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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 anatomic or pathologic 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—A 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 two 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 two animals. Graft delivery was visualized with post-procedure 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 post-operative morbidity was observed in either animal.

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Disclaimers: 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 manuscript. Dr. Boulis was also a paid employee of Above & Beyond in 2015, which funds ALS research. Pete Piferi is an employee of MRI Interventions, Inc., which supplied devices and software for this study.
Conclusion—This report supports the proof-of-principle for transplantation and visualization of pharmacologic or biological agents into the spinal cord of a large animal under the guidance of MRI.

Keywords
Image-guided neurosurgery; interventional MRI; magnetic resonance imaging; percutaneous; spinal cord; stereotactic techniques; targeting; Cell therapy; molecular imaging; swine

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 (ALS), Spinal Cord Injury (SCI), and Multiple Sclerosis (MS). 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 multi-planar imaging capabilities of MRI. Interventional MRI is the method of choice in many centers for guiding implantation of deep brain stimulation (DBS) electrodes[15–17].

Moreover, image-guided approaches have been used to access the spine and spinal cord. Computed Tomography (CT) and MRI are used in the clinic to guide percutaneous cordotomy[18–20] and other procedures in the spine and spinal cord[21–24]. Pre-clinical 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 disc[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 MS 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 re-injection into the same spinal cord segments. The use of MRI and this targeting system also allows for real
time adjustment of 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 Intuitional 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 (Ferraheme, AMAG Pharmaceuticals), an ultra-small superparamagnetic iron oxide nanoparticle (SPIO), previously used for cellular tracking with MRI[33,34]. Immediately following mechanical passage, the re-forming 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 hours. 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 (4mm 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 (Figure 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 2cm. 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 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) located outside the fringe field[35].

In Vitro Assessment

A 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 (Figure 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 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 Osirix DICOM viewer.

Magnetic Resonance Imaging

All images and procedures were done with a clinical 3T MRI scanner (Magnetom TRIO, Siemens Medical, Malvern, PA) with a bore length of 140cm and diameter of 60cm. Structural images were acquired using sagittal T2-weighted turbo spin echo (TSE) (TE/TR=101/3400 msec, Resolution = 0.73x0.73x2 mm) and T1-weighted 3D spin echo sequences (TE/TR=2.45/7 msec, Resolution = 0.8x0.8x0.8 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 2D multi-slice sequence was utilized (TE/TR=10/159 msec, Flip Angle=30°, Averages=4, Resolution = 0.9×0.9×1.5 mm).

Anesthesia

The pigs were fasted for 12 hours prior to induction anesthesia with Ketamine (35 mg/kg, IM) and Acepromazine (1.1 mg/kg, IM). 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, headfirst position under general anesthesia. Appropriate sterile preparation and draping was 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 4mm 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 2D TSE, sagittal T1 3D, and axial T2* GRE 2D 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 trajectory. 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 2.5×10⁶ hNPCs or pNPCs in 25 μL was infused into the spinal cord at a rate of 5μL/minute for each animal. The needle remained in the cord for 2 minutes following injection to minimize reflux and repeat GRE imaging was performed to confirm cell graft delivery. The catheter and cannula were removed and post-operative scanning was performed.

Post-Operative 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 μm intervals and stained with Prussian Blue (PB) reagent for microscopic Iron and counter-stained with Eosin. Immunohistochemical staining for detection of grafted human cells using a primary mouse monoclonal anti-human nucleus (HuNu) 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 (Figure 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 (Figure 1C), ferumoxytol SPIO nanoparticles in 25 μL saline infused, and a hypointense focus was observed at the targeted injection site (Figure 1D). Five independent targeting/accuracy trials were performed (Figure 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 two-level L1–L2 laminectomy including dural opening three weeks earlier. The laminectomy was used to simulate the scenario of a previous surgical transplantation (Figure 2A). The second pig was a naïve pig without previous surgery (Figure 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 (Figure 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 was selected using the ClearPoint software (Figure 3C). In the naïve pig with no laminectomy, a trajectory through the interlaminar space was utilized (Figure 3D). Successful alignment of the inserted cannula to the planned trajectory was confirmed with T2*-weighted MRI after the cannula was advanced to the ligamentum flavum (Figure 4A). The injection needle was inserted into the spinal cord through the cannula and placement was confirmed with MRI (Figure 4B, E). A single graft of 2.5×10⁶ 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 (Figure 4C, F). Furthermore, the graft was observed after the needle was removed (Figure 4D). The procedure duration was four hours for the pig with laminectomy and six hours in the naïve pig. Trajectory planning was approximately 1.5 hours and needle insertion/injection 1 hour in both animals.

Behavioral Assessment

Pre- and post-operative behavioral assessment was completed. Following recovery from anesthesia, the pigs showed no signs of distress and ambulated within two hours. No deficits were observed in the general neurological exam, 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 (Figure 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 (Figure 5B). Iron precipitates were not located on the contralateral side or in non-injected areas of the spinal cord. Furthermore, numerous GFP-positive grafted pNPCs were observed at the site of the blue iron precipitates (Figure 5C).

Discussion

The results of this study provide preliminary evidence for a novel, minimally invasive approach aimed at achieving delivery of pharmacologic or biological agents directly 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 DBS electrodes using the ClearPoint system and sub-millimeter 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 anatomic or pathologic sites in the spinal cord while concurrently reducing procedural morbidity. MRI is the only imaging modality that allows for direct visualization of spinal cord anatomic 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. A pig with and without previous surgical laminectomy were chosen to assess the feasibility of both inter-laminar 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 one of the two 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 two 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 pharmacologic or biological therapeutics to the spinal cords of patients with ALS, SCI lesions, intraspinal tumors, or MS plaques.

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References


Figure 1. In Vitro Assessment of an MR-Compatible Spinal Injection System with a Phantom Model

The MRI compatible injection system (A) 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 spine. The injection needle of the MR-Compatible SmartFlow catheter protrudes through a ceramic guide cannula (insert). The system is mounted to a MRI spine phantom model for in vitro assessment. Under the guidance of T1-weighted MRI (3T 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 (B). 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). For targeting accuracy studies, the tip of a plastic cannula (yellow asterisk) was targeted and the injection needle (yellow arrowhead) was advanced to target based on the ClearPoint software calculations. Radial and axial error were calculated based on coronal (E) and axial (F) T2*-weighted MRI.
Figure 2. MR-Guided Transplantation Strategy in the Porcine Spinal Cord

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, B). A trajectory (dotted arrow) through the scar tissue (solid arrow) was utilized. A trajectory (dotted arrow) through the interlaminar space was utilized to gain access to the cord in a naïve pig with no previous laminectomy (C, D).
Figure 3. MR-Compatible Spinal Injection System Placement and Targeting/Trajectory Planning In Vivo

The pigs (n = 2) were sterile prepared in the MR scanner and the MRI compatible injection system was securely fastened to the spine with percutaneous lamina posts (A). Two body flex radiofrequency coils were placed on either side of the system (B). 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 on 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).
Figure 4. MR-Guided Spinal Cord Transplantation In Vivo
Representative T2*-weighted images from MR-guided spinal cord injection show the SmartFlow catheter (arrow) advanced to the surface of the spinal cord (A) in the pig with previous laminectomy. The titanium stylet was removed from the ceramic guide cannula and the injection needle was inserted into the spinal cord (B). A graft of $2.5 \times 10^5$ pig neural stem cells with ferumoxytol nanoparticles was injected into the cord (C). The needle was removed and a hypointense focus (arrowhead) representative of the graft was observed in the cord at the target site (D). In the animal without laminectomy, the cannula (arrow) was guided through the interlaminar space and the injection needle inserted into the cord (E). Once in the cord, a graft of $2.5 \times 10^5$ human neural stem cells labeled with ferumoxytol nanoparticles was injected. The graft was observed as a hypointense focus on T2*-weighted MRI (F).
Figure 5. Histological Confirmation of Graft Delivery into the Spinal Cord
Representative light microscopy images of histochemical staining for iron deposits with prussian blue reagent. Characteristic blue iron precipitates indicative of the ferumoxytol label were observed in the central grey mater/ventral horn of the pig with no laminectomy (A). However, no surviving human cells were observed (not shown). In the pig with previous laminectomy, blue iron precipitates were observed in the central white mater medial to the ventral horn (B). A representative photomicrograph showed a GFP+ pig neural progenitor cell graft in the medial white mater (C), which correlated to the region containing iron precipitates. Scale bars: 1 mm; (Inserts) 100 μm.