Original Article

Effect of blood and artificial saliva contamination on marginal adaptation and sealing ability of different retrograde filling materials: A comparative analysis

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Abstract

Objective: The objective of this study was to evaluate the effect of blood and artificial salivary contamination of different root-end filling materials on microleakage using a confocal laser scanning microscope and on marginal adaptation using a scanning electron microscope.

Materials and Methods: Eighty noncarious single-rooted teeth with mature apices were taken. After retro-cavity preparation, they were randomly assigned into two major groups ($n = 40$). They were contaminated with blood and artificial saliva, respectively. Each major group was divided randomly into four subgroups ($n = 10$) and filled as follows: Subgroup A, Biodentine; Subgroup B, bioactive bone cement; Subgroup C, Cention N; and Subgroup D, Bio-C Repair. The samples were sectioned transversely at 1 and 2 mm from the root apex and checked under a confocal laser scanning microscope for microleakage and under an scanning electron microscope for marginal adaptation. The average mean values were calculated. Independent samples *t*-tests, paired *t*-tests, and one-way analysis of variance with Tukey's *post hoc* tests were done to analyze the data.

Results: All the tested materials showed marginal gaps and dye leakage. The Bio-C Repair group showed the least mean marginal gap and dye leakage values, followed by bioactive bone cement, Biodentine, and Cention N, respectively, in both blood and artificial saliva contamination. However, the mean marginal gaps and dye leakage between the major groups were statistically insignificant.

Conclusion: In an overall comparison, Bio-C Repair was found to be superior in terms of marginal adaptation and sealing ability under the test conditions.

Keywords: Artificial saliva; Bio-C Repair Biodentine; bioactive bone cement; blood; Cention N; CLSM; scanning electron microscope

INTRODUCTION

"Seal is the deal." One of the ideal requirements for a filling material is its ability to seal the cavity to prevent any percolation of fluids or ingress of bacteria. Even in surgical endodontics, the same rule applies. The main surgical steps involved in surgical endodontics are curettage, root-end resection and preparation, and root-end filling. The placement of a root-end filling is one of the key steps in managing the root end, as it provides a physical seal.[1]

Various materials have been proposed and tested in the pursuit of meeting the ideal requirements of a root-end

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filling material. These include conventional materials such as amalgam, gutta-percha, Cavit, GIC, IRM, Super EBA, and gold foil, all of which have varying degrees of "biocompatibility, sealing ability, and moisture resistance.[2]

In recent years, various new materials have been introduced in dentistry. Biodentine was advocated as a "dentin substitute" in 2010. It has improved physical properties such as a short setting time and the ability to penetrate through opened dentinal tubules and crystallize interlocking with dentin, thereby providing good mechanical and sealing properties. As a retrograde filling material, it shows a better sealing ability and bioactivity than MTA.[3]

Polymethyl methacrylate (PMMA), also known as bone cement, is widely used in orthopedic surgery. It has good handling and working properties, a fast-setting time, and good marginal adaptation, making it a suitable material for various endodontic treatments.[4,5] Despite its favorable properties, bone cement exhibits a few drawbacks including its brittle nature and its inability to directly bond to the tooth structure. This necessitates its modification by the addition of bioactive filler particles like MTA. This modification in the cement is known as bioactive bone cement.

A UDMA-based alkasite restorative material known as Cention N introduced with this has a shrinkage stress reliever with a low modulus of elasticity and improved marginal integrity. It utilizes an alkaline filler that is capable of releasing acid-neutralizing ions. A study carried out by Shailendra *et al*. showed that Cention N is as biocompatible as MTA.[6]

Recently introduced Bio-C Repair material is formulated in a ready-to-use threaded syringe. It has added advantages such as easy handling and insertion. Various studies have shown that it has good cell viability and cell adhesion and thereby its bioactivity.[7,8]

Ideally, the placement of filling material should be carried out in a moisture-free environment. However, in a clinical surgical scenario, there is a high chance of contamination through blood or saliva.[9] The marginal adaptation and sealing ability of retrograde filling materials play a crucial role in such conditions and determine the long-term success of such treatment.

To date, no other studies have compared the marginal adaptation and sealing ability of aforementioned materials in the presence of blood and artificial saliva.

This study intended to compare and evaluate the marginal adaptation using a scanning electron microscope and sealing ability using a confocal laser scanning microscope of the root-end filling material, namely Biodentine, bioactive bone cement, Cention N, and Bio-C Repair, in the presence of blood and artificial saliva.

MATERIALS AND METHODS

Approval from the ethical committee of the institute was obtained for this study (Ref No. SSDCRI/IEC/2021-2022/5/2), and the study was conducted accordingly.

Eighty noncarious single-rooted teeth with mature apices were taken, cleaned, and stored in 0.9% saline until further use. Decoronation was done till the cementoenamel junction and working length was determined using a 15K file (Mani, Inc., Tochigi, Japan). Chemo-mechanical preparation was done till F3 (ProTaper) using an E Connect Endo motor. Then, obturation was done, and the roots were placed at room temperature with 100% humidity for 1 week. A 3-mm root-end resection was done using a diamond disc at a 90° angle to the long axis of the tooth. A diamond-coated ultrasonic tip (ED11) was used to prepare a 3-mm retrograde cavity. (A periodontal probe was used to measure the depth of the cavity).

Eighty specimens were divided into two major groups:

1. Forty retrograde cavities were contaminated with human blood ($n = 40$) and then divided into four subgroups as follows:
   - Group A: Retrograde cavities were filled with Biodentine (Septodont) ($n = 10$)
   - Group B: Retrograde cavities were filled with Bioactive bone cement (Surgical Simplex P, Stryker) ($n = 10$)
   - Group C: Retrograde cavities were filled with Cention N (Ivoclar) ($n = 10$)
   - Group D: Retrograde cavities were filled with Bio-C Repair (Angelus) ($n = 10$)
2. Forty retrograde cavities were contaminated with artificial saliva (wet mouth) ($n = 40$) and then divided into four subgroups as follows:
   - Group E: Retrograde cavities were filled with Biodentine (Septodont) ($n = 10$)
   - Group F: Retrograde cavities were filled with Bioactive bone cement (Surgical Simplex P, Stryker) ($n = 10$)
   - Group G: Retrograde cavities were filled with Cention N (Ivoclar) ($n = 10$)
   - Group H: Retrograde cavities were filled with Bio-C Repair (Angelus) ($n = 10$).

With the help of small pluggers, each material was condensed into a cavity. Then, all specimens were stored in an incubator for 24 h to allow retrograde filling materials to be set.

Bioactive bone cement

Preparation of bioactive bone cement

Preliminary research showed the optimal MTA and silane coupling agent concentration required to modify bone cement without compromising its handling characteristics.[10]

Powder modification

0.4 mg of MTA and 0.6 mg of bone cement were mixed at a ratio of 60:40 until all of the MTA particles were mixed with the polymer powder.

Liquid modification

One milliliter of monomer liquid was combined with one drop of the silane coupling agent (Angelus) and mixed thoroughly.

The liquid and powder of the modified bone cement were combined in a 2:1 ratio.[11]

Dye penetration

The external surface (except for the region of root-end filling) of all the specimens was covered with two coats of nail varnish, to prevent penetration of the dye through the dentinal tubules and the accessory canals. All the specimens were then immersed in 0.5% aqueous rhodamine dye (5 mg of rhodamine B powder mixed in 100 ml of distilled water) for 48 h. After 48 h, the specimens were rinsed under running water for 5 min and allowed to dry.

Tooth sectioning

All the teeth were then sectioned to get a 1-mm section sample at 1 and 2 mm from the tip. Then, the samples were tested for the following:

Microleakage testing

The extent of dye leakage was measured using a confocal laser scanning microscope (Zeiss LSM 510; Carl Zeiss, Jena, Germany). These CLSM images were analyzed for dye penetration [Figures 1 and 2].

Marginal adaptation assessment

The marginal gaps between the retrograde cavity walls and the retro-filling material were assessed under a scanning electron microscope (JEOL-JSM-IT-300). These scanning electron microscope (SEM) images were analyzed for marginal gaps between the root-end filling material and the cavity wall [Figures 1 and 2].

Statistical analysis

Data were analyzed using IBM SPSS version 20 software (IBM SPSS, IBM Corp., Armonk, NY, USA) at a significance level of 5%. Independent samples *t*-tests, paired *t*-tests, and one-way analysis of variance with Tukey's *post hoc* tests were done to analyze the study data.

RESULTS

The means and standard deviation of values of blood and artificial saliva-contaminated groups are shown in Tables 1 and 2, respectively.

- All the root-end filling materials in both blood and artificial saliva-contaminated cavities showed both gaps in terms of marginal adaptation and microleakage
- All materials exhibited higher mean marginal gap values and dye leakage in artificial saliva contamination than in the presence of blood contamination. This was not statistically significant
- The lowest mean marginal gap and dye leakage were shown by the Bio-C Repair group
- The Cention N group showed the highest mean gaps and dye leakage, followed by the Biodentine group.

Table 1: Comparison between the four subgroups in the blood-contaminated group

Parameter (mm)	Group	n	Mean	SD	SE	95% CI for mean		*F*	*P*
						Lower bound	Upper bound		
Confocal 1	Biodentine	10	391.6200[a]	103.40551	32.69969	317.6482	465.5918	1.05	<0.001*
	Bioactive bone cement	10	288.6900[b]	42.86059	13.55371	258.0294	319.3506		
	Cention N	10	451.6200[b,c]	115.11168	36.40151	369.2741	533.9659		
	Bio-C Repair	10	133.8910[a,c]	210.16982	66.46153	−16.4554	284.2374		
Confocal 2	Biodentine	10	351.6200[a]	75.88463	23.99683	297.3354	405.9046	8.16	<0.001*
	Bioactive bone cement	10	250.6900[b]	68.86256	21.77625	201.4287	299.9513		
	Cention N	10	431.6200[b]	73.79272	23.33531	378.8319	484.4081		
	Bio-C Repair	10	113.8910[a,b]	159.50011	50.43836	−0.2085	227.9905		
SEM 1	Biodentine	10	3.36890[a]	0.383188	0.121175	3.09478	3.64302	33.01	<0.001*
	Bioactive bone cement	10	2.33250[a,b]	0.930997	0.294407	1.66650	2.99850		
	Cention N	10	4.06680[b]	0.763540	0.241453	3.52060	4.61300		
	Bio-C Repair	10	1.09860[a,b]	0.654599	0.207002	0.63033	1.56687		
SEM 2	Biodentine	10	3.10360[a]	0.701287	0.221766	2.60193	3.60527	8.74	<0.001*
	Bioactive bone cement	10	2.14630[a,b]	0.573487	0.181352	1.73605	2.55655		
	Cention N	10	3.91940[b]	0.869047	0.274817	3.29772	4.54108		
	Bio-C Repair	10	1.23210[a,b]	0.559151	0.176819	0.83211	1.63209		

*Statistical significance. $P \leq 0.05$ considered statistically significant, groups with similar superscripts in the mean value have significant differences in Tukey's *post hoc* tests for multiple pairwise comparisons. SD: Standard deviation, SE: Standard error, CI: Confidence interval, SEM: Scanning electron microscope

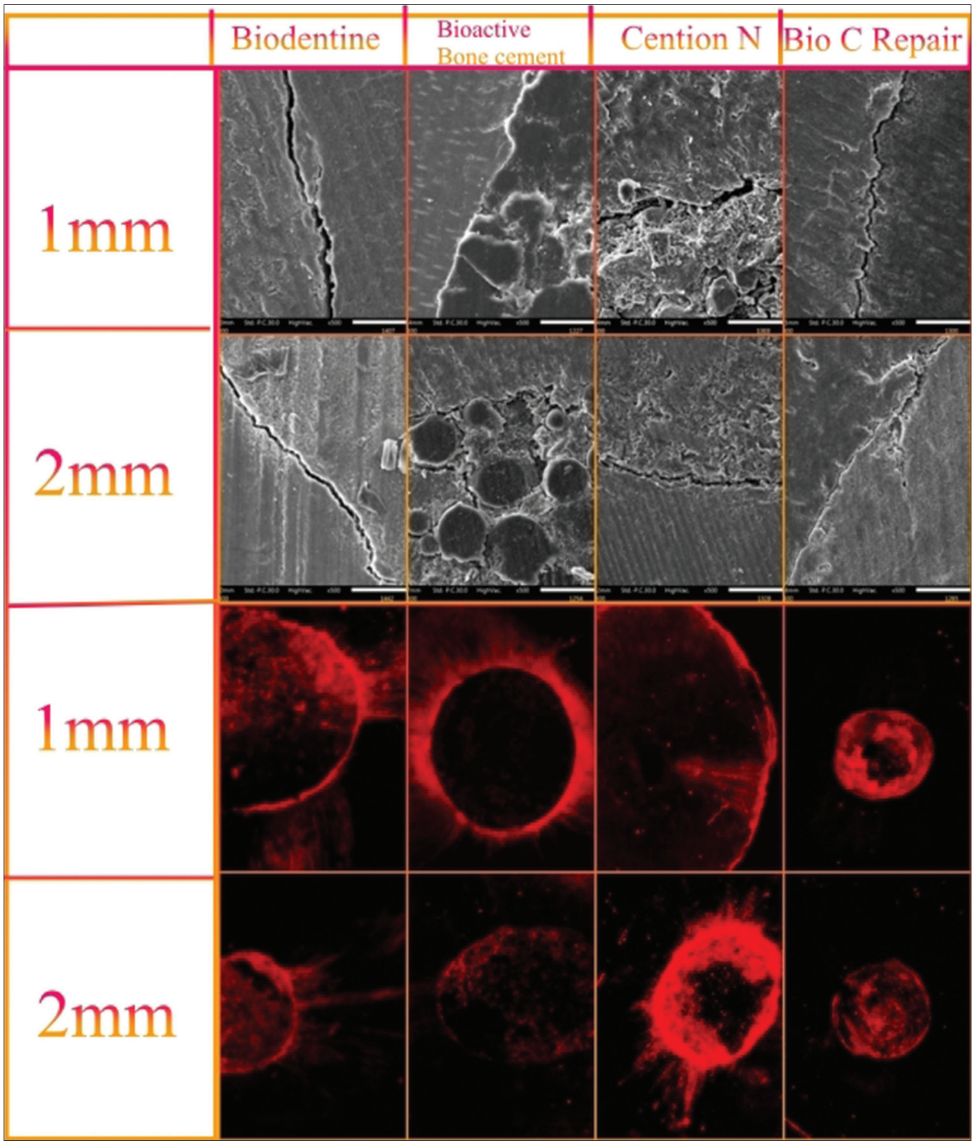

Figure 1: Scanning electron microscope and CONFOCAL images of blood-contaminated cavities with four different retrograde materials at 1- and 2-mm levels

Table 2: Comparison between the four subgroups in the artificial saliva group

Parameter (mm)	Group	*n*	Mean	SD	SE	95% CI for mean		*F*	*P*
						Lower bound	Upper bound		
Confocal 1	Biodentine	10	431.62[a]	62.25600	19.68708	387.0847	476.1553	27.86	<0.001*
	Bioactive bone cement	10	317.70[a,b]	76.00099	24.03362	263.3382	372.0738		
	Cention N	10	521.17[b]	86.19952	27.25868	459.5126	582.8394		
	Bio-C Repair	10	180.92[a,b]	118.26373	37.39827	96.3202	265.5218		
Confocal 2	Biodentine	10	401.62[a]	42.55753	13.45787	371.1762	432.0638	38.7	<0.001*
	Bioactive bone cement	10	277.70[a,b]	82.42044	26.06363	218.7460	336.6660		
	Cention N	10	481.17[b]	53.33751	16.86680	443.0206	519.3314		
	Bio-C Repair	10	160.92[a,b]	94.75887	29.96538	93.1346	228.7074		
SEM 1	Biodentine	10	3.800[a]	1.229273	0.388730	2.92063	4.67937	20.06	<0.001*
	Bioactive bone cement	10	2.700[b]	0.674949	0.213437	2.21717	3.18283		
	Cention N	10	4.600[b]	1.074968	0.339935	3.83101	5.36899		
	Bio-C Repair	10	1.50[a,b]	0.707107	0.223607	0.99417	2.00583		
SEM 2	Biodentine	10	3.20[a]	0.918937	0.290593	2.54263	3.85737	30.01	<0.001*
	Bioactive bone cement	10	2.40[b]	0.516398	0.163299	2.03059	2.76941		
	Cention N	10	4.40[a,b]	0.966092	0.305505	3.70890	5.09110		
	Bio-C Repair	10	1.30[a,b]	0.483046	0.152753	0.95445	1.64555		

*Statistical significance. $P \leq 0.05$ was considered statistically significant, groups with similar superscripts in the mean value have significant differences in Tukey's *post hoc* tests for multiple pairwise comparisons. SD: Standard deviation, SE: Standard error, CI: Confidence interval, SEM: Scanning electron microscope

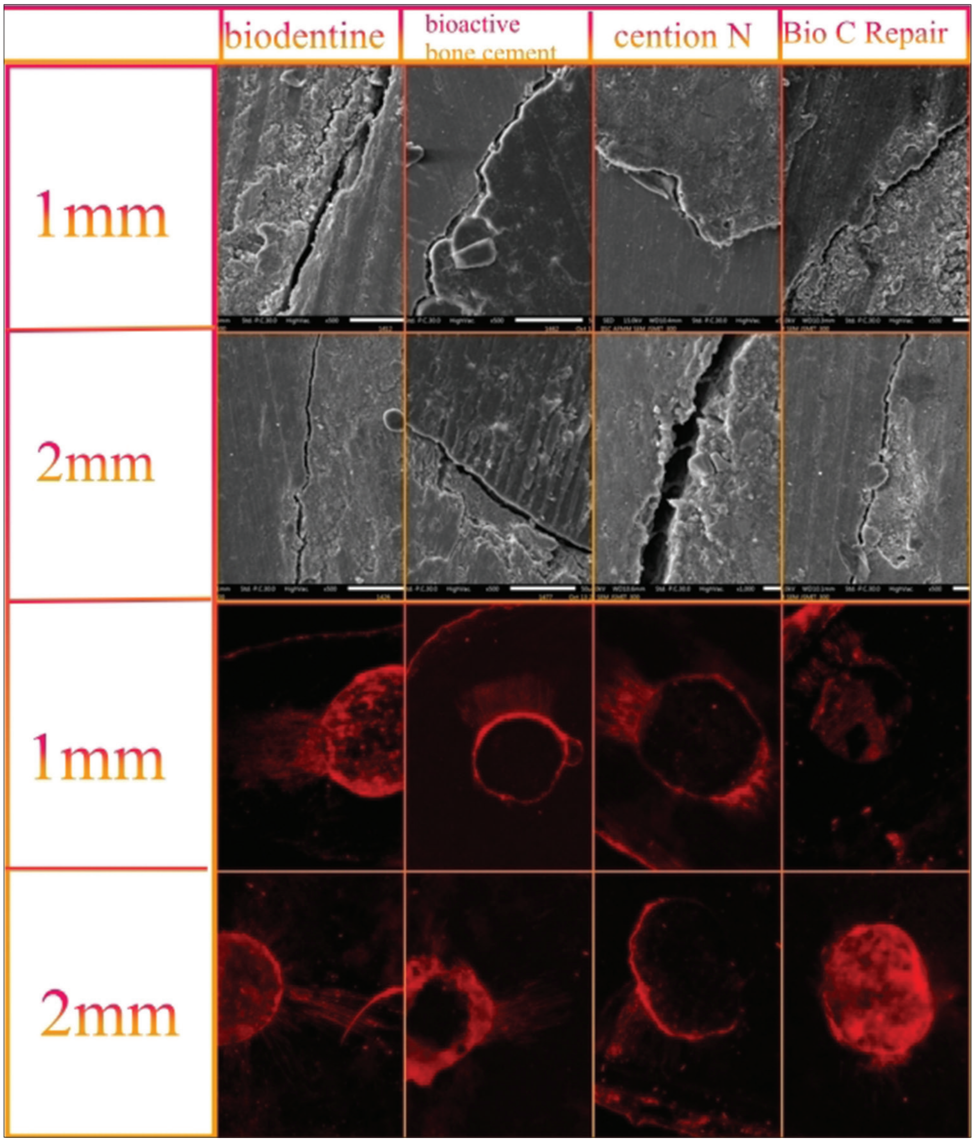

Figure 2: Scanning electron microscope and CONFOCAL images of artificial saliva-contaminated cavities with four different retrograde materials at 1- and 2-mm levels

DISCUSSION

Simply cutting off the root apex and filling the canals does not achieve a goal because the real objective of endodontic surgery is to create an impervious seal for the root canal system.

For a successful outcome, the proper management of the resected root end during periradicular surgery plays a critical role. Marginal adaptation is the degree of approximation of filling material to the dentine surface. Sealing ability and the quality of the seal obtained by the filling materials is of paramount importance for a successful long-term treatment outcome.

In the present study, Group-IV BIO C REPAIR showed the least microleakage and mean marginal gap values under tested conditions. The probable reason for least microleakage and marginal gap values can be attributed to the low solubility rate (below 3#) and low volumetric loss (below 1%) of bio c repair.[12] The low solubility and volumetric loss in addition to the dimensional expansion demonstrated by Bio-C Repair may be related to its hydration, water sorption, and particle size.[13] The second lowest microleakage values and mean marginal gap values are shown by bioactive bone cement as its characteristics are unaffected by moisture or blood contamination.[11] The results of this study are in agreement with a study conducted by Mir *et al.* (2018),[14] where the sealing ability of bioactive bone cement was found to be superior to MTA, and also in agreement with a study conducted by Saji *et al*.,[15] where the least microleakage was demonstrated by PMMA bone cement when compared with MTA and Biodentine.

Biodentine showed higher microleakage values and mean marginal gap values. This could be due to its solubility, which is higher than 3% and which may be related to the presence of a water-soluble polymer in its composition.[16] Because of its high solubility, Biodentine has caused greater volumetric loss leading to marginal gaps and microleakage. These results were in agreement with the studies conducted by Thanavibul *et al*.,[17] Nekoofar *et al*.,[18] and Shalabi *et al*.[19]

Cention N, an alkasite restorative material, is similar to ormocer or compomer materials, which are regarded as a subgroup of composite resin. Cention N contains alkaline glass fillers which release hydroxide, calcium, and fluoride ions which neutralizes acidic conditions. Cention N is a dual-curing material with a short setting time. Its biocompatibility is at par with MTA. Due to the advantages of the material, we used Cention as a retrograde filling material.[6,20]

The results of the present study showed that Cention N showed a higher marginal gap and dye leakage among all tested materials in both blood and artificial saliva contamination. This may be due to the fact that contamination of the cavity surfaces with moisture can block the required contact of the adhesive and adherent. Absorption of salivary constituents by the cavity walls results in a decrease of the surface energy and makes the surface unfavorable for adhesion. In addition, water content may result in incomplete polymerization of the adhesive monomers. Hatirli and Boyraz[21] stated that saliva contamination significantly increases microleakage in the Cention N group.

The transverse section used in our study was chosen over the longitudinal section because it allows the visualization of the restoration-dentin interface around the circumference.[22]

3-mm root-end tip resection, perpendicular (90°) to the long axis of the tooth, was performed in our study to eliminate apical ramifications and lateral canals, thus reducing the number of open dentinal tubules and leakage at the resected root,[23] and the retrograde cavities were prepared using diamond-coated ultrasonic retrotips – ED 11 retrotip (woodpecker) as they were found to be advantageous than burs.[24]

In the present study, both parameters, microleakage and marginal adaptation, were assessed using the same sample. First, dye penetration was assessed under a confocal laser scanning microscope followed by gold sputtering of the sample and placement under SEM to analyze the marginal adaption. Both microleakage and marginal adaptation were evaluated only at 1-mm and 2-mm levels; the 3-mm level section was not considered, as it would encroach upon the junction between the retrograde filling material and the gutta-percha.

The findings of this study show that the Bio-C Repair group had a better sealing ability and marginal adaptation, followed by bioactive bone cement under both blood and artificial saliva contamination. Biodentine showed higher mean marginal values and dye leakage than anticipated in comparison with other studies. This could be because of the difference in the methodology used. Cention N showed the highest mean marginal values and dye leakage. As there are no studies in literature considering the marginal adaptation and sealing ability in the presence of blood and artificial saliva of Cention N, no direct comparisons can be made. Therefore, additional studies are required in different clinical conditions with a larger sample size.

CONCLUSION

Within the limitations of this study, the following conclusions can be drawn.

1. All the tested materials in this study showed some degree of marginal gap and dye leakage in both blood and artificial saliva-contaminated samples
2. The lowest mean marginal gap value and dye leakage were shown by the Bio-C Repair group followed by bioactive bone cement.

Furthermore, long-term clinical studies are required to confirm and evaluate these findings as many factors in the oral environment may affect the properties of the materials used.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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