Introduction
Matrix-based articular cartilage repair have revealed a great potential for cartilage defects (chondral or osteochondral defects). However, one major problem is still the non-invasive monitoring of cell behavior in vivo, especially the precise localization of the transplanted cells in the target tissue and the examination of proliferation and differentiation.

In this study, human mesenchymal stem cells (MSCs) were labeled with very small superparamagnetic iron oxide nanoparticles (VSOPs) (Fig. 1) and embedded in collagen type I hydrogels. These three-dimensional constructs were detected in vitro using high-resolution magnetic resonance imaging (MRI).

Materials and Methods
VSOP-labeled bone marrow derived MSCs were analyzed in vitro on their proliferation potential and induction of apoptosis compared to unlabeled control MSCs. Stability of labeling during expansion of cells was determined with the iron specific Prussian blue staining. Collagen-type-I-hydrogels (Arthrotek GmbH, Esslingen, Germany) with different concentrations of labeled and unlabeled MSCs were imaged in vitro with a Bruker Avance-500 MR spectrometer. MRI was performed at different time points using a 11.7 T high-field MR spectrometer. In all experiments 2D and 3D FLASH-sequences with echo times of 3 to 25 ms and repetition times of 30 to 800 ms were employed. Nominal spatial resolutions of up to (78 µm)² were achieved.

Results
1. Viability of MSCs after labeling
The measurements show no significant difference in viability between labeled and unlabeled MSCs. 48 hours after labeling these cells were tested by annexin V-Cy3 detection. As shown in Fig. 2A and B, no induction of apoptosis after labeling could be observed. Iron-specific Prussian blue staining revealed uptake of VSOPs in the cytoplasm of MSC (Fig. 2D).

2. Proliferation of labeled and unlabeled MSCs
There was only little or no inhibition of proliferation of labeled MSCs compared to the unlabeled controls. This was shown in growth curves (Fig. 3A + B) and WST proliferation assays (Fig. 3C). The small differences between the curves in Fig. 3B were proved by determination of cumulative population doubling during expansion of the cells over a longer period of time. As shown in Fig. 3D, in long-term cultivation no differences in proliferation could be observed. However, in cell culture a pronounced decrease of VSOP-particles/cell could be observed after 6-9 cell divisions. After embedding of magnetically labeled MSCs in collagen type I hydrogels, where no proliferation takes place, the cells could be detected with a MR spectrometer over more than 20 weeks (Fig. 4 + 5). Controls with unlabeled cells were also visible in the MR images, but didn’t exhibit the typical hypointensities in the vicinity of the VSOPs (Fig. 4A+B, neglecting image artifacts due to air bubbles). Furthermore, gels with low cell numbers (5x10⁵ cells/ml) were monitored (Fig. 4C+D).

Discussion
The results of our study reveal that MSCs labeled with VSOPs can be successfully detected in collagen type I hydrogels in vitro using high-field MRI. Further investigations have to show whether VSOP-labeling of MSCs have an influence on the differentiation of cells and how long after labeling cells can be visualized and monitored reliably in vivo.

References

MR imaging of VSOP labeled Human Mesenchymal Stem Cells in a Collagen Type I Hydrogel for cartilage repair

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