CHAPTER 3

MATERIALS AND METHODS

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3.1 Fabrication of specimens

Sixty disc-shaped specimens of 10mm diameter and 2mm thickness were made from each resin composite listed in Table 3.1. The composite resin was packed into a perspex split mould (Figure 3.2) and lightly condensed into the mould using plastic instrument (Figure 3.4-a). The upper surface of the mould was then covered with a matrix strip (Figure 3.4-b) (Ruwa matrix strip, Kemdent, USA) and a microscopic glass slide was placed (Figure 3.4-c) prior to curing with a visible light curing unit (Spectrum 800, Dentsply/ Caulk USA). Excess material was extruded using finger pressure on top of the glass slides. The composite was cured through the glass slide for 40 seconds (Figure 3.4-d). The intensity of the light cure unit (500mW/cm^2) was monitored periodically throughout the experiment using a built in light intensity radiometer. The tip of the light curing unit (8mm in diameter) was placed against the glass slide to ensure uniform distance from light source to the resin composites. The matrix strip was discarded after each used. All procedure was carried out at room temperature.

Table 3.1 Resin composites restorative material used in the study

Resin composites (Manufacturer)	Resin	Filler	Batch No.
Spectrum®TPH® (Dentsply/Caulk,USA)	Bis-GMA-adduct Bis-EMA TEGDMA	Microhybrid <1.5µm & 0.04	0407000895 Exp 07 2007
Filtek™Z350 (3M ESPE, Dental Products, St.Paul Minn, USA)	Bis-GMA UDMA TEGDMA Bis-EMA	Nanohybrid 5-20nm & 0.6-1.4 µm	20051226 Exp 11 2008
Ceram•X mono (Dentsply/Caulk,USA)	Bis-GMA UDMA TGDMA	Nanohybrid < 50nm	0510000198 Exp 03 2008
Ceram•X duo-enamel shade (Dentsply/Caulk,USA)	Bis-GMA UDMA TGDMA	Nanohybrid < 50nm	0508000430 Exp 12 2007

a b c d

Figure 3.1 Resin composites restorative materials

a- TPH Spectrum

b- Filtek Z350

c- Ceram•X- Mono

d- Ceram•X- Duo-enamel shade

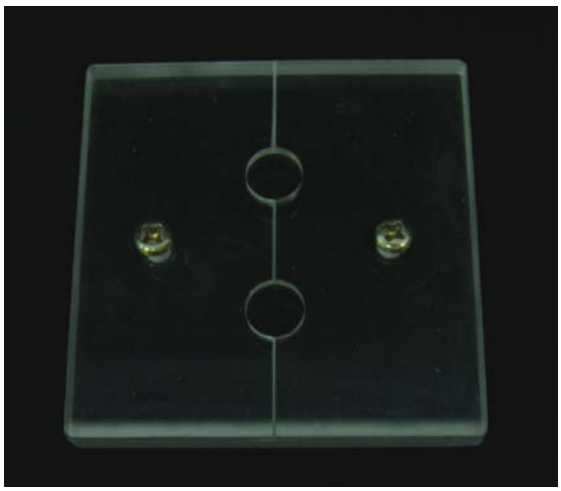
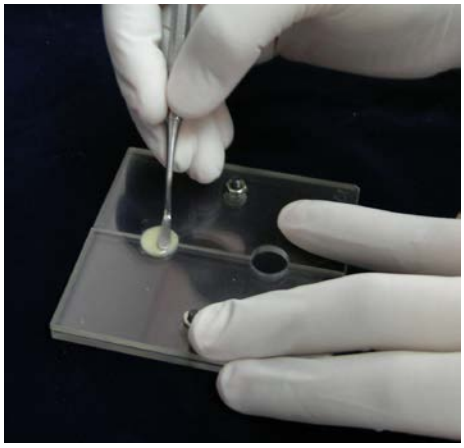
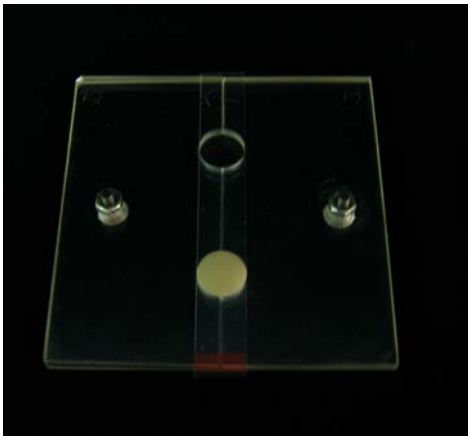


Figure 3.2 Perspex split mould

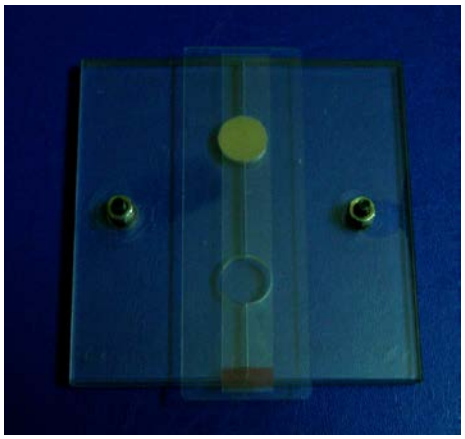
Figure 3.3 Matrix strip



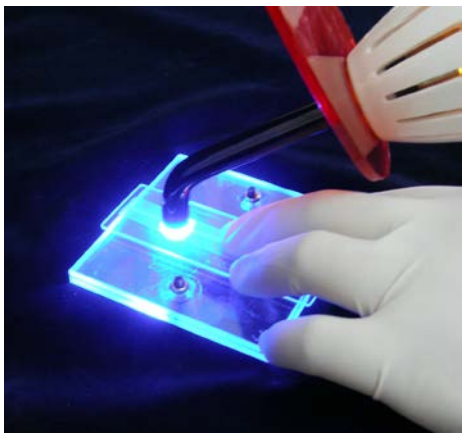
a) Resin composite was lightly condensed into the mould using a plastic filling instrument



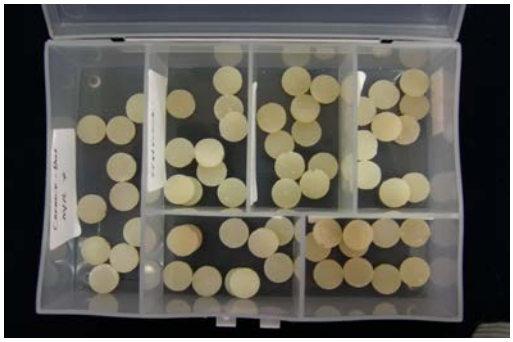
b) The upper surface of the mould was then covered with a matrix strip.



c) The upper surface of the mould then covered with a microscopic glass slide.



d) The composite then was cured through the upper side of the glass slide.



e) The specimens after curing

Figure 3.4 Procedure of specimen fabrication

3.2 Polishing of specimens

The top cured surface was polished with Sof-Lex pop-on polishing discs (3M ESPE Dental products, St.Paul, Minn, USA) (Figure 3.5) according to the manufacturer's instructions. The medium grit was used at 10,000rpm for 15 seconds followed by fine grit at 30,000rpm for 20 seconds and then superfine grit at 30,000rpm for 30 seconds. The polishing procedures were carried out using a slow speed handpiece according to the manufacturer's instructions (Figure 3.6).

A unidirectional constant motion and light pressure was applied during polishing. Samples were kept dry while polishing in order to produce a smooth and uniform finish. After each step of polishing, all specimens were thoroughly flushed with water and air-dried before the next step until final polishing. After all the specimens were polished, they were ultrasonically cleaned with distilled water in biosonic cleanser for five minutes and then placed in distilled water stored in an incubator with a temperature of 37º C for 24 hours.

3.3 Microhardness test

After 24 hours, all the specimens were blot dry and divided to six groups randomly. The top polished surface of each specimen of the groups were then measured for surface microhardness prior to immersion in mouthrinses at a load of 200g for 15 seconds using Vickers microhardness tester, model Shimadzu (Shimadzu Corp, Kyoto, Japan) (Figure 3.9). The microhardness tester was calibrated prior to testing in order to ensure reliable data is obtained. The specimens were positioned centrally beneath a digital microhardness tester (Figure 3.10) and indentation was made on the best focused surface of the composite disc. Three indentations were made from each specimen. Each indentation was made on a surface that has not been indented before. The dimensions of

the indentations were measured using the eyepiece of the microscope of the hardness tester and the hardness value was automatically calculated in Vickers Hardness Number Formula (VHN).

The six groups of the specimens were then immersed in 20ml of five types of mouthrinses and distilled water (Table 3.2), (Figure 3.7 and Figure 3.8) and stored for 24 hours in an incubator at a temperature of 37º C. Then all the specimens were blotted dry and the surface microhardness was measured again.

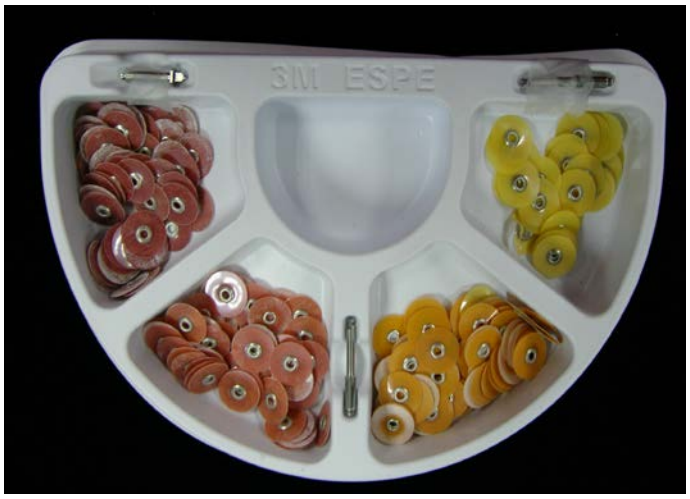


Figure 3.5 Soflex polishing discs

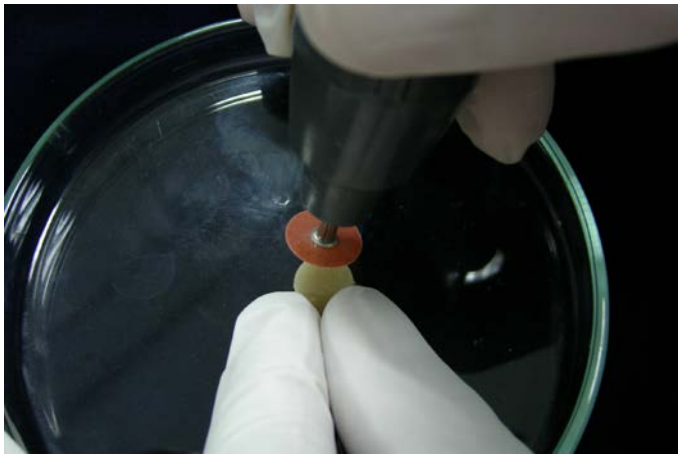


Figure 3.6 Polishing the specimen using soflex polishing disc with slow speed handpiece

Table 3.2 Mouthrinses used in the study

Mouthrinses	Manufacturer	Composition	Alcohol content
Listerine antiseptic mouthwash	Warner-Lamber Co.Morris Plains. NJ,USA	Water Ethanol 27% Menthol 0.042% Eucalyptol 0.09% Thymol 0.064% Metyl salicylate 0.06% Benzoic acid 0.15%	27%
Oral B- Tooth and gum care alcohol-free mouth rinses	Oral B Laboratories, Belmont,Ca,USA	Cetylpyridium chloride 0.053% Sodium fluoride 0.05% Sodium benzoate 0.025% Metylparaben 0.1% Polyparaben 0.01% Others- purified water, glycerine, flavour	0%
Mouthrinse X	Oral Biology Dept, Dental Faculty, University of Malaya	Plants extract (guava leaves, mint leaves and mango leaves) Sterile water	0%
Mouthrinse Y			
Mouthrinse Z			
Distilled water			0%

a b c d e f

Figure 3.7 Mouthrinses used in the study

a- Listerine

b- Oral-B

c- Mouthrinse X

d- Mouthrinse Y

e- Mouthrinse Z,

f- Distilled Water

Figure 3.8 Specimens in mouthrinses

Figure 3.9 Shimadzu Vickers microhardness testing machine

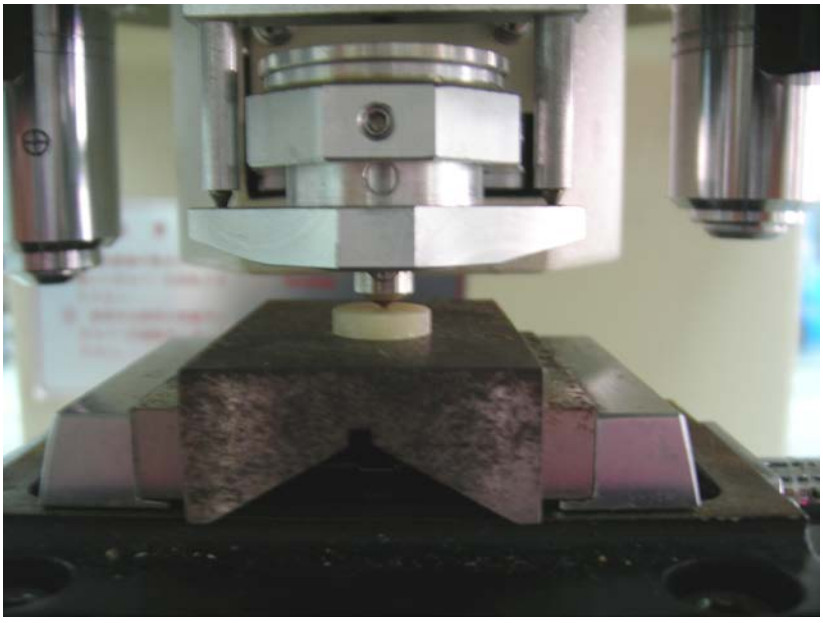


Figure 3.10 Indenter on the specimen

3.4 Surface analysis

Surface analysis using Atomic Force Microscopy (AFM) (Ambios Technology Universal- Scanning Probe Microscope SPM™) (Figure 3.11) was afterthought to evaluate surface changes of specimens after immersion in six different solutions. AFM used the scanning probe microscopy to probe the surfaces at atomic resolution. The system generates 3D topography and allows surface analysis of samples all in one system. The lateral scan range was set at 40µm. One specimen was randomly chosen from each group of composite after immersion in various mouthrinses and distilled water. The specimen was placed still on the measuring table and the scanning using contact mode and the detection was done using deflection of the cantilever probe. The surface analysis in three-dimensional and surface roughness measurement in Ra was calculated and presented by the software of AFM.

Figure 3.11 Universal-Scanning Probe Microscope (USPM™)

3.5 Research design and data analysis procedure

3.5.1 Research design

In this experimental research, two research designs were employed. The first, a univariate between-subjects research design with one independent variable (IV) and one dependent variable (DV). The IV was composites type comprising of four levels (TPH Spectrum, Filtek Z350, Ceram•X Mono and Ceram•X Duo-enamel shade and the DV was Vickers surface hardness. The second was a univariate between-subjects research design with two independent variable (IV) and one dependent variable (DV). The IV were composites type comprising of four levels (TPH Spectrum, Filtek Z350, Ceram•X Mono and Ceram•X Duo-enamel shade and the second IV were solution type comprising of six levels (Listerine, Oral-B, three experimental herbal-based mouthrinses and distilled water). The DV was Vickers surface hardness.

3.5.2 Data analysis procedure

The statistical procedure used for the first research design will be comparing the means of the different levels of the IVs using the One-way Analysis of Variance (ANOVA). After which the initial mean VHN readings (before immersion in mouthrinses) will be randomized using SPSS Version 13.0 to constitute the control group for this study.

Data collected for the test groups will be subjected to SPSS Descriptives to evaluate assumptions to meet univariate analysis of variances. Two main effects will be tested in this study i.e. composites and mouthrinses. Dunnet t-double sided test will be used to compare the difference between control (VHN before immersion) and test group (VHN after immersion). Post-hoc test will be carried out for multiple comparisons between subject effects.