Research Article

Evaluation of The Effect of Three Different Mouthwashes on The Surface Microhardness And Colour Stability of A Bioactive Restorative Material – An in Vitro Study

Dr. Vijay Mathai[1] , Dr. Anujin Manoharan[2*] , Dr. Rajesh Sasidharan Nair[3] , Dr. Sarah Renjelina Christopher[4] , Dr. Vijil Vimala Ravi[5] , Dr. Theodore Rajah Thambiraj[6]

[1]Professor, Department of Conservative Dentistry and Endodontics, Sree Mookambika Institute of Dental Sciences, Kulasekharam, Tamilnadu, India., ORCID:0000-0002-7013-5782, Email:drvijaymathai@gmail.com
[2]Post Graduate Student, Department of Conservative Dentistry and Endodontics, Sree Mookambika Institute of Dental Sciences, Kulasekharam, Tamilnadu, India. ORCID:0009-0007-9459-0564
Email: anujinm8@gmail.com
[3]Professor & HOD, Department of Conservative Dentistry and Endodontics, Sree Mookambika Institute of Dental Sciences, Kulasekharam, Tamilnadu, India, ORCID:0009-0003-0718-7496, Email:drrajeshaparna72@gmail.com
[4]Professor, Department of Conservative Dentistry and Endodontics, Sree Mookambika Institute of Dental Sciences, Kulasekharam, Tamilnadu, India, ORCID:0009-0004-9370-5292, Email:sarah.c.martin111@gmail.com
[5]Reader, Department of Conservative Dentistry and Endodontics, Sree Mookambika Institute of Dental Sciences, Kulasekharam, Tamilnadu, India, ORCID:0000-0002-6731-8131, Email:vijil24mk@gmail.com
[6]Senior Lecturer, Department of Conservative Dentistry and Endodontics, Sree Mookambika Institute of Dental Sciences, Kulasekharam, Tamilnadu, India, ORCID:0009-0005-3096-0525, Email:theodore.theo2@gmail.com
Corresponding author: Dr. Anujin Manoharan, Post Graduate Student, Department of Conservative Dentistry and Endodontics, Sree Mookambika Institute of Dental Sciences, Kulasekharam, Tamilnadu, India. ORCID:0009-0007-9459-0564 , Email: anujinm8@gmail.com

Abstract

Context: The performance of bioactive restoratives clinically is highly dependent on their ability to withstand exposure to chemical agents in the oral cavity.

Aims: To evaluate the effect of three different mouthwashes on the surface microhardness and colour stability of the bioactive restorative material ACTIVA™ BioACTIVE.

Materials and Methods: Seventy-two disc-shaped samples of ACTIVA™ BioACTIVE (8 mm diameter, 1 mm thickness) were fabricated and randomly assigned to four groups of 18 samples each. Group 1 (artificial saliva – control), Group 2 (Listerine® Cool Mint®), Group 3 (Sensodyne Complete Protection+), and Group 4 (Himalaya Active Fresh Miswak). Following a continuous 24-hour immersion at 37°C, surface microhardness was measured using a Digital Vickers hardness tester. Colour stability (ΔE) was assessed via spectrophotometry using the CIE L*a*b* system.

Statistical Analysis: Data were analysed using descriptive statistics, one-way ANOVA, and Tukey HSD post hoc tests with the significance threshold set at $p \leq 0.05$.

Results: Significant differences were observed across all experimental groups ($p < 0.001$). Group 1 showed the highest stability. Among the mouthwashes, Listerine caused the greatest reduction in microhardness and the highest colour change. Sensodyne and Himalaya exhibited significantly better microhardness retention, while Sensodyne demonstrated superior colour stability.

Conclusion: Alcohol-containing Listerine mouthwash significantly compromised the surface microhardness and colour stability of ACTIVA™ BioACTIVE. Non-alcoholic Sensodyne and herbal Himalaya mouthwashes were more compatible, maintaining material properties within acceptable clinical limits.

Keywords: ACTIVA™ BioACTIVE, colour stability, Himalaya Miswak, Listerine® Cool Mint®, mouthwashes, Sensodyne Complete Protection+, Spectrophotometer, surface microhardness, Vickers hardness tester.

Key Message: Alcohol-containing mouthwashes significantly reduce the surface microhardness and colour stability of ACTIVA™ BioACTIVE restorative material. Alcohol-free and herbal mouthwashes showed better compatibility and maintained material properties within clinically acceptable limits.

Introduction

Dental caries continues to be a prevalent chronic disease and restorative dentistry has shifted focus from extensive tooth preparation towards minimally invasive procedures utilizing bioactive materials.[1] Although glass ionomer cements (GICs), composite resins, and amalgam have long served as conventional dental materials, each possesses distinct limitations. Amalgam compromises aesthetics, while composite restorations are frequently challenged by polymerization shrinkage and the development of secondary caries.[2] Glass ionomer cements (GICs) are compromised by deficient mechanical properties and low resistance to wear.[3]

To overcome these drawbacks, recent research has focused heavily on bioactive restorative materials that establish a dynamic mineral exchange with surrounding tooth structure. The use of a novel bioactive resin matrix and reactive glass fillers is designed to provide the release and re-supply of calcium, phosphate, and fluoride ions. ACTIVA™ BioACTIVE represents a groundbreaking material that incorporates features of both GICs and composites.[4,5] It is a dynamic material that releases ions in response to pH drops.[6]

The long-term clinical success of these restorations depends heavily on their surface microhardness and colour stability, which dictate resistance to wear and aesthetic longevity.[7,8] Restorative materials in the oral cavity may be exposed to mouthwashes, which may soften the resin matrix or dissolve the filler particles.[9,10] The surface properties may be greatly influenced by the presence of substances like alcohol, essential oil, and pH differences within the common formulations like Listerine Cool Mint with alcohol, Sensodyne Complete Protection+ with

desensitizing agent, or Himalaya Active Fresh Miswak. Chemical reactions between these active ingredients and the organic phase of the resin matrix trigger structural alterations that increase staining susceptibility.[11]
The aim of this in vitro study was to evaluate the effect of these three commercially available mouthwashes on the surface microhardness and colour stability of ACTIVA™ BioACTIVE restorative material. The null hypothesis was that these mouthwashes would have no effect on the surface microhardness and colour stability of ACTIVA™ BioACTIVE restorative material.

Materials and Methods

This study obtained the institutional ethical clearance with approval [Institutional Ethical Clearance Number omitted for blinding/double-blind review]. The study utilised three commercial mouthwashes: Listerine® Cool Mint®, Sensodyne Complete Protection+, and Himalaya Active Fresh Miswak. Detailed specifications are provided in Table 1.
Seventy-two disc-shaped samples (8 mm diameter, 1 mm thickness) were fabricated using ACTIVA™ BioACTIVE (Pulpdent, Watertown, USA) in Teflon moulds (Metallography India, Mumbai, India). To ensure smooth surfaces, the material was sandwiched between Mylar strips (Samit Dental Product, New Delhi, India) and pressed with a glass slide. Samples were polymerised for 20 seconds using a Woodpecker I-LED Curing Light (Guilin Woodpecker Medical Instrument Co., Ltd., Guangxi, P.R. China) at 385-515 nm and 1000 mW/cm^2. With the help of a 1 mm thick glass slide, the distance between the light source and the sample was standardised. Void-free discs were polished using a Shofu Composite Polishing Kit (Shofu Dental GmbH, Ratingen, Germany) sequentially from coarse to superfine, then stored in artificial saliva for 24 hours before testing.
Samples were randomly assigned to four groups of 18 samples each,
Group 1 (Control): 5 mL artificial saliva (Aritech Chemazone Private Limited, Haryana, India)
Group 2: 5 mL Listerine Cool Mint (Johnson & Johnson, New Brunswick, NJ, USA)
Group 3: 5 mL Sensodyne Complete Protection+ (GSK Consumer Healthcare, Brentford, United Kingdom)
Group 4: 5 mL Himalaya Active Fresh Miswak (Himalaya Wellness Company, Bengaluru, India).
Samples were submerged in their respective media at 37º C for 24 hours, rinsed with distilled water, and returned to artificial saliva. Following this the samples were subjected to Surface microhardness test and colour stability analysis.
Surface microhardness was evaluated using a NEXUS 400™ Digital Vickers hardness tester (INNOVATEST, Maastricht, The Netherlands). Five indentations per sample were made with a 100 g load and a 15 seconds dwell time. Perpendicular diagonals were measured at 20 x magnification to calculate the average Vickers Hardness Number (VHN).
Colour variations were assessed using a Cary 5000 extra-oral spectrophotometer (Agilent Technologies, California, USA) from 380 to 780 nm at a 1 nm interval. Total colour change (ΔE_{ab}) was determined using the formula:
$\Delta E_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$
Results were evaluated against a perceptibility threshold of 0.8 and an acceptability threshold of 1.8. Data processing was conducted via SPSS Version 25 (IBM Statistics, Chicago, USA). One-way ANOVA and Tukey HSD post hoc tests determined significant differences between groups ($p \leq 0.05$).

Results

Descriptive statistics for surface microhardness and colour stability (ΔE) are presented in Tables 2 and 3, respectively. One-way ANOVA indicated highly significant differences across all experimental groups for both surface microhardness ($p < 0.001$) and colour stability ($p < 0.001$). Group 1 (Control) demonstrated the highest stability, with the highest mean microhardness (13.300) and the lowest mean colour change (0.4372).
Among the experimental solutions, Group 2 (Listerine Cool Mint) had the greatest impact, resulting in the lagest reduction in surface microhardness (11.222) and the highest mean colour change (1.0078). Post-hoc analysis confirmed that the changes in Group 2 were statistically significant compared to the Group 1 control ($p < 0.001$). Although all the groups of mouthwash showed a statistically significant decrease in the microhardness in comparison with the control ($p < 0.001$), Groups 3 (Sensodyne Complete Protection+) and 4 (Himalaya Active Fresh Miswak) demonstrated significantly higher surface microhardness than Group 2 (Listerine Cool Mint) ($p < 0.001$), with no significant difference between Groups 3 and 4 ($p = 0.786$).
In terms of colour stability, Group 4 developed a significant difference when compared to Group 1 ($p < 0.001$); however, Group 3 demonstrated good colour stability, showing no significant difference from the control group ($p = 0.409$). Group 3 had better colour stability in comparison to Group 4, as per the post hoc test ($p = 0.001$). While the colour changes in Group 4 crossed the perceptibility threshold ($\Delta E > 0.8$), both Groups 3 and 4 exhibited colour changes below the clinical acceptability limits ($\Delta E < 1.8$).

Discussion

According to the present study, Sensodyne Complete Protection+ mouthwash caused the least change in surface microhardness, followed by Himalaya Active Fresh Miswak mouthwash and Listerine® Cool Mint® mouthwash. Sensodyne Complete Protection+ mouthwash caused the least change in the colour, followed by Himalaya Active Fresh Miswak mouthwash and Listerine® Cool Mint® mouthwash. Thus, the study hypothesis that there would be no effect on the surface microhardness and colour stability of the bioactive restorative material ACTIVA™ BioACTIVE by the use of the three different mouthwashes was rejected.

The regular use of mouthwash on restorations modifies their physical and optical properties. The use of alcohol, aromatics, or oxidizing agents in mouthwashes can react with the resin matrix and fillers present in restorative materials.[12] The compatibility will be influenced by the chemical nature of the mouthwash rather than its baseline pH level. As compared to alcohol-containing mouthwashes, alcohol-free mouthwashes have less polymer degradation, and pigment uptake due to the absence of solvent action on the resin matrix. In addition, natural plant pigments found in herbal mouthwashes can induce surface discolouration and these formulations lack the harsh chemical solvents that affect the resin matrix.[13]

Resin degradation and structural failure at the filler-resin interface are signaled by a reduction in surface microhardness, which refers to the material's ability to resist abrasion and penetration. A considerable decline in microhardness of composites has been found using mouthwashes that have alcohol and whitening agents.[12,14]

This study employed the Vickers hardness test over the Knoop and Brinell tests. By averaging calculations of two perpendicular diagonals, the pyramidal diamond-shaped indenter with a 136° angle forms a symmetrical imprint for accurate measurement. In addition, for a specific testing range, the Vickers hardness test is independent of loads.[15] Conversely, the Knoop indenter uses an elongated rhomboidal pyramid with a 7.11:1 diagonal ratio, where elastic recovery after unloading can alter residual indentation geometry and distort hardness estimations.[16] The Brinell macrohardness test was excluded because its large, spherical steel indenter tends to cause catastrophic structural failure in brittle biomaterials rather than a clean surface indentation.[17]

Aesthetic longevity is greatly dependent on colour stability, making it a crucial parameter. The use of mouthwashes makes teeth more susceptible to discolouration; highly pigmented rinses and chlorhexidine formulations produce exacerbated effects.[13,18] This study used spectrophotometry for colour stability evaluation using CIE L*a*b*.

A 24-hour static immersion protocol was employed in this study. Clinically, if a patient rinses for 4 minutes daily, the total time spent rinsing in one year equals 1,460 minutes (4 minutes x 365 days), which correlates closely with the experimental 24-hour continuous immersion of 1,440 minutes (24 hours x 60 minutes).[19] The static immersion technique was chosen to isolate chemical impact on surface properties while excluding extraneous mechanical loads and temperature changes. Thermal stress from thermocycling was omitted to isolate the pure chemical impact of the mouthwashes on the surface of the material. Continuous immersion is methodologically sufficient to isolate chemical erosion from physical fracturing.[12] Tracking dye discolouration is a purely chemical, sorption-dependent process requiring static incubation to observe stain affinity rather than mechanical wash-out effects caused by thermal cycling.[20]

The factors contributing to the surface degradation caused by Listerine mouthwash are the presence of alcohol and the acidic pH of the solution; alcohol acts as a plasticiser, weakening the resin matrix, while acid hastens the process of degradation of the resin-to-filler interface. This is in accordance with the findings of studies carried out by Manzoor et al. and Karademir et al., where composite restorations exposed to Listerine showed a marked reduction in microhardness values.[21,14] Due to the presence of alcohol and specific colourants in the solution, the value of discolouration was above the threshold value of 0.8. Ulusoy et al. found that Listerine caused a clinically appreciable discolouration in composites.[22] Álvarez-Horna J et al. stated that Listerine® Cool Mint® caused a significant colour difference in composite material.[23] In a study by Karademir et al. it was found that Listerine did not cause significant colour changes in bulk-fill composites.[14]

The mean microhardness of Sensodyne Complete Protection+ (Group 3) was 12.289. Although the reduction in microhardness was statistically significant in relation to the control group, there was a lesser effect because of the absence of alcohol. Sensodyne proved to be compatible with the optical properties of the substance in the colour stability test, since its mean value was 0.5789, lower than the perceptibility threshold of 0.8. In a study done by Büyükgöze Dindar and Tekbaş Atay, it was found that Sensodyne cool mint mouthwash did not cause any significant discolouration.[24] Mean microhardness of Himalaya Active Fresh Miswak (Group 4) was 12.100. Similar levels of surface protection from Groups 3 and 4 can be observed compared to conventional high alcohol antiseptic mouth rinses, based on the absence of any statistically significant difference between the two groups ($p = 0.786$). It is likely that the natural dyes and extracts from herbs used by the Miswak plant account for the colour difference value of 0.9444 achieved by the Himalaya Miswak, which was above 0.8 but safely below the clinical acceptability limit of 1.8.

However, this study has its own limitations. The study was done under in vitro conditions that cannot completely simulate the salivary flow rate, temperature changes, oral clearance, and biofilms. These factors can influence the behaviour of the restorative materials in vivo. Mouthwashes are not in contact with the restoration for a long time, and it is also diluted by saliva. But the continuous immersion in the mouthwash for a long time can exaggerate the effects. Only one bioactive material was tested against the mouthwashes. So, the results cannot be generalised to other restorative materials like conventional composite materials and Glass Ionomer Cement. Furthermore, even though the colour assessment using ΔE values is standardised, it may not portray the clinical perception of colour under different circumstances such as lighting conditions and vision of the individuals. Finally, patient-related factors such as dietary habits, oral hygiene practices, and saliva composition were not considered.

Table 1: Mouthwashes used in the study

Mouthwashes	Manufacturer	Composition
LISTERINE® Cool Mint® Mouthwash	Johnson & Johnson Pvt. Ltd	Purified water, Sorbitol, Alcohol, Poloxamer 407, Benzoic Acid, Sodium Saccharin, Eucalyptol, Flavour, Methyl Salicylate, Thymol, Sodium Benzoate, Menthol, Cl 42053
Sensodyne Complete Protection+ Mouthwash	GSK Consumer Healthcare	Aqua, Glycerol, Sorbitol,Potassium Nitrate, PEG-60,Hydrogenated Castor Oil, Poloxamer 407, Sodium Benzoate, Flavour, Disodium Phosphate, Methylparaben, Propylparaben, Monosodium Phosphate, Sodium Fluoride, Sodium Saccharin, CL 42090.
Himalaya Active Fresh Miswak Mouthwash	Himalaya drug company Pvt. Ltd.	Aqua, Glycerin, Salvadora persica Stem Extract, Punica Granatum Fruit Extract, Azadirachta indica Leaf Extract, Propylene Glycol, Sorbitol, Hydrogenated Castor Oil, Benzyl Alcohol, Sodium Benzoate, Flavour, Citric Acid, Sodium Saccharin, Cetylpyridinium Chloride, Sodium Fluoride, Menthol, Ethyl Menthane Carboxamide, Cl15885, Cl14700.

Table 2: Descriptive Statistics of Surface Microhardness of ACTIVA™ BioACTIVE Restorative Material in Different Study Groups

Groups	Surface Microhardness		p value
	Mean	Standard Deviation	
1	13.300	0.6860	< 0.001*
2	11.222	0.5547	
3	12.289	0.6694	
4	12.100	0.4899	

*** (p value ≤ 0.05 is statistically significant)**

Table 3: Descriptive Statistics of Colour Change (ΔE) of ACTIVA™ BioACTIVE Restorative Material in Different Study Groups

Groups	Colour Change (ΔE)	p value

	Mean	Standard Deviation	
1	0.4372	0.19204	< 0.001*
2	1.0078	0.23898	
3	0.5789	0.30127	
4	0.9444	0.33618	

*** (p value ≤ 0.05 is statistically significant)**

Conclusion

Within the limitations of this in vitro study, the alcohol-containing Listerine mouthwash caused significant reductions in microhardness and colour stability of ACTIVA™ BioACTIVE restoratives. However, Sensodyne and Himalaya oral rinses managed to preserve aesthetics and structural stability within acceptable clinical limits.

Authors' Contributions:

1. Dr. Vijay Mathai: Concepts, Design, Definition of intellectual content, Data acquisition, Manuscript preparation, Manuscript editing, Manuscript review
2. Dr. Anujin Manoharan: Design, Literature search, Clinical studies, Experimental studies, Manuscript preparation, Manuscript editing, Manuscript review, Guarantor.
3. Dr. Rajesh Sasidharan Nair: Concepts, Design, Literature search, Data acquisition, Data analysis, Statistical analysis, Manuscript review.
4. Dr. Sarah Renjelina Christopher: Definition of intellectual content, Clinical studies, Experimental studies, Data acquisition, Data analysis, Statistical analysis, Manuscript preparation, Manuscript editing, Manuscript review.
5. Dr. Vijil Vimala Ravi: Design, Literature search, Clinical studies, Manuscript editing, Manuscript review.
6. Dr. Theodore Rajah Thambiraj: Concept, Experimental studies, Data analysis, Manuscript editing, Manuscript review.

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