Innovations

Comparative Study of Colour Stability and Tensile Strength of Conventional Room Temperature Vulcanized Silicone and Modified Room Temperature Vulcanized Silicone by Incorporating Silicon **Dioxide Nanoparticles from Bamboo Extract**

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Abstract:

Introduction: Room temperature vulcanizing silicone, or RTV silicone, has gained popularity in maxillofacial clinics due to its notable advancements, which are available as platinum-cured polydimethylsiloxane. Previous investigations have demonstrated improved mechanical and optical qualities when silicon dioxide nanoparticles are added to room-temperature vulcanized silicone. Bamboo has a lot of silica. Thus, the goal of this study is to incorporate silicon dioxide derived from bamboo to increase the tensile strength and color stability of room-temperature vulcanized silicone. Evaluate the differences between modified RTV silicone that contains silicon dioxide from the bamboo extract and conventional RTV silicone in terms of color stability and tensile strength. Materials and procedure: Sixty silicone RTV samples were made. Using epoxy molds measuring 150 mm X150 mm X 2 mm, six of which were of conventional RTV silicone (Group A) and six for each group, which were of bio-silicone dioxide modified RTV silicone (Group B) at various concentrations (0.5%, 1%, 2%, and 3%). Followed by tensile strength testing on the samples. Then a 1000-hour natural weathering process, the samples' color stability was assessed. Result: Statistical analysis is done using SPSS software. To minimize Type I errors, an ANOVA test ($\alpha = 0.01$) is used to determine whether group means differ substantially for color stability. The null hypothesis, equal means, is disproved if p < 0.01. The F-test for variance equality is used in advance to ensure variances are similar. In the case of tensile strength, an ANOVA test ($\alpha = 0.05$) finds differences between the means of five groups. If the ANOVA shows significance (p< 0.05), a Tukey HSD post-hoc test is used to identify particular group differences. Conclusion: This study showed that 0.5% bio-silica-modified RTV silicone provides the optimum tensile strength and color stability during an artificial aging process of 1000 hours, making it the ideal concentration for enhanced performance.

Keywords: Banchloshan, Bio-Silica, Sio₂ Nanoparticles, Room Temperature Vulcanized Silicone, Maxillofacial Prosthesis

Introduction:

Maxillofacial prosthodontics uses materials like silicones to reconstruct lost facial features due to their versatility. [1] Maxillofacial prostheses can be made from several materials, including porcelain, natural rubber, gelatin, latex, acrylics, and silicones in anoplasty. [2]. Room temperature vulcanizing silicone, or RTV silicone, has gained popularity in maxillofacial clinics due to its notable advancement over previous polymers, which are available as platinum-cured polydimethylsiloxane. [3] Despite their flexibility and aesthetically pleasing appearance, silicones are prone to deterioration and discoloration. UV rays, air contaminants, and extended contact with skin all contribute to this poor color stability, degradation. [3] Low tear and tensile strength.^[4] Although attempts to extend longevity by integrating stronger reinforcing elements have been attempted, their success has been limited [5]. The type and amount of fillers in the silicone network, as well as the degree of crosslinking in the network, determine the fundamental features of silicone elastomer. Elastomer cross-linkingis also influenced by the kind, composition, the kind of fillers; the reinforcing material; the curing temperature; and the polymerization method [6].

A number of techniques were attempted to enhance the color stability of maxillofacial silicones, including the inclusion of opacifiers, oil paint, and nanooxides, with differing degrees of success. The addition of nano-oxides to the elastomers produced the best results out of all of these techniques. [7] Previous studies have demonstrated that adding silicon dioxide nanoparticles to room-temperature vulcanized silicone improves its mechanical and optical characteristics [5].

Silicon dioxide nanoparticles can be extracted synthetically or from a natural source such as sugarcane [8] or bamboo stem. Bamboos are rich sources of silica. So, bamboo extract can be a source of silicon dioxide. Nanoparticles extracted from bamboo leaf and stem are used as coatings to increase the material's resistance to microorganisms, hydrophobicity, wettability, thermal stability, flameretardant qualities, and ultraviolet (UV) resistance as mentioned in previous studies [20]. As bamboo extracts are also used in cosmetics and skin care [9], it is thus proven biocompatible with the skin [10]. Bamboo stem extract is more efficient as it contains a higher amount of silica, so it can be used as a replacement for the synthetic silica.

'Banshlochan' is the siliceous concretion found in the hollow internodes of Bambusa arundinacea. It has a concentration of silica (Si) of 85.78%. Intake of Banshlochan is used to treat asthma, cough, poisoning, paralysis, and other debilitating illnesses. Additionally, it is utilized as an astringent, cooling, expectorant, sweet, acrid, diuretic, haemostatic, cardiac, febrifuge, and tonic. [11]

Although they have shown some promise, attempts to extend the lifespan of RTV silicones by adding reinforcing materials—like nanoparticles—have not been very successful. Prior research has shown that adding silica nanoparticles to RTV silicone enhances its mechanical and optical qualities [12]. Nevertheless, synthetic silica continues to be the most common source, and little research has been done on biocompatible, alternate sources of silica that would provide greater sustainability and performance. There are no previous studies that incorporating the bio-silica from bamboo into RTV silica is done. In this study, we are utilizing Banshlochan/Vanshlochan, an ayurvedic product, as the source for bamboo extract, from which we are extracting silicon dioxide nanoparticles.

Thus, this study aims to compare and enhance the color stability and tensile strength of traditional RTV silicone and silicon dioxide-modified RTV silicone. The CIE L*a*b* and elongation tests are used to accomplish this.

Methodology:

This study was done in the Prosthodontics Department of Tagore Dental College and Hospital, Tamil Nadu, India.

Materials required:

- The Room-Temperature Vulcanized Silicone (Siloczest liquid silicone rubber (LSR-105), which has part A and part B (1:1))
- Intrinsic stain, burnt senna shade from Siloczest (human Indian skin tone).
- Epoxy Silicone resin mold measuring 150x150x2mm.
- Silicon dioxide nanoparticles (bio-silica) (Banshlochan/Vanshlochan)
- Vacuum mixer (500 rpm) and weighing machine

Sample preparation:

A sample size of six in each group was determined in order to establish a mean reduction difference between 0% concentration and 0.5%, 1%, 2%, and 3% concentration of bio-silica at 5% risk and 95% power is calculated. For each parameter (color stability and tensile strength) a sample size of thirty was taken into consideration. Consequently, a total sample size of sixty was attained. (table 1)

Bio-silica preparation:

Banshlochan was processed by the sol-gel method to produce silicon dioxide nanoparticles and was calcinated at 650°C for 2 hours and then stirred for 1 h at 100 °C. After filtering the banshlochan, the residue was repeatedly cleaned with distilled water. Then 8 mL of NaOH (1M) was added to the residue, and it was continuously stirred for one hour at 100°C. [13]

When the combination was filtered, sodium silicate was obtained. For the gelling procedure, HCl 1 M was used to titrate the solution. Following a 24-hour aging period, the aqua gel was repeatedly cleaned with distilled water and allowed to dry. An agate mortar is used to grind the silica xerogel into powder.[13]

Sample preparation:

For the volume of the 150x150x2 mm slab, 49.5 g of RTV silicone is taken (1 ml = 1.10 g/cm³), and intrinsic stain is added in 3 drops to the samples. Bio-silica nanoparticles obtained are added at 0%, 0.5% (0.2 mg), 1% (0.49 mg), 2% (0.99 mg), and 3% (1.48 mg) to samples. And mixed using a vacuum mixer [14] (Figure a) and poured in slabs (Figure b).

Sample artificial aging:

A total of 1000 hours were spent exposing thirty samples to artificial aging processes, including exposure to sunlight, pollutants, dirt, and dust [15]. At last, all of the specimens were stored in a dark environment with 50% relative humidity for one full day (for 24hrs).

Testing procedure:

Test of tensile strength:

The 30 dumbbell-shaped samples were cut according to ASTM-D412 (2021) standardization, and the samples were installed in the universal testing machine (UTM) with a 1 KN load cell under the following conditions: 23±2°C, 50±5% R.H., 6 control, and an elongation test. A universally standardized machine was then used to measure tensile strength. The formula Load = Stress (Nm-2)/Initial cross-sectional area (mm-2) was used to calculate the tensile strength and percentage elongation.[15]

Color stability test:

Color stability is evaluated by calculating ΔE using the CIEL*a*b* system [1,5] D65/10*, and ΔE was calculated using the formula $\Delta E = ([\Delta L]^2 + [\Delta a]^2 + [\Delta b]^2)^{\frac{1}{2}}$. categorizing as: $\Delta E < 1$ (indisputable), $1 < \Delta E < 3.3$ (acceptable), and $\Delta E > 3.3$ (undesirable). NBS units are quantified as NBS= $\Delta E \times 0.92$, with interpretations trace (0.0-0.5), minor (0.5 to 1.5), perceptible (1.5 to 3.0), significant (3.0 to 6.0), a lot (6.0 to 6.0)to 12.0) and extremely much (>12) [15]. In the experiment, 6 control and 24 test slabs undergo initial and final tests of CIEL*a*b*after 1000 hours of exposure to UV, dirt, and pollution, following ASTM D2244 (2016) under environmental conditions of 23±2°C and 50±5% R.H.

Statistical analysis:

For color stability, an ANOVA test with alpha = 0.01 to determine whether the group means differ in any statistically significant way. An F-test for variance equality is done to compare two independent samples' variances that are significantly different.

An ANOVA test with alpha = 0.05 is performed for tensile strength, and then a posthoc test, or Tukey HSD test, is carried out to determine whether groups differ from one another after determining whether there are any statistically significant differences between the means of the five groups that were present.

Results:

The formula $\Delta E = ([\Delta L]2 + [\Delta a]2 + [\Delta b]2) \frac{1}{2}$ yields the color difference between the original and final CIE L*a*b* test, with average ΔE of 1.46, 1.7, 2.45, 2.91 and 3.34

B1, B3, for Group A, B2, and В4 accordingly. All five groups displayed a clinically acceptable color shift, according to a comparison of the ΔE values.

Graph 1 shows a slow rise in the ΔE value and an increase in concentration, with Group A and Group B1 having nearly identical values.

According to the National Bureau of Standards (NBS) units, multiplying the ΔE value with 0.92 (Δ Ex 0.92) revealed that the average values for Groups A, B1, B2, B3, and B4 were 1.34, 1.56, 2.25, 2.67, and 3.07, respectively. Groups B2, B3, and B4 show significant color variations, but Groups A and B1 show only minor color changes.

Graph 2, shows a slow rise in the Δ Ex0.92 value and an increase in concentration, with Group A and Group B1 having nearly identical values.

Table 2 shows There is a significant difference between the four groups (0.5%, 1%, 2%, and 3%). But expect 0.5% of the other three to be highly significant with control groups. According to the ANOVA results, there is a highly significant difference between the group means (F=1083.51, p=0.000), which disproves the null hypothesis that there is no difference.

From Table 3, we infer that F-statistics is 0.17, which shows a relatively low ratio of within-group variation to variation across groups. P-value = 0.04, At the α = 0.01 level, this result is not statistically significant since p > 0.01 (the selected significance threshold), indicating that there is insufficient evidence to support a difference between the two groups.

The tensile strength results showed average values 3, 3.21, 2.64, 2.57 and 2.12 (Mpa) for Groups A, B1, B2, B3 and B4 respectively, which is plotted in Graph 3.

Graph 3 shows increased tensile strength in Group B1 with 0.5% concentration when compared to the control and all other groups.

When comparing the groups' mean tensile strength, the ANOVA test yielded a pvalue less than the 0.05 alpha level, suggesting that the groups differ significantly from one another. The Tukey's Honest Significant Difference (HSD) post-hoc test findings are displayed in Table 4, which compares group means for the dependent variable ("Values") among various groups. Following an ANOVA, Tukey's HSD aids in determining significant differences between group means.

Table 4 shows Group A (0%) differs significantly from Group B2- 1%, B3-2%, and B4-3% (p < 0.05), but not between B2-1% and B3-2% (p > 0.05). Positive values indicate higher means in the first group and negative in the second. B2-1%, B3-2%, and B4-3% have higher means than Group A, with no difference between B1-0.5% and Group A.

Table 5, This table shows the means for each group and their homogeneous subsets based on the Tukey HSD test at the 0.05 significance level. In Subset 1, groups B4-3%, B3-2%, and B2-1% are homogeneous. In Subset 2, groups A 0% and B1-0.5%are also homogeneous.

Inferred from overall Tukey results, groups spanning subsets (e.g., B4-3% vs. A 0%) may exhibit substantial differences. This is plotted in the graph 4.

Discussion:

Maxillofacial prostheses serve both functional and aesthetic purposes, and they play a crucial role in helping affected individuals regain their quality of life. Silicone is the most widely used material to restore lost facial structures, although research is still being done to overcome its shortcomings and develop a substance that could be termed the "ideal maxillofacial prosthetic material." [16]

According to a 2010 survey, silicone-based maxillofacial prostheses have a relatively short lifespan of 7 to 24 months, primarily depending on the retention technique (implant or adhesive).[17]Some of the most common reasons for replacing a prosthesis are silicone tearing, inadequate maintenance, and color changes.^[18] Silicone elastomers' mechanical characteristics are influenced by numerous factors, such as the presence of fillers, cross-link density, and the molecular weights of polymer chains. A new class of polymer materials with greater flexibility due to the strength of the nano-oxides has been produced by introducing nanoparticles (NPs) into the polymer matrix [19, 20, 21].

Introducing reinforcing compounds to materials to find solutions for their mechanical shortcomings was one of the numerous studies carried out. The addition of nano-TiO2, ZnO, and CeO2 to silicone elastomer was one such endeavour. [22]

According to Mahovi'c Poljalcek et al., the addition of smaller amounts of SiO₂ (1%) was more effective in preventing the aging of fluorescent coatings than coatings that contained TiO₂ nanoparticles with photocatalytic capabilities.^[23] The deformation elasticity test findings showed that adding nanoparticles, particularly SiO₂, to the coating greatly (by 40%) increased the unaged samples' bending resistance.[24] Jun Che et al. and

Tarannum et al. experiment results showed that bamboo extract can filter UV rays [25, 26], while Sihama et al. found that adding natural nanoparticles improved the mechanical qualities of maxillofacial silicone.[5]

Vanshlochan, an ayurvedic product from bamboo extract, which is utilized in this process to extract nanoparticles, has a major component (85.78%) of silica [11]. Numerous studies reported in the literature were conducted in vitro, simulating some of the principal degradative agents that have been identified as catalysts for the depletion of mechanical properties and discoloration. In research on accelerated natural aging and induced aging, UV radiation is considered to be the most important of these.

This study shows, that there is no significant difference between the tensile strength of 0.5% incorporated RTV silicone and the control group (0%). This is in agreement with a study by Mustafa S et al., which found that the tensile strength of nano-SiO₂ group increased significantly [9]. However, in the present study, only 0.5% showed an equivalent result to control; as the concentration increases, the tensile strength decreases. Therefore, the optimal concentration for improved RTV silicone tensile strength is 0.5% bio-silica.

Limitation:

The study's limitations include a limited sample size that could restrict the findings' generalizability and a relatively short duration that makes it challenging to assess the long-term effects of aging. Increasing the study's duration and sample size might yield accurate information on the longevity and performance of maxillofacial prostheses. Banshlochan can be purified to eliminate the trace metals present in it to provide better results.

Conclusion:

The color stability of 0.5% bio-silica-modified RTV silicone is superior to that of other concentrations (average $\Delta E = 1.70$), with no significant difference observed when compared to conventional RTV silicone (average $\Delta E = 1.46$), and the color change between initial and final results was low in 0.5%. The remaining three concentrations demonstrate clinically acceptable color changes. Similar to other concentrations, 0.5% modified RTV silicone displays a higher tensile strength (average 3.8 MPa), showing no significant difference from the control group.

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References:

- 1. K K G, K HK, Ravichandran R, V P, S K, Jinesh KB, Mathew OP, Ananthakumar S, Mohammed ASP. Effect of titanium dioxide nanocoating on the colour stability of room temperature vulcanizing maxillofacial silicone-an invitro study. Clin Oral Investig. 2023 Dec;27(12):7799-7807.(www.link.springer.com)
- 2. Karthikeyan I. A review on prosthetic rehabilitation of maxillofacial region. Anaplastology 2014;3:1173-2161.(www.longdom.org)
- 3. Tetsuya TAKAMATA, B. Keith MOORE and Varoujan A. CHALIAN. An Evaluation of

- Color Changes of Silicone Maxillofacial Materials after Exposure to Sunlight. Dental Materials journal 8 (2): 260-270, 1989. (www.jstage.jst.go.jp)
- 4. Mitra A, Choudhary S, Garg H, H G J. Maxillofacial prosthetic materials- an inclination towards silicones. J Clin Diagn Res. 2014 Dec;8(12):ZE08-13. (www.jcdr.net)
- 5. Sihama Issa Salih, Jawad Kadhim Oleiwi and Hajir Mohammed Ali Modification of Silicone Rubber by Added Pmma and Natural Nanoparticle used for Maxillofacial Prosthesis Applications. Vol. 14, No. 4, February 2019 Issn 1819-6608 (www.arpnjournals.org)
- 6. Shakir DA, Abdul-Ameer FM. Effect of nano-titanium oxide addition on some mechanical properties of silicone elastomers for maxillofacial prostheses. (www.sciencedirect.com)
- 7. Sonnahalli NK, Chowdhary R. Effect of nanoparticles on color stability and mechanical and biological properties of maxillofacial silicone elastomer: A systematic review. J Indian Prosthodont Soc 2020; 20:244-54. (www.ipsonline.in)
- 8. Khotseng, L. Extraction and Synthesis of Silicon Nanoparticles (SiNPs) from Sugarcane Bagasse Ash: A Mini-Review. Appl. Sci. 2022, 12,2310. (www.mdpi.com)
- 9. Choi, Moon-Hee et al. "Antioxidative and Anti-Melanogenic Activities of Bamboo Stems (Phyllostachys nigra variety henosis) via PKA/CREB-Mediated MITF Downregulation in B16F10 Melanoma Cells." International journal of molecular sciences vol. 19,2 409. 30 Jan. 2018, (www.mdpi.com)
- 10. Vasave, H., Wasule, D.D., & Bobade, R. (2019). Bamboo's Extract for Rejuvenating Skin.(www.ijsdr.org)
- 11. Sagarika Parida, Kedar Kumar Rout Method of Identification and Standardisation of 'Vamsalochana' (Bamboo Manna) Indian Drugs 51(01) January 2014. (www.indiandrugsonline.org)
- 12. Wu, Lianfeng & Wang, Xianming & Ning, Liang & Han, Jianjun & Wan, Zhong & Lu, Min. (2016). Improvement of Silicone Rubber Properties by Addition of Nano-SiO 2 Particles. Journal of applied biomaterials & functional materials. 14. 10.5301/jabfm.5000298. (www.journals.sagepub.com)
- 13. Ni'mah YL, et al. (2023). Synthesis of Silica Nanoparticles from Sugarcane Bagasse by Sol-Gel Method. Nanoparticle. 4(1):10.(www.jnanoparticle.com)
- 14. Hatamleh MM, Watts DC. Porosity and color of maxillofacial silicone elastomer. J Prosthodont. 2011;20: 60-6. (www.onlinelibrary.wiley.com)
- 15. Gandhi DS, Sethuraman R. Comparative evaluation of tensile strength, tear stability hardness of conventional strength, color and trisnorbornenylisobutyl polyhedral silsesquioxane modified room temperature vulcanizing maxillofacial silicone after a six month artificial aging period. J Indian Prosthodont Soc 2022;22:328-37.(www.j-ips.org)
- 16. Hatamleh M.M., Haylock C., Watson J., Watts D.C. Maxillofacial prosthetic rehabilitation in the UK: A survey of maxillofacial prosthetists' and technologists' attitudes and opinions. Int. J. Oral Maxillofac. Surg. 2010;39:1186–1192. (www.ijoms.com)

- 17. Gradinariu AI, Racles C, Stoica I, Stelea CG, Simionescu AA, Jehac AE, Costan VV. Silicones for Maxillofacial Prostheses and Their Modifications in Service. Materials (Basel). 2024 Jul 4;17(13):3297(www.mdpi.com)
- 18. Mehta S, Nandeeshwar DB. A spectrophotometric analysis of extraoral aging conditions on the color stability of maxillofacial silicone. J Indian Prosthodont Soc 2017;17:355-60. (www.j-ips.org)
- 19. Han Y, Kiat-amnuay S, Powers JM, Zhao Y. Effect of nano-oxide concentration on the mechanical properties of a maxillofacial silicone elastomer. The Journal of Prosthetic Dentistry. 2008 Dec 1;100(6):465-73.(www.clinicalkey.com)
- 20. Bellamy K., Limbert G., Waters M., Middleton J. An elastomeric material for facial prostheses: synthesis, experimental and numerical testing aspects. Biomaterials. 2003;24(27):5061-5066. (www.sciencedirect.com)
- 21. Hayashi S., Fujikiand K., Tsubokawa N. Grafting of hyperbranched polymers onto ultrafine silica: postgraft polymerization of vinyl monomers initiated by pendant initiating groups of polymer chains grafted onto the surface. React Funct Polym. 2000;46(2):193-201. (www.sciencedirect.com)
- 22. Khanand A., Alam M. Synthesis, characterization and analytical applications of a new and novel 'organic-inorganic' composite material as a cation exchanger and Cd (II) ion-selective membrane electrode: polyaniline Sn(IV) tungstoarsenate. React Funct Polym. 2003;55(3):277–290.(www.sciencedirect.com)
- 23. Mahovi'c Polja'cek, S.; Tomašegovi'c, T.; Leskovšek, M.; Stankovi'c Elesini, U. Effect of SiO2 and TiO2 Nanoparticles on the Performance of UV Visible Fluorescent Coatings. Coatings 2021, 11, 928. (www.mdpi.com)
- 24. Sun, Haoxian; Li, Xuhong; Li, Haitao; Hui, David; Gaff, Milan; Lorenzo, Rodolfo; (2022) Nanotechnology application on bamboo materials: A review. Nanotechnology Reviews , 11 (1) pp. 1670-1695. 10.1515/ntrev-2022-0101. (www.doaj.org)
- 25. J. Chen, Q. Luo, Y. Zhou, et al., Color stability of dried pork meat slices containing bamboo leaf extract and L-cysteine under drying temperature variation, Food Sci. Anim. Prod. 1 (2023) 9240009. (www.semanticscholar.org)
- 26. Afrin, T., Tsuzuki, T., & Wang, X. (2012). UV absorption property of bamboo. The Journal of The Textile Institute, 103(4), 394–399. (www.semanticscholar.org)

Table 1: Sample Distribution for Tensile Strength and Color Stability Tests Group A- Control Group B-Test

	Group	Sub-Group	Sub- Group	Sub- Group	Sub-
	A	B1	B2	В3	Group
					В4
Concentration	0%	0.5%	1%	2%	3%
of Bio-silicon					
Dioxide					
No. of samples	6	6	6	6	6
for tensile					
strength					
No. of. samples	6	6	6	6	6
for color					
stability					

TOTAL: 60 samples

Table 2: Anova Results for Source of Variation between Groups

Source of	Sum of	Degrees of	F-Value	P-Value	Result
Variation	Squares	Freedom			
Between	8.81	3	1083.51	0.000	Highly
groups					Significant

^{*}p-value is <0.01 is significant

Table 3: Comparison of B1 0.5% and Control A 0% using F-test (α = 0.01)

Variable	Test B1 0.5%	Control	F	P-Value	Result
		A 0%		(A=0.01)	
Mean±SD	1.71±0.012	1.45±0.029	0.17	0.04	No Significant
n	6	6			Difference

^{*}p- value is <0.01 is significant

Table 4: Tukey HSD Post-Hoc Test Results Comparing Group Means for Dependent Variable "Values"

Name of	Name of the	Mean	Standard	Significance	95% Con	fidence
the Groups (I)	Groups (J)	Difference	Error		Inte	r v al
		(I-J)				
					Lower	Upper
					Bound	Bound
Group A (0%)	Group- B1-0.5%	02500	.22645	1.000	6901	.6401
	Group- B2-1%	.95333*	.22645	.002	.2883	1.6184
	Group- B3-2%	1.00833*	.22645	.001	.3433	1.6734
	Group- B4-3%	1.57500*	.22645	.000	.9099	2.2401
Group B1	Group- A-0.%	.02500	.22645	1.000	6401	.6901
(0.5%)	Group- B2-1%	.97833*	.22645	.002	.3133	1.6434
	Group- B3-2%	1.03333*	.22645	.001	.3683	1.6984
	Group- B4-3%	1.60000*	.22645	.000	.9349	2.2651
Group B2 (1%)	Group- A-0.%	95333*	.22645	.002	-1.6184	2883
	Group- B1-0.5%	97833*	.22645	.002	-1.6434	3133
	Group- B3-2%	.05500	.22645	.999	6101	.7201
	Group- B4-3%	.62167	.22645	.075	0434	1.2867
Group B3 (2%)	Group- A-0.%	-1.00833*	.22645	.001	-1.6734	3433
	Group- B1-0.5%	-1.03333*	.22645	.001	-1.6984	3683
	Group- B2-1%	05500	.22645	.999	7201	.6101
	Group- B4-3%	.56667	.22645	.122	0984	1.2317
Group B4 (3%)	Group- A-0.%	-1.57500*	.22645	.000	-2.2401	9099
	Group- B1-0.5%	-1.60000*	.22645	.000	-2.2651	9349
	Group- B2-1%	62167	.22645	.075	-1.2867	.0434
	Group- B3-2%	56667	.22645	.122	-1.2317	.0984

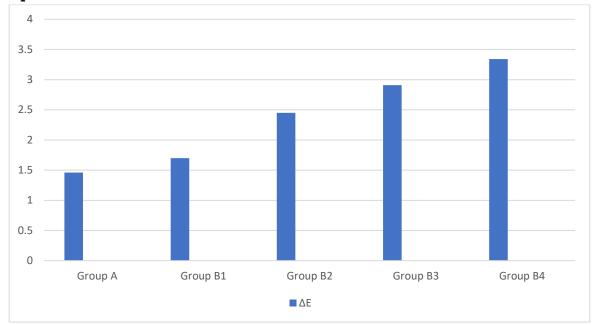
At the 0.05 level, the mean difference is statistically significant.

Table 5: Tukey HSD Subset Analysis for Group Means (Alpha = 0.05)

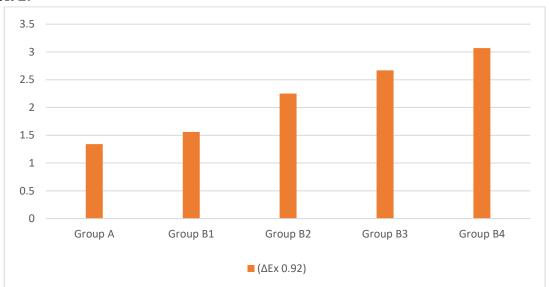
•		, -	` - /		
Name of the N		Subset for Alpha=0.05			
Groups		1	2		
Group-B4-3%	6	1.8750			
Group-B3-2%	6	2.4417			
Group-B2-1%	6	2.4967			
Group-A-0%	6		3.4500		
Group-B1-0.5%	6		3.4750		
Significance		.075	1.000		

Group means in homogenous subsets are shown.

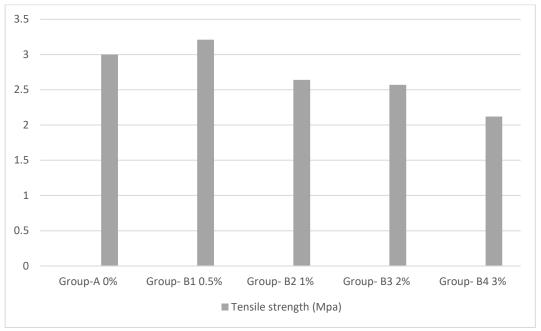
Graph 1



Graph 2:



Graph 3:



Graph 4: Comparison of Group Means with Tukey HSD Results

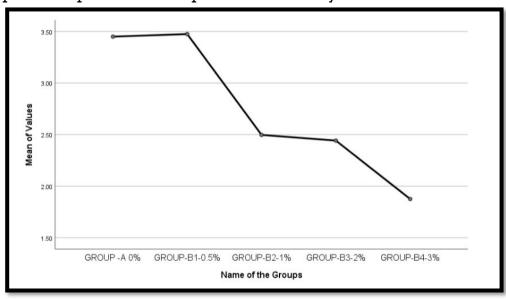




Figure (a)



Figure (b)

CRIS Guidelines (Checklist for Reporting In-vitro Studies)*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	la	Identification as an in vitro/laboratory study in the title	1
	16	Structured summary of trial design, methods, results, and conclusions	1
ntroduction			
Background and	2a	Scientific background and explanation of rationale	1-2
bjectives	2b	Specific objectives or hypotheses	2
Methods			
nterventions	3	The intervention for each group, including how and when they were actually administered, with sufficient detail	
		to allow replication	3
Outcomes	4	Completely defined pre-specified primary and secondary outcome measures, including how and when they	
		were assessed	3-4
ample size	5	How sample size was determined	2
Randomisation:			
Sequence	6	Method used to generate the random allocation sequence	NA
generation			
Allocation	7	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers),	NA
concealment mechanism		describing any steps taken to conceal the sequence until interventions were assigned	
теснанат			
Implementation	8	Who generated the random allocation sequence, who enrolled teeth, and who assigned teeth to intervention	NA
Blinding	9		4 (those
		If done, who was blinded after assignment to interventions (for example, care providers, those assessing outcomes) and	assessing
		how	outcome)
Statistical methods	10		5
		Statistical methods used to compare groups for primary and secondary outcomes	
Results			
Numbers analysed	lla	For each group, number of 'items' (drugs) included in each analysis and whether the analysis was by original assigned groups	5-7 (Graph1,2,3
Outcomes and	116	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as	6,8
estimation		95% confidence interval)	(Table 2,3,4,5)
Discussion			
imitations	12a	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	10
eneralisability	12b	Generalisability (external validity, applicability) of the trial findings	10
nterpretation	12e	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	10
Other information			
rotoco1	24	Where the full trial protocol can be accessed, if available	NA
unding	25	Sources of funding and other support (such as supply of drugs), role of funders	Title page

^{*}This checklist was developed by the authors based on the following sources:

Krithikadatta J, Gopikrishna V, Datta M. CRIS Guidelines (Checklist for Reporting In-vitro Studies): A concept note on the need for standardized guidelines for improving quality and transparency in reporting in-vitro studies in experimental dental research. J Conserv Deve. 2014; 17(4):301–304.

^{2.} Faggion CMJr. Guidelines for reporting pre-clinical in vitro studies on dental materials. J Evid Based Dent Pract. 2012;12(4):182–189.

^{3.} www.consort-statement.org.