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Ion Release and Antibacterial Activity of Calcium- Fluoride Releasing Self-Adhesive Resin Cement and Conventional Self-Adhesive Resin Cement: An In-Vitro Study

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Aim: To investigate the ion release and antibacterial activity of calcium-fluoride releasing self-adhesive resin cement and conventional self-adhesive resin cement.

Materials and methods: Forty specimens were prepared and divided into two groups according to the cement type: (Group I) calcium-fluoride releasing self-adhesive resin cement (TheraCem®) and (Group II) conventional self-adhesive resin cement (Nova Resin). Each group was separated into 2 subgroups; According to the type of test that were performed: subgroup for ion release assessment of fluoride, calcium and phosphorus (n= 10) and subgroup for Antibacterial assessment against Streptococcus Mutans (n= 10). A direct contact test was carried out to assess bacterial growth. Calcium and phosphorus ions were analyzed using inductively coupled plasma mass spectrometry (ICP-MS) whereas fluoride ions release was analyzed by ion chromatography (I C). Data was collected and IBM SPSS Statistics for Windows, Version 23.0 used for analysis. using the student's *t*-test, Two-Way ANOVA test and Spearman's correlation coefficient. The significance level was set at $P \leq 0.05$.

Results: For ion release test, Group I showed statistically significant higher mean F, Ca and P ion release than Group II ($P < 0.001$). For antibacterial activity test, Group II exhibited a statistically significant difference in mean bacterial count when compared to Group I. ($P < 0.001$).

Conclusion: calcium- fluoride releasing self-adhesive resin cement (TheraCem®) has more antibacterial properties than conventional self-adhesive resin cement (Nova Resin) since it releases calcium, phosphorus and fluoride ions.

Keywords: Calcium- Fluoride releasing self-adhesive resin cement, Conventional resin cement, Ion release, Antibacterial activity.

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Introduction

Since cements function as sealing agents along the finishing lines of prepared abutments for fixed partial dentures, improper cementation can lead to microleakage of oral fluid, potentially causing bacterial complications and the formation of secondary caries.[1]

Researchers have found substantial evidence that fluoride, phosphorus and calcium can prevent cavities. Thus, embedding antibacterial properties in dental adhesives can improve the remineralization process and suppress the growth and activity of microorganisms linked to caries at the tooth-restoration interface.[1]

All fluoridated adhesives will aid in preventing demineralization and enhancing remineralization if they continue to release fluoride [2]. Even a small quantity of fluoride delivered continually around the tooth can minimize demineralization of the tooth's tissue.[3]

Deionized water is regarded as a more specific medium than artificial saliva and other acidic media for assessing the release of Ca and F ions from the examined cement samples.[1]

A micro gap between the prepared tooth margin and fixed prostheses allows plaque deposition and cariogenic bacterial colonization, resulting in secondary caries [4] .Streptococcus Mutans were selected as it is the primary causative agent for dental caries, which remains one of the most prevalent diseases affecting humans. Additionally, Streptococcus Mutans is routinely employed in assessing the antibacterial efficacy of restorative materials.[1]

As a result, the goal of this research was to evaluate fluoride, calcium and phosphorus ions and antibacterial activity of two self-adhesive resin cements.

Materials and Methods

Specimens' preparation

Forty disc-shaped samples of the two resin cement (n =20) were constructed by filling a split teflon mold (6 mm height × 4 mm diameter) for Ion Release test in accordance with Nicholson, et al.2021[5] and (3 mm height × 6 mm diameter) for Direct contact test for antibacterial activity following Gao , et al.2023 [6]. After application of separating medium using disposable tips until the mold is filled.

After placement of the cement in the mold, Mylar matrix strips were placed on the top of the specimen to obtain a smooth surface. The recommended time for curing resin cement following the manufacturer's instructions was 20 seconds. All specimens were removed from the mold after setting and finished with finishing strips.

Study design and sample grouping

Samples were divided into two major groups based on the cement being tested: (Group I) Calcium-Fluoride Releasing Self-Adhesive resin cement (TheraCem®) and (Group II) Conventional Self-adhesive resin cement (Nova Resin). Materials manufacturer, Composition, brand name and lot number were represented in (Table 1). samples were divided into two subgroups in relation to the type of test that were performed: Ion Release Test (n=10), Antibacterial Test (n=10).

Ion Release Test

Ten specimens for each resin cement were prepared and a nylon thread was inserted into each cement specimen during setting. After final setting, each specimen individually was immersed and suspended vertically by nylon thread in centrifuge tubes filled with 20 mL of deionized water following Nicholson, et al.2021[5]. This procedure is repeated at different periods (1, 14 and 28 days). Afterwards, stored samples were gathered and analyzed using Inductively coupled plasma mass spectrometry (ICP-MS) for Calcium and Phosphorus ions and Ion chromatography (IC) for Fluoride ions

Table 1: Materials composition and their Manufacturer

Material Name	Composition	Manufacturer	Lot Number	Trade Name
Self-Adhesive Resin Cement (Nova Resin)	Powder: Calcium Aluminum Fluorosilicate, Barium Oxide, Zinc Oxide. Liquid: Polyacrylic Acid, Acid itaconic, Acid Copolymer Aqua Solution, Tartaric Acid, Paraban, Deionized Water.	Imicryl, Konya, Turkey	23A343	Nova Resin
Self-Adhesive Resin Cement (Theracem)	Calcium Base (Filler) 20-50% Glass (Filler) 30 – 50% Dimethacrylates 20 – 50% 10 methacryloyloxydecyl di-hydrogen phosphate (MDP) Ytterbium Fluoride 5 – 15% Amorphous Silica 1 – 5% Initiator 1 –10%	BISCO, Schaumburg, U.S.A.	2300111767	Theracem
Deionized water.	Ultra-Pure Water DNase / RNase - Free Deionized Water	B - 4800 verviers, Belgium	OMB080	BIO - CHEM

Direct contact test for antibacterial activity

Following Demirel, et al.2019 [7], Streptococcus Mutans (Strain SG - 1, accession: PP412038), provided by Assiut University Mycological Centre (AUMC), was used in this investigation. Each disc of the cement was put into a test tube filled with 20µL bacterial suspension (1.2×10^{8}CFU/ml) and placed above each sample. The tube was then incubated vertically. During incubation, the evaporation of the suspension liquid provides those bacteria had direct contact with the examined substance. Then 3ml of tryptic soy broth was added to each tube and stirred for a few minutes. After 48 hours of incubation at 37°C, colonies were recorded and CFU/mL calculated.

Statistical analysis

The numerical data was provided as means and standard deviations (SD). The U test was used for comparison between two groups. A Two-Way ANOVA test was utilized to evaluate the influence of material type, time and interactions on mean ion release. Significance was set at $P \leq 0.05$.

Results

Ion Release

Fluoride (F)

As general data revealed, Group II showed statistically significantly lower mean F ion release than Group I ($P \leq 0.05$).

Regarding (Group I), there was a statistically significant change in mean F ion release by time. Pair-wise comparisons revealed that there was a statistically significant increase in mean F ion release at one day (149.2 ± 1.6) followed by a statistically significant decrease in mean F ion release after 14 days (5.9 ± 0.3) then there is non-statistically significant change in mean F ion release after 28 days (7.7 ± 1.6)

Regarding (Group II) there was a statistically significant increase in mean F ion release at one day (51.9 ± 1.4) followed by a statistically significant decrease in mean F ion release after 14 days (11.4 ± 0.9) followed by a statistically significant increase in mean F ion release after 28 days (17.8 ± 1.9).

Calcium (Ca)

Regarding (Group I) there was a statistically significant change in mean Ca ion release by time. Pair-wise comparisons revealed that there was a statistically significant increase in mean Ca ion release at one day (33.9 ± 0.4) followed by a statistically significant decrease in mean Ca ion release from 14 days (3.2 ± 0.6) to 28 days (4.4 ± 0.3).

Regarding (Group II) there was a statistically significant change in mean Ca ion release by time. Pair-wise comparisons revealed that there was a statistically significant increase in mean Ca ion release at one day (36.4 ± 1.9) followed by a statistically significant decrease in mean Ca ion release from 14 days (1.5 ± 0.3) to 28 days (2 ± 0.6).

Phosphorus (P)

Group I showed statistically significant change in mean P ion release by time. Pair-wise comparisons revealed a statistically significant increase in mean P ion release at one day (127 ± 1.4) followed by a significant decrease in mean P ion release after 14 days (16.8 ±1.9) and 28 days (7.8 ±0.9).

Moreover, group II revealed a statistically significant change in mean P ion release by time. Pair-wise comparisons revealed that there was a statistically significant increase in mean P ion release at one day (64.7 ± 2) followed by a statistically significant decrease in mean F ion release after 14 days (23.7 ± 1.9) However, the mean P ion release after 28 days (26.6± 1.5) showed statistically significant higher value compared to 14 days (Table 2) (Figure 1).

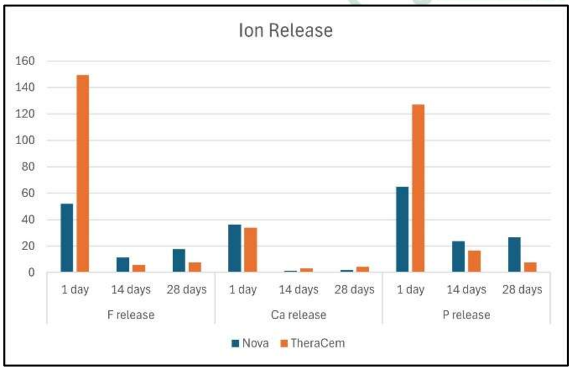

Figure 1: Column chart represents mean and SD values for F, Ca and P ion release on different time intervals

Table 2: Mean and standard deviation (SD) values of F, Ca and P ion release at different time intervals.

	Time Interval (Day/s)	F ion		Ca ion		P ion	
		Mean	SD	Mean	SD	Mean	SD
Group I	1	158.5	1.5	19.9	1.9	242.7	1.9
	14	244.1	1.7	30.9	1.4	174.1	1.9
	28	167.5	1.4	27.7	1.8	213.9	1.9
Group II	1	140.3	1.5	10.2	1.8	146.3	1.3
	14	211.6	1.6	11.1	1.1	132.3	1.8
	28	171.6	1.9	21.7	1.2	144.3	1.9

Results of Antibacterial activity

Group II showed statistically significant higher mean bacterial counts (6.26 ± 0.46) than Group I (1.94 ± 0.32) (Figure 2).

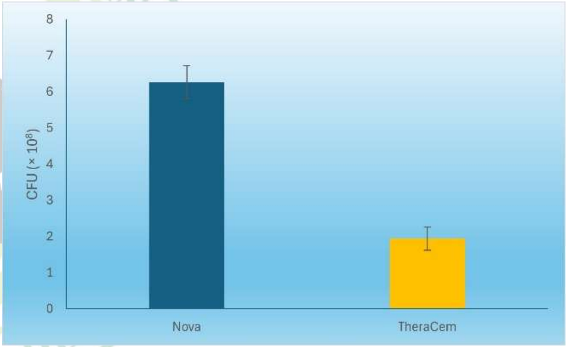

Figure 2: Column chart represents mean and SD values for bacterial counts in the two groups.

Correlation between Ca, F and P ion release and bacterial counts

There was no statistically significant correlation between Ca, F, P ion release and bacterial counts at all observation periods except after one day where there was a statistically significant inverse (negative) correlation between F ion release and bacterial counts (Correlation coefficient = -0.715, P <0.001). An increase in F ion release after one day is associated with a decrease in bacterial counts and vice versa (Table 3) Figure (3).

Table 3: Descriptive statistics and results of Spearman's correlation coefficient for correlation between Ca, F and P ion release and bacterial counts

Ion	Time (day)	Correlation coefficient (ρ)	*P*-value
Ca	1	-0.051	0.830
	14	-0.365	0.113
	28	-0.362	0.116
F	1	-0.715	<0.001*
	14	0	1
	28	0.356	0.123
P	1	-0.398	0.082
	14	-0.039	0.870
	28	-0.024	0.919

**: Significant at $P \leq 0.05$*

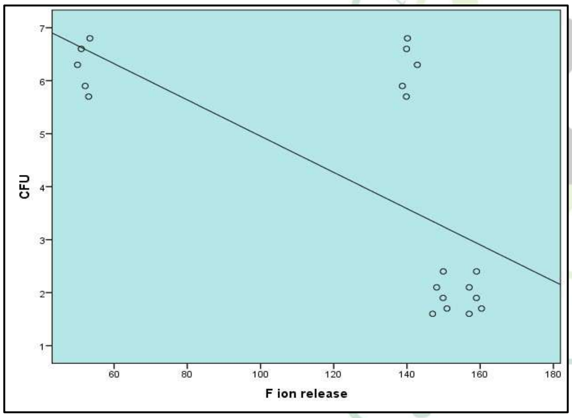

Figure 3: Scatter diagram representing inverse correlation between ions release and bacterial counts

Discussion

Self-adhesive cement has received a broad appeal due to its reduced working time. Currently, various adhesive cements offer optimum adhesion and uniform film thickness, Roesch-Ramos et al., 2022.[8]
Calcium-fluoride-releasing self-adhesive resin cement is considered a superior type of resin cement due to their ability to release ions like Ca, F and P. This ion release property has various positive consequences, including inhibition of bacterial development, remineralizing tooth structure, and reducing secondary caries formation, Conti et al. 2023. [9]

In the current study, we utilized calcium-fluoride releasing resin cement (TheraCem) that has been claimed to generate crystalline calcium hydroxide, potentially strengthening the mechanical interaction between the cement and dentin. It has antibacterial properties and may release Ca and F ions, which help remineralize tooth structures. As a result, it is deemed a bioactive substance, Tavangar et al., 2022.[10] and Youssef et al., 2023. [11]

TheraCem is a self-adhesive resin cement featuring Ca^{+} and phosphate ions Mostafa et al., 2021.[12]. Their release is reliant on the filler given by the cement; therefore, the existence of this element is related to the cement's major components and the fluoride ion. All these ions contribute to the remineralization process , Zahra'a et al., 2021.[13]

Inductively coupled plasma mass spectrometry (ICP-MS) is a potent chemical analysis instrument that employs plasma to ionize materials, allowing them to achieve greater accuracy and sensitivity in detecting ions in biological samples without requiring complicated sample preparation, Moirana et al.,2021.[14]

The ion chromatography (IC) method was preferred over gas chromatography and ion-selective electrodes because it can detect free fluoride ions and monitor low levels of fluoride, which other approaches cannot Aparajitha et al., 2021. [15]

Deionized water was chosen as a medium in our research because it is widely accessible and efficiently exhibits fluoride release from materials without the interference of minerals or organic molecules observed in other solutions. It is one of the most specific ways to estimate fluoride emission from dental materials. Additionally, more fluoride is emitted in deionized water than in artificial saliva, Al samollyet al.,2021.[16]

This study revealed that after one day of immersion in deionized water, Group II had a statistically significant lower mean F ion release than group I. After 14 and 28 days, group II demonstrated statistically significantly greater mean F ion release than group I. This finding is consistent with Raafat et al., 2018.[17], who discovered that extended contact with the storage media increases fluoride ion escaping through cement pores and fractures.

Our findings demonstrated that there was a statistically significant increase in mean Ca ion release after 14 days, followed by a statistically significant reduction after 28 days. However, the mean Ca ion release after 28 days showed a statistically significant higher value than the one-day amount of Ca ion release, suggesting that theracem's high calcium ion release may be therapeutic for the pulp, as calcium may promote growth factor-mediated deposition of pulpal dentin Huang et al.,2020.[18]

In this research, we apply the direct contact test, which is a quantitative approach for assessing the influence of direct contact between the material and bacteria on microbial survival in a standardized vitro environment. This test is useful since it is not impacted by the cement's diffusivity or dissolution, allowing it to overcome some of the drawbacks of the agar diffusion method Kato et al., 2023.[19]

The most prevalent cause of failure in fixed dental restorations is secondary caries along the margins AlSahafi et al., 2020.[20].Streptococcus Mutans got selected as the test microorganism because it causes secondary caries. Streptococcus Mutans have been used in various studies to evaluate the antibacterial characteristics of dental materials Demirel, et al., 2019.[21] and Kar et al., 2021.[22]

The current study's results against Streptococcus Mutans employing a direct contact test revealed that group I (Theracem)had higher antibacterial activity compared to group II (Nova resin).

Group I (TheraCem) had a statistically significant higher mean F ion release compared to group II (Nova resin). As stated by Gamal et al., 2024.[1], the fluoride content of restorative materials could be related to their antibacterial activity. Numerous studies have revealed that fluoride ions are effective as well as helpful in preventing and managing caries at particular doses. Fluoride suppresses the growth of oral streptococci in vitro.

The current study's antibacterial results against Streptococcus Mutans are in line with the study of Lila-Krasniqi et al., 2022.[23], who discovered that group I (Theracem) has antibacterial properties against Streptococcus Mutans and plaque development, as well as protecting properties for secondary caries, which are primarily owing to continuous fluoride releasing effect.

In line with Aučinaitė, et al., 2023.[4] , the anticariogenic effect of fluoride is well established because of their capacity to remineralize tooth structure. Fluoride may potentially interfere with Streptococcus Mutans' metabolic function. Fluoride inhibits bacterial enolase. Fluoride prevents bacterial growth and acid production by interfering with their metabolism.

Conclusion

Within the limitations of this study, we can conclude that:

1. Calcium – fluoride releasing self-adhesive resin cement (TheraCem®) has antibacterial activities greater than conventional self-adhesive resin cement (Nova Resin).
2. Greater quantities of calcium, phosphorus, and fluoride ions are released by calcium-fluoride releasing self-adhesive resin cement (TheraCem®), particularly during the early stages of immersion.

Funding: Not applicable.

Data availability: Data is available upon request.

Declarations

Ethics approval and consent to participate: This study was approved by the Ethical Committee of Faculty of Dentistry, Minia University RHDIRB2017122004 under the protocol number (802), 2023.

Competing interests: None.

References

1. Gamal SM, Masoud GI, El-Dessouky RA. Evaluation of time-dependent ion-release and antibacterial activity of three adhesive resin cements. Tanta Dental Journal. 2024;21(1):21-8.
2. Kawashima M, Yamaguchi S, Mine A, Li H, Imazato S. Novel testing method to evaluate the mechanical strength of self-adhesive resin cements with reflection of cement thickness. Dental Materials Journal. 2021;40(5):1235-42.
3. Elbanna A, Elmesellawy MY, El-Refai DA. Antibacterial behavior of calcium fluoride-modified tricalcium silicate cement against different oral bacterial strains. Ain Shams Dental Journal. 2024;34(2):76-87.
4. Aučinaitė R, Trumpaitė-Vanagienė R, Versockienė J, Lastauskienė E. Evaluation of the Antibacterial Activity Against Streptococcus mutans and Solubility of Different Dental Luting Cements In Vitro. Materials Science. 2023;29(1):111-8.
5. Nicholson JW, Coleman NJ, Sidhu SK. Kinetics of ion release from a conventional glass-ionomer cement. Journal of Materials Science: Materials in Medicine. 2021;32:1-10.
6. Gao Z, Xu Y, Kan Y, Li H, Guo R, Han L, et al. Comparison of antibacterial activity and biocompatibility of non-leaching nitrofuran bone cement loaded with vancomycin, gentamicin, and tigecycline. Journal of Orthopaedic Surgery and Research. 2023;18(1):569.
7. Dimitriadi M, Petropoulou A, Zafiropoulou M, Zinelis S, Eliades G. Degree of Conversion and Mechanical Properties of Modern Self-Adhesive Luting Agents. Applied Sciences. 2021;11(24):12065.
8. Roesch-Ramos L, Mora A, Moreno-MarÍN F, Ruiz M. In vitro study of the film thickness of six resin cements. ECORFAN Journal Bolivia. 2022:29-34.
9. Conti G, Veneri F, Amadori F, Garzoni A, Majorana A, Bardellini E. Evaluation of Antibacterial Activity of a Bioactive Restorative Material Versus a Glass-Ionomer Cement on Streptococcus Mutans: In-Vitro Study. Dentistry Journal. 2023;11(6):149.
10. Tavangar MS, Safarpour A, Torabi Parizi A, Shafiei F. Evaluating the shear bond strength and remineralization effect of calcium silicate-based and conventional self-adhesive resin cements to caries-affected dentin. Clinical and Experimental Dental Research. 2022;8(6):1630-7.
11. Youssef YB, Shalaby MM, Radwan SA. Assessment of Shear Bond Strength of Different Veneering Materials to Polyetherketonketon (PEKK). Egyptian Dental Journal. 2023;69(3):2153-64.
12. Mostafa NA, Mohammed SL, Thabet YGE. Effect of Different Materials of Primary Telescopic Crowns on The Frictional Fit of Pekkton Partial Denture Frameworks in Kennedy Class I. Ain Shams Dental Journal. 2021;22(2):105-11.
13. Zahra'a A, AL-Naimi AM, editors. Accumulative calcium ion release & EDX analysis from new generation self– adhesive resin cement. 1st International Ninevah Conference on Medical Sciences (INCMS 2021); 2021: Atlantis Press.
14. Moirana RL, Kivevele T, Mkunda J, Mtei K, Machunda R. Trends towards Effective Analysis of Fluorinated Compounds Using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Journal of Analytical Methods in Chemistry. 2021;2021(1):8837315.
15. Aparajitha R, Selvan PS, Ahamed AS, Bhavani S, Nagarajan V. Comparative evaluation of long-term fluoride release and antibacterial activity of an alkasite, nanoionomer, and glass ionomer restorative material–An in vitro study. Journal of Conservative Dentistry and Endodontics. 2021;24(5):485-90.
16. Alsamolly W, Sadek GEDM. Comparative assessment of antibacterial activity and fluoride release potential of Cention-N and different bioactive restorative materials: An in vitro study. Egyptian Dental Journal. 2021;67(3):2795-803.
17. Raafat R. Antibacterial effect of different types of glass ionomer restorations on streptcocus mutans. Egyptian Dental Journal. 2018;64(1-January (Fixed Prosthodontics, Dental Materials, Conservative Dentistry & Endodontics)):329-34.
18. Huang CT, Blatz MB, Arce C, Lawson NC. Inhibition of root dentin demineralization by ion releasing cements. Journal of Esthetic and Restorative Dentistry. 2020;32(8):791-6.
19. Kato G, Gomes PS, Neppelenbroek KH, Rodrigues C, Fernandes MH, Grenho L. Fast-setting calcium silicate-based pulp capping cements—integrated antibacterial, irritation and cytocompatibility assessment. Materials. 2023;16(1):450.
20. AlSahafi R, Balhaddad AA, Mitwalli H, Ibrahim MS, Melo MAS, Oates TW, et al. Novel crown

cement containing antibacterial monomer and calcium phosphate nanoparticles. Nanomaterials. 2020;10(10):2001.
21. Demirel G, Eryılmaz M, Şeberol H, Gür G. In vitro antibacterial activity of self-etch bio-active dental adhesives after artificial aging. European Oral Research. 2019;53(1):32-7.
22. Kar SK, Tripathi A, Jhonson M. Comparative Study of Antibacterial Activity of Five Luting Cements on Strains of Streptococcus mutans: An In Vitro Study. Journal of the California Dental Association. 2021;49(1):27-34.
23. Lila-Krasniqi ZD, Halili RB, Hamza V, Krasniqi S. Evaluation of antimicrobial effectiveness of dental cement materials on growth of different bacterial strains. Medical Science Monitor Basic Research. 2022;28:e937893-1.