

A pilot study on professional cleansing protocols for PMMA resins produced by CAD/CAM and 3D printing technologies: Effects on surface roughness and color stability

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ABSTRACT

Objectives: This in vitro pilot study evaluated the effects of two professional cleansing protocols on surface roughness and color stability of various resins for complete dentures.

Methods: 50 resin specimens were prepared and divided into five groups (n = 10): Probase Hot (PH, Ivoclar AG), IvoBase CAD (IBC, Ivoclar AG), Ivotion Dent (ID, Ivoclar AG), Dima Print Denture Base (DPDB, Kulzer), and Dima Print Denture Teeth (DPDT, Kulzer). After a 7-day immersion in coffee solution, two cleansing protocols were applied: a chemical one using an alkaline solution, and a combined mechanical+chemical one involving an acid-based cleaning agent with a rotating needle device followed by the same chemical protocol. Surface roughness was measured before and after cleaning using a structured light profilometer (Confovis) connected to a microscope (Eclipse LV150N, Nikon). Color stability was assessed with a colorimeter (Easy_Color, SmartVision). One specimen per group underwent SEM analysis at baseline and after both protocols. The data analysis was performed by using Kruskal-Wallis test and post-hoc Dunn test for comparison.

Results: Surface roughness was not significantly affected by either protocol (p>0.05). All ΔE values were below the perceptibility threshold ($\Delta E < 1.2$), except for ID after both the chemical ($\Delta E = 2.28$) and the mechanical+chemical protocol ($\Delta E = 2.39$) and DPDB after both the chemical ($\Delta E = 2.06$) and the mechanical+chemical protocol ($\Delta E = 2.33$).

Conclusions: Surface roughness and color stability of PMMA resins were not affected by the tested cleansing protocols, so they could be used by clinician during the periodically control visit.

Clinical Significance: The proposed cleansing protocols help preserve the long-term aesthetic and functional integrity of dentures, enhancing patient satisfaction and oral health.

1. Introduction

Polymethylmethacrylate (PMMA) is the most widely used material for the fabrication of complete removable dental prostheses (CRDPs). Traditionally, compression molding and heat-polymerized techniques were traditionally PMMA fabrication methods [1].

The introduction of digital techniques have rationalized both clinical protocols and manufacturing processes for CRDPs, allowing the fabrication of CRDPs with subtractive (milling) and additive (3D-printing)

processes [2,3].

For materials employed in CRDPs, including PMMA, it is essential that they satisfy defined mechanical, physical, and biological requirements to guarantee adequate performance and longevity under the demanding conditions of the oral environment.

A crucial aspect to ensure the maintenance of CRDPs is the cleansing. An improper cleansing of CRDPs has not only been associated with the formation of plaque biofilm or oral pathologies but has also been demonstrated to have systemic implications [4–8].

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Table 1
PMMA materials and cleansing agents used in the study.

PMMA resins			
Resin	Manufacturer	Fabrication technique	Composition
Probase Hot (PH)	Ivoclar AG, Schaan, Liechtenstein	Heat-curing resin	Powder: Polymethyl methacrylate, softening agent, benzoyl peroxide, pigments Liquid: Methyl methacrylate, dimethacrylate (linking agent), catalyst
IvoBase CAD (IBC)	Ivoclar AG, Schaan, Liechtenstein	Milling	Polymethyl methacrylate, co-polymer for impact toughness modification, pigments
Ivotion Dent (ID)	Ivoclar AG, Schaan, Liechtenstein	Milling	Highly cross-linked PMMA, pigments
Dima Print Denture Base (DPDB)	Kulzer, Hanau, Germany	3D Printing	Esterification products of 4,4'-isopropylidenediphenol, ethoxylated and 2-methylprop-2-enoic acid 7,7,9-trimethyl-4,13-dioxo-3,14-dioxa-5,12- diazahexadecane-1,16-diyl bismethacrylate Propylidynetrimethyl trimethacrylate Diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide Mequinol
Dima Print Denture Teeth (DPDT)	Kulzer, Hanau, Germany	3D Printing	Esterification products of 4,4'-isopropylidenediphenol, ethoxylated and 2-methylprop-2-enoic acid 7,7,9-trimethyl-4,13-dioxo-3,14-dioxa-5,12- diazahexadecane-1,16-diyl bismethacrylate Propylidynetrimethyl trimethacrylate Diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide Mequinol
Cleansing solution			
Solution	Manufacturer	Description	Composition
Help:ex discolor f	Renfert, Hilzingen, Germany	Alkaline solution	(1-(3-Methoxypropoxy)propan-1-ol; (2-Methoxymethyletoxy)-propanol; Dipropylene glycol methyl ether 2-Aminoethanol Potassium hydroxide
Help:ex plaque p	Renfert, Hilzingen, Germany	Acid-based cleansing agent in a powder form	Sulphamic acid

Several types of cleansing protocols can be used to remove biofilms from the surface of CRDPs. These protocols are generally classified as professional or home-care. Professional protocols involve the application of specialized products by the clinician during in-office procedures, whereas home-care protocols are carried out by patients at home as part of their daily oral hygiene routine.

Moreover, cleansing protocols include mechanical and chemical procedures. Mechanical protocols can be performed with brushes, microwave irritation, rotational motion of needles and ultrasonic devices. Chemical agents include products based on sodium hypochlorite (NaOCl), peroxides, neutral peroxides with enzymes, or acid-based agents. In literature, the most effective way to remove the biofilm from CRDPs is the combined use of mechanical and chemical cleansing protocols [9].

Cleansing protocols should reduce or remove biofilm without altering physical and mechanical properties of PMMA [10,11]. However, several studies [12,13] demonstrated that the prolonged use of denture cleansers can interfere with physical and mechanical properties, including the surface roughness [14,15] and color stability [16,17] of denture resins.

Several studies demonstrated a lower surface roughness of milled resin compared to 3D printing and conventional resins [18–20]. Khan et al [21] reported in their systematic review a higher resistance to color change for milled denture bases compared to 3D printing and conventional resins. Since the resin manufacturing method results in differing roughness and color-stability characteristics, even the application of an identical cleaning protocol may influence the mechanical properties of PMMA [22].

Given the limited evidence available in the literature regarding the effects of combined chemical and mechanical protocols on the physical and mechanical properties of PMMA, the aim of this in vitro study was to evaluate the effects of two professional cleansing protocols on the surface roughness and color stability of PMMA resins for CRDPs fabricated using digital CAD/CAM and conventional 3D printing technologies.

The first null hypothesis tested was that both cleansing protocols

would not affect surface roughness of PMMA resins.

The second null hypothesis tested was that both cleansing protocols would not affect color stability of PMMA resins.

2. Materials and methods

All types of PMMA resin materials and denture cleanser agents used in this study, including manufacturer information and composition, are listed in Table 1.

Five types of PMMA resins for CRDPs with different fabrication techniques were tested (n = 10): one heat-polymerized resin (PH; Probase Hot, Ivoclar AG, Schaan, Liechtenstein), two CAD/CAM resins (IBC: IvoBase CAD; ID: Ivotion Dent white color A2, Ivoclar AG, Schaan, Liechtenstein) and two 3D-printed resins (DPDB: Dima Print Denture Base; DPDT: Dima Print Denture Teeth A2; Kulzer GmbH, Hanau, Germany).

A total of 50 resin specimens of standardized dimension (10 × 10 × 3 mm) were obtained and tested.

Specimens belonging to the PH group were produced as follows: initially wax patterns were made to the required specimen size (10 × 10 × 3 mm) and then two flaskswere filled using type II gypsum (Gibraltar-Plaster, HenrySchein, Melville, NY, USA). After removing the wax model with hot water, two layers of gypsum-resins insulator (Separating Fluid, Ivoclar AG, Schaan, Liechtenstein). A mixture of heat polymerized acrylic resins was prepared according to the manufacturer's instructions with a proportion of 22.5 g of powder and 10 ml of liquid and at dough stagepacked into the mold. The flask was closed and placed in the hydraulic press at 8 MPa pressure. The flask was then placed in cold water, heated up to 100°C and boiled for 45 min according to the manufacturer's instructions.

Specimens produced with a milling technique were obtained from pre-polymerized PMMA discs starting from a standard tessellation language (.STL) file.

The discs were sliced using a milling machine (Programill PM7, Ivoclar AG, Schaan, Liechtenstein) and after milling was completed, the

Table 2
Treatments descriptions.

Treatment	Treatment description
Baseline P0	/
7-day immersion in a coffee solution P1	Immersion in a coffee solution (Nescafé Classic, Nestlé S.A., Vevey, Switzerland) with a water/coffee ratio of 120ml/4g. The coffee solution was replaced every 24 h.
Chemical cleansing protocol (CH) P2	Immersion of the specimens in an alkaline solution (help:ex discolor f, Renfert GmbH, Hilzingen, Germany) for 10 min. After the immersion, the specimens were rinsed with water and dried with absorbent paper.
7-day immersion in a coffee solution P3	Immersion in a coffee solution (Nescafé Classic, Nestlé S.A., Vevey, Switzerland) with a water/coffee ratio of 120ml/4g. The coffee solution was replaced every 24 h.
Mechanical+chemical cleansing protocol (MECH-CH) P4	Immersion of the specimens in the cleaning bowl of a rotating needle device (SYMPRO, Renfert GmbH, Hilzingen, Germany) filled by water and a sachet of an acid-based cleaning agent (help:ex plaque p, Renfert GmbH, Hilzingen, Germany) for 15 min. Then the specimens were rinsed with water and dried with absorbent paper. After the mechanical treatment, the specimens were immersed in an alkaline solution (help:ex discolor f, Renfert GmbH, Hilzingen, Germany) for 10 min. After the immersion, the specimens were rinsed with water and dried with absorbent paper.

specimens were cut off from the PMMA bank using an acrylic trimming disc bur.

Specimens produced with an additive technique were fabricated with a 3D printer (Cara Print 4.0 Pro, Kulzer GmbH, Hanau, Germany) starting from a standard tessellation language (STL) file.

3D printing was done with an incremental layer thickness of 100µm in a horizontal orientation. After the printing process was completed, the specimens were thoroughly cleaned by rinsing in 96% ethanol once for 3 min. After drying the specimens, they were post-cured in an ultraviolet light device (UV Everes Curo, Sweden & Martina, Padua, Italy) for 10 min to complete the curing process.

The surfaces of the specimens were polished in accordance with the manufacturer's instructions. Initially, a white goat-hair brush in combination with fine wet pumice (Pomice, Simed Srl, Settimo Torinese, Italy) was employed. Subsequently, a deerskin brush in combination with a polishing paste (Universal Polishing Paste, Ivoclar AG, Schaan, Liechtenstein) was used. All polishing procedures were performed by the same trained operator.

Before the cleansing protocols, the specimens were immersed in a staining coffee solution coffee (Nescafé Classic, Nestlé S.A., Vevey, Switzerland). The solution was prepared with a water/coffee ratio of 120ml/4g according with producer's guidelines.

Coffee was added to boiling water and stirred at room temperature until cooled to 37°C before immersion. The coffee solution was replaced every 24 h. This period may correspond to 34 to 67 months of clinical service, based on an average daily exposure of 5 to 10 min to coffee [23].

The first cleansing protocol (P2) was performed after a 7-day immersion in a coffee solution and it involved the immersion of the specimens in an alkaline solution (help:ex discolor f, Renfert GmbH, Hilzingen, Germany) for 10 min according to the manufacturer's guidelines. After the immersion, the specimens were rinsed with running water for 10 seconds and dried with absorbent paper in order to test color stability and roughness.

The second cleansing protocol (P4) was performed after a 7-day immersion in a coffee solution. The specimens were placed in the cleaning bowl of a rotating needle device (SYMPRO, Renfert GmbH, Hilzingen, Germany) filled by water and a sachet of an acid-based

cleaning agent (help:ex plaque p, Renfert GmbH, Hilzingen, Germany) was put in the bowl according to the manufacturer's guidelines. After 15 min of cleaning, the device was stopped, the specimens were rinsed with water and dried with absorbent paper. After the mechanical treatment, the specimens were immersed in an alkaline solution (help:ex discolor f, Renfert GmbH, Hilzingen, Germany) for 10 min according to the manufacturer's guidelines. After the immersion, the specimens were rinsed with water and dried with absorbent paper in order to test color stability and roughness.

The choice of a sequential combined protocol was primarily due for practical reasons since the same specimens were used throughout the study to simulate a realistic clinical scenario. In addition, in routine professional cleaning sessions, clinicians typically attempt an initial chemical treatment alone, followed-if necessary-by a combined chemical-mechanical intervention to remove more persistent stains from the prosthesis. This sequence therefore reflects both the product's intended use and common clinical practice.

Each treatment and procedures are resumed in Table 2.

Morphological analyses were carried out on the surface of the specimens at baseline (P0), after the chemical cleansing protocol (P2) and after the mechanical+chemical cleansing protocol (P4) using a Confovis structured light profilometer connected to an optical microscope (Eclipse LV150N, Nikon, Tokyo, Japan). For each specimen, an area of 0.8 × 0.8mm was analyzed from which the surface roughness values defined according to the standard UNI EN ISO 25178-2:2012 [24].

The arithmetic mean height or Sa parameter is defined as arithmetic mean of the height of the surface, calculated according to the equation [23]:

$$Sa = \frac{1}{A} \iint_A |z(x,y)| dx dy$$

Where A is the area considered and z is the height of the coordinate point x and y.

For each group, one specimen was randomly selected for SEM analysis (Feg SEM, Nova NanoSEM 450, FEI) to morphologically characterize the surface: SEM analysis was conducted at baseline (P0), after the chemical cleansing protocol (P2) and after the mechanical+chemical cleansing protocol (P4). Specimens were put on a stub, were not sputter-coated and imaged at a × 500, × 1000, × 2000 magnification (vacuum setting:LVD, acceleration voltage: 8.00kV and working distance: 5.8).

Multiple regions of the same specimen were examined to minimize potential bias and ensure a representative evaluation of the surface morphology.

Color analyses were performed at baseline (P0), after the first protocol (P2) and after the second protocol (P4).

The color coordinates (L*: lightness, a*: red/green, and b*: yellow/blue) were obtained with a latest generation of live-video comparative spot digital colorimeter (Easy_Color, SmartVision, Udine, Italy). The Commission Internationale de l'Eclairage (CIE) settings were as follows: observer degree: 2; ΔE type: ΔE_{ab} CIELAB; Illuminant: D; Temperature: 50. White and black calibration was performed as suggested by the manufacturer at the beginning of the test and calibration was performed every 10 measurements. Each specimen was placed on a viewing platform equipped with a LED light source and covered with a black cap. The position and dimensions of the analysis area (1.73 × 0.86 mm) were precisely adjusted and customized. The reference Lab* values were then compared to the current readings, and the system automatically recorded the ΔE values [25].

Roughness and color measurements were conducted by a single, trained operator. Both setups ensured that measurements could be repeated at the exact same location and with the same measurement size, either on the same specimen for multiple readings or on different specimens, with all measurements performed under ambient conditions

Table 3

Surface roughness: Median, Min and Max values (Sa, μm) of materials evaluated at baseline (P0), after CH (P2) and after MECH-CH (P4). PH, Probase Hot. IBC, IvoBase CAD. ID, Ivotion Dent. DPDT, Dima Print Denture Teeth. DPDB, Dima Print Denture Base. P0, Baseline. P2, after chemical cleansing protocol (CH). P4, after mechanical+chemical cleansing protocol (MECH-CH).

	PH			IBC			ID			DPDT			DPDB		
	Median	Min	Max	Median	Min	Max	Median	Min	Max	Median	Min	Max	Median	Min	Max
P0	0.077	0.042	0.093	0.067	0.037	0.118	0.087	0.078	0.102	0.079	0.055	0.115	0.07	0.046	0.094
P2	0.06	0.034	0.116	0.0855	0.038	0.114	0.0825	0.064	0.089	0.0825	0.034	0.116	0.0825	0.034	0.116
P4	0.0535	0.041	0.094	0.096	0.059	0.125	0.0905	0.081	0.104	0.0925	0.054	0.12	0.071	0.044	0.109

Table 4

Dunn's Post Hoc test for comparison of surface roughness among the groups.

Group	Group	p-value
DPDT	DPDB	0.0045
ID	DPDB	0.0075
IBC	DPDB	0.4221
ID	IBC	1.0000
ID	DPDT	1.0000
PH	DPDB	1.0000
IBC	DPDT	1.0000
PH	IBC	0.0771
PH	ID	0.0006
PH	DPDT	0.0003

at 21°C and 50% relative humidity.

Statistical analyses were performed with a statistical software program (JMP Pro, v17; JMP Statistical Discovery LLC Campus Drive Cary, NC, USA).

A preliminary Shapiro-Wilk test was conducted to confirm the normal distribution of the data. The assumption of homogeneity of variances was tested with Bartlett test and Levene test. Since both roughness and color stability had a non-homoscedasticity, a Kruskal-Wallis test ($\alpha = 0.05$) was performed. The p-value was below the respectable critical threshold of 0.05, so post-hoc Dunn's test was conducted to determine statistically significant differences.

3. Results

3.1. Surface roughness

Surface roughness values of the PMMA resins tested are presented in Table 3 and 4, Fig. 1 and Fig. 2.

No significant interaction of cleansing protocol applied on surface roughness was found ($P > 0.05$).

DPDB shown a lower roughness compared to DPDT ($p = 0.0045$) and ID ($p = 0.0075$), also PH shown a lower roughness compared to ID ($p = 0.0006$) and DPDT ($p = 0.0003$), while there was not a statistically significant difference between DPDB and PH and between IBC and all the other groups ($p > 0.05$).

An example of the measurements made with the profilometer for a DPDB specimen is shown in Fig. 2.

3.2. Color stability

Table 5 shows color differences analysis (ΔE) as the differences of E before and after cleansing protocols (P2-P0, P4-P0 and P4-P2).

All materials tested presented an imperceptible color difference after the cleansing protocols, except for ID after CH ($\Delta E = 2.28$) and MECH-CH ($\Delta E = 2.39$) and DPDB after CH ($\Delta E = 2.06$) and MECH-CH ($\Delta E = 2.33$).

4. Discussion

The first null hypothesis, that the chemical or mechanical-chemical cleansing protocols evaluated in this study did not affect surface roughness of PMMA resins, was accepted. Any significant effect of the above-mentioned cleansing protocols on surface roughness of PMMA resins was found ($p > 0.05$).

Coffee is an acidic beverage rich in chromogenic compounds, such as tannins and polyphenols, that can bind to the organic components of denture materials, contributing to surface discoloration. Coffee (Nescafé), used as the staining medium in the present study, is characterized by an acidic pH, ranging from approximately 4.7 to 5.4, consistent with commonly reported values for coffee beverages, which

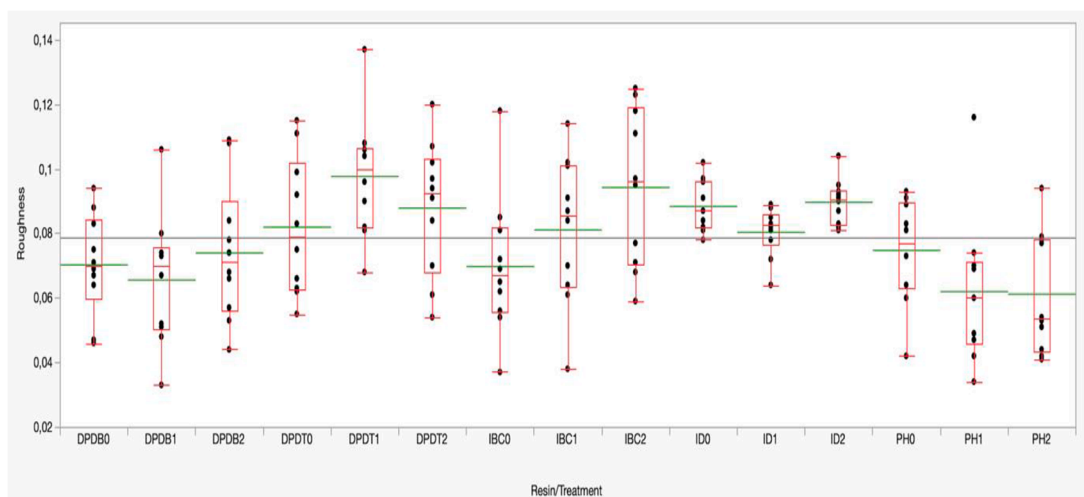


Fig. 1. Box plot of roughness data related to cleansing protocols and resin type. PH, Probase Hot. IBC, IvoBase CAD. ID, Ivotion Dent. DPDT, Dima Print Denture Teeth. DPDB, Dima Print Denture Base. P0, Baseline. P2, after chemical cleansing protocol (CH). P4, after mechanical+chemical cleansing protocol (MECH-CH). (Kruskal-Wallis test and Dunn test for comparison)

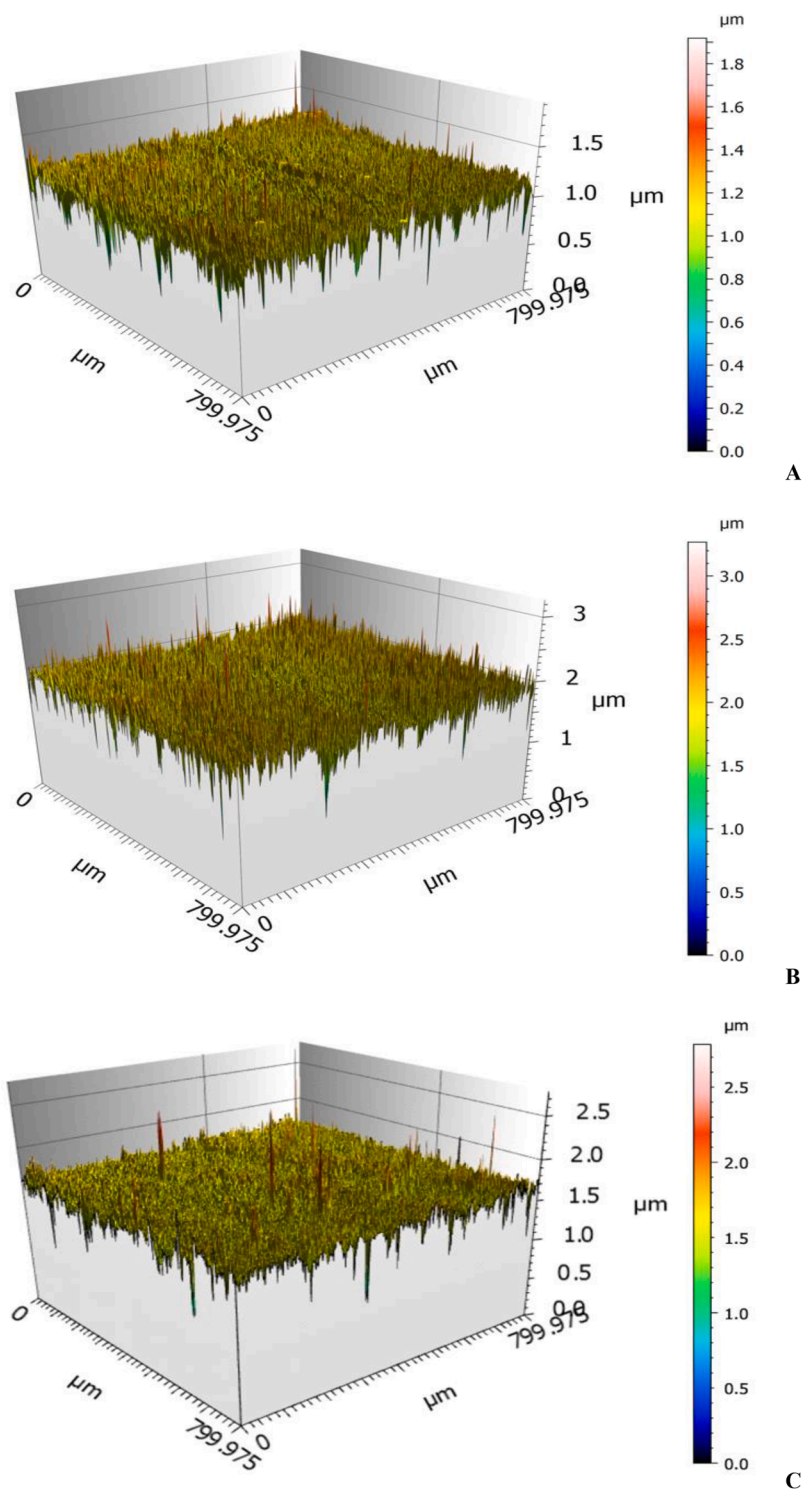


Fig. 2. Roughness images of a DPDB specimen after P0-baseline(A), P2-after CH cleansing protocol (B) and P4-after MECH-CH cleansing protocol (C).

may transiently lower oral pH after consumption and represent a clinically relevant acidic challenge.

Alkaline cleaners enhance stain removal because their basic pH promotes the solubilization and breakdown of these acidic and pigment-rich deposits through saponification and dispersion mechanisms, making alkaline formulations particularly effective against stains derived from acidic chromogens. In this context, Help:ex discolor F - used for the chemical protocol (P2) - a strongly alkaline cleansing solution with a reported pH ranging from approximately 11.5 to 14.0, may facilitate the removal of coffee-related discolorations. Conversely, Help:ex plaque P -

used for the chemical+mechanical protocol (P4) - which reaches a near-neutral pH after neutralization according to the manufacturer's instructions, reflects conditions compatible with routine professional cleaning procedures. These pH values fall within ranges that can be considered clinically acceptable when considering the buffering capacity of saliva, which typically restores physiological pH levels shortly after acidic challenges [26]. Furthermore, according to the manufacturer's guidelines, the selected alkaline cleanser is specifically recommended for dissolving or removing persistent stains-such as those caused by tar, coffee, and tea-from CRDPs.

Table 5

Color difference analysis (ΔE) after and before the cleansing protocols. PH, Probase Hot. IBC, IvoBase CAD. ID, Ivotion Dent. DPDT, Dima Print Denture Teeth. DPDB, Dima Print Denture Base. P0, Baseline. P2, after chemical cleansing protocol (CH). P4, after mechanical+chemical cleansing protocol (MECH-CH).

	PH	IBC	ID	DPDT	DPDB
$\Delta E1$ (P2-P0)	0.70	0.11	2.28	0.18	2.06
$\Delta E2$ (P4-P0)	0.88	0.04	2.39	0.25	2.32
$\Delta E2$ (P4-P2)	0.29	0.08	0.11	0.07	0.26

Findings of this in vitro study support those of Peracini et al. [27] and Paranhos et al. [28], who observed that an alkaline cleanser had no effect on surface roughness of resins for CRDPs. Moffa et al. [29] reported no significant differences in the surface roughness of resins for CRDPs after cleaning with mechanical brushing alone or in combination with chemical cleaning (alkaline peroxide- based denture cleanser and/or chlorhexidine digluconate).

On the other hand, Costa et al. [12] and Al-Thobity et al. [14] demonstrated that alkaline and acid-based cleansers significantly increased the surface roughness of PMMA resins. Davi et al. [30] reported that treatment with alkaline chemical solutions altered the surface morphology of polymeric denture base resins by inducing the release of plasticizers. Ozyilmaz et al. [13] revealed that cleansing with an acid-based solution induced a significantly lower effect on surface roughness as compared to the alkaline cleansers.

Steinmassl et al. [31] reported that most CRDPs fabricated by digital technologies, in particular by subtractive processes, have smoother surfaces as compared to CRDPs produced by conventional techniques. Moreover, Şahin et al. [18], Anderson et al. [19] and Menon et al. [20] reported a lower roughness of CRDPs produced by milling techniques after cleansing protocols compared to resins produced by 3D printing and conventional techniques. These findings are in contrast with the results of this in vitro study, since the lowest roughness values were observed for 3D-printed resin DPDB and traditional resin PH as compared to ID (milled resin) and DPDT (3D printed resin).

All polishing procedures were performed by the same trained operator in order to minimize operator-dependent variability and the same standardized protocol was used for all groups to allow a direct comparison among materials.

With regard to the higher roughness observed in the milled group, in contrast with the results of most literature, surface features inherent to the milling process, such as machining marks, may be more difficult to eliminate completely through conventional polishing and could have contributed to the higher roughness values.

Printing layer thickness is a critical parameter in additive manufacturing, as it directly influences surface topography and optical behavior. A smaller layer thickness generally results in a smoother surface by reducing the staircase effect, whereas larger layers may increase surface irregularities and light scattering. In the present study, all 3D-printed specimens were fabricated using a standardized layer thickness of 100 μm and a horizontal build orientation, in order to minimize variability related to printing parameters.

Despite identical printing settings, significant differences in surface roughness were observed between the two 3D-printed resins. DPDB, the pink denture base resin, exhibited lower roughness values compared to DPDT, the white denture base resin ($p = 0.0045$). Increased surface roughness can enhance diffuse reflection, thereby altering perceived color and brightness. In this context, the lower roughness of DPDB may have contributed to more uniform light reflection compared to DPDT and the milled CAD/CAM resin (ID), which showed higher roughness values ($p = 0.0075$).

Nevertheless, the highest surface roughness values measured when using the two cleansing protocols remained below 0.2 μm , which is the reported threshold value for plaque accumulation [32]. Although, it

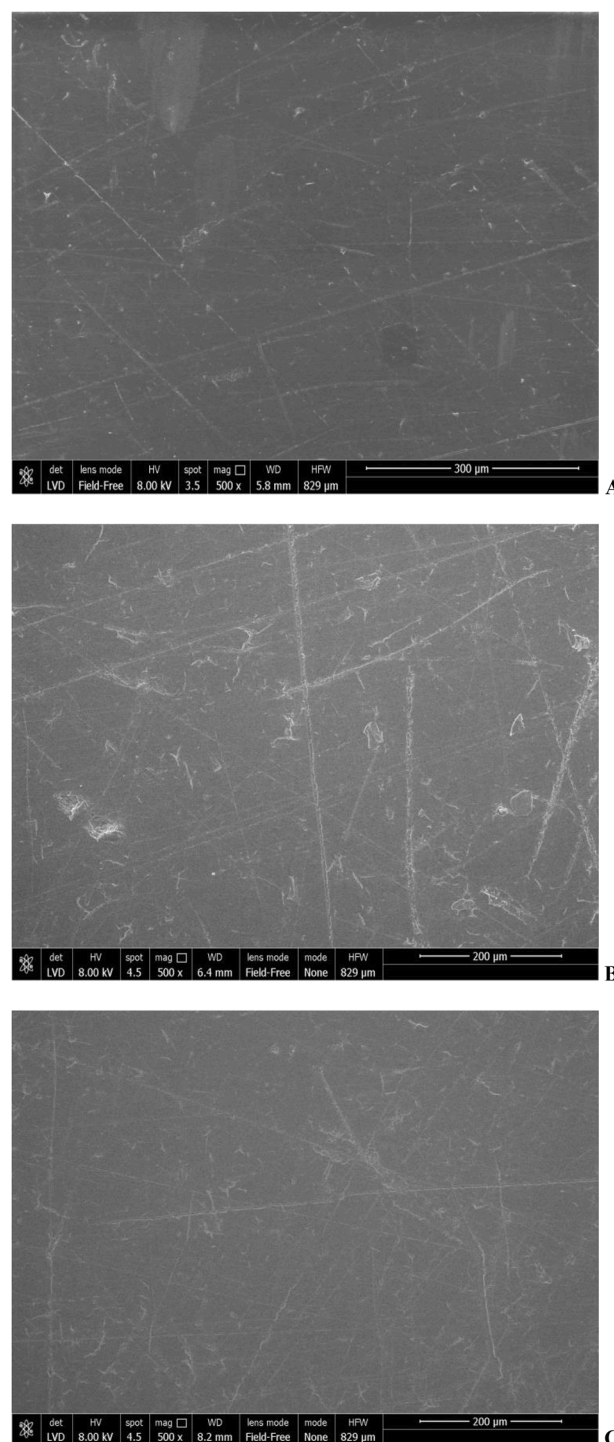


Fig. 3. DPDT analyzed by SEM $\times 500$ mag at baseline (A), after CH (B) and after MECH-CH (C). After MECH-CH, a slight increase in rectilinear scratches can be detected on the surface probably due to the mechanical action of the rotating needle device.

should be noted that, in this in vitro study, chewing and thermal cycles were not performed and the presence of saliva was not considered, and these factors could have influenced the results.

However, it has to be point out that interpretation of data can be affected by different set ups, since different solutions used in different concentrations and application protocols may lead to different results. In fact, there is not a standardization of cleaning protocols, since there is a wide variety of cleanser solutions, concentrations and immersion

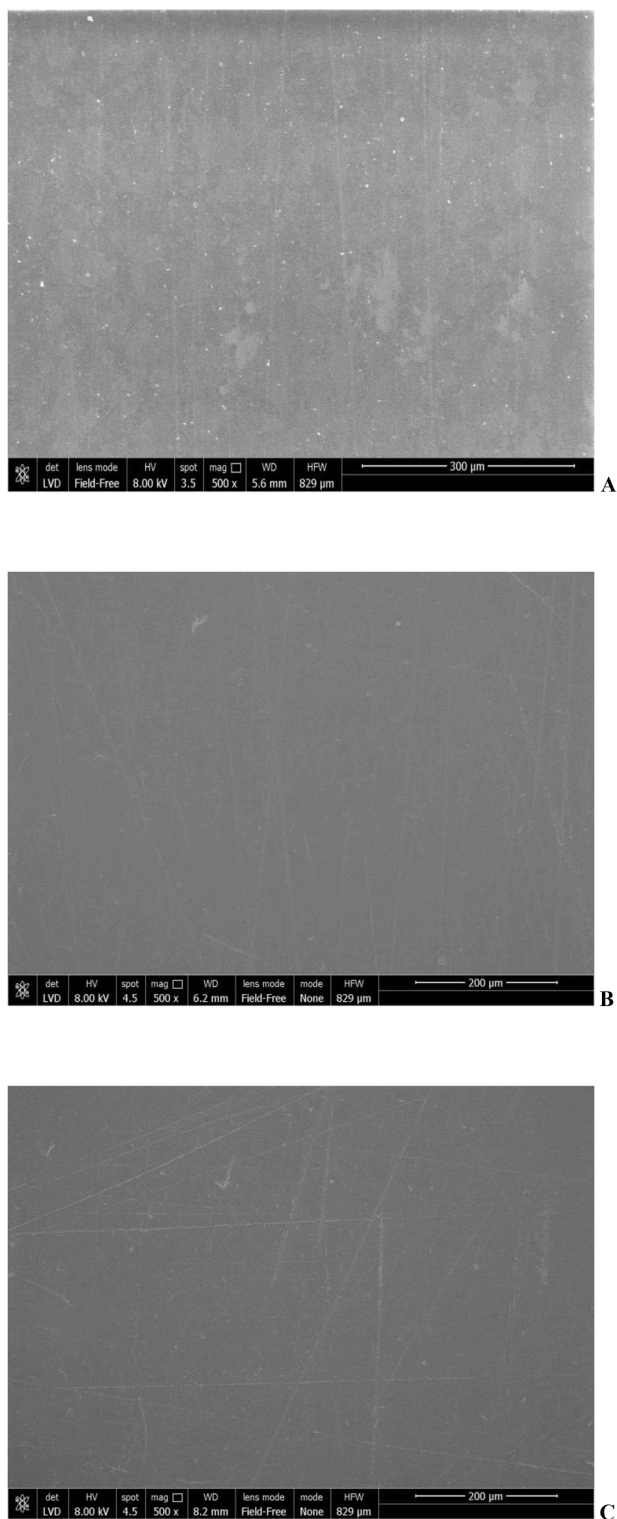


Fig. 4. IBC analyzed by SEM $\times 500$ mag at baseline (A), after CH (B) and after MECH-CH (C). After MECH-CH, a slight increase in rectilinear scratches can be detected on the surface probably due to the mechanical action of the rotating needle device.

durations. Moreover, this *in vitro* study investigated two cleansing protocols which can be performed by the clinician not daily but approximately once a year, while most of the studies in literature rely on daily cleaning protocols that can be carried out by patients.

SEM analysis revealed no differences in surface morphology between

specimens evaluated at baseline and after CH and MECH-CH protocols.

As presented in Figs. 3 and 4, after MECH-CH, a slight increase in rectilinear scratches can be detected probably due to the mechanical action of the rotating needle device.

The second null hypothesis, that the chemical or mechanical-chemical cleansing protocols evaluated in this study did not affect color stability of PMMA resins, was accepted.

Regarding the shade selection, for the tooth-colored materials, the shade A2 was used for all specimens. This choice was made to ensure the highest possible level of standardization within the limitations of the commercially available materials. However, it was not feasible to fully standardize the color across all materials. In particular, the gingival portions (base) of the materials are supplied by the manufacturers in predefined shades, and a direct chromatic match between base and tooth materials from different production technologies is not currently achievable.

The rationale for comparing tooth and base materials directly lies in the clinically relevant objective of this study, which was to evaluate the interaction between professional cleansing protocols and materials fabricated using different manufacturing technologies (conventional heat-curing, CAD/CAM milling, and 3D printing), as these combinations reflect daily clinical practice.

A limit of the study could be represented by the intrinsic shade differences among the materials, which may have influenced the absolute color measurements. Nevertheless, the primary outcome of this study focused on color changes (ΔE) within each material over time, rather than absolute baseline color values, thereby minimizing the impact of initial shade differences.

$\Delta E1$ was calculated as a difference between ΔE after (P2) and before CH cleansing protocol (P0). $\Delta E2$ was calculated as a difference between ΔE after (P4) and before MECH-CH cleansing protocol (P0). $\Delta E3$ was calculated as a difference between ΔE after CH cleansing protocol (P2) and after MECH-CH cleansing protocol (P0). According to ISO/TR 28642 Dentistry-Guidance on Color Measurement (2016 Edition), color differences of $\Delta E < 1.2$ are imperceptible to the human eye while values of $\Delta E > 2.7$ are considered clinically unacceptable [33]. $\Delta E1$, $\Delta E2$ and $\Delta E3$ of all the resins tested was under the above-mentioned range ($\Delta E < 2.7$). Therefore, all materials tested presented an imperceptible color difference, except for ID after CH ($\Delta E = 2.28$) and MECH-CH ($\Delta E = 2.39$) and DPDB after CH ($\Delta E = 2.06$) and MECH-CH ($\Delta E = 2.33$).

When comparing color differences after the chemical cleaning protocol (CH) with baseline values (E1), the absence of a visually perceptible color change indicates that the protocol was effective in removing stains, as the specimens did not exhibit clinically detectable differences compared to their initial condition at delivery. Conversely, ID and DPDB specimens showed measurable color differences, suggesting a comparatively reduced cleaning efficacy, although the observed ΔE values remained below the established clinical perceptibility threshold of $\Delta E = 2.7$.

A similar trend was observed when comparing the combined mechanical and chemical cleaning protocol (MECH-CH) with baseline values (E2). In this case, non-perceptible color differences again indicate satisfactory cleaning performance, whereas ID and DPDB specimens exhibited detectable changes, pointing to a lower, yet still clinically acceptable, stain removal effectiveness.

Finally, the comparison between MECH-CH and CH alone (E3) revealed a slight additional color difference, suggesting that the inclusion of the mechanical component may contribute to further stain removal, albeit to a limited extent.

The lower color stability observed for ID may be explained, as previously discussed, by surface features inherent to the milling process, such as machining marks, which may be more difficult to eliminate completely through conventional polishing procedures.

Conversely, the lower color stability of the 3D-printed specimens cannot be attributed to a single cause but is more likely the result of multiple interacting factors associated with additive manufacturing

techniques, such as the higher polarity, quantity of residual monomers, high solubility, and water sorption are additive factors that could influence the color stability of 3D-printed materials [34]. Layer thickness may also affect color perception by modifying surface microgeometry and light scattering. These findings suggest that, beyond layer thickness, material composition and pigment characteristics play a crucial role in color stability. White or lightly pigmented resins may be more susceptible to chemical degradation and water sorption, which can exacerbate color changes even in the absence of pronounced mechanical wear.

In contrast, the lower surface roughness observed for DPDB may have contributed to reduced light scattering and greater resistance to color changes following isolated chemical treatment. However, when mechanical treatment was combined with chemical exposure, surface alterations became sufficient to induce perceptible color variation, highlighting the synergistic effect of surface degradation and optical changes. Another limitation of the study is related to the number of samples in each group. Future studies should include a larger number of samples.

In any case, according to the above ISO standard, ΔE_s values obtained in this study were in the range so they can be considered all clinically acceptable. The results of the present *in vitro* study are in agreement with those of Schmutzle et al. [9], Al-Thobity et al. [14], Sousa Porta et al. [16] and Arruda et al. [17], which found no significant differences after cleansing protocols even if different set ups, agents and durations have been tested.

5. Conclusions

Based on the findings of this *in vitro* study, the following conclusions can be drawn:

1. The surface roughness of the PMMA resins tested was not significantly affected by the chemical and mechanical+chemical cleansing protocols evaluated in this study.
2. The color stability of all PMMA resins tested was not significantly affected by chemical and mechanical+chemical cleansing protocols.

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CRediT authorship contribution statement

Martina Bonvicini: Writing – original draft, Methodology, Investigation, Formal analysis. **Davide Silvestri:** Writing – review & editing, Formal analysis. **Antonio Arena:** Writing – review & editing, Formal analysis. **Francesca Zicari:** Writing – review & editing, Formal analysis. **Adolfo Di Fiore:** Supervision. **Luca Bortolotti:** Investigation, Formal analysis. **Luca Lusvardi:** Supervision, Resources. **Carlo Monaco:** Validation, Supervision, Resources, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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