Microhardness and cervical microleakage of healthy and periodontally involved dentin at class II cavities restored with conventional and bulk-fill resin composites

Horieh Moosavi[1] DDS, MS
Sara Majidinia[2] DDS, MS
Seyed Ali Banihashem Rad[3] DDS, MS
Mohammad Jafari Giv[4] DDS
Fatemeh Namdar[5] DDS, MS

[1] Associate Professor, Department of Restorative and Cosmetic Dentistry, School of Dentistry, Mashhad University of Medical Sciences, Mashhad, Iran
[2] Assistent Professor, Department of Restorative and Cosmetic Dentistry, School of Dentistry, Mashhad University of Medical Sciences, Mashhad, Iran
[3]Associate Professor, Department of Periodontics, School of Dentistry, Mashhad University of Medical Sciences, Mashhad, Iran
[4] Dentist, Dental Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[5] Assistant Professor, Dental Materials Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

Corresponding Author:
Fatemeh Namdar
Dental Materials Research Center,
Mashhad University of Medical Sciences
Vakil-Abad Blvd, Mashhad 91735, Iran
Tel.: +989155081447
Fax:+985138829500
E-mail: F.namdar90@gmail.com;
namdarf2@mums.ac.ir

Summary

***Aims*: The purpose of this *in-vitro* research was to compare microhardness and cervical microleakage of healthy and periodontally involved dentin after restoration with conventional and bulk-fill composites.**

***Methods*: For microhardness test, 20 human molars were collected (10 healthy and 10 for periodontal reasons). Dentinal disks from cemento-enamel junction were prepared. Each specimen received 9 indentations (3 for each superficial, median and deep dentin) and an average of them was recorded as the Vickers hardness number (VHN) of each area. For microleakage test, 20 healthy teeth and 20 extracted for the periodontal disease were collected. A standardized box-only cavity was prepared in every tooth. The samples of each group were randomly allocated to two subgroups of 10 and restored as follows: in the experimental group, the cavities were filled by a 4-mm layer of Tetric N-Ceram bulk-fill resin composite (BRC). In the control group, the conventional resin composite (Tetric N-Ceram) was inserted incrementally and then light cured. After 1000 cycles of thermocycling, cervical microleakage was evaluated by dye extraction technique. Data were submitted to ANOVA, t-test, and Mann-Whitney U test (α = 0.05).**

***Results*: VHN values of healthy dentin at all depths were more than periodontally involved dentin. The most microleakage was seen in periodontally involved teeth filled with conventional composite, but there were no significant differences between different groups.**

***Conclusions*: Although periodontal disease had a significant effect on the reduction of VHN, but no significant effect was observed on the cervical microleakage after using different resin composites.**

Key Words: bulk-fill composite, dentin, microhardness, microleakage.

Introduction

Free radical polymerization of methacrylate-based resin composites leads to volumetric shrinkage (1) and defect formation at the interface between tooth and restoration, recurrent caries, increased sensitivity and cuspal deflection (2). Recently, bulk-fill resin composites (BRCs) have been introduced by enhanced mechanical properties and time-saving (3). These composites are claimed to be used for increments of up to 4 mm thickness, without adverse effects on the degree of conversion (4).

Mechanical and chemical properties of root dentin exposed to periodontal disease may present significant alterations considering the difference in microbial flora involved in cervical dentin caries and therefore affect dentin bond strength in cervical areas (5). Because of the modifications in the tooth structures exposed to periodontal diseases, the microhardness value is thought to be different between normal and periodontal involved dentin.

The lack of enamel at the deep gingival margin of class II cavities leads to unstable adhesion of com-

posite resin to dentin or cementum. Also, dentin has less mineralized content and more water when compared to enamel (6). Furthermore, in the gingival margin of class II cavities, polymerization shrinkage and shrinkage stresses may exceed the adhesive-dentin bond strength which leads to gap formation and microleakage (7). Although BRCs have shown low polymerization shrinkage and high curing depth, concerns are about the ability of these composites to completely adapt to cervical cavosurface margins and internal surfaces of class II cavities (3).
Previous researches have shown that collagenous matrix breakdown by Host-derived dentinal matrix proteinases following periodontal disease, especially chronic periodontitis, can affect the adhesive properties of composite resin to dentin (8-10). Additionally, due to different microbial flora in periodontal disease, mechanical and chemical properties of radicular dentin exposed to oral cavity can be different from healthy dentin and this influences the bond strength to cervical dentin. Since the use of bulk-fill composites are expanding and also the overall age of the community and the presence of cervical lesions following periodontal diseases are increasing, therefore we conducted this laboratory research to evaluate whether a bulk filling technique affects gingival microleakage in healthy and periodontally involved dentin of class II cavities. Null hypotheses of this study were: 1) there wouldn't be any significant difference in microhardness of the healthy and periodontally involved dentin; 2) type of dentin (healthy and periodontally involved) and composite resin (bulk-fill or conventional) wouldn't influence the cervical microleakage of class II cavities.

Materials and methods

Sixty extracted, caries-free human permanent molars without cracks and restoration (30 healthy human third molar teeth, 30 ones extracted due to severe chronic periodontitis disease) were collected in this study. Ten samples of each group were randomly allocated for measurement of Vickers microhardness and 20 specimens for microleakage assessment. After extraction, all teeth were stored in thymol solution at 4°C for less than one month. Healthy impacted or semi-impacted third molars were obtained by surgery. Also, permanent molars suffering from severe chronic periodontitis with more than 5 mm periodontal pockets were selected from individuals with age 41 to 50 years. After removing residual periodontal tissues, the specimens were cleaned with pumice. An informed consent was obtained from donors under the protocol approved by the Ethics Committee for Human Studies and registry no.IR.mums.sd.REC. 1394. 68.

Measurement of Vickers microhardness

Twenty extracted teeth (10 healthy human third molars and 10 extracted due to severe chronic periodontitis disease) were used for measurement of Vickers microhardness. Dentinal discs were obtained just from cemento-enamel junction (CEJ) area with 2 mm thickness using a low-speed diamond saw (IsoMet 4000, Buehler, USA) (Fig. 1). After polishing by silicon paper of 1000, 1500, 2000 grit, Vickers indentations (Sinowon Manual, China) were performed for dentin surfaces (superficial, median and deep) with a force of 100 g for 20 s (Fig. 2). Each specimen received 9 indentations (3 for each superficial, median and deep dentin) and an average of the readings of each area at different groups was recorded as the Vickers hardness number (VHN) of a region.

Microleakage assessment

As previously stated, forty extracted teeth (20 healthy and 20 extracted due to severe chronic periodontitis disease) were used for this test. A standardized box only cavity was prepared on the mesial surface of each tooth with a new fissure diamond (008) bur mounted in a high-speed dental handpiece and air and cooling water spray. The buccolingual extension and axial depth of the cavities were 3 mm and 1.5

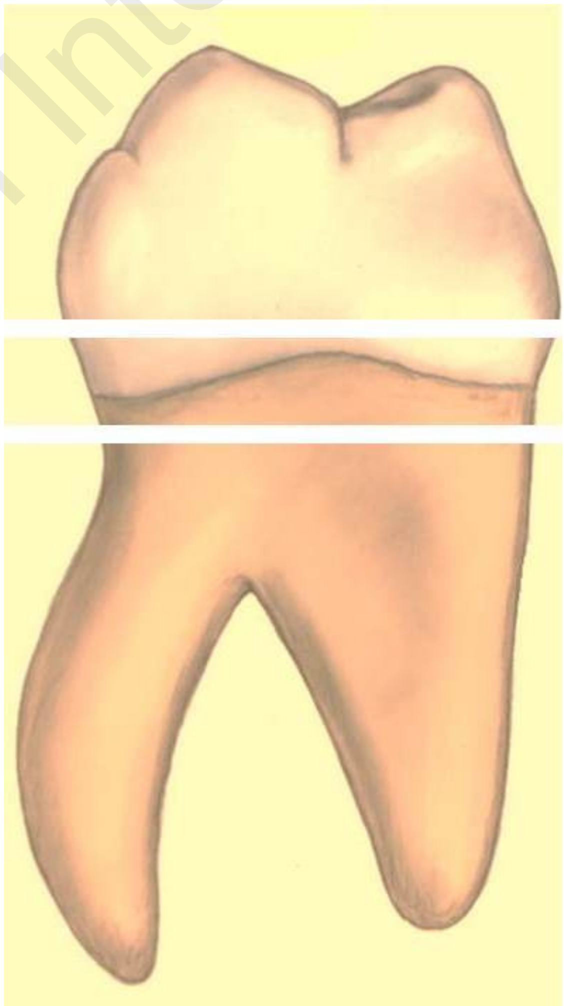

Figure 1. Dentinal discs obtained from CEJ area.

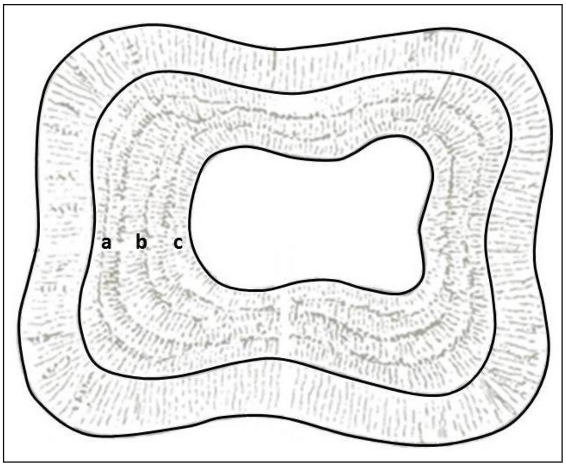

Figure 2. Cross sectional view of dentin surfaces (a) superficial, (b) median and (c) deep dentin for evaluation of Vickers microhardness.

mm, respectively. The cervical margin was left as a butt joint and located 0.5 mm below the CEJ. All dimensions were checked with the periodontal probe and digital caliper to ensure uniformity among preparations. The cavities were prepared uniformly by an expert operator. The dentin and enamel surfaces were conditioned with 37.5% phosphoric acid (Ivoclar Vivadent, Schaan, Liechtenstein) for 30 and 15 seconds, respectively. Rinsing was done for 15 seconds and the remaining water was eliminated by absorbing cotton. The adhesive Tetric N-Bond (Ivoclar Vivadent, Schaan, Liechtenstein) was applied based on the manufacturer's instruction and photo-activated for 20 seconds using the light curing device (1200 mW/cm^2, Bluephase C8, Ivoclar Vivadent, Schaan, Liechtenstein). Matrix application was done with Tofflemire stainless steel matrices. Then the samples of each group of healthy and periodontally involved dentin were randomly divided to two subgroups of 10 and restored as follows:

In the experimental subgroup, a 4-mm layer of Tetric N-Ceram BRC (Ivoclar vivadent, Schaan, Liechtenstein) was placed into the cavity and packed against the metallic matrix and then light activated for 40 seconds in continuous mode. Conventional Tetric N-Ceram resin composite (Ivoclar vivadent, Schaan, Liechtenstein) was inserted incrementally in preparations of the control subgroup while the thickness of each layer was approximately 2 mm and photo-activated for 40 seconds. Materials' details and manufacturers' specifications are shown in Table1.

The restored specimens were then subjected to artificial aging. The samples were immersed in distilled water baths at the temperature of 5°C and 55°C for 1000 cycles. The storage period in each temperature was 20 seconds and the transferring period was 10 seconds.

Dye extraction technique

All restored teeth were covered by two layers of nail polish up to one millimeter from the cervical margin and then were submerged in methylene blue solution for two days. Subsequently, the root of each specimen was cut just a lower level from the penetration area by a diamond saw mounted on the low speed cutting machine (IsoMet, Buehler, USA) (Fig. 3) and the remaining crown was then stored in a bottle containing 1000 µl of nitric acid (65% by weight) for three days. Then the bottles were centrifuged (Versatile SIGMA, Montreal Biotech, Montreal, Quebec, CA) for 5 minutes at 14,000 rpm, and 100 µl of every specimen was evaluated by using the spectrophotometer (CECIL Instruments, Cambridge, UK) at 550 nanometer. The spectrophotometer findings indicate the light absorption of the methylene blue in the interface of dentin and resin that present the microleakage score of the restoration.

Statistical analysis

The differences in hardness measurements were analyzed with two-way ANOVA and Tukey test. Statistical analysis of microleakage was done by independent-samples t-test and Mann-Whitney U test using SPSS version 14.0 (Chicago, Illinois, USA). The significance level was considered 0.05.

Results

Vickers microhardness values

Average VHN values of healthy dentin at all depths

Table 1. Materials' details and manufacturers' specifications.

Name	Composition	Filler wt%, vol%
Tetric N-Ceram Bulk fill	Dimethacrylates (Bis-GMA, Bis-EMA, UDMA), barium glass, itterbium trifluoride, mixed oxide and prepolymer, additives, catalysts, stabilizers, pigments	80% (including 17% prepolymers), 60%
Tetric N-Ceram Conventional	Bis-EMA,UDMA, TEGDMA, Ba-Al-Fl-borosilicat Glass, Ba Glass, YbF_3	80-81%wt

Bis-GMA, Bisphenol-A diglycidyl ether dimethacrylate; Bis-EMA, Bisphenol-A ethoxylated dimethacrylate; TEGDMA, Triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate.
- Prepolymer includes monomer, glass filler and ytterbium fluoride.

Figure 3. Representation of the penetration area after covering the specimen with two layers of nail varnish.

Table 2. Comparison of VHN values (Mean±SD) between different depths of dentin.

	Dentin Type	VHN	P-value
Superficial	Healthy	89.3±19.4[b]	0.071
	Periodontally involved	75.3±11.9[a]	
Median	Healthy	78.0±18.8[a]	0.096
	Periodontally involved	65.2±12.5[c]	
Deep	Healthy	78.0±7.9[a]	0.288
	Periodontally involved	74.7±10.3[a]	

The same letters indicate no significant differences.

were more than periodontally involved dentin, but the differences between them were not statistically significant (Table 2). VHN values at superficial area of healthy dentin were more than other depths, but influences of dentin depth were not statistically significant. In periodontally involved teeth, superficial and deep dentin showed the same microhardness values.

Microleakage assessment
However cervical microleakage scores of conventional resin composite bonded to both healthy and periodontally involved dentin were more than BRC, but the differences between two resin composites were not statistically significant (Fig. 4). Additionally, periodontally involved dentin showed higher microleakage scores compared to healthy dentin but statistical analysis presented no significant differences between two groups.

Discussion

The results of this research showed that the VHN values of superficially dentin were significantly higher

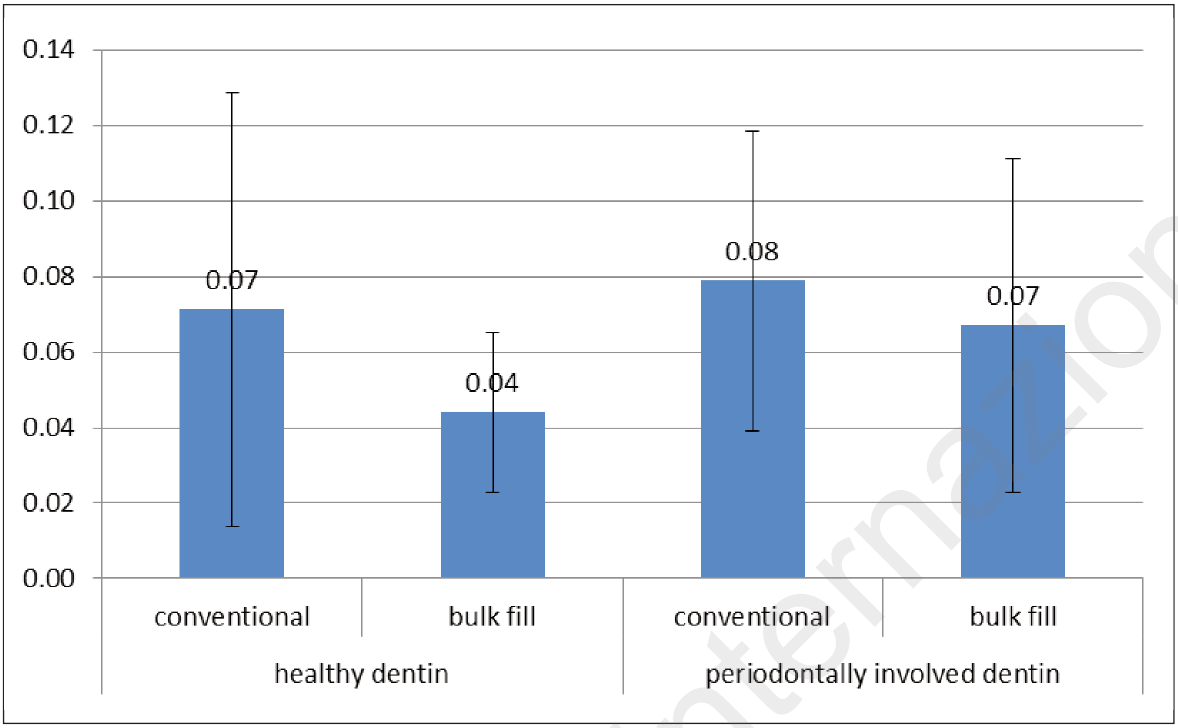

Figure 4. Cervical microleakage of two resin composites (conventional and bulk fill) at healthy and periodontally involved dentin.

than median and deep healthy dentin. Previous studies have shown that the microhardness values of dentin must be demonstrated in terms of the region in dentin. Some studies have reported that the superficial dentin was harder than the inner dentin and the hardness decreased from the dentino-enamel junction (DEJ) to the pulp (11, 12). Kinney et al. demonstrated that the hardness reduction as a function of depth was related to the lower stiffness of inter-tubular dentin because of non-homogeneous distribution of the inorganic content within the organic matrix (12). Pashley et al. reported that the enhanced tubular density at interior dentin corresponded to decreased hardness (13). Another study stated that hardness reduction with the location might be related to differences in the hardness of intertubular dentin, not to more quantity of tubules (14). Fuentes et al. (11) reported VHN=63 for deep sound dentin and stated since that the dentin tubules are not oriented randomly, properties may depend on their direction. The mean VHN scores reported in this study for healthy dentin were in ranging from 78 to 89.3 and were somewhat higher than previous studies (57-62 VHN). The dissimilarity between reported values is due to the method of specimen preparation and indentation techniques employed (5, 15, 16).

Based on our findings, the dentin of teeth suffering from severe chronic periodontitis showed less VHN compared to healthy dentin. In agreement with our results, Riffle et al. and Emslie et al. found that the cervical root dentin showed less microhardness scores in patients who suffer from periodontal disease or with exposure of cementum (17, 18). They presented that the microhardness values had reverse relationship with periodontal pocket depth (17, 18). Lee et al. showed that the host-activated dentin proteinases causes the destruction of collagen matrix following periodontal disease and may reduce the dentin hardness of periodontally involved teeth (10).

The results of this study validated the second research hypothesis, as there were not any significant differences in cervical microleakage of class II cavities concerning the type of dentin (healthy or periodontally involved teeth) and placement techniques (bulk-fill or incrementally). Least cervical microleakage was also observed at healthy dentin of class II cavities filled with Balk-fill resin composite.

Polymerization shrinkage and contraction stress can induce adhesive failure and gap formation in the composite restorations. Also, placement technique, photo-activation method, stress relieving capacity and C-factor are effective on the magnitude of the stress (4). El-Damanhoury et al. showed a significant reduction in shrinkage stress of BRC compared to conventional resin composites while maintaining the depth of curing at the 4 mm thickness. They concluded that this could support the use of these composites to fill deep cavities with high C-factor (4).

The mechanisms used to decrease contraction stress and to enhance the curing depth are different between bulk-fill resin composites. Some manufacturers enhance light transmission through the resin composite by using monomers, pigments, fillers with the same refractive indices. Others decrease the inorganic content to enhance the light transmission and depth of curing, although this approach compromises the mechanical properties (19). Another approach to achieve deeper polymerization is the use of the additional or photo initiators like Ivocerin that is the patented light activator of Tetric N-Ceram BRC and is responsible for complete curing of the restoration. Ivocerin is more light reactive than camphorquinone, acts as a polymerization booster and allows polymerization in deeper increments without compromising the physical properties of the resin composite (20, 21).

Tetric N-Ceram BRC has the specially patented filler that relieves contraction stresses (20). The manufacturer of this material claimed that due to the low elastic modulus of this patented filler, the shrinkage stress reliever expands slightly during polymerization and plays like a spring amongst the glass fillers with the higher modulus of elasticity. Also, Tetric N-Ceram bulk fill has prepolymerized fillers which attenuate elastic modulus and their translucency helps to pass the photons through the substance. Finally, polymerization shrinkage and contraction stress in Tetric N-Ceram BRC decrease during polymerization, obtain a good marginal seal and allow increments up to 4 mm to be inserted (20, 22, 23). However, in our research none of the two resin composites was able to inhibit perfectly cervical microleakage.

In line with our findings, some research concluded that any significant differences were not recorded in the cervical microleakage scores for incrementally compared to the bulk fill composites restored teeth (7, 24).

Most class II cavities extend up to or beyond the CEJ so that the cervical margins will be placed at the dentin surfaces and may cause a weak marginal seal and microleakage between the dentin and composite resin (25). Cavosurface margins restricted to enamel can preserve marginal seal of cavity preparation because of the strong adhesion between the enamel and resin adhesive. Additionally, the internal stress of the composites materials at dentin surfaces are often more than the bond strength and subsequently gap formation occurs at the resin-dentin interface (26).

Past investigations have demonstrated that the mineral substance of dentin plays an essential role in the bond strength of composite to dentin (27). Yoshiyama et al. explained that when the dentin was undergoing chemical and structural changes naturally, fewer resin tags formed because of the existence of mineral sclerotic casts in the dentin tubules. Also, a limited resin infiltration layer was seen at the external margins of the natural lesions. They explained that the lower bond strength between resin and dentin structures in natural lesions was because of the long term exposure to the oral cavity and, thereby, the presence of unknown substances in dentin and thinner hybrid layer (28).

Dye extraction technique has employed to assess the marginal integrity and the ability of dental materials to seal the cavosurface margins. This method is simple and presents the characteristic of micro-leakage quantitatively (29). Therefore it was utilized to evaluate microleakage in the current research. In spite of the restrictions of this investigation, although periodontal disease had a significant effect on the dentin microhardness reduction, but no significant outcome was observed on the cervical leakage after the use of conventional and BRCs. More research about long-term clinical evaluation of BRCs and periodontally involved teeth are required.

Conclusions

VHN values of healthy dentin were more than periodontally involved teeth at all depths. Conventional resin composite showed more cervical microleakage than BRCs bounded to both healthy and periodontally involved dentin, but the differences between two resin composites were not statistically significant. Additionally, periodontally involved dentin showed higher microleakage scores compared to healthy dentin, although statistical analysis showed no significant differences between them.

References

1. Bagis Y, Baltacioglu I, Kahyaogullari S. Comparing microleakage and the layering methods of silorane-based resin composite in wide Class II MOD cavities. Operative Dentistry. 2009;34(5):578-585.
2. Phillips RW, Avery DR, Mehra R, Swartz ML, McCune RJ. Observations on a composite resin for Class II restorations: three-year report. The Journal of prosthetic dentistry. 1973;30(6):891-897.
3. Leevailoj C, Cochran M, Matis B, Moore B, Platt J. Microleakage of posterior packable resin composites with and without flowable liners. Operative Dentistry. 2001;26(3):302-307.
4. El-Damanhoury H, Platt J. Polymerization shrinkage stress kinetics and related properties of bulk-fill resin composites. Operative dentistry. 2014;39(4):374-382.
5. Rautiola C, Craig R. The microhardness of cementum and underlying dentin of normal teeth and teeth exposed to periodontal disease. Journal of Periodontology. 1961;32(2):113-123.
6. Demarco F, Ramos O, Mota C, Formolo E, Justino L. Influence of different restorative techniques on microleakage in class II cavities with gingival wall in cementum. Operative dentistry. 2001;26(3):253-259.
7. Patel P, Shah M, Agrawal N, Desai P, Tailor K, Patel K. Comparative evaluation of microleakage of class II cavities restored with different bulk fill composite restorative systems: An in vitro study. J Res Adv Dent. 2016;5(2):52-62.
8. Shinkarenko T, Rumiantsev V, Egorova E, Eliseeva T. Matrix metalloproteinases in periodontitis. Stomatologiia. 2013;92(2):77.
9. Sorsa T, Tjäderhane L, Konttinen YT, Lauhio A, Salo T, Lee HM, et al. Matrix metalloproteinases: contribution to pathogenesis, diagnosis and treatment of periodontal inflammation. Annals of medicine. 2006;38(5):306-321.
10. Lee W, Aitken S, Sodek J, McCulloch C. Evidence of a direct relationship between neutrophil collagenase activity and periodontal tissue destruction in vivo: role of active enzyme in human periodontitis. Journal of periodontal research. 1995;30(1):23-33.
11. Fuentes V, Toledano M, Osorio R, Carvalho RM. Micro-

hardness of superficial and deep sound human dentin. Journal of Biomedical Materials Research Part A: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials. 2003;66(4):850-853.
12. Kinney J, Balooch M, Marshall S, Marshall G, Weihs T. Atomic force microscope measurements of the hardness and elasticity of peritubular and intertubular human dentin. Journal of biomechanical engineering. 1996;118(1):133-135.
13. Pashley D, Okabe A, Parham P. The relationship between dentin microhardness and tubule density. Dental Traumatology. 1985;1(5):176-179.
14. Marshall Jr GW, Marshall SJ, Kinney JH, Balooch M. The dentin substrate: structure and properties related to bonding. Journal of dentistry. 1997;25(6):441-458.
15. Chuenarrom C, Benjakul P, Daosodsai P. Effect of indentation load and time on knoop and vickers microhardness tests for enamel and dentin. Materials Research. 2009;12(4):473-476.
16. Yassen GH, Eckert GJ, Platt JA. Effect of intracanal medicaments used in endodontic regeneration procedures on microhardness and chemical structure of dentin. Restorative dentistry & endodontics. 2015;40(2):104-112.
17. Riffle A. The dentin: its physical characteristics during curettage. The Journal of Periodontology. 1953;24(4):232-241.
18. Emslie R, Stack M. The micro hardness of roots of teeth with periodontal disease. Dent Pract Dent Rec. 1958;9:101-113.
19. Corral-Núnez C, Vildósola-Grez P, Bersezio-Miranda C, Campos A-D, Fernández Godoy E. State of the art of bulk-fill resin-based composites: a review. Revista Facultad de Odontología Universidad de Antioquia. 2015;27(1):177-196.
20. Jang J, Park S, Hwang I. Polymerization shrinkage and depth of cure of bulk-fill resin composites and highly filled flowable resin. Operative dentistry. 2015;40(2):172-180.
21. Furness A, Tadros MY, Looney SW, Rueggeberg FA. Effect of bulk/incremental fill on internal gap formation of bulk-fill composites. Journal of dentistry. 2014;42(4):439-449.
22. Scientific documentation of Tetric N-Ceram Bulk Fill. Retrieved online 11 September 2018. http://www.ivoclarvivadent.in/en-in/p/all/tetric-n-ceram-bulk-fill
23. Hirata R, Clozza E, Giannini M, Farrokhmanesh E, Janal M, Tovar N, et al. Shrinkage assessment of low shrinkage composites using micro-computed tomography. Journal of Biomedical Materials Research Part B: Applied Biomaterials. 2015;103(4):798-806.
24. Moorthy A, Hogg C, Dowling A, Grufferty B, Benetti AR, Fleming G. Cuspal deflection and microleakage in premolar teeth restored with bulk-fill flowable resin-based composite base materials. Journal of Dentistry. 2012;40(6):500-505.
25. Litonjua LA, Andreana S, Bush PJ, Tobias TS, Cohen RE. Noncarious cervical lesions and abfractions: a re-evaluation. The Journal of the American Dental Association. 2003;134(7):845-850.
26. Feilzer A, De Gee A, Davidson C. Setting stress in composite resin in relation to configuration of the restoration. Journal of Dental Research. 1987;66(11):1636-1639.
27. Spencer P, Wang Y, Walker M, Swafford J. Molecular structure of acid-etched dentin smear layers-in situ study. Journal of Dental Research. 2001;80(9):1802-1807.
28. Yoshiyama M, Sano H, Ebisu S, Tagami J, Ciucchi B, Carvalho R, et al. Regional strengths of bonding agents to cervical sclerotic root dentin. Journal of dental research. 1996;75(6):1404-1413.
29. NIK IR, NASERI EB, Majidinia S, NIK SR, GIV MJ. Effect of Chlorhexidine and Ethanol on Microleakage of Composite Resin Restoration to Dentine. Chin J Dent Res. 2017;20(3):161-168.