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BIOCOMPATIBILITY AND MARGINAL ADAPTABILITY OF BIOCERAMIC PUTTY MATERIAL AS ROOT END FILLING MATERIAL (IN VITRO STUDY)

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ABSTRACT

Purpose: This investigation aimed to assess the Biocompatibility and Marginal Adaptability of Bioceramic Putty material as root end filling material compared with Mineral Trioxide Aggregate (MTA).

Methodology: For the marginal adaptability test, Thirty-six human extracted single rooted teeth were used. Samples were randomly distributed into 3 groups (n=12) based on the root end filling materials (Cerkamed Bio-MTA, Well-Root Putty, and Gutta percha). The tested materials were mixed and applied in accordance with the manufacturer's instructions. After complete setting, they were immersed into 1% Rhodamine B fluorescent dye for 24h then marginal adaptability was evaluated using confocal laser microscope measuring dye penetration in microns. For biocompatibility test, thirty-six discs with 2 mm thickness and 2 mm diameter were used. The discs were randomly distributed into 3 groups (n=12) consistent with the tested materials. Biocompatibility of the tested samples was measured against hFB (human fibroblast cells) for 24h and 48h using the MTT Cell Viability Assay.

Results: For the marginal adaptability test, there were no statistically significant distinctions among Cerkamed Bio-MTA and Well-Root Putty. For biocompatibility test, Well-Root Putty samples showed significant cytotoxicity on both time intervals.

Conclusion: Neither Cerkamed Bio-MTA and Well-Root putty showed 100% marginal adaptability but they were close to each other. Cerkamed Bio-MTA and Gutta percha did not affect cell viability whereas; Well-Root Putty showed cytotoxic effect on fibroblastic cells.

KEYWORDS: Cerkamed Bio-MTA, Laser confocal Microscope, MTT Assay, Well-Root Putty.

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INTRODUCTION

Conventional root canal therapy is capable of successfully repairing periapical tissue in the vast majority of cases. In cases with intricate anatomy, infection might continue with a multitude of bacteria even after a root canal obturation is sufficient. Bacteria in peri-radicular tissues cause periapical lesions because they evade the host's natural defenses and the chemical treatments used to treat root canal inflammation. Subsequent treatments, involving periapical surgery, may be necessary; this procedure often entails inserting a root end filling material to isolate the pulp chamber from the surrounding tissues and fix any damage.

The ideal root end filling material should possess certain properties involving biocompatibility, high marginal adaptability, ability to permit or induce alveolar bone repair and cementogenesis, antimicrobial activity, ease of manipulation, resistance to dissolution, corrosion resistance, and not staining the tooth or peri-radicular tissue.

Mineral Trioxide Aggregate (MTA), which comes with dicalcium and tricalcium silicate, tricalcium aluminate, calcium sulphate dehydrate, and bismuth oxide, is currently the root-end filling cement that is utilized most often. MTA forms a colloidal gel with PH (12.5) through a hydration reaction when mixed with water then creates a hard structure after several hours. Unfortunately, MTA is not easy to manipulate, has long setting time, and has high initial sensitivity to moisture as it may be washed out easily if the unset material is exposed to immediate irrigation.

Nowadays many different materials are in action as retrograde filling materials with more ease of manipulation that enable them to compete with MTA and Biodentine. These materials include Bioceramic putty that comes in the form of paste consistency facilitating its manipulation and application in contrast to MTA.

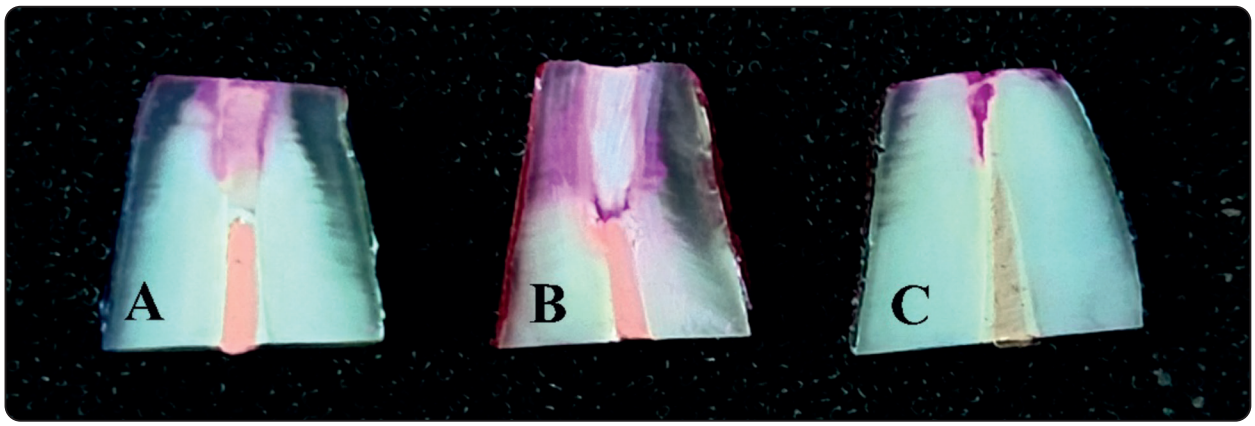
Therefore, conducting a study to evaluate the biocompatibility and marginal adaptability of Well-Root bioceramic putty as root end filling material contrasted with Cerkamed Bio-MTA was thought to be beneficial.

MATERIALS AND METHODS

Marginal Adaptability Test

Sample preparation and grouping:

Teeth were sectioned longitudinally in bucco-lingual direction parallel to the long axis of the teeth into 3 thirds using a diamond disk (0.2mm thickness) then the middle sections only were used for testing. The sections were tested using Leica DMi8 Confocal laser Microscope using Spiral cuts. The maximum amount of dye penetration was measured from the start of the retro-cavity to the highest amount of dye penetration in microns using Leica analysis software **as shown in figures (1, 2).**



Fig, (1) Rhodamine B dye Penetration through tested Materials (A) Cerkamed Bio-MTA (B) Well-Root Putty (C) Gutta Percha

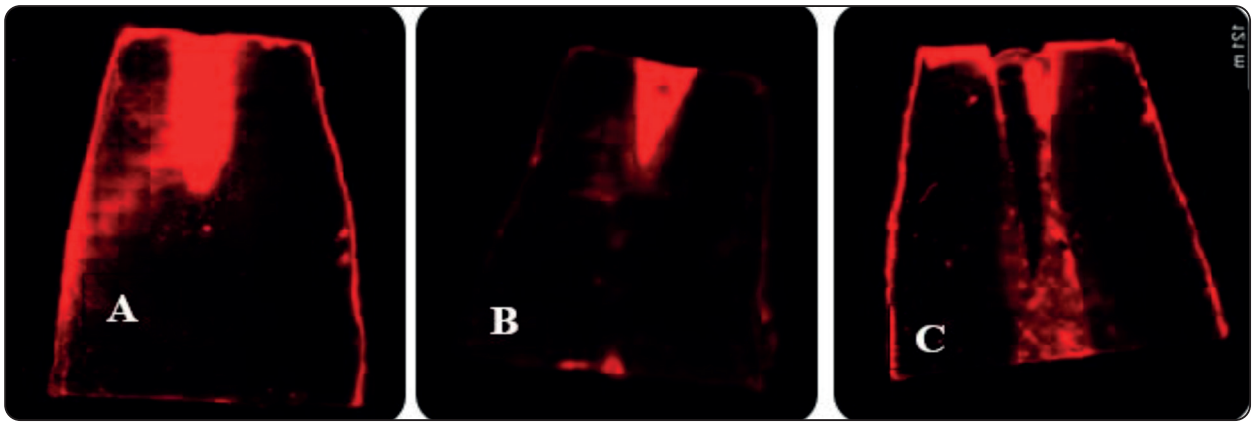


Fig. (2) Confocal Laser Microscope Image comparing dye penetration between (A) Cerkamed Bio-MTA (B) Well-Root Putty (C) Gutta percha

Biocompatibility Test

Cell Culture

The Brazilian cell bank (BCRJ, Rio de Janeiro, Brazil) provided the human primary fibroblast cells (hFB cell line). A medium called Dulbecco's Modified Eagle Medium (DMEM) was utilized for cell culture. As an additional supplement, the medium included 2 milliliters of L-glutamine, 10% fetal bovine serum (FBS), 100 units/ml of penicillin G sodium, 100 units/ml of streptomycin sulphate, and 250 mg/ml of amphotericin B. Humidified air containing 5% CO_2 was utilized to maintain cells at sub-confluency at 37 degrees Celsius.

Cytotoxicity activity:

The MTT (3-[4, 5-dimethylthiazole-2-yl]-2, 5-diphenyltetrazolium bromide) Cell Viability Assay was employed to assess the cytotoxicity of the tested samples against hFB cells for 24h and 48h. The MTT assay is primarily based on the active mitochondrial dehydrogenase enzyme of living cells, which cleaves the tetrazolium rings of the yellow MTT and forms dark blue insoluble formazan crystals that are largely impermeable to cell membranes. This process results in the accumulation of the formazan crystals within healthy cells. The crystals are liberated as a result of the solubilization of the cells, which are subsequently solubilized. The quantity of viable cells is directly proportional to the concentration of soluble formazan dark blue color. The absorbance at 570 nm was utilized to quantify the degree of MTT reduction.

Reagents preparation:

MTT solution was prepared by adding 5mg/ml of MTT in 0.9 percent NaCl.

Procedure:

A 96-well microplate with a flat bottom was used to plate cells (1x104 cells/well), which were then left to settle for 24h. Utilizing sterile Teflon molds, the samples were made as per the manufacturer's instructions, with discs measuring two millimeters in diameter and two millimeters in length. The Cerkamed BioMTA sample was weighed, mixed, and adjusted at 100 μg/mL final concentration. The Well-Root bioceramic putty was left to set in humid condition then cut into six slices (1mg ± 10%). The Gutta percha was cut into six slices (1mg ± 10%). Each slice was applied to one well.

All samples were applied for 24h and 48h at 37° C, in a humidified 5% CO_2 atmosphere based on previous study.[2] The medium containing the samples was removed after incubation, and 20 L of MTT solution was added per well before

being incubated for a further four hours. After dissolving the MTT crystals in 180 μL of acidified isopropanol per well, the plate was left to thaw at room temperature. The absorbance at 570 nm was then measured using a microplate ELISA reader (FLUOstar OPTIMA, BMG LABTECH, Ortenberg, Germany). The cells that were treated with cytotoxic agents had a relative viability of below 100 percent, and the data were presented as a percentage in contrast to the untreated cells.

Calculation:

The percentage of relative viability was calculated with the following equation:

$$\frac{\text{Optical Density of treated cells}}{\text{Optical Density of controlled untreated cells}} \times 100$$

Statistical Analysis

The categorical data was evaluated utilizing Fisher's exact test and provided as frequency and percentage values. We displayed numerical data as means and standard deviations (SD). By examining the data distribution and utilizing the Kolmogorov-Smirnov and Shapiro-Wilk tests, we checked them for normalcy. Intergroup comparisons and repeated measures were conducted using one-way ANOVA followed by Tukey's post hoc test, as the data exhibited parametric distribution. For comparisons within groups, we employed analysis of variance (ANOVA) with a Bonferroni post hoc test. At $p < 0.05$, the level of significance was established. R, a statistical analysis program for Windows, version 4.1.2, was utilized for the statistical analysis.[3]

RESULTS

Marginal Adaptability test

There were significant variances among Gutta Percha vs. both Cerkamed Bio-MTA and Well-Root Putty. Whereas; there were no statistically significant differences between Cerkamed Bio-MTA vs. Well-Root Putty. The mean of Dye penetration for Gutta percha was the highest mean followed by Cerkamed Bio-MTA & Well-Root Putty materials. The mean differences of dye penetration for Gutta percha was higher than those for Cerkamed Bio-MTA & Well-Root Putty by 3707.3 & 4003.2; respectively & vice versa **as shown in table (1)**.

Biocompatibility test

Using MTT assay, the samples showed different cytotoxic and cell proliferation effects **as shown in figure (3).** Cerkamed BioMTA samples and Gutta Percha samples showed compatible behavior with the cells with non-significant cytotoxicity on both time intervals. On the other hand, Well-Root Putty samples showed significant cytotoxicity on both time intervals. On comparing, the biocompatibility

TABLE (1) Comparison between Materials regarding Dye penetrations in Microns

Dye penetration in microns	N	Mean	SD	Median	Range		F	P Value	Sig.
					Min.	Max.			
MTA	12	3832.1	1090.5	3544.4	3028.2	6969.9			
Putty	12	3536.1	576.0	3355.2	2701.4	4511.7	53.3	<0.001	HS
Gutta Percha	12	7539.3	1357.2	8021.4	3455.1	8324.2			

One-Way ANOVA Test, ***Different Letters = S, Same letters = NS. P>0.05= NS, P<0.05=S, P<0.01=HS***

of each sample on both time points there was no significant variance in cell viability ($p > 0.05$). On comparing the different samples after 24h and 48h, Well-Root Putty samples showed significant lower viability compared to both Cerkamed Bio-MTA and Gutta percha ($p < 0.001$) but there was no significant distinction in cell viability among both Cerkamed BioMTA samples and Gutta Percha samples on both time intervals ($p > 0.05$).

The mean of MTT for Gutta Percha at 24h was the highest mean followed by Cerkamed Bio-MTA and Well-Root Putty whereas; the mean of MTT for Cerkamed Bio-MTA at 48h was the highest mean followed by Gutta Percha & Putty **as shown in table (2).**

The mean difference for Well-Root Putty at 24h; was lower than those for Gutta Percha & Cerkamed Bio-MTA by 73.7 & 57.7; respectively and vice versa. While; the mean difference for Well-Root Putty at 48h; was lower than those for Cerkamed Bio-MTA & Gutta Percha by 61.0 & 54.8; respectively and vice versa **as shown in table (3).**

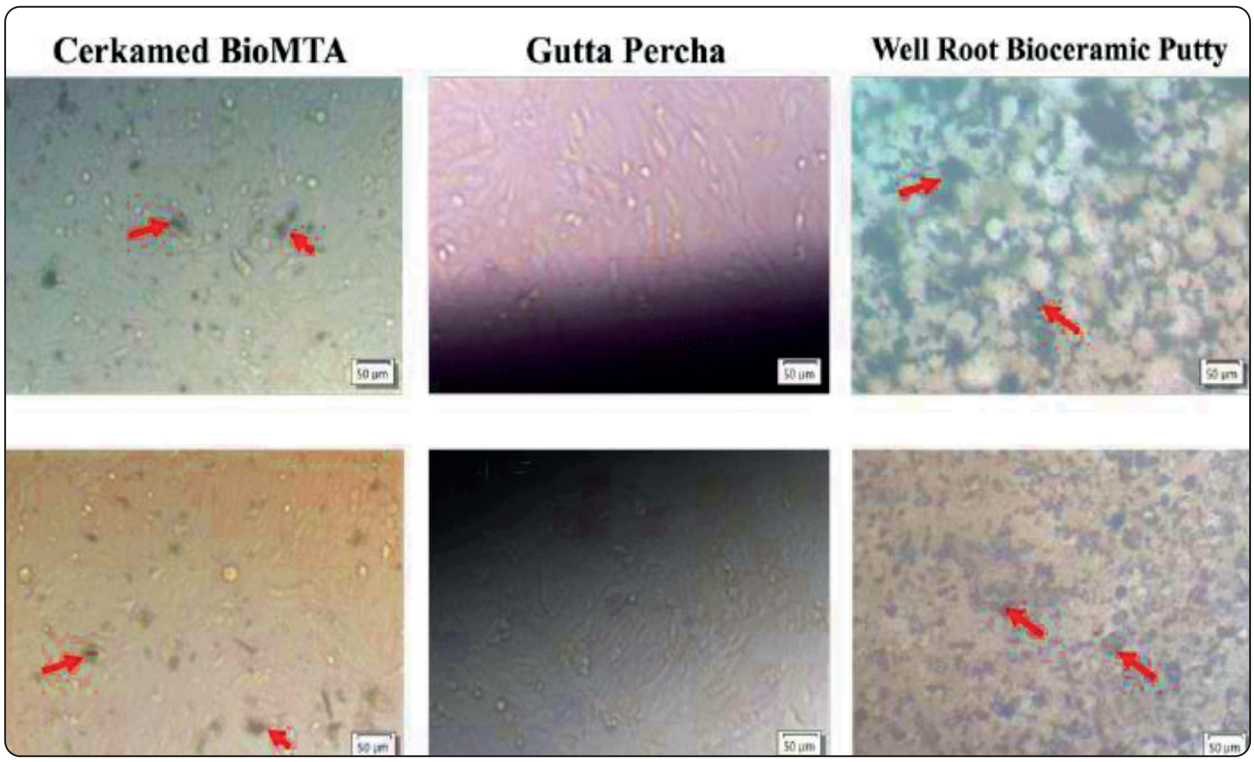


Fig. (3) Dark field photos of cells treated with Cerkamed BioMTA, Gutta Percha, and Well Root Bioceramic Putty samples at both 24 and 48 hrs. The photos show normal cell distribution and normal morphology in both Cerkamed BioMTA and Gutta Percha samples at both time points. Well Root Bioceramic Putty show altered cell morphology and distribution of sample all over the cells. The red arrows are pointing to the samples' distribution around cells. The magnification is 10X and the scale bar is 50 μm.

TABLE (2) Comparison between Materials Using MTT Assay.

MTT	Material	Mean	SD	Median	Range		F	P Value	Sig.
					Min.	**Max.**			
24 hrs.	**MTA**	96.5	12.3	96.5	84.2	108.9	38.4	<0.001	HS
	Putty	38.9	4.8	41.1	33.4	42.2			
	Gutta Percha	112.5	13.3	108.1	102.0	127.5			
48 hrs.	**MTA**	98.5	19.1	103.8	77.3	114.4	21.3	0.002	HS
	Putty	37.5	7.7	34.2	32.0	46.3			
	Gutta Percha	92.3	7.3	89.4	86.8	100.5			

One-Way ANOVA Test*, Different Letters = S, Same letters = NS. P>0.05= NS, P<0.05=S, P<0.01=HS*

TABLE (3) Comparison between Time Intervals of Materials regards MTT Assay readings.

MTT	**Time**	**Mean**	**SD**	**Median**	**Range**		**t**	**P Value**	**Sig.**
					Min.	**Max.**			
MTA	**24 hrs.**	96.5	12.3	96.5	84.2	108.9	0.12	0.917	NS
	48 hrs.	98.5	19.1	103.8	77.3	114.4			
Putty	**24 hrs.**	38.9	4.8	41.1	33.4	42.2	0.19	0.864	NS
	48 hrs.	37.5	7.7	34.2	32.0	46.3			
Gutta Percha	**24 hrs.**	112.5	13.3	108.1	102.0	127.5	2.23	0.157	NS
	48 hrs.	92.3	7.3	89.4	86.8	100.5			

One-Way ANOVA Test*, Different Letters = S, Same letters = NS. P>0.05= NS, P<0.05=S, P<0.01=HS*

DISCUSSION

Marginal Adaptability test

The Marginal adaptability of retrograde filling materials is the corner stone and the key indicator that determines the success of repairing process by preventing infiltration of microorganisms in the site of the retrograde cavity.

In our study, we used Cerkamed Bio-MTA (Cerkamed, Stalowa Wola, Poland) as it exhibits a reduced setting time and has the same desirable properties compared with traditional MTA. Cerkamed Bio-MTA, in accordance with the manufacturer, has working time about 4 minutes when mixed with its liquid, and sets completely in about 3hrs. It also contains high content of calcium

ions that remineralizes the tooth tissue besides high amount of silicon and calcium compounds that support tissue regeneration after wall perforation and intra-canal resorption. It contains hydroxyapatite, a natural component of bones, which integrates the compound perfectly into the bone structure.

In our study, we aimed to compare Well-Root putty (VERICOM Co., LTD, Korea) with Cerkamed Bio-MTA, as it is a new bioceramic material in the putty form that was introduced to the market without enough information about its marginal adaptability, Biocompatibility and other physical properties.

In our study, we used single rooted teeth with single canals to facilitate the application of the tested materials with no limitations of accessory penetration of the dye through ismuths.

In this study, we depended on dye penetration test because of its ease of performance as in contrast to the other available techniques. It was conducted using confocal laser microscope with 1% Rhodamine b fluorescent dye. However, this technique is believed to have certain drawbacks, such as the smaller molecular size of the dye molecules in comparison to microorganisms, which results in a measurement of the deepest point reached by the dye rather than the absorbed volume of the sample. A material that can prevent the penetration of small molecules (dye) should be able to prevent the penetration of larger substances, such as microorganisms and their byproducts, consistent with **Torabinejad et al**.[1]

In literature, most researchers sectioned the teeth buccolingually into 2 halves, but we sectioned teeth buccolingually into three equal thirds with average thickness (1mm) for each then examined the middle third because of the inability of confocal laser microscope to get accurate readings in thick sections. Confocal laser microscope was adjusted to give readings using tile spiral 2D scanning instead of Z-stack 3D scanning to exclude any penetration of the dye except from the beginning of the retrograde cavity where the dye was applied, and to give more area of surface scanning for the entire slice.

In our study, the results for Marginal adaptability showed that there were no significant variances between Cerkamed Bio-MTA and Well-Root Putty. Whereas; there was significant variances comparing both materials vs. Gutta Percha.

These results of the Marginal adaptability of MTA and Bioceramic Putty are in agreement with many previous studies in the literature.[1, 4-11] while some studies[12] reported that MTA had better sealing ability than bioceramic putty due to its better marginal adaptation.

Biocompatibility test

Biocompatibility is a critical property because the biological effects of the set cements on the surrounding tissues are determined by the discharge of leached components. The gold standard for comparing novel root end filling materials is MTA, which is renowned for its exceptional biocompatibility. MTA is bioactive, as it possesses both inductive and hard tissue conductive properties. Nevertheless, certain clinicians subjectively report experiencing challenges in manipulating MTA as a result of its granular consistency and extended setting time. Consequently, numerous new bioceramic materials with comparable biological components have been created to address the limitations of MTA.

In our study, we used MTT assay to determine the biocompatibility of both Cerkamed Bio-MTA and Well-Root Putty. A semi-quantitative cytotoxicity colorimetric assay based on MTT can measure the percentage of live cells, which makes it easier to compare the materials that were utilized. Any material is considered cytotoxic if it reduces cell viability by over thirty percent. following live cells reduce the yellow MTT salt utilizing mitochondrial activity, they generate formazan crystals that can be dissolved in an organic solvent and are either blue

or purple. These results are acquired following the reduction process.

In our study, Cerkamed Bio-MTA did not present any cytotoxic effect on fibroblastic cells; on the other hand, Well-Root putty had greater cytotoxicity compared to Cerkamed Bio-MTA and the positive control Gutta percha that was proven as inert material. The null hypothesis, which posited that there were no significant distinctions among MTA and other forms of calcium silicate-based cements, is rejected by these results.

In our study, fresh extracts of the set materials were used, however particles of cements was observed at the bottom of wells especially in Well-Root Putty which may have interfered with the obtained data. Perhaps the composition of the material or issues encountered when obtaining a full batch of the material could explain why Well-Root Putty demonstrated lower cell survival than Cerkamed Bio-MTA.

The results of our study are in agreement with many previous studies. [2, 13, 14] On the other hand, some studies [15-17] reported that there was no significant difference between MTA and Bioceramic cements.

CONCLUSION

Based on our results, and within the limitations of this in-vitro study, it can be concluded that:

- Neither of the test materials showed 100% marginal adaptability. There was no significant variation between Cerkamed Bio-MTA and Well-Root putty.
- All the test materials showed statistically significant differences according to cell viability on human fibroblast cells. Cerkamed Bio-MTA and Gutta percha did not affect cell viability whereas; Well-Root Putty showed some cytotoxic effect on fibroblastic cells.

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