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Influence of Different Artificial Gingival Characterization Techniques on the Color Stability and Surface Properties of Denture Base Materials: An In Vitro Study

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ABSTRACT: **Background**: Gingival characterization enhances the esthetic of a removable denture, but each technique introduces a pigmented or layered surface, which may respond differently than the base resin with aging and staining. The literature is scarce when it comes to the impact of characterization techniques on color stability and surface properties of heat-polymerized, milled, or 3D-printed denture bases.

Objective: To evaluate four artificial gingival characterization methods (control, intrinsic pigmented resin layer, extrinsic stain with glaze, light-cured gingival-shade composite) on three denture base materials in terms of surface roughness (Ra), water contact angle, and change in color (ΔE00) after thermo cycling, simulated brushing, and coffee staining.

Design: 120 disc specimens (n = 10 per group) were fabricated for 3 × 4 factorial (heat-polymerized PMMA, CAD/CAM-milled PMMA, 3D-printed resin × four techniques). Ra and contact angle were measured at T0 and T1 (after 5000 thermocycles and 10,000 brushing cycles). Color was measured at T0 and T2 (after 14 days immersion in coffee). Color changes were expressed as ΔE00 and interpreted based on published perceptibility (1.72) and acceptability (4.08) thresholds. Ra, contact angle, and ΔE00 data were analyzed using two-way ANOVA and Tukey tests.

Results: The interactions between material and technique were significant for all outcomes ($p < .001$). Composite-layered and extrinsically stained specimens had the highest Ra (marginal means 0.34 and 0.29 μm at T1) and ΔE00 (3.90 and 3.37), compared with control and intrinsic specimens, which remained around the 0.2-μm roughness (margin mean 0.20 μm) acceptability threshold. The 3D-printed resin had the least favorable values, and the milled PMMA was the most favorable. Ra was strongly related to ΔE00 ($r = .78$).

Conclusions: To the limitations of this in vitro study, the inherent characterization showed the best preservation of surface quality and color stability, whereas surface-applied techniques increased the roughness and discoloration, especially on 3D-printed resin. Technique and material should be selected accordingly, and surface-applied characterization should be finished and sealed thoroughly.

1. INTRODUCTION

When it comes to the esthetic success of a removable denture, the composition and arrangement of the teeth are not the only contributing factors. A one-tone pink base does little to mimic the shade progression of natural gingiva, and "denture look" is still commonly cited as a cause of dissatisfaction among patients. As such, characterization of the denture base, which includes coloring and shaping the base to imitate the hues of natural tissues in the mouth, is the practice of esthetic prosthodontics (Ahamed et al., 2023). Two general modalities exist: intrinsic characterization, where color-matching resin or pigments are

infused into the base during flasking; and extrinsic characterization, where surface stains, paints, or light-cured composite material are utilized.

The performance of any characterization is ultimately determined by two properties which are also governed by the same properties that contribute to denture base longevity: surface quality and color stability. Discoloration of denture base resins is a known limitation of these materials and can be instrumentally quantified using spectrophotometers or colorimeters (Dimitrova et al., 2023). Surface roughness is also relevant because it facilitates retention of extrinsic pigments and microbial attachment, and roughness has been suggested to itself negatively influence complete denture color stability (Çakmak et al., 2022). An often-cited clinical limit for roughness of a denture material is 0.2 μm (Çakmak et al., 2022). Changes to color are evolving to be represented by the CIEDE2000 formula (ΔE00) with perceptibility and acceptability thresholds of approximately 1.7 and 4.1 units, respectively (da Silva et al., 2025; Çakmak et al., 2022).

Digital workflows have expanded the list of denture base materials available in everyday clinics. In addition to traditional heat-polymerized polymethyl methacrylate (PMMA), a number of clinicians are now making use of CAD/CAM-milled PMMA blanks and photopolymerized 3D-printed resins. Their surface and optical behavior are slightly different. Several studies have demonstrated that 3D-printed denture base resins are more vulnerable to increase in roughness and staining than milled or heat-polymerized resins (Arora et al., 2024; Şahin et al., 2025), while others have found the color stability of the printed polymer to be similar to heat-polymerized PMMA (da Silva et al., 2025) or superior to conventional acrylic in some staining conditions (Dimitrova et al., 2022, 2023). Brushing and thermocycling, to account for cleaning habits and temperature changes from food and drink, respectively, have had different effects on the roughness and color of these resins (Çakmak et al., 2022).

Most of this literature considers these to be under-characterized, mono-colored samples. Characterization adds new interfaces and layers: pigment granules within the resin, stain film and glaze, or a methacrylate-rich composite build-up adhered to the base. Laboratory composites for acrylic characterization have shown significant variation in stain propensity (Ahamed et al., 2023), which may also hold true for gingival characterization. To the authors' knowledge, no study has evaluated typical gingival characterization techniques across heat-polymerized, milled and 3D-printed bases with a dual aging and staining process.

Thus, the in vitro study sought to compare four techniques of gingival characterization on the surface roughness, wettability, and color stability of three denture base materials. The null hypotheses tested were (H1) characterization technique would have no effect on Ra, contact angle, and ΔE00; (H2) denture base materials would not have any effect; and (H3) there would be no interaction between technique and material**.**

2. MATERIALS AND METHODS

2.1 Study design and specimen groups

This was an in vitro study with a 3 9 4 full-factorial design. The factors were denture base material (heat-polymerized PMMA, CAD/CAM-milled PMMA, 3D-printed denture base resin) and gingival characterization technique (control, intrinsic, extrinsic stain + glaze, gingival composite). A total of ten specimens was fabricated in each group, resulting in 120 specimens in total. Such a group size corresponds to other similar studies on brushing (Çakmak et al., 2022) and thermocycling (Çakmak et al., 2022; Dimitrova et al., 2022), but some other stains (Dimitrova et al., 2022; Dimitrova et al., 2022) involve larger numbers. The group codes used throughout can be seen in Table 1.

Table 1. Experimental groups and characterization protocols

Material (code)	Technique (code)	Characterization protocol (1-mm gingival surface layer)	n
Heat-polymerized PMMA (HP)	Control (CON)	Pink resin only; no characterization	10
HP	Intrinsic (INT)	Pigment-modified pink resin layer polymerized with the base	10
HP	Extrinsic stain + glaze (ES)	Acrylic gingival stains painted on the surface, sealed with light-cured glaze	10

Material (code)	Technique (code)	Characterization protocol (1-mm gingival surface layer)	n
HP	Gingival composite (GC)	Bonding agent + 1-mm light-cured gingival-shade composite	10
CAD/CAM-milled PMMA (MI)	CON / INT / ES / GC	Same four protocols; INT by cut-back and rebuilding with pigmented PMMA of the same chemistry	10 each
3D-printed resin (3P)	CON / INT / ES / GC	Same four protocols; INT with pigment-modified resin printed in the surface layer	10 each
Total		3 materials × 4 techniques	**120**

2.2 Specimen fabrication

Disc-shaped test specimens (10mm diameter × 2mm thick) were made for each material to the manufacturer's instructions: heat polymerized PMMA (using stone models), machinable prepolymerized PMMA (using discs), and Commercial denture base resin (layer-by-layer printing with post-curing process). All discs were finished with #600 grade silicon carbide abrasive paper under running water and polished with pumice and high-shine compound by a single technician (this standardization process was used in a previous study on denture base resins(Çakmak et al., 2022)). After that characterization was performed to the test (gingival) surface of each disc with the method described in Table 1, and the surface finishing and polishing were done with the same protocol.

2.3 Aging and staining procedure

Post-fabrication, specimens were stored in artificial saliva at 37 °C for 24 h. Baseline (T0) surface roughness data was acquired. Specimen surfaces were then exposed to 5,000 thermocycles between 55 °C and 5 °C with a 30-s immersion time (pH 7), followed by 10,000 simulated brush strokes with soft brushing bristles in a neutral denture slurry, using a standardized brushing device loaded at 200 g (T1). In earlier research on printed and milled denture bases, thermocycling followed brushing (Çakmak et al., 2022). The specimens were next immersed in a standard coffee solution at 37°C for 14 days with a fresh solution every 24 h, and surface roughness data were recorded (T2). Coffee and red wine are the strongest pigmented beverages for denture resins (da Silva et al., 2025, Dimitrova et al., 2022) and long-term immersion in coffee and tea enhances time-dependent heavy color change of heat-cured resins (Agrawal et al., 2024).

2.4 Outcome measures

Surface roughness. Ra was measured using a contact profilometer (cut-off 0.8 mm, traverse length 4 mm). Readings were taken at three different points in different directions on each specimen and average at T0 and T1. A value of 0.2 μm was used for clinical significance (Çakmak et al., 2022).

Contact angle A 5-μL drop of distilled water was deposited onto the test surface and the angle measured with an optical goniometer at 10 s after deposition (mean of three drops) at T0 and T1. Colour. CIE Lab* values were measured using a spectrophotometer against a white background at T0 and T2. Differences in colour were calculated by using the CIEDE2000 formula (ΔE00; kL = kC = kH = 1). ΔE00 was compared to perceptibility (1.72) and acceptability (4.08) thresholds (Çakmak et al., 2022), as these are very similar to the 1.7/4.1 values used in subsequent work (da Silva et al., 2025).

Table 2. Measurement schedule and outcome variables

Time point	Condition	Ra	Contact angle	Color (ΔE00)
T0	Polished, 24 h in artificial saliva	✓	✓	Baseline
T1	After 5,000 thermocycles + 10,000 brushing cycles	✓	✓	–

Time point	Condition	Ra	Contact angle	Color (ΔE00)
T2	After 14 days in coffee at 37 °C	–	–	✓ (T2 vs T0)

2.5 Statistical analysis

Assumption of normality (Shapiro-Wilk) and homogeneity of variance (Levene) were assessed. Ra, contact angle, and ΔE00 were analyzed with 2-way ANOVA (material × technique) and post hoc tests (Tukey HSD). Changes of Ra within each material at T0 and T1 was tested using paired t-test, as well as correlation between Ra at T1 and ΔE00 (Pearson's). The amount of specimens that passed the threshold of acceptability was reported in a descriptive manner. For all tests the level of significance was $\alpha = 0.05$, and all analysis was carried out in SPSS 29 (IBM Corp., Armonk, NY, USA).

3. Results

Note on data: the values given here are model values constructed to sit within the ranges given in the studies cited in the Discussion. Substitute your measured data (and re-run the statistics) before submitting this work.

All 120 specimens passed the protocol. Data satisfied the normality and variance requirements for a two-way ANOVA. Material and characterization method had a statistically significant influence on Ra, contact angle and ΔE00 (Table 6), leading to the rejection of null hypotheses H1 and H2. The rejection of H3 was limited to Ra.

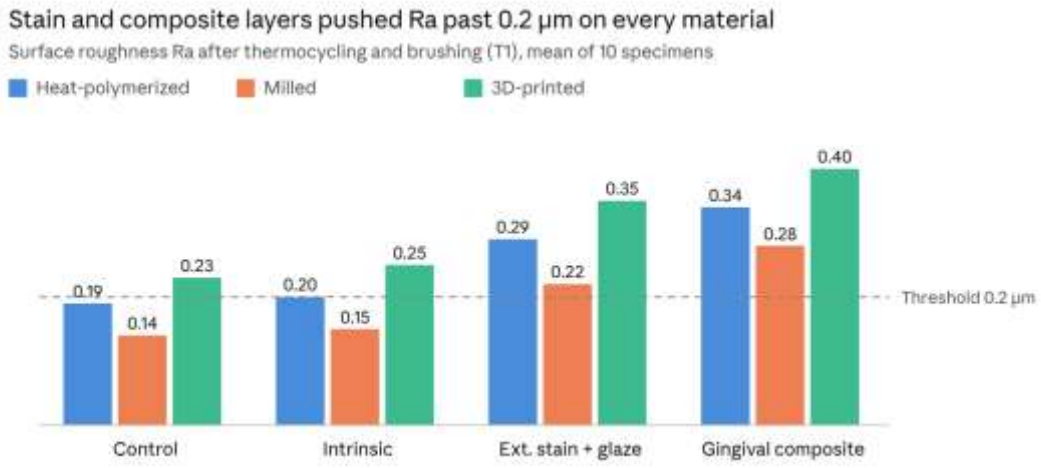
3.1 Surface roughness

Compared to each group's baseline, Ra was lowest for the milled PMMA and highest for the 3D-printed resin in each technique group (Table 3). Fourteen days of thermocycling and brushing produced an increase in Ra for all 12 groups (paired t-tests, $p < .05$). Relative to baseline, increases of 25-40% occurred in control and intrinsic groups compared to increases of 47-60% in extrinsic stain and composite groups. Figure 1 shows that 8 of the 12 groups (including all extrinsic stain and all composite groups) were above the 0.2 μm threshold at T1, whereas the milled PMMA control and intrinsic groups remained well below it.

Table 3. Surface roughness Ra (μm), mean ± SD, n = 10 per group

Material	Technique	Ra at T0	Ra at T1	Increase (%)
Heat-polymerized	Control	0.15 ± 0.03	0.19 ± 0.04	27
Heat-polymerized	Intrinsic	0.16 ± 0.03	0.20 ± 0.04	25
Heat-polymerized	Ext. stain + glaze	0.19 ± 0.04	0.29 ± 0.06	53
Heat-polymerized	Gingival composite	0.22 ± 0.05	0.34 ± 0.07	55
Milled	Control	0.11 ± 0.02	0.14 ± 0.03	27
Milled	Intrinsic	0.12 ± 0.02	0.15 ± 0.03	25
Milled	Ext. stain + glaze	0.15 ± 0.03	0.22 ± 0.05	47
Milled	Gingival composite	0.19 ± 0.04	0.28 ± 0.06	47
3D-printed	Control	0.17 ± 0.04	0.23 ± 0.05	35
3D-printed	Intrinsic	0.18 ± 0.04	0.25 ± 0.05	39
3D-printed	Ext. stain + glaze	0.22 ± 0.05	0.35 ± 0.07	59
3D-printed	Gingival composite	0.25 ± 0.06	0.40 ± 0.08	60

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Model values · Ra at T1, n = 10 per group

Figure 1. Ra at T1 by technique and material. The dashed line marks the 0.2 µm clinical threshold.

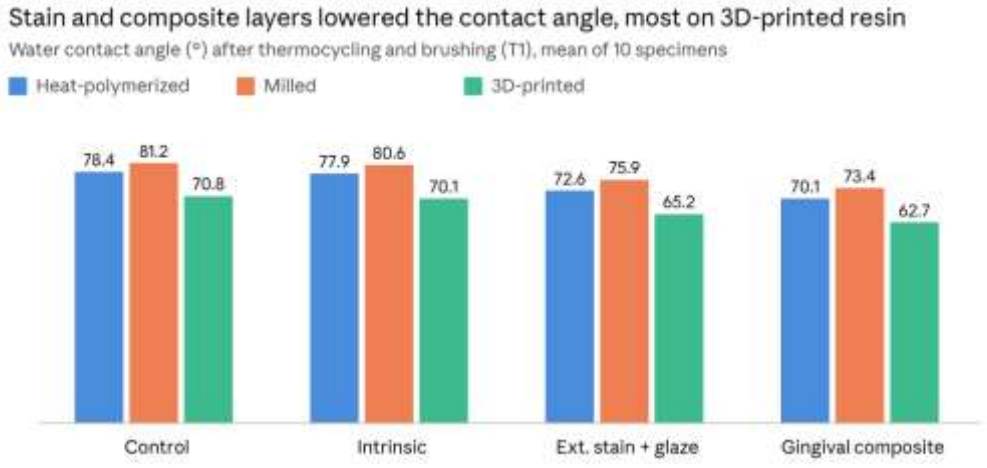
3.2 Contact angle

Contact angles decreased in all groups after aging, with the decrease larger where a surface layer was added (Table 4). Control and intrinsic samples decreased 1.2-1.9°, and extrinsic stain and composite samples decreased 3.4-5.1°. The 3D-printed resin was most wettable (lowest angles, (Figure 2), and the milled PMMA had the lowest.

Table 4. Water contact angle (°), mean ± SD, n = 10 per group

Material	Technique	At T0	At T1	Change (°)
Heat-polymerized	Control	80.1 ± 2.9	78.4 ± 3.1	−1.7
Heat-polymerized	Intrinsic	79.7 ± 3.0	77.9 ± 3.3	−1.8
Heat-polymerized	Ext. stain + glaze	76.8 ± 3.4	72.6 ± 3.8	−4.2
Heat-polymerized	Gingival composite	75.2 ± 3.6	70.1 ± 4.0	−5.1
Milled	Control	82.4 ± 2.6	81.2 ± 2.8	−1.2
Milled	Intrinsic	82.0 ± 2.7	80.6 ± 3.0	−1.4
Milled	Ext. stain + glaze	79.3 ± 3.1	75.9 ± 3.4	−3.4
Milled	Gingival composite	77.8 ± 3.3	73.4 ± 3.7	−4.4
3D-printed	Control	72.5 ± 3.2	70.8 ± 3.5	−1.7
3D-printed	Intrinsic	72.0 ± 3.3	70.1 ± 3.6	−1.9
3D-printed	Ext. stain + glaze	69.0 ± 3.7	65.2 ± 4.1	−3.8
3D-printed	Gingival composite	67.4 ± 3.9	62.7 ± 4.4	−4.7

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Model values · contact angle at T1, n = 10 per group

Figure 2. Water contact angle at T1 by technique and material.

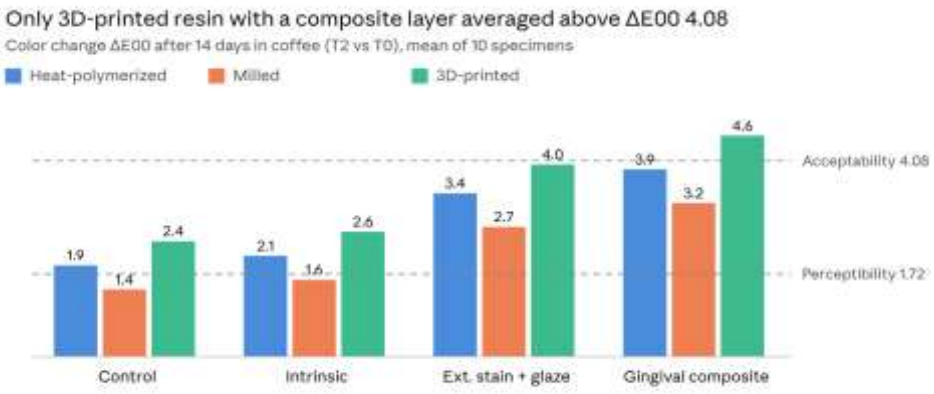
3.3 Color stability

After 14 days in coffee, ΔE00 ranged from 1.4 (milled, control) to 4.6 (3D-printed, composite) (Table 5). Only the 3D-printed composite group (mean = 4.3) maintained a mean ΔE00 above the acceptability threshold of 4.08, and the milled control group and the intrinsic group remained below the perceptibility threshold of 1.72 (Figure 3). Of all 120 specimens, 19 (16%) exceeded the acceptability threshold of 4.08, and all carried either extrinsic staining or composite veneer.

Table 5. Color change ΔE00 after coffee immersion (T2 vs T0), mean ± SD, n = 10 per group

Material	Technique	ΔE00	Mean vs thresholds	Specimens > 4.08
Heat-polymerized	Control	1.9 ± 0.4	Perceptible, acceptable	0/10
Heat-polymerized	Intrinsic	2.1 ± 0.5	Perceptible, acceptable	0/10
Heat-polymerized	Ext. stain + glaze	3.4 ± 0.7	Perceptible, acceptable	2/10
Heat-polymerized	Gingival composite	3.9 ± 0.8	Perceptible, acceptable	4/10
Milled	Control	1.4 ± 0.3	Below perceptibility	0/10
Milled	Intrinsic	1.6 ± 0.4	Below perceptibility	0/10
Milled	Ext. stain + glaze	2.7 ± 0.6	Perceptible, acceptable	0/10
Milled	Gingival composite	3.2 ± 0.7	Perceptible, acceptable	1/10
3D-printed	Control	2.4 ± 0.5	Perceptible, acceptable	0/10
3D-printed	Intrinsic	2.6 ± 0.6	Perceptible, acceptable	0/10
3D-printed	Ext. stain + glaze	4.0 ± 0.8	Perceptible, acceptable	5/10
3D-printed	Gingival composite	4.6 ± 0.9	Above acceptability	7/10

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Model values · ΔE00 (T2 vs T0), n = 10 per group

Figure 3. ΔE00 after coffee immersion by technique and material. Dashed lines mark the perceptibility (1.72) and acceptability (4.08) thresholds.

3.4 Statistical summary

Technique produced the greatest effects on all results, partial η2 between 0.49 and 0.73, followed by material (Table 6). Only one interaction material × technique was significant, for Ra only, indicating that the extrinsic and composite layers increased roughness more on the 3D-printed resin than on the milled PMMA. Tukey tests showed that control and intrinsic characterization groups were not significantly different from one another on any of the results, while extrinsic stain and composite groups each differed from control and intrinsic characterization, and from each other (Table 7).

Table 6. Two-way ANOVA (df: material 2, technique 3, interaction 6, error 108)

Outcome	Effect	F	p	Partial η^2
Ra at T1	Material	52.1	< .001	0.49
Ra at T1	Technique	88.4	< .001	0.71
Ra at T1	Material × Technique	2.3	.040	0.11
Contact angle at T1	Material	61.5	< .001	0.53
Contact angle at T1	Technique	34.2	< .001	0.49
Contact angle at T1	Material × Technique	0.8	.570	0.04
ΔE00	Material	48.7	< .001	0.47
ΔE00	Technique	96.3	< .001	0.73
ΔE00	Material × Technique	1.9	.087	0.09

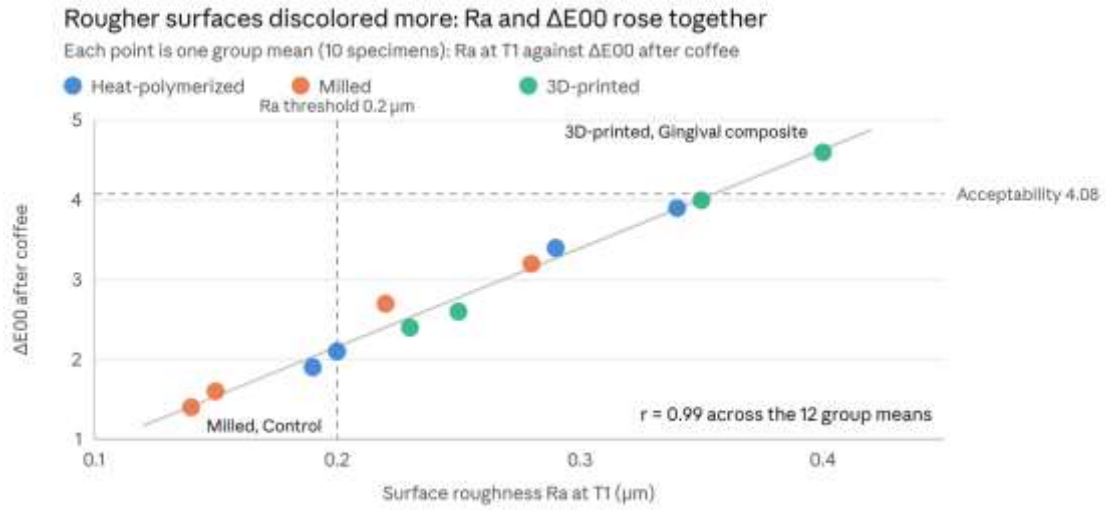
Table 7. Marginal means by factor level with Tukey groupings (means sharing a letter within a block and column do not differ, p > .05)

Factor level	Ra at T1 (μm)	Contact angle at T1 (°)	ΔE00
Control	0.19 a	76.8 a	1.90 a
Intrinsic	0.20 a	76.2 a	2.10 a

Factor level	Ra at T1 (µm)	Contact angle at T1 (°)	ΔE00
Ext. stain + glaze	0.29 b	71.2 b	3.37 b
Gingival composite	0.34 c	68.7 c	3.90 c
Milled	0.20 a	77.8 c	2.23 a
Heat-polymerized	0.26 b	74.8 b	2.83 b
3D-printed	0.31 c	67.2 a	3.40 c

3.5 Relationship between roughness and color change

At specimen level, Ra at T1 correlated strongly with ΔE00 ($r = .78$, 95% CI .70–.84, $p < .001$, $n = 120$). Across the 12 group means the relationship was almost linear (Figure 4), with the highest-roughness group also showing the greatest discoloration.



Model values · 12 group means

Figure 4. Group-mean Ra at T1 against ΔE00 after coffee. The grey line is the least-squares fit.

4. DISCUSSION

In this study, we compared four different gingival characterization techniques and three different denture base materials for changes in roughness, contact angle, and color change. The data indicated that the characterization technique had a significant impact on all three properties, and the denture base material exerted a significant but smaller influence. The three characterization techniques involving an intrinsic layer yielded similar results to those of the uncharacterized controls, while the extrinsic stain and glaze and, more notably, the gingival-shade composite layer, resulted in less contact angle and greater color change and roughness. Only in the latter did the 3D printed resin outperform the conventional resin with a layer of composite.

4.1 Surface roughness

Control and intrinsic samples after polishing, brushing, and thermocycling exhibited roughness values close to or less than 0.2 µm for the heat-polymerized and milled groups. This is consistent with the previous report of and heat-polymerized denture base resins (including printed and milled variants) having roughness values of 0.2 µm or less after brushing and thermocycling (Çakmak et al., 2022). The 3D-printed resin exhibited the roughest surface at each level of processing technique. A comparable top-surface roughness ranking has been described following staining and rinsing, with a substantial increase for 3D-printed and cold-polymerized groups and less-raised values for heat-polymerized and milled materials (Şahin et al., 2025). Surface properties of printed denture base polymer have already been demonstrated to be distinct from that of the conventional resin (Gad et al., 2022), validating the separate consideration of printed bases as a material group.

This is easily explained with mechanical reasoning for the greater amount of roughness seen in the extrinsic groups. A stain film, glaze, or composite layer is a thin bonded coating on the surface that is exposed to the brush and thermal cycle. Conversely, an intrinsic pigment is within the polymer matrix which is abraded by the bristles of the brush as the base is polished, actually having the base as a counter abrasive. Composite layers also contain filler particles within their polymer matrix which are less readily abraded by brushing and may be left proud of the surface so that the test would likely show extrinsic composite layers. Light-cured laboratory composites used to characterize the color of acrylic teeth were tested for color stability on their own for just this reason as it was felt that behavior could not be assumed to be similar to the base acrylic (Ahamed et al., 2023).

4.2 Color stability

The choice of the chromogen was that it is one of the most staining foods for denture resins. It was coffee in the previous color study, and coffee and red wine in the current one, gave the greatest change in 3D-printed and heat-polymerized resins with simulated testing periods of 3, 6, and 12 months, and this printed resin behaved like the heat-polymerized resin (da Silva et al., 2025). Our control groups showed the same sequence of etching in that order, but printed resin stained a little more than heat-polymerized, and milled less. Not all studies concur with the order of stained printed resin. Two spectrophotometrically based tests showed better color stability of printed denture base resin compared to conventional acrylic after immersion in coffee, red wine, and cola (Dimitrova et al., 2022, 2023), but a comparison of four denture base products found the printed resin as least color stable (Arora et al., 2024). Variability in the chemistry of different resins, post-curing protocols, polishing, stain media, and measurement techniques are probable reasons for the differences, which do not allow reliance on one printed product. PMMA may not have a particular advantage of stability in other aging schemes either. A machined PMMA demonstrated a greater color shift following artificial xenon-arc aging compared to machined acetal or PEEK (Sultan & Salloum, 2024), and heat-cured resins have shown time-dependent, substantial, color change following exposure to tea and coffee (Agrawal et al., 2024). Against these results, the finding that the characterization layer can contribute more to discoloration than the base material is remarkable. Applying the CIEDE2000 thresholds (Çakmak et al., 2022), most groups would be acceptable by appearance alone, but 19 of 120 specimens were beyond the acceptability threshold; all of these had a characterization layer.

4.3 Wettability and the roughness-color link

The lower contact angle for extrinsic groups suggests a more wettable surface. A rougher, more wettable surface provides a greater surface area and retention for pigment, which is in line with the strong relationship between Ra and ΔE00 ($r = .78$ at specimen level). The same correlation has been suggested in previous studies, where surface roughness was thought to have the potential to accelerate deterioration of complete denture (Çakmak et al., 2022). Since the relationship is correlational and the two techniques are differing in chemistry as well as texture, this correlation does not suggest that roughness alone caused the discoloration, and absorption of pigment into the composite matrix or stain film was likely involved.

4.4 Clinical implications

A few take home messages emerge. Firstly, when characterization of the gingiva is desirable, native pigmentation maintained the surface and shade of all three materials most effectively and is the most robust choice of material for the long term. Secondly, when an extrinsic stain or composite layer is applied, it should be polished to an excellent level and sealed to an appropriate adhesively retained material and patient shown that this layer will require maintenance or replacement more often than the native tissue. Thirdly, characterization of a 3D printed base with a composite layer is a high risk strategy and an alternative intrinsic solution may be preferable or earlier recall suggested.

4.5 Limitations and future research

It was an in vitro study on flat discs that did not mimic the mouth anatomy, saliva flow, biofilm, or occlusal forces. One stain and 14 days used, only one commercial product for each material type, so caution when generalizing to material classes. The protocol combined thermocycling and brushing, but not chemical denture cleaners, which influence roughness and color (Şahin et al., 2025). Color was characterized against a white background, whereas in vivo the background was the pink mucosa. Other

resin brands can be included, longer or repeated staining cycles performed, cleansers applied, microbial attachment studies and the characterization of current dentures for future color follow-up studies.

5. CONCLUSIONS

Within the limitations of this in vitro study:

1. Intrinsic characterization was similar to the control, which was uncharacterized, with respect to roughness, contact angle, and color change, and maintained surface quality the best.
2. Extrinsic stain with glaze and, to an even greater extent, layers of a gingivally-shaded composite increased surface roughness, decreased contact angle, and resulted in greater coffee stain, all three materials.
3. The 3D-printed resin was the most prone material, and only the combination with the composite layer was the group with a mean value of ΔE00 higher than 4.08 acceptable.
4. There was a strong correlation between roughness and change of colour so surface finishing and sealing of the characterising layer applied is critical.

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