



Experimental Glass Ionomer Cement Containing Gallic acid: Antibacterial Effect and Fluoride Release an *in vitro* Study

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Abstract

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AIM: The assessment of antibacterial effect and fluoride release on glass ionomer cement (GIC) modified by different concentrations of gallic acid (GA) and their effect on working and setting time.

METHODS: Four groups were tested, Group I (control non modified group) and Groups II-IV represent GIC modified by GA in three different concentrations (125 mg/ml, 62.5 mg/ml, and 31.25 mg/ml GA powder/GIC liquid, respectively). Antibacterial effect against *Streptococcus Mutans* (*S. Mutans*) was determined after 24 h by broth dilution method. Fluoride release was evaluated after 3 time intervals 24, 48, and 96 h using spectrophotometer. Working and setting time were measured in all groups by indentation method.

RESULTS: Increasing the concentration of GA significantly increases the antibacterial effect. For all time intervals, the highest fluoride release was observed in Group IV and the lowest were in Group I. After 24 h Groups II, III, and IV were significant to Group I, while after 48 and 96 h Group IV was significant to Group I. In addition, working and setting time significantly increased with increasing the GA concentration.

CONCLUSION: GA improves the antibacterial effect of GIC against *S. Mutans* and also improves the fluoride release. The increase in working and setting time of GA modified groups were still within the limit given by ISO 9917-1:2007 specifications.

Introduction

Dental caries is one of the most widespread infectious diseases in the world [1]. It is multifactorial disease which requires various factors such as the presence of fermentable sugar, host factors, and the presence of cariogenic microbial flora such as *Streptococcus Mutans* (*S. Mutans*) and *Lactobacillus Casei* [1]. Atraumatic restorative treatment (ART) is a procedure that involves the removal of carious tooth tissue using hand instruments and restoring the cleaned cavity with an adhesive restorative material, such as glass ionomer cement (GIC) [2]. This procedure is rapidly gaining acceptance, especially for very young children who experience extreme fear or anxiety. GIC have certain features that are superior to other restorative materials including; chemical adhesion to the tooth structure, cariostatic action by long term release of fluoride, capability of absorbing, and storing fluoride and ability to remineralize dental tissues [3]. Moreover, it has coefficient of thermal expansion and elastic modulus similar to dentin. But also it is subjected to criticism due to its high viscosity, lack of sufficient strength and weak antibacterial action of fluoride [2], [3].

Procedures employed in the cavity preparation for the treatment of dental caries lesion do not eliminate all the causative bacteria. The bacteria left in the dentin possibly leads to loss of marginal seal, secondary caries, and consequently pulp disease [4].

Many modifications were made on glass ionomer by incorporating different additives such as chlorhexidine, nano-silver, nano-hydroxyapatite, casein, and bioactive glass particles to improve its antibacterial properties [5], [6], [7], [8]. Recently, natural extracts have gained even more attention as active agents into oral care products and dental materials. Many natural extract such as chitosan and propolis have potential antimicrobial activities which could lead to the production of safe, economical, and efficient alternative materials to be used in caries management [9], [10].

Gallic acid (GA) is a well-known natural antioxidant that is basically a secondary polyphenolic metabolite with $C_6H_2(OH)_3COOH$ chemical formula. It is extracted from various plants such as oak bark, tea leaves, apple peels, grapes, strawberries, pineapples, bananas, and caesalpinia mimosoides plant [11], [12]. In a previous study, GA exhibited an obvious antibacterial activity against *Salmonella Typhi* and *Staphylococcus aureus* [13].

In another study, it was proved that GA can inhibit the growth of cariogenic bacteria such as *S. Mutans*, *Streptococcus Sobrinus*, *Actinomyces viscosus*, *Lactobacillus Casei*, *Lactobacillus acidophilus*, and periodontopathic bacteria such as *Porphyromonas gingivalis* and *Fusobacterium nucleatum*. It also can inhibit the in vitro formation of *S. Mutans* biofilms [14].

No previous study was conducted to modify the GIC by GA. Hence, the present study was a trial to modify conventional GIC by different concentrations of GA. The null hypothesis was that the modification of GIC with GA might not improve its antibacterial effect and adversely affect fluoride release, working, and setting time.

Methods

Materials

The materials used in the study were, water based powder and liquid GIC (Ketac Cem radiopaque, 3M ESPE, Germany), and GA powder (Sigma-Aldrich, USA).

Methods

Grouping of samples

Four groups were prepared according to a previous pilot study; Group I conventional GIC powder and liquid, Group II- IV GIC powder mixed with GIC liquid modified by GA in three different concentrations 125 mg/ml, 62.5 mg/ml, and 31.25 mg/ml, respectively, for each group.

GA liquid preparation

For each Group II-IV GA powder was measured using sensitive balance (Kern ABJ-NM/ABS 220-4N, UK) according to the previously mentioned concentrations and inserted into a tube, then GIC liquid was dispensed by using micropipette and mixing done by a vortex mixer (VM-300, Taiwan) for 10 s.

Antibacterial test

Sample preparation

A total number of 20 disc shaped samples, $n = 5$ for each group were prepared in a split mold 10 mm diameter \times 2 mm thickness. The molds were inserted on a glass slab which was covered by a celluloid matrix, for each sample 331 mg of GIC powder was mixed with 100 μ l of GIC liquid prepared previously for each group. The mix was packed into the

mold, covered with celluloid matrix strip and another glass slab. Then, a 50 gm static load was applied on the top of the glass slab for 30 s. After 15 min samples were removed from its molds, checked for defects and stored in an incubator (Bernareggio, Italia), at $36 \pm 1^\circ\text{C}$ in relative humidity for 24 h to ensure complete setting. After that, samples were finished with finishing discs (Sof-Lex, 3M ESPE, USA) and exposed to ultraviolet radiation to prevent contamination during the antibacterial test.

Inoculum preparation

The inoculum was prepared by growing *S. Mutans* in Luria Bertani broth (LB broth media) which is the common media for growth *S. Mutans*. A 0.5 McFarland standard was prepared by mixing 0.05 mL of 1.175% barium chloride dihydrate ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$), with 9.95 mL of 1% sulfuric acid (H_2SO_4). The suspension of *S. Mutans* in LB broth media was adjusted to achieve turbidity equivalent to the prepared McFarland standard and comparison was done with spectrophotometer (Prietest analyzer, Germany). This results in a suspension containing approximately $1-2 \times 10^8$ colony forming units/mL for bacteria.

Broth dilution test procedure

All test procedures were performed in aseptic conditions, a total of 20 weatherman tubes were filled with 3 ml of LB broth media, sterilized, and mixed with 20 μ l of the prepared inoculum. Each sample was placed in a tube, and then tubes were sealed with cotton plugs and covered by a parafilm to prevent the contamination.

Another 2 tubes were added as a positive and negative control, 3 ml broth media were added to each tube. For the positive control tube 20 μ l of the standard inoculum was added, then both tubes were sealed as mentioned before (Figure 1).

All tubes were incubated for 24 h at $36 \pm 1^\circ\text{C}$ before evaluating the absorbance with spectrophotometer.

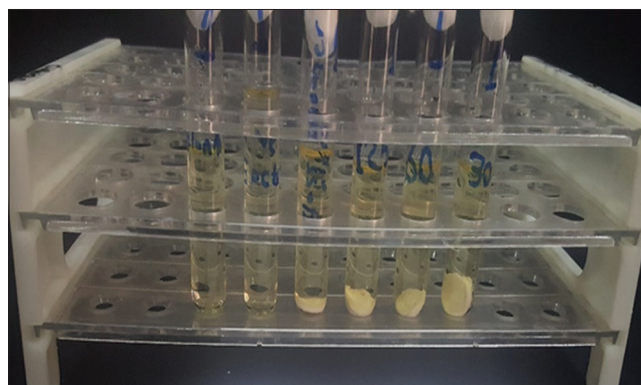


Figure 1: Samples of each group with +ve and -ve control

Fluoride release test

A total of 40 samples, $n = 10$ for each group were prepared in a split mold 8 mm diameter \times 2 mm thickness. Within the preparation of samples, a nylon thread was inserted into each during packing. The samples were stored in a relative humidity at $36 \pm 1^\circ\text{C}$ for 24 h to ensure complete setting. After that, samples were suspended individually in well-sealed tubes containing 10 ml deionized water and incubated at $36 \pm 1^\circ\text{C}$ to be tested after 24 h. The immersed deionized water was collected for each tube and buffered by 2 ml Spands reagent (Hach Company World Headquarters, Loveland, CO, USA) then, measured using a previously calibrated spectrophotometer (Hach DR 4000, Loveland, CO, USA). Fluoride ions detected in the solution were expressed in ppm (mg/L).

All the discs were dried gently by a tissue and reimmersed in another 10 ml deionized water and the previous procedures were repeated for each sample at 48 and 96 h time intervals.

Working and setting time measurement

Working time and setting time tests were performed regarding ANSI/ADA specification No. 66. For working time a total of 40 samples, $n = 10$ for each group were prepared in a mold 9.5 mm diameter \times 4.8 mm thickness. Test was carried out using a custom made device which has a flat end indenter with a diameter 2 ± 0.05 mm and mass of 28 ± 0.25 g. The indenter tip was fixed at distance approximately 5 mm above the surface of the sample (Figure 2). After 2 min from starting the mix, the indenter was carefully applied perpendicular onto the surface of the sample to make an indentation. The procedure was repeated every 10 s until the indenter failed to make an indentation when viewed using a magnifying lens ($\times 10$). The indenter was cleaned after each application. For each sample, the time elapsing between the start of mixing till the failure to make an indentation was recorded as the working time.

Another 40 samples, $n = 10$ for each group were prepared in the same mold for setting time measurement. The same device was used with a flat end indenter with a diameter 1.0 ± 0.01 mm and mass of 400 ± 5 g. The indenter tip was fixed at distance approximately 5 mm above the surface of the sample. After 2.5 min from starting the mix, the indenter was carefully applied perpendicular onto the surface of the sample to make an indentation. The procedure was repeated every 30 s equal interval, until the indenter failed to make an indentation when viewed using a magnifying lens ($\times 10$). For each sample, the time elapsed until failure of indentation or to penetrate was recorded as setting time.



Figure 2: The custom made device used for working time measurement

Statistical analysis

Collected data were analyzed using GraphPad Prism computer software version 8 (California, USA). All data were quantitative variables, so illustrated as mean and standard deviation. One-way analysis of variance test was done for pairwise comparisons followed by Tukey's *post hoc* analysis to detect significance between groups, differences between groups were considered significant when $p < 0.05$.

Results

The antibacterial test results are presented in Table 1 and Figure 3. Group I showed a non-significant difference regarding bacterial turbidity comparing to +Ve control group. Interestingly, Groups II, III, and IV significantly lowered levels of bacterial turbidity comparing to Group I and +Ve control group.

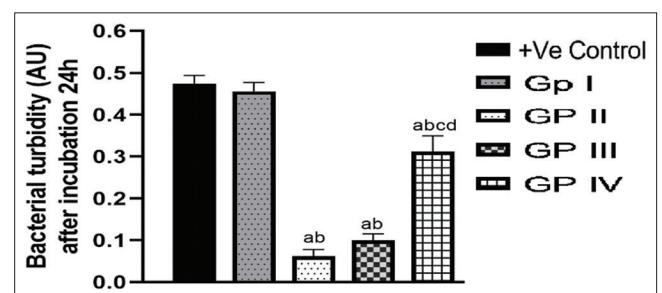


Figure 3: Bar chart representing mean and significance for antibacterial test.

Table 1: Mean, SD and decreasing percent of mean between different groups comparing to + control for antibacterial test

Groups	Mean bacterial turbidity (AU)	SD	Decreasing percent of mean comparing to+Ve Control
+Ve Control	0.47	0.045	0
Group I	0.46	0.047	2.1
Group II	0.06 ^{ab}	0.036	87.2
Group III	0.10 ^{ab}	0.035	78.7
Group IV	0.31 ^{abcd}	0.084	34

AU: Absorbance unit, ^aSignificantly different from+Ve Control, ^bSignificantly different from Group I, ^cSignificantly different from Group II, ^dSignificantly different from Group III, p < 0.05, SD: Standard deviation.

Fluoride release test results are represented in Table 2 and Figure 4. Regarding 24 h interval results, Groups II, III, and IV showed a significant increase in fluoride release as compared to Group I.

Table 2: Mean, SD, and increasing percent of mean between different groups comparing to Group I for fluoride release test

Time	Groups	Mean fluoride release (ppm)	SD	Increasing percent of mean comparing to GP I
24 h	Group I	4.55	0.685	0
	Group II	5.84 ^a	1.180	22
	Group III	5.94 ^a	0.988	23.4
	Group IV	6.41 ^a	0.490	29
48 h	Group I	3.53	0.780	0
	Group II	3.66	1.151	3.6
	Group III	4.35	1.184	18.9
	Group IV	4.88 ^a	0.606	27.7
96 h	Group I	2.36	0.615	0
	Group II	2.67	0.983	11.6
	Group III	2.91	0.864	18.9
	Group IV	3.95 ^a	1.257	40.2

^aSignificantly different from group I, p = 0.002 for 24 h results, p = 0.031 for 48 h results and p = 0.033 for 96 h results, SD: Standard deviation.

Regarding 48 and 96 h interval results, Group IV showed a significant increase in fluoride release as compared to Group I, while Groups II and III did not show any significant difference on fluoride release when compared with the Group I. In addition, for all time intervals there was no significant difference between Groups II, III, and IV.

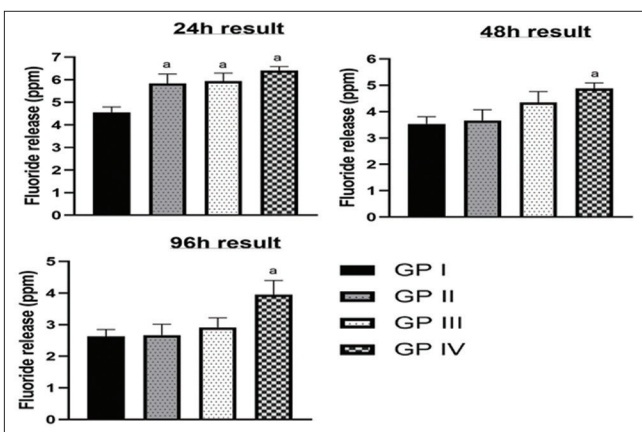


Figure 4: Bar chart representing mean and significance for fluoride release test

Working and setting time test results are represented in Tables 3 and 4, Figures 5 and 6, respectively. All modified groups showed a significant increase in working and setting times as compared to Group I. In addition, they were significantly different to each other.

Table 3: Mean, SD, and increasing percent of mean between different groups comparing to Group I for working time test

Groups	Mean working time (Seconds)	SD	Increasing percent of mean comparing to Group I
Group I	262.7	8.028	0
Group II	365.8 ^a	4.104	28.1

Group III	350.6 ^{ab}	6.059	25.1
Group IV	327.9 ^{abc}	5.384	19.9

^aSignificantly different from Group I, ^bSignificantly different from Group II, ^cSignificantly different from Group III, p < 0.05, SD: Standard deviation.

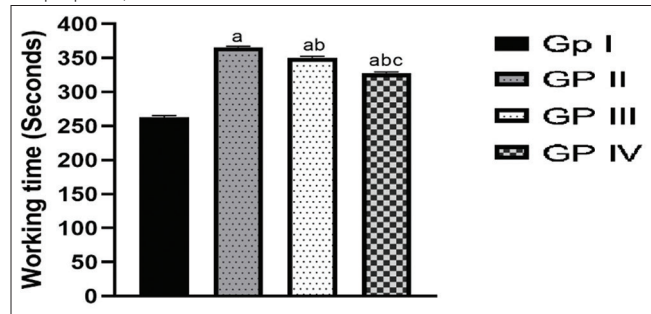


Figure 5: Bar chart representing mean and significance for working time test

Discussion

GICs have been the most commonly used water based cements for final cementation of dental crowns, bridges, orthodontic brackets, and ART. Several properties make GIC a material of choice among which, their ability to bond chemically to enamel and dentin, biocompatibility and ability to release fluoride ions over a prolonged period of time [2], [3].

Table 4: Mean, SD, and increasing percent of mean between different groups comparing to Group I for setting time test

Groups	Mean setting time (Seconds)	SD	Increasing percent of mean comparing to Group I
Group I	399.8	9.589	0
Group II	519.9 ^a	7.415	23.1
Group III	495.3 ^{ab}	8.206	19.3
Group IV	469.6 ^{abc}	7.763	14.9

^aSignificantly different from Group I, ^bSignificantly different from Group II, ^cSignificantly different from Group III, p < 0.05, SD: Standard deviation.

The previous studies have shown that GIC release approximately 10 ppm of fluoride during the first 24 h following insertion into the cavity [15]. However, microorganisms have been found to be viable for at least a period of 2 years under the GIC. For this reason, the accuracy of ART may be questionable [16], so modification of GIC with a strong antibacterial agent could give a great benefit.

GA is a natural herbal extract, which has a history of safety and effectiveness against bacteria compared to other chemical agents as proved in a previous studies [13], [14], [17], [18], [19]. In the present study, GA was used to boost the antibacterial effect of

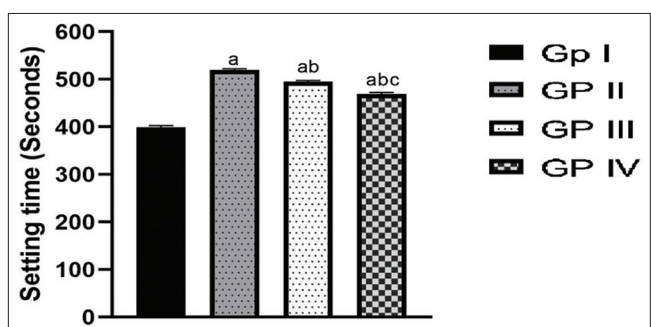


Figure 6: Bar chart representing mean and significance for setting time test

GIC. It was added in to the liquid of the GIC in three different concentrations according to the pilot study, based on minimal three prospective concentrations, which found to be effective against *S. Mutans* that is the main pathogen in dental caries [20].

Broth dilution method was used as it is a direct contact method for assessment of the antibacterial effect, which has better simulation to the oral cavity situation than other testing methods [21].

According to the results of antibacterial testing, addition of GA with different concentrations significantly decrease the antibacterial turbidity for group II-IV respectively as compared to +ve control group, also all groups were significant to each other. The results supported previous studies which confirm the antibacterial effect of GA against gram +ve and -ve bacteria that increase with increasing its concentration [13], [14], [17], [18], [19].

The antibacterial mechanism was explained by the ability of GA to make irreversible changes in the bacterial cell membrane properties. It might lead to change in the surface charge, occurrence of local rupture or pore formation that lead to consequent leakage of essential intracellular constituents [17].

GICs are the most widely used fluoride releasing restorative materials in dentistry. Fluoride release can be affected by many factors such as cement solubility, acidity, presence of surface coatings, fluoride concentration in glass particle, and modification of GIC [22]. Increasing the acidity, increases the dissolution of the GIC leading to greater elution of ions including the fluoride (F^-), hydrogen (H^+), and aluminum ions (Al^{+3}). Hence, the release of fluoride in high acidic conditions occurs with complexation [22], [23]. The Al^{+3} may result in species such as AlF_4^- and H^+ may cause the formation of either the complex HF_2^- or undissociated HF. None of these possible fluoride species yields free fluoride ions, so they are not detectable with fluoride ion selective electrodes [24], [25]. This explained the increase in fluoride release for all modified groups and its decrease by increasing GA concentration.

At different time interval, the highest fluoride release was after 24 h and significantly decreased gradually after 48 and 96 h. It could be explained by the greater ionic movement after 24 h interval. This phenomena is called burst effect which is attributed to the rapid dissolution of fluoride from the outer surface into the solution by reaction of the polyacrylic acid with the fluoride containing glass particles during the setting reaction [26], [27]. However, the drop of the fluoride levels that occurred in the subsequent days might be caused by the slower particles dissolution and release through the material pores [26], [27].

Setting reaction of GICs is an acid base reaction, the reaction involves the dissolution of the glass particle's surface by the polyacrylic acid, followed by the release of metal ions such as Ca^{2+} and Al^{3+} ions.

Then Ca^{2+} react with the carboxylate groups of the polyacrylic acid, resulting in the formation of calcium polyacrylate matrix at the end of initial setting [25], [27].

A small amount of complexing agent has been found to alter the setting behavior of GIC. Tartaric acid (TA) has been shown to be the most effective of these additives, since it tend to prolongs working time [25], [28]. In the presence of TA, metal ions are still extracted from the aluminosilicate glass, but on release, they apparently react preferentially with the TA to form the tartrate and this delays the formation of the polysalt and prolong the working time [28], [29], [30].

In this study, working and setting time increase with increasing the GA concentration, it can be explained by the simulation of behavior of GA and TA due to similarity in composition $C_6H_2(OH)_3COOH$ and $COOH(CHOH)_2COOH$, respectively. GA may lead to hindrance in the crosslinking of the calcium with the polyacid chain, which may have delayed the initial matrix formation [30]. However, the delay in working and setting time still within the limit given by ISO 9917-1:2007 specifications, according to which the net setting time should be within 90 to 480 s. A slight increase in initial setting time is beneficial for the dentist as it offers more time for manipulation of cement.

The reaction of TA with the metal ions forms the tartrate. The reaction may also occur with fluoride ions which yield a bounded ion and in the presence of high concentrations of GA; it may react with the released ions like the TA and affect the fluoride release [31].

Conclusion

It was concluded that, the addition of GA to GIC improve the antibacterial effect against *S. Mutans* and also improve the fluoride release. The increase in working and setting time of GA modified groups were still within the limit given by ISO 9917-1:2007 specifications.

References

1. Heng C. Tooth decay is the most prevalent disease. Fed Pract. 2016;33(10):31-3. PMID:30766141
2. Leal S, Bonifacio C, Raggio D, Frencken J. Atraumatic restorative treatment: Restorative component. Monogr Oral Sci. 2018;27:92-102. <https://doi.org/10.1159/000487836> PMID:29794453
3. John F. McCabe, Yan Z, Al Naimi OT, Mahmoud G, Rolland SL. Smart materials in dentistry. Aust Dent J. 2011;56(1):3-10.
4. Botelho MG. Inhibitory effects on selected oral bacteria of

- antibacterial agents incorporated in a glass ionomer cement. *Caries Res.* 2003;37:108-14. <https://doi.org/10.1159/000069019> PMID:12652048
5. Khoroushi M, Keshani F. A review of glass-ionomers: From conventional glass-ionomer to bioactive Glass-ionomer. *Dent Res J (Isfahan).* 2013;10(4):411-20. PMID:24130573
 6. Takahashi Y, Imazato S, Kaneshiro AV, Ebisu S, Frencken JE, Tay FR. Antibacterial effects and physical properties of glass-ionomer cements containing chlorhexidine for the ART approach. *Dent Mater.* 2006;22(7):647-52. <https://doi.org/10.1016/j.dental.2005.08.003> PMID:16226806
 7. Moshaverinia A, Ansari S, Moshaverinia M, Roohpour N, Darr JA, Rehman I. Effects of incorporation of hydroxyapatite and fluoroapatite nanobioceramics into conventional glass ionomer cements. *Acta Biomater.* 2008;4(2):432-40. <https://doi.org/10.1016/j.actbio.2007.07.011> PMID:17921077
 8. Paiva L, Fidalgo TK, da Costa LP, Maia LC, Balan L, Anselme K, *et al.* Antibacterial properties and compressive strength of new one-step preparation silver nanoparticles in glass ionomer cements (NanoAg-GIC). *J Dent.* 2018;69:102-9. <https://doi.org/10.1016/j.jdent.2017.12.003> PMID:29253621
 9. Apurva M, Ramesh KP, Natesan M. Antibacterial effect and physical properties of chitosan and chlorhexidine-cetrimide modified glass ionomer cements. *J Indian Soc Pedod Prev Dent.* 2017;35(1):28-33. <https://doi.org/10.4103/0970-4388.199224> PMID:28139479
 10. Elgamily H, Ghallab O, El-Sayed H, Nasr M. Antibacterial potency and fluoride release of a glass ionomer restorative material containing different concentrations of natural and chemical products: An in-vitro comparative study. *J Clin Exp Dent.* 2018;10(4):e312-20. <https://doi.org/10.4317/jced.54606> PMID:29750090
 11. Yoon CH, Chung SJ, Lee SW, Park YB, Lee SK, Park MC. Gallic acid, a natural polyphenolic acid, induces apoptosis and inhibits proinflammatory gene expressions in rheumatoid arthritis fibroblast-like synoviocytes. *Joint Bone Spine.* 2013;80(3):274-9. <https://doi.org/10.1016/j.jbspin.2012.08.010> PMID:23058179
 12. Li K, Guilin G, Junxiang Z. Antibacterial activity and mechanism of a laccase catalyzed chitosan gallic acid derivatives against *Escherichia coli* and *Staphylococcus aureus*. *J Food Control.* 2018;9(2):234-43.
 13. Anchana C, Aphiwat T, Rakariyatham N. Antimicrobial gallic acid from *Caesalpinia mimosoides* Lamk. *J Food Chem.* 2007;100(3):1044-8.
 14. Kang MS, Oh JS, Kang IC, Hong SJ, Choi CH. Inhibitory effect of methyl Gallate and Gallic acid on oral bacteria. *J Microbiol.* 2008;46(6):744-50.
 15. Mazzaoui SA, Burrow MF, Tyas MJ. Fluoride release from glass ionomer cements and resin composites coated with a dentin adhesive. *Dent Mater.* 2000;16(3):166-71. [https://doi.org/10.1016/s0109-5641\(00\)00003-8](https://doi.org/10.1016/s0109-5641(00)00003-8) PMID:10762676
 16. Schriks MC, van Amerongen WE. Atraumatic perspectives of ART: Psychological and physiological aspects of treatment with and without rotary instruments. *Community Dent Oral Epidemiol.* 2003;31(1):15-20.
 17. Borges A, Ferreira C, Saavedra MJ, Simoes M. Antibacterial activity and mode of action of ferulic and gallic acids against pathogenic bacteria. *Microb Drug Resist.* 2013;19(4):256-65. <https://doi.org/10.1089/mdr.2012.0244> PMID:23480526
 18. Shao D, Li J, Tang R. Inhibition of gallic acid on the growth and biofilm formation of *Escherichia coli* and *Streptococcus mutans*. *J Food Sci.* 2015;80(6):1299-305. <https://doi.org/10.1111/1750-3841.12902> PMID:25974286
 19. Kang J, Liu L, Liu M, Wu X, Li J. Antibacterial activity of Gallic acid against and its effect on biofilm *Shigella flexneri* formation by repressing gene expression. *J Food Control.* 2018;7(1):147-54.
 20. Lemos JA, Palmer SR, Zeng L, Abranches J. The biology of *Streptococcus mutans*. *Microbiol Spectr.* 2019;7(1):1110-28.
 21. Balouiri M, Sadiki M, Koraichi S. Methods for in vitro evaluating antimicrobial activity: A review. *J Pharm Anal.* 2016;6(2):71-9. <https://doi.org/10.1016/j.jpha.2015.11.005> PMID:29403965
 22. Divya KP, Khijmatgar S, Chowdhury A. Factors influencing fluoride release in atraumatic restorative treatment (ART) materials: A review. *J Oral Biol Craniofac Res.* 2019;9(4):315-20. <https://doi.org/10.1016/j.jobcr.2019.06.015> PMID:31334004
 23. Wiegand A, Buchalla W, Attin T. Review on fluoride releasing restorative materials, fluoride release and uptake characteristics, antibacterial activity and influence on caries formation. *Dent Mater.* 2007;23:343-62. <https://doi.org/10.1016/j.dental.2006.01.022> PMID:16616773
 24. Jackson GE. The existence of AlF_4^- in aqueous solution and its relevance to phosphorylase reaction. *Inorg Chem Acta.* 1988;151(4):273-6.
 25. Sidhu SK, Nicholson JW. A review of glass-ionomer cements for clinical dentistry. *J Funct Biomater.* 2016;7(3):16. <https://doi.org/10.3390/jfb7030016> PMID:27367737
 26. Attar N, Turgut MD. Fluoride release and uptake capacities of fluoride-releasing restorative materials. *Oper Dent.* 2003;28(4):395-402. PMID:12877425
 27. Neelakantan P, John S, Anand S, Sureshbabu N, Subbarao C. Fluoride release from a new Glass-ionomer cement. *Oper Dent.* 2011;36(1):80-5. <https://doi.org/10.2341/10-219-LR> PMID:21488733
 28. Craig R, Powers J. *Restorative Dental Materials.* 14th ed. London, UK: Mosby, Elsevier; 2019.
 29. Anusavice KJ, Shen C, Rawls R. *Phillips' Science of Dental Materials.* 13th ed. Amsterdam, Netherlands: Elsevier; 2021.
 30. Prosser HJ, Richards CP, Wilson AD. NMR spectroscopy of dental cements II. The role of tartaric acid in Glass-Ionomer cements. *J Biomed Mater Res.* 1982;16(4):431-45.
 31. Nicholson JW, Brookman PJ, Lacy OM, Wilson AD. Fourier transform infrared spectroscopic study of the role of tartaric acid in Glass-Ionomer dental cements. *J Dent Res.* 1988;67(12):1451-4.