Original Article

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Dentistry Section

Effect of Chlorhexidine Mouthwash, Povidone-iodine Gargles and Herbal Mouth Sanitiser on Colour Stability and Surface Roughness of Conventional Nanohybrid Composite- An In-vitro Study

NIDHI SURENDRA PISAL[1], NIMISHA CHINMAY SHAH[2], NAMITA NITIN GANDHI[3], MEETKUMAR S DEDANIA[4], AJAY SINGH RAO[5]

ABSTRACT

Introduction: Chlorhexidine is commonly used to treat dental diseases as a broad-spectrum topical antibacterial medication. Povidone-iodine is a water soluble blend of molecular iodine and the solubiliser polyvinyl pyrrolidone. Amsarveda (pharmaceutical company) developed a natural mouth sanitiser with liposomal curcumin.

Aim: Comparative evaluation of the effect of chlorhexidine mouthwash, povidone-iodine gargle and herbal mouth sanitiser on colour stability and surface roughness of conventional nanohybrid composite.

Materials and Methods: This in-vitro study was conducted in the Department of Conservative Dentistry and Endodontics at KM Shah Dental College and Hospital, Vadodara, Gujarat, India, from November 2020 to June 2021. Total 60 extracted human permanent maxillary premolar teeth were selected. Class V restorations were performed on the extracted teeth with nanohybrid composite resin. The samples were divided into three groups by computer randomisation. The three test solution groups (n=20 each) ie., group A- chlorhexidine 0.2% mouthwash, group B has povidone-iodine (2%), group C- herbal mouth sanitiser. The colour change and surface roughness of all the samples were measured before and 15 days after immersion into the test solutions. The data was collected and statistically analysed using one-way Analysis of Variance (ANOVA) and Tukey's post-hoc test.

Results: The ΔE values for all three groups show a statistically significant difference (p-value <0.001). The mean baseline and after 15 days values for surface roughness in groups A and B showed a statistically significant difference (p-value <0.001). Whereas, no statistically significant difference in group C (p-value=0.654).

Conclusion: Based on the results of the present study, it can be stated that the povidone-iodine gargle showed more surface roughness and colour change of conventional nanohybrid composite followed by chlorhexidine mouthwash and herbal mouth sanitiser.

Keywords: Discolouration, Mouthrinse, Profilometer, Resin composite, Spectrophotometer

INTRODUCTION

The most common choice of materials for anterior and posterior restorations is composite. Resin composite should resist colour change and polish maintains its aesthetic property over a long period of time [1]. Composite discolouration is an undesired effect, even though it is unavoidable in the oral environment. It can be due to various colouring agents present in food. These can change the colour of composite resins through absorption and/or adsorption when consumed for long period of time [2,3]. While deciding on a restorative material, surface roughness and colour stability of aesthetic dental materials are often disregarded over other physical and mechanical properties [1].

Mouthrinses are routinely used formulations for cleansing the mouth, primarily before dental surgical procedures, due to their ability to lower the microorganisms in the oral cavity [4-6]. Although there is no scientific proof that mouthwashes help prevent Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2) transmission, the American Dental Association (ADA) and the Centers for Disease Control and Prevention (CDC) have suggested using preprocedural mouthwashes before oral therapies [7]. However, regular usage of mouthrinses may be harmful to oral and dental tissues [8,9].

Chlorhexidine is commonly used to treat dental diseases as a broad-spectrum topical antibacterial medication. It is known to discolour oral tissues and restorations, especially when combined with dietary variables. Povidone-iodine is a water-soluble blend of molecular iodine and the solubiliser polyvinyl pyrrolidone. It is used for one to two minutes of mouth rinsing like chlorhexidine before dental treatment [10]. Similar to mouthwashes, recently mouth sanitiser have been introduced. Amsarveda (pharmaceutical company) developed a natural mouth sanitiser with liposomal curcumin. As per the claims of the manufacturer, it can be swirled in the mouth for a minute and could be used every 2-4 hours. It is stated that any pathogene is entrapped and deactivated by it. This mouth sanitiser contains aloevera extract, berberis aristata extract, green tea extract, liposomal curcumin, ocimum sanctum extract, *andrographis paniculate* extract and *Glycyrhhiza glabra* extract.

Aloevera extract prevents the formation of microorganisms that can cause infections in humans, soothes wounds, and lowers dental plaque. Berberis aristata extract, green tea extract, liposomal curcumin, and ocimum sanctum extract are anti-inflammatory, antioxidant-rich, and antiviral in nature [11].

However, considering the rise in the usage of mouthwashes, the researches comparing composite discolouration due to mouthwashes are minimal [12,13]. Previous in-vitro studies have researched about the discolouration of composites due to various beverages and foodstuffs [2,13,14]. But not much has been explored with the discolouration of composites due to regular use

of mouthrinses, especially povidone-iodine gargle and herbal mouth sanitiser which can be recommended during Coronavirus Disease-2019 (COVID-19) times. Thus, the aim is to evaluate and compare the effect of chlorhexidine mouthwash, povidone-iodine gargle and herbal mouth sanitiser on colour stability and surface roughness of conventional nanohybrid composite before and after 15 days time interval. The null hypothesis was that there will be no difference between effect of chlorhexidine mouthwash, povidone-iodine gargle, and herbal mouth sanitiser on colour stability and surface roughness of conventional nanohybrid composite.

MATERIALS AND METHODS

This in-vitro study was conducted in the Department of Conservative Dentistry and Endodontics at KM Shah Dental College and Hospital, Vadodara, Gujarat, India, from November 2020 to June 2021. The approval was obtained from the Institutional Ethics Committee (SVIEC/ON/Dent/SRP/20118).

Inclusion and Exclusion criteria: Intact mature premolar teeth extracted for orthodontic purpose were included in the study. The teeth with caries, attrition, any hypoplastic defect, cracks, and previous restorations were excluded from the study.

Sample size calculation: A minimum 42 samples (14 per group) should be taken for present study to achieve 95% confidence interval and 80% power. Using the formula,

$$N=2\times(Z\times SD/d^2)$$

where, Z=3.24, SD=0.12, d=0.15.

Thus, the final sample size was 60 extracted human permanent maxillary premolar teeth.

Procedure

The extracted teeth were disinfected in 0.5% chloramine-T trihydrate solution for one week and were cleaned off calculus and periodontal tissue using an ultrasonic scaler. All teeth were stored in normal saline at 4° until further processing. All the teeth were treated by the principal investigator. For all extracted teeth, the operator prepared Class V cavities with 3 mm (mesiodistal), 2 mm (occluso-gingival), and 1.5 mm depth at the occlusal margin and 1 mm depth at the cervical margin on the buccal surface. For restoration of cavities, selective enamel etching was done with 37% phosphoric acid (Prime Dental) and rinsed with distilled water for one minute. Then, universal bonding agent (Tokuyama Palfique bond) was applied to the enamel and dentinal walls of the cavity and light-cured for 20 seconds. This was followed by the incremental layering of nanohybrid resin composite (Palfique LX5, Tokuyama, Japan) and then light-cured for 20 seconds. The curing light used during this study was of GC D Light Duo (Tokyo, Japan) (Dual Wavelength) (400-480 nm). The light intensity of the unit was regularly checked using the curing light intensity meter. The final cure of composite was completed through the application of glycerine which improves surface resistance and allows for better finishing and polishing procedures [15]. After the completion of the composite placement, the standard finishing and polishing regime was carried out by the Shofu Super Snap Rainbow Kit.

The extracted teeth were stored in artificial saliva for 24 hours after the finishing and polishing regimen. The colour change and surface roughness of all the samples were measured after composite restoration and before immersion into the test solution. A spectrophotometer (Vita Easyshade Advance 5.0 digital shade guide) was used for evaluating colour stability. The surface roughness of the material was tested using a SJ-210 profilometer (Mitutoyo, Japan). The samples were then divided into three groups by computer randomisation on www.randomizer.org.

- Group A (n=20)- Chlorhexidine 0.2% mouthwash (Dr. Reddy's Clohex ADS liquid)
- Group B (n=20)- Povidone-iodine Germicide gargle 2% (Betadine)
- Group C (n=20)- Herbal mouth sanitiser (Amsarveda Mouth sanitiser)

The samples were stored in a 3 mL test tube in artificial saliva (wet mouth). The samples of group A were immersed in the test solutions for one to two minutes twice a day for 15 days. The samples of group B were immersed in the test solution according to the manufacturer's instructions. The samples of group C were immersed in the test solution for one minute three to four times a day for 15 days. The solutions have different advised usage time and frequencies; hence the samples were immersed in these for different duration and frequency. Thermocycling of the samples of all groups was done two times in a day for 15 days, at 5°C±2°C to 55°C±2°C with 30 seconds dwell time, and five second transfer time. After 15 days the samples were stored in artificial saliva and evaluated for surface roughness and colour change. The colour stability and surface roughness evaluators and statistician were blinded.

STATISTICAL ANALYSIS

All the data was collected and statistically analysed using one-way Analysis of Variance (ANOVA) and Tukey's post-hoc test using Statistical Package for the Social Sciences (SPSS) software version 21.0. (IBM, Chicago, IL, USA).

RESULTS

Evaluation of colour stability: The ΔE values for all three groups show a statistically significant difference (p<0.001) [Table/Fig-1] which suggests a colour change in all three groups. The highest ΔE value was seen with group B (povidone-iodine) and the least in group C (herbal mouth sanitiser). On intergroup comparison, it was found that the ΔE values for group A vs group B and group B vs group C, showed a statistical significance (p-value <0.001) and, no statistically significant difference was found between group A vs group C [Table/Fig-2].

Variables	Group A (n=20)	Group B (n=20)	Group C (n=20)	p-value (One-way ANOVA)
Baseline L*	71.66±1.04	72.28±0.84	71.92±1.08	0.151
Baseline a†	0.67±0.02	0.66±0.02	0.66±0.02	0.368
Baseline b‡	24.63±0.96	24.95±1.48	24.28±0.84	0.188
15 days L*	77.4±0.46	78.34±0.19	77.32±0.74	<0.001
15 days a†	0.84±0.08	0.96±0.08	0.81±0.07	<0.001
15 days b‡	29.76±0.59	30.74±0.56	27.38±0.33	<0.001
ΔL	-5.74±1.21	-6.06±0.75	-5.4±0.61	0.016
Δa	-0.17±0.08	-0.3±0.08	-0.14±0.07	<0.001
Δb	-5.13±1.08	-5.79±1.6	-3.1±0.82	<0.001
ΔE	4.84±0.96	5.38±0.5	4.2±0.44	<0.001

[Table/Fig-1]: Intragroup comparison of mean values of group A, B and C for colour change.
L*: indicates lightness ranges between 0 (dark) and 100 (White);
†a: is the red/green coordinate; ‡b: is the yellow/blue coordinate;
p-value <0.05 was considered significant

Variables	Post-Hoc Tukey test		
	Group A vs Group B difference (p-value)	Group A vs Group C difference (p-value)	Group B vs Group C difference (p-value)
ΔL	-0.62 (0.129)	-0.26 (0.689)	0.36 (0.492)
Δa	0.01 (0.358)	0.01 (0.582)	0 (0.922)
Δb	-0.32 (0.644)	0.35 (0.591)	0.67 (0.154)
ΔE	-0.94 (<0.001)	0.08 (0.875)	1.02 (<0.001)

[Table/Fig-2]: Intergroup comparison of mean difference values for colour change between groups.
p-value <0.05 was considered significant

Evaluation of surface roughness: The mean baseline and after 15 days' values for surface roughness in group A and group B showed a statistically significant difference (p-value <0.001) and no

statistically significant difference in group C (p-value=0.654) [Table/Fig-3]. On intergroup comparison, it was found that the mean difference values for group A vs group C, and group B vs group C showed statistical significance, thus, chlorhexidine mouthwash and povidone-iodine gargle used in COVID-19 era can cause surface roughness of composite restorations and herbal mouth sanitiser can be used as a mouthrinse. Also, there was no statistically significant difference between group A and group B which suggests that chlorhexidine and povidone-iodine both cause significant surface roughness [Table/Fig-4]. Povidone-iodine gargle showed more surface roughness and colour change amongst the three groups followed by chlorhexidine mouthwash and herbal mouth sanitiser.

Group	n	Mean	Standard deviation	t-value	p-value
Group A					
Baseline	20	0.26	0.02	-11.13	<0.001
After 15 days	20	0.5	0.11		
Group B					
Baseline	20	0.2	0.02	-12.22	<0.001
After 15 days	20	0.47	0.1		
Group C					
Baseline	20	0.24	0.08	0.46	0.654
After 15 days	20	0.23	0.06		

[Table/Fig-3]: Intragroup comparison of mean of baseline and after 15 days values for surface roughness.
p-value <0.05 was considered significant

Surface roughness -Ra (in µm)	Post-Hoc Tukey test		
	Group A vs Group B difference (p-value)	Group A vs Group C difference (p-value)	Group B vs Group C difference (p-value)
At baseline	0.06 (<0.001)	0.02 (0.281)	-0.04 (0.036)
After 15 days	0.03 (0.52)	0.27 (<0.001)	0.24 (<0.001)
Difference (15 days- baseline)	-0.03 (0.576)	0.25 (<0.001)	0.27 (<0.001)

[Table/Fig-4]: Intergroup comparison of mean difference values for surface roughness between groups.
p-value <0.05 was considered significant

DISCUSSION

In clinical setting, tooth coloured restorative materials have been widely employed to satisfy patients' aesthetic needs [16]. Restoration of teeth with recently developed nanocomposites is the most significant input of nanotechnology to the dental fraternity. Colour stability is one of the qualities of composite resins that must stand the test of time [17]. Aesthetics is given a high priority in modern dentistry. The oxidation of tertiary amine results in surface staining in nanohybrid composites. This produces a yellowish discolouration. The colour stability of a composite restoration is related to its surface roughness. Surface roughness can contribute to higher plaque retention and stain absorption than a smoother surface [18].

The aetiology of composite discolouration is complex, with proprietary mouthrinse being one of the contributing factors [19]. The hardness, wear, and colour stability of composites may be affected by low pH of the mouthrinse. Furthermore, alcoholic components are not the sole element causing softening of resin and alcohol-free mouthrinses can impair colour stability composites; a study has revealed that color stability can be reduced by both alcohol-containing and alcohol-free mouthrinses [20]. The mouthrinses emulsifiers and organic acids may cause surface deterioration of resin composite materials [21].

In the current study, the povidone-iodine group showed the highest surface roughness and colour change. The composition of the povidone-iodine germicidal gargle, as per manufacturer consisted of povidone-iodine 2% w/v, absolute alcohol content 8.38% v/v, and mint flavour aqueous base. Essential oil's (including eucalyptol, thymol, menthol, and methyl salicylate) as an active ingredient in some mouthrinses. Therefore, essential oil's active ingredient in addition to high alcohol content and low pH of the mouthrinse containing alcohol and essential oil's may have played a role in the increased colour change and surface roughness of the nanohybrid resin composites used in this study [22]. A study by Bayraktar Y et al., evaluated the effect of SARS-CoV-2 effective mouthwashes on the staining, translucency, and surface properties of a nanofill resin composite. The chlorhexidine group displayed the lowest ΔE3 value, povidone iodine and cetylpyridinium hydrochloride group were found statistically similar to the chlorhexidine group [23].

Chlorhexidine mouthwash consists of chlorhexidine gluconate 0.2%, sodium fluoride 0.5% and zinc chloride 0.09%. It has been hypothesised that tannin-containing dietary components have a significant chromatogenic potential, especially when combined with chlorhexidine. Denatured proteins and iron from food include thiol groups, which give sulphur and eventually create iron sulphide, which causes the stain [24]. A study has revealed that chlorhexidine-containing mouthrinse having 0.2% of chlorhexidine gluconate could cause perceptible colour change in composites [25].

The COVID-19 outbreak and pandemic are creating significant worry in all countries' healthcare systems. According to Moosavi MS et al., using mouthwash before dental operations reduces the risk of viral transmission to the dental team via aerosol and saliva [26]. Amsarveda (pharmaceutical company) developed a mouth sanitiser with natural extracts [11]. No studies have yet evaluated the discolouration potential of this mouth sanitiser. This group showed the least colour change and surface roughness on composite. The less discolouration could be because of the non alcoholic contents of the mouth sanitiser.

CHX has a high ionic concentration, which may cause the release of soluble components from the resin composites, increasing surface roughness. Similarly, Abo EN and Yousef M have also reported increased surface roughness of resin composites exposed to mouthrinses containing CHX [27].

In this study, a Digital spectrophotometer- Vita Easyshade 5 was used for measuring colour stability. ΔE is appropriate for small colour changes evaluation and repeatable, sensitive, objective method [28]. The oral environment determines the performance of a material. For example, mouthwashes in contact with restorations influence physical properties due to their compositions [19,29]. Some studies focussed on effects of different exposure protocols of mouthrinses on surface characteristics of a few aesthetic restorative materials [12,30]. The results of a study by Miranda Dde A et al., showed that those mouthwashes containing alcohol or hydrogen peroxide present an increased chance of change in the superficial roughness and hardness of the tested composites. The Knoop microhardness and surface roughness of the tested composites depends on the time period of immersion [31].

A statistical significant difference between the colour stability and surface roughness of the three groups was obtained. Thus, the null hypothesis of this study was rejected.

Limitation(s)

The limitation of the study is that as it is an in-vitro study, it is not possible to mimic oral conditions completely including the effect of food and beverages. Thus, it is difficult to extrapolate the result of this study to in-vivo conditions.

CONCLUSION(S)

This in-vitro study demonstrated the povidone iodine gargle showed more surface roughness and colour change of conventional nanohybrid composite amongst the three groups followed by chlorhexidine mouthwash and herbal mouth sanitiser. Within the limitations of the study, it can be stated that the herbal mouth sanitiser group performed better than the povidone iodine and chlorhexidine group and can be considered for future in-vivo evaluation and use.

Declaration: This manuscript is purely for research purpose and no brand is being promoted here.

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PARTICULARS OF CONTRIBUTORS:
1. Postgraduate Student, Department of Conservative Dentistry and Endodontics, K. M. Shah Dental College and Hospital, Sumandeep Vidyapeeth, Vadodara, Gujarat, India.
2. Professor and Head, Department of Conservative Dentistry and Endodontics, K. M. Shah Dental College and Hospital, Sumandeep Vidyapeeth, Vadodara, Gujarat, India.
3. Postgraduate Student, Department of Conservative Dentistry and Endodontics, K. M. Shah Dental College and Hospital, Sumandeep Vidyapeeth, Vadodara, Gujarat, India.
4. Senior Lecturer, Department of Conservative Dentistry and Endodontics, K. M. Shah Dental College and Hospital, Sumandeep Vidyapeeth, Vadodara, Gujarat, India.
5. Reader, Department of Conservative Dentistry and Endodontics, K. M. Shah Dental College and Hospital, Sumandeep Vidyapeeth, Vadodara, Gujarat, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Nimisha Chinmay Shah,
Professor and Head, Department of Conservative Dentistry and Endodontics,
K. M. Shah Dental College and Hospital, Sumandeep Vidyapeeth,
Vadodara, Gujarat, India.
E-mail: nshah7873@gmail.com

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