In vivo trimodal ultrasound, photoacoustic, and magnetic resonance imaging to guide and monitor stem cell therapies in the spinal cord

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Background, Motivation and Objective

Stem cell therapy is a promising option to treat amyotrophic lateral sclerosis (ALS), the most common adult-onset neurodegenerative disease of motor neurons in the spinal cord. However, several factors are inhibiting clinical translation: 1) intra-operative real-time guidance of the stem cell injection; and 2) postoperative stem cell tracking. Thus, we have synthesized photomagnetic nanocubes (PMNCs) to safely label stem cells for intraoperative, ultrasound/photoacoustic (US/PA) image-guided delivery of stem cells and postoperative US/PA/MRI tracking of stem cells *in vivo*.

Statement of Contribution/Methods

PMNCs (Fig. 1A) with optical and magnetic properties were produced in-house via a seed-mediated method. Mesenchymal stem cells (MSCs) were labeled with PMNCs (PMNC-MSCs) by incubation in cell culture for 24 h (Fig. 1B). PMNC-MSCs were suspended at 10k cells/μl, a clinically relevant concentration. Per the clinical procedure, a lumbar laminectomy was performed in nude rats to remove the bone and expose the underlying spinal cord. PMNC-MSCs (5 μl) were injected directly into the spinal cord (Fig. 1C). The muscle and skin were sutured back over the spinal cord, but the bone was not replaced. US/PA images were acquired using the Vevo LAZR or Phocus Mobile (20 MHz; 680-970 nm wavelength). T2-weighted MR images (TE = 33 ms; TR = 4250 ms) were acquired using a Bruker Pharmascan 7T with a 60 mm coil. *In vivo* images were acquired at 3, 5, 7, and 10 days post-injection.

Results/Discussion

Phantom studies confirmed PMNC-MSCs produced PA and MR contrast, with detection limits of ~80 and ~300 cells/ μl, respectively. In intraoperative studies, US depicted spinal cord anatomy (Fig. 1D), while PA imaging (750 nm) visualized needle placement (Fig. 1E) in real-time to guide the injection (Fig. 1F), which could improve procedure safety and efficacy. The average PA signal increased with injection volume (Fig. 1G), indicating potential for quantitative feedback. At 10 days post-injection, US/PA and MR images still detected PMNC-MSCs (red arrows), and good agreement was observed between modalities (Fig. 1H – K). Our results demonstrate the ability of US/PA/MRI to assist clinicians and researchers throughout treatment by providing a customizable tool for intraoperative guidance and postoperative, longitudinal monitoring of stem cells in the spinal cord.

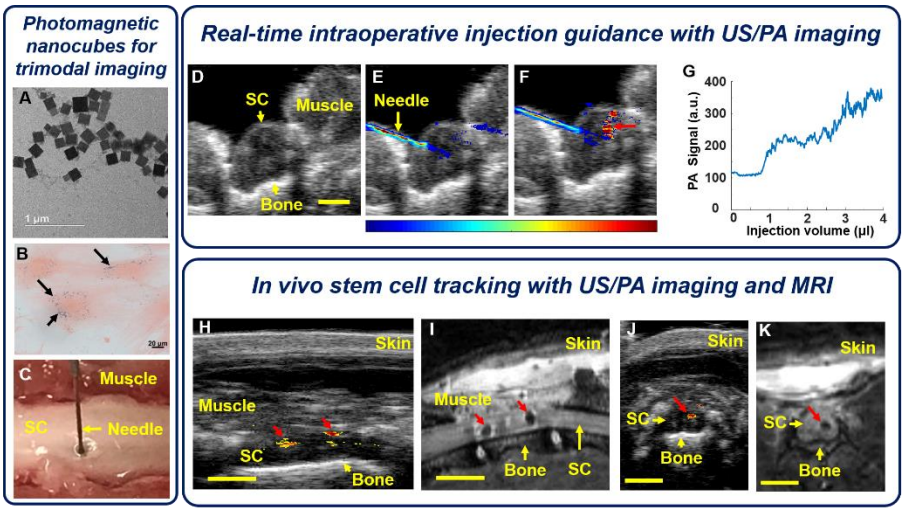

Figure 1. TEM of PMNCs (A). Histology of PMNC(blue)-MSCs (eosin stained) (B). Photo of the surgical procedure, per the clinical protocol (C). SC = spinal cord. Intraoperative image-guidance, *ex vivo* (D - G). US showed anatomy, but the needle was not visible (D). PA imaging guided needle placement (D) and PMNC-MSC (red arrows in all panels) delivery in real-time (E). PA signal increased with PMNC-MSC injection volume (G). *In vivo (H – K) US/PA* (H, J) and MR (I,K) images at 10 days post-injection. Sagittal (H, I) and axial views (J, K). Scale bar = 3 mm.