COMPARATIVE STUDY ON THE EFFECT OF FOUR DISINFECTANT SOLUTIONS ON WETTABILITY OF ELASTOMERIC IMPRESSION MATERIALS: AN IN-VITRO STUDY

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Abstract

An in-vitro study was undertaken to evaluate effect of four recommended commercially available disinfectant solutions on the wettability of three types of elastomeric impression materials at 10 and 30 minutes time intervals. The impression materials evaluated were addition silicone, condensation silicone and polyether. The disinfectants used were 2% glutaraldehyde, 0.5% sodium hypochlorite, 0.05% iodophor and 0.25% benzalkonium chloride. 100 samples of each impression material was made and subjected to 10 different immersion protocols including distill water as control. The surface wettability (contact angle) of all samples was measured using the contact angle analyzer (Digidrop, Contact angle meter, GBX products, France). The data obtained was statistically analyzed. Among the elastomeric impression materials evaluated, wettability wise polyether proved to be the best impression material followed by addition silicone and condensation silicone. Disinfection with 2% glutaraldehyde had shown lowest contact angle values followed by 0.5% sodium hypochlorite solution, 0.25% benzalkonium chloride and 0.05% iodophor respectively.

Key words – Disinfection, hydrophilic, wettability, contact angle, elastomers.

Introduction

Impression making is a routine procedure in all dental surgeries and most often contamination of dental impressions with varying amount of blood and saliva does occur. Therefore these impressions have the potential to transmit serious diseases to all dental personnel who routinely handle them. Furthermore it has been shown that contaminated impressions can cross-infect stone casts that have been poured against them. It is therefore imperative that the standard protocols for disinfecting dental impressions are followed for all patients. Under everyday conditions, it is mainly chemical disinfection processes, which are suitable. [1,2]

Silicone impression materials, polyether and zinc oxide eugenol are popular among dentists and are used for majority of impression making procedures. Apart from being dimensionally and chemically stable, an impression material should possess surface properties that allow it to be easily wetted by a standard mix of gypsum product. Inadequate wetting of an impression results in the incorporation of air bubbles and voids in stone casts, which play a pivotal role in success or failure of prosthesis. [3,4] There is a need to disinfect the impressions, especially after removing from oral cavity. But these disinfectants may alter the wettability property of some of the impression materials rendering them more or less wettable by slurry of gypsum. To date, little information is available on the changes in impression material wettability as a function of exposure to a disinfectant.

Nowadays most dental schools and hospitals as well as an increasing number of practitioners and laboratories disinfect their impressions before using them for the construction of casts. Guidelines about the proper procedures are continuously issued by major authoritative bodies, manufacturers, various researchers and standard textbooks [1-6]. However, the recommendations are often not detailed enough and in many cases they contradict each other, concerning what particular solution, method or application time is suitable for the disinfection of each impression material. The available literature review revealed various combinations of impression materials/disinfecting solutions with different time intervals and concentrations creating confusion [6-17].

The present study was therefore undertaken to evaluate the effect of four recommended commercially available disinfectant solutions on the wettability of four different types of impression materials with two different recommended time intervals (10 minutes and 30 minutes).

Materials and methods

In this study four impression materials were evaluated i.e addition silicone (Betasil® vario light, Müller-Omicron, Germany), condensation silicone (Alphasil® perfect light, Müller-Omicron, Germany), polyether (Impregnum ™ soft, 3M ESPE®, USA) and zinc oxide eugenol (DPI® Impression paste, DPI® dental products, DPI India). All materials used in this study were low-viscosity materials in an effort to achieve a homogeneous void-free mix. For the fabrication of the samples, a custom fabricated mould having a diameter of 30 mm and width of 3mm was used (Figure 1). The impression materials were dispensed and mixed as per manufacturer's directions. The custom made metal mould was cleaned with 99% ethanol and placed on a glass slab over which a polyethylene strip of same size had been placed. The appropriately mixed impression materials were loaded in the mould. Care was taken during filling of the mould to avoid incorporation of air bubbles. Another polyethylene strip & clean glass slab was placed immediately on the over filled mold. A clamp was used to apply uniform pressure on top of the overfilled mould. Impression samples were allowed to set for time duration as prescribed by the manufacturer before separation from the mould. All the samples were inspected and those with visible defects were discarded and remade. The impression material samples were handled with forceps and the operator wore nitrile gloves throughout the procedures to avoid any contamination. Samples for each impression material were prepared (100 disc shaped flat surface samples for each impression material).

Four ADA recommended disinfectants were used in this study. The disinfectants used were 2% glutaraldehyde, 0.5% sodium hypochlorite, 0.05% iodophor and 0.25% benzalkonium chloride. The water was obtained from a distilled water plant and used as the control.

The impression materials were grouped into four groups and disinfectants were categorized into four categories with control distilled water forming the fifth category. Samples of each group were prepared using the custom fabricated mould. After preparation of the samples for different impression materials, they were disinfected in requisite concentration of disinfectants, thus forming ten sets of different protocols as shown in Table 1. 10 samples of respective impression material was subjected to immersion disinfection in all four selected disinfectant and distilled water for two time durations i.e 10 minutes and 30 minutes respectively. After completion of disinfection protocol of each impression material, they were evaluated for their wettability. This was carried out by measuring the contact angle for each sample using a contact angle analyzer (Figure 2).

For measuring contact angle a saturated solution of calcium sulphate dihydrate was used.[7,8,11] Before and after disinfection, the samples were rinsed for 10 seconds. To determine the contact angle equal size drops of saturated solution of calcium sulphate dihydrate i.e 0.05 ml were dispensed over the surface of the sample using a calibrated microburette. The image of the drop of the solution contacting the sample (interface) was captured automatically by the camera within 30 seconds. Two contact angle readings were taken for each drop at either ends of the image (right and left) of the drop by the computer software. Average of both the readings was calculated to get the final reading of contact angle for each sample by the computer software.

The data obtained was statistically analyzed and the mean and standard deviation were calculated for each group. 'Three-way- ANOVA' was carried to test the contributions of the three Main Effects (Emersion Time, Impression Material and Disinfectant Solutions) and their Interactions. We have also compared two treatment combinationswith smallest mean contact angles, namely Time-10 minutes x Material PE x Disinfectant DW and Time-10 minutes x Material PE x Disinfectant 2% GD, using Students-t test. For statistical analysis, the statistical software "MINITAB-1513" was used.

Results

The experiment consists of three factors: A = Immersion Time at two levels (10 minutes and 30 minutes), B = Impression Material at four levels (AS, CS, PE and ZE) and C = Disinfectant at five levels (2%GD, 0.5%SH, 0.05%IO, 0.25%BC and control(DW))., resulting in 40 Treatment combinations.

Table-2 shows mean/ Std Dev of contact angles for all 40 different combinations of impression materials and disinfectants for each immersion time: 20 combinations of (Materials x Disinfectants) with each immersion time.

Combinations of materials and disinfectants with 10 minutes immersion time have lower mean values compared to the same combinations with the immersion time of 30 minutes except in case of CS* IO.

In the 10 minutes group the least mean ± SD is 33.57± 1.26 for PE* DW(Control) combination. The highest mean ±SD is 103.26 ± 1.43 for CS*IO.

In the 30 minutes group the trend is similar to that of 10 minutes group. The lowest mean ±SD is again for PE* DW(Control) as 36.34 ± 0.13 but the highest mean ± SD (102.35±0.49) is observed for CS*BC.

In both of the time groups the combinations of CS with different Disinfectants produce the highest mean values (74.74 with DW to 103.26 with IO in 10 minutes group and 77.06 with DW to 102.35 with BC in 30 minutes group). Combinations of PE and Disinfectants produce the smallest ones (33.57 with DW to 57.22 with IO in 10 minutes group and 36.34 with DW to 58.38 with IO in 30 minutes group)

For each material mean contact angles are the lowest for disinfectant DW and the highest for IO in both Immersion Time groups.

Table-3 shows Number of observations (N), Mean and Std Dev(S.D.) of contact angle values for each factor at different levels. These values provide overall contribution of factors at different levels and will be needed for post ANOVA pair wise comparisons of levels within each Main Effect (Factor).

Statistics for Immersion Time in respect of each interval are based on 200 observations on all 20 combinations of materials and disinfectants. For Immersion Time of 10 minutes mean ± S. D. equals 73.56 ± 19.82. For Immersion Time of 30 minutes the values are as 76.95 ± 19.65. Data are represented by a Box Plot in (Figure 5).

In case of Impression Material for each level (Material), the mean and S.D . are based on 100 observation on all 10 combinations of Time and Disinfectants. For Addition Silicone (AS) Mean ± S. D. equals 79.29 ± 9.36. For Condensation Silicone(CS), Polyether(PE) and Zinc oxide Eugenol (ZE) Means ± Std. Dev. . are (95.33 ± 10.23), (46.83 ± 8.64) and (80.57 ± 6.65) respectively. CS shows the highest mean c-angle (95.33) followed by ZE(80.57), AS(78.29) and PE(46.83). The data by impression material are presented in Figure 6.

As regards different disinfectant solutions Means and standard deviations are given by (75.05 ± 22.62), (76.55 ± 18.72), (82.95 ± 16.05), (80.97 ± 17.37) and (60.76 ± 15.75) due to GD, SH, IO. BC and DW respectively. IO has the highest mean value (82.95) followed by BC(80.97). SH(76.55), GD(75.05) and DW(60.76) being the lowest. The data by disinfectant are presented in Figure 7**.**

For testing the significance of different sources of variation namely three main effects (factors), three second order interaction and one third order interaction procedure suggested by Satterthwaite, F. E. explained in Experimental Designs (Cochran, W. G. and Cox, G. M.) is used to test the significance of the main effects(Factors) in the presence of (significant) interactions. The results of ANOVA are in given in Table-4.

The Third Order Interaction is highly significant ($F = 18.12$, $P = 0.0001$). The interaction (Time x Material) is not significant ($F = 0.49$, $P = 0.1322$). This means that change in the immersion time does not influence the performance of materials. The significance of the third order interaction is result of the last two second order interactions.

However Interaction (Immersion Time x Disinfectant) as well as interaction (Materials x Disinfectant) are significant ($F = 5,39$, $P = 0.0130$ and $F = 12.13$, $P = 0.0001$). Significance of Interaction (Time* Disinfectant) indicates duration of immersion influences the effect of disinfectants. Similar explanation holds for the interaction (Material * Disinfectant).

Net effect of immersion time is significant at 5% level ($F = 6.95$, $P = 0.0486$) indicating that mean contact angles change with the change. in the duration of immersion time. However mean contact angle has increased by only 3.29 from 73.56 to 76.95 for an increase 20 minutes in the immersion time (Table-3). From practical consideration this may not be significant.

Net effect of Disinfectant is significant at 1% level ($F = 11.98$, $P = 0.0097$) implying significant differences in the means of contact angle form one disinfectant to another. Mean contact angles vary from a minimum of 60.76^0 due to DW to a max of 82.95 due to IO. GDS and SH are practically equal with mean values of 75.05 and 76.55 respectively. So is the case with respect to IO and BC: means equal to 82.96 and 60.97 respectively.

Net effect of Impression Material is significant with $F=113.74$ and $P = 0$. This implies highly significant differences in the means of contact angle form one material to another. The least mean contact angle is for PE= 46.83^0 and highest due to CS = 95.33^0. As and ZE are practically equal with means equal to 79.36 and 80.57 respectively.

Thus statistical analysis shows that - Best Emersion Time = 10 minutes, best material as PE and best disinfectant as DW. Considering the net effects of the three Factors and their interactions the best choice has to be one of the combinations of these three.

From Table-2 we find that there is a huge difference of 69.49^0 in mean contact angles from 103.06^0 for the combination (10 min x CS x 0.05%) to 33.57^0 for the combination (10 min x PE x DW). The best option is (10 minutes PE x DW) with (Mean ± SD as $33.57^0 \pm 1.26^0$) closely followed by (10 min x PE x 2% GD) with (Mean ± SD as 37.70 ± 0.87).These two smallest combinations are compared using Students-t test. Results are in Table-5. Difference of even 4.13^0 is statistically highly significant (T-Value = 26.85, p-Value = 0.00001). Thus statistically the best choice comes out to be the combination (Time-10 minutes x Material PE x Disinfectant DW)

Discussion

Dental impressions have the potential to transmit serious diseases to all dental personnel who routinely handle them. The routine procedure of rinsing impressions under tap water immediately after removal from the mouth eliminates gross contamination along with saliva and blood but not all microorganisms are removed and they can be a source of infection. There appears to be a great deal of conflict surrounding the impression disinfection techniques being used by dental offices and laboratories. Although various Governmental and private organizations like ADA (American Dental Association); OSHA (Occupational and Safety Hazards Organization); CDC (Centre for Disease Control, Government of United States, Department of labor) and dental literature provide guidance about how specific impression materials should be disinfected to balance the goals of safety and accuracy, they cannot offer definitive answers to the problems at hand because there is no faultless universal disinfectant. [1-4]

Disinfection eliminates virtually all recognized pathogenic microorganisms but not necessarily all microbial forms, on inanimate objects. Different methods for disinfection of impression materials have been suggested that includes argon radiofrequency glow discharge, ethylene oxide, autoclave, microwave, ultraviolet radiation and chemical disinfectant solutions. Among the different methods mentioned above, chemical disinfection is more commonly employed. Various agents used for chemical disinfection include alcohols, chlorine and chlorine compounds, formaldehyde, glutaraldehyde, ortho-phthalaldehyde, hydrogen peroxide, iodophors, peracetic acid, phenolics, and quaternary ammonium compounds. Disinfectants are available as spray and immersion solutions in different concentrations.[5-6]

Immersion disinfection is the most reliable method as it guarantees that all surfaces of impression and impression tray will come into contact with disinfectant solution. In 1991 ADA released new guidelines that recommended immersion disinfection for all the impression materials including zinc oxide eugenol impression material, addition silicone, condensation silicone and polyether, provided recommended time of disinfection is used.[6] The contact time for the various products used as disinfectants showed variations from 3 to 30 minutes.[4] Published reports in the dental literature often are varied regarding the time of immersion and concentrations. [1,2] Traditionally the iodophors, chlorine compounds, glutaraldehydes, phenols, benzalkonium chloride have required exposure time ranging from 10 to 30 minutes. The preferred concentrations of commonly used disinfectants are as follows - Sodium hypochlorite : 0.5 to 1%, glutaraldehyde : 2 to 3.2%, povidine-iodine : 0.1%, formaldehyde : 4%, chlorhexidine : 0.5 %, ethanol : 50%, formalin : 10%, benzalkonium chloride : 0.25%.[2]The manufacturer's recommended exposure time for given disinfectant should be interpreted as the minimum exposure time. However that time may be exceeded if necessary but may not be reduced. Published reports in the dental literature often are varied regarding the time of immersion and concentrations. Therefore this study was taken up to find out the reliable time duration and concentration ideally suited for disinfection of routinely used impression materials.

Although elastomeric impression materials offer number of advantages for routine clinical procedures, one of the drawbacks of these materials is poor wettability. In spite of repeated claims by the manufacturer that their material is superior and hydrophilic, we routinely encounter impression surface defects due to poor wettability. Wettability is defined as the ability of a liquid to spread over the surface of the solid. Contact angle or wetting angle is the angle formed at the interface between the droplet and the horizontal surface. A liquid is considered to be wetting a surface when contact angle is less than 90 degrees and is considered non-wetting when contact angle is more than 90 degrees. Thus an impression material is considered hydrophilic if the contact angle is less than 90 degrees. [3, 4] There is an ongoing effort by dental manufacturers to create impression materials with improved wetting properties. Disinfection solutions may alter the surface characteristics of these newer materials. [8-20]

When the impression materials were evaluated for mean contact angles after immersion in various disinfectants for 10 and 30 minutes immersion time intervals(Table 2), it was seen that the mean contact angle values for Impregnum ™ soft (polyether) showed highest wettability compared to Betasil® vario light (addition silicone) and Alphasil® perfect light (condensation silicone) after being subjected to 2% glutaraldehyde, 0.5% sodium hypochlorite, 0.05% iodophor and 0.25% benzalkonium chloride including control (distilled water). It was seen that overall for all disinfection protocols the subgroups of polyether showed the better wettability results followed by addition silicone and condensation silicone. All the elastomeric impression materials evaluated in this study were found to be hydrophilic since their contact angle values were less than 90 degrees when subjected to control (distilled water). However the contact angle values between polyether and addition silicone differed substantially, implying polyether is more hydrophilic than addition silicone. Zinc oxide eugenol also can be considered as hydrophilic as the contact angle value was less than 90 degrees and remained below 90 degrees after being subjected to all the disinfectant protocols. A higher immersion interval or exposure period caused significant increase in the contact angle values of the irrespective of impression materials and disinfectants. Among all disinfectant solutions, for glutaraldehyde and sodium hypochlorite, there were highly significant differences in wettability factor depending on the time of immersion.10 minutes immersion produced lower contact angle values. It was seen that all impression materials subjected to control (distilled water) also showed increase in contact angles based on immersion period, however this increase here may not be clinically significant as the differences were less and all of impression materials had contact angle less than 90 degrees making them hydrophilic. From these results it is imperative that immersion time interval should be carefully selected for usage of these disinfectants.

Since practically for all combination of materials and disinfectants the contact angle values differed significantly between two immersion time intervals, 'three–way-ANOVA' test was carried out for different combinations of impression materials and disinfectants along with control (distilled water) for each immersion time interval separately, to evaluate if the performance of different impression materials differ statistically significantly from one another as well as to find the same about different disinfectants. Since the test showed significant differences among impression materials as well as different disinfectants, pair wise comparison for impression materials and disinfectants including control was done using the 'Tukey's simultaneous test' procedure(Table 4). From the results of three way ANOVA test (Table 3), we can see that there were significant differences in the mean contact angles among all impression materials. Results show that highly significant difference between the levels of all main factors as well as all interaction between the main effects: Time of immersion, Cast Materials and Disinfectants. For all main effects P values are practically zero. Mean Contact Angle (76.95) due to 30 minutes duration is significantly higher compared to Mean Contact Angle (73.56) due to 10 minutes duration. For the 10 minutes immersion interval, the subgroups subjected to sodium hypochlorite gave the lowest contact angle values. Therefore while using sodium hypochlorite as a disinfectant; the recommended time of immersion is 10 minutes. Disinfectant glutaraldehyde closely followed sodium hypochlorite as far as wettability is concerned and 10 minute immersion produced lower contact angles. So it is suggested to use 10 minutes of immersion in 2% glutaraldehyde solution for effective disinfection without affecting wettability. When impression materials with different disinfectant solutions were evaluated for wettability factor, it was seen that polyether had shown the least contact angle values indicating hydrophilicity, followed by addition silicone. For condensation silicone, the contact angle values were quite high after immersion disinfection indicating hydrophobicity and therefore, least preferred compared to addition silicones and polyether. All the disinfectants influenced the wettability of zinc oxide eugenol and contact angle values increased after immersion disinfection.

Disinfection protocols for impression materials should be routinely followed in dental surgeries to avoid cross infection. But care should be taken so that these procedures are not creating dimensional inaccuracies leading to a faulty cast. Selection of a disinfectant for impressions is an individual choice, but to avoid dimensional changes, time of immersion should be taken care of. Based on this study 10 minutes disinfection with either 0.5% sodium hypochlorite or 2% glutaraldehyde produced better results compared to 30 minutes immersion. Therefore 10 minutes disinfection protocol with any of the disinfectant solutions mentioned before is recommended for routine disinfection of elastomeric impression materials and zinc oxide eugenol impression paste.

Silicone impression materials are available in various consistencies. In this study, only some of the low viscosity impression materials were evaluated. It is unreasonable to expect similar results with various other brands of impression materials with various consistencies subjected to different immersion protocols with disinfectant solutions. The objective of this study was only to evaluate the effect of disinfectant solutions on wettability of elastomeric impression materials. The other factors like dimensional changes and effect of different concentrations of various disinfectant solutions on wettability were not investigated. Further research can be taken up to overcome these limitations.

Conclusions

Within the limitations of this study, the following conclusions can be drawn:

1. All disinfectant solutions decreased the wettability (increased contact angle values) of all the impression materials evaluated irrespective of immersion time interval.

2. The mean contact angle values of condensation silicone were highest among elastomers for both 10 minutes and 30 minutes immersion time interval followed by addition silicone and polyether. The contact angle values of zinc oxide eugenol samples were higher than addition silicone and polyether.

3. Among all disinfectant solutions, immersion in 2% glutaraldehyde and 0.5% sodium hypochlorite revealed highly significant differences in contact angle values compared to 0.05% iodophor and 0.25% benzalkonium chloride depending on the duration of immersion.10 minutes immersion produced lower contact angle values.

4. The mean contact angle values differed significantly depending on the immersion time interval among all impression materials. The results were highly significant for the 10 minutes immersion interval. Disinfection with 0.5% sodium hypochlorite solution had shown lowest contact angle values followed by 2% glutaraldehyde, 0.25% benzalkonium chloride and 0.05% iodophor respectively.

5. The contact angle values increased considerably when subjected to 30 minutes of immersion with different disinfectants and the results were highly significant . Disinfection with 2% glutaraldehyde had shown lowest contact angle values followed by 0.5% sodium hypochlorite solution, 0.25% benzalkonium chloride and 0.05% iodophor respectively.

6. Among the elastomeric impression materials evaluated, wettability wise polyether proved to be the best impression material followed by addition silicone and condensation silicone.

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TABLE-1: Disinfection protocol for GROUP AS (Addition silicone impression material)

Method	Subgroups	Disinfection protocol	No. of Samples
I	AS,GD,10	Addition silicone specimens immersed in 2% gluteraldehyde for 10 minutes	10
II	AS,GD,30	Addition silicone specimens immersed in 2% gluteraldehyde for 30 minutes	10
III	AS,SH,10	Addition silicone specimens immersed in 0.5 % sodium hypochlorite for 10 minutes	10
IV	AS,SH,30	Addition silicone specimens immersed in 0.5 % sodium hypochlorite for 30 minutes	10
V	AS,IO,10	Addition silicone specimens immersed in 0.05% iodophor for 10 minutes	10
VI	AS,IO,30	Addition silicone specimens immersed in 0.05% iodophor for 30 minutes	10
VII	AS,BC,10	Addition silicone specimens immersed in 0.25% benzalkonium chloride for 10 minutes	10
VIII	AS,BC,30	Addition silicone specimens immersed in 0.25% benzalkonium chloride for 30 minutes	10
IX	AS,DW,10	Addition silicone specimens left untreated without disinfection immersed in distilled water for 10 minutes	10
X	AS,DW,30	Addition silicone specimens left untreated without disinfection immersed in distilled water for 30 minutes	10

NOTE: Combinations are similar for CS, PE and ZE.

TABLE-2: MEAN / STD. DEV: CONTACT ANGLE; (N = 10 FOR EACH CELL)

Immersion	Impression	Disinfectants				
Time	**Materials**	**2% GD**	**0.5% SH**	**0.05% IO**	**0.25% BC**	**Control(DW)**
10 Minutes	**AS**	72.55/ 1.3	74.98/ 1.98	87.82/ 1.46	84.87/ 1.16	62.50/ 0.85
	C S	97.20/ 0.79	93.26/ 0.53	103.26/ 1.43	101.67/ 0.94	74.74/ 0.57
	PE	37.70/ 0.87	44.21/ 1.12	57.22/ 0.82	53.80/ 1.67	33.57/ 1.26
	ZE	84.53/ 0.57	78.19/ 1.22	82.40/ 2.22	80.13/ 1.96	66.62/ 0.49
30 Minutes	**AS**	76.55/1.42	86.09/ 1.33	88.30/ 2.33	85.20/ 2.81	64.04/ 0.57
	C S	102.56/ 0.71	101.81/ 0.74	99.42/ 1.35	102.35/ 0.49	77.06/ 0.99
	PE	41.50/ 2.67	50.90/ 1.24	58.38/ 1.37	54.66/ 1.67	36.34/ 0.13
	ZE	87.77/ 1.66	82.94/ 0.47	86.78/ 1.36	85.08/ 0.36	71.25/ 0.44

TABLE-3: OVERALL MEAN AND STD DEV: EACH FACTOR (LEVEL WISE)

Factors	Levels	N	Mean	S. D.
Immersion Time	**10 Minutes**	200	73.56	19.82
	30 Minutes	200	76.95	19.65
Impression Material	**Addition Dsilicon (AS)**	100	79.25	9.36
	Condensation Silicon (CS)	100	95.33	10.23
	Polyether (PE)	100	46.83	8.64
	Zinc Oxide Eugenol (ZE)	100	80.57	6.65
Disinfectant	**2% Glutaraldehyde (GD)**	80	75.05	22.62
	0.5% Sodium Hydrochloride (SH)	80	76.55	18.72
	0.05% Iodophor (IO)	80	82.95	16.05
	O,25% Benzal. Chloride (BC)	80	80.97	17.37
	Control (DW: Distilled Water)	80	60.76	15.75

TABLE-4: GLM: THREE FACTOR ANALYSIS

FACTOR	TYPE	LEVELS	VALUES
TIME (Minutes)	Fixed	2	10, 30
MATERIAL	Fixed	4	AS, CS, PE, ZE
DISINFECTANT	Fixed	5	BC, DW, GD, IO, SH

ANOVA

SOURCE	DF	SS	MS	F	P
Time	1	1147.9	1147.9	**6.95**	**0.0486****
Material	3	124867.0	41622.3	**113.74**	**0#**
Disinfectant	4	24280.8	6070.2	**11.98**	**0.0097****
Time* Material	3	43.1	14.4	**0.49**	**0.1322NS**
Time* Disinfectant	4	619.9	155.0	**5.39**	**0.0130***
Material* Disinfectant	12	4221.8	351.8	**12.13**	**0.0001******
Time* Material *Disinfectant	12	347.6	29.0	**16.12**	**0.0001******
Error	360	646.9	1.8		
Total	399	156175.1			

NOTE: NS: Not Significant; *: Significant at 5%; **: Significant at 1%; *: Significant at 0.1%; ****: Significant at 0.01%; #: Significant at 0%**

TABLE-5: Student's t Test Between Time-10 Minutes. x Material PE x Disinfectant DW and Time-10 minutes x Material PE x Disinfectant 2% GD

Combinations	Mean ± SD	Diff (Means)	t-Value	p-Value
10Min X PE X DW	$33.57^0 \pm 1.26^0$	4.13	26.85	0.00001
10Min X PE X 2% GD	37.70 ± 0.87			

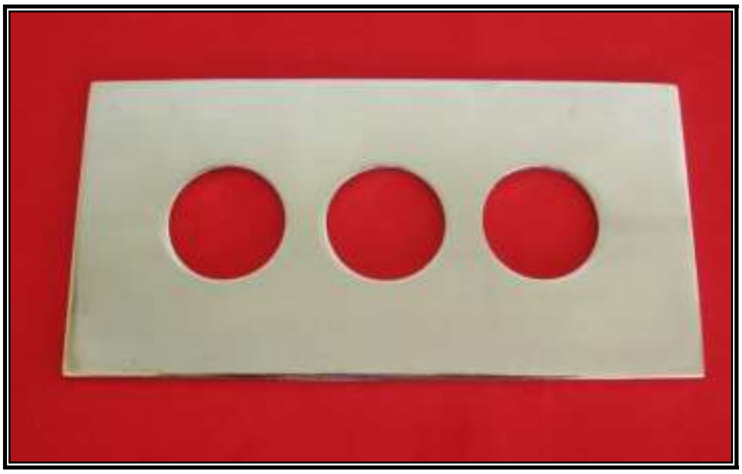

Fig. 1 Custom fabricated mould

Fig. 2 Contact angle analyzer (Digidrop, Contact angle meter, GBX products, France)