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Effect of the fluoride content on the bioactivity of calcium silicate-based endodontic cements

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Abstract

This study was aimed at investigating the effect of the fluoride content (added as NaF) on the *in vitro* bioactivity of an experimental calcium silicate-based cement (wTC-Bi) obtained from white Portland cement. To this purpose, wTC-Bi and fluoride-doped wTC-Bi cements (i.e. FTC-Bi and F10TC-Bi with fluoride contents of 1% and 10% w/w, respectively) were aged in Dulbecco's Phosphate Buffered Saline (DPBS) and were comparatively analysed by micro-Raman and IR spectroscopy to investigate the presence of deposits on the surface of the cements and the composition changes of the cement as a function of the storage time. Commercial White ProRoot MTA was analyzed as reference.

All the tested cements showed the formation of a calcium phosphate deposit already after 5 h of soaking. Fluoride-doped cements demonstrated a higher bioactivity than the undoped wTC-Bi cement. This result was explained in relation to the different solubility of the deposit formed on the cements: a B-type carbonated apatite on the undoped cements and a less soluble fluoride containing B-type carbonated apatite on the fluoride-doped cements. The NaF content was found to influence the apatite forming ability; actually, the cement richer in NaF, i.e. F10TC-Bi showed a lower bioactivity than FTC-Bi, which contained only 1% w/w of NaF. This result may be explained in relation to the lower hydration rate of the former, which showed the formation of lower amounts of CSH, ettringite and portlandite phases.

Keywords: B. Chemical properties; B. Spectroscopy; D. Apatite; D. Silicate; E. Biomedical applications

1. Introduction

Portland cements are hydraulic materials manufactured by roasting at about 1400 °C clay and limestone. White Portland cements are mainly composed of tricalcium silicate (alite, $3CaO \cdot SiO_2$), dicalcium silicate (belite, $2CaO \cdot SiO_2$) and minor amounts of tricalcium aluminate ($3CaO \cdot Al_2O_3$). Calcium sulphates are generally added to Portland cements to adjust the setting time, i.e. to avoid a rapid desiccation of the paste and the brittleness of the cement: a too fast setting time impedes an effective and complete hydration of the calcium silicate particles of the cement.

In the last two decades, calcium silicate-based cements have received an increasing consideration in endodontics because they are hydraulic cements that set in the presence of water and biological fluids, an important property for dental cements. Therefore, in the 1990s, a new material, MTA (mineral trioxide aggregate) was developed as root-end filling material. As reported by the patent [1], MTA is a type I ordinary Portland cement with a 4:1 addition of bismuth oxide (radiopacifier).

It is well established that the hydration of MTA-based cements involves a number of chemical reactions that take place simultaneously. The hydrolysis of calcium silicates produces a nanoporous gel of calcium silicate hydrates (CSH phase) and calcium hydroxide (portlandite). The presence of the latter makes the hydrated cement highly alkaline [2] and leads to a calcium release in the surrounding environment. From the reaction between tricalcium aluminate and gypsum with water, ettringite ($3CaO \cdot Al_2O_3 \cdot 3CaSO_4 \cdot 32H_2O$) is also produced.

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MTAs and calcium silicate-based cements appear very promising materials due to their well-documented bioactivity [3–9]. According to the European Society for Biomaterials (ESB) Consensus Conference of 1987 [10], a bioactive material must be able "to induce specific biological activity". This definition has been subsequently refined so that, according to Kokubo and Takadama [11], bioactive materials are considered as bone bonding materials, i.e. as materials that form bone-like apatite upon immersion in a serum-like solution. In other words, they are able to accelerate heterogeneous apatite crystallization in a solution supersaturated towards hydroxyapatite [11]. Actually, the deposition of hydroxyapatite-like calcium phosphates on the biomaterial surface may facilitate the direct bonding to bone and represents an essential requirement for osteoconduction and osteoinduction [12]. Moreover, bioactive calcium silicate cements may induce remineralisation of partially demineralised dentine [13,14].

Although false positive and false negative results can be occasionally obtained [15], the use of abiotic calcifying media that simulate body fluids in inorganic ion concentrations (i.e. simulated body fluids, SBFs) is a currently accepted method to study the mechanism of biomaterials calcification. The study of the bioactivity *in vitro* is useful to predict the apatite formation ability *in vivo*.

Fluoride plays an important role in tissue mineralization processes, supplying clinical benefits. It may remineralize enamel and softened dentine, reducing caries formation [16]; actually, fluoride ions have been traditionally incorporated into dental resins and glass ionomer cements to increase the remineralisation properties of filling materials [17]. Several studies have demonstrated the high activity of fluorine on osteoblastic cells and have reported that micromolar fluoride concentrations are effective to improve the cell attachment and the subsequent cell activities and to stimulate proliferation of osteoblast progenitor, osteoblast and osteoblast-like cells [18,19], improving mineralization and bone formation *in vivo* [20].

With regards to calcium silicate cements, Gandolfi et al. reported that the addition of fluoride (1% w/w) causes an increase in expansion, setting time [21], long-term apical sealing ability [22], and an improvement of the biological behavior [23].

In this context, our study was aimed at investigating the effect of the fluoride content on the in vitro bioactivity of an experimental calcium silicate-based cement (wTC-Bi) obtained from white Portland cement. To this purpose, wTC-Bi and fluoride-doped wTC-Bi cements (with fluoride contents of 1% and 10% w/w) were aged in a SBF solution, i.e. Dulbecco′s Phosphate Buffered Saline (DPBS) and were comparatively analysed by micro-Raman and IR spectroscopy to investigate the presence of deposits on the surface of the cements and the composition changes of the cement as a function of the storage time. Commercial White ProRoot MTA was analyzed as reference. Since this cement contains bismuth oxide as radiopacifer, also the experimental cements were added with this component.

2. Materials and methods

2.1. Cements preparation and ageing experiments

To prepare the experimental wTC-Bi, FTC-Bi and F10TC-Bi, the Portland cement powder (CEM I white Aalborg, Aalborg, Denmark) was thermally and mechanically treated, added with 5% w/w calcium chloride and 17% w/w bismuth oxide. Sodium fluoride 1% w/w or 10% w/w was added to wTC-Bi to produce two experimental fluoride-doped cements, identified as FTC-Bi and F10TC-Bi, respectively.

The cement powder was mixed with DPBS (Cambrex Bio Science Verviers s.p.r.l., Belgium, cat. n.BE17-512) for 15 s (powder/liquid ratio 3:1 w/w), then layered on a plastic coverslip (Thermanox, diameter 1.2 cm) to obtain standard disks. Mechanical vibrations were used to make the disk surfaces flat and regular, with a $1.1 \pm 0.1\ cm^2$ exposed surface area.

White ProRoot MTA (Dentsply, Tulsa, OK, USA) disks were prepared analogously by mixing for 30 s on a glass slab the calcium-silicate mineral powder with the provided liquid, i.e. deionized water, following the manufacturer directions.

After preparation, the disks were immediately immersed in 5 mL of DPBS (15 mL of medium for 1 g of cement paste) and maintained at 37 °C. The samples were soaked in DPBS for different times (i.e. 5 hours, 1 day, 7, 14 and 28 days). DPBS is a physiological-like buffered (pH 7.4) Ca- and Mg-free solution composed of (mmol L^{-1}): K^+ (4.18), Na^+ (152.9), Cl^- (139.5) and PO_4^{3-} (9.56, sum of $H_2PO_4^-$ (1.5) and HPO_4^{2-} (8.06)). The storage media were renewed every week.

2.2. Spectroscopic measurements

Micro-Raman spectra were recorded in situ on the samples maintained in their storage media, to prevent any transformation. The spectra were obtained using an argon laser (Innova Coherent 70; Coherent Inc., Santa Clara, CA, USA) operating at 514 nm and a Jasco NRS-2000C micro-Raman spectrometer (Jasco Inc., Easton, MD, USA) equipped with a 160 K frozen digital CCD detector (Spec-10: 100B, Roper Scientific Inc. Trenton, NJ, USA) employing a microscope of 20 × magnification. Under these conditions, laser spot size was about 5 μm. All the spectra were recorded in back-scattering conditions with 5 cm^{-1} spectral resolution and a power on the sample of about 50 mW. The unhydrated powders of the cements were analyzed as control.

IR spectra were recorded using a Nicolet 5700 FTIR (Thermo Electron Scientific Instruments Corp., Madison, WI, USA), equipped with a Smart Orbit diamond attenuated total reflectance (ATR) accessory and a DTGS detector; the spectral resolution was 4 cm^{-1}. The ATR area had a 2 mm diameter. Under this instrumental setup, the IR radiation penetration into the cement was about 2 μm. The aged samples were analyzed after air-drying at room temperature.

To minimize problems deriving from the possible sample inhomogeneity, five Raman and IR spectra at least were recorded on each specimen area (i.e. upper surface, inner fractured