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Antimicrobial potency of alkali ion substituted calcium phosphate cements

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Abstract

Potassium and sodium containing nanoapatite cements were produced by the reaction of mechanically activated $CaNaPO_4$ (CSP), $CaKPO_4$ (CPP) and $Ca_2KNa(PO_4)_2$ (CPCP) with a 2.5% Na_2HPO_4 solution. The cements exhibited clinically acceptable setting times of approximately 5 min and compressive strengths of 5–10 MPa. The antimicrobial properties of the cements were tested with the agar diffusion test using *Streptococcus salvarius*, *Staphylococcus epidermis* and *Candida albicans*. All types of alkali ion containing cements showed a significantly higher antimicrobial potency with inhibition zones of approx. 4–11 mm than a commercial calcium hydroxide cement which resulted in small inhibition zones around the cement samples of a maximum of 1.5 mm. The antimicrobial properties of all the cements were not found to diminish even after longer incubation times. This behaviour was attributed to the formation of soluble alkaline metal phosphates during setting which increased the pH value in the agar gel around the alkali containing calcium phosphate cement to 8.5–10.7 compared to 6.5–8.0 for the $Ca(OH)_2$ product. The high antimicrobial potency of alkali–calcium phosphate cements may find an application in dentistry as pulp capping agents, root fillers or cavity liners.

Keywords: Calcium phosphate cement; Mechanical activation; Antimicrobial properties; pH value

1. Introduction

Calcium hydroxide ($Ca(OH)_2$)-based materials are commonly used as antimicrobial materials in dental applications, e.g. as cavity liners, pulp-capping agents or for the treatment of infected root canals. $Ca(OH)_2$ can be applied either as suspension in an inert solvent (water, glycerine) or self-setting cements may be formed by the reaction of $Ca(OH)_2$ and salicylate ions to form calcium salicylate (with an unreacted $Ca(OH)_2$ component) or by adding $Ca(OH)_2$ to light-cured methacrylate-based resins [1]. The antimicrobial and anti-inflammatory potency of these materials originates from the release of hydroxyl ions which raise the pH value of the surrounding environment to approximately 12–12.5 after dissolution. This high pH value may kill bacteria by damaging the cytoplasmic membrane and DNA and denaturing proteins [2]. However, $Ca(OH)_2$ cements have some drawbacks in addition to being weak. Whilst suspensions or salicylate-based systems release sufficient $Ca(OH)_2$ for significant antibacterial properties [3] and the stimulation of secondary dentine [4], they are thought to be soluble [5]. Light-cured systems are less soluble, however, there is little evidence of considerable antibacterial effect by them [3]. A different route to combine long-term stability and sufficient antibacterial activity is the use of calcium phosphate cements (CPC) that set to form nanocrystalline, insoluble hydroxyapatite; antimicrobial properties have been introduced into these cements by either adding antibiotics to the liquid cement phase [6–8] or by using an excess of CaO as

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cement reactant [9]. In this paper, we demonstrated that a strong antimicrobial activity is also obtained from CPCs if strong basic tertiary alkali phosphates are formed during the setting reaction of sodium and potassium substituted calcium phosphates. $CaKPO_4$ (CPP) or $Ca_2KNa(PO_4)_2$ (CPSP) have been used by Bermudez et al. [10] and Driessens et al. [10,11] in conjunction with α-tricalcium phosphate for cements that precipitate nanocrystalline apatites in a broad range $0.8 < Ca/P < 1.5$. More recently, we could show that single component and strongly basic cements are available from CPSP after mechanically activating (MA) and thereby amorphising the compound during high-energy ball milling [12]. This method was used in this paper to prepare self-setting cements from CPP, CPSP and $CaNaPO_4$ (CSP) with a near neutral pH disodium phosphate solution as liquid. The antimicrobial properties of the cements compared with a commercial dental $Ca(OH)_2$/salicylate cement were tested using the agar diffusion test with clinically relevant bacteria, namely, *Streptococcus salvarius*, *Staphylococcus epidermis* and *Candida albicans*.

2. Materials and methods

CPP and CSP were synthesised by heating a mixture of monetite (DCPA; Mallinckrodt Baker, Griesham, Germany) and potassium or sodium carbonate (both Merck, Darmstadt, Germany) in a 2:1 molar ratio to 1050 °C for 24 h followed by quenching to room temperature in a desiccator. CPSP was produced similarly from a mixture of DCPA, potassium carbonate and sodium carbonate in a 4:1:1 molar ratio. The sintered cakes were crushed with pestle and mortar and passed through a 355 μm sieve. Milling of CPP, CSP and CPSP for 24 h was performed in a planetary ball mill (PM400 Retsch, Germany, 400 mm diameter, unidirectionally) at 250 rpm with 500 ml agate jars, 200 agate balls (10 mm) and a load of 75 g powder and 125 ml ethanol (99.9%), per jar. The ground powders were dried in a vacuum oven at 60 °C. Particle size distributions were determined using a laser particle size analysis (L300, Horiba, Kyoto, Japan) after dispersing 100 mg of the powder particles in 200 ml isopropanol by applying ultrasound for 15 min.

Single component cements were produced by the mixture of ground powders and 2.5 wt% Na_2HPO_4 solution at a powder to liquid ratio (PLR) of 2 g/ml. Cylindrical samples (12 mm height, 6 mm diameter) for mechanical testing were prepared as described previously by means of a cantilever device [13] at a pressure of 9 MPa for 5 s, followed by a constant load of 0.7 MPa for 2 h. Samples ($n = 9$ per condition) were tested in compression after additional 22 h storage in water at 37 °C at a crosshead speed of 1 mm/min using a static mechanical testing machine Zwick 1440 (Zwick, Ulm, Germany) and a 5 kN load cell. The initial setting time of the cements was measured in a humidity chamber at 37 °C and >90% relative humidity using the Gilmore needle test with a needle of 113.98 g and 2.117 mm diameter. In order to compare hydroxide release of these cements with a commercial $Ca(OH)_2$ product, Life (Kerr, Italy) and 600–700 mg of hardened CPCs ($n = 3$) were boiled in 100 ml of water for up to 460 min. The pH of the water was measured and the water was refreshed after 1, 10 and 100 min and then every 60 min boiling thereafter.

The antimicrobial properties of the cements were tested using the agar diffusion test [14] using *S. salvarius*, *S. epidermis* and *C. albicans*. Uncompacted cement discs with a diameter of 10 mm and a height of 5 mm were made at a PLR of 2 g/ml for the materials using silicon matrices. Cements were set at 37 °C and 90% relative humidity for 4 h. The agar gel for *C. albicans* was prepared by dissolving 22.2 g Pepton/Trypton Difco™ (Becton and Dickinson, USA), 11.1 g yeast extract (AppliChem, Darmstadt, Germany), 16.7 g Trypticase™ Soy Agar II with 5% sheep blood (Becton and Dickinson, USA) and 100 ml glucose solution (5%) in 1000 ml water at 60 °C. The gels for *S. epidermis* (10 g Pepton/Trypton, 2 g yeast extract, 15 g agar and 5 g NaCl) and *S. epidermis* (29.5 g fluid thioglycollate medium USP/EP (Becton and Dickinson, USA) and 15 g agar) were prepared similarly. All gels were autoclaved at 134 °C for 2 h prior to filling them into 100 mm petri dishes. Since the antimicrobial activity of the cements originates from ion diffusion into the agar gel volume, the thickness of the gel was adjusted to a constant 2 mm. In all, 10 μl of the bacteria were cultivated in 2 ml medium (similar composition as described for gel preparation of each strain, except prepared without agar) for 24 h at 37 °C, diluted 1:4 with water and 50 μl of this suspension was homogeneously dispersed on every agar plate. The preset cement sample (10 × 5 mm) was placed on the middle of the agar plate and the inhibition zone around the cement sample was measured after 1, 3, 6 and 10 d at 37 °C. The pH variation in the gel around the cement samples was measured at 1 mm increments by means of a micro-pH-electrode. Statistical significance was performed by means of a one-way analysis of variance (ANOVA), followed by a Tukey post hoc test using SPSS (v 13.0).

3. Results

Mechanical activation of the sodium and potassium substituted calcium phosphates by ball milling over a period of 24 h resulted in hardening cement pastes with a neutral pH 2.5% Na_2HPO_4 solution, while the raw materials showed nearly no setting reaction with an aqueous phase. Compressive strengths were found to be in the range from 5 MPa (CPP) up to 11 MPa (CPSP) after 24 h setting and initial setting times were in the range 5–6 min (Table 1) while the $Ca(OH)_2$/salicylate cement showed a compressive strength of 19 MPa and a setting time of 3.5 min. All cements hardened under alkaline conditions since the pH values measured in cement pastes were found to be approximately 11–12 (Fig. 1), a lower pH value of approximately 10.5 was found for the $Ca(OH)_2$/salicylate cement paste. The reaction product had in all cases an apatitic structure as shown by the typical HA peaks at $2\theta = 25.9°$ (0 0 2) and between 31.0–34.0° [(3 −2 1), (2 −1 2), (3 0 0), (2 0 2)] in