Physico-chemical characterization of a new thermo-responsive fluoride-releasing poloxamer-based gel.

Thermo-responsive fluoride-releasing gel

ABSTRACT

Objective: The purpose of this study is to evaluate physico-chemically the fluoride-releasing efficiency of a new smart thermo-responsive material to be used as fluoridating agent

Study design: Three different gel materials containing sodium monofluorophosphate (Na_2PFO_3) or sodium fluoride (NaF) were studied. Different formulations of the smart material were characterized by rheological measurements, selecting the best for dental practice in terms of viscosity and fluoride-release efficiency.

Fluoride release was tested on human tooth with analysis combining complex viscosity measurements to characterize the material and two complementary analytical methods: 1) XPS analysis, and 2) fluoride ion selective potentiometric electrode (ISE).

Results: all the test formulations presented maximum viscosity values at the temperature of the oral cavity (37° C). Fluoridating treatments increased the fluoride content on the surface, and also within the tooth, only when NaF was used as doping agent The formulation containing 22% of organic fraction presented the optimal balance between high viscosity values and good solubility of NaF.

The gel containing Na_2PFO_3 had a very slight fluoridating effect.

Conclusions: The biocompatible polymeric gel containing NaF increased the fluoride content not only on the surface but also within the structure of the tooth

INTRODUCTION

The effective role of fluoride in the prevention of dental demineralisation has been widely documented over the last 80 years [1,2]. Many studies report substantial reductions in dental caries in developed countries after the use of both systemic and topical fluoride applications became widespread [3-5]. In 2011, the Scientific Committee on Health and Environmental Risks (SCHER) stated that water fluoridation, as well as topical fluoride applications (*e.g.* fluoridated toothpaste or varnish) appear to prevent caries, primarily on permanent dentition.

Systemic fluoride intake during the pre-eruptive phase appears to give remarkable benefits on dental tissue mineralization, principally preventing decay in dental grooves and interproximal areas; while the effect of continued systemic exposure of fluoride from any source is questionable once the permanent teeth have erupted. [6,7]

Topical fluoride application during the tooth eruption period also appears to have good results: immediately after eruption, the teeth have not yet completed their hard tissue mineralization [8]. A slightly porous enamel, as is found in recently-erupted teeth, is more prone to acid attack. Topical application is very helpful in preventing caries even when teeth are partially erupted [9]. Conversely, maintaining low fluoride concentrations for long periods of time is the best way to prevent decay in erupted teeth [10-12].

In this case, SCHER agrees that topical application of fluoride is most effective in preventing tooth decay, sustaining the fluoride levels in the oral cavity, and helping to prevent caries. Fluoride remains in the oral cavity after topical application, in the form of deposits of calcium-fluoride complexes covered by a protein layer. These deposits are detectable in the saliva, on tooth surfaces, and also in bacterial biofilms. When the oral pH decreases, the slow release of fluoride from protein-covered deposits begins. Augmented fluoride concentration allows the re-mineralization of tooth surfaces, preventing caries.

For the above reasons, in the last 20 years, the topical use of fluorides (e.g. fluoride toothpastes, mouthrinses, gels, and varnishes) has become more popular than systemic use (e.g. fluoride tablets, addition of fluoride to drinking water); in particular, fluoride toothpastes are the most widespread topical fluoride-releasing device [13,14].

In this context, the paper presents an innovative thermo-responsive fluoride-releasing dental material, based on a smart poloxamer, capable of releasing fluoride when applied to the teeth; it is “quasi-solid” at the temperature of the oral cavity, but “semi-liquid” at lower temperatures typical of washing steps.

Due to their temperature-dependent physical state, poloxamers doped with fluoride might be an interesting product for delivering topical fluoride.

MATERIALS AND METHODS

Three different fluoride-releasing gel materials were studied: a poloxamer-based gel (P1) containing sodium monofluorophosphate (Na_2PFO_3), a poloxamer-based gel (P2) containing sodium fluoride (NaF), and a commercial gel (C, Neutral Fluorine, Dental Medical) for comparison purposes.

Gel preparation and different formulations

The home-made gels were prepared by simply mixing the Poloxamer 407 (Poly(ethylene glycol)-block-poly(propylen glycol)-block-poly(ethylene glycol); reference standard according to the European Pharmacopeia and the National Formulary of the United States Pharmacopeia - USP/NF) with water at different weight percentages of the organic fraction (see Table 1, first column). After preliminary physico-chemical characterization (see below), the formulation containing 22% of organic fraction (P), which presented the proper balance between high viscosity and good solubility of the doping agents, was chosen for the subsequent doping studies.

The doping was done on two aliquots of P formulation, obtaining the two fluoridating devices:

- P1: 1.14% in weight of sodium monofluorophosphate (Na_2PFO_3), corresponding to 0.15% of NaF;
- P2: 0.15% in weight of sodium fluoride (NaF, Fluka, ≥ 99.0%).

The prepared gels were kept in a refrigerator, prior to being used in the fluoridating tests.

Gel formulation characterization

The different gel formulations (before doping) were characterized by rheological measurements, to study their viscous behavior in function of the temperature.

The measurements were done using a Rotational Rheometer Physica MCR300, equipped with 25 mm parallel plates. Complex viscosity was measured from 5°C to 45°C using a plate gap of 1 mm, constant strain set at 5%, and a constant frequency of 10Hz.

In order to evaluate the temperature at which the different polymeric formulations presented the pseudo-transition phase (from liquid to "semi-solid", gel) an empirical new method (magnetic stirrer method) was adopted. The formulation in a 100 ml beaker containing a magnetic bar and a thermometer was placed on a magnetic stirrer. The temperature was raised until the magnetic bar instantaneously stopped moving, indicating that the "semi-solid, gel" state had been achieved. The values obtained with this method were compared with those obtained by rheological measurements.

Fluoride release measurements in water

In this study, the measurements were performed by direct potentiometry under constant agitation, on an Amel 338 potentiometer, using a Fluoride Ion Selective Electrode (F-ISE, Amel, Italy) with a single crystal of LaF_3 doped with EuF_2, as solid-state membrane, and a saturated calomel as reference electrode. The working solution comprised 20 ml TISAB IV (Total Ionic Strength Adjustment Buffer) in 80 ml of deionized water (Milli-Q, Millipore). TISAB IV consisted of sodium tartrate dihydrate (Sigma Aldrich, Primary Standard, 23 g), TRIS (Fluka, SRM, 24.2 g) and hydrochloric acid (Fluka, 37%, 8.4 ml) in 100 ml deionized water. The ISE calibration was run using a 0.005 M sodium fluoride (Fluka, ≥ 99.0%) standard solution, obtaining an experimental slope of 54.9 mV.

The release of fluoride over time from different gel materials was monitored, placing a small container filled with gel directly in the working solution at 37°C, under continuous stirring. The container had a volume of 1.65 ml and the surface of gel exposed to the solution was 50.24 mm^2.

Ex vivo fluoride release measurements

Teeth (T1 and T2) were obtained from two young female patients (22 and 20 years old) who, for orthodontic treatment, required the surgical removal of a third molar under local anesthesia. The teeth presented integrity of the enamel surfaces, and absence of traumatic injuries, cavities, and erosion of the enamel. Access to the third molar was from the buccal aspect, and bone removal, where required, was with a round bur under continuous irrigation, preserving tooth integrity.

After rinsing with deionized water and a toothbrush, T1 and T2 were each divided into three fragments: T1F1 (0.4288 g), T1F2 (0.2567 g) and T1F3 (0.2331 g); T2F1 (0.2515 g), T2F2 (0.3740 g) and T2F3 (0.1937 g).

Analysis and fluoridating treatment of tooth T1

Fragments T1F1 and T1F2 were characterized by XPS analysis, to determine the surface quantity of fluoride (and other elements) before and after the fluoridating treatments. After a preliminary analysis, the two fragments were immersed for 15 minutes in two different gels: P1, the new poloxamer-based gel doped with sodium monofluorophosphate (Na_2PFO_3), and C, the commercial gel by Neutral Fluorine, Dental Medical. After treatment the two fragments were washed with deionized water and a toothbrush, and dried by rapid immersion in acetone.

The three fragments, treated (T1F1 and T1F2) and untreated (T1F3), were then dissolved by immersion in a stirred solution of 5 ml concentrated perchloric acid (60%) for 24 hours, in a 25 ml polyethylene flask. After complete dissolution, the flasks were filled with deionized water. 1 ml of each sample was added to a solution containing 5 ml of TISAB IV and 19 ml of deionized water and the resulting solution was analyzed by potentiometric method, with the standard addition technique, using a 0.005 M sodium fluoride standard solution.

Analysis and fluoridating treatment of tooth T2

All the fragments were characterized by XPS analysis before and after treatment, to determine the surface quantity of fluoride (and other elements) and subsequently dissolved in perchloric acid, for potentiometric analysis as described above.

Fragment T2F1 was untreated; fragment T2F2 was immersed for 15 minutes in the fluoridating gel P2, the new poloxamer-based gel doped with sodium fluoride (NaF); fragment T2F3 was immersed for 15 minutes in the fluoridating gel P2, rinsed with deionized water and then again treated by immersion for a further 15 minutes in the P2 gel. After treatment, the fragments were washed with deionized water and a toothbrush, and dried by rapid immersion in acetone.

XPS Analysis

XPS measurements were performed in an M-Probe Instrument (SSI) equipped with a monochromatic Al Ka source (1486.6 eV) with a spot size of 200x´-750 μm and a pass energy of 25 eV, providing a resolution for 0.74 eV.

The energy scale was calibrated with reference to the $4f_{7/2}$ level of a freshly evaporated gold sample, at (84.00 ± 0.1) eV, and with reference to the $2p_{3/2}$ and 3s levels of copper, at (932.47 ± 0.1) and (122.39 ± 0.15) eV, respectively.

With a monochromatic source, an electron flood gun was used to compensate the buildup of positive charge on the insulator samples during the analyses: a value of 10 eV was selected for measurements on these samples. For all samples, the C1s peak level was taken as internal reference at 284.6 eV. The accuracy of the reported binding energies (BE) may be estimated as ± 0.2 eV. The quantitative data were also carefully checked, and were reproduced several times; the percentage error is estimated at ± 1%

RESULTS

Physico-chemical characterization

Figure 1 presents the rheological measurements of the polymeric formulations tested. All gels show sigmoidal thermoresponsive behavior, characterized by minimum viscosity (the formulation behaves as a liquid) at temperature below 18°C, and maximum viscosity (the formulation behaves as a gel) for temperature above 33°C. Repeated heating and cooling of the formulations does not affect the shape of the curves. The typical sigmoidal shape of the rheological curves enables a "phase pseudo-transition" (gelation point, T_{PT}) temperature to be established, by determining the position of the maximum of the first derivative curve (Figure 1 shows as an example the 25% case).

The gelation point temperatures were also determined using the new empirical method described above; the two methods gave similar results (Figure 2 and Table 1). They indicate that the linear dependence of T_{PT} decreases as the percentage of organic fraction in the gel increases (*i.e.* with decreasing percentages of water).

In particular, all the test formulations presented maximum viscosity values at the temperature of the oral cavity (37° C), allowing them to be used as smart fluoridating matrixes in dental practice. Among the different compositions studied, the second part of the research examined the formulation containing 22% of organic fraction, which presented the optimal balance between high viscosity values and good solubility of the doping active agents.

Fluoride release in water

As Figure 3 shows, the fluoride release over time in water from the P2 gel, which contains NaF as source of fluoride ions, was characterized by an activation period (7 min), after which the gel began to release fluoride into the solution.

After approximately 11 minutes, the fluoride concentration reached a plateau, followed by a second slow growth period. A period of 15 minutes thus appeared an appropriate exposure time in dental treatment, and ex-vivo treatments were performed for this time.

P1 gel release was one order of magnitude lower than that of P2, indicating the poor fluoride dispensing capability of this material, containing Na_2PFO_3 as fluoride source.

Indeed, Na_2PFO_3 is usually added to toothpastes as long-term fluoride-releasing matter, whereas for the present purpose it is of no use. It was not possible to study the release in water from the commercial gel, as it dissolves in aqueous matrixes.

Ex vivo fluoride release

The ex-vivo experiments of fluoride release from the gel, and fluoride intake by the tooth, were studied by an ad-hoc protocol that took into account the initial results of this study. The procedure was described in detail above. The results of ISE electrochemical analysis and XPS measurements are summarized in Table 2.

The results obtained with the two analysis methods must be interpreted in a different ways: whereas XPS analysis provides an estimation of fluoride content on the surface of the tooth, ISE measurements, performed on a fully dissolved sample, determine the total fluoride content. It appears from these results that fluoridating treatments increased the fluoride content on the surface, and also within the tooth, only when NaF was used as doping agent (P2 and C). The gel containing sodium monofluorophosphate had a very slight fluoridating effect, confirming the results of release in water. Of the gels containing NaF, the new polymer (P2) doubled the fluoride concentration in the tooth. Moreover, the fluoride released by the P2 gel easily entered inside the tooth, as confirmed by the low percentage of surface fluoride measured by XPS. On C2 gel treatment, the result was the opposite. This difference may be due to the different nature of the gels: P2, which is semi-solid at the temperature of the oral cavity, is closely attached to the tooth surface, allowing more regular diffusion of the active agent from gel to tooth.

Lastly, repetition of the treatment (with P2 gel) further increased the fluoride content, suggesting that a number of short applications, rather than a single long treatment, might be preferable in dental practice.

DISCUSSION

In 2010 Walsh et al. reviewed 75 studies, confirming the benefits of using fluoride toothpaste in preventing caries in children and adolescents over placebo, but the effect was only significant for fluoride concentrations above 1000 ppm [15,16]. Most commercial toothpastes have fluoride concentration between 1100 and 1450 ppm. Toothpaste with lower fluoride content (< 600ppm) might reduce fluoride ingestion by young children, and minimize the risk of fluorosis; however, studies have found no evidence of any caries-reducing effect [17]. This type of medical device presents some drawbacks: whereas in adults (with fully developed spitting reflex) less than 10% of the toothpaste is ingested, the estimated intake in children may be as high as 40%; ingestion has been reported to be 48% in 2 to 3 year olds, 42% in 4 year olds, 34% in 5 year olds, and 25% in 6 year olds; in children aged between 8 and 12 years, ingestion is reported to be around 10% [18].

The only scientifically-proven risk of fluoride use is the development of fluorosis, which may occur with fluoride ingestion during tooth and bone development. Fluorosis of the permanent teeth occurs when a sufficient quantity of fluoride is ingested for a sufficient period of time while the tooth enamel is undergoing mineralization. Fluorosis is the result of subsurface hypomineralization and porosity between the developing enamel rods [19]. This risk exists in children below 8 years, and the most susceptible period for permanent maxillary incisor fluorosis is between 15 and 30 months of age [20-22].

Toxic levels of fluoride are possible, particularly in children, as a result of ingesting large quantities of fluoride supplements. The toxic dose is 5 to 10 mg of elemental fluoride per kilogram of body weight; lethal doses in children have been calculated to be between 8 and 16 mg/kg [23]. The European Food Safety Authority, Panel on Dietetic Products, Nutrition and Allergies (EFSA NDA panel) considers that an intake of less than 0.1 mg F/kg BW/day in children up to 8 years old leads to no significant occurrence of "moderate" forms of fluorosis in the permanent teeth [24].

Other topical products include mouthrinses, varnishes and gels. The efficacy of mouthrinses in preventing caries appears to be low [4,25]. Varnishes and gels are used by professional dental workers for caries prevention on permanent teeth and to treat early enamel carious lesions in the primary dentition [12,26,27]. Particularly during their eruption

(approximately from 5 to 7 and from 11 to 14 years old), the posterior permanent teeth can be at high risk of developing carious lesions. In these periods, topical application of fluoride-containing varnishes and gels can be useful to prevent caries. Varnishes and gels may contain high levels of fluoride (from 20000 to 50000 ppm) in different chemical formulations: 1) Sodium Fluoride; 2) Phosphate Fluoride; 3) Stabilized Stannous Fluoride, which shows interesting antimicrobial activity but can also pigment the tooth surface. The high fluoride concentrations in these products may increase the risk of ingestion of large quantities of fluorides. Enhanced control of these materials, particularly during treatment of younger patients, is therefore fundamental.

The poloxamer family comprises more than 30 non-ionic, amphiphilic ABA-type block copolymers, in which A is poly(ethylene glycol) (PEG) and B is poly(propylene glycol) (PPG). Their physical state (liquid, paste, solid) is governed by their molecular weight and block ratio. Poloxamers are well tolerated (non-toxic), although at high concentrations some side-effects, including hypercholesterolemia and hypertriglyceridemia, suggest that the polymer concentration should be kept to a minimum [28]. Poloxamer 407 (Pluronic® F127) has a lower critical solubility temperature (LCST) at biologically-relevant temperatures (25°C at 20% wt.), a feature making it the most popular candidate of the series for biomedical applications. Drug loading is readily achieved by simple mixing [29]. Polymeric gels capable of absorbing and releasing cations and anions for different purposes are widely used, and have been studied with electroanalytical techniques [30]

These gels are “solid” and adhere to the tooth surface at 37°C, thus ensuring perfect and continuous contact between gel and oral part to be treated, and avoiding accidental ingestion. Furthermore, the polymeric materials can be rapidly removed by simply rinsing with cold water during mechanical oral suctioning.

The strong fluoridating capability of a smart thermo-responsive material has been demonstrated, through analytical measurements performed on ex-vivo tooth samples.

The biocompatible polymeric gel presented here can not only increase the fluoride content on the surface and within the treated tooth, but also presents important features able to facilitate dental practice and enhance efficiency. The thermo-responsive behavior offers the dental practitioner a material that is easily manipulated outside the oral cavity (when it is in its liquid form), and that sticks to the tooth surface at the oral

cavity temperature, as the fluoridating process takes place, avoiding the risk of dispersion and ingestion of the active material.

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LEGENDS

Figure 1. Rheological measurements of different polymers, at different percentages of organic fraction. - - - - - First derivative plot for 25% content.

Figure 2. Phase transition temperatures at different percentages of organic fraction in the polymeric gel material, determined by magnetic stirrer method (solid circles) and by rheological measurements (open circles).

Figure 3. Release of fluoride ions in water from P2 gel, containing NaF as source of fluoride ions.