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PROGRAMA DE MESTRADO PROFISSIONAL EM ODONTOLOGIA

GUSTAVO OSSAMU BLATT OHIRA

AVALIAÇÃO *IN VITRO* DO DESGASTE DE DENTES ARTIFICIAIS EM MÁQUINA DE ESCOVAÇÃO SIMULADA

AN *IN VITRO* EVALUATION OF ARTIFICIAL TEETH WEAR IN SIMULATED BRUSHING MACHINE

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Orientador: Profª. Drª. Núbia Inocencya Pavesi Pini.

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Maringá, ___ de _____ de 2022.

BANCA EXAMINADORA

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Prof. Dr. _____

UNINGÁ

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Prof. Dr. _____

Instituição

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Prof. Dr. _____

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DEDICATÓRIA

Dedico este trabalho a minha esposa **Fernanda**, profissional brilhante em sua área que, por vezes, abdica de oportunidades de crescimento profissional em prol da nossa família. Amor, você é meu alicerce e ter você ao meu lado desde o início da minha formação foi fundamental em todas as tomadas de decisões - que foram muitas. Obrigado por sempre me trazer de volta nas horas de euforia e me colocar para cima nos momentos difíceis.

Dedico também está conquista aos meus pais, **Masanao** e **Maria Lourdes**. Eles são meus exemplos, sempre priorizaram os recursos da família para o pagamento de bons colégios e cursos complementares. No fundo queremos ser iguais aos nossos pais, e o mestrado também foi por eles.

Dedico esta etapa ao meu irmão **Eduardo**, colega de profissão e exímio ortodontista. A profissão nos aproximou e da mesma forma nos distanciou. Queria poder estar mais próximo dele e compartilhar de outros assuntos que não os odontológicos. Ter ele ao meu lado sem surtar de vez comigo mostra o quanto nossa ligação é forte.

Por último dedico essa vitória a minha princesa **Marcela**. Ela é um anjo, com um coração enorme. São muitas horas na escola ou com a tia Cássia. Ela não tem culpa da vida que escolhemos. Espero que um dia ela entenda que não foi por mal.

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Agradeço aos professores do programa de prótese dentária, **Fernanda, Daniel, Aline e Núbia** que não mediram esforços para nos entregar um curso de qualidade.

Aos meus velhos e novos amigos **Alberto, Diogo, Everton, Fabiano Brites e Fabiano Gava**, companheiros de profissão e mestrado. Os dias foram mais leves com vocês.

Aos meus pacientes, a minha companheira de trabalho **Beatriz** e aos meus colegas de profissão que souberam entender e adequar a rotina nas semanas que eu me ausentei para o mestrado.

“A gente muda o mundo com a mudança da mente e quando a mente muda a gente anda pra frente”.

Gabriel o Pensador

RESUMO

INTRODUÇÃO: O processo de escovação em dentes artificiais provoca desgastes que podem resultar em alterações estéticas. O objetivo desse estudo, *in vitro*, foi avaliar o desgaste abrasivo provocado por uma escovação simulada em cinco dentes artificiais: Premium (Kulzer®); Delara (Kulzer®); Biotone (Dentsply®); Artiplus IPN (Dentsply®) e Trilux (Vipi®) em três veículos diferentes: Colgate total 12®; Elmex® e Grupo Controle (sem dentifrício). **MATERIAL E MÉTODOS:** Foram confeccionados cento e oitenta corpos de prova, apenas incisivos centrais e laterais foram incluídos no estudo. Para o teste de escovação simulada, foi utilizado o equipamento ODEME MEV 3T - 10XY® (Jaraguá do Sul/Santa Catarina/Brasil) e dez mil ciclos foram aplicados em cada amostra o que correspondem a um ano de escovação. A análise de massa foi feita em Ti (teste inicial – antes da escovação) e Tf (teste final – após a escovação) através de três registros de pesagem com o auxílio da balança analítica de precisão MARTE AD500® (Santa Rita do Sapucaí / Minas Gerais / Brasil) e a média entre os valores foi utilizada para análise estatística. A análise de cor foi realizada em três momentos: antes da escovação, após a escovação e após o manchamento com Nescafé (Nestlé®) por meio do espectrofotômetro Easyshade® (Bad Säckingen, Alemanha). Foi confeccionada uma matriz em silicone para que o local de medida de cor fosse padronizado para cada espécime. Cada medida de cor foi realizada por três vezes e o resultado utilizado foi a média destes três valores para luminosidade (L*), coordenada vermelho-verde (a*) e coordenada amarelo-azul (b*). A análise da área de desgaste foi realizada por meio do escâner de bancada CEREC Omnicam AF Dentisply Sirona® (Bensheim / Alemanha) em duas mensurações, antes e após o protocolo de escovação. Os arquivos em formato *STL* foram sobrepostos por meio do software Ortho Analyzer 3Shape® (Copenhage / Dinamarca) e a diferença da área desgastada foi computada. **RESULTADOS:** Artiplus apresentou maior desgaste superficial quando escovado com dentifrício (p=0,0019). Para a coordenada L* apenas o dente Premium não apresentou diminuição significativa da luminosidade. Para a coordenada a* os dentes Premium e Biotone não apresentaram diferenças estatísticas em relação à análise inicial independente do protocolo de higiene.

Ainnda em a* os dentes Trilux e Biotone tiveram redução significativa dos valores em relação a análise inicial. Para Delara e Premium, após a coloração não foram encontradas diferenças. Para a coordenada b*pode-se observar que após a escovação e após a coloração houve redução desse parâmetro com significância em relação à análise inicial. Em relação a análise do peso, nem a escovação e nem o protocolo de manchamento resultaram em diferença significativas em relação a medida inicial. **CONCLUSÃO:** No presente estudo os dentes Premium e Biotone apresentaram uma boa estabilidade após escovação simulada e manchamento com Nescafé. Artiplus apresentou um desgaste significativo quando escovado com dentifrício.

Palavras-chave: Dentifrícios. Desgaste dos dentes. Escovação dentária.

LISTA DE FIGURAS

LISTA DE TABELAS

LISTA DE ABREVIATURAS E SIGLAS

Ar – Dente Artiplus

Bi – Dente Biotone

De – Dente Delara

Pr – Dente Premium

Tr – Dente Trilux

Cr – Grupo controle água destilada

Col – Grupo dentifrício colgate

El – Grupo dentifrício Elmex

INPEN® - Rede de polímeros interpenetrados

INCOMP - Técnica de injeção e compressão de camadas

PVC – Policloreto de vinila

RDA – Abrasividade relativa da dentina

ADA – Associação odontológica americana

ISO – Organização internacional para padronização

Ti – Tempo Inicial

Tf – Tempo Final

STL – Formato de arquivo *"Standard Triangle Language"*

SUMÁRIO

1. INTRODUÇÃO

Os implantes osseointegrados representam um marco na reabilitação total de arcos edêntulos, por meio deles é possível entregar uma reabilitação fixa para substituir as estruturas perdidas pelo paciente (dente, periodonto de proteção, periodonto de sustentação) de forma funcional (ADELL et al., 1981; BRANEMARK; SVENSSON; VAN STEENBERGHE, 1995). As próteses fixas sobre quatro ou mais implantes, construídas em uma estrutura metálica, se bem indicadas e confeccionadas, apresentam índices elevados de sucesso ao longo dos anos (ADELL et al., 1981; BRANEMARK; SVENSSON; VAN STEENBERGHE, 1995). A resina acrílica e os dentes de estoque são os materiais mais utilizados na confecção de próteses fixas de arco total, eles apresentam boas características estéticas a um custo acessível (ESQUIVEL et al., 2020). Outro material de cobertura para as próteses totais fixas, são as cerâmicas odontológicas, elas oferecem maior lisura e resistência ao desgaste em relação aos acrílicos (BAJRAKTAROVA-VALJAKOVA et al., 2018). Em contrapartida, são materiais de difícil reparo e com custo elevado de produção (MALO et al., 2012).

Todos os materiais utilizados para próteses estão sujeitos ao desgaste de sua estrutura. Esse desgaste pode ser decorrente do atrito com o antagonista durante a mastigação, fala e deglutição, os quais são aspectos fisiológicos. (UEHARA et l., 2019); assim como, pode ser decorrente de abrasão, quando outros objetos causam esse desgaste (UEHARA et l., 2019), como por exemplo a abrasão da escova durante a escovação. A composição dos materiais de cada tipo de prótese fará com que esse desgaste seja maior ou menor (PARANHOS et al., 2013). A menor resistência ao desgaste dos materiais acrílicos influencia diretamente na estabilidade do tratamento protético (STOBER et al., 2020). Os padrões oclusais afetados pelo desgaste dos dentes artificiais interferem de forma direta na estética e função do tratamento reabilitador (KAMONWANON et al., 2015). Desgastes oclusais mais pronunciados são notados quando o antagonista é dente natural ou cerâmica (ESQUIVEL et al., 2020). Alterações na composição dos dentes artificiais modificam propriedades mecânicas, aumentando a resistência ao desgaste (PARANHOS et al., 2013). Um exemplo para melhorar a dureza e resistência à fissura são os dentes artificiais de resina acrílica reticulados com uma rede de polímero interpenetrante, dupla reticulação e misturas

de polímeros (UEHARA et l., 2019). A incorporação de uma carga inorgânica na matriz de polímero é mais um método para aumentar a resistência ao desgaste (KAMONWANON et al., 2015).

O desgaste dos dentes artificiais pelo processo de higienização das próteses acarreta perda de estrutura o que modifica as propriedades ópticas dos dentes artificiais, evidenciando as camadas mais internas (UEHARA et l., 2019). A mudança de coloração afeta diretamente na aceitação do tratamento pelo paciente (MONTAYAGHENI et al., 2020; NEPPELENBROEK et al., 2016). Técnicas corretas de higienização favorecem a estabilidade de cor ao diminuir a velocidade de perda das estruturas (PARANHOS et al., 2013).

O sabão neutro é descrito como um adjuvante à escovação, visto que é efetivo contra boa parte dos microrganismos (PARANHOS et al., 2013; SALLES et al., 2007). Também apresenta pouca abrasividade o que contribui para uma perda mais lenta das estruturas (PARANHOS et al., 2013; SALLES et al., 2007). Porém, em estudos comparativos, o sabão líquido neutro mostrou-se menos efetivo para remoção do biofilme quando comparado a dentifrícios (PARANHOS et al., 2013; SALLES et al., 2007).

Em próteses fixas, os dentifrícios apresentam sabor agradável e, por esse motivo, têm melhor aceitação que o sabão líquido (PARANHOS et al., 2013). Os dentifrícios específicos para próteses totais ou mesmo os convencionais têm mudado a sua configuração para promover a limpeza dos dentes/prótese com uma abrasividade cada vez menor (POLICASTRO et al., 2016).

Dentifrícios muito abrasivos podem promover o desgaste dos dentes artificiais e impactar negativamente na estética e na função da prótese (MOON; POWERS; KIAT-AMNUAY, 2014). A perda de estrutura aumenta a porosidade da prótese e a susceptibilidade ao manchamento, o papel do dentifrício para a escovação das próteses com dentes artificiais e base em resina acrílica deve contemplar a correta limpeza da prótese com o mínimo de abrasividade (MOON; POWERS; KIAT-AMNUAY, 2014; SATOH et al., 1990).

Outro fator importante que pode contribuir para alterações cromáticas nos dentes artificiais é a exposição a substâncias corantes. O café, em especial, pode

interagir negativamente com as peças protéticas, acelerando o processo de depreciação do material (HIPÓLITO et al., 2013)

Diante de tantas opções de higienização e a importância de se conhecer àquelas com menor deterioração dos dentes artificiais, o presente trabalho pretende avaliar o desgaste de cinco tipos de dentes artificiais expostos à escovação com agentes de limpeza de diferentes composições e avaliar a diferença de cor antes e após manchamento com café.

2. OBJETIVOS

2.1. Objetivo Geral

O objetivo do presente estudo é comparar o desgaste e a estabilidade da cor de cinco tipos de dentes artificiais submetidos à escovação com diferentes agentes de limpeza.

2.2. Objetivos Específicos

- Mensurar a diferença de peso dos espécimes frente a escovação com diferentes dentifrícios e água destilada;
- Avaliar a área de desgaste por meio do escaneamento das amostras;
- Avaliar a diferença de cor com espectofotômetro (Easyshade) após a escovação simulada;
- Avaliar a diferença de cor com espectofotômetro (Easyshade) após manchamento com Nescafé®
- Comparar os grupos estudados.

3. ARTIGO

O artigo apresentado foi escrito de acordo com as normas da revista ***Journal of Esthetic and Restorative Dentistry*** (anexo 1).

RESEARCH ARTICLE

An *in vitro* evaluation of artificial teeth wear in simulated brushing machine.

Gustavo O Blatt Ohira Msc[1], Núbia Inocencya Pavesi Pini PhD[1].

1. School of Dentistry, Restorative Dentistry, University of Uningá, Brazil.

Correspondence: Gustavo O B Ohira, Vila Nova St, 168 – Centre – Pomerode/SC – Brazil. Zip code:89107000.

Email: ohiraodontologia@gmail.com

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CONFLICT OF INTEREST

The authors declare no conflict of interest

Abstract

Objective: To compare the wear and color stability of five artificial teeth submited to simulated brush with different cleaning agents

Material and Methods: One hundred and eighty specimens were made, only central and lateral incisors were included in the study. For the simulated brushing test, the ODEME MEV 3T - 10XY® equipment (Jaraguá do Sul / Santa Catarina / Brazil) was used and ten thousand cycles – corresponding to one year of brushing – were applied to each sample. The mass analysis was performed in Ti (initial test - before brushing) and Tf (final test - after brushing) through three weighing records with the aid of a MARTE AD500® analytical precision balance (Santa Rita do Sapucaí / Minas Gerais Gerais / Brazil) and the mean between the values was used for statistical analysis. Color analysis was performed at three times: before brushing, after brushing and after staining with Nescafé coffee (Nestlé®), using the Easyshade® spectrophotometer (Bad Säckingen, Germany). A silicone matrix was performed standardized for each specimen. Each color measurement was performed three times and the result used was the average of these three values for luminosity (L*), red-green coordinate (a*) and yellow-blue coordinate (b*). The analysis of the wear area was performed using the CEREC Omnicam AF Dentisply Sirona® benchtop scanner (Bensheim / Germany) in two measurements, before and after the brushing protocol. The files in STL format were overlaid using the Ortho Analyzer 3Shape® software (Copenhage / Denmark) and the difference in the worn area was computed.

Results: Artiplus showed greater surface wear when brushed with toothpaste ($p=0.0019$). For the L* coordinate, only the Premium tooth did not show a significant decrease in luminosity. For the a* coordinate, Premium and Biotone teeth did not show statistical differences in relation to the initial analysis independent of the hygiene protocol. Still in a*, the Trilux and Biotone teeth had a significant reduction in values in relation to the initial analysis. For Delara and Premium, after staining, no differences were found. For the b* coordinate, it can be observed that after brushing and after staining there was a significant reduction in this parameter in relation to the initial analysis. Regarding the weight analysis, neither brushing nor the staining protocol resulted in significant differences in relation to the initial measurement.

Conclusion: Premium Tooth and Biotone will perform well against simulated brushing and staining. Artiplus showed greater surface wear when brushed with toothpast.

Clinical Significance: Coffee and brushing can change the appearance of artificial teeth.

Keywords: Dentifrices. Tooth Wear. Toothbrushing.

INTRODUCTION

The artificial teeth made with acrylic resin are the material most used in total implant supported prosthesis or total and partial removable. They are advantageous once they are easy to work and present acceptable aesthetics and accessible cost[1]. However, these materials are more susceptible to wear and staining[2,3]. Another option to these clinical situations is to use ceramics, which present higher surface smoothness and abrasive resistance, but are not repairable and higher cost[4,5].

The lack of resistance of the acrylic resin may lead to the wear of the artificial tooth due to several factors, including the process of hygiene. This is important once the wear modifies the optical properties of the material, changing tooth color[6-8] and favoring the staining[7], once the surface wear of the artificial tooth will expose internal layers of the tooth[6], which are not smoothness and brightness[8]. This is important since changing the color of the prosthesis directly affects the patient's acceptance of the treatment[7,8]. In this sense, some industries are worried in improving the physical properties of the artificial tooth, changing their composition or the method of organization of the polymer reticulation[6] and including inorganic particles into the polymeric matrix[9]. Besides, it is important to evaluate the correct method of hygiene, to avoid the loss of color stability and loss of structural integrity of the material[10].

Commonly, the neutral soap is instructed to be used by patients who use removable prosthesis as a good method of cleaning, since it presents antimicrobial properties and it is not abrasive[10,11]. However, in case of fixed prosthesis, its use is not possible and the patients are instructed to use toothpastes. Several toothpastes are available in the market with larger variability of compounds, flavors and abrasivity.

Even for the conventional toothpastes, there is an effort of the studies and industries in changing their composition in order to become less abrasive and more adequate to be used[2,3,12].

This study aimed to evaluate the susceptibility of different artificial teeth made with acrylic resin and used for dental prosthesis to the abrasiveness of different protocols of hygiene and to the susceptibility of staining. The null hypothesis is that there is no difference neither in relation to the artificial tooth used nor in relation to the methods of cleaning tested concerning wear, color change and staining susceptibility.

MATERIALS AND METHODS

Experimental design

The specimens of this *in vitro* study were central and lateral incisors from five types of acrylic resin artificial teeth: Pr - **Premium** *(Heraeus Kulzer GmbH/Hanau, Germany);* De - **Delara** *(Heraeus Kulzer GmbH/Hanau, Germany);* Bi - **Biotone** *(Dentsply/Petrópolis, Brasil);* Ar - **Artiplus IPN** *(Dentsply International/Pennsylvania, USA)* e Tr - **Trilux** *(Vipi/Pirassununga, Brasil).* For each type of tooth, thirty-six samples were prepared and randomly allocated into three groups, according to the hygiene protocol (n=12): DW - Distilled water; ET - Elmex toothpaste (Elmex Kariesschutz) and CT - Colgate toothpaste (Colgate Total 12 Clean Mint). All samples were mechanical brushed in a brushing machine. Afterwall, the samples were submitted to staining using a coffee solution. The samples were evaluated concerning weight loss, color changing and surface wear. The materials used in the study are listed in **Table 1 and 2.**

Table 1. Structural Features.

Teeth	**Model / Color**	**Identification number**	**Characteristics**
Artiplus IPN	A2 / L50	0000422018	Polymethyl methacrylate; butylene glycol dimethacrylate; high quantity of INPEN® material (network of interpenetrated polymers).
Biotone IPN	62 / 2N	0000422018	Polymethyl methacrylate; high performance density crosslinked resin.
Delara	A2 / O47	(10)201389518700	Polymethyl methacrylate; reticulated matrix of crosslink; INCOMP technology (layer injection and compression technique).
Premium	A2 / O6	(10)2013912443400	Polymethyl methacrylate; multiple polymer matrix; microfill combined with viscoelastic nanofill (nano® pearls); INCOMP technology (layer injection and compression technique).
Trilux	2A / F4 S	0000012020	Polymethyl methacrylate; double cross-linking; organically modified ceramics; polymerized ethylene glycol dimethacrylate.

Table 2. Composition of toothpastes and distilled water.

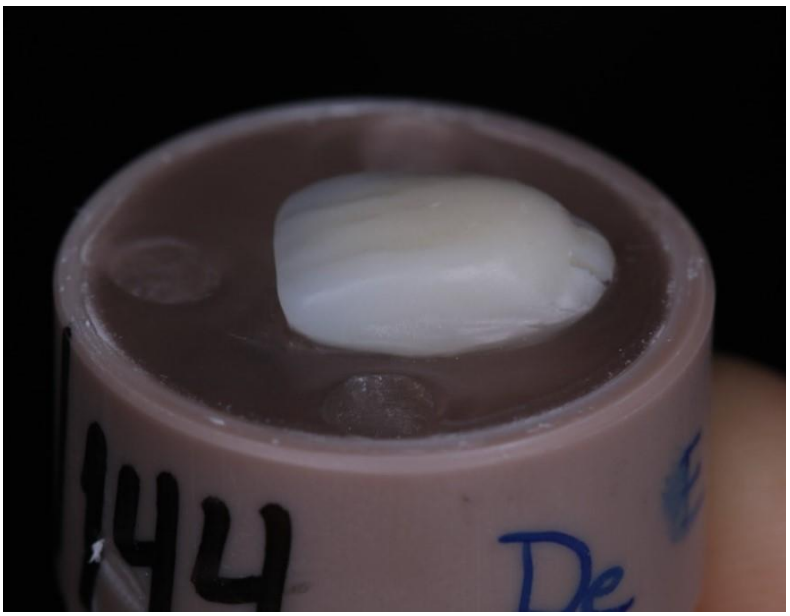
Material	**Composition**	**Manufacturer**
Colgate Total 12	Sodium Fluoride (1450 ppm Fluorine), Triclosan 0.3%, Water, Glycerin, Sorbitol, Hydrated Silica, Sodium Lauryl Sulfate, PVM/MA Copolymer, Flavor, Carrageenan, Sodium Saccharin, Sodium Hydroxide, White Dye CI 77891	Colgate
Elmex	Aqua, Hydrated Silica, Sorbitol, Hydroxyethylcellulose, Olaflur, Aroma, Saccharin, CI 77891, Limonene.	Elmex
Distilled water	Deionized water	Asfer

Specimens preparation

Each tooth was included into Polyvinyl chloride (PVC) tubs with 2.5 cm of diameter and 1.5 cm of length (Tigre / Brazil) with transparent and self-cured acrylic resin (Jet Clássico / São Paulo, Brazil). In each sample, two grooves were made in the cervical portion of the artificial tooth and other three in the acrylic resin to maintain the reference area of wear, allowing the superposition of initial and after brushing profiles. **(Figure 1 and 2).**

Figure 1. Front view specimen: tooth in elevated position to the acrylic; markings on the cervical of the tooth and on the base of the acrylic to favor the overlapping of the specimens in software before and after the simulated brushing process.

Figure 2. Front view specimen: tooth in elevated position to the acrylic; markings on the cervical of the tooth and on the base of the acrylic to favor the overlapping of the specimens in software before and after the simulated brushing process.



Hygiene Protocol

The specimens were submitted to the brushing machine using toothbrushes as recommended of the American Dental Association (ADA) and of ISO/TR 14569-1:2007, as follow: flat bristle head 27 mm x 10 mm and bristles of diameter 0,2 mm (Oral-B PRO-SAÚDE nº 40 P&G do Brasil, Manaus, AM)[13] . Brushing was performed by using an automatic brushing machine (MEV2; Odeme Dental Research) **Figure 3.** using 10000 strokes at a frequency of 2 Hz with a representative 2-N brushing load. The brushing was performed with distilled water (DW) or with two toothpastes – Elmex (ET) or Colgate (CT), in a slurry with distilled water a proportion of 1:3[14]. After the brushing protocol, the specimens were washed with tap water and a 24h period was waited to perform the analysis of the physical properties.

Figure 3. Equipment ODEME MEV 3T - 10XY® - allows the simultaneous simulation of brushing in ten specimens.

Staining Protocol

After brushing, the specimens were submitted to staining with coffee solution for 7 days.The coffee solution was prepared at a ratio of 3.6-g instant coffee (Nestlé Brasil Ltda) for each 300 mL of boiling distilled water and the specimens were immersed in this solution only after the beverage had cooled to room temperature **Figure 4.**

Figure 4. Samples immersed for seven consecutive days in ice cube trays with Nescafé solution.

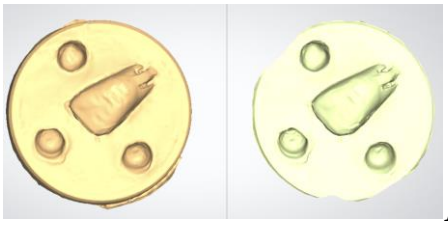
Analysis of physical properties

The specimens were evaluated concerning surface wear, color change and weight loss. Each analysis was performed before and after brushing and, in case of color change and weight loss, the analysis were also performed also after staining.

The analysis of wear was performed using a manual scanner (CEREC Omnicam AF Dentisply Sirona®, Bensheim, Alemanha) to obtain STL archives to

analyze in a specific software (Ortho Analyzer 3Shape®, Copenhage / Dinamarca). The images from the initial and after the brushing protocol were superposed to quantify the surface loss. In the pilot study, a value of 0.1 mm was determined as an ideal to evaluate wear throughout the chromatic map **Figure 5.**

Figure 5. A: Image of the same specimen before and after brushing; **B:** Demarcation of points for overlapping; **C:** Superimposed images showing a central wear island (shades of darker blue - denote wear close to 0.1 mm according to the lateral scale).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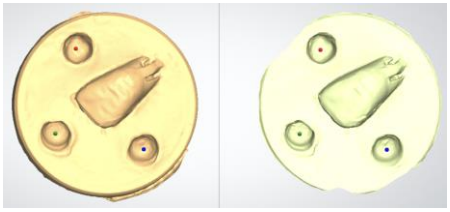
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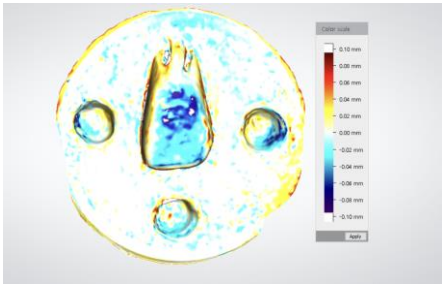
C

The weight analysis was performed using a precision digital scale (MARTE AD500® (Santa Rita do Sapucaí / Minas Gerais / Brasil). Each sample was individually measured in a controlled ambient in triplicate, being the result considered the mean of the three measurements.

The color was evaluated with a clinical spectrophotometer (VITA Easyshade; VITA Zahnfabrik) using an individual evaluation index fabricated from a polyvinyl siloxane material (Variotime; heraeus kulzer). The index consisted of a putty matrix with a Ø6-mm hole to be positioned at the middle of the buccal surface of each evaluated specimen to standardize location **Figure 6.** For each specimen, three analysis were performed and the mean of the values obtained was considered. Color measurements were recorded in the CIELab system, which defines color as L* for lightness, ranging from 0 (black) to 100 (white); and a* and b* for chromatic characteristics, ranging from red (+a*) to green (-a*) and yellow (+b*) to blue (-b*)[15]. The color was measured 3 times for each evaluation, and the mean L*, a*, and b* values were calculated. The CIELab color change (ΔE_{ab}) was calculated from $\Delta E_{ab} = \sqrt{(L_1 - L_2)^2 + (a_1 - a_2)^2 + (b_1 - b_2)^2}$. Color differences[16,17] were also calculated by using the CIEDE2000 formula: $\Delta E_{00} = [(\Delta L'/K_L S_L)^2 + (\Delta C'/K_c S_c)^2 + (\Delta J'/K_H S_H)^2 + R_T(\Delta C'/K_C S_C)(\Delta H'/K_H S_H)]^{½}$, where ΔL', ΔC', and ΔH' are the differences in lightness, chroma, and hue, respectively, between the baseline and the subsequent color readings; R_T is the rotation function corresponding to chroma and hue difference interaction in the blue region; S_L, S_C and S_H are weighting terms for adjustment of the total color difference for variation in perceived magnitude with variation in the location of the color coordinate difference between 2 color measurements; and K_L, K_C and K_{Ho} are correction terms for the experimental conditions[18]. The sequence of ΔE_{00} calculation was carried out as previously described[19].

Figure 6. A: Index in addition silicone to guide the color taking. B: Color taking with Easyshade.


A

B

Statistical analysis

The results were submitted to an exploratory analysis. Afterall, the data of L*, b, ΔE_{ab} and ΔE_{00} were assessed using the generalized linear model to analyse the differences between time, type of tooth and type of hygiene protocols. The a values and the surface wear were submitted to the Kruskal Wallis and Dunn tests to compare types of tooth and hygiene protocols; and to the Friedman and Nemenyi test to evaluate differences among time of analysis. The level of significance was set at 5%. All the analyses were performed using R software (R Core Team (2021); R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

The results of surface wear are presented in **Table 3,** in which just the results for volume 1 are presented, once the other area of analysis showed the same pattern of results. Comparing the hygiene protocols, the tooth Artiplus was the one that showed higher amount of surface wear when brushed with toothpastes with statistically significant difference in relation to brushing with distilled water (p=0.0019). In relation to the comparison between the different teeth, when using toothpastes, this tooth presented higher wear than Biotone and Premium, when brushed with Elmex (p=0.0011) and in case of Colgate, it showed higher surface wear than Biotone (p=0.0335).

Table 3: Wear volume as a function of tooth and brushing protocol.

Variable	Tooth	Brushing protocol						p-value
		Distilled Water		Elmex		Colgate		
		Average (SD)	Median (minimum and maximum value)	Average (SD)	Median (minimum and maximum value)	Average (SD)	Median (minimum and maximum value)	
Volume 1	Trilux	0.03 (0.10)	0.00 (0.00- 0.34) Aa	0.09 (0.10)	0.07 (0.00- 0.29) Aab	0.07 (0.14)	0.02 (0.00- 0.49) Aab	0.0938
	Biotone	0.00 (0.00)	0.00 (0.00- 0.00) Aa	0.00 (0.01)	0.00 (0.00- 0.05) Ab	0.03 (0.04)	0.00 (0.00- 0.14) Ab	0.1871
	Artiplus	0.04 (0.07)	0.00 (0.00- 0.21) Ba	0.22 (0.24)	0.15 (0.03- 0.94) Aa	0.29 (0.37)	0.14 (0.00- 1.25) Aa	0.0019
	Delara	0.09 (0.16)	0.00 (0.00- 0.46) Aa	0.21 (0.32)	0.02 (0.00- 0.97) Aab	0.10 (0.11)	0.08 (0.00- 0.40) Aab	0.7127
	Premium	0.06 (0.13)	0.00 (0.00- 0.45) Aa	0.05 (0.09)	0.00 (0.00- 0.26) Ab	0.10 (0.11)	0.08 (0.00- 0.40) Aab	0.3098
p-valores			0.3055		0.0011		0.0335	

Different letters (capital letters horizontally and lowercase letters vertically comparing the teeth in each variable) indicate statistically significant differences ($p \leq 0.05$).

Considering the analysis of weight **Table 4.**, it can be seen that the artificial teeth are stable in relation to this parameter irrespective of the treatment applied (type of hygiene protocol with or without staining). Significative differences were found when comparing the type of tooth, being Premium and Trilux similar and with higher mass than the other teeth tested.

Table 4: Mass as a function of tooth, brushing protocol and time.

Time	Tooth	Brushing protocol					
		Distilled Water		Elmex		Colgate	
		Average (SD)	Median (minimum and maximum value)	Average (SD)	Median (minimum and maximum value)	Average (SD)	Median (minimum and maximum value)
Initial	Trilux	6.55 (0.59) Aa	6.53 (5.68-7.39)	6.41 (0.64) Aa	6.29 (5.51-7.44)	6.44 (0.64) Aa	6.39 (5.43-8.14)
	Biotone	6.29 (0.53) Ab	6.41 (5.29-6.89)	6.05 (0.40) Ab	5.96 (5.37-6.64)	5.89 (0.50) Ab	5.66 (5.32-6.95)
	Artiplus	6.07 (0.26) Ab	6.04 (5.70-6.50)	5.94 (0.35) Ab	6.03 (5.01-6.28)	6.01 (0.28) Ab	6.06 (5.58-6.39)
	Delara	6.14 (0.35) Ab	6.10 (5.46-7.00)	6.14 (0.18) Ab	6.14 (5.87-6.44)	6.23 (0.40) Ab	6.33 (5.38-6.65)
	Premium	6.48 (0.28) Aa	6.50 (6.11-6.92)	6.50 (0.43) Aa	6.42 (6.05-7.52)	6.50 (0.36) Aa	6.38 (5.92-7.25)
After brushing	Trilux	6.55 (0.59) Aa	6.53 (5.68-7.39)	6.41 (0.64) Aa	6.29 (5.50-7.43)	6.53 (0.56) Aa	6.41 (5.98-8.14)
	Biotone	6.29 (0.53) Ab	6.4 (5.29-6.88)	6.04 (0.35) Ab	6.00 (5.37-6.64)	5.94 (0.51) Ab	5.68 (5.32-6.95)
	Artiplus	6.07 (0.26) Ab	6.04 (5.70-6.50)	5.94 (0.35) Ab	6.03 (5.01-6.28)	6.01 (0.28) Ab	6.06 (5.58-6.39)
	Delara	6.14 (0.35) Ab	6.10 (5.46-7.00)	6.14 (0.18) Ab	6.14 (5.87-6.44)	6.23 (0.39) Ab	6.33 (5.38-6.65)
	Premium	6.47 (0.28) Aa	6.50 (6.11-6.92)	6.50 (0.43) Aa	6.42 (6.05-7.52)	6.50 (0.36) Aa	6.38 (5.92-7.24)
After staining	Trilux	6.56 (0.59) Aa	6.54 (5.69-7.39)	6.42 (0.64) Aa	6.30 (5.51-7.44)	6.45 (0.64) Aa	6.40 (5.44-8.15)
	Biotone	6.30 (0.53) Ab	6.41 (5.30-6.89)	6.05 (0.40) Ab	5.97 (5.38-6.64)	5.90 (0.50) Ab	5.67 (5.33-6.96)

Time	Tooth	Brushing protocol					
		Distilled Water		Elmex		Colgate	
		Average (SD)	Median (minimum and maximum value)	Average (SD)	Median (minimum and maximum value)	Average (SD)	Median (minimum and maximum value)
	Artiplus	6.08 (0.26) Ab	6.05 (5.72-6.52)	5.95 (0.35) Ab	6.05 (5.02-6.29)	6.02 (0.27) Ab	6.07 (5.59-6.4)
	Delara	6.15 (0.35) Ab	6.12 (5.47-7.01)	6.16 (0.18) Ab	6.15 (5.90-6.46)	6.24 (0.40) Ab	6.34 (5.39-6.66)
	Premium	6.49 (0.28) Aa	6.52 (6.13-6.93)	6.51 (0.43) Aa	6.43 (6.07-7.53)	6.52 (0.36) Aa	6.40 (5.94-7.26)

There was no significant difference between the times ($p>0.05$). Different letters (capitals horizontally and lowercase vertically comparing the teeth at each brushing time and protocol) indicate statistically significant differences ($p\leq0.05$). p(tooth)<0.0001; p(brushing)=0.4220; p(tooth x brushing)=0.7836; p(time)=0.1584; p(time x tooth)=0.6336; p(time x brushing)=0.1837; p(time x tooth x brushing)=0.8333

In relation to the color analysis, the results of each CIELab coordinate are presented in the tables 5, 6, and 7 respectively. Considering the L* coordinate **Table 5,** it can be seen that after brushing, most groups, with exception of the tooth Premium brushed with toothpastes, showed significant decrease in the luminosity in relation to the initial analysis. However, after staining, all groups were statistically different from the initial and after brushing analysis. The comparison between the teeth, before any treatment (initial analysis) showed differences in relation to this parameter, as follows Premium > Delara = Biotone > Artiplus > Biotone. After brushing, it can be seen that for most groups, brushing with distilled water resulted in reduction in luminosity when brushing with toothpaste, with significant difference in relation to Elmex for Trilux, Artiplus, Delara and Premium; and in relation to Colgate for Biotone and Premium ($p<0.05$). After staining, the teeth were altered to the same extent when comparing the hygiene protocols. Comparing the different teeth, Premium was the one that showed lesser alteration of luminosity when compared to the others ($p<0.05$). The luminosity presented in decreasing values considering the hygiene protocol and staining was: DW - Premium > Biotone > Delara > Artiplus = Trilux; Elmex - Premium > Biotone = Delara > Trilux > Artiplus, and Colgate - Premium > Biotone = Delara > Artiplus = Trilux.

Table 5: L value of the CIELab system as a function of the tooth, brushing protocol and time.

Time	Tooth	Brushing protocol					
		Distilled Water		Elmex		Colgate	
		Average (SD)	Median (minimum and maximum value)	Average (SD)	Median (minimum and maximum value)	Average (SD)	Median (minimum and maximum value)
Initial time	Trilux	73.88 (2.12) Ad	73.85 (71.30 -79.10)	74.93 (1.26) Ad	74.75 (73.50 – 77.90)	73.96 (1.43) Ad	74.00 (72.20 – 76.30)
	Biotone	77.85 (0.89) Ab	77.80 (76.10 - 79.10)	77.35 (1.36) Ab	77.35 (75.20 – 79.4)	77.64 (1.04) Ab	77.65 (76.40 – 79.70)
	Artiplus	76.13 (0.69) Ac	76.05 (75.00 – 77.10)	76.10 (1.06) Ac	75.70 (75.10 – 79.00)	75.58 (1.29) Ac	76.00 (73.30 – 76.90)
	Delara	77.57 (0.41) Ab	77.55 (77.00 – 78.50)	77.26 (0.56) Ab	77.45 (75.90 – 77.90)	77.22 (0.75) Ab	77.20 (75.80 – 78.60)
	Premium	78.75 (0.69) Aa	78.75 (77.70 – 79.80)	79.47 (0.80) Aa	79.70 (77.70 – 80.50)	79.96 (0.77) Aa	79.95 (78.70 – 81.00)
After brushing	Trilux	*72.28 (2.08) Bd	72.10 (69.00 – 77.20)	*73.74 (1.34) Ad	73.40 (72.40 - 76.90)	73.44 (1.25) ABe	73.50 (71.30 – 75.10)
	Biotone	*76.28 (0.92) Bb	76.30 (74.40 – 77.80)	*76.63 (1.41) ABb	76.72 (74.60 – 78.93)	*77.20 (1.15) Ab	77.27 (75.70 – 79.30)
	Artiplus	*74.73 (0.98) Bc	74.70 (72.40 – 76.00)	*75.60 (0.92) Ac	75.45 (74.60 – 77.90)	*74.86 (0.78) Bd	74.70 (73.80 – 76.00)
	Delara	*76.13 (0.56) Bb	76.20 (75.20 77.10)	*76.78 (0.61) Ab	76.85 (75.70 – 77.80)	*76.43 (0.72) ABc	76.40 (75.30 – 77.90)
	Premium	*78.37 (0.71) Ca	78.25 (77.30 – 79.60)	79.36 (0.75) Ba	79.40 (78.00 – 80.60)	80.06 (0.82) Aa	80.00 (78.70 – 81.10)
After staining	Trilux	*$69.01 (2.30) Ad	69.75 (64.40 – 72.20)	*$69.66 (0.99) Ac	69.40 (68.30 – 71.50)	*$70.91 (3.62) Ac	69.65 (66.00 – 78.30)
	Biotone	*$74.00 (1.31) Ab	74.10 (72.40 – 76.70)	*$74.39 (1.74) Ab	74.7 (71.7 – 77.3)	*$73.94 (3.32) Ab	75.05 (67.3 – 77.9)

Time	Tooth	Brushing protocol					
		Distilled Water		Elmex		Colgate	
		Average (SD)	Median (minimum and maximum value)	Average (SD)	Median (minimum and maximum value)	Average (SD)	Median (minimum and maximum value)
	Artiplus	*$69.83 (2.67) Ad	70.60 (65.20 – 72.90)	*$67.78 (2.36) Bd	67.65 (64.7 – 73.5)	*$69.99 (2.53) Ac	69.9 (64.8 – 74.3)
	Delara	*$72.71 (1.95) Bc	73.20 (68.10 – 75.10)	*$74.73 (1.53) Ab	75.4 (72.2 – 76.7)	*$73.94 (1.55) ABb	73.75 (72.2 – 77.8)
	Premium	*$76.12 (1.32) Aa	76.10 (73.00 – 78.10)	*$76.33 (1.16) Aa	76.15 (74.4 – 78.3)	*$76.31 (2.16) Aa	76.6 (71.5 – 79.5)

*Differs from the initial time under the same tooth conditions and brushing protocol ($p \leq 0.05$). $ Differs from the time after brushing under the same tooth conditions and brushing protocol ($p \leq 0.05$). Different letters (capitals horizontally and lowercase vertically comparing the teeth at each brushing time and protocol) indicate statistically significant differences ($p \leq 0.05$). p(tooth)<0.0001; p(brushing)=0.0387; p(tooth x brushing)=0.2183; p(time)<0.0001; p(time x tooth)<0.0001; p(time x brushing)<0.0001; p(time x tooth x brushing)=0.0017.

Analysing the a* coordinate **Table 6**, in general, the teeth Premium, Biotone and Delara showed higher a* values with significant difference in relation to Trilux and Artiplus ($p<0.05$). It can be seen that after brushing the Premium and Biotone teeth was the one that did not show statistically significant difference in relation to the initial analysis irrespective of the hygiene protocol. Considering the Elmex toothpaste, Trilux was the one group that showed reduction in a* with significance to the initial condition ($p<0.0001$). The staining protocol resulted in decrease of the a* values with significant difference in relation to initial analysis for Trilux and Biotone, irrespective of the hygiene protocol. The Artiplus group stained was different just from the condition after brushing. For Delara and Premium, after staining, no differences were found comparing it with their initial or after brushing results. Comparing the tooth, Premium, Delara and Biotone, in general, were the groups which showed lower reduction in a*.

Table 6: A-value of the CIELab system as a function of the tooth, brushing protocol and time.

Time	Tooth	Brushing protocol						p-value
		Distilled Water		Elmex		Colgate		
		Average (SD)	Median (minimum and maximum value)	Average (SD)	Median (minimum and maximum value))	Average (SD)	Median (minimum and maximum value)	
Initial	Trilux	-1.91 (0.41)	-1.75 (-2.90 – 1.50) Acd	-1.78 (0.27)	-1.75 (-2.40 - 1.20) Acd	-2.14 (0.49)	-2.00 (-2.80 - 1.50) Acd	0.2724
	Biotone	4.79 (1.17)	4.78 (3.07 – 6.60) Aa	4.71 (1.44)	4.15 (3.00 - 6.97) Aa	5.06 (1.20)	4.75 (3.67 – 7.30) Aa	0.6961
	Artiplus	-2.79 (0.17)	-2.80 (-3.10 - 2.60) Ad	-2.63 (0.16)	-2.65 (-2.99 - 2.49) Ad	-2.75 (0.14)	-2.70 (-3.00 - 2.60) Ad	0.0811
	Delara	-0.74 (0.2)	-0.80 (-1.00 - 0.40) Abc	-0.59 (0.27)	-0.69 (-1.19 - 0.29) Abc	-0.66 (0.14)	-0.60 (-1.00 - 0.50) Abc	0.3056
	Premium	0.41 (0.37)	0.40 (-0.40 – 1.20) Aab	0.02 (0.34)	0.00 (-0.69 - 0.79) Bab	0.23 (0.43)	0.20 (-0.40 1.00) ABab	0.0244
p-value			<0.0001		<0.0001		<0.0001	
After brushing	Trilux	-1.68 (0.43)	*-1.50 (-2.70 -1.20) Acd	-1.53 (0.26)	*-1.59 (-2.19 - 1.00) Acd	-1.74 (0.34)	*-1.75 (-2.20 -1.30) Acd	0.4935
	Biotone	4.65 (1.17)	4.65 (2.80 – 6.40) Aa	4.46 (1.43)	4.03 (2.77 – 6.67) Aa	5.12 (1.14)	4.85 (3.67 – 7.27) Aa	0.4223
	Artiplus	-2.43 (0.15)	*-2.45 (-2.70 -2.20) Bd	-2.34 (0.31)	-2.30 (-3.00 - 1.90) ABd	-2.23 (0.13)	*-2.20 (-2.40 -2.10) Ad	0.0333
	Delara	-0.63 (0.17)	*-0.60 (-0.90 -0.40) Bbc	-0.38 (0.25)	-0.35 (-0.80 - 0.10) ABbc	-0.23 (0.17)	*-0.20 (-0.60 – 0.00) Abc	0.0003
	Premium	0.17 (0.45)	0.25 (-1.00 – 0.90) Aab	-0.08 (0.42)	0.05 (-1.00 – 0.40) Aab	0.32 (0.47)	0.40 (-0.50 1.00) Aab	0.0597
p-value			<0.0001		<0.0001		<0.0001	

Time	Tooth	Brushing protocol						p-value
		Distilled Water		Elmex		Colgate		
		Average (SD)	Median (minimum and maximum value)	Average (SD)	Median (minimum and maximum value))	Average (SD)	Median (minimum and maximum value)	
After staining	Trilux	-1.59 (0.3)	*-1.60 (-2.23 - -1.03) Abc	-1.31 (0.38)	*-1.37 (-1.77 - 0.43) Abc	-1.67 (0.55)	*-1.53 (-2.97 -1.10) Abc	0.1993
	Biotone	3.94 (1.09)	*4.03 (2.37 - 5.30) Aa	3.54 (1.25)	*3.10 (1.87 - 5.37) Aa	3.81 (1.32)	$3.87 (1.73 - 6.10) Aa	0.7187
	Artiplus	-1.76 (1.62)	*-2.12 (-2.70 - 3.30) Ac	-2.68 (0.22)	$-2.65 (-3.10 – 2.27) Bc	-3.06 (1.89)	$-2.53 (-9.00 - 1.93) Bc	0.0019
	Delara	-0.21 (2.25)	-0.72 (-1.67 - 6.80) Aab	-0.5 (0.44)	-0.55 (-1.20 - 0.13) Aab	-0.93 (0.63)	$-0.68 (-2.03 - -0.27) Ab	0.2285
	Premium	-1.42 (3.44)	-0.47 (-8.60 - 1.83) Abc	-0.6 (0.89)	-0.72 (-1.83 – 0.90) Ab	-0.11 (1.41)	-0.27 (-2.40 - 2.60) Aab	0.5628
p-value			<0.0001		<0.0001		<0.0001	

* It differs from the initial time under the same tooth conditions and brushing protocol (p≤0.05). $ Differs from the time after brushing under the same tooth conditions and brushing protocol (p≤0.05). Different letters (capitals horizontally and lowercase vertically comparing the teeth at each brushing time and protocol) indicate statistically significant differences (p≤0.05).

For the b* coordinate **Table 7**, the comparison among the teeth before any treatment (initial analysis) showed the follow order for this parameter: Biotone > Premium > Trilux = Delara > Artiplus. It can be seen that after brushing and after staining there was reduction in this parameter with significance in relation to the initial analysis. There were no differences between the hygiene protocols after brushing, when the same tooth was compared ($p>0.05$). After staining, significant reduction for b* was verified for Artiplus (DW > CT > EL) and Delara (EL > CT). Comparing the teeth, in the initial condition the b* values are presented as Biotone > Premium > Delara = Trilux > Artiplus. After brushing, this sequence was maintained, adding difference between Delara and Trilux. With the staining protocol, Artiplus and Trilux were the tooth that showed higher reduction in b*, while Artiplus was for EL and Delara for CT.

Table 7: B-value of the CIELab system as a function of the tooth, brushing protocol and time.

Time	Tooth	Brushing protocol					
		Distilled Water		Elmex		Colgate	
		Average (SD)	Median (minimum and maximum value)	Average (SD)	Median (minimum and maximum value)	Average (SD)	Median (minimum and maximum value)
Initial	Trilux	22.11 (3.63) Ac	23.15 (11.90-25.90)	23.97 (1.57) Ac	23.75 (21.30-26.70)	22.90 (3.55) Ac	23.65 (14.30-26.60)
	Biotone	37.81 (4.06) Aa	37.70 (32.10-44.00)	38.73 (4.27) Aa	38.03 (33.4-44.43)	38.80 (3.71) Aa	37.73 (33.87-45.30)
	Artiplus	19.34 (0.92) Ad	19.30 (18.00-21.00)	19.37 (1.34) Ad	19.40 (17.20-21.90)	19.08 (1.08) Ad	19.05 (17.30-21.30)
	Delara	22.25 (1.48) Ac	22.55 (19.70-24.20)	23.11 (1.42) Ac	23.15 (20.50-25.30)	22.17 (0.94) Ac	22.30 (19.80-23.50)
	Premium	27.93 (2.74) Ab	27.90 (22.10-32.50)	27.11 (2.78) Ab	28.30 (22.20-30.00)	28.13 (3.19) Ab	28.25 (22.20-32.80)
After brushing	Trilux	*21.13 (3.90) Ac	22.35 (10.20-24.50)	*23.13 (1.75) Ac	23.10 (20.10-25.70)	22.77 (2.41) Ac	23.00 (17.50-25.90)
	Biotone	*36.97 (4.13) Aa	36.40 (31.10-43.30)	*37.65 (4.55) Aa	37.45 (31.17-43.50)	*37.90 (3.74) Aa	37.17 (32.57-44.37)
	Artiplus	*18.16 (1.1) Ad	18.05 (16.10-20.00)	*18.33 (1.39) Ae	18.60 (16.30-21.10)	*18.16 (0.92) Ae	18.10 (16.90-20.20)
	Delara	*21.47 (1.28) Ac	21.65 (19.20-23.00)	*21.43 (1.25) Ad	21.55 (19.00-23.70)	*20.95 (0.76) Ad	21.15 (19.10-21.90)
	Premium	*27.41 (3.05) Ab	28.00 (20.30-31.80)	*26.05 (3.25) Ab	27.55 (19.20-29.10)	*27.19 (3.38) Ab	27.75 (21.20-31.90)
After staining	Trilux	*$18.74 (1.91) Ac	18.73 (15.17-21.50)	*$18.23 (2.55) Ac	18.63 (14.17-21.53)	*$17.26 (4.84) Ab	18.78 (9.57-23.87)
	Biotone	*$33.96 (3.67) Aa	35.57 (28.03-37.80)	*$32.35 (4.79) Aa	31.67 (22.77-37.93)	*$33.64 (4.73) Aa	33.78 (27.13-40.87)

Time	Tooth	Brushing protocol					
		Distilled Water		Elmex		Colgate	
		Average (SD)	Median (minimum and maximum value)	Average (SD)	Median (minimum and maximum value)	Average (SD)	Median (minimum and maximum value)
	Artiplus	*17.65 (2.34) Ac	18.32 (14.23-20.43)	*$13.49 (2.14) Cd	13.27 (10.53-18.73)	*$15.88 (2.29) Bb	16.65 (11.80-18.23)
	Delara	*20.20 (2.95) ABbc	20.32 (14.53-23.83)	*21.12 (2.08) Ab	21.58 (17.67-24.43)	*$17.51 (4.25) Bc	17.73 (8.87-22.73)
	Premium	*$23.03 (6.77) Ab	23.12 (12.43-35.10)	*$20.49 (5.61) Abc	21.50 (10.63-27.50)	*$22.38 (5.84) Ab	21.43 (12.07-33.90)

* It differs from the initial time under the same tooth conditions and brushing protocol (p≤0.05). $Differs from the time after brushing under the same tooth conditions and brushing protocol (p≤0.05). Different letters (capitals horizontally and lowercase vertically comparing the teeth at each brushing time and protocol) indicate statistically significant differences (p≤0.05). p(tooth)<0.0001; p(brushing)=0.3529; p(tooth x brushing)=0.0472; p(time)<0.0001; p(time x tooth)<0.0001; p(time x brushing)=0.0013; p(time x tooth x brushing)=0.0045.

For ΔE_{ab} **(Table 8)** calculated after brushing and the initial condition, the protocol with DW, in general, showed higher variation with significant difference in relation to CT for Biotone and EL for Artiplus. Comparing the tooth, there were no differences between them for DW, there was difference between Delara and Premium, with Delara presenting the higher variation and for CT, Trilux presented the higher variation with statistical difference in relation to the other teeth. The ΔE_{ab} considering the conditions after staining and brushing, showed difference between the hygiene protocol just for Artiplus, which showed the highest variation when brushed with Elmex and for Delara, which showed the lesser variation for this toothpaste. Comparing the different teeth with the same hygiene protocol, there were differences between Premium (highest variation) and Delara (lower variation) for DW and EL.

Table 8: Delta Eab of the CIELab system depending on the tooth and brushing protocol.

Variable	Tooth	Brushing protocol					
		Distilled Water		Elmex		Colgate	
		Average (SD)	Median (minimum and maximum value)	Average (SD)	Median (minimum and maximum value)	Average (SD)	Median (minimum and maximum value)
Delta Eab Initial-after brushing	Trilux	1.96 (0.48) ABa	2.03 (1.31- 2.87)	1.52 (0.36) Bab	1.57 (0.99- 2.09)	2.36 (2.86) Aa	1.68 (0.58- 11.22)
	Biotone	1.84 (0.37) Aa	1.84 (1.32- 2.79)	1.38 (0.91) ABab	1.05 (0.37- 3.29)	1.07 (0.35) Bb	1.04 (0.48- 1.86)
	Artiplus	1.88 (0.69) Aa	1.77 (1.17- 3.85)	1.27 (0.38) Bab	1.15 (0.83- 2.22)	1.42 (0.43) ABb	1.54 (0.67- 1.98)
	Delara	1.73 (0.56) Aa	1.78 (0.93- 2.89)	1.78 (0.43) Aa	1.66 (1.04- 2.79)	1.59 (0.47) Ab	1.59 (0.81- 2.33)
	Premium	0.95 (0.62) Ab	0.72 (0.3- 2.16)	1.19 (0.91) Ab	0.86 (0.33- 3.16)	1.07 (0.37) Ab	1.03 (0.57- 1.92)
p-valores		p(tooth)<0.0001- p(brush)=0.2993- p(interaction)=0.0252					
Delta Eab After brushing – After staining	Trilux	5.56 (1.83) Aab	5.54 (2.85- 8.21)	6.54 (2.43) Aa	6.04 (1.92- 11.09)	7.30 (4.09) Aa	5.65 (2.65- 13.26)
	Biotone	4.77 (1.05) Aab	5.16 (2.48- 5.83)	6.25 (3.94) Aa	5.24 (2.48- 17.22)	6.73 (3.83) Aa	6.24 (1.84- 11.99)
	Artiplus	5.44 (2.92) Bab	4.05 (2.26- 10.63)	9.35 (2.60) Aa	9.49 (2.91- 12.49)	5.70 (3.37) Ba	6.53 (1.22- 11.65)
	Delara	4.48 (2.85) Ab	3.71 (1.37- 10.35)	2.68 (1.52) Bb	2.15 (0.56- 4.96)	5.36 (2.91) Aa	4.36 (2.47- 10.92)
	Premium	7.10 (3.66) Aa	7.86 (1.91- 12.00)	7.36 (3.88) Aa	7.55 (1.14- 14.41)	7.26 (4.87) Aa	6.37 (2.94- 20.24)
p-value		p(tooth)<0.0001- p(brush)=0.1403- p(interaction)=0.0199					

Different letters (capital letters horizontally and lowercase letters vertically comparing the teeth in each variable) indicate statistically significant differences (p≤0.05).

Regarding ΔE_{00} **(Table 9)** calculated after brushing and the initial condition, the comparison between hygiene protocols showed higher variation for DW with Biotone, and for DW in relation to EL for Artiplus. The comparison between the teeth showed that Premium presented the lower variation for DW and CT, while the brushing with EL resulted in the variation as follows: Premium = Biotone < Artiplus = Delara < Trilux. In relation to the ΔE_{00} obtained from the comparison between the conditions after staining and brushing, it can be seen that Artiplus showed the highest variation when brushed with EL, while Delara showed the lowest variation with this toothpaste. Comparing the teeth, some differences were found as follows: DW - Artiplus = Premium > Biotone and EL - Artiplus > Premium = Trilux = Biotone > Delara. The brushing with CT resulted in no difference between groups.

Table 9: Delta E00 of the CIELab system depending on the tooth and brushing protocol.

Variable	Tooth	Brushing protocol					
		Distilled Water		Elmex		Colgate	
		Average (SD)	Median (minimum and maximum value)	Average (SD)	Median (minimum and maximum value)	Average (SD)	Median (minimum and maximum value)
Delta E00 Initial-after brushing	Trilux	1.35 (0.35) Aa	1.33 (0.90- 2.11)	1.02 (0.27) Aa	0.99 (0.68- 1.52)	1.42 (1.57) Aa	1.06 (0.47- 6.33)
	Biotone	1.19 (0.23) Aa	1.18 (0.89- 1.69)	0.70 (0.47) Bb	0.46 (0.26- 1.68)	0.52 (0.24) Bc	0.45 (0.25- 1.16)
	Artiplus	1.26 (0.48) Aa	1.16 (0.84- 2.66)	0.80 (0.20) Bab	0.77 (0.55- 1.31)	0.99 (0.25) ABb	1.01 (0.51- 1.41)
	Delara	1.15 (0.37) Aa	1.13 (0.65- 1.90)	0.95 (0.23) Aab	0.93 (0.56- 1.51)	1.00 (0.30) Aab	0.93 (0.57- 1.57)
	Premium	0.55 (0.34) Ab	0.44 (0.22- 1.19)	0.64 (0.53) Ab	0.42 (0.18- 1.79)	0.55 (0.17) Ac	0.53 (0.38- 0.99)
p-value		p(tooth)<0.0001- p(brush)=0.0071- p(interaction)=0.0059					
Delta E00 After brushing – After staining	Trilux	3.65 (1.32) Aab	3.78 (1.83- 5.79)	4.14 (1.56) Ab	3.94 (1.41- 7.3)	4.64 (2.36) Aa	3.57 (1.95- 7.98)
	Biotone	2.43 (0.68) Ab	2.40 (1.58- 3.54)	2.91 (1.72) Ab	2.67 (1.16- 7.19)	3.57 (2.15) Aa	3.21 (1.09- 6.85)
	Artiplus	4.19 (2.54) Ba	2.97 (1.67- 9.20)	6.75 (1.86) Aa	6.77 (2.13- 8.99)	4.33 (2.78) Ba	4.81 (0.92- 10.28)
	Delara	3.27 (2.30) Aab	2.50 (0.94- 8.21)	1.78 (1.09) Bc	1.35 (0.42- 3.30)	3.53 (1.76) Aa	2.99 (1.62- 6.77)
	Premium	4.39 (2.77) Aa	3.83 (0.99- 9.13)	4.17 (2.07) Ab	4.11 (0.71- 7.66)	4.36 (2.69) Aa	3.58 (1.98- 11.78)
p-value		p(tooth)=0.0968; p(brush)<0.0001; p(interaction)=0.0169					

Different letters (capital letters horizontally and lowercase letters vertically comparing the teeth in each variable) indicate statistically significant differences ($p \leq 0.05$).

In relation to the analysis of weight **(Table 4)**, neither the brushing nor the staining protocol resulted in significant differences in relation to the initial measurement for any tooth. There were no differences between the hygiene protocols in any condition (initial, after brushing and after staining). The comparison between teeth showed that the Trilux and Premium presentes higher weight than the other teeth.

DISCUSSION

The null hypothesis of the study were rejected once some differences were found concerning type of artificial teeth and methods of hygiene when analyzing the physical properties tested (wear, color and staining susceptibility). In general, the teeth were similar in relation to the surface wear after brushing with slight differences between them. Brush with distilled water did not result in alteration on the tooth surface of any material. However, brushing with toothpaste changed the tooth surface, producing some differences when comparing the different artificial teeth used. Artiplus was the tooth which showed higher surface wear as comparing it in relation to the other hygiene protocols, as comparing the different teeth brushed with toothpastes. This result may indicate that the inclusion of a higher amount of INPEN® material, as indicated by the fabricant as a network of interpenetrated polymers, may be not too stable to the abrasive challenge as brushing. Artiplus is of the same fabricant of Biotone (Dentisply Sirona) and it presents this material as the top of the categorie. However, Artiplus presented higher wear than Biotone, irrespective of toothpaste used. Despite the differences found in relation to this analysis, no differences were found considered the influence of the hygiene protocol in the analysis of weight, indicating that this parameter was stable during the tests applied (abrasion and staining). The differences found when comparing the different teeth concerning this analysis may be related to the composition of the tooth or its fabrication method.

Considering the color parameters, some important differences were found. In general, luminosity (variation between black and white) is a standard property used to compare the color of different materials, in this case artificial teeth, once it indicates how lighter the tooth is[20]. In relation to it, comparing the teeth used, the Premium

presented higher luminosity. This tooth is considered the top of the categorie being indicated by their fabricant as a more natural and resistant tooth. Once L is an important property, this tooth may be considered lighter than the others. The other coordinates indicate the variation in color, being a* from green to red, and b* from yellow to blue, considering from the negative to positive[21]. Some differences concerning these parameters were found between teeth but they are probably related to the composition and fabrication method of the teeth.

The challenges proposed (abrasion and staining) significantly influenced the color analysis. Concerning L, all brushing protocols resulted in reduction of this coordinate, excepting the Premium teeth cleaned with both toothpastes. Comparing the kind of teeth used, this was the one which showed the lowest reduction in luminosity. When considering the brushing with distilled water, it can be understood that the physical impact of the toothbrushes under the tooth surface was enough to produce this color alteration, included the Premium teeth. For this material, considered with superior properties, the use of toothpaste did not produce any deleterious effects, indicating that the ingredients from toothpastes as surfactants, humectants and other cleaning agents may reduce the effect of the bristles on their surface. For all groups, the staining protocol significantly influenced the reduction of luminosity in comparison to the initial time and after brushing condition, which indicates that the deposition of pigments from coffee solution clearly impacts the lighting appearance of the tooth.

Considering the a* coordinate, it can be visualized that for the teeth which altered their values, there was an increase in them, indicating an alteration in direction to the red axis, which is an expression of the darkening effect. This is clearly observed for the Trilux group, irrespective of the cleaning method used, and for Artiplus and Delara brushed with distilled water of Colgate toothpaste. Elmex was selected for this study

as the less abrasive between the most commonly toothpastes used by the patients which showed beneficial effects in not inducing the darkening of the teeth. After staining, the Biotone also showed increase in a* leading to the conclusion that this tooth, which is the cheapest material, after brushing, became more prone to the retention of pigments.

For the b* coordinate the results are very clear. All groups showed reduction in valued of b* indicating a yellowish effect, as after brushing as after brushing+staining, irrespective the brushing protocol. Curiously, Biotone was the tooth who showed higher values of b*, followed by Premium (top of the market). Comparing these both materials, it can be seen that they presented a very similar composition with differences mainly in relation to the method of application and organization of the polymers inside artificial teeth.

Studies evaluating the cleaning method of the denture prosthesis are important once their quality may impact on the health of the patient. It is already known that brushing with toothpaste and toothbrush, which is recommended for patients with fixed prosthesis, impact on the color and gloss of the tooth, changing its appearance, and increase its surface roughness, which can lead to a higher retention of biofilm[22-24]. Once there are available in the market different artificial materials varying physical characteristics and price, it is important to compare them to help clinicians in choosing the best option of material for their patients.

The ΔEab and ΔE00 analysis showed that, considering the condition after brushing and initial, caused variation in tooth color for all tooth, being the Premium the one that showed the lowest and significant alteration in relation to all the other tooth for DW ($p<0.0001$); in relation to Delara for Elmex and in relation to Trilux for Colgate ($p<0.0001$). Despite to result in slight numerical variation after brushing, which is not

considered clinically important, these results together with the abovementioned (L*, a* and b*) demonstrate that the brushing protocols alter the tooth surface. Toothbrushing with toothpaste has been associated with surface roughness of prosthetic dental materials[23] and, as a consequence, the optical behavior of the material is all changed, as visualized in this study. After staining, the variation of ΔEab and ΔE00 were more pronounced for all groups. Curiously, Delara and Premium were statistically significant when treated with DW and Elmex, being Delara the group that presented the lowest variation. This is an important result to be observed once both material are from the same fabricant, with different purposes between them. Delara is classified as a more accessible material, with inferior quality in relation to the Premium. However, the present results show that Delara is less prone to staining.

Considering the protocol using Colgate, it can be seen that no differences were found among the groups. Comparing the protocols, different behavior were observed for some tooth: Biotone the highest variation using Elmex, while with this same toothpaste, Delara showed the lowest variation (ΔEab and ΔE00). The slight differences found between the brushing protocols, with toothpaste or DW, are probably related to the toothpastes selected. Both, Elmex and Colgate are classified as medium abrasiveness according to the Relative Dentin Abrasivity (RDA=70). Besides, these materials present neutral pH and abrasive particles regular-shaped in a small-diameter, which are commonly in presence of hydrated silica, and, using of soft brushes, like as in this study, result in a polished surface[25,26].

A limitation of the study is the lack of simulation of the buccal environment, using, as example, human or artificial saliva. However, all the conditions established in the experimental design followed previous studies, in order to allow comparability. There is a lack of studies comparing the different prosthetic artificial teeth available in the

market and, in this sense, this present study worried in joining the most used materials from the market and to compare them, in order to opportunize to the clinician to know about the differences between them, as well as their advantages and disadvantages.

CONCLUSIONS

Based on the findings of this study is possible to conclude that:

- The brushing protocols tested was able to produce surface wear and color alteration in the artificial tooth used.

- The brushing and staining protocols caused the yellowish effect in the materials used, once significant alterations were found for L*, a* and b*;

- The staining protocols caused significant color alteration which could be clinically perceptible, with some differences between the materials used.

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ANEXO 1

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4. Abstract structured (intro/methods/results/conclusion) or unstructured
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8. Tables (each table complete with title and footnotes);
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A structured abstract of no more than 200 words must be provided for each article. Footnotes, references, and abbreviations are not used in the abstract.

For original research articles, the abstract should include the following headings and sections: (1) Objective. This section includes a statement of the problem and the purpose of

the study, (2) Materials and Methods. This section should include materials, methods and statistical analyses employed in the study. (3) Results. (4) Conclusions.

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For systematic literature review articles, the abstract should include the following headings and sections: (1) Objective. This section should include a statement of the topic to be reviewed and a description of the search strategy of relevant literature (search terms and databases), (2) Materials and Methods. This section should contain inclusion criteria (language, type of studies i.e. randomized controlled trial or other, duration of studies and chosen endpoints). (3) Results. This section should include evaluation of papers and level of evidence. (4) Conclusions.

For general review articles the abstract should include the following headings and sections: (1) Objective. This section should include a statement of the topic to be reviewed. (2) Overview. This section should include a brief summary of the findings of the review. (3) Conclusions.

In addition to Abstracts, all papers should include the following:

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In a few sentences, please indicate the clinical importance and implications of the research or clinical technique discussed, and if applicable, its relevance to esthetic dentistry.

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Add at least five keywords that reflect the primary content of the paper.

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Clinical and laboratory/fundamental research papers

Well written and properly structured research hypotheses are the central core of every section of a research manuscript. All research should be hypothesis-driven and clinical and laboratory research manuscripts must state proper research hypotheses, based on the pre-existing knowledge and scientific background supplied in the Introduction. The research hypothesis does not have to be assumed to be correct – it is perfectly acceptable if the research hypotheses are invalidated, as long as the authors provide substantive preliminary rationale for initiating the test, and subsequent information identifying factors that influenced the outcome. The null hypothesis should not be the framework of a paper based on the scientific method. Null hypotheses are applicable only when the collected data are structured for statistical analysis.

Introduction: Provide sufficient background and listing of pre-existing knowledge (references) that support the anticipated outcome of the work. As a general rule, no new references should be introduced past this section. The only exception are references used in supporting Materials and Methods. Do not use author names in the paper: instead of, e.g., Smith at al. reported..., use One study (or similar) reported that ___.34

(where 34 corresponds to the reference by Smith et al.)

State the purpose of your research. This portion should be presented as a paragraph on its own. Within this paragraph, describe the major experimental factors, parameters being measured, and experimental control.
Lastly, clearly state the research hypotheses, labeled as such, and provide a numerical listing of each hypothesis. This listing is key to the paper. The same sequence of hypothesis testing will be used to structure the Materials and Methods, Results, Discussion, and the Conclusions sections.

Materials and Methods: Follow the sequence of the listing of the research hypotheses in describing parameter testing. The detail level in this section should be such that someone experienced in the art and science of those methods could easily reproduce the same experiment in their laboratory.

Describe methods of statistical analysis and provide justification of sample size from pilot testing. The pre-set level of a Type 1 statistical error (the alpha) should be mentioned here as well. Usually, testing is performed at a pre-set alpha of 0.05, meaning that a significant difference exists with 95% confidence.

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Results: Present the results of the findings in the same sequence as the experimental parameters described in the Materials and Methods. If parametric statistical methods were used, provide the initial normality and equivalence of variation results. If those tests are not passed, indicate such and also provide what non-parametric analyses was used instead.

Present the data only once – in either tabular or graphical format. Using either method, provide an embedded coding system to identify groups that were identified as statistically not different if appropriate. Indicate the significance level of each major experimental factor, as well as any interaction terms (p-values).

Discussion: Without repeating the purpose of the research, start this section with addressing individual research hypotheses, ideally in separate, sequential paragraphs. Start with a sentence indicating if the experimental data upheld or invalidated the corresponding research hypothesis. After that, compare and contrast the current findings related to this hypothesis with work performed by others in the field (references from the Introduction). Provide insights as to why or why not similar information was found.

After addressing individual research hypotheses, put together the knowledge gained from these findings into one coherent theme. Discuss the clinical/research significance of the findings or the significance of this new knowledge over that in the existing literature. This is where the author is allowed to speculate for the first and only time.

Provide a paragraph on the study limitations. Applying the research findings outside of the experimental design needs to be taken with caution. Lastly, provide insight as to what types of research need to be done as a consequence of the new knowledge found in the current project.

Conclusions should contain no speculative statements – only the facts as they are limited to what the data reveal about the tested research hypotheses, following their order. It is good to preclude the listing of conclusions with "Within the limitations of this current study, it was concluded that:"

1. Address Research Hypothesis #1
2. Address Research Hypothesis #2
3. and so on.

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