Microanalysis For Remineralization Potential of Surface Pre-Reacted Glass (S-PRG) Restorative Material Versus Resin Modified Glass Ionomer: (In Vitro Study)

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Abstract

Aim: This study aimed to assess the microanalysis for remineralization potential of surface pre-reacted glass (S-PRG) restorative material versus Resin-modified glass ionomer.

Subjects and methods: twenty-seven caries-free, freshly extracted, human permanent premolar teeth were used. Non-beveled trapezoidal class V cavities were performed in the gingival one-third of the buccal surface. The teeth were separated into three equal groups (nine=9). Group (1) was used as a negative control without any restorations. Group (2) was restored with S-PRG filler (Giomer; Beautifil II), and Group (3) was restored with RMGI (Fuji II LC). All restored teeth were cut buccolingually into two sections in a vertical plane parallel to the tooth's long axis. Remineralization of the tooth structure (calcium, phosphorus, and fluoride) was assessed by SEM/ (EDX) after one day, 1 month, 3 months, and 6 months, respectively.

In comparison between different groups, the results revealed that: The Demineralized dentine model was significantly the lowest, the Fuji II LC group was significantly the highest, and the Giomer group revealed an insignificant difference between both of them after 1 day. Meanwhile, Fuji II LC was significantly higher than Giomer at all other time intervals.

Conclusions: Glass ionomers are still the gold standard for remineralization to tooth tissues. The minerals uptakes of tooth tissues are time-dependent properties.

Keywords: Remineralization potential, S-PRG filler, RMGI, Energy Dispersive X-ray.

Dental caries is the most common oral condition, which affects people of all ages**(1).** It leads to discomfort, impairs functionality, and reduces one's quality of life. Dental caries can cause damage to the surface of teeth. It undergoes stages of demineralization and remineralization prior to infiltrating deeper levels of tooth lesions**(2).**

Fluoride stimulates the synthesis of fluorapatite in the presence of calcium and phosphate ions which are formed during enamel demineralization. This plays a vital role in the remineralization and correction of developing caries. The enamel's surface has small pores that allow the fluoride to be deposited **(3).**

Glass ionomer cements have anti-cariogenic properties that can be related to fluoride release. Current glass ionomer cements achieve a low ion release, which is insufficient to inhibit the tooth structure's demineralization. Novel GICs or modified existing ones have been used to achieve longer-lasting anti-cariogenic effects **(4).** Various fluoride-containing restorative products exist, such as Resin-modified glass ionomers (RMGI) and Giomers. These products were developed to solve the challenge associated with traditional glass ionomer cement and resin composite and preserve their clinical usage**(5).** RMGIC is still the exclusive material clinically prescribed to repair decaying non-biting areas in individuals who are at high risk to have caries due to its increased fluoride release capabilities **(6).** Giomer is a modified resin composite that includes pre-reacted glass providing it the potential to induce tooth remineralization **(4).** For the past several years, attention has been focused on the idea of using surface pre-reacted glass ionomer (S-PRG) fillers with multi-ions release capabilities in restorative materials. Additionally, the acid that causes tooth demineralization is believed to be neutralized by the ion exchange provided by Giomer fillings**(7).**

Materials and methods:

Ethical approval:

The protocol of this study was approved by the Council of Conservative Dentistry Department – Faculty of Dentistry – October 6 University and the ethical issues were reviewed and revised by the Research Ethics Committee – Faculty of Dentistry – October 6 University on January 2022(Approval No.RECO6U/ 12-2022).

Sample size calculation:

A power analysis was formed to have adequate power to apply a statistical test by adopting an alpha level of (0.05) a beta of (0.2) i.e. power=80% and an effect size (f) of (0.637) calculated based on the results of a study by ***Faul et al. (2007)***, **(8).** The predicted readings for remineralization potential from a total of (27) extracted teeth (9 samples per group) were nine readings for the demineralizing samples, and 36 readings for each restorative material to reach 72 readings at four intervals for both materials. Sample size calculation was done by using G*Power version 3.1.9.7.

Preparation of the specimens:

Twenty-seven healthy, caries-free, human permanent lower premolars were collected after recent extraction. Each tooth was placed separately in a 2.5 mm cylindrical acrylic block for stabilization. Standardized non-beveled class V cavities with trapezoidal shaped and dimensions of 3mm & 2mm mesiodistal width, 2mm in the occulso- gingival height, and 1.5mm depth were prepared using diamond bur installed at high speed with contra angled coolant hand-piece in the gingival one-third of the buccal surfaces. To assure uniform size, the cavity's dimensions were measured using William's graded probe**(9).** Then the teeth were randomly separated into 3 equal groups (n=9) regarding for restorative material. The composition and manufacturers of these materials were listed in **Table (1)**. ***According to El-Bahrawy et al. (2020),*** The cavities of the first group (control);(G1) had a 15-second etching procedure using 37% orthophosphoric acid, followed by washing using an air-water spray and a gentle 10-second air-drying procedure. Finally, no restoration was placed into the cavities**(10).** The cavities of the second group (G2); were etched in the same manner as the control group. Then 2-3 coats of BeautiBond universal adhesive system, were placed to etched enamel and dentin using a micro brush for 15 seconds while being gently agitated and air-thinned for 5 seconds. After that, the Giomer (Beautifil II) restorative material was packed in oblique increments into the cavities by a composite applicator. Then cured each increment for 20 seconds using a woodpecker light cure unit (*1000 mW/cm2 1200 mW/cm2*) at zero distance. The cavities of the third group (G3); were etched in the same manner as the control group. The RMGIC (Fuji II LC) capsule was triturated into an amalgamator and then blended for 10 seconds. Then the RMGI capsule was forcibly applied to the cavities using the special gun to ensure the absence of air bubbles. The polymerization took place for 6 minutes. All restored teeth were cut buccolingually into two sections in a vertical plane parallel to the tooth's long axis. According to ***Mosallam et al. (2021),*** pH cycling was performed on the samples by being kept in artificial saliva with a pH value of 7.2 for 23 hours, which most closely approximates the normal levels of saliva in the oral cavity **(11).** The specimens (after being cleaned with 1 ml of distilled water) were put back into a fresh vial with 1 ml of orange juice for just one hour. Daily replenishment of the beverages was done by using standardized milliliters. The remineralization potential of tooth structure (calcium, phosphorus, and fluoride) was assessed after one day, 1 month, 3 months, and 6 months, respectively by using Energy Dispersive X-ray.

Results:

III.Remineralization of hard tooth tissue:

a.Effect of time on the remineralization of dental tooth tissue :

The mean and standard deviation of remineralization of hard tooth tissue at different intervals of both groups were found in Table (2) and Figure (1). Comparison between different intervals to evaluate the effect of time was performed by using One Way ANOVA test followed by Tukey`s Post Hoc test.

Micro analysis for ions uptake from S-PRG (Giomer) group:

The mean value and standard deviation of fluoride ions were (2.43±0.52) after one day, followed by (7.46±0.72) after 1 month, then (5.69±0.77) after 3 months, and (4.86±0.52) after 6 months, respectively. The results revealed that, after 1 month it was significantly the highest and after 6 months it was significantly the lowest. There was an insignificant difference between the Demineralized dentine model (1.92±0.56) and Giomer after 1 day.

The mean value and standard deviation of phosphorus ions were (9.04± 0.61) after one day, followed by (16.89±0.81) after 1 month, then (14.86±0.85) after 3 months, and (12.77± 0.74) after 6 months, respectively. The results revealed that, after 1 month it was significantly the highest and after 6 months it was significantly the lowest. There was a significant difference between the Demineralized dentine model (7.95±0.59) and Giomer after 1 day.

The mean value and standard deviation of calcium ions were (19.03±0.50) after one day, followed by (31.47±0.83) after 1 month, then (27.44±0.94) after 3 months, and (25.48± 0.75) after 6 months, respectively. The results revealed that, after 1 month it was significantly the highest and after 6 months it was significantly the lowest. There was a significant difference between the Demineralized dentine model (17.79±0.64) and Giomer after 1 day.

Micro analysis for ions uptake from RMGI (Fuji II LC) group:

The mean value and standard deviation of fluoride ions were (3.01±0.40) after one day, followed by (9.07±0.78) after 1 month, then (7.77±0.71) after 3 months, and (6.43± 0.65) after 6 months, respectively. The results revealed that, after 1 month it was significantly the highest and after 6 months it was significantly the lowest. There was a significant difference between the Demineralized dentine model (1.92±0.56) and after 1 day of Fuji II LC.

The mean value and standard deviation of phosphorus ions were (9.96±0.59) after one day, followed by (17.67±0.76) after 1 month, then (15.54±0.77) after 3 months, and (13.64± 0.85) after 6 months, respectively. The results revealed that, after 1 month it was significantly the highest and after 6 months it was significantly the lowest, with P value= 0.52. There was an insignificant difference between the Demineralized dentine model (7.95±0.59) and Fuji IILC after 1 day.

The mean value and standard deviation of calcium ions were (20.05±0.54) after one day, followed by (35.69±0.78) after 1 month, then (28.61±0.60) after 3 months, and (26.60± 0.61) after 6 months, respectively. The results revealed that, after 1 month it was significantly the highest and after 6 months it was significantly the lowest, with P value = 0.91. There was an insignificant difference between the Demineralized dentine model (17.79±0.64) and Fuji II LC after 1 day.

b.Effect of material on the remineralization of dental tooth tissue:

The mean and standard deviation of remineralization of hard tissue at all intervals regarding the Demineralized dentine model, S-PRG (Giomer), and RMGI (Fuji II LC) groups were found in Table (3) and Figure (2). Comparison between different groups was performed by using One Way ANOVA test which revealed that:

The mean value and standard deviation of fluoride ions:

- After one day (T1): Demineralized dentine model (1.92±0.56) was significantly the lowest, Fuji II LC (3.01±0.40) was significantly the highest while Giomer (2.43±0.52) revealed insignificant difference with both of them, p value= 0.0005.
- After one month (T2): Fuji II LC (9.07±0.78) was significantly higher than Giomer (7.46±0.72), as P value=0.0003.
- After 3 months (T3): Fuji II LC (7.77±0.71) was significantly higher than Giomer (5.69±0.77), as P value<0.0001.
- After 6 months (T4): Fuji II LC (6.43 ±0.65) was significantly higher than Giomer (4.86±0.52), as P value<0.0001.

The mean value and standard of phosphorus ions:

- After 1 day (T1): Demineralized dentine model was significantly the lowest (7.95±0.59), Fuji II LC was significantly the highest (9.96±0.59), while Giomer revealed a significant difference with both of them (9.04±0.61), p value< 0.0001.

- After one month (T2): Fuji II LC (17.67±0.76) was significantly higher than Giomer (16.89±0.81), as P value=0.05.
- After 3 months (T3): Fuji II LC (15.54±0.77) was significantly higher than Giomer (14.86±0.85), as P value= 0.09.
- After 6 months (T4): Fuji II LC (13.64±0.85) was significantly higher than Giomer (12.77±0.74), as P value=0.03.

The mean value and standard of calcium ions:

- After 1 day (T1): Demineralized dentine model was significantly the lowest (17.79±0.64), Fuji II LC was significantly the highest (20.05±0.54), while Giomer revealed a significant difference with both of them (19.03±0.50), p value<0.0001.
- After one month (T2): Fuji II LC (35.69±0.78) was significantly higher than Giomer (31.47±0.83), as P value<0.0001.
- After 3 months (T3): Fuji II LC (28.61±0.60) was significantly higher than Giomer (27.44±0.94), as P value=0.006.
- After 6 months (T4): Fuji II LC (26.60 ±0.61) was significantly higher than Giomer (25.48±0.75), as P value=0.003.

Discussion:

Dental caries is a widespread condition that affects teeth, causes discomfort and anguish, limits function, and demineralizes tooth tissues **(12).** Dentine is mostly made up of inorganic minerals that consist of hydroxyapatite and non-crystalline amorphous calcium phosphate. When these minerals are exposed to bacterial acids, they break down, which causes dentin demineralization **(13)**. ***Dhananjaya et al. (2021),*** described how the demineralizing process begins as an insignificant lesion and then proceeds to the oral tissues **(14).** Remineralization is a natural healing process that places minerals back into the crystal lattice of hydroxyapatite (HAP). It happens under neutral pH conditions when mineral ions are re-deposited from saliva within the carious lesion, which leads to the creation of newer ones, which are bigger and harder to acidic disintegration **(12).**

GICs have undergone a number of compositional changes, since their inception in an effort to reduce any unfavorable characteristics that might restrict their therapeutic use. Added to that, an easy-handling material supplied in capsules simplifies handling and enhances the qualities of the materials **(15).** As a result of adding resin monomers to an aqueous solution of polyacrylic acid, a more durable RMGI was developed **(16).**

Giomer is a fluoride-releasing material that contains pre-reacted glass fillers. PRG fillers are formed by the acid-base reaction between fluoroalumino -silicate glass (FASG) and polyalkenoic acid (PAA) when combined with water forming a wet siliceous hydrogel **(17).** The surface reaction of PRG consists of a three-layered structure with a multifunctional fluoro-boro-aluminosilicate glass core, pre-reacted glass-ionomer phase, and an outer SiO_2 coating layer **(18).** Two different commercially available ion-releasing restorative materials, S-PRG filler (Beautifil II) and RMGI (Fuji II LC) were selected in this study due to their ion-releasing properties as well as their suggested capability of remineralization. Only sound lower premolars with normal anatomical structure were carefully examined under (X 2.5) magnification to exclude any defect at the surface, thus providing standardization and avoiding any confounding factors. Remineralization potential was assessed using Energy Dispersive X-ray (EDX), which allowed an accurate estimation of the present minerals along the tooth/restoration interface to be detected **(19).** EDX is a reliable as well as accurate method for determining the major elemental composition present in the material **(20).** The findings of this study revealed that, the mean values for fluoride, phosphorus, and calcium ions of RMGI (Fuji II LC) were significantly higher than those of S-PRG (Giomer; Beautifil II) in terms of the remineralization potential of tooth tissues. This may be attributed to that, when the acid-base reaction of RMGI begins, the setting of the material is initiated resulting in releasing ions. The released calcium, aluminium, fluoride ions, and other ions like phosphorus can exchange with the moist oral environment **(21).** Glass ionomer is regarded as a bioactive material that releases ions (fluoride, calcium, and phosphorus) into nearby tissues. Additionally, it promotes remineralization based on creates fluoro-apatite crystals, which are harder than hydroxyl-apatite **(22).** The amount of fluoride, given to remineralization of the tooth structure, can increase due to the increased fluoride released by glass ionomers under acidic conditions **(23).** The primary mechanism for GIC's bonding is an ionic chemical link that develops between the liquid cement's component and the calcium found in the tooth structure's hydroxyapatite. Fluoride ions make teeth more resistant to caries by converting hydroxyapatite crystals into fluorapatite, resulting in hard tissues **(10).**

Resin-modified glass ionomers keep their surface pH very low. Low surface pH may promote the leaching of ions, leading to high bursts of ion release from RMGI. On the other hand, the ions released from Giomers are demonstrated to be extremely minimal when compared to RMGICs due to the presence of pre-reacted glass fillers. Also, Giomers must be attached to the tooth structure with an adhesive that can prevent ions diffusion to stimulate remineralization **(4).** Moreover, the Giomers' monomers have a low potential for absorbing water and releasing ions. So that, the extensive acid-base reaction that take place in RMGI is the major cause for the high amount of ions release **(24).** ***Dhananjaya et al. (2021),*** found that, the high mean areas of remineralization were observed when RMGIC was applied in comparison with the Giomer **(14)**. ***Soi et al. (2013),*** also found that, fluoro-apatite crystals, which are stronger than hydroxyapatite crystals, were formed in higher amounts from GIC restorations than Giomer restorations **(25)**. ***Özveren et al. (2018),*** showed that, the Giomer demonstrated a lower calcium wt% phosphorus wt% ratio, which indicates less remineralization than RMGI **(26).** ***Sajini et al. (2022),*** concluded that Giomer exhibited a lower percentage in the calcium: phosphorus ratio than bioactive restorative materials **(27)**. ***Yli-Urpo et al. (2004),*** concluded that RMGIC seems to have a better potential to remineralize damaged dentin due to the presence of bioactive glass particles **(28).**

However, these data were in contradiction with ***Shinonaga et al. (2018),*** who reported that, no phosphorus ion was found in the Giomer group that was submerged in water. Additionally, neither the Giomer nor the GIC groups that were submerged in water had calcium ions. Different results were obtained due to using different methods and different specimen sizes **(29).**

In my study, there was a noticeable difference between S-PRG (Giomer) and RMGI when their remineralization potential was compared at various time intervals. Remineralization potential after one month was significantly the highest and significantly the lowest after six months. This might be attributed to a two-step process (an early wash-out phase and then a sustained diffusion-based phase). The timing of releasing the ions might be related to the chemical content and method of material mixing. The “early burst” phase can last for up to four weeks **(30).** The incorporation of a poly-HEMA hydrogel in the RMGI content promotes more water sorption and causes higher solubility and a larger release of hydroxyl ions during the first month. The slower ions released from RMGI during the following months may be caused by how slowly glass fillers dissolve through cement pores **(10).** Also, the decrease of ions from Giomer during the following months could be related to a lack of acid-base reaction, an insufficient glass matrix layer, or porosity. This result was agreed with ***Sagmak et al**. (2020),* who conducted that, RMGI liberated a high amount of fluoride level on 28 days in comparison with on one day **(31).** On the other side, the result obtained in this study was in disagreement with ***Choudhary et al. (2015),*** and ***Harhash et al. (2017***), assessed the GIC and Giomer's abilities to release fluoride ions, and they found that the level of fluoride after 7 days was significantly higher than after 21 days **(32),(33).**

Conclusions:

1. Glass ionomers are still the gold standard for remineralization to tooth tissues.

2. The minerals uptakes of tooth tissues are time-dependent properties.

Recommendations:

1. Further in vitro studies are recommended to investigate other types of ion-releasing materials with other aging protocols like thermocycling.

2. Further in vitro studies are recommended to perform a correlation between ions uptake and ions release processes.

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Table (1):

Material	**Specification**	**Composition**	**Manufacturer**	**Lot number**
BeautiBond	Self -etching Universal Adhesive light bonding system, the latest generation	Special HEMA – free formulation of phosphoric and carboxylic acid monomer. Ceramics (alumina, zirconia) or metal surfaces to enhance bonding	SHOFU INC Dental GmbH, Japan. www.shofu.com	062142

Giomer restoration Shade (A3)	Nano hybrid radiopaque bioactive composite	1-Bis-GMA 2-UDMA 3-Bis-MPEPP 4- TEGDMA. 5-83.3 wt% Fluoro-silicate glass	SHOFU Dental GmbH, Japan. www.shofu.com	032114
Fuji II LC Shade (A3)	Light cured Resin modified glass-ionomer cement	1-hydroxyethyl methacrylate 2-Polyacrylic acid, 3-water. 4-58 wt% Fluoro-aluminumsilicate	GC Corporation, Tokyo, Japan. www.dentacarts.com/gc/	2201191

Table (2): Mean and standard deviation of both groups regarding remineralization of hard tissue at all intervals:

Effect of time		F-		P-3		Ca+2	
		M	SD	M	SD	M	SD
Demineralized dentine model		1.92 [a]	0.56	7.95 [a]	0.59	17.79 [a]	0.64
S-PRG (Giomer)	After 1 day	2.43 [a]	0.52	9.04 [b]	0.61	19.03 [b]	0.50
	After 1 month	7.46 [b]	0.72	16.89 [c]	0.81	31.47 [c]	0.83
	After 3 months	5.69 [c]	0.77	14.86 [d]	0.85	27.44 [d]	0.94
	After 6 months	4.86 [c]	0.52	12.77 [e]	0.74	25.48 [e]	0.75
	P value	<0.0001*		<0.0001*		<0.0001*	
Demineralized dentine model		1.92 [a]	0.56	7.95 [a]	0.59	17.79 [a]	0.64
RMGI (Fuji II	After 1 day	3.01 [b]	0.40	9.96 [a]	0.59	20.05 [a]	0.54
	After 1 month	9.07 [c]	0.78	17.67 [a]	0.76	35.69 [a]	0.78

LC)	After 3 months	7.77 [d]	0.71	15.54 [a]	0.77	28.61 [a]	0.60
	After 6 months	6.43 [e]	0.65	13.64 [a]	0.85	26.60 [a]	0.61
	P value	<0.0001*		0.52		0.91	

M: mean *SD: standard deviation*

P: probability level which is significant at $P \leq 0.05$

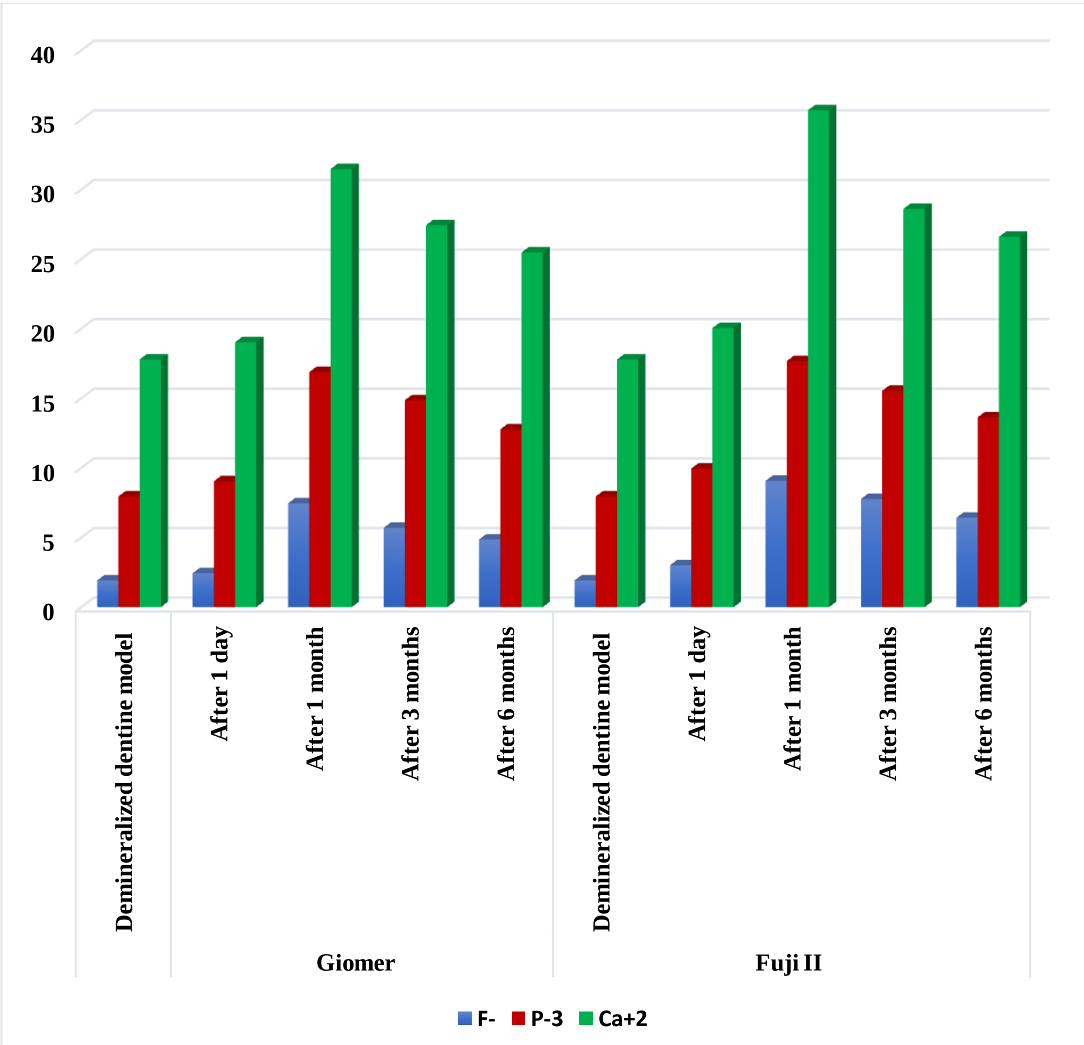

Figure (1): Mean value of remineralizing minerals of hard tooth tissue

Table (3): Mean and standard deviation of both groups regarding remineralization of hard tissue at all intervals:

Effect of material		Demineralized dentine model		S-PRG (Giomer)		RMGI (Fuji II LC)		P value
		M	SD	M	SD	M	SD	
F-	After 1 day	1.92 a	0.56	2.43 ab	0.52	3.01 b	0.40	0.0005*
	After 1 month	----	----	7.46	0.72	9.07	0.78	0.0003*
	After 3 months (T3)	----	----	5.69	0.77	7.77	0.71	<0.0001*
	After 6 months (T4)	----	----	4.86	0.52	6.43	0.65	<0.0001*
P	After 1 day	7.95 a	0.59	9.04 b	0.61	9.96 c	0.59	<0.0001*
	After 1 month	----	----	16.89	0.81	17.67	0.76	0.05
	After 3 months	----	----	14.86	0.85	15.54	0.77	0.09
	After 6 months	----	----	12.77	0.74	13.64	0.85	0.03*
Ca	After 1 day	17.79 a	0.64	19.03 b	0.50	20.05 c	0.54	<0.0001*
	After 1 month	----	----	31.47	0.83	35.69	0.78	<0.0001*
	After 3 months	----	----	27.44	0.94	28.61	0.60	0.006*
	After 6 months	----	----	25.48	0.75	26.60	0.61	0.003*

M: mean *SD: standard deviation*

P: probability level which is significant at $P \leq 0.05$

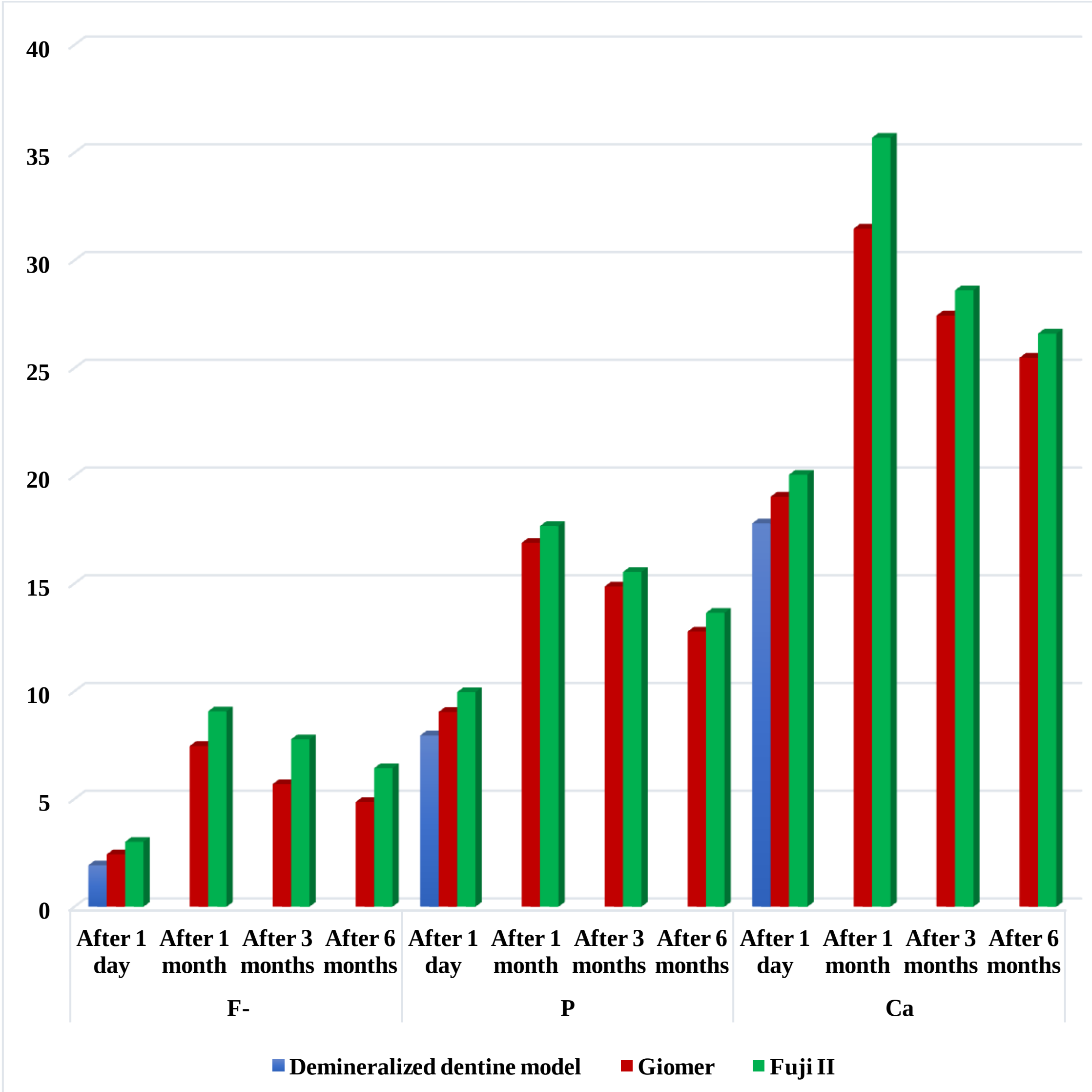

Figure (2): Mean value of remineralizing minerals of hard tooth tissues