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Fluoride Release from a Resin-modified Glass-ionomer Cement in a Continuous-flow System: Effect of pH

ABSTRACT

Fluoride is added to many dental restorative materials, including glass-ionomer cements, for the specific purpose of leaching fluoride into the surrounding tissues to provide secondary caries inhibition. During the caries process, an acidic environment attacks the dental tissues as well as the glass-ionomer cement. We hypothesized that pH significantly affects the rate of release of fluoride from the glass-ionomer cement. A continuous-flow fluoride-measuring system that monitors the amount of fluoride released over time was used to determine the release of fluoride from a resin-modified glass-ionomer cement (KetacFil®). The results show that the release rate began with a fast burst of fluoride which quickly diminished to low levels in 3 days. Under neutral pH conditions, the rate of fluoride release at 72 hrs was significantly slower than at pH 4.

KEY WORDS: fluoride, fluoride kinetics, glass-ionomer cement, fluoride release, controlled delivery systems.

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INTRODUCTION

Because fluoride is an effective cariostatic agent, many dental materials have been designed to provide fluoride to the oral environment (Toumba and Curzon, 1993; Castioni *et al*., 1998; Eichmiller and Marjenhoff, 1998). It has been hoped that long-lasting timed-release materials such as glass-ionomer cements could provide sufficient amounts of fluoride at the site of the restoration to prevent further demineralization and to assist in remineralization. In spite of anecdotal reports of efficacy, no controlled clinical study of any filling material has been shown to reduce caries incidence in the general population (Randall and Wilson, 1999). Only recently has an anticaries effect from glass-ionomer cements in xerostomic patients been demonstrated (Haveman *et al.*, 2003). All patients in this study had impaired salivary flow, and the caries reduction was observed over a period of 2 yrs. These results are in contrast to the lack of similar results for patients with normal salivary function (stimulated flow rate of 6 mL/min; Dawes and Macpherson, 1992) and bring to question what factors influence fluoride release from glass ionomers. Current knowledge of what controls the release of fluoride from glass-ionomer cements includes the effects of pH and, to a lesser extent, artificial saliva (Carvalho and Cury, 1999). Most other physiologic and environmental influences are unknown (reviewed by Wilson and Nicholson, 1993).

The methods used for the assessment of fluoride release have varied among laboratories, making inter-laboratory comparisons difficult if not impossible (Cranfield *et al.*, 1982). The most utilized method for the assessment of the release rate of fluoride from dental restorative materials has been *via* batch (static) methods. Typical regimes for the batch methods are to place material samples in a volume of leaching solution for a period of time, followed by an analysis of the solution. Some of these experiments have been extended for as long as 2 yrs (Forsten, 1990).

A continuous-flow cell apparatus and method have been designed that allow for the in vitro assessment of physiologically relevant variables such as salivary flow rate, residual volume, surface area of the restoration, and saliva composition. This method mimics more closely the *in vivo* situation, because saliva flows continuously through the mouth, and any fluoride released from materials into the saliva is removed from the mouth as the saliva is swallowed. The results garnered *via* this method are directly relevant to an understanding of the factors that govern the release of fluoride *in vivo*. The purpose of this paper is to present the flow-cell method and results of studies to determine the kinetics of fluoride release from glass-ionomer cements as a function of pH. The purpose of these experiments was to determine the amount of fluoride that is released from glass-ionomer cement at neutral and cariogenic pH under conditions of continuous flow. The hypothesis tested was that pH significantly affects the rate of release of fluoride from the glass-ionomer cement.

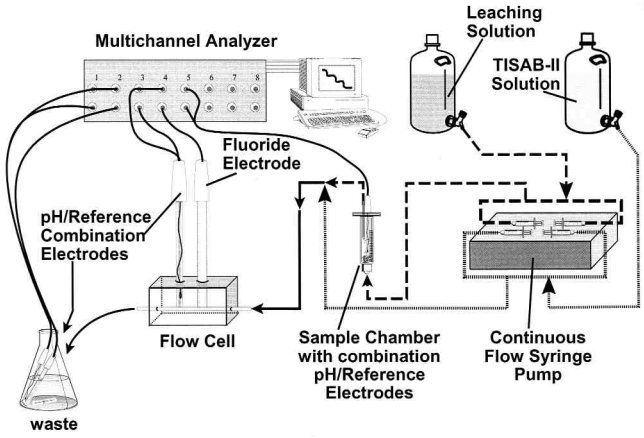

Figure 1. Diagram of the continuous-flow system.

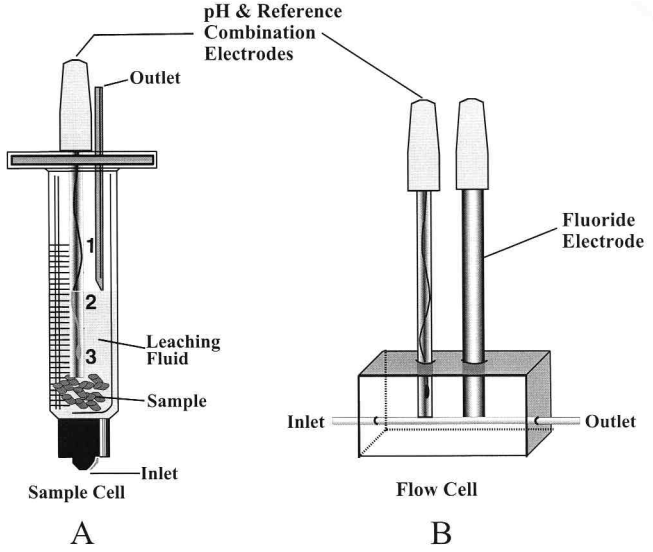

Figure 2. Diagrams of **(A)** the sample cell and **(B)** the flow cell.

MATERIALS & METHODS

Continuous-flow Measurement System

The measurement system (Fig. 1) consists of three basic components: (1) the continuous-flow syringe pump, which can deliver a constant flow of fluids for extended periods of time through the sample chamber (Fig. 2A); (2) the fluoride-measurement cell (Fig. 2B), where the continuous measurement of fluoride concentration in the fluid stream occurs; and (3) the data collection system that records the output of the electrometer as a function of time. There is a pH/reference combination electrode placed in the sample chamber to monitor the pH of the leaching fluid as it passes over the sample. There are pH/reference combination and fluoride-ion-selective (F-ISE) electrodes placed in the waste container. These additional electrodes provide a system check of the cumulative fluoride concentration for the complete experiment, and are used as verification of the experimental run. An eight-channel high-impedance electrometer (Model MS314A, Elchema, Inc., Pottsdam, NY, USA) connected to a data acquisition board (Model VF910, Real Time Device, Inc., State College, PA, USA) in a PC computer running the data program (ELECTRODE.EXE) collected the data.

The continuous-flow syringe pump was capable of delivering a controlled volume of fluid for extended periods of time. The dual-syringe head filled one syringe as the opposite syringe was dispensing fluid. Check valves prevented the filling syringe from drawing fluid from the sample side of the pump, while other check valves prevented dispensing fluid to the fill reservoirs. Another pair of syringes on the pump head delivered the same volume of TISAB II solution to the system at the same rate as the leaching solution was delivered. In this way, a 1:1 dilution of the sample into TISAB by volume was achieved.

The leaching fluid was delivered at a constant rate to the sample chamber, where the sample to be leached was encountered. The volume of the sample chamber can be adjusted, as can the amount of sample placed in the chamber. The volume of the sample chamber divided by the flow rate is defined as the residence time. After the leaching solution passed over the sample and out of the sample chamber, an equal volume of TISAB II was introduced into the sample stream just prior to the measurement cell.

The measurement cell was equipped with a F-ISE and a reference electrode (Fig. 2). The fluoride concentration was measured continuously as the solution passed through the cell. The solution was then collected in a waste container, where the cumulative fluoride concentration was monitored by another F-ISE/reference-electrode pair. Additionally, a combination pH electrode was placed in the sample chamber so that we could obtain data about the sample's effect on the pH of the leaching solution. Preliminary experiments showed that the typical drift was less than 2 mV *per* day and that the drift was both up and down, centering about a mean value. A two-millivolt drift represents a relative standard uncertainty of 3% in the measured value.

Standardization of the F-ISE

Standardization of the F-ISE/reference-electrode pair in the flow cell was done before, during, and after each experimental run. The flow cell was designed such that it could be removed from the system during electrode standardization. With the flow cell removed from the system, fluoride standards were injected into the flow cell with a syringe, and the data were recorded. After standardization, the flow cell was then replaced in the measurement system, and the monitoring program was re-started. This process usually took less than 30 min. The other F-ISE/reference-electrode pairs were standardized in the usual method [Orion] in 10-mL beakers containing the fluoride standards.

Measurement Validation

We validated the measurements by calculating the area under the curve of the flow cell data (kinetic) and comparing that with the cumulative fluoride concentration in the waste container. These data curves overlapped completely throughout the experiment, even though the concentrations measured in the flow cell and the waste container were different at almost all times.

Specimen Preparation

Commercially available glass-ionomer cement samples were made following the manufacturer's recommended methods (Ketac-Fil®, Espe America, Inc., Norristown, PA, USA). Individual delivery

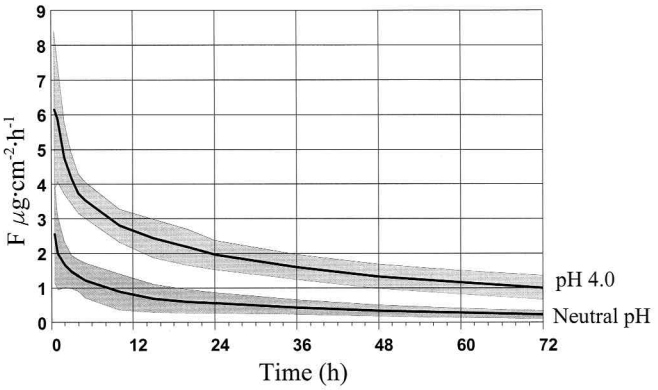

Figure 3. Fluoride released from glass-ionomer cement over time under conditions of cariogenic and neutral pH. The solid line is the mean of 5 measurements, and the shaded area around the line is ± 1 standard deviation.

ampoules were activated and placed in a triturater for 10 sec. The mixed cement paste was then quickly dispensed into a Teflon® mold and allowed to cure for 7 min. We made the mold by drilling 2-mm-diameter holes into a sheet of Teflon® 1.75 mm thick. Clean glass slides were used to cover the top and bottom of the mold while the samples were curing. The cylinders (2 mm diameter x 1.75 mm tall) were then removed from the mold and stored at 100% humidity for at least 24 hrs before the leaching experiments began. The surface area of the cylinders was determined by calculation to be 5.33 x 10^{-3} cm^2·cylinder^{-1} or 0.494 cm^2·(g of cylinders)$^{-1}$ of glass-ionomer cement.

Experimental Procedures

For all the experimental runs, 1 g of cured glass-ionomer cement (Ketac-Fil®) pellets (0.494 cm^2/g) was placed in a 4-mL leaching chamber. The sample surface area was approximately equivalent to the exposed surface area of 4 glass-ionomer restorations and was necessary if we were to achieve sufficient fluoride concentrations in the leach fluid to be measurable by the F-ISE. The leaching fluid (distilled H_2O at neutral pH or 50 mmol/L KCl titrated to pH 4.0 with concentrated HCl) was passed over the glass-ionomer cement sample at a rate of 0.25 mL/min for up to 10 days at room temperature.

RESULTS

Glass-ionomer cement samples were exposed to leaching solution for at least 5 days, and experiments were repeated 5 times at each pH. In all experiments, a large release of fluoride was observed initially for about 10 hrs, followed by a long period of substantially slower fluoride release. Fig. 3 shows that this release was significantly higher throughout the runs at pH 4 than under neutral conditions ($p < 0.05$ at all time points). At 72 hrs, samples leached under neutral pH conditions released fluoride at a rate of (0.34 ± 0.18) μg F cm^{-2} h^{-1} (mean ± SD). Samples leached under pH 4 released fluoride significantly faster at a rate of (1.5 ± 0.55) μg F cm^{-2} h^{-1} ($p < 0.05$, *t* test). At 5 days, the release rate was much lower at (0.09 ± 0.02, n = 2) μg $L^{-1}mm^{-2}$ under neutral conditions but still (1.0 ± 0.37) μg F cm^{-2} h^{-1} at pH 4.0 leaching.

DISCUSSION

Langenbucher (1969) listed the disadvantages of static methods for the determination of timed-release characteristics and dissolution behavior of solid drugs: (1) Flow conditions in the liquid medium depend on a great many parameters, such as vessel dimensions, fluid volume, stirring rate, and the position of the sample in the fluid; (2) fluid volume must be fixed and thus determines the dissolution kinetics; and (3) the dissolution of the solid causes an increase of its concentration in the liquid from zero up to either the saturation limit or the complete dissolution of the solid. This concentration profile is different from the *in vivo* process, where the dissolved material is removed continuously by the flow of physiological fluids (saliva). Tingstad and Riegelman (1970) expand on the disadvantages with the following list: (1) lack of flexibility of static systems to control variables; (2) lack of homogeneity caused by both large volumes and agitation methods; (3) a variable concentration gradient as the solid dissolves that is not similar to *in vivo* concentration profiles; (4) a method of agitation that is semi-quantitative and relates poorly to theoretical dissolution rate equations; and (5) the fact that the data obtained tend to obscure the details of the dissolution process. These authors state that static batch methods "...produce data expressed as an integral function. That is, since the dissolved molecules are accumulating in the solution, the resultant data represent an integral function of the dissolution process rather than a differential function."

The continuous-flow method described here allows many different variables to be evaluated either singly or by group so that their synergistic effects on the release of fluoride from timed-release materials can be determined. Examples include the ability of the system to evaluate the effects of saliva composition (pH, calcium, fluoride, ionic strength, etc., as simulated by the leaching fluid) on the dynamic release of fluoride; the flow rate of saliva across the restoration; the surface area of the restoration; and the amount of time required to replace saliva with fresh saliva (residence time). Possibly the most intriguing feature of the continuous-flow method is that it is flexible enough to allow for the evaluation of non-steady-state environments such as the change in pH as plaque generates acids, or intermittent high concentrations of fluoride introduced either by a rinse or from toothbrushing.

During the first 10 hrs, there was a burst of fluoride release that then declined to a slow, steady release of fluoride (Fig. 3). After the initial burst, the release characteristics could be described by the linear relationship between the logarithm of the flux *vs.* time under either pH 4 or neutral conditions. The logarithm of the fluoride flux from the glass-ionomer cement was significantly correlated to time, as described by the following general equation:

$$\ln(J_F) = (-1/t)t + \ln A \qquad \text{(Eq. 1)}$$

where: J_F is the flux of fluoride *per* unit surface area (μg F·cm^{-2}·h^{-1}); (-1/*t*) is the rate of change of the flux *per* unit area (μg F·cm^{-2}·h^{-2}); t is time (h), and *A* is a constant representing the initial fluoride release rate (μg F·cm^{-2}·h^{-1}), as calculated from the linear regression fit of the data from 15 hrs to 72 hrs. The slope (-1/*t*) of Eq. 1 is related to the dissolution and diffusion of fluoride through the matrix of the glass-ionomer cement.

The slopes (-1/*t*) of the fluoride flux from the glass-ionomer resin at pH 4 and neutral conditions were remarkably

similar at (0.0153 ± 0.0008) μg $F \cdot cm^{-2} \cdot h^{-2}$ and (0.0196 ± 0.0005) μg $F \cdot cm^{-2} \cdot h^{-2}$, respectively. The small size and similar magnitude of the coefficients indicate that the limiting mechanism in fluoride release was the diffusion of fluoride ions through the matrix material and not the effect of the bathing solution composition. The difference in the calculated flux at time zero represents the effect of the bathing solution on the initial dissolution of fluoride ions from the surface of the glass-ionomer cement, where pH 4 liberates much more fluoride initially than does neutral bathing solution. The relationship found in Eq. 1 can be used to predict the length of time the glass-ionomer cement can release defined amounts of fluoride without recharging. For example, assuming that the minimum flux of fluoride release needed to maintain a clinically relevant concentration of fluoride at the restoration is 0.1 μg $F \cdot cm^{-2} \cdot h^{-1}$, then one can calculate the length of time the glass-ionomer cement will succeed in meeting or exceeding this flux. Under pH 4 conditions, it will proceed for 246 hrs, and under neutral conditions, it will proceed for 133 hrs before the fluoride-release rate reaches the arbitrary minimum rate.

These predictions are based on results obtained when the bathing solutions are much simpler than the composition of whole saliva. It is interesting to speculate on what the effects of saliva would be on the fluoride-release rate from glass-ionomer cements. Because the results of this study indicate that the rate of release of fluoride (after the initial burst) from glass-ionomer cements is diffusion-controlled and not related to the solution chemistry, the only effects that saliva could have would be surface modifications that effect the rate of diffusion from the cement. It is anticipated that salivary protein could form a pellicle over the cement. This pellicle could act as a diffusion barrier as seen in enamel (Zahradnik *et al.*, 1976; Tung and Brown, 1983; Carey *et al.*, 1991) and slow the diffusion of fluoride from the cement, and it could bind fluoride as it passes through the membrane. Thus, it is anticipated that this diffusion barrier would have the effect of slowing the rate of fluoride release.

The glass-ionomer cement released more fluoride when the environment was at lower pH, thus providing the greatest amount of fluoride when it would be most needed to prevent secondary caries. The hypothesis that a greater amount of fluoride is released and at a greater rate at lower pH was partially supported by the observation that the amount of fluoride released was significantly higher throughout the runs at pH 4 by a factor of 3 to 4 times greater than at neutral conditions ($p < 0.05$ at all time points). However, after the initial burst period, the rate of release was not pH-dependent. More studies are required for a better understanding of this phenomenon and for identification of the rate-limiting step for the release of fluoride into the environment.

In these experiments, the release rate of fluoride after 3 days in continuous flow was much lower than those reported by others after many months' exposure in batch methods (reviewed by Eichmiller and Marjenhoff, 1998). These findings underscore the necessity for the release of fluoride from dental restorative materials to be evaluated under conditions that model the environment of the mouth if any beneficial effects or limitations that the released fluoride might have are to be fully appreciated.

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DISCLAIMER

Certain commercial materials and equipment are identified in this paper to specify the experimental procedure. In no instance does such identification imply recommendation or endorsement by the National Institute of Standards and Technology or the ADA Foundation, or that the material or the equipment identified is necessarily the best available for the purpose.

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