ISSN 0973-2063 (online) 0973-8894 (print)
Bioinformation 20(10): 1368-1373 (2024)

BIOINFORMATION
Discovery at the interface of physical and biological sciences
BIOMEDICAL INFORMATICS
www.bioinformation.net
Volume 20(10)
Research Article

Received October 1, 2024; Revised October 31, 2024; Accepted October 31, 2024, Published October 31, 2024
DOI: 10.6026/9732063002001368

BIOINFORMATION 2022 Impact Factor (2023 release) is 1.9.

Declaration on Publication Ethics:
The author's state that they adhere with COPE guidelines on publishing ethics as described elsewhere at https://publicationethics.org/. The authors also undertake that they are not associated with any other third party (governmental or non-governmental agencies) linking with any form of unethical issues connecting to this publication. The authors also declare that they are not withholding any information that is misleading to the publisher in regard to this article.

Declaration on official E-mail:
The corresponding author declares that lifetime official e-mail from their institution is not available for all authors

Comments from readers:
Articles published in BIOINFORMATION are open for relevant post publication comments and criticisms, which will be published immediately linking to the original article without open access charges. Comments should be concise, coherent and critical in less than 1000 words.

Disclaimer:
The views and opinions expressed are those of the author(s) and do not reflect the views or opinions of Bioinformation and (or) its publisher Biomedical Informatics. Biomedical Informatics remains neutral and allows authors to specify their address and affiliation details including territory where required. Bioinformation provides a platform for scholarly communication of data and information to create knowledge in the Biological/Biomedical domain.

Edited by Neelam Goyal & Shruti Dabi
E-mail: dr.neelamgoyal15@gmail.com & shrutidabi59@gmail.com; Phone +91 98188 24219
Citation: Wahab *et al.* Bioinformation 20(10): 1368-1373 (2024)

Anti-microbial and micro-leakage properties of orthodontic cement

Afreen Wahab*, Chanamallappa Ganiger, Renuka Pawar, Sandesh Phaphe & Yusuf Ronad

Department of Orthodontics and Dentofacial Orthopaedics, School of Dental Sciences, Krishan Vishwa Vidyapeeth (Deemed to be University), Karad, Satara, Maharashtra, India; *Corresponding author

Affiliation URL:
https://kvv.edu.in/

Author contacts:
Afreen Wahab - E - mail: afugujarat@gmail.com
Chanamallappa Ganiger - E - mail: channapparganiger@gmail.com
Renuka Pawar - E - mail: drrenukapawar@hotmail.com
Sandesh Phaphe - E - mail: sandeshphaphe2541@gmail.com

ISSN 0973-2063 (online) 0973-8894 (print)
Bioinformation 20(10): 1368-1373 (2024)

Yusuf Ronad - E - mail: dryusufr14@gmail.com

Abstract:
Glass Ionomer Cement (GIC) is used for cementing orthodontic bands because of its anti-cariogenic property, which is attributed to the release of fluoride. Therefore, it is of interest to assess the antimicrobial property (AM-P) and micro-leakage (ML) of GIC incorporated with different concentration of N-acetylcysteine (N-AC) & copper nanoparticle (Cu-NP). Our study composed of 5 groups i.e. group I is control with different concentration of N-AC & Cu-NP involving each group with 8 samples. We found that, group V showed the highest ZOI, while the ML was seen highest for group I with a score of 2.2 ± 1.09 and the least score was recorded for Group III (0.8± 0.37), thus addition of 2% Cu-NP and 15% N-AC resulted in minimal ML. We conclude that increase in concentration of N-AC & CU-NP AM-P efficiency also increases; on the other hand, increase in the concentration of N-AC & Cu-NP did not decrease the ML.

Keywords: Glass Ionomer Cement (GIC), antimicrobial property (AM-P), micro-leakage (ML), N-acetylcysteine (N-AC), copper nanoparticle (Cu-NP).

Background:
Research have found that, Orthodontics and Dentofacial Orthopedics, is an integral discipline within the realm of dentistry, focus on the intricate interplay between dental and facial structures **[1]**. This specialized field is dedicated to diagnosing, preventing, and treating anomalies of tooth and jaws, recognizing the profound impact of these irregularities on both aesthetics and functions. Through the use of various orthodontic techniques, devices, and personalized treatment plans, practitioners aim to not only achieve an aesthetically pleasing smile but also to optimize the overall facial harmony **[1].** Originally, removable orthodontic appliances (ROA) were employed to achieve the desired tooth movements(TM), but such appliances cannot cause bodily TM. Fixed orthodontic appliances (FOA) were introduced which involves bonding of anterior teeth and banding of posterior teeth with cements, this has since gained global acceptance (GGA). The problem with FOA is that they increase the surface area where bacteria can adhere, making it harder to maintain effective oral hygiene (OH). Thus, biofilms are more likely to develop at the tooth interface adjacent to fixed appliances (FA). This will causes more adherence of bacteria like *Streptococcus mutans* (SM) and Lactobacillus species(LB-S), this will lowers the oral pH below the critical level leads into formation of acids, like citric acid(CA) which will cause decalcification of enamel which eventually causes white spot lesions(WSL) observed by Zachrisson in 1977 **[1]**. Studies have also found that, SM and LB-S are linked to the onset and progression of WSL, later leading into dental caries (DC) and periodontal diseases (PD-D) by colonizing of bacteria around the teeth. Therefore, enamel (E) decalcification (DCF) is frequently observed in areas with plaque build-up, especially in orthodontic patients **[2]**. WSLs are evident as subsurface enamel porosity (SS-EP) that manifests as chalky opacities around brackets on the tooth surface. WSL are one of the most common adverse effects (AE) of FOT. The first dental cement utilized for band cementation (BC) was $ZnPO_4$. This cement is no longer employed due to its weak adhesion (WA) and tendency to dissolve in the oral environment (OE). A significant breakthrough in DC was achieved by Wilson and Kent in 1972 with the invention of GIC. Studies have shown that, N-AC is a mucolytic compound having wide margin of safety as a therapeutic. This is mainly used to reduce the viscosity of mucus in pulmonary compromised patients (PCP) **[3].** Nanotechnology(NT) has been utilized to enhance the effectiveness of dental materials(DM).CU-NP, in particular, have been investigated for their AB-P & ability to prevent ML. Research shows that, increased concentration of Cu-NP can enhance adhesive-dentin interfaces (ADI), improving their resistance to ML **[3]**. The final outcome of a FOT is to obtain the best possible esthetic along with the restoration of harmonious occlusion (HO). After de-bonding (DB), the enamel condition should be preserved close to its original form. E-DCF can cause irreversible damage and would lead to disruption of intact enamel surface **[4].** Therefore, it is of interest to assess AM and ML using different concentration of N-AC &Cu-NP in GIC.

Materials and Methods:
The current in-vitro study was conducted in a total 5 bottles of GIC using 3M ESPE KETAC CEM GLASS. Subsequently, they were divided into 5 groups named Group I (Conventional GIC), while experimental groups like Group II (GIC + 2% Cu-NP & 10% *N*-AC), Group III (GIC + 2% Cu-NP & 15% *N-AC*) Group IV (GIC + 2% Cu-NP & 15% *N-AC*) and Group V (GIC + 3% Cu-NP & 15% *N-AC*). For preparation of the samples, SM strain was procured, cultured with selective media and stock was prepared. Sterile Muller Hinton Agar(S-MHA) was poured into plates, with the depth of the medium set at around four milli-meters. The plates were incubated IB) for 30 minutes in an incubator to eliminate excess moisture from the surface once solidified. Isolated specimens were used for sensitivity testing. Approximately 5-6 colonies of SM-S were selected and inoculated (IO) into nutrient broth (NB) using a wire loop (WL). The broth culture (BT-C) was then incubated at 35-37˚ C for 2-5 hours. A sterile cotton swab(S-CS) was dipped into the diluted IO and then swabbed onto MHA-plates (PL). Excess inoculum was removed with another CS. The Petri dishes (PD) were closed and left at room temperature for 5-10 min to allow the IO to dry, aiming for confluent growth. Well was prepared at seven mm diameter in each plate with the help of syringe, in order to keep distance for zone of inhibition (ZOI). Remaining agar was discarded. Disc on each well which have been inoculated was cultured with SM-S. All PL were incubated at 37˚C for 24-48

ISSN 0973-2063 (online) 0973-8894 (print)
Bioinformation 20(10): 1368-1373 (2024)

hours. After incubation(IC), the area of inhibition around each well was observed as shown in (**Figure 1 & 2).**

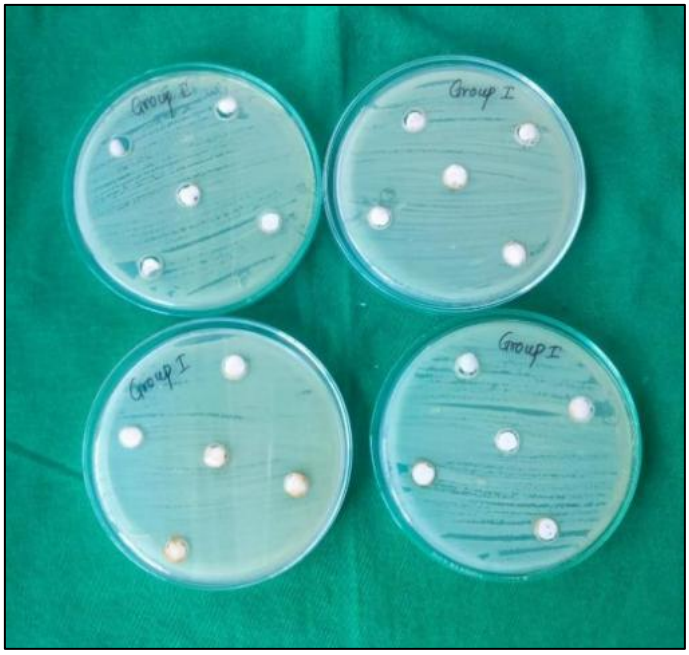

Figure 1: IC-C plates of group I

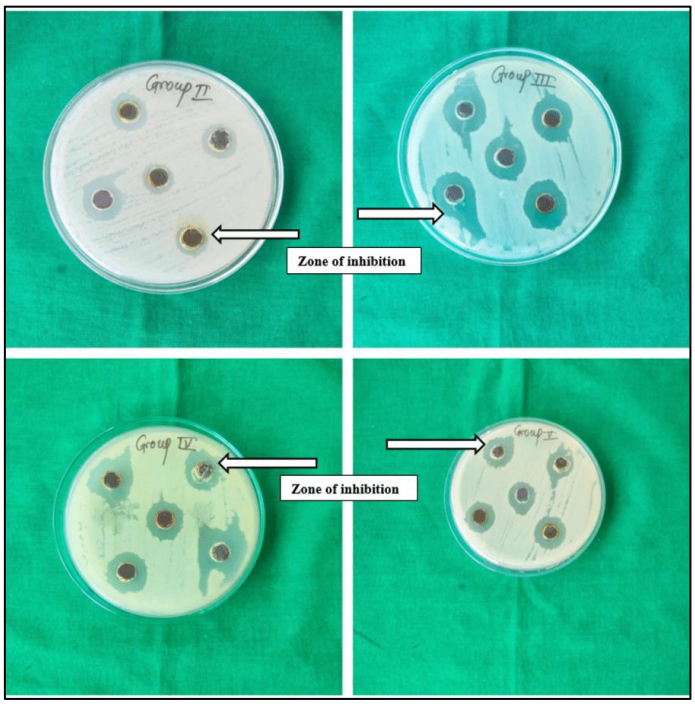

Figure 2: IC-C-PL of group II, III, IV and V showing ZOI

The diameter (DIA) of the IH - ZOI caused by the sample against bacteria was measured. Using a digital caliper (DG-CL), the DIA-IH-ZOI (specimens + IB-Z) was measured in mm after 48 hours, and the average measurement was recorded as the day 1 value. The bacterial population usually dies due to the release of toxic metabolites if cultures were kept for a long duration. Hence, on day two, fresh agar plates (FAPL) were used and cultured, and the specimens were transferred, and IC and IN-Z was calculated. For ML evaluation, 40 PM teeth obtained from the Department of Oral and Maxillofacial Surgery (OMFS) were separated into 5 groups, with each group containing 8 teeth. The teeth underwent cleaning with pumice (PU), followed by rinsing with distilled water, and thorough drying with compressed air. Subsequently, bands were selected, pinched, and optimally adapted to the crown of each tooth. Then for each tooth band cementation was done for respective groups and then placed in distilled water for 24 hours to prevent dehydration. After cementation, the samples was subjected to 5000 thermo cycles in thermo cycling unit which would simulate six months of temperature changes in oral environment as shown in (**Figure 3)**.

Figure 3: Samples of each groups kept in thermo-cycling unit

The apices of all teeth with bands were sealed using sticky wax to prevent dye seepage, and nail polish was applied over the tooth surfaces to prevent dehydration, leaving a 1 mm gap around the bands. Afterward, all teeth were immersed in water once the nail polish had dried. To assess ML all samples were

ISSN 0973-2063 (online) 0973-8894 (print)
Bioinformation 20(10): 1368-1373 (2024)

immersed in a 0.5% methylene blue solution (MBS) for 24 hours at room temperature as shown in (**Figure 4)**.

Figure 4: Samples of each group immersed in 0.5% methylene blue solution

After removal, teeth were rinsed in tap water to remove superficial dye using a brush. Next, the samples were dried and encased in self-curing acrylic blocks (SC-AB), extending up to the occlusal surface of the bands. Using a low-speed diamond saw, the blocks were bisected in the labio-lingual direction (LL-D). Longitudinal sections from the middle portion of each tooth were cut at the occlusobuccal (OB) and occlusolingual surfaces (OL-S) as shown in (**Figure 5).**

Figure 5: Sectioning of tooth using a low-speed diamond saw

Table 1: Grading system

Score 0	**No dye penetration**
Score 1	Dye penetration to the extent of one occlusal third of the sealant-enamel joint surface
Score 2	Dye penetration to the extent of one middle third of the sealant-enamel joint surface
Score 3	Dye penetration to the extent of one apical third of the sealant-enamel joint surface

The specimens were examined under a stereomicroscope(SM) (20x magnification) to evaluate dye penetration (DP) along the cement-enamel interface (CEI). Each section was graded at both the buccal and lingual margins of the bands between the interfaces **(Table 1).**

Statistical analysis:

All analysis was performed using IBM SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, USA). Kolmogorov Smirnov and Shapiro wilk tests were applied. An inter-group comparison was conducted to compare AM-E & ML prevention among the 5 groups using Kruskal Wallis, ANOVA test followed by Mann Whitney U test for pairwise comparison. The mode of failure among the five groups was assessed using the Chi-square test.

Table 2: Comparison of am- resistance (r) (zoi- based)

Tests of Normalit							
	Group	**Kolmogorov-Smirnov**			**Shapiro-Wilk**		
		Statistic	df	Sig.	Statistic	df	Sig.
Zone of inhibition	Group I	.	6	.	.	6	.
	Group II	0.358	6	0.016 S	0.781	6	0.040 S
	Group III	0.248	6	0.200*	0.789	6	0.047 S
	Group IV	0.401	6	0.003 HS	0.687	6	0.005 HS
	Group V	0.266	6	0.200*	0.917	6	0.485

Table 3: Comparison of ml (based on dp)

Tests of Normality							
	Group	**Kolmogorov-Smirnov**			**Shapiro-Wilk**		
		Statistic	df	Sig.	Statistic	df	Sig.
Microleakage (labial)	Group I	0.367	5	0.026 S	0.684	5	0.006S
	Group II	0.492	6	0.000 HS	0.496	6	0.000 HS
	Group III	0.504	7	0.000 HS	0.453	7	0.000 HS
	Group IV	0.31	6	0.074	0.805	6	0.065
	Group V	0.349	5	0.046 S	0.771	5	0.046 S
Microleakage (lingual)	Group I	0.231	5	0.200*	0.881	5	0.314
	Group II	0.319	6	0.056	0.683	6	0.004
	Group III	0.504	7	0.000 HS	0.453	7	0.000 HS
	Group IV	0.209	6	0.200*	0.907	6	0.415
	Group V	0.367	5	0.026 S	0.684	5	0.006 HS

Results:

Table 2 shows that, the mean score of Group I is 0.1±0.00, group II is 2.63±0.16, group III is 2.19±0.13, group IV is 3.41±0.52 and group V is 4.11±0.14. The mean score is highest for group V and least for group I. Kruskal Wallis ANOVA test revealed highly statically significant difference between the groups (p = 0.000). **Table 2** shows that, both the variables showed high significance in group IV i.e. 0.003 and 0.005. **Table 3** shows that, the mean L-ML score of group I is 2.0±1.09, group II is 1.16±0.40, group III is 0.85±0.37, group IV is 1.83±1.32 and group V is 1.4±0.89. No significant difference observed between the groups. The mean lingual microleakage score of group I is 2.2±0.83, group II is 1.5±0.54, group III is 1.14±0.37, group IV is 1.66±1.21 and group V is 1.80±1.09. Thus found, no significant difference observed between the groups. **Table 3** showed, highly significance for group I, II &III at labial side while on the other hand, group II, III & V showed highly significance at lingual side.

Discussion:

Orthodontic treatment (OT) involves the use of braces, bands, and wires to move teeth into better positions within the jaw. This procedure is crucial not only for aesthetic purposes but also to enhance function and overall oral health (OH). It primarily involves attaching brackets and bands and applying the desired forces by inserting wires into the bracket slots **[5].** The major drawback seen with FO appliances is biofilm retention (BF-RT) and plaque accumulation (PQ-AM) that eventually leads to high oral microbial load (MB-L). As a result, it can lead to WSL, PD problems, E-DCF that can damage intact enamel surface (IES) **[5].** Hence, to prevent this harm to tooth structure integrity (TSI), various oral prophylaxis methods have been introduced for instance, chemical methods such as 0.2% CHX mouthwash, electric brushes (E-B), professionally teeth cleaning methods include use of U/S scalers. However, caries still remains to be the most prevalent condition amongst patients undergoing FOT. According to the literature, the bacteria involved for DC and PD-D are SM-S and LB-S **[6]**. Over the past two decades, GIC have gained popularity for band cementation (BD-CM) due to their capacity to adhere well to enamel and metal, as well as their ability to release and absorb fluoride (F). This cement has the inherit property of anti-cariogenic (A-CG) and AM activity by the release of F ions that helps in restraining the bacterial growth (B-G). Although having superior properties compared to most of the D- CM materials, GIC still presents with poor AM-P in an aqueous environment and marginal seal quality (MSQ) **[6].** Nanomaterials (NM) were introduced and widely used in dentistry to enhance the properties of GIC. One such NP widely used is Cu-NP. For a long time, Cu has shown AM effects **[6].** Gutiérrez *et al.* found that addition of Cu-NP copper nanoparticles did not affect several mechanical properties tested and higher concentration of Cu-NP produced ADI that are more resistant to ML. The Cu-NP significantly increased AM-A and also enhance the BS on the teeth interface, therefore inhibiting ML **[7].**

In our study, *N*-AC (10%&15%) & Cu-NP (2% and 3%) by weight were incorporated into GIC at different concentration to enhance its AM-P and reduce ML following BD-CM. 5 bottles of GIC powder, each containing 10 grams, were obtained and divided into 5 groups. Group I served as the control group with conventional GIC. Group II consisted of GIC + 2% Cu-NP and 10% *N*-AC. Group III involved GIC + 2% Cu-NP & 15% *N*-AC. Group IV contained + 3% Cu-NP & 10% *N*-AC, while Group V included GIC + 3% Cu-NP & 15% *N*-AC. The NP and *N*-AC were accurately weighed using an analytical scale for incorporation. Then the powder was placed into amalgam capsules (AM-CP) and submitted to the action of amalgamator (AMG) with vibration for 6 seconds. Moreover, SM-S was obtained and cultured on selective media to establish a viable stock. Next, SMHA was carefully poured into Petri plates (PP), ensuring a consistent depth(C-DP), and left to solidify. Afterward, 5-6 colonies of SM-S were meticulously selected for sensitivity testing (ST). These colonies were inoculated into a nutrient-rich broth (NRB) and placed in an appropriate incubation (ICB) environment for several hours to allow for growth. After an ICB period of 24-48 hours, the BT-C was spread evenly onto MHA-PL & allowed to dry naturally to remove excess moisture. Subsequently, each well was IC with SM-S-C. The PL was then placed in a controlled IB set at the optimal temperature for bacterial growth (BG). Following IB, the plates were carefully examined to observe any ZOI around the wells, indicating the effectiveness of the test substances against BA. DG-CP was used to measure both the specimens and the diameter of the ZOI after 48 hours. Each tooth was sectioned buccolingually into two halves using a stereomicroscope at 20X magnification, and dye penetration along the cement-enamel interfaces was evaluated. Scoring for dye penetration was conducted from both buccal and lingual margins of the bands following the grading system described by Souza SD *et al*. **[8]**.

The present study shows statistically significant differences (p-value < 0.05) in all the groups except control group i.e. group I. However, the values for subsequent groups (Group II, III, and IV) did not adhere to a normal distribution. Group V (GIC incorporated with 3% Cu-NP & 15% N-AC) displayed the highest ZOI, with an average value of 4.11±0.14 which indicates highest concentration of N-AC & Cu-NP has more efficient AM-A. Whereas, Group IV (GIC incorporated with 3% Cu-NP &10% N-AC) showed ZOI with a mean value of 3.41 ± 0.524 which indicates the AM-A less than the group V. There was minimal variation in the AM effectiveness between group II & III. Our study also found varying levels of AM-A among different formulations of GIC modified with N-AC & Cu-NP. Group I exhibited minimal AM-A with the smallest ZOI (0.1 mm), indicating limited effectiveness. Conversely, Groups II, III, IV & V showed increased AM-A with mean zone diameters of approximately 2.6 ± 0.16 mm, 2.1 ± 0.13 mm, 3.4 ± 0.52 mm, and 4.1 ± 0.14 mm, respectively. The results indicate that higher concentration of Cu-NP & N-AC corresponded to greater AM efficacy, particularly group V, demonstrating the highest AM-A. These findings align with previous studies that have shown

enhanced antibacterial properties (AB-P) of modified GIC formulations containing similar additives **[9].** Significant differences were observed in pairwise comparisons between group II, IV&V (p = 0.004), underscoring the influence of formulation on AM effectiveness. Overall, the study underscores the potential of modifying GIC with Cu-NP & N-AC to enhance its AM-P, suggesting promising applications in DM aimed at reducing BG and improving clinical outcomes. ML tests were done using DP were conducted across all sample groups. The highest ML value was found with group I with a score of 2.2 ± 1.09 and the least score was recorded for group III (0.8± 0.37) with p value = (0.17) being highly significant this is might be because of Cu-NP having increased surface area that improves the physio-mechanical, hence more prevention in seepage of oral fluids. Uysal *et al.* **[10]** examined the degree of micro-leakage at the interfaces of the cement and enamel and between the cement and the band. The results obtained showed that the BD-CM using conventional GIC exhibited notably elevated levels of ML at both the cement-band (CM-BD) and CEI. The most significant finding was observed in group III for both the labial and lingual surfaces (p=0.00), with a mean ML score of 0.85 ± 0.37. This suggests that the addition of 2% Cu-NP and 15% N-AC resulted in minimal ML. However, there was no statistical difference observed for group IV (p=0.65) and group V. This lack of difference could be attributed to the possibility that increasing the concentration of N-AC & Cu-NP may not further reduce ML efficacy **[11]**.

The mean counts of S. mutans, aerobic and anaerobic lactobacilli, and total bacteria surrounding orthodontic bands cemented with Fuji I containing 8wt% nHA were significantly lower compared to those around orthodontic bands cemented with pure Fuji I (P < 0.05).The study concluded that the incorporation of 8wt% nHA into GI cement may improve its antibacterial properties for the cementation of orthodontic bands, reduce the accumulation of cariogenic bacteria and potentially lower the incidence of caries in orthodontic patients **[12].** ZMgO nanoparticles made dental cements much more antibacterial, which means they can resist bacterial microleakage and likely secondary caries. It is suggested that zMgO nanoparticles be used in cements because they have antibacterial properties that make cavities and gum infections less likely to happen again **[13].** A positive effect on reducing microleakage bands surrounding orthodontic bands was revealed by 15% nano-HA-modified banding GIC **[14]**.

Conclusions:

Modified Glass Ionomer Cement (GIC) exhibited the highest zone of inhibition suggesting strong antimicrobial property. Conventional GIC showed the highest micro-leakage score; whereas GIC + 2% copper nano-particle & 15% N-acetylcysteine demonstrated the lowest value. Therefore, we conclude that, as the concentration of N-AC & Cu-NP increases, AM efficiency also increases.

References:

[1] Mizrahi E. *American journal of orthodontics*.1982 **82**:62. [PMID: 6984291]
[2] Mitchell L. *British journal of orthodontics*.1992 **19**:199. [PMID: 1390575]
[3] Rasmussen K *et al. Letters in applied microbiology*. 2016 **62**:30. [PMID: 26518358]
[4] Amin F *et al. Materials*.2021 **14**:6260. [PMID: 34771787]
[5] Sidhu SK and Nicholson JW. *Journal of functional biomaterials*.2016 **7:**16. [PMID: 27367737]
[6] Alobiedy AN *et al. Journal of Engineering*. 2019 **25**:72. [DOI:10.31026/J.ENG.2019.02.05]
[7] Gutiérrez MF *et al. Journal of dentistry*. 2017 **61**:12. [PMID: 28438559]
[8] Souza SDFC *et al. Journal of Applied Oral Science.* 2012 **20**:329. [PMID: 22858699]
[9] Norevall LI *et al. European Journal of Orthodontics*. 1996 **18**:373. [PMID: 8921659]
[10] Uysal T *et al. American Journal of Orthodontics and Dentofacial Orthopedics*. 2010 **137**:534. [PMID: 20362915]
[11] Mazzaoui SA *et al. Journal of dental research*.2003 **82**:914. [PMID: 14578505]
[12] Shirazi M et al. Dental Research Journal. 2024 21:1.[PMID: 38425324]
[13] Naguib GH et al. Bioactive Materials. 2022 8:49.[PMID: 34541386]
[14] Enan ET & Hammad SM. The Angle Orthodontist. 2013 83:981.[PMID: 23745977]