Flexural Strength of CAD/CAM Denture Base Materials: Systematic Review and Meta-analysis of *In-vitro* Studies

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Introduction: Digital complete dentures fabrication techniques are expanding. This study aimed to review flexural strength (FS) of milled and 3D-printed denture base materials to answer the study question: is FS of computer-aided designing/computer-aided manufacturing (CAD/CAM) denture base comparable to conventional heat-polymerized materials? Materials and Methods: Search was done within different databases for articles published between January 2010 and June 2021 using specific keywords. Articles of *in-vitro* studies in English language with methods following International Standards Organization standardization/ ADA specifications for flexural testing of conventional and CAD/CAM (milled or printed) polymethyl methacrylate (PMMA) materials were included. Results: Out of the 61 studies, 9 were processed for data extraction and only 7 underwent meta-analysis. Two, six, and one study showed high, moderate, and low risk of bias, respectively. Random-effects model was used for analysis and resulted in the average FS of 120.61 MPa [95% confidence interval (CI): 109.81-131.41] and 92.16 MPa (CI: 75.12–109.19) for CAD/CAM milled and heat-polymerized PMMA, respectively. Conclusion: Subtractive CAD/CAM technique of denture fabrication showed satisfactory FS values, whereas additive CAD/CAM method was comparable to conventional heat-polymerized technique with lower value, requiring further investigations and improvement. The clinical use of milled denture bases is an acceptable substitution to heat-polymerized PMMA, making the denture fabrication an easier and faster process.

Keywords: 3D-printed denture base, CAD/CAM denture base, flexural strength, PMMA

INTRODUCTION

S cientists are on the path to find a technique that will eliminate or reduce the downsides seen with conventional denture fabrication technique that has been used for more than eight decades and improve the mechanical, physical, and optical properties of the polymethyl methacrylate (PMMA) material.^[1] This improvement maybe brought up by the introduction of computer-aided designing/computer-aided manufacturing (CAD/CAM) technology.^[2] In this technique, the obtained information from the patient is converted into digital data using specific computerized

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software.^[3,4] Based on this information, the denture is designed digitally and saved in STL (Standard Triangle Language) format upon which the denture is produced in a subtractive (milling) or additive (3D-printing) technique.^[5-9]

Conventional method of fabrication involves lengthy procedures of technique-sensitive material

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manipulation, complex and lengthy procedures of denture waxing, investing, dewaxing, packing, and polymerization of heat-polymerized PMMA that undergoes a degree of polymerization shrinkage resulting in slight inaccuracy of final denture.[1,10,11] In comparison, the digital process can accomplish similar results with less number of clinical visits, allow visualization of residual ridge and denture to be made from different aspects, permit digital record keeping, and improve patient satisfaction. Additionally, CAD/ CAM milled dentures provide better adaptation and retention of the denture.[3,12-19] Milled dentures are produced by subtractive trimming from readymade prepolymerized pucks of resin that were fabricated under strict conditions of elevated temperature and pressure to produce highly dense material. This process reduces further polymerization during denture fabrication and allows the production of dentures with adequate mechanical properties in thinner sections. This permits better speech and higher patient comfort.^[3,5,13,17,20]

Digitally fabricated dentures are produced by either additive (3D-printing) or subtractive (milling) methods. Milling of acrylic dentures is widely spreading,^[19] but 3D-printing affords a variety of advantages over milling, such as being economical^[21] and enabling concurrent manufacturing of many products.^[10] In contrast, the accuracy of dentures made by subtractive manufacturing is reliant on the milling tools number, size, and configuration,^[22] whereas additively manufactured dentures reproducibility is developing with the technology.

Since the use of 3D-printing technology is still in its initial stages and is continuously evolving, there is a deficit of reported data regarding mechanical properties of these materials. Only few studies reported the FS of 3D-printed resin being lower than other types of PMMA.^[23] Others^[24] reported that pure 3D-printed PMMA had lower FS value that improved upon reinforcement with fillers.

Regardless of the advantages associated with CAD/ CAM technology, scientific evidences regarding mechanical properties of CAD/CAM fabricated prostheses are lacking. Hence, this review was prepared to evaluate and compare the FS of milled and 3D-printed PMMA denture base material to heatpolymerized acrylic resin.

MATERIALS AND METHODS

This systematic review was prepared inline with the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement and the Patient or Population, Intervention, Control or Comparison, and Outcome (PICOS) approach. The PICOS question was: is the flexural strength (FS) of CAD/CAM (milled or 3D-printed) denture base materials comparable with the conventional heat-polymerized denture base materials? Where the participants groups were complete denture base materials, and intervention groups are CAD/CAM denture bases, in comparison to conventional heat-polymerized denture bases with the outcome of included studies being the flexural strength. To answer this question, studies complying to the following inclusion criteria were included in the analysis.

Inclusion criteria comprised full-length *in-vitro* studies, written in English language, and comparing between FS of CAD/CAM and heat-polymerized denture base materials using ISO standardization/ADA specifications for testing. Articles not meeting these criteria were excluded. Search was done using PubMed, Google Scholar, EM-BASE, Scopus, and Web of Science databases for articles published between January 2010 and June 2021 with the last search date being July 7, 2021. The search keywords were CAD/CAM denture base, PMMA, flexural strength, CAD/CAM, computer-aided, computer-assisted, digital, 3D-printing, milled, complete denture/s, and a combination of two or more of them.

The review was done in three steps. First, two independent reviewers evaluated the titles of all acquired articles according to the inclusion criteria, and discrepancies were resolved by discussion. Secondly, abstracts of the selected titles were screened and those of interest were marked for full-text analysis. At the third step, selected full-text articles were examined and the data were tabulated in a template. Among the extracted data are the author's names, year or publication, materials used, brand names and manufacturers, specimen dimensions, sample size, storage medium, results, and outcome.

The authors modified the Cochrane risk of bias tool to fit the objective of the study. The risk of bias of included studies (n=9) was evaluated by two authors using an adaptation of the methods applied in a previous systematic review.^[25] During this process, parameters were reported as yes (when mentioned/available) or no (when absent).^[26]

JASP 0.14.1 was used for statistical analysis. In the meta-analysis, presence of heterogeneity in the data was checked first to select the proper model for further analysis. Random-effects model was employed in cases in which heterogeneity in data was found, whereas fixed-effects model was used otherwise. Visual inspection of forest plots and χ^2 tests were used to test the presence of heterogeneity. Possibility of publication bias was evaluated by Egger's test and visual inspection of funnel plots.

RESULTS

The initial search of the databases provided (n=61) studies [Figure 1]. Titles irrelevant to the research question, duplicate titles, and publications in languages other than English were excluded This process yielded 25 studies that were further scrutinized to ensure meeting inclusion criteria. At the end, nine studies^[13,23,27-33] were included for data extraction and analysis. Table 1 presents a summary of extracted data.

Figure 2 summarizes the risk of bias for the included studies. Out of the nine included studies, six^[13,23,28-31] showed medium risk, two^[27,32] showed high risk of bias, and one^[33] showed low risk of bias. The risks of bias reported were mainly due to missing information regarding sample size calculation and blinding of examiners.

Out of the nine final included articles, eight^{[13,27-^{33]} compared FS of milled denture base with heatpolymerized denture base, whereas only one study compared milled, 3D-printed, and heat-polymerized denture bases.^[23] The nine included studies followed ISO standardization/ADA specification regarding specimen dimensions and testing for three-point bending test. For the denture base materials used, a wide range of pre-polymerized CAD/CAM discs,}

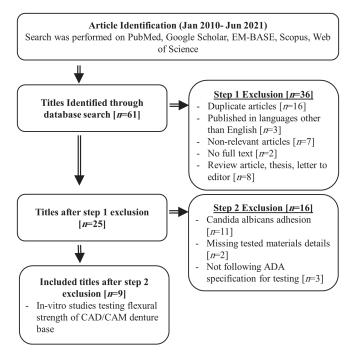


Figure 1: Article inclusion and exclusion process

3D-printing material, and heat-polymerized PMMA were used [Table 1]. Seven studies^[13,27-31,33] were included in the meta-analysis, whereas two studies did not either mention the means^[23] or presented the results in a chart^[32] making data extraction difficult.

Included in the meta-analysis of milled denture bases are seven studies with 10 total treatment groups, one of which was eliminated for being an outlier. For heatpolymerized method, seven studies were included with height total observations. Visual inspection of forest plots and χ^2 tests for both groups (milled and heatpolymerized) revealed the presence of heterogeneity $(I^2 > 75\%, P$ -value<0.001) [Figures 3 and 4]. Hence, the random-effects model was used. The average FS for milled PMMA was 120.61 MPa [95% confidence interval (CI): 109.81-131.41] and for heat-polymerized 92.69 MPa (95% CI: 73.58-111.79). The shape of the funnel plots for both groups (milled and heatpolymerized) was not clear to draw any conclusion about the presence of publication bias. Therefore, Egger's test was used and the results were insignificant (P-value=0.55, 0.976), respectively, which suggests the absence of publication bias.

According to the meta-analysis results, milled materials showed higher FS value when compared with heatpolymerized. In between milled materials, three studies^[30,31,33] milled specimens to desired dimensions using milling machines, whereas the other six studies^[13,23,27-29,32] cut the pre-polymerized acrylic resin blocks using diamond saw with no variation in results averages. One study^[23] assessed the FS of 3D-printed resin, and the results were presented as minimum and maximum values with no mention of the mean. However, the 3D-printed material recorded the lowest FS value between all tested PMMA materials.

DISCUSSION

High FS of resin base material is a prime factor for denture durability and prevention of failures under load.^[13,34] During denture life, it is exposed to repeated cycles of masticatory forces. This repeated action may lead to denture cracking, fracture, and failure.^[35] This failure is potentiated in the presence of poor fit, improper design, or notches.^[36] In such situations, the fracture of the denture happens after the maximum flexure fatigue is exceeded. The FS test is defined as maximum stress experienced by a material at its point of yielding and is considered a collective reflection of the material's tensile, shear, and compressive strengths.^[37] The three-point bending test has been adopted by the ISO as the recommended test for FS of polymers^[38]

Author /year	Fabrication	Brand (manufacturer)	Specimen preparation	Dimensions (mm)	Sample size	Storage	Mean±SD (MPa)	Outcome
Srinivasan et al. ^[13]	CAD/CAM	AvaDent Puck Disc (AvaDent Global Dental Science)		<u>× /</u>	<i>n</i> =5	Water (37°C, 24 h)	121±2	CAD/CAM showed improved mechanical properties than heat- polymerized PMMA
	Heat- polymerized	Aesthetic Red, (CANDULOR)	Conventional				96±4	
Prpić <i>et al</i> . ^[23]	CAD/CAM	IvoBase CAD disc PMMA (Ivoclar Viviadent)	Cut	64×10×3.3	<i>n</i> =10	Water (37°C, 50±1 h)	Max. 103.60 Min. 69.06	Milled PMMA showed bette FS than heat polymerized and 3D-printed acrylics do. 3D-printed had lowest F strength
		Interdent CC disc PMMA (Interdent					Max. 119.1 Min. 103.7	Strongth
		d.o.o.) Polident Pink CAD/ CAM disc basic (Polident d.o.o.)	,				Max. 116.4 Min. 97.0	
	3D-printed	NextDent Base (NextDent B.V.)	Printed				Max. 84.5 Min. 60.0	
	Heat- polymerized	ProBase Hot (Ivoclar Vivadent) Paladon 65 (Kulzer GmbH) Interacryl Hot (Interdent d.o.o.)	Conventional				Max. 100.7 Min. 71.8 Max. 88.5 Min. 62.2 Max. 110.6 Min. 84.1	
	Polyamide	Vertex ThermoSens Polyamide (Vertex Dental BV)					Max. 80.50 Min. 51.99	
Al-Dharrab ^[27]	CAD/CAM	Interdent CC disc PMMA (Interdent d.o.o.)	Cut	65×10×3	n=10	Distilled water (37°C, 24 h)	34.05±2.32	Milled PMM had lower FS than heat- polymerized PMMA.
	Heat- polymerized	Polident Pink CAD/ CAM disc basic (Polident d.o.o.)	Conventional				62.38 ± 1.73	
Arslan <i>et al</i> . ^[28]	CAD/CAM	M-PM Disc (Merz Dental GmbH)	Cut	$64 \times 10 \times 3.3$ subjected to 5000 TC procedure (5–55°C; 30 s dwell time)	(10 TR 10 not TR)	Distilled ,water (37°C, 48±2 h)	122.47±5.54 (TR) 114.52±5.81	PMMA had

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Author /year	Fabrication	Brand (manufacturer)	Specimen preparation	Dimensions (mm)	Sample size	Storage	Mean±SD (MPa)	Outcome
		AvaDent Puck Disc (AvaDent Global Dental Science) Polident Pink CAD/ CAM Disc (Polident d.o.o.)					118.32 ± 4.66 (TR) 106.78 ± 3.37 133.43 ± 5.9 (TR) 125.85 ± 3.92	
	Heat- polymerized	Promolux (Merz Dental GmbH)	Conventional				108.95 ± 5.63 (TR) 98.83 ± 6.33	
Pacquet et al. ^[29]	CAD/CAM	IvoBase CAD disc (Ivoclar Vivadent)	Cut	65×10×2.5		Distilled water (37°C, 24±2 h)	87.98±7.37	FS of milled PMMA was lower than heat- polymerized but higher than injection
	Heat-	ProBase Hot	Conventional				97.31±4.96	molded.
	polymerized Injected	(Ivoclar Vivadent) IvoCap injection molding (Ivoclar Vivaden)	then cut Injection molding	40×4×2			79.35±10.1	
Aguirre et al. ^[30]	CAD/CAM	Vertex PMMA, AvaDent Original shade (Global Dental Science)	Milled	64×10×3.3		Distilled water (37±1°C, 7 days)	146.6±6.6	Milled PMMA exhibited higher FS than heat- polymerized and injection- molded PMMA.
	Heat-	Lucitone 199	Conventional				116.6±3.1	I WIWIA.
	polymerized Injected	(Dentsply Sirona) SR IvoCap High impact (Ivoclar Vivadent)	Injection molding				86.7±7.1	
Al-Dwairi et al. ^[31]	CAD/CAM	AvaDent Puck Disc (AvaDent Global Dental Science)	Milled	65×10×3		Distilled water (37±1°C, 7 days)	123.11±9.47	Milled PMMA exhibited significantly higher FS compared with heat- polymerized.
		Tizian Blank PMMA (Schütz Dental)					130.67±10.48	
	Heat- polymerized	Meliodent heat cure (Heraeus Kulzer)	Conventional- short cycle				93.33±8.64	
Perea-Lowery et al. ^[32]	CAD/CAM	L-Temp Pink (Degos Dental GmbH)	Cut	65×10×3.2		Half dry- stored, and half in water (37°C, 30 days)	NS	Material and storage had significant influence on FS.

Table 1: Continued									
Author /year	Fabrication	Brand (manufacturer)	Specimen preparation	Dimensions (mm)	Sample size	Storage	Mean±SD (MPa)	Outcome	
								Satisfactory FS of milled PMMA. Heat- polymerized had higher FS compare with milled MMA.	
		IvoBase CAD disc (Ivoclar Vivadent)					NS		
		Temp Basic Tissue (Zirkonzahn)					NS		
	Heat- polymerized	Paladon 65 (Kulzer GmbH)	Conventional- short cycle ther cut	1			NS		
Hada <i>et al.</i> ^[33]	CAD/CAM	Lucitone199 dentur base disc (Dentsply Sirona)		64×10×3.3	n=10	Purified water (37°C, 50 h)	105.1±2.2	Milled PMMA showed high FS compared with heat- polymerized	
	Heat- polymerized	Acron (GC)	Conventional- long cycle then milled				87.9±5.0	polymerized	

FS: flexural strength, NS: not stated, TR: thermal cycling

with acceptable clinical value set to be not less than 65 MPa (ISO 1567).^[38]

Resins available to fabricate denture bases are polymerized using a variety of ways including heat, chemical, visible light, and microwave energy. Although the process is rapid, it is never complete, and a percentage of free monomer is still detected after polymerization.^[39] In addition, heat-polymerized dentures may contain porosities due to uncontrolled heating, improper mixing, monomer evaporation, or inadequate pressure during polymerization. Additionally, bases may have crazing as a result of water incorporation during polymerization or warpage due to incorporated stresses.^[40,41] Volumetric and linear shrinkages are inherent to heat-polymerized resin; therefore, occlusal adjustments are normally required after de-flasking. Similarly, carving of a post-dam groove in the cast is partially indicated to compensate for the shrinkage occurring after polymerization that may cause the lift-off the palatal section of the denture.

As CAD/CAM PMMA pucks are manufactured in extremely controlled environment, they are produced in a highly dense state with minimal shrinkage, porosity, or free monomers.^[3,30] The milling process utilizes prepolymerized pucks, which eliminates processing errors. The literature has reported better attributes of milled denture bases, including mechanical and biological properties, in addition to good surface characteristics.^[4]

FS, among others, may indicate the degree of polymerization of the resin, whereas lower degree of conversion will result in inferior mechanical properties including FS.^[42] Consequently, higher FS values reported for milled specimens can be linked to higher degrees of conversion.^[12] Therefore, one can assume that the equipment used to pre-polymerize pucks is capable of polymerizing the acrylic resin to higher degree than conventional heat-polymerized.^[30]

According to the meta-analysis, milled denture base resins showed high FS (120.61 MPa) compared with heat-polymerized PMMA (92.16 MPa). The difference was mainly attributed to milling the denture out of a completely pre-polymerized PMMA puck.^[43,44] When CAD/CAM pucks are produced, longer chains of polymer are created, which means higher rate of conversion and minimal residual monomer reducing its plasticizing effect and improving the

A	Author, Year of	Sample Size Calculation	Control Group	Specimens standardization ADA/ISO	Stated clearly testing method	Statistical Analyses Carried Out	Reliable Analytical Methods or Statistical Indicators	Blinding of Assessors	Risk of Bias	
	Srinivasan et al., 2018 (13)	No	Yes	No	Yes	Yes	Yes	No	Moderate	
	Prpić et al., 2020 (23)	No	Yes	Yes	Yes	No	Yes	No	Moderate	
	Al-dharrab Ayman, 2017 (27)	No	Yes	No	No	Yes	Yes	No	High	
	Arslan et al., 2018 (28)	No	Yes	Yes	No	Yes	Yes	No	Moderate	
	Pacquet et al., 2019 (29)	No	Yes	Yes	No	Yes	Yes	No	Moderate	
	Aguirre et al., 2020 (30)	No	Yes	Yes	Yes	Yes	Yes	No	Moderate	
	Al-Dwairi et al., 2018 (31)	No	Yes	Yes	Yes	Yes	Yes	No	Moderate	
	Perea-Lowery et al., 2020 (32)	No	Yes	No	Yes	No	Yes	No	High	
	Hada et al., 2021 (33)	Yes	Yes	Yes	Yes	Yes	Yes	No	Low	
	Low risk of bias			signed where the parameter was reported, and prmation was absent or unclear. The risk of bias						
	Moderate risk of bias									
	High risk of bias	-				received as 7=Low.				
В	Blindin	g of Ass	sessors							
	Reliable Analytical Methods or Statis	stical Ind	licators							
	Statistical Analys									
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	Specimens standardiz									
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	Sample									
	0% 20% 40% 60% 80% 100%									
	□N/A ■Unclear ■Low ■High									

Figure 2: Risk of bias

FS, minimal porosity, less internal stress and flaws, minimal intermolecular distances, and higher material resistance.^[12,14,29,30,32,45,46]

The conventional processing technique induces linear shrinkage (0.45-0.9%),^[45] whereas digital processing virtually eliminates further shrinkage. Murakami *et al.*^[47] recommended processing heat-polymerized PMMA at high pressure to increase molecular weight of polymer matrix and to decrease residual monomer and internal voids.^[12,47]

The reported results of FS of milled denture base material varied.^[13,23,27-33] Al-Dharrab^[27] and Pacquet *et al.*^[29] reported superiority of heat-polymerized

PMMA than milled PMMA. Srinivasan *et al.*,^[13] Aguirre *et al.*,^[30] and Hada *et al.*,^[33] all concluded that milled PMMA had better mechanical properties than heat-polymerized PMMA. Perea-Lowery *et al.*,^[32] showed a significant variation in mechanical properties of the tested materials with heat-polymerized PMMA showing the highest FS value. However, the performance of all resins was satisfactory. These findings suggest the applicability of using heat-polymerized PMMA for denture fabrication as CAD/CAM resins did not show better mechanical properties. Al-Dwairi *et al.*,^[31] exhibited in a previous study better FS, impact strength, and flexural modulus of milled PMMA when compared with heat-polymerized resin. At another

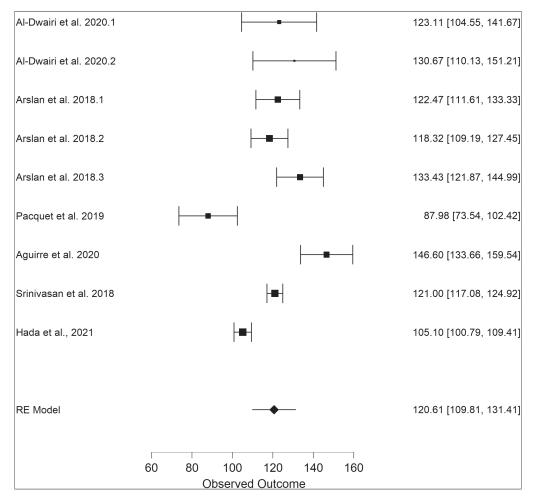


Figure 3: Forest plot for milled PMMA

level, Steinmassl *et al.*^[12] failed to detect the source of the residual monomer or report significant difference between different PMMA types.

Prpić et al.^[23] reported the highest FS with milled PMMA followed by heat-polymerized then lastly 3D-printed PMMA. This study was the only one included in this review that evaluated FS of 3D-printed PMMA. Although the 3D-printed material used had the lowest FS value, it met the ISO recommendation for FS (65 MPa)^[38] and is suggested as an option for the fabrication of denture bases. The variation in the reported values could be associated with material's structure.^[23] The downside of the 3D-printed acrylic is the low double-bond conversion in comparison with other types of acrylic resins, which in turn could affect the mechanical properties.^[48] Due to the limited number of studies, additional research is recommended to reach conclusions regarding mechanical properties of 3D-printed denture base PMMA^[23] and to evaluate its applicability for the fabrication of dentures with properties comparable to those of milled or heat-polymerized PMMA.

Photopolymerization of solvent-free resins offers economical and countless uses in dentistry. For optimal clinical performance, the resins should possess high curing rate, storage stability, low viscosity, and adequate biological properties and results in a final product with high mechanical and physical properties.^[49,50] Total conversion of the monomer is not guaranteed after polymerization, and systemic side effects may occur as a result of exposure to residual monomer or degradation products.^[48] To correlate this to clinical situation, structurally poor acrylic resin may cause cytotoxic effects to oral tissues and impede protein synthesis of epithelial cells.^[51]

The degree of double bond conversion during polymerization is an important indicator of physical and mechanical properties of the resulting resin. It refers to the amount of double bonds between carbon atoms that are transformed into single bonds; i.e., monomer is converted to polymer.^[52,53] The result of incomplete conversion means that free monomer is suspended to the end product with possible leach-out and tissue irritation.^[54] For 3D-printed materials,

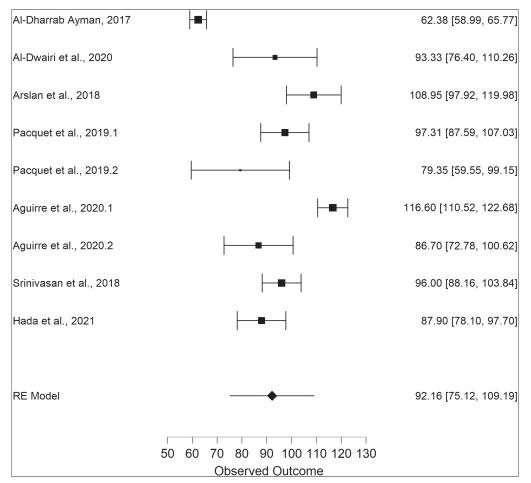


Figure 4: Forest plot for heat-polymerized PMMA

no definitive conclusion exists to correlate between conversion rate and biological effects.^[55] Therefore, new formulations must be carefully used due to the variation in constituents, structure, or functionality. One should take into consideration that the extent of polymerization is dependent on multiple factors such as the composition including the concentration of photoinitiator, specimen geometry, and curing environment.^[52]

Looking at the acrylic specimens from a different perspective, only two studies used scanning electron microscopy for analysis of fractured surface^[31] or to detect surface characteristics.^[28] Generally, brittle fractures will show compact and smooth appearance of fracture site, whereas rough and irregular surface suggests intermediate (brittle to ductile) fracture modes.^[56,57] The fractographic appearance of milled and heat-polymerized materials predominantly showed brittle fractures. Confined zones of intermediate fractures were seen with milled materials.^[31] Arslan *et al.*^[28] found that conventional PMMA exhibited more pores and surface irregularities than milled PMMA.

Steinmassl *et al.*^[12] were the first to report variation of physical properties between different brands of milled PMMA after reporting different densities of dentures made from four brands. These results might be linked to variations in the initial processing of PMMA to produce the pre-polymerized pucks.

Clinically, higher mechanical strength of milled PMMA may allow for thinner denture bases/palatal plates that comprise more natural speech and feel and higher degree of patient comfort and satisfaction, although evidence is still lacking till the moment.^[12,13] If future studies confirm the longevity of the milled PMMA strength after cyclic fatigue, they would be considered valuable alternatives in cases presenting with high functional loads, cases with repeated denture fractures,^[30] overdentures without metal reinforcement,^[32] implant-supported overdentures in cases with limited inter-arch space,^[58] or hollowed and lightweight maxillo-facial prostheses for oncology patients.^[13]

Finally, the authors would like to report the limitations of this study such as the low number of included studies; only one of the included studies did thermocycling and another one evaluated 3D-printed denture bases. Therefore, further studies evaluating the FS and other mechanical properties of milled and 3D-printed PMMA in oral environment-simulated conditions are necessary. Fractography analysis of fracture sites to determine the behavior of material failure is also recommended.

CONCLUSIONS

Based on the reviewed literature, most of the studies reported satisfactory FS of milled specimens in comparison to heat-polymerized PMMA. 3D-printing of denture bases was comparable to conventional heat-polymerized PMMA, but showed lower FS value necessitating further investigations. Until further research and advancement in material composition and property of 3D-printed resin, milled and heatpolymerized PMMA resins remain the materials of choice to fabricate denture bases with high flexural strength.

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CONFLICTS OF INTEREST

The authors have no conflicts of interests to declare.

AUTHORS CONTRIBUTIONS

Both the authors contributed to study design, data acquisition and interpretation, and manuscript writing and finalization.

ETHICAL POLICY AND INSTITUTIONAL REVIEW BOARD STATEMENT Not applicable.

PATIENT DECLARATION OF CONSENT

Not applicable.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available within the manuscript and its supplementary materials.

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