Hot-spot 19F imaging of stem cell transplantation into the intrathecal space in a large animal model

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Target audience: Physicians and scientists interested in image-guided stem cell treatment of spinal cord-related disorders.

Purpose: There is a continuous search for minimally invasive, yet efficient, methods of cell delivery to the central nervous system. The spinal cord is a particularly demanding target. Of the many disorders to be addressed, we are focusing on amyotrophic lateral sclerosis, with the pivotal respiratory function with majority of the muscles innervated by the cervical spinal cord. The intra-arterial route to the spinal cord is technically cumbersome and risky, while the intraparenchymal route is highly invasive, requiring laminectomy and needle puncture of the complex structure of the spinal cord. We have shown in small animal models that cerebrospinal fluid (CSF) may serve as a medium with which to target the spinal cord (Fig. 1A). In the clinical setting, CSF space may be accessed by the minimally invasive introduction of a catheter via lumbar puncture. However, that localization is highly challenging due to the uncertainty of cell distribution and the difficulties of proton imaging because of omnipresent magnetic field inhomogeneity. Fluorine imaging has been considered the method of choice, but due to relatively low sensitivity and deep location of the cervical intrathecal space large quantities of contrast agent are required for acceptable detection signal. The purpose of this study was to evaluate applicability of injectable ^{19}F-labeled hyaluronic acid hydrogel for targeted, image-guided intrathecal injection of stem cells.

Materials & Methods: Iron oxide labeling of stem cells: Mesenchymal stem cells were treated overnight with 20 µg/ml of Molday ION-Rhodamine B (BioPAL, Inc.). Prior to transplantation, cells were harvested and suspended in 10 PBS, or a HyStem hydrogel (Glycosan Biosystems), 1.0x10^6 cells/ml. Preparation of fluorine hydrogel: After the HyStem hydrogel components and the proper amount of fluorine were mixed, gelation occurs. Capillary tubes were used to determine the level of solidification. As the gelation process extended, less and less of the hydrogel composite could move up the capillary tube. Measuring the distance moved by the capillary enabled the creation of a gelation index, which was indicative of the process of solidification. Phantom imaging: ^{19}F emulsion V-sense (Celsense) was mixed with HyStem hydrogel at a ratio of 1:10 and the labeled hydrogel was placed in 5 mm NMR tubes for the ^{19}F-MRI experiments, performed on a vertical bore 17.6 T NMR spectrometer (Bruker) equipped with a Micro 2.5 gradient system. A 25 mm diameter ^{19}F-^{1}H/^{17}O volume coil was used for RF transmission and reception. A RARE sequence was used to acquire the ^{19}F-MRI images using the following parameters: TR/TE=5000/8 ms; slice thickness (ST) of 6 mm; FOV 2x2 cm; and a matrix of 64x64.
Cell suspension within a fluorine hydrogel: The harvested cells were suspended in a Hystem component of the hydrogel, and then, 30 minutes prior to transplantation, a cross-linker (Extralink) and fluorine were added at a proportion of 1:10, and the cell suspension was shaken to avoid precipitation.
Intrathecal transplantation: Under fluoroscopic guidance, the catheter was introduced *via* a lumbar puncture to the cervical intrathecal space. Then, the animal was moved to the MR scanner and pre-transplantation images were obtained. After cell injection, another set of MR images was obtained.
In vivo MRI on pigs was performed on a clinical 3T MRI scanner (TIM-Trio, Siemens). To detect iron oxide-labeled cells injected intrathecally on the cervical level, the standard SWI sequence was applied with the following parameters: TR/TE=2400/30 ms; 3 mm ST; FOV 73x93 cm; and matrix 240x256. For fluorine imaging, a custom ^{19}F 4-channel phased array surface coil and a TrueFISP sequence (TR/TE=3.7/1.85 ms; 5 mm ST; 32 NA; 21x25 cm FOV, Matrix 108x128) were used. For anatomical reference, a sagittal T2w-TSE sequence (TR/TE=2400/12 ms; 3 mm ST; 35 echo train) was applied with geometry identical to the ^{19}F scan.
Ex vivo MRI of the spinal column explant was performed on a horizontal 11.7T Bruker Biospect MRI scanner equipped a 20mm ^{19}F/^{1}H double-tuned transceiver surface coil. A RARE sequence was used to acquire ^{19}F MRI images with the following parameters (TR/TE=1600/7.5 ms, ST=1mm, FOV=20x38 mm, and a matrix of 64x64). For anatomical reference, a T2w RARE sequence (TR/TE=5000/10 ms, ST=1mm, FOV=20x38 ,and a matrix of 128x128) was used.

Results: We showed that fluoroscopy guided placement of a catheter in the cervical intrathecal space, after its introduction by lumbar puncture is feasible. Susceptibility weighted MRI for detection of iron oxide-labeled cells failed to visualize any hypointense signals when cells were injected as suspension in saline (Fig.1B, C) indicating their untargeted dispersion within the intrathecal space. Injection of iron oxide-labeled stem cells suspended in the hydrogel resulted in new hypointensities (Fig. 1D, E, arrowheads), but the images were of poor quality due to magnetic field inhomogeneity within the intrathecal space. To achieve the high fluorine signal we used the advantage of hydrogel application for loading with ^{19}F nanoparticles by mixing the ^{19}F emulsion and the hydrogel components at various ratios, and then we selected the conditions providing the highest contrast load without the compromise of the gelation process. We have also verified ^{19}F signal in a phantom experiment (Fig. 1F). We then performed a study with intrathecal stem cell injection suspended in optimized ^{19}F hydrogel in pigs and demonstrated that following placement of the catheter as shown on an X-ray image (Fig. 1G) distribution of injected hydrogel can be precisely visualized using ^{19}F MRI (Fig. 1H). Detailed distribution of the ^{19}F labeled hydrogel was further confirmed by *ex vivo* MRI (Fig. 1I).

Conclusion: The use of stem cells suspended in a fluorinated hydrogel enables their very precise deployment within the cervical intrathecal space. Since fluorine nanoparticles are a clinical-grade product, the method can be directly transferred to the clinical setting.

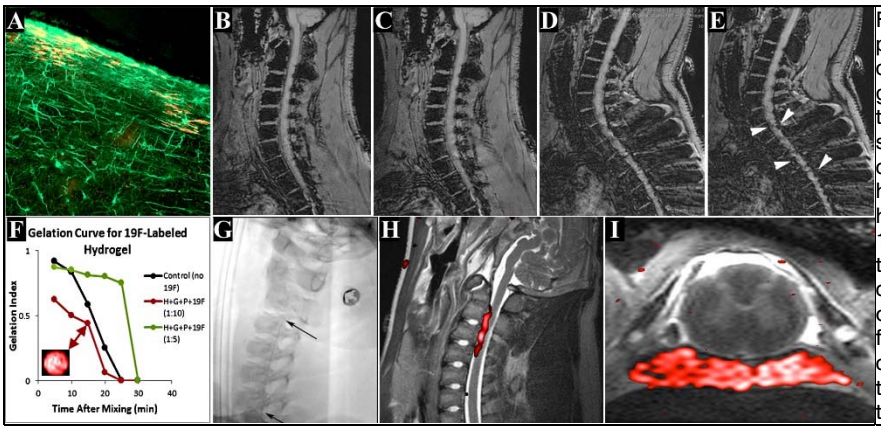

Fig. 1 Fluorine imaging within the intrathecal space. A) Glial progenitor cells migrating from intrathecal space to the spinal cord in mice as a proving feasibility of this delivery route – green color indicates transplanted cells. B) Pre- and C) post-transplantation images in pigs with stem cells suspended in saline. D) Pre- and C) post-transplantation images of stem cells suspended in hydrogel (arrowheads indicate new hypointensities due to injected cells. F) Gelation curve of the hydrogel depending on the Gel/19F emulsion ratio, inset is a 19F MRI of the phantom at a ratio of 10:1 (arrow). G) X-ray of the neck with the upper arrow pointing to the position of the catheter tip within the cervical intrathecal space. H) 19F MRI overlaid on T2 scan. The red color indicates the "hot-spot" fluorine signal of the hydrogel on the *in vivo* MRI. It corresponds topographically to the position of the catheter tip. I) The *ex vivo* MRI demonstrates details of the location of the fluorinated hydrogel inside the intrathecal space.