

Evaluation of Different Restorative Materials after Exposure to Chlorhexidine

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Abstract: Objectives: To evaluate the effect of chlorhexidine solution on the micro-hardness and surface roughness of three different restoratives (nanoionomer, Nano Ceramic composite and giomer). Methods: Three different restorative materials [Ketac N100 (KN), 3M/ESPE, Ceram X (CX), Dentsply and Beautiful II (BII), Shofu] were tested in this study. Fifty discs (5mm diameter x 3mm thickness) of each tested material were prepared, ten specimens were used as control while the other 40 specimens were divided into four subgroups (n=10); first and second subgroups were immersed in an artificial saliva for one week and one month respectively. Third and fourth subgroups were subjected to 0.2% Chlorhexidine Digluconate [(CHX), Colgate Periogard] for one week and one month respectively, as they were immersed in CHX for 1 min. 3 times daily and immersed in artificial saliva after each chlorhexidine exposure. Five specimens of each subgroup were tested for surface roughness using Quanta Environmental Scanning Electron Microscope while other five specimens were tested for microhardness using Vickers Micro-Hardness Tester. Data were statistically analyzed using Three-way ANOVA and Tukey's post-hoc test ($P \leq 0.05$). Results: Microhardness mean values were 86.9, 83.3 and 72.8 for KN, CX and BII respectively. However, these values significantly decreased after 1 month. Exposure to CHX for one month showed statistically significant highest mean surface roughness values. However, CX (138.2) showed the statistical significant highest mean surface roughness values, this was followed by BII (122.9) which showed lower value. KN showed the statistical significant lowest mean surface roughness values. Conclusions: Under the conditions of this study it was concluded that the long term exposure to 0.2% Chlorhexidine Digluconate had resulted in gradual increase in surface roughness and gradual decrease in micro-hardness of the tested materials.

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1. Introduction:

Currently caries is, more evidently than ever, regarded as an infectious disease process characterized by episodes of de-and re-mineralization, Diab et al.,(2007). Thus, medical model of treatment with caries control measures and re-mineralization methods of initial carious lesions have been promoted. Mouthrinses containing chlorhexidine and/or fluoride are considered the simplest vehicle for chemoprophylaxis.

After more than 30 years of use in the dental practice, clinical efficacy of chlorhexidine is documented. It is considered the gold standard against which other antiplaque and gingivitis agents due to its dicationic nature, which affords the agent the property of persistence of antimicrobial effect at the tooth surface, Jones (2000).

Once the carious lesion becomes cavitated, surgical treatment is indicated. Tooth-colored restorative materials have been widely used to restore such lesions in order to satisfy patients' esthetic demands. Several types of resin composites with different physical and mechanical characteristics are available in the dental market, Celik et al.,(2008). They are classified according to the size, shape, and distribution of filler particles. Among the most widely used

composites are the microfills and microhybrids. By the introduction of nanotechnology in dentistry, nanocomposites with nanometer-sized filler particles have been developed. These nanocomposites have increased filler loading. Thus offering many advantages, such as reduced polymerization shrinkage, improved mechanical and optical characteristics, and better gloss retention, Moszner and Salz (2001); Mitra et al., (2003); Moszner and Klapdohr (2004);Terry (2004).

Moreover, the wear resistance of these nanocomposites has been reported to be equal or even greater than that of microfill and microhybrid composite resins.

However, after restoration, the frequent use of both alcohol-containing and alcohol-free mouth rinses could affect the hardness of these resin composite restorations, Diab et al., (2007). Since, the hardness is related to material's strength and rigidity; it has great consequence on the clinical durability of restorations. Moreover, the repeated use of mouth rinses could affect the maintenance of a highly polishable surface. Hence, the presence of any roughness on the surface of the composite restoration can allow adsorption of extrinsic stains, thus it may cause discoloration of such esthetic materials. Therefore, surface roughness

is considered another factor that affects the clinical longevity of esthetic fillings.

Unfortunately, there is limited information on whether the resin-based materials are resistant to chlorhexidine in terms of surface roughness and hardness. Therefore, the purpose of this study was to investigate the influence of exposure to chlorhexidine solution and storage in artificial saliva on the surface roughness and microhardness of three different restorative materials (nanoionomer, nano ceramic composite and giomer).

2. Materials and Methods:

2.1. Materials:

2.1.1. Sample:

Three different restorative materials [Ketac N100 (KN), 3M/ESPE, Ceram.X Mono+, (CX), Dentsply and Beautiful II (BII), Shofu] were tested in this study (table 1) 50 discs (5 mm diameter x 3 mm thickness) of each tested material were made.

Each disc was fabricated by carefully inserting an increment of tested restorative material using a nitride plated resin-composite instrument (Aescolap, FIG.21B, Germany) into a circumferential Teflon mold with 5mm of internal diameter and 3mm of height positioned onto a 0.051 mm thick transparent polyester film strip (Mylar, DuPont, Wilmington, Del.) over a glass slide. The first increment was light cured for 20 seconds using Optilux 501 (Kerr Corp, Orange, CA).

The second increment was inserted then another 0.051 mm thick transparent polyester film strip was applied on top of the Teflon mold filled with the tested material. An additional glass slide was placed over the previously positioned polyester film strip, and a 1 kg weight applied during 1 min. to extrude the excess material and to obtain a uniformly smooth specimen surface. Afterwards, the weight was removed and the second increment of tested restorative material light cured for 20 seconds through the polyester film strip. The output light intensity was continuously monitored with a radiometer (SDS Demetron, Orange, CA) to ensure a constant value of 600 mW/cm². The top surface of the disc against which the load was applied was marked by a notch from the side to be examined for surface roughness and hardness.

After fabrication of the discs, ten specimens of each tested material were divided randomly into 2 subgroups (n=5). Both subgroups were examined immediately, one for surface roughness and the other for microhardness. These both subgroups were used as control. The other 40 specimens of each tested material were divided randomly into four subgroups (n=10);

- 1) Immersed in an artificial saliva for a week
- 2) Immersed in an artificial saliva for a month.
- 3) Exposed to 0.2% Chlorhexidine Diguconate [(CHX), Colgate Periogard] daily for a week.
- 4) Exposed to 0.2% Chlorhexidine Diguconate [(CHX), Colgate Periogard] daily for a month.

2.2. Methods:

2.2.1. The protocol of the work:

A special protocol was followed in order to expose the specimens to CHX. The specimens were immersed in CHX for 1 min. 3 times daily and immersed in artificial saliva after each chlorhexidine exposure. This protocol was done at room temperature and repeated for a week and a month. A specially designed device was fabricated (fig. 1) to allow the specimens to be placed in the same solutions for the same period at the same time. CHX and the artificial saliva were changed after each specimens' exposure.

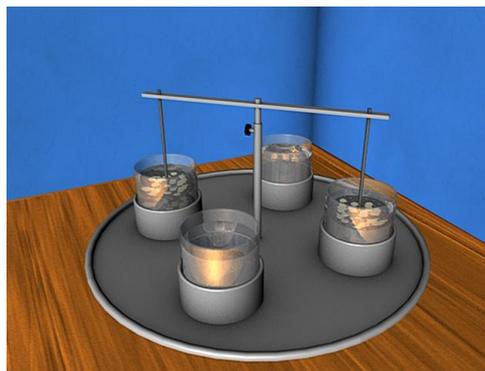


Figure 1: the chlorhexidine exposure device.

3. Results:

3.1. Surface roughness results:

The results of the three-way ANOVA showed that, the regression model is fit to describe the relationship between the studied variables. The results showed that material, exposure to chlorhexidine, storage periods and the interaction between the three variables had a statistically significant effect on mean surface roughness values ($P \leq 0.05$).

3.2. Effect of the material

To compare the effect of the tested material on the surface roughness values, Tukey's test was used and the results were presented in figure (3), and showed that Ceram X had the statistically significantly highest mean roughness value. This was followed by Beautiful II which showed lower value. Ketac N showed the statistically significantly lowest mean surface roughness value ($P \leq 0.05$).

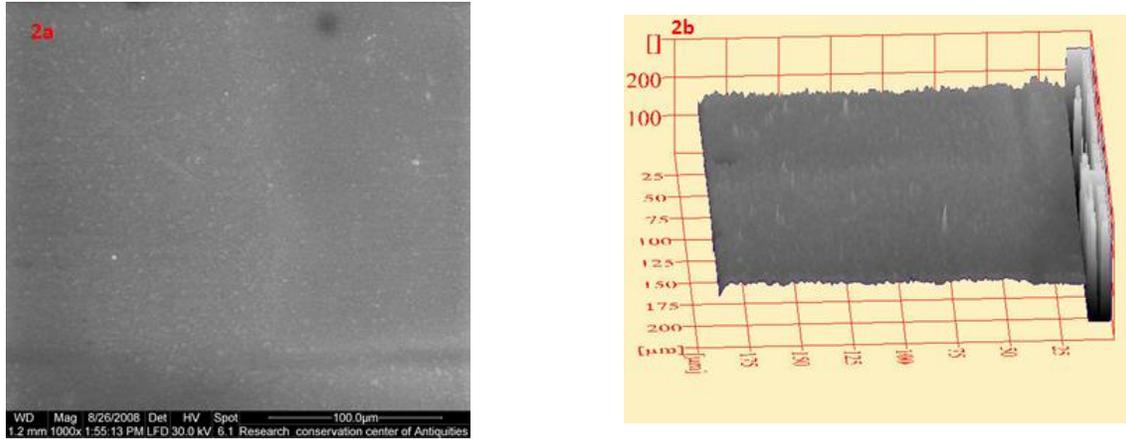


Figure (2): a. scanning photomicrograph of Ceram.X specimen after storage in artificial saliva for a week, b. image analysis divided the scanning photomicrograph into peaks.

Table (1): Manufacturers, manufacturers’ nstructions and compositions of the used

Materials	Principal components	Manufacturer’s Instructions	Manufacture
KetakN100 Nano-ionomer (Light-Curing Glass Ionomer Restorative)	De-ionized water, Blend including HEMA, methacrylate-modified polyalkenoic acid (Vitrebond Copolymer—VBPC) and acid-reactive fluoroaluminosilicate glass, Nanoparticles and Nanoclusters (69% by Wt).	<ol style="list-style-type: none"> 1. Dispense the necessary amount of Ketac Nano restorative into the Teflon mold 2. Allow Ketac Nano restorative to rest for approximately 60 seconds after placement in preparation permits the paste to become firmer and less tacky. Waiting time should not exceed the total working time (2.5 minutes). This procedure is repeated with each increment. 3. After this waiting period, light cure each increment of the material for 20 sec 	3M/ESPE, St. Paul, U.S.A
Ceram.XMono+ (Nano Ceramiccomposite)	10-25% Methacrylate modified polysiloxane (Organically modified ceramic), Dimethacrylate resin, Fluorescence pigment, UV stabilizer, Stabilizer Camphorquinone, Ethyl-4(dimethylamino) benzoate, Barium-aluminium-borosilicate glass Silicon dioxide nano filler, Iron oxide pigments and titanium oxide pigments and aluminiumsulfosilicate pigments	<ol style="list-style-type: none"> 1. Dispense the necessary amount of material from the Syringe onto a mixing Pad. 2. Pack the necessary amount of the material required for incremental placement (in 2 mm layers or less) and protect the remaining dispensed material from light. 3. Light cure each increment for 20 sec 	Dentsply Caulk, Milford, DE, USA
Beautiful II (Nano-hybrid resin based giomer material)	<p>Matrix: 16.7wt% of resin (Bis-GMA and TEGDMA).</p> <p>Filler structure: Surface Pre-Reacted Fluoroboroaluminosilicate Glass Filler, Nano Filler, Multi Fluoroboroaluminosilicate Glass Filler (68.6 vol% and 83.3 wt%)</p>	<ol style="list-style-type: none"> 1-Dispense the necessary amount of material from the syringe onto the mix pad. 2-Pack the necessary amount of the material required for incremental placement (in 2 mm layers or less) and protect the remaining dispensed material from light. 3. Light cure each increment for 20 sec. 	SHOFU INC., Kyoto, Japan

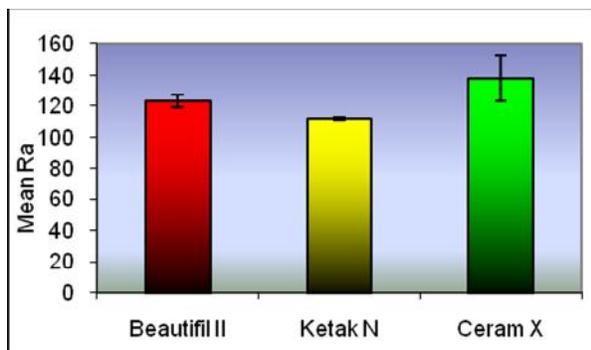


Figure (3): Bar graph representing comparison between mean surface roughness (Ra) values of the three tested materials

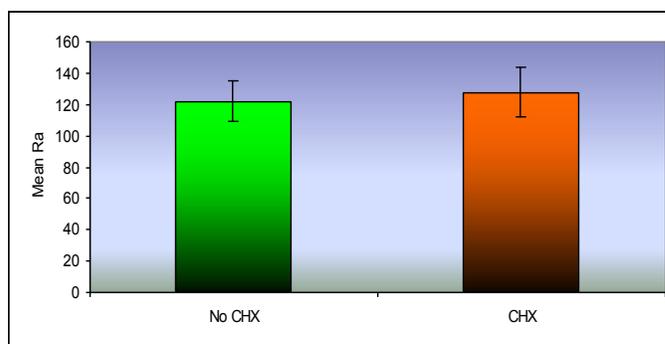


Figure (4): Bar graph representing comparison between mean surface roughness (Ra) values with and without chlorhexidine (CHX) exposure

3.3.Effect of chlorhexidine (CHX):

Tukey's test indicated that, subgroups that were exposed to chlorhexidine (CHX) showed statistically significant higher mean surface

roughness values than those that were stored in artificial saliva (fig. 4).

Table (2): Comparison between surface roughness values with different interactions:

Material	Mouth wash	Time	Mean	SD	<i>P</i> -value
Beautifil II	Control	Control (Base line)	120.35 ^d	3.4	<0.001*
	Artificial saliva (no CHX)	1 week	120.17 ^d	4.7	
		1 month	122.36 ^d	3.1	
	CHX	1 week	121.39 ^d	3	
1 month		130.22 ^c	2.8		
Ketak N	Control	Control (Base line)	110.17 ^e	4.1	
	Artificial saliva (no CHX)	1 week	110.92 ^e	3.7	
		1 month	112.22 ^e	4.1	
	CHX	1 week	111.98 ^e	2.6	
1 month		113.11 ^e	3.7		
Ceram X	Control	Control (Base line)	119.17 ^d	4	
	Artificial saliva (no CHX)	1 week	130.02 ^c	4.7	
		1 month	152.83 ^a	3.7	
	CHX	1 week	136.26 ^b	2.9	
1 month		152.96 ^a	3		

3.4.Effect of storage periods:

Figure (5) compares between surface roughness values at different storage periods. It shows that, there was a statistically significant increase in the mean surface roughness values by time since, the statistically significant highest mean surface roughness values was found after 1 month period. Whereas, the lowest mean surface roughness values were found at base line (control) subgroups

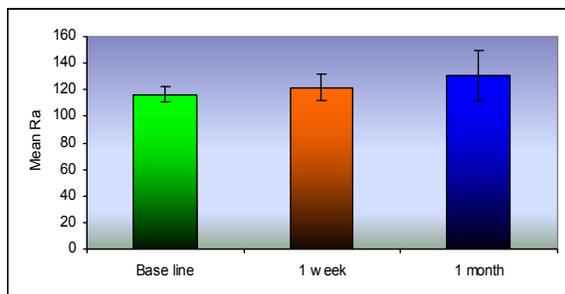


Figure (5): Bar graph representing mean surface roughness (Ra) values at different storage periods

3.5.Effect of different interactions

Table (2) shows that, there was no statistically significant difference between the Ceram X subgroup that was stored in artificial saliva for one month and the Ceram X subgroup that was exposed to CHX for one month which showed the statistically significantly highest means surface roughness values. No statistically significant difference was found between the following subgroups; Control ketak N subgroup, Ketak N subgroups that were stored in artificial saliva for one week and one month and Ketak N subgroups that were exposed to CHX for one week and one month. However, Ketak N subgroup that was exposed to CHX for a month showed the lowest means surface roughness values.

3.6.Microhardness results:

The results of the three-way ANOVA showed that, the regression model is fit to describe the relationship between the studied variables ($P \leq 0.05$). The results showed that material, storage periods and the interaction between the three variables had a statistically significant effect on mean microhardness values. Whereas, the use of CHX mouth wash had no statistically significant effect on mean microhardness values.

3.7.Effect of the material

To compare the effect of the tested material on the microhardness values, Tukey's test was used and the results were presented in figure (6), and showed that, there was no statistically significant difference between Ketak N and Ceram X which showed the statistically significant highest mean microhardness values. Beautifil II showed the statistically significant lowest mean microhardness values ($P \leq 0.05$).

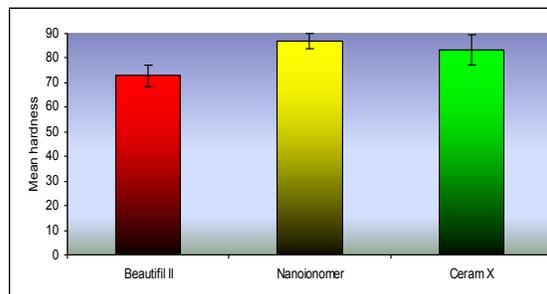


Figure (6): Bar graph representing mean microhardness values of the three tested materials

3.8.Effect of Chlorhexidine (CHX):

Figure (7) shows that, chlorhexidine had no effect on the microhardness of the three tested materials, since there was no statistically significant difference between mean microhardness values of the chlorhexidine exposed and non-exposed specimens.

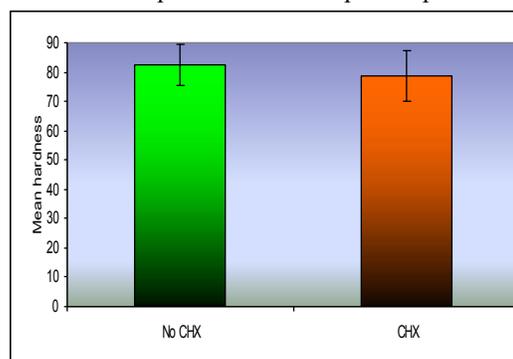


Figure (7): Bar graph representing mean microhardness values with and without CHX exposure

3.9.Effect of storage periods:

Tukey's test indicated that, there was a statistically significant decrease in mean microhardness values after 1 month. Meanwhile, no statistically significant difference found in the mean microhardness values between the control and one week storage subgroups (fig. 8).

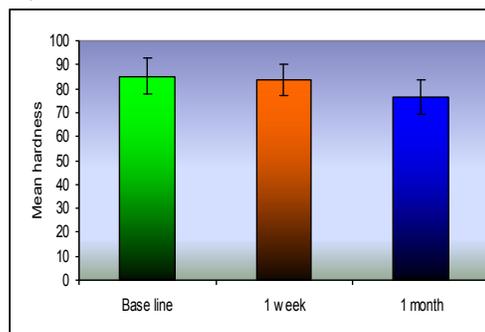


Figure (8): Bar graph representing mean microhardness values at different storage periods

3.10. Effect of different interactions:

Table (3) shows that, there was no statistically significant difference between the Ketak N control subgroup, Ketak N subgroup that was stored in artificial saliva for a week, Ketak N subgroup that was exposed to CHX for a week and Ceram X control

subgroup which showed the statistically significantly highest mean microhardness values. Meanwhile, Beautifil II subgroup that was exposed to CHX for a month showed the statistically significant lowest mean microhardness values

Table (3): Comparison between microhardness with different interactions

Material	Mouth wash	Time	Mean	SD	P-value
Beautifil II	Control	Control (Base line)	76.6 ^f	8.9	<0.001*
	Artificial saliva (no CHX)	1 week	75.5 ^f	10	
		1 month	71 ^g	7.2	
	CHX	1 week	74.7 ^e	9.1	
		1 month	66.1 ^h	6.9	
	Ketak N	Control	Control (Base line)	89.6 ^a	
Artificial saliva (no CHX)		1 week	89 ^a	10.1	
		1 month	84.9 ^b	8.4	
CHX		1 week	88.5 ^a	9.2	
		1 month	82.7 ^c	8.9	
Ceram X		Control	Control (Base line)	89.5 ^a	
	Artificial saliva (no CHX)	1 week	87.3 ^b	8	
		1 month	78 ^d	9.3	
	CHX	1 week	86.7 ^b	10.1	
		1 month	74.8 ^c	6.8	

*: Significant at $P \leq 0.05$, Means with different letters are statistically significantly different according to Tukey's test.

4. Discussion:

One of the most important prerequisite of restorative dental materials is their ability to withstand the exposure to various substances in the mouth for a considerable period of time without any change in their clinical performance⁸. Surface damage to restorative materials resulted from the chemical environment of the oral cavity had been reported by some investigations, Yap et al.,(2005). Chlorhexidine was used in this study since, in clinical dentistry it is the primary antimicrobial agent for chemical control of plaque and gingivitis, Van Strifp et al.,(1997).

A special 0.2% Chlorhexidine Digluconate exposure protocol was followed in our study in order to mimic the clinical situation in the oral cavity. Clinically, the patient was advised to use chlorhexidine mouth wash for one minute twice or three times per day. Therefore, in this study, the specimens were immersed in CHX for 1 min. 3 times daily and immersed in artificial saliva after each chlorhexidine exposure. Normally, it would not be used for more than 7 days at a time. Since, immediate and noticeable alteration in the bacterial flora had been shown after 3-day exposure to chlorhexidine, which is maintained for 7 days, however, there is a rapid return to baseline levels by 14 days, Franco et al.,(2003). Therefore, it may be used over a longer period of time. When the exposure to chlorhexidine is

increased to 9 days, the effect on the microbial flora is increased to 11 weeks and more, Franco et al.,(2003). Consequently in our study, CHX exposure protocol followed was conducted successively for one week and one month. After each storage period, the influence of chlorhexidine on three different restorative materials in terms of surface roughness and microhardness was evaluated. No previous studies in the literature were found to assess the effect of chlorhexidine on the surface roughness and microhardness of different aesthetic restorative materials. This motivated us to perform the present research. Moreover, the effect of storage in artificial saliva on surface roughness and microhardness of the three tested restorative materials was also examined.

Surface roughness evaluation in dental literatures was performed using different methods including optical profilometry, Janusa et al.,(2010) and laser scanning microscope (VK-8500, Keyence, Osaka, Japan), Hosoya et al.,(2011). However, in the present study, a novel method was performed using Quanta Environmental Scanning Electron Microscope (QESEM) and specific computer software. QESEM allows us to perform accurate and precise quantitative evaluation for the surface roughness of the tested materials.

When evaluating the effect of CHX and artificial saliva on the surface roughness of the three tested

materials, the results of this study revealed that, exposure to chlorhexidine increased the surface roughness of the three tested restorative materials more than storage in artificial saliva. This could be attributed to the difference in the pH value between CHX and artificial saliva. The pH of 2% chlorhexidine alone was inside the optimum range for its antimicrobial action, which is from 5 to 7, Basrani et al.,(2004); Freire et al.,(2010). However, in our study we used 0.2% chlorhexidine which is a diluted form. Consequently, it will have a higher pH values. Meanwhile, the pH of the artificial saliva was 7. This result was in agreement with other researchers who found that pH-challenge of different storage media had an intense effect on the properties of tested materials including surface hardness, fluoride release, wear rates and caries inhibition ability, Carvalho and Cury (1999).

Although, Machado et al.,(2011) used different concentration for CHX, they found that immersion in a chlorhexidine solution increased the surface roughness of some tested materials. This can be attributed to the higher ionic concentration of CHX solution in comparison with water or artificial saliva which might lead to more release of the soluble components such as plasticiser and decrease the sorption of water, Kazanji and Watkinson (1988); Parker et al.,(1997). Furthermore, the length of CHX exposure time could attribute to the increased surface roughness. Although our specimens were exposed to CHX for only 1 min. 3 times daily, they were not washed with water after each CHX exposure but only immersed in artificial saliva for the intervening periods. This is because of the manufacturer's recommendations which instructed not to rinse the oral cavity after being rinsed thoroughly with Colgate Periogard 0.2 % Solution. This means that, the specimens remained exposed to CHX for the whole day but in a diluted concentration.

Meanwhile, Yeh et al.,(2011), used different materials and solutions, and found that, acidulated phosphate fluoride agent (60 Second Taste Gel) following the application mode of their study, could increase the surface roughness of Premisa, Filtek Z350, and Grandio. This detrimental effect was attributed to the erosion of resin matrix and silane-treated interfaces as well as dissolution of inorganic fillers. Similarly, Borges et al.,(2011) reported that, acidic medium resulted in elution of byproducts in all materials.

Carvalho and Cury(1999)found that, pH-exposure increased the amount of fluoride released than water and the lowest value was detected in artificial saliva. Also, Turssi et al.,(2002) revealed that the surface characteristics of resin-based restoratives subjected to a pH-cycling model (demineralizing solution for 6

hours and artificial saliva for 18 hours) was significantly higher compared with both distilled deionized water and artificial saliva. This can be attributed to the difference in the pH values of the used solution in comparison to artificial saliva, Peris et al.,(2007). However, our results was in contradiction with Yap et al.,(2005), who reported that the nanofill composite and minifill composite was not significantly influenced by dietary solvents with different pH more than distilled water.

When comparing the surface roughness of each tested material, statistical analysis showed that, Ceram X had the highest mean roughness value. This was followed by Beautifil II which showed lower value. While Ketak N showed the statistically significant lowest mean surface roughness value. This could be attributed to the differences in the chemical composition, such as the type and amount of the resin monomers and the composition of the inorganic content, Pearson and Longman (1989);Khokhar et al.,(1991); Borges et al.,(2011), as well as, the degree of water sorption and hydrophilicity of the matrix resin included in the tested materials.

Ceram X, contains organically modified ceramic (ormocer) nanoparticles which are different from conventional polymers because they have an inorganic backbone based on silicon dioxide and are functionalized with polymerizable organic units to produce 3-dimensional compound polymers, Manhart et al.,(1999). According to manufacturer's data, these nanoceramic particles are inorganic-organic hybrid particles. Therefore, due to the composition differences between the three tested materials, they were not equally susceptible to changes in the surface roughness. Furthermore, some solutions might be able to promote extraction of non-reacted components in resin-based restorative materials, Borges et al.,(2011) resulting in increased surface roughness. This result was in agreement with Jung et al.,(2007)who demonstrated that Ceram-X did not yield better surface quality than did the other nanofill composites, Filtek Supreme and Tetric Evoceram. This difference was explained with the low volumetric filler content since the filler concentration of Ceram X is 76% by weight and 57% by volume of the material, Celik et al.,(2008).

Beautifil II resin matrix is composed of bis- GMA and TEGDMA. Bis-GMA was reported to show more water sorption than other resin matrices such as urethane dimethacrylate, Khokhar et al.,(1991). This water absorption may be higher than the loss of plasticiser. Therefore, an increase in volume may have occurred, Kazanji and Watkinson (1988) making the surface smoother. That is why Beautifil II showed lower surface roughness value than Ceram.X. Meanwhile, exposure of the fluoroaluminosilicate

glass nanoparticles and nanoclusters fillers present in Ketak N on the surface could lead to lower surface roughness.

In the present study, chlorhexidine had no effect on the microhardness of the three tested materials. This could be due to absence of alcohol in the used chlorhexidine mouth rinse. Since, it was reported that, resin-based restoratives are susceptible to chemical softening and reduction in the hardness by mouth rinses with high alcohol content, Diab et al.,(1991);Gürdal et al.,(2002). This result was in agreement with Rios et al.,(2008) who found that, there were no differences in the microhardness among the materials and between the saliva and the erosive challenge.

On the other hand, this result was in contradiction with some research which could be due to the use of different immersion liquid with different pH values. Gürkan et al.,(1997) revealed that regardless of alcohol concentration, both alcohol-containing and alcohol-free mouth rinses could affect the hardness of resin-restorative materials. Also, Mohamed-Tahir et al.,(2005) found that, the microhardness of the compomer and giomer materials were more affected by pH than the composite material that was evaluated. They attributed this difference to the significant compositional differences among the studied materials. Moreover, Hamouda(2011) indicated that all tested materials presented lower surface hardness as a result of storage in low pH beverages than the same materials stored in deionized water.

When comparing the microhardness of the three tested material regardless of the immersion solution, the results showed that, although Ceram X showed the highest mean microhardness values, there was no statistically significant difference between Ketak N and Ceram X. Meanwhile, Beautifil II showed the lowest mean microhardness values. This could be attributed to the differences in the resin matrix, filler particles' size, type and loading of the tested materials, Catelan et al.,(2010). Also, Bis-GMA that was present in Beautifil II was reported to result in more water sorption than other resin matrices, Okte et al.,(2006). Water molecules, may act as a plasticiser following diffusion into the polymer, thus progressively relaxing the polymer chains and subsequently lowering the hardness of the material, Campanha et al.,(2011).

The results of this study indicated that, storage for a month significantly increased the surface roughness values and decreased microhardness values. This can be explained by that, the whole hydrolytic degradation mechanism of the resin-based restoratives is a diffusion rate dependent process. This process is affected by polymer type, filler load and type, and surface treatment of the particles, Ferracane (2006).

After immersion of resin-based restorative materials in solutions, the resin matrix may swell, decreasing the frictional forces between polymer chains, Ferracane et al.,(1998). Additionally, tensile stresses are generated at the resin-filler interfaces, straining the bonds in the inorganic component. Thus, the frictional forces between filler and resin matrix are increased, easing the pull-out of the filler particles, Soderholm (1981).

These results were in contradiction with de Moraes et al.,(2008), who revealed that 6 months storage period had no significant effect on surface roughness and hardness at the subsurface layer of the tested materials. They attributed unchanged hardness of the subsurface layer as a function of aging to increased conversion of monomer and/or further post-curing cross-linking reactions in the resin phase of the tested restoratives in the course of time. Meanwhile, unchanged surface roughness during the course of the experiment was due to that, the water was changed monthly not daily. Enhanced degradation could be expected if the storage medium had been more frequently renewed.

5. References:

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