dental materials xxx (2018) xxx.e1–xxx.e10

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.intl.elsevierhealth.com/journals/dema

Synthesis of silver-containing calcium aluminate particles and their effects on a MTA-based endodontic sealer

Luiza Helena S. Almeida[a], Rafael R. Moraes[a], Renata D. Morgental[a], Sérgio S. Cava[b], Wellington Luiz O. Rosa[a], Patrícia Rodrigues[b], Anderson S. Ribeiro[c], Marcus Só[d], Fernanda G. Pappen[a,*]

[a] Graduate Program in Dentistry, School of Dentistry, Federal University of Pelotas, Brazil
[b] School of Materials Engineering, Federal University of Pelotas, Brazil
[c] Graduate Program in Chemistry, School of Chemistry, Laboratório de Metrologia Química (LabMeQui), Federal University of Pelotas, Brazil
[d] Graduate Program in Dentistry, School of Dentistry, Federal University of Rio Grande do Sul, Brazil

ARTICLE INFO

Article history:
Received 7 August 2017
Received in revised form 29 March 2018
Accepted 9 May 2018
Available online xxx

Keywords:
Calcium aluminate
Calcium aluminate and silver
Calcium ions release
Silver ion release
Flow
pH
Radiopacity
Antibiofilm
Root canal sealers

ABSTRACT

Objective. To synthetize calcium aluminate (C3A) and silver-containing C3A particles (C3A + Ag) testing their effects on the properties of a MTA-based endodontic sealer in comparison to an epoxy resin- and a calcium silicate-based sealer.
Methods. Pure C3A and C3A + Ag particles were synthesized by a chemical method and characterized using XRD to identify crystalline phases. SEM/EDS analysis investigated morphology, particle size, and elemental composition of particles. Setting time, flow, radiopacity, water sorption and solubility of commercial and modified sealers were evaluated according to ISO 6876/2012. The pH and ions release were measured using a pHmeter and a microwave induced plasma optical emission spectrometer. The inhibition of biofilm growth was evaluated by confocal laser scanning microscopy (CLSM). Data were rank transformed and analyzed by ANOVA and Tukey test ($P < 0.05$).
Results. The C3A particles showed an irregular grain agglomerated structure with voids and pores. In C3A + Ag particles, Ag modified the material morphology, confirming the deposition of Ag. The physicochemical properties of the modified MTA-based sealer were similar to the commercial material, except for the significant increase in Ca^{+2} release. However, there was no Ag release. Setting time, flow, radiopacity, water sorption and solubility were adequate for all materials. All the materials showed alkaline pH. Antibiofilm effect was improved in the presence of C3A particles, while the biofilm inhibition was lower in the presence of Ag.
Significance. The modified sealer presented improved antibiofilm properties and calcium release, without dramatic effects on the other characteristics. It is expected a positive effect in its antimicrobial behavior.

* *Corresponding author at*: Graduate Program in Dentistry, Federal University of Pelotas, Rua Gonçalves Chaves 457 Room 507, Pelotas, RS 96015-560, Brazil.
E-mail address: fernanda.pappen@ufpel.edu.br (F.G. Pappen).

https://doi.org/10.1016/j.dental.2018.05.011

1. Introduction

Properties of root canal sealers have an impact on the quality of root canal filling and subsequently on the endodontic outcome [1]. These materials should present adequate physicochemical properties, such as setting time, radiopacity, flow, water sorption and solubility. Ideal sealers also would have antimicrobial properties and stimulate the repair of the affected periapical tissues [2]. Many materials are currently used as root canal sealers, including zinc oxide–eugenol, calcium hydroxide, glass-ionomer, silicone, polymer resins [1,3] and more recently, calcium silicate [4,5]. Although no material could gather all the desired characteristics, new root canal sealers are constantly being developed in attempts to improve their physical, chemical, and biological properties.

The ability to induce mineralized tissue deposition, present biocompatibility and antimicrobial properties are among the largest endodontic challenges. The mechanism of repair stimulation by deposition of mineralized tissue as well as antimicrobial action may depend on the presence of antibacterial components, pH, and the ability to release calcium ions (Ca^{2+}) [2]. Calcium silicate-based sealers, such as mineral trioxide aggregate (MTA) based sealers, were introduced in attempt to combine biological and sealing properties. However, MTA-based sealers have demonstrated lower pH values than other materials [6], with a severe cytotoxic effect [7] and intense inflammatory effects on bone and subcutaneous connective tissue [8]. In addition, the antibacterial activity of MTA-based sealers remains a limitation, since the material presents an antibacterial activity before setting that is not sustained after setting [6,9].

Calcium aluminate — $3CaO{\cdot}Al_2O_3$ (C3A) particles have been used recently for many biomedical applications [10]. In endodontic sealers, the addition of C3A would increase the chemical reaction of hydration, which is based on dissolving the calcium aluminates with the subsequent precipitation of hydrated compounds. This may result in a continuous process of dissolution/precipitation, prolonging the release of Ca^{2+} that could increase the reparative capacity induced by the sealer [10]. In addition, release of Ca^{2+} may lead to a gradual increase in the pH and consequently to a large number of osteogenic cells acting in linear closure of bone defects, likely forming a more compact mineral bridge [10]. Similarly, the addition of silver to endodontic materials has been tested to inhibit microbial development and prevent infections [11].

The aim of this study was to synthesize and characterize C3A particles with or without Ag and investigate their effects on physical–chemical and biological properties of a MTA-based endodontic sealer. Other conventional sealers were tested for comparison. The hypothesis tested was that the incorporation of C3A + Ag would improve Ca^{2+} release and impart antibiofilm properties to an MTA-based sealer without drastic effects on other properties.

2. Materials and methods

2.1. Study design and materials tested

This *in vitro* investigation was designed for synthesizing pure C3A and C3A + Ag particles and testing their effects on the physicochemical and biological properties of a commercial MTA-based endodontic sealer (MTA Fillapex; Angelus, Londrina, PR, Brazil). Other sealers were tested as received for comparison: an epoxy resin-based sealer (AH Plus; Dentsply De Trey Gmbh, Konstanz, Germany) and a calcium silicate-based sealer (EndoSequence BC Sealer; Brasseler USA, Savannah, GA, EUA). These materials were selected since EndoSequence BC have similar composition to the material modified in this study, presenting excellent physicochemical and biological properties [12,13]. AH Plus is considered a gold-standard endodontic sealer, with low solubility and disintegration [14,15] and adequate dimensional stability [12]. However, this sealer has no bioactive properties [16] or osteogenic potential [17]. Particles were synthesized containing 1 mol% or 5 mol% Ag and were added at 5 wt% or 10 wt% to the MTA-based sealer. The concentrations of particles added were defined in pilot studies. In total, 9 groups were tested, as shown in Table 1. The sealers were manipulated according to the manufacturers' directions. Particle characterization involved analyses using X-ray diffraction (XRD), scanning electron microscopy (SEM), and energy-dispersive X-ray spectroscopy (EDS). When testing the sealers, the physicochemical response variables were setting time, flow, radiopacity, water sorption (W_{SR}) and solubility (W_{SL}). Antimicrobial response variables included the total biofilm and viable bacteria biovolume grown on the sealers.

2.2. Synthesis and characterization of the inorganic particles

C3A and C3A + Ag powders were synthesized by means of a polymeric precursor method using a variation of a previously described method [18]. In this method, chelates are formed between metal cations and the carboxylic acid from citric acid dissolved in aqueous solution to obtain homogeneous and polycrystalline single phases. Addition of ethyleneglycol leads to the formation of an organic ester, followed by polymerization. Thermal treatments were performed in two stages: at 400 °C to remove organic materials, and at 1000 °C to obtain crystalline phases. In this work, by controlled addition of calcium, aluminum and silver nitrates, we obtained the different particles: C3A (no Ag present), C3A + 1 mol% Ag, and C3A + 5 mol% Ag. The Ag content was based on previous studies that showed that at least 1 mol% of Ag increased the antibacterial activity of MTA powder without affecting other properties [19]. The resulting particles were characterized using XRD to identify crystalline phases (diffractometer XRD-6000; Shimadzu, Tokyo, Japan) using Cu Kα1 (λ = 1.5406 Å) in a range of 10–80° and 0.02°. SEM/EDS analysis (JSM 6610; Jeol, Tokyo, Japan) investigated filler morphology, particle size, and elemental composition of the resulting powders.