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Two novel amino acid-coated super paramagnetic nanoparticles at low concentrations label and promote the proliferation of mesenchymal stem cells

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We identified two amino acid-coated magnetic nanoparticles that promoted mesenchymal stem cell growth without the need for transfection agents by increasing the proportion of cells in the S phase. Because of the low concentration, high labelling efficiency and promotion of cell growth, these two nanoparticles may be potential labelling biomaterials for cell tracking.

Mesenchymal stem cells (MSCs) can proliferate and differentiate into various kinds of cells. Because of the low immunogenicity, easy acquisition and multilineage differentiation potential, MSCs have been reported to benefit cell therapy applications such as spinal cord injury [1], rheumatoid arthritis [2], heart disease and joint pain [3] in many clinical trials. Bone-marrow MSCs (BMSCs) have become promising sources for cell-based therapy to replace injured or dying cells with new and functional stem cells. However, the efficiency of BMSC transplantation is low because of low cell viability and cell tracking and an inappropriate environment, which results in high retention of donor cells and low engraftment and reduced transplantation efficiency [4]. Thus, an effective, non-invasive and non-toxic technique is needed to monitor the in vivo behavior of implanted BMSCs and understand the fate of these cells.

Many biological agents have been studied for cell tracking systems. Because of the small size but large surface-to-volume ratio, superparamagnetic properties and increased reactivity, superparamagnetic iron oxide (SPIO) has been shown to have good labelling materials for cell tracking [5]. However, many magnetic nanoparticles (NPs) have potential cytotoxicity in BMSCs, for a major obstacle with their use in cell-based therapy. To reduce the toxicity of magnetic materials, bare SPIO is usually coated by other agents, for example, organic or other biological targeted materials, metals and carbon. After the chemical modification, the toxicity in multicellular organisms is decreased and the complexity for toxicology evaluation is increased. Thus, surface-coated NPs with enhanced functions and high biocompatibility are needed.

In our previous studies, we synthesized 2 magnetic NPs (MNPs) based on an iron oxide (Fe_3O_4) core that were surface-coated with lysine or glycine (MNPs@Gly: Fe_3O_4@3-glycidoxypropyltrimethoxysilane@glycine; MNPs@Lys: Fe_3O_4@3-glycidoxypropyltrimethoxysilane@Lysine). The 2 NPs could efficiently remove several kinds of anionic and cationic dyes from wastewater under severe conditions [6, 7]. They reduced BMSC viability and induced cell apoptosis at high concentrations (MNPs@Gly: 15 μg/ml; MNPs@Lys: 50 μg/ml) [8]. However, we wondered about the effect of the MNPs at low concentrations and whether they could be used as labelling agents for cell tracking in BMSCs.

We first examined the cell viability after treatment with these 2 MNPs at low concentrations. Both MNPs@Gly and MNPs@Lys at 1 μg/ml increased the viability of BMSCs (Fig. 1A and C). At < 1 μg/ml, the MNPs might be not induce changes in protein in the signal pathway, such as phosphorylation, ubiquitination or protein level changes, and thus not increase cell viability. At > 1 μg/ml, other apoptosis pathways might be activated and induce BMSC apoptosis[8]. Therefore, the MNPs only at 1 μg/ml increased cell viability. Cell viability can be increased via proliferation promotion or death inhibition. Therefore, we examined the effect of the MNPs on BMSC apoptosis and necrosis. Cell morphology revealed no apoptotic or necrotic cells (Fig. 1B and D). To further confirm the cell apoptosis and necrosis, we used Hoechst 33258 staining and LDH assay. Normal and MNP-treated groups showed no cell nucleus condensation or fragments (Fig. 1B and D). LDH release in BMSCs did not differ between normal and MNP-treated groups (Fig. S1). From these results, we concluded that the increased cell viability induced by 1 μg/ml MNPs was not caused by death inhibition but rather promotion of cell growth. Thus, MNPs@Gly and MNPs@Lys at 1 μg/ml could promote the proliferation of BMSCs in vitro.

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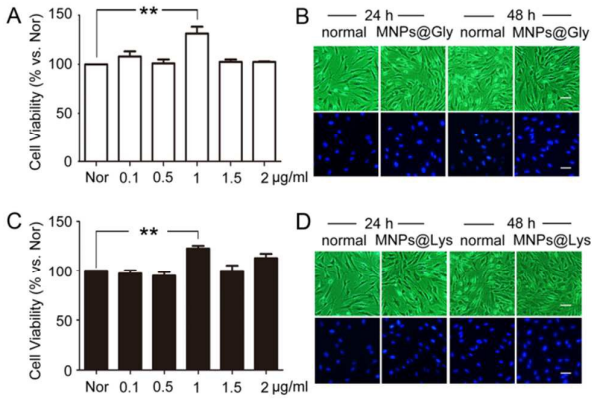

Fig. 1 Cell viability and morphological changes of bone-marrow mesenchymal stem cells (BMSCs) treated with MNPs@Gly and MNPs@Lys. (A) WST-8 assay of cell viability of BMSCs treated with 0.1, 0.5, 1, 1.5 or 2 μg/ml MNPs@Gly for 48 h. Data are mean±SEM percentage compared with the normal group (**P < 0.01 vs normal, n = 3). (B) Hoechst 33258 staining of morphological changes of BMSCs and changes in the cell nucleus with MNPs@Gly treatment at 1 μg/ml for 24 and 48 h. Scale bars: 20 μM. (C) WST-8 assay of cell viability of BMSCs treated with 0.1, 0.5, 1, 1.5 or 2 μg/ml MNPs@Gly for 48 h. Data are mean±SEM percentage compared with the normal group (**P < 0.01 vs normal, n = 3). (D) Hoechst 33258 staining of morphological changes of BMSCs and changes in the cell nucleus with MNPs@Lys treatment at 1 μg/ml for 24 and 48 h. Scale bars: 20 μM.

Currently, cell-based therapies use scaffolds to maintain cells for implantation for several purposes such as neural stem cells for cell replacement therapy in spinal cord injury[9] and stroke-damaged rat brain[10]. However, these scaffolds are inorganic materials and do not have any biological activity, so they may function as barriers for bone or tissue regeneration. In this regard, MSC transplantation without a scaffold is an ideal approach, and cell transplantation without scaffolds is used more for repair [11, 12]. Additionally, the use of minimally invasive technology is becoming popular. Therefore, the efficiency of cell transplantation must be improved by injection of a small quantity of BMSCs instead of a large numbers of cells. However, cell-based therapies require a certain number of cells, because in clinical trials, most BMSCs function by secreting growth factors. Thus, the treatment effect would be improved if the NPs could also promote BMSC proliferation after labelling the cells. Most synthesized SPIO used for labelling cannot induce MSC proliferation. These NPs have no inhibitory effect on proliferation of MSCs from different sources but cannot increase cell viability [4, 13-17]. An amine-surface-modified SPIO at 50 μg/ml could induce MSC proliferation and label cells efficiently [18]. As well, ferucarbotran, an ionic SPIO could increase MSC growth at 30 μg/ml [19]. Although these NPs could induce cell proliferation, the concentrations used were much higher than we used, for better superiority in cell labelling.

Because of their characteristics, many SPIO NPs are difficult to be phagocytized into cells. To increase the efficiency of intracellular SPIO uptake, transfection agents (TAs) were used, including lipofectamine, superFect, poly-L-lysine (PLL) and protamine [20]. However, the TA could decrease the migration capacity and colony-formation ability [5]. Therefore, using NPs directly without TA in clinical trials is preferred. In our study, about 90% of BMSCs were labelled after treatment with the 2 MNPs at 1 μg/ml without any TA (Fig. 2B). Many coated SPIO NPs have been synthesized for use as good labelling materials for cell tracking. The labelling efficiency of aminopropyltriethoxysilane-modified SPIO was about 80% after treatment with human MSCs (hMSCs) for 24 h at 12.5 μg/ml combined with the TA polyamine PLL hydrobromide [15]. In another report, several sodium hyaluronate and dopamine-coated SPIO NPs were synthesized. The average labelling efficiency was from 61% to 71%. The highest percentage of labelled rat MSCs was just over 90% [21]. Additionally, the labelling efficiency of an amine surface-modified SPIO was > 95% in hMSCs. However, the concentration used in that study was 25 μg/ml[18]. Our Prussian blue staining revealed that about 90% of BMSCs were labelled after treatment with the 2 MNPs at 1 μg/ml without any TA. Thus, compared with other reported SPIO NPs, the labelling efficiency of our 2 MNPs is fairly high. Both NPs were distributed in the cytoplasm and around the nucleus (Fig. 2A). No clumps were found adhering to the cell surface or the bottom of the culture well. Thus, MNPs@Gly and MNPs@Lys are potential labelling biomaterials for cell tracking.

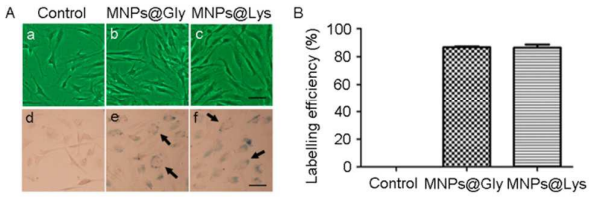

Fig. 2 Labelling efficiency of 1 μg/ml MNPs in BMSCs for 24 h. Control: BMSCs not treated with any MNPs; MNPs@Gly: cells treated with 1 μg/ml MNPs@Gly; MNPs@Lys: cells treated with 1 μg/ml MNPs@Lys. (A) (a-c) Phase-contrast microscopy of morphological changes of BMSCs. (d-f) Prussian blue staining of increased Fe accumulation in BMSCs. Scale bar: 20 μM (arrows show the positive-stained cells). (B) Quantitative analysis: about 90% of BMSCs were efficiently labelled at 1 μg/ml NPs (n=3).

The level of Fe is related to the cell cycle progression. Decreased Fe level in cells can lead to the cell cycle stopping at the G1/S phase [19]. Because 2 coated NPs could increase cell

viability, we next used PI staining and flow cytometry to examine cell cycle distribution. Treatment with MNPs@Gly and MNPs@Lys for 24 h significantly increased cells in the S phase, which suggested that the MNPs accelerated the cell cycle (Figs. S2 and S3). Cells treated with naked uncoated Fe_3O_4 MNPs showed no increase in proportion of cells in the S phase. Therefore, MNPs@Gly and MNPs@Lys promoted cell growth by regulating cell cycle progression. Further investigations are needed to show which genes or proteins are involved in the cell-cycle progression modulation induced by the 2 MNPs.

Conclusions

Based on our previous work, we investigated the effects of the amino acid-coated magnetic NPs MNPs@Gly and MNPs@Lys on BMSCs at low concentrations. Both MNPs@Gly and MNPs@Lys at 1 μg/ml could promote the proliferation of BMSCs in vitro by increasing the proportion of cells in the S phase. The labelling efficiency of these two NPs in BMSCs at 1 μg/ml was high without any TA. Because of the low concentration, high labelling efficiency and promotion of cell growth, MNPs@Gly and MNPs@Lys could be potential labelling biomaterials for cell tracking.

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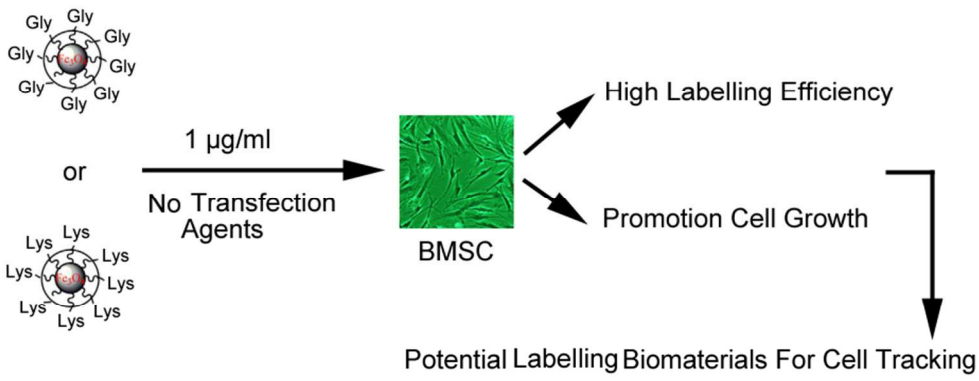

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