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Antimicrobial properties and dentin bonding strength of magnesium phosphate cements

G. Mestres [a,b], M. Abdolhosseini [c], W. Bowles [d], S.-H. Huang [d], C. Aparicio [d], S.-U. Gorr [c], M.-P. Ginebra [a,b,*]

[a] Biomaterials, Biomechanics and Tissue Engineering Group, Department of Materials Science and Metallurgy, Technical University of Catalonia, Av. Diagonal 647, 08028 Barcelona, Spain
[b] Biomedical Research Networking Center in Bioengineering, Biomaterials and Nanomedicine, CIBER-BBN, Spain
[c] Department of Diagnostic and Biological Sciences, University of Minnesota School of Dentistry, Minneapolis, MN, USA
[d] Department of Restorative Sciences, University of Minnesota School of Dentistry, Minneapolis, MN, USA

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ABSTRACT

The main objective of this work was to assess the antimicrobial properties and the dentin-bonding strength of novel magnesium phosphate cements (MPC). Three formulations of MPC, consisting of magnesium oxide and a phosphate salt, $NH_4H_2PO_4$, NaH_2PO_4 or a mixture of both, were evaluated. As a result of the setting reaction, MPC transformed into either struvite ($MgNH_4PO_4{\cdot}6H_2O$) when $NH_4H_2PO_4$ was used or an amorphous magnesium sodium phosphate when NaH_2PO_4 was used. The MPC had appropriate setting times for hard tissue applications, high early compressive strengths and higher strength of bonding to dentin than commercial mineral trioxide aggregate cement. Bacteriological studies were performed with fresh and aged cements against three bacterial strains, *Escherichia coli*, *Pseudomonas aeruginosa* (planktonic and in biofilm) and *Aggregatibacter actinomycetemcomitans*. These bacteria have been associated with infected implants, as well as other frequent hard tissue related infections. Extracts of different compositions of MPC had bactericidal or bacteriostatic properties against the three bacterial strains tested. This was associated mainly with a synergistic effect between the high osmolarity and alkaline pH of the MPC. These intrinsic antimicrobial properties make MPC preferential candidates for applications in dentistry, such as root fillers, pulp capping agents and cavity liners.

1. Introduction

The local release of antimicrobial agents is an efficient strategy for treating infections in hard tissues, namely bones and teeth, where the restricted accessibility to the site of infection is a challenge [1,2]. Thus, the development of biomaterials that release antimicrobial agents, such as antibiotics, inorganic ions and antibodies, to locations where specifically needed has attracted much attention [3].

The ideal material for the local treatment of infections in mineralized tissues should present, along with antibacterial properties, potential for enhancing mineralization by osteoconduction or even osteogenesis, depending on the specific application. Furthermore, the clinical demand of injectable biomaterials has been growing during recent years in direct relation to the increasing use of minimally invasive surgical techniques in orthopedics and dentistry. In this context, the development of inorganic cements with bioactive potential and antibacterial properties poses an attractive challenge. Inorganic cements, more specifically calcium phosphate cements, have been investigated extensively for local delivery of antibiotics [4]. However, this approach also has associated issues, such as the control and reproducibility of the kinetics of drug release and the risk of developing bacterial resistance when the released doses of antibiotics are too low. Moreover, the large increase in the number of antibiotic-resistant bacterial strains has prompted renewed interest in the use of inorganic antibacterial agents [5]. Antimicrobial calcium phosphate cements have been developed either by doping the cement with silver ions [6] or by adding an excess of calcium oxide or basic tertiary alkali phosphates as reactants [7,8]. The later ensures that release in excess of alkaline groups (e.g., OH^-, PO_4^{3-}) is accomplished. Mineral trioxide aggregate (MTA) is one more example of an inorganic cement with antimicrobial properties. MTA is a variant of Portland cement, used for endodontic treatments [9]. MTA is composed of calcium silicate and calcium aluminate, and its antibacterial potency has been attributed to either its basic pH [9] or its ability to induce the formation of reactive oxygen species [10]. As extensively revised by Parirokh and Torabinejad [11], results on the antimicrobial properties of MTA are controversial, which might be due

* Corresponding author at: Biomaterials, Biomechanics and Tissue Engineering Group, Department of Materials Science and Metallurgy, Technical University of Catalonia, Av. Diagonal 647, 08028 Barcelona, Spain. Tel.: +34 93 4017706; fax: +34 93 4016706.
E-mail address: maria.pau.ginebra@upc.edu (M.-P. Ginebra).

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to chemical variations in the formula for different commercial MTA cements [12]. These alkaline mineral cements have found general application as antimicrobial materials in dentistry, e.g., as cavity liners or for treating root canal infections.

More recently, a novel family of inorganic cements based on magnesium phosphates has been developed. These cements have attractive properties for dental and orthopedic applications, such as high strength [13], high adhesion properties [14] and high bone regeneration capacity [15,16]. Moreover, a preliminary study consisting of inoculating *Streptococcus sanguinis* in broth medium that was previously in contact with magnesium phosphate cements (MPC) suggested a bacteriostatic or bactericidal effect, depending on the composition of the specific cement [13].

The aim of this work was to evaluate the potential dental application of three MPC formulations by focusing on three main aspects of the MPC: (i) physicochemical properties; (ii) bonding strength to dentin; and (iii) antimicrobial properties in fresh and aged state against three bacterial strains, using several bacteriological assays.

2. Materials and methods

2.1. Cement preparation and characterization

Three different MPC formulations were prepared, consisting of a combination of magnesium oxide (MgO; Merck, ref. n. 105867) and ammonium dihydrogen phosphate ($NH_4H_2PO_4$; Panreac, ref. n. 131126.1210) or sodium dihydrogen phosphate (NaH_2PO_4; Fluka ref. n. 71496) or an equimolar mixture of both. The molar ratio of MgO to phosphate salt was 3.8, since it is known that an excess of magnesium ensures the completion of the reaction and enhances strength development [17,18]. The MgO was calcined at 1475 °C for 12 h to decrease its reactivity [19,20]. The calcined MgO and the phosphate salts were milled separately in a planetary ball mill to a mean size of 5.2 ± 0.3 μm for the MgO, 275 ± 14 μm for the $NH_4H_2PO_4$ and 186 ± 98 μm for the NaH_2PO_4. Sodium borate decahydrated (borax, $Na_2B_4O_7{\cdot}10H_2O$; Fluka, ref. n. 72000) was added as a retardant agent (6 wt.%) to increase setting time and decrease exothermy during the cement setting reaction [13]. Previously, borax particles were also milled to a mean size of 16 ± 1 μm. The cement powder was mixed with distilled water in a liquid to powder ratio of 0.13 ml g^{-1}. Depending on the phosphate salts used ($NH_4H_2PO_4$, NaH_2PO_4 or both) the resulting cements were coded as NH_4-MPC, Na-MPC and NH_4+Na-MPC, respectively.

The setting times of the cement pastes were determined with Gillmore needles [21]. The cohesion time, which is the time that a cement requires to no longer disintegrate when immersed in an aqueous solution, was evaluated by visual inspection of a cement disk soaked in distilled water [22]. Setting and cohesion times were evaluated in triplicate. The compressive strength of the MPC was measured in cement cylinders (6 mm in diameter, 12 mm high), which were prepared in Teflon molds, and soaked in Ringer's solution at 37 °C for 7 days. The mechanical testing was performed in a universal testing machine (Adamel Lhomargy DY 32/34) equipped with a load cell of 10 kN, at a cross-head speed of 1 mm min^{-1}. Ten specimens were tested for each formulation. The crystalline phases of the mixed reagents and of the cements set for 7 days in Ringer's solution were analyzed by X-ray powder diffraction (XRD; PANalytical, X'Pert PRO Alpha-1) using Bragg–Brentano geometry and Cu K_α radiation. Step scanning was performed with an integration time of 50 s, using a 2θ scan step of 0.017° between 4° and 50°.

2.2. Cement–dentin bonding strength

The bonding strength between cement and dentin was evaluated by a push-out test using single-root human teeth. The study was performed with the three MPC formulations, and gray MTA (ProRoot®, Dentsply, Maillefer) was used as a control. Human extracted teeth were obtained as clinical waste specimens without identifying data. The project was exempt (exemption #4, University of Minnesota, Minnesota, USA) from Institutional Review Board (IRB) review. Before use, each tooth was placed in 5% sodium hypochlorite solution (NaOCl; Acros Organics, ref. n. 419552500) for 1 h for surface disinfection and periodontal ligament removal, followed by storage in 1% NaOCl solution at 4 °C for a period no longer than 1 month. The tests were performed using the coronal third of the root canal. The teeth were perpendicularly embedded in resin and cut with a low-speed diamond saw (Buehler Isomet) through the apical and coronal areas. Root canals were enlarged using size 2 and 3 Gates Glidden drills (Dentsply Maillefer), mounted using a slow-speed dental hand-piece. The diameter of the root canal was progressively increased to 1.1 mm. At each instrument change, the root canal was irrigated with 0.5 ml of 5% NaOCl, using a 27-gauge needle. At the end of canal instrumentation, 0.5 ml of 17% EDTA solution (Acros Organics, ref. n. 118430010) was used for 3 min to remove the smear layer, as EDTA is a chelating agent [23]. Each specimen was finally irrigated with 0.5 ml of 5% NaOCl for 1 min, followed with distilled water for 1 min more, and dried with paper points (Dentsply Maillefer). The teeth were obturated by placing cement into the root canal and condensing it with endodontic pluggers. After being stored in a humid environment for 24 h, the roots were sectioned horizontally into 2-mm slices, using a low-speed diamond saw. The same operator conducted all procedures.

The push-out test was performed in a universal testing machine (MTS 810, Eden). A 1-mm-diameter pin was used to push the cement perpendicularly from the apical part of the root at a speed of 0.5 mm min^{-1}, until the pin was displaced 1 mm. Cement/dentin bonding strength was determined by dividing the maximum load by the bonding surface. The bonding surface between cement and dentin was calculated as $2\pi rh$, where r is the radius of the cement (mm), and h is the thickness of the root slice (mm). Three teeth were used for each cement formulation, and four slices were taken from each; therefore, 12 specimens were tested per cement formulation.

2.3. Microbiological studies

2.3.1. Bacterial culture conditions

The antimicrobial properties of the MPC were assessed against *Escherichia coli*, strain DH5α (Invitrogen, Carlsbad, CA), *Pseudomonas aeruginosa*, strain PAO1, and *Aggregatibacter actinomycetemcomitans*, strain JP2. The latter two strains were kind gifts from Dr. Donald Demuth, University of Louisville (Kentucky, USA). *E. coli* is frequently used as a model organism in microbiology studies; *P. aeruginosa* is a strain commonly found in caries, endodontic infections or implant-related infections that notoriously form biofilms [24–27]; and *A. actinomycetemcomitans* is frequently found in bone infections such as periodontitis [28].

E. coli and *P. aeruginosa* were grown in Luria Bertani broth (LB; Difco, ref. n. 244620), and *A. actinomycetemcomitans* in Brain Heart Infusion broth (BHI; Bacto, ref. n. 237500). *E. coli* and *P. aeruginosa* were grown by shaking bacteria overnight at 37 °C in aerobic conditions. *A. actinomycetemcomitans* were maintained at 37 °C for 2 days in an anaerobic chamber (Coy Laboratory Products), where oxygen and hydrogen were purged down to 100 ppm and 1%, respectively, using nitrogen and a biological atmosphere mixture (5% carbon dioxide in nitrogen) [29]. The density of bacterial cultures was quantified by spectroscopy as the optical density at 600 nm (od_{600}) after performing colony counts on different bacterial suspensions. od_{600} was converted to colony forming units (CFU) as follows: $od_{600} = 1$ contained $\sim 10^9$ CFU ml^{-1} *E. coli*; 10^{10}