



Magnesium and osteoarthritis: from a new perspective

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Abstract: Magnesium (Mg) as an essential trace element in bone metabolism has been paid to great attention in recent years according to its osteogenic effect and critical role in bone mineralization process. However, the biological influence of Mg to cartilage metabolism had not been taken in consideration seriously, because articular cartilage is believed to be lacking of mineral ingredient, and most body Mg content is stored in bone (50–60%) and soft tissue (40%) rather than in cartilage. In this review, we retrospect the original works which previously detected Mg based calcium (Ca)-phosphate composites in cartilage tissue, and analyzed the correlation between mineral crystals and arthropathy in osteoarthritis (OA). It indicates that Mg deficiency in cartilage tissue lead to chondrocytes lesions caused by uncontrolled growing of high Ca content crystals in cartilage tissue. Additionally, Mg ions directly promote chondrocytes differentiation and viability, however, the mechanism is not understood yet. We make hypothesis and probable explanation for Mg ions' exotic characters in chondrogenic effect based on the existent incomplete studies of Mg ions in cell energy metabolism, cytoskeletal rearrangement and integrin deformation. Mg has already been applied into the OA treatment in animal model due to its significantly protective effect on cartilage tissue. In future studies, clinical administration of Mg (oral administration or local infiltration) will be a promising therapy that conservatively treats OA with a thorough understanding of mechanism of Mg ions' biological effect on cartilage tissue repair.

Keywords: Magnesium (Mg); osteoarthritis (OA); chondrocytes; stem cells

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Introduction

Osteoarthritis (OA) is a multi-factor induced musculoskeletal disorder with low self-repairing ability due to its dense extracellular matrix (ECM) with sparsely distributing blood vessels, nerves, stem cells and highly specialized cells called chondrocytes. Among all the risk factors related to OA, such as local risk factors (muscle weakness, excess physical

activities, joint injury, mal-alignment of joint, leg length inequality etc.), modifiable systemic risk factors (obesity, unhealthy diets), non-modifiable systemic risk factors (age, gender, ethnicity, genetics), age is one of the strongest predictors of OA (1,2).

In China, the largest developing country with a population of 1.3 billion in the world, had already entered into the aged society since 2010 with 25.3% of the

population comprised individuals aged 50 years or older (3). Based on the uniformed inclusion criteria and quantitative index, which including 6,218 questionnaires and 5,334 sample X-ray films, the prevalence rate of primary OA was 48.7% (1,086/2,230) in 50–59 years, 62.2% (754/1,213) in 60–69 years and was 62.1% (391/630) in 70 years and over in China 2010 (4). It can be foreseen that the prevalence of age-related OA will be fast growing in the near future in China, and the efficacious preventing and treatment ways for OA would be in great demanding in the next decades. It urges us to understand the mechanism of age-related OA which is still poorly understood. Disturbed biomechanical practices and weak muscle strength, with biological changes such as enduring inflammation, as well as chondrocytes malfunction and inferior self-renewing capability of cartilage progenitor cells are likely combined contributing factors in age-related OA (5). Nevertheless, very few insights had been put into the tissue mineral changes in cartilage metabolism. For example, calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), selenium (Se), zinc (Zn), copper (Cu), and iron (Fe) are well contained in the joint tissue and play respective roles in cartilage normal function and progress of OA and rheumatoid arthritis (RA) (6,7). Mg is an important element which had been overlooked in the musculoskeletal disorders for a long time until the significant osteogenic effect of Mg is reported in recent years (8-10).

In articular cartilage, it is distributing with some tiny minerals in this non-mineralized hard tissue. Mg is considered existing in the hyaline cartilage matrix as Mg-substituted tricalcium phosphate (TCP) commonly named whitlockite $[(Ca,Mg)_3(PO_4)_2]$ (11-13). These cuboid crystals have been described in both normal and pathologic articular cartilage tissues determined by electron and X-ray diffraction. Its frequent existence in cartilage tissue bring questions about Mg's role in cartilage generation, physiological cartilage metabolism and pathogenicity in OA cartilage (14).

It was reported that Mg-deficiency in young rodents elicited an apparent decrease in the number and the size of the proximal tibial articular chondrocytes with a statistically significant decrease in the width of the articular cartilage (15). It also showed a lack of the orderly chondrocyte columns arrangement, a reduction in the proteoglycans amount and a statistically significant decrease of width in growth plate of the proximal tibia, as well as a highly significant decrease of the trabecular bone volume and a statistically significant decrease of the osteoblastic surface in the proximal metaphysis of the tibia (15).

Some other studies had been found that Mg deficiency in immature dogs/rats led to significant cartilage lesions with distinct alterations in ultrastructure and chondrocytic fibronectin staining (16-18). Histomorphometry demonstrated significantly decreased distal femur articular cartilage chondrocyte density and decreased tibia growth plate width in experimental animals compared to controls, ECM of both articular cartilage and growth plates in experimental animals contained reduced amounts of proteoglycans and reduced levels of SOX9 in rats following a 6-month dietary Mg restriction (19). Within a relative short time of Mg intake restriction (28 days), cartilage lesion was observed in juvenile rats rather than in aged rats (20,21). In human beings, several epidemiological studies reported that serum Mg (Mg intake) was inversely and significantly associated with radiographic knee OA, joint space narrowing (JSN) and RA (22-25). All the studies strongly indicate a potential role of Mg in the genesis and prevention of OA.

Here we details the topic about the pivotal role of Mg in pathogenesis and prevention of OA by reviewing the main published Mg-related studies in OA, RA, cartilage and bone metabolism.

Existence of Mg in the cartilage tissue

Ca phosphate crystals, including hydroxyapatite, carbonated apatites, octacalcium phosphate and Mg-substituted whitlockite have been reported in human articular cartilage and synovial fluid (11,26-28). In contrast to Mg-substituted whitlockite, the other Ca containing crystals with high Ca/P ratio in cartilage and periarticular tissue was strongly associated with osteoarthritic reaction and degenerative joint disease (11,14). For instance, articular deposition of basic Ca phosphate [(BCP), a term restricted to various mixtures of carbonate substituted apatite, octacalcium phosphate, and TCP] is associated with an exaggerated form of OA (14,29,30). In addition, Ca pyrophosphate dihydrate (CPPD) crystal deposition disease affects articular cartilage and is a frequent concomitant of severe OA (30). These crystals stimulate synthesis and secretion of cytokines and proteases by phagocytic cells like synovial lining cells and chondrocytes. They also induce mitogenesis in synovial lining cells, thereby increasing the number of cells able to secrete cytokines and proteases in response to crystals shed into the joint fluid (14).

The Ca moiety in BCP and CPPD crystals appears to play the important roles in eliciting biologic responses by

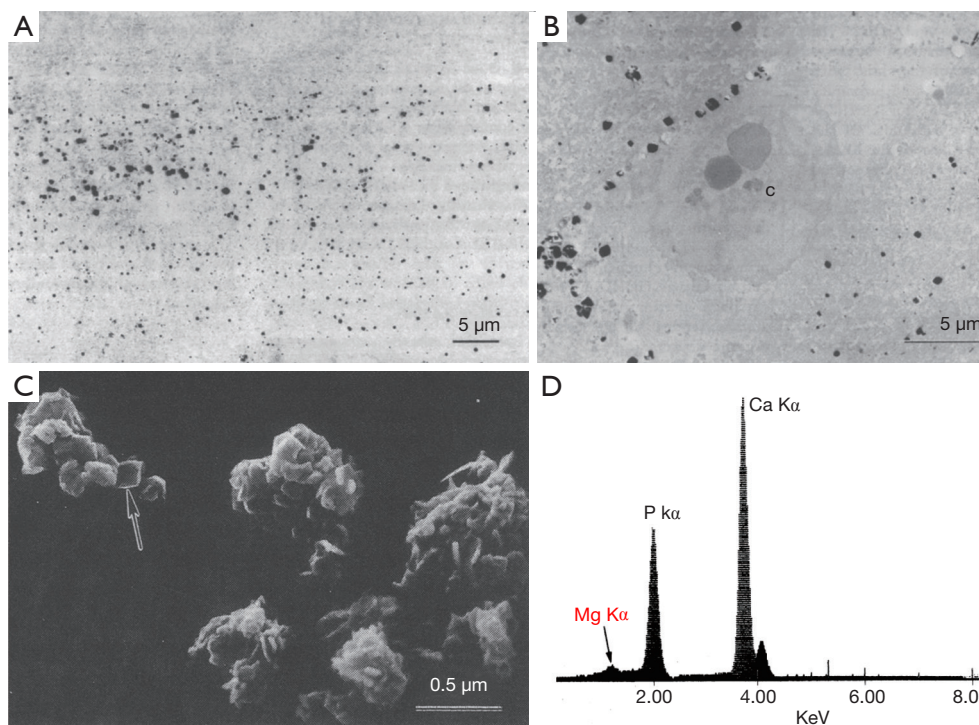


Figure 1 Presence magnesium (Mg) whitlockite crystals in cartilage tissue. (A-D) Superficial zone femoral head articular cartilage, processed using both the anhydrous and standard TEM techniques and observed unstained. Crystals are distributed both as a band below the articular surface (11-13).

phagocytic cells. Particulates that do not contain Ca do not stimulate mitogenesis after phagocytosis (14). Whitlockite appears to share some of the same biologic properties, such as enhancing mitogenesis and synthesis and secretion of matrix metalloproteinase 1 and 3 (MMP1 and MMP3) of phagocytic cells in response to these crystals (14). However, the biologic responses to whitlockite are attenuated compared with Ca-containing crystals that are devoid of Mg. Mitogenesis and proteases synthesis induced by whitlockite, which has approximately 9.2% atomic substitution of Mg for Ca, is less than that induced by the Mg-free β -TCP (14).

Potential explanations for the reduced mitogenesis and proteases synthesis of phagocytic cells in response to the whitlockite crystals may be due to the interference of Mg with the early and rapid cytosolic influx of Ca by lessening crystal-membrane interactions, or interference with mitogen-activated protein (MAP) kinase induction and MAP kinase cascade (14). However, the postulation has not been proven by detailed research experiments yet. What have been known by now is that Mg ions have direct inhibiting effect on aberrant mineralization of Ca rich crystals (*Figure 1*).

Redeeming effect of Mg^{2+} on chondrotoxicity caused by chondrocalcinosis

Researchers have explored the role of the Mg ions in biomineralization for decades. In 1984, Nancollas (31) found that Mg^{2+} appreciably retard the OCP growth rate and strongly inhibit thermodynamically stable hydroxyapatite crystallization (32,33). They further proved that Mg^{2+} at concentrations ranging from 0.0099 to 0.99 mM reduce the hydroxyapatite growth rate by 51% and 93% (32). After finding that inhibitory effect of Mg^{2+} is enhanced by synergization with CO_3 anions, Cao proposed that Mg^{2+} induce a significant inhibitory effect on the larger crystal growth of hydroxyapatite by forming a surface complex at the active growth site of newly formed small hydroxyapatite crystals and bonding to small crystals to prevent unceasing precipitation of Ca-P (34,35). It has been suggested that Mg^{2+} kinetically hinders nucleation and subsequent growth of hydroxyapatite by competing for structural sites with chemically similar but larger Ca^{2+} (31,36).

Mg deficiency in cartilage tissue would lead to the Ca-rich BCP, CPPD and finally hydroxyapatite crystallization, which is characterized as articular chondrocalcinosis

commonly occur in elderly people with OA (37,38).

Typical observations of Mg deficiency induced cartilage lesion includes (I) bundle-shaped, electron-dense aggregates on the surface and in the cytoplasm of chondrocytes; (II) detachment of the cell membrane from the matrix and necrotic chondrocytes; (III) reduction of the ECM; and (IV) swelling of cell organelles such as mitochondria (16-18). These electron-dense, bundle-like aggregates occurring physiologically in the pericellular spaces from proliferating chondrocytes is prone to be the Ca containing BCP and CPPD crystals (18). The synovial lining cells producing inflammation factors such as tumor necrosis factor α (TNF- α) which is known to induce interleukin 1 (IL-1) and their synergistic effects have been described in the cartilage tissue with Mg deficiency may be caused by the BCP and CPPD crystals (39,40). The released cytokines cause the impairment of proteoglycan synthesis, chondrocytes injury and breakdown of cartilaginous matrix by induction of MMPs. In accordance with this mechanism, increased plasma levels of IL-1 and TNF- α are also observed in Mg-deficient rats compared to controls (39,40). In Mg deficient state, after triggering the inflammatory response cascades, elevation of TNF- α would promote the release of IL-6 and interferons, synthesis of nitric oxide (NO), substance P (SP) and IL-4. Immune cells and synovial cells would be activated and stimulate the production of prostaglandins and several cytokines which synergetically involved in inflammation (31,37).

The character of tissue lesion of Mg-deficiency induced cartilage is similar with that after cartilage toxicant quinolone treatment in rats (18). It is also reported that quinolone-induced chondrotoxicity is possibly associated with the Mg-chelating properties of quinolones. Mg²⁺ can bind to the drugs of quinolone class, thus reducing the concentration of biologically active ionized Mg²⁺ in the cartilage tissue (18). It indicates that Mg²⁺ concentration disturbance in local cartilage tissue could be one of the chief pathogenic causes in the arthropathy process.

Mg is the fourth abundant cation in the body and the second most common intracellular cation after potassium. It plays important roles in animals as a central ion of adenosine triphosphatase (ATPase) and making it pivotal for numerous physiological functions (41,42). Mg²⁺ is required for a very wide range of cellular reactions, including all phosphoryl transfers related to DNA and protein synthesis. The increase in cytosolic free Mg²⁺ which initiated by the binding of growth factors to their receptors in the cell membrane leads to up regulation of Mg- adenosine

triphosphate (ATP) ²⁻ which accounts for the downstream protein synthesis of the phosphatidylinositol (PI) 3-kinase cascade, promotes cell mitosis and proliferation (43). The importance of cytosolic Mg²⁺ for cell proliferation is explained via the membrane, cytosolic Mg²⁺, mitosis model (MMM) (44,45). However, cytosolic Mg²⁺ affects chondrocytes and cartilage progenitors not only on the cell proliferation and viability, but also on their chondrogenic differentiation.

Profitable effect of Mg²⁺ on chondrocytes viability and chondrogenic differentiation

The addition of magnesium sulfate (MgSO₄) along with local anesthetic agents was found resulted in greater human chondrocytes viability than when cells are treated with a local anesthetic alone (46). In another study, chondrocytes were cultivated in the presence of quinolones and in Mg-free medium show severe alterations in cytoskeleton and decreased ability to adhere to the culture dish (32). With Mg²⁺ supplementation, the number of attached cells increased to 40–70% that of control cells in Mg-free medium (32). Human chondrocytes viability is greater in the presence of Mg²⁺ than selected local anaesthetics, this effect possibly is due to the antagonizing character of Mg²⁺ to chondrocyte NMDA-receptor which mediating cytosolic Ca²⁺ evoking (33,47). In studies of Dou *et al.*, after incubating pure metal Mg microspheres with chondrocytes, glycosaminoglycans (GAG) content, collagen type I/II/X and aggrecan were significantly increased in chondrocytes under proper concentration of Mg²⁺ conditions (48). Meanwhile, AH Martínez-Sánchez found that Mg extracts could induce human umbilical cord perivascular cells (HUCPCs) which exhibiting a high mesenchymal stem cell potential, differentiate into chondrocytes with type II collagen (COL2A1), aggrecan, SOX9 up regulated, and synthesis of cartilage-like ECM (49).

In our study, high concentration of Mg ions (from 2 to 10 mM) promotes osteogenic and chondrogenic but rather than adipogenic differentiation of tendon-derived stem cells (TDSCs) and bone marrow stem cells (BMSCs) in respective inducing medium *in vitro*. This phenomenon is jointly incorporate with high cellular ATP production. To be noticed, the stem cell didn't undergo differentiation in simple Mg containing medium (without the inducing ingredients in the medium). Our findings are in accordance with other groups in certain extent (50-52). For example, Feyerabend *et al.* found Mg concentrations up to 10 mM

lead to an increase of the proliferation rate, higher degree of chondrocyte differentiation with increased GAG production, the absence of collagen type I (COL1A1) gene expression and higher melanoma inhibitory activity (MIA) expression (51). Yoshizawa *et al.* also found that osteogenic differentiation of BMSCs is enhanced at 5 and 10 mM $MgSO_4$, and collagen type X mRNA (COL10A1, an ECM protein deposit during bone healing and hypertrophic process in chondrocytes) expression is increased at 10 mM $MgSO_4$ in both medium with and without osteogenic factors (53). It also found that Mg^{2+} profoundly enhances alkaline phosphatase (ALP) gene expression and osteogenic differentiation activity in hBMSCs even at a relatively low concentration (2.5 mM) (54). In Zheng's group, Mg ions were found to enhance the proliferation and redifferentiation of chondrocytes and the osteogenic differentiation of osteoblasts at specific concentrations, respectively (52). These studies show that besides its favorable effect to chondrocyte viability, Mg^{2+} also performs the promoting effect on stem cells differentiation. Overall, the predominant action of cellular Mg is related to ATP utilization, and thus it exists in all cells primarily as Mg-ATP (55). Through interaction with universally required ATP in cell, Mg may influence most life metabolic processes including cartilage and bone modeling (56).

All the effects of Mg ions on stem cells, chondrocytes, osteoblast, osteoclasts and other lining cells such as fibroblasts and T cells may share a common mechanism based on cytosolic Mg^{2+} actions. We notice that along with Mg^{2+} entering into the cytoplasm, cellular ATP content is augmenting in our recent study (57). After incubating with high concentration Mg^{2+} , stem cells enhance its adhesion to the substrate with focal adhesion kinase (FAK) phosphorylated in first 24 hours. Then in the following 1 to 2 weeks, stem cells are programmed into chondrogenic and osteogenic differentiation. In contrast to Ca^{2+} , which is responsible for fast cellular reactions, Mg shows gradually and very well controlled, long-term influence on many cellular reactions (51).

Being different from biochemical molecular which inducing chondrogenic and osteogenic differentiation on stem cells, the influence of Mg^{2+} on cell metabolic pattern and cell stiffness may dictate the fate of stem cell in more basic or downstream way (58,59). Mg^{2+} has the intrinsic capability to co-regulate cell energy metabolism, cytoskeleton arrangement, protein synthesis and, indirectly, the onset of DNA synthesis. So we postulate that the mechanism of chondrogenic effect of Mg^{2+} on cartilage

progenitor cells and chondrocytes may be dependent on the inner pathway related to ATP production and/or cytoskeleton arrangement in these cells.

Intracellular functions of Mg ions

The pathway of Mg^{2+} transmembrane shipping into chondrocytes is not clear yet. Nonetheless, Mg^{2+} transport from extracellular fluid to cytoplasm had been deeply studied in the past two decades. Genetic screenings on human diseases and microarray-based expression studies have resulted in the identification of numerous Mg^{2+} transporting proteins. In eukaryocytes, membrane Mg channels are a broad range of transmembrane proteins with similar structures and amino sequences. They are generally encoded as *SLC41A1*, *SLC41A2*, *Mrs2*, *ACDP2*, *Paracellin-1*, *Claudin 16*, *TUSC3*, *MagT1*, *TRPM6*, and *TRPM7* genes, which have same role in prokaryotic cells (60,61). Among them, *TRPM7* is ubiquitously expressed in all tissues, and it is proved to combine with *TRPM6* to form a *TRPM6/TRPM7* hetero-oligomerization and create Mg transmembrane conduction in distal convoluted tubule (62). Channel MagT1 is also expressed in all tissues and is considered responsible for mammalian cell Mg^{2+} uptake (61,63).

Under resting conditions, Mg^{2+} slowly moves across the cell membrane with a turnover of several hours. Yet, increasing or decreasing the extracellular Mg^{2+} will change significantly total intracellular Mg^{2+} content (64). In general, intracellular Mg primarily fulfills various intracellular physiological functions as a cofactor of DNA, RNA, ATP, and almost all 600 enzymes (55,65). Mg has two general types of interaction with an enzyme: (I) binding to an enzyme substrate in the reaction of kinases with Mg-ATP, thereby forming a complex with the enzyme; and (II) binding directly to the enzyme, thereby altering its structure and/or serving as catalytic player.

Mg²⁺ affects mitochondria activities

In a typical mammalian cell, 90% of total cytosolic Mg^{2+} is bound to ATP in the cytosol or sequestered within nuclei, mitochondria and endoplasmic reticulum (ER) (65). Numerous experimental and clinical data have suggested that Mg^{2+} deficiency can induce elevation of intracellular Ca^{2+} concentrations, formation of oxygen radicals such as reactive oxide species (ROS), proinflammatory agents and changes in membrane permeability and transport

processes in cells (66,67). It was found that cardiomyocytes are exposed to hypoxia for 1 hour and reoxygenation for 2 hours, and ROS level is increased 100–130% during reoxygenation alone. Yet the ROS level is further down regulated to 60% by increasing extracellular Mg^{2+} concentration to 5 mM at reoxygenation (68).

Mitochondria is a major source of intracellular Mg^{2+} , half of the mitochondria Mg^{2+} is localized in the mitochondria matrix, 40% of which is present in the intermembrane space and the remaining 10% are equally bound at the outer and inner membrane level (~5% each) (65). A change in external Mg^{2+} modulates the rate of oxidative phosphorylation (OxPhos) and respiratory rate in mitochondria through modulation in the activities of the succinate and glutamate dehydrogenases in intact rat heart mitochondria (69). The myocardial protective effect of Mg^{2+} is mainly by inhibiting intracellular Ca^{2+} overload through blocking L-type Ca^{2+} channel, and recovering mitochondrial membrane potential by opening the mitochondrial K_{ATP} channel during hypoxia (70).

In studies of stem cells, endogenous ROS generated from the mitochondrial electron transport chain (ETC) complex III is required to initiate directed adipogenesis of MSCs (71). Unlike adipogenesis, osteogenesis cannot tolerate ROS. In fact, antioxidant enzymes such as superoxide dismutase and catalase are simultaneously up regulated with OxPhos in osteoblasts to prevent ROS accumulation (58,72). In contrast to adipogenesis and osteogenesis, MSCs undergoing chondrogenesis have significantly reduced O_2 consumption and OxPhos, indicating a shift towards increased glycolysis. Furthermore, hypoxia inhibits MSCs osteogenesis, whereas chondrogenesis is unaffected (73,74).

Taken together, these studies indicate that subtle manipulation of oxidative consumption and energy productive ways by intracellular Mg^{2+} directly influence the differentiation of MSCs either into osteoblasts, adipocytes or chondroblasts. Actually, Mg^{2+} is found regulating proliferation and differentiation in stem cells by altering mitochondria function in latest study (75,76). However, the detailed effect of Mg ions on cell energy metabolism and its consequences in stem cell fate need to be explored in future studies.

Mg affects cytoskeleton arrangement

Besides biochemical signals regulating MSCs commitment, biomechanical signals directly and indirectly played important roles in regulating a stem cell fate (77).

Cytoskeletal contractility by actin-actomyosin system is an important mechanical regulator of directing stem cell differentiation. Inhibiting the cytoskeletal contractility by reducing Rho-associated kinase (ROCK), nonmuscle myosin II and FAK activities lead to adipogenesis (77). The chondrogenic and osteogenic differentiation of MSCs probably correlates with the FAK activity and the cytoskeletal contractility. The contractile cytoskeleton consists of actin, myosin, microtubules and intermediate filaments (78). Remodeling of the actin cytoskeleton through actin dynamics is involved in a number of biological processes (79). The cellular contractility induces downstream events including the recruitment of adhesion molecules and kinases such as the mechanosensitive FAK, zyxin and talin, subsequently triggering the activation of Rho GTPases (80). In eukaryotic cells, the lipid membrane is connected to the actin cortex via the family of members of the ezrin-radixin-moesin (ERM) linker proteins, including ezrin, radixin and moesin (81).

MSCs exhibit inherent plasticity in terms of their ability to differentiate into different lineage including chondrocytes and osteoblasts (82,83). MSCs are softer in quiescent state than in differentiated state which is likely to influence cellular functions including mechano-transduction and migration (83). The increased stiffness of differentiated cells is resulted from the increased membrane-cortex adhesion, and the differentiated cells exhibited greater F-actin density and slower actin remodeling (83). Polymerization of actin is regulated by a series of kinases, such as GTPase RhoA, ROCK, and LIM kinases, which phosphorylates the actin depolymerizing protein cofilin and stabilizes actin filaments (84). Stabilizing polymerized actin filaments increases hMSCs viability and osteoblast and chondroblast differentiation (85). Activation of actin depolymerization and consequently reducing the cell tension by cytochalasin D or inhibition of ROCK activity mimics the phenotype of poor spread cells, resulting in adipogenesis (85). This effect of cytochalasin D is due to its promotion of ATPase activity in cells, which is contrary to effect of Mg^{2+} in our studies for dorsal root ganglia neurons (57).

ATP hydrolysis occurs on F-actin in two subsequent reactions, cleavage of ATP followed by the slower release of Pi. ATP is hydrolyzed (at the rate of $1/3.3 \text{ s}^{-1}$) following the elongation of filaments at the growing end of filaments, whereas the Pi release is 100 times slower (86). As a result, newly polymerized filaments consist of stable ADP-Pi actin (F-ADP-Pi), whereas the older filaments contain mainly ADP actin (F-ADP), which disassembles more rapidly (86).

Actin dynamics also depends on the identity of the bound divalent cation, physiologically Mg^{2+} , associated with the bound nucleotide (87). Effect of Mg^{2+} on actin polymerization is not studied thoroughly yet, however, the action between Mg^{2+} and non-muscle myosin is well studied.

Previous studies with motor proteins such as myosin traditionally focus on Mg^{2+} as a cofactor in ATP binding, hydrolysis, and product release in the force-generating mechanochemical cycle (88). In recent studies, Mg^{2+} inhibit class I, II, V myosins and class VII myosins in a Mg^{2+} dependent manner (0.3–9.0 mM free Mg^{2+}) in both ATPase and motility assays (88–90). The results demonstrate that Mg^{2+} alters key steps in mechanochemical cycle by coupling the nucleotide and actin-binding regions. It alters the structural transition that limits ADP dissociation from actomyosin. Because the ADP release step is rate-limiting in myosin, increasing free Mg^{2+} concentration slows the myosin ATPase activity as well as sliding velocity. The mechanism of altering detachment is likely due to Mg^{2+} exchange in the active site, although it is unclear how Mg^{2+} impacts attachment. Higher concentrations of free Mg ions stabilize the tension-bearing actin myosin ADP state and shift the system from the production of rapid movement toward the generation of tension.

The total tension present in the plasma membrane (i.e., the “apparent” membrane tension) has a minor contribution from the surface tension of the lipid bilayer and a substantial contribution from the molecular contacts that afford adhesion to the underlying actin cytoskeleton (88). To prevent large changes in tension is the inner request of stem cells in quiescent state, and the plasma membrane must maintain continuous interactions with the cytoskeleton (80). In this process, MSCs are particularly susceptible to membrane blebbing, membrane-cytoskeleton structure is highly dynamic and continuously remodeling in undifferentiated MSCs (83). The increased tension indicates the increased critical pressure for membrane-actin cortex detachment or bond strength in MSCs following differentiation. Meanwhile, this “blebability” is reduced during chondrogenic differentiation with significant increases in instantaneous and equilibrium moduli (83).

Under quantitative confocal microscopy, the differentiated cells have longer recovery times indicative of a more stable actin cortex with slower turnover compared to hMSCs in review of actin organization and dynamics (91). This is in agreement with the observations of increased membrane-cortex adhesion which in turn influences cell

mechanics by reducing bleb formation (83). Here we point out the reduced membrane bleb formation along with chondrogenic and osteogenic effect of Mg^{2+} on MSCs is a probable mechanism of Mg^{2+} suppression to osteoclasts whose function are mainly dependent formation of blebbing and vesicles (92,93).

Interaction between extracellular Mg^{2+} and integrins

Cells adhere to the underlying ECM substrate by employing membrane-bound integrins. A large complex network of adhesion molecules has been shown crucial for stem cells and force-mediated differentiation, some of the important structural proteins in cell adhesion include integrin, talin, vinculin and FAK (80). Integrin structurally contains three distinct divalent cation-binding sites. Two binding sites where integrin interacts with its ligand are occupied by Mg^{2+} , presence of Mg^{2+} is essential for integrin-ligand binding and their presence is required for cell adhesion (59,94).

From *in vitro* investigations on chondrocytes, it is known that several integrins are present on chondrocytes and that the cell-matrix interaction is mediated by integrin receptors of the $\beta 1$ subfamily (18). These receptors recently be demonstrated to exist in human cartilage, and they are reduced in rat chondrocytes after ofloxacin treatment (95). Because binding of integrins and its receptors require divalent cations, particularly Mg^{2+} , integrins' function could be impaired in Mg deficiency or by Mg^{2+} -chelating agents such as quinolones in cartilage tissue (95,96). So it had been speculated that impairment of cell-matrix interaction through integrins activation is one of the possible mechanism in Mg-deficiency induced arthropathy.

Mg^{2+} is involved in direct activation and the regulation of signal cascades of integrins (97,98). In chondrocytes, at least eight different types of integrins are expressed and play the important roles in differentiation and the interaction of chondrocytes with the ECM (99). Mg^{2+} is found increased adhesion of hMSCs to collagen, and this effect is inhibited by neutralizing antibodies for integrin $\alpha 3$ and $\beta 1$. In this study, Mg^{2+} also promotes synthesis of cartilage matrix during *in vitro* chondrogenesis of synovial MSCs, however, the chondrogenic differentiation effect of Mg^{2+} is diminished by neutralizing antibodies for integrin $\beta 1$ but not for integrin $\alpha 3$ (100). It was also reported that high concentration of Mg^{2+} promotes proliferation of human bone marrow-derived stromal cells (hBMSCs) via integrins $\alpha 2$ and $\alpha 3$, but not $\beta 1$ (54). It is fascinating that whether

intracellular Mg^{2+} or extracellular Mg^{2+} which directly binding to cell membrane integrin domain which protruding out of the cytoplasm (101), is enrolled in the differed differentiation pathway in stem cells. In further studies, it is pivotal to explore the effect between intracellular/extracellular Mg^{2+} and integrin regulation to understand the role of Mg^{2+} on chondrogenic differentiation (51).

Mg in treatment of OA and future direction

Based on its chondrocyte benevolent effects of Mg^{2+} , Mg salts have been studied in the OA treatment in recent years, actually only in the bench now and still far from the bed. CH. Lee *et al.* firstly intra-articularly injected $MgSO_4$ in the OA Wistar rats caused by intra-articular injection of collagenase (500 U) in the knee, and Mg^{2+} significantly reduced the severity of cartilage degradation in the OA knee (102). OA rats receiving intra-articular $MgSO_4$ injections showed a significantly lower degree of cartilage degeneration than the rats receiving saline injections, synovitis phenomenon was also suppressed after $MgSO_4$ treatment (102). Mechanical allodynia and thermal hyperalgesia showed significant improvement in the OA + $MgSO_4$ group as compared to the OA group (102). It had been considered that the inflamed state of arthritic knees is characterized by the presence of neural transmitters such as SP and glutamate in the inflamed region and synovial fluid (103). These neural transmitters in the knee joint result in thermal hyperalgesia and mechanical allodynia, which conducted through N-methyl-D-aspartic acid (NMDA) receptor on synoviocytes and chondrocytes (104). Mg^{2+} acts as an antagonist at the glutamate subtype of NMDA receptors and blocks NMDA-induced currents in a voltage-dependent manner by blocking receptor channel effects (105,106). Dietary restriction of Mg intake lowers the mechanical nociceptive thresholds in rats, which can be reversed by the NMDA receptor antagonist, MK-801 (102). Local intra-articular administration of $MgSO_4$ modulates chondrocyte metabolism through inhibition of cell NMDA receptor phosphorylation and apoptosis, attenuates the development of OA and concomitantly reduces nociception (102). Even in normal rat knee joint, intra-articular injection of $MgCl_2$ solution had no significant adverse effect of inflammation and cartilage degeneration compared to saline injection (107).

Mg^{2+} is a potential therapeutic agent in the treatment of the OA. Further research is needed not only to better define the administration mode of Mg^{2+} on OA but also to clarify

the role of Mg^{2+} as a NMDA antagonist in OA treatment. In our recent published work, Mg^{2+} significantly promotes replasticity in sensory neurons, and increases up regulation of neural transmitters (e.g., calcitonin gene-related peptide) which facilitates osteogenesis and bone fracture healing (57). It reminds us that the biological effects of Mg^{2+} are multi-systems and far-ranging to neuron, myocyte, osteoblast, osteoclast, fibroblast, chondrocyte, epithelial cell, progenitor cell and stem cell. In cartilage, there is few sensory nerves and neural peptides, the direct effect of Mg^{2+} on chondrocytes and progenitor cells should be well studied.

At the present, drug treatments for OA are pain alleviating and/or antagonizing to inflammation factors (e.g., TNF- α , IL-1 β /6/8), most of conservative treatments cannot even slow the progression of OA. At the later stage of OA, patients usually have to receive total knee/hip replacement surgery. So both clinicians and researchers are trying to find the efficacious treatment to prevent the progression and cure the OA in the early stage. It was found that MSCs are recruited and aggregated into the damaged tissue site and differentiate into chondrocytes in the beginning of OA (108,109). However, the stem cells gradually lost their differential capability during the persisting inflammation (110). As we review above, Mg ions can promote the chondrogenic differentiation on MSCs. Meanwhile, Mg ions also perform its significant inhibiting effect on the inflammation activities to osteoclasts (93). Our previous study showed that the expression of nucleostemin (a stem cell nucleus marker which is well maintained in quiescence and mitosis and down-regulated in differentiation) in cartilage was decreased after Mg treatment in rat OA model. It indicates Mg^{2+} may well keep the viability and differential potent of cartilage stem cells. That makes Mg a new prospective drug in the conservative treatment for OA through locally administrating Mg ions in the lesioned cartilage.

The fascinating effect of Mg in musculoskeletal system enlightens us to explore the existence of stem cells or chondrocyte progenitors in cartilage tissue, and search for the cause accounting for the losing capability of these cells in cartilage repair. Thoroughly understanding the mechanism of Mg ions' curing effect on OA would inspire the new findings for cartilage self-repairing processing, and design feasible ways to treat early OA.

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Footnote

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