). The platform contained adjustable post fasteners that allowed for translation in the rostral/caudal plane and multiple locking points in the medial/lateral plane. The ClearPoint SmartFrame device (MRI Interventions Inc.) was rigidly attached to the injection platform and was maneuvered in the rostral/caudal and medial/lateral planes on a rail system (Fig. 1a). The SmartFrame allowed for targeting and controlled advancement of the custom-built injection needle (MRI Interventions Inc.) using the ClearPoint targeting software to calculate a trajectory. The SmartFrame was modified by shortening the device lock at the top of the device to create a lower profile by 2 cm. A ceramic guide cannula with a titanium stylet was used to pass through the soft tissues and provide access to the spinal cord. The infusion needle (SmartFlow catheter, MRI Interventions Inc.) was composed of fused silica glass and inserted through the guide cannula after stylet removal. The internal diameter of the needle tip was 200 µm with a stepped tip design and had a single continuous lumen from the distal tip to the proximal connector of the integrated extension tube. When inserted through the

Fig. 1. In vitro assessment of an MR-compatible spinal injection system with a phantom model. **a** The MR-compatible injection system utilizes: (1) MR-compatible SmartFlow catheter (MRI Interventions), (2) modified ClearPoint device (MRI Interventions), and (3) MR-compatible spine platform with percutaneous lamina posts for fastening to the spine. The injection needle of the MR-compatible SmartFlow catheter protrudes through a ceramic guide cannula (*inset*). The system is mounted to an MRI spine phantom model for in vitro assessment. **b** Under the guidance of T1-weighted MRI (3-tesla full-body scanner, Siemens) and the ClearPoint

software (MRI Interventions), the catheter (arrow) was advanced to the surface of the spinal cord in the phantom model. The injection needle was inserted into the spinal cord (**c**) to the planned target (red circle), and an infusion of ferumoxytol iron oxide nanoparticles was performed, as seen with gradient echo T2*-weighted MRI (**d**). **e** For targeting accuracy studies, the tip of a plastic cannula (yellow asterisk) was targeted, and the injection needle (yellow arrowhead) was advanced to the target based on the ClearPoint software calculations. Radial and axial errors were calculated based on coronal (**e**) and axial (**f**) T2*-weighted MRI.

guide cannula, the infusion needle extended 10.5 mm beyond the tip of the cannula, ensuring the guide cannula did not enter the spinal cord parenchyma. 10 ft of extension tubing from the infusion needle allowed the injection to be done in the scanner bore with a microprocessor-controlled syringe pump (Tritech Research Inc., Los Angeles, CA, USA) located outside the fringe field [35].

In vitro Assessment

An MR spinal cord phantom model was constructed from a solid foam human T₉–L₂ spine model (1325-13, Sawbones) with either an agar gel spinal cord for feasibility studies or a plastic cannula tip in the spinal canal for accuracy/targeting studies. The phantom was submerged in water in a clear box (Fig. 1a). The MR-compatible injection system was attached to the phantom, and multiple injections were performed under MR guidance with the ClearPoint software to establish system workflow. Five separate procedures (mounting, targeting, trajectory planning, and infusion needle insertion) targeting the plastic cannula tip were performed to calculate the mean radial and axial error of the system. Error was calculated by measuring distance from needle tip to target in coronal and axial T2*-weighted images with an Osirix DICOM viewer.

Magnetic Resonance Imaging

All images and procedures were done with a clinical 3-tesla MRI scanner (Magnetom TRIO, Siemens Medical, Malvern, PA, USA) with a bore length of 140 cm and diameter of 60 cm. Structural images were acquired using sagittal T2-weighted turbo spin echo (TE/TR = 101/3,400 ms, resolution = 0.73 × 0.73 × 2 mm) and T1-weighted 3-dimensional spin echo sequences (TE/TR = 2.45/7 ms, resolution = 0.8 × 0.8 × 0.8 mm, 96 slices). The T1-weighted images were used for fiducial identification and trajectory planning. For cannula visualization and graft identification, a gradient echo (GRE) T2*-weighted axial 2-dimensional multislice sequence was utilized (TE/TR = 10/159 ms, flip angle = 30°, averages = 4, resolution = 0.9 × 0.9 × 1.5 mm).

Anesthesia

The pigs were fasted for 12 h prior to induction anesthesia with ketamine (35 mg/kg, i.m.) and acepromazine (1.1 mg/kg, i.m.). The pigs were maintained for the duration of the procedure on isoflurane (1.5–2.5%, inhaled) mixed with oxygen.

In vivo MR-Guided Spinal Cord Injection

Two female Göttingen minipigs were enrolled in the study. The pigs were placed in the MRI scanner table in the prone, head-first position under general anesthesia. Appropriate sterile preparation

Fig. 2. MR-guided transplantation strategy in the porcine spinal cord. **a, b** Potential cannula trajectories for transplantation of neural stem cells into the spinal cord were designed for an animal with a previous laminectomy (solid arrow) and dural opening. A trajec-

tory (dashed arrow) through the scar tissue (solid arrow) was utilized. **c, d** A trajectory (dashed arrow) through the interlaminar space was utilized to gain access to the cord in a naïve pig with no previous laminectomy.

and draping were done over the thoracolumbar spine. The rostral and caudal portions of the injection platform were fastened to the vertebra above and below the target site. Palpation was used to determine the location of spinous processes for attachment [35]. The aluminum posts were advanced to the surface of the lamina through a 1-cm dermal incision. The titanium lamina screws with a 4-mm anchor were advanced through the posts and fastened into the lamina. A fiducial grid was placed over the skin on the target site (SmartGrid, MRI Interventions Inc.). The SmartFrame was attached to the injection platform. Two phased-array body coils were sterile-draped and placed beside the injection platform. Pre-entry sagittal T2 2-dimensional turbo spin echo, sagittal T1 3-dimensional, and axial T2* GRE 2-dimensional images covering the fiducial markers and spinal cord region of interest were acquired. The L₁–L₂ spinal cord segment was targeted. The pre-entry images were imported to the ClearPoint software to acquire the target (ventral horn of the spinal cord), plan an initial trajectory, and set the cannula entry point on the skin using the fiducial grid.

The animal was moved out of the scanner bore and the skin was nicked through the fiducial grid to mark the planned entry point, and the grid was removed. Due to the thickness of the porcine skin, a 1-cm dermal incision was made over the entry point. Stepwise angular and planar adjustments were made with repeat imaging to align the cannula with the final planned trajectory in near real time. The ceramic guide and stylet were advanced to depth at the ligamentum flavum. The titanium stylet was removed, and imaging was performed to confirm the cannula was on the correct trajec-

tory. Once the final trajectory was determined to be on target, the injection needle was advanced through the cannula and into the spinal cord ventral horn. Final targeting was assessed with T2* GRE imaging. A single injection of 2.5×10^6 hNPCs or pNPCs in 25 μ L was infused into the spinal cord at a rate of 5 μ L/min for each animal. The needle remained in the cord for 2 min following injection to minimize reflux, and repeat GRE imaging was performed to confirm cell graft delivery. The catheter and cannula were removed, and postoperative scanning was performed.

Postoperative Management and Behavioral Assessment

Oral cyclosporine (10 mg/kg) was administered for immunosuppression from the day of surgery to euthanasia. The pigs underwent general neurological examination/observation before and following the procedure. Sensory evaluation took place in the form of a tactile stimulus to the interdigital space. Behavioral assessment of motor function was performed daily. Gait and motor function were assessed according to the Tarlov scale [36].

Euthanasia, Tissue Processing and Histology

The pigs were euthanized 21 days after transplantation. Transcardiac perfusion with 0.9% NaCl solution followed by 4% paraformaldehyde was performed. The fixed spinal cord was excised and frozen. The cord was sectioned axially at 50- μ m intervals and stained with Prussian blue reagent for microscopic iron and counter-stained with eosin. Immunohistochemical staining for detection of grafted human cells using a primary mouse monoclonal

Fig. 3. MR-compatible spinal injection system placement and targeting/trajectory planning in vivo. **a** The pigs ($n = 2$) were sterile prepared in the MR scanner, and the MR-compatible injection system was securely fastened to the spine with percutaneous lamina posts. **b** Two body flex radio-frequency coils were placed on either side of the system. A trajectory through the skin and into the ventral horn of the spinal cord was calculated with T1-weighted MR images (**c**) with the ClearPoint software to go through scar tissue in a pig with a previous laminectomy (not shown) or through the interlaminar space (**d**). The center of the green crosshair shows the planned trajectory through the interlaminar space avoiding the spinous process of the rostral vertebra (yellow asterisk) and the edge of the caudal lamina (yellow arrow).

anti-human nucleus antibody (MAB1281; EMD Millipore; 1/250) and detection of grafted pig cells using a primary mouse monoclonal anti-GFP antibody (AB3080; EMD Millipore; 1/250) was performed on every 6th section with cresyl violet background stain. Images were captured using a Nikon E400 microscope supplied with NIS-Elements imaging software (Nikon Instruments Inc.). The current study was not designed to investigate the therapeutic efficacy or biological properties of transplanted cell grafts, which has been established in previous studies [29–31].

Results

In vitro Assessment

A spinal cord phantom model was used to establish workflow and evaluate the accuracy of the MR-guided spinal cord injection system. The system was mounted to the phantom, and T1-weighted images (Fig. 1b) were used to plan a trajectory to a target in the spinal cord with the ClearPoint software. The injection needle was inserted along the planned trajectory into the target in the spinal cord (Fig. 1c), ferumoxytol SPIO nanoparticles in 25

μL saline infused, and a hypointense focus was observed at the targeted injection site (Fig. 1d). Five independent targeting/accuracy trials were performed (Fig. 1e, f), and the mean error was 0.42 ± 0.34 mm (range 0.14–1.01 mm) in the radial plane and 1.06 ± 0.51 mm (range 0.48–1.67 mm) in the axial plane.

Cell Transplantation into the Spinal Cord under MR Guidance

Two pigs received MR-guided transplantation of cells into the spinal cord. The first pig had a previous 2-level L₁–L₂ laminectomy including dural opening 3 weeks earlier. The laminectomy was used to simulate the scenario of a previous surgical transplantation (Fig. 2a). The second pig was a naïve pig without previous surgery (Fig. 2b). Percutaneous stem cell injection into the spinal cord was initiated through placement of the spinal injection system while the pig was on the MR scanner table (Fig. 3a, b). The ventral horn in the spinal cord was targeted using a blend of T2*-weighted GRE and T1-weighted images. A trajectory traversing the soft tissue into the spinal cord target

Fig. 4. MR-guided spinal cord transplantation in vivo. **a** Representative T2*-weighted images from MR-guided spinal cord injection show the SmartFlow catheter (arrow) advanced to the surface of the spinal cord in the pig with previous laminectomy. **b** The titanium stylet was removed from the ceramic guide cannula, and the injection needle was inserted into the spinal cord. **c** A graft of 2.5×10^5 pig neural stem cells with ferumoxytol nanoparticles was injected into the cord. **d** The needle was removed, and a hypointense

focus (arrowhead) representative of the graft was observed in the cord at the target site. **e** In the animal without laminectomy, the cannula (arrow) was guided through the interlaminar space and the injection needle inserted into the cord. **f** Once in the cord, a graft of 2.5×10^5 human neural stem cells labeled with ferumoxytol nanoparticles was injected. The graft was observed as a hypointense focus on T2*-weighted MRI.

was selected using the ClearPoint software (Fig. 3c). In the naïve pig with no laminectomy, a trajectory through the interlaminar space was utilized (Fig. 3d). Successful alignment of the inserted cannula to the planned trajectory was confirmed with T2*-weighted MRI after the cannula had been advanced to the ligamentum flavum (Fig. 4a). The injection needle was inserted into the spinal cord through the cannula, and placement was confirmed with MRI (Fig. 4b, e). A single graft of 2.5×10^6 SPIO-labeled GFP-pNPCs (previous laminectomy) or hNPCs (no laminectomy) was injected into the cord, and a hypointense focus representative of the negative contrast produced by the ferumoxytol-labeled graft was observed at the target site in the spinal cord with T2*-weighted MRI (Fig. 4c, f). Furthermore, the graft was observed after the needle had been removed (Fig. 4d). The procedure duration was 4 h for the pig with laminectomy and 6 h in the naïve pig. Trajectory planning was approximately 1.5 h and needle insertion/injection 1 h in both animals.

Behavioral Assessment

Pre- and postoperative behavioral assessment was completed. Following recovery from anesthesia, the pigs showed no signs of distress and ambulated within 2 h. No deficits were observed in the general neurological exami-

nation, sensory evaluation, or motor assessment for the duration of the study (21 days).

Histology

To validate the MR results and confirm the intraspinal injection location of SPIO-labeled cell grafts, the spinal cord was excised and serially stained for the presence of microscopic iron using the Prussian blue reagent. Scattered blue iron precipitates were observed in the ventral horn of the pig that received hNPCs (Fig. 5a). However, human nuclei were not observed at the site. Characteristic blue iron precipitates were located in the white matter medial to the ventral horn of the spinal cord in the pig that received GFP-pNPCs (Fig. 5b). Iron precipitates were not located on the contralateral side or in noninjected areas of the spinal cord. Furthermore, numerous GFP-positive grafted pNPCs were observed at the site of the blue iron precipitates (Fig. 5c).

Discussion

The results of this study provide preliminary evidence for a novel, minimally invasive approach aimed at achieving delivery of pharmacological or biological agents di-

Fig. 5. Histological confirmation of graft delivery into the spinal cord. Representative light microscopy images of histochemical staining for iron deposits with Prussian blue reagent. **a** Characteristic blue iron precipitates indicative of the ferumoxytol label were observed in the central gray matter/ventral horn of the pig with no laminectomy. However, no surviving human cells were observed

(not shown). **b** In the pig with previous laminectomy, blue iron precipitates were observed in the central white matter medial to the ventral horn. **c** A representative photomicrograph showed a GFP+ pNPC graft in the medial white matter, which correlated with the region containing iron precipitates. Scale bars = 1 mm; insets 100 μ m.

rectly into spinal cord parenchyma under MRI guidance. This is the first published report of MRI-guided intraspinal stem cell transplantation in a live animal. Most importantly, the use of a large animal model and clinical MRI scanner make this procedure directly applicable to clinical translation. Intraoperative MR targeting, trajectory planning and cannula guidance are well established in the brain for the implantation of deep brain stimulation electrodes using the ClearPoint system, and submillimeter accuracy has been achieved [37]. The in vitro accuracy achieved in this study is comparable to previous reports [37, 38].

Successful translation of stem cell-based therapies for spinal cord disease requires optimization of many parameters, including the delivery method. The method that delivers the most cells to the target site with the least invasive approach would be ideal. Ongoing and completed clinical trials have employed intravascular, intrathecal, and intraparenchymal delivery approaches to transplant cell therapies to the spinal cord. Minimal adverse events, mostly transient sensory deficits, have been observed in these trials [1]. Direct intraparenchymal delivery represents the most straightforward approach for delivering cells to the target site. Optimizing the intraparenchymal approach with MR-guidance could allow for increased accuracy with direct visualization and targeting of anatomical or pathological sites in the spinal cord while concurrently reducing procedural morbidity. MRI is the only imaging modality that allows for direct visualization of spinal cord anatomical landmarks and pathology.

The safety profile for intraparenchymal transplantation of cellular therapeutics is established in large animal models and clinical trials [2–9]. While MR guidance could reduce the need for an open surgical procedure and allow for percutaneous delivery, it raises other concerns, including: cerebrospinal fluid leakage or hemorrhage of vasculature from incidental needle puncture; inaccurate targeting due to displacement of the cord from the resistance of the dura mater to needle puncture; and limited range of transplantation sites due to the vertebra. However, the advantages of this approach are direct targeting to pathology, confirmation of needle location at target site with MRI, and decreased invasiveness compared to the current intraparenchymal delivery procedure. Furthermore, this approach could allow for repeat injection in areas of spinal cord that have been operatively exposed and injected previously. Repeat surgical exposure is challenging due to scar tissue formation, loss of tissue planes, and potential adhesion of spinal cord to dura mater. Pigs with and without previous surgical laminectomy were chosen to assess the feasibility of both interlaminar injection and injection through scar tissue from previous laminectomy. This would emulate two clinical transplantation possibilities.

Extensive preclinical studies in large animal models must be conducted to evaluate the safety and accuracy of percutaneous, MR-guided spinal cord cell graft transplantation. The purpose of this study was not to investigate the survival, engraftment, or function of the transplanted therapeutic product, and grafted neural stem cells were observed in only 1 of the 2 animals. It is possible that

the cells injected in the other animal were rejected by the host immune system, which is likely due to xenograft immune rejection, and/or the injection was not adequately delivered into the spinal cord due to reflux, cannula clogging, or off-target injection. A limitation of this report is the use of only 2 animals for injection, the procedural length, and quality of in vivo MR images. These difficulties were due to complications with RF coil placement, pig placement in the prone position, respiratory motion, and adapting software designed for brain applications to the spinal cord. The current procedure length is comparable to initial non-MR-guided, surgical transplantation approaches [3]. For future studies, an integrated RF coil, a device for properly positioning/holding the pig, and modified software will be employed. However, the main objective of this study was to provide proof of principle for the procedure.

Conclusion

We describe the first MRI-guided, percutaneous stem cell transplant into the pig spinal cord. This supports the proof of principle for transplantation of stem cells into the spinal cord of a large animal under the guidance of MRI. This MRI-guided, minimally invasive approach

could be used clinically to directly deliver pharmacological or biological therapeutics to the spinal cords of patients with amyotrophic lateral sclerosis, spinal cord injury lesions, intraspinal tumors, or multiple sclerosis plaques.

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Disclosure Statement

Nicholas M. Boulis, MD, is the inventor of surgical devices to enable safe and accurate injection of the human spinal cord. Neuralstem Inc. has purchased an exclusive license to these technologies. Dr. Boulis received an inventor's share of this fee and has the rights to royalty payments for distribution of this technology. These technologies are not used in this paper. Dr. Boulis was also a paid employee of Above & Beyond in 2015, which funds amyotrophic lateral sclerosis research. Pete Piferi is an employee of MRI Interventions Inc., which supplied devices and software for this study.

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