ANTIBACTERIAL PROPERTIES, FLUORIDE RELEASE AND RECHARGEABILITY OF PIT AND FISSURE SEALANTS*: AN IN-VITRO STUDY

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ABSTRACT:
Despite the recent declines in dental diseases in developed nations due to better dental awareness and prevention, the high prevalence of dental caries among school children still poses a significant health problem. Fluoride releasing sealants are effective in arresting pit and fissure caries by reducing enamel solubility and acid production by bacteria that initiate caries. Studies reveal that initial fluoride release is relatively intense but declines quickly reaching extremely low levels in the absence of any procedure of any fluoride recharge. Some recent studies have shown that Glass ionomers have the capacity to absorb fluoride from tooth paste or fluoride solutions and subsequently release it. There is minimal data available about the antimicrobial activity of fluoride releasing sealants as well as the rechargeability of these sealants. Hence the present study evaluated the fluoride release and antibacterial properties of two conventionally used pit and fissure sealants, (Glass ionomer sealant, and Composite resin sealant) and their rechargeability with a fluoride dentifrice (Kidodent®). Results showed that Composite resin sealant had a higher antibacterial activity when compared to Glass ionomer sealant. Glass ionomer sealant had a much higher immediate fluoride release than Composite resin sealant. However both sealants showed significant rechargeability when exposed to fluoride tooth paste.

Key words: Pit and fissure sealants, Antibacterial activity, Fluoride release, Rechargeability

INTRODUCTION:
Caries develops when bacterial plaque cannot be removed from the deeper regions of the fissure. Over 80% of caries that occurs in children and teenagers is in the occlusal surface.¹ To prevent fissure caries, the concept of altering the pit and fissure morphology as a means of reducing the susceptibility of occlusal surfaces to dental caries has been in vogue for over 100 years.²

Sealants form a physical barrier between the tooth surface and the oral environment that reduces carious lesions caused by Streptococcus mutans.³ A more recent innovation has been the introduction of fluoride releasing sealants. The rationale is that the sealant acts as a reservoir from which the added fluoride is gradually released into the oral cavity to inhibit...
enamel demineralization and to enhance remineralization. Hence, a dual advantage is obtained.\(^3\)

It is postulated that sealants possessing antibacterial properties are advantageous. Since microleakage cannot be avoided, antibacterial properties of fissure sealant materials may contribute to the prevention of caries. It has been suggested that fluoride release by the sealants is able to produce the inhibition of S. mutans. \(^1\)

Studies reveal that initial fluoride release is intense relatively but declines quickly reaching extremely low levels in the absence of any procedure of fluoride recharge.\(^4\) Some recent studies have shown that Glass ionomers have a capacity to absorb fluoride from tooth pastes or fluoride solutions and subsequently release it. \(^5\)

Glass ionomers, introduced to dentistry in the early 1970s, possess many properties that support their consideration in a wide variety of clinical applications. The two primary advantages of glass ionomer restoratives include adhesion to dentin and enamel and potential to increase the tooth’s resistance to secondary caries due to sustained fluoride release.\(^6\) The Glass ionomer sealant (Fuji VII), used in our study is moisture tolerant, acid resistant, and possesses a unique “pink” color.

Composite resin systems have been developed over several decades and serve the dentist in several applications. Recently fluoride has been added to composite resin system to confer anticariogenic properties. In general the composites do not leach fluoride as do the glass ionomer restorative materials from the matrix phase but fluoride is released from the filler particles.\(^5\) The composite resin sealant (Clinpro) used in our study has a high fluoride release, a unique pink color which changes to white color after polymerization.

Fluoride Dentifrices have been used widely due to their cariostatic properties and remineralization potential. Ability of the restorative materials to uptake fluoride during brushing, and then releasing in between brushings would provide a mechanism for potential caries inhibition.\(^7\)

Various studies have focused on retention and fluoride release of pit and fissure sealants. \(^8,9\) However not many studies have been done regarding the Antibacterial activity, fluoride release and fluoride rechargeability of pit and fissure sealants in our scenario. Thus the present investigation evaluated the Antibacterial activity, immediate fluoride release from glass ionomer sealant and composite resin sealant as well as their rechargeability with a fluoride dentifrice (Kidodent),\(^8\) which contains sodium monofluorophosphate -0.38 % w/w in a flavored gel base specially formulated for children below 6 years of age with fluoride content of 550 ppm which has been launched for the first time in India.
MATERIALS AND METHODS:

ANTIBACTERIAL ACTIVITY

The antibacterial effect of sealants on Mutans Streptococci was assessed by measuring the inhibition zones produced around the sealants on trypticase soy agar plates. The inhibition zones were measured in millimeters. The experiment was done in triplicate. \cite{10} (Fig 1)\& (Photograph no 1).

FLUORIDE RELEASE

Conventionally used Glass ionomer sealant (Fuji VII GC) and composite resin sealant (Clinpro 3M ) were evaluated for fluoride release and recharge ability by using Kidodent tooth paste( 550 ppm).

Teflon molds were fabricated with inside dimensions of 5mm diameter by 3mm height into which the sealants were placed and polymerized in two layers, for 20 seconds each by using visible light curing unit. The discs were rinsed and transferred to tubes containing 10 ml of deionized water. Fluoride release was measured and recorded after a 24 hour intervals by using an Orion microprocessor ion analyzer (model no 901) thereafter, fluoride release was measured at 24 hours intervals for the next two weeks. \cite{11} (Fig 2)

RECHARGE \cite{12}

At the end of the two week period, the discs were randomly divided into two groups, test and control groups. Each disc in the test group was exposed to an external fluoride source, Kidodent tooth paste, daily, by immersing the disc in 2 ml of the tooth paste for five minutes followed by rinsing and drying the specimen and then storing in deionized water This was done to simulate daily tooth brushing by children for a period of at least 5 minutes. Fluoride ion released in to the de ionized water was measured by using fluoride ion analyzer for the next two weeks. Results were subjected to statistical analysis by using Students paired t test (Fig 3.)

RESULT:

The mean of the inhibition zones produced by Composite resin sealant showed a wider zone of inhibition zone (6.3mm) compared to that of glass ionomer (3.5 mm) which was statistically very highly significant.\(p<.001)\).

The mean cumulative fluoride release from day 1 to day 14 for glass ionomer sealant ranged between 8.2 ppm to 0.634 ppm and for Composite resin sealant ranged between 0.375 ppm to 0.055 ppm respectively. The mean cumulative fluoride release after 24 hours in the glass ionomer sealant was as high as 8.2 ppm, whereas in composite resin sealant it was only 0.375 ppm which was statistically very highly significant. The fluoride release dropped drastically to 3 ppm and 0.212 ppm for glass ionomer sealant and composite resin sealant respectively on day 2, thereafter periodic fluctuation in discharge of fluoride was observed for
both sealants. Glass ionomer however showed an increase in fluoride release compared to composite resin sealant which was statistically significant.

There was a marked increased fluoride release after recharging with fluoride tooth paste in both the sealants which was statistically significant. The fluoride release from glass ionomer sealant increased from 0.634 ppm to 1.444 ppm from day 14 to day 15 and in composite resin sealant the fluoride release increased from 0.055 to 0.503 ppm from day 14 to day 15 respectively i.e on the first day after recharge. It was observed that that fluoride release from both sealants was greatest during the first two days of recharge which was statistically significant. Thereafter the fluoride release showed a gradual decrease with periodic fluctuations for the next 10 days (upto day 24). Overall, Glass ionomer sealants (Fuji VII) showed a significant higher net fluoride release than composite resin sealant (Clinpro) upto 27 days after recharge.

The fluoride release of control samples in both the sealants remained close to the baseline values (i.e. from day 14 upto day 28) with periodic fluctuations which was statistically not significant.

**DISCUSSION:**

Caries develops when bacterial plaque cannot be removed from the deeper regions of the fissure. Over 80% of caries that occurs in children and teenagers is in the occlusal surface. Sealants form a physical barrier between the oral environment and deep fissures that contribute to caries prevention.[1]

Our study evaluated the antibacterial activity of the two pit and fissure sealants, Glass ionomer sealant and composite resin sealant. It was seen that glass ionomer sealant and the composite resin sealant exhibited definite inhibition zone against S Mutans. However, composite resin sealant (Clinpro) showed a higher inhibitory effect which was statistically very highly significant. This could be attributed due to the high fluoride release of the composite resin sealant.

A similar study was conducted to evaluate the antibacterial activity of three fluoride releasing sealants, namely Helioseal, Fluoroshield, and Teeth mate FTM on the strains of Mutans Streptococci. They showed that Teethmate FTM was the only sealant which showed the inhibitory activity against the strains of Mutans Streptococci tested.[4]

Fluoride and pit and fissure sealants have been widely accepted and well documented as effective caries preventive measure.[13,14,15] Low but slightly elevated levels of fluoride in saliva and plaque helps to prevent and reverse caries by inhibiting demineralization and enhancing remineralization.[15] Chemical studies reveal that initial fluoride release from glass ionomer cements is relatively intense but declines quickly reaching extremely low levels in the absence of any procedure of fluoride recharge.[5]

In the present study, it was observed that immediate mean cumulative fluoride release of both the sealants was high during first three days, however Glass ionomer sealant (Fuji VII) showed a much higher fluoride release during the first two weeks as compared to Composite resin sealant (p=0.001). These findings are in the accordance with findings of Wailan D Chan et al, and Dhull et al who found that the amount and rate of fluoride released from short term elution process is much higher for the Glass ionomer Cements and resin modified Glass ionomer cements than compared to the commercial composites.\[16,17\]

There was a significant drop in the fluoride release from day 1 to day 2 in the Fuji VII group i.e. from 8.0 ppm to 3.0 ppm (graphs 1&2) which gradually decreased till day 14 whereas in the Clinpro sealant, the drop in the fluoride release from day 1 to day 2 was only from 0.375 ppm to 0.212 ppm, which was considerably less than a similar drop in Glass ionomer sealant, thereafter fluoride levels steadily declined up to 14 days. This observation is in agreement with the findings of Schwartz et al, and Willian D Chan et al. In Glass ionomer restorative materials there is said to be an acid base reaction. The set material consist of unreacted glass core in a matrix of polyacid which leaches Ca, Al, F ions. The initial fluoride burst of fluoride ions release is due to high concentration of fluoride ions that remain in matrix immediately after setting reaction concludes. Later the long term release of fluoride which is at a lower rate is due to the unreacted glass core particles.\[17,18\]

Whereas in composites, there is no acid base reaction; the only source of fluoride would come from the Glass filler particles resulting in a slow diffusive release. This could explain the difference in the fluoride release between Glass ionomer sealant (Fuji VII) and Composite resin sealant (Clinpro).\[19,23\]

At the end of a two week period, when the sealants were exposed to a fluoride tooth paste, both the sealants showed a considerable increase in fluoride release as compared to baseline values (on day 14) which was statistically significant. It is in accordance with earlier study where the fluoride level boosted above baseline for a period of 2 weeks following a single exposure to fluoridated tooth paste.\[13,22\] In the present study the amount of fluoride release after continuous recharge for both the sealants were greatest in the first two days after which, periodic fluctuations were seen. However, the fluoride levels were much higher than the baseline values for both sealants on all the days providing a contineous source of fluoride ions.

Fluoride uptake may be more from the surface rather a bulk diffusion effect. It has been expected that the maturity of the cement would effect its fluoride uptake because as the cement ages the ionic matrix becomes more cross linked. A more cross linked matrix would
impede the required diffusion process.[20]

When the net fluoride releases of the two sealants were compared after recharge, the glass ionomer sealant released significantly more fluoride than composite resin sealant up to 25th post exposure day. This is in accordance with Takashaki et al and Mousaninasab et al who found that, the resin composites released significantly less fluoride than the Glass ionomer cements.[12,21]

However, it was interesting to observe that Glass ionomer sealants showed a remarkable increase in fluoride release on days 26, 27 and 28 in both the control and the recharged samples. This could be attributed to post maturation slow long term fluoride release of the sealants. However, a similar sharp increase in fluoride release was also observed in recharged samples of composite resin sealant (Clinpro) on days 25, 27, and 28. This finding needs to be further investigated.

When the fluoride release from the control samples of both the sealants were compared (i.e samples without recharge) it was observed that fluoride release by the composite resin sealant was much less than the glass ionomer sealant (Fuji vii). Fluoride release by Glass ionomer sealant even without recharge was higher than composite resin sealant (Clinpro) with recharge.

Fluoride release is one of the advantages of pit and fissure sealants containing fluoride. Our study supports the rechargeability of Glass ionomer sealant and Composite resin sealant with an external fluoride source such as a fluoride tooth paste with glass ionomer sealant showing higher rechargeability. This implies that these materials can act as rechargeable fluoride reservoirs delivering low levels of fluoride to the oral cavity after the ion source has been removed.

CONCLUSION:

Composite resin sealant showed higher antibacterial activity compared to Glass ionomer sealant.

Glass ionomer sealant showed a continuous fluoride release which increased further after recharging with fluoride tooth paste.

Composite resin sealant showed a minimal fluoride release which increased slightly after recharging with fluoride tooth paste.

REFERENCES:


2. Chestnutt IG, Schafer F, Jacobson AP and Stephen KW. The prevalence and


FIGURES:

FIGURE 1

Sealants placed on trypticase soy agar plate with inhibition zones

GRAPH 1

Comparison of the immediate fluoride release and the fluoride release after fluoride recharge of glass ionomer sealant (Fuji VII) and composite resin sealant (CLINPRO) at various time intervals

Graph 2
Comparison of fluoride release of Fuji VII, CLINPRO without recharge at various time intervals
FIGURE 2

ANTIBACTERIAL ACTIVITY OF SEALANTS

- Standard Indicator Bacterial Strains (Mutans streptococci)
- Tested strains were grown and sub cultured once a week on Trypticase soy agar plate aerobically
- Grown in Brain heart infusion broth for 18 hours at 37 degree centigrade
- 200 microlitres of an overnight culture broth of an indicator strain was added to 3 ml to sterile saline poured onto surface of TSA plates
- The sealants were polymerized by a visible light source for 40 seconds.
- Sealants were then placed on the pre designated area of about 3 mm in diameter onto surface of TSA plates previously inoculated with S mutans and
- Inhibition zones were recorded in millimeters
FIGURE 3

STUDY DESIGN TO MEASURE THE IMMEDIATE FLUORIDE RELEASE

Disc shaped samples of the sealants were fabricated in a Teflon mold with inside dimensions of 5mm diameter by 3mm height.

The sealants were polymerized in two layers, for 20 seconds each by using visible light curing unit.

30 such discs of each material were fabricated & stored in 10ml of deionized water at 37 degree centigade in separate polystyrene test tubes.

Initial fluoride release was measured and recorded after a 24 hour interval, thereafter fluoride release was measured at 24 hour intervals for the next two weeks.
FIGURE -3

STUDY DESIGN TO MEASURE THE FLUORIDE RELEASE AFTER RECHARGING OF SEALANTS

Fluoride release was analyzed after 24 hours, and thereafter repeated at 24 hour intervals for the next two weeks.

**GIC sealant (Fuji VII)**

- Control (15)
- No recharge
- Exposed to fluoride tooth paste

**Composite resin sealant (Clinpro)**

- Control (15)
- No recharge
- Exposed to fluoride tooth paste

Test (15)