Degradation of Resin Restorative Materials by Streptococcus Mutans: A Pilot Study

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Objective: *To evaluate the degradation of three resin based restorative materials by S Mutans.* ***Study design:*** *Class I cavity was prepared in extracted premolars and were randomly divided into 3 groups (Group I – Conventional composite (CC), Group II – Resin Modified GIC and Group III–Giomer). Teeth were then restored by respective restorative material and equally divided in two subgroups (Control and Experimental). Experiment subgroup samples were then incubated in 2 ml of BHI with 1:10 dilution of SM (MTCC-497) grown overnight in BHI whereas control subgroup samples were incubated in BHI without SM. The incubation solution was collected at 2,14 and 30 days interval, and the analysis for identification and quantification of Bis-HPPP was done by High performance Liquid Chromatography.* ***Results:*** *Statistical analysis of the collected data revealed a statistically increased Bis HPPP production in the presence of SM in all the tested materials, with minimum in Resin Modified GIC and a maximum in Conventional Composite (CC).* ***Conclusion****: SM degrades the resin based restorative materials & among the tested materials Resin Modified GIC appears to be most Biostable.*

Key words: Resin, S mutans, degradation, High performance liquid chromatography.

INTRODUCTION

The choice between resin composite and amalgam restorations has been widely driven by esthetic and health concerns. Adverse health effects from exposure to mercury in dental amalgams and the desire for improved esthetics has led to a steady and rapid increase in composite resin restorations.[1] However high fracture rate, reduced longevity, prevalence of secondary caries, and bacterial proliferation associated with biodegradation of resin composites have also been an issue and a focus of research in recent times.[2]

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Nearly 70% composite restorations need to be replaced for failed restorations[3] in an average replacement time of 5.7 years.[4]Recurrent or secondary caries is among one of the primary causes (31-70%) for composite restoration replacement which occurs due to compromised restoration-tooth interface.[5]

Past research on resin materials has focused on physical processes that may have led to degradation. These physical processes are classified either under material loss and uptake (sorption, extraction, dissolution and mineralization) or physical changes (softening, stress cracking, fatigue fracture etc).[6]On the other hand, biochemical processes leading to degradation have seldom been discussed in literature[7].

In resin modified glass ionomer cement the carboxylate groups of glass ionomer cement are replaced by methacrylate group to enable the cement to set by both acid base reaction and photochemical reaction.[8] Similarly Giomers comprise of prereacted glass ionomer fillers added to conventional formulation of composites, the conventional composites have polymeric matrix, the resin monomers in the matrix are predominated by the complexed methacrylate resins and are primarily based on coupling of chemical content through ester linkages. These ester linkages are vulnerable to hydrolysis by esterase activity present in the oral cavity. The result of biodegradation could be deterioration of resin composites especially at the interface. This in turn releases degradation

products such as methacrylic acid (MA), triethylene glycol (TEG) and bishydroxy-propoxy-phenyl-propane (BisHPPP).[9]Saliva and bacteria infiltrate the interface spaces and exaggerate the effects of biodegradation, thus undermining the restoration and contributing to recurrent caries, hypersensitivity and pulpal inflammation.[10]

Human saliva has enzymes namely Cholesterol esterase-like (CE-like) and Pseudocholinesterase (PCE) which have potential to degrade the resin based composite restoration[1]producing several byproducts. BisHPPP produced as one of the by-products is the marker of esterase mediated resin degradation. Cariogenic bacteria Streptococcus mutans also contains esterases similar to saliva which can potentially degrade the resin based restorative materials similarly.[11] The purpose of this study is to compare and evaluate the degradation of resin based restorative materials by Streptococcus mutans.

MATERIALS AND METHOD

After ethical approval from Institutional Ethical Committee, 30 sound premolars extracted for orthodontic treatment purpose were obtained, teeth were then thoroughly cleaned , autoclaved at121 degree C,16lbs for 20 minutes and placed in normal saline for the study. Class I cavity was prepared and the samples were randomly divided into three groups and restored using respective restorative materials abiding to manufacturers instructions (Group I–Conventional Composite–Filtek Z250, Group II–Resin modified glass ionomer cement – Hybond Resi glass and Group III -Giomer-Beautifill II. Samples from each group were further divided into two subgroups A (experimental) and B (control).

Samples from IB,IIB and IIIB were incubated in individual sterile vials containing 2ml of Brain heart infusion and samples from IA, IIA, IIIA were incubated in 2 ml of BHI with 1:10 dilution of S mutans MTCC 479 grown overnight in BHI till the cell count reached a level of 10^9 cells in per ml of BHI. Incubation solutions were collected every 48 hours from each group and replaced with fresh solutions and this procedure was repeated for 30 days. Individual solution collected was centrifuged at 5000 rpm for 20 minutes to separate the cells present within the solution and then analysis for isolation and quantification of Bis HPPP (bis-hydroxy-propoxy-phenyl-propane) degradation product was done on 2nd ,14th and 30th day by High-performance liquid chromatography[12] (HPLC).

RESULTS

A trend of increasing BisHPPP release throughout the incubation period was observed for all groups. One way ANOVA showed highly significant difference (p = 0.000)among IA, IIA and IIIA at different time intervals.(Table 1).One way ANOVA showed highly significant difference (p = 0.000) among IB, IIB and IIIB at different time intervals.(Table 1).Tukey's post hoc test showed that BisHPPP release was significantly higher in IA and IB followed by IIIA and IIIB and least for IIA and IIB.

DISCUSSION

Cariogenic bacteria (S. mutans) present in saliva contain esterase enzymes that can potentially degrade the resin based restorative materials. This study represents a clear vulnerability of resin based restorative materials to one of the most prominent oral bacteria.

Human saliva has been known to have esterase enzymes in it, these esterases have affinity towards the ester linkages present in the resin monomers and cleave the ester bonds present in the Bis GMA producing several byproducts, of which BisHPPP is considered as the marker of esterase mediated resin degradation.

Esterases are linked to virulence and pathogenesis of the microorganisms. Esterase secreted from Group A Streptococcus is a virulence factor that contributes not only to severe invasive infection but is also linked with degradation potential.[13] Esterase-mediated degradation was seen to occur in all restorative materials in the present study however the extent of degradation varied. Material chemistry appeared to be a critical factor in determining material's biochemical stability. The reason for difference in BisHPPP release among the restorative materials can be attributed to the resin content of the same. On comparing the patents filed for these materials, Resin content was found minimal in Resin modified glass ionomer cement and maximum in conventional composites.

Bis-HPPP byproduct formation was also observed in the control groups, this could be due to the ester linkages present in the resin

Table 1: BisHPPP release(µg/ml) at different time intervals from experimental and control groups.

Groups	Time Interval					
	2nd Day		14th Day		30th Day	
	A	B	A	B	A	B
Group I (mean ±SD)	1.4371 ± 0.0054	0.8442 ± 0.0041	7.6153± 0.0082	4.4029± 0.0045	10.4154 ± 0.0045	5.6813± 0.1717
Group II(mean ± SD)	0.5777 ± 0.0058	0.1251 ± 0.0040	4.8852± 0.0040	1.4934 ± 0.0039	6.8265 ± 0.0038	1.7370± 0.0040
Group III(mean ± SD)	0.9032 ± 0.0040	0.4370± 0.0042	6.8172± 0.0043	2.7844 ± 0.0037	9.3365 ± 0.0038	3.5757± 0.0038
One way ANOVA	F= 35979.918, P= 0.000 (<0.05) Sig. Diff.	F= 38455.011, P= 0.000 (<0.05) Sig. Diff.	F= 286828.641, P= 0.000 (<0.05) Sig. Diff.	F= 645431.416, P= 0.000 (<0.05) Sig. Diff.	F= 1021353.782,P= 0.000 (<0.05) Sig. Diff.	F= 1978.909, P= 0.000 (<0.05) Sig. Diff.
Tukey Post Hoc Test	GroupI>GroupIII>GroupII		GroupI>GroupIII>GroupII		GroupI>GroupIII>GroupII	

monomer which get cleaved and undergo hydrolysis in the presence of water. However the significantly higher Bis-HPPP production in experimental group suggests that this hydrolytic degradation gets further amplified in the presence of S. mutans.

Borge *et al* [14] evaluated the degradation of different resin based materials after a caries challenge and suggested that a high caries challenge environment (low pH) degrades the tested materials, Similar results were obtained by Fucio *et al* [15] where they studied the effect of S mutans biofilm over various restorative materials and concluded that a thirty-day-old biofilm promote degradation of restorative materials seen as negative effect on the surface morphology , surface roughness and hardness. Gregson *et al* [16] also concluded that bacteria like S mutans are capable enough to cause changes in surface topography of the composite resin which in turn contributes to the secondary caries and changes in esthetic properties. Kermanshahi *et al* [8] studied the esterase mediated degradation at resin dentin interface and showed that the degradation occurred at the interface created micro-gaps, which was sufficient enough to help bacterial penetration and in turn leading to secondary caries[8]. Mahar *et al* [11] also concluded that S. mutans has esterase activities at levels sufficient enough to degrade resin composites and adhesives thus compromising the resin-dentin interface and contributing to the progression of secondary caries.

CONCLUSION

Based on the above findings it can be suggested that the S mutans degrades resin based restorative materials and among the three resin based restorative materials resin modified glass ionomer cement appears to be most bio-stable. However a long term evaluation of restorative materials for degradation by S mutans is required. However,manufacturers should consider the Bio-stability of materials as prime concern without sacrificing/compromising the physical/chemical properties of the same.

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