An in-vitro scan electron microscope comparative study of dentine-Biodentine interface

Jameel M. A. Sulaiman, B.Sc., M.Sc. (1)
Maha M. Yahya, B.D.S., M.Sc. (2)
Wiaam M.O. Al-Ashou, B.D.S., M.Sc. (2)

ABSTRACT

Background: This research was an in-vitro SEM comparative study of Dentine – Biodentine ™ interface.
Materials and Methods: Sixty three freshly extracted human molars, Biodentine ™ (Septodont, France), MTA (ProRoot, Tulsa, Brazil), GIC (MediFil, Promedica, Germany), light microscope, scaler and pumice, high speed hand piece, diamond bur, Scan Electron Microscope: VEGA\\ Easy Probe. TESCAN – Germany. The study was performed first at the University of Mosul, College of Dentistry to dental models were brought the sixty-three of the specialty dental health center in Mosul. The teeth was prepared by cleaning, cutting, and removing all the caries and examined under light microscope and decayed teeth was excluded .Then the teeth was divided randomly into three main groups (A, B, C) and each major group was divided into three sub groups: (A_1, A_2, A_3) was filled with (Biodentine ™), (B_1, B_2, B_3) was filled with (MTA) and (C_1, C_2, C_3) was filled with the (GIC). Each subset contains seven (7) samples. All groups were filled according to the manufacturer instructions, and then restored at 37°C and 100% humidity. After storage periods of (7, 14, 28) days, the teeth were sectioned mesio-distaly using a low speed diamond saw (Isomet, Buehler Ltd.), and examined under SEM at the University of Technology-Nano Research Center in Baghdad.
Results: Under the condition of this in vitro study, examination with SEM showed that the marginal gaps between the experimental materials and the dentine is time dependant, with the best results was observed between Biodentine and dentine interface.
Conclusion: The marginal gaps between the experimental materials and the dentine are time dependent.
Keywords: Interface, Biodentine ™, MTA, SEM. **(J Bagh Coll Dentistry 2014; 26(1):42-48).**

الخلاصة

الأهداف : يهدف البحث إلى استخدام تقنية المجهر الاليكتروني لإجراء دراسة مقارنة للوسط البينيلمادةعاج السن الطبيعي مع المادة الصناعية المثيلة لها. **المواد وطرائق العمل**: ثلاثة وستون (63) عينة من الأسنان الطبيعية ، ثلاثة مواد مختلفة من الحشوات السنية الـ(Biodentine ™) ، ومادتي الـ (MTA) والـ (GIC) ، المجهر الاليكتروني الماسح :(SEM VEGA\\ Easy Probe. TESCAN - Germany)، مجهر ضوئي ، مقحلة أسنان(Scaler) ومسحوق (Pumice) لمعالجة الأسنان، رأس قاطع من الماس، بالإضافةإلى أدوات يدوية عالية السرعة، جهاز تجفيف من الرطوبة. أجريت الدراسة أولا في جامعة الموصل كلية طب الأسنان بعد أن تم جلب نماذج الأسنان الثلاثة والستون من المركز الصحي التخصصي للأسنان في الموصلثم بدأ العمل لتحضير العينات للعمل البحثي لتشمل التنظيف والقص وإزالة جميع التسوسات وفحصها بالمجهر الضوئي لغرض التخلص من الأسنان المنخورة . قسمتالأسنان عشوائيا إلى ثلاثة مجاميع رئيسية وهي(A, B, C)والمجموعة الرئيسية الواحدةإلى ثلاثة مجاميع ثانوية (A_1,A_2,A_3) لمادة الـ (Biodentine ™) ، (B_1,B_2,B_3) لمادة الـ (MTA) و (C_1,C_2,C_3) لمادة الـ (GIC) . وكل مجموعة ثانوية تحتوي على سبعة(7) عينات. ثم بدأت المعالجة بالحشوات السنية المخصصة لهذه الدراسة وهي مادة الـ (Biodentine ™) فرنسي الصنع من شركة (Septodont)، ومادة الـ (MTA) برازيلي من شركة (ProRoot, Tulsa) اما مادة الـ (GIC)– ألماني من شركة (MediFil, Promedica)، بعدها بدأت عميلة الخزن بالماء المقطر ولفترات زمنية مختلفة بالأيام وهي (7, 14, 28) لكل مجموعة ، ثم جففت جميع العينات بجهاز تجفيف للتخلص من الرطوبة وتم تقطيعها ثم بعدها أرسلت للتصوير بجهاز المجهر الاليكتروني الماسح (SEM)في الجامعة التكنولوجية – مركز بحوث النانوتكنولوجي في بغداد.
النتائج:أظهرت نتائج هذه الدراسة أن مقدار الفجوة الحاصلة بين عاج السن والمواد المفحوصة تعتمد على الزمن وان أفضل النتائج لوحظت بين مادة الـ (Biodentine ™)وسطح عاج السن.
الاستنتاجات: إن مقدار الفجوة السطحية بين المواد المفحوصة وسطح عاج الأسنان مرتبط بعامل الزمن.

INTRODUCTION

Torbinejad first developed mineral trioxide aggregate (MTA) as a surgical root repair material in 1993 (1). Subsequently, significant interest has been shown in MTA, due to its compatibility (2) and potential bioactivity (3). More recently, a new calcium-silicate restorative material called Biodentine ™ has been introduced by Septodent, to be used not only as an endodontic repair material but also as a coronal restorative material for dentin replacement.

Biodentine ™ consists of a powder and liquid in apipette. The powder mainly contains tricalcium and diecalcium silicate, the principle component of Portland cement and MTA, as well as calcium carbonate zirconium dioxide serves as contrast medium (4).

(1)Lecturer. Department of Basic Sciences. College of Dentistry, University of Mosul.
(2)Lecturer. Department of Conservative Dentistry. College of Dentistry, University of Mosul.

The liquid consist of calcium chloride in an aqueous solution with an admixture of modified poly carboxylate. The powder is mixed with the liquid in a capsule in a toturator for 30 seconds, sets in about 12 to 16 minutes (5).

Biodentine ™ can be used for the treatment of root perforation or for the pulp floor, internal and external resorption, apexification, retrograde root canal obturation, pulpotomy, and also for temporary sealing of cavities and cervical filling (6).

Biodentine ™ with Active Biosilicate Technology announced by dental material manufacturer Septodent in September of 2010, and made available in January of 2011, Biodentine ™ is a calcium silicate based material used for crown and root repair treatment, repair of perforation or desorption's, apexification and root-end filling. The material has indications similar to calcium silicate based materials e.g. MTA, Septodent claimed that Biodentine ™ is not mutagenic (7) and that it can resist microleakage (8).

Biodentine ™ shares both its indications and mode of powder in capsule and liquid in a pipette. The powder mainly contains tri calcium and dicalcium silicate, the principle component of Portland cement as well as calcium carbonate, zirconium dioxide serves as a contrast medium [9]. The liquid consists of calcium chloride in aqueous solution with an admixture of polycarboxylate. The powder is mixed with the liquid in a capsule in the triturate for 30 seconds. Once mixed Biodentine ™ sets in about 12 minutes. During the setting of cement calcium hydroxide is formed. The consistency of Biodentine ™ reminds of that of phosphate cement [10, 11].

The aim of this present study is to investigate the marginal interfaces created between Biodentine ™, MTA, GIC and Dentine. The sealing ability of these materials is assessed in-vitro through SEM observation of the tooth-cement interface.

MATERIALS AND METHODS

Sixty three freshly extracted human molars were used for this study. After visual inspection with a light microscope to ensure that the teeth did not show any caries or cracks, the teeth were cleaned and polished with scaler and pumice. One standardized class I cavity in the occlusal surface were prepared on each tooth. All manipulations and restorations were performed by a single experienced operator to prevent variations due to operator's skill. Cavities were prepared with a high speed handpiece, using a diamond bur under heavy water spray. The diamond bur was replaced after every four preparations.

All internal line angles were rounded. The overall dimensions and depths of cavities were standardized as follows: occlusal floor width 4mm, length 5mm, depth 2.5mm. The occlusal floor ended in dentine, just below the dentino-enamel junction. The teeth were immediately and randomly divided into nine groups (7 teeth for each) according to the filling material used for the restoration of the occlusal cavities and the time of storage as follow:

Group A: filled with Biodentine and subdivided into three sub groups (A_1, A_2, A_3) with seven teeth for each.

A_1: Restored with Biodentine and stored for 7 days

A_2: Restored with Biodentine and stored for 14 days

A_3: Restored with Biodentine and stored for 28 days.

Group B: filled with MTA and subdivided into three groups (B_1, B_2,B_3) with seven teeth for each subgroup.

B_1: Restored with MTA and stored for 7 days

B_2: Restored with MTA and stored for 14 days.

B_3: Restored with MTA and stored for 28 days.

Group C: filled with Glass Ionomer cement and subdivided into three groups (C_1, C_2, C_3) with seven teeth for each subgroup.

C_1: Restored with Glass Ionomer cement and stored for 7 days.

C_2: Restored with Glass Ionomer cement and stored for 14 days.

C_3: Restored with Glass Ionomer cement and stored for 28 days.

All groups were filled according to the manufacturer instructions, and then restored at 37 °C and 100% humidity.

After storage periods, the teeth were sectioned mesio-distally using a low speed diamond saw (Isomet, Buehler Ltd.), thus passing through the center of the restoration. Then the sectioned specimens were cleaned with 10% orthophosphoric acid (H_3SO_4) for 3 to 5 seconds and quickly rinsed with air water spray for 15 seconds to remove the smear layer. Later all the specimens were dehydrated by increasing concentration of ethyl alcohol [C_2H_5OH] (30%, 50%, 70, 90% and 100%).

Once the specimens were dehydrated with various concentrations of alcohol, they were mounted with silver paste on metallic stubs and gold coated with sputtering system under vacuum desiccation and then examined under SEM (VEGA Easy Probe – Germany),at acceleration voltage of 10 to 30 KV.

The internal gaps between the dentinal surface and dentine substitute materials were observed under scanning electron microscope.

Representation photomicrographs were taken at a magnification power of (1000-1200) X. The internal gaps at different levels were measured in each photomicrograph and mean was taken. The values obtained in microns and the data were calculated and analyzed statistically using ANOVA and Duncan's multiple range test at ($p<0.05$).

RESULTS

Under the condition of this in vitro study, examination with SEM showed that the interface between group A (Biodentine ™) and human dentin were approximately in intimate contact after 28 days of storage (i.e. the gap observation was 1µm). While during the first week the mean diameter of the gaps between Biodentine ™ and the tooth structure was (8.1± 0.888 µm) and the

interface became more intimate after two weeks, the mean diameter of the gaps was (3.16 ± 0.7638µm). And the difference between Biodentine ™ groups at different time was statistically highly significant ($p<0.01$) as seen in Figure (1), Table (1).

The result of this in vitro study showed that in group B (MTA) the mean diameter of the gaps was (54.467 ± 4.313 µm), (6.0 ± 1.0 µm) and (3.333 ± 1.528 µm) was statistically highly significant ($p<0.01$) as seen in Figure (1), Table (2), for subgroup (B_1,B_2 and B_3) respectively. Group B_3represent the lowest mean of gaps which was not significantly differenced from groups B_2 ($P>0.05$). Group B_1 showed the highest mean of the gaps, and the difference was significant when compared with the group B_2 and B_3 ($P<0.05$) as seen in Figure (1), Table (2).

The results also showed that in group C (Glass Ionomer) the mean diameter of the gaps was (7.27 ± 0.86 µm), (25.0 ± 6.26µm) and (64.0 ± 33.81µm) for subgroup (C_1, C_2 and C_3) respectively, and the difference was statistically significant between these groups($P<0.05$)as seen in Figure (1), Table (3).

Duncan's multiple range test table(4) showed that at 7 days storage period Glass Ionomer Group represent the lowest mean of the gaps (7.267 ± 0.862) µm which was significantly different($P<0.05$)when compared with Biodentine ™ group (8.1 ± 0.889µm) and MTA group(54.467 ± 4.313µm) ,and difference was not statistically significant between Biodentine ™ and MTA($P>0.05$).

Duncan's multiple range test table (5) showed that at 14 days storage period Biodentine ™ Group represent the lowest mean of the gap (3.167 ± 0.764 µm) which was not significantly different ($P>0.05$) when compared with MTA group (6.0 ± 1.0 µm) and Glass Ionomer group (25.0 ± 6.264 µm) which showed the highest mean of gaps and the difference was highly significant when compared with Biodentine ™ and MTA group ($p<0.01$).

Duncan's multiple range test table (6) showed that at 28 days storage period Biodentine ™ Group represent the lowest mean of the gap (1.0 ± 0.000 µm) which was not significantly different when compared with MTA group (3.33 ± 1.53 µm) and Glass Ionomer group (64.00 ± 33.81µm) which showed the highest mean of gaps and the difference was significant ($p<0.05$) when compared with Biodentine ™ and MTA group.

Duncan's multiple range test table (7), figure (2), showed that at (7, 14, 28) days storage period Groups (A, B, C) was highly significantly different ($p<0.01$). There was no significant between A_1, A_2, A_3, B_2, B_3 and C_1 ($P<0.05$), and there was no significant between B_1 and C_3, but there was a significant between C_2 and the other subgroups.

DISCUSSION

The quality and durability of the interface is a key factor for the survival of a restorative material in clinical conditions; the marginal adaption and the intimate contact with the surrounding material (dentine, enamel and dental material) are determinative features [5,13]. In the present study this was investigated by scan electron microscope (SEM) at magnification (1000-1200) X to assess the interfacial seal between enamel and dentine and three restorative materials (Biodentine ™, GIC and MTA). SEM represents a valid tool for evaluation of the marginal integrity in in-vitro studies [14, 15]. It is a widely used morphological examination of different interface [16]. Additionally it is used to obtain a quantitative evaluation of the extent of the marginal gaps [17-19]. Under the condition of this in-vitro study, examination with SEM should that the interface between Biodentine ™ and human dentine are approximately in intimate contact after 28 days of storage (the gap was 1 µm) observer between Biodentine ™ and the tooth structure, while during the first week the mean diameter of the gap between Biodentine ™ and the tooth structure was (8.1 ± 0.888 µm) and the interface become more intimate after two weeks, i.e. the mean diameter of the gaps was (3.1667 ± 0.7638µm).

Santos *et al* [20] observed that the interfacial gap of Biodentine ™ - dentine may be compared to the hard tissue layer shown to be formed when using Pro-Root MTA which is considered as a precipitation of Hydroxyapatite. Goldberg *et al* [21] observed that SEM microphotograph showed the occurrence of a cohesive failure with Biodentine ™ cement with alteration of the tooth-biomaterial interfaces, hence providing evidence for the quality of the micromechanical adhesion occurring during the SEM preparation

Table (6) showed that there is a direct contact (without a gap), between Biodentine ™ and the natural dentine. The cracks observed in side Biodentine ™ caused by dehydration due to SEM sample preparation under vacuum [22]. This cohesive failure dose not affected the dentine – Biodentine ™ interface, which indicate the quality of the micro-mechanical adhesion [23, 24].

In comparison of interface of Biodentine ™- Dentine in tables (4), (5) and (6), the interfaces were very similar in all of the subgroups (A_1, A_2, A_3), while in group (B) the mean diameter of the gaps were gradually decreasing with time. The interface between MTA and Dentine became more

intimate after (28) days of storage. The possible reason for the decrease in diameter of gaps is the slight expansion of MTA upon setting [25, 26]. The marginal adaptation of MTA has been assessed using SEM [27, 28], the long term seal was measured over a (12) weeks and (12) month period. These studies reported good results with MTA; this may be because of its moisture tolerance and long setting time [29, 30].

In the present study, group C with GIC (C_1, C_2, C_3) showed a large gap between the GIC and tooth structure, and this gap is increasing with time. During setting, GIC absorb a considerable amount of water, which may affect their sealing ability and physical properties. Silica hydrogel forming around the glass particles is likely to act as a fluid reservoir. It also tends to undergo some amount of shrinkage during the setting which can cause loss of the marginal integrity [31,32]. Glass Ionomer (GIC) is a material with universal properties as dentist substitute; its ability to exhibit chemical bond to tooth structure provides an excellent marginal seal. However the marginal seal is compromised because of its dissolution in tissue fluids and its technique sensitivity [33].

As conclusions; all of the studied materials exhibited some degree of marginal gaps that are time dependent. A positive correlation was found between the marginal adaptation and time of storage. Biodentine ™ and MTA exhibited similar performances that are better than GIC under conditions of this study.

REFERENCES

1. Lee SJ, Monsef M, Torabinejad M. Sealing ability of a mineral trioxide aggregate for repair of lateral root perforations. J Endod 1993; 19: 541–4.
2. Camilleri J, Montesin FE, Papaioannou S, McDonald F, Pitt Ford TR. Biocompatibility of two commercial forms of mineral trioxide aggregate. Int Endod J 2004; 37: 699-704
3. Tay FR, Pashley DH, Rueggeberg FA, Loushine RJ, Weller RN. Calcium phosphate phase transformation produced by the interaction of the Portland cement component of white mineral trioxide aggregate with a phosphate-containing fluid. J Endod 2007; 33:1347-51.
4. Atmeh AR, Chong EZ, Richard G, Festy F, Watson TF. Dentin-cement interfacial interaction: calcium silicates and polyalkenoates. J Dent Res 2012; 91(5): 454-9.
5. Laurent P, Camps J, De Méo M, Déjou j, About I. Induction of specific cell responses to a Ca_3SiO_5 – based posterior restorative material. J Dent Mater 2008; 24: 1486-94.
6. Dammaschke T, Leidinger J, Schäfer E. Long-term evaluation of direct pulp capping-treatment outcomes over an average period of 6.1 years. Clin Oral Investig 2010; 14: 559-67.
7. Harmand O, assessment of the genotoxicity Ames test (salomonellahyphimurium and E. Coil). Report RG EN RA EXT-RD 941055. Biodentine scientific file, page 22.
8. Tran V, Pran V, Colon P. Microleakage of new restorative calcium (Biodentine) oral presentation, mentioned in "Biodentine publications and communication 2005-2010" by Septodent (Dec. 2012, 12) P. 64.
9. Orosco FA, Bramant CM, Garcia RB, Bernardineli N, De Morase IG. Sealing ability, marginal adaptation their correlation using three root-end filing materials as optical plags. JAPP Oral Sci 2010; 18:127-134.
10. Koubi G. A Chemical study of a new Ca_3SiO_5 – based material induced as dentine substitute. Abstract in Clin Oral Invest, Sevillia 2009.
11. Shayegan A. Biodentine: Anew Material used as pulp-capping agent in primary pig teeth poster at IADT 16th World Congress Dental Traumatology. Verona 2010.
12. Tran V. Microleakage of anew restorative Calcium based cement (Biodentine). Oral presentation PDF IADR, London 2010.
13. Watson TF, Cook RJ, Festy F, Pilecki P, Sauro SE. Optical imaging techniques for dental biomaterials interfaces. In: Dental biomaterial interfaces. Int Endod J 2008; 41: 977-86.
14. Ferrari M, Vichi A, Grandini S. Efficacy of different adesive techniques on bonding gto root canal walls: an SEM investigation. J Dent Mater 2001; 17: 422-9.
15. Gwinnett AJ, Kanca J. Interfacial morphology of resin composite and shining erosion lesions. Am J Dent 1999; 5: 315-7.
16. Atmeh A R, Chong E Z, Richard G, Festy F, Watson T F. Dentin-cement interfacial interaction: calcium silicates and polyalkenoates. J Dent Res 2012 91(5): 454-9.
17. Han L, Okiji T. Uptake of calcium and silicon released from calcium silicate-based endodontic materials into root canal dentin. Int Endod J 2011; 44: 1081-7.
18. Weller RN, Tay KC, Garrett LV, Mai S, Primus CM, Gutmann JL, et al. Microscopic appearance and apical seal of root canals filled with gutta-percha and Pro Root Endo Sealer after immersion in a phosphate containing fluid. Int Endod J 2008; 41: 977-86.
19. Young AM, Sherpa A, Pearson G, et al. Use of Raman spectroscopy in the characterization of the acid–base reaction in glass-ionomercements. Biomaterials 2000; 21(19): 1971-9.
20. Santos AD, Moraes JC, Araujo EB, Yukimitu K, Valerio Filho WV. Physio-chemical properties of MTA and a novel experimental cement. Int Endod J 2005; 38: 443-7.
21. Goldberg M, Pradelle-Plasse N, Tran XV, Colon P, Laurent P, Aubut V, About I, Boukpessi T, Septier D, Biocompatibility or cytotoxic effects of dental composites. Oxford: Cox moor Publishing; 2009. pp. 181-203.
22. Gondim E, Zaia AA, Gomes BP, Ferraz CC, Teixeira FB, Souza-Filho FJ, Investigation of the marginal adaptation of root-end filling materials in root-end cavities prepared with ultrasonic tips. Int Endod J 2009; 36: 491-9.
23. Zanini M, Sautier JM, Berdal A, Simon S. Biodentine induces immortalized Murine pulp cell differentiation into odontoblast - like cells and stimulates biominerlaization. Int Endod J 2012; 38(9): 1-7.
24. Grech L, Mallia B, Camilleri J. Characterization of set intermediate restorative material, biodentine, bioaggregate and a prototype calcium silicate cement

for use as root-end filling materials. Int Endod J 2013; 46(7): 632-41.
25. Storm B, Eichmiller FC, Tordik PA, Goodell GG. Setting expansion of gray and white mineral trioxide aggregate and Portland cement. J Endod 2008; 34(1): 80-2.
26. Parirokh M, Torabinejad M. Mineral trioxide aggregate: A comprehensive Literature Review Part 1: Chemical – Physical Ant: bacterial properties. J Endod 2010; 36(1): 16-27.
27. Torabinejad M, Watson TF, Pitt Ford TR. Sealing ability of mineral trioxide aggregate when used as a root end filling material. J Endod 1993; 19(12): 591-5.
28. Torabinejad M, et al. Dye leakage of four root end filling materials: Effects of blood contamination. J Endod 1994; 20: 159-63.
29. Torabinejad M, Falah A, Kettering JD, Pitt Ford TR. Bacterial leakage of mineral trioxide aggregate as a root end filling material. J Endod1995; 21: 109-12.
30. Torabinejad M, Wilder P, Kettering JD, Pitt Ford TR. Comparative investigation of marginal adaptation of mineral trioxide aggregate and other commonly used root-end filling materials. J Endod1995; 21: 295-9.
31. Inoue S, Yoshimura M, Tinkle JS, Marshall FJ. A 24-week study of the microleakage of four retrofiling materials using a fluid filtration method. J Endod 1991; 17: 369-75.
32. Banomyong D, Palamara JEA, Messer HH, Burrow MF. Sealing ability of occlusal resin composite restoration using four restorative procedures. Eur J Oral Sci 2008; 116(6): 571-8.
33. Zaia AA, Nakagawa R, Quadros De, et al. An in vitro evaluation of four materials as barriers to coronal microleakage in root-filled teeth. J Endod 2002; 35; 729-34.

Table 1: Duncan's Multiple Range Tests for difference in the gaps between the dentin and the Biodentine ™ at different time intervals.

Sub - Group	Number	Mean	Std. Deviation	Duncan's test
A_1	7	8.1000	0.888	A
A_2	7	3.1667	0.7638	B
A_3	7	1.0000	0.0000	C

One-Way Analysis of Variance

Source	DF	SS	MS	F - test	P - value
Factor	2	79.442	39.721	86.77	0.000
Error	6	2.747	0.458		
Total	8	82.189			

Table 2: Duncan's Multiple Range Tests for difference in the gaps between the dentin and the MTA at different time intervals.

Sub - Group	Number	Mean	Std. Deviation	Duncan's test
B_1	7	54.467	4.313	A
B_2	7	6.000	1.000	B
B_3	7	3.333	1.528	B

One-Way Analysis of Variance

Source	DF	SS	MS	F	P
Factor	2	4970.75	2485.37	339.89	0.000
Error	6	43.87	7.31		
Total	8	5014.62			

Table 3: Duncan's Multiple Range Tests for difference in the gaps between the dentin and the Biodentine ™ at different time intervals.

Sub - Group	Number	Mean	Std. Deviation	Duncan's test
C_1	7	7.27	0.86	A
C_2	7	25.000	6.26	A
C_3	7	64.000	33.81	B

One-Way Analysis of Variance

Source	DF	SS	MS	F	P
Factor	2	5054	2527	6.41	0.032
Error	6	2366	394		
Total	8	7420			

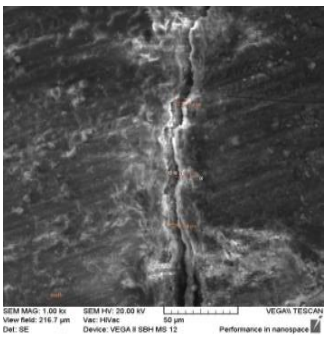
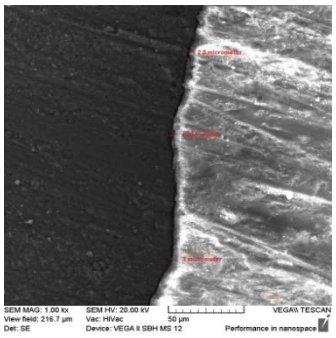
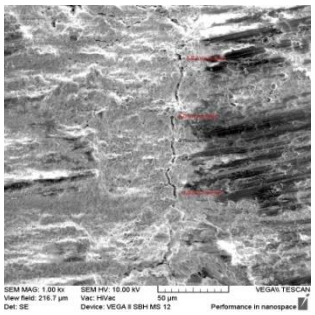
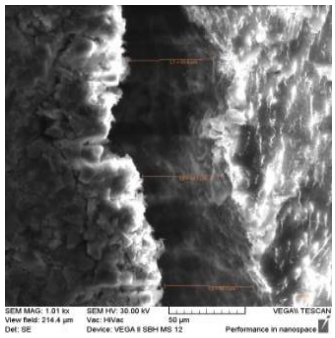
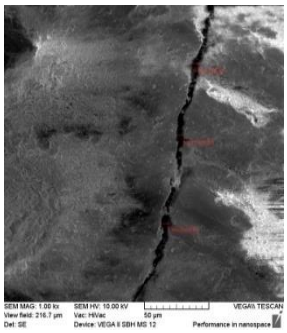
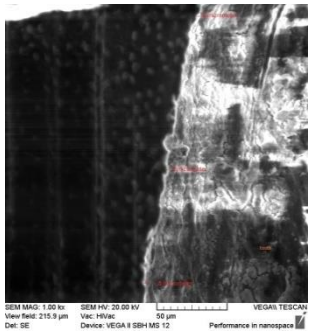
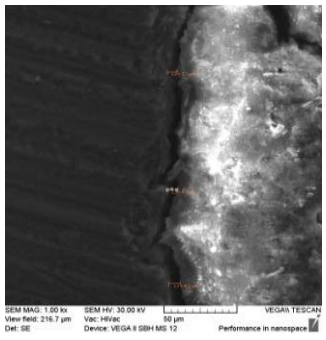
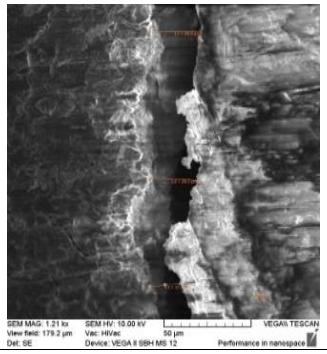
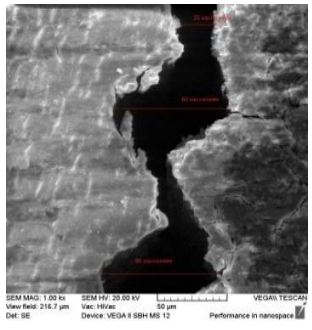
Group Material	7- days - Storage	14- days - Storage	28- days - Storage
Group -A Biodentine™	Sub-group (A_1)	Sub-group (A_2)	Sub-group (A_3)
			
Group - B MTA	Sub-group (B_1)	Sub-group (B_2)	Sub-group (B_3)
			
Group - C GIC	Sub-group (C_1)	Sub-group (C_2)	Sub-group (C_3)
			

Fig. 1: SEM images at magnification (1000-1200) X for the interspaces gap between tested materials and dentine

Table 4: Duncan's Multiple Range Tests for difference in the gaps between the Biodentine ™ and MTA and Glass Ionomer cement at 7 days.

Material	Number	Mean	Std. Deviation	Duncan's test
Biodentine ™	7	8.1000	0.889	A
MTA	7	54.467	4.313	B
Glass Ionomer	7	7.267	0.862	A

One-Way Analysis of Variance

Source	DF	SS	MS	F	P
Factor	2	4378.40	2189.20	326.15	0.000
Error	6	40.27	6.71		
Total	8	4418.68			

Table 5: Duncan's Multiple Range Tests for difference in the gaps between the Biodentine ™ and MTA and Glass Ionomer cement at 14 days.

Material	Number	Mean	Std. Deviation	Duncan's test
Biodentine ™	7	3.167	0.764	A
MTA	7	6.000	1.000	A
Glass Ionomer	7	25.000	6.264	B

One-Way Analysis of Variance

Source	DF	SS	MS	F	P
Factor	2	845.7	422.9	31.07	0.001
Error	6	81.6	13.6		
Total	8	927.4			

Table 6: Duncan's Multiple Range Tests for difference in the interface gap between the Biodentine ™ and MTA and Glass Ionomer cement at 28days

Material	Number	Mean	Std. Deviation	Duncan's test
Biodentine ™	7	1.00	0.00	A
MTA	7	3.33	1.53	A
Glass Ionomer Cement	7	64.00	33.81	B

One-Way Analysis of Variance

Source	DF	SS	MS	F	P
Factor	2	7655	3827	10.03	0.012
Error	6	2291	382		
Total	8	9946			

Table 7: FOR ALL

Sub - Group	Number	Mean	Std. Deviation	Duncan's test
A_1	7	8.1000	0.888	A
A_2	7	3.1667	0.7638	A
A_3	7	1.0000	0.0000	A
B_1	7	54.467	4.313	C
B_2	7	6.000	1.000	A
B_3	7	3.333	1.528	A
C_1	7	7.27	0.86	A
C_2	7	25.000	6.26	B
C_3	7	64.000	33.81	C

One-Way Analysis of Variance

Source	DF	SS	MS	F	P
Factor	8	13693	1712	12.77	0.000
Error	18	2413	134		
Total	26	16106			

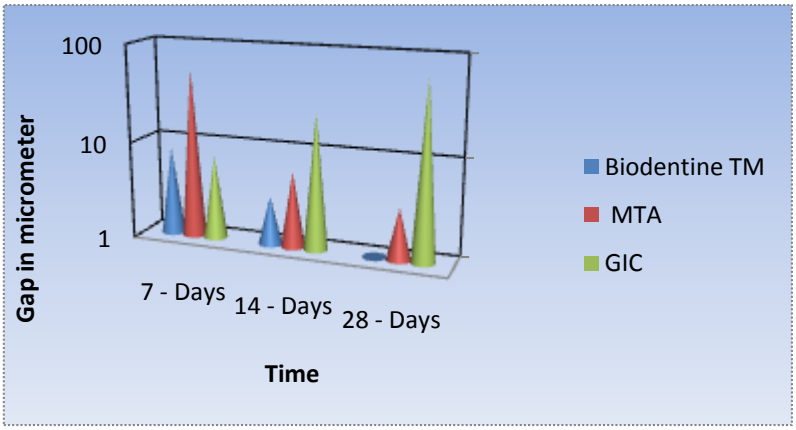


Figure 2: Gap Distance with time storage for Biodentine ™, MTA and GIC.