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Original Article

EFFECT OF CASTOR OIL AS POLYETHERETHERKETONE DENTURE BASE CLEANSER AGAINST *CANDIDA ALBICANS* COUNT AND SURFACE ROUGHNESS

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ABSTRACT

Objective: To evaluate the effectiveness of a 10% castor oil solution as a denture cleanser by comparing its ability to reduce *Candida albicans* counts and its effect on the surface roughness of thermoplastic nylon and polyetheretherketone (PEEK) denture base materials, relative to an alkaline peroxide cleanser.

Methods: Thirty polished nylon and PEEK specimens (20 mm diameter, 2 mm thickness) were sterilized using ultraviolet (UV) light for 5 minutes and inoculated with *Candida albicans* (ATCC 14053). Specimens were then immersed for 20 minutes in one of the following solutions (n = 5 per group): A – Nylon + distilled water, B – Nylon + alkaline peroxide, C – Nylon + 10% castor oil, D – PEEK + distilled water, E – PEEK + alkaline peroxide, F – PEEK + 10% castor oil, Adherent cells were suspended and cultured on Sabouraud dextrose agar at 37 °C for 48 hours, after which colonies were counted. Surface roughness (µm) was measured using a profilometer before and after 122 hours of immersion in alkaline peroxide, 10% castor oil, or distilled water.

Results: Alkaline peroxide produced the lowest *Candida albicans* counts; however, 10% castor oil resulted in a significantly greater reduction than distilled water. One-way ANOVA showed a highly significant effect of cleansers on *Candida albicans* counts for both nylon and PEEK specimens ($p < 0.0001$). For PEEK immersed in 10% castor oil, paired t-test analysis revealed no significant change in surface roughness ($p = 0.061$).

Conclusion: The 10% castor oil solution effectively reduced *Candida albicans* counts and produced minimal changes in the surface roughness of PEEK denture base material. Castor oil shows promise as a biocompatible denture cleanser, with particularly favorable outcomes for PEEK-based dentures.

Keywords: Castor oil, Polyetheretherketone, *Candida albicans* count, Surface roughness

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INTRODUCTION

In the 1950s, thermoplastic nylon was initially used as denture base material. These thermoplastic materials have the advantages of no residual monomers, non-toxicity, non allergenic materials and good biocompatibility [1]. On the other hand, it is reported that these materials have some problems, such as high water absorption, high surface roughness, bacterial contamination, discoloration and more difficult to be polished due to their low melting point [2]. Therefore, to overcome these problems, Polyetheretherketone (PEEK) is introduced with its more favorable properties. PEEK is an organic thermoplastic polymer material that belongs to polyaryletherketone's group [3]. This material can be combined with other materials such as ceramic, carbon, or fiber material to increase the material's properties as an alternative to metal [4]. BioHPP® is PEEK material with the addition of ceramic particles that aimed to improved the polish-ability and increased the mechanical strength. BioHPP® has ceramic microparticles with a size of about 0.3-0.5 microns and covers 20% of the total volume [3, 4].

Denture cleansers are widely used to control denture plaque formation and prevent colonization by *Candida albicans* and other species. Continuous and prolonged use of denture cleanser can affect some properties of denture base materials such as reduce in gloss, higher surface roughness and color change, which have an important role in denture aesthetics and durability [5]. The ideal denture cleanser should easy to use, effective to remove organic and inorganic substances from the surface of denture, have bactericidal properties, fungicidal properties and not cause changes in the properties of the denture base [6]. A commonly used denture cleanser agent is alkaline peroxide in tablet form. This cleaner is easy to purchase and easy to use (Polident®). Alkaline peroxide is effective in dissolving plaque due to its bactericidal and fungicidal properties [6]. However, the use of alkaline peroxide cleaning agents has been reported to cause changes in denture base surface morphology due to the release of active oxygen.[7] There is no report about the recommended denture cleanser for PEEK denture base.

Many plants have been known and studied to have antifungal, antivirus and antimicrobial effects from alkaloids, flavonoids, steroids and saponins such as *Riccinus communis, Agave attenuate, Psidium guajava, Murraya koenigii, Musa acumianta,* etc. [8-10]. Natural products have many advantages such as its cost, non-toxicity, feasibility, acceptability, and almost no side-effect. These are caused by the phytochemicals presents in herbs with wide range effect like anti-inflammatory, antiviral, antibacterial, antifungal, antioxidant, wound healing, etc. [11]. *Riccinus communis* has been used in dentistry such as endodontics and periodontology because of its biocompatibility, antimicrobial and anti-inflammatory activity. *Riccinus communis* 's seeds can be made into oil form and called castor oil [12-14]. This oil has potential to be used in denture hygiene as it acts like a powerful detergent [12]. Castor oil contains ricinoleic acid, which has been used in dentistry because its anti-inflammatory and antimicrobial properties that can break glucose molecules from the cell walls of pathogenic microbes and fungi as well as a taste and odor that patients can tolerate [13-15]. The denture should be immersed in the denture cleanser daily for 20 min as the effective immersion time [14].

Surface roughness of denture base is of particular interest, where existing studies have shown a relationship between surface roughness, plaque accumulation and *Candida albicans* attachment [16]. Schubert's study also showed an increase in the number of *Candida albicans* attachments as surface roughness increased and the Ra parameter was reported as an appropriate surface roughness parameter to assess microbial attachment to polished materials. Selective selection of cleaning agents is essential to prevent plaque accumulation, *Candida albicans* attachment and minimize changes in surface roughness.

Microbial and fungal plaque attachment is influenced by the surface structure, surface roughness and porosity of a denture base material. Surface roughness greatly influences the formation of biofilm as well as making biofilm removal difficult. Microbial and fungal attachment can increase along with the increased of the surface roughness [16]. Fernandes' research stated that *Candida* biofilm growth was the highest on thermoplastic nylon samples and the use of cleaning solutions can reduce the number of *Candida*. Durkan's research results stated that there was a significant increase in surface roughness in the thermoplastic nylon group soaked in alkaline peroxide solution. Badaro's research results showed the highest increase in surface roughness in 0.5% sodium hypochlorite solution, followed by 0.25% sodium hypochlorite and the lowest castor oil 10% [13-17]. Until now, research on the use of castor oil as a cleanser is still lacking specifically for PEEK.

Objective

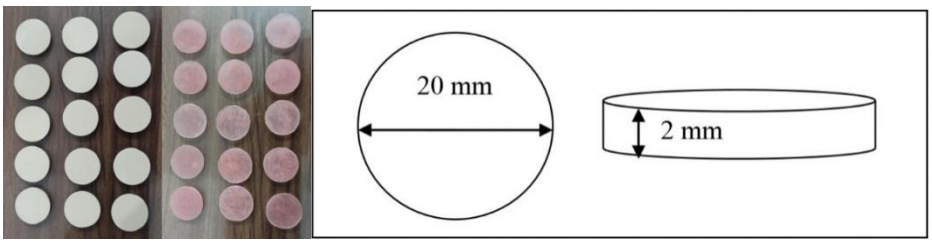
To evaluate castor oil solution as denture cleanser, comparing it to alkaline peroxide based on *Candida albicans* count and surface roughness of thermoplastic nylon and *polyetheretherketone* denture bases.

MATERIALS AND METHODS

This study used 30 samples divided into 6 groups (n=5), namely 15 thermoplastic nylon (Yamahachi, Japan) and 15 polyetheretherketone (BioHPP Bredent, Germany) samples each. The *wax up* samples were made using a stainless steel master mold with a diameter of 20 mm and a plate thickness of 2 mm. The sample was made by injection technique and then polished to a high gloss. Castor oil 10% solution was made by mixing 10 ml of pure castor oil into 10 ml of tween 80 in a beaker glass and stirring with a glass stirrer with a one-way motion until an emulsion corpus was formed. Then add distilled water to the emulsion up to 100 ml and stir until homogeneous. Alkaline peroxide solution was prepared by dissolving 1 polident tablet into 200 ml distilled water in a glass beaker. The number of *Candida albicans* test was done first then continued with surface roughness test using the same sample within the same group.

The number of *Candida albicans* was carried out by initially contaminating *Candida albicans* colonies that had been cultured from American Type Culture Collection (ATCC) 14053 with 2.0 Mc Farland standards using VITEK machine. Samples was sterilized by using an ultraviolet (UV) chamber for 5 min according to previous studies because it was claimed effective method. Then grouping thermoplastic nylon samples after immersion with distilled water (group A), thermoplastic nylon after immersion with alkaline peroxide (group B), thermoplastic nylon after immersion with castor oil 10% (group C), PEEK after immersion with distilled water (group D), PEEK after immersion with alkaline peroxide (group E) and PEEK after immersion with castor oil 10% (group F). Each group consist of 5 samples immersed for 20 min with each sample transferred into a test tube containing 10 ml of Sabouraud Dextrose Broth (SDB) solution. Afterward, 0.1 ml of Candida albicans suspension derived from samples was added in SDB solution and then vibrated with a vortex. The solution was then inoculated on Sabouraud Dextrose Agar (SDA), which were then incubated for the final 48 h at 37 °C. The number of *Candida albicans* was counted using a colony counter (Interscience*)* in colony-forming units per milliliter (CFU/ml).

Surface roughness measurements of each sample were carried out before and after immersion of thermoplastic nylon with distilled water (group A), thermoplastic nylon with alkaline peroxide (group B), thermoplastic nylon with castor oil 10% (group C), PEEK with distilled water (group D), PEEK with alkaline peroxide (group E) and PEEK with castor oil 10% (group F) which were immersed for 5 days 1 h 40 min (assuming immersion of 20 min per day for 365 days which was 20 min times 365 d = 7.300 min = 121 h and 40 min = 5 d 1 h 40 min) in an incubator. Castor oil and alkaline peroxide was refreshed every 24 h. Surface roughness was measured using a profilometer (Mitutuoyo SJ 210, Japan) on one flat surface of the sample for 3 times and mean surface roughness was calculated. The profilometer settings was done according to International Organization for Standardization (ISO) 1997 (cutoff wavelength 0.8 mm, traverse length 10 mm and speed 0.5 mm/s). Microbial testing has been approved by Health Research Ethic Comitee of Universitas Sumatera Utara (No. 178/KEPK/USU/2023).



(A) **(B)** **(C)**

Fig. 1: (A) Representative samples of PEEK, (B) Representative samples of Nylon, (C) Diameter, thickness and shape of the sample

RESULTS

The results of the univariate test showed that the PEEK group in alkaline peroxide showed the smallest number of *Candida albicans* at 4.2±2.28 CFU/ml and the largest in the thermoplastic nylon group in distilled water at 407±8.155 CFU/ml (table 1). The univariate test showed that the PEEK group in 10% castor oil had the least difference in surface roughness increase of 0.007±0.006 µm and the thermoplastic nylon group in alkaline peroxide had the most surface roughness increase of 0.076±0.018 µm (table 2). The results of statistical analysis through a one-way Analysis of Variance (ANOVA) on the number of *Candida albicans* from the statistical test results in group A, group B and group C obtained a significance level of $p<0.0001$, which indicated that there was an effect of soaking between group A, group B and group C (table 3). The results of statistical analysis through a one-way ANOVA on the number of *Candida albicans* from the statistical test results in group D, group E and group F obtained a significance level of $p<0.0001$, which indicated that there was an effect of soaking between group D, group E and group F (table 4).

The effect of immersing thermoplastic nylon denture base material in *10%* alkaline peroxide and castor oil solution on surface roughness was obtained through a one-way ANOVA statistical test using the difference in surface roughness before and after. The statistical test results obtained a p value<0.0001, which indicated the effect of immersion among the three groups (table 5). A post hoc test with Least Significant Difference (LSD) was conducted and it showed no difference in surface roughness between distilled water and alkaline peroxide. The effect of immersion of PEEK denture base material in 10% alkaline peroxide and castor oil solution on surface roughness was obtained through a one-way ANOVA statistical test using the difference in surface roughness before and after. The statistical test results obtained a p value<0.0001, which indicated the effect of immersion among the three groups (table 6). A post hoc test with LSD was conducted and it showed differences between all groups. PEEK after immersion with castor oil 10% (group F) showed the least change in surface roughness with $p = 0.061$ (table 7).

Table 1: Mean number of *Candida albicans* in thermoplastic nylon and *Polyetheretherketone* in a solution of distilled water, alkaline peroxide and *castor oil* 10%

Sample	Candida albicans count (CFU*/ml)					
	Group A	Group B	Group C	Group D	Group E	Group F
1	421	9	201	394	8	42
2	406	11	167	262	3	80
3	400	16	204	329	2	92
4	403	18	211	370	4	72
5	405	10	197	346	4	98
$\bar{X}$ ±SD**	407±8.155	12.8±3.962	196±17	340.2±50.14	4.2±2.28	76.8±21.936

A-nylon and distilled water B-Nylon and alkaline peroxide,, C-Nylon and castor oil 10%, D-Polyetheretherketone and distilled water, E-Polyetheretherketone and alkaline peroxide, F-Polyetheretherketone and castor oil 10%, *CFU = Colony Forming Units, **SD = Standard Deviation

Table 2: Mean values of surface roughness difference of thermoplastic nylon and *polyetheretherketone* in distilled water, alkaline peroxide and *castor oil* 10% solution

Sample	Surface roughness (µm)					
	Group A	Group B	Group C	Group D	Group E	Group F
1	0.067	0.108	0.027	0.030	0.050	0.011
2	0.059	0.072	0.021	0.025	0.046	0.000
3	0.055	0.063	0.028	0.038	0.053	0.012
4	0.062	0.076	0.028	0.026	0.039	0.014
5	0.071	0.062	0.025	0.035	0.027	0.001
$\bar{X}$ ±SD*	0.062±0.006	0.076±0.018	0.025±0.002	0.030±0.005	0.043±0.010	0.007±0.006

A-nylon and distilled water B-Nylon and alkaline peroxide,, C-Nylon and castor oil 10%, D-Polyetheretherketone and distilled water, E-Polyetheretherketone and alkaline peroxide, F-Polyetheretherketone and castor oil 10%, * SD = Standard Deviation

Table 3: Effect of immersion of thermoplastic nylon denture bases in distilled water, alkaline peroxide and *castor oil* 10% solution on the number of *Candida albicans*

	Candida albicans count (CFU*/ml)			One-way ANOVA**
Sample	Group D	Group E	Group F	p
1	421	9	201	
2	406	11	167	
3	400	16	204	0.0001
4	403	18	211	
5	405	10	197	
$\bar{X}$ ±SD***	407.00±8.15	12.80±3.96	196.00±17.00	

A-nylon and distilled water B-Nylon and alkaline peroxide,, C-Nylon and castor oil 10%, *CFU = Colony Forming Units, ** ANOVA = Analysis of Variance, *** SD = Standard Deviation

Table 4: Effect of immersion of PEEK denture material in distilled water, alkaline peroxide and *castor oil* 10% solution on the number of *Candida albicans*

	Candida albicans count (CFU*/ml)			One-way ANOVA**
Sample	Group D	Group E	Group F	p
1	394	8	42	
2	262	3	80	
3	329	2	92	0.0001
4	370	4	72	
5	346	4	98	
$\bar{X}$ ±SD***	340.20±50.14	4.20±2.28	76.80±21.93	

D-Polyetheretherketone and distilled water, E-Polyetheretherketone and alkaline peroxide, F-Polyetheretherketone and castor oil 10%, *CFU = Colony Forming Units, **ANOVA = Analysis of Variance, ***SD = Standard Deviation

Table 5: Effect of immersion of thermoplastic nylon denture base in distilled water, alkaline peroxide and *castor oil* 10% solution on surface roughness

Group	Sample quantity	Surface roughness difference (µm)	One-way ANOVA*
	n	$\bar{X}$±SD	p
A	5	0.062±0.006	
B	5	0.076±0.018	0.0001
C	5	0.025±0.002	

A-nylon and distilled water B-Nylon and alkaline peroxide,, C-Nylon and castor oil 10%, *ANOVA = Analysis of Variance,

Post hoc test with LSD

Group	n	p
A-B	5	0.091
A-C	5	0.0001
B-C	5	0.0001

Table 6: Effect of immersion of PEEK denture base in distilled water, alkaline peroxide and *castor oil* 10% solution on surface roughness

Group	Sample quantity	Surface roughness difference (µm)	One-way ANOVA*
	n	X̄±SD	p
D	5	0.030±0.005	
E	5	0.043±0.010	0.0001
F	5	0.007±0.006	

D – Polyetheretherketone and distilled water, E – Polyetheretherketone and alkaline peroxide, F – Polyetheretherketone and castor oil 10%, * ANOVA = Analysis of Variance

Post hoc test with LSD

Group	n	p
D-E	5	0.029
D-F	5	0.001
E-F	5	0.0001

Table 7: Effect of immersion of PEEK denture base in castor oil before and after immersion in terms of surface roughness

Sample	Surface roughness (µm)		Paired T-test
	Before immersion	After immersion	p
1	0.076	0.087	
2	0.084	0.084	
3	0.082	0.094	0.061
4	0.083	0.097	
5	0.070	0.071	

DISCUSSION

Thermoplastic nylon is a denture base material that has the advantages of elastic properties and gum-like color but the disadvantages of water absorption, surface roughness after use and more complex laboratory procedures. This surface roughness will play a role in the retention of food and microorganisms [18]. The difference in the number of *Candida albicans* in alkaline peroxide and *castor* oil 10% is due to differences in solution pH and differences in the mechanism of action of the cleaning solution. Alkaline peroxide is alkaline and castor oil is acidic. The pressure caused by high pH can cause cell wall damage so that fungi are easier to develop at acidic pH compared to alkaline pH [19]. The use of distilled water as a control showed the highest number of *Candida albicans* among the other groups and this was expected [13].

PEEK's structure has good chemical resistance because to the presence of aryl rings formed by ketones and ethers at opposing ends of the ring. Its chemically stable structure causes increased orbital electron delocalization along the macromolecule, providing PEEK highly unreactive and resistant to chemical, thermal, and post-irradiation degradation [3, 4] Vylkova's research stated that *Candida albicans* poorly grew at very alkaline condition in the presence of a non-fermentable carbon source [20]. This is why the result of number of *Candida albicans* in this research is lower in alkaline peroxide group than castor oil 10% because of the alkaline solution which *Candida albicans* was immersed. Alkaline peroxide when mixed with water and dissolved in water causes the sodium perborate content to decompose releasing oxygen. This oxygen will oxidize the organic deposits contained in the plaque which results in a cleaning action on the denture base. This can effectively remove organic deposits and kill microorganisms [17, 21]. In this research, it was found that alkaline peroxide was more superior in terms of eliminating *Candida albicans* than castor oil 10% and distilled water as control.

Castor oil can be seen as a potential denture cleanser agent because it is able to work as a detergent agent and antimicrobial properties also non-toxic effect on oral tissues [14, 15]. The ricinoleic acid will break the cell wall with glucose molecule inside and cause an oxidation reaction, which will cause lipophilic properties and cause loss of cytoplasm and lysis of the cell wall [22]. *C. albicans* actively neutralizes the environment from either acidic or alkaline pHs. Under acidic conditions, this species can raise the pH from 4 to>7 in less than 12 h. The change in pH is caused by the release of ammonia from the cells produced during the breakdown of amino acids [20].

In this research, we found that castor oil 10% could eliminate the *Candida albicans* significantly both in PEEK and nylon group compare to the control group. Ritonga PWU and Bidaya EE's research also showed a decrease in the number of *Candida albicans* disinfected with 10% castor oil solution [23]. Salles' research stated that soaking dentures in a 10% *castor oil* solution for 20 min could eliminate microorganisms such as *Escherichia coli, Candida albicans,* and *Candida glabrata* [24].

Polyetheretherketone denture bases have good chemical properties, good physical stability and have higher resistance to acid-base solutions than thermoplastic nylon denture bases [2-4]. In this research, we found that the polishability of PEEK material was better than polyamide. The hygroscopic nature of polyamide can lead to increased surface roughness, so the longer immersion will cause degradation of the resin structure. The surface roughness of polyamide thermoplastic resin is not only affected by the active content, but also the immersion time of polyamide. The longer the polyamide is immersed in the cleaning solution which can cause an increase in absorption of water or liquid, the higher the surface roughness of the polyamide [25]. Another factor is also influenced by the active content contained in alkaline peroxide which can affect the properties of polyamide. If alkaline peroxide dissolves in water, it will produce H_2 O_2. Hydrogen peroxide will decompose into $2H_2$ O+2 (O) (oxygen). Free radicals from hydrogen peroxide can cause disruption of polyamide bonds due to the entry of oxygen (O-), which does not have an electron pair into the polymer chain and oxidation occurs which results in physical changes in polyamide such as roughness [25].

Castor oil has good characteristics as a denture cleaning solution. It has strong detergent action and antimicrobial ability with the ability to break down glucose molecules from the cell walls of pathogenic fungi, and has no strong odor [14, 15, 24]. The main composition of *Ricinus communis* seeds are fatty acids and neutral lipids. Ricinoleic acid is a monounsaturated fatty acid that has detergent action as an anti-inflammatory and antimicrobial [10]. It was expected from this study that the use of castor oil 10% could minimize the occurrence of changes in surface roughness as PEEK material immersed in castor oil showed the least changes. Badaro's research also stated that a 10% castor oil/*R. communis* solution showed minimal changes in surface roughness [14, 15, 24]. Polyamide denture base also showed minimal changes in surface roughness during the test, that might be because of the advantage of oil-based denture cleanser. This is in accordance with Rowe's statement, which stated that oil-based cleaning agents will reduce the occurrence of changes in

material properties as one of the advantages of castor oil [26]. Until the time this research was done, there was still no report about castor oil 10% as denture cleanser for PEEK and polyamide denture base. The limitation of this study are the absence of real-time aging and the use of distilled water instead of artificial saliva, which may limit the generalizability.

CONCLUSION

Based on the results of this study, it can be concluded that the PEEK denture base immersed in 10% castor oil solution showed the least change in surface roughness (paired t-test of PEEK in 10% castor oil before and after immersion showed a value of p = 0.061) and was able to eliminate the number of *Candida albicans* effectively (one-way ANOVA both PEEK and nylon in 10% castor oil for *Candida albicans* count also showed a value of p<0.0001). Based on this surface roughness value, the PEEK base showed excellent polish ability, fulfilling one of the requirements of an ideal denture base. 10% Castor oil demonstrated potential efficacy as denture cleanser but requires further validation through future studies.

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Nil

AUTHORS CONTRIBUTIONS

Haslinda Z Tamin: Research concept, Supervision, Design, Critical Review, William Wijaya: Data Collection, Analysis, Interpretation, Literature Review, Writing the article, Materials, Ricca Chairunnisa: Data Collection, Design, Analysis, Literature review, Critical Review, Urip Harahap: Research concept, Supervision, Design, Critical Review

CONFLICT OF INTERESTS

Declared none

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