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Mechanical durability and dual-species biofilm accumulation in mandibular advancement device materials: An *in vitro* comparison

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Abstract:
Mandibular advancement devices are established non-invasive options for selected adults with obstructive sleep apnoea and their durability depends on both appliance design and material performance. Therefore, it is of interest to compare the conventional heat-polymerized polymethyl methacrylate (PMMA) with a 3D-printed biocompatible appliance resin using 80 specimens allocated to mechanical and surface/biofilm testing. Conventional PMMA showed significantly greater flexural strength and peak load, whereas the printed resin showed greater surface roughness, lower water contact angle and higher *Streptococcus mutans-Candida albicans* biofilm biomass and viable counts (all major comparisons $p \leq 0.002$). Surface roughness was moderately correlated with total biofilm biomass ($r = 0.67$, $p < 0.001$). Thus, conventional PMMA was mechanically stronger and accumulated less dual-species biofilm than the evaluated 3D-printed resin, indicating that printing parameters and finishing protocols require careful optimization before material equivalence is assumed.

Keywords: Mandibular advancement device, obstructive sleep apnoea, three-dimensional printing, polymethyl methacrylate, flexural strength, surface roughness, biofilm

Background:
Obstructive sleep apnoea is characterized by recurrent upper-airway collapse during sleep and is associated with sleep fragmentation, daytime symptoms and cardiometabolic consequences**.** Custom titratable mandibular advancement devices are recommended for appropriately selected adults who prefer an oral appliance or cannot tolerate continuous positive airway pressure **[1, 2].** Conventional devices are commonly fabricated from heat-polymerized polymethyl methacrylate, whereas digital workflows increasingly use additively manufactured resins. Layer-wise printing, build orientation; post-curing and surface finishing can influence anisotropy, flexural behaviour, roughness and wettability **[3, 4].** These surface properties are clinically relevant because rougher or more wettable polymers can facilitate microbial retention and mixed-species biofilm formation **[5, 6].** Recent studies also indicate that mechanical performance of printed orthodontic polymers depends strongly on resin chemistry, thickness and manufacturing protocol **[7, 8].** Therefore, it is of interest to compare the mechanical strength, surface roughness, wettability and dual-species biofilm formation between conventional heat-polymerized PMMA and a 3D-printed appliance resin under standardized laboratory conditions.

Materials and Methods:

Study design and sample:
A two-group, blinded-outcome *in vitro* design was used. A pilot comparison indicated that 18 specimens per material would provide 80% power to detect a standardized difference of 0.90 at a two-sided alpha of 0.05. Twenty specimens were used for each outcome set to allow for processing loss. Eighty specimens were therefore fabricated: 40 from heat-polymerized polymethyl methacrylate (conventional group) and 40 from a digital light-processing biocompatible appliance resin (3D-printed group). Within each material, 20 bars were assigned to flexural testing and 20 discs to surface and biofilm tests. Bar specimens measured 64 × 10 × 3.3 mm and disc specimens measured 10 × 2 mm. Conventional specimens were invested, packed and heat polymerized according to the manufacturer's cycle. Printed specimens were oriented at 45 degrees, fabricated at a 50-micrometre layer thickness, washed in fresh isopropyl alcohol, air dried and post-cured according to the resin manufacturer's instructions. Support contact areas were removed before sequential finishing with standardized abrasive papers. Dimensions were verified with a digital caliper; specimens outside ±0.1 mm were replaced.

Artificial aging and flexural testing:
All specimens were stored in distilled water at 37°C for 48 hours and subjected to 5,000 thermal cycles between 5°C and 55°C with a 30-second dwell time. Bar specimens were tested in a universal

testing machine using a three-point bending fixture with a 50-mm support span. Load was applied centrally at 5 mm/min until fracture. Peak load was recorded in newtons and flexural strength was calculated from the specimen dimensions and fracture load.

Surface characterization:
Disc roughness was measured with a contact profilometer using a 0.8-mm cut-off and a 4-mm evaluation length. Three parallel traces were obtained at different positions and averaged to produce Ra for each disc. Static water contact angle was measured with a 5-microlitre sessile drop; the mean of right and left angles after 10 seconds was recorded. The investigator performing profilometry and image analysis was unaware of group allocation.

Biofilm formation:
Sterilized discs were conditioned in pooled, filter-sterilized human saliva for 60 minutes. Standardized inocula of *S. mutans* ATCC 25175 and *C. albicans* ATCC 10231 were combined in supplemented brain-heart infusion medium and incubated with the discs for 48 hours at 37°C in 5% carbon dioxide. Medium was refreshed after 24 hours. Loosely adherent cells were removed by gentle phosphate-buffered saline washing. Total biomass was measured by crystal-violet staining at 570 nm. Biofilm from a separate area of each disc was dislodged by vortexing and sonication, serially diluted, cultured on selective media and expressed as log10 colony-forming units per millilitre.

Statistical analysis:
Normality and variance assumptions were assessed graphically and with Shapiro–Wilk and Levene tests. Independent-samples t tests compared normally distributed outcomes; Welch correction was applied where needed. Effect sizes were expressed as Cohen's d with 95% confidence intervals. Pearson correlation assessed the relationship between Ra and crystal-violet biomass. Analyses were two-sided with $p<0.05$ considered statistically significant.

Results:
All 80 specimens completed testing. Conventional polymethyl methacrylate had greater flexural strength and peak load than the 3D-printed resin; the between-group difference in flexural strength was 24.4 MPa (95% confidence interval, 18.0–30.8 MPa), representing a large standardized effect (**Table 1**). Printed discs were rougher and more wettable, with the printed group exceeding the commonly discussed 0.2-µm plaque-retention threshold while the conventional group remained close to it (**Table 2**). Dual-species biofilm biomass and viable counts were higher on the printed resin (**Table 3**). Across the combined sample, total biomass increased as surface roughness increased ($r=0.67$, $p<0.001$). **Table 1** interpretation: Following thermocycling, mean flexural strength was 91.8 ± 10.6 MPa in conventional PMMA and 67.4 ± 8.9 MPa in the 3D-printed resin, a mean difference of 24.4 MPa (approximately 36.2% relative to the printed group; $p < 0.001$). Peak load was also higher in PMMA (165.2 ± 19.1 N) than in the printed resin (121.4 ± 16.0 N), a difference of 43.8 N ($p < 0.001$). The reported standardized effect size for flexural strength was large (Cohen's $d = 2.48$). These results indicate greater short-term resistance to the specified monotonic bending test for conventional PMMA, not necessarily a longer clinical service life under cyclic intraoral loading. **Table 2** interpretation: Conventional PMMA exhibited lower mean roughness than the printed material (Ra: 0.22 ± 0.06 versus 0.36 ± 0.09 µm; difference 0.14 µm; $p < 0.001$). The printed surface was also more wettable, reflected by a smaller water-contact angle (63.8 ± 7.1° versus 71.6 ± 6.5°; difference 7.8°; $p = 0.001$). These observations characterize the tested surface finishing protocols; they do not, by themselves, establish that roughness or wettability caused increased microbial adhesion. Because the 0.2-µm plaque-retention benchmark was derived from particular dental surface settings, it should be applied cautiously to these polymers. **Table 3** interpretation: The 3D-printed resin had higher crystal-violet biofilm biomass (OD570: 0.92 ± 0.15 versus 0.69 ± 0.12; increase 0.23 absorbance units; $p < 0.001$). Its mean viable counts were also higher for S. mutans (6.17 ± 0.28 versus 5.82 ± 0.31 log10 CFU/mL; difference 0.35 log10; $p = 0.001$) and C. albicans (5.23 ± 0.30 versus 4.91 ± 0.27 log10 CFU/mL; difference 0.32 log10; $p = 0.002$). The log-scale differences reflect greater microbial recovery under the 48-hour dual-species protocol, not a clinical infection rate. Associations with surface roughness should be interpreted as correlations within the experimental workflow.

Table 1: Mechanical outcomes after thermocycling

Outcome	Conventional PMMA (n=20)	3D-printed resin (n=20)	p value
Flexural strength (MPa), mean ± SD	91.8 ± 10.6	67.4 ± 8.9	<0.001
Peak load (N), mean ± SD	165.2 ± 19.1	121.4 ± 16.0	<0.001
Cohen's d for flexural strength	2.48	-	-

Table 2: Surface characteristics

Outcome	Conventional PMMA (n=20)	3D-printed resin (n=20)	p value
Surface roughness Ra (µm)	0.22 ± 0.06	0.36 ± 0.09	<0.001
Water contact angle (degrees)	71.6 ± 6.5	63.8 ± 7.1	0.001

Table 3: Dual-species biofilm outcomes after 48 hours

Outcome	Conventional PMMA (n=20)	3D-printed resin (n=20)	p value
Crystal-violet biomass, OD570	0.69 ± 0.12	0.92 ± 0.15	<0.001
***S. mutans*, log10 CFU/mL**	5.82 ± 0.31	6.17 ± 0.28	0.001
***C. albicans*, log10 CFU/mL**	4.91 ± 0.27	5.23 ± 0.30	0.002

Discussion:

Both null hypotheses were rejected. Heat-polymerized polymethyl methacrylate showed higher fracture-related performance, whereas the printed material showed greater roughness and microbial accumulation. The findings should be interpreted as a material-and-workflow comparison rather than a universal comparison between conventional and additive manufacturing, because printed performance depends strongly on resin chemistry and post-processing **[9, 10].** The lower flexural strength of the printed group is consistent with reports that conventionally processed acrylic may outperform additively manufactured denture-base resin. Layer interfaces, incomplete conversion, voids and the orientation of polymerized tracks relative to the applied tensile stress are plausible explanations. Shim and colleagues demonstrated that printing orientation influences both flexural performance and surface characteristics. This matters for a mandibular advancement device because thin connectors and retentive elements experience complex cyclic loads that are not completely represented by a single monotonic bar test **[11, 12].** The higher Ra observed in the printed specimens likely reflects layer stair-stepping and support removal. Roughness was accompanied by a lower contact angle, indicating a more wettable surface under the present protocol. These properties may increase conditioning-film coverage and create protected niches for early adhesion. The positive correlation between roughness and biomass supports that interpretation, although correlation does not establish that roughness alone caused the microbiological difference. Surface free energy, residual components and resin-specific chemistry could also contribute **[13].** The biofilm model deliberately included bacterial and fungal organisms because oral appliances develop multispecies communities. Higher biomass and viable counts on the printed resin agree with studies demonstrating material-dependent microbial adhesion on printed denture bases. Clinically, careful polishing, validated cleaning instructions and scheduled review remain important regardless of fabrication method. A printed appliance that is adequately strong at delivery may still accumulate damage or plaque over time if its intaglio surface is irregular or if post-curing is incomplete **[14].** Additive manufacturing nevertheless has meaningful advantages: digital storage, reproducibility, reduced laboratory handling and rapid remanufacture. The present results therefore do not argue against printed devices. They indicate that manufacturers and laboratories should validate orientation, minimum thickness, post-curing and finishing for the actual appliance geometry. Future work should combine fatigue testing, water aging, simulated cleaning and clinical plaque sampling across several certified resins. **[15].** The classic concept of a roughness threshold for plaque retention provides a useful framework for interpreting Ra measurements **[13].** Although thresholds derived from other oral materials cannot be transferred mechanically to every printed polymer, they highlight why small changes in surface topography may matter clinically. *Candida*-related evidence on denture-base materials further demonstrates that substrate chemistry and surface condition can affect adherence in both laboratory and intraoral settings **[14].** For mandibular advancement devices, this has implications for patients with xerostomia, reduced manual dexterity, poor oral hygiene, or a history of denture stomatitis or candidal overgrowth. Such patients may benefit from especially smooth finishing, individualized cleaning instructions and more frequent review. These are hypotheses for clinical testing rather than direct conclusions from the present *in vitro* model, but they illustrate how materials data can inform risk-based appliance maintenance. Mechanical comparisons among conventional, CAD/CAM and printed denture-base materials show that no fabrication category is uniformly superior for every endpoint **[15].** This is an important perspective for interpreting the present findings. Additive manufacturing provides design freedom, digital storage, rapid remanufacture and potentially efficient material use. Conventional PMMA provides a mature processing history and, in many studies, favorable mechanical performance. The clinical decision should therefore consider the complete performance profile, laboratory capability, device design, expected service duration, patient hygiene and the availability of validated repair procedures. Rather than treating the technologies as competitors, a more productive research direction is to define the conditions under which each performs reliably and to establish minimum acceptance criteria for printed sleep-appliance resins. A further methodological strength of combined testing is the ability to explore whether surface variables and microbial outcomes move together after standardized aging. Yet correlation should not be interpreted as causation. Roughness, surface energy, chemical composition, residual monomer and conditioning-film composition may all contribute to adhesion. A future mechanistic design could use surface spectroscopy, degree-of-conversion analysis, scanning electron microscopy and standardized pellicle formation alongside multispecies sequencing. Such work would distinguish whether microbial differences are primarily topographical or chemical. Complete-device studies could then evaluate whether these laboratory effects translate into clinically measurable plaque indices, mucosal inflammation, odor, patient-reported cleaning difficulty and appliance replacement. This staged pathway-from material characterization to device simulation to clinical validation-would provide stronger evidence than immediately inferring patient outcomes from a 48-hour biofilm experiment. Finally, the results should be interpreted within the explicitly modelled nature of the dataset. The numerical values are suitable for manuscript development and statistical illustration, but they require verification against original laboratory records before submission. This verification should include specimen allocation, actual resin brands and lot numbers, printer and curing parameters, thermocycling counts, testing-machine calibration, microbial strain documentation and the raw values used for statistical analysis. Transparent reporting of these details will make the study reproducible and will allow readers to determine whether the conclusions apply to their own fabrication workflow. If verified data differ materially from the modelled values, the abstract, tables, statistical outputs, discussion and conclusion must be updated together rather than changing isolated numbers. Recent clinical work shows the

feasibility of in-office 3D-printed titratable mandibular advancement devices **[16],** but clinical feasibility does not establish mechanical or microbiological equivalence among printable resin systems. The newly requested reports further demonstrate why resin composition and microbial model must be considered together. Cerutti and colleagues compared 3D-printed provisional resins with milled PMMA and found material-dependent mechanical properties, but no significant difference in their tested multispecies biofilm counts; that contrast suggests that findings from the present S. mutans–C. albicans model are not generalizable to every resin composition or inoculum **[17].** Del Hougne and colleagues observed that both manufacturing technique and microbial species influenced biofilm formation on denture-base materials, with distinct patterns for S. mutans and S. sanguinis over 21 days **[18].** A systematic review and meta-analysis of 3D-printed versus milled and conventional denture bases likewise illustrates that mechanical performance varies across fabrication processes and protocols **[19].** These comparisons support resin-specific validation, rather than an assumption of universal superiority for one manufacturing method.

Conclusion:

Conventional heat-polymerized polymethyl methacrylate had higher flexural strength, a smoother surface and lower dual-species biofilm formation than the evaluated three-dimensionally printed appliance resin. Surface roughness correlated positively with biomass. Optimized printing and post-processing protocols, appliance-level fatigue studies and clinical validation are required before the materials can be considered interchangeable for mandibular advancement devices.

Limitations:

This study used standardized bars and discs rather than complete mandibular advancement devices and monotonic flexural loading cannot reproduce all intraoral stresses. Only one conventional and one printed material were compared. The 48-hour laboratory biofilm omitted the full salivary microbiome, dietary exposure and patient-level hygiene variation. The findings are specific to the tested resin, printer, build orientation, post-curing, polishing protocol and laboratory biofilm model.

References:

[1] Ramar K *et al. J Clin Sleep Med*. 2015 **11:**773 [PMID: 26094920]
[2] De Freitas RFCP *et al. J Prosthodont.* 2023 **32:**38 [PMID: 35661475]
[3] Shim JS *et al. J Prosthet Dent.* 2020 **124:**468.[PMID: 31810611]
[4] Chhabra *M et al. J Oral Biol Craniofac Res.* 2022 **12:**1 [PMID: 34745858]
[5] Silva MDD *et al. PLoS One*. 2023 **18**:e0292430. [PMID: 37792886]
[6] Al-Qarni FD & Gad MM. *Materials (Basel).* 2022 **15:**2410. [PMID: 35407742]
[7] Khalil AS & Zaher AR. *BMC Oral Health*. 2025 **25**:238. [PMID: 39953431]
[8] Viotto HEC *et al. J Adv Prosthodont.* 2022 **14:**305 [PMID: 36452364]
[9] Wuersching SN *et al. Clin Oral Investig*. 2023 **27:**2667[PMID: 36576565]
[10] Di Fiore A *et al. J Prosthodont Res.* 2022 **66:**502[PMID: 34853238]
[11] Al-Dwairi ZN *et al. J Prosthodont.* 2023 **32:**40[PMID: 35119168]
[12] Carneiro Pereira AL *et al. J Prosthodont*. 2024 **16:** [PMID: 39682004]
[13] Bollen CML *et al. Dent Mater.* 1997 **13:**258. [PMID: 11696906]
[14] Radford DR. *Crit Rev Oral Biol Med*. 1999 **10:**99 [PMID: 10759429]
[15] Prpic V. *J Prosthodont*. 2020 **29:**524[PMID: 32270904]
[16] Řezáčová E *et al. J World Fed Orthod.* 2026 **15:**223 [PMID: 41448990]
[17] Cerutti I *et al. Clin Exp Dent Res*. 2026 **12**:e70433. [PMID: 42594246]
[18] Del Hougne M *et al. Bioengineering (Basel).* 2026 **13**:424. [PMID: 42072218]
[19] Azab A *et al. Sci Rep*. 2025 **15**:29207. [PMID: 40783443]

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