Fluorine imaging has been considered the method of choice, but due to relatively low sensitivity and deep location of the cervical intrathecal space large 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 19F-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 cross-linker from 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: 19F 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 19F-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 19F-/1H- O volume coil was used for RF transmission and reception. A RARE sequence was used to acquire the 19F-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.

**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 hypointensities 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 19F nanoparticles by mixing the 19F 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 19F signal in a phantom experiment (Fig. 1F). We then performed a study with intrathecal stem cell injection suspended in optimized 19F 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 19F MRI (Fig. 1H). Detailed distribution of the 19F 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.