Evaluation of Microleakage of Nanoionomer and Nanocomposite Restorations, immersed in Fruit Drink, Fresh Fruit Juice and Soft Drink – An *in vitro* Study

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Aim: *To evaluate microleakage of Nanoionomer (3M ESPE Ketac™ N100 Light cured Nanoionomer Restorative) and Nanocomposite (3M ESPE Filtek™ Z350 XT Universal Restorative) restorations, immersed in fruit drink, fresh fruit juice and soft drink.* ***Study design:*** *Eighty caries free maxillary premolars extracted for orthodontic purpose were used for the study. Class V cavities were prepared and restored with Nanocomposite on buccal surface and Nanoionomer on the palatal surface. The teeth were thermocycled following the restoration. The experimental groups comprised of 72 teeth (3 groups comprising 24 teeth each for fruit drink, fresh fruit juice and soft drink), while remaining 8 formed the control group. Each of experimental group was further divided into three subgroups (low, medium and high immersion).The teeth were finally immersed in Rhodamine B dye, sectioned and evaluated under stereomicroscope. Statistical analyses used were Mann-Whitney test and ANOVA test.* ***Results:*** *The teeth showed statistically significant microleakage as the immersion regime increased. Soft drink group showed highest microleakage followed by fresh fruit juice and fruit drink. Nanocomposite exhibited more microleakage but the comparison was not statistically significant.* ***Conclusion:*** *The three beverages used in the study affected the microleakage of both restorative materials significantly. The microleakage scores increased as the frequency of the immersions increased. Soft drink caused highest microleakage followed by fresh fruit juice and fruit drink.*

Key words: microleakage, beverages, Nanoionomer, Nanocomposite.

INTRODUCTION

The concept of health has prevailed for centuries and dietary habits are apparently changing with modernization. It has been reported that contemporary fluid consumption patterns of children are now more diverse than in past years, since carbonated soft drinks and fruit juices have replaced much of the previous consumption of water and milk among children. [1]

The potential acidity and cariogenicity of beverages consumed by young children and adolescents has been the subject of many studies in the past 30 years. Excessive contact of the tooth structure with acidic food leads to loss of dental hard tissues. Thus it can be assumed that restorative materials, when subjected to low pH environment in the oral cavity, leads to degradation of its surface and marginal integrity. [2]

Microleakage may be defined as the clinically undetectable passage of bacteria, fluids, molecules or ions between a cavity wall and the restorative material applied to it.[3] Clinical experiences that are associated with leakage are staining around the margins of restorations, postoperative sensitivity, secondary caries, restoration failure, pulpal pathology or pulpal death, partial or total loss of restoration.[4, 5]

A wide variety of restorative materials are available nowadays, the recent ones being Nanoionomers and Nanocomposites. Today, nanotechnology has become a popular discipline in science and technology. Nanotechnology is the production of functional materials and structures in the range of 0.1 to 100 nanometers by various physical and chemical methods. Inclusion of nanofiller and nanoclusters of filler material provides enhanced esthetics, improved polishability and enhancement of certain physical characteristics of the restorative material in the mouth.[6]

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There is no literature reporting the effects of beverages on the marginal integrity of these newer restorative materials. Therefore, the current study was carried out with the following aims and objectives:

1. To evaluate the effect of a fruit drink, a fresh fruit juice and a soft drink on the microleakage, if any, in Nanoionomer and Nanocomposite.
2. To compare the effect of different immersion regimes on the microleakage.
3. To compare the microleakage between Nanoionomer and Nanocomposite.

MATERIALS AND METHOD

This study was carried out in the Department of Paedodontics and Preventive Dentistry, Terna Dental College, Nerul, Navi Mumbai. The study was approved by the Institutional Review Board – Ethics Committee. The restorative materials tested were 3M ESPE Ketac™ N100 Light cured Nanoionomer Restorative (Nanoionomer) and 3M ESPE Filtek™ Z350 XT Universal Restorative (Nanocomposite). The beverages tested in the current study were a fruit drink (Frooti, *Parle Agro Co*.), a freshly prepared fruit juice (Orange) and a soft drink (Coca Cola, *Coca-Cola Co*.).

80 human maxillary premolars with no signs of caries, extracted for orthodontic purpose were used. Class V cavities were prepared on the buccal and lingual surfaces of the teeth, 1mm above the cemento-enamel junction. The cavity preparation was standardized using a William's graduated periodontal probe to be 3mm in length, 2mm in width and 2mm in depth. The cavities on the buccal surface were restored with Nanocomposite, while those the lingual surface was restored with Nanoionomer. The restorations were done in strict accordance with the manufacturer's guidelines. The restored teeth were stored at room temperature in water for 1 week. During this period the teeth were subjected to 200 thermocycles between 5°C and 55°C. Dwell time was 1 minute with 10 seconds transit between baths.[7]

Out of the 80 prepared tooth samples, 72 were equally divided into three groups of 24 each. Remaining 8 were used as control. Each group was further subdivided as given in Table 1.

- Group I – Fruit drink (24 teeth)
- Group II – Fresh fruit juice(24 teeth)
- Group III – Soft drink (24 teeth)

The samples were subjected to the various immersion regimens. For low immersion regime the restorations were subjected to one immersion lasting five minutes per day. For medium immersion regime they were subjected to 5 immersions per day. For high immersion regime the samples were subjected to 10 immersions per day. Each immersion lasted for five minutes, and the immersions were evenly distributed over a 12 hour period. The whole procedure was carried out for 8 days. Before and after each immersion the samples were copiously rinsed in 0.1M phosphate buffered saline (pH 7.2). When not exposed to the immersion regime, they were stored in deionised water at room temperature.[8]

At the end of the test period the apices of the teeth were sealed with sticky wax, and all tooth surfaces except a 1mm wide zone around the margins of the restoration was painted with nail varnish. The teeth were then immersed in Rhodamine B solution for 24 hours, rinsed, dried, and invested in clear resin. Each tooth was sectioned bucco-lingually through the centre of the restoration with help of a low speed water cooled diamond disc.

Table 1 – Distribution of sample in group and subgroups.

Group	Low Immersion (1/Day)	Medium Immersion (5/Day)	High Immersion (10/Day)
Group I (Fruit drink)	8	8	8
Group II (Fresh fruit juice)	8	8	8
Group III (Soft drink)	8	8	8
Group IV (Control)	8 (No Immersion regimes)		

The specimens thus obtained were examined under 40X magnification in stereomicroscope (*Motic Co. SMZ-143 series*) to evaluate the microleakage. Dye penetration was graded based on the extent of penetration along the walls of the restoration. This was scored using criteria similar to the one used by Staninec and Holtz (1988).[9]

Scores:

Score 0- No dye penetration (Figure 1)

Score 1- Dye penetration along occlusal wall but less than half way to axial wall (Figure 2)

Score 2- Dye penetration along occlusal wall but more than half way to the axial wall (Figure 3)

Score 3- Dye penetration along occlusal wall upto and along axial wall (Figure 4)

The samples were randomly cross examined for the evaluation of the scores by an independent investigator to eliminate the bias. The statistical analysis was done using SPSS (version 16.0) software. Scores obtained were analysed using Mann Whitney test and was considered statistically significant at $p = 0.05$ or less. Between the groups and the two restorative materials, the comparison was done using ANOVA test.

RESULTS

The results and observations of microleakage were summarized as follows:

NANOIONOMER: The frequency, mean score and median of all groups for Nanoionomer is depicted in Table 2.

Intragroup Comparison: When specimens were compared between the three immersion regimes, all the specimens scored higher microleakage as the number of immersion intervals increased. p values of the comparison between different immersion regimes are depicted in Table 3.

Intergroup Comparison: When Low immersion regimes of the three groups were compared with each other, the result was not statistically significant. When Medium immersion regimes of the three groups were compared with each other, the result was not significant. When High immersion regimes of the three groups were compared with each other, the result was significant between Group

Figure 1 – Score 0 (under 40X magnification)

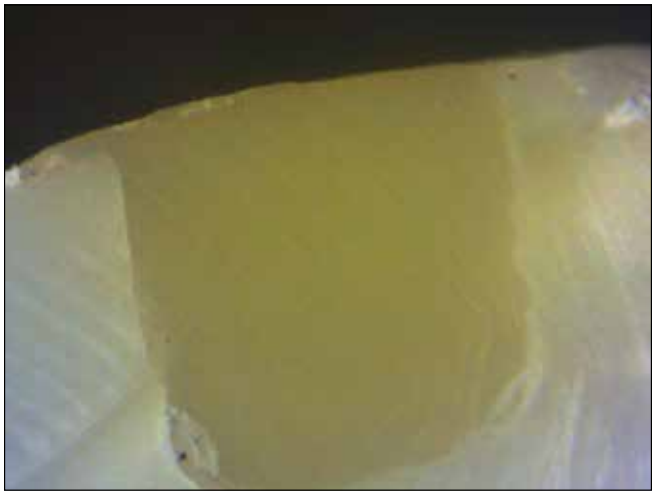


Figure 2 – Score 1 (under 40X magnification)

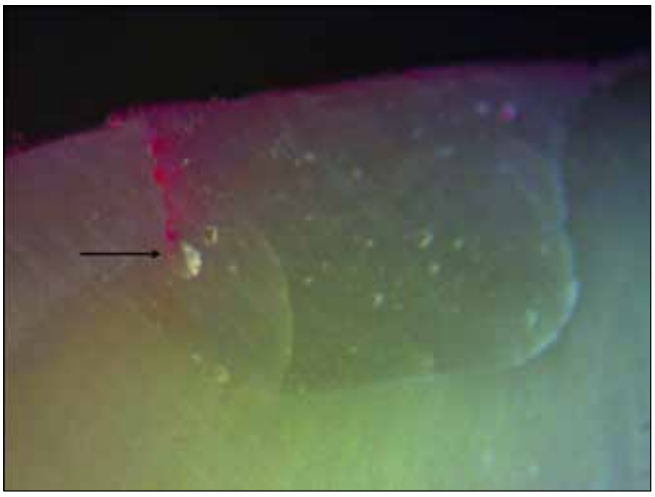


Figure 3 – Score 2 (under 40X magnification)

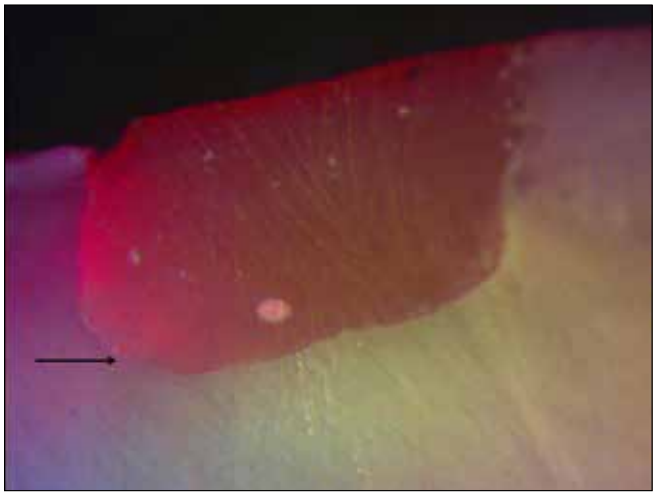


Figure 4 – Score 3 (under 40X magnification)

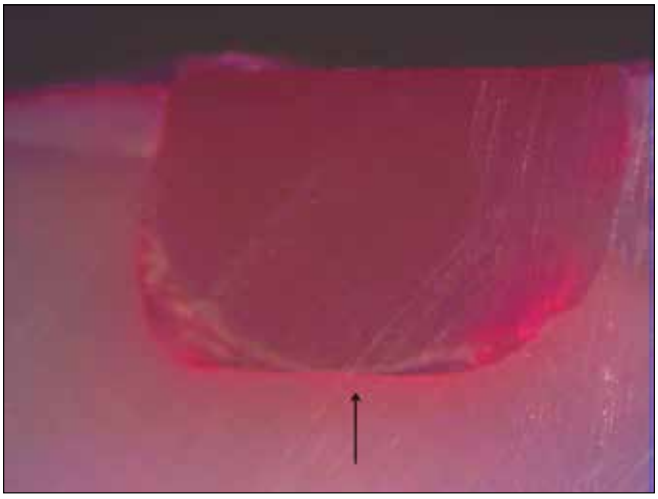


Table 2 - Descriptive statistics on the microleakage pattern of Nanoionomer following immersion for varying periods of time in Fruit drink, Fresh fruit juice, Soft drink and Control.

Groups	Micro-leakage Scores	Low Immersion		Medium Immersion		High Immersion	
		No	Mean Median	No	Mean Median	No	Mean Median
Group I (Fruit drink)	0	6	0.25 0	4			
	1	2		4	0.5 0.5	6	1.25 1
	2	2				2	
	3						
Group II (Fresh fruit juice)	0	5		2		3	
	1	3		4		3	
	2		0.38 0	2	1 1	2	1.88 2
	3						
Group III (Soft drink)	0	4					
	1	4	0.5	5	1.38 1	4	2.5 2.5
	2		0.5	3			
	3					4	
No Immersion regime							
Group IV (Control)	0	7					
	1	1					
	2		0.12 0				
	3						

Table 3 - Intragroup comparison for Nanoionomer showing p-value (S- significant, NS- not significant)

Group	Low v/s Medium	Medium v/s High	Low v/s High
Group I (Fruit drink)	**p =** 0.440 **NS**	**p =** 0.038 **S**	**p =** 0.005 **S**
Group II (Fresh fruit juice)	**p =** 0.130 **NS**	**p =** 0.083 **NS**	**p =** 0.002 **S**
Group III (Soft drink)	**p =** 0.021 **S**	**p =** 0.005 **S**	**p =** 0.0001 **S**

I (fruit drink) and Group III (soft drink), whereas it was not significant for other comparisons. (Figure 5)

NANOCOMPOSITE: The frequency, mean score and median of all groups for same is depicted in Table 4.

Intragroup Comparison: When specimens were compared between the three immersion regimes, all the specimens scored higher microleakage as the number of immersion intervals increased. p values of the comparison between different immersion regime are depicted in Table 5.

Intergroup Comparison: When Low immersion regimes of the three groups were compared with each other, the result was not significant. When Medium immersion regimes of the three groups were compared with each other, the result was not significant. When High immersion regimes of the three groups were compared with each other, the result was significant between Group I (fruit drink) and Group III (soft drink), whereas it was not significant for other comparisons. (Figure 6)

COMPARISON BETWEEN NANOIONOMER AND NANOCOMPOSITE: When mean scores of Nanoionomer and Nanocomposite were compared, Nanocomposite showed higher scores than Nanoionomer in all the three groups (fruit drink, fresh fruit juice and soft drink). However the comparison was not statistically significant.

DISCUSSION

Pediatric restorative dentistry has evolved a long way from the age old amalgam restorations to the present day state of the art Nanomaterials.[10] Nanotechnology can provide more stable and natural interface between the mineralized hard tissues and these advanced restorative biomaterials.[11, 12] However, the recently introduced nanofilled restorative materials have not been extensively researched.

Microleakage remains a problem with the commonly used restorative materials. Gap formation and concomitant leakage of bacterial fluids, molecules and ions are brought about by dimensional change such as polymerisation shrinkage, thermal expansion, incomplete hygroscopic expansion and chemical degradation.[2]

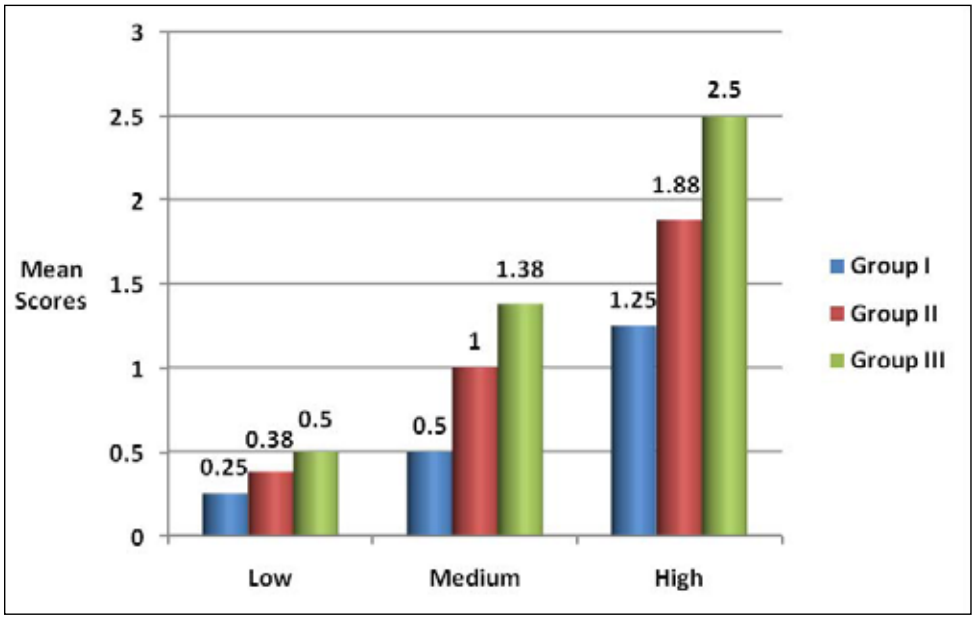
In the last decade, consumption of beverages has increased dramatically, especially among the children and adolescents.[13] The erosive potential of beverages has been reported in both in vivo and in vitro studies. However, many erosion studies have employed extremely long immersion regimes ranging from 15 minutes to 72 hours in eroding solutions, which might not be representative of the normal consumption pattern.[14] There are a number of possible limitations in the above studies, as these studies do not accurately depict the actual impact of the frequency or the prolonged and continuous exposure to beverages. Accordingly, a more realistic consumption pattern put forward by Maupome *et al* in 1998 was followed.[8]

The results of the current study showed generalized increase in microleakage scores with increase in frequency of immersion in all test beverages, for both Nanoionomer and Nanocomposite.

In fruit drink (Group I), both Nanoionomer and Nanocomposite showed statistically significant microleakage in Medium and High immersion regimes, when compared to Low immersion regime. The comparison was not statistically significant between Low and Medium immersion regime.

Fruit drinks contain citric and ascorbic acids and also have

Figure 5 – Intergroup comparison: Graph showing comparison of mean scores in immersion regime of different groups for Nanoionomer.



stabilizing agents which maintains pH of the drink. Thus, these drinks pose high risk of demineralization, ultimately leading to erosion and also degradation of the restorative material.[15, 16]

In fresh fruit juice (Group II), the microleakage scores increased as the frequency of immersion increased for both Nanoionomer and Nanocomposite. This was in accordance with the study conducted by Maganur *et al*, in which the microleakage scores for Filtek™ flow and Vitremer™ increased with increase in frequency of immersion in Orange juice.[17] In our study, when comparison was made between low, medium and high immersion, the result was statistically significant between low against high and medium against high. It was not significant between low and medium immersion group. This may imply that although orange juice causes microleakage in low frequency, it may be clinically significant at higher frequency only.

Repeated intake of fresh fruit juice leads to a marked reduction in the salivary pH. Studies evaluating fresh fruit juices have reported that orange juice is rich in citric acid, and has a pH of 3.98. This pH is just below the critical pH of 4 needed to cause enamel erosion and microleakage of restorative materials.[18]

In soft drink (Group III) also, the microleakage scores increased with increase in frequency of immersion for both Nanoionomer and Nanocomposite. In this group particularly, the comparison between all three immersion regimes were statistically significant (p value < 0.05 between low and medium; medium and high; and low and high). Out of which, the comparisons between low and high immersion regime was highly significant (p value < 0.001). This was again in accordance with the study conducted by Maganur *et al,* in which the microleakage scores for Filtek™ flow and Vitremer™ increased with increase in frequency of immersion in Cola drink group.[17] Similarly in the study conducted by Narsimha VV comparing Dyract AP and Fuji II LC under various immersion regime in a cola drink, statistically significant difference was observed between the two of all the three regimes.[19] In this study, there was statistically significant difference ($p<0.01$) between low and medium as well as low and high immersion regimes. However, there was no statistically significant difference between the medium and high immersion regimes.

This can be attributed to the fact that the Cola drinks have an inherent acidity due to the presence of both orthophosphoric acid and carbonic acid, which tends to increase enamel demineralization, erosion and the microleakage around the restoration.[20]

To compare the ability of the test beverages to cause marginal leakage, the subgroups within a test group was compared to the corresponding subgroup in the other groups. For example, specimens under low immersion in Group I were compared with specimens under low immersion in Group II and Group III respectively. The generalized result observed during this comparison was that Group III (soft drink) showed higher microleakage than Group II (Fresh fruit juice), followed by Group I (fruit drink), for both Nanoionomer and Nanocomposite. In simpler terms, soft drink caused highest microleakage followed by fresh fruit juice than fruit drink. However the result was statistically significant between fruit drink and soft drink only.

The two materials i.e. Nanoionomer and Nanocomposite were also compared to each other under different immersion regimes. In our study, in control group, both the materials showed similar microleakage scores. Under different immersion regime in test beverages, Nanocomposite exhibited more microleakage than Nanoionomer in all groups. However, the result was not statistically significant. In the study by Maganur *et al*, Filtek™ flow (flowable composite) showed higher microleakage scores than Vitremer™ (Resin modified Glass ionomer cement).[17] While in the study conducted by Narsimha , Dyract AP (Polyacid modified composite resin) and Fuji II L.C (resin modified glass ionomer restorative material) revealed similar microleakage scores.[19] In another study, Sabdi *et al* concluded that Filtek Z250 has significantly lower degree of microleakage after acid exposure compared to conventional Glass ionomer cement, Resin modified GIC and silver amalgam.[21]

All the beverages used in the present study were acidic in nature

Figure 6 – Intergroup comparison: Graph showing comparison of mean scores in immersion regime of different groups for Nanocomposite

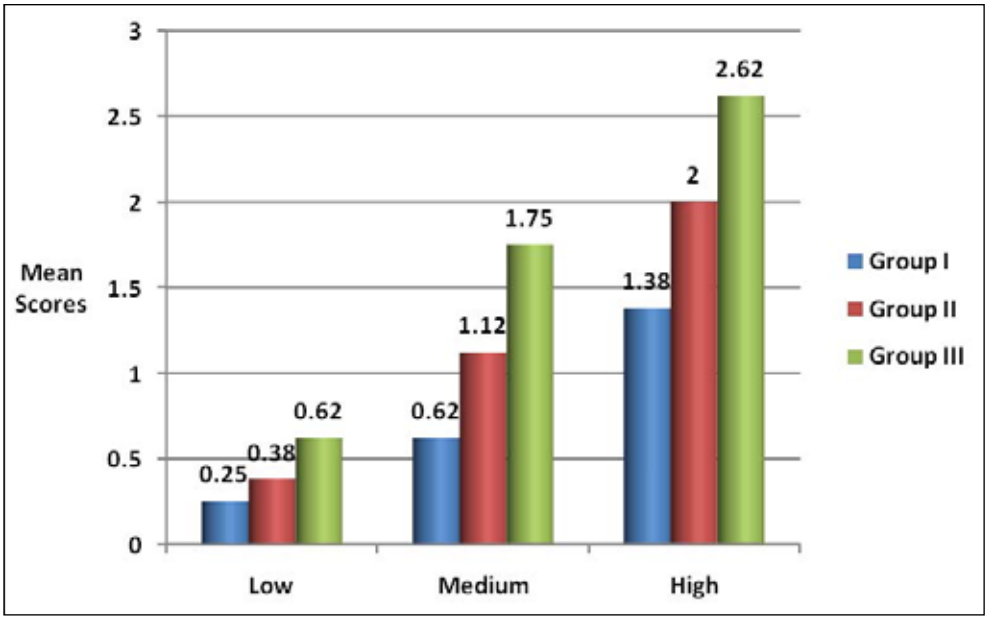


Table 4- Descriptive statistics on the microleakage pattern of Nanocomposite following immersion for varying periods of time in Fruit juice, Fresh fruit juice, Soft drink and Control.

Groups	Micro-leakage Scores	Low Immersion		Medium Immersion		High Immersion	
		No	Mean Median	No	Mean Median	No	Mean Median
Group I (Fruit juice)	0	6	0.25 0	3	0.62 1	5	1.38 1
	1	2		5		3	
	2						
	3						
Group II (Fresh fruit juice)	0	5	0.38 0	1	1.12 1	2	2 2
	1	3		5		4	
	2			2		2	
	3						
Group III (Soft drink)	0	3	0.62 1	3	1.75 2	3	2.62 3
	1	5		4			
	2			1		5	
	3						
		No Immersion regime					
Group IV (Control)	0	7	0.12 0				
	1	1					
	2						
	3						

Table 5 - Intragroup comparison for Nanocomposite showing p-value (S- significant, NS- not significant)

Group	Low v/s Medium	Medium v/s High	Low v/s High
Group I (Fruit drink)	p = 0.234 NS	p = 0.038 S	p = 0.003 S
Group II (Fresh fruit juice)	p = 0.05 S	p = 0.05 S	p = 0.001 S
Group III (Soft drink)	p = 0.007 S	p = 0.028 S	p = 0.0001 S

and affected the marginal integrity of the restorative materials used. Efforts should be taken to modify beverages by either adding or deleting certain components so as to reduce their harmful effects on teeth and restorative materials. The components used to modify the beverages are calcium (with or without phosphate ions), citrate and fluoride.[22] Also, appropriate diet counselling should be provided which is tailored for a particular individual to limit the intake of these beverages.

It would be inappropriate to deduce the findings of the present study to the conditions existing in vivo in humans. In the oral cavity, any drink or foodstuff will be instantaneously mixed with saliva, with a subsequent change in its pH. In addition, acidic drinks have also been shown to stimulate salivary secretion, which in turn facilitates the buffering systems.[23] Therefore, we recommend further studies combining both qualitative and quantitative evaluations, which will indicate more precisely the effects of beverages on the clinical integrity of the restorative materials in the oral environment.

CONCLUSION

- The three beverages used in the study affected the microleakage of both restorative materials significantly.
- The microleakage scores increased as the frequency of the immersions increased.
- Soft drink caused highest microleakage followed by fresh fruit juice and fruit drink. However the comparison was statistically significant between fruit drink and soft drink only.
- Nanocomposite exhibited more microleakage than Nanoionomer in all groups, however the comparison was statistically not significant.

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