RESEARCH PAPER

Dissociation of magnesium oxide and magnesium hydroxide nanoparticles in physiologically relevant fluids

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Abstract Magnesium oxide (MgO) and hydroxide [Mg(OH)₂] are conventionally considered insoluble in water and stable at high temperatures. However, in this study, we found significant dissociation of MgO and Mg(OH)₂ into ions when they were immersed in different physiologically relevant solutions in the form of 20-nm and 10-nm nanoparticles respectively, under standard cell culture conditions in vitro, i.e., a 37 °C, 5% CO₂/95% air, sterile, humidified environment. The change in Mg²⁺ ion concentrations and pH measured in the physiologically relevant solutions (e.g., Dulbecco's modified Eagle's Medium (DMEM), simulated body fluid (SBF), relevant chloride solutions, and deionized water) confirmed their dissociation. Possible mechanisms and contributing factors for dissociation of MgO and Mg(OH)₂ nanoparticles were discussed. The evidence suggests that nucleophilic substitution of OH[−] by Cl[−] in Mg(OH)₂ is energetically unfavorable and it is more likely that Cl[−] plays a role in the stabilization of intermediate forms of MgO and Mg(OH)₂ as it

dissociates. The pH and buffering capability of the immersion solutions might have played the most significant role in dissociation of these nanoparticles when compared with the roles of chloride (Cl[−]), proteins, and different buffering agents. This article provided the first evidence on the dissociation of MgO and Mg(OH)₂ nanoparticles in physiologically relevant conditions and elucidated possible factors contributing to the observed behaviors of these nanoparticles in vitro, which is important for their potential medical applications in vivo.

Keywords Magnesium oxide (MgO) nanoparticles · Magnesium hydroxide [Mg(OH)₂] nanoparticles · Biofluids · Dulbecco's modified Eagle's Medium (DMEM) · Simulated body fluid (SBF) · HEPES buffer · Chloride (Cl[−]) solutions

Introduction

Magnesium oxide (MgO) and magnesium hydroxide [Mg(OH)₂] have been largely used in industrial applications (Amundsen et al. 2000; Fruhwirth et al. 1985; Johnson and Liu 2013), and the majority of literature on them focused on their microstructure, properties, and performance in non-biological environments. MgO is very stable at high temperatures in oxidizing and reducing atmospheres up to 2300 °C and 1700 °C, respectively and is a model for crystalline cubic structure, with a high degree of perfection (Amundsen et al. 2000). Its stability at these conditions led to the statements in most

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textbooks that MgO is generally “stable” and “relatively inert” as metal oxides (Atkins 2010; Cotton and Wilkinson 1988). However, the conditions under which MgO is “stable” and “relatively inert” were not clearly discussed. It is also well accepted that MgO is hygroscopic and hydrates at room temperature to form $\text{Mg}(\text{OH})_2$ (Láska et al. 1993; Mejias et al. 1999; Refson et al. 1995), which explains why MgO is rarely found in natural mineral deposits. The hygroscopic nature of MgO and its transition to $\text{Mg}(\text{OH})_2$ are often mentioned alongside a statement in textbooks that MgO is generally stable (Fruhworth et al. 1985; Láska et al. 1993; Refson et al. 1995). Conventionally, $\text{Mg}(\text{OH})_2$ is known to be insoluble in water and stable at temperatures up to 300 °C (Amundsen et al. 2000).

Recently, MgO and $\text{Mg}(\text{OH})_2$ have attracted increasing attention for biomedical applications because of their bioactivity (Fielding et al. 2014; Kum et al. 2014; Patel et al. 2013; Pourdanesh et al. 2014) and distinct effects on bone marrow-derived mesenchymal stem cells (BMSCs) and pathogenic bacteria (Wetteland et al. 2016). MgO and $\text{Mg}(\text{OH})_2$ usually form at the interface of Mg-based biodegradable implants with cells and tissues as major degradation products, during passivation and degradation of metallic Mg and its alloys. Mg-based metallic alloys are a promising class of biodegradable metals for medical implant applications (Liu 2011; Lock et al. 2014; Tie et al. 2016). Clinically, MgO and $\text{Mg}(\text{OH})_2$ have long been used as a dietary supplements to improve bone density (Carpenter et al. 2006; Stendig-Lindberg et al. 1993). In physiological conditions, both MgO and $\text{Mg}(\text{OH})_2$ release magnesium ions (Mg^{2+}), and Mg^{2+} ions demonstrated osteoconductivity in published works (Yoshizawa et al. 2014a; Yoshizawa et al. 2014b). MgO nanoparticles significantly increased BMSC adhesion and proliferation in low concentrations, e.g., at 200 $\mu\text{g}/\text{mL}$ (Wetteland et al. 2016) and exhibited antimicrobial properties (Sawai 2003; Sawai and Yoshikawa 2004; Wetteland et al. 2016), which is beneficial for reducing the risks associated with infections of implanted materials. However, the fundamental properties of MgO and $\text{Mg}(\text{OH})_2$ in physiologically relevant conditions, including their dissociation in the nanoparticle form, have not yet been well studied. It is necessary to build a library on the fundamental properties of MgO and $\text{Mg}(\text{OH})_2$ in physiological environments to further explore their applications in medicine. Specifically, the objective of this study was to investigate the dissociation of MgO and $\text{Mg}(\text{OH})_2$

nanoparticles when immersed in various physiologically relevant fluids under standard cell culture conditions in vitro, and characterize their changes in microstructure and composition. The standard in vitro cell culture condition, i.e., a 37 °C, 5% CO_2 /95% air, sterile, humidified environment was used in this study to elucidate why the behaviors of MgO and $\text{Mg}(\text{OH})_2$ nanoparticles in our previous in vitro cell culture experiments (Wetteland et al. 2016) differed from the solubility data found in standard chemistry literature.

In this study, Dulbecco's modified Eagle's Medium (DMEM) was selected to represent widely used cell culture media. DMEM with or without supplement of serum proteins were included to determine whether the proteins could affect the dissociation of MgO and $\text{Mg}(\text{OH})_2$ in the media, which could provide important insight into the effects of proteins on the degradation process of Mg-based biodegradable metals because MgO and $\text{Mg}(\text{OH})_2$ represent the major intermediate degradation products of Mg-based biodegradable implants. Simulated body fluid (SBF) was selected to mimic human body fluid since the ion concentrations in SBF are equivalent to those found in human blood plasma (Oyane et al. 2003). One of the buffers in SBF is 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES). HEPES buffer is a synthetic biological buffer that is effective in regulating pH between 6.8 and 8.2. HEPES regulated the local pH at the surface of Mg, which increased the degradation rate of Mg (Dezfuli et al. 2014; Ferguson et al. 1980). Considering the specific activity of HEPES, a solution of HEPES in water was included, to elucidate which component in SBF might have the greatest effect on MgO and $\text{Mg}(\text{OH})_2$ dissociation.

It has been suggested in literature that Cl^- is a key contributor to the corrosion of Mg-based metals by interacting with $\text{Mg}(\text{OH})_2$ at the surface of the metal. It was hypothesized that Cl^- ions may initiate the dissociation of MgO and $\text{Mg}(\text{OH})_2$. To evaluate the effects of Cl^- ions on the dissociation of MgO and $\text{Mg}(\text{OH})_2$, two solutions with different Cl^- sources, MgCl_2 and NaCl, were included in this study. The use of MgCl_2 allowed for the delivery of Cl^- without the addition of another component, considering that MgO and $\text{Mg}(\text{OH})_2$ are expected to release Mg^{2+} ions. NaCl should provide another excellent source of Cl^- ions because previous studies showed that intermediate species of MgO formed in the solution with NaCl (Johnson and Liu 2013; Peng et al. 2014). Thus, NaCl was used as a

comparison to MgCl_2 to evaluate if the cations, Mg^{2+} versus Na^+ , would have different effects on dissociation of the nanoparticles. The concentration of the chloride salts was set at 103 mM, the same concentration of Cl^- found in SBF. A higher concentration of MgCl_2 at 200 mM was included to explore the possible effects of higher chloride concentrations on dissociation of MgO and $\text{Mg}(\text{OH})_2$. Deionized water (DI) was included as a control because it does not have physiologically relevant ions and proteins. Ion concentrations and ionic strengths for cDMEM, DMEM, SBF (Kokubo et al. 1990), HEPES, NaCl (103-mM Cl^-), MgCl_2 (103-mM Cl^-), and MgCl_2 (200-mM Cl^-) are summarized in Table 1.

Materials and methods

Preparation of MgO and $\text{Mg}(\text{OH})_2$ nanoparticles

MgO nanoparticles were procured from US Research Nanomaterials Inc. (US3310, 99+% purity, 20 nm in diameter). In order to mimic the in vivo conditions under which the nanoparticles are used and make the results comparable to other in vitro studies, MgO nanoparticles were sterilized in a glass container via heating at 200 °C in an oven for 1 h prior to immersion in

physiologically relevant solutions. MgO cannot be disinfected through UV irradiation because the UV affects the surface chemistry of the MgO particles by causing adsorption of O_2 and production of superoxide ions (Ito et al. 1985; Yanagisawa 1981). MgO is also recommended not to be autoclaved for sterilization because of its hygroscopic nature. MgO nanoparticles were sterilized prior to characterization and immersion experiments in vitro so that the particles would be in the same condition for each experiment. The sterilized MgO nanoparticles were weighed in the amount of 0.6, 3.0, and 6.0 mg in sterile microcentrifuge tubes to obtain the concentrations of interest. After weighing, 3 mL of respective solution was added to each tube to obtain MgO nanoparticles in the concentrations of 200, 1000, and 2000 $\mu\text{g/mL}$ in each type of immersion solutions. These concentrations were selected based on the previous study with MgO nanoparticles (Wetteland et al. 2016).

$\text{Mg}(\text{OH})_2$ nanoparticles (US3320, 99+% purity, 10 nm in diameter, US Nanomaterials, Inc.) were sterilized in a glass jar and heated in an oven at 100 °C for 1 h. This temperature was selected to be much lower than the dehydration temperature of $\text{Mg}(\text{OH})_2$ at 300 °C to ensure that transition to MgO would not occur. $\text{Mg}(\text{OH})_2$ nanoparticles were weighed in the amount of 0.6, 3.0, 6.0, and 9.0 mg in sterile microcentrifuge

Table 1 Ion concentrations and ionic strengths for the immersion solutions of cDMEM, DMEM, SBF (Kokubo et al. 1990), HEPES, NaCl (103-mM Cl^-), MgCl_2 (103-mM Cl^-), and MgCl_2 (200-mM Cl^-). Values for FBS within cDMEM were estimated from the average measurements reported in literature (Gstraunthaler and Toni 2013). Values for P/S within cDMEM were provided by Hyclone GE Healthcare Life Sciences. HEPES has been shown not to influence ionic strength in literature (Stellwagen et al. 2008)

Ions (mM)	cDMEM	DMEM	SBF	HEPES	NaCl (103-mM Cl^-)	MgCl_2 (103-mM Cl^-)	MgCl_2 (200-mM Cl^-)
Na^+	153.38	154.46	142.01		103		
K^+	14.76	5.51	2.00				
Mg^{2+}	0.80	0.81	1.50			51.5	100
Ca^{2+}	2.30	1.80	2.50				
Cl^-	117.74	118.63	103.08		103	103	200
HCO_3^-	39.64	44.04					
CO_3^{2-}			27.00				
HPO_4^{2-}			1.00				
SO_4^{2-}	0.81	0.81	0.50				
HEPES			50	50			
Ionic strength (M)	0.170	0.168	0.148	~0	0.103	0.154	0.3

tubes. After weighing, 3 mL of respective solution was added to each tube to obtain $\text{Mg}(\text{OH})_2$ nanoparticles in the concentrations of 200, 1000, 2000, and 3000 $\mu\text{g/mL}$ in each type of immersion solutions. The concentration of 3000 $\mu\text{g/mL}$ $\text{Mg}(\text{OH})_2$ had a similar molar concentration to that of MgO at 2000 $\mu\text{g/mL}$. Specifically, 3000 $\mu\text{g/mL}$ of $\text{Mg}(\text{OH})_2$ is equivalent to 51.44 $\mu\text{mol/mL}$ (51.44 mM) and 2000 $\mu\text{g/mL}$ of MgO is equivalent to 49.62 $\mu\text{mol/mL}$ (49.62 mM). This was included to make the results for MgO and $\text{Mg}(\text{OH})_2$ comparable to each other in both mass and molar concentrations.

Preparation of physiologically relevant solutions

Preparation of non-supplemented and complete DMEM

Dulbecco's modified Eagle's Medium (DMEM, Corning) is a common medium for in vitro culture of adherent cells and was purchased from Fisher Scientific. It is common to supplement DMEM with 10% fetal bovine serum (FBS, Hyclone) and 1% penicillin/streptomycin (P/S, Hyclone) to obtain complete DMEM (cDMEM). Both DMEM without supplements and cDMEM with supplemental proteins from FBS and P/S were included for the immersion study to understand the effects of DMEM versus cDMEM. Non-supplemented DMEM is referred to as "DMEM." All solutions were prepared in a laminar flow hood to maintain sterility.

Preparation of SBF

To evaluate the dissociation of MgO and $\text{Mg}(\text{OH})_2$ in a body fluid, revised simulated body fluid (SBF) was prepared as an immersion medium to mimic human blood plasma, following established protocols (Iskandar et al. 2013). Briefly, 700 mL of deionized (DI) water was placed in a 1000-mL polypropylene beaker; then, 5.377 g of NaCl , 0.420 g of NaHCO_3 , 2.332 g of Na_2CO_3 , 0.224 g of KCl , 0.228 g of $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$, 0.305 g of $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 11.928 g of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, Sigma Aldrich) in 100 mL of DI water, 0.277 g of CaCl_2 , and 0.071 g of Na_2SO_4 were added sequentially into the beaker. Please make sure that each component was fully dissolved before adding the next. The solution was mixed and titrated with approximately 0.8 mL of 1-M NaOH solution to bring the pH to 7.4. The pH was confirmed with a pre-calibrated pH meter

(Symphony SB70P, VWR). If pH was not at the target of 7.4, it was adjusted with 1-M NaOH to increase it or HEPES to decrease it. The solution was then transferred to a 1000-mL volumetric polypropylene flask and the total volume was brought up to 1000 mL by adding DI water. In a sterile laminar flow hood, the SBF solution was sterilized via vacuum filtration (Nalgene PES Filter 168, Thermo Scientific).

Preparation of HEPES

HEPES is a zwitterionic buffering agent that can maintain a biological pH between 6.8 and 8.2 and is not dependent on CO_2 concentration in the incubator or air. A HEPES solution in DI water was made to match the same concentration of HEPES found in SBF. Specifically, 1.193 g of HEPES was added to 100 mL of DI water to achieve the concentration of 50 mM. The HEPES solution was sterilized using the same vacuum filtration method (Nalgene PES Filter 168, Thermo Scientific) in a sterile laminar flow hood.

Preparation of NaCl solution

NaCl solution was used as an immersion medium because NaCl is one of the key physiological salts and it can provide a suitable source of Cl^- ions for comparison with MgCl_2 solutions. A solution containing 103 mM of Cl^- was prepared by dissolving NaCl in DI water, in the same manner as that for MgCl_2 solutions described below. As previously mentioned, 103 mM is the concentration of Cl^- in SBF, and SBF mimics ionic concentrations of human blood plasma.

Preparation of MgCl_2 solution

MgCl_2 solutions were used as the Cl^- source at two concentrations of 103- and 200-mM Cl^- in deionized water (DI, QGARD00D2, Millipore). The concentration of 103 mM was selected because this is the same concentration of Cl^- found in SBF. To evaluate the effects of excess Cl^- , on nanoparticle dissociation, MgCl_2 solution at the concentration of 200 mM was also included as an immersion medium. Both solutions were prepared the day prior to use in the immersion experiment. To obtain an initial stock solution, 3 g of $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ was added to a glass bottle of 100 mL of DI, which was stirred with a stir bar to dissolve all salt clumps. The MgCl_2 solution was sterilized by filtration through a

vacuum filter (Nalgene PES Filter 168, Thermo Scientific) in a laminar flow hood. The Mg^{2+} concentration was measured using inductively coupled plasma-optical emission spectrometry (ICP-OES, Optima 8000, Perkin Elmer). The measured Mg^{2+} concentration was 135 mM. The expected concentration from 3 g of $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ in 100 mL of DI was 147.6 mM. This discrepancy could occur if the $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ had been further hydrated in ambient environment before use. Therefore, it is helpful to check the ion concentration using ICP-OES before dilution and then adjusting to the desired concentration by necessary dilutions. In MgCl_2 , the ratio of Mg^{2+} to Cl^- is 1:2. Therefore, the resulting Cl^- concentration in the stock solution was 270 mM. Dilutions were made so that the resulting Cl^- concentration would be 103 and 200 mM for the dissociation study.

Dissociation of MgO and $\text{Mg}(\text{OH})_2$ nanoparticles by immersing in respective solutions

The sterilized solutions with respective MgO or $\text{Mg}(\text{OH})_2$ nanoparticles at the concentrations of 200 $\mu\text{g/mL}$, 1000 $\mu\text{g/mL}$, 2000 $\mu\text{g/mL}$, and 3000 $\mu\text{g/mL}$ (for $\text{Mg}(\text{OH})_2$ only) were thoroughly suspended through pipetting and placed into the wells of sterile 12-well polystyrene culture plates (Corning, Falcon® 351143) in a biosafety-II laminar flow hood to maintain sterility and keep consistency with standard cell culture practices. The respective solutions of 3 mL without nanoparticles were included as the control group, referred to as the 0 $\mu\text{g/mL}$ group. The 12-well polystyrene culture plates were then placed in an incubator (MCO-19AIC, Sanyo Scientific) and incubated under standard cell culture conditions, i.e., 37 °C and 5% CO_2 , for 24 h to allow nanoparticles to dissociate in the conditions that closely mimic a physiological environment. After 24 h of incubation, the plates were removed from the incubator. The respective solutions with remaining particles were collected from each well and transferred into 15-mL conical tubes. Each tube was then centrifuged at 3000 rpm for 2 min to collect the particles as a pellet; the supernatant from each tube was then transferred into a new 15-mL conical tube without disturbing the pellet. To rinse the particles, the pellet was suspended in 100% pure ethanol and centrifuged at 3000 rpm for 1 min. The ethanol was then aspirated out and new ethanol was added for storing the particles for further analyses of microstructure and composition. MgO and $\text{Mg}(\text{OH})_2$ are both insoluble in ethanol (Press 2017).

Analyses of respective solutions after immersion with MgO and $\text{Mg}(\text{OH})_2$

Measuring pH of each solution

The pH of each immersion solution was measured immediately after separation from the particles, to minimize the changes due to temperature and atmospheric CO_2 , using a pre-calibrated pH meter (Symphony SB70P, VWR).

Measuring Mg^{2+} ion concentrations using ICP-OES

Mg^{2+} ion concentration in each solution was measured after immersion of nanoparticles using inductively coupled plasma-optical emission spectrometry (ICP-OES, Optima 8000, Perkin Elmer). Each solution collected after immersion was diluted to 1:200 in DI water. The solutions without nanoparticles were used to establish the baseline concentrations. This baseline concentration was subtracted from the measured Mg^{2+} ion concentrations for the groups with 200–2000 $\mu\text{g/mL}$ (or 3000 $\mu\text{g/mL}$ for $\text{Mg}(\text{OH})_2$) nanoparticles, and the reported values are the concentrations of Mg^{2+} released from MgO or $\text{Mg}(\text{OH})_2$ during immersion in respective solutions.

Characterization of MgO and $\text{Mg}(\text{OH})_2$ nanoparticles before and after immersion

Analyses of microstructure and composition using SEM and EDS

After culture, the collected particles from the MgO group of 2000 $\mu\text{g/mL}$ concentration and $\text{Mg}(\text{OH})_2$ group of 3000 $\mu\text{g/mL}$ concentration for each solution were mounted on a pin mount and coated with platinum and palladium (Pt/Pd) using a sputter-coater (108 Auto, Cressington) for analyses using scanning electron microscopy (SEM; FEI XL30) and energy dispersive x-ray spectroscopy (EDS; attached to SEM). MgO and $\text{Mg}(\text{OH})_2$ before immersion were also characterized using SEM and EDS. Most samples were imaged using a secondary electron detector with a spot size of 3, a working distance of 10 mm, at an accelerating voltage of 30 kV and a magnification of $\times 10,000$. However, MgO and $\text{Mg}(\text{OH})_2$ in the cDMEM solution were imaged at an accelerating voltage of 5 kV, because the presence of proteins resulted in melting/burning of the samples at 30 kV.

Analyses of crystal structures using X-ray diffraction

After culture, the collected particles from the MgO group of 2000 $\mu\text{g/mL}$ concentration and $\text{Mg}(\text{OH})_2$ group of 3000 $\mu\text{g/mL}$ concentration were transferred in 100% ethanol to a zero-diffraction silicon wafer (SiZero24D10C1, MTI). After the ethanol evaporated at room temperature, the particles on a silicon wafer were analyzed using X-ray diffraction (XRD, Empyrean, PANalytical). The XRD spectra for MgO and $\text{Mg}(\text{OH})_2$ before and after immersion were obtained using Cu K α radiation (45 kV, 40 mA) at a step size of 0.006° and dwelling time of 50 s using a PIXcel 1D detector (PANalytical). Phase identification was performed using the HighScore software (PANalytical).

Statistical analysis

All experiments were run in triplicate. All data sets were analyzed using one-way analysis of variance (ANOVA) followed by the Tukey highest significant difference post hoc test. Statistical significance was considered at $p < 0.05$.

Results

Morphological changes in MgO and $\text{Mg}(\text{OH})_2$ nanoparticles after immersion in respective fluids

Exposure and immersion in each type of media resulted in significant changes in the morphologies of remaining particles of MgO and $\text{Mg}(\text{OH})_2$, as shown in Fig. 1. Prior to immersion in the physiological solutions, both MgO and $\text{Mg}(\text{OH})_2$ appeared as spherical particles in the nanometer size range. Both MgO and $\text{Mg}(\text{OH})_2$ appeared to be more aggregated after immersion in cDMEM, likely because of interactions of the nanoparticles with proteins and ions in the media. $\text{Mg}(\text{OH})_2$ showed similar morphology after immersion in DMEM in comparison with the nanoparticles after immersion in cDMEM, but MgO appeared different with flake-shaped microstructures like a sponge mesh. After immersion in SBF, MgO and $\text{Mg}(\text{OH})_2$ showed similar morphology to the respective nanoparticles after immersion in DMEM; that is, MgO showed flake-shaped microstructures while $\text{Mg}(\text{OH})_2$ showed aggregated microstructure. After immersion in HEPES solution,

Fig. 1 SEM images of (A–I) MgO and (A'–I') $\text{Mg}(\text{OH})_2$ nanoparticles before and after immersion in respective physiologically relevant solutions for 24 h. SEM images with the scale bar of 2 μm were all taken at an original magnification of $\times 10,000$. For the SEM images in the insets (I) and (I') that have the scale bar of 200 nm, the original magnification was $\times 150,000$

MgO showed flaky microstructures that organized into a flower morphology, while $\text{Mg}(\text{OH})_2$ showed layered flaky microstructures. After immersion in

NaCl solution, both MgO and Mg(OH)₂ showed particulate agglomerates embedded with some thin-sheet structures, and interestingly, MgO sample seemed to have nanostructured meshes within the agglomerates, which was not observed with Mg(OH)₂ sample. After immersion in MgCl₂ solutions with the concentrations of 103 mM and 200 mM, MgO showed interlaced flaky flower-like structures, while Mg(OH)₂ showed agglomerates of needle-shaped structures embedded in some thin-sheet structures. After immersion in DI water, similar microstructures were observed for both MgO and Mg(OH)₂, when comparing to their respective counterpart after immersion in MgCl₂ solutions. In general, MgO appeared to have more dramatic change in the morphologies when comparing before and after immersion in respective solutions than that of Mg(OH)₂.

Changes in crystalline phases and elemental compositions in MgO and Mg(OH)₂ after immersion in respective fluids

The XRD spectra of MgO before immersion confirmed the characteristic peaks of MgO phase with some small peaks of Mg(OH)₂ phase. The XRD results revealed that two characteristic peaks of MgO shifted from 42° and 62° to 44° and 64°, respectively, after immersion in respective solutions, indicating crystal structural change, as shown in Fig. 2. A peak of Mg(OH)₂ phase at 38° was detected after MgO were immersed in most testing solutions for 24 h, except in HEPES solution. Additionally, a small peak of MgCl₂ phase was found after MgO were immersed in MgCl₂ solution with 200-mM Cl⁻. The residual MgO particles after culturing in HEPES solution resulted in no detectable peaks, indicating loss of its crystalline phase.

Fig. 2 XRD spectra of MgO nanoparticles before and after immersion in respective physiologically relevant solutions

The XRD spectra of MgO before immersion confirmed the characteristic peaks of $\text{Mg}(\text{OH})_2$ phase. After 24 h of immersion in respective physiological solutions, the remaining $\text{Mg}(\text{OH})_2$ particles still exhibited most characteristic peaks of $\text{Mg}(\text{OH})_2$, with some degree of decrease in peak intensity in all testing solutions except HEPES, as shown in Fig. 3. In the HEPES solution, almost all characteristic peaks for $\text{Mg}(\text{OH})_2$ disappeared and small peaks of residual HEPES were detected. After $\text{Mg}(\text{OH})_2$ nanoparticles were immersed in MgCl_2 , a small peak corresponding with $\text{Mg}_2(\text{OH})_3\text{Cl}$ phase was detected.

EDS analyses of the MgO and $\text{Mg}(\text{OH})_2$ nanoparticles could complement the XRD results and provide useful information on the changes in elemental compositions before and after immersion, as shown in Tables 2 and 3 and Fig. 4. Before immersion, as expected, MgO and $\text{Mg}(\text{OH})_2$ nanoparticles only showed the presence of Mg and O with respective atomic ratios of 1:1 and 1:2.

After immersion in cDMEM, MgO sample showed the presence of Mg, O, C, P, Ca, Na, and S; the atomic percent for Mg was only 8.58%. After immersion in DMEM, MgO sample showed the presence of Mg, O, C, Cl, and Na with 23.32% Mg. In contrast, $\text{Mg}(\text{OH})_2$ sample showed the presence of Mg, O, C, and Na after immersion in cDMEM, where the atomic percent of Mg was 77.85%; for $\text{Mg}(\text{OH})_2$ immersed in DMEM, Mg, O, C, P, Ca, Cl, Na, and S were observed, where Mg content was 20.38%. After immersion in SBF, both MgO and $\text{Mg}(\text{OH})_2$ samples showed the presence of Mg, O, C, P, Ca, and Na, with 9.66% and 3.36% Mg, respectively. After immersion in HEPES solution, MgO yielded residual particles with Mg, O, C, and S detected, while $\text{Mg}(\text{OH})_2$ yielded residual particles with Mg, O, and C detected. After immersion in each chloride solution, the elements from the respective salts were present along with Mg and O for MgO and $\text{Mg}(\text{OH})_2$, as expected.

Fig. 3 XRD spectra of $\text{Mg}(\text{OH})_2$ nanoparticles before and after immersion in respective physiologically relevant solutions

Table 2 EDS analyses of MgO nanoparticles before and after immersion in physiologically relevant solutions. The EDS analyses were performed on the corresponding SEM images in Fig. 1. The MgO column is the elemental compositions of respective nanoparticles before immersion

MgO sample before and after immersion in respective solutions (atomic %)									
Element	cDMEM	DMEM	SBF	HEPES	NaCl (103-mM Cl ⁻)	MgCl ₂ (103-mM Cl ⁻)	MgCl ₂ (200-mM Cl ⁻)	DI	MgO
Mg	8.58	23.32	9.66	8.37	36.55	34.59	24.20	34.18	46.18
O	15.19	47.99	31.76	27.43	62.21	64.52	47.81	65.82	53.82
C	70.14	27.39	54.74	63.85	0	0	27.07	0	0
P	2.98	0	2.24	0	0	0	0	0	0
Ca	2.16	0	1.31	0	0	0	0	0	0
Cl	0	1.18	0	0	0.51	0.90	0.92	0	0
Na	0.73	0.13	0.30	0	0.74	0	0	0	0
S	0.22	0	0	0.35	0	0	0	0	0
O/Mg ratio	1.77	2.06	3.29	3.28	1.70	1.87	1.98	1.93	1.17

After immersion in DI water, only Mg and O were detected for both MgO and Mg(OH)₂ samples, and the O content in the post-immersion MgO sample increased but decreased in Mg(OH)₂ sample when compared with the compositions of their counterparts before immersion.

Release of Mg²⁺ ions and pH change after immersion of MgO and Mg(OH)₂ in respective fluids

Figure 5 shows the changes in Mg²⁺ ion concentrations in cDMEM, DMEM, SBF, HEPES, chloride solutions, and DI water after culture with MgO and

Mg(OH)₂. The theoretical Mg²⁺ ion concentrations under the assumption of complete dissociation of MgO or Mg(OH)₂, i.e., 100% dissociation, were calculated and plotted as the black bars in respective conditions for comparison. Generally, Mg²⁺ concentration increased as the initial concentrations of MgO and Mg(OH)₂ increased. At 200 µg/mL, both MgO and Mg(OH)₂ showed 100% dissociation in all solutions. The solutions that showed the values exceeding the 100% dissociation level are attributed to the deviation in initial mass measurement of the nanoparticles and in Mg²⁺ concentration measurement with ICP-

Table 3 EDS analyses of Mg(OH)₂ nanoparticles before and after immersion in physiologically relevant solutions. The EDS analyses were performed on the corresponding SEM images in Fig. 1. The Mg(OH)₂ column is the elemental compositions of respective nanoparticles before immersion

Mg(OH) ₂ sample before and after immersion in respective solutions (atomic %)									
Element	cDMEM	DMEM	SBF	HEPES	NaCl (103-mM Cl ⁻)	MgCl ₂ (103-mM Cl ⁻)	MgCl ₂ (200-mM Cl ⁻)	DI	Mg(OH) ₂
Mg	77.85	20.38	3.36	4.56	37.22	38.58	41.43	40.36	35.57
O	15.98	42.25	13.17	20.37	62.13	61.35	57.87	59.64	63.43
C	4.58	34.01	80.72	75.06	0	0	0	0	0
P	0	1.69	1.89	0	0	0	0	0	0
Ca	0	0.93	0.38	0	0	0	0	0	0
Cl	0	0.08	0	0	0.20	0.07	0.16	0	0
Na	1.63	0.49	0.07	0	0.45	0	0	0	0
S	0	0.17	0	0	0	0	0	0	0
O/Mg ratio	0.21	2.07	3.92	4.47	1.67	1.59	1.40	1.48	1.78

Fig. 4 Graphical representation of the elemental compositions of (A) MgO and (B) Mg(OH)₂ nanoparticles, quantified from EDS analyses before and after they were immersed in respective physiologically relevant solutions for 24 h

OES. In Fig. 5A, MgO at 1000 µg/mL and 2000 µg/mL showed statistically higher dissociation when immersed in cDMEM, DMEM, and SBF than that in HEPES buffer, NaCl, MgCl₂ (103 and 200 mM), and DI water. Moreover, the dissociation of 1000 µg/mL and 2000 µg/mL MgO reached about 100% level in cDMEM, DMEM, and SBF. The dissociation of

2000 µg/mL of MgO in NaCl (103-mM Cl⁻) was greater than that in MgCl₂ (103- and 200-mM Cl⁻) and DI water. In Fig. 5B, at the concentration of 1000 µg/mL, Mg(OH)₂ showed statistically greater Mg²⁺ ion concentration and dissociation in SBF than that in the solutions of just chloride salts. At the concentration of 2000 µg/mL, Mg(OH)₂ showed

Fig. 5 Mg²⁺ ion concentrations after immersion of (A) MgO and (B) Mg(OH)₂ nanoparticles in respective physiologically relevant solutions for 24 h. The black bars show the theoretical values of Mg²⁺ ion concentrations that were calculated under the assumption of 100% dissociation of respective nanoparticles. Experimental values are mean ± standard deviation; *n* = 3. *p < 0.05

statistically greater Mg²⁺ ion concentration and dissociation in SBF and HEPES than that in all the other solution. and the dissociation of Mg(OH)₂ was statistically greater in cDMEM and DMEM than that in the solutions of chloride salts and DI water. At the concentration of 3000 μg/mL, Mg(OH)₂ showed statistically greater Mg²⁺ ion concentration and dissociation in cDMEM, DMEM, SBF, and HEPES than that in the solutions of just chloride salts and DI water. and the dissociation of Mg(OH)₂ in HEPES and SBF was statistically greater than that in cDMEM and DMEM. In general, at the concentration of 3000 μg/mL, dissociation of Mg(OH)₂ showed the trend of high to low in the order of HEPES > SBF > (cDMEM, DMEM) >

NaCl > (MgCl₂, DI water). None of the groups with 1000, 2000, or 3000 μg/mL of Mg(OH)₂ reached the 100% dissociation.

Figure 6 shows the pH of the respective solutions after 24 h of culture with different concentrations of MgO and Mg(OH)₂. In the 0 μg/mL condition, as shown in Fig. 6A and B, the pH of cDMEM, DMEM, and SBF was between 7.6 and 8.2, and the pH of HEPES, NaCl, and MgCl₂ (103-mM and 200-mM Cl⁻) was between 4.9 and 5.3. The pH of DI water was 5.6–5.8. At 200 μg/mL, the pH of cDMEM and DMEM was approximately 8.1–8.3 and the pH of SBF was 7.8 for both MgO and Mg(OH)₂. The pH of the HEPES solution was 6.6 when cultured with Mg(OH)₂ and 7 with MgO. The pH of the NaCl

Fig. 6 The pH values of respective physiologically relevant solutions before and after immersion of (A) MgO and (B) Mg(OH)₂ nanoparticles for 24 h. Values are mean $\pm$ standard deviation; $n = 3$. * $p < 0.05$

solution was 7.7 after immersion with both nanoparticles. When immersed with MgO, as shown in Fig. 6A, the pH of MgCl₂ solutions (103-mM and 200-mM Cl⁻) was 8.4 and 8, respectively. When immersed with Mg(OH)₂, as shown in Fig. 6B, the pH of MgCl₂ solutions (103-mM and 200-mM Cl⁻) was 8.5 and 7.9, respectively. For both MgO and Mg(OH)₂, the pH of DI water was 8.1. Generally, for each solution, its pH continued to increase with increasing concentrations of MgO or Mg(OH)₂, up to 2000 $\mu\text{g/mL}$ and 3000 $\mu\text{g/mL}$, respectively. When the nanoparticles increased to 1000, 2000, and 3000 $\mu\text{g/mL}$, the pH of different solutions followed a similar trend as that at 200 $\mu\text{g/mL}$. At 2000 $\mu\text{g/mL}$ of MgO and 3000 $\mu\text{g/mL}$ of Mg(OH)₂, the pH of cDMEM, DMEM, SBF, and

HEPES was statistically lower than that of three chloride solutions and DI water, likely because the former had buffering effects and the latter did not.

Discussion

Microstructural and compositional changes after immersion of MgO and Mg(OH)₂ nanoparticles in physiologically relevant fluids

MgO nanoparticles were hydrated to form Mg(OH)₂ when immersed in respective solutions tested in this study, which is expected as this reaction is well

established (Fruhworth et al. 1985; Láska et al. 1993; Refson et al. 1995). After MgO was cultured in respective fluids, the characteristic peaks of Mg(OH)_2 were found in the XRD spectra (Fig. 2) and the atomic ratio of O/Mg increased from ~ 1 to ~ 2 for most of fluids and ~ 3 for SBF and HEPES (Table 2, Fig. 4A), which confirmed the formation of Mg(OH)_2 . Moreover, the ordered flower-like microstructures after immersion of MgO nanoparticles in respective fluids (Fig. 1) were very similar to nano- Mg(OH)_2 reported in literature (Jadhav 2014; Li et al. 2013). Previous studies suggested that the transformation of MgO to Mg(OH)_2 might occur along a shared crystal face (Fruhworth et al. 1985; Láska et al. 1993). However, based on our post-culture sample characterization, it is also likely that Mg(OH)_2 precipitated from the dissociated ions. It is speculated that both MgO to Mg(OH)_2 transformation and re-deposition of Mg(OH)_2 from ions might have occurred in culture. Interestingly, after immersion of MgO in HEPES, no crystalline phase was detected (Fig. 2), even though flower-like microstructures were still observed in SEM image (Fig. 1). This is possibly related to the high O/Mg ratio of ~ 3 after MgO was cultured in HEPES and distinct interactions between MgO and HEPES buffer. The small MgCl_2 peak was detected after immersion of MgO samples in the higher concentration of MgCl_2 solution but not in the lower concentration counterpart, suggesting that the peak of MgCl_2 was more likely from salt residue of the immersion solution, rather than nucleophilic substitution of O^{2-} or OH^- by Cl^- . Actual nucleophilic substitution of OH^- by Cl^- is unlikely, because the energy in Mg=O and Mg-OH bonds is greater than that in Mg-Cl , making nucleophilic substitution by chloride energetically unfavorable.

Mg(OH)_2 showed little change in the crystal structure after immersion in most physiological solutions except in HEPES (Fig. 3). In HEPES, most peaks for Mg(OH)_2 phase disappeared and some peaks of HEPES were detected, likely because of the distinct interactions between Mg(OH)_2 and HEPES. The small peak of $\text{Mg}_2(\text{OH})_3\text{Cl}$ detected in Mg(OH)_2 exposed to MgCl_2 solutions suggested that Cl^- could bind to the intermediate MgOH^+ formed during Mg(OH)_2 dissociation. It is possible that the presence of Cl^- stabilized the charge in MgOH^+ groups, which could decrease the adsorption of H^+ to nearby Mg(OH)_2 , thus enhancing dissociation of Mg(OH)_2 . Fruhwirth et al. described formation of MgOH^+ as a rate limiting step in the dissociation of MgO (Fruhworth et al. 1985), which is likely a rate

limiting step for the dissociation of Mg(OH)_2 as well, considering that MgO readily hydrates to form Mg(OH)_2 . Previous studies indicated that metallic magnesium (Mg) degrades more rapidly in solutions containing NaCl (Johnson and Liu 2013; Peng et al. 2014). While direct nucleophilic substitution is unlikely as energetically unfavorable, the interaction between Cl^- and MgOH^+ is a possible explanation for the increased degradation of metallic Mg in chloride containing solutions.

The EDS results for MgO and Mg(OH)_2 in cDMEM, DMEM, and SBF showed the presence of expected elements from the components within these solutions. The carbon (C) and sulfur (S) detected after immersion in HEPES are likely from the HEPES itself. The sodium and chloride detected after immersion in NaCl solution, as well as the chloride detected after immersion in MgCl_2 solutions, are all considered to be residues from the salt solutions. Importantly, the amount of chloride detected was very low (1% or less) after immersion of MgO or Mg(OH)_2 in NaCl or MgCl_2 solutions, which indicated that 99% or more of remaining particles did not contain chloride and supported that formation of MgCl_2 was unlikely. It is more likely that the chloride detected in EDS was from salt residue of NaCl and MgCl_2 solutions.

Metallic Mg alloys has attracted significant interest for orthopedic implant applications because of its bone-like mechanical properties, biodegradability, and biocompatibility (Cipriano et al. 2017a; Cipriano et al. 2015). However, Mg-based implants have a rapid degradation rate, which greatly limits their clinical translation. Modifying metallic Mg surface with MgO and Mg(OH)_2 has been attempted to reduce the degradation rate of Mg (Cipriano et al. 2017b; Cipriano et al. 2015), but the interactions of MgO and Mg(OH)_2 with physiological solutions are not well understood. Previous studies showed that HEPES increased the degradation rate of metallic Mg (Dezfuli et al. 2014). As Mg degrades, a passivation layer of MgO and Mg(OH)_2 forms on its surface. It is likely that the interactions of MgO and Mg(OH)_2 with HEPES disrupted the passivation layer and thus increased degradation of Mg. Previous studies suggested that the presence of Cl^- ions accelerated the degradation of Mg (Mueller et al. 2009) because chloride ions replaced hydroxide in the Mg(OH)_2 that formed on the surface during Mg degradation; the formation of highly soluble MgCl_2 was

believed to be responsible for the release of Mg^{2+} . However, $\text{Mg}(\text{OH})_2$ (solubility 1.25×10^{-2} g/L) (Dean 1998) has been known to have significantly lower solubility than MgCl_2 (solubility ~ 5.5 g/L), which made the replacement of the hydroxide groups by chloride energetically unfavorable. Since the 1930s, $\text{Mg}(\text{OH})_2$ has been produced by a precipitation method on a large scale, that is, by adding $\text{Ca}(\text{OH})_2$ to a solution of MgCl_2 to form $\text{Mg}(\text{OH})_2$, which further demonstrated that the conventional explanation (Amundsen et al. 2000; Cotton and Wilkinson 1988) on the role of chloride attack on Mg degradation and $\text{Mg}(\text{OH})_2$ dissociation should be reconsidered. This study on the dissociation of MgO and $\text{Mg}(\text{OH})_2$ in the presence of Cl^- provided new insight on the degradation processes of metallic Mg in physiologically relevant fluids.

Mg^{2+} release and pH change in physiologically relevant fluids induced by MgO and $\text{Mg}(\text{OH})_2$ nanoparticles

The change in Mg^{2+} ion concentration measured by ICP-OES is a more reliable indicator of the dissociation of MgO and $\text{Mg}(\text{OH})_2$, while microstructural and compositional characterization by SEM, EDS, and XRD provided valuable information on the change in the solid phases after immersion in the relevant solutions. At 200 $\mu\text{g/mL}$, both MgO and $\text{Mg}(\text{OH})_2$ nanoparticles showed 100% dissociation, i.e., fully degraded, in all solutions. Interestingly, the respective solubility of MgO (0.0086 g/L = 8.6 mg/L = 8.6 $\mu\text{g/mL}$) and $\text{Mg}(\text{OH})_2$ (1.25×10^{-2} g/L = 12.5 mg/L = 12.5 $\mu\text{g/mL}$) reported in conventional textbooks (Amundsen et al. 2000; Cotton and Wilkinson 1988; Dean 1998) was much lower than 200 $\mu\text{g/mL}$ (i.e., 0.2 g/L). These conventional solubility values were determined at 25 °C in ambient conditions in distilled water; in contrast, the immersion solutions were kept at 37 °C under standard cell culture conditions (sterile, humidified, 5% CO_2) in this study. Temperature, environment, and compositions of immersion solutions are all possible factors contributed to the observed differences. Moreover, the nano-sized MgO and $\text{Mg}(\text{OH})_2$ particles have higher surface area to volume ratio and greater surface energy, which could also increase dissociation reactions in the immersion solutions. The conventional methods for measuring the solubility of a compound rely on visual inspection of precipitation with human eyes (Bhattachar et al. 2006; Wolthuis et al. 1960); in contrast, the ICP-OES was used

to in this study quantify Mg^{2+} ions, which provides greater accuracy for measuring dissociation of MgO and $\text{Mg}(\text{OH})_2$. It is likely the differences in the compositions of culture media, pH values, particle sizes, experimental environments (temperature, $\text{CO}_2\%$, humidity), and methods of measurement that have resulted in the higher than expected dissociation of MgO and $\text{Mg}(\text{OH})_2$ in aqueous culture media than their solubility data in water that were previously reported in conventional chemistry textbooks.

At the concentrations of 1000 $\mu\text{g/mL}$ and above, the dissociation of MgO in cDMEM, DMEM, and SBF was far greater than that in the NaCl, MgCl_2 , and DI water solutions. In comparison, the dissociation of $\text{Mg}(\text{OH})_2$ in cDMEM, DMEM, SBF, and HEPES was greater than that in the NaCl, MgCl_2 , and DI water solutions in average at the concentrations of 1000 $\mu\text{g/mL}$ and was statistically greater at the concentrations of 2000 and 3000 $\mu\text{g/mL}$. Previous studies on the hydration kinetics and dissolution of MgO at room temperature showed a non-linear pH effect. The dissociation of MgO and $\text{Mg}(\text{OH})_2$ was reported to be heavily influenced by pH and temperature (Fedorockova and Raschman 2008; Fruhwirth et al. 1985; Mejias et al. 1999). Both MgO and $\text{Mg}(\text{OH})_2$ are basic solids. At a pH less than 5, MgO can freely dissociate as $\text{MgO} + 2\text{H}^+ \rightarrow \text{Mg}^{2+} + \text{H}_2\text{O}$, with the rate dependent upon the concentration of H^+ and Mg^{2+} already present in the solution. At a pH of 7–8.5, MgO can dissociate in an overall reaction of $\text{MgO} + \text{H}_2\text{O} \rightarrow \text{Mg}^{2+} + 2\text{OH}^-$ (Fruhwirth et al. 1985); the dissociation rate in this case is dependent upon OH^- adsorption and subsequent Mg^{2+} and OH^- desorption. The proposed mechanism was that adsorption of protons would produce MgOH^+ , which was vulnerable to OH^- attack. The buffering capacity of cDMEM, DMEM, and SBF could keep their pH in the physiological ranges between 7.2 and 8.2, which would increase the dissociation of $\text{Mg}(\text{OH})_2$ according to this proposed mechanism. Meanwhile, $\text{Mg}(\text{OH})_2$ could also precipitate, which would counteract the dissociation. Since the pH of HEPES, NaCl, MgCl_2 , and DI water was acidic, the former reaction is more likely. Since the pH of physiologically relevant fluids such as DMEM and SBF are usually buffered at a pH of 7.4 that falls between the 7 and 8.5 ranges, the latter reaction is more likely. DMEM consists of salts in similar concentrations as in SBF, bicarbonate buffer, and amino acids. The pH and buffering capability within DMEM likely caused the dissociation of MgO and $\text{Mg}(\text{OH})_2$ to continue, in agreement

with literature (Fruhworth et al. 1985); the dissociation of MgO in SBF was not significantly different from that in cDMEM or DMEM, which further supported this notion.

The dissociation of $\text{Mg}(\text{OH})_2$ in SBF and HEPES was significantly higher than that in cDMEM and DMEM at the concentration of 2000 $\mu\text{g/mL}$ and above, likely because the HEPES buffer present in the former two solutions but absent in the latter two solutions caused the difference. $\text{Mg}(\text{OH})_2$ readily dissociated in the presence of HEPES alone, which might have been facilitated by the acidic pH of the HEPES-only solutions. Additionally, HEPES was known to have an affinity for metallic ions in blood plasma (Dezfuli et al. 2014), which further supports the possibility of HEPES-mediated dissociation of $\text{Mg}(\text{OH})_2$. However, the dissociation behaviors of MgO and $\text{Mg}(\text{OH})_2$ in HEPES may not reflect how they would behave in vivo, considering that HEPES is a synthetic buffer.

Interestingly, the dissociation of MgO and $\text{Mg}(\text{OH})_2$ nanoparticles showed no statistically significant difference after immersion in cDMEM versus DMEM, indicating that the presence of FBS and P/S was not a major factor affecting their dissociation. However, previous studies have shown that magnesium ions could bind to serum albumin (Kroll and Elin 1985), which is present in FBS and thus in cDMEM, but not in DMEM. Logically, the binding of Mg^{2+} ions to proteins would remove free Mg^{2+} from solution, thereby driving the dissociation of MgO and $\text{Mg}(\text{OH})_2$ nanoparticles in cDMEM to a higher level than that in DMEM. Indeed, at 2000 $\mu\text{g/mL}$ MgO and 3000 $\mu\text{g/mL}$ $\text{Mg}(\text{OH})_2$, the average Mg^{2+} ion concentration in cDMEM was higher than that in DMEM, indicating greater dissociation in cDMEM than that in DMEM, even though the statistical significance was not detected. The effects of protein- Mg^{2+} binding on the dissociation of MgO and $\text{Mg}(\text{OH})_2$ nanoparticles become more pronounced when the nanoparticle concentrations increased, and, thus, it is possible to detect significantly greater dissociation in cDMEM than in DMEM when the MgO and $\text{Mg}(\text{OH})_2$ concentrations exceed 2000 $\mu\text{g/mL}$ and 3000 $\mu\text{g/mL}$, respectively. This requires further investigation.

MgO and $\text{Mg}(\text{OH})_2$ nanoparticles showed no increase in dissociation in the chloride solutions when compared to the DI water. This is to be expected, because the energy of the bonds in MgO and $\text{Mg}(\text{OH})_2$ is greater than that of MgCl_2 , indicating that substitution is not energetically favored. Additionally, the presence of Mg^{2+} has also been

shown to decrease dissociation of MgO, which is consistent with diffusion kinetics (Fruhworth et al. 1985); this might be the reason that greater average Mg^{2+} release was observed in the NaCl solution than in the MgCl_2 solutions with the same Cl^- concentration.

Conclusion

Nano-sized MgO and $\text{Mg}(\text{OH})_2$ dissociated significantly in physiologically relevant solutions that mimic the in vivo conditions inside the body, even though MgO and $\text{Mg}(\text{OH})_2$ have conventionally been regarded as stable and insoluble compounds. While biological fluids and environments are extremely complex and dynamic, it is likely that pH still plays the most significant part in the dissociation of MgO and $\text{Mg}(\text{OH})_2$ nanoparticles. The buffering capacity of physiological solutions likely drives the reactions toward further dissociation of MgO and $\text{Mg}(\text{OH})_2$ nanoparticles. It is unlikely that Cl^- can go through nucleophilic substitution on MgO or $\text{Mg}(\text{OH})_2$, but it is likely that Cl^- plays a role in stabilizing the intermediate forms of MgO and $\text{Mg}(\text{OH})_2$ during dissociation. This study provided the first evidence on the behaviors of MgO and $\text{Mg}(\text{OH})_2$ nanoparticles in physiologically relevant conditions in vitro and revealed the possible factors contributing to their dissociation, which is important for their potential medical applications in vivo. Additionally, the results on the dissociation behaviors of MgO and $\text{Mg}(\text{OH})_2$ in physiologically relevant conditions are valuable for understanding the in vivo degradation process of metallic Mg and Mg alloys for bioresorbable implant applications.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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