The effect of adding bioactive glass on the fluoride release of resin-modified glass-ionomer cement in simulated body fluid

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Abstract

Glass ionomer cements (GICs) originally designed for use as dental materials have a number of advantages over acrylic bone cements. These include non-exothermic setting reaction with no thermal damage to surrounding tissues, minimal shrinkage during gelation, the possibility of incorporation drugs and growth factors within the cement and the ability to release fluoride. It is already known that fluoride increase trabecular bone density and it is used in the treatment of osteoporosis to inhibit bone resorbtion. However, fluoride release process is complex and affected by variables related to the cement formulation, manipulation, the mixing process and elution medium. This study was made to examine the change of the cement powder composition on the fluoride release profile in simulated body fluid for clinical efficacy in respect of orthopedic surgery. Therefore, the effects of adding different content of bioactive glass to the new cured cement on elution of fluoride and its kinetic were investigated.

Keywords: *Resin-modified glass-ionomer cement; Bioactive glass; Simulated body fluids; Apatite, Osseo-conductive.*

Introduction

Ionomeric materials are already successfully use for dental applications because of their properties which include a rapid set, adhesion to enamel and dentine, and release of fluoride ions which are thought to confer resistance against dental caries [1, 2]. Furthermore, these properties accompanied with non-exothermic setting reaction with no thermal damage to surrounding tissues, minimal shrinkage during gelation and the possibility of incorporation drugs and growth factors within the cement make these materials suitable for using as orthopedic cement and bone substitutes in oral and maxillofacial surgery [3, 4].

In the past few years, further developments in the field of glass ionomer cements (GICs) has led to introduction of light-activated hybrid GIC versions creating the resin-modified glass ionomer cements (RMGICs). In this new category of biomaterials, hydrophilic monomers and photo-initiators were added to the polyacrylic acid and ion-leachable glass in order to improve the physical and mechanical properties of conventional GICs. Resin-modified glass-ionomer cements are made of the same materials as conventional glass-ionomers, including water, basic glass powder, and polyacrylic acid. They also contain 2-hydroxyethyl methacrylate (HEMA) and photosensitive initiators for facilitating free radical polymerization when exposed to blue light from a conventional dental curing lamp [5,6].

Numerous studies have documented the fluoride release of conventional GICs and resin-modified glass ionomers [7,8]. However, the study of fluoride release from GICs has been extensive, but mainly related to the dental application of GICs such as elution in salvia, de-ionized water, acetic acid, lactic acid and citric acid [9-11]. It is already known that fluoride increase trabecular bone density and it is used in the treatment of osteoporosis to inhibit bone resorbtion. Fluoride ions have also been shown to produce dose-dependent fluorapatite crystallization during osteogenisis [12,13]. It is thus possible to speculate that a greater volume of bone should form in association with implanted GICs.

Bioactive glasses (BGs) are usually considered as bone bioactive ceramics which bond to surrounding osseous tissue and enhance bone tissue formation [14, 15]. Accordingly, it is speculated that hybridization of resin-modified glass-ionomer cement and bioactive glass may improve the bio-reactivity of the current cement with surrounding bone. However, the fluoride release process is complex and affected by variables related to the cement formulation, manipulation, the

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mixing process and elution medium [9]. This study was made to examine the change of the cement powder composition on the fluoride release profile in simulated body fluid for clinical efficacy in respect of orthopedic surgery. Therefore, we measured the [F^-] release data of the novel cured ionomer cement in simulated body fluid (SBF) for further clinical applications. Furthermore, the effect of adding different content of bioactive glass to the cement structure on elution of fluoride was investigated.

2. Materials and Methods

In the present study, the same resin-modified glass-ionomer (RMGI) powder formulation which was synthesized used in our previous investigations [16], was utilized for measuring the amount of fluoride release in simulated body fluid. Furthermore, the bioactive glass (BG) composition which was reported by Hench and coworkers [14] was chosen as the composition of bioactive glass (SiO_2 53%, Na_2O 23%, CaO 20% and P_2O_5 4%). The mixture of reagent grade chemicals were put into platinum crucible and melted at 1400^{o}C for 2h in an electronic furnace. The molten glass was rapidly poured into iced water to produce a granular frit. Subsequently, the obtained frits were ground and sieved to remove particles over 45μm.

For cement preparation different ratio of BG particles were mixed with RMGI powders (Table 1).

Table 1. The properties of different cement used in this study

Sample Code	Bioactive glass (%wt)	Handling time (s)	Light exposure time from each side (s)
A	0	65	120
AB-15	15	50	100
AB-30	30	30	60

Afterwards, the prepared cement powder and a commercial resin-modified glass-ionomer liquid, Fuji II LC (Improved; batch number 609211, GC Corp., Tokyo, Japan), were mixed thoroughly at room temperature. Five cylindrical specimens (8 mm in diameter × 2 mm thick) of each material were prepared by using Teflon split molds. After being filled, each mold was sandwiched between two glass plates (separation with celluloid strips), excess of material removed and then the material was cured using a photo-curable lamp (Farazmehr, Esfahan, Iran). Then the final cements were ejected from the mould after 15 min in 100% humidity, and kept in incubator at 37^{o}C for 24 h prior to testing.

Fourier Transform Infrared Spectroscopy (FTIR; Bruker Vector 33, Germany) was used to determine the extent of acid- base reaction after 24 hours of irritation. The dried cement powders (three specimens were prepared for each material) before immersion (1 mg) were mixed with KBr powder (300 mg) and pressed to form a disk for infrared analysis. All measurements were at a 4 cm^{-1} resolution. The net peak area ratio of the carboxylate salts formed (C=O str of COOM, 1600–1500 cm^{-1}) to the unionized carboxyls (C=O str of COOH, 1740–1735 cm^{-1}) was used to express numerically the acid–base reaction rate [17, 18].

For fluoride release measurement, five disk-shaped specimens (8× 2 mm^2) were prepared from each material. The samples were placed in plastic test tubes containing 25 ml simulated body fluid (SBF) and kept at 37 ^{0}C in an incubator for different periods of time up to 28 days**.** The SBF was prepared by dissolving the reagent-grade NaCl (Merck, 6400, Germany), $NaHCO_3$ (Merck, 6323, Germany), KCl (Merck, 4935, Germany), $K_2HPO_4 \cdot 3H_2O$ (Merck, 5099, Germany), $MgCl_2.6H_2O$ (Merck, 5833, Germany) , Na_2SO_4 (Merck, 13462, Germany), $CaCl_2$ (Merck, 2387, Germany) into de-ionized water, and buffered with Tris (Hydroxy-methyl-amino-methane) ($(CH_2OH)_3CNH_2$; Merck, 8387, Germany) and Hydrochloric acid (HCl; Merck, 314, Germany) to pH 7.4 at 37^{o}C [16]. At the time of fluoride measurement, each specimen was removed from its container and the storage solution decanted for analysis. The specimen was returned to a new container with a fresh 25 ml SBF solution, and storage was continued. Fluoride measurements have been carried out using ion selective electrode potentiometer (Mettler Toledo-MA 235 Ion analyzer, 152153000 Mettler Toledo fluoride selective electrodes, and 373-90-WTE-ISE-57 Mettler Toledo reference electrode). The equipment was calibrated immediately before each measurement by using fresh standard solutions ranging from 0.1 to 100 ppm fluoride. Equal volume of total ionic strength adjustment buffer (TISAB) was added to the collected solutions to provide constant background ionic strength, decomplex fluoride, and adjust the solution pH. The concentration (ppm) of each solution was directly read out on the instrument display and the final results were reported as cumulative fluoride release (μg/cm^2) and fluoride release rate (μg/cm^2/day) taking into account the surface area and solution volume of each specimen. The comparison of fluoride release data was carried out using ANOVA (α=0.05). The non-linear regression was performed using Matlab 7.8.0 (R2009a) software.

3. Results

3.1 FTIR Spectroscopy

Fig. 1 reveals the infrared reflectance spectra of the resin-modified glass-ionomer cement before exposure in SBF in the range of 1800-1400 cm^{-1} at room temperature. The C=O stretching vibration of ester group of poly HEMA and COOH group in polyacid absorb in the region 1740-1700 cm^{-1}. Moreover, the bands in the region 1600-1500 cm^{-1} due to the asymmetric stretching vibration of COO^- of carboxylic acid salts, were observed [18,19]. It can be seen from this figure that by increasing the amount of bioactive glass to the cement structure, the intensity of carboxylate salts peak (C=O str of COOM, 1600-1500 cm^{-1}) increased. Table 2. depicts the ratio of the carboxylate salts formed (C=O str of COOM, 1600-1500 cm^{-1}) to the remaining unionized carboxyl groups (C=O str of COOH, 1740 cm^{-1}).

Table 2. The COOM/COOH ratios of the studied cements before soaking in SBF solution

Sample	COOM/COOH (±SD)
A	0.0693 (±0.015)
AB-15	0.2792 (±0.040)
AB-30	0.7009 (±0.079)

The results show that the amounts of COOM/COOH ratio become greater by increasing the amount of bioactive glass to the experimental cement structure.

3.2. Fluoride release profile

The fluoride release profile is described in terms of the cumulative release over elapsed time. The cumulative fluoride release (μg/cm^2) of the experimental materials as a function of time is shown in Table 3 for up to 28 days.

Table 3. Mean and standard deviation of the cumulative fluoride release from different samples in SBF for 28 days

Time(day)	A	AB-15	AB-30
1	40.81 (16.08)	44.44 (4.23)	35.09 (7.52)
2	59.97 (17.23)	63.41 (11.03)	43.36 (8.62)
3	79.97 (16.89)	82.51 (10.57)	48.43 (9.83)
4	94.35 (18.43)	97.39 (10.36)	52.74 (10.62)
5	119.91 (18.41)	126.3 (12.93)	61.27 (10.25)
8	126.83 (19.10)	135.09 (7.54)	67.86 (9.88)
10	139.79 (21.32)	144.51 (9.42)	70.76 (9.82)
12	154.31 (21.43)	161.48 (11.11)	77.94 (9.15)
15	167.94 (21.20)	174.08 (10.52)	80.99 (9.10)
16	186.10 (20.65)	196.73 (15.41)	86.58 (9.65)
22	192.76 (20.81)	206.85 (14.65)	89.40 (9.87)
24	194.86 (21.53)	213.02 (14.44)	92.55 (9.18)
26	202.90 (21.46)	220.08 (15.18)	94.88 (9.07)
28	210.12 (21.28)	227.01 (15.21)	96.86 (8.85)

The evaluation of data presented in Table 3 shows that there are significant difference between fluoride release data of each formulation ($p < 0.05$) and the effectiveness of the soaking time on fluoride release data. In which, the cumulative amount fluoride released after 28 days in SBF gives the following result:

AB-15> A> AB-30

3.3. Fluoride release kinetics

The cumulative fluoride release profile for each material studied was fitted to equations using nonlinear regression. Table 4 summarized the equations that have been suggested in the literature to describe the cumulative release of fluoride as a function of time [20-26].

In the present study the authors used the Matlab 7.8.0 (R2009a) curve fitter to apply these equations to the experimental data. The curve fitter uses the Trust-Region algorithm to find the coefficients (parameters) of the independent variable(s) that gives the best fit between the equation and the data. This algorithm seeks the values of the parameters that minimize the sum of the squared differences between the observed values and predicted values of the dependent variable. The adequacy of these equations (Table 5) was determined based on the r-square (r^2) and root mean squared error (RMSE). As shown in Table 5, for A and AB-15 samples the best equation to describe the cumulative fluoride release was Eq. (3). While for AB-30 cements the best equation was Eq.(2).

Table 4. Equations describing the fluoride release kinetics

Equation no.	*Equation*	References
1	$[F]_c = a + b\sqrt{t} + ct$	[20,21]
2	$[F]_c = [F]_I(1-e^{-b\sqrt{t}}) + \beta\sqrt{t}$	[22]
3	$[F]_c = [F]_I(1-e^{-bt}) + \beta\sqrt{t}$	[23]
4	$[F]_c = [F]_I /(t_{1/2}+ t) + \beta\sqrt{t}$	[24]
5	$[F]_c = [F]_I /(t_{1/2}+ t) + \alpha t$	[25]
6	$[F]_c = [F]_I t /(t_{1/2}+ t) + \beta\sqrt{t}$	[26]

3.4. Fluoride release rates

The fluoride release profile in terms of release rates is shown in Fig. 2 for all materials studied. The rates were obtained by taking the first derivative of the determined equation (Table 5) from the regression analysis with respect to time, and substituting the regression parameters.

Table 5.Comparison of the equations given in Table 4 for their adequacy of presenting cumulative fluoride release

Materials		Eq. (1)	Eq. (2)	Eq. (3)	Eq. (4)	Eq. (5)	Eq. (6)	Best eq.	Parameters	Value
A	r^2 RMSE	0.9879 7.405	0.9867.95	0.9923 6.133	0.974310.77	0.6091 42.04	0.9887 7.139	3	$[F]_I$ b β	69.39 0.2362 26.39
AB-15	r^2 RMSE	0.9892 7.532	0.9891 7.568	0.9922 6.425	0.98269.592	0.6556 42.62	0.9916.874	3	$[F]_I$ b β	50 0.2598 33.52
AB-30	r^2 RMSE	0.9927 2.484	0.9965 1.652	0.9931 2.415	0.95126.437	0.7821 13.6	0.9955 1.964	2	$[F]_I$ b β	50 0.6611 9.155

***r^2 is correlation coefficient and RMSE is root mean squared error (The smaller the RMSE, the better the fitting).**

As we can see in Fig. 2, all specimens have initially high fluoride release (13 μg/cm^2/day or above), but then it declines rapidly (burst effect). After that, the fluoride release sustain at a lower level for a relatively long time. The A and AB-15 samples have longer time (up to 10 days) of fluoride release above 5 μg/cm^2/day, AB-30 specimens, which have approximately 3 days of fluoride release above 5 μg/cm^2/day.

4. Discussion

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The release of fluoride from ionomer cements is governed by various intrinsic and extrinsic factors. The intrinsic factors are: composition, powder/liquid ratio, mixing time, temperature, specimen geometry, permeability, surface treatment, and finishing. The extrinsic factors include type of storage medium (pH, temperature, ionic strength, viscosity, and the presence of complexing ions), experimental design (volume of storage medium, frequency of medium change, stirring), and analytical methods [27, 28]. In the present study fluoride release data of the experimental cements revealed that there were a high initial concentration and a declining amount over time. Furthermore, our present findings showed that adding bioactive glass increased the fluoride release to some extent. The results showed that the greatest release occurs on the initial days, after which the amount diminished gradually until a relatively constant rate was reached at 8 days for AB-30 and 14 days for A and AB-15 samples. The initial high release rate is due to the surface wash-off effect, a time-dependent step, whilst constant fluoride release occurs as a result of diffusion through pores and cracks. Diffusion through the bulk occurs during the maturation of specimens and presents long-term continuing reaction [29] .The duration of this study was too short to make any inference regarding the occurrence of this process.

The total amount (Table 3) and the rate of fluoride release (Fig. 2) in this study indicated that the AB-15 samples shows high amount of fluoride elution during soaking in SBF. This was due to the presence of alkali metal ions (here sodium) which facilitate an ion exchange mechanism between the cement and environment, by increasing the mobility of fluoride ions within the cement matrix. It is generally held that in glass-ionomer cements, different cations could affect fluoride release from these cements [9,30]. In which increasing the alkali metal content of the constituent glass, not only increased the rate and amount of mono-valent cation release from the cement matrix, but also the rate and amount of fluoride release [30]. As mentioned above, in the present study the fluoride release in SBF well obeyed Eq. (3) with the A and AB-15 cements and did Eq. (2) with AB-30 samples. The first process corresponding to the right-hand first terms of Eqs. (2) and (3) is associated with a short-term initial elution occurring rapidly, but ceasing after some time (Fig. 3). The two parameters characteristics for this process are the total amount of fluoride to be released $[F]_I$ and the so-called half-life time $t_{1/2}$ which gives the time needed to release half of this total amount. Fig.4. illustrates the values for the parameter $[F]_I$ of Eqs.(2) and (3) representing process I for different studied cements.

When $[F]_I$ is considered, marked differences are noted in which (A> AB-15>AB-30). From Fig.4, it is seen that this quantity differ significantly at the 95% confidence level for the sample containing bioactive glass (AB-15 and AB-30) and sample without bioactive glass (A). The comparison suggests that a short-term process due to the surface wash-off and dissolution is significantly related to the amount of acid-base reaction in the cement structure. However, the IR Spectra of the samples (Fig.1 and Table 2) revealed that adding bioactive glass to the cement structure enhanced the amount of acid-base reaction. This fact suggests that the more basic bioactive glasses promoted the acid–base reaction in cured ionomer cements during setting process. According to literature [27-30], the short-term fluoride-release process would be related to the elution of relatively loosely bound fluoride from the cement matrix. As the amount of such fluoride is merely determined by the acid-base reaction during the setting process, one can expect that the presence of bioactive glass would have effect on its elution.

The second process corresponding to the right-hand second term of Eqs.(2) and (3), i.e. ß√t, is associated with a prolonged and more and slowly occurring elution which would be responsible for the long-term release of fluoride. A comparison of the kinetic parameter ß (which is expected to be linearly related to the diffusion coefficients) of process II according to Eqs.(2) and (3) for different samples is made on Fig. 5. As can be seen from this figure, the ß value differ statistically significantly from each other (AB-15>A> AB-30). The course of process II is illustrated in Fig.6 where the cumulative amount of fluoride released by this process is shown for the formulations considered. The findings revealed that by adding bioactive glass to the cement structure the diffusion rate of fluoride increased to some extent. In which, the AB-30 samples shows a small amount of fluoride release in SBF in compare with the AB-15 cements. As mentioned above, this may be based on the fact that a more tightly bound is created in the AB-30 samples. Consequently, fluoride has low diffusion rate through the organic cross linked matrix because of a strong interaction-complexation with aluminum and calcium ions linked to the polyalkenoate chains in cement matrix.

5. Conclusion

This study has shown that:

1. The extent of the acid–base reaction during the setting process of cured ionomer cements was enhanced by increasing the amount of bioactive glass in cement structure.
2. Among all the materials studied, cumulative fluoride release is adequately described by a two-term equation. It consists of an initial fluoride release via a rapid dissolution process (first term) followed by diffusion over an extended period of time (second process).
3. By adding bioactive glass to the cement structure the diffusion rate of fluoride increased to some extent. In which addition of 15 wt% bioactive glass to the studied cement showed the highest fluoride release data in compare with the other cements.

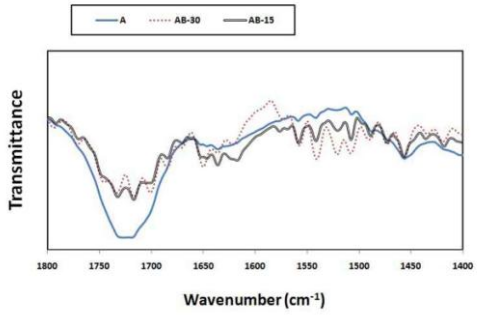

Fig.1. The change in the IR spectra of cements after adding bioactive glass

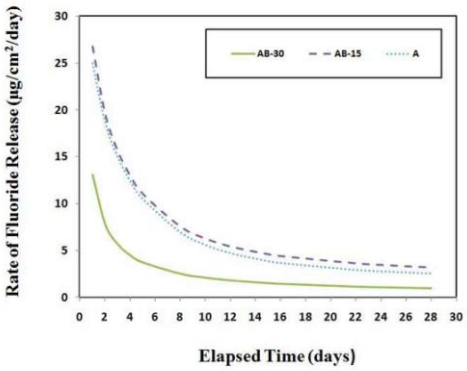

Fig.2. Rate of fluoride release for the experimental cements

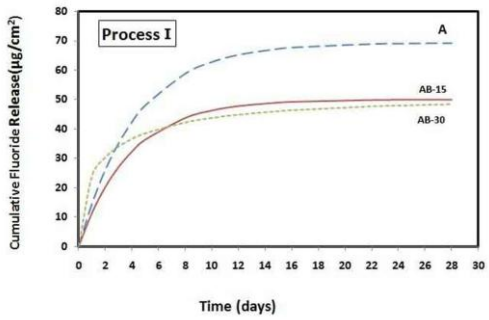

Fig.3. Cumulative amount of fluoride released by process I according to Eqs.(2) and (3) on the basis of a fit of the fluoride release of five individual samples of the materials mentioned.

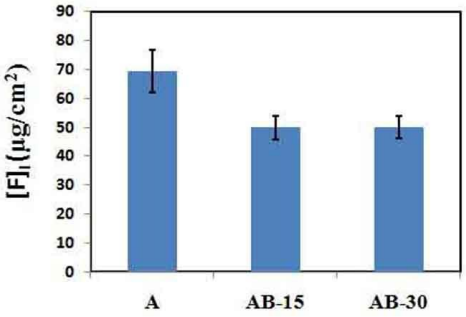

Fig.4. Values for the parameter $[F]_I$ of Eqs.(2) and (3) representing process II for different fluoride releasing materials. The error bars represent the 95% confidence interval.

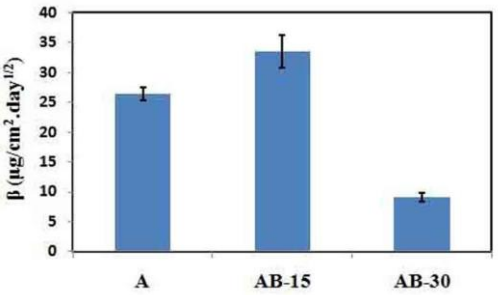

Fig.5. Values for the parameter ß of Eqs.(2) and (3)representing process II for different fluoride releasing materials. The error bars represent the 95% confidence interval.

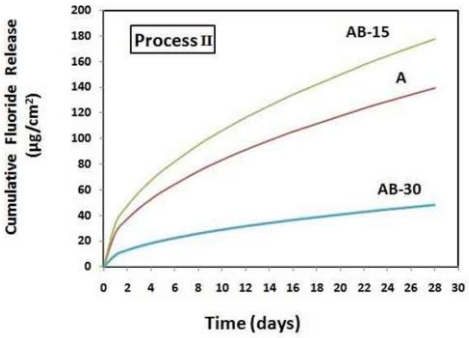

Fig.6. Cumulative amount of fluoride released by process II according to Eqs.(2) and (3) on the basis of a fit of the fluoride release of five individual samples of the materials mentioned.

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