In vitro effect of human saliva on the output of fluoride from controlled-release devices

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Abstract

This study was designed to determine the in vitro fluoride output from controlled-release devices in stimulated, whole human saliva, and to assess changes in the salivary calcium concentration following immersion of the devices. Twenty fluoride (F) controlled-release devices of the hydroxyethyl methacrylate (HEMA)/methyl methacrylate (MMA)-type were employed. Each was designed to release 1–2 mg F per day. All devices were placed individually in 10.0 ml of deionized water for 3 days to assess baseline F output. Seven devices with markedly high or low output were discarded. During days 4–13, three of the remaining 13 devices were placed individually in 10.0 ml of stimulated, whole saliva donated by three volunteers. All devices were returned to deionized water during days 14–17. All solutions were replaced daily with fresh solutions, and each test tube was inverted once every 24 hr. The study was conducted at 19–21°C. Fluoride concentration of the deionized water and human saliva was assessed by ion-specific electrode; calcium concentration of the saliva was assessed pre- and postimmersion by flame atomic absorption spectrometry. The fluoride output of the devices placed in saliva decreased to 36% of their baseline rates ($P < 0.001$). The calcium concentration of the human saliva decreased from a mean preimmersion value of 3.58 mg/100 ml (± 0.63 SD) to a postimmersion value of 2.64 (± 1.12 SD; $P < 0.001$). These results suggest an interaction between the fluoride released by the devices and calcium in the saliva. This interaction may lead to the formation of CaF_2 within or on the surface of the device, reducing the F output. (Pediatr Dent 16:410–12, 1994)

Introduction

Fluoride at the enamel surface can reduce the degree of demineralization and enhance remineralization during an acid challenge.[1,2] One means of theoretically elevating salivary fluoride levels is using an intraoral device designed to release controlled amounts of fluoride over prolonged periods.[3,4] The type of device employed in most investigations consists of a copolymer core of hydroxyethyl methacrylate (HEMA) and methyl methacrylate (MMA) in a 50:50 ratio into which NaF has been incorporated. The core is surrounded by a 30:70 HEMA/MMA copolymer membrane that controls the rate of fluoride release for up to several months.[5]

Though the fluoride release rate is presumed to be constant, there are some indications that this may not be the case under all conditions. Intraperitoneal devices implanted in rats were found to release fluoride at 31% of their initial rates.[6] Whitford et al.[7] determined the fluoride release from HEMA/MMA devices in deionized water, isotonic saline, parotid ductal saliva, plasma, and a $CaCl_2$ solution with a calcium concentration of 10 mg/100 ml. Fluoride output decreased slowly in deionized water and saline over a 5-day period to 86 and 76% of the initial values, respectively. The release rates in the $CaCl_2$ solution, saliva, and plasma declined abruptly to relatively constant levels that were 46, 27, and 19% of the initial rates in water, respectively. The calcium concentration of the solutions decreased, leading to speculation that the released fluoride was precipitating as CaF_2 and possibly binding with other cations within or on the devices.

Adair et al.[8] studied the fluoride output of HEMA/MMA devices placed in artificial saliva with calcium concentrations of 0, 4.5, 8.0, and 12.0 mg/100 ml. The fluoride release rates decreased to 29, 16, 12, and 10% of their respective baseline outputs. When the devices were returned to deionized water, the fluoride output rebounded to 70–105% of the baseline values.

The results from several clinical studies have shown both increases and decreases in salivary fluoride concentration in humans wearing HEMA/MMA devices.[9–12] Saturation of the solution with respect to fluoride within the HEMA/MMA devices is likely to lead to CaF_2 precipitation within or on the device whenever calcium is present in the ambient solution. This precipitate could reduce the output of fluoride from the device.

The purpose of this project was to determine the fluoride output from HEMA/MMA controlled-release devices in samples of human saliva and changes in the salivary calcium concentration following immersion of the devices.

Methods and materials

This study employed 10x5x3-mm rectangular devices of the HEMA/MMA type containing NaF blended into the core and designed to release approximately 1–2 mg fluoride per 24 hr (Southern Research Corporation, Birmingham, AL). The study was divided into

three segments. In Segment 1, 20 devices were placed in individual test tubes containing 10.0 ml of deionized water for the first 3 days. Devices with markedly high or low fluoride output were discarded.

The remaining 13 devices were assigned to two groups during Segment 2 (days 4–13). Group I consisted of 10 devices that were individually maintained in 10.0 ml of deionized water. Group II comprised three devices that were placed individually in 10.0 ml of stimulated whole saliva donated by three volunteers. Saliva was stimulated by having the individuals chew a 1x1-in. square of Parafilm® (American Can Co, Greenwich, CT).

During Segment 3 (days 14–17), all devices were again placed in deionized water. The study was conducted at 19–21°C. Each test tube was inverted once during each 24-hr period, and again just prior to withdrawing solution for analysis. Throughout the study all solutions were replaced daily with fresh solutions and portions were stored for analysis. Fluoride was analyzed using the ion-specific electrode (model 9409, Orion Research, Boston, MA) after the addition of an equal volume of Total Ionic Strength Adjustment Buffer (TISAB; Fischer Scientific, Fair Lawn, NJ). In addition, saliva samples from days 4, 5, and 6 were also analyzed after overnight diffusion using Whitford's modification[13] of the Taves acid-HMDS (hexamethyldisiloxane) method.[14] This method quantitatively releases fluoride from insoluble compounds including calcium fluoride and fluorapatite. Salivary calcium was analyzed by flame atomic absorption spectrometry (Varian Spectra 20 — Varian Techtron Pty Ltd, Mulgrave, Victoria, Australia) and a matrix of 5000 ppm KCl.

The data are expressed as mean ± SD for daily fluoride output (mg) and salivary calcium concentration (mg/100 ml). Differences in fluoride release rates among the groups within the segments of the study were examined by one-factor analysis of variance (ANOVA). The Fischer PLSD test was used as the post hoc test. An alpha value of 0.05 was selected *a priori* as the indicator for statistical significance.

Results

The mean fluoride release rates into water (Group I) and into whole human saliva (Group II) for each segment are shown in the Figure. There were no differences between the groups during Segment 1 (days 1–3), when all devices were in deionized water. During Segment 2 (days 4–13), the average fluoride output from the devices in saliva was markedly depressed to 36% of the baseline value ($P < 0.001$). During Segment 3 (days 14–17), when all devices were in deionized water again, the fluoride release rates from the devices in Group II increased slightly, but remained less than 50% of the initial release rate. The difference in fluoride output was still significantly depressed ($P = 0.006$). A comparison of the analytical results using the TISAB-buffered and acid-HMDS diffusion methods revealed no statistically significant differences.

Table. Daily average Ca^{++} concentration (mg/100 ml) of saliva prior to and following 24-hr immersion of controlled-release devices

Donor	*Preimmersion [Ca]*	*Postimmersion [Ca]*
1	3.30 (0.15)	2.49 (1.04)*
2	3.66 (0.73)	2.81 (1.32)
3	3.77 (0.77)	2.64 (1.10)
All	3.58 (0.63)	2.64 (1.12)†

Data are expressed as mean ± SD.

* $P < 0.05$.

† $P < 0.001$.

The Table shows that the averages of the initial salivary calcium concentrations of the three subjects ranged from 3.30 to 3.77 mg/100 ml in Segment 2. The preimmersion average for all subjects was 3.58 mg/100 ml. The average concentrations after the controlled-release devices had been in the saliva samples for 24 hr ranged from 2.49 to 2.81 mg/100 ml. The postimmersion average for all subjects was 2.64 mg/100 ml, a statistically significant reduction in calcium concentration ($P < 0.001$).

Discussion

Immersion of controlled-release devices in stimulated, whole saliva significantly depressed their fluoride release rates even during the first 24-hr period. These 24-hr data, while not shown in the Figure, were calculated separately. These results were similar to those of other studies[7,8] that determined the fluoride release rates of identical devices in parotid ductal saliva, plasma, a $CaCl_2$ solution, and artificial saliva with graded calcium concentrations (0–12 mg/100 ml). Each of these studies demonstrated a sharp decrease in fluoride release rates during the first 24 hr that was sustained as long as the devices remained in the test solutions. The significance of this study is the demonstration of a similar effect for whole human saliva, including the data on calcium loss from solution after immersion of the devices.

One mechanism for the decrease in fluoride release from the HEMA/MMA devices appears to involve an interaction between fluoride and calcium. In our study and that of Whitford et al.,[7] measurable decreases in the calcium concentrations of the test solutions were observed. It was hypothesized that the ion product for calcium and fluoride within or at the surfaces of the controlled-release devices exceeded the solubility product ($3x10^{-11}$), so that calcium fluoride was precipitated in and/or on the surfaces of the devices and would block some of the aqueous channels for fluoride diffusion.[7] The present findings are consistent with this hypothesis.

Calcium, however, is not a necessary factor in the reduction of fluoride release from HEMA/MMA de-

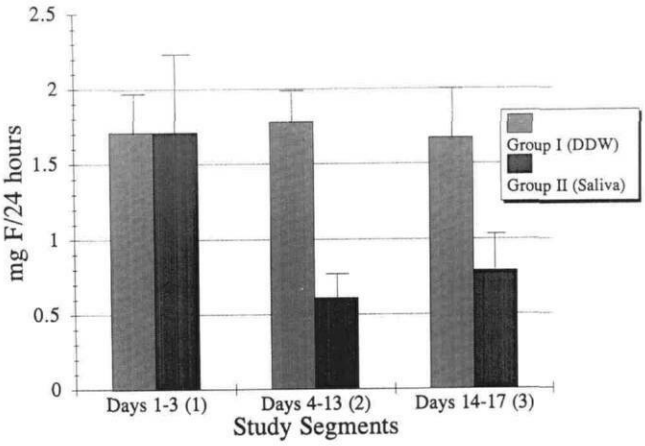

Figure. Mean fluoride release rates (mg F/24 hr) for controlled-release devices during the three study segments. Error bars represent standard deviation. All devices were in deionized water (DDW) during segments 1 and 3.

vices. In the study by Adair et al.,[8] one of the artificial saliva test solutions contained no calcium salts, but the release of fluoride into the calcium-free solution still was reduced significantly. The reductions in the release rates, however, were directly proportional to the calcium concentrations of the test solutions (0, 4.5, 8, and 12 mg/100 ml). It was hypothesized that the rate reduction in the calcium-free solution was due, at least in part, to the formation and precipitation of magnesium-fluoride salts (the $MgCl_2 \cdot H_2O$ concentration of the artificial saliva was 0.59 g/L).

A second mechanism might involve adsorption of salivary proteins onto the devices that could limit the rate of fluoride release. Adsorbed proteins also could coat any calcium fluoride that formed on the surface of the devices and subsequently limit its dissolution.

This study involved saliva from only three individuals. Previous studies,[7,8] however, demonstrated nearly identical responses of the controlled-release devices in calcium-containing solutions. Based on our experience with these studies, we considered these saliva samples adequate to demonstrate the effect.

Conclusions

The results from our study and those reported previously[6–8] indicate that the process of fluoride release from HEMA/MMA controlled-release devices can be affected by several variables. The fluoride release rate provided by manufacturers typically is determined in deionized water and assumed by investigators to remain constant. It is important to recognize that the rate is likely to be considerably smaller within the oral cavity or in test solutions containing components that may interact with fluoride.

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