EGYPTIAN DENTAL JOURNAL
www.eda-egypt.org
VOL. 71, 2385:2392, JULY, 2025
PRINT ISSN 0070-9484 • ONLINE ISSN 2090-2360
FIXED PROSTHODONTICS AND DENTAL MATERIALS
Submit Date : 08-05-2025 • Accept Date : 30-06-2025 • Available online: 10-07-2025 • DOI : 10.21608/edj.2025.381860.3465

EFFECT OF MODIFIED GLASS IONOMER WITH MORINGA NANO POWDER/CHITOSAN ON MECHANICAL AND ANTIBACTERIAL PROPERTIES: AN IN VITRO STUDY

Huda Ahmed Amin EL Gendi *, Ayman Sabbah** *and* Lamis Enaba***

ABSTRACT

Glass ionomer cements (GICs) are restorative materials that have been commercially accessible for more than 50 years, and their extensive use is owing to their many benefits. However, GICs have several limitations. The poor mechanical qualities of GICs are the most significant drawbacks restricting their use.

This study aimed to examine the impact of incorporating moringa nano-powder (M/NP) and Chitosan (CH) solution into GIC on antimicrobial activity, compressive strength, and surface hardness. A significant improvement in antimicrobial capability was observed with dual alterations. The enhancement in the surface hardness was also a result of the synergistic effect of the dual modification. In contrast, there was a decrease in the compressive strength. In conclusion, the antibacterial and surface hardness characteristics of GIC were successfully boosted by adding moringa NP and Chitosan on the expense of compressive strength reduction.

KEYWORDS: glass ionomer cement, moringa, Chitosan, mechanical properties, nanoparticles.

INTRODUCTION

Glass ionomer cements (GICs) have been used extensively in clinical dentistry as a base, luting, and restorative material since they were founded in 1972. Calcium fluoro-aluminosilicate glass powder and an aqueous mixture of tartaric and acrylic acids make up the conventional GIC. Its clinical advantages include chemical adherence to the hard tooth structure, fluoride release, antibacterial properties, anti-cariogenic nature, and the ability to promote the remineralization of carious lesions. Numerous studies have demonstrated the bactericidal effects of conventional GICs. GICs' mode of action against

* Prosthodontic Department, Faculty of Dentistry, Deraya University, Minya, Egypt.
** Pediatric Dentistry, Public Health and Orthodontics Department, Faculty of Dentistry, Misr International University, Cairo, Egypt.
*** Prosthodontic Department, Faculty of Dentistry, Misr International University, Cairo , Egypt.

bacteria is unknown, but some possibilities have been proposed. The hypothesis that fluoride ions leached from GICs are the cause of the bacterial inhibitory effect is the most supported. Moreover, prior studies on the acidity of polyacrylic acid (PAA) have demonstrated its antibacterial action. According to a different theory, GICs' Zn component of GICs possesses antibacterial properties. It is still up for debate whether traditional GICs' antibacterial activity is effective against S. mutans, a major contributor to dental caries development [1].

Oral biofilms are generally functionally structured and organized, balancing normal flora and pathogenic bacteria, such as S. mutans. Therefore, treatment of GICs with various potent antibacterial agents may have significant clinical implications, assuming that there are no adverse effects on different adhesive and/or physical characteristics. Recently, there has been a surge in interest in nanomaterial research because of its potential for use in dentistry and medicine [2].

Not only traditional treatments derived from plant extracts, such as extracts from the leaves and seeds of the moringa plant, are beneficial therapies, but they are also less costly and hazardous than medications. Therefore, M. oleifera leaves have the potential to be used as both a culinary and medicinal item [3].

Dental caries is intricately linked to cariogenic biofilms with a high concentration of Streptococcus Mutans [4]. Changes in the oral environment, such as sucrose consumption and a pH drop, may affect bacterial homeostasis, which could increase cariogenic bacteria. The cariogenic bacteria Lactobacillus acidophilus, Streptococcus Sobrinus, and Streptococcus mutans may produce a cariogenic biofilm. Because of its tolerance, S. Mutans is particularly linked to dental caries among these bacteria. Mutans also use glucosyltransferases to produce glucans from sucrose because of their rapid metabolism and high acidity. Biofilm production is associated with glucan, which is related to the biofilm proteins and bacteria present in biofilms formed by antimicrobial agents [5].

Elemental analysis showed the existence of Proanthocyanidins in moringa. Mikarimi et al. [6], found that plant-derived proanthocyanidins may be helpful natural agents for non-invasive dentistry and promote carious enamel lesions' remineralization. To our knowledge, no studies have reported using Moringa Oleifera leaves (MOL) as a reinforcing filler.

In the same context, Chitosan (CH) has shown an efficient antibacterial effect against various bacteria, yeasts, and fungi. Chitosan is a class of partially or fully deacetylated chitin molecules and is an immunostimulatory, antibacterial, antifungal, biodegradable, and biocompatible cationic polyelectrolyte. It has been demonstrated that Chitosan is antibacterial against various bacteria, filamentous fungi, and yeasts[1]. In addition, Chitosan exhibits negligible toxicity to mammalian cells and possesses a lethal antibacterial effect on gram-positive and negative bacteria [7,8]. It has previously been reported that Chitosan may change the liquid phase of GICs, significantly enhancing the mechanical and antibacterial properties at an ideal chitosan content (10% v/v). Moreover, Chitosan stimulated the release of fluoride ions [9].

This study aimed to examine the impact of incorporating moringa nano-powder (M/NP) and Chitosan (CH) solution into GIC on antimicrobial activity, compressive strength, and surface hardness. We hypothesize that the addition of Moringa nano-powder (M/NP) and Chitosan (CH) to GIC will enhance its antibacterial activity against oral pathogens (Lactobacillus acidophilus, Streptococcus mutans, Streptococcus aureus and Staphylococcus aureus) and improve surface hardness, though it may compromise compressive strength.

MATERIALS AND METHODS

1. Sample size calculation

The power of the sample was calculated by a power analysis program (G*Power v3.1.9.2; Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany). The calculated sample was (n = 8), where (effect size [f] = 3.96, Beta level (β) = 0.95, and power = 95% at α = 0.05). Accordingly, eight specimens per group were tested against each bacterial strain (n = 8/group/strain) which means 64 specimens. For mechanical testing, 16 specimens were fabricated per test.

2. Specimen grouping

"Ninety six glass ionomer cement (GIC) specimens were fabricated and categorized as Group I, representing control, where GIC was unmodified and Group II comprised M/NP-chitosan-modified glass ionomer. Each group had eight specimens tested for compressive strength, microhardness, and antimicrobial activity.

3. Specimen fabrication

An industrial silicon mold, having 4 cuboid-shaped housings, was prepared. The study utilized a commercially available restorative glass ionomer cement for posterior teeth with high-strength characteristics (GC Gold Label Glass Ionomer High Strength Posterior Restorative; GC Corporation, Tokyo, Japan). To modify the GIC powder, we used moringa purchased from the National Research Center with a 21 nm particle size. The liquid of the GIC was modified by low molecular weight/viscosity chitosan (CH) (Loba-Chemie Pvt. Ltd. Mumbai, India). Chitosan was dissolved in (0.1 mol/L) acetic acid, followed by precipitation in 0.1 mol/L NaOH to be purified. Washing was conducted with 70% ethanol after precipitation, and freeze-drying was implemented thereafter. The control group specimens were manufactured by mixing the unmodified GIC at 3.6 g/1. g powder/liquid (P/L) ratio to follow the manufacturer's instructions. The study group was prepared by mixing 3% (w/w) M/NP with the GIC powder and then was shaken to get a homogenous mix. The GIC liquid was also modified by adding a chitosan solution (CH) at 10% (v/v), shaken gently for 5 min at room temperature, and then manipulated to the nano-moringa-modified GIC powder to prepare the specimen.

4. Preparation Method:

4.1. Moringa preparation:

Moringa Oleifera nano-powder (21 nm particle size) was sourced from the (National Research Center, Dokki, Cairo, Egypt). The GIC powder was modified with 3% milled moringa by mixing and shaking the two powders. The 3% (w/w) concentration of M/NP was selected based on prior studies demonstrating optimal mechanical reinforcement without agglomeration [10, 11].

4.2 Chitosan preparation

Low molecular weight/viscosity chitosan (CH), purchased from (Loba-Chemie Pvt. Ltd. Mumbai, India), was utilized to modify the GIC liquid. Chitosan was purified by dissolving in 0.1 mol/L acetic acid and then precipitating in 0.1 mol/L NaOH. The precipitate was washed with 70% ethanol and freeze-dried [9].

5. Testing.

5.1. Modified GIC specimens' preparation

The prepared GIC specimens were 15 mm in diameter and 2 mm in thickness and then replicated using an industrial silicon mold. The mold was filled with GIC mixture, then covered with a glass slab, and left at room temperature for 24 h to set. After setting, the specimens were exposed and stored for another 24 h at 37°C and 100% humid atmosphere before being sterilized with ethylene oxide.

5.1.1 Microorganisms Preparation

The microorganisms tested in this study were (Lactobacillus acidophilus ATCC 4356, Streptococcus mutans ATCC 25175, Streptococcus aureus ATCC 29213 and Staphylococcus aureus ATCC 6538).

5.1.2: Agar well diffusion test.

The agar plates containing the Mueller-Hinton agar were seeded with 1.8 x 10^8 colony-forming units (CFU) per milliliter of test bacteria with an optical density (OD600) of 0.5. After incubation for 24 h at 37°C, inhibition zones were monitored. The inhibition zones surrounding the wells were measured in millimeters, considering only those greater than 6 mm diameter. The inhibition zones obtained are the average of three replicates for each experiment. Based on the manufacturer's instructions, GIC was mixed and poured into the wells of the agar plates. The agar diffusion technique was applied to test the antimicrobial activity of the specimens against Staphylococcus aureus, Streptococcus mutans, and Lactobacillus bacteria[12].

5.2.1 Compressive strength (Mechanical Properties)

The compressive strength (CS) was evaluated following established ISO standards [13]. The GIC materials of the control and study groups were packed into round industrial silicon molds (25 mm in length, 2 mm in thickness, and 2 mm in width). Until the molds completed their first setting, they were packed with GIC and covered with glass slides. The specimens (n = 8 per group) were set up and then submerged in distilled water at 37°C for 24 hours prior to testing. Until the specimen failed, the CS was measured at a crosshead speed of 1 mm/ min. The Instron model 3345 was used to calculate and record data using the Blue Hill universal Instron software. The compressive strength value was measured in MPa by calculating ($CS = 4P/ \pi D2$), where P represents the load (N) at fracture, and D was for the specimen's diameter in (mm).

5.2.2 Micro Hardness (Mechanical Properties)

Disc-shaped specimens (6 mm diameter and 3 mm thickness) were created after replicating (n = 8 per group) using an industrial silicon mold. After setting, the specimens were submerged in distilled water for 24 h before testing at 37°C. The surface hardness was determined using a Vickers microhardness tester (Tukon.1102, Wilson hardness tester, Buehler, Germany) with a diamond-indenter weighing 100 g and a dwell-time of 10 s applied to nine different locations on each specimen's surface. The Vickers hardness number (VHN) was calculated by averaging three readings for each specimen.

6. Statistical methods:

The data was coded and entered using the Statistical Package for the Social Sciences (SPSS) version 26 (IBM Corp., Armonk, NY, USA). Data were tested for normality using the Shapiro-Wilk test. Descriptive statistics of the normally distributed data were presented using the mean and standard deviation, whereas non-parametric data were summarized using the median and interquartile range. For normally distributed data, groups were compared using unpaired t-tests, while the Mann-Whitney test was employed for the non-parametric data. The threshold for statistical significance was $p < 0.05$ [14].

RESULTS

Agar well diffusion test

The antimicrobial activities of groups 1 and 2 against Lactobacillus, Streptococcus, and Staphylococcus aureus were determined using the disc diffusion method, as shown in Figure (1).

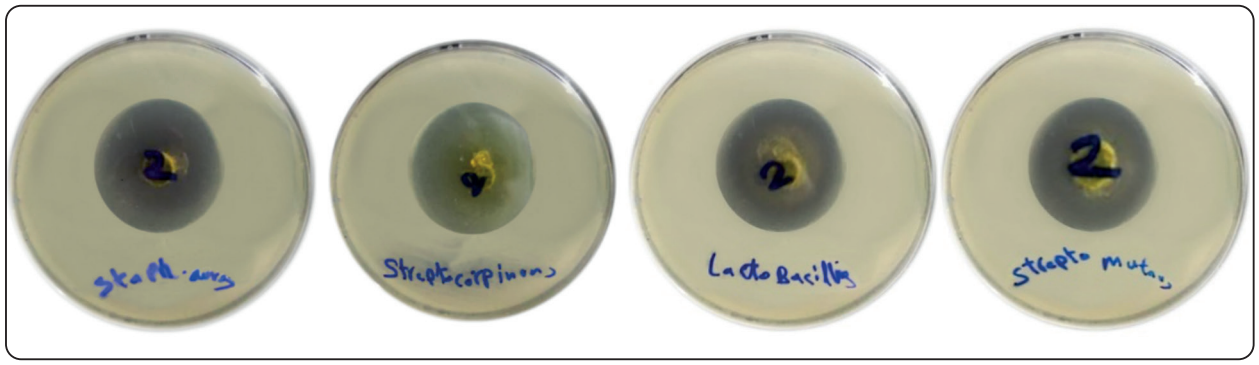

Fig. (1) Antimicrobial activities of moringa nanoparticles against (Lactobacillus acidophilus ATCC 4356, Streptococcus mutans ATCC 25175, Streptococcus aureus ATCC 29213 and Staphylococcus ATCC 6538)

The antibacterial activity assessment of group 2 showed no growth of the microorganisms mentioned.

Compressive strength

The results of the CS showed that for the non-modified GIC group (group 1), the Mean and Standard Deviation were 82.15 and 33.31 MPa. While the Mean and Standard Deviation for group 2 are 65.05 and 24.96 MPa. There was a significant difference in the CS values between the non-modified GIC (group 1) and M/NP-chitosan-modified GIC (group 2).

Micro Hardness

The results of the surface microhardness for all groups are presented in Figure (2).

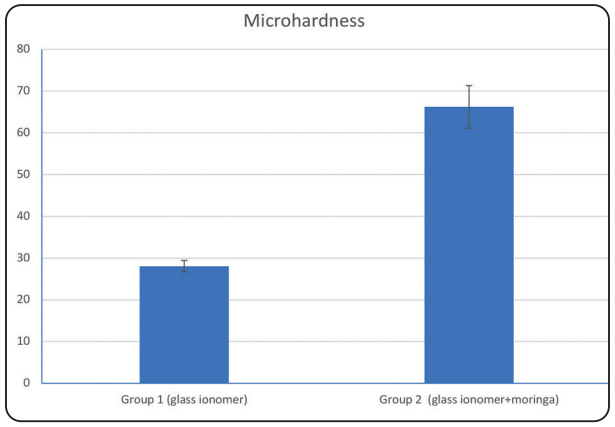

Fig. (2) Surface microhardness for groups 1 & 2

The results of the microhardness testing showed that the Mean and Standard Deviation for group 1 were 28.07 and 1.35 (VHN), respectively. The Mean and Standard Deviation for Group 2 were 66.22 and 5.11 (VHN), respectively. There was a significant difference in the VHN values between the non-modified GIC (group 1) and M/NP-chitosan-modified GIC (group 2).

DISCUSSION

The present study assessed the effects of dual modification of GIC with Moringa nano-powder (M/NP) and Chitosan (CH) on its antibacterial activity, surface hardness, and compressive strength. The findings presented a substantial improvement in antibacterial capabilities and surface hardness while experiencing a decrease in compressive strength. These outcomes align with our hypothesis that M/NP and CH would provide a synergistic benefit to antibacterial performance and surface hardness at the expense of compressive strength. Accordingly, the alternative hypothesis was accepted.

The dual-modified GIC possessed vigorous antibacterial activity against S. mutans, S. aureus and L. acidophilus (complete inhibition in agar-diffusion test). This finding can be attributed to the combined effects of M/NP and CH. The antibacterial potential of Moringa likely occurs from at least a portion of the bioactive compounds it contains

(i.e., proanthocyanidins may disrupt bacterial cell membranes and metabolic pathways [6]). M/NP had a small particle size (21 nm) and a relatively high surface area to volume ratio, allowing deeper penetration into the area of bacterial colonization [15,16]. CH is polycationic and causes cell membrane disruption by binding to negatively-charged phospholipids, leading to the leakage of intracellular components [17,18]. The influence of synergies between M/NP and CH likely increased the resulting effects, as CH has mucoadhesive properties, which may help retain Moringa nanoparticles at the agar media longer than M/NP alone [19, 20]. The results of this investigation coincide with previous studies that have demonstrated increased antibacterial activity for CH-modified GICs against oral bacteria [1, 9].

Despite the improved antibacterial activity and hardness, the compressive strength (CS) of the modified GIC decreased by 21% (65.05 MPa vs. 82.15 MPa). There are a few potential reasons for the decreased CS, including increased porosity. The hydrophilic behavior of CH and M/NP can cause increased water absorption, thus creating voids that weaken the structure of the cement [20-24]. Secondly, interference with acid-base reaction. The alkaline nature of CH can partially neutralize PAA, thus decreasing the efficiency of the setting of the glass ionomer [15]. Lastly, particles' agglomeration affect the GIC mix. The considerable effort to homogenize M/NP and GIC powder will most likely result in nanoparticle agglomeration, which can form concentrated points of stress, making the material more vulnerable to fracture [21]. It is crucial to address the high standard deviation in compressive strength (33.31 MPa for control, 24.96 MPa for modified GIC) may reflect inherent variability in nanoparticle dispersion. Future studies should optimize mixing protocols to reduce agglomeration.

The results here differ from previous studies showing that incorporating nanoparticle additives improved the mechanical properties of GIC [2, 23]. The difference in results showcases the critical nature of additive selection: for example, hydrophilic, non-silicate nanoparticles like M/NP may have decreased strength properties, whilst hydrophobic bioceramics have been shown to improve them, e.g., hydroxyapatite (HA) [2].

The hardness values of the modified GIC were 136% higher than that of the control (66.22 VHN vs. 28.07 VHN). There are two proposed reasons for this. Firstly, nanoparticles as reinforcing agents: M/NP's fill the interstitial space in the GIC matrix for a more densified microstructure. The smaller nanoparticles serve as nucleation points for PAA crosslinking, which fortifies the glass-polymer network [21]. In addition, CH - PAA interactions: CH hydroxyl and acetamide groups generate hydrogen bonds with the carboxylate groups in PAA, strengthening interfacial adhesion between the glass particles and the polymer matrix [21]. Zhao et al. [23] have also demonstrated increased hardness of GICs modified with silica nanoparticles.

The dual-modified GIC has the potential to be used in non-load-bearing situations (e.g., Class V restorations, fissure sealants) where its antibacterial and surface hardness properties are priorities, but this affords limited use or confidence in stress-bearing scenarios because of its lower compressive strength. Future studies using dual-modification of GIC should study optimal concentrations of M/NP and CH to obtain ideal antibacterial/mechanical properties. Moreover, studies should focus on fluoride release and biocompatibility characteristics in the long-term stage. Evaluation against multispecies colonies under cariogenic conditions (e.g., low pH and exposure to sucrose) should also be considered.

CONCLUSION

The dual modification of GIC with Moringa nano-powder (3% w/w) and Chitosan (10% v/v) significantly enhanced antibacterial activity against

S. mutans, S. aureus, Staph. aureus and L. acidophilus and improved surface hardness by 136%. However, compressive strength decreased by 21%, likely due to increased porosity from hydrophilic additives. While this modified GIC shows promise for non-load-bearing applications, further optimization is needed to balance mechanical and antibacterial performance.

REFERENCES

1. Marwa A. Ibrahim, Balasankar Meera Priyadarshini, Jennifer Neo, Amr S. Fawzy, Characterization of Chitosan/TiO2 Nano-Powder Modifled Glass-Ionomer Cement for Restorative Dental Applications. Journal of Esthetic and Restorative Dentistry . 2017. 29: 146-156.
2. Moshaverinia A, Ansari S, Moshaverinia M, et al. Effects of incorporation of hydroxyapatite and fluoroapatite nanobioceramics into conventional glass ionomer cements (GIC). Acta Biomaterials. 2008;4:432–40.
3. Awaad AS, El-meligy RM, Qenawy SA, Atta AH, Soliman GA. Anti-inflammatory, antinociceptive and antipyretic effects of some desert plants. J Saudi Chem Soc. 2011. 15:367-373.
4. Lee SH, Kim YJ. A comparative study of the effect of probiotics on cariogenic biofilm model for preventing dental caries. Arch Microbiol. 2014. 196:601-609.
5. Su-Kyung Jwa Efficacy of Moringa oleifera Leaf Extracts against Cariogenic Biofilm . Prev. Nutr. Food Sci. 2019;24(3):308-312.
6. M. Mirkarimi, S. Eskandarion, M. Bargrizan, A. Delazar, M.J. Kharazifard, Remineralization of artificial caries in primary teeth by grape seed extract: an in vitro study, J. Dent. Res. Dent. Clin. Dent. Prospects . 2013. 7 (4) 206–20610.
7. Sara H. Younis , Raneem F. Obeid , Mohamed M. Ammar . Subsurface enamel remineralization by Lyophilized Moringa leaf extract loaded varnish . Helyion . 2020 : 6 e 05054 .
8. Kong M, Chen XG, Xing K, Park HJ. Antimicrobial properties of Chitosan and mode of action: a state of the art review. Int J Food Microbiol 2010;144:51–63.
9. IbrahimM, Esguerra R, Neo J, Fawzy AS. Characterization of antibacterial and adhesion properties of chitosan-modified glass ionomer cement. J Biomater Appl 2015;30:409–19.
10. Mashiar Rahman1, M. Mominul Islam Sheikh1, Shamima Akhtar Sharmin1, M. Soriful Islam1, M. Atikur Rahman1, M. Mizanur Rahman2,3 and M. F. Alam1: Antibacterial Activity of Leaf Juice and Extracts of Moringa oleifera Lam. against Some Human Pathogenic Bacteria. CMU. J. Nat. Sci. (2009) Vol. 8(2)
11. Elsaka SE, Hamouda IM, Swain MV. Titanium dioxide nanoparticles addition to a conventional glass- ionomer restorative: influence on physical and antibacterial properties. J Dent 2011;39:589–98.
12. National Committee for Clinical Laboratory Standards(NCCLS). 2002. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard. National Committee for Clinical Laboratory Standards, Wayne, Pennsylvania, USA.
13. ISO. ISO 9917-1: dentistry-water-based cements-part 1: powder/liquid acid-base cements. Geneva, Switzerland: International Organization for Standardization; 2007.
14. Chan YH (2003): Biostatistics102: Quantitative Data – Parametric & Non-parametric Tests. Singapore Med J.;44(8): 391-396.
15. Moshaverinia A, Roohpour N, CheeW, Schricker C. A review of polyelectrolyte modifications in conventional glass-ionomer dental cements. JMater Chem 2012; 22:2824–33.
16. Wetzel B, Rosso P, Haupert F, Friedrich K. Epoxy nanocomposites—fracture and toughening mechanisms. Eng Fract Mech 2006;73:2375–98.
17. Hayashi Y, Ohara N, Ganno T, et al. Chitosan-containing gum chewing accelerates antibacterial effect with an increase in salivary secretion. J Dent 2007;35:871–4.
18. Chung YC, Su YP, Chen CC, et al. Relationship between antibacterial activity of chitosan and surface characteristics of cell wall. Acta Pharmacol Sin 2004; 25:932–6.
19. 19.Yadav AV, Bhise B. Chitosan: a potencial biomaterial effective against typhoid. Curr Sci 2004;87:1176–8.
20. Pedro AS, Cabral-Albuquerque E, Ferreira D, Sarmento B. Chitosan: an option for development of essential oil delivery systems for oral cavity care. Carbohydr Polym 2009;76:501–8.
21. Amin F, Rahman S, Muhammad S, Farshid S.: Effect of Nanostructures on the Properties of Glass Ionomer Dental Restoratives/Cements: A Comprehensive Narrative Review. Materials (Basel). 2021 Nov; 14(21): 6260.

22. Khalil M, Hussein LA.: Influence of Antimicrobial Nanoparticles on Flexural Strength and Hardness of Polymethylmethacrylate. Macedonian Journal of Medical Sciences. 2021 Dec 08; 9(D):314-318.

23. Zhao J and D. Xie.: Effect of Nanoparticles on Wear Resistance and Surface Hardness of a Dental Glass-ionomer Cement. Journal of Composite Materials, 1 November 2009.

24. Spinola M, Barbosa P, Torres C and Bresciani E.: Mechanical Assessment of Glass Ionomer Cements Incorporated with Multi-Walled Carbon Nanotubes for Dental Applications. Oral 2021, 1, 190–198.