

ORIGINAL

Comparative evaluation of the flexural strength of two different CAD/CAM-milled PMMA for long-term fixed provisional restorations

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Aim: To determine and compare the flexural strength (FS) of heat-cured poly methyl methacrylate (PMMA) resin, CAD/CAM-milled PMMA resin, and CAD/CAM-milled PMMA resin reinforced with graphene.

Materials and methods: In accordance with ISO 10477:2018, thirty rectangular samples with measurements of 25 mm (l) x 2 mm (b) x 2 mm (h) were manufactured for the purpose of FS evaluation (Dentistry-polymer-based crown and veneering materials). PMMA resin samples that were heat-cured (Group-I; n = 10), CAD/CAM-milled (Group-II; n = 10), and CAD/CAM-milled PMMA resin reinforced with graphene (Group-III; n = 10) were produced and sorted according to the kind of material. Before testing, the samples were kept for a full day in distilled water. For each of the thirty samples, a 3-point bend test was conducted utilizing a universal testing machine at a crosshead speed of 1.0mm/min till breakage. Utilizing a One-way ANOVA and the *post hoc* Tukey HSD test, the FS data were tabulated and statistically analyzed.

Results: For the test samples in Group I, the mean flexural strength was 550.29 MPa. For Group II the mean flexural strength was 1481.966 MPa. For Group III the mean FS was 1447.45 MPa. A comparative assessment of Group I test samples, Group II test samples, and Group III test samples showed that the mean FS is highly significant ($P < 0.001^{**}$). On multiple comparisons, the mean FS difference between Group I test samples and Group II test samples was observed to be highly significant ($P < 0.01^{**}$). The mean FS difference between Group I test samples and Group III test samples was observed to be highly significant ($P < 0.01^{**}$). The mean FS difference between Group II and Group III was observed to be insignificant ($P > 0.05^{*}$).

Conclusion: CAD/CAM-milled PMMA resin showed the maximum FS followed by CAD/CAM-milled PMMA resin reinforced with graphene and heat-polymerized PMMA. Therefore, CAD/CAM PMMA-based polymers could be utilized for long-term provisional restorations in comparison to heat-polymerized poly methyl methacrylate (PMMA) resin.

Keywords: computer-aided design, graphene, Polymethylmethacrylate

Introduction

Provisional restorations are indicated for tooth-supported fixed partial dentures and implant-supported restorations. Provisional restorations have immediate protective, functional, and stabilizing benefits, but they are also helpful for diagnostics, where the occlusal, functional, and

design aspects are developed to determine the best course of action before permanent restorations are fabricated (1).

The biological, esthetic, and mechanical requirements for fixed prosthodontic restorations should all be met by a provisional restoration. For complete mouth rehabilitation situations, provisional restorations should last for a long time if additional therapy such as intensive periodontal treatment,

orthodontic stabilization, or evaluation of alterations in the vertical dimension of the occlusion is needed (2). When long-term fixed provisional restorations replace several teeth, material strength and stability are essential for long-span and long-term provisional treatment.

Several materials are being utilized for the production of provisional restorations. Commonly used materials include autopolymerizing resin, dual curing resin, bis-GMA resin, bis-acryl resin, visible light-cured resin, urethane dimethacrylate resin, and heat-cured PMMA resin (1, 2). Heat-cured Provisional materials made of PMMA resin continue to be the preferred material since they exhibit more flexural strength than those made of other resins. Heat-cured poly methyl methacrylate (PMMA) resin could function satisfactorily for long-term provisional restorations as it has the advantages of greater flexural strength, wear resistance, color stability, maintenance of surface finish, and resistance to polymer breakdown compared to the other resins (3).

Heat-cured PMMA resin is more popular due to its manipulation, ease of handling, and ease of stability and repairability in an oral environment. Nonetheless, heat-cured PMMA's mechanical qualities have been deemed insufficient, and its common downsides include volumetric shrinkage, dimensional alterations, fracture susceptibility, residual monomer, the potential for surface and subsurface cavities, and poor marginal adaptability (4).

The shortcomings of traditional heat-cured PMMA resin have been addressed in recent years by the development of CAD/CAM PMMA-based polymers. The chemical structure of PMMA-based (CAD/CAM) polymers is comparable to that of traditional PMMA materials (5). CAD/CAM PMMA-based polymers, on the other hand, have better material qualities since they are less soluble in water, dense, highly cross-linked, and homogeneous. One potential explanation for the high FS of CAD/CAM PMMA-based polymers could be their lack of porosity and voids (5).

CAD/CAM PMMA-based polymers can be milled to a precise outline from a dense blank of pre-polymerized acrylic resin to a very minimal thickness without compromising its strength or crack propagation during the milling process. Hence, CAD/CAM PMMA offers increased physical, mechanical, and esthetic properties in comparison to traditional heat-cured PMMA resin. CAD/CAM PMMA-based polymers offer several advantages such as a decrease in residual monomer content, improved optical properties, better color stability, and ease of fabrication of provisional restorations by eliminating conventional impression and fabrication procedures, thereby, improving patient comfort (5, 6).

To increase mechanical stability, CAD/CAM PMMA-based polymers have been reinforced with graphene. Because of its special mechanical qualities, graphene has generated a lot of attention in a variety of scientific domains, including dentistry. The term “graphene” refers to a flat monolayer of carbon atoms that are incredibly strong and elastic, and are

firmly arranged into a two-dimensional honeycomb lattice. Graphene oxide and reduced graphene oxide are two of graphene's derivatives (6).

Graphite can be oxidized to produce graphene oxide. It offers a variety of functional groups (like hydroxyl, carboxyl, and epoxy groups) that can be combined with other materials and biomolecules to form graphene oxide, allowing for the use of these combinations in a variety of polymer-based nanocomposites with a broad range of uses (3, 6). Composites with improved mechanical properties can be created by blending materials and polymers linked to graphene. Interestingly, the improvements are still noticeable at low filler loadings in the polymer matrix. Polymer physicomaterial characteristics may be greatly enhanced with graphene.

Graphene has been used widely for periodontal tissue regeneration, being coated on the surface of implants to improve osseointegration (7–9). It has good anti-bacterial properties and has shown excellent biocompatibility. Due to these enhanced capabilities, graphene is being incorporated into dental materials such as metals, ceramics, and polymers. The addition of graphene and carbon fillers has been shown to significantly increase the flexural strength of PMMA polymers (10, 11).

Many studies have shown that the incorporation of graphene into PMMA resin leads to unesthetic dark discoloration of the material which would be unsuitable for the fabrication of the prosthesis (9–11). Therefore, to overcome this discoloration, graphene is reinforced into PMMA during industrial manufacturing of CAD/CAM PMMA blank.

Flexural strength is of paramount importance, especially for long-span and long-term fixed prosthodontic restorations. When establishing mechanical strength, stiffness, and temporary restorative material rigidity, flexural strength is essential.

The main method used to assess any modifications, additions, or reinforcements made to PMMA-based polymers is flexural strength. When a test specimen is subjected to flexural loading, its FS—also referred to as its modulus of rupture or bending strength—is measured as the force per unit area at the fracture site. Both four-point and three-point bend loading can be used in flexural testing. The FS of various PMMA resin polymers is more commonly assessed using the three-point bend test (12).

The FS of bis-acrylate composite resin (Protemp), CAD/CAM PMMA, and traditional heat-cured PMMA have all been examined and compared in several research (5, 13–21). Bis-acrylate composite resin has the disadvantage of discoloration and susceptibility to fracture compared to conventional heat-cured resin in long-term use. Nevertheless, limited studies were reported with CAD/CAM PMMA reinforced with graphene.

In light of the foregoing discussion, the current *in vitro* study's objective is to assess and contrast the FS of three

various materials: CAD/CAM-milled PMMA, heat-cured PMMA resin, and CAD/CAM-milled PMMA reinforced with graphene, for application in long-term fixed provisional restorations. The current research null hypothesis is that there shouldn't be any discernible variations in the flexural strength of PMMA that has been heat-cured and PMMA polymers that have been CAD/CAM milled.

Materials and methods

In accordance with ISO 10477:2018, rectangular specimens measuring 25mm(l)x2mm(b)x2mm(h) were created (n = 30). These specimens were used to assess the flexural strength (Dentistry-polymer-based crown & veneering materials).

To create heat-cured PMMA resin test samples, a CAD/CAM-milled PMMA sample measuring 25mm x 2 mm x 2 mm was utilized to make a custom putty silicone mold. Pouring the melted wax into the silicone mold allowed for the creation of wax patterns. Following the cooling process, the wax was removed and the wax designs were submerged in a type III dental stone-filled metal dental flask. The stone was permitted to be set, followed by the application of separating medium on the set dental stone. An additional increment of dental stone was mixed to fill the base of the dental flask. The flask was tightened with the help of a metal clamp and was allowed to set. The flask was later placed in a boiling water bath for 15 minutes for wax elimination. The flask was removed from the hot water bath and opened, separating medium was applied to the mold cavity and other dental stone surfaces. For the purpose of polymerizing the heat-cured PMMA resin using the compression molding process, the monomer and polymer (DPI tooth molding powder) were measured out and manually combined as per the directions of the manufacturer. The mix was allowed to reach the dough stage and was packed into the mold by using finger pressure. The flask was closed and tightened in a bench press and was permitted to bench cure for 20 minutes. The flask was placed in the curing unit and was allowed to polymerize at 74°C for approximately 2 hours followed by 100°C for 1 hour. Following the polymerization cycle, the samples were taken out of the dental flask polished, and finished using acrylic trimmers and aluminum oxide abrasive sheets (600, 800 grit). The samples' dimensions (25mm x 2mm x 2mm) were confirmed with a digital vernier caliper. The samples were kept in distilled water for 24hrs prior to testing. Thus, test "samples of heat-cured PMMA resin (n = 10) were obtained.

In order to attain the CAD/CAM-milled PMMA test samples (n = 10)", a stereolithography (STL) file was virtually generated utilizing the CATIA software (Dassault Systèmes) to the appropriate dimensions (25mm x 2mm x 2mm). A 10mm thick CAD/CAM PMMA blank (BiLKIM CO. LTD, Turkey) was utilized to mill 10 test samples utilizing CAD/CAM milling machine (D15, Yenadent,

Vierzon, France), (Figures 1A–C). Acrylic trimmers and aluminum oxide abrasive sheets were used for the samples' finishing and polishing processes (600, 800 grit). A digital vernier caliper was used to confirm the samples' dimensions (25 × 2 × 2 mm). Before testing, the samples were kept for a full day in distilled water. Thus, test samples



FIGURE 1 | (A) CAD/CAM PMMA blank. **(B)** Manufacturer packaging CAD/CAM PMMA resin blank (front cover). **(C)** Manufacturer packaging CAD/CAM PMMA resin blank (back cover).

(n = 10) of PMMA resin that had been CAD/CAM milled were produced.

In order to produce the CAD/CAM-milled PMMA reinforced with graphene test samples (n = 10), a stereolithography (STL) file was virtually generated utilizing the CATIA software (Dassault Systèmes) to the necessary dimensions (25mm x 2mm x 2mm). A 14mm thick CAD/CAM PMMA blank reinforced with graphene (G-CAM, Graphenano Dental, Spain), (Figures 2A–C), was utilized to mill 10 test samples utilizing CAD/CAM milling machine (D15, Yenadent, Vierzon, France). The samples underwent polishing and finishing processes with aluminum oxide abrasive sheets and acrylic trimmers (600, 800 grit). A digital vernier caliper was utilized to confirm the samples' dimensions (25mm x 2mm x 2 mm). Before testing, the samples were kept for a full day in distilled water. Thus, test samples of CAD/CAM-milled PMMA resin (n = 10) were obtained.

Thirty test samples in all were made according to the kind of material that was examined in this investigation, which were categorized into 3 groups:

Group I: Heat-cured poly methyl methacrylate (PMMA) resin samples (n = 10).

Group II: CAD/CAM-milled PMMA resin samples (n = 10).

Group III: CAD/CAM-milled PMMA resin reinforced with graphene samples (n = 10).

The flexural strength of each of the thirty samples was assessed separately in a universal testing machine utilizing the 3-point bend test (ASTM D 790, Instron 3369). The equipment used to hold the sample featured vertical supports with a 15 mm support span, on which the sample had been mounted. Once the sample was inserted, it was loaded at its center at a crosshead speed of 1mm/min until it fractured. The universal testing machine computer software captured and displayed the load-deflection curve and the ultimate load to failure (Figures 3A–C). The FS data (σ) were estimated in Mega Pascals (MPa) using the FS formula, and the maximum load at fracture was noted in newtons (N). $\sigma = 3Fd/2wh^2$, where w (mm) represents the calculated width at the sample center, h (mm) is the height at center of a sample, d (mm) represents “the distance between vertical support spans, and F (N) represents the maximum load at fracture”. For each of the thirty test samples, the flexural strength values were measured in Mega Pascals (MPa). Using the statistical software program SPSS version 21, the findings were tabulated and statistical analysis was performed. Estimates of the standard deviation & mean were made using the data from each sample for every research group. One-way ANOVA (Analysis of Variance) was utilized to analyze the data, and then the *post hoc* Tukey HSD test was performed. At the 5 percent significance threshold, statistical significance was taken into account.



FIGURE 2 | (A) CAD/CAM PMMA resin reinforced with Graphene blank. **(B)** Manufacturer packaging of CAD/CAM PMMA resin reinforced with Graphene blank (front cover). **(C)** Manufacturer packaging of CAD/CAM PMMA resin reinforced with Graphene blank (back cover).

Results

The mean FS of Group I heat-cured PMMA resin test samples (550.29 MPa), Group II CAD/CAM-milled PMMA resin

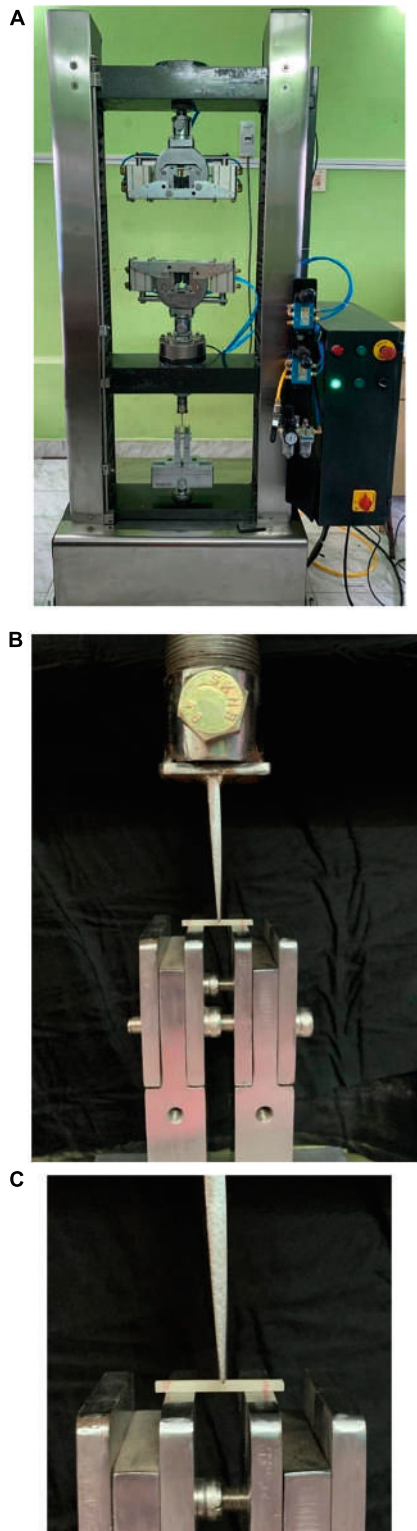


FIGURE 3 | (A) Universal testing machine. **(B)** Placement of test sample on sample holding apparatus. **(C)** Application of load at the center of the sample.

test samples (1481.966 MPa), and Group III CAD/CAM-milled PMMA resin reinforced with graphene test samples (1447.45 MPa) were evaluated.

TABLE 1 | Basic data and mean flexural strength of Group I (heat-cured PMMA resin) test samples.

Sample No.	Flexural Strength (MPa)
1	543.27
2	551.4
3	547.16
4	554.4
5	556.92
6	553.24
7	546.71
8	545.4
9	549.23
10	555.17
Mean/Standard Deviation (S.D)	550.29/4.61311

TABLE 2 | Basic data and mean flexural strength of Group II (CAD/CAM-milled PMMA resin) test samples.

Sample No.	Flexural Strength (MPa)
1	1646.06
2	1646.06
3	1455.89
4	1646.02
5	1462.6
6	1455.92
7	909.93
8	1473.23
9	1480.24
10	1643.71
Mean/Standard Deviation (S.D)	1481.966/220.0925

A statistically significant ($P < 0.01^{**}$) comparison of mean FS of test samples from Group I (heat-cured PMMA resin), Group II (CAD/CAM-milled PMMA resin), and Group III (CAD/CAM-milled PMMA resin reinforced with graphene) was made.

On multiple comparisons, the mean FS of Group I (heat-cured PMMA resin) test samples was less than that of Group II (CAD/CAM-milled PMMA resin) test samples and the difference was observed to be statistically highly significant ($P < 0.01^{**}$). The mean FS of Group I (heat-cured PMMA resin) test samples was less than that of Group III (CAD/CAM-milled PMMA resin reinforced with graphene) test samples and the difference was observed to be statistically highly significant ($P < 0.01^{*}$). The mean FS of Group III (CAD/CAM-milled PMMA resin reinforced with graphene) test samples was less than that of Group II (CAD/CAM-milled PMMA resin) test samples, but the difference was found to be statistically insignificant ($P > 0.05^{*}$), (Tables 1–5).

TABLE 3 | Basic data and mean flexural strength of Group III (CAD/CAM PMMA resin reinforced with graphene) test samples.

Sample No.	Flexural Strength (MPa)
1	1463.13
2	1280.24
3	1477.32
4	1463.16
5	1448.71
6	1463.16
7	1475.42
8	1477.31
9	1477.16
10	1448.89
Mean/Standard Deviation (S.D)	1447.45/59.76915

Discussion

In difficult instances requiring rigorous periodontal treatment, orthodontic stability, or vertical dimension assessment, the abutment preparation and restorative cementation may take days, weeks, or months (2).

Often in such conditions, patients require placement of provisional restorations for extended periods of time to maintain occlusal stability, function, and esthetics till the fabrication of the planned definitive restorations. Long-term temporary restorations must be able to resist both functional and parafunctional stresses and allow the patient to maintain good oral hygiene (22–25).

Heat-cured acrylic resins are commonly used in the fabrication of provisional restorations as they possess acceptable strength, wear resistance, and resistance to fracture. However, in clinical situations that warrant long-span and long-term fixed provisional restorations, flexural strength is considered a key parameter that determines the final success of the prosthesis. Prior research has demonstrated that water sorption negatively impacts the FS of typical provisional materials, and the main drawback of resin-based provisional restorations is their comparatively low strength (5, 21, 26–29).

Studies have shown that, when compared to pure PMMA materials, the abrasive wear resistance and tensile and fatigue strength of heat-cured acrylic resin PMMA materials exhibit highly significant rises with 3wt% and 5wt% of zirconium nanoparticles. Similar outcomes were observed in the work of Shirkavand et al. (24) when PMMA was treated with titanium dioxide nanoparticles, leading to a notable improvement in tensile strength.

Acrylic resins supplemented with 0.25 percent or 0.5 percent pristine nano clay increased the FS, fracture toughness, and flexural modulus, according to Shakeri et al. (25) findings. The flexibility of PMMA and its overall mechanical performance were positively impacted

by electrospun nanofibers made of polyvinyl alcohol. To enhance the qualities of the matrix materials, carbon-based nanomaterials like graphene and carbon nanotubes were also added to or utilized as reinforcements in PMMA resins. Graphene and carbon nanotubes in varying amounts were added to a PMMA matrix by Swami et al. (11) via sonification of the nanoparticles in the monomer liquid. The outcomes demonstrated that the inclusion of even minute amounts of single-wall nanotubes considerably increases the impact strength of the PMMA resin. A limitation of the addition of carbon nanotubes and graphene to PMMA is the unaesthetic discoloration of the resin material, which is considered unsuitable for restorative treatment. The discoloration may be due to agglomeration and non-homogeneous distribution of the nanoparticles as they are manually added to the resin. One possible solution to overcome this discoloration could be the incorporation of nanoparticles into PMMA during industrial manufacturing. Hence, in the current research, CAD/CAM PMMA resin reinforced with graphene was included for calculation of its FS.

The Flexural strength of provisional restorative materials may vary within the parent chemical material viz PMMA and between different chemical groups (autopolymerizing resin, heat-cured PMMA resin, light-cured resin, and CAD/CAM PMMA-based polymers) of parent material (2). Because of this, estimating the flexural strength of temporary materials using only their generic composition can occasionally be confusing. The chemical structure of CAD/CAM PMMA-based polymers is comparable to that of traditional PMMA materials. On the other hand, because CAD/CAM PMMA-based polymers are more homogeneous, highly cross-linked, and have lower water solubility & sorption, they have better mechanical properties (5). Furthermore, until they are employed, CAD/CAM PMMA-based polymers are kept in the air to guarantee that the post-polymerization process happens in combination with the relaxing phenomenon (6).

Al-Dwairi et al. (4) examined the flexural modulus, impact strength, and FS of 2 brands of CAD/CAM PMMA—AvaDent and Tizian Blank PMMA—as well as a traditional heat-cured PMMA. The outcomes showed that, when compared to the traditional heat-cured groups, the “CAD/CAM PMMA specimens had better flexural modulus, flexural strength, and impact strength.” The current study’s outcomes are the same as those of the research conducted by Al-Dwairi ZN et al. In the current research, the FS of CAD/CAM PMMA resin (1481.966 MPa) > the FS of CAD/CAM PMMA resin reinforced with graphene (1447.45 MPa) > the FS of heat-cured PMMA resin (550.29MPa)

Alp et al. (5) used the 3-point bend test to assess the FS of conventional intermediate resin materials with CAD/CAM PMMA-based polymers. The findings demonstrated that the bis-acrylate composite resin’s FS was inferior to that of the PMMA-based polymers. The traditional PMMA resin showed the least amount of flexural strength.

TABLE 4 | Comparative evaluation of the mean flexural strength of Group I (heat-cured PMMA resin) test samples, Group II (CAD/CAM-milled PMMA resin) test samples, and Group III (CAD/CAM-milled PMMA resin reinforced with graphene) test samples.

Groups	No. of Samples	Mean Flexural Strength (MPa)	Standard Deviation (S.D)	p-Value
Group I	10	550.29	4.61311	0.000
Group II	10	1481.966	220.0925	
Group III	10	1447.45	59.76915	

P-value < 0.01**; Highly significant.

Following thermocycling, Çakmak et al. (27) assessed the FS of various CAD/CAM polymethyl methacrylate (PMMA)-based polymers, as well as traditional interim resin materials, polyethyl methacrylate (PEMA), and auto polymerized bisacrylate composite resin, both with and without a surface sealant. The FS of PMMA-based CAD/CAM polymers was found to be greater than that of traditional intermediate resin materials, which is in line with the findings of the current investigation.

In a study by Rayyan et al. (21) the color stability, wear resistance, fracture resistance, surface hardness, water sorption, and microleakage of interim restorations created using CAD/CAM were compared with those constructed manually. Interim crowns made of CAD/CAM were said to have stable mechanical and physical characteristics, making them suitable for long-term interim restorations.

Abdullah et al. (28) evaluated the internal fit, marginal gap, strength of fracture, and mechanism of fracture of CAD/CAM provisional crowns with those of direct provisional crowns. While there was no difference in the manner of fracture across the groups, there was a statistically significant difference in the internal gap, strength of fracture, and marginal gap for every group. It was determined that in comparison to direct provisional restorations, CAD/CAM-manufactured provisional crowns show greater fit and strength.

Peñate et al. (29) examined the fracture strengths and marginal fit of interim FDPs made with various materials using a direct approach. The fracture strengths of interim prostheses made with CAD/CAM technology and interim prostheses reinforced with glass fiber were found to be identical, according to their findings. The least fracture-resistant interim FDPs were the unreinforced ones.

CAD/CAM PMMA-based polymers' Flexural Strength had been compared had been compared to traditional provisional resins in a number of experiments (5, 26–28). Nevertheless, there is a paucity of scientific information regarding the flexural strength of PMMA and PMMA-based polymers supplemented with nanoparticles when it comes to long-term fixed temporary restorations.

The Flexural Strength of various fixed provisional restorative materials has been evaluated by several researchers and the most common methods employed for evaluating flexural strength are the three-point bend

TABLE 5 | Comparative evaluation of the mean flexural strength difference of Group I (heat-cured PMMA resin) test samples, Group II (CAD/CAM-milled PMMA resin) test samples, and Group III (CAD/CAM-milled PMMA resin reinforced with graphene) test samples.

Group (I)	Group (J)	Mean Flexural Strength Difference (I-J)	Sig.
Group I	Group II	–931.67600*	0.000
	Group III	–897.16000*	0.000
Group II	Group I	931.67600*	0.000
	Group III	34.51600	0.829
Group III	Group I	897.16000*	0.000
	Group II	–34.51600	0.829

test and the four-point bend test. Bend tests are thought to be significant because they replicate the direction in which occlusal force transmission occurs in a clinical setting (12).

According to Tripathi et al., improper stress transfer properties and the filler agglomeration effect cause mechanical strength to deteriorate when graphene oxide additions exceed 1%. This could be a possible reason for the decrease in flexural strength value of Group III (CAD/CAM-milled PMMA resin reinforced with graphene) test samples (1447.45 MPa) when compared with Group II (CAD/CAM-milled PMMA resin) test samples (1481.966 MPa). The study found a highly significant difference between the FS of heat-cured PMMA along CAD/CAM-milled PMMA polymers, rejecting the null hypothesis (30, 31).

The current research has some limitations. The *in vitro* design of the study could not simulate the different intraoral conditions and thus, FS values of CAD/CAM PMMA resin reinforced with graphene were found to be slightly less than those of CAD/CAM PMMA resin.

Evaluating the FS of a dental material after aging, thermocycling, and cyclic loading can elicit a thorough understanding in assessing the clinical performance of the respective material for its long-term use. While the flexural strength test has proved to be a valuable tool in assessing clinical performance, more extensive clinical research is necessary to determine the best fixed temporary restorative material for extended usage.

PMMA polymers that were CAD/CAM milled showed higher flexural strength. To strengthen the conclusions

drawn from this work, more research on the effects of adding different reinforcements to CAD/CAM polymers, with a larger sample size and *in vivo* simulations, is advised.

Conclusion

According to the study's findings, PMMA resin that was CAD/CAM milled and reinforced with graphene demonstrated the highest flexural strength, whereas the former showed the lowest. The least FS has been demonstrated by heat-cured PMMA resin.

Author contributions

MT: collection of literature, concept and design, data collection, manuscript preparation and editing. VM: collection of literature, concept and design, data collection, manuscript preparation and editing. JS: sample preparation, collection of articles, manuscript preparation. HR: manuscript editing, review. VJ: - manuscript review. AS: manuscript review. All authors contributed to the article and approved the submitted version.

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