Long term VSOP labeling:
Human adult progenitors differentiate into mature neurons

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Magnetic resonance imaging (MRI) is a unique non-invasive technique to monitor *in vivo* procedures with non-limited depth penetration. High-resolution imaging requires a sufficient cytoplasmatic incorporation of magnetic contrast agents into the cells. Commonly used and safe contrast agents are very small superparamagnetic iron oxide particles (VSOPs). However the effects of VSOP incorporation on stemness and neuronal differentiation potential of human adult neural stem cells (haSCs) remain unknown, providing essential information for regenerative-medicine-based treatments of CNS disorders.

CULTURING AND MAGNETIC LABELING

Human adult hippocampal progenitor cells (haSCs) were obtained from a patient with temporal lobe epilepsy submitted to epilepsy surgery [1]. Mouse embryonic stem cells (eSCs, CRL-1934, ATCC, Manassas, USA) were co-cultured as described in [2].

Cells were magnetically labeled using very small superpara-magnetic iron oxide particles (VSOPs, C200, Ferropharm, Teltow, Germany). These sterile VSOPs consist of a 5 nm iron oxide core coated by monomer citrate yielding a diameter of 9 nm.

haSCs and eSCs were magnetically labeled with 0.5 mM VSOPs for 4 hours [3]. The uptake of VSOPs was evaluated using Prussian blue staining and phase contrast microscopy. The rate of positive cells was determined as the proportion of cells with blue cytosolic particles compared to the total number of cells identified by red nuclear counterstaining.

For neuronal differentiation, cells were cultured in differentiation media containing SHH, FGF-8, and ascorbic acid. Cells were differentiated for 15 days.

HISTOLOGICAL ANALYSIS OF VSOP-LABELED haSCs

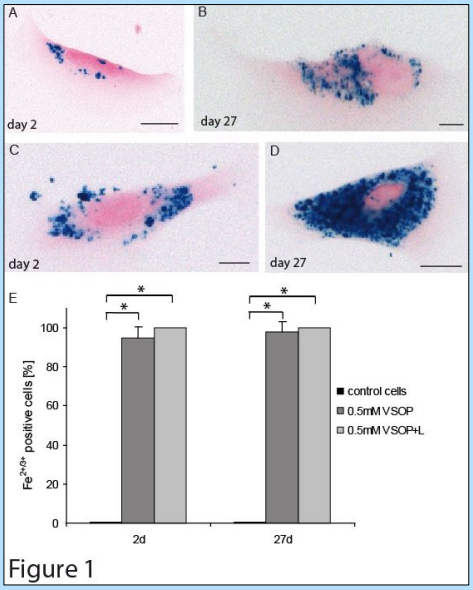

Figure 1:
Intracytoplasmatic VSOP uptake into haSCs following 4h incubation with 0.5 mM VSOPs. Cells were fixed with 4 % PFA and iron was visualized using Prussian blue staining on day 2 (A, C) and on day 27 (B, D) after labeling. (A+B) Fixed and stained cells after incubation with 0.5 mM VSOPs, (C+D) lipofection with 0.5 mM VSOPs. Scale bar is 10µm. (E) Intracytoplasmatic VSOP uptake into 90-100% of human neural progenitor cells. Data were analyzed and presented as means ± s.e.m. Differences were considered significant at $P < 0.01$.

TRANSCRIPTIONAL ANALYSIS OF STEMNESS, NEURAL AND NEURONAL MARKERS

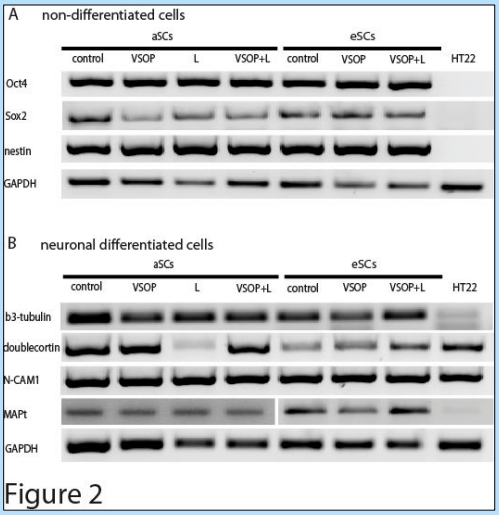

Figure 2: VSOP labeling and lipofection of non-differentiated stem cells; RT-PCR analysis of pluripotency markers revealed no impact on stemness (A). (B) Subsequent neuronal differentiation resulted in a neuronal phenotype expressing various neuronal markers as measured by RT-PCR. Mature mouse hippocampal cells HT22 served as a negative control for pluripotency markers and as a positive control for mature neuronal markers.

CONCLUSIONS

Application of an efficient labeling protocol resulted in a stable uptake of high amounts of magnetic contrast agent, even at low VSOP concentrations sufficient for *in vivo* applications.

Histological staining for iron-oxide revealed a stable vesicular incorporation for at least one month. By advising tailored labeling strategies, cellular viability remained unaffected, a prerequisite towards clinical application of this method.

Transcriptional analysis (RT-PCR) of pluripotency markers (Oct4, Sox2) revealed no impact of VSOP incorporation on stemness. Subsequent neuronal differentiation of the labeled populations resulted in a neuronal phenotype, expressing various neuronal markers (β3-tubulin, DCX, N-CAM1, MAPt) as measured by RT-PCR.

[1] Siebzehnrubl FA. *et al.*, *Exp. Brain Res*, 2007
[2] Bibel M. *et al., Nat. Neurosci.*, 2004.
[3] Stroh A. *et al., Mol. Imaging*, 2009.

This work was supported by the Bavarian State Ministry for Sciences, Research and the Arts, „ForNeuroCell“. Thanks to H. Pilgrimm and V. Ntziachristos.