EGYPTIAN DENTAL JOURNAL
www.eda-egypt.org
VOL. 69, 2379:2388, JULY, 2023
PRINT ISSN 0070-9484 • ONLINE ISSN 2090-2360
CONSERVATIVE DENTISTRY AND ENDODONTICS
Submit Date : 10-04-2023 • Accept Date : 02-05-2023 • Available online: 10-07-2023 • DOI : 10.21608/edj.2023.205151.2515

EVALUATION OF MICROBIAL BIOFILM ADHESION AND SURFACE MECHANICAL PROPERTIES OF MODIFIED ALKASITE WITH TITANIUM DIOXIDE NANOPARTICLES

Tayseer Mohamed Maaly *, Mahmoud Saad Abdel-Hlim** *and* Mohammed Samir El Nawawy***

ABSTRACT

Objective: To assess the ability of modified alkasite with TiO_2 nanoparticles (NPs) to prevent microbial biofilm adhesion without compromising the color and surface mechanical properties of the new formulations.

Materials and Methods: The Powder of TiO_2 NPs was added to alkasite powder in ratios of 3, 5 and 7% (w/w). Crystal violet assay (CV) method was used for assessment of microbial biofilm adhesion, micro hardness was measured by Vickers diamond indentor, color changes of the modified alkasite powder were measured by a spectrophotometer, and surface roughness values were recorded using digital microscope. Three dimensional images were taken for all groups. One and two-way ANOVA were used for the statistical analysis, followed by Tukey's test.

Results: Increased the ratio of titanium dioxide nanoparticles added to alkasite significantly decreased microbial biofilm formation without compromising other properties.

Conclusion: The alkasite restorative material modified by 7% nano-titania significantly decreased microbial biofilm formation without any effect on the color and mechanical properties.

KEYWORDS: Alkasite, biofilm formation, color measurement, Nano titania, Microhardness.

INTRODUCTION

Composites have recently become the most widely applied restorative materials in dentistry, because of their capability to preserve tooth structure for cavity preparation, good aesthetics and used for direct-filling technique.[1] GIC has long-term stability, cost-efficacy, and ease of use. It is often accomplished in bulk without using of adhesive, however, it has poor mechanical abilities, inappropriate for stress-bearing situations, encouraging the improvement of resin-based composites.[2,3]

* Assistant Professor, Dental Biomaterials Department, Faculty of Dentistry, Zagazig University
** Microbiology and Immunology Department Faculty of Pharmacy Zagazig University
*** Assistant Professor, Operative Dentistry Department, Faculty of Dentistry, Mansoura University

Recently, the permanent restoration of posterior teeth has been carried out using the new filling material Cention N. It is a member of the Alkasites species.[4] Alkasite is a subgroup of the composite class, resemble compomer materials, It makes use of alkaline fillers that release ions that neutralize acids.[5] The pH during acid attacks is controlled by cention N because it rises hydroxide ions concentrations and their release. Demineralization can be prevented as a result. Moreover, a significant amount of calcium and fluoride ions are released, helping to remineralize tooth enamel.[4]

Any surfaces in the oral cavity that are in direct contact with surrounding the environment, such as the teeth's surfaces, mucosa, and restoration materials, are susceptible to biofilm formation. Secondary caries is brought on by the biofilm growth on the surface of composite, which harms the material and surface and allows bacteria to injury the teeth surface into space between the restoration and the teeth.[6]

Despite the fact that there have been several studies devoted to creating antimicrobial compounds to solve this issue, the majority of these efforts have been unsuccessful since antibacterial medicines quickly degrade, which results in low efficacy and safety issues.[7]

Several tests have been conducted to develop the antibacterial capabilities of GICs, including the addition of quaternary ammonium salt and chlorhexidine gluconate to GICs. Regrettably, this bactericide addition had negative effects on the mechanical strength of GICs. Thus, it's crucial to pick bactericidal agents and figure out the specific quantity needed to modify GICs.[8]

When used in dental restorations, NPs can provide a number of benefits. Its smaller size gives them a greater specific surface area, which results in distinct characterization when compared to bulk-size particles.[9] This improvement inspired the researchers to include several NPs, such as titanium dioxide, zirconium dioxide and silicon dioxide, into dental biomaterials to increase their characteristics.[10, 11]

Mechanical and physical qualities have been considerably enhanced by the addition of nanoparticles.[12] Due to its exceptional qualities, such as its high microhardness, appropriate antibacterial capabilities, and inexpensive cost, TiO_2 nanoparticles are preferred in the dental industry.[10] Due to its biocompatibility and chemical durability, it has been widely used in organic degradation processes.[13]

The surface microhardness and roughness of dental restorative materials must be considered. Both internal and external influences can affect the surface roughness of dental materials. Rough surfaces accumulate more plaque than smooth surfaces, and the material is more easily worn. Alteration of the roughness of the surface of restoration is a precursor to bacterial colonization and a risk factor for developing gingival illnesses in the future.[14]

The current work assessed how the incorporation of the titanium dioxide nanopowder to alkasite affected the development of microbial biofilms and the mechanical qualities of the surface. The null hypothesis was that adding titania nanoparticles to alkasite not alter its mechanical characteristics or the microbial biofilm adhesion

MATERIALS AND METHODS

Sample Size Calculation

Sample size determined by an earlier study [15], suitable sample size was 7 as a group, while mean ± standard deviation was 0.051±0.003, the mean ± standard deviation of other group was 0.06±0.007, at an 80% power and a 0.05 type I error probability. The entire sample size increased to 9 to recompense 20% drop out. G Power 3.1.9.7 was used for calculation of sample size.

I. Specimens preparation

Materials that were used in the study are listed in Table 1. At ratios of 3, 5, and 7% (w/w), Cention N powder and TiO_2 nanopowder were combined to create experimental powders. The following groups were created after the powder was combined for 10 minutes with a mortar and pestle and a balance was used for meaurement (TS4000, Ohaus, Pine Brook, NJ, USA) with accuracy of 0.0001 g

Group (I) Conventional Cention N powder

Group (II) Cention N modified with 3% (w/w) TiO_2-NP

Group (III) Cention N modified with 5% (w/w) TiO_2-NP.

Group (IV) Cention N modified with 7% (w/w) TiO_2-NP

TABLE (1) Materials used in the study.

Material	Composition	Manufacturer
Cention N	Powder: silicate glass filler and calcium fluorosilicateand Liquid contains Urethanedimethacrylate	Ivoclar Vivadent Schaan FL-9494 Liechtenstein
Titanium Dioxide	Nano-powder of Titanium Dioxide with anatase phase, average size 21 mm, 99.5%.	Sigma Aldrich, St. Louis, MO, USA

The ingredients' powder and liquid were combined in accordance with the manufacturer's instructions. All prepared specimens employed the suggested powder/liquid (P/L) ratio, which was 1.8/1 for the Cention N. For a homogenous consistency, one scoop of powder and one drop of liquid were mixed with a plastic spatula using a folding motion on the mixing pad and mixing slab. Before sterilization, specimens were preserved at 37°C with 100% humidity for 24 hours. Afterwards, all samples were sterilised at 55°C.

II. Biofilm assessment by crystal violet assay (CV) method

Specimens used for testing biofilm formation were prepared with (diameter of 4 mm and a thickness of 6 mm) using a Teflon mold. To investigate the capacity of mixed biofilm growth on various discs, the crystal violet (CV) assay was modified slightly. E. coli, Staphylococcus aureus, and Candida albicans standard strains (ATCC 10538, 6538, and 10231, respectively) were cultivated on Muller Hinton broth (MHB) for E. coli and S. aureus, and MHB with 2% glucose for C. albicans, then incubated at 300C for 1-2 daysA fresh aqueous suspension with 0.5 McFarland turbidity was made from an overnight culture and diluted 1/100 in fresh broth. The examined discs were inserted in various wells of a 12-well tissue culture plate, and each well received 1 mL of mixed inoculum suspension as a negative control. Wells that contained simply the discs and media were not used. Thereafter, under stationary circumstances, the plate was incubated for 48 hours at 37 °C. The contents of each well were aspirated, and the discs were then cleaned three times in PBS, fixed for 20 minutes in 1 mL of 99% methanol, decanted, dried by air, and stained for 15 minutes in 1 mL of 2% CV. After carefully removing any excess stain, the discs were allowed to air-dry before the bonded stain was gently removed using 500 L of 33% glacial acetic acid. Optical density (OD) of stained adherent biofilm was determined with microtitre plate ELISA reader at wavelength of 630 nm.[16] The ability of biofilm formation on tested discs were classified into four groups based on the measured ODts compared to OD of negative control (ODc): non adherent (N), weakly (W) adherent, moderately (M) adherent, and strongly (S) adherent.[17]

III. Color measurement

The dimensions of the specimens were prepared (4 mm thick and 8 mm in diameter). The colours of all specimens were measured using a reflective

spectrophotometer. (X-Rite, model RM200QC, Neu-Isenburg, Germany). The specimens and the instrument were perfectly lined up, and a 4 mm aperture size was chosen. Measurements were made in relation to the CIE standard illuminant D65 on a white background using the CIE L*a*b* colour space. Baseline values were determined by measuring the colour of conventional alkasite specimens. Specimens of modified alkasite were tested for colour alterations (E) and estimated by the following formula.:

$$\Delta E_{CIELAB} = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})\ ½$$

Where: L* = lightness (0-100), a* = (change the color of the axis red/green) and b* = (color variation axis yellow/blue)

IV. Surface roughness test

A Teflon mold with split disc shape measuring with 8 mm diameter and 2 mm thickn was employed. Using a digital microscope (***U500x Digital Microscope, Guangdong, China***) with an integrated camera, each specimen was captured on camera. A vertically positioned, 2.5 cm away from the samples digital camera with a resolution of 3 Mega Pixels was used to take pictures. Each image has a resolution of 1280 by 1024 pixels when it was captured. To identify and standardise the area of roughness measurement, digital microscope images were adjsuted to 350 x 400 pixels by Microsoft Office Picture Manager. A 3D picture of the specimens' surface profile was subsequently produced. For each specimen, five 3D photos were taken at a 10 mm 10 mm size in the middle and on each side. Depending on the size of the characteristic bacteria expected to cling to repair surface in vivo, this area was selected. Average heights (Ra) were measured and expressed as average height in millimetres.

V. Microhardness Test.

To create disc-shaped specimens, a Teflon mold measuring 8mm diameter and 2 mm thickness was used. Micro-hardness of the surface specimens was assessed by a Vickers diamond indenter and a 20X objective lens on a digital exhibition (Model HVS-50, Laizhou Huayin Testing Instrument Co., Ltd., China). The specimens' surfaces were subjected to a 100g load for 15 seconds. On the surface of each specimen, three indentations were made, evenly spaced around a circle and not more than 0.5 mm apart from one another. By using the scaled microscope that was built in to quantify the indentations' diagonal length. Vickers values were converted into micro-hardness values. The following equation was used to determine micro-hardness:

$$HV=1.854\ P/d^2$$

Where, **HV** is Vickers hardness in Kgf/mm^2, **P** is the load in Kgf and **d** is the length of the diagonals in mm

IV. Statistical analysis

Statistical analysis was performed with SPSS 16 (Statistical Package for Scientific Studies), examination of the given data was achieved using Shapiro-Wilk test and Kolmogorov-Smirnov test for normality which discovered that data were originated from normal data. Accordingly, comparison between different groups was performed by using One Way ANOVA test followed by Turkey's Post Hoc test for multiple comparisons.

RESULTS

I. Microbial Biofilm Adhesion

Biofilm forming capacity of microbial strains on all groups are shown in Table (2), Fig. (1). Means and standard deviations of the microbial biofilm formation of various groups are shown in Table 3. A graphical representation of biofilm adhesion results are shown in Fig. (2). Group I revealed significantly the highest value, (0.69±0.03), followed by group II (0.246 ± 0.005), then group III (0.195±0.009), while group VI was significantly the lowest (0.14±0.005). Tukey's analysis revealed significant difference between all groups as $P < 0.0001$.

II. Color change measurement

Means and standard deviations of color change values (ΔE) between the unmodified alkasite and the three modified groups can be seen in Table 3. Fig. (3) Denotes a graphical representation of color changes results. Group II showed the lowest (ΔE) value (14.39±1.46), while group IV showed the highest value (15.60±1.85) without significant difference as P=0.41.

III. Surface roughness:

Mean and standard deviations of surface roughness values for the evaluated alkasites are shown in Table 3. Fig. (4) Represents a graphical illustration of surface roughness results. The results showed that group III, 5% (w/w) nano titania-enriched alkasite, presented the highest mean value (0.2537±0.0011), and the original alkasite displayed the lowest one (0.2530±0.0016). ANOVA test revealed insignificant difference among all groups as P=0.73. Three dimensional images of digital microscope for all groups are shown in Fig. (5). conventional

IV. Micro-hardness

Mean and standard deviations of the surface microhardness and (Kg/mm2) for all groups are displayed in Table 3. A graphical illustration of surface microhardness results is shown in Fig. (6). The control group, i.e., conventional alkasite, represented the lowest value (80.85 ±4.79), and group III, 5% (w/w) nano titania-modified alkasite, displayed the highest mean (86.26 ±4.26). One-way ANOVA revealed insignificant difference between all groups as P=0.07.

TABLE (2) Biofilm forming capacity of microbial strains on all groups.

Material	Absorbance (ODt) at 570 nm	Absorbance (ODc)	Biofilm Forming Capacity
1	0.697	0.211	M
2	0.195	0.163	W
3	0.246	0.201	W
4	0.148	0.163	N

M: moderate biofilm forming, W: weak biofilm, N: non-biofilm. ODc: optical density of negative control (well with disc and media only), ODt: optical density of microbial biofilm

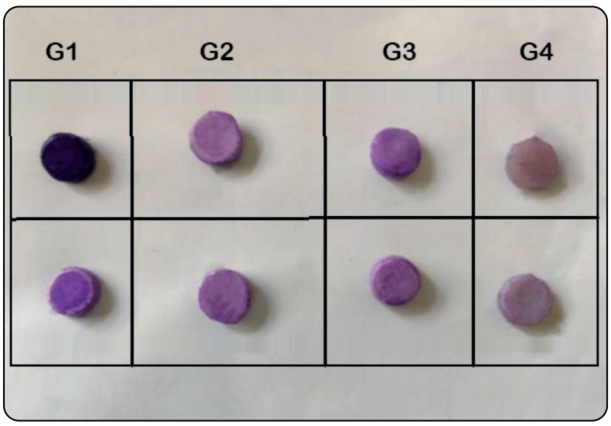

Fig. (1) Biofilm formation on all groups against negative ones.

TABLE (3) Mean and standard deviation of biofilm adhesion, color changes, surface roughness, microhardness of all groups and comparison between them.

Group	Microhardness (Kg/mm2)	Biofilm adhesion	Surface roughness (µm)	Color change
Gr I	80.85± 4.79[a]	0.697±0.035 [a]	0.2530± 0.0016[a]	Baseline
Gr II	83.35±4.57 [a]	0.246±0.005 [b]	0.2536±0.0013[a]	14.39±1.46 [a]
Gr III	86.26± 4.26[a]	0.195±0.009 [c]	0.2537± 0.0011[a]	14.85±2.30 [a]
Gr IV	85.81±5.03 [a]	0.148±0.005 [d]	0.2531±0.0016 [a]	15.60±1.85 [a]
P value	0.07 ns	<0.0001*	0.73 ns	0.41 ns

Ns: non-significant difference as P>0.05. *Significant difference as P<0.05.
Means with different superscript letters were significantly different as P<0.05.
Means with the same superscript letters were insignificantly different as P>0.05.

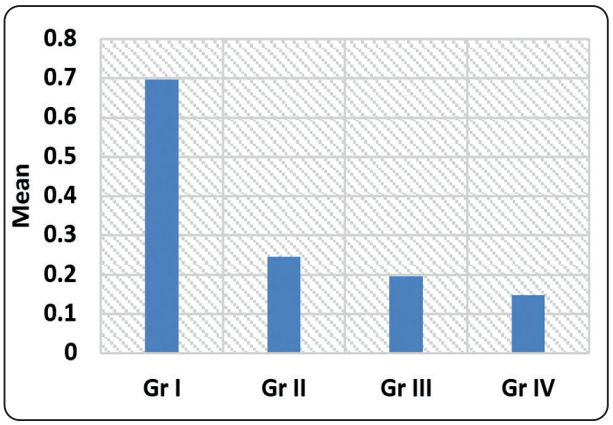

Fig. (2) Bar chart representing biofilm adhesion of all groups.

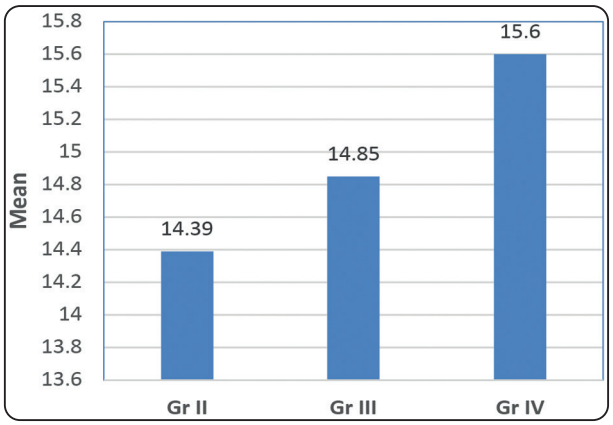

Fig. (3) Bar chart representing color changes of modified groups.

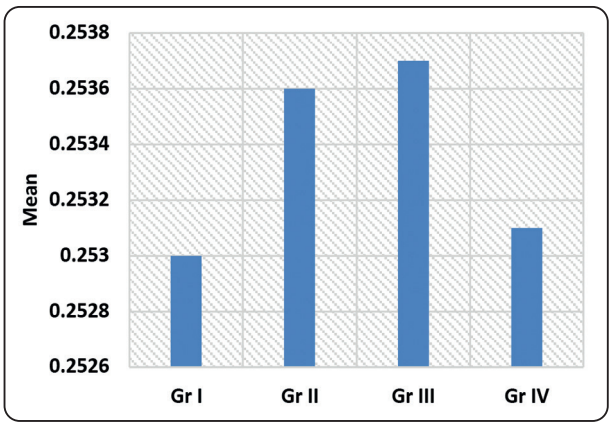

Fig. (4) Bar chart representing surface roughness of all groups.

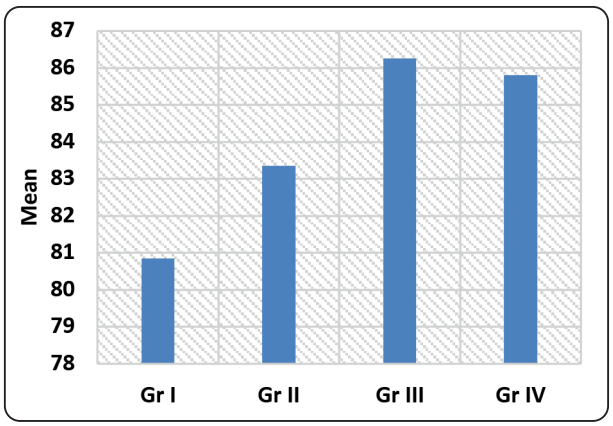

Fig. (6) Bar chart representing hardness of all groups.

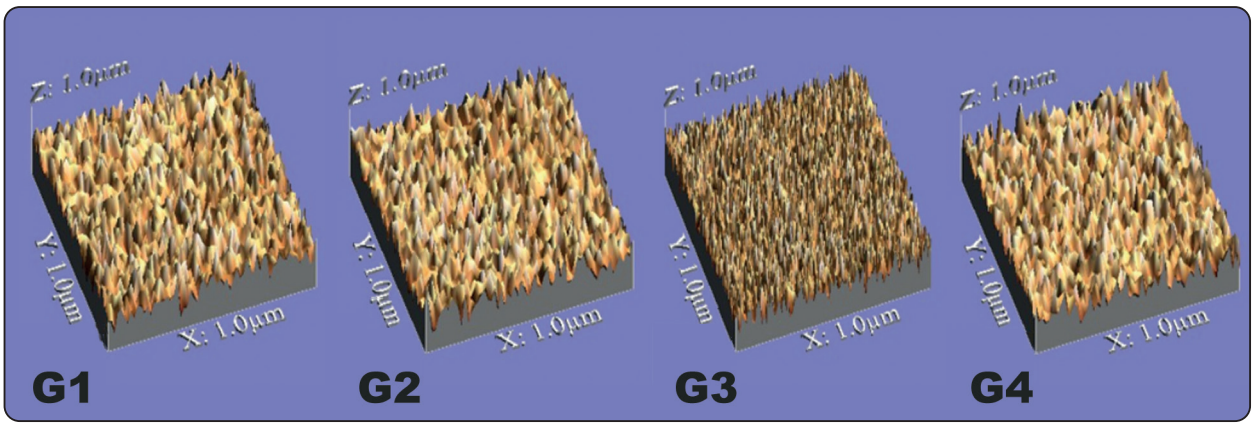

Fig. (5) Digital images of the surface topography for all groups.

DISCUSSION

The antibacterial qualities of dental materials are among the most essential biological characteristics taken into account when choosing their application. Biofilm development can be better avoided by using materials with antibacterial qualities. Dental and periodontal disorders are caused by biofilm, which develops onto surfaces in the oral cavity that are enclosed by the acquired pellicle so creating a complex, active microbial media.[18]

There has been a lot of trials done to make dental materials antibacterial. Ag, Cu, TiO_2, ZnO, chitosan, and quaternary ammonium polyethylenimine (QPEI) NPs are just a few examples of the numerous nanomaterial that have shown successful at controlling biofilms and being merged into polymer matrices by way of filler particles.[19, 20]

The amount and diffusion of nanoparticles were found to be the primary factors in enhancing properties of composite resin, while decreasing the size and increasing the filler volume will result in rise in the surface hardness and compressive strength of the composite. Nanoparticles were used to develop modified nano-composite with better physical and mechanical properties.[21]

The current study evaluated modification of cention N with TiO_2 with three ratios 3%, 5% and 7%. Based on the most common concentrations considered in the earlier studies, two concentrations (3 wt.% and 7 wt.%) of nanoparticles were preferred also; it was established that ratio above 7 wt.% could cause massive color change of modified nanocomposite. [22]

TiO_2 nanoparticle use has a strong chance of reducing the development of white spots since bacteria are less likely to acquire resistance to it.[11] In the present study, addition of nano titania in different ratios into alkasite decreased microbial biofilm adhesion to the restoration with significant results. The result is in agreement with previous study[23] that TiO_2 nanoparticle use has a strong chance of reducing the development of white spots since bacteria are less likely to acquire resistance to it. Modified composite with more than 5% NPs dramatically decreased S. mutans and S. sanguinis, and this effect grew as the proportion of the nanoparticles in the composites increased.[23] This result is in agreement with the finding of a previous study [24] that TiO_2 nanoparticles had antibacterial activity toward the bacteria E. coli, Staph. aureus, P. aeruginosa, C. albicans, and B. subtilis and that TiO_2 NPs were suitable for use as inorganic antimicrobial agents.

The small size of TiO_2NP (21 nm) and large surface area may allow the TiO_2 particles to diffuse inside the bacterial cell and cause intracellular damage.[25] Based on the antibacterial mechanism, hydroxyl free radicals and peroxide should specifically be detected by TiO_2NPs as species of reactive oxygen (ROS).[26]

The dental fillings success and patient satisfaction are significantly influenced by the color stability of a restorative material.[27] Previous study[10] concluded that the addition of ZrO_2, TiO_2, and SiO_2 to light-cured RBCs could develop their mechanical and surface properties, but one of the most frequent drawbacks observed was regarding the reinforcement of light-cured RBCs via NPs is the color changes. Other study stated that NPs made of reparative materials demonstrated better optical characteristics because the NPs dimension is less than the visible light's wavelength. [28]

To evaluate changes in colour in dental materials, the CIE L*, a*, and b* colour evaluation method is regarded as an appropriate instrument.[29]

In our research, the color changes was measured using a digital spectrophotometer because it is thought to be a reliable and adaptable measurement device.[30] The results showed that addition of NPs has no significant effect on the change of the color of unmodified alkasite, low ratio (3 wt%) showed

less color change than high ratio (7 wt%) which in agreement with a previous study.[30]

Surface roughness of all groups was recorded using USB digital microscope The optical methods be liable to realize the need for quantitative characterization of surface topography lacking contact.[31] Addition of 3 and 5% nano titania into alkasite increased the surface roughness with no significance and this result is in agree with Aref and Abdallah.[15] While 7% modified material showed no increase in surface roughness. Compared to other nanofillers, titanium dioxide is more stable structurally and creates fewer clumps of particles. The PH and structure of the nanoparticles influence their ability to aggregate. Low PH in titanium dioxide nanoparticles reduces their ability to clump together.[32] TiO_2 groups had clinically acceptable surface roughness and a creamy white tint that offers the restoration process an appealing appearance.[33]

The results of our work exhibited an insignificant rise in surface hardness with adding of both 3, 5 % ratios of TiO_2. While modified alkasite with 7 % nanotitania showed lower hardness value than 5 % modified one with no significance. The reason for the increase in surface hardness is because TiO_2 NPs are packed inside, creating a denser surface with fewer vacancies and increasing the resistance to permanent indentation.[15] Several investigations showed that composite resin treated with TiO_2 significantly increased in hardness and FS. [34,35] Because there wasn't enough ionomer to grasp the comparatively high ratio of TiO_2 nanoparticle powders, the mechanical characteristics of glass ionomer cement that contains the ration of 7% (w/w) TiO_2 nanoparticles decreased.[11]

Another study discovered that composite resin enhanced with 1 weight percent of TiO_2 was significantly harder than unfilled composite. It was found that ZrO_2 at a concentration of 7 % may help to advance the mechanical properties of composite resin, but TiO_2 is best used at low concentrations (3 %).[22] Particles clump together as the TiO_2 content rises. The agglomerated chemicals may serve as stress concentration points in the matrix, which would be bad for the material's mechanical characteristics.[36]

One of the biggest difficulties in manufacturing nano-composite has been preventing agglomeration. TiO_2 aggregates most likely produce certain micro-pores and micro-cracks as structural flaws that are absent from TiO_2 nanoparticles at low concentrations.[37]

CONCLUSIONS

Within the restrictions of this analysis, it could be determined that 7% (w/w) nano titania-modified alkasite restorative material exhibited high resistance to biofilm adhesion while maintaining the color measurement and surface mechanical properties. Additional researches are needed to evaluate the impact of this modification on other mechanical properties, such as compressive strength, fracture toughness

REFERENCES

1. Parolia A, Adhauliya N, de Moraes Porto I C, Mala K. A comparative evaluation of microleakage around class V cavities restored with different tooth colored restorative materials. Oral Health and Dental Management.2014;13:120–6.

2. Mazumdar P, Das A, Guha C. Comparative evaluation of hardness of different restorative materials (restorative GIC, Cention N, nanohybrid composite resin and silver amalgam)-an in vitro study. Int J of Adv Reaarch. 2018; 6 (3):826–32.

3. Kielbassa A M, Oehme E P, Shakavets N, Wolgin M. In vitro wear of a (resin-coated) high-viscosity glass ionomer cement and a glass hybrid restorative system, J. Dent. 2020;105:103554.

4. Cention 2016. N Ivoclar Vivadent AG Research & Development Scientific Service. N Ivoclar Vivadent AG Research & Development Scientific Service, pages 0–58.

5. Chole D, Shah H, Kundoor S, Bakle S, Gandhi N, Hatte N. In Vitro Comparision of Flexural Strength of Cention-N, Bulk-Fill Composites, Light-Cure Nanocomposites, and

Resin-Modified Glass Ionomer Cement & quot. IOSR Journal of Dental and Medical Sciences (IOSR-JDMS) 2018:17(10):79–82.

6. Cao, W.; Zhang, Y. Development of a novel resin-based dental material with dual biocidal modes and sustained release of Ag+ ions based on photocurable core-shell AgBr/cationic polymer nanocomposites. J. Mater. Sci. Mater. Med. 2017, 28.
7. Al-Dulaijan, Y.A.; Cheng, L.; Weir, M.D.; Melo, M.A.S.; Liu, H.; Oates, T.W.; Wang, L.; Xu, H.H.K. Novel Rechargeable Calcium Phosphate Nanocomposite with Antibacterial Activity to Suppress Biofilm Acids and Dental Caries. J. Dent. 2018;72: 44–52
8. Marti, L.M., E.R. Azevedo, M.D. Mata, E.M.A. Giro and A.C.C. Zuanon, 2014) Addition of chlorhexidine gluconate to a glass ionomer cement: a study on mechanical, physical and antibacterial properties. Braz Dent J., 25:33-37
9. Al-Dulaijan, Y.A.; Cheng, L.; Weir, M.D.; Melo, M.A.S.; Liu, H.; Oates, T.W.; Wang, L.; Xu, H.H.K. Novel Rechargeable Calcium Phosphate Nanocomposite with Antibacterial Activity to Suppress Biofilm Acids and Dental Caries. J. Dent. 2018, 72, 44–52. .
10. Azmy, E.; Al-Kholy, M.R.Z.; Al-Thobity, A.M.; Gad, M.M.; Helal, M.A. Comparative Effect of Incorporation of ZrO2 , TiO2 , and SiO2 Nanoparticles on the Strength and Surface Properties of PMMA Denture Base Material: An In Vitro Study. Int. J. Biomater. 2022, 5856545.
11. Elsaka S E, Hamouda I M, and Swain M V, "Titanium dioxide nanoparticles addition to a conventional glass-ionomer restorative: influence on physical and antibacterial properties," Journal of Dentistry. 2011; (39): 589–98.
12. Antibacterial Properties of Nanoparticles in Dental Restorative Materials. A Systematic Review and Meta-Analysis January 2020
13. Makvandi P, Gu J T, Zare E N. "Polymeric and inorganic nanoscopical antimicrobial fillers in dentistry," Acta Biomaterialia.2020;101: 69–101.
14. K. Soygun, A. Soygun, and M. C. Dogan, "The effects of Chitosan addition to glass ionomer cement on microhardness and surface roughness, "Journal of Applied Biomaterials and Functional Materials," vol. 19, Article ID 2280800021989706, 2021.
15. Neven S. Aref and Reham M. Abdallah. Evaluation of Mechanical Properties and Antibacterial Activity of Nano TitaniaEnriched Alkasite Restorative Material: An In Vitro Study: The Open Dentistry Journal.2022;16.
16. El-Ganiny, A.M., Shaker, G. H., Aboelazm, A. A., El-Dash, H. A. Prevention of Bacterial Biofilm Formation on Soft Contact Lenses Using Natural Compounds. J Ophthalmic Inflamm Infect. 2017;7(1):11.
17. Stepanović, S., Vuković, D., Hola, V., Bonaventura, G. D., Djukić, S., Ćirković, I., Ruzicka, F. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. Apmis, 2007;115(8):891-899
18. Mangal et al 2020. Zhang N, Melo MAS, Weir MD, Reynolds MA, Bai Y, Xu HHK. Do dental resin composites accumulate more oral biofilms and plaque than amalgam and glass ionomer materials? Materials (Basel) 2016; 9(11)E888
19. Liu, Y. et al. Nanotechnology-based antimicrobials and delivery systems for biofilm-infection control. Chem. Soc. Rev.2019;48, 428–446.
20. Padovani, G. C. et al. Advances in dental materials through nanotechnology: facts, perspectives and toxicological aspects. Trends Biotechnol. 2015;33, 621–636.
21. Rafid M., Al Badr Effect of addition ZrO2 nanoparticles to dental composites on the physical and mechanical properties. International Journal of Scientific and Engineering Research .2018;9(6):1288–1293.
22. Emad Azmy, Mohamed Reda Zaki Al-Kholy, Mohamed Fattouh, Laila Mohamed Mohamed Kenawi, and Mohamed Ahmed Helal mpact of Nanoparticles Additions on the Strength of Dental Composite Resin: International Journal of Biomaterials Volume 2022; 1165431,9 pages.
23. Sabriye Pişkin. ArzuPalantöken, and Müge Sari Yılmaz. International Conference on Emerging Trends in Engineering and Technology (ICETET'2013) Dec. 7-8, 2013; Patong Beach, Phuket (Thailand)..,
24. Wetzel, B., P. Rosso, F. Haupert and K. Friedrich, 2006. Epoxy nanocomposites fracture and toughening mechanisms. Eng Fract Mech 2006; 73:2375–98
25. Wang, H., B. Tang, X. Li and Y. Ma, 2011. Antibacterial properties and corrosion resistance of nitrogen doped TiO2 coatings on stainless steel. J Mat Sci Technol. 2011; 27:309-16..
26. Wang, H.; Zhu, M.; Li, Y.; Zhang, Q.; Wang, H. Mechanical Properties of Dental Resin Composites by Co-Filling

Diatomite and Nanosized Silica Particles. Mater. Sci. Eng. C 2011, 31, 600–605.

27. Sahin, O.; Koroglu, A.; Dede, D.Ö.; Yilmaz, B. Effect of Surface Sealant Agents on the Surface Roughness and Color Stability of Denture Base Materials. J. Prosthet. Dent. 2016, 116, 610–616

28. Erdemir, U.; Yıldız, E.; Eren, M.M. Effects of Sports Drinks on Color Stability of Nanofilled and Microhybrid Composites after Long-Term Immersion. J. Dent. 2012, 40, e55–e63.

29. Arikawa, H.; Kanie, T.; Fujii, K.; Takahashi, H.; Ban, S. Effect of Filler Properties in Composite Resins on Light Transmittance Characteristics and Color. Dent. Mater. J. 2007, 26, 38–44

30. Nasim, I.; Neelakantan, P.; Sujeer, R.; Subbarao, C.V. Color Stability of Microfilled, Microhybrid and Nanocomposite Resins—An in Vitro Study. J. Dent. 2010, 38, e137–e142

31. Ossama B. Abouelatta. 3D Surface Roughness Measurement Using a Light Sectioning Vision System. Proceedings of the World Congress on Engineering 2010 Vol I

32. Hajipour MJ, Katharina MF, Ashkarran AA, Dorleta J .Antibacterial properties of nanoparticles. Trends in Biotech.2012;10:1016-20

33. Comparative evaluation of surface roughness in composite resins incorporated with silver and Titanium dioxide nanoparticles. IJSDR.2019;4:85-90.

34. Sabzi M., Mirabedini S. M., Zohuriaan-Mehr J., Atai M. Surface modification of TiO2 nano-particles with silane coupling agent and investigation of its effect on the properties of polyurethane composite coating. Progress in Organic Coatings 2009;65(2):222–228.

35. Xia Y., Zhang F., Xie H., Gu N. Nanoparticle-reinforced resin-based dental composites. Journal of Dentistry. 2008;36(6):450–45

36. Salih Durdu, Gizem Cihan, Emine Yalcin , Atilgan Altinkok. Characterization and mechanical properties of TiO2 nanotubes formed on titanium by anodic oxidation Volume 47, Issue 8, 15 April 2021, Pages 10972-10979.

37. Shankar Sharma, Naveen Kumar, Peter R. Makgwane . TiO2/SnO2 nano-composite: New insights in synthetic, structural, optical and photocatalytic aspects. Inorganica Chimica Acta.2022;529:120640.