Original Article



Investigation of Short and Long Term Effects of Various Mouthwashes on the Color Stability of Hybrid Composites Çeşitli Ağız Gargaralarının Hibrit Kompozitlerin Renk Stabilitesi Üzerine Kısa ve

Uzun Dönem Etkilerinin İncelenmesi

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ABSTRACT

Objective: The color stability of dental composite restorations is an important criteria for clinical success. This study aimed to investigate the long-term effects of various mouthwashes on the staining of direct composites.

Methods: Disc-shaped samples were prepared by using 4 different commercially available hybrid composites (Clearfil Majesty, Kuraray; Charisma Smart, Heraeus Kulzer; Quadrant Universal, Cavex; Brilliant EverGlow, Coltene) and divided randomly into five groups according to mouthwashes: Sensodyne, Oral B 3D White Luxe Glamorous Shine, Listerine, Colgate Plax, Meridol. Initial colors of specimens were measured by using a spectrophotometer (Vita Easyshade V, Germany). Specimens were immersed in the mouthwashes and stored in an incubator set at 37 °C for 12, 60, and 120 hours, equivalent to daily use of mouthwash for 1, 5, and 10 years, respectively. Subsequently, the color change value of different materials was calculated as ΔE^*ab . The data were analyzed by ANOVA and paired sample t-tests.

Results: The mouthwash type and application time affected the color change values (p=0.00). The most color change (ΔE^*) was observed in Colgate Plax, followed by Meridol. The least ΔE^* was observed in Sensodyne after 1 year and in Oral B after 5 and 10 years. The materials showing the least and most ΔE^* after 1-year mouthwash application were Brilliant and Quadrant, respectively. The least and most ΔE^* after 5 and 10 years were observed in Brilliant and Charisma groups, respectively.

ÖZ

Amaç: Dental kompozit restorasyonların renk stabilitesi klinik başarı için önemli bir kriterdir. Bu çalışmanın amacı çeşitli gargaraların direkt kompozitlerin renk stabilitesi üzerindeki uzun dönem etkilerini araştırmaktır.

Yöntemler: Disk şeklidenki örnekler 4 farklı hibrit kompozit (Clearfil Majesty, Kuraray; Charisma Smart, Heraeus Kulzer; Quadrant Universal, Cavex; Brilliant EverGlow, Coltene) ile hazırlanarak, gargaralara göre rastgele beş gruba ayrıldı: Sensodyne, Oral B 3D White Luxe Glamorous Shine, Listerine, Colgate Plax, Meridol. Numunelerin başlangıç renkleri bir spektrofotometre (Vita Easyshade V, Almanya) kullanılarak ölçüldü. Numuneler 37 °C'ye ayarlanmış bir inkübatörde 1, 5 ve 10 yıl boyunca günlük gargara kullanımına eşdeğer olan 12, 60 ve 120 saat süreyle gargaralar içerisinde saklandı. Ardından farklı materyallerin renk değişim değeri ΔE^* ab olarak hesaplandı. Veriler ANOVA ve eşleştirilmiş örnek t-testleri ile analiz edildi.

Bulgular: Gargara çeşidi ve uygulama süresi renk değişimi değerlerini etkilemiştir (p=0,00). En fazla renk değişimi (ΔE^*) değerleri Colgate Plax'ta gözlendi ve bunu Meridol izledi. Bir yıllık uygulama sonrası en düşük ΔE^* değerleri Sensodyne grubunda, 5 ve 10 yıllık uygulama sonrasında ise Oral B grubunda gözlendi. Bir yıllık gargara uygulamasından sonra en düşük ve en yüksek ΔE^* değerleri gösteren materyaller sırasıyla Brilliant ve Quadrant'tır. Beş ve 10 yıllık uygulama sonrası en düşük ve en yüksek ΔE^* değerleri ise sırasıyla Brilliant ve Charisma gruplarında gözlendi.

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©Copyright 2022 by the Bezmiâlem Vakıf University Bezmiâlem Science published by Galenos Publishing House. Received: 01.09.2021 Accepted: 30.11.2021 **Conclusion:** The staining in composite restorations caused by mouthwashes varies depending on the structural properties of the resin composite, the pH of the mouthwashes, and exposure time.

Keywords: Coloration, color measurement, composite resins, mouthwashes, spectrophotometry

Introduction

The increase in aesthetic expectations has resulted in the development of various tooth-colored restorative material compositions for clinical use. Therefore, various direct resin composites with different particle size, shape, and distribution of fillers have been developed and available on the market (1). The filler particles in the resin composite directly affect the properties such as surface roughness, gloss, wear resistance, and polymerization shrinkage (2). Despite all the improvements, concerns about color stability, longevity, and durability of resin composite restorations still remain.

Dental biofilm formation is the main factor for the initiation and progression of oral infectious diseases such as gingivitis, periodontal inflammation, and caries (3). Mechanical methods such as tooth brushing and interdental cleaning are effective for plaque removal but are directly dependent on personal skills. Besides, it is difficult to provide oral hygiene with effective brushing in disabled or traumatized patients. Various studies have shown that the use of auxiliary methods such as mouth rinsing can be effective in preventing plaque accumulation (4,5). However, frequent usage of mouthwashes can have detrimental effects on dental tissues and restorative materials (6).

Despite the constant improvements in the composition of resin composites, substances such as saliva, food, liquids, and

Sonuç: Kompozit restorasyonlarda ağız gargaralarının neden olduğu renklenme, rezin kompozitin yapısal özelliklerine, gargaraların pH değerine ve gargaraya maruz kalma süresine bağlı olarak değişmektedir.

Anahtar Sözcükler: Renklenme, renk ölçümü, kompozit rezin, ağız gargaraları, spektrofotometri

mouthwashes can result in increased solubility (7). Additionally, mouthwashes trigger a decrease in oral pH associated with an increase in sorption and solubility, causing surface degradation and thus discoloration of the composite resin material (2). Previous studies stated that mouthwashes and antiseptics used for oral infection control and antimicrobial activity can cause external discoloration of dental hard tissues and restorations (8-12). However, only a few focused on the newly developed mouthwashes and the discoloration of hybrid composites. Therefore, the amount of discoloration that may occur as a result of exposure to different types of resin composite restorations to different antimicrobial agents is still an issue that needs to be investigated. Accordingly, this study aimed to examine the effects of five mouthwashes on four different aesthetic restorative materials during different periods of time by analyzing color stability. The tested null hypotheses were that: sustainable color stability of different restorative materials after immersion in numerous mouthwashes (1) would not be affected by increasing exposure time (2) and would not demonstrate a difference between the different composite materials or mouthwashes.

Methods

The direct composites used in the current study were included in four hybrid resin composites and presented in Table 1. A3 shade was selected for each brand. A total of 100 disk-shaped specimens were prepared in polytetrafluoroethylene molds

Table 1. The rescolative materials used in the present study and their compositions						
Material (manufacturer)	Composition	Туре				
Clearfil Majesty Esthetic (Kuraray Medical Inc., Tokyo, Japan)	Silanated barium glass filler (40% by volume) Pre-polymerized organic filler Bis-GMA* Hydrophobic aromatic dimethacrylate di-Camphorquinone	Nano-hybrid composite				
Charisma Smart (Heraeus Kulzer GmbH, Hanau, Germany)	BIS-GMA* matrix and contains approximately 59% filler by volume with a particle size of 0.005-10 μm Barium Aluminum Fluoride glass, highly dispersive silicon dioxide	Micro-hybrid composite				
Quadrant Universal LC (Cavex, Holland BV, Netherlands)	Methacrylate-based monomers (24.5% by volume) Silica, silicate glass and fluoride containing fillers (75.0% by volume) Polymerisation catalysts Inorganic pigments	Hybrid composite				
Brilliant EverGlow (Coltene/Whaledent AG Altstatten, Switzerland)	Methacrylates, dental glass, amorphous silica, zinc Oxide (range of dimensions of inorganic filler particles: 0.02- 1.5 µm)	Submicron hybrid				
*Bis-GMA: Bisphenol A-glycidyl methacrylate						

 Table 1. The restorative materials used in the present study and their compositions

(thickness: 2 mm and diameter: 5 mm) and divided into four groups according to the restorative material they were prepared from (n=25 from each material).

Resin composites were packed into these molds according to the manufacturer's instructions. Each mold was located on a flat glass surface separated with a mylar strip that was positioned on the glass to prevent the resin composite from adhering to the surface. Following condensation, another mylar strip was placed on the top surface to avoid oxygen inhibition layer formation and then gently pressed with a glass plate to extrude excess material. Then, each specimen was light-cured for 40 s on each side with a power density of 1,000 mW/cm² (Valo LED, Ultradent Products Inc., South Jordan, UT, USA) from the nearest distance perpendicular to the surface. The light output was measured with a radiometer (SDI LED Radiometer, Bayswater, Australia) to provide standardization. To obtain equal thickness in each group, the thickness was checked with a digital caliper (Powerfix Electronic Digital Caliper, Padget Services, London, England).

After polymerization, the specimens surfaces were polished with polishing discs in a decreasing gradient (SwissFlex, Coltene, Altstatten, Switzerland). At the end of these procedures, the debris was removed from surface with ultrasonic cleaning and then specimens were stored in distilled water for 24 h at 37 °C.

The initial colors were measured on a CIE L*a*b* color scale with a spectrophotometer (Vita Easyshade V, Vita Zahnfabrik, Bad Sackingen, Germany). Before measurement, the specimens were thoroughly dried and placed in contact with the measuring probe of the spectrophotometer. Color measurements were repeated three times on a standard white background and averaged. Prior to each measurement, the device was calibrated.

The pH of five different mouthwash types was recorded by using a digital pH meter (Hanna HI 83141, USA). Three measurements were taken from each mouthwash and averaged. Subsequently, 25 specimens from each group were randomly

divided into five subgroups (n=5) and different mouthwashes were applied to each subgroup (Table 2). The specimens were then packed in 20 mL of the mouthwashes in capped containers to prevent evaporation and were stored in an incubator set at 37 °C for 12 hours, equivalent to daily use of mouthwash for one year (1,12). At the end of the one-year test period, the specimens were immersed in distilled water and the color measurements were repeated. The color change value ΔE^*ab was calculated according to the following formula:

 $\Delta E^*ab = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$

where L* stands for lightness, a* for green-red (-a=green; +a=red) and b* for blue-yellow (-b=blue; +b=yellow). Values of $\Delta E > 3.3$ were considered clinically unacceptable.

The same measurements were repeated after 60 and 120 hours which were equivalent to daily use of mouthwash for 5 and 10 years, respectively. The data were collected and subjected to statistical analysis.

Before the sampling procedure, a power analysis was conducted for sample size calculation. When 80% power and error probability α =0.05 were accepted, and the losses of specimens were taken into consideration, it was determined that 5 specimens in each subgroup were required.

Statistical Analysis

The results of color measurements were analyzed by using statistical software, SPSS 22.0 (SPSS Inc., Chicago, IL, USA). The normality of the distributions was confirmed by Skewness, Kurtosis, and the Shapiro-Wilk test. Means and standard deviations were given as descriptive statistics and analysis of variance (ANOVA) was used to evaluate the effect of mouthwashes and material type on color change. Besides, paired sample t-test was used for intragroup comparisons of the different time results. The significance limit was set at p < 0.05.

Table 2. The mouthwashes used in the present study								
Mouthwashes	Composition	Alcohol content	PH	Manufacturer				
Sensodyne Cool Mint	Aqua, Glycerin, Sorbitol, Potassium Nitrate, PEG-60 Hydrogenated Castor Oil, Poloxamer 407, Sodium Benzoate, Aroma, Disodium Phosphate, Methylparaben, Propylparaben, Sodium Phosphate, Sodium Fluoride, Sodium Saccharin, CI 42090.	Alcohol free	6.82	GlaxoSmithKline, Brasil				
Oral B 3D White Luxe Glamorous Shine	Aqua, Alcohol, Glycerin, Disodium Pyrophosphate, Tetrasodium Pyrophosphate, Polysorbate 80, Aroma, Poloxamer 407, Sodium Saccharin, Sodium Fluoride, Sucralose, CI 42090.	Containing alcohol	6.88	Procter & Gamble, Weighbridge, UK				
Listerine Cool Mint	Aqua, Propylene Glycol, sorbitol, poloxamer 407, benzoic acid, sodium saccharin, eucalyptol, methyl salicylate, aroma, thymol, menthol, sodium benzoate, sodium fluoride.	Alcohol free	5.82	Johnson&Johnson Inc., USA				
Colgate Plax	Aqua, Glycerin, Propylene Glycol, Sorbitol, Poloxamer 407, Flavor, Cetylpyridinium Chloride, Potassium Sorbate, Sodium Fluoride, Sodium Saccharine, Menthol, CI 42051.	Alcohol free	4.5	Colgate-Palmolive, New York, NY, USA				
Meridol	Aqua, Xylitol, PVP, PEG-40 Hydrogenated Castor Oil Olaflur, Aroma (mint-aniseed-eucalyptus), Stannous Fluoride, Sodium Saccharin, CI 42051.	Alcohol free	4.2	GABA Group, Basel, Switzerland				

Table 2. The mouthwashes used in the present study

Results

The means and standard deviations of the color change (ΔE^*) values of the various hybrid composites in five different mouthwash types were shown in Table 3. As a result of the variance analysis, it was detected that the material, the mouthwash type, and application time affected the color change values (p=0.00). In general, it was observed that the color changes caused by mouthwashes, except for Colgate Plax, were within the limits accepted (ΔE <3.3). When the exposure time to mouthwashes was prolonged, the color change values increased mostly, although there was no statistically significant difference in Sensodyne and Oral B subgroups.

In all material groups, the most color change was observed in the Colgate Plax group, followed by Meridol. While the mouthwashes causing the least color change after 1 year of application were Sensodyne and Oral B after 5 and 10 years of application, respectively. The material showing the least color change after 1, 5, and 10 years of mouthwash application was Brilliant, whereas the material that showed the most color change after 1-year application was the Quadrant. On the other hand, the most color change after 5 and 10 years of mouthwash application was observed in the Charisma group.

Discussion

Preservation of the color stability of aesthetic restorative materials is one of the most important features in terms of durability. This property indicates inconsistency between various restorative materials and the color instability is one of the main reasons for the replacement of aesthetic restorations (13).

Intrinsic factors including the matrix, filler composition and size, addition of minor pigments, and the photoinitiator system can affect the color stability of resin composite restorations (5,12). Besides, incomplete polymerization causes a significant influence on color stability (14,15). The increase in particle size of aesthetic restorative materials results in increased water absorption through the polymer chains, affects the bonds between the matrix and

Table 3. The means and standard deviations of the color change (ΔE^*) values of the restorative materials in five mouthwashes

Mouthwashes	Restorative materials	1 year colour change (ΔE*) mean ± SD	5 years colour change (ΔE*) mean ± SD	10 years colour change (ΔE*) mean ± SD
	Clearfil	0.36±0.23 ^{A,a}	1.24±0.93 A,a	1.76±0.95 ^{A,b}
Sensodyne Cool Mint	Charisma	0.59±0.35 ^{A,a}	0.82±0.27 A,a	1.09±0.22 ^{A,a}
	Brillant	0.42±0.23 A,a	1.12±0.45 ^{A,b}	1.33±0.33 A,b
	Quadrant	0.88±0.33 ^{A,a}	1.12±0.38 ^{A,a}	1.00±0.35 ^{A,a}
	р	0.050	0.681	0.161
Oral B 3D White Luxe Glamorous Shine	Clearfil	0.81±0.28 ^{A,a}	1.06±0.25 ^{A,ab}	1.24±0.26 A,b
	Charisma	0.77±0.17 ^{A,a}	1.17±0.25 ^{A,b}	1.18±0.31 A,b
	Brillant	0.54±0.11 ^{A,a}	0.99±0.27 ^{A,b}	1.01±0.31 A,b
	Quadrant	0.76±0.16 ^{A,a}	0.85±0.21 A,a	0.92±0.17 A,a
	р	0.136	0.247	0.244
Listerine Cool Mint	Clearfil	0.76±0.25 ^{A,a}	1.40±0.43 ^{A,b}	1.94±0.44 ^{A,c}
	Charisma	0.96±0.41 ^{A,a}	1.13±0.31 ^{A,a}	1.34±0.53 AB,a
	Brillant	0.83±0.40 ^{A,a}	1.33±0.35 ^{A,b}	1.27±0.14 ^{в,ь}
	Quadrant	0.82±0.30 ^{A,a}	1.06±0.46 ^{A,a}	0.88±0.18 ^{B,a}
	р	0.815	0.489	0.003*
Colgate Plax	Clearfil	2.99±1.29 A,a	3.24±0.46 A,a	3.20±0.67 A,a
	Charisma	3.91±0.76 ^{A,a}	5.91±0.70 ^{B,b}	5.50±0.69 ^{B,b}
	Brillant	2.76±0.93 A,a	3.47±0.90 A,a	3.91±1.92 AB,a
	Quadrant	3.60±0.31 ^{A,a}	4.69±0.29 ^{C,b}	5.07±0.51 AB,c
	р	0.194	0.000*	0.017*
Meridol	Clearfil	1.43±0.54 ^{A,a}	2.07±0.10 ^{A,b}	2.18±0.14 ^{A,b}
	Charisma	1.16±0.38 ^{A,a}	1.01±0.57 ^{B,a}	2.69±0.41 AB,b
	Brillant	1.38±0.70 ^{A,a}	1.75±0.46 AB,a	1.83±0.45 AC,a
	Quadrant	2.22±0.88 ^{A,a}	2.11±0.45 A,a	2.38±0.59 ^{A,a}
	р	0.095	0.003*	0.038*

Different capital letters indicate statistically significant differences (*p<0.05) between different resin composites for the same mouthwash. Different small letters indicate statistically significant differences (*p<0.05) between different time intervals for the same material.

filler particles, and leads to an uneven surface during polishing and susceptibility to external staining (8,16). Additionally, it has been reported in various studies that the color stability depends on the brand and shade of the material, the radiation time and intensity, and the finishing technique (8). Therefore, the same color shades of different resin composite materials were included in the present study, and standard polishing procedures were followed after equal time polymerization with the same lightcuring device.

Color stability can be determined both visually and by specific instruments such as colorimeter or spectrophotometer (16). The methodology used in the current study was similar to previous studies using spectrophotometry (10,15). CIE L*a*b* system is used to investigate color change (ΔE) since it has advantages such as sensitivity, objectivity, and repeatability. Few studies stated that ΔE values higher than 2 could be detected clinically (17). On the other hand, according to most studies, a ΔE value of 3.3 is the limit and higher values are considered clinically unacceptable (18,19). However, values between 2.2 and 4.4 are clinically acceptable for the Healthy Lifestyles Program of the Commissioned Corps of the United States Public Health Service and higher values can also be acceptable depending on the study design (20).

External discoloration may occur due to poor oral hygiene, diet, and smoking habits, and the use of mouthwashes is also considered one of the external factors that threaten the color stability of aesthetic restorations (21). The usage of mouthwashes to control caries and periodontal disease has become popular. Consequently, the current study aimed to assess the color stability of various aesthetic composite materials after subjected to 1, 5, and 10 years of mouth rinsing. It has been reported in the literature that storing resin composites in mouthwash for 12 hours is equivalent in time to 1 year of 2 minutes daily use (1). Therefore, the specimens were stored in mouthwashes for 12, 60, and 120 hours and it was aimed to provide an effect equivalent to the 1, 5, and 10-years exposure. The mouthwashes were changed every 4 hours to maintain their effectiveness.

Previous studies have stated that the smoothest surface of restorations is achieved by polymerizing the resin composites in direct contact with a mylar strip and any additional polishing procedures can lead to an increase in surface roughness (22,23). Therefore, in some studies, polishing was not preferred (14,24). However, finishing and polishing may be required in clinical conditions even if mylar strips are used. In this study, the same polishing processes were applied to all specimens in order to imitate the clinical conditions appropriately.

As is known, the presence of alcohol and low pH of the mouthwashes can affect the surface integrity of the composite materials, promote organic degradation and affect stain resistance (25). Sensodyne caused the least color change after 1 year period, as expected due to its high pH and alcohol-free content (26). In the present study, Oral B 3D White Luxe Glamorous Shine was the only alcoholic mouthwash and caused a similar effect on color change (ΔE) to Sensodyne and Listerine. This situation

was explained by Oral B 3D White Luxe Glamorous Shine had the highest pH value among the studied mouthwashes. Even though Listerine did not contain alcohol, it was reported in several studies that due to its low pH (5.82), it could cause biodegradation of resin composites and erosion resulting in staining (1,27). However, in the present study, the color stability of resin composites was not affected by alcohol content, and there was no significant difference among the Sensodyne, Listerine and Oral B 3D White Luxe Glamorous Shine (p=0.537 and p=0.910, respectively). Colgate Plax and Meridol, which had the lowest pH values, showed significantly higher color change than the other mouthwashes. Considering the mouthwashes that caused the most and the least color change were alcohol-free, it could be stated that alcohol content was not the only factor that had a softening effect on resin composites.

The effect of mouthwashes on the color change of resin composites can also be material-dependent, and the discoloration susceptibility of the material can be attributed to the degradation caused by water sorption (17). Water sorption of a resin composite material is dependent on the quality of the bond between matrix and filler. Extra water sorption may expand the resin component, hydrolyze the silane and result in microcrack formation. The mouthwash solutions can cause staining by promoting microcrack formation at the interface between the filler and matrix (28). In addition, resin composites containing fewer fillers are more prone to staining. Besides, it was reported that the materials containing urethane dimethacrylate in the resin matrix presented higher color stability than materials containing other dimethacrylate types due to the low viscosity and the water sorption properties (29). Since all composite materials used in this study were hybrids and their contents and particle sizes were similar, no statistically significant difference was observed between materials in 1-year of application, even though Brilliant showed the lowest, and Quadrant showed the highest color change. However, after 10-years of Meridol and Colgate Plax application, the Clearfil Majesty Esthetic group exhibit significantly lower color change values than the Charisma Smart group. This situation can be explained by the nano-hybrid structure of Clearfil Majesty Esthetic as well as the micro hybrid structure of Charisma Smart. Thereby, both null hypotheses were partially rejected. The most discolored material after 5 and 10 years of mouthwash application was Charisma smart which contained the bigger filler particles among the composite materials used in the present study, proving the importance of the particle size.

In previous studies, the short-term effects of mouthwashes on composite resins have been widely investigated (1,5,8). However, owing to the developments in restorative materials and adhesive dentistry, the longevity of resin composites has increased. Previous studies have shown that at least 60% of resin composite restorations can last for more than 10 years when the appropriate materials are applied correctly (30,31). On the other hand, studies showing the long-term effects of mouthwashes are very limited (32). Studies have shown that the long term use of mouthwashes containing high concentrations of alcohol may have detrimental oral effects such as epithelial detachment, keratosis, mucosal ulceration, petechiae, and oral cancer (33). However, it has been controversial if the use of alcohol-based rinsing increases the risk of oral cancer, oropharynx or other head and neck cancers (34). In previous studies, there was no consensus on whether it was a risk factor for cancer. The results of the studies in the literature were inconsistent. Therefore, alcohol-free mouthwashes were mostly preferred in the present study. Even though long-term use of mouthwashes is not recommended, the fact that these products are commercially available on the market and patients can easily buy and use without a prescription have created difficulties against control. In the present study, the effects of mouthwashes on the color stability of restorative materials after 1, 5, and 10 years of application were investigated and it was concluded that increased exposure time of mouthwashes also increased color change.

It has been reported that the washing effect of saliva, water, and different beverages consumed can reduce the staining caused by mouthwashes. Within the limitations of the present *in vitro* study, the relationship between mouth rinsing-induced staining, nutrition, and aging on resin composites could not be examined. Therefore, further *in vivo* studies are required to evaluate discoloration potential of different mouthwashes on various restorative materials.

Conclusion

The findings of the present study indicate that mouthwashes, which play an important role in maintaining periodontal health, may cause staining in resin composite restorations. The amount of this effect may vary depending on the structural properties of the resin composite, factors such as the color, consistency, and pH of the mouthwashes, and exposure time. All resin composites used in the present study showed color difference after immersion in mouthwashes but these differences were not visually perceptible after 1 year of application. However, after 10 years of application, clinically unacceptable staining was observed in some groups. The clinician should consider this situation, examine the color compatibility of existing resin composite restorations with dental tissues during routine controls, and replace discolored restorations when necessary.

Ethics

Ethics Committee Approval: Since the present study named as "investigation of short and long term effects of various mouthwashes on the color stability of hybrid composites" was not conducted on humans or animals, ethics committee approval is not required as only experimental studies were conducted on the material.

Peer-review: Externally peer reviewed.

Authorship Contributions

Surgical and Medical Practices: M.B.D., Concept: M.B.D., M.T.A., Design: M.B.D., M.T.A., Data Collection or

Processing: M.B.D., Analysis or Interpretation: M.B.D., Literature Search: M.B.D., M.T.A., Writing: M.B.D., M.T.A.

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