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Review

Magnetic cell delivery for the regeneration of musculoskeletal and neural tissues

Naosuke Kamei [a, b], Nobuo Adachi [a], Mitsuo Ochi [c, *]

[a] Department of Orthopaedic Surgery, Graduate School of Biomedical & Health Sciences, Hiroshima University, Hiroshima, Japan
[b] Medical Center for Translational & Clinical Research, Hiroshima University Hospital, Hiroshima, Japan
[c] President of Hiroshima University, Higashihiroshima, Japan

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ABSTRACT

Magnetic targeting is a cell delivery system using the magnetic labeling of cells and the magnetic field; it has been developed for minimally invasive cell transplantation. Cell transplantation with both minimal invasiveness and high efficacy on tissue repair can be achieved by this system. Magnetic targeting has been applied for the transplantation of bone marrow mesenchymal stem cells, blood CD133-positive cells, neural progenitor cells, and induced pluripotent stem cells, and for the regeneration of bone, cartilage, skeletal muscles, and the spinal cord. It enhances the accumulation and adhesion of locally injected cells, resulting in the improvement of tissue regeneration. It is a promising technique for minimally invasive and effective cell transplantation therapy.

Contents

1. Introduction

Magnetic cell labeling has widely been used for the isolation of specific cell populations or *in vivo* cell tracking using magnetic resonance imaging (MRI) [1,2]. Recently, a delivery system of magnetically labeled cells, termed magnetic targeting, was developed for minimally invasive and effective cell transplantation [3,4]. In this system, magnetically labeled cells were locally injected into the body and controlled using an external magnetic field. This

Abbreviations: MRI, Magnetic resonance imaging; MSC, mesenchymal stem cell; SPIO, superparamagnetic iron oxide; iPS, induced pluripotent stem; T, tesla; IKDC, International Knee Documentation Committee; KOOS, Knee Injury and Osteoarthritis Outcome Score.

* Corresponding author.
E-mail address: ochim@hiroshima-u.ac.jp (M. Ochi).

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system prevents the diffusion of transplanted cells and promotes the accumulation and adhesion of the transplanted cells to the damaged tissue. This system achieves both minimal invasiveness of cell transplantation and high efficacy for regenerative therapy. Magnetic targeting has been applied for the transplantation of bone marrow mesenchymal stem cells (MSCs), blood CD133-positive cells, neural progenitor cells, and induced pluripotent stem (iPS) cells for the regeneration of bone, cartilage, skeletal muscles, and the spinal cord in animal models or a clinical trial [5–14].

2. Magnetic labeling of cells

In an early stage of development, rat neural progenitor cells were magnetically labeled with mono-sized magnetic beads composed of styrene-acryl polymers as a core coated with magnetic ferrite thin film (diameter: 300 nm, Ferri sphere 100C, Nippon Paint, Tokyo, Japan) [14,15]. In the second step, superparamagnetic iron oxide (SPIO) nanoparticles were used for the magnetic cell labeling as clinically applicable agents [16]. SPIO nanoparticles have been used as contrast agents for MRI [17]. In addition, they have also been used for cell labeling in *in vivo* cell tracking using MRI [18]. Ferucarbotran is a clinically approved SPIO-coated carboxydextran with a diameter of about 45–60 nm [19]. Ferucarbotran was used for the magnetic labeling of bone marrow MSCs. In a preclinical study using a mini-pig, SPIO particles were incorporated into the cytoplasm of cultured bone marrow MSCs after a 12-h incubation with ferucarbotran-containing culture medium (97.5 μg Fe/mL, Bayer Healthcare Co Ltd, Osaka, Japan) and protamine sulfate (5 μg/mL, Mochida Seiyaku Ltd, Tokyo, Japan) [7]. In a clinical trial, ferucarbotran (49 μg Fe/mL, Resovist®; FUJIFILM RI Pharma Co. Ltd., Tokyo, Japan) was used for the magnetic labeling of human bone marrow MSCs without protamine sulfate [13]. In an animal study using a nude rat and a human iPS cells, the iPS cells were magnetically labeled using ferucarbotran (98.2 μg Fe/mL, Resovist®) without protamine sulfate [12]. In another study, CD133-positive cells isolated from human peripheral blood using antibody-coupled magnetic beads and magnetic cell sorting were utilized for the magnetic targeting [10].

3. Magnetic targeting in animal models

3.1. Bone

Oshima et al. have shown the efficacy of magnetic targeting of bone marrow MSCs using a rabbit forelimb bone defect model. In this study, a rabbit forelimb bone defect was bridged with an artificial bone having interconnected porous hydroxyapatite. Magnetic targeting of bone marrow MSCs enhanced the infiltration of MSCs into the artificial bone and bone formation [6]. Kodama et al. have also demonstrated the enhancement of bone formation in a non-healing femoral fracture rat model using the magnetic targeting of bone marrow MSCs. Magnetically labelled and luciferase-positive bone marrow MSCs were injected into the bone fracture site in the presence or absence of a magnetic field. As observed using *in vivo* bioluminescence imaging, magnetic targeting was found to enhance the proliferation and survivability of the transplanted MSCs. Radiological and histological evaluations showed that magnetic targeting improved bone repair four and eight weeks after treatment [20].

3.2. Articular cartilage

The intra-articular injection of bone marrow MSCs has been reported to improve the repair of osteochondral defects of the knee [21]. Kobayashi et al. have applied the magnetic targeting of bone marrow MSCs for articular cartilage repair. Magnetically labeled bone marrow MSCs were injected into a rabbit knee joint with an osteochondral defect in the patella. Magnetic targeting markedly enhanced the accumulation of injected MSCs into the osteochondral defect site. The accumulation of injected MSCs was also observed via arthroscopic evaluation using a swine patella osteochondral defect model [5]. Magnetic targeting of human MSCs in human osteochondral tissue was also examined *in vitro*. Bone marrow aspirates and degenerated osteochondral tissues of the knee were collected during the surgery for total knee arthroplasty, with the acknowledgment of the ethical committee of Hiroshima University. Osteochondral fragment pieces were attached to the sidewall of a tissue culture flask. Magnetically labeled human MSCs in cultured medium were then poured into the flasks in the presence or absence of a magnetic field. Magnetic targeting enabled the formation of a magnetically labeled cell layer on the osteochondral tissues [22]. Using a rabbit model, Mahmoud et al. have demonstrated that the magnetic targeting of bone marrow MSCs showed efficient cartilage repair even in case of a severe chronic osteochondral defect [23]. Kamei et al. have reported the magnetic targeting of bone marrow MSCs in a mini-pig patella cartilage defect model using a bulk superconducting magnet system. Magnetically labeled mini-pig bone marrow MSCs retained their chondrogenic differentiation potential. *In vitro* studies showed that the adhesion rate of the MSCs transplanted to the cartilage defect site was increased by the magnetic targeting, compared to the case for the gravity adhesion technique. Additionally, the adhesion rate of the transplanted MSCs was similar in four different magnetic flux densities and exposure times (0.6 T (T), 10 min; 0.6 T, 60 min; 1.5 T, 10 min; 1.5 T, 60 min). An *in vivo* study demonstrated that magnetic targeting promoted the accumulation of transplanted MSCs into the cartilage defect site and cartilage repair, compared to the case for the gravity adhesion technique of MSCs or non-treatment [7]. For safety evaluation, the *in vivo* kinetics of transplanted MSCs after the injection of magnetically labeled MSCs into the knee joint in the presence or absence of a magnetic field was examined using human bone marrow MSCs and a nude rat knee osteochondral defect model. Assessments using *in vivo* and *ex vivo* fluorescent imaging, immunofluorescent staining and reverse transcription-polymerase chain reaction revealed that transplanted MSCs were not present in any major organs (brain, heart, lungs, liver, kidneys, spleen) after intraarticular administration, regardless of magnetic targeting [24].

The magnetic targeting of iPS cells has also been utilized for articular cartilage repair. Magnetically labeled human iPS cells embedded in collagen hydrogel were transplanted into the osteochondral defect site in the knee of a nude rat in the presence or absence of a magnetic field. Tumor formation was observed in the knee joint after the transplantation of iPS cells in the absence of a magnetic field. However, the magnetic targeting of iPS cells improved the histological cartilage repair, without tumor formation [12].

3.3. Skeletal muscle

Natsu et al. have reported that the transplantation of rat bone marrow MSCs into the skeletal muscle injury site enhanced muscle repair [25]. Nakabayashi et al. have applied the magnetic targeting of bone marrow MSCs for the treatment of a skeletal muscle injury model. Magnetically labeled bone marrow MSCs isolated from luciferase transgenic rats were injected into the injured tibialis anterior muscle in the presence or absence of a magnetic field. As shown by bioluminescence imaging, magnetic targeting enhanced the *in vivo* proliferation of the transplanted MSCs within 72 h after transplantation. Histological and mechanical assessments revealed that the magnetic targeting improved the repair of injured skeletal muscles [9]. In the magnetic targeting of bone marrow MSCs, the

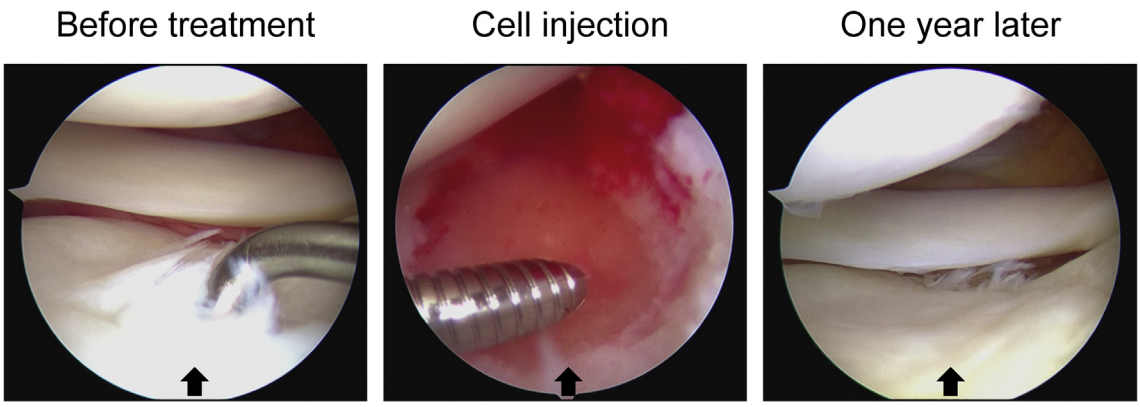

Fig. 1. Arthroscopy at one year after surgery showed complete coverage of a cartilage defect.

optimum strength and time of exposure to the magnetic field for cell retention at the transplantation site and muscle repair were investigated using human bone marrow MSCs and a nude rat muscle injury model. The optimum magnetic field conditions were examined using a combination of one of three magnetic flux densities (0.6 T, 1.5 T, and 3 T) and one of three exposure times (1 min, 10 min, and 60 min). A magnetic flux density of 1.5 T and an exposure time of 10 min were efficient conditions to induce the retention of transplanted cells and muscle repair [26].

Shi et al. have reported that the transplantation of human peripheral blood CD133-positive cells having angiogenic potential enhanced the repair of skeletal muscles [27]. CD133-positive cells are isolated using the CD133 antibody coupled to magnetic beads and magnetic cell sorting. Therefore, CD133-positive cells, have a magnetic attraction property. Ohkawa et al. have applied the magnetic targeting of CD133-positive cells for skeletal muscle repair. Human peripheral blood CD133-positive cells were injected into the injured tibialis anterior muscle of a nude rat in the presence or absence of a magnetic field. Magnetic targeting of CD133-positive cells enhanced angiogenesis and myogenesis at the injury site, resulting in the improvement of histological and mechanical muscle repair [8].

3.4. Spinal cord

Neural progenitor cells were reported to enhance axon growth in organ cultures of the brain cortex and spinal cord through the release of neurotrophic factors [28,29]. Magnetic targeting enhanced the effect of neural progenitor cells on axon growth [14].

Bone marrow MSCs have been used for the treatment of spinal cord injury in animal models and clinical trials [30,31]. Satake et al. have reported the migration of bone marrow MSCs injected into the subarachnoid space of the lumbar spine to the injured thoracic spinal cord [32]. Magnetic targeting could be used for promoting the migration of MSCs injected into the subarachnoid space of the lumbar spine to the injured thoracic spinal cord in a rat model [16]. In addition, the magnetic targeting of MSCs improved the functional recovery after spinal cord injury in a rat model [11].

Peripheral blood CD133-positive cells have also been used for the repair of injured spinal cord [33]. *In vivo* whole animal imaging revealed that the magnetic targeting of human peripheral blood CD133-positive cells injected into the subarachnoid space of the lumbar spine promoted the migration of transplanted cells to the injured thoracic spinal cord. The magnetic targeting of CD133-positive cells enhanced axon growth and functional recovery after spinal cord injury [10].

4. Clinical trial

The magnetic targeting of autologous bone marrow MSCs in 5 patients with a focal articular cartilage defect in the knee was approved by the Ministry of Health, Labour, and Welfare of Japan. A 1.0-T compact neodymium magnet was attached at a suitable position around the knee joint to allow the magnetic force to be as perpendicular to the surface of the lesion as possible. Then, 1×10^7 magnetically labeled autologous bone marrow MSCs were injected into the knee joint in a visual field of an arthroscope. The targeting efficacy was assessed by MRI T2 mapping; the clinical outcomes were assessed using the International Knee Documentation Committee (IKDC) Subjective Knee Evaluation and the Knee Injury and Osteoarthritis Outcome Score (KOOS). No serious adverse events were observed during the treatment or in the follow-up period (one year). MRI T2 mapping showed that the cartilage defect areas were almost completely filled with cartilage-like tissue. Clinical outcome scores 48 weeks post operation were significantly better than those observed before operation. Arthroscopy in 3 patients who provided informed consent showed a complete coverage of their cartilage defects (Fig. 1) [13]. In this clinical study, a technical issue of the magnetic targeting was identified. The magnetic force is weakened at the lesion part in the case where the lesion is far from the magnet. Although the magnetic field generating device should be improved for applying the magnetic targeting to any part of the body, the magnetic targeting has the possibility of applicability for the regeneration of various organs.

5. Conclusion

Magnetic targeting can be utilized for the transplantation of various types of cells and the treatment for various kinds of musculoskeletal and neural tissue disorders. It is a promising technique for minimally invasive cell transplantation.

Conflicts of interest

The authors declare no competing interests.

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