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Concentration-dependent behaviors of bone marrow derived mesenchymal stem cells and infectious bacteria toward magnesium oxide nanoparticles

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ABSTRACT

This article reports the quantitative relationship between the concentration of magnesium oxide (MgO) nanoparticles and its distinct biological activities towards mammalian cells and infectious bacteria for the first time. The effects of MgO nanoparticles on the viability of bone marrow derived mesenchymal stem cells (BMSCs) and infectious bacteria (both gram-negative *Escherichia coli* and gram-positive *Staphylococcus epidermidis*) showed a concentration-dependent behavior *in vitro*. The critical concentrations of MgO nanoparticles identified in this study provided valuable guidelines for biomaterial design toward potential clinical translation. BMSCs density increased significantly when cultured in 200 μg/mL of MgO in comparison to the Cells Only control without MgO. The density of BMSCs decreased significantly after culture in the media with 500 μg/mL or more of MgO. Concentrations at or above 1000 μg/mL of MgO resulted in complete BMSCs death. Quantification of colony forming units (CFU) revealed that the minimum bactericidal concentration (MBC) of MgO for *E. coli* and *S. epidermidis* was 1200 μg/mL. The addition of MgO nanoparticles into the cultures increased the pH and Mg^{2+} ion concentration in the respective culture media, which might have played a role in the observed cell responses but not the main factors. *E. coli* and *S. epidermidis* still proliferated significantly at alkaline pH up to 10 or with supplemental Mg^{2+} dosages up to 50 mM, indicating bactericidal properties of MgO are beyond the effects of increased media pH and Mg^{2+} ion concentrations. MgO nanoparticles at a concentration of 200 μg/mL provided dual benefits of promoting BMSC proliferation while reducing bacterial adhesion, which should be further studied for potential medical implant applications. The use of free MgO nanoparticles yielded detrimental effects to BMSCs in concentrations above 300 μg/mL. We recommend further study into MgO nanoparticle as a coating material or as a part of a composite.

Statement of Significance

This article reports the quantitative relationship between the concentration of magnesium oxide (MgO) nanoparticles and its distinct biological activities towards mammalian cells and infectious bacteria for the first time. The effects of MgO nanoparticles on the viability of bone marrow derived mesenchymal stem cells (BMSCs) and infectious bacteria (both gram-negative *Escherichia coli* and gram-positive *Staphylococcus epidermidis*) showed a concentration-dependent behavior *in vitro*. The critical concentrations of MgO nanoparticles identified in this study provided valuable guidelines for biomaterial design toward potential clinical translation.

1. Introduction

This article reports the quantitative relationship between the concentration of magnesium oxide (MgO) nanoparticles and its distinct biological activities towards mammalian cells and

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infectious bacteria for the first time. The effects of MgO nanoparticles on the viability of bone marrow derived mesenchymal stem cells (BMSCs) and infectious bacteria (both gram-negative and gram-positive) showed a concentration-dependent behavior *in vitro*. The critical concentrations of MgO nanoparticles identified in this study provided valuable guidelines for biomaterial design toward potential clinical translation.

The bioactivity and mechanical properties of MgO have recently attracted significant interest for potential biomedical applications, from bone repair to biosensing [1–5]. For bone repair, Nygren et al. observed that the thickness of compact bone increased by 25% after implanting MgO powder paste into bone marrow cavity of rat tibia for 3 weeks as compared with sham-operated controls. This suggested that MgO had stimulatory effects on bone healing and regeneration [6]. Clinically, MgO has also been used to improve bone mineral density when taken orally as a dietary supplement [7]. Moreover, MgO is beneficial for improving mechanical properties of poly (methyl methacrylate) (PMMA)-based bone cement. For example, MgO was incorporated into PMMA cement to enhance the fracture toughness of bone-PMMA interface [8]. Additionally, MgO powder was reported to exhibit antimicrobial properties against both gram-negative and gram-positive bacteria *in vitro*, such as *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) [9,10]. These appealing properties of MgO make it a promising material for treating bone-related diseases and injuries, such as osteoporosis (a disease that decreases bone density and increases risk for bone fracture), osteomyelitis (bone infection), bone fracture, or other bone loss induced by trauma or disease.

MgO nanoparticles can further enhance the aforementioned properties considering its high surface area to volume ratio due to its nanometer size, as has been shown for other metal oxide nanoparticles [11]. Before taking advantage of their desirable properties for clinical applications, it is important to determine how MgO nanoparticles affect cellular functions, specifically the functions of relevant bone cells. BMSCs are multipotent stem cells that can differentiate into a variety of mesodermal cells, including osteoblasts (bone forming cells), and play critical roles in bone healing [12,13]. Thus, BMSC culture provides an excellent model system for studying the cytocompatibility of MgO nanoparticles and determining clinically relevant concentration range.

The culture methods to determine material-cell interactions are not yet standardized. Previous studies have used co-seeding and sequential seeding to study the interactions between BMSCs and other cell lines [14–16]. We were interested in applying the same techniques to study the interactions between BMSCs and MgO nanoparticles. Co-seeding involves seeding the BMSCs with MgO simultaneously. Sequential seeding involves culturing BMSCs for 24 h prior to exposure to MgO. Through these methods we aimed to model two analogous *in vivo* scenarios: the interactions between MgO nanoparticles and migrating cells (co-seeding) and the interaction between MgO nanoparticles and well-established cells within the extracellular matrix (sequential seeding).

In addition, to realize the coupled benefits of MgO nanoparticles in promoting bone growth and antimicrobial activity, it is essential to determine how MgO nanoparticles affect viability of infectious bacteria, especially the species causing orthopedic implant infection and osteomyelitis. *E. coli* is one of the most common gram-negative bacteria that cause orthopedic implant infection [17]. *Staphylococcus epidermidis* (*S. epidermidis*) is one of the most common gram-positive infectious bacteria found in osteomyelitis [17,18]. Thus, we are interested in evaluating the antimicrobial activity of MgO nanoparticles with both bacteria types. It is also important to include both gram-negative and gram-positive bacteria in the study to comprehend overall antimicrobial properties of a material. Minimum bactericidal concentration (MBC) is crucial for fighting clinically relevant bacterial infections and developing antimicrobial biomaterials and surfaces because MBC indicates the lowest concentration required to kill bacteria. Specifically, the MBC is the concentration that results in $\geqslant$90% decrease in the colony forming units (CFU), with respect to the seeding density. MBC is not yet established for MgO nanoparticles. Krishnamoorthy reported that the minimum inhibitory concentration (MIC) of MgO nanoparticles was 500 μg/mL for *E. coli* and 1000 μg/mL for *S. aureus* using a microtitre plate-based assay with resazurin (7-hydroxy-10-oxidophenoxazin-10-ium-3-one, $C_{12}H_7NO_4$) as an indicator for bacterial growth [19]. The previously reported MIC provided the basis for our study to determine the MBC of MgO nanoparticles for *E. coli* and *S. epidermidis*.

Therefore, the objective of this study was to investigate the effects of MgO nanoparticles on the viability of BMSC, *E. coli*, and *S. epidermidis*, and to determine the critical cytotoxic concentrations (CCC) for BMSCs and MBCs for bacteria. To better understand the factors that may affect the CCC and MBCs, we also analyzed the media and broth with respect to the change of pH and Mg^{2+} ion concentrations.

2. Materials and methods

2.1. Preparation and characterization of MgO nanoparticles

MgO nanoparticles were procured from US Research Nanomaterials Inc. (US3310). The vendor reported that MgO nanoparticles had a 99+% purity, a diameter of 20 nm, a specific surface area of >60 m^2/g, a polyhedral morphology, a bulk density of 0.145 g/cm^3, and a true density of 3.58 g/cm^3. The MgO nanoparticles for BMSC and bacterial cultures were sterilized through heating at 200 °C in an oven for one hour. MgO cannot be sterilized 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 [25,26]. MgO nanoparticles were sterilized prior to characterization so that the particles would be in the same condition during characterization as they were in *in vitro* experiments.

MgO nanoparticles were characterized before their use in cell and bacteria cultures. The morphology of MgO nanoparticles was visualized using a field emission scanning electron microscope (SEM; Philips XL30), with a secondary electron detector, at an accelerating voltage of 30 kV, a working distance of 10 mm, and an original magnification of 250,000×. MgO particle size and distribution were quantified based on SEM images using the quantitative image analysis tools in ImageJ. The morphology and crystal structure of the MgO nanoparticles were also confirmed using transmission electron microscopy (TEM; Titan Themis 300) at an accelerating voltage of 120 kV. Elemental composition of the MgO nanoparticles was confirmed using the energy dispersive X-ray spectroscopy (EDS; EDAX Leap detector attached to Philips XL30 SEM) at a spot size of 3 and an accelerating voltage of 30 kV. Crystalline phase of MgO nanoparticles was characterized using X-ray diffraction (XRD; Empyrean, PANalytical). The XRD spectrum for MgO was obtained using Cu Kα 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).

2.2. BMSC culture with MgO nanoparticles and analyses

2.2.1. Bone marrow derived mesenchymal stem cell culture

Rat BMSCs were extracted from the femur and tibia of juvenile Sprague Dawley rats according to the established protocol approved by the Institutional Animal Care and Use Committee (IACUC) at the University of California at Riverside [27,28]. Briefly, the ends of the dissected long bones were cut using a scalpel and