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Bismuth-doped injectable calcium phosphate cement with improved radiopacity and potent antimicrobial activity for root canal filling

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ABSTRACT

A bismuth-doped injectable calcium phosphate cement (BD-ICPC) with improved radiopacity, potent antimicrobial activity and sealability was developed by introducing bismuth salicylate basic (BSB) into the powder phase of the calcium phosphate cement (CPC). The results showed that the radiopacity and sealability of BD-ICPC were improved compared with pure ICPC. Although BSB had a retarding effect on the setting rate of the cement, the addition of BSB reduced the viscosity and yield stress of BD-ICPC, thus enhanced its injectability. It was noteworthy that BD-ICPC had a potent antimicrobial activity with improved sealability. In addition, BD-ICPC afforded a uniform and tight adaptation to the root canal wall. These results indicate that BD-ICPC possesses a combination of good in vitro radiopacity, high injectability, potent antimicrobial activity, improved sealability and tight adaptation to the root canal. It is expected to be used as a novel root canal filling material.

1. Introduction

Complete and impervious obturation of the root canal system is of prime clinical importance for the long-term success of endodontic treatment. It has been recommended that an ideal root canal filling should provide a complete, three-dimensional filling and prevent microorganism leakage [1]. However, incomplete filling of the root canal is considered to be a major cause of the failure of endodontic surgery. Passages allowing bacterial, fluid and chemical substance penetration are apt to be created when the root canal is not well obturated [2]. Nearly 60 percent of root canal filling materials available cannot meet the clinical requirements because of incomplete obturation [3–5]. A material must have a high sealing ability for root filling applications, to prevent leakage of microorganisms and their by-products around the margins of the material, leading to overall failure of the restoration.

The injectability of root canal fillings is closely related to their sealability [6]. An injectable material can penetrate into irregular canals and completely obturate small lateral canals. Thus, in the past few years many different injectable materials have been introduced to improve the sealability of root canals. With good injectability and setting in situ, injectable acrylic cements and calcium hydroxide pastes have been widely used as root canal fillings [7]. However, acrylic cements have some shortcomings, such as an exothermic reaction and poor adhesion to the surface of bone. Calcium hydroxide pastes also have disadvantages, such as a low setting rate and dissolution, which leads to loss of the filling [8], so they can only be used as temporary fillings. Injectable calcium phosphate cement (ICPC) not only possesses a combination of good injectability, fast setting in situ and easy shaping to complicated geometries [9–11], but also shows high biocompatibility and good bioactivity. It may be an alternative injectable root canal filling.

Both overfilling and underfilling of injectable cements into the root canal often occur and cause serious problems during endodontic treatment [12–15]. For instance, serious neurological complications have been reported after overfilling of the root canal with endodontic cement [16]. Nerve root pain, the most common associated adverse event, is usually caused by microleakage between the root canal wall and the filling. To fully fill the root canal the obturation procedures are performed visually with close fluoroscopic monitoring [17]. Radiography enables the filling of the canal to be monitored at later stages during root canal therapy. A root canal filling should be radiopaque enough that overfilling or underfilling of material in the root canal and the location can be easily detected. In addition, voids, improper contours, root fracture and other important diagnostic structures should be visible on X-ray images [18,19]. However, due to intrinsic radiopenetration or weak

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radiopacity, root canal fillings often cannot be detected clearly by radiography. Therefore, radiopaque agents have been introduced into root canal fillings. Barium sulfate and zirconium dioxide are the two most common radiopaque agents used in bone cements. However, many reports have highlighted deleterious effects, such as causing bone resorption, decreasing the mechanical properties of fillings and damaging biological functions [20–22]. Therefore, it is necessary to develop novel radiopaque agents for use in root canal fillings.

Taking into account the good radiopacity offered by bismuth oxide and organic bismuth compounds [23,24], bismuth salicylate basic (BSB), a commonly used medical additive, was introduced as an alternative radiopaque agent in this work. It is noteworthy that BSB has long been in clinic because of its high effectiveness and low toxicity in the treatment of a variety of microbial infections, including *Helicobacter pylori*, diarrhea, peptic ulcers and other gastrointestinal disorders [25–29]. Moreover, bismuth-based compounds are stable and insoluble, which may improve the long-term sealability of ICPC in the root canal. Chemically inert bismuth oxide has been added as a radiopaque agent to Portland cement, known as mineral trioxide aggregate (MTA), used for over a decade as a dental material [30]. However, the addition of BSB to ICPC as a root canal filling material has not been investigated before.

The purpose of this study was, first, to develop a bismuth-doped injectable calcium phosphate cement (BD-ICPC) by introducing BSB directly into calcium phosphate cement (CPC) powders and, second, to investigate the radiopacity, injectability, setting time, phase composition, microstructure, antimicrobial activity, sealability and adaptation to the root canal wall.

2. Materials and methods

2.1. Materials and preparation

All calcium phosphates used in this experiment were prepared in our laboratory – the preparation methods can be obtained from the relevant literature [10]. Briefly, tetracalcium phosphate (TTCP) [$Ca_4(PO_4)_2O$] was synthesized by a solid to solid reaction between calcium phosphate and calcium carbonate at a temperature of 1500 °C for 8 h. Dicalcium phosphate dihydrate (DCPD) ($CaHPO_4{\cdot}2H_2O$) was prepared from diammonium phosphate [$(NH_4)_2HPO_4$] and calcium nitrate [$Ca(NO_3)_2$] in an acidic environment, and anhydrous dicalcium phosphate (DCPA) ($CaHPO_4$) was obtained by removing the water of crystallization from DCPD at 120 °C. The CPC powder was composed of TTCP and DCPA in an equivalent molar ratio. The CPC powder was then mixed with 0, 5, 10, 15, 20 and 25 wt.% BSB (Shanghai Mingtai Medicine Co., China) to form the blended CPC powders by dry grinding for 24 h in a ball mill. The BD-ICPCs were prepared by mixing the blended CPC powders homogeneously with deionized water at a fixed powder to liquid (P/L) ratio of 2 g ml^{-1}. Pure ICPC without BSB was used as a control.

2.2. Radiographic assessment

Three cylinders (5 mm diameter × 5 mm) of each cement were used for X-ray radiopacity assessment. Standardized radiographic images of all samples were taken with an X-ray device (MXR-160A, Shenzhen X-ray Electric Co., China) after exposure at 60 keV and 10 mA for 0.7 s. The distance between the cement and the film was fixed at 10 mm. The film was processed in an automatic processor (ZW2003A, Zhongtian Medical Machine Co., China) at 30 °C for 4 min. The relative X-ray radiopacities of pure ICPC, BD-ICPCs and an extracted tooth were judged visually.

The image contrast between the sample region and the surrounding black region was used to evaluate the X-ray radiopacity of the cements, which can be calculated according to the following equation [31]:

$$V = (G_1 - G_2)/G_1 \quad (1)$$

where V represents the image contrast and G_1 and G_2 represent the gray scale values of the sample region and the surrounding black region, respectively. The image processing software Adobe Photoshop® (CS3 extended version, Heshan Great Brighten Umbrella Manufacturing Co., China) was used to determine the gray scale values of the sample region and the surrounding black region, as reported by Kjellson et al. [32]. Ten pairs of regions, as far apart as possible, were selected at random in both the sample and the black surrounding region to measure their gray scale values. Each region was averaged over an area of 5 × 5 pixels. Experiments were carried out on three specimens for each cement. The results are expressed as means ± standard deviation. One-way analysis of variance (ANOVA) was used for statistical analysis of the measured image contrast. A value of $P < 0.05$ was considered to be statistically significant.

2.3. Rheological properties and injectability

The rheological properties (including viscosity and stress) of pure ICPC and all BD-ICPCs were determined with a rheometer (RS600, Thermo Hakke, Germany). The samples were poured into the plate assembly of the rheometer. To avoid undesired effects of different mechanical histories, the samples were allowed to shear at an identical rate of 100 s^{-1} for 20 s to establish a baseline shear history at 37 °C. Afterwards, viscosity curves for $\eta \sim \dot{\gamma}$ and stress curves for $\tau \sim \dot{\gamma}$ were determined over the range of shear rates 0.01–100 s^{-1}. The yield stress was maximum at 0 shear rate according to the $\tau \sim \dot{\gamma}$ curves.

The injectabilities of pure ICPC and all BD-ICPCs were measured following a previously described procedure [33]. The mixing time for pure ICPC and the BD-ICPCs was 1 min. The syringe was fitted with a 0.7 mm inner diameter needle and a 3 kg compressive load was applied vertically to the top of the plunger. The percentage injectability was calculated by division of the mass expelled from the syringe by the total amount of cement charged into the syringe in 2 min.

2.4. Setting time and compressive strength

The setting times of pure ICPC and all the BD-ICPCs were measured using a Vicat apparatus according to ASTM Test Method C 187–98. The Vicat apparatus consisted of a frame bearing a movable rod (300 g in mass) with a 1 mm diameter stainless steel needle fitted at the end. The BD-ICPCs were placed in a glass tube (6 mm diameter × 10 mm) and stored at 37 °C in a 100% humidity chamber to set. The needle was carefully lowered vertically onto the surface of the setting cement and allowed to remain there for 5 s. The cement was measured at intervals of 0.5 min. The time at which the needle could penetrate <1 mm into the cement was taken as the setting time.

Steel cylindrical molds (6 mm diameter × 10 mm) were used to prepare pure ICPC and all the BD-ICPCs for compressive strength testing. After pouring into the steel molds the cement was pressed with a steel column (5 mm diameter × 5 mm^3) under a stress of 700 kPa for 5 s to eliminate air bubbles. After setting at 37 °C in a 100% humidity chamber for 3 days, the cement was removed from the mold. The compressive strength of the cement was measured at a loading rate of 1 mm min^{-1} using a universal testing machine (AG-2000A, Shimadzu, Japan). Each cement was tested three times and the average values are given.