An effective strategy of magnetic stem cell delivery for spinal cord injury therapy

Dmitry Tukmachev,[ab] Oleg Lunov,[*c] Vitalii Zablotskii,[c] Alexandr Dejneka,[c] Michal Babic,[d] Eva Sykova,[ab] Sarka Kubinova,[*ab]

[a]Institute of Experimental Medicine, ASCR, Prague, Czech Republic

[b]Department of Neuroscience, 2nd Faculty of Medicine, Charles University, Prague, Czech Republic

[c]Institute of Physics, ASCR, Prague, Czech Republic

[d]Institute of Macromolecular Chemistry, ASCR, Prague Czech Republic

Supplementary Information

1. Cell preparation

The preparation of GFP-positive mesenchymal stem cells (MSCs) and labelling with poly-*L*-lysine-coated SPION was done in accordance to previously published methods.[1, 2] To summarise, MSCs were obtained from 4-week-old green fluorescent protein transgenic Sprague-Dowley rats, TgN (acro/act-EGFP) 4Osb. The animals were deeply anesthetized, the femurs and tibias were dissected and the bone marrow was plated on Petri dishes in medium containing DMEM (PAA Laboratories GmbH,Pasching,Austria), 10% FBS (PAA Laboratories GmbH, Pasching, Austria), and Primocin™ (100 μg mL^{-1}) (Lonza Cologne AG, Koln, Germany). Cells were allowed to adhere; non-adherent cells were removed after 48 days by replacing the medium. Adherent cells were cultivated at 37 °C in a humidified atmosphere containing 5 % CO_2, and the medium was changed twice a week. After reaching near-confluency, the cells were harvested by a trypsin/EDTA solution. After 2-3 passages, the cells were labelled with SPION and transplanted into the animals.

2. Cell labelling with SPIONs

Poly-*L*-lysine-coated (PLL) SPIO nanoparticles were used in this study for cell labelling. The nanoparticles were prepared and characterized as described elsewhere.[2] The diameter of the dry-state particles measured by TEM was 6.2 nm. The hydrodynamic diameter of the used particles, measured by dynamic light scattering, was D_h = 141 ± 10 nm, PDI = 0.52 ± 0.1, zeta potential was ζ = 47 ± 4 mV. The saturation magnetization of neat particles $M_s \sim 70$ A·m^2·kg^{-1} was obtained by measuring the magnetization curves with SQUID magnetometer. Because the weight of the coating agent is 1 % of the weight of iron oxide, the reduction of M_s by coating can be omitted. The coating of maghemite nanoparticles surface of with PLL was investigated using a Thermo Nicolet Nexus 870 FTIR spectrometer (Madison, WI, USA) in an H_2O-purged environment with DTGS (deuterated triglycine sulfate) detector. The Golden Gate single-reflection ATR system (Specac Ltd., Orpington, Great Britain) was applied to measure the ATR spectra of powdered samples over a wavenumber range 400–4000 cm^{-1}. Typical parameters were as follows: 256 sample scans, resolution 4 cm^{-1}, Happ-Genzel apodization, KBr beamsplitter. The samples for measurements were prepared by freeze-drying of PLL-coated nanoparticles purified by multiple centrifugation and redispersion in ultrapure water (four times at 14 000 rpm for 1 h).

Cultures of MSCs were incubated with SPION (50 μL per 10 mL of culture medium, i.e., 15.4 μg of iron per 1 mL media) 72 hours prior to the experiments. After this, the nanoparticles were washed out and the labelled cells were implanted into the animals.

3. Balloon-induced compression lesion model

The use of animals in this study was approved by the ethics committee of the Institute of Experimental Medicine AS CR (Prague, Czech Republic). A balloon compression lesion was performed in a total of 15 male Wistar rats (280-400 g). In short, the animals were anesthetized with 2 % isoflorane (Forane®, Abbott Laboratories, Queenborough, Great

Britain) and shaved on the back from *C7* to *Th1*. Under sterile conditions the skin was cut in the midline from *Th7-Th12*. The soft tissue was removed, as well as the spinous processes of vertebrae *Th8-Th11*. A catheter was filled with saline and connected to a Hamilton syringe. The catheter was inserted into the epidural space and advanced cranially for 1 cm, so that the centre of the balloon rested at the *Th8-Th9* level of the spinal cord. The balloon was rapidly inflated with 15 μL of saline for 5 min. The catheter was then deflated and removed.

4. Cell transplantation

One week after the induction of the lesion, 5 x 10^5 cells in 50 μL of phosphate-buffered saline were injected intrathecally at the *L5-L6* level, at a distance of 10 cm from the lesion site (Fig. 1A and B). Subsequently, as was described in the main manuscript, the external magnetic system was placed around the rat, under the top of the vertebral column at *Th8–Th9* above the lesion site, for 2 h to improve cell retention and attachment (Fig. 1A and B). Three groups of animals were used with five animals in each group; SPION-labelled MSCs exposed to MF, non-labelled MSCs exposed to MF and SPION-labelled MSCs without exposure to MF. Cell quantification was performed 24 hours after cell transplantation in longitudinal sections.

5. Histology and image analysis

Twenty-four hours after cell transplantation, the animals were intracardially perfused under deep anesthesia (pentobarbital 150 mg kg^{-1}; Sigma, St Louis, MO) with 4 % paraformaldehyde in 0.1 M PBS. The spinal cords were dissected and histologically processed. Cell quantification was performed in longitudinal sections (20 μm) using a fluorescent microscope (Carl Zeiss, Rochester, NY). GFP-positive MSCs were counted in 360 μm thick upper segments of an area 36 mm^2 (18 × 2 mm), with the epicentre of the lesion in the centre of the segment. Image quantifications were performed using ImageJ software (NIH). Cell number was calculated by normalizing corrected total cell fluorescence (CTCF) of the full area of interest to average fluorescence of a single cell. The net average CTCF

intensity of a pixel in the region of interest was calculated for each image utilizing a previously described method.[3] The region placed in an area without fluorescent objects was used for background subtraction. CTCF was determined as the sum of pixel intensity for a single image with the subtracted average signal per pixel for a region selected as the background. Averages of normalized intensity values of at least 10 morphologically indentical cells were calculated to determine the mean fluorescence of a single cell.

6. Cell staining

In order to visualize the SPION labelling in cell culture, cells were fixed in paraformaldehyde and phosphate buffer (PBS) for 15 min, washed with 0.1M PBS and then stained for iron using potassium ferrocyanide (Lachema, Brno, Czech Republic) to produce ferric ferrocyanide (Prussian blue) according to a standard staining protocol. To detect the infiltration of macrophages in the lesion, immunofluorescent staining for CD68 (ED1) (Serotec, Oxford, UK) was performed.

7. Mathematical modelling

The magnetic field and force distributions were calculated with the help of the explicit analytical expressions for magnetic field induction generated by a cylindrical permanent magnet, magnetized along its symmetry axis. For homogeneously magnetized cylinder of the radius, a and length L, the axial (B_z) and radial (B_ρ) components of the magnetic field induction can be calculated as[4]:

$$B_z = -\frac{\mu_0}{4\pi} M \int_0^{2\pi}\int_0^{a}\left(\frac{R(L/2-z)}{(R^2+(L/2-z)^2+\rho^2-2R\rho\cos\Phi)^{3/2}}+\frac{R(L/2+z)}{(R^2+(L/2+z)^2+\rho^2-2R\rho\cos\Phi)^{3/2}}\right)dRd\Phi \qquad (1)$$

and

$$B_\rho = -\frac{\mu_0}{4\pi} M \int_0^{2\pi}\int_0^{a}\left(-\frac{R(2\rho-2R\cos\Phi)}{2(R^2+(L/2-z)^2+\rho^2-2R\rho\cos\Phi)^{3/2}}+\frac{R(2\rho-2R\cos\Phi)}{2(R^2+(L/2+z)^2+\rho^2-2R\rho\cos\Phi)^{3/2}}\right)dRd\Phi \qquad (2)$$

where Φ is the azimuthal angle, z is the coordinate along the symmetry axis of a cylinder, ρ is the radial coordinate, M is the remanent magnetization and μ_0 is the permeability of free space. The vector field of the magnetic gradient shown in Fig. 2 was calculated using equations.1 and 2.

8. Statistical Analysis

The statistical significance of differences in cell counts in the spinal cord lesions between the groups was determined using ANOVA Fisher's LSD and Newman-Keuls tests. Differences were considered statistically significant statistically at $*P < 0.05$.

References

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