Brazilian Dental Science

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UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Instituto de Ciência e Tecnologia
Campus de São José dos Campos

ORIGINAL ARTICLE

DOI: https://doi.org/10.4322/bds.2025.e4568

Comparative study of conventional and 3D printed denture base polymers: effect of low-pressure plasma as a surface treatment method

Estudo comparativo de polímeros para bases de próteses dentárias convencionais e impressas em 3D: efeito do plasma de baixa pressão como método de tratamento de superfície

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How to cite: Meki AA, Khalaf M, Elsayed HM, El-Hossary F, Ammar M, Elsalam EBA. Comparative study of conventional and 3D printed denture base polymers: effect of low-pressure plasma as a surface treatment method. Braz Dent Sci. 2025;28(1):e4568. https://doi.org/10.4322/bds.2025.e4568

ABSTRACT

Objective: To compare flexure strength (FS) and surface roughness properties of conventional heat polymerized (CHP) and three-dimensionally printed (3DP) denture base resins and study the effect of plasma surface treatment on these properties. **Material and Methods**: Rectangular resin samples (65 × 10 × 3.3 mm^3) were fabricated from two material groups: CHP and 3DP resins (N=24/material group). Each group was divided into control and treated groups (n=12/subgroup) to study their flexural strength and surface roughness properties. A comparative evaluation of these properties was performed between the control groups at first. Afterwards, treated groups were exposed to low pressure atmospheric plasma treatment and were compared with control (untreated) samples regarding changes in their properties both before and after plasma treatment. **Results**: The surface properties of control CHP groups showed higher FS ($p<0.0001$) and lower surface roughness ($p=0.0002$) than the 3DP group. Generally, when compared to the control group of each material, the plasma-treated CHP group and the treated 3DP group showed significant increase in FS ($p<0.0001$). Surface roughness significantly increased in treated group of CHP ($p<0.0001$) but had no significant change in treated 3DP group ($p=0.068$). **Conclusion**: Conventional heat polymerized denture base resins possess superior flexural strength and lower surface roughness compared to 3D printed resins. Plasma surface treatment is an effective method to strengthen both CHP and 3DP denture base resins and roughen (micro-etch) CHP resin surfaces toward further chemical reactions.

KEYWORDS

Acrylic denture base; Plasma; Polymethyl methacrylate; Surface treatment; Three-dimensional printing.

Resumo:

Objetivo: Comparar a resistência à flexão (RF) e as propriedades de rugosidade superficial de resinas para bases de próteses dentárias polimerizadas por calor convencionais (CHP) e impressas em três dimensões (3DP) e estudar o efeito do tratamento de superfície com plasma nessas propriedades. **Material e Métodos**: Amostras de resina retangulares (65 × 10 × 3,3 mm^3) foram fabricadas a partir de dois grupos de materiais: resinas CHP e 3DP (N=24/grupo de material). Cada grupo foi dividido em grupos controle e tratados (n=12/subgrupo) para estudar suas propriedades de resistência à flexão e rugosidade superficial. Uma avaliação comparativa dessas propriedades foi realizada entre os grupos controle inicialmente. Posteriormente, os grupos tratados foram expostos ao tratamento com plasma atmosférico de baixa pressão e foram comparados com as amostras

controle (não tratadas) em relação às mudanças em suas propriedades antes e depois do tratamento com plasma. **Resultados**: As propriedades de superfície dos grupos CHP controle mostraram maior RF (p<0,0001) e menor rugosidade superficial (p=0,0002) do que o grupo 3DP. Geralmente, quando comparado com o grupo controle de cada material, o grupo CHP tratado com plasma e o grupo 3DP tratado mostraram aumento significativo na RF (p<0,0001). A rugosidade superficial aumentou significativamente no grupo tratado de CHP (p<0,0001), mas não apresentou alteração significativa no grupo 3DP tratado (p=0,068). **Conclusão**: As resinas para bases de próteses dentárias polimerizadas por calor convencionais possuem resistência à flexão superior e menor rugosidade superficial em comparação com as resinas impressas em 3D. O tratamento de superfície com plasma é um método eficaz para fortalecer as resinas para bases de próteses dentárias CHP e 3DP e tornar as superfícies da resina CHP mais rugosas (micro-jateamento) para reações químicas adicionais.

Palavras-chave

Base de prótese acrílica; Plasma; Polimetilmetacrilato; Tratamento de superfície; Impressão tridimensional.

INTRODUCTION

Polymethyl methacrylate (PMMA) has become the dominant biomaterial for the fabrication of prostheses in dental laboratories and clinics due to their favorable mechanical and aesthetic properties, simple processing techniques, cost-efficiency, and reduced toxicity. It has superseded previous denture base materials due to its reduced volumetric shrinkage, better mechanical and surface properties, reduced residual monomers and surface porosity [1,2].

With the current technologies advancing at a rapid pace and the integration of CAD-based technology in dental fields, complete dentures can be fabricated using computer-aided design/computer-aided manufacturing (CAD/CAM) technology, either by computerized milling (subtraction) from a single block or three-dimensional (3D) printing (addition) techniques using raw liquid resin [3].

The additive manufacturing techniques (AMT), also called rapid prototyping, were introduced to dental fields to enhance the final product quality and eliminate some of the drawbacks of the milling techniques regarding final waste products and the wear of cutting burs. The fabrication process includes a distinct layered-manufacturing method using unpolymerized liquid resin in an accurate printing machine. Afterward, a mandatory photo-polymerization step is required to enhance mechanical properties and avoid distortion [4].

This technique has gained popularity due to its precision, reduced time, standardized production, waste minimization, and lower infrastructure costs along with producing finer details (undercuts and better anatomy). Still, in this processing method, incomplete polymerization (residual monomers) may occur before the photo-polymerization step causing dimensional changes and affecting strength and surface texture of the final product [5].

Since it is very difficult to produce a material that meets all ideal requirements alone, the general attitude is to apply some improving treatments to these materials to augment their properties and utilize their full potential in various fields. Among these treatment methods is the use of plasma surface treatment. Low pressure plasma is a well-introduced treatment method for dry surfaces due to its simplicity, tunability and solvent-free aspect. Also, its peerless ability to modify polymer surfaces and modify the surface energy of the denture base surface, thereby improving bonding, biocompatibility, mechanical properties, chemical stability and surface texture [6] without affecting the main bulk properties and its implementation of green chemistry principles [7].

Plasma parameters are important for the treatment process and they depend on principal factors including type of gas used, operating pressure, input power, location of the sample from the plasma source [8]. In the field of applied plasma science, it is recommended to assess these conditions before determining the most suitable parameters for each treatment process.

Many different types of gas-plasmas have been cited in the literature including oxygen, ammonia, helium, and argon for modification of polymer surfaces. The resulting effect on the material mainly depends on the type of gas

used [9]. Crosslinking of a polymer outer surface can also be enhanced by gas plasma [10].

For an effective prosthetic care and patient satisfaction, denture base material must possess a sufficient flexural strength (FS) to withstand occlusal forces during mastication. A standard method to measure the FS of denture bases is the three-point bending test stated in ISO standards [11]. Also, surface roughness is of great importance in denture base materials and should be kept within acceptable clinical values to be used safely in the oral cavity [12].

To the best of our knowledge, there have been no studies on the impact of plasma surface treatment on 3DP denture base materials. Since printed denture bases have shown reduced FS in previous studies [13] and plasma treatment has been used to improve polymer surface properties [9], this current study was conducted to evaluate the impact of plasma treatment on FS and surface roughness of CHP and 3DP denture base material in the aim of improving 3DP resin properties in denture bases.

The first null hypothesis, regarding control groups, was that CHP group would be superior in both FS and surface roughness values. The second null hypothesis was that plasma surface treatment would significantly affect surface roughness and FS in both materials.

MATERIAL & METHODS

Specimen's fabrication and grouping:

A total of 48 specimens from both materials were fabricated and divided into control and treated subgroups (figure 1). Six specimens were used for each test.

Conventional heat-polymerized specimens

A total of 24 bar shaped samples were prepared by investing modeling wax specimens with the dimensions of ($65 \times 10 \times 3.3\ mm^3$) as per ISO: 20795-1:2013 for three point bending testing [11]. Investment was done and mold space was prepared by dewaxing. Then, acrylic pink powder and liquid (lot number 224008064, Acrostone Dental & Medical Supplies, Cairo, Egypt) were proportioned, mixed, packed, and polymerized in a thermostatically controlled water bath, complying with the manufacturer's instructions, and as described in previous studies [13]. After polymerization using a long-curing cycle (74 °C for 8 hours) and slow bench cooling, deflasking was done and samples were retrieved and finished.

3D printed specimen fabrication

Designing software (MasterCAM® CNC software v8.1) was used to virtually design

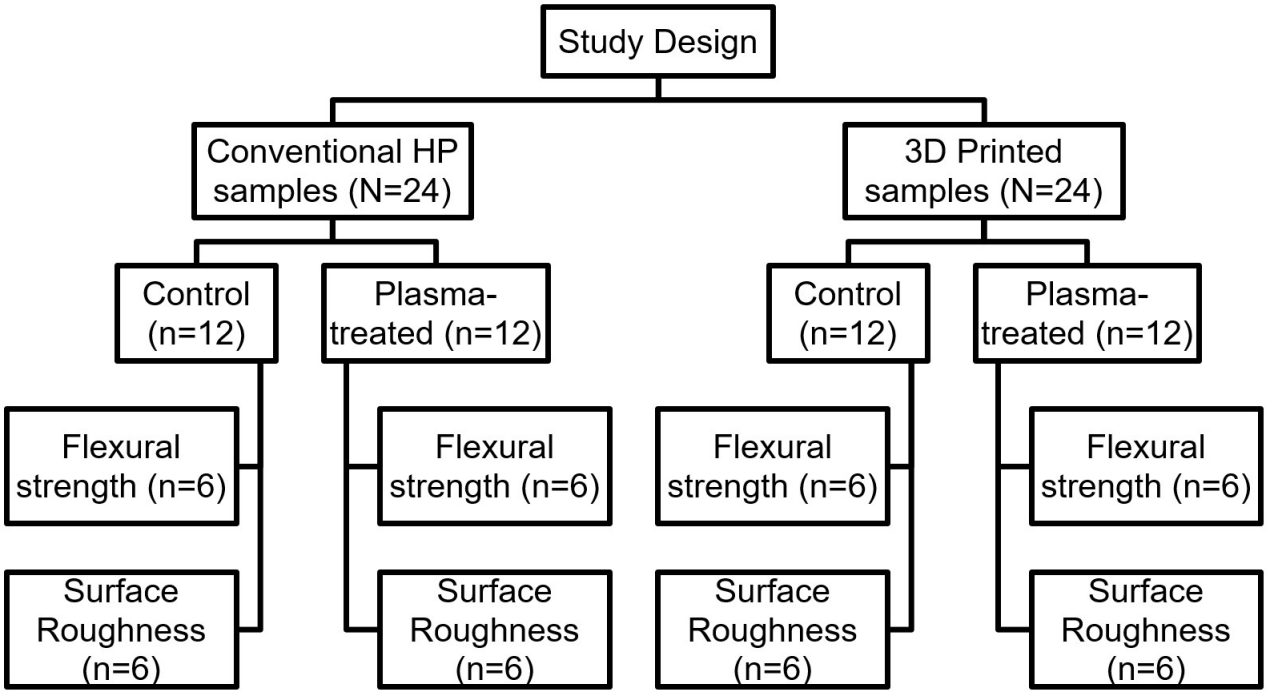

Figure 1 - Organizational chart illustrating the grouping of specimens and tests performed.

specimens with the same dimensions. The STL design file was exported to the CAM software (Chitubox Basic software v1.9.3) and positioned at 0° on the platform with a 50 μm of layer thickness complying with the manufacturer's instructions and similar to previous studies [14]. The STL file was copied, arranged, and sliced by the same software and then exported to the 3D printer (Phrozen shuffle LCD Resin 3D Printer, phrozen shuffle tech Co LTD, Taiwan) with ultraviolet light source having a wavelength ranging from 380 to 420 Nm. Pink liquid resin (lot number WU082N02, Next Dent, Denture 3D+, Vertex Dental, Netherlands) was shaken and placed in resin tanks. After printing, specimens were removed and cleaned using two-step wash in Ethanol >90% (lot number Eoo58111, OctoPharma, Ethyl alcohol, Egypt) in an ultrasonic cleaner (CD-4820, Codyson, China) for 4 minutes and then for another one minute to remove any remaining uncured resin [14]. Post-polymerization was done for >20 minutes [15] using an ultraviolet curing unit (Bre. Lux. Power Unit, Bredent, Germany) in a temperature of >60°C, while submerged in glycerin [16]. All samples' dimensions in this study were verified using digital calipers (Carbon-fiber composite digital caliper, Total Inc. China).

Plasma treatment:

Plasma used in this study is low pressure plasma using a homemade reproducible experimentally arranged system of direct current (DC) glow discharge plasma system (Figure 2-3) similar to another used in previous studies [17].

The vacuum cell consists of two parallel moving electrodes surrounded at 2.5 cm by a cylindrical tube made of Pyrex glass. Each electrode is a brass disk with a diameter of 5 cm and a thickness of 1.5 cm. A double-stage rotary vacuum pump (Speedivac 2, Edwards High Vacuum, Crawley, UK) was used to maintain a base pressure of 4×10^{-2} bar. The vacuum tube was connected to open air via a needle valve (Edwards capsule dial gauge CG 16K) by which the air gas flow can be controlled to adjust the gas pressure inside the tube. A vacuum gauge was connected to measure the gas pressure inside the tube. To generate the plasma, both electrodes were connected to a DC power supply working up to 0.45 kV and a variable load resistance controlled the current. Specimens were positioned on the center of the lower electrode, transversely in the direction of gas flow. Samples were exposed to the generated

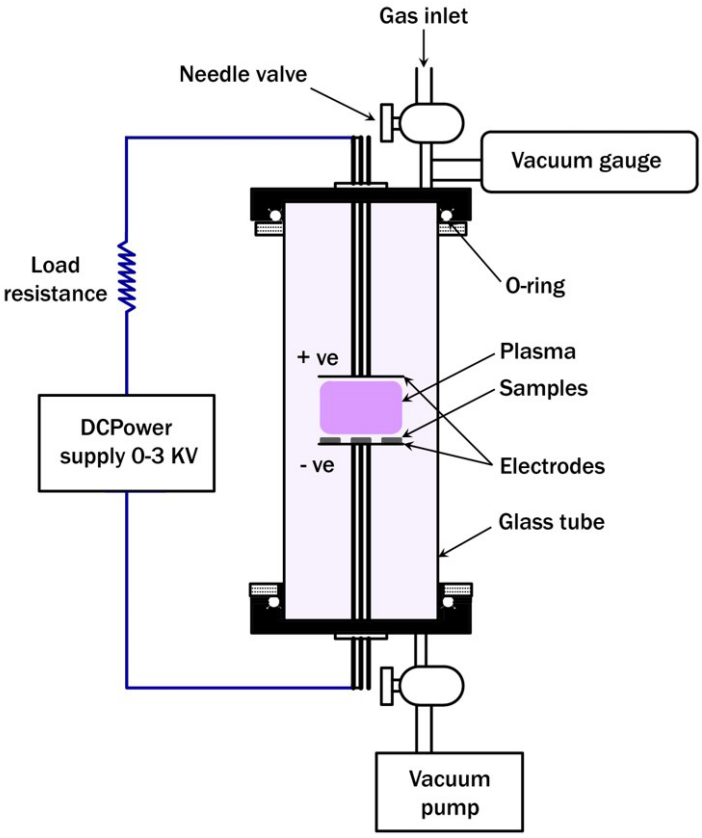

Figure 2 - A circuit representation of the device arrangement.

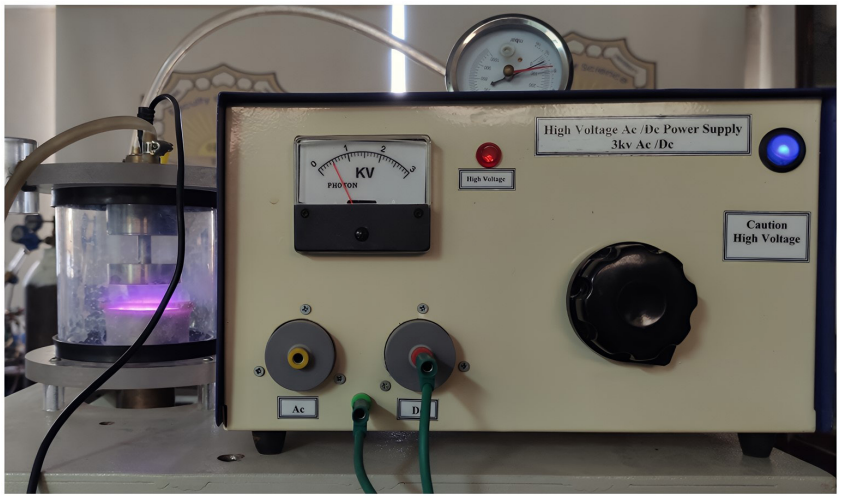

Figure 3 - DC-glow discharge plasma system including DC power supply and Vacuum chamber.

plasma (homogenous uniform glow) for 20 min. After the exposure time, the tube was kept locked to restore pressure and dissipate the heat for 10 minutes. The samples were handled carefully and isolated in a dry place to minimize the effect of surface aging [18]. A pilot study was performed on a separate set of samples, with different plasma exposure times to determine the best plasma parameters for the required test without affecting the specimen's bulk.

Specimens Testing

Flexural properties (three-point bending test)

A total of 48 rectangular samples of the four subgroups were tested using a universal testing machine (Model 3345; Instron Industrial Products, Norwood, USA) at room temperature, as per ISO: 20795–1:2013 [11]. Each sample was horizontally mounted in the loading fixture (two parallel supporting rods with span length of 50 mm) and connected to the testing machine and a Bi-beveled chisel (2 mm width) with a 5 kN of load force. Then, samples were compression loaded until fractured by a crosshead at a steady rate of displacement (1 mm/minute). Data was recorded by computer software (Bluehill Lite Software Instron® Instruments). The limiting stress at which failure or instability is imminent is represented by FS and its value for each sample was calculated by the formula: $(FS = 3FL/2wh^2)$ - where; FS is the flexural strength (MPa), F is the load (N) at fracture, L is the span between supporters (mm), w is the sample width (mm) and h = sample height (mm).

Non-contact surface roughness testing:

Non-contact technique was done as mentioned in the literature [19]. A digital microscopic camera (U500x, Guangdong, China), with a resolution of 3 Mega Pixels, was used to capture the samples while connected with compatible computer. The camera was placed vertically at 2.5 cm away from the samples. Light was obtained using an 8-LED lamp, with a color index close to 95%. Images were recorded at maximum resolutions and cropped to 350 × 400 pixels to standardize area of roughness measurement. Analysis of the cropped images was done using WSxM software for scanning probe microscopy (SPM) on Windows. (Ver. 5, Nanotec, Electronica, SL). System calibration was made using a ruler. For each specimen, multiple images were collected in the central and side areas. Average heights (Ra) were calculated and expressed in micrometers (μm).

Statistical analysis

Data were collected, tabulated, statistically analyzed, presented as descriptive statistics, and tested for normality using Shapiro-Wilk tests. A two-tailed independent-sample t-test was used to compare between control groups and evaluate the difference between the control and treated subgroups of the same material. The confidence interval was set at 95% and the significance level to 0.05. A P-value ≤ 0.05 was considered statistically significant. Statistical analysis was achieved using GraphPad Prism™ software (version 9.5 for Windows; GraphPad Inc., California, USA).

RESULTS

Before plasma treatment, the mean FS of control CHP groups (82.30 ±2.48) was higher than control 3DP groups (66.14 ±2.6). Statistical data analysis showed a highly significant difference in FS between the control groups ($p<0.0001$) (Table I). The mean surface roughness of control 3DP groups (0.244±0.024) was higher than control CHP group (0.14±0.037). The analysis showed a highly significant difference in roughness between the control groups ($p=0.0002$) (Table II, Figure 4).

Table I - Mean, standard deviations (SD), and significance of FS in PMMA groups

Groups	Mean ±SD (MPa)		*p*-value*
	Control	Treated	
CHP group	82.30 ±2.48	95.13 ±1.95	<0.0001*
3DP group	66.14 ±2.6	99.3 ±9.26	<0.0001*
p-value*	<0.0001*		

*Significant at (p≤0.05).

After plasma treatment, when FS test results were examined, the treated CHP group (95.13±1.95) showed higher FS than control group (82.30±2.48). The analysis showed a highly significant difference between treated and control CHP groups ($p<0.0001$). Treated 3DP groups (99.3 ±9.26) showed a significantly higher FS than control groups (66.14 ±2.6). The analysis showed a highly significant difference between treated and control 3DP groups (p<0.0001) (Figure 5-B).

Also, when surface roughness results were examined, treated CHP group (0.28±0.016) showed higher surface roughness than control group (0.14±0.037). The analysis showed a highly significant difference between treated and control groups ($p<0.0001$). And treated 3DP groups (0.27±0.019) showed a higher FS than control groups (0.24±0.024). Yet, statistical analysis showed an insignificant difference between treated and control groups ($p=0.068$) (Figure 5A). A 3D surface analysis image scan of control and treated groups is shown in figure (6).

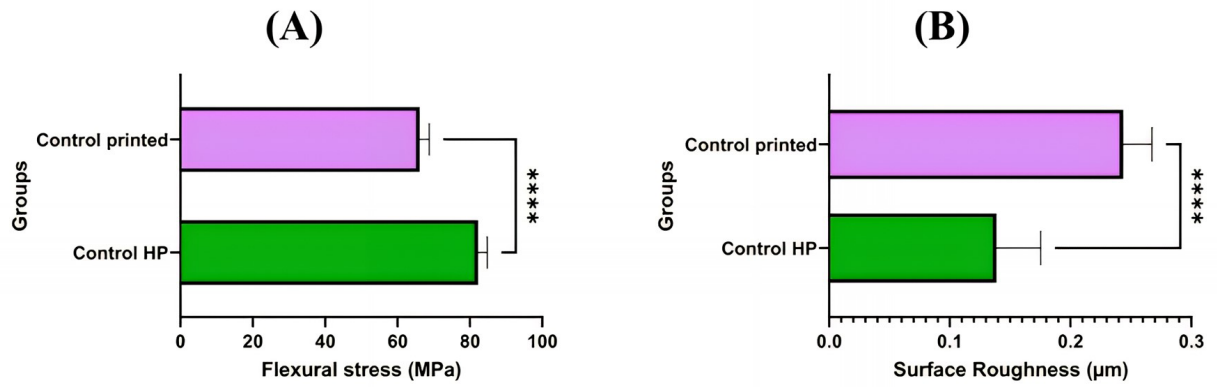

Figure 4 - Statistical analysis (Mean and standard deviation) of A) Flexural strength and B) Surface roughness between control groups.

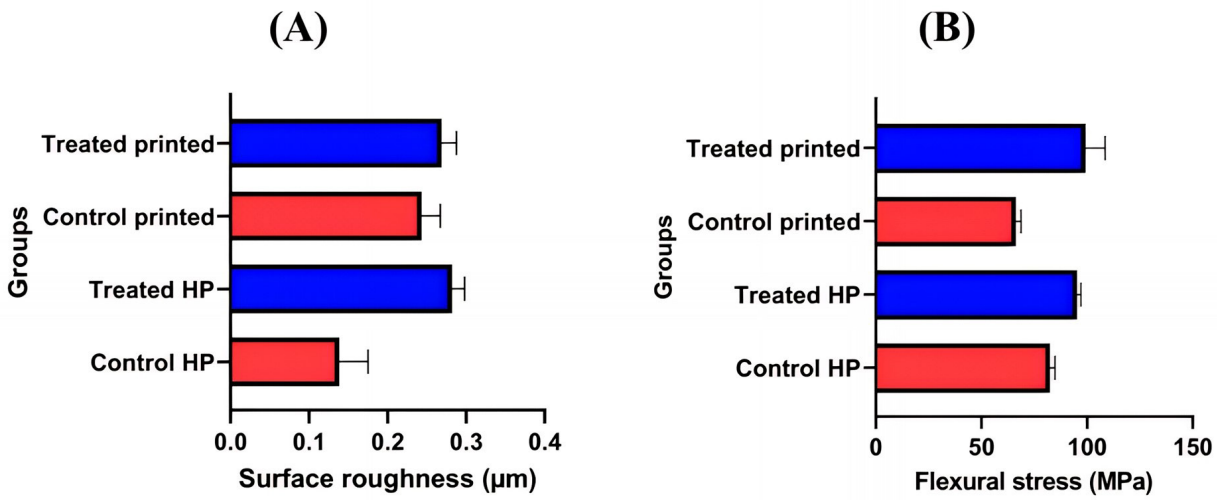

Figure 5 - Statistical analysis (Mean and standard deviation) of (A) Surface roughnes and (B) Flexure strength between control and treated groups.

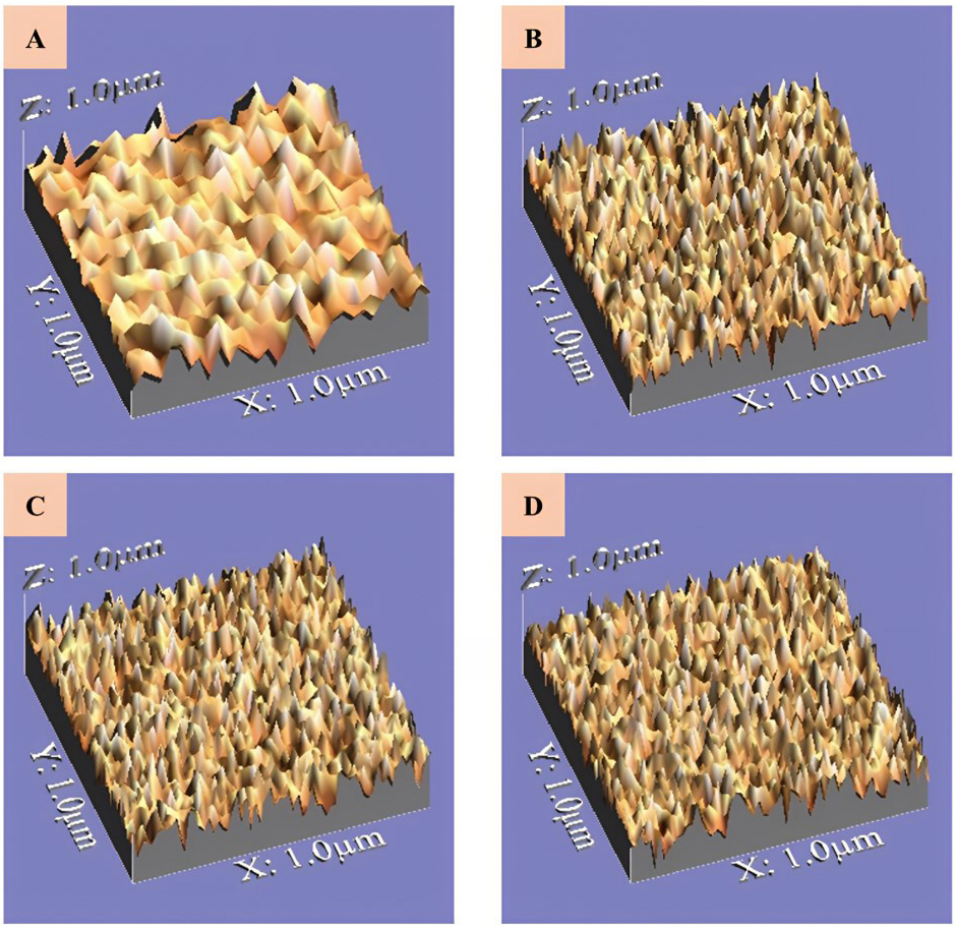

Figure 6 - showing 3D surface analysis image scan of control and treated groups A) CHP control group, B) CHP treated group, C) 3DP control group, D) 3DP plasma treated group.

Table II - Mean, SD and significance of surface roughness in PMMA groups

Groups	Mean ±SD (µm)		*p*-value*
	Control	Treated	
CHP group	0.14±0.037	0.28±0.016	<0.0001*
3DP group	0.24±0.024	0.27±0.019	0.068
p-value*	0.0002*		

*Significant at (p≤0.05).

DISCUSSION

The first null hypothesis was partially rejected as CHP control group showed lower roughness values. The second null hypothesis was partially rejected as plasma surface treatment did not significantly affect surface roughness of 3DP group.

Earlier studies of plasma treatment to PMMA samples were done only to CHP resins. Hence, this study included laboratory-based 3D printed dental resin samples used for denture base fabrication. In this study, control 3DP samples demonstrated the lowest FS values and yet, they were above the ISO recommendations for minimum FS of polymers used for acrylic denture bases (65 MPa) [11]; which supports the manufacturer's claim of its suitability as denture base materials.

During the polymerization process, the degree of double bond (terminal aliphatic C=C) conversion into single covalent bonds between carbon atoms (C-C) causes the change of material from liquid to solid forms. Furthermore, the degree of conversion (DOC) is an important indicator of the mechanical and physical properties of the resulting resin. Printed resins have incomplete (lower) DOC in comparison with other types of resins [20]. This indicates the presence of free suspended monomers to the end-product with possibility of leach-out and tissue irritation and eventually affects the mechanical properties due to the relatively weak bond between successive printed layers [20].

In this study, control CHP group showed a higher FS values when compared to 3DP control group. This could be attributed to the high-

temperature long-cycle polymerization process used during fabrication of CHP samples [3] which gives them a better chance of curing and improves the fusion between cross-linking agents and polymer chains. Consequently, this decreases residual monomers, increases molecular weight, and minimizes the chances of internal porosity, crack propagation and material plasticity.

The decreased FS values of 3DP samples could be due to the use of acrylic ester monomers that have relatively low DOC [21] as the abovementioned. Another explanation could be the water-polymer bonds formed during layered polymerization technique due to water sorption properties [4]. When water molecules diffuse into resin polymer, they form interpolymeric spaces that force polymer chains away from each other with water molecules in between them. This will reduce adhesion forces between successive layers and cause swelling of the denture base, hence decreasing both strength and surface smoothness, and increasing plasticizing effects [4].

This also has been verified by Gad et al. [21] who found some voids using SEM at fracture side of printed samples caused by evaporation of water particles incorporated between layers of printed samples. These voids decrease interfacial bonding between layers and adversely affect mechanical performance of printed samples and initiate fractures.

This result agrees with earlier studies using the same brand of liquid resin (NextDent Denture 3D+). Chhabra et al. [1], Fouda et al. [22] and Gad et al. [21] reported similar results, and their FS values were somewhat similar to our results and in compliance with ISO recommendation. Also, Al-Dwairi et al. [23] concluded the same results which were slightly higher than the recorded in our study. Al-Qarni and Gad [13] showed similar results, but their flexure values of 3DP resins were lower than ISO requirements. This difference can be explained by variations in building parameters and post polymerization process [5]. Prpić et al. [24] showed the same results using another product of the same brand (NextDent Base).

Other studies of different brands showed opposite results. Interestingly, Temizci et al. [3] concluded in their study that FS in the 3DP group was higher than milled group and HP group. In addition, Di Fiore et al. [25] reported in their study an insignificant difference in FS values between CHP and 3DP samples; with a slight increase in flexure values in 3DP samples. These unusual results could be explained by the difference in material brands used during each study, the difference in build orientation, layer thickness and\or printer used during sample processing [5].

Surface roughness test results showed higher roughness values of the control 3DP group than the control CHP groups. This high roughness value could be a result of the voids formed by water sorption properties of printed samples [21] and/or a natural sequel to the layered building of objects that forms micro-stepping surface, also known as layer lines [5]. Although this surface topography is inevitable, printing parameters such as building orientations and layer thickness were taken into consideration in this study.

It has been proven in the literature that, during printing, the lower the layer thickness, the higher the DOC, hence the lower residual (uncured) monomers in the final product. Also, a zero degree of build orientation results in fewer layers per specimen and improves their object's details (surface smoothness) [26] and FS as much as possible when the printed layers are subjected to vertical loads [21]. Another issue in additive printing techniques that could be a source of surface roughness is the possibility of forming partially cured particles when inadequate post-polymerization step is not properly achieved. These particles may dislodge from the surface and form microscopic porosities [5].

The results of the current study agree with previous similar studies; Both Poker et al. [14] and Gad et al. [21] concluded similar results using the same product by a non-contact profilometer scan. Additionally, Meirowitz et al. [27] in their study of Candida albicans adhesion to denture base fabrication methods, they reported that the 3DP samples showed higher surface roughness than both CHP and milled samples. Furthermore, using a contact profilometer, Falahchai et al. [28], Di Fiore et al. [25] and Helal et al. [12] reported similar results despite the difference of building parameters. On the contrast to our findings, Al-Dwairi et al. [23] reported an insignificant increase in surface roughness in the CHP group in comparison to the 3DP group of the same resin brand.

Low-pressure plasma treatment causes free radicals that result in four chemical modifying effects on the polymer surface micro-environment,

these are (1) surface cleaning (removal of organic contamination), (2) micro-etching (degradation/ablation), (3) surface activation (modification of the surface functional groups) and (4) cohesive strengthening of the surface by cross-linking (branching of polymer chains) in near surface molecules that stabilize the surface mechanically [8].

The longer treatment time in the current study was beneficial to slow down the ageing process of the polymers in the air, increase stability of plasma polymer films (PPFs) and maintain their gained properties for a longer time. This is achieved by enhancing surface cross-linking and preventing interface enthalpy (polar group reorientation and surface restructuring) [29]. This is in agreement with a previous study by Vesel and Mozetic [18] reporting that longer plasma treatment times increase surface crystallinity, and sequentially slow the ageing process due to the limited surface mobility of chains at polymer surfaces.

Among the factors affecting the strength of polymers is the cross-linking of polymeric chains, as it has a crucial impact on the deformation behavior (strain hardening) of the polymer resulted by restricting the motion of polymer chains, hence giving strength to the polymer [30]. Another factor is the crystallinity degree, as the crystalline phase enhances intermolecular bonding leading to regularly aligned chains (lamellae), leading to higher strength and hardness features [31].

Studies show that plasma treatment of polymers improves cross-linking by branching of near surface molecules, along with the degree of surface crystallinity, and increasing surface roughness (etching). when they are exposed to appropriate plasma density [32]. Similarly, Yun et al. [33] reported an increase in polymer cross linked structure and degree of crystallinity upon exposure to plasma within the PPFs which restricts chain mobility and increases mechanical properties of the polymers. Motaal et al. [17] revealed that plasma treatment increased the flexural strength of repaired CHP resins with auto polymerized resins.

In contrast, Pan et al. [34] and Jassim [35] reported a decreased strength of polymer after plasma surface treatment. This might be related to different types of plasma parameters used and/or different brands of polymers.

The application of plasma on polymers removes low molecular weight polymers by breaking primary chemical bonds (chain cleavage) by ion bombardment and transforming them into high molecular weight surface polymers by cross-linking reactions between remaining chains and formation of PPFs on the outer surface, which enhances surface stability in comparison to conventional polymer coatings [29]. Cross-linking of polymers can improve mechanical properties, bond strength at the surface and their ability to resist the heat by forming a very thin cohesive layer [10].

The increased flexural strength values in the CHP group in this study can be elucidated based on the abovementioned cross-linking phenomenon of plasma and its effect on polymer degree of crystallinity, thus, increasing mechanical properties of polymers by hindering their molecular chains movement [31]. Yet, this result disagrees with a previous study by Jassim [35] reporting a decreased flexural strength of CHP acrylic after plasma treatment. The probable cause of this difference is the use of argon plasma in the previous study [35].

From a physical point of view, plasma treatments increase roughness values (etching) in polymers as a result of ion bombardment into the polymer surface, which consequently forms minute peaks and valleys in micro surface levels (figure 6), hence increasing surface roughness [36]. Also, this can be ascribed to the fact that oxygen containing plasma treatment is considered an effective etching technique to polymeric surfaces that creates roughness by preferential ablation of the amorphous residues between the crystal domains effect [9,31].

The increase in roughness of CHP samples after O_2 containing plasma treatment agrees with a previous study by Masood and Mahamed [9] reporting similar roughness results due to the formation of carbon-containing groups (C-O-C, C=O, C=C) on the surface which was analyzed using FTIR spectrum. Resulting in activating the treated surface toward further chemical reactions (surface etching) [9]. Yildirim et al. [6] also reported similar roughness results using argon plasma. However, this disagrees with Jassim [35] who reported in a previous study a decrease in roughness of CHP acrylic after argon plasma treatment. Chytrosz-Wrobel et al. [31] reported

similar roughness results on other medically relevant polymers after O_2 plasma treatment.

This difference in surface topography can improve mechanical interlocking and the increase surface area available for molecular or chemical reactions. Clearly, the rough geometry of the interface provides an increased adhesion strength using mechanical and chemical mechanisms [37]. Another observation of the results is that treated 3DP samples showed no significant effect on roughness property in comparison to control group. The author attributed this to the existing inherent roughness resulting from the layered manufacturing process used [14].

CONCLUSION

The current study indicated that 3DP resins possess lower FS properties and higher surface roughness than CHP resins. Plasma surface treatment significantly increased FS and surface roughness in the treated CHP group. Plasma treated groups of 3DP resins showed a high increase in FS almost comparable to CHP resins, without affecting their surface roughness when compared to control groups.

Author's Contributions

AAM: Conceptualization, Methodology, Software, Validation, Formal Analysis, Investigation, Resources, Data Curation, Writing – Original Draft Preparation. MK: Resources, Data Curation, Writing – Original Draft Preparation, Writing – Review & Editing. HME: Resources, Data Curation, Writing – Review & Editing. FEH: Writing – Review & Editing, Visualization, Supervision. MA: Writing – Review & Editing, Visualization, Supervision. EBAE: Writing – Review & Editing, Visualization, Supervision.

Conflict of Interest

The authors have no conflicts of interest to declare.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Regulatory Statement

This study was designed as an experimental in-vitro controlled study without using any living tissues. Hence, no ethical approval was necessary.

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Date submitted: 2024 Nov 01
Accept submission: 2025 Jan 22