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PERSPECTIVES

Magnesium-based implants: Beyond fixators

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Summary The mystery behind magnesium-induced bone formation is revealed by linking the neuropeptide(s) from sensory nerves with the osteogenic differentiation of stem cells in the periosteum. Zhang and colleagues' study sheds light on the development of magnesium pills or calcitonin gene-related peptide-delivery system for the prevention or treatment of osteoporosis.

The translational potential of this article: For the first time, magnesium is shown to be beneficial for fracture healing at the weight-bearing site. In addition, calcitonin gene-related peptide-delivery system will also be another translational direction, as the promotive role of calcitonin gene-related peptide in fracture healing is supportive. These cost-effective and innovative treatment approaches will definitely bring a reduction not only in the suffering of patients, but also in the economic burden for their families and our society.

In most cases of bone-fracture caused by aged-related osteoporosis and the occurrence of bone trauma, fixation devices are needed to hold the fractured bones in place through a surgical approach. Clinicians have realised that there are major drawbacks of conventional bone fixation devices made of permanent rigid metals, such as "stress shielding", which results in bone loss and thus inevitable secondary surgery to remove the fixation devices. In recent decades, many studies have been conducted to develop biodegradable metallic implants to overcome the limitations of permanent devices [1—3]. Magnesium, apart from its biodegradable capacity, with mechanical strength similar to the cortical bone, is brought to the spotlight with great interest. Recently, two clinical studies have demonstrated the safety, efficacy, and feasibility of pure magnesium or magnesium-based alloy for the fixation of bony flap or bone fractures occurring at nonweight-bearing sites [4,5]. However, the underlying cellular and molecular mechanisms remain tantalisingly unclear. In addition, neither the effect nor the feasibility of magnesium in fixing bone fractures at weight-bearing sites was investigated in the previous studies. Therefore, understanding these is the prerequisite for the further successful application of magnesium-based materials for supporting the treatment of orthopaedic disorders.

In a recent article published in *Nature Medicine*, Zhang et al [6] reported their original findings, focusing on the

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mechanisms underneath the beneficial effect of magnesium on bone formation as well as identifying the translational potential of an innovative magnesium-containing intramedullary nail (Mg-IMN) in fixing the fractured long-bone (i.e., femur, weight-bearing site). It is typical translational research, that is, a from 'bench to bedside' study. Implantation of pure magnesium into rat distal femur triggered dramatic new bone formation at the periosteal region, rather than the endosteal region of the cortical bone. According to the anatomical characteristics of long bone, there are sensory nerve endings [~80% of fibres are positive for calcitonin gene-related peptide (CGRP)] and periosteum-derived stem cells (PDSCs) densely distributed in the fibrous membrane (i.e., periosteum) covering the outer layer of long bone; therefore, the intriguing magnesium-induced periosteal bone formation was likely attributed to the unique structure of periosteum. Indeed, in this study, no new bone was formed in the area with periosteum stripped after magnesium implantation. In addition, the effect of magnesium was significantly abrogated by either CGRP receptor antagonist or knock-down of the calcitonin receptor-like receptor or receptor-activity modifying protein 1 *in vivo*, confirming that a CGRP receptor is of particular importance for magnesium-induced periosteal bone formation. Enzyme-linked immunosorbent assay and immunofluorescence results indicated that CGRP expression was significantly higher in magnesium-implanted samples compared with stainless steel-implanted ones. All these results suggest that CGRP and its receptor are indispensable in the aforementioned interesting phenomenon.

When conducting *in vitro* experiments, the authors firstly found evidence that the entry of magnesium ions mediated by two important ion channels (magnesium transporter 1 and transient receptor potential cation channel subfamily M member 7) could lead to an increase in the content of synaptic vesicles (miniscule sacs within the cell where CGRP are stored, Figure 1) and consequently increased CGRP release [6]. In primary isolated PDSCs, CGRP promotes osteogenic differentiation via calcitonin receptor-like receptor- and receptor-activity modifying protein 1-dependent activation of cyclic adenosine monophosphate-responsive element binding protein 1 and Osterix (Figure 1).

To our knowledge, for the first time, we are able to comprehensively understand how magnesium ions stimulate sensory neurons to secret CGRP and how CGRP promotes the PDSCs to differentiate toward osteogenic lineage. However, a number of interesting questions deserve more exploration. First, as shown in the μX-ray fluorescence images, the magnesium concentration is relatively higher at the endosteal region compared with the periosteal region [6], suggesting higher local pH. Does inappropriate higher pH inhibits cell proliferation at the endosteal region? If yes, this will serve as another explanation as to why new bone is dramatically formed at the periosteal region, rather than the endosteal region. Second, substance P was also increased after magnesium implantation albeit with less drastic response than CGRP. What is the contribution of substance P in magnesium-induced bone formation? Third, is there any direct effect (promotion or inhibition) of magnesium on the regulation of stem cell differentiation? It has been previously reported that an interference screw made of pure magnesium can promote graft incorporation and attenuate peritunnel bone loss through accumulating bone morphogenetic protein 2 (BMP-2) in an animal model with anterior cruciate ligament reconstruction [7], suggesting that the released magnesium may also increase bone formation in trabecular bone, in which bone marrow mesenchymal stem cells play a central role. Future studies are required to offer additional insights into the mechanisms underlying magnesium-induced bone formation.

Zhang et al [6] have also made a great breakthrough in the translational application of Mg-IMN for treating the challenging osteoporotic fracture created at long bone (femur). Importantly, macrobiomechanical test results show that about 30% enhancement in terms of maximum compressive load in femora received Mg-IMN implantation compared with those with stainless steel. This outcome is of great clinical significance. Additionally, Mg-IMN was able to promote the formation as well as the remodelling of fracture callus, which is likely to be attributed to the sole effects of CGRP exhibited at different stages. As magnesium degradation products are reported to inhibit the activity of osteoclasts [8], which is crucial for the bone remodelling; together with the findings from Zhang and colleagues' study, at least, we can rule out the possibility that magnesium itself directs the bone remodelling at a late stage of fracture healing. To investigate the microscale alternations thoroughly in both the mechanical properties and crystal structure, advanced techniques, including microindentation testing, Raman microscopy, and atomic force microscopy, will be applied in future studies. While supplementation of CGRP did not affect the proliferation of PDSCs, it did affect the proliferation of osteoblasts *in vitro* and *in vivo*, explaining why fracture callus was larger in the Mg-IMN group compared with the IMN group. However, it remains to be addressed what the key elements determining the different responses of PDSCs and osteoblasts are, even though under the same treatment with CGRP. In addition, it is well established that angiogenesis is an indispensable early event for fracture repair. Zhang et al [6] have not provided direct evidence regarding to the status of angiogenesis. Based on available literature in which CGRP is characterised as a vasodilator [9], magnesium itself can also enhance the expression of vascular endothelial growth factor [7]; thus, it is reasonable to hypothesise that the angiogenesis may be also significantly enhanced in Mg-IMN treated fractured bone. To test this hypothesis, tube formation test and micro-computed tomography-based angiography will be helpful techniques. All this evidence suggests the pleiotropic functions of magnesium in the enhancement of bone formation and bone-fracture healing.

While the overall outcome is encouraging, the potential side-effect is worthy of our attention. As aberrant expression of CGRP induces migraine, would the magnesium-elevated CGRP increase pain? It is impressive that this concern is originally raised by the authors. They found that the elevation of CGRP was "single-pulsed", peaking (8-fold increase) at 4 days after receiving magnesium implantation, without aberrant nerve sprouting. Neither limb idleness index nor print area differed between the Mg-IMN and IMN group, suggesting no increase in pain.

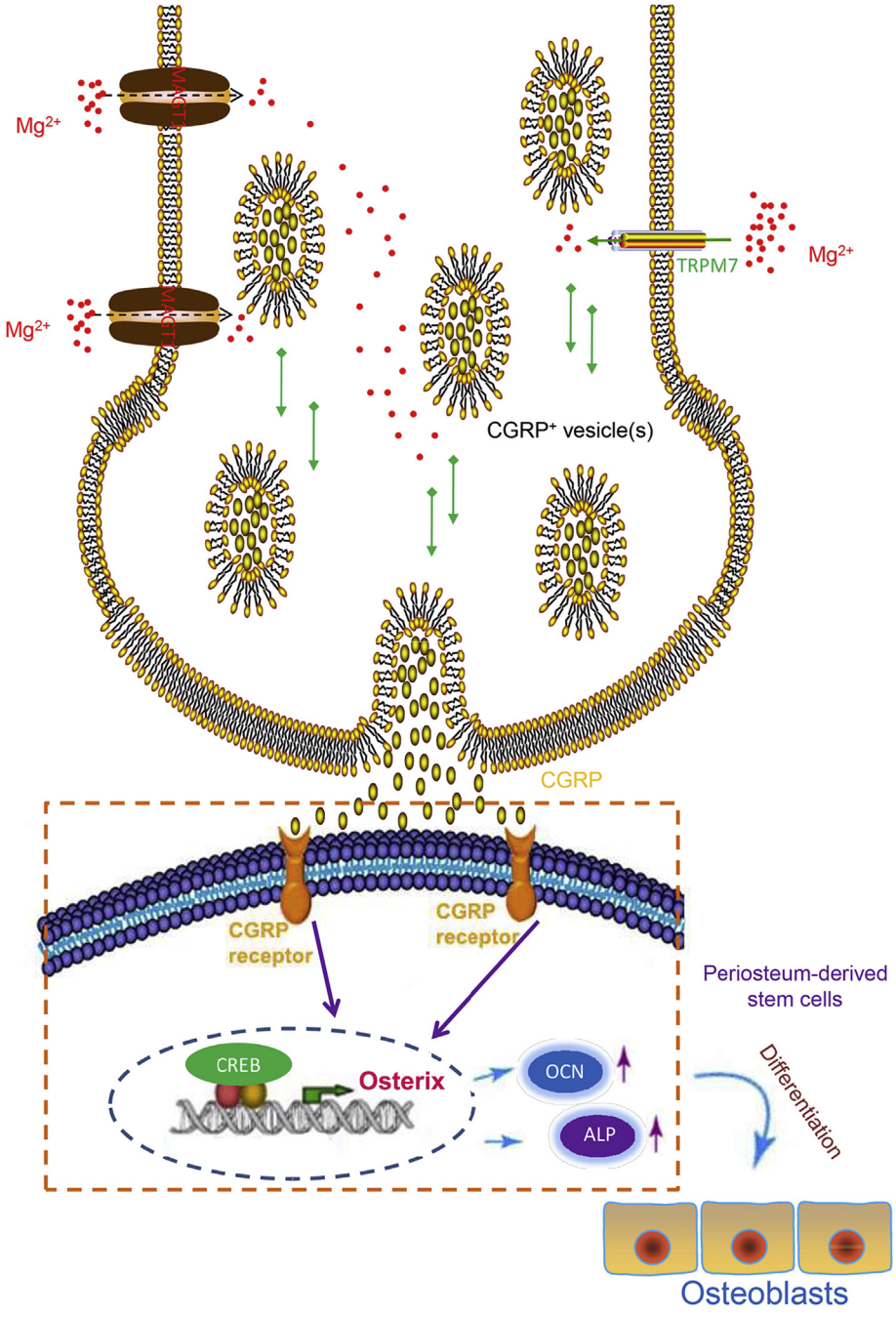

Figure 1 Molecular and cellular mechanisms involved in magnesium-enhanced bone formation and bone fracture healing. Mg^{2+} releasing from the implant is diffused into the periosteal region and transported into the sensory neurons by magnesium transporter 1 (MAGT1) and transient receptor potential cation channel subfamily M member 7 (TRPM7). Elevation of intracellular Mg^{2+} further leads to increased accumulation and transportation of CGRP+ synaptic vesicles in the nerve ending. Higher extracellular CGRP binds to CGRP receptor expressed on the surface of periosteum-derived stem cells (PDSCs), which activates the phosphorylation of cAMP response element binding protein (CREB) in the nuclei of PDSCs. The activation of CREB specifically targets Osterix, a known important transcriptional factor for the osteogenic differentiation of stem cells, following with higher osteocalcin (OCN) and alkaline phosphatase (ALP). Through connecting these aforementioned signalling pathway elements, PDSCs are promoted to differentiate toward osteoblastic lineage, presenting with significantly more new bone formation in the periosteal region.

Magnesium may also have other potential indications. For example, it is well known that hydrogen gas is formed during *in vivo* degradation of magnesium and its alloys. Inhalation of 2% hydrogen gas during 2-hour ischaemia improved brain injury in rats, presenting with less infarct area compared with the rats without hydrogen gas inhalation [10], implicating that magnesium implants (with mesh-like structure) may be helpful not only for repairing a calvarial defect, but also for reducing brain injury (if any) through the diffusion of hydrogen gas into the surrounding brain tissue, which is accompanied with the

gradual degradation of magnesium. It is worthwhile for us to perform preclinical studies to further test if magnesium-mesh is an alternative material to the conventional titanium-mesh towards the biological repair of calvarial defects.

In conclusion, magnesium-based materials are promising bioactive devices to be widely used in orthopaedic fields, no longer limiting to fix fractured bones at nonweight-bearing sites. With the rapid development of multiple techniques in improving the properties to a greater extent and the ongoing efforts to be made by working with biomaterials experts and clinicians, the dream of avoiding a second removal surgery will become a reality in the near future. Zhang and colleagues' study [6] also sheds light on the development of magnesium pills or CGRP-delivery system for the prevention or treatment of osteoporosis. Without doubt, these types of cost-effective and innovative treatment approaches will bring a reduction not only in the suffering of patients, but also in the economic burden for their families and our society.

Conflicts of interest

The author confirms that there are no conflicts of interest or funding support.

References

[1] Zberg B, Uggowitzer PJ, Loffler JF. MgZnCa glasses without clinically observable hydrogen evolution for biodegradable implants. Nat Mater 2009;8:887—91.

[2] Gu X, Zheng Y, Zhong S, Xi T, Wang J, Wang W. Corrosion of, and cellular responses to Mg-Zn-Ca bulk metallic glasses. Biomaterials 2010;31:1093—103.

[3] Witte F. The history of biodegradable magnesium implants: a review. Acta Biomater 2010;6:1680—92.

[4] Lee JW, Han HS, Han KJ, Park J, Jeon H, Ok MR, et al. Long-term clinical study and multiscale analysis of *in vivo* biodegradation mechanism of Mg alloy. Proc Natl Acad Sci U S A 2016; 113:716—21.

[5] Zhao D, Huang S, Lu F, Wang B, Yang L, Qin L, et al. Vascularized bone grafting fixed by biodegradable magnesium screw for treating osteonecrosis of the femoral head. Biomaterials 2015;81:84—92.

[6] Zhang Y, Xu J, Ruan YC, Yu MK, O'Laughlin M, Wise H, et al. Implant-derived magnesium induces local neuronal production of CGRP to improve bone-fracture healing in rats. Nat Med 2016;22:1160—9.

[7] Cheng P, Han P, Zhao C, Zhang S, Wu H, Ni J, et al. High-purity magnesium interference screws promote fibrocartilaginous entheses regeneration in the anterior cruciate ligament reconstruction rabbit model via accumulation of BMP-2 and VEGF. Biomaterials 2016;81:14—26.

[8] Zhai Z, Qu X, Li H, Yang K, Wan P, Tan L, et al. The effect of metallic magnesium degradation products on osteoclast-induced osteolysis and attenuation of NF-kappaB and NFATc1 signaling. Biomaterials 2014;35:6299—310.

[9] Brain SD, Williams TJ, Tippins JR, Morris HR, MacIntyre I. Calcitonin gene-related peptide is a potent vasodilator. Nature 1985;313:54—6.

[10] Ohsawa I, Nishimaki K, Yamagata K, Ishikawa M, Ohta S. Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals. Nat Med 2007;13: 688—94.