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Antibiofilm activity of nanosized magnesium fluoride

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ABSTRACT

The ability of bacteria to develop antibiotic resistance and colonize abiotic surfaces by forming biofilms is a major cause of medical implant-associated infections and results in prolonged hospitalization periods and patient mortality. This raises the urgent need to develop compounds that can inhibit bacterial colonization of surfaces. In this study, we present an unreported microwave-based synthesis of MgF_2 nanoparticles (Nps) using ionic liquid. We demonstrate the antimicrobial activity of these fluoride nanomaterials and their ability to restrict biofilm formation of common bacterial pathogens. Scanning and transmission electron microscopic techniques indicated that the MgF_2·Nps attach and penetrate into the cells. Flow cytometry analysis revealed that the Nps caused a disruption in the membrane potential. The MgF_2·Nps also induced membrane lipid peroxidation and once internalized can interact with chromosomal DNA. Based on these findings we further explored the possibility of using the MgF_2·Nps to coat surfaces and inhibit biofilm formation. A microwave synthesis and coating procedure was utilized to coat glass coupons. The MgF_2 coated surfaces effectively restricted biofilm formation of the tested bacteria. Taken together these results highlight the potential for developing MgF_2 nanoparticles in order to inhibit bacterial infections.

1. Introduction

The increased resistance of bacteria to antibiotic therapy is a growing concern for doctors and medical officials world-wide. In the last 2 decades, bacteria have developed resistance to almost all the commercially-available antibiotics, and the number of new antibiotics expected to enter the market is limited. One of the modes by which bacteria exert this resistance is their ability to develop biofilms [1]. Biofilms are bacterial communities encased in a self-produced hydrated polymeric matrix. Biofilm development is known to follow a series of complex but discrete and well-regulated steps [2,3]: (i) microbial attachment to the surface; (ii) growth and aggregation of cells into microcolonies; (iii) maturation and production of a protecting extracellular matrix; and (iv) dissemination of progeny cells for the formation of new colonies.

An important characteristic of microbial biofilms is their innate resistance to immune system- and antibiotic-killing [1,4]. This has made microbial biofilms a common and difficult to treat cause of medical infections [3,5,6]. It has been estimated that over 60% of bacterial infections currently treated in hospitals are caused by bacterial biofilms [5]. A major contribution to this statistic comes from the fact that biofilms are a major cause of infections associated with medical implants. The number of implant-associated infections approaches 1 million/year in the US alone and their direct medical costs exceed $3 billion annually [7].

The inherent resistance of biofilms to killing and their pervasive involvement in implant-related infections has prompted the search for surfaces/coatings that inhibit bacterial colonization. One approach comes from recent progress in nanotechnology, which offers an opportunity for the discovery of compounds with antimicrobial activity as well as the use of "nano-functionalization" surface techniques. Recent examples include the direct antibacterial properties of colloidal ZnO nanoparticles towards a broad range of microorganisms [8,9] or the selective targeting of gold nanorods towards pathogenic bacteria and killing them by applying photothermal treatment [10]. Other examples include the functionalization of biomaterials with antibacterial properties by coating [11], impregnation [12–14] or embedding nanomaterials [15,16].

Fluorides are well known for their antimicrobial activity [17,18]. This activity is mediated via three major mechanisms: (i) the formation of metal-fluoride complexes, especially with aluminum and beryllium cations, which interact with F-ATPase and nitrogenase enzymes inhibiting their activities [19]; (ii) the formation of HF, which disturbs the proton movement through the cell membrane

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[20], and, finally, (iii) F^- or HF can directly bind and inhibit specific cellular enzymes. For example, enolase (an important enzyme in glycolysis) is known to be inhibited by a complex of F^- and Mg^{+2} at micromolar concentrations in low pH [21].

In this study, we report on the antibiofilm activity of metal fluoride nanomaterials. We utilized a simple and fast microwave based synthesis method to synthesize MgF_2 nanoparticles (MgF_2·Nps), and characterized their activity against two common nosocomial biofilm-forming pathogens, i.e. *Escherichia coli* and *Staphylococcus aureus*.

2. Experimental procedure

2.1. Magnesium fluoride nanoparticles synthesis and surface coating procedure

We utilized a previously published microwave methodology developed by our group [22]. However, the protocol of the previous work did not include the synthesis of MgF_2 which is briefly described herein. A 10 g of a room-temperature ionic liquid solvent, 1-butyl-3-methylimidazolium tetrafluoroborate ($BMIBF_4$, Solvent Innovation GmbH) and the magnesium acetate tetrahydrate precursor salt (1 g, Acros Organics) were heated in a domestic microwave reactor system (2.45 GHz, Kenwood 900 W) for 2 min. At the end of the reaction the products were washed several times with acetonitrile, methanol (all ACS grade, BioLab) and finally double-distilled water to remove the ionic liquid and other organic impurities. The washed nanoparticles were than dried under vacuum. The product was characterized by X-ray diffraction on a Rigaku X-ray diffractometer model 2020, CoKα; $\lambda = 1.78892$ Å. The structural morphology was studied using transmission electron microscopy (TEM, JEOL 1200Ex) with an accelerating voltage of 80 kV.

The glass surfaces were coated by adding coupons cut from standard microscope glass slides directly into the chemical reaction medium in the microwave oven. After the reaction the coupons were washed several times with methanol and double distilled water (DDW) to remove the ionic liquid. The integrity of the coated surface imaged with an AFM microscope (MultiMode NanoScope V AFM).

2.2. Bacterial cultures and growth conditions

E. coli C600 and *S. aureus* FRF1169 were grown in Tryptic Soy Broth (TSB, Difco) and Tryptic Soy Broth 66% supplemented with glucose 0.2% (TSB-Glu) media respectively at 37 °C. Cell growth was followed by measuring the absorbance for 24 h at an optical density (OD_{595}) using a microplate reader (Synergy 2, BioTek Instruments).

2.3. Static biofilm formation assay

Overnight cultures of tested bacteria were diluted 1:100 in fresh media and grown for 4 h at 37 °C with shaking (250 rpm). Water-insoluble compounds were assayed in a modified macrodilution broth format. Compounds (~0.023–1.5 mg) were added to sterile polypropylene tubes (Greiner Bio-One) and the appropriate volume of a solution containing approximately 1.0×10^7 CFU/ml of *E. coli* or *S. aureus* in media were added. Tubes containing Mg^{+2}, F^- standard solutions or MgF_2 commercial salt (Sigma Aldrich) served as controls. A 100 µl of the tested cell suspension was added to each well in 96-well plate and was incubated for 24 h at 37 °C. Following incubation, the wells were washed twice with DDW to remove non attached cells and stained with 1% Crystal-violet (CV, Sigma) for 15 min at room-temperature. Stained wells were then washed five times with DDW and the remaining CV was eluted by the addition of absolute ethanol for 15 min. The biofilm biomass was then determined by measuring the absorbance at OD_{595}.

2.4. Scanning electron microscopy of bacterial samples

Samples of *S. aureus* and *E. coli* cultures treated for 4 h with MgF_2 nanoparticles (1 mg/ml) were fixed with glutaraldehyde and paraformaldehyde for 1 h. Following this incubation the samples were washed three times with phosphate buffer saline (PBS) without Ca^{+2} and Mg^{+2}. Samples were then immersed for 1 h in titanic acid and glutamate solution in a 4:5 ratio concentrations respectively. Samples were then

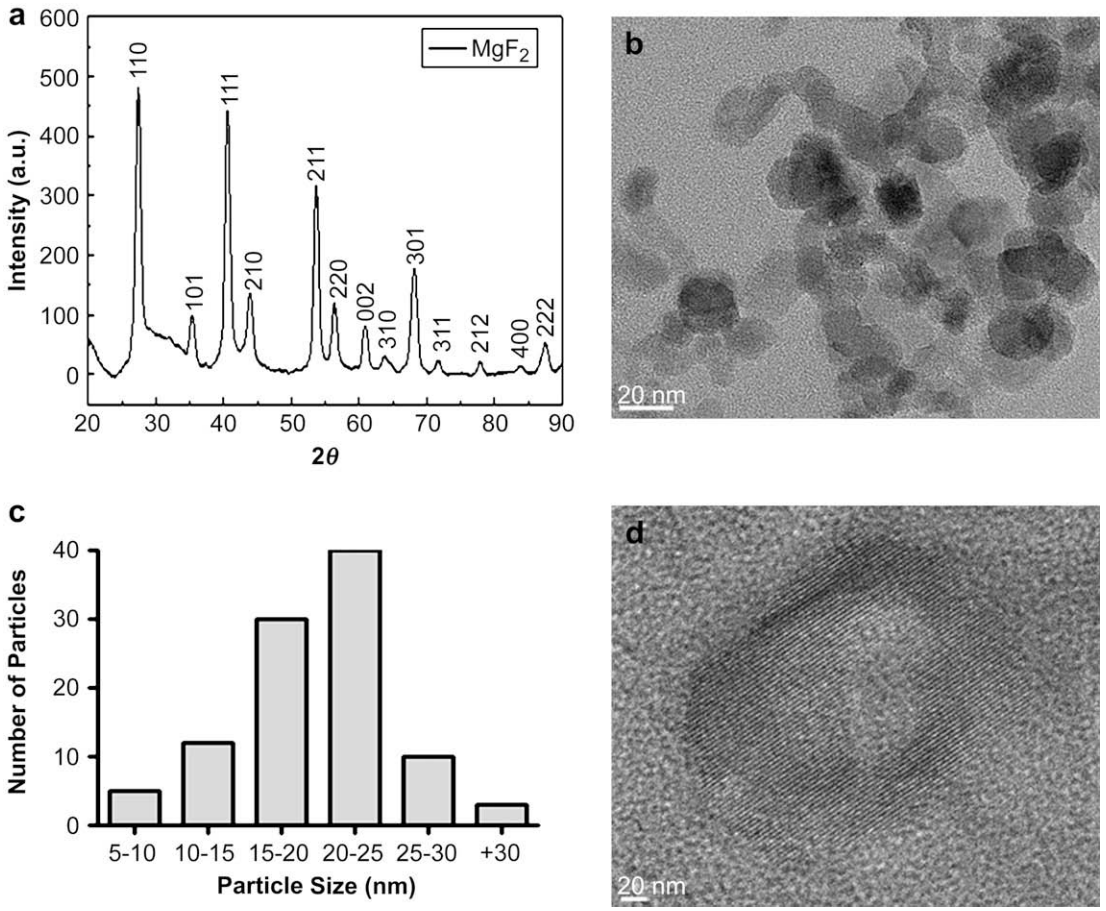

Fig. 1. MgF_2·Nps characterization. (a) Powder X-ray diffraction (XRD) patterns of MgF_2·Nps; (b–c) HR-TEM micrographs and (d) nanoparticles size distribution. The MgF_2·Nps examined were a product of a 2 min microwave reaction as described in the Material and methods section.