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Salivary Indicators for a Healthy Mouth: An In Vivo Study

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Abstract

The study aimed to assess salivary properties like salivary pH, buffering capacity and *S.mutans* count before and after restoration of carious lesions of primary teeth in children aged 6-12 years using three different restorative materials i.e. GIC, SS crown and composite, and divided into three groups of ten each and restored with the above materials respectively. 5ml of stimulated saliva was collected after thorough oral prophylaxis, pH was recorded using pH strips and buffering capacity by salivary buffering capacity test and *S.mutans* count was done by inoculating 1ml of saliva in MSB agar plate and counted after 3 days. The second and the third samples were collected on fifteenth and thirtieth day of restorations and assessed for the same. The analysed results showed a statistically significant increase in both salivary pH and buffering capacity and a decrease in *S.mutans* count in all the three groups when compared to the baseline data. No significant difference was seen between the groups. Hence, concluded that restoration of the tooth itself improves the salivary properties like salivary pH and buffering capacity and decreases the *S.mutans* count, irrespective of the materials used.

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Introduction

Dental caries is one of the most common human diseases, with a high prevalence in the early mixed dentition worldwide (Schipper, 2007; Smith, 2013; Ng MW a, b., 2014, 2012; Ai JY., 2012).

It results from an imbalance between multiple risk factors and protective factors in addition to interplay of three principles factors: host, microflora and substrate over the time. Although not directly involved, but past caries experience, social and behavioural factors can also aid in caries risk identification.

The flow of saliva, its buffer capacity and presence of fluoride play an important role in caries prevention (Sonbul, 2010). Stimulation of saliva results in a flushing effect that clears oral debris and noxious agents, dilutes and eliminates sugars and other substances, increases buffer capacity and balances demineralization/remineralization and antimicrobial activity (Anderson 2003).

Saliva is a clinically informative, biological fluid that is useful for novel approaches to prognosis, laboratory or clinical diagnosis, and monitoring and management of patients with both oral and systemic diseases.

It is easily collected and stored and ideal for early detection of disease as it contains specific soluble biological markers (Stookey, 2008).

Salivary diagnostics has evolved into a sophisticated science, and serves as a subset of the larger field of molecular diagnostics, now recognized as a central player in a wide variety of biomedical basic and clinical areas (Stookey, 2008).

Carious surfaces alter conditions in oral cavity by increasing bacterial adherence, favouring plaque retention, decreasing carbohydrate clearance, and increasing acid production (Malamud *et al.*, 2011).

Glass ionomer cement have the ability to neutralize the salivary acid by buffering the lactic acid via the release of chemical ions and also has an antibacterial effect (Mayanagi *et al.*, 2011).

The antibacterial effects of composites for filling are mainly relevant to inhibition of plaque accumulation on the surface of the materials and tooth around the restoration (Subramanyam *et al.*, 2016).

The systematic removal of all carious lesions and a thorough prophylaxis are sometimes considered to be effective methods for bringing the disease under control (Mayanagi *et al.*, 2011; Subramanyam *et al.*, 2016; I. Nedeljkovic *et al.*, 2016).

However, there is little clinical or experimental evidence to support this concept (I. Nedeljkovic *et al.*, 2016; Massler., 1969; Winter *et al.*, 1973).

Hence our study evaluates and compares the efficacy of restorative materials like glass ionomer cement, composites and stainless-steel crowns on the salivary pH, buffer capacity and *S.mutans* count.

Materials and Methods

The study was conducted on thirty children aged between 6-10 years, who visited the Department of Pedodontics & preventive Dentistry, AECS Maaruti College of Dental Sciences & Research Centre, Bangalore.

Inclusion criteria

1. Caries active children with at least two to three decayed teeth
2. Children with prior consent

Exclusion criteria

1. Medically compromised children.
2. Non-restorable tooth surfaces

The children were divided into three groups of ten each. Group I - restored with GIC, group II – restored with SSC (Stainless steel crowns) and group III – composite restorations 5ml of stimulated saliva was collected after thorough oral prophylaxis. The pH of the saliva was recorded using pH strips. About 1ml of saliva was inoculated into *Mitis -Salivaris- Bacitracin* (MSB) agar plate using a micropipette and inoculation loops. The buffering capacity was tested using salivary buffering capacity test.

Caries was excavated with spoon excavator and cavity preparation done using high speed airotor hand piece and no. 4 round bur followed by restoration with respective restorative material. Saliva samples were collected after 15 days and thirty days of the restoration and assessed for the same. The data obtained were tabulated and subjected to statistical analysis using One Way ANOVA test and inter group comparison was made using Tukeys multiple posthoc procedures.

Results and Discussion

The pH of the saliva increased after 15 days and 30 days of restoration in all the three groups which were statistically significant. In group I, pH improved from 6 (baseline) to 7.9, group II from 6.05 to 7.85 and in group III from 6.4 to 7.65 after 30 days. However, the difference between the groups was not statistically significant.

Table 2 represents the buffering capacity of saliva. There is significant improvement in the buffering capacity on the 15th and 30th day after restoration as compared to the baseline sample. The buffer capacity of group I showed a mean increase of 1.2 + 0.48 after 30 day, group II & III showed an increase of 1.9 + 0.39 & 1.9 + 0.77 respectively after 30 days. There was no statistically significant difference seen between the groups on the 15th day of restoration, but showed statistically significant difference between group I & II and group I

& III on the 30th day where GIC showedgreater increase in the buffer capacity.

Table 3 shows statistically significant reduction in the *S.mutans*count at both 15th and 30th day interval. In group I the *S.mutans* count had reduced by 3.42 + 0.77 30 days after restoration. In group II the reduction was 3.55 + 1.21 and in group III, the reduction was 3.93 + 0.93. The difference between the groups at different time interval was not statistically significant.

Unstimulated saliva is essential for the health and wellbeing of the oral cavity and also bestows a strong protective effect to the oral cavity, against dental caries (Doyle, 1973; Amerongen *et al.*, 2004).

The functions of saliva include lubricating the oral tissues, protecting the oral soft tissues from abrasion during mastication, facilitating the digestion of carbohydrates, antibacterial activity against foreign microorganisms, flushing the oral cavity to remove food particles and debris from the tissues, and chemically maintaining an environment rich in calcium, phosphate and acid buffering agents (Anderson 2003).

More than 700 oral microbial species have now been identified, making oral flora one of themost complex microbial communities in the human body (Kedjarune *et al.*, 1997; Aas., 2005). Saliva could act as an oralcirculating fluid for bacterial transmission and act as a reservoir for bacterial colonization (Paster *et al.*, 2001).

Bacteria, including anaerobic species, can survive in saliva and utilize salivary constituents for growth (Greenstein *et al.*, 1997; De Jong *et al.*, 1984; Bowden., 1997).

Table.1 Analysis of Salivary pH before and after restoration and comparison between the three groups (I, II, III) byone-way ANOVA

Groups	Before restoration		15 days		30 days		Changes from before restoration to					
							15 days			30 days		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	P value	Mean	SD	P value
Group I	6.00	0.94	7.50	0.85	7.90	0.74	1.50	0.97	0.0009*	1.90	1.20	0.0007*
Group II	6.05	0.72	7.25	0.49	7.85	0.41	1.20	0.48	0.0001*	1.80	0.54	0.0001*
Group III	6.40	0.66	7.25	0.35	7.65	0.78	0.85	0.58	0.0012*	1.25	0.89	0.0014
Pair wise comparisons by Tukeys multiple posthoc procedures												
Group I vs Group II	p=0.9890		p=0.6263		p=0.9846		p=0.6176			p=0.9678		
Group I vs Group III	p=0.4986		p=0.6263		p=0.6818		p=0.1207			p=0.2681		
Group II vs Group III	p=0.5849		p=0.9999		p=0.7815		p=0.5214			p=0.3843		

*p<0.05, # applied paired t test

Table.2 Analysis of Salivary buffer capacity before and after restoration and comparison between the three groups (I, II, III) by paired t test

Groups	Before restoration		15 days		30 days		Changes from before restoration to					
							15 days			30 days		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	P value	Mean	SD	P value
Group I	1.30	0.63	2.15	0.88	2.50	0.67	0.85	0.47	0.0003*	1.20	0.48	0.0001*
Group II	1.25	0.89	2.55	0.83	3.15	0.85	1.30	0.42	0.0001*	1.90	0.39	0.0001*
Group III	1.40	0.66	2.55	0.64	3.30	0.48	1.15	0.47	0.0001*	1.90	0.77	0.0001*
Pair wise comparisons by Tukeys multiple posthoc procedures												
Group I vs Group II	p=0.9875		p=0.5056		p=0.1035		p=0.0895			p=0.0291*		
Group I vs Group III	p=0.9506		p=0.5056		p=0.0372*		p=0.3226			p=0.0291*		
Group II vs Group III	p=0.8923		p=0.9990		p=0.8765		p=0.7462			p=0.9999		

*p<0.05, # applied paired t test

Table.3 Analysis of *S.mutans* count before and after restoration and comparison between the three groups (I, II, III) byone-way ANOVA

Groups	Before restoration		15 days		30 days		Changes from before restoration to					
							15 days			30 days		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	P value	Mean	SD	P value
Group I	5.40	1.75	3.10	1.30	1.98	1.03	2.30	0.74	0.0001*	3.42	0.77	0.0001*
Group II	4.76	1.48	2.76	0.79	1.21	0.46	2.00	0.98	0.0001*	3.55	1.21	0.0001*
Group II	5.50	1.16	2.94	0.52	1.57	0.44	2.56	0.69	0.0001*	3.93	0.93	0.0001*
Pair wise comparisons by Tukeys multiple posthoc procedures												
Group I vs Group II	p=0.6032		p=0.6894		p=0.0523		p=0.6906			p=0.9569		
Group I vs Group III	p=0.9884		p=0.9205		p=0.4005		p=0.7597			p=0.4932		
Group II vs Group III	p=0.5142		p=0.8996		p=0.4947		p=0.2906			p=0.6662		

*p<0.05, # applied paired t test

Carious tooth surfaces may induce intraoral changes, such as increased plaque accumulation, elevated bacterial colonization, reduced carbohydrate clearance, and increased acid production and also contributes to constant reinfection of the patient's mouth by maintaining the microbiological risk of development of new lesions (Loesche, 1986). Aminabadi *et al.,* (2013) observed that the increase in pH has a linear relationship with the number of eliminated carious tooth surfaces and concluded that saliva quality can be substantially improved by eliminating dental caries (Krasse., 1986). Windowati *et al.,* (2013) observed patients with high caries risk have significantly lower salivary pH compared to patients with low caries risk.

Glass ionomer cements are capable of elevating the pH to the level which could arrest the caries (Windowati *et al.,* 2013). GIC inhibits the pH fall due to slow but steady release of fluoride from GIC, especially at acidic conditions (Humphrey, 1950). Several studies support these findings (Windowati *et al.,* 2013; Humphrey., 1950; Krishnamurthy., 2012; Topcuoglu *et al.,* 2012).

In our study, we have also found significant improvement in the buffer capacity in the GIC group which can be attributed to the attack at the matrix releases both poly acrylic acid and metal ions. Consequently, the storage solution becomes a mixture of lactic acid and metal lactates, the classic combination that creates a buffer solution (Krishnamurthy, 2012).

Conventional composite shows no buffering ability. Buffering capacity directly influences the demineralization process of the adjacent tooth tissue, but it was also demonstrated that the inability of composite to increase the local pH facilitates the growth of aciduric and cariogenic bacteria (Willershausen *et al.,* 2003). This is in contrast to the results of our study which can be attributed to the fact that, the smoother surface of composite restorations decreases the bacterial adhesion and thereby depleting the bacterial reservoir (Imazato, 2003).

Stainless steel crown (SSC) was introduced by Humphrey in 1950 (Seale, 2002). Since 1950, SSCs have been widely used for the restoration of grossly destructed carious primary teeth and those teeth requiring pulp therapy or where other restorative materials are likely to fail. The SSC is easy to place, economical and it has the excellent durability (Braff, 1975). Braff *et al.,* (1975) stated that SSCswere significantly superior to multisurface amalgams in the restoration of primary molars. Willershausen (2003) showed a potential positive inhibitory effect of stainless steel crown restorations as compared to composite fillings with respect to the oral bacterial colonization (Nicholson, 1999). The present study shows a significant improvement in the salivary pH and buffer capacity and reduction in *S.mutans* count in the Stainless Steel crown group which may be due to lack bacterial adhesion sites leading to depletion of the bacterial reservoir.

The present study, compared saliva before and after eliminating carious lesions. There was a significant increase in salivary pH and buffering capacity, and a significant decrease in the *S.mutans* count at the end of the study in all the three groups. Studies have shown an inverse relation, between the salivary pH and buffer capacity and the number of bacteria present in the saliva (Kesel *et al.,* 1958; Elliot *et al.,* 1964; Ryu *et al.,* 2010).

Keene *et al.,* (1976) and Loesch *et al.*,(1977) showed a reduction in the number of sites of *S.mutans* and also a decrease in the proportion of S. mutans to total streptococci, thus making the oral environment more alkaline by increasing the salivary pH which was correlating with our results.

In conclusion, Removal of the caries itself improves the salivary properties like salivary pH, buffer capacity and reduces the *S.mutans* count, irrespective of the materials used.

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