ISSN: 2581-5989
PubMed - National Library of Medicine - ID: 101738774

International Journal of Dental Science and Innovative Research (IJDSIR)

IJDSIR : Dental Publication Service

Available Online at: www.ijdsir.com

Volume – 5, Issue – 2, April - 2022, Page No. : 454 - 463

Comparative evaluation of remineralizing ions release at acidic and neutral pH from glass ionomer cement, bioactive glass material and Alka site material- An in-vitro study

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Citation of this Article: Dr. Akanksha Garg, Dr. Ritika Malhotra, Dr. Manish Bhalla, Dr. Geetika Datta, Dr. Sonvita Debnath, Dr. Dzuthohulu Thirah, "Comparative evaluation of remineralizing ions release at acidic and neutral pH from glass ionomer cement, bioactive glass material and Alka site material- An in-vitro study", IJDSIR- April - 2022, Vol. – 5, Issue - 2, P. No. 454 – 463.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Background: Fluoride-releasing restorative materials have been investigated in order to minimise the risk of secondary caries as well as remineralise the underlying lesion. Apart from fluoride, the release of sodium, phosphate and silicate ions also make the cement a bioactive material.

Aim: The present study is designed to evaluate and compare the release of remineralizing ions at acidic and neutral ph. from GIC, bioactive glass and bulk-fill Alka site material under in-vitro conditions.

Materials and methodology: The study was conducted in 120 freshly extracted human premolar teeth. The procedure involved disinfection of specimen using 0.1% thymol solution and thereafter storage in normal saline for 24 hours. A standardized cavity of 2mm depth, 3mm length and 1.6mm width was prepared in all the sample teeth. The samples (*n* = 120) were randomly divided into the three groups: group 1**:** (*n* = 40): Glass ionomer cement (GIC), group 2: (*n* = 40): Bioactive Glass restorative material (BAG), and, group 3: (*n* = 40): Alka site restorative material (ARM). The thermal cycling test comprised of 500 cycles in water of 20 seconds each, ranging between 5°C and 55°C. Next, each group of 40 samples were further divided into 2 subgroups on the basis of pH (acidic pH – 4, neutral pH – 7) of the solution used for testing the remineralization potential of the samples. At the end of the first 24 hours and at 7-, 14- and 21-days testing for ions was done.

Observations and results: Acidic medium showed a higher concentration of fluoride, phosphate, hydroxyl and calcium ions release as compared to neutral medium ($p<0.005$). The release of fluoride, phosphate and hydroxyl ions at the end of 21 days from Cention-N was significantly higher as compared to GIC and bioactive glass material ($p<0.005$). The ion release increased as the time duration increased from 24 hours to 21 days.

Conclusion: BAG and ARM may be used as alternatives to GIC because of their evident remineralizing potential.

Keywords: Remineralisation, GIC, Bio glass, Alka site, Secondary caries.

Introduction

Dental caries or tooth decay continues to be one of the leading chronic lifestyle diseases worldwide [1]. The prevalence of dental caries as 27% to 64% in 12-year-old children and 26% to 83% in adults [2]. It is a well-known fact that, dental caries involves interactions between the tooth structure, the microbial biofilm formed on the tooth surface and sugars, as well as salivary and genetic influences' [3,4]. Secondary caries on the other hand, is largely a complication following dental restorations. It is defined as 'lesions at the margins of existing restorations' or 'caries associated with restorations or sealants [5,6]. It is considered 'complicated' as secondary caries can be either causally associated with a defective restoration or causally associated with an intact restoration or not causally associated with the restoration at all, but simply primary caries that was left behind adjoining the existing restorations. The pathogenesis of secondary caries is a result of misbalance in the demineralisation-remineralisation cycle, the former being dominant. Another very important factor that modifies the pathogenesis is the property of the dental restorative material at the restorative margin. Prevention of secondary caries or lowering the risk of it is largely responsible on location of the restoration, caries susceptibility of the patient, operator skill and dental restorative materials used [7,8,9].

Fluoride-releasing restorative materials have been investigated in order to minimise the risk of secondary caries as well as remineralise the underlying lesion [10]. One of the commonly used cements with wide range of applications and sustained release of fluoride is glass polyalkenoate cement or glass-ionomer cement (GIC). It is proven that GIC provides an 'early burst' of fluoride release followed by sustained lower-level diffusion-based release [11]. It is interesting to note that fluoride release increases in the acidic medium. The drawbacks of GIC like poor compressive strength and marginal leakage are compensated in the recently developed bioactive glass containing material (BAG). Bioactive glass is made up of amorphous sodium calcium phosphosilicate and is a highly reactive material in the oral cavity. BAGs experience ionic dissolution and glass degradation when they come into contact with body fluids or simulated body fluid via the interchange of H^+ ions in the solution and Na^+ and Ca^{2+} from the glass network.

Another new fluoride-releasing bulk-filling restorative material is metal-free Alka site restorative material. Alka site refers to a new category of restorative material, somewhat similar to compomer or ormocers and is a subgroup of the composite. Alka sites utilize an alkaline filler which is capable of releasing acid-neutralizing ions [12]. The active component is a calcium fluorosilicate glass which shows degradation behaviour similar to bioactive glasses [13]. The absence of phosphate in this material helps in the increased formation of fluorite unlike other bioactive glasses [14]. The material releases Ca^{2+} and F^{-} and forms "apatite like" phase upon immersion containing orthophosphate. In acidic medium calcium fluoro-silicate glass particles undergo glass degradation at the surface but to a lesser extent at the neutral pH [15].

The aim of this in vitro cross-sectional study was to evaluate and compare the release of remineralizing ions in acidic and neutral medium from glass ionomer cement, bioactive glass and bulk-fill Alka site materials at 24 hours, 7, 14 and 21 days.

Materials and method

The present study was carried out in the Department of Pedodontics and Preventive Dentistry following review and clearance by the Ethical Committee Board of the University.

Sample size estimation: Sample size was calculated using G Power Software (version 3.0.10). Based on the calculated effect size of 0.26, 5% level of precision, 95% confidence level and 80% power of the study.

120 freshly extracted human teeth were included in the study. The specimens were then disinfected using 0.1% thymol solution and thereafter stored in normal saline for 24 hours Figure 1A, B [16].

Preparation of standardized cavity: A standardized cavity of 2mm depth, 3mm length and 1.6mm width was prepared using modified SF-41 ISO 109/010 bur.

The samples (n = 120) were thereafter randomly divided into the following three study groups using draw of lots: Group 1: (n = 40): Glass ionomer cement (GIC) (Ketac 3M, ESPE), Group 2: (n = 40): Bioactive Glass restorative material (BAG) (Pulp dent Activa), and Group 3: (n = 40): Alka site restorative material (ARM) (CENTION-N) Figure 2. All the teeth were restored using the respective restorative materials. The materials were mixed by single researcher as per the manufacturer's instructions. A layer of nail varnish was then used to coat the samples.

The restored specimens were stored in water at 37°C for 24 hours before subjecting them to thermocycling (500 cycles in water of 20 seconds each, ranging between 5°C and 55°C) to simulate the oral environment. Next, each group of 40 samples were further divided into 2 subgroups using draw of lots. The sub-grouping was done on the basis of pH (acidic pH – 4, neutral pH – 7) of the solution used for testing the remineralization potential of the samples. 120 plastic containers were arranged and half of them were filled with 5ml of acidic buffer and the rest with 5ml of neutral buffer solution Figure 3,4. The samples were then suspended into the solution containing containers Figure 5. At the end of the first 24 hours the containers were thoroughly shaken and 5 sample containing containers from each of the 6 subgroups were sent to laboratory to check for remineralizing ion release. Similar procedure was carried out at the end of 7, 14 and 21 days. The storage medium of the containers was changed every 24 hours of the experiment time so as to avoid ion saturation of the buffer solution that may hinder further ion release from the restorative material.

The data collected was entered in the excel spreadsheet using Microsoft Excel Software (Version17.0) and was transferred to Statistics Package for Social Sciences (SPSS) version 26.0.

Results

Comparison of release of remineralizing ions from GIC, BAG and ARM materials in acidic and neutral medium was done (Table 1) where, a significant difference was found in ion release among the three groups. The highest fluoride release was seen in GIC at all the observed time intervals whereas the highest hydroxyl, phosphate and fluoride release was seen in ARM.

Discussion

The present study has been based on restorative materials that have recently caught attention due to their biomimetic properties and remineralization potential at tooth-restoration interfaces thereby limiting secondary caries.

The results of the present study demonstrate the release of fluoride, hydroxyl, phosphate and calcium ions from GIC in acidic and neutral medium at different time intervals. Acidic medium showed a higher concentration of respective ion release as compared to neutral medium. The ion release increased with time from 24 hours to 21 days. Several scientific researches have confirmed that fluoride release from glass ionomer cements is characterised by a quick initial release, followed by a rapid decline in the rate of fluoride release after a short period [17-19]. To further corroborate this finding, in a study by Attin T et al. (1999), the capacity of glass ionomer sealants to act as a fluoride reservoir in the oral cavity while maintaining a low fluoride level in oral fluids was demonstrated [20]. Moreover, the amount of fluoride released by ‘fresh' GIC, which is similar to the material against a cavity wall, is more than twice that of matured specimens, according to laboratory tests [21]. The present study confirms all the previous research findings in terms of its initial burst and later sustained release. Aside from the aqueous nature and pH of the oral environment, a variety of other factors, such as surface erosion and component leaching, can have a significant impact on the behaviour of a restorative material and its ion leaching properties El-Mallakh BF and Sarkar NK (1990) discovered that the amount of fluoride released from various GICs varied significantly depending on the storage medium [22]. Fluoride release into the acidic medium has been found to be significantly higher than in neutral buffers, which could be attributed to increased erosion of the resin-modified GIC at low pH. This increased release of fluoride at acidic conditions may reduce secondary caries risk, as has been observed in vitro by various authors [23-25]. GIC is a good choice of restorative material in treating patients at increased risk for caries because of its capacity to release and store fluoride. The release of hydroxide ions from a restorative material may help to neutralise excess acidity during acid attacks by cariogenic flora, preventing demineralization [26]. However, the release of hydroxyl ions from GIC is not in significant amounts as per the present study results. The results of the present study are in accordance with previously published literature which demonstrates release of hydroxyl from GIC to be negligible [14]. The phosphate and calcium ion release from GIC was negligible but increased from 24 hours to 21 days in acidic medium as well as in neutral medium. Nicholson JW et al. (2021) discussed from their observations that when stored at 20–22 °C, glass-ionomer cement has been shown to release sodium, silicon, aluminum, and phosphorus under neutral and acidic conditions, and calcium under acidic conditions [27]. The authors also stated that sufficient phosphorus was released during the short storage time experiments with daily water changes. However, the release was negligible in the long-term storage experiment with monthly changes of the storage water.

The mean fluoride concentration release from BAG at 24 hours in acidic medium was 0.09 + .01 which increased to 1.86 + 0.06 at 7 days and 2.66 + 0.05 at 21 days. In neutral medium, the fluoride concentration at 24 hours was 0.05 + .01 which increased to 1.20 + 0.07 at 7 days and then further to 1.87 + 0.12 at 21 days. Bio glass dissolution is determined by the breakdown of the Si-O-Si link, which results in a rapid increase in fluoride ion concentration between days 0 and 3, followed by a gradual drop [28,29]. This shows that chemical makeup of bio glass has a significant impact on its fluoride release profile. Davis et al. (2014) found that composites containing silicon-rich bio glass particles had considerably increased long-term fluoride release [30]. Mneimne et al. (2011) reported that bio glass particles containing phosphate or fluoride might induce apatite development faster and at a lower pH [31]. Hence the results of the present study are in accordance with the previously published scientific literature. This release if compared to that from GIC is significantly less at 24 hours and at 7 days as GIC shows an initial burst in fluoride release. This is in accordance with study by May E and Donly KJ (2017) but in another research by Nagi SM et al. (2018) the authors concluded that there lies no significant difference in release of fluoride between conventional RMGIC and ACTIVA. However, it was additionally reported that the former exhibited better recharge and release properties [32,33]. The release of hydroxyl, calcium and phosphate was observed to increase over time although the quantity may not be ideal enough to cause tooth remineralization. Similar findings were reported by According to Tiskaya M et al. (2019), Activa released very few ions, including fluoride, when immersed in Tris Buffer solution (pH 7.3) and artificial saliva (pH 7), but more ions, including large amounts of aluminum when using the same media. This suggests that the glasses in Activa are acid degradable fluoro-aluminosilicate glasses, similar to those found in glass ionomer cements [34]. When F-containing bioactive glass (BAG) is added to RB-ILMs, the BAG works like an ion source, releasing Ca, P, F, and other ions when the BAG filler comes into contact with fluid, even at neutral ph. This allows for controlled F release. As a result, the hydrophilicity and acidic nature of RBILM resin matrices had an impact on enhancing F release [17,34,35]. The present study shows that at the end of 21 days the release of fluoride from Cention N is highest and significantly more from that released from GIC or Activa, thereby corroborating the previous findings. The results of the study done by Ruengrungsom C et al. (2020) demonstrated that all groups released more fluoride ion in acidic pH than in neutral pH, indicating that when conditions become acidic due to cariogenic difficulties, Cention-N will release more fluoride ion. This could be because lowering the pH of the solvent causes more surface dissolution of the materials, which increases fluoride ion release [36]. In a study by Gupta N et al. (2020), the authors reported that when compared to Cention-N (light-cure) and GIC, Cention-N (self-cure) had the largest fluoride ion release in acidic pH [14]. The release of hydroxyl ions was prominent compared to phosphate and calcium over the 21-day period, although the release of all three ions was higher in acidic medium. In a study by Ruengrungsom C et al. (2020), the authors confirm that Cention N had excellent capacity for Ca release and rechargeable ability of Ca/P. This result is similar to that of the present study.[37] The authors also stated that in acidic pH, Cention-N showed a very strong alkalizing potential. This could be due to the hydroxyl and calcium ions released by Cention-N alkaline glass fillers, which can have a direct effect on the pH levels in the oral cavity, allowing excess acidity caused by cariogenic bacterial activity to be neutralized [36]. In another study by Kasraei S et al. (2021), it was shown that Cention N has the maximum alkanizing potential owing to its high phosphate release when compared to RMGIC and Activa [37,38].

The smaller sample size and limited time of observation can be considered as drawbacks. However, the materials were tested in both neutral and acidic media which confirms clarity of the results observed. Further studies should be conducted with longer periods of observations to confirm the remineralizing potentials of the newer restorative materials when compared to the gold standard, glass ionomer cement.

Conclusion

The following conclusions were derived from the observations from the present study:

1. The release of ions from three restorative materials was higher in acidic medium when compared to neutral medium.
2. GIC releases highest amount of fluoride ions compared to BAG and ARM at the end of 24 hours.
3. ARM release maximum fluoride in the overall observation period of 21 days followed by GIC and BAG.
4. ARM released hydroxyl and phosphate ions in significant amounts compared to GIC and BAG in 21 days.
5. Release of calcium from all three restorative materials was found to be in trace amounts.

References

1. Marcenes W, Kassebaum NJ, Bernabe E, Flaxman A, Naghavi M, Lopez A, Murray CJ. Global burden of oral conditions in 1990-2010: a systematic analysis. J Dent Res. 2013; 92:592–7
2. Shah N, Pandey RM, Duggal R, Mathur VP, Parkash H, Sundaram KR. Oral Health in India. A Report of Multi-centric Study. Director General of Health Services, Ministry of Health and Family Welfare, Government of India and WHO collaborative programme. December 2007. Available at: http://www.whoindia.org/en/ section20/ Section 30_1525. htm. Last accessed on 07.12.2020.
3. Selwitz RH, Ismail AI, Pitts NB. Dental caries. Lancet. 2007 Jan 6;369(9555):51-9.
4. Pitts NB, Zero DT. White paper on dental caries prevention and management. FDI World Dental Federation, http://www.fdiworlddental.org/sites/default/ files/media/documents/2016-fdi_cpp-white_paper.pdf (2016).
5. Machiulskiene V, Campus G, Carvalho JC, Dige I, Ekstrand KR, Jablonski-Momeni A, Maltz M, Manton DJ, Martignon S, Martinez-Mier EA, Pitts NB, Schulte AG, Splieth CH, Tenuta LMA, Ferreira Zandona A, Nyvad B. Terminology of Dental Caries and Dental Caries Management: Consensus Report of a Workshop Organized by ORCA and Cariology Research Group of IADR. Caries Res. 2020;54(1):7-14.
6. Mjör IA, Toffenetti F. Secondary caries: a literature review with case reports. Quintessence Int. 2000 Mar;31(3):165-79.
7. Demarco FF, Collares K, Coelho-de-Souza FH, Correa MB, Cenci MS, Moraes RR, Opdam NJ. Anterior composite restorations: A systematic review on long-term survival and reasons for failure. Dent Mater. 2015 Oct;31(10):1214-24.
8. Opdam NJ, Bronkhorst EM, Roeters JM, Loomans BA. A retrospective clinical study on longevity of posterior composite and amalgam restorations. Dent Mater. 2007 Jan;23(1):2-8.
9. Schwendicke F, Krüger H, Schlattmann P, Paris S. Restoration outcomes after restoring vital teeth with advanced caries lesions: a practice-based retrospective study. Clin Oral Investig. 2016 Sep;20(7):1675-81.
10. Exterkate RA, Damen JJ, ten Cate JM. Effect of fluoride-releasing filling materials on underlying dentinal lesions in vitro. Caries Res. 2005 Nov-Dec;39(6):509-13.

11. De Witte, A.M.; De Maeyer, E.A.; Verbeeck, R.M.H.; Martens, L.C. Fluoride release profiles of mature restorative glass ionomer cements after fluoride application. Biomaterials 2000, 21, 475–82.

12. Scientific Documentation; Cention N, Ivoclar Vivadent AG; research and development scientific service, issue; October 2016.

13. http://refhub.elsevier.com/S0109 5641(19)30877-2/sbref0005

14. Gupta N, Jaiswal S, Nikhil V, Gupta S, Jha P, Bansal P. Comparison of fluoride ion release and alkalizing potential of a new bulk-fill Alka site. J Conserv Dent. 2019 MayJun;22(3):296-9.

15. Sulieman RT. Microleakage of root canal sealed with temporary endodontic sealing materials. Tikrit J Dent Sci 2013; 1:24-9.

16. Marinelli CB, Donly KJ, Wefel JS, Jakobsen JR, Denehy GE. An in vitro comparison of three fluoride regimens on enamel remineralization. Caries Res. 1997;31(6):418-22.

17. Wiegand A, Buchalla W, Attin T. Review on fluoride-releasing restorative materials-- fluoride release and uptake characteristics, antibacterial activity and influence on caries formation. Dent Mater. 2007 Mar;23(3):343-62.

18. eidlich P, Miranda LA, Maltz M, Samuel SM. Fluoride release and uptake from glass ionomer cements and composite resins. Braz Dent J. 2000;11(2):89–96.

19. Yap AU, Tham SY, Zhu LY, Lee HK. Short-term fluoride release from various aesthetic restorative materials. Oper Dent. 2002;27(3):259–65.

20. Attin T, Buchalla W, Siewert C, Hellwig E. Fluoride release/uptake of polyacid modified resin composites (compomers) in neutral and acidic buffer solutions. J Oral Rehabil. 1999 May;26(5):388-93.

21. Forsten L. Fluoride release and uptake by glass ionomers. Scand J Dent Res 1991; 99:241—5.

22. el Mallakh BF, Sarkar NK. Fluoride release from glass-ionomer cements in de-ionized water and artificial saliva. Dent Mater. 1990 Apr;6(2):118-22.

23. Donly KJ, Ingram C. An in vitro caries inhibition of photopolymerized glass ionomer liners. ASDC J Dent Child. 1997 Mar-Apr;64(2):128-30.

24. Hsu CY, Donly KJ, Drake DR, Wefel JS. Effects of aged fluoride-containing restorative materials on recurrent root caries. J Dent Res. 1998 Feb;77(2):418-25.

25. Nagamine M, Itota T, Torii Y, Irie M, Staninec M, Inoue K. Effect of resin-modified glass ionomer cements on secondary caries. Am J Dent. 1997 Aug;10(4):173-8.

26. Nicholson JW, Aggarwal A, Czarnecka B, Limanowska-Shaw H. The rate of change of pH of lactic acid exposed to glass-ionomer dental cements. Biomaterials. 2000; 21:1989–93.

27. Kishore G, Sai-Sankar AJ, Pratap-Gowd M, Sridhar M, Pranitha K, Sai-Krishna VS. Comparative evaluation of fluoride releasing ability of various restorative materials after the application of surface coating agents: An in vitro study. J Clin Diagn Res. 2016;10(12):38–41.

28. Shah FA, Brauer DS, Hill RG, Hing KA. Apatite formation of bioactive glasses is enhanced by low additions of fluoride but delayed in the presence of serum proteins. Mater Lett. 2015; 153:143–7.

29. Davis HB, Gwinner F, Mitchell JC, Ferracane JL. Ion release from, and fluoride recharge of a composite with a fluoride-containing bioactive glass. Dent Mater. 2014;30(10):1187–94.

30. Mneimne M, Hill RG, Bushby AJ, Brauer DS. High phosphate content significantly increases apatite formation of fluoride-containing bioactive glasses. Acta Biomater. 2011;7(4):1827–34

31. May E, Donly KJ. Fluoride release and re-release from a bioactive restorative material. Am J Dent. 2017 Dec;30(6):305-8.

32. Nagi SM, Moharam LM, El Hoshy AZ. Fluoride release and recharge of enhanced resin modified glass ionomer at different time intervals. Futur Dent J. 2018 Dec 1;4(2):221- 4.

33. Tiskaya M, Al-Eesa NA, Wong FSL, Hill RG. Characterization of the bioactivity of two commercial composites. Dent Mater. 2019 Dec;35(12):1757-68.

34. https://www.pulpdent.com/activa-bioactive-overview/#bioactive-properties

35. Asmussen E, Peutzfeldt A. Long-term fluoride release from a glass ionomer cement, a compomer, and from experimental resin composites. Acta Odontol Scand. 2002 Mar;60(2):93-7.

36. Ruengrungsom C, Burrow MF, Parashos P, Palamara JEA. Evaluation of F, Ca, and P release and microhardness of eleven ion-leaching restorative materials and the recharge efficacy using a new Ca/P containing fluoride varnish. J Dent. 2020 Nov; 102:103474.

37. Kasraei S, Haghi S, Valizadeh S, Panahandeh N, Nejadkarimi S. Phosphate Ion Release and Alkalizing Potential of Three Bioactive Dental Materials in Comparison with Composite Resin.

Legend Tables and Figures

Table 1: Comparison of release of remineralizing ions of GIC, BAG and ARM materials in acidic and neutral medium

Time interval	F^-			Oh^-			Po_4^{3-}			Ca^{2+}		
	GIC	BAG	ARM	GIC	BAG	ARM	GIC	BAG	ARM	GIC	BAG	ARM
ACIDIC MEDIUM												
24 hours	0.12 + .013	0.15 + 0.01	0.1 ± 0.01	0.03 + .001	0.53 + 0.014	1.48 ± 0.012	1.53 + .064	1.46 + 0.11	0.64 ± 0.08	1.14 + 0.11	1.54 + 0.07	38.1 ± 0.79
p-value	0.0001			0.0001			0.0001			0.0001		
7 days	3.28 + 0.15	1.86 + 0.06	1.86 ± 0.13	0.38 + 0.008	0.97 + 0.012	1.44 ± 0.01	1.87 + 0.05	2.06 + 0.11	2.50 ± 0.14	1.88 + 0.01	2.18 + 0.05	116.70 ± 1.20
p-value	0.0001			0.0001			0.0001			0.0001		
14 days	4.62 ± 0.18	3.66 ± 0.09	4.38 ± 0.26	0.81 ± 0.005	0.21 ± 0.005	0.93 ± 0.05	2.06 ± 0.09	2.39 ± 0.06	3.56 ± 0.08	2.80 ± 0.07	3.54 ± 0.07	122.34 ± 0.68
p-value	0.0001			0.0001			0.0001			0.0001		

21 days	6.48 ± 0.08	4.96 ± 0.05	6.28 ± 0.13	0.18 ± 0.008	0.13 ± 0.013	0.52 ± 0.01	2.46 ± 0.03	2.52 ± 0.06	4.32 ± 0.16	3.16 ± 0.06	3.89 ± 0.07	124.9 ± 0.56
p-value	0.0001			0.0001			0.0001			0.0001		
NEUTRAL MEDIUM												
24 hours	0.08 ± .01	0.10 ± 0.01	0.07 ± 0.008	0.01 ± 0.00	0.016 ± 0.008	0.034 ± 0.005	1.27 ± 0.04	0.96 ± 0.05	0.36 ± 0.049	0.77 ± 0.04	1.08 ± 0.08	31.5 ± 1.17
p-value	0.001			0.0001			0.0001			0.0001		
7 days	2.66 ± 0.18	1.20 ± 0.07	1.22 ± 0.15	0.03 ± 0.0008	0.26 ± 0.015	0.03 ± 0.007	1.65 ± 0.029	1.87 ± 0.07	1.83 + 0.12	1.49 ± 0.06	1.91 ± 0.067	110.54 ± 0.57
p-value	0.0001			0.681			0.003			0.0001		
14 days	4.10 ± 0.06	3.16 ± 0.04	3.54 ± 0.089	0.03 ± 0.0008	0.022 ± 0.010	0.02 ± 0.007	1.85 ± 0.05	2.13 ± 0.039	3.06 ± 0.11	2.43 ± 0.08	3.11 ± 0.07	117.96 ± 0.62
p-value	0.0001			0.111			0.0001			0.0001		
21 days	6.13 ± 0.04	4.15 ± 0.12	5.58 ± 0.08	0.036 ± 0.004	0.022 ± 0.008	0.029 ± 0.005	2.25 ± 0.11	2.30 ± 0.03	3.89 ± 0.06	2.90 ± 0.07	3.38 ± 0.06	121.18 ± 0.58
p-value	0.0001			0.012			0.0001			0.0001		

Figure 1: (a) samples stored in thymol solution, (b) samples stored in normal saline

Figure 2: (a) glass ionomer cement, (b) bioactive glass, (c) bulk-filling Alka site material, (d) normal saline, thymol, buffer solutions and distilled water

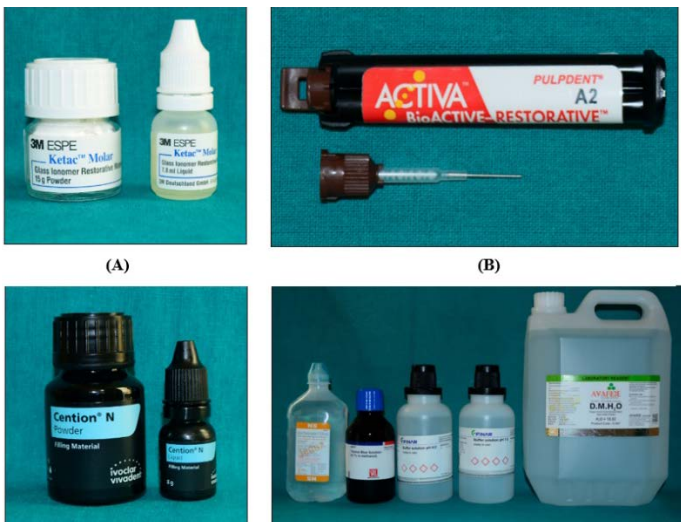

Figure 3: (a) acidic buffer solution being filled in plastic containers, (b) neutral buffer solution being filled in plastic containers

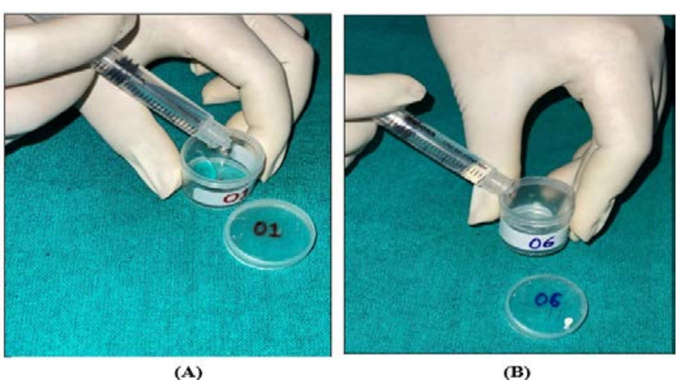

Figure 4: plastic containers filled with acidic and neutral buffer solution respectively

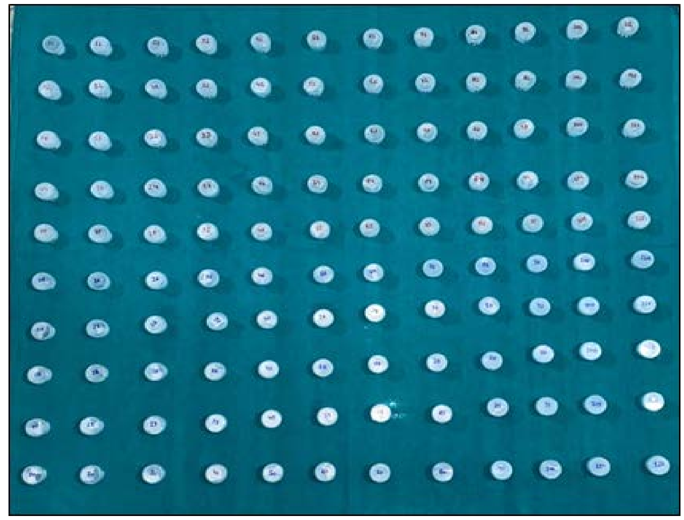

Figure 5: (a) prepared specimens; (b) specimens suspended in plastic containers filled with acidic and neutral buffer solution respectively; (c) specimens suspended in plastic containers with acidic and neutral buffer solution respectively