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Antimicrobial Effect of Three Recently Developed Restorative Materials Against Streptococcus Mutans and Lactobacillus Casei-an In-vitro Study.

Abstract

Objective : The aim of the study was to determine the antibacterial efficacy of three different restorative materials against Streptococcus mutans and Lactobacillus casei.

Materials and methods : In the present study agar well diffusion method was applied to evaluate and compare antimicrobial efficacy of Fuji IX (GCTokyo,Japan), Hi-dense (ShofuInc,Kyoto,Japan) and Beautifil II (ShofuInc,Kyoto,Japan) against Streptococcus mutans and Lactobacillus casei. Zone of inhibition was measured after at different time intervals that is 24hr,48hr,72hr and 7th day.

Results: Hi-dense restorative material demonstrated the best antimicrobial efficacy against Streptococcus mutans followed by Fuji IX and Beautifil II. Most effective antibacterial restorative material against Lactobacillus caseiwas Fuji IX.

Conclusion: All the tested restorative materials in present study demonstrated significant antimicrobial efficacy.

Key words: Antibacterial activity,Beautifil II, Fuji IX, Hi-Dense,Lactobacillus casei, Streptococcus mutans

Introduction:

Dental caries is the most predominant disease diagnosed in the oral cavity of humans. As indicated by an systematic assessment for the Global Burden of Disease Study 2010, Worldwide, around 36% of the people have dental caries in their permanent teeth and in primary teeth it influences about 9% of the populace.[1] Individuals are susceptible to caries throughout their lifetime.[2] One of the major risk factor associated with dental caries is the prevalence of microbes Streptococcus mutans and Lactobacillus casei in the oral cavity. According to researches Streptococcus mutans and Lactobacilli had an ability to grow in an acidic environment and convert sugar supplied in the diet into organic acids through rapid metabolism.[3]Streptococcus mutansis the main microorganism that initiates caries and plays important role of enamel decay.[4] The Lactobacillus microbes are essential in further advancement of caries, particularly in dentin. Carious lesions form where oral biofilms are enabled to evolve and remain on teeth for long period of time. Risk of caries is associated withphysical, biological, environmental, behavioural and lifestyle-related components. Procedures employed in the therapy of dental caries such as caries excavation and cavity preparation do not eliminate all the microorganisms from the cavity. The microbes remaining in the dentin and possible loss of marginal seal may result in secondary caries, and subsequently to pulp diseases. In such procedures, restorative cements with antimicrobial properties are used to promote the remineralization of the tissue and to reduce the viability of residual bacteria, thus preventing the occurrence of secondary caries.[5]

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Amongst the dental restorative materials used in dentistry, glass ionomer cements have shown promising antibacterial effects due to continuous release of fluoride ions. These fluoride ions help in remineralization of initial carious lesion. Glass-ionomer cements are also able to inhibit the growth of some oral bacterial species because of their initial low pH.[6]The same antimicrobial effect has been shown by a new generation of restorative materials like resin-modified glass ionomer cements, compomers and giomers. By modifying glass ionomer cement, the manufacturers strive not only to improve their mechanical and aesthetic properties, but to increase their antibacterial activity as well.[7]

Materials and Method:

In the present study bacterial strains of Streptococcus mutans(ATCC 25175) and Lactobacillus casei (ATCC 393) were employed to evaluate the antimicrobial efficacy of three restorative materials by agar well diffusion method.

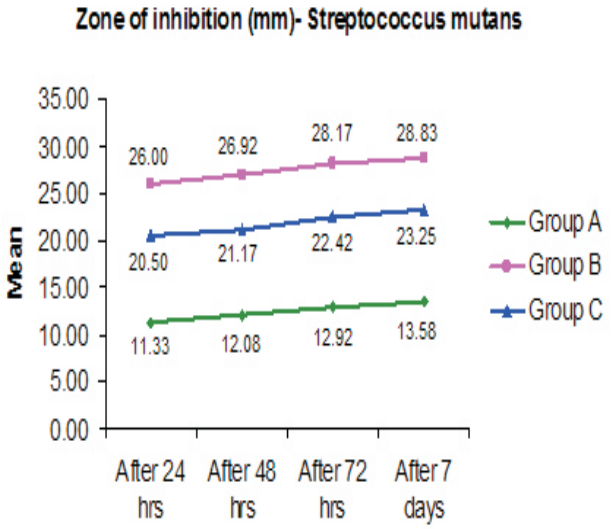
Strains of the selected microorganisms were revived in brain heart infusion agar plate for 24 hour at 370 C . After incubation reactivated, bacterial colonies were dissolved in 0.85% NaCl to prepare bacterial inoculum of a turbidity of 0.5 Mcfarlandstandard, 10 µl of this inoculum suspension was pipetted onto a sterile brain heart infusion agar plate with a sterile cotton swab to get a lawn culture. Wells of 7 mm in depth and 5 mm in diameter were bored in the agar media. Total 36 wells were prepared on 12 agar plates for both bacterial strains which were divided into three groups according to restorative materials. All the three tested restorative materials were mixed according to manufacturer's instructions and placed inprepared wells in a sterilized manner.Plates were left at room temperature for 30 minutes and then incubated at 37°C for 7 days. Results were obtained by measuring the diameter of microbial inhibition zones at the 24 hour, 48 hour, 72 hour and 7th day of incubation. All the test were repeated 4-5 times. Raw data collected was subjected to statistical analysis.

Results:

Data were summarized as Mean ± SD (standard deviation). Groups were compared by two factor repeated measures analysis of variance (ANOVA) and the significance of mean difference within (intra) and between (inter) the groups was done by Tukey's post hoc test after ascertaining normality by Shapiro-Wilk's test and homogeneity of variances by Levene's test. A two-tailed (a=2) p value less than 0.05 ($p<0.05$) was considered statistically significant. Analyses were performed on SPSS software (PSAW, windows version 18.0).

Period	Group A	Group B	Group C
After 24 hrs	11.33 ± 0.89	26.00 ± 0.95	20.50 ± 1.38
After 48 hrs	12.08 ± 0.79	26.92 ± 0.90	21.17 ± 1.19
After 72 hrs	12.92 ± 0.79	28.17 ± 1.53	22.42 ± 1.38
After 7 days	13.58 ± 0.90	28.83 ± 1.53	23.25 ± 1.29

Table 1: zone of inhibition (Mean ± SD, n=12) of three groups (Group A:Beautifil II, Group B: Hi-Dense, Group C: GC Fuji IX) against S. mutansover the different time periods

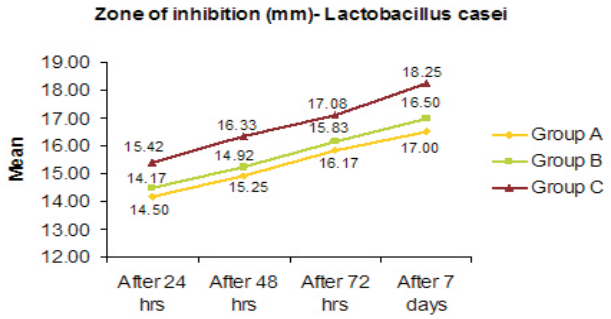


Graph 1. Mean zone of inhibition of three groups over the different time periodsagainst S. mutans.

The S. mutans zone of inhibition (mm) of three groups (Group A, Group B and Group C) over the periods (after 24 hrs, 48 hrs, 72 hrs and 7 days) is summarized in Table :1 and also depicted in graph:1. Mean S. mutans zone of inhibition increase linearly with time in all groups and the increase was found highest in Group B followed by Group C and Group A (Group B > Group C > Group A).

Period	Group A	Group B	Group C
After 24 hrs	14.17 ± 0.83	14.50 ± 0.80	15.42 ± 1.00
After 48 hrs	14.92 ± 0.67	15.25 ± 0.87	16.33 ± 0.89
After 72 hrs	15.83 ± 1.03	16.17 ± 1.03	17.08 ± 1.00
After 7 days	16.50 ± 0.90	17.00 ± 1.21	18.25 ± 1.36

Table 2: zone of inhibition (Mean ± SD, n=12) of three groups Group A:Beautifil II, Group B: Hi-Dense, Group C: GC Fuji IX) against L. casei over the different time periods



Graph 2. Mean zone of inhibition of three groups over the different timeperiods against L. casei.

The L. casei zone of inhibition (mm) of three groups (Group A, Group B and Group C) over the periods (after 24 hrs, 48 hrs, 72 hrs and 7 days) is summarized in Table :2 and also shown in Graph:2 . Mean L. casei zone of inhibition increase linearly with time in all groups and the increase was found highest in Group C followed by Group A and Group B (Group C > Group A> Group B).

Discussion:

Dental caries is still a prevalent disease which is recognized as the primary cause of oral pain and tooth loss.[8] It is a common public oral disease which hinders the achievement and maintenance of oral health in all age groups. In a report by WHO in 2005 have mentioned that the problem of oral disease still persists despite great improvements in the oral health of population in several countries.[9]

Procedures applied in therapy of dental caries do not eliminate all the micro-organisms from the prepared cavity. The desirable events such as pulp injury and pulp necrosis are frequently associated with the presence of these residual bacteria and the ingress of new microbes through microleakage. Therefore, the restorative material used for such treatment should possess antibacterial activity against cariogenic bacteria.[10]

Antimicrobial properties of various restorative materials have been evaluated, in the past, but none of them have been able to completely eliminate cariogenic bacteria's from the prepared cavity. Therefore the present study was undertaken to evaluate the antimicrobial efficacy of recently introduced restorative materials.

In the present study, Lactobacillus casei and Streptococcus mutans were taken to evaluate the antimicrobial efficacy of the material as they are most common cariogenic bacteria isolated from the tooth affected with caries. Much work has been done to ascertain the initiation and progression of caries in human population. In one such study by Karpinski TM et al.[3]it was observed that Streptococcus mutans is responsible for the initiation of carious lesion and theLactobacillus are the ones which leads to further progression. These microorganisms have been vastly experimented for antimicrobial efficacy of restorative material in past by many researchers.[11-12]

Once the initiation of caries is there, the ultimate goal is to limit its progression and restoring the affected tooth so that it can regain its normal form and function. This can be achieved by removing the infected portion through cavity preparation and restoring it with suitable restorative material.

In past many restorative materials have been tried to restore the teeth but the drawback being microleakage at the tooth restoration interface which in turn lead to development of secondary caries.[13]Secondary or recurrent dental caries is by far the most frequent reason for replacement of restorations.[14]To overcome these drawbacks many restorative materials have been developed in the modern era such as Glass ionomer cement, which possess antimicrobial efficacy as well as bond to the cavity margins via chemico mechanical bonding.[15]

The methodology of studies concerning the antimicrobial properties of dental materials presented in the literature varies greatly, which in turn hampers the comparison of study results. Most authors, including us, have carried out their experiments using the agar diffusion method.

In the present study, Hi-dense (Group B) showed mean zone of inhibition diameter (Table:1)of 26mm, 26.92mm, 28.17mm and 28.83 mm after an incubation period of 24hrs, 48hrs, 72hrs and 7 days respectively against Streptococcus mutans. The widest zone of inhibition shown by Hi-dense in case of S. mutans can be due to the fact that higher amount of fluoride release during setting of mixed cement. Moreover the higher values can also be attributed to the silver content in its composition which is believed to poses antimicrobial properties. While other factors being physical properties and consistency of the mix. The present research is in agreement with earlier study done by Mahuli S A et al.[16]who compared the antimicrobial efficacy of metal modified glass ionomer cement with FUJI IX, they observed thatHi-dense showed maximum zone of inhibition against S.mutans at all observed time.

On the other hand in case of Lactobacillus casei, Group B (Hi-Dense) showed the mean zone of inhibition (Table:2)of 14.17mm, 14.92mm, 15.83 and 16.50 mm after an incubation period of 24hrs , 48hrs , 72 hrs and 7 days respectively. The observed zone of inhibition was least in case of Lactobacillus casie because of the fact that it has the ability to decrease environment PH values and make it more acidic so as to survive. The results of this study are in accordance with the study done by uczaj-Cepowicz Eet.al.[17] who concluded that the silver reinforced glass ionomer haven't inhibited growth of the standard Lactobacillus caseistrain.

In the present study Group A (Beautifil II) showed the mean zone of inhibition diameter in case of S. mutanswas 11.33mm, 12.08mm, 12.92mm and 13.58mm at 24 hrs, 48hrs, 72hrs and 7th day while for Lactobacillus caseiit was observed 14.50mm, 15.25mm, 16.17mm and 17.00mm at 24hrs,48hrs,72hrs and 7th day respectively. The mean zone of inhibition is observed to be increasing with the time elapsed after setting of cement is due to the inherent property of giomers that release the fluoride ions over the period of time after setting. Also they can uptake fluoride from the environment which in turn serves as reservoir and results in long term leach of fluoride ions from the restoration. Similar results were observed by HotwaniK etal.[18] in their study, they concluded that beautiful II released higher fluoride as compared to conventional glass ionomer at all given time intervals and the amount of fluoride release increases with time after setting of cement.[18]

The mean diameter of inhibitory zone (Table:1) in Group C(GC FUJI IX) was observed to be 20.50mm, 21.17mm, 22.42mm and 23.25mm at 24hrs , 48hrs, 72hrs and 7 days respectively for S. mutans while the inhibitory zone diameter (Table:2) in case of L.Casie was monitored to be 15.42mm, 16.33mm, 17.08mm and 18.25mm at 24hrs,48hrs, 72hrs and 7 days respectively. The zone of inhibition increases with time can be due to the inherent property of GC Fuji IX which includes its chemical composition, release of fluoride and other ions and low pH value during setting of mix. Marczuk-Kolada Getal.[12] in their study observed higher release of fluoride on the seventh day of the study which is in accordance with the results of current research.

According to the current state of knowledge, fluoride-releasing adhesives, including glass ionomer cements, should be considered as an important group of restorative materials. Many authors emphasize the significance of their antibacterial activity, resulting largely from the release of fluoride ions.

Conclusion :

Within the limitations of this in vitro investigation, it can be concluded that:

- All three tested restorative cements that were Beautifil II, Hi-dense and Fuji IX showed antibacterial activity with differences according to the material and bacterial strain.
- Furthermore, Hi-dense restorative material demonstrated maximum zone of inhibition followed by Fuji IX and Beautifil II against Streptococcus mutans.
  Hi-Dense >Fuji IX>Beautifil II
- Fuji IX represented maximum zone of inhibition against Lactobacillus casei as compared to Beautifil II and Hi-dense

Fuji IX>Beautifil II >Hi-Dense

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