In vitro fluoride release from a different kind of conventional and resin modified glass-ionomer cements

Mediha Selimović-Dragaš[1*], Lajla Hasić-Branković[2], Fehim Korać[3], Nermin Đapo[4], Amina Huseinbegović[1], Sedin Kobašlija[1], Meliha Lekić[5], Šahza Hatibović-Kofman[6]

[1]Department of Preventive and Pediatric Dentistry and [2]Department of Restorative Dentistry, Faculty of Dentistry, University of Sarajevo, Bolnička 4a, 71 000 Sarajevo, Bosnia and Herzegovina. [3]Department of Physical Chemistry, Faculty of Sciences, University of Sarajevo, Zmaja od Bosne 33-35, 71 000 Sarajevo, Bosnia and Herzegovina. [4]Department of Psychology, Faculty of Philosophy, University of Sarajevo, Franje Račkog 1, 71 000 Sarajevo, Bosnia and Herzegovina. [5]Department of Medical chemistry, Faculty of Medicine, Čekaluša 90, 71 000 Sarajevo, University of Sarajevo, Bosnia and Herzegovina. [6]Divisions of Orthodontics & Paediatric Dentistry, Schulich School of Medicine & Dentistry, University of Western Ontario, London, Canada.

Abstract

Fluoride release is important characteristic of glass-ionomer cements. Quantity of fluoride ions released from the glass-ionomer cements has major importance in definition of their biological activity. The objectives of this study were to define the quantity of fluoride ions released from the experimental glass-ionomer cements and to define the effect of fluoride ions released from the experimental glass-ionomer cements on their cytotoxicity. Concentrations of the fluoride ions released in the evaluated glass-ionomer cements were measured indirectly, by the fluoride-selective WTW, F500 electrode potential, combined with reference R503/D electrode. Statistical analyses of F^- ion concentrations released by all glass-ionomers evaluated at two time points, after 8 and after 24 hours, show statistically higher fluoride releases from RMGICs: Vitrebond, Fuji II LC and Fuji Plus, when compared to conventional glass-ionomer cements: Fuji Triage, Fuji IX GP Fast and Ketac Silver, both after 8 and after 24 hours. Correlation coefficient between concentrations of fluoride ion released by evaluated glass-ionomer cements and cytotoxic response of UMR-106 osteoblast cell-line are relatively high, but do not reach levels of biological significance. Correlation between concentrations of fluoride ion released and cytotoxic response of NIH3T3 mouse fibroblast cell line after 8 hours is high, positive and statistically significant for conventional GICs, Fuji Triage and Fuji IX GP Fast, and RMGIC, Fuji II LC. Statistically significant Correlation coefficient between concentrations of fluoride ion released and cytotoxic response of NIH3T3 cell line after 24 hours is defined for RMGIC Fuji II LC only.

KEY WORDS: fluoride release, glass-ionomers, resin modified glass-ionomers, cytotoxicity.

INTRODUCTION

The ability of glass-ionomer cements to release fluoride has been known for a long time [1] and has been a significant factor in their increasing use in dentistry. Both *in vivo* [1,2] and *in vitro* [2-4] studies have shown that the release of fluoride ions can continue over a long period of time. Fluoride ions released by glass-ionomer cements helped in reduction of demineralization of adjacent enamel, enhancement of his remineralization and prevention of secondary caries by inhibition of microbial growth and metabolism [5,6]. Fluorides represent the basic component of glass powder and if it is to be efficiently extracted by the polyacid it has to be in crystalline form as fluorite [5]. Two mechanisms have been proposed by which fluoride may be released from glass-ionomer cements. One mechanism is short term reaction presented by rapid dissolution from outer surface into solution while second is more gradual, presented with the sustained diffusion of ions trough the bulk cement [6]. Quantity of fluoride ions released from the glass-ionomer cements has major importance in definition of their biological activity. It is claimed that the fluoride release of resin modified glass-ionomer cements (RMGICs) is comparable to that of conventional glass-ionomer cements (GICs) [4]. In view of the complex chemistry and physicochemistry of GICs and RMGICs differences in the processes responsible for the fluoride release can be expected [7]. The objectives of this *in vitro* study were to define the quantity of fluoride ions released from the experimental glass-ionomer cements and to define the effect of fluoride ions released from the experimental glass-ionomer cements on their cytotoxicity.

* Corresponding author: Mediha Selimović-Dragaš,
Department of Preventive and Pediatric Dentistry,
Faculty of Dentistry, University of Sarajevo, Bolnička
4a, 71 000 Sarajevo, Bosnia and Herzegovina
Phone: +387 33 214-249 (138); e-mail: mselimovic96@gmail.com

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MATERIALS AND METHODS

Materials

Three conventional glass-ionomer cements: GC Fuji IX GP fast, GC FUJI Triage (GC Corporation) and Ketac Silver (3M/ESPE) and three resin modified glass-ionomer cements: GC Fuji II LC, GC Fuji plus (GC Corporation) and Vitrebond (3M/ESPE) were used as an experimental materials in this study.

Procedures

The concentration of fluoride ions of eluates of each experimental GICs and RMGICs was assayed by means of an electrode potential of ion specific electrode (WTW, F 500) in combination with referent electrode R503 / D, for all ion selective electrodes series 500. The electrode was calibrated with three standard solutions of 0.00001 g/L; 0.001 g/L and 0.1 g/L of fluoride. Materials were prepared at room temperature according to manufacturer's instructions, packed into open siliconized rings (internal diameter 4mm. and height 2 mm) between two celluloid sheets. Resin modified glass-ionomer cements: GC Fuji II LC, GC Fuji Plus (GC Corporation) and Vitrebond (3MESPE) were polymerized for 40 sec. on each surface with light activation lamp Elipar ™ FreeLight L (3MESPE) [8]. Chemically cured, conventional glass-ionomer cements: GC Fuji IX GP Fast, GC FUJI Triage (GC Corporation) and Ketac Silver (3M/ESPE), were allowed to set under transparent matrix strips for 7 minutes. The whole sample consisted of 108 discs, 18 discs for each experimental material. Discs were divided in three groups and each group was divided in two subgroups, consisted of three specimens of each experimental material. After 24 hours the test specimens were immersed in a polypropylene container PP, 25x90mm/30ml (Semikem: Cat. No.15.0597) completely covered with 5 ml distilled, deionized water. The containers were hermetically closed. First measurement of fluoride concentration in eluates of three samples of each tested material was conducted after 8 hours. The other three specimens of each experimental glass-ionomer cement were eluted for 24 hours at room temperature until the moment of second measurement of fluoride concentration. After 8 hours for the first measurements, and after 24 hours for the second measurements, 5ml of TISAB solution (total ionic strength adjustment buffer solution- Modell: TISAB; Best.Nr.:140 100; WTW D-82362 Weilheim) was added to each container. The dishes were hermetically closed and agitated at the speed of 60 Hz for two minutes. Elutes, prepared by this way, were set for 30 minutes in order to achieve stabile solution before measurements [9]. A fluoride ion selective electrode WTW, F500, combined with reference R503/D electrode were used to quantify the amount of fluoride ion released from each specimen into the buffer solution. The fluoride ion concentrations of eluates of each experimental GICs were measured in triplicate. A total amount of fluoride released (expressed in micrograms of fluoride released per gram of solution) into the buffer solution, after 8 hours and 24 hours was calculated from the calibration curve [10]. Each data point was the average of three samples. Concentration of free F^- ions was determined by potentiometric methods based on mathematic formula:

$$E = E^o + \frac{RT}{nF} \ln c_{F^-}$$

Based on this formula and data obtained during the experiment, calibration curve for Fluoride selective electrode was constructed. For quantitative determination of F^- ions in the eluates (μg/g) standard calibration curve were obtained by plotting the peak heights of known concentration of the Fluor solutions and calibration curve for Fluoride selective electrode constructed previously. Calibration diagram constructed that way (Figure 1.) gave us a mathematic formula for calculation of concentration of Fluoride ions express in μg/g which is:

$$x = \frac{112{,}77 - y}{42{,}325}$$

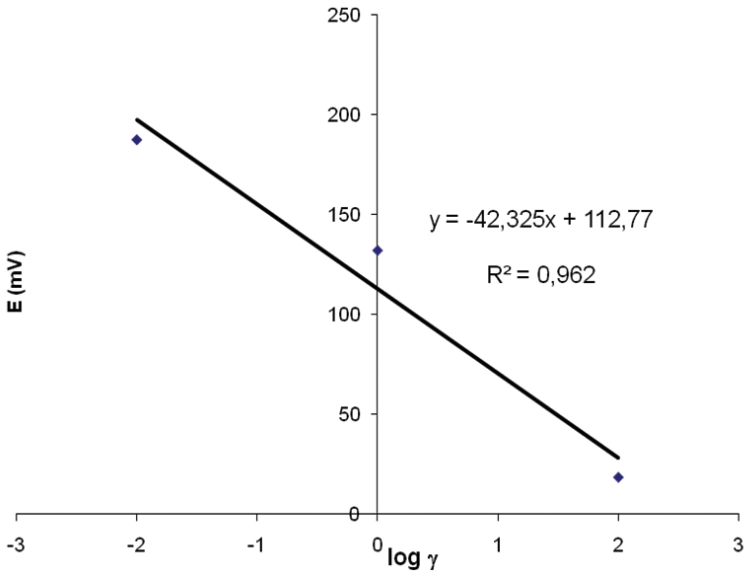

FIGURE 1. Calibration diagram for the calculation of F^- ions (γ, μg/g)

TABLE 1. Descriptive statistic values of concentration of F^- ions (μg/g) in the 8 and 24 hours eluates

Material	N	Min	Max	Mean	SD	Skewness		Kurtosis	
						Statisc	Std. Error	Statistic	Std. Error
Fuji Triage_8h	9	0.098	0.682	0.333	0.200	0.205	0.717	-0.618	1.400
Fuji Triage_24h	9	0.144	0.812	0.392	0.201	0.988	0.717	1.478	1.400
Fuju LCII_8h	9	0.133	1.261	0.624	0.332	0.582	0.717	0.647	1.400
Fuju LCII_24h	9	0.312	2.092	1.265	0.568	-0.484	0.717	-0.627	1.400
Ketac Silver_8h	9	0.022	0.303	0.150	0.106	0.114	0.717	-1.348	1.400
Ketac Silver _24h	9	0.050	0.369	0.229	0.133	-0.676	0.717	-1.680	1.400
Fuji IX fast_8h	9	0.023	12.875	4.518	4.654	0.734	0.717	-0.751	1.400
Fuji IX fast_24h	9	0.027	8.894	3.425	3.726	0.620	0.717	-1.671	1.400
Fuji Plus_8h	9	0.243	1.318	0.855	0.385	-0.321	0.717	-1.528	1.400
Fuji Plus_24h	9	0.920	3.112	1.731	0.738	0.706	0.717	-0.254	1.400
Vitrebond_8h	9	0.625	23.808	9.962	9.146	0.514	0.717	-1.423	1.400
Vitrebond_24h	9	1.897	33.359	12.114	10.590	1.131	0.717	0.684	1.400

Values of concentration of F^- ions (μg/g) released from experimental conventional and resin modified glass-ionomer cements were much higher in the 24 hours eluates for all experimental materials.

Statistical analysis

The collected data were analyzed by SPSS 15.0 statistics software program for Windows. Wilcoxon test was used to determine significant differences between the concentrations of fluoride ions of each experimental material released after 8 hours and 24 hours of elution time. Statistical differences in concentration of fluoride ions released by experimental conventional GICs and RMGICs for both elution times (8 hours and 24 hours) were evaluated by Kruskal Wallis test and Mann-Whitney test. Cytotoxicity and F^- release were evaluated for significant differences by Spearman's rank correlation coefficient.

RESULTS

Fluoride release

Table 1. shows descriptive statistical values of released fluoride ions from the respective GICs and RMGICs . Distributions of results do not deviate significantly from symmetry. To assess the equality of variances in six groups of experimental materials Levene's test was used and statistical difference between the variances in the experimental materials was found (F_1 (5, 48) =20.86, p=0.0001; F_2 (5. 48) =13. 39, p=0.0001). Considering a statistical difference between the homogeneity of variances, for the evaluation of statistical difference in the amount of released F^- ions between two time points (8 and 24 hours) and for the six different GICs non-parametric statistical tests were used. The Wilcoxon signed-rank test was used to test statistical difference in the amount of released F^- ions between two time points (8 and 24 hours) and statistical difference is significant Z= -2.897, p=0.004 so we can conclude that the amount of released F^- ions for all experimental GICs was greater after 24 hours (Figure 2.). RMGIC Vitrebond released significantly more F^- ions at each time interval than all other experimental GICs and RMGICs (Figure 3).

Statistical test of differences of the amount of released F^- ions between two time points (8 and 24 hours) showed that RMGICs Vitrebond, Fuji II LC and Fuji plus released significantly more fluoride ions at both time points (8 and 24 hours) comparing with conventional GICs Fuji Triage, Fuji IX GP fast and Ketac Silver. Kruskal Wallis test showed statistically significant differences for 8 hours and for 24 hours, χ^2_{8h} (5)=28.542, p=0,0001; χ^2_{24h} (5)=32.193, p=0.0001. RMGIC Vitrebond (3M/ESPE) released the greatest amount of F^- ions which is significantly different comparing to all other GICs and RMGICs. Ketac Silver (3M/ESPE) released the smallest amount of F^- ions which is

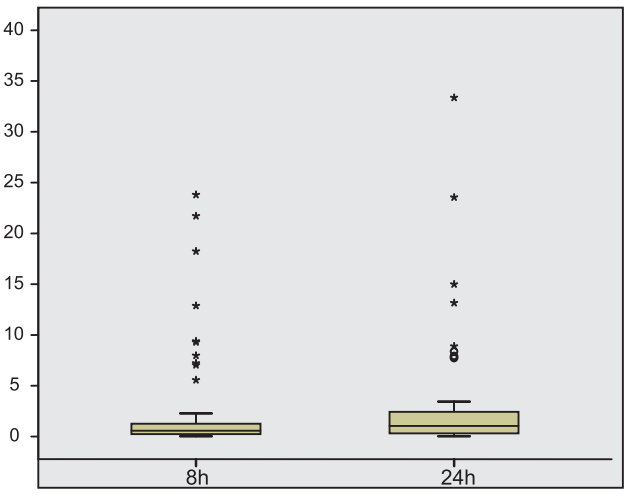

FIGURE 2. Box-plot distribution of the amount of released F^- ions between two time points (8 and 24 hours). Median of the amount of released F^- ions after 8 hours is C=0.568, which is lower comparing with the median of the amount of released F^- ions after 24 hours (C= 1.0485).

TABLE 2. Pairs of statistical differences of the amount of released F^- ions for all experimental materials after 8 hours and after 24 hours

Time	Material	Statistical difference
8h	Fuji Triage (A)	B, E, F
	Fuji II LC (B)	C, F
	Ketac Silver (C)	D, E, F
	Fuji IX GP Fast (D)	C
	Fuji Plus (E)	F
	Vitrebond (F)	A, B, C, E
24h	Fuji Triage (A)	B, E, F
	Fuji II LC (B)	C, F
	Ketac Silver (C)	E, F
	Fuji IX GP Fast (D)	F
	Fuji Plus (E)	F
	Vitrebond (F)	A, B, C, D, E

RMGIC Vitrebond (3M/ESPE) released the greatest amount of F^- ions which is significantly different comparing to all other GICs and RMGICs. Conventional GIC Ketac Silver (3M/ESPE) released the smallest amount of F^- ions.

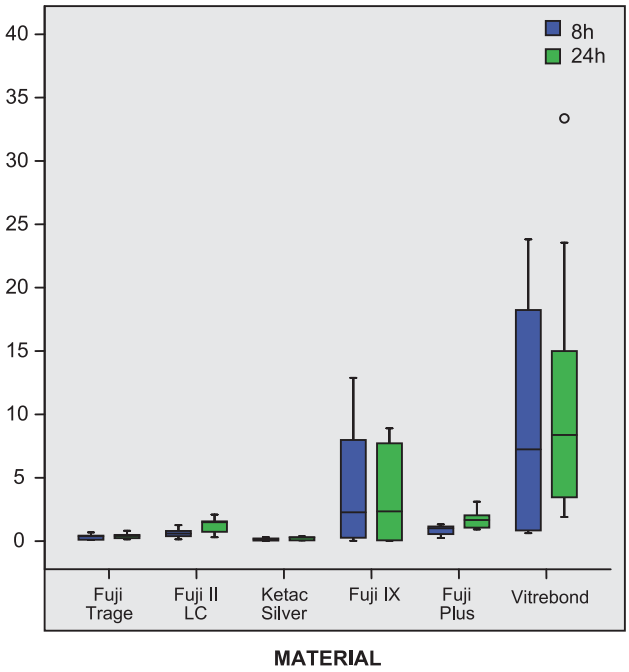

FIGURE 3. Box-plot distribution of the amount of released F^- ions after 8 hours and after 24 hours. Median of the amount of released F^- ions from RMGIC Vitrebond is significantly higher comparing with all other GICs for the both time points (8 and 24 hours).

significantly different comparing to RMGICs Fuji plus and Vitrebond for both time points (8 and 24 hours) and GIC Fuji IX GP Fast after 8 hours, only (Table 2.).

Correlation between cytotoxicity and fluoride release

With the aim to investigate relationship between F^- released after 8 hours and cytotoxic reaction of UMR106 – osteoblast-like cells for all experimental GICs, Spearman's rank correlation coefficient were obtained. Spearman's correlation coefficient indicated a moderate, negative and significant correlation between F^- released after 8 hours and cytotoxic reaction of UMR-106 – osteoblast-like cells for all experimental GICs (ρ=-0.518). Similar correlation was find between F^- released after 24 hours and cytotoxic reaction of UMR-106 – osteoblast-like cells for all experimental GICs (ρ= -0.611). Spearman's rank coefficient between F^- released after 8 hours and cytotoxic reaction of NIH3T3 mouse fibroblast cells indicated a low, negative and statistically insignificant correlation (ρ = -0,127) while correlation between F^- released after 24 hours and cytotoxic reaction of NIH3T3 mouse

TABLE 3. Matrices of correlation of F^- release from experimental GICs and cytotoxic response of UMR-106 – osteoblast-like cells and NIH3T3 mouse fibroblast cells.

Material	F^- release 24 hrs	Cytotoxicity UMR-106 8hrs	Cytotoxicity UMR-106 24hrs	Cytotoxicity NIH3T3 8hrs	Cytotoxicity NIH3T3 24hrs
Fuji Triage F^-8hrs	0.750*	-0.050		0.717*	
Fuji Triage F^-24hrs			-0.167		0.400
Fuji II LC F^- 8hrs	0.800(**)	-0.267		0.817(**)	
Fuji II LC F^-24hrs			-0.383		0.750(*)
Ketac Silver F^-8hrs	0.653	0.433		0.200	
Ketac Silver F^-24hrs			-0.209		0.536
Fuji IX GP Fast F^-8hrs	0.767(*)	-0.067		0.800(**)	
Fuji IX GP Fast F^-24hrs			-0.100		0.367
Fuji Plus F^-8hrs	-0.385	-0.583		-0.217	
Fuji Plus F^-24hrs			0.285		-0.561
Vitrebond F^-8hrs	0.533	-0.083		-0.233	
Vitrebond F^-24hrs			0.167		0.250

** ρ statistically significant at 0.01; * ρ statistically significant at 0.05

No material, neither conventional nor resin modified glass-ionomer cement showed statistically significant correlation coefficient between F^- released in the both time points (8 and 24 hours) and cytotoxic reaction of UMR-106 – osteoblast-like cells. Correlation coefficient demonstrated a high, positive and significant correlation between F^- released after 8 hours and cytotoxic reaction of NIH3T3 mouse fibroblast cells for conventional GIC Fuji Triage (ρ = 0.717), and Fuji IX GP Fast (ρ = 0.800) as well as RMGIC Fuji II LC (ρ = 0.817). Spearman's rank correlation coefficient between F^- released after 24 hours and cytotoxic reaction of NIH3T3 mouse fibroblast cells was statistically significant for RMGIC Fuji II LC only (ρ = 0.750).

fibroblast cells was negative and significant (ρ = -0.342). Table 3. shows that, although some values of correlation coefficient are relatively high, no one reach the level of statistical significance of 0.05 probably because of insufficient numbers of specimens in the sample (n=9). No material, neither conventional nor resin modified glass-ionomer cement showed statistically significant correlation coefficient between F^- released after 8 hours and cytotoxic reaction of UMR-106 – osteoblast-like cells although correlation coefficient of conventional GIC Ketac Silver (ρ = 0.433), and RMGIC Fuji Plus (ρ = -0.583) are relatively high. Correlation between F^- released after 24 hours and cytotoxic reaction of UMR-106 – osteoblast-like cells for each experimental GICs was low and not statistically significant. The values of correlation coefficient between F^- released after 8 hours and cytotoxic reaction of NIH3T3 mouse fibroblast cells were much higher. Spearman's rank correlation coefficient demonstrated a high, positive and significant correlation between F^- released after 8 hours and cytotoxic reaction of NIH3T3 mouse fibroblast cells for conventional GIC Fuji Triage (ρ = 0.717), and Fuji IX GP Fast (ρ = 0.800) as well as RMGIC Fuji II LC (ρ = 0.817). Spearman's rank correlation coefficient between F^- released after 24 hours and cytotoxic reaction of NIH3T3 mouse fibroblast cells was statistically significant for RMGIC Fuji II LC only (ρ = 0.750).

DISCUSSION

Fluoride release by GICs and RMGICs is an important property of those materials and plays a major role in its selection for specific clinical application [5]. It seems that fluoride ions released from GICs act in a dose-dependant manner. *In vitro*, at relatively high concentration, fluoride acts as an enzyme inhibitor. *In vivo*, eliminates microorganisms, those which remains in the cavity after preparation, and reinforces demineralized enamel and dentin [11].The pattern of released fluoride is that the greatest amount of fluoride being released during the first days, than decreasing to the nearly constant level [3]. The present *in vitro* study evaluated the quantity of fluoride ions released from the three conventional glass-ionomer cements: GC Fuji IX GP fast, GC FUJI Triage (GC Corporation) and Ketac Silver (3M/ESPE) and three resin modified glass-ionomer cements: GC Fuji II LC, GC Fuji plus (GC Corporation) and Vitrebond (3M/ESPE) and defined the effect of fluoride ions released from the experimental glass-ionomer cements on their cytotoxicity. Results obtained in this study showed that the rate of F^- released from RMGIC comparing with conventional GIC at both time points (8 and 24 hours) were significantly higher. Kan et al. [9] concluded that the greatest amount of F^- release occurs in the first 24 hours, which was confirmed in present study, were was the difference in fluoride release for all experimental materials significantly greater after 24 hours (p= 0.004). In the present study, the greatest amount of F^- released was showed by RMGIC Vitrebond (3M/ESPE) and those results were significantly higher comparing with all experimental materials for both time points (8 and 24 hours). Results of this *in vitro* study coincide with the results of Mitra S.B., who has demonstrated that Vitrebond was capable of long-term fluoride release without any degradation of physical properties over the time [10]. In the present *in vitro* study, the amount of F^- in RMGIC Fuji II LC (GC Corporation) 24 hours. eluate was 1,265 ppm and demonstrated statistically significant correlation with the cytotoxic response of NIH3T3 mouse fibroblast cells after 8 hours. (ρ= 0.817) and 24 hours immersion (ρ = 0.750). Results obtained in this study demonstrated that there was no statistically significant relation with the cytotoxic response of UMR-106 - osteoblast like cells and the amount of F^- in RMGIC Fuji II LC (GC Corporation) eluate after 8^{th} and 24^{th} hours immersion. Fuji II LC in the present study demonstrated moderate cytotoxicity which coincide with the results of Kan et al. [9], who pointed out that Fuji II LC behaved more like a resin composite. The fact that brand formulation and material type may influence the amount of F^- ion released [12], was confirmed in present study where RMGIC Vitrebond (3M/ESPE) released significantly greater amount of F^- ions comparing with other experimental RMGICs. Capability of glass-ionomer cements to act as a fluoride ion reservoir presents an important advantage in the process of prevention of secondary caries around restorative margins as well as surrounding surfaces [3]. Salar et al. [13] showed that GC Fuji Triage, as a glass-ionomer sealant, have a relatively high fluoride content and released enough fluoride to local environment for a longer period of time which increased resistance to caries in the adjacent enamel. Results obtained in the study Marković et al. [14] showed that the amount of fluoride released by GC Fuji Triage in saline medium is much higher compared to the Fuji IX GP and Fuji II LC. Results obtained in present study showed that the GC Fuji Triage after 24 hrours liberated 0.392 ppm of F^- which was less comparing with Fuji IX GP and Fuji II LC. Those discrepancies in the results obtained in those two studies could be explained with the fact that present study measured the amount of fluoride released by GICs and RMGICs specimens 24 hours after the setting period, in order to avoid rapid dissolution from outer surface of the freshly mixed specimens into solution. Results of this *in vitro* study coincide with the results of Forsten [4] who pointed out that the pattern of fluoride release from RMGICs was similar to that of conventional GIC with the greatest release of fluoride ions at the beginning, followed

by continuing release of F^- ions over some time period. The results obtained in present study indicated that Ketac-Silver® (3M/ESPE) released 0.2841 ppm F^- after 24 hours immersion, which was the least amount of F^- released in this study. Those results corresponded with the results of Forsten [4] who indicated that the amount fluoride released from the Ketac-Silver® is smaller than that of other conventional GICs. Those findings have been confirmed in clinical experience because secondary caries is not as rare in connection with the Ketac-Silver® as with other conventional GICs [4]. Conventional GIC Fuji IX GP Fast (GC Corporation) with the average of 2.3454 ppm of F^- ions released after 24 hours showed minor cytotoxic effect which suggested that cytotoxicity cannot be explained by F^- release alone. With the exception of Fuji II LC (GC Corporation), the present *in vitro* study demonstrated that the concentration of F^- released by experimental RMGICs and GICs after the both 8 and 24 hours immersion had no significant effect on cytotoxic response of UMR-106 – osteoblast-like cells and NIH3T3 mouse fibroblast cells. It is more likely that cytotoxic response of cell lines used in present study occurred due to unidentified toxic components which were leached out during the immersion time. However, additional, more complex experimental studies comprising large number of factors could provide more valid conclusion. Having in mind that liberation of soluble components of experimental GICs can occur during the polymerization process or later on, further investigation should be based upon identification of severely cytotoxic leachable substances and their quantification.

CONCLUSION

Based on the results obtained in the present *in vitro* study it was concluded that:

Statistical analyses of F^- ion concentrations released by all glass-ionomers evaluated at two time points, after 8 and after 24 hours, show statistically higher fluoride releases from RMGICs: Vitrebond, Fuji II LC and Fuji Plus, when compared to conventional glass-ionomer cements: Fuji Triage, Fuji IX GP Fast and Ketac Silver, both after 8 and after 24 hours. Correlation coefficient between concentrations of fluoride ion released by evaluated glass-ionomer cements and cytotoxic response of UMR-106 osteoblast cell-line and NIH3T3 mouse fibroblast cell line are relatively high, but do not reach levels of biological significance. According to the methodology employed in the present study, it can be concluded that experimental GICs liberated F^- as well as other soluble components, which diffused into the culture medium. These components cannot be dismissed as possible cytotoxic factors which contribute to the cytotoxicity observed in this study.

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DECLARATION OF INTEREST

The authors declare no conflict of interest.

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