# EFFECT OF DIFFERENT CHLORHEXIDINE BASED MOUTHWASHES ON HARDNESS OF RESIN BASED DENTAL COMPOSITES.AN IN-VITRO STUDY

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## **ABSTRACT**

**Objective:** The objective of this study was to evaluate the influence of two commercially available chlorhexidine based mouthwashes of different concentrations on hardness of two resin based dental composites.

Materials and Methods: A total of 90 disc shaped specimens were fabricated from Ceram-X (Dentsply) and Filtek Z-350 (3-M ESPE) composite restorative materials. After initial hardness testing (Baseline), samples of each type of composite were randomly divided into three groups (n=30). The specimens were then stored in Mouthwash – 1, Mouthwash – 2 and Distilled water (DW). All the specimens were stored in an incubator at 37oC during the storage period. Specimens were tested for hardness at baseline, after one week and then after 4 weeks of storage span. Surface hardness measurements were done using a WOLPERT Micro Vickers tester 402-MVD (Hylec Controls, Australia). Three indentations were made with 300 g force for 30 seconds on each sample and average value of the indentations was taken as Vickers Hardness Number (VHN).

**Results:** The VHN values of Composite -1 specimens were significantly decreased upon storage in Mouthwash-1 when compared at baseline (57.36  $\pm$  0.80) to one week (54.18  $\pm$  1.57) and four weeks (53.91  $\pm$  1.57) of storage. (p-value < 0.001). Similarly, there was significant decrease in VHN values of specimens upon storage in Mouthwash-2 when they were compared at baseline (56.29  $\pm$  1.29) to one week (54.35  $\pm$  1.81) and four weeks (53.60  $\pm$  1.70) of storage (p-value < 0.001). The VHN values of Composite-2 significantly decreased upon storage in Mouthwash-1 when they were compared at baseline (57.33  $\pm$  1.49) to one week (54.35  $\pm$  1.81) and four weeks (53.60  $\pm$  1.70) of storage (p-value < 0.001). The VHN values of Composite-2 specimens were a significantly decreased upon storage in Mouthwash-2 when they were compared at baseline (57.41  $\pm$  1.68) to one week (53.13  $\pm$  1.76) and four weeks (52.73  $\pm$  1.69) of storage in CMW (p-value < 0.001).

**Conclusions:** Both the restorative materials exhibited decrease in hardness upon immersion in chlorhexidine based mouthwashes of different concentrations.

**Key words:** Hardness, Chlorhexidine, Resin based composites.

### **INTRODUCTION**

A significant breakthrough in contemporary dentistry is the improvement of light-cured resin based

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procedures¹. Present day dental composites possess superior physical and mechanical properties attributed to advancement in nano-science which is implemented in modifying the filler particles of the dental composites². Resin based composites have been tested both in-vitro and in-vivo settings for many parameters to become the material of choice for restoration of teeth³. However the dietary pattern of individuals and constant variation of pH and temperature in the oral

composite materials for direct and indirect restorative

environment may affect the surface properties of dental tissues and restorations<sup>4</sup>.

Ilie et al<sup>5</sup> and Hamouda et al<sup>6</sup> have documented in their studies that the surface of the restorative materials placed on the tooth may also be affected by the chemical action of various types of food, drinks and oral hygiene maintenance products. Among the other oral hygiene maintenance products, chlorhexidine based mouthwashes are considered to be the most effective, widely prescribed and used preparation to control plaque and gingivitis<sup>7</sup>. It is claimed that chlorhexidine was the first antimicrobial agent shown to control plaque and chronic gingivitis<sup>8</sup>. Having superior bactericidal effectiveness against gram positive as compared to gram negative bacteria, it also possess anti yeast properties<sup>9</sup>.

Hardness is the major mechanical property of materials<sup>10</sup>. This property may represent a composite resin's mechanical strength and resistance to intra-oral softening<sup>11</sup>. Gürgan et al<sup>12</sup> have reported that both alcohol contained and alcohol free mouthwashes may influence the hardness of the restorative materials. As hardness is related to material's strength and rigidity, it has implication on the longevity of restorations<sup>13</sup>.

Although the use of chlorhexidine based mouth rinses is widespread, studies investigating the effect of these mouth rinses on the hardness of restorative materials have reported conflicting results. Penugonda et al<sup>14</sup> attributed the presence of alcohol in mouthwashes responsible for the softening of composites. In another study Yap et al<sup>15</sup> showed that the effect of mouth rinses on hardness and wear depends on the restorative material used. Cavalcanti et al<sup>16</sup> found nearly similar values of Knoop hardness number when composites were used at different time intervals of exposure to curing light after storage in different mouth rinses.

Although the effect of the mouth rinses on the restorative materials may be different depending on many factors that may not be simulated in-vitro, routine in-vitro testing of such materials is suggested for a new product<sup>17</sup>. Based on this idea, an in-vitro study was conducted to observe the effect of most widely used commercially available mouth rinses in Pakistan on the hardness of two resin composite materials.

The reason of conducting such study was that every dental professional should have a knowledge of the effect of the use of chlorhexidine based mouthwashes on the surface properties of the newly developed restorative materials, as the ultimate prescriber of mouth rinses is a dentist and he/she is the person who is responsible if the durability of the placed material is affected by the regular use of such products. The values of hardness of the test material (Composite-1) were compared with those of another material (Composite-2).

The objective of the study was to compare the hardness of the two composite materials after immersion in two mouthwashes of different chlorhexidine concentrations.

The alternate hypothesis of the study was that the storage of composite materials in mouthwashes based on chlorhexidine of different concentrations affects the hardness of resin composites.

### **MATERIALS AND METHODS**

The experimental study was carried out at the Departments of Operative Dentistry and Science of Dental Materials, Dr. Ishrat-ul-Ebad Khan Institute of Oral Health Sciences, Dow University of Health Sciences, Karachi. Hardness testing was carried out in the Department of Materials Engineering at N.E.D. University of Engineering and Technology, Karachi, Pakistan.

The materials under investigation in the present study were M-2 shade of Ceram-X (Dentsply) quoted as Composite-1(Fig.1) and A-2 shade of Filtek Z-350 (3-M ESPE) quoted as Composite-2.

90 specimens were prepared from Composite-1., similarly 90 specimens were made from Composite-2. The specimens were prepared by inserting the composite material into a round Teflon mould (8 mm in diameter and 2 mm deep) (Fig. 1), covered with mylar strip and pressed between glass plates (Fig. 2,3,4).

Specimens were polymerized with a LED unit, BLUEDENT LED POWER SMART (BG Light Limited, Plovdiv, Bulgaria) for 20 seconds according to manufacturer's recommendation with a light intensity of 800 mW/cm2 from the upper and lower surfaces of the specimens. The LED unit had light tip diameter of 8 mm.

The distance between the light and the specimen were standardized by using a glass slide of 1 mm width. Afterwards, all specimens were removed from the mould (Fig. 5) and stored in distilled water for 24

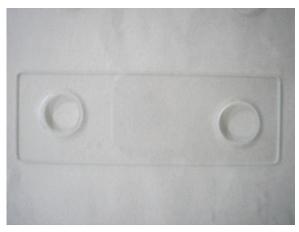


Figure - 1: Teflon mould put over even glass surface.



Figure - 2: Composite material inserted into the mould cavity



Figure - 3: Composite material condensed in the mould cavity

hours at 37°C in an incubator.

After recording the baseline hardness the specimens of each group were to be tested for the same



Figure - 4: Composite material covered with Mylar strip and pressed between glass plates.



Figure - 5: Cured disc shape specimen removed from the mould.

properties at two time intervals i.e. after 1 week and after 4 weeks of storage in distilled water, Protect Mouthwash (Roomi enterprises) quoted as Mouthwash 1 containing 0.12% Chlorhexidine and Clinica Mouthwash (Platinum Pharmaceuticals) quoted as Mouthwash 2 containing 0.2% Chlorhexidine. The specimens were subjected to hardness testing according to ASTM E384 - 10e2 (Standard Test Method for Knoop and Vickers Hardness of Materials). Vickers microhardness measurements were done using a WOLPERT Micro Vickers tester 402-MVD (Hylec Controls, Australia). The specimens were individually fixed in the apparatus and positioned in such a way that the specimen surface was perpendicular to the indenter.

A load of 300 g was applied to the surface of each specimen for dwell time of 30 seconds. Three indentations were made on top surfaces of each specimen.

The distance between each indentation was ≥ 4 larger diagonals of the indenter. This precaution was taken to prevent areas of plastic deformation produced by neighboring indentations from altering the hardness values. The average of the three readings was taken and hardness values were calculated. Pre and post-storage hardness measurements were done on the same surface of each specimen.

#### STATISTICAL ANALYSIS

Statistical Package for the Social Sciences (SPSS-16) was used for Data Analysis. Mean and standard deviation of the baseline hardness (VHN) and the post storage hardness (VHN) values of the groups were obtained and compared using One-way analysis of variance (ANOVA) Post-hoc Scheffe's test was used to analyze where the difference actually lies in the test groups. Level of significance was kept at < 0.05 for all the statistical tests.

### **RESULTS**

# Comparisons of mean hardness (VHN) values of Composite-1 specimens

Table 1. shows comparison of Vicker's hardness (VHN) baseline value and values after one and four weeks storage of Composite-1 specimens in distilled water, Mouthwash-1 and Mouthwash-2.

The VHN values of specimens were found to have no statistically significant difference at baseline (57.43  $\pm$  0.72), one week (57.19  $\pm$  0.80) and four weeks (57.06  $\pm$  0.60) of storage in distilled water (p-value = 0.501).

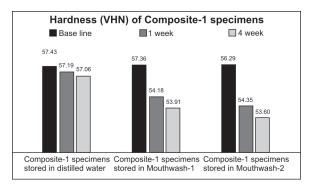


Figure - 6: Graphical representation of mean hardness (VHN) values of Composite-1 specimens at baseline, one week and four weeks storage in distilled water, Mouthwash-1 and Mouthwash-2

One-way ANOVA showed that there was a statistically significant decrease in VHN values of specimens when they were compared at baseline (57.36  $\pm$  0.80), one week (54.18  $\pm$  1.57) and four weeks (53.91  $\pm$  1.57) of storage in Mouthwash-1 (p-value < 0.001).

Post-hoc Scheffe's test showed that the statistically significant difference in VHN values lies at baseline Vs 1 week (57.36  $\pm$  0.80 Vs 54.18  $\pm$  1.57, p-value = 0.03), and baseline Vs 4 weeks (57.36  $\pm$  0.80 Vs 53.91  $\pm$  1.57, p-value < 0.001) of storage in Mouthwash-1. There was no statistically significant difference in VHN values between 1week and 4 week (54.18  $\pm$  1.57 Vs 53.91  $\pm$  1.57, p-value = 0.910) storage in Mouthwash-1.

One-way ANOVA showed that there was a statistically significant decrease in VHN values of specimens when they were compared at baseline (56.29  $\pm$  1.29), one week (54.35  $\pm$  1.81) and four weeks (53.60  $\pm$  1.70) of storage in Mouthwash-2 (p-value < 0.001).

Post-hoc Scheffe's test showed that the statistically significant difference in VHN values lies at baseline Vs 1 week (56.29  $\pm$  1.29 Vs 54.35  $\pm$  1.81, p-value = 0.03), and baseline Vs 4 weeks (56.29  $\pm$  1.29 Vs 53.91  $\pm$  1.57, p-value < 0.001) of storage in Mouthwash-2. There was no statistically significant difference in VHN values between 1 week and 4 week (54.35  $\pm$  1.81 Vs 53.60  $\pm$  1.70, p-value = 0.861) storage in Mouthwash-2.

# Comparisons of mean hardness (VHN) values of Composite-2 specimens

Table 2. shows comparison of Vicker's hardness (VHN) baseline value and values after one week and four weeks storage of Composite-2 specimens in distilled water, Mouthwash-1 and Mouthwash-2 solutions.

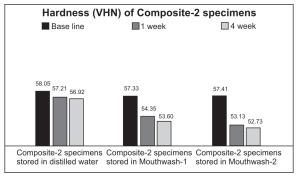


Figure - 7: Graphical representation of mean hardness (VHN) values of Composite-2 specimens at baseline, one week and four weeks storage in distilled water, Mouthwash-1 and Mouthwash-2

Table 1. Comparison of Mean Hardness (VHN) of Composite-1 specimens

Storage solution of Composite-1 specimens	Storage time			1
	Baseline	One week	4 weeks	p-value
Distilled Water	$57.43 \pm 0.72$	57.19 ± 0.80	57.06 ± 0.60	0.501
Mouthwash-1	$57.36 \pm 0.80$	54.18 ± 1.57	53.91 ± 1.57	< 0.001
Mouthwash-2	56.29 ± 1.29	54.35 ± 1.81	53.60 ± 1.70	< 0.001

Level of Significance: < 0.05

Test of Significance: One way ANOVA

Table 2. Mean Hardness (VHN) of Composite-2 specimens

Storage solution of Composite-2 specimens	Storage time			e volue
	Baseline	One week	4 weeks	p-value
Distilled Water	58.05 ± 1.41	57.21 ± 1.51	56.92 ± 1.27	.198
Mouthwash-1	57.33 ± 1.49	54.35 ± 1.81	53.60 ± 1.70	< 0.001
Mouthwash-2	57.41 ± 1.68	53.13 ± 1.76	52.73 ± 1.69	< 0.001

Level of Significance < 0.05

Test of Significance: One way ANOVA

One-way ANOVA showed that the VHN values of specimens has no statistically significant difference at baseline (58.05  $\pm$  1.41), one week (57.21  $\pm$  1.51) and four weeks (56.92  $\pm$  1.27) of storage in control solution i.e. distilled water (p-value = 0.198).

One-way ANOVA showed that there was a statistically significant decrease in VHN values of specimens when they were compared at baseline (57.33  $\pm$  1.49), one week (54.35  $\pm$  1.81) and four weeks (53.60  $\pm$  1.70) of storage in Mouthwash-1 (p-value < 0.001).

Post-hoc Scheffe's test showed that the statistically significant difference in VHN values lies at baseline Vs 1 week ( $57.33 \pm 1.49$  Vs  $54.35 \pm 1.81$ , p-value < 0.001), and baseline Vs 4 weeks ( $57.33 \pm 1.49$  Vs  $53.60 \pm 1.70$ , p-value < 0.001) of storage in Mouthwash-1. There was no statistically significant difference in VHN values between 1 week and 4 week storage in Mouthwash-1 ( $54.35 \pm 1.81$  Vs  $53.60 \pm 1.70$ , p-value = 0.609).

One-way ANOVA showed that there was a statistically significant decrease in VHN values of specimens when they were compared at baseline (57.41  $\pm$  1.68), one week (53.13  $\pm$  1.76) and four weeks (52.73  $\pm$  1.69) of storage in Mouthwash-2 (p-value < 0.001).

Post-hoc Scheffe's test showed that the statistically significant difference in VHN values lies at baseline Vs 1 week (57.41  $\pm$  1.68 Vs 53.13  $\pm$  1.76, p-value < 0.001), and baseline Vs 4 weeks (57.41  $\pm$  1.68 Vs 52.73  $\pm$  1.69, p-value < 0.001) of storage in Mouthwash-2.

There was no statistically significant difference in VHN values between 1 week and 4 week storage in Mouthwash-2 (53.13  $\pm$  1.76 Vs 52.73  $\pm$  1.69, p-value = 0.872).

# Comparison of mean hardness (VHN) values of Composite-1 with Composite-2 specimens

Table 3. shows comparison of mean hardness (VHN) values of Composite-1 with Composite-2 specimens after storage in Mouthwash-1 and Mouthwash-2 for 1 week and 4 weeks. One way ANOVA showed no significant difference in mean VHN values of Composite-1 with Composite-2 specimens after 1 week storage in Mouthwash-1 (54.18  $\pm$  1.57 Vs 54.35  $\pm$  1.81, p-value = 0.975).

There was no significant difference in mean VHN values of Composite-1 with Composite-2 specimens after 1 week storage in Mouthwash-2 (54.35  $\pm$  1.81 Vs 53.13  $\pm$  1.76, p-value = 0.721).

There was no significant difference in mean VHN values of Composite-1 with Composite-2 specimens after 4 week storage in Mouthwash-1(53.91  $\pm$  1.57 Vs 53.60  $\pm$  1.70, p-value = 0.831).

There was no significant difference in mean VHN values of Composite-1 with Composite-2 specimens after 4 week storage in Mouthwash-2 (53.60  $\pm$  1.70 Vs 52.73  $\pm$  1.69, p-value = 0.753).

### **DISCUSSION**

The requirement of an ideal restorative mate-

rial is that it should function in the same manner as dental hard tissue under different masticatory loads and changing oral environment<sup>18</sup>. Moreover, it should closely resemble in appearance and color of the tissue that it is replacing<sup>18</sup>. New materials are developed so that their properties come closer to dental tissues in the harsh oral environment<sup>19</sup>. In the present study an important properties i.e. hardness of two composite materials is analyzed before and after storage in two commercially available chlorhexidine based mouthwashes.

Hardness is associated to the strength, rigidity and intraoral softening of the restorative material in service<sup>20</sup>. Therefore, any chemical softening resulting from the use of mouthwash would have implications on the clinical durability of the material. Factors contributing to hardness of a material are the storage time, degree of conversion, chemistry of storage media and the chemical composition of the material itself<sup>21,22,23</sup>.

Depth of cure of composite material may also be related to hardness of the material<sup>24</sup>. When Zhu<sup>25</sup> and his colleagues investigated the factors that influences depth of cure; they found that when curing 2 mm or greater thicknesses of composites, light intensity and exposure duration become by far the most important influence above either shade or filler types of composite material<sup>25</sup>. Keeping this in consideration, the width of the composite specimens was kept at 2 mm in the present study.

Gürgan et al<sup>12</sup> reported that both alcohol-containing and alcohol-free mouthwashes affect the hardness of resin composites. Similarly, Penugonda et al14 stated that the alcohol content in mouthwash may affect composite hardness, whereby this softening effect was directly related to the percentage of alcohol in the mouthwash. However, Gürdal et al<sup>17</sup> argued that alcohol content in mouthwashes had no effect on the hardness of restorative materials. In the present study, on chemical analysis the mouthwashes that were used, were found to have 7.65% alcohol in Mouthwash-1, 16.45% alcohol in Mouthwash-2, and the effect of the mouthwash on surface hardness of both the composites were also different from the effect of distilled water on the test materials. In the present study, when the VHN values of specimens stored in distilled water were compared with the VHN values of specimens stored in mouthwashes, both the restorative materials showed softening. But there were no significant decrease in VHN values observed when comparison was made between the two mouthwashes used for the same storage period of each restorative material. So alcohol content may be a factor in softening of materials but its When the VHN values of the two materials were compared after storage in distilled water at baseline, one week and 4 weeks, there was no significant decrease in hardness, suggesting that storage in water does not affect the hardness of the material. Significant decrease in hardness in both the materials occurred when baseline VHN values were compared with 1 week storage in mouthwash values, suggesting that significant softening occurred in one week time of storage. No considerable softening was seen in the test materials from 1 week to 4 week storage period; this suggests that mean hardness values are not significantly decreased by storage in mouthwashes beyond 1 week time. This may be due to the reason that more absorption of water or other constituents of mouthwashes occurs in the first week of immersion.

Cavalcanti et al<sup>16</sup> tested the microhybrid composite (Spectrum TPH) for Knoop hardness after storage in distilled water, Listerine, Reach, Plax and Periogard. They found no interaction between products and storage time. Though, the hardness values decreased with the increasing storage time but KHN values were not significantly different. In the present study VHN values are taken instead of KHN values and two restoratives used contain nano particles instead of microhybrid particles in the Cavalcanti study. The comparison of hardness values was done after storage in distilled water as compared to other mouthwashes. The VHN values also decreased in the present study but there was a significant decrease in VHN values with increasing storage time. Difference in the chemical composition of the restoratives and mouthwashes used by Cavalcanti and the present study may be a reason of conflicting results seen.

When the hardness values of Composite-1 specimens and Composite-2 specimens were compared, although more softening was seen in Composite-2 specimens as compared to that of Composite-1; the result was not significant. The chemistry of Composite-1 and Composite-2 are different but the closeness in hardness values of the two different types of specimens suggests that the chemical composition have negligible influence on hardness of the restorative materials. The slight variation in hardness between the two composites can probably be explained by the

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small differences in chemistry of fillers used in the two products. Composite-1 contain nano-ceramic filler particles and the filler particles of composite-2 are nano-agglomerated<sup>26,27</sup>.

Carvalho et al<sup>28</sup> showed that the low pH of solutions may induce phenomena of sorption and hygroscopic expansion in composite restorative materials, as methacrylic acid is produced in due course resulting in enzymatic hydrolysis. In the present study, the pH value of mouthwash containing 0.2% chlorhexidine (Mouthwash-2) was 5.71 which is lower than that of mouthwash containing 0.12% chlorhexidine (Mouthwash-1, pH = 6). The pH value of distilled water used was 6.9. The lower pH of Mouthwash-2 may have changed the polymeric matrixes of the composite more than those which were stored in Mouthwash-1, by catalysis of ester groups from dimethacrylate monomer present in its composition. The hydrolysis of these ester groups may have formed alcohol and carboxylic acid molecules, which speed up the degradation of the composites, due to the decrease of pH inside the resin matrix. The significance of filler loading is also important here, since the more the loading the lesser the resin matrix, hence less effect of pH on VHN values observed.

This might also explain lower VHN values obtained for composite specimens stored in Mouthwash-2 as compared to those specimens that were stored in Mouthwash-1 or distilled water.

### **CONCLUSION**

Form this study it was concluded that:-

- Both composite-1 and composite-2 exhibited decrease in hardness upon immersion in chlorhexidine based mouthwashes of different concentrations.
- Immersion in distilled water had no significant effect on hardness of both composite-1 and composite-2.

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