Magnesium enhances adherence and cartilage formation of synovial mesenchymal stem cells through integrins

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Introduction:

We previously reported that more than 60% of synovial mesenchymal stem cells (MSCs) placed on osteochondral defect adhered to the defect in 10 minutes and promoted cartilage regeneration (1). The efficiency of adherence is considered to depend on interaction between cells and extracellular matrix (ECM), in which integrins play important roles. Magnesium affects functions of integrins in cells from various sources (2), and integrins are involved in differentiation of MSCs (3). We examined whether magnesium enhanced adherence and chondrogenesis of synovial MSC through integrins.

Materials and Methods:

Human synovium and cartilage were harvested during knee surgery. Rabbits were also used in this study. Adherent colony-forming cells from synovium were used for analyses.

Results:

Magnesium increased adhesion of human synovial MSCs to collagen coated slides (Fig. 1A) and human osteochondral defects (Fig. 2A). These effects were inhibited by neutralizing antibodies for integrin α3 and β1 (Fig. 1B, 2B). Magnesium also promoted synthesis of cartilage matrix during in vitro chondrogenesis of synovial MSCs, which was diminished by neutralizing antibodies for integrin β1 (Fig. 3). An in vivo study in rabbits showed that magnesium promoted adherence of synovial MSCs in 1 day and cartilage formation in 4 weeks (Fig. 3).

Discussion:

Higher concentration of magnesium increased adherent property of synovial MSCs through integrin α3 and β1, and improved the efficiency of adherence when cells were placed on cartilage defects. Furthermore, higher concentration of magnesium promoted synthesis of cartilage matrix in synovial MSC through integrin β1. The addition of magnesium to synovial cell suspension will improve cartilage regeneration therapy by using synovial MSCs.

Conclusion:

Magnesium enhanced adherence of synovial MSCs and promoted synthesis of cartilage matrix through interaction between integrin and ECM, resulting in improvement of cartilage regeneration.

References:


Fig 1. Magnesium effect on adherence of human synovial MSCs to collagen coated slides. A, Microscopic photos and adhering cell number. Synovial MSCs suspended in PBS at indicated concentrations of magnesium were placed for 10 minutes. Non adherent cells were washed away. Bars = 100μm. Values are the mean ± SD (n = 3). B, Microscopic images and quantification of adherent cells pretreated with neutralizing antibodies suspended in PBS containing 10 mM magnesium.

Fig 2. Magnesium effect on adherence of human synovial MSCs to human cartilage defects. A, Effects of magnesium and neutralizing antibodies on adherence of synovial MSCs. Top side view of the cartilage defects without fluorescence (upper, bars = 2.5 mm) and with fluorescence (middle, bars = 0.5 mm). B, Quantification of fluorescence intensity. Values are the mean ± SD (n = 3). * = p < 0.05 compared to the intensity of 0 mM magnesium.

Fig 3. Magnesium effect on in vitro chondrogenesis of human synovial MSCs. A, Pellets cultured in chondrogenic medium at indicated concentrations of magnesium. Macroscopic images at 1 week (scale = 1mm) (upper). Wet weight of pellets (lower) * = p < 0.05 compared to 0.8 mM magnesium. B, Pellets pretreated with or without neutralizing antibodies. Macroscopic images of pellets (left), histologies stained with safranin-o (middle, bars = 10μm) and weight of pellets. Values are the mean ± SD (n = 3). * = p < 0.05.

Fig 4. In vivo analysis for investigating magnesium effect on adherence and cartilage regeneration in rabbits. A, Rabbit cartilage defect. Synovial MSC suspensions in the presence or absence of 5 mM magnesium were placed on cartilage defects, the position was maintained for 10 minutes (upper), and the defect was observed 1 day after the transplantation (lower, bar = 2 mm). B, Fluorescent images of cartilage defect for DiI labeled MSCs (upper, bars = 1 mm). C, Quantification of fluorescence intensity in the cartilage defect at 1 day. Values are the mean ± SD (n = 3). * = p < 0.05. D, Sagittal sections of regenerated cartilage stained with safranin-o and immunostained with type II collagen. Bars = 1 mm. E, Higher magnified histologies stained with toluidine blue in metachromatic area (upper, bars = 50 μm), and the ratio of chondrocytes with lacunae to all chondrocytes in areas of metachromasia (lower). Values are the mean ± SD (n = 3). * = p < 0.05.