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Effect of incorporating various concentrations of reduced Graphene Oxide (rGO) nanoparticles on the Shore D hardness of 3D-printed denture base resin: An *in vitro* Study

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Abstract

Background: Three-dimensional (3D) printing is increasingly used for denture base fabrication; however, concerns remain regarding various mechanical properties of 3D-printed denture materials. To address these limitations, nanotechnology has been explored as a promising approach for enhancing material properties. In this context, reduced graphene oxide (rGO), owing to its favourable mechanical properties, has been used for various biomedical applications.

Aim: To evaluate the surface hardness of a 3D-printed denture base resin incorporating different concentrations of rGO.

Materials and methods: A total of 25 standardized disc-shaped specimens were fabricated using a 3D-printing process and divided into five groups with 5 in each. The groups included a control (0 wt%) and four experimental groups containing 0.005 wt%, 0.01 wt%, 0.1 wt%, and 0.25 wt% reduced graphene oxide (rGO), respectively. Specimens were designed using CAD software and fabricated using a DLP-based 3D-printer. Raman spectroscopy was performed to confirm the characteristic peaks of rGO within the resin matrix. Shore D hardness was measured using a manual analogue durometer in accordance with ASTM D2240 standards. Statistical analysis was performed using one-way ANOVA, followed by post hoc tests.

Results: An increase in Shore D hardness was observed in Group 2a (0.005 wt%) and Group 2b (0.01 wt%), both significantly higher than the control, whereas a decrease was noted in Group 2c (0.1 wt%) and Group 2d (0.25 wt%), which were significantly lower than the control. All intergroup comparisons were statistically significant ($p < 0.0001$).

Conclusion: The findings indicate that the effect of rGO incorporation on shore D hardness is concentration-dependent, but shows an inverse relationship, where lower concentrations result in greater improvement, while higher concentrations lead to reduced performance. The optimal concentrations were found to be 0.005 wt% and 0.01 wt%.

Keywords: 3D-Printing, Denture base resin, Nanoparticles, Reduced Graphene Oxide, Shore D Hardness.

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1. Introduction

Polymethyl methacrylate (PMMA) has long been considered the material of choice for denture base fabrication due to its favourable biological properties, ease of processing, and acceptable aesthetics. However, despite these advantages, PMMA exhibits certain inherent limitations, including reduced hardness, susceptibility to wear, and fracture under functional stresses. These shortcomings may compromise the long-term clinical performance of denture prostheses, particularly under repeated masticatory loading [1,2].

In recent years, the application of three-dimensional (3D) printing in prosthodontics has expanded rapidly, offering a digital approach for denture base fabrication with improved accuracy, reproducibility, and workflow efficiency. This technique minimizes manual errors and allows precise duplication of prosthetic designs. However, despite these advantages, the mechanical performance of 3D-printed denture base resins remains a concern. These limitations are primarily attributed to the layer-by-layer fabrication process and variations in polymerization, which can result in inferior mechanical properties compared to conventional materials [3,4].

Among the various mechanical properties, hardness is a key parameter that reflects a material's resistance to surface indentation under an applied load. Hardness in polymeric materials is commonly evaluated using Shore hardness scales, which comprise multiple durometer scales designed for materials with varying stiffness ranges. Among these, Shore A is used for soft, elastomeric materials such as silicone-based liners, whereas Shore D is employed for rigid polymers such as polymethyl methacrylate (PMMA) [5]. As denture base resins, particularly 3D-printed resins, are relatively rigid in nature, Shore D hardness is widely employed for their evaluation. Clinically, lower Shore D hardness may increase susceptibility to wear, thereby compromising the longevity of the prosthesis. These concerns highlight the need for modifications to improve the Shore D hardness of 3D-printed denture base resins [6,7].

To address these limitations, several strategies have been explored, including modification of polymer composition, optimization of processing techniques, and incorporation of reinforcing fillers. Among these, the incorporation of nanoparticles such as zirconia, titanium dioxide, and silica has been widely investigated, with studies demonstrating improvements in mechanical properties through enhanced stress transfer, reduced crack propagation, and restriction of polymer chain mobility [8,9]. However, these nanoparticles may exhibit agglomeration at higher concentrations and can affect aesthetics due to increased opacity. More recently, attention has shifted toward carbon-based nanomaterials such as graphene oxide (GO) and reduced graphene oxide (rGO), owing to their high mechanical strength and large surface area [10,11]. However, GO has been associated with issues such as agglomeration and non-uniform dispersion, which may negatively affect material properties [12,13].

Reduced graphene oxide (rGO), a derivative of graphene oxide, exhibits a partially restored graphitic structure along with residual functional groups that facilitate better interaction with polymer matrices. This structural characteristic allows improved dispersion and effective reinforcement. Additionally, rGO has demonstrated promising mechanical properties and favourable biocompatibility, making it a suitable candidate for dental material applications [14]. However, to the best of current knowledge, there is no literature evaluating the effect of rGO incorporation in 3D-printed denture base resins, particularly with respect to Shore D hardness. Therefore, the present study aimed to assess the influence of rGO nanoparticles on the Shore D hardness of a 3D-printed denture base resin. As characterization of such carbon-based nanomaterials is essential, techniques such as Raman spectroscopy are commonly employed to confirm their structural features [15].

Additionally, green synthesis methods, in which plant-derived extracts act as natural reducing and stabilizing agents during nanoparticle synthesis, have gained increasing attention in recent years. This approach offers advantages such as reduced use of hazardous chemicals, eco-friendly processing, and improved biocompatibility. Consequently, the incorporation of green-synthesized nanoparticles into dental materials has been proposed as a promising strategy to enhance mechanical properties while maintaining biological safety [16]. However, evidence regarding the incorporation of green-synthesized rGO into 3D-printed denture base resins remains limited. Hence, this study aimed to investigate the effect of various concentrations of rGO on the Shore D Hardness of 3D-printed denture base resins.

2. Materials and methods

The present *in vitro* study was carried out in the Department of Prosthodontics in collaboration with the Department of Nano Sciences and Technology, JSS Academy of Higher Education and Research (JSS AHER), Mysuru. This study obtained Institutional Ethics Committee (IEC approval no. 54/2024) approval and was conducted in accordance with institutional guidelines for *in vitro* research. The study methodology consisted of a series of steps from material preparation to hardness testing.

2.1 Sample size determination

Sample size was calculated using JMP software (version 19, SAS Institute Inc., Cary, NC, USA) at a 95% confidence level ($\alpha = 0.05$) and 80% power. A total of 25 standardized specimens, each measuring 12 mm in diameter and 6 mm in thickness, were prepared for the study. The specimens were equally distributed into five groups with 5 in each ($n = 5$) based on the concentration of reduced graphene oxide (rGO) incorporated into the 3D-Printed denture base resin (3D Accuprint Denture, D Tech, Mumbai, India). Group 1 served as the control group without rGO incorporation, while Groups 2a, 2b, 2c, and 2d represented experimental groups containing rGO at concentrations of 0.005 wt%, 0.01 wt%, 0.1 wt%, and 0.25 wt%, respectively.

2.2 Green-synthesis of rGO nanoparticles

Reduced graphene oxide (rGO) nanoparticles used in this study were synthesized from graphene oxide through a green reduction approach employing a plant-derived extract. The phytochemicals present in the extract functioned as natural reducing as well as stabilizing agents, facilitating the conversion of graphene oxide to reduced graphene oxide. The synthesis was carried out in collaboration with the Department of Nanosciences and Technology, JSS AHER, Mysuru. The resulting rGO nanoparticles were obtained in the form of a fine black powder and were stored in airtight containers to prevent contamination and moisture exposure until further use.

2.3 Incorporation of rGO nanoparticles into denture base resin

The synthesized rGO nanoparticles were incorporated into a commercially available 3D-printable denture base resin at predetermined concentrations of 0.005 wt%, 0.01 wt%, 0.1 wt%, and 0.25 wt%. The required quantity of nanoparticles for each concentration was precisely measured using an analytical weighing balance (Sartorius AG, Gottingen, Germany). The concentrations were selected based on preliminary pilot testing. Initial concentrations of 0.1 wt%, 0.25 wt%, 0.5 wt%, and 1 wt% were evaluated, among which the higher concentrations (0.5 wt% and 1 wt%) showed reduced Shore D hardness. Based on these findings, lower concentrations of 0.01 wt% and 0.005 wt% were subsequently selected for the study.

The nanoparticles were then gradually introduced into the resin matrix. To ensure uniform dispersion and minimize particle agglomeration, the mixture was initially subjected to magnetic stirring at approximately 150–200 rpm for 30 minutes at room temperature to facilitate homogeneous blending. This was followed by ultrasonication at a frequency of 33 kHz for a duration of 1 hour. This two-step homogenization process enhanced the distribution of rGO nanoparticles within the resin matrix [17].

2.4 CAD-design and 3D-printing of specimens

Test specimens were digitally designed using computer-aided design (CAD) software to ensure dimensional accuracy and standardization. Disc-shaped specimens with dimensions of 12 mm in diameter and 6 mm in thickness were fabricated in accordance with ASTM D2240-03for denture base materials [18]. The finalized designs were converted into standard tessellation language (STL) files and subsequently imported into the 3D-printing software. Specimen fabrication was performed using a digital light processing (DLP)-based stereolithography printer (ASIGA MAX UV, 385 nm; ASIGA, Sydney, Australia), which polymerizes the resin layer-by-layer under controlled light exposure.

2.5 Cleaning and post-polymerization of specimens

Following fabrication, the printed specimens were subjected to a cleaning procedure to remove any residual uncured resin present on the surface. This was achieved by immersing the specimens in 90% isopropyl alcohol for approximately 5 minutes. Subsequently, the specimens were placed in a UV light curing unit (Asiga flash curing unit, Sydney, Australia) for post-polymerization, which was carried out for a duration of 20–25 minutes. This step ensured complete polymerization and enhanced the mechanical properties of the material. After post-curing, the specimens were disinfected by immersion in 70% ethanol for 5 minutes. The disinfected specimens were then removed and allowed to air dry at room temperature prior to mechanical testing [19].

2.6 Raman spectroscopic characterization

Raman spectroscopic analysis was performed to confirm the presence and structural integrity of reduced graphene oxide within the resin matrix. The analysis was carried out using a Raman spectroscope (Horiba Xplora plus, Horiba

Scientific, France), and spectra were recorded over a wavenumber range of 500–4000 cm^{-1} under ambient conditions. The obtained spectra were examined for characteristic vibrational bands corresponding to graphitic carbon structures, including the D and G bands associated with rGO. Variations in peak intensity and position were analysed to assess the distribution and incorporation of rGO at different concentrations within the denture base resin [15].

2.7 Shore D Hardness testing

The surface hardness of the prepared specimens was evaluated using a Shore D hardness tester (Manual Analogue Durometer, Excel Industries, Kerala, India) (Figure 1) in accordance with ASTM D2240 standards [18]. Each specimen was placed on a stable, flat surface to ensure accurate measurement conditions. The durometer indenter was placed perpendicular to the specimen surface with uniform pressure. Three readings were taken at different points on each specimen, and the average value was recorded as the final Shore D hardness.

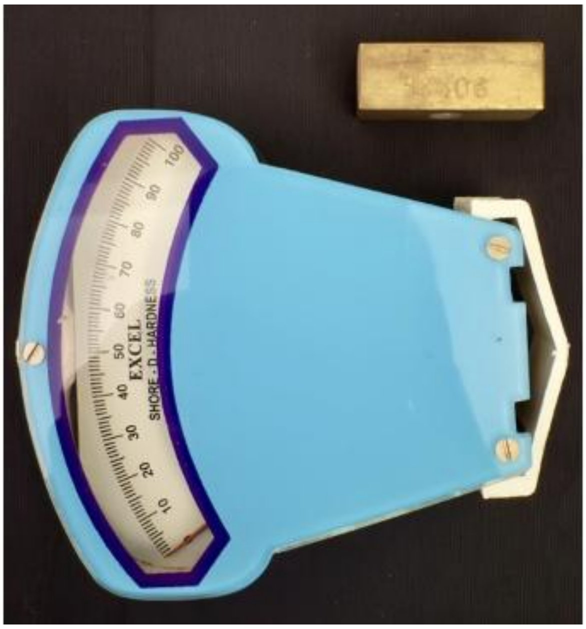

Figure 1. Manual Analogue Shore D Hardness Tester.

2.8 Statistical Analysis

The collected data were compiled and subjected to statistical analysis using IBM SPSS Statistics (version 22.0; IBM Corporation, Armonk, NY, USA). Descriptive statistics, including mean and standard deviation, were calculated for each group. Intergroup comparisons were performed using one-way analysis of variance (ANOVA) to identify statistically significant differences among the groups. When significant differences were observed, multiple comparisons were carried out using the least significant difference (LSD) post hoc test. The level of statistical significance was set at $p < 0.05$.

3. Results

3.1 Raman spectroscopic evaluation of green-synthesized reduced graphene oxide

Raman spectroscopic analysis showed the presence of reduced graphene oxide (rGO) in the denture base resin specimens. A peak was observed around 1590–1600 cm^{-1}, corresponding to graphitic carbon (G band). Other peaks appeared in the range of 2930–2970 cm^{-1}, related to C–H vibrations, and between 600–660 cm^{-1}, indicating C–C skeletal vibrations. A broad band was also seen between 3200–3400 cm^{-1}, suggesting the presence of residual oxygen-containing groups. Raman spectra were obtained for specimens incorporated with different concentrations of rGO, where (a) represents 0.005%, (b) 0.01%, (c) 0.1%, and (d) 0.25%, as shown in Figure 2.

The position of the peaks did not show noticeable changes between groups, indicating that the structure of rGO remained stable after incorporation. Variations in Raman peak intensity were observed among the groups. At lower concentrations, there was a greater peak intensity, but as the rGO content increased, there was a progressive decrease, with the highest concentration group showing the lowest intensity. Differences in the concentration and distribution of nanoparticles inside the resin matrix could be the cause of this intensity variance.

3.2 Shore D hardness evaluation

The mean Shore D hardness values of all groups are presented in Table 1. Group 1 (control) demonstrated a mean hardness of 84.2 ± 0.5. Among the experimental groups, Group 2a showed the highest hardness (86.1 ± 0.6), followed by Group 2b (85.3 ± 0.7). A reduction in Shore D hardness was observed in Group 2c (82.6 ± 0.4), with the lowest value recorded in Group 2d (81.1 ± 0.5). One-way analysis of variance (ANOVA) revealed a statistically significant difference in Shore D hardness among the groups (F = 389.703, $p < 0.0001$) (Table 1). Post hoc analysis demonstrated that all pairwise comparisons between the groups were statistically significant ($p < 0.0001$) (Table 2). This indicates that each group differed significantly from every other group in terms of Shore D hardness. Group 2a exhibited the highest hardness values, whereas Group 2d showed the lowest. Overall, the results indicate that the incorporation of rGO had a significant influence on Shore D hardness, with an increase observed in Groups 2a and 2b, followed by a decline in Groups 2c and 2d.

Table 1. Descriptive statistics of Shore D hardness values

Groups	Mean	Standard Error	F - Ratio	Significance (p–value)
Group 2a	86.120	0.10257	389.703	<.0001*
Group 2b	85.320	0.10257		
Group 2c	82.620	0.10257		
Group 2d	81.120	0.10257		
Group 1	84.200	0.10257		

*Significant difference

Table 2. Post hoc pairwise comparisons performed using the Least Significant Difference (LSD) test

Groups		Mean Difference	Standard Error	p - value
Group 1	Group 2a	1.920	0.1450517	<.0001*
	Group 2b	1.120	0.1450517	<.0001*
	Group 2c	1.580	0.1450517	<.0001*
	Group 2d	3.080	0.1450517	<.0001*
Group 2a	Group 2b	0.800	0.1450517	<.0001*
	Group 2c	3.500	0.1450517	<.0001*
	Group 2d	5.000	0.1450517	<.0001*
Group 2b	Group 2c	2.700	0.1450517	<.0001*
	Group 2d	4.200	0.1450517	<.0001*
Group 2c	Group 2d	1.500	0.1450517	<.0001*

*Significant difference

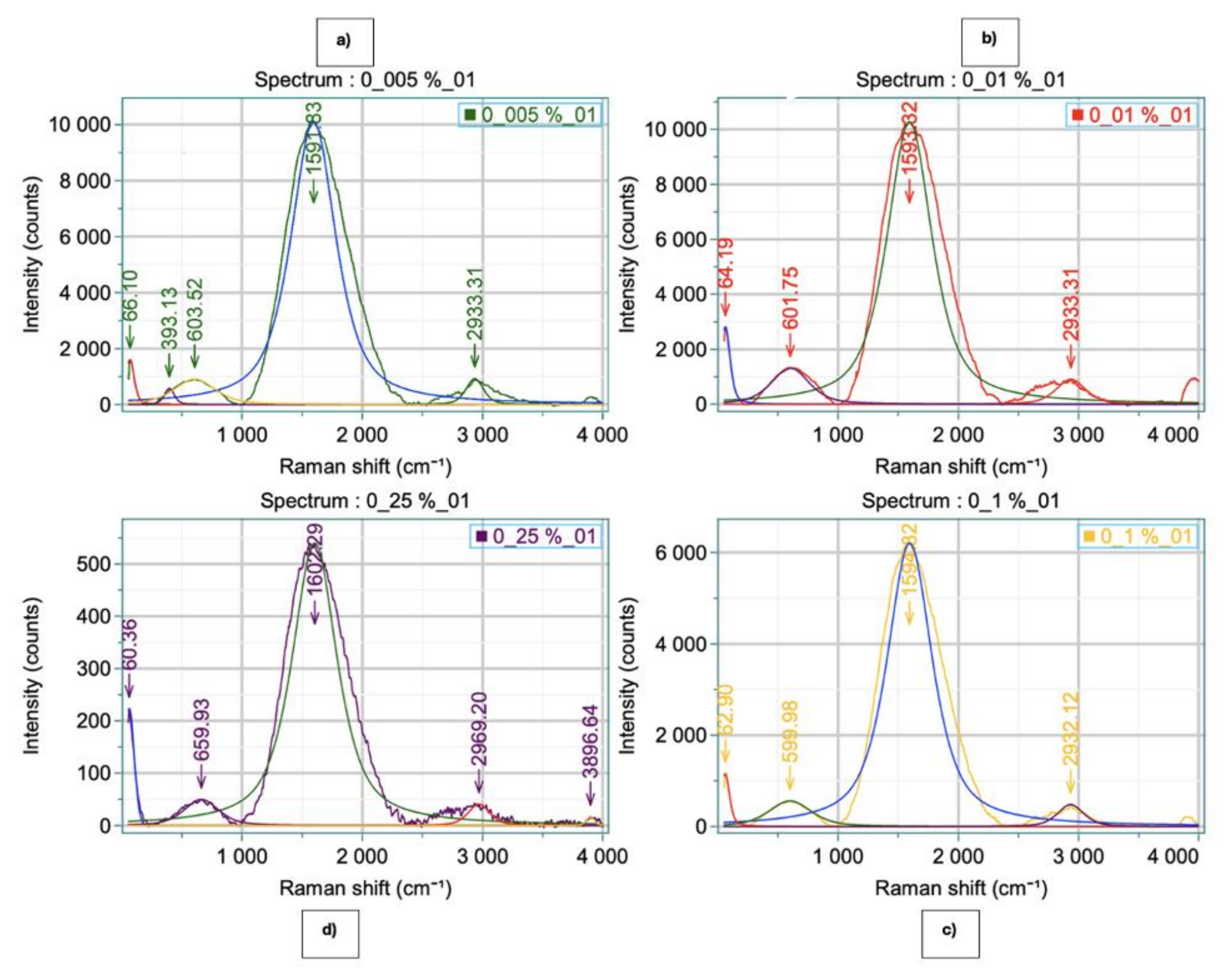

Figure 2. Raman spectra of 3D-printed denture base resin modified with different concentrations of green-synthesised reduced graphene oxide incorporated. Where the concentrations include: a. 0.005%, b. 0.01%, c. 0.1%, and d 0.25%.

4. Discussion

Shore D hardness was selected in this study as it reflects the resistance of rigid denture base materials to surface deformation under functional loading, which is directly related to wear resistance and clinical durability. This parameter is particularly relevant for 3D-printed resins, as their layer-wise fabrication and variations in polymerization can influence surface integrity and mechanical behaviour [7]. To ensure reproducibility, specimens were fabricated using a standardized CAD-based 3D-printing protocol with controlled parameters [7,9,20]. However, limitations such as reduced mechanical strength, wear susceptibility, and layer-wise fabrication inconsistencies highlight the need for strategies to enhance the mechanical properties of 3D-printed denture base resins [21].

Among the strategies explored, the incorporation of nanoparticles has been widely investigated to enhance the hardness of denture base resins, with several studies reporting improved mechanical performance at optimized concentrations. Gad *et al.* demonstrated that zirconia nanoparticles improve hardness at lower concentrations due to better filler–matrix interaction and increased resistance to indentation [10]. Similarly, Asar *et al.* reported improved hardness with silica nanoparticles; however, these benefits were not maintained at higher concentrations, where agglomeration and non-uniform dispersion adversely affected mechanical properties [22].

A comparable trend has also been observed in 3D-printed systems. Jassim *et al.* demonstrated improved Shore D hardness with cerium zirconium oxide nanofibers in 3D-printed denture base materials [23]. Badogu *et al.* reported enhanced hardness in 3D-printed acrylonitrile butadiene styrene (ABS) reinforced with zirconia (ZrO_2) nanoparticles [24]. These findings collectively suggest that nanoparticle concentration and dispersion are critical factors influencing the mechanical performance of nanoparticle-modified 3D-printed resins.

Based on these observations, attention has shifted towards carbon-based nanomaterials such as graphene oxide (GO) and reduced graphene oxide (rGO). These materials are known for their high strength and their ability to interact effectively with the polymer matrix, which may improve resistance to indentation [25,26]. Graphene oxide has been studied as a reinforcing filler in PMMA denture base materials, including those produced using additive manufacturing. At lower concentrations, improved hardness has been reported, whereas at higher concentrations, a decline in hardness has been observed, possibly due to agglomeration and interference with the polymerization process [12,27–29].

In contrast, reduced graphene oxide (rGO), formed by the partial reduction of graphene oxide, presents a comparatively more stable structure with fewer oxygen functionalities while still retaining sufficient sites for interaction with the polymer matrix [30]. This structural balance may promote better dispersion within the resin matrix and more effective reinforcement, which can contribute to improved resistance to indentation, particularly at lower concentrations [14]. However, despite these potential advantages, the use of rGO in 3D-printed denture base resins has not been extensively investigated, especially in relation to Shore D hardness. Therefore, the present study aimed to evaluate the effect of incorporating rGO nanoparticles on the Shore D hardness of a 3D-printed denture base resin, highlighting the novelty of this approach.

In addition to material selection, green synthesis methods have gained increasing attention for nanoparticle preparation due to their eco-friendly nature and potential biocompatibility advantages. In these approaches, plant-derived extracts act as natural reducing and stabilizing agents during the synthesis process [16,31]. However, there is limited evidence on the use of green-synthesized nanoparticles in PMMA denture base resins with respect to Shore D hardness. In the present study, graphene oxide was synthesized using a hydroalcoholic extract of Garcinia cambogia fruit rind through a green synthesis route, and subsequently reduced to obtain green-synthesized reduced graphene oxide (rGO), which was used for further characterization and incorporation into the denture base resin.

To characterize rGO within the resin matrix, Raman spectroscopy was employed, as it enables the identification of characteristic vibrational bands associated with graphitic structures [15]. In the present study, Raman spectroscopic analysis confirmed characteristic peaks of rGO within the denture base resin across all experimental groups. The peaks corresponding to graphitic structures were observed without any significant shift in position, indicating that the structural integrity of rGO was preserved after incorporation. However, variations in peak intensity were noted, which may reflect differences in nanoparticle distribution within the resin matrix at different concentrations. These findings are consistent with previous studies reporting similar Raman spectral features of graphene-based nanomaterials [32,33].

The results of Shore D hardness testing demonstrated a concentration-dependent effect of rGO incorporation but with an inverse relationship.

An improvement in hardness was observed at lower concentrations (0.005 wt% and 0.01 wt%), with the highest value recorded at 0.005 wt%. This enhancement may be attributed to uniform dispersion of nanoparticles within the polymer matrix, which improves resistance to surface indentation and restricts localized deformation. Similar improvements at lower nanoparticle concentrations have been reported in graphene-reinforced polymer systems [34,35]. At higher concentrations (0.1 wt% and 0.25 wt%), a reduction in hardness was observed. This decrease may be associated with agglomeration of nanoparticles, leading to non-uniform distribution and formation of stress concentration sites within the material [12,13]. In addition, higher nanoparticle loading may interfere with light transmission during the photopolymerization process in DLP-based printing systems, resulting in incomplete curing and reduced surface properties [35].

The present study has some limitations, as only Shore D hardness was evaluated and the sample size could have been larger to obtain a more representative average. The study was carried out under *in vitro* conditions. Further studies evaluating additional mechanical properties and long-term performance are recommended to better establish the clinical applicability of rGO-reinforced 3D-printed denture base resins..

5. Conclusion

Within the limitations of this study, the incorporation of green-synthesized reduced graphene oxide (rGO) into 3D-printed denture base resin demonstrated a concentration-dependent effect on surface hardness. The addition of rGO at 0.005 wt% and 0.01 wt% resulted in improved hardness, whereas higher concentrations (0.1 wt% and 0.25 wt%) did not show similar improvement. These findings highlight the importance of optimizing nanoparticle concentration to achieve effective reinforcement without compromising material properties, supporting the potential of rGO in enhancing the performance of 3D-printed denture base resins.

Conflicts of interest: The authors declared no conflicts of interest.

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