Nano-magnesium phosphate hydrogels: efficiency of an injectable and biodegradable gel formulation towards bone regeneration

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The use of autografts is considered as gold standard for the treatment of bone tissue defects caused by tumor, disease or trauma. The second surgery for autologous bone harvesting is often linked to donor site morbidity, pain, blood loss and infections (1). To circumvent these issues, synthetic bone grafts made of either organic biopolymers such as poly lactic-co-glycolic acid (PLGA) or collagen (2) and inorganic replacement materials like calcium orthophosphate (CaP) bioceramics are commercially available and frequently used in the clinic (3). The variety of such materials in terms of composition and application form (e.g., pastes, blocks, granules) is huge but most of them do not fulfill all main criteria for adequate bone substitutes: osteogenesis, osteoinductivity, osteoconductivity and osseointegration. Only autologous bone seems to comply with those demands (4).

In this perspective the research article from Laurenti *et al.* (5) exactly responds to this issue. Especially, if looking for alternatives to traditional inorganic scaffolds with limited injectability and biodegradability (2,6). Laurenti *et al.* presented a material formulation comprising three topics being currently of crucial interest within the research area of bone tissue engineering (TE): two-dimensional (2D)-nanomaterials, hydrogels and magnesium phosphate (MgP) minerals. In brief, MgP-nanosheets were fabricated and successfully formed a physically crosslinked long-term stable hydrogel which was injectable through a needle with a very small inner diameter. Without shearing the gel, it behaved like a solid material (*Figure 1*), whereby the application of a mechanical load resulted in shear-thinning with a dramatic increase of injectability. The authors proved that this material had osteogenic properties and could accelerate bone healing and osseointegration though it contained no osteoinductive supplements like cells or growth factors.

Nanomaterials are of great interest in many different research areas as they have unique size-dependent properties (7). In biomaterial engineering, they offer a good possibility to mimic the hierarchical architecture of native extracellular matrix (e.g., nanofibers) or might be used for the delivery and release of small bioactive molecules such as growth factors or DNA (2). Hydrogels are physically or chemically crosslinked polymer chains that are able to bind high amounts of water depending on properties like their mesh size (8). They can be designed in a way such that they are similar to native extracellular matrix where cells feel comfortable in (9). A lot of different hydrogel formulations have been shown to be biocompatible and they can be synthesized on the basis of biopolymers such as gelatin and alginate (8).

Recently, MgP minerals have attracted a broad interest in terms of bone regeneration as a competitive alternative for CaP ceramics. They are likewise biocompatible (10), but hydrated products of MgP-cements have a higher degradation potential (10,11), as released Mg^{2+}-ions are known to oppress the precipitation of worse soluble re-precipitates (11,12). Magnesium further has an impact

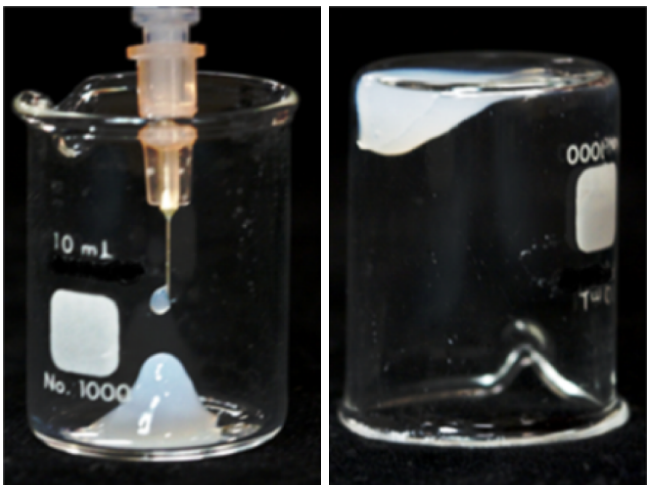

Figure 1 Injectability of the nano-MgP-gel (left) and solid-like behavior without applying shear forces (right). Reprinted with permission from (5). Copyright [2016] American Chemical Society.

on bone remodeling: it was shown to promote osteoblast differentiation and inhibit osteoclastogenesis in a dose-dependent manner (13). Magnesium represents 1.6 wt.% of human bone (14) which contains 50–60% of the total magnesium amount in the body (15). The synthesis of the MgP-nanosheets represents a major advantage within the discussed publication because a simple precipitation route was chosen. Usually, the synthesis of 2D-nanomaterials is linked to time-consuming processes with toxic and expensive chemicals or high pressures and temperatures (e.g., exfoliation). Here, the raw materials simply consisted of brucite [$Mg(OH)_2$] dissolved in phosphoric acid (H_3PO_4) and merged with sodium hydroxide (NaOH) solution. Mixing both, a MgP colloidal suspension with the precipitate $Mg_xNa_y(HPO_4)_z(PO_4)_T{\cdot}nH_2O$ was obtained. *Figure 2A* depicts the phase diagram within the system $Mg(OH)_2$-NaOH-H_3PO_4, but stable colloidal suspensions only formed in the red area whereat formulation A (molar ratio $Mg(OH)_2/NaOH/H_3PO_4$ =0.13/0.52/0.3) and B (molar ratio =0.18/0.45/0.37) showed long-term stabilities of 2–4 years without mineral phase transformations. In those cases, the mineral composition of the precipitate was close to newberyite ($MgHPO_4{\cdot}3H_2O$) in terms of the Mg/P ratio (1–1.15) and crystal water amount ($3 \leq n \leq 4$). The resulting crystals had a laminar morphology with high aspect ratio and a thickness of 4–7 nm (*Figure 2B*).

MgP-nanocrystals showed attractive interactions with both negatively and positively charged glass surfaces which confirmed the presence of both charges within the crystals (*Figure 2C*). This enabled the formation of physically crosslinked gels with water through electrostatic and van der Waals interactions. Within the gel, the nanoparticles formed a three-dimensional (3D)-honeycomb network with partially overlapping aggregates (*Figure 2C*). Gels with a MgP-nanosheet content of 5–10 wt.% behaved like thixotropic fluids with shear-thinning effect with high viscosities at low shear rates and vice versa which is beneficial for injectability and minimally invasive surgeries. Notably, the gel was injectable through a small gauge insulin needle by applying 9–18 N at a velocity of 0.3 mm/min.

In vitro studies suggested good cell viability of human fibroblasts (*Figure 2D*) especially for colloidal suspensions based on formulation B which had a pH close to physiological conditions (7.8). In differentiating osteoblasts from mouse bone marrow cells, this suspension induced the upregulation of osteogenesis related genes such as alkaline phosphatase (early osteogenesis) and osteocalcin (bone mineralization). Simultaneously, an increase in nanocrystal porosity during cell culture indicated their bioresorbability. Using a rat tibia model, an accelerated bone healing and osseointegration was demonstrated. Micro-computed tomography (μCT) scans revealed that the defect treated with MgP-nanosheet colloidal suspension was completely filled with new bone already 2 weeks after surgery compared to the control group. Simultaneously, a dense bone-implant contact was supported (*Figure 2E*). Histology and histomorphometric analysis confirmed these observations, as osteoblast differentiation, collagen synthesis and mineralization were promoted by the new bone void filler. At the same time, the *in vivo* resorption of the gel seemed to be fully accomplished after 3 days.

In summary, the material formulation presented by Laurenti *et al.* (5) seems to have several superior characteristics compared to conventional synthetic bone substitutes, e.g., an enhanced bone healing capacity by activating both osteoblasts and osteoclasts as well as thixotropic paste behavior for minimal invasive application through thin needles. This is in our view a significant step forward and a paradigm shift in developing novel bone replacement formulations, e.g., for orthopedic or craniofacial applications.

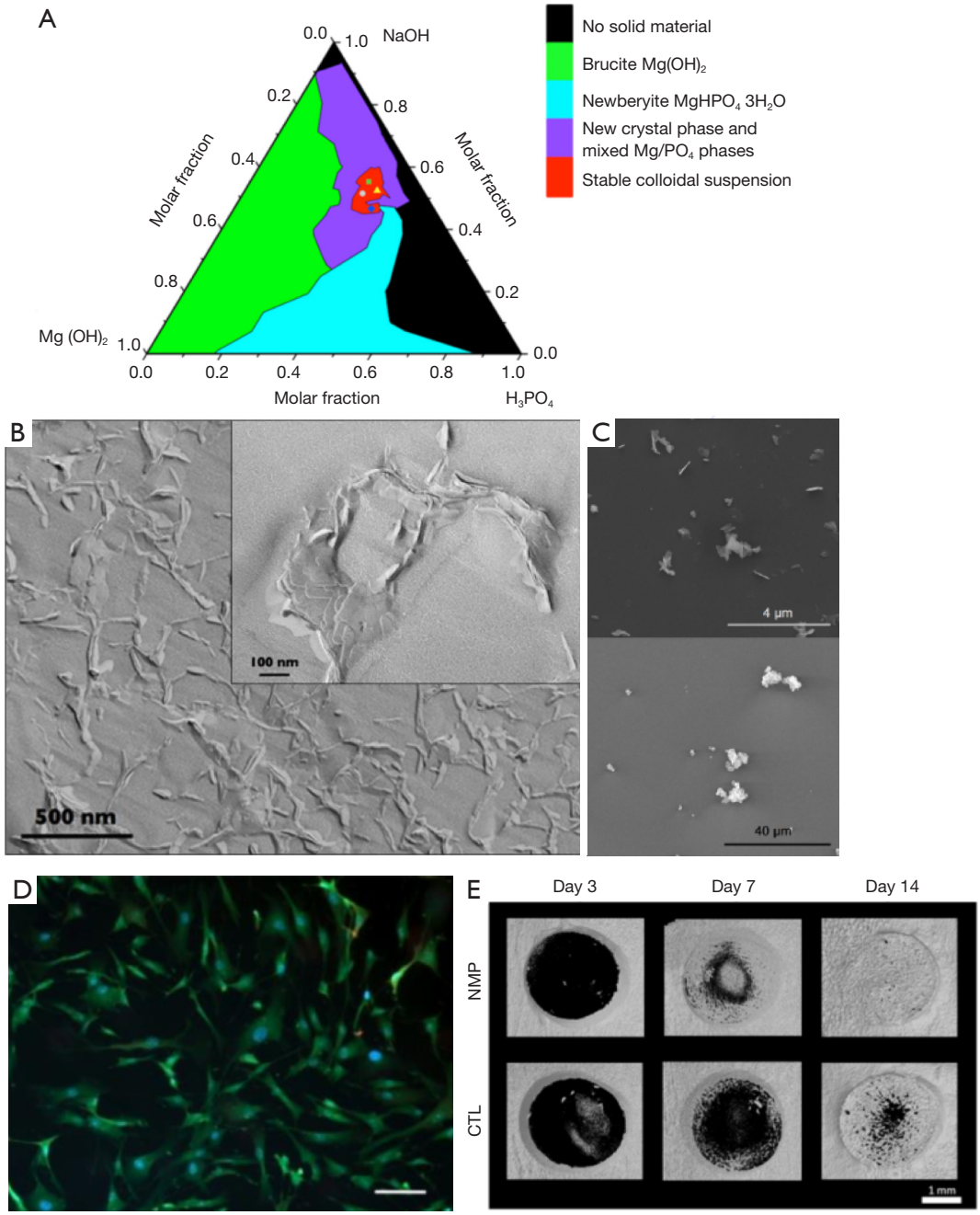

Figure 2 Physico-chemical and biological properties of 2D MgP-sheets and descending hydrogels. (A) Diagram of the system $Mg(OH)_2$-NaOH-H_3PO_4 whereat stable colloidal suspensions only formed in the red area and a [molar ratio $Mg(OH)_2$/NaOH/H_3PO_4 =0.13/0.52/0.3] and b (molar ratio =0.18/0.45/0.37) showed long-term stabilities; (B) TEM micrographs of replica of a colloidal suspension containing 5% MgP-nanoparticles; (C) resulting MgP-nanosheets showed attractive interactions with both negatively (above) and positively (below) charged surfaces; (D) Live-dead assay on human fibroblasts for formulation B after 4 days and (E) µCT of bone defects after treatment of the rat tibia with MgP-nanosheet containing colloidal suspension (above) in comparison to the empty defect control (below). Reprinted with permission from (5). Copyright [2016] American Chemical Society.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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