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Fluoride release and antibiofilm of Alkasite restorative materials

Rouaa K. Obaees[1*], Emad F. Alkhalidi[2], Suhad M. Hamdoon[3]
[1,2,3]College of Dentistry, University of Mosul, Mosul, Iraq; Rouaakh93@gmail.com (R.K.O.).

Abstract: This invitro study assessed fluoride ion release and antibiofilm activity of Alkasite materials against two types of bacterial spp. (*Streptococcus mutans* and *Lactobacillus plantarum*). Three different ion-releasing materials (Cention N, Cention Forte and Fuji IX GP) were used to make four groups. A total of 96 disc-shaped samples were prepared in polyethene mould, 32 samples (n=8) were used for evaluating fluoride ion release the samples were placed in vial containing 5ml of deionized water, and the measurements were recorded in four periods (1,7,21 and 28) days by utilizing an ion-selective electrode (ISE). 64 samples were used to evaluate the anti-biofilm activity of the materials groups against (*S. mutans* and *L.plantarum*), by using crystal violet assay for quantitative biofilm assessment (n=5 of each group for each bacterial spp.) and by SEM for the qualitative evaluation (n=3 of each group for each bacterial spp). Fuji IX GP registered the highest amount of fluoride release on 1st day of the observation period than Cention N and No primer was added to Cention Forte. On the 28th day, Primer added Cention Forte recorded the highest mean value of fluoride release among all of the groups. Primer-added Cention Forte has significantly more biofilm accumulation of both (*Streptococcus mutans* and *Lactobacillus plantarum*) than Cention N and No primer-added Cention Forte. Fuji IX showed significantly higher *Streptococcus mutans* biofilm accumulation compared to Cention N. Alkasite restorative materials showed a low potential for biofilm accumulation regarding both *Streptococcus mutans* and *Lactobacillus plantarum.*

Keywords: *Antibiofilm, Cention Forte, Cention N, Flouride, Alkasite, Fuji IX.*

1. Introduction

Dental caries, considered one of the most spread worldwide diseases, which stranded as the third largest non-infectious disease by the World Health Organization (WHO) [1]. It is a biofilm-dependent oral disease [2]. Dental caries can be viewed as a disruption in between demineralization and remineralization equilibrium in the hydroxyapatite part of the teeth frequent sugar consumption can induce biofilm acidities and promote the shift toward the pathogenic bacteria, such as *S. mutans*, that can survive in an acidic environment, a prolonged period of low pH-induced tooth demineralization and finally, leading to frank cavity formation [3].

Streptococcus mutans is considered the primary bacterium responsible for initiating caries on the other hand *Lactobacilli* are considered the main microbes responsible for both the progression of caries and also secondary caries formation, the acid-producing microbes able to initiate the process of demineralization, the formation of secondary caries might take place at the interface between the tooth and restoration [4].

Different kinds of direct filling materials are available in contemporary dental practices starting from amalgam and through to the up-to-date bulk fill composite [5]. Each type of these materials has its advantages and disadvantages which identify their specificity in the use form, favorable restorative materials are those products which prevent both bacterial growth and surface colonization [6].

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* Correspondence: Rouaakh93@gmail.com

Amalgams have been used for several years owing to their good mechanical properties, but because it has several limitations including poor esthetics, complex cavity preparation, not adhering to dental tissues, and mercury toxicity, the selection priorities are then directed to their alternatives [7, 8]. Despite the excellent esthetics and the convenient operation qualities of dental resin composite nevertheless, in the long-term of this restoration, the growing cariogenic bacteria will create biofilms on its surface, particularly in the gap that formed due to the polymerization shrinkage, causing secondary caries and leading to treatment failure [1]. Glass ionomer cement (GIC) has been broadly used as base luting, liners, and also as restorative materials because of their qualities such as biocompatibility, their ability to adhere chemically to the tooth, and the ability to release fluoride, but this material also has its pros like mechanical abilities are poor (inappropriate to be used at stress bearing situation) [9].

Much research in the modern dentistry field has focused on the development of various types of restorative materials and different treatments produced for dental caries [10]. Because of the limitations of the majority used restorative materials for direct restorations, the demand for another alternative is required, a material with characteristics of good esthetic, acceptable mechanical qualities, F^- F-release, convenient application technique, and also cost-effective are the main goals of the targeted product [8].

Recently, new and enhanced materials for restorations have been introduced Cention N is a direct basic filling material, it falls under the alkasite material group, it is a subgroup of composite material, the same as ormocer and compomers, and it comes in the form of powder and liquid, unlike the conventional resin composite since the monomer phase of this material does not contain any (Bis-Gma, TEGDMA and HEMA) [11]. It is also comparable to glass ionomer cement (GICs), it can release fluoride, and also perform as an aesthetic filling restorative material. Cention N has improved aesthetic qualities, reasonably more transparent when it was compared to other products of glass ionomers, moreover, it has superior compressive strength so this material combines the best qualities of GIC and amalgam [12]. Cention N has various applications when compared to its equivalents, this material contains alkaline fillers that may act as acid-neutralizing particles due to their ability to release (F^-, Ca^{+2} and OH^-). Cention N constituted (78%) weight inorganic fillers. The alkaline glass particles represent (24%) of the weight of the final product and these fillers release fluoride ions that could be comparable to those that are liberated by the traditional glass ionomer [13].

A new alkasite material introduced in (2021) Cention Forte comes in capsulated form, the manufacturer claimed that this material also can release fluoride (F^-), calcium (Ca^{+2}), and hydroxide (OH^-) ions, Cention Forte provided along with a primer that designed to be utilized especially with Cention Forte as recommended by the manufacturers, it is a self-curing and self-etching primer that offers the excellent foundation to enhance the bond between Cention Forte and the tooth [14, 15]. Both of the alkasite materials are provided with self and light-curing properties, they are radiopaque basic filling materials that are used to restore anterior and posterior teeth that can be used to fill class (I, II, and V) cavities in both permanent and primary teeth.

Fluoride is known for its role as an anti-cariogenic agent that falls under several mechanisms including fluorapatite formation which is less soluble compared to the original hydroxyapatite crystals of the tooth, it has a role in the enhancement of the remineralization process, it also interferes with the formation of ionic bonds at time of pellicle and microbial plaque formation, and its inhibitory role on the growth and metabolism of bacteria [9]. The effectiveness of ion-releasing restorative materials in preventing caries lesions, especially under acidic conditions pH of (5.5), is dependable on the amount and duration of the fluoride ions that are released by these materials. An enhanced fluoride release after the restoration setting is preferred because it helps reduce the survival ability of the microbes that could be left in the inner part of the carious dentin after the cavity has been prepared [16, 17]. Moreover, a delayed and continuous fluoride release helps prevent new caries by creating an unfavourable environment in which microorganisms will be not able to survive [18].

This study was directed to evaluate the anti-biofilm activity of two types of Alkasite restorative materials (Cention N, Cention Forte and Cention Forte with its designated (Primer)) against two types

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of mono-species cariogenic bacterial mono-species biofilm (*S.mutans* and *L.plantarum*) and their fluoride release potentials at different periods in comparison with high viscosity conventional GIC (Fuji IX GP).

2. Materials and Methods

Restorative materials used in the study are listed with their compositions in Table (1).

Table 1.
The materials used in this assessment.

Materials name	Compositions
Cention N (Ivoclar Vivadent, Schaan, Liechtenstein)	Powder: fillers (Ytterbium trifluoride, Isofiller, barium aluminium silicate glass, calcium barium aluminium fluorosilicate glass and calcium fluorosilicate (alkaline glass) filler, initiator and pigments. Liquid:dimethacrylates (aliphatic aromaticUDMA, (UDMA)) and initiators
Cention Forte (Ivoclar Vivadent, Schaan, Liechtenstein)	Powder: inert barium alumino-boro-silicate glass, ytterbium fluoride, calcium fluoro-alumino-silicate glass, and reactive SiO2-CaO-CaF2-Na2O glass fillers Liquid: aliphatic-aromatic UDMA, DCP, UDMA, and PEG-400-DMA Initiator system including hydroperoxide, Ivocerin, and acyl phosphine oxide
Cention Primer (Ivoclar Vivadent, Schaan, Liechtenstein)	Methacrylate-modified polyacrylic acid, MDP, HEMA, Bis-GMA D3MA, silicon, ethanol, dioxide, potassium hydroxide and campherquinone
Fuji IX GP (GC corporation, Tokyo, Japan)	Fluoroaluminosilicate glass, Polyacrylic acid and polybasic carboxylic acid

Grouping of samples: A total number of 96 samples were constructed using a standardized specially constructed Polyurethane mould with a height of (2 mm) and inner diameter of (5 mm), in which the materials were packed to construct a disc-shaped sample The samples then were divided according to the parameter of evaluation into 32 specimens for fluoride release and 64 specimens for antibiofilm activity (N=32 for each bacteria type).

For fluoride release measurement (n=8):
Group I- Cention N
Group II- No primer added Cention Forte
Group III- Primer added Cention Forte
Group IV- Fuji IX (control positive)
For biofilm evaluation (n=8 for each bacterial species):
Group I- Cention N
Group II- No primer added Cention Forte
Group III- Primer added Cention Forte
Group IV- Fuji IX (control positive)

The Sterile mould was placed on the middle of a sterile microscope glass slide and a mylar strip was placed in between, each type of restorative material was loaded into the mould with a slight overfilling of the mixed material. Every single type of restorative material was manipulated according to the manufacturer's guideline instructions.

For the Primer added Cention Forte (group III) one primer drop was dispensed into a sterile dispenser and (activate the primer with the disposable brush for 5 seconds) then an inter-proximal carver instrument (composite filling instrument), was dipped into the self-curing primer for one second. each side of the instrument was wiped for three seconds on the edge of the dispenser to remove the excess primer. The primer on the instrument was adapted on the uncured specimen top surface with some pressure to ensure a smooth and flat surface. An additional mylar strip has been used to cover the

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top surface of the specimens to inhibit the oxygen-inhibiting layer construction, above the second mylar strip a microscope glass slide was placed. The mould then was compressed with (500 gm) weight for 30 seconds to allow the excess material to extrude out, good packing and the consistent surface of the specimens. All the specimens were allowed to be set without any light cure device at room temperature for fifteen minutes.

Specimen storage for fluoride ion release measurements: Eight-disc-shaped specimens of each restorative material group were individually immersed in tightly sealed screw-caped plastic containers with 5 ml deionized water (PH 6.8). The samples were stored in an incubator at 37 C with 95% relative humidity. The measurements were recorded at each (1, 7, 21 and 28) days incubation period. At each measurement time, every individual specimen was removed carefully from the container and their storage solution was used for analysis, the samples were replaced into a new sterile screw-caped test tube that contained fresh (5 ml) deionized water and incubated at 37 ºC with 95 % relative humidity for the next measurement time, this procedure was repeated at every measurement time interval.

Fluoride ion release measurement: Fluoride ion release measurement was performed by using Ion Selective Electrode (ISE) (CRISON INSTRUMENTS, S.A. E- 08328 ALELLA- Barcelona). The ion selective electrode device was calibrated at each measurement time with (NaF) standard solution including a serial of dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} ppm F^-), respectively after the calibration process of the device the released fluoride ions were measured after removing the sample from the solution at each time interval of (1, 7, 21 and 28) days with a good shaking of the testing solution for (5 seconds) afterwards the glass electrode was inserted into the solution. The concentration of the released fluoride ions in each solution was taken in ppm. All the measurements took place at constant room temperature.

2.1. Biofilm Assessment

Microorganisms' isolation and identification: The two most important cariogenic bacterial species: *S. mutans* and *L. plantarum* (were gratefully obtained from the Laboratory of Microbiology of Basic Dental Science at the collage of Dentistry/university of Mosul (isolated by a researcher team) and identified (morphologically using the appropriate selective culture media and genetically by PCR). For re-cultivation of each stored bacterium and to ensure purity a single colony is picked up by mean of an inoculating needle and transferred to sterile brain heart infusion broth tubes, cultivated at 37 °C for 18 hours then cultured on their appropriate selective medium (Mitis salivarius agar and Rogosa agar) for *S. mutans* and *L. plantarum* respectively.

Bacterial inoculum preparation: A single colony of *S. mutans* and *L. plantarum* from each pure selective agar plate was used to inoculate the TSB broth tube, incubated under anaerobic conditions at 37°C for 18 hours, to obtain turbidity equal to 0.5 McFarland standards scale (1.5×10^8 CFU /ml). The turbidity of the broth was evaluated visually by comparing the *S. mutans* and *L. plantarum* (TSB) cultures with tube 0.5 McFarland against a striped black-white card behind them.

Microbiological analysis: The antibiofilm activity of tested restorative materials and control positive were assessed on each bacterial spp. (*S. mutans and L. plantarum*) by:

1. Quantitative biofilm assessment by Crystal violet assay.
2. Qualitative biofilm assessment using Scanning Electron Microscope (SEM).

The total number of samples used in microbiological work was 64 discs 20 discs for each bacterium were used for CVA and 12 discs for each bacterium for SEM analysis. The samples were tested just after their settings time, the specimens were placed in the wells of a sterile flat bottom of a 96-well plastic microtiter plate. 10 µL of the adjusted TSB microbial suspension of each bacteria spp. (*S. mutans* and *L. plantarum*) was transferred carefully with the aid of a sterile micropipette to the surface of each of the material discs. The microtiter plates containing discs loaded with 10 µl microbial broth were sealed and incubated in a candle jar at 37 °C for 30 minutes to ensure direct contact between the discs and the bacteria then a 190 µL of sterile Tryptic Soy Broth (TSB) was added to each well. Afterwards, the

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microtiter plates containing the samples were then incubated at 37 ◦C in anaerobic conditions for 24 hours.

Biofilm assessment (Crystal violet assay): The evaluation and comparison of the bacterial biofilm formed by *S. mutans* and *L. plantarum* on tested material and the control positive discs upright surfaces assessed by utilizing crystal violet (CV) staining assay. Forty samples were used After 24 hours of the incubation period, each disc was removed from the microtiter plate wells and placed into a new 96-well plate, washed three times with 200 µl of sterile deionized water pH (6.8) times then washed with 200 µl phosphate buffer saline (PBS) pH 7.4 to remove the loosely bound bacteria. Afterwards, the samples were left to dry at room temperature for half an hour. Thereafter, stained with 200µl of 0.1 % crystal violet and incubated for 20 minutes at room temperature. Immediately afterwards, the same washing process was performed, to remove the excess stain, then the specimens were left to dry at room temperature, and the samples were transferred to new 96 flat bottom well plates. To elute the bound crystal violet absorbed by the adherent bacterial biofilm a 200µl of 30% glacial acetic acid was added on each disc. the discs were removed and the stain of the formed biofilm was assessed by measuring the absorbance at 570 nm wavelength with a microtiter plate reader. The absorbance of the eluted stain is equal to the amount of *S. mutans* and *L. plantarum* biofilm formed on the specimen's surface.

Scanning Electron Microscope (SEM): Qualitative evaluation of the anti-biofilm effect of Alkasite materials and High viscosity GIC cement against two cariogenic bacterial species (*S. mutans* and *L. plantarum*) was performed by Scanning electron microscope (SEM) using 24 discs sample 12 discs for each bacterium 3 discs for each material 2 of them incubated with the test bacterium and one disc incubated with broth only. After 24 hours of incubation of (*in vitro* mono species biofilm formation), broth was drowned from each well by micropipette, and the samples with each bacterial biofilm were washed with 200 µl deionized distilled water and phosphate buffer saline (pH 7.4) 2-3 times along with vortex to dislodge loosely bounded cells, then the discs were transferred to a new sterile 96 flat bottom microtiter plate and left to dry at room temperature. The samples then were fixated by adding 200 µl of 2.5% glutaraldehyde for 2 hours. Thereafter each specimen was placed in a sterile screw-capped test tube and each tube was then left to dry overnight at 37◦C in an incubator. Then the samples received a gold coat to be examined with SEM that operates at 15 Kilovolts (KV) at a magnification of (13000). To avoid sample selection bias consistent selections of image location between multiple samples were used in our study, the representative images of each bacterial biofilm on each material sample surface were then recorded, and a descriptive analysis for the samples was performed.

Statistical analysis: Statistical analysis was performed using (SPSS) v.20.0 for Windows. Data for fluoride ion release was analyzed by using one-way (ANOVA) followed by the Duncan test for intergroup analysis test. For antibiofilm evaluation and comparison, the data was analyzed by using the Kruskal Wallis test followed by Dunne's pairwise comparison for inter-group analysis. The (p-value ≤0.05) was considered to be statistically significant.

3. Results

Fluoride ion release analysis: Means and standard deviations for fluoride ion release in (ppm) among groups within each observation period are listed in Table (2). The result of the presented study showed that there was a statistically significant difference among the groups in the amount of the released fluoride ions at each measurement time interval in the following order:

At the period of 1st day, the fluoride release was significantly ($p \leq 0.05$) higher among the groups in the following order control positive IV group >II group > I group > III group.

At the 7th day measurement interval, the fluoride ion release was recorded as statistically significant ($p \leq 0.05$) higher among the groups in the following order II group > I group > Control positive IV group > III group.

On the 21st day of the measurement period, the fluoride ion release was recorded as statistically significant ($p \leq 0.05$) higher among the groups in the following order group II > group III > group I > group IV.

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At the 28th day of the measurement period the fluoride ion release was statistically significant ($p \leq 0.05$) higher among the groups in the following order group III > group II > group I> group IV.

Table 2.
Means and standard deviations for fluoride ion release in (ppm) among groups within each observation period.

Period	Materials groups	Mean	±SD	F-test	P-value	Duncan groups
1st day	Group I	2.9260	0.14654	219.499	0.000001	C
	Group II	3.2695	0.22478			B
	Group III	2.0713	0.15372			D
	Group IV	4.8366	0.31498			A
7th day	Group I	6.0895	0.14124	232.528	0.000001	B
	Group II	7.5305	0.14605			A
	Group III	4.5349	0.40058			D
	Group IV	5.0804	0.19140			C
21st day	Group I	5.8045	0.44102	206.32	0.000001	C
	Group II	7.487	0.31593			A
	Group III	6.2463	0.38720			D
	Group IV	4.1114	0.15218			B
28th day	Group I	2.2278	0.18292	359.436	0.000001	A
	Group II	2.9075	0.21533			B
	Group III	4.3510	0.23330			C
	Group IV	1.1538	0.15905			D

This means values with different letters vertically have significant differences at $p \leq 0.05$. GrI=Cention N, Gr II=No primer added Cention Forte, Gr III=Primer added Cention Forte and Gr IV=Fuji IX GP (control positive).

Biofilm assessment Analysis: Means of absorbance represent biofilm formation by Crystal violet (CV) stain assay, the standard deviation for comparisons of the anti-biofilm effect of the materials groups and the positive control against *S. mutans* and *L. plantarum* presented in Table (3). Kruskal Wallis test result showed there were statistically significant differences in the means of absorbance among groups in their effect against *S. mutans* (0.017) and *L. plantarum* (0.024) biofilm formation. Post-hoc comparison Dunn's test was used to detect which pairs of groups were different significantly.

There was a significant difference in terms of *S. mutans* biofilm CV absorbance mean value between Cention N and Fuji IX (control positive), and also between Cention N and Primer added Cention Forte. Also, Primer-added Cention Forte showed a significantly higher mean value of *S. mutans* biofilm CV absorbance compared to No primer-added Cention Forte. No statistically significant difference in the mean value of *S. mutans* biofilm CV absorbance between primer-added Cention Forte and Fuji IX. Moreover, the difference was not significant between No primer added Cention Forte and Fuji IX. Also, the difference was not statistically significant between Cention N and No primer added Cention Forte. There was no statistically significant difference in terms of *L. plantarum* biofilm CV absorbance mean value among all the groups except for Primer added Cention Forte which has a mean value of *L. plantarum* biofilm CV absorbance that was significantly higher than Cention N and No primer added Cention Forte.

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Table 3.
Mean and standard deviation for comparing the mean antibiofilm effect against *S. mutans* and *L. plantarum* of four material groups at an incubation period of 24 hours.

Groups	Mean	± SD	P- value	Bacterial ssp.	Comparison
Group I	0.41567	0.004726	0.017*	*S. mutans*	A
Group II	0.42500	0.005292			AB
Group III	0.86033	0.004163			C
Group IV	0.51800	0.009539			BC
Group I	0.36567	0.054629	0.024*	*L. plantarum*	A
Group II	0.39400	0.019287			A
Group III	0.70833	0.041199			B
Group IV	0.44500	0.004583			AB
Different letters vertically referring to significant differences at p≤0.05					
(n=5 for each group against each single bacterial ssp.).					
Gr I=Cention N, Gr II=No primer added Cention Forte,					
Gr III=Primer added Cention Forte and Gr IV= Fuji IX (control positive).					

3.1. Scanning Electron Microscopy Analysis (SEM)

No microorganisms were applied to all the samples, the primer of Primer added Cention Forte has a similar look that mats the topography, Fuji IX appeared with black lines representing cracks on the surface of each sample surface that incubated for 24 hrs with TSB only. The micrograph of each sample surfaced after in vitro mono species biofilm formation of *S mutans* which showed marked microcolonies formation, particularly on the surface of Primer added Cention Forte and also microbial biofilm accumulation around the cracks of Fuji IX. The micrograph of each sample surface after in vitro mono species biofilm formation of *L.plantarum* showed the greatest accumulation of *L. plantarum* appeared on the surface of Primer added Cention Forte and Fuji IX (Figure 1).

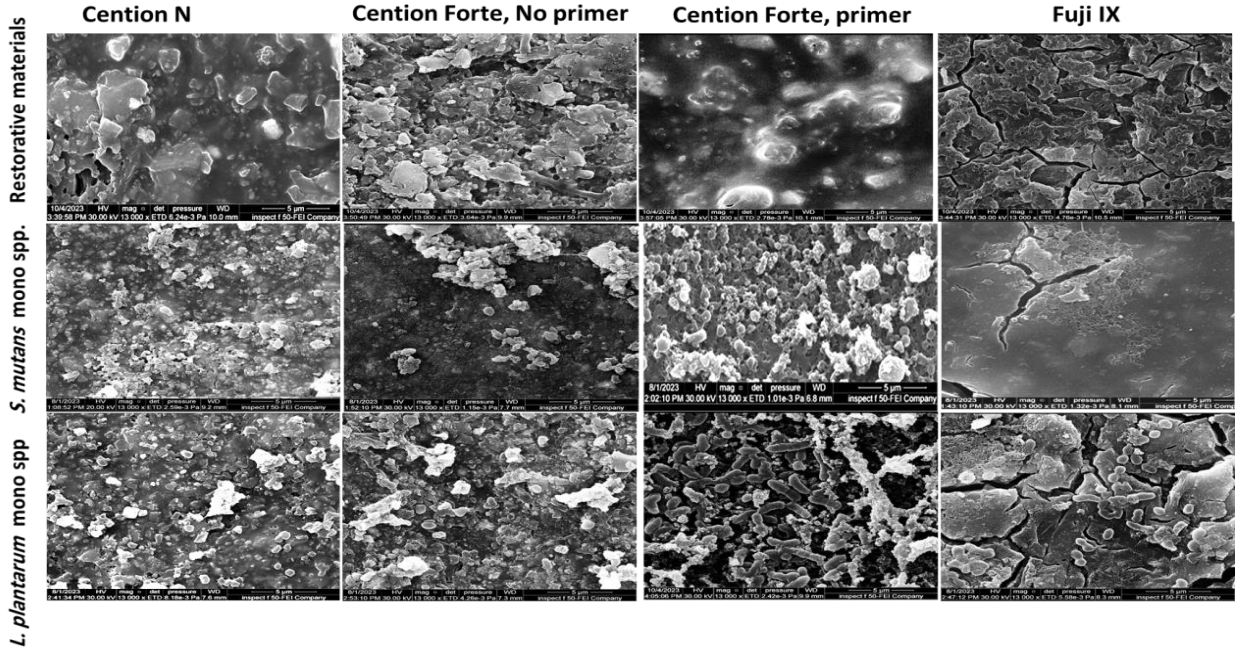

Figure 1.
A representative SEM image for restorative materials samples, *S mutants* mono spp., *L. plantarum* mono spp. Centurion N, No primer added Cention Forte, Primer added Cention Forte and Fuji IX.

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4. Discussion

In this study, we used an ion-selective electrode to measure the released fluoride ions from the restorative material regarding its accuracy, and simplicity and it is considered to be more convenient [19]. The storage medium was changed only at the measurement intervals of the observation periods (1, 7, 21 and 28 days) in previous studies several authors have been changing the storage solution regardless of the measurement intervals. McCabe (1998) mentioned that daily changing of the storage solution might minimize or even inhibit fluoride ions release by equilibration of the immersion solution [19].

The result of this study proved that there is continuous F^- ion released from all tested materials in 28 days with different patterns of each material type. According to the result of the presented study, there was a significant difference in the amount of fluoride released among the materials at each immersion period so the first null hypothesis was rejected. The results revealed an increase in fluoride ions released from Fuji IX from day 1st to day 7th (which was the highest amount compared to other days of the immersion period) that underwent reduction over time. The same pattern of fluoride release was seen in Tiwari [20] in which the authors studied the Antibacterial Activity and fluoride ion release of different types of glass ionomer, Compomer and Zirconomer [20].

Cention N and No primer added Cention Forte also showed an increase in fluoride release from the 1st day to the 7th day of the observation period this pattern of fluoride release of alkasite materials (Cention N) was also seen in a study conducted by Felz, et al. [21] the author compared fluoride ion release and antibacterial activity of four different restorative materials, their result revealed that there was an increase in fluoride release from Cention N on the 7th day and it was the highest amount compared to other days of the observation period [21]. During the first day of immersion, Fuji IX released a significantly higher amount of fluoride ion in comparison to Cention N and No primer was added to Cention Forte. Other researchers also pointed out such phenomenon showing that conventional GICs provide an initial fluoride ion burst effect [22, 23].

The GIC initial burst effect can be possibly due to the initiation of the (acid-base) reaction and the attack on fluroaluminosilicate particles by polyacrylic acid which leads to the ion release [24]. Also, the conventional GICs contain FAS fillers that are non-silanated which going to make the material easily hydrolyzed resulting in the initial burst [25, 26]. The burst effect can be brought on by the initial and superficial rinsing of the material surface and could be related to the high water solubility and sorption of the GIC [15, 27].

Cention N and No primer added Cention Forte have significantly lower fluoride ion release by the end of the 1st day than Fuji IX. This could be explained due to the differences in the filler types since, the fillers in Cention N and No primer added Cention Forte are surface modified (silanized fillers) therefore, becoming resistant to deterioration and may lead to a decrease in the number of fluoride ions that are released into the storage medium [28]. Also, Alkasite materials because of the calcium phosphate and calcium fluoride that form on its surface which has the presence of a (0.5-nm) thick surface layer which is resistant to being rinsed with deionized water (D.I) [29].

Additionally, the setting reaction of Alkasite materials is similar for Compomer and Giomer throughout our making a network of resin and forming covalent bonds with nonreactive and reactive silanized filler kind that will lead the material to become more resistant to hydrolysing [26]. The capability of the ion-releasing restorative material to release ions depends on the material's permeability to water, a sufficient amount of water absorption is necessary for the ions to be released from the filler particles and leached into the surrounding storage medium [30]. Primer added Cention Forte released significantly the lowest amount of fluoride ions at (the 1st day and 7th day) observation periods compared to all other materials, this could be explained due to the presence of the coat (Cention Forte Primer) which acted as a chemical-physical barrier for the ions to be released, because filler particles were isolated from the aqueous solution by the adhesive primer coat layer.

Alkasite materials (Cention N and No primer added Cention Forte) have registered significantly higher fluoride release in comparison to Fuji IX on the 7th day of the observation period. Alkasite filling

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materials are capable of releasing F^- gradually over time. This result was consistent with a study by Singh, et al. [31] the authors explained the reason that Alkasite material has a greater ratio of powder to liquid and the great amount of alkaline glass in the final product of the Alkasite material could be the cause of the significantly higher amount of fluoride ions release over a longer period [31]. Additionally, the spike in fluoride ions released from Alkasite materials in both groups (Cention N and No primer added Cention Forte), at the end of the 7thday, explained by Singbal et al. (2022) this might as a result of the unreacted barium aluminium fluorosilicate glass and calcium fluorosilicate glass particles within the self-cured cured material [27].

The result of this study showed that No primer added Cention Forte registered a significantly higher amount of fluoride release when compared to Cention N at all observation periods, it might be explained due to the differences in the alkaline fillers between the two materials (not mentioned by the manufactures). The high amount of fluoride ion release registered in this study might not be decisively related to any of Cention Forte's special features due to the complexity of the material composition and the information regarding their substances proprietary to date still insufficient. Fuji IX has expressed significantly less amount of fluoride ion release when compared to Alkasite material (Cention N and No primer added Cention Forte) at (the 7th, 21st and 28th day) observation period, because as the time increased the reaction of the acid-base will form a silicic gel on the partially reacted FAS fillers surface. This gel makes the fillers stick well to the GICs matrix and protects them from hydrolysis by making the cement less soluble [32].

According to El-Bahrawy and Attia [33] the F^- ions released from GICs occur throughout three phases starting from surface loss, diffusion through cracks and pores, and finally bulk diffusion, so after the washout effect of the GIC in the first 24 hours that will follow with a slower release of F^- ions throughout the subsequent days could be explained due to slower dissolution of their glass particles through the cement pores and fractures. Bulk fluoride diffusion occurs during the maturation period as a constant contact between the GIC material with the deionized water [33]. Primer-added Cention Forte expressed significantly higher fluoride ion release in comparison to Cention N and Fuji IX on (the 21st day), also the result revealed Primer added Cention Forte registered significantly higher fluoride ion release among all of the three other groups on (the 28th day) observation period, this could be related to Cention Forte primer (Coat) contain elevated concentrations of acidic monomers that contributed to the hydrophilicity of the primer.

The monomers with ester bonds like hydroxyl ethyl methacrylate (HEMA) are highly prone to hydrolysis with time in the presence of water, which might be the reason for an increased F^- release from the material [22]. Ion release is influenced by several experimental factors such as the solution of the storage type, the frequency of changing the storage solution, the storage medium composition and pH value [25]. Deionized water was used rather than artificial saliva considering its high viscosity and the presence of ions in artificial saliva. These ions affect the release of F^- from the restorative materials. Therefore, they will lead to an incorrect assessment of the released F [21].

All of the materials registered the lowest amount of F^- release on the 28th day of the observation period this could be related to the depletion of the ions, as the period increases, leading to F^- released in small quantities [29]. The antimicrobial properties of a dental material are considered within the most required biological characteristics that should be taken into consideration when determining their application. When using a material with antimicrobial properties the development of bacterial biofilm could be avoided [34]. The Crystal violet staining assay is based on measuring the absorbance levels of CV stain of adhered biofilm that formed on the material sample surface, it is considered one of the most commonly used techniques for biofilm formation determination in vitro studies [35].

One of the factors that contribute to the failure of dental restorations is highly related to biofilm accumulation at the tooth-restoration interface, which will cause surface roughening and deterioration of the restoration, which ultimately can lead to microleakage and so restoration failure due to the formation of secondary caries [36, 37]. According to the result of this study, there was a significant

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difference among the four materials regarding their antibiofilm activity against bot bacterial spp, thus the second null hypothesis was rejected.

Moreover, the result of this study showed that Cention N and No primer added Cention Forte exhibited the highest antibiofilm activity against each bacterial species (*S. mutans* and *L. plantarum*). For antibiofilm activity against *S. mutans*, only Cention N showed a significant difference in comparison with Fuji IX, the difference was not statistically significant between No primer added Cention Forte and Fuji IX, however both Cention N and No primer added Cention Forte showed no significant difference in term of *L. plantarum* formed biofilm with Fuji IX (control positive). Both Cention N and No primer added Cention Forte has statistically significant higher antibiofilm activity against both species compared to Primer added Cention Forte.

The reason that Cention N showed a statistically significant difference compared to Fuji IX in their antibiofilm activity against *S. mutans* while No primer added to Cention Forte did not differ statistically could be related to the differences in the mixing procedure, in Cention N (Hand mixing) which can be related to the formations of bubbles and porosities and more ions will be diffused from the material after mixing compared to No primer added Cention [38]. Although there was no statistically significant difference between, no primer added Cention Forte and Fuji IX in terms of their antibiofilm activity against *S. mutans* and no significant difference between the three materials (Cention N, No primer added Cention Forte and Fuji IX) in terms of their antibiofilm activity against *L. plantarum*. Still, Alkasite materials showed a mean value with the lowest biofilm CV absorbance than Fuji IX (control positive).

This could be explained due to both materials (Alkasites and Fuji IX GP) releasing fluoride ions, these ions are well known to have an inhibitory effect on the cariogenic biofilm through several mechanisms including their (acid production, formation of extracellular polymeric substance (EPS) and acid tolerance). As a result of the metabolic activity of the bacteria acidic conditions will be created and that will promote the releasing of free F^-, the released F^- will react with H^+ and form HF (weak electrolyte) the formed HF will efficiently decrease the acidic environment created by the bacterial metabolism by impacting on the bacterial metabolism both in a direct way (inhibition of ATPase and enolase) and in indirect way (intracellular acidification) [39-41]. Alkasite material (Cention N and No primer added Cention Forte) contains alkaline glass fillers that release not only F^- but also calcium and hydroxide ions [30].

In a previous study done by Gupta, et al. [29] to compare the fluoride release and the alkalinizing potentials of Cention N and conventional GIC cement in both neutral and acidic PH at multiple periods the authors concluded that Cention N has significantly higher alkalinizing potentials. Also, the material released a significantly larger amount of F^- in the acidic condition compared to GIC [29]. In a study done by Ruengrungsom, et al. [42] to evaluate (Ca^{+2}, F- and P) release and recharge in neutral and acidic conditions of Cention N, high viscosity GIC and several ion-releasing restorative materials the authors concluded that Cention N in lactic acid storage solution showed a high potential for calcium ion release [42]. The release of F, OH^- and Ca^{+2} ions from Alkasite materials and as a result of the acidic environment created by the bacterial biofilm metabolic activity will be released in a great amount leading to an elevation of the pH of the formed biofilm on the material surface creating a local environment that is less favourable to the bacterial colonization, furthermore, prevent adjacent hard tooth structure demineralization [43]. According to Hamza, et al. [44] Cention Forte showed no difference in terms of antibiofilm activity in comparison to different types of GICs or even the conventional composite resin, this difference could be related to the use of complex biofilm in their investigation since the samples were incubated in a saliva pool as source for the biofilm while, in this assessment single species biofilms were utilized for both (*S. mutans* and *L. plantarum*) [44]. According to Previous research done by Park, et al. [45] the authors evaluated and compared the bacterial adhesion of *S. mutans* to Cention N, RMGIC and (bulk-fill) composite, and the study results revealed that Cention N has the lowest bacterial adhesion values independently on surface roughness due to the F^- released from this material has a direct effect the S. mutans metabolism [45].

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Moreover, another study done by Daabash, et al. [46] evaluated the adhesion of *S. mutans* and the related surface characteristics of several ion-releasing restorative and their result revealed that Cention N material has the lowest mean value of biofilm formation compared to the other tested material and there was no relation between the material surface characteristics and bacterial colonization and other factors would have a more significant effect on bacterial colonization such as the ions release by Cention N [46]. Primer-added Cention Forte group showed the lowest antibiofilm activity against both bacterial spp. (significantly higher mean value of biofilm CV absorbance than Cention N and No primer added Cention Forte. Not significant but showed a higher mean value of biofilm CV absorbance compared to Fuji IX (control positive)). Cention primers contain both HEMA and BisGMA these monomers can potentially elevate the glycosyltransferase activity, which leads to increased formation of the bacterial extra-cellular matrix, therefore bacterial adhesion would be maximized and a mature biofilm will be formed on the surface of Primer added Cention Forte [47, 48]. In addition, the primer acted as a chemical and physical barrier for the ions to be released from the material because filler particles will be isolated from the surrounding solution by the coating layer and that will decrease the ions to be liberated from the material [22, 30]. Resulting in a higher biofilm formation on the Primer added Cention Forte surface. Results of the surface topography of the materials samples were in agreement with antibiofilm activity for both bacterial species in all of the tested groups.

The findings of this investigation should be interpreted as the same as any in-vitro investigation limitation. The first limitation was using a standardized formed sample while there would be more variation in the clinical situation for the shape and size of the filling. The other limitation we utilized the single species of *S. mutans* and *L. plantarum* biofilm. Still, the application of this model provided conditions that could be useful and reproducible for cariogenic biofilms. Therefore, future (in-vitro) studies with the application of the multispecies biofilm and in-vivo (clinical) studies are most needed for the examination of the material within conditions that will be closer to representing the oral environment.

5. Conclusion

Alkasite materials (Cention N and No primer added Cention Forte) release a higher amount of F^- than Fuji IX at all times except for the first day. Cention N was more effective against *S. mutans* biofilm accumulation than Fuji IX. Two alkasite materials (Cention N and No primer added Cention Forte) revealed comparable antibiofilm activity against *L. plantarum* to Fuji IX. The application of the designated coat (Primer) on Cention Forte diminished its fluoride release along with its antibiofilm activity against the two types of cariogenic bacterial species.

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The authors confirm that the manuscript is an honest, accurate, and transparent account of the study; that no vital features of the study have been omitted; and that any discrepancies from the study as planned have been explained. This study followed all the ethical practices during writing.

Competing Interests:

The authors declare that they have no competing interests.

Authors' Contributions:

All authors contributed equally to the conception and design of the study. All authors have read and agreed to the published version of the manuscript.

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