The Saudi Dental Journal (2023) 35, 1014–1022

King Saud University

The Saudi Dental Journal

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Remineralization ability of different root canal sealers

Raghad A Al-Askary, Wiaam M.O. AL-Ashou *, Sawsan H. Al-Jubori

Department of Conservative Dentistry, College of Dentistry, University of Mosul, Mosul, Iraq

Received 14 June 2023; revised 7 August 2023; accepted 13 August 2023
Available online 18 August 2023

KEYWORDS

Sealer;
Biocompatibility;
Remineralization;
Bioceramic

Abstract *Aims:* This research was designed to contrast the biocompatibility and remineralization ability of different sealers (BioRoot, MTA-FillApex and GuttaFlow-Bioseal).

Method: Twenty rabbits were used in this study, they were randomly divided into 4 groups equally depending on the observation time"3,7,14, and 28 days" post-implantation. Each rabbit was generally anesthetized,"7cm"long incision was made on the skin of the right and left sides of the ventral aspect of the mandible of each rabbit, 4 bony cavities of approximately"5mm"in depth and"2mm"in diameter (2 cavities on the left side and 2 cavities on the right side of mandible of each rabbit) were made in the cortical surface of the buccal alveolar bone. The sealers mixed depend on manufactural instructions and immediately insert into the prepared cavities (in the right side the BioRoot and MTA-FillApex were placed while on the left side, GuttaFlow-Bioseal was placed in one cavity and the other cavity was left unfilled as control).The same volume of each sealer was placed in the corresponding cavity using disposable syringes. After each observation period, the animals were sacrificed and bone biopsy from the tested area was taken and examined histologically using Olympus light microscopy at"400X"magnification.

Results: The obtained data were analyzed through non-parametric statistical tests using SPSS software version"22".Kruskal-Wallis test and Mann - Whitney test were utilized at"0.05"levels of significance to evaluate the results. GuttaFlow-Bioseal displayed excellent biocompatibility in comparison to other groups indicated by low inflammatory tissue reaction at all evaluation intervals. While the BioRoot group represented better osteo-conductivity although statistically not significant than GuttaFlow-Bioseal group.

* Corresponding author at: College of Dentistry, University of Mosul, Mosul, Iraq.
E-mail addresses: raghadadnan_77@uomosul.edu.iq (R.A Al-Askary), wiaamalashou@uomosul.edu.iq (W.M.O. AL-Ashou), dr.sawsanahmed@uomosul.edu.iq (S.H. Al-Jubori).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sdentj.2023.08.004

Conclusion: BioRoot and GuttaFlow-Bioseal showed higher osteo-conductivity and biocompatibility than MTA-FillApex. However, all sealer used in this study were well tolerated by bone tissue and might accelerate bone repair.

1. Introduction

The goal of root canal (RC) obturation is to fill the root canal systems with a seal that is dimensionally stable and biocompatible (Orstavik, 2005). The use of endodontic sealers along with gutta-percha (GP) become standard in root canal obturation (Jung et al., 2019) due to the absence of adhesion between GP and the internal walls of the root canal (Camargo et al., 2017), and to fill the spaces left inside the canal system to obtain fluid-tight seal and to restrain the entry and aggregation of irritants which is the main cause of endodontic failure (Jung et al., 2019).

The sealers are prepared to dwell inside the RC. However, some extrusion through the apical narrowing and even from other communication between RC and surrounding environments including lateral canals and auxiliary foramen may occur (Troiano et al., 2018, Sfeir et al., 2021); as a consequence, tissue fluid may readily infiltrate the RC system; causing the sealer material to degrade and its′ components leak out to the surrounding tissue and hence may be penetrated to periodontal tissue and alveolar bone; causing local inflammatory response in periapical tissues and negative outcomes (Braga et al., 2015, Pawińska et al., 2015, Poggio et al., 2017). The subsequences of inflammatory reactions of the sealer or its component with the per-radicular tissue may cause phagocytosis and cause inactivation of alkaline phosphatase, which is required for bone formation. Therefore, the sealer's biological properties specifically the biocompatibility and bioactivity are critical as the sealer's physical properties (Lodiene et al., 2008, Camilleri, 2015). Therefore, to overcome the toxic effects associated with traditional sealers like zinc–oxide eugenol and resin base sealers (Troiano et al., 2018, Cosme-Silva et al., 2019, Eid et al., 2021) great efforts make by companies to produce sealing materials with bioactivity and biocompatibility in addition to good physical properties. One type of this sealer is bioceramic sealer which is the most advanced kind of sealing material (Braga et al., 2015, Raghavendra, et al., 2017, Seo et al., 2019).

Many studies revealed that most bioceramic-based sealers have high biocompatibility and osseo-conductivity even when the sealers unintentionally extruded from the apical foramen in RC obutration; this may attribute to the formation of Ca $(OH)_2$, and $Ca_3(PO_4)_2$, which consider the main inorganic ingredient of the teeth and bone (Ha et al., 2017; Jung et al., 2018, Sfeir et al., 2021).

BioRoot RCS, is a bioceramic sealer that was marketed in 2015. This sealer was modified from Biodentine and disposed of a powder-liquid system. The powder contains tricalcium silicate, povidone and zirconium oxide, while the liquid consist of water, calcium chloride, and polycarboxylate (Raghavendra, et al., 2017, Eid et al., 2021, Al-Ali et al., 2022).

MTA-Fillapex, is a sealer that combines the physiochemical features of resin based sealer as well as the biological properties of MTA calcium silicate -based sealer. It is composed of MTA, salicylate, natural and diluting resins, nanoparticulated silica, bismuth trioxide and pigments (Camilleri, 2015, Prado et al., 2018).

GuttaFlow Bioseal is a silicone -based RC sealer that contains a bioactive glass (sodium-oxide, phosphorus-oxide, calcium-oxide, and silica) as well as GP, polydimethylsiloxane, platinum and zirconium-oxide (Gandolfi et al., 2016, Reszka et al., 2019, Attash and AL-Ashou, 2022).

This research aimed to contrast biocompatibility (inflammation response) and mineralization ability of different RC sealers (BioRoot RCS, MTA Fillapex and GuttaFlow Bioseal). The null hypothesis was that biocompatibility and mineralization weren't induced by these sealers.

2. Materials and methods

The biocompatibility study complied with the "ANSI /ADA Specification No. 41:2005 and ISO 10993–6:2016 (Recommended Standard Practices for the Biological Evaluation of Dental Materials/ Tests for local effects after implantation)".

2.1. Selection of the animal model

Twenty healthy male albino rabbits, aged approximately (4–5) months and weighing (1.5 ± 0.25) kg, were used in this research. Using the rabbits in this study was agreed officially with the research ethics committee with reference No.(UoM. Dent/H.DM.21/23) in (1/3/2023), "College of Dentistry/ University of Mosul/Iraq".

The rabbits were homed in an animal house in the college of veterinary medicine, at (23 ± 2 °C) rooms with a 12 h day-night cycle; they gave a free approach to water and were fed the same diet throughout the study time thus closely preserving their habitual situation. Examinations of rabbits' health were being done by vet. physician. The rabbits were then supplied with water only 12 h before operation. They were classified randomly into 4 equal groups (five rabbits each) depending on the scarifying periods (3, 7, 14, & 28) days post- implantation (Moretton et al., 2000, Saghiri et al., 2015).

2.2. Surgical implantation procedures

Each rabbit was intramuscularly generally anesthetized using a rodent anesthesia cocktail. Waiting for 15 min until the rabbit lost consciousness. 0.25 ml of 3% lidocaine local anesthesia was infiltrated into the site of operation. The extraoral submandibular area was shaved, washed with tap water and disinfected with 10% povidone-iodine. Seven cm incision was made longitudinally on the skin of the right and left sides of the ventral aspect of the mandible of each rabbit with the aid of sterile surgical blade No. 10. The margins of the incision were

retracted and the connective tissue separated by a sterile blunt end scissor and the periosteum was incised to expose the mandibular molar area bone (Saghiri et al., 2015, Zhang et al., 2015).

Four bony cavities of approximately 5 mm in depth and 2 mm in diameter (2 cavities on the left side and 2 cavities on the right side of the mandible of each rabbit) were made in the cortical side of the buccal alveolar bone using a sterile low-speed hand piece with No. 8 carbide round bur under constant copious cooling with saline solution to avoid overheating that may cause local tissue necrosis. The cavities were then washed with sterile saline solution to remove debris and control bleeding and then dried with sterile paper points, 5 mm space left between the adjacent cavities (Tassery et al., 1997, Moretton et al., 2000).

The sealers (Table 1) were mixed following the manufactural instructions and inserted immediately to the prepared cavities (on the right side the BioRoot sealer anteriorly and MTA FillApex posteriorly was placed while in the left side GuttaFlow Bioseal was placed anteriorly in one cavity and the other posterior cavity was left unfilled as control). The same volume of every sealer was placed in the respective cavity using disposable syringes.

Next to implantation; the wounds′ margins were joined and sutured, then disinfected with oxytetracyclin aerosol spray (OTC). After the anesthesia recovery, the animals were monitored by vet. doctor every six hours to identify any local, systemic and, behavioral abnormalities such as edema, purulent exudation, suture dehiscence, and lack of appetite; and maintained on the same diet and water. The sutures were removed seven days' post-surgery (Moretton et al., 2000, Zhang et al., 2015).

2.3. Termination time and histological preparation

After each observation period (3, 7, 14 & 28) days next to implantation, five animals were euthanized via an anesthetic using 3 ml of "5% Ketamine hydrochloride" and "2 ml of 2% Xylazin" intramuscularly in the thigh. Then the skin at the ventral surface of the mandible of each rabbit was disinfected, incision, and undermined and the bony tissue was isolated from the mandible bone. The bone biopsy that consisted of the implanted cavity with 2 mm safety margin was immersed immediately in 10% formalin at pH 7.0 for 24 h at room temperature. Then the bone biopsy was removed from the formalin, washed with running water, underwent demineralization in 10% formic acid, and then dehydration and clearing with xylene, then embedded in coded paraffin wax according to the tested material and the scarifying time. Subsequently, serial sections in the buccolingual direction measuring 4 μm in thickness were cut in block samples by a rotary microtome (Saghiri et al., 2015, Zhang et al., 2015, Quintana et al., 2019). The sectioned tissue slices were then mounted on a labeled glass slides and stained with hematoxylin and eosin, after that the slides were examined by two oral histo-pathologist blinded to the sealers used and scarifying periods using "Olympus" light microscopy.

Table 1 Composition and manufacturer of the tested sealers.

Sealers	Compositions	Manufacturers
BioRoot™ RCS	Powder: tricalcium silicate, zirconium- oxide, povidone. Liquid: aqueous solution of calcium-chloride and polycarboxylate.	Septodont, Saint-Maur-des Fosses, France
MTA Fillapex	Salicylate resin, natural resin, diluting resin; nano particulated silica; MTA; bismuth-oxide; pigments.	Angelus (Londrina, PR, Brazil)
GuttaFlow-Bioseal	Gutta -percha, bioactive glass, zinc- oxide; polydimethyl siloxane; zirconia, barium sulfate; color pigments; platinum catalysis; micro silver.	(Coltene Whaledent, GmBH Co. KG, Langenau, switzerland)

2.4. Histopathological assessment of intraosseous specimens

Intraosseous inflammatory tissue reactions to the implanted materials ware evaluated at 40X magnification and scored according to the FDI criteria (Stanford, 1980).

Grade 0: "No inflammatory cells or existing of less than 5 cells".

Grade 1: "Mild inflammation 5 – 25 inflammatory cells".

Grade 2: "Moderate inflammation 25 – 125 inflammatory cells".

Grade 3: "Severe inflammation of > 125 inflammatory cells".

New bone formation at the implanted cavities was evaluated at 100X magnification (Moretton et al., 2000; Saghiri et al., 2015) as follows:

Grade 0: "Absence of new bone formation".

Grade I: "Slight, existing bony islets and coverage of less than 25% of the material surface with a bone".

Grade II: "Moderate, coverage at least 50% of the material surface with bone".

Grade III: "Extensive; complete coverage of the material surface with bone or the formation of an osseous bridge around the material".

The data obtained were analyzed through non-parametric statistical tests using SPSS software version 22. Kruskal-Wallis and Mann -Whitney tests were performed at 5% levels of significance to evaluate the results.

3. Results

3.1. Histopathological analysis

Representative histopathological images of testing sealers at each scarifying periods (3,7,14, and 28) days are explained in Fig. 1 (a-d).

3.1.1. Three days scarifying time

The control group/ empty bony cavity, shows mild to moderate acute inflammatory cell infiltration. The cavity is filled by granulation tissue with a large number of fibroblasts. No bone formation and no blood vessel formation.

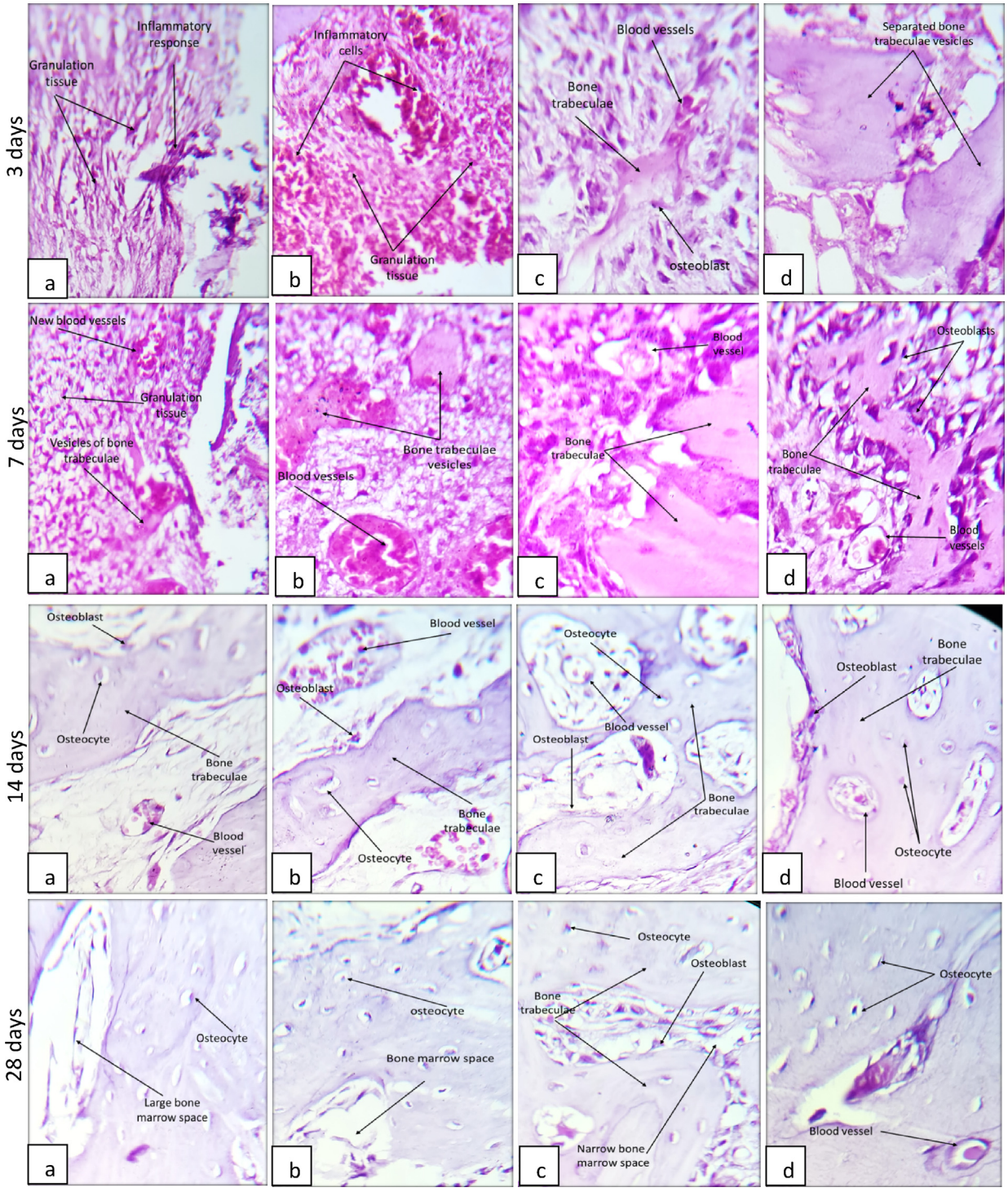

Fig. 1 Histo-microscopical image illustrated the bone tissues inflammatory reaction and bone remineralization in control group /empty cavity (a), MTA Fillapex sealer group (b), GuttaFlow bioseal sealer group (c), and BioRoot sealer group (d) at 3, 7, 14 & 28 days scarifying periods at "40x" magnification.

For MTA Fillapex sealer; moderate to severe acute inflammatory cell infiltration. Full filled of granulation tissue formation. No bone formation and no blood vessels formation.

For GuttaFlow bioseal sealer; mild inflammatory cell infiltration. Start of fine bone trabecula formation with an accumulation of osteoblast around it.

For BioRoot sealer; mild to moderate inflammatory cell infiltration. The bone formation is more prominent than in GuttaFlow bioseal with few new blood vessels formation.

3.1.2. Seven days scarifying time

The control group show mild inflammatory cell infiltration with very small vesicles of bone formation with small new blood vessels and filled the space with granulation tissues.

For MTA Fillapex sealer; mild to moderate acute inflammatory cell infiltration was evident with small vesicles of bone formation with new blood vessels and larg number of fibroblast and granulation tissues formation.

For GuttaFlow bioseal sealer; there is no sign of inflammation. Good bone formation with very few blood vessel formation.

For BioRoot sealer; absent of mild inflammatory cell infiltration was evident. Good continuous bone trabecular formation with a large number of osteoblast. There is good angiogenesis that lead to new blood vessels formation, and there is lesser amount of granulation tissues in the bony space.

3.1.3. Fourteen days scarifying time

The control group showed an absent of inflammatory cell infiltration with thin continuous bone trabecular formation with wide bone marrow and few number of blood vessels. There is a large number of osteoblast with the start of the appearance of osteocyte.

For MTA Fillapex sealer, same as for the control group but absent to mild inflammation with smaller bone marrow and good angiogenesis.

For GuttaFlow bioseal sealer; there is no sign of small bone marrow space. Large numbers of osteocytes in lacunae with large number of osteoblast on the surface of bone and good blood vessel formation.

For BioRoot sealer; is the same as for GuttaFlow bioseal sealer group but with more obvious bone formation and narrower bone marrow and a larger number of osteocyte and blood vessels formation.

3.1.4. Twenty-eight days scarifying time

The inflammatory response subsided with time and no inflammatory reaction was observed in all groups. All groups give the same histological picture by complete and normal bone healing and the further healing was just remolding.

3.2. Statistical analysis

Mean ± Standard Deviation of inflammatory tissues reactions and bone remineralization around the experimental sealers at various scarifying periods were explained in Table 2.

The experimental groups revealed various inflammatory bony tissues response. However; all experimental groups illustrated declined in the inflammatory bony tissue response with time proceeding next to implantation till no inflammatory reactions in the bony tissues at 28 days.

Kruskal-Wallis and Mann-Whitney tests were performed at 0.05 levels of significance to evaluate and compare the influence of each sealer on the intensity of inflammatory tissues reaction and bone remineralization at the scarifying period after implantation Table 3 and Table 4.

3.2.1. Three days scarifying periods

The results show no significant differences between control and BioRoot groups $p > 0.05$ in the inflammatory response. However, the MTA Fillapex showed statistically significantly ($p \leq 0.05$) higher inflammatory response while the GuttaFlow bioseal showed statistically significantly ($p \leq 0.05$) less inflammatory response.

For bone formation; the GuttaFlow bioseal showed a higher bone remineralization ability but with no statistically significant differences from BioRoot group ($p > 0.05$) but statistically significantly ($p \leq 0.05$) higher than both of the control and MTA Fillapex groups that also show no significant differences between them ($p > 0.05$).

3.2.2. Seven days scarifying period

There are statistically significant differences among the groups on the inflammatory reaction at 7 days after implantation. However, MTA Fillapex showed statistically significantly ($p \leq 0.05$) higher inflammatory response followed by the control group, then the BioRoot groups while the GuttaFlow bioseal showed a statistically significant ($p \leq 0.05$) less inflammatory response.

For bone formation; the GuttaFlow bioseal and BioRoot groups showed no statistically significant differences between them ($p > 0.05$). However, they showed statistically significantly ($p \leq 0.05$) higher bone remineralization ability than both of the control and MTA Fillapex groups which also show no statistically significant differences between them ($p > 0.05$).

3.2.3. Fourteen days scarifying period

The results show no statistically significant differences among the control, BioRoot, and MTA Fillapex groups ($p > 0.05$) in the inflammatory response. However, the GuttaFlow bioseal showed statistically significantly ($p \leq 0.05$) less inflammatory response among all groups. For bone formation; same as for seven days observation period.

3.2.4. Twenty-eight days scarifying period

Since all implanted groups represented the same tissues reactions score (0) at 28 days after implantation, therefore no statistical comparison was done.

For bone formation; although there were no statistically significant differences among the groups ($p > 0.05$). The GuttaFlow bioseal showed the faster bone remineralizing ability then followed by BioRoot, MTA-Fillapex, and the control group respectively.

4. Discussion

The obturating materials may impact preapical-tissues via direct contact or via filtrated substances which are passed to the surrounding tissue by the dentinal tubules, apical foramin and lateral or accessory canals. For that reason, obturating materials should have biocompatibility and own the capacity to permit or stimulate bone repair(Lodiene et al., 2008, Al-Ali et al., 2022).

Bioactive and biocompatible sealers can enhance inflamed tissue to recognize and healing of the wound in apical-periodontitis (Cintra et al., 2017, Seo et al., 2019). The purpose of this research was to estimate the biocompatibility and bone

Table 2 Mean and standard deviation of the inflammatory tissues reaction and bone remineralization for testing groups at different observation periods.

Inflammatory Tissues Reaction				
Observation period	Control group	MTA Fillapex	GuttaFlow bioseal	BioRoot sealer
3 day	1.30 ± 0.48	2.40 ± 0.51	1.00 ± 0.00	1.30 ± 0.48
7 day	1.00 ± 0.00	1.50 ± 0.52	0.00 ± 0.00	0.60 ± 0.51
14 day	0.00 ± 0.00	0.30 ± 0.48	0.00 ± 0.00	0.00 ± 0.00
28 day	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Bone Remineralization				
Observation period	Control group	MTA Fillapex	GuttaFlow bioseal	BioRoot sealer
3 day	0.00 ± 0.00	0.00 ± 0.00	0.30 ± 0.48	0.40 ± 0.51
7 day	0.30 ± 0.48	0.40 ± 0.51	1.60 ± 0.51	1.50 ± 0.52
14 day	1.60 ± 0.51	1.50 ± 0.52	2.70 ± 0.48	2.80 ± 0.42
28 day	2.80 ± 0.42	2.90 ± 0.31	3.00 ± 0.00	3.00 ± 0.00

Table 3 Kruskal- Wallis and Mann- Whitney tests for comparison the mean inflammatory reaction of different groups after implantation periods.

Materials Groups	N	Mean ± SD	Chi-Square	P-value
3 Day				
Control group	10	1.30 ± 0.48[b]	24.352	0.000*
MTA Fillapex	10	2.40 ± 0.51[a]		
GuttaFlow bioseal	10	1.00 ± 0.00[c]		
BioRoot sealer	10	1.30 ± 0.48[b]		
7 Day				
Control group	10	1.00 ± 0.00[b]	28.556	0.000*
MTA Fillapex	10	1.50 ± 0.52[a]		
GuttaFlow bioseal	10	0.00 ± 0.00[d]		
BioRoot sealer	10	0.60 ± 0.51[c]		
14 Day				
Control group	10	0.00 ± 0.00[b]	9.489	0.023*
MTA Fillapex	10	0.30 ± 0.48[a]		
GuttaFlow bioseal	10	0.00 ± 0.00[b]		
BioRoot sealer	10	0.00 ± 0.00[b]		

mineralization ability of BioRoot, GuttaFlow Bioseal, and MTA FillApex root canal sealers over time.

The widely accepted procedure to estimate biocompatibility is the implantation experiments, as in this procedure the materials contact directly with sub-cutaneous tissues or bone. This technique was more convenient since the processes of healing cannot be simulated in the in vitro tests "cell cultures". In this study, intraosseous implantation was utilized as sealing materials when clinically used may become in contact with surrounding bone (Orstavik, 2005, Ha et al., 2017, Haji et al., 2022).

The periods of implantation utilized in the current research were coordinated with many of the researches performed on tissue reaction to materials that are implanted in bone tissues or sub-cutaneous connective tissues (Tassery et al., 1997, Santos et al., 2019, Hoshino et al., 2020).

All animals in this study kept in good health condition during the whole periods of the implantation. Examination of the implant sites by macroscopic showed satisfactory healing of the wound without any infection at all tested periods.

In this study the overall severity of inflammatory reactions declined with time which indicates that all the tested sealers had satisfactory biocompatibility, this was consistent with the results of previous studies (Dimitrova-Nakov et al., 2015, Santos et al., 2019, Gaudin et al., 2020).

The highest inflammation grades for all groups on the third day after implantation may be related to different factors, involving trauma from the surgical procedure, incomplete setting of the sealers, solubility, and high pH as registered in many types of research (Quintana et al., 2019, Hoshino et al., 2020).

According to the result of this study at (3,7,14) days after implantation, the MTA-Fillapex shows the highest level of inflammatory response in comparison to other groups. While the Guttaflow- bioseal showed the least inflammatory response.

MTA-Fillapex contains salicylate resin and diluted resin in its composition which might impair its biocompatibility, in addition the mixture of the tricalcium-silicate with the salicylate resin rising flowability and increase the setting time and consequently increase solubility. Studies showed that MTA Fillapex is more soluble after setting (Cintra et al., 2017, Poggio et al., 2017, Saygili et al., 2017). On the other hand, the presence of bismuth oxide as a radiopacifier in MTA-

Table 4 Kruskal-Wallis and Mann-Whitney tests for comparison the mean of bone remineralization of different groups after implantation periods.

Materials Groups	N	Mean ± SD	Chi-Square	P-value
3 Day				
Control group	10	0.00 ± 0.00^{b}	8.610	0.035*
MTA Fillapex	10	0.00 ± 0.00^{b}		
GuttaFlow bioseal	10	0.30 ± 0.48^{a}		
BioRoot sealer	10	0.40 ± 0.51^{a}		
7 Day				
Control group	10	0.30 ± 0.48^{b}	23.732	0.000*
MTA Fillapex	10	0.40 ± 0.51^{b}		
GuttaFlow bioseal	10	1.50 ± 0.52^{a}		
BioRoot sealer	10	1.60 ± 0.51^{a}		
14 Day				
Control group	10	1.50 ± 0.52^{b}	25.123	0.000*
MTA Fillapex	10	1.60 ± 0.51^{b}		
GuttaFlow bioseal	10	2.70 ± 0.48^{a}		
BioRoot sealer	10	2.80 ± 0.42^{a}		
28 Day				
Control group	10	2.80 ± 0.42^{ab}	3.865	0.276^{NS}
MTA Fillapex	10	2.90 ± 0.31^{ab}		
GuttaFlow bioseal	10	3.00 ± 0.00^{a}		
BioRoot sealer	10	3.00 ± 0.00^{a}		

10 = The mean of the ten slides would be evaluated for each group. * Differences is statistically significant at "$P \leq 0.05$"; NS Not significant.

Fillapex, whereas for GuttaFlow bioseal and BioRoot sealers is the zirconium oxide may lead to more tissue inflammatory response for MTA Fillapex since zirconium oxide had no cytotoxic effect on human cell differentiation and consider more biocompatible than bismuth oxide (Saraiva et al., 2018, Benetti et al., 2019, Delfino et al., 2020).

In this study, MTA Fillapex didn′t show negative effects on bone mineralization but also it had no significant simulative effect on healing when comparison with the control group.

On day 14,milder inflammatory response was seen, this my due to the fact that the freshly mixed material has greater irritating effects and is potentially cytotoxic than completely set materials and this concede with other findings (Pawińska et al., 2015, Collado- Gonzalez et al., 2017, Al-Ali et al., 2022).

All sealer groups show bone mineralization activity since all sealer used in this study contain calcium-silicate materials, which form calcium- hydroxide and calcium-silicate hydrogel when come in contact with water, that encourages the formation of mineralized tissues because it is ability to react with phosphate to form hydroxyapatite (Braga et al., 2015, Saraiva et al., 2018, Cosme-Silva et al., 2019, Benetti et al., 2019).

The result represented that BioRoot sealer group, showed better organized and thicker collagen fibers with higher amounts of newly formed trabecular bone and wide blood vessels with large numbers of osteoblasts and osteocytes. Although it statistically not significant than GuttaFlow bioseal group.

It may be speculated that BioRoot RCS may produce a higher amount of calcium hydroxide during the hydration reaction which may in turn stimulates the calcification enzymes of osteoblasts to form of the CaPs. In addition, it's capacity for ions releasing and leaching of Ca^{++} during the process of hydration lead to high pH value that explains its biocompatibility and provides an environment without bacteria that could have a helpful effect on the healing of the tissue (Camilleri, 2015, Gaudin et al., 2020, Haji et al., 2022).

Furthermore, BioRoot RCS's has high solubility, it may be possible that it's not only biocompatible, but it also releases certain components into the adjacent tissues that could encourage tissue repair "bioactivity" extended period (imitrova-Nakov et al., 2015, Jung et al., 2018).

GuttaFlow Bioseal contains bioactive glass and releases bioactive ions like (Ca^{++} and OH^{-}) and this might induce proliferation and the adhesion of osteoblasts, resulting in a faster repair of hard tissue injuries. GuttaFlow Bioseal also lake of resin in its composition and had an alkalinizing effect and this alkalinity might contribute to its osteogenic potential, biocompatibility, and antibacterial properties (Gandolfi et al., 2016; Collado-Gonzaĺez et al., 2017; Saygili et al., 2017; Reszka et al., 2019).

According to the results of this study, the hypothesis was rejected since all the sealers used in this study were inducing the bone mineralizing ability and representing good tissues biocompatibility.

5. Conclusion

Under the conditions of current research, GuttaFlow-Bioseal has demonstrated superior biocompatibility defined by the lowest inflammatory reactions scores at all examination intervals. Also this study revealed that better osteo-conductivity can be obtained with BioRoot sealer group, although statistically not significant in GuttaFlow-bioseal group.

Reduction of inflammatory response and formation of new bone and bony islets were noted after 28 days for all sealer groups.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We would like to thank "College of Dentistry at Mosul University" for its support and continuous help.

Regulatory Statement

"This study was conducted under all the provisions of the local human subjects oversight committee guidelines and policies of the Faculty of Dentistry, Mosul University, Iraq. The approval code for this study is": (UoM.Dent/H.DM.21/23) in (1/3/2023).

References

Al-Ali, H., Alnema, M., AL-Ashou, W.M., 2022. Evaluation of the cytotoxicity of four different root canal sealers on human periodontal ligament fibroblast cells. IJDMSR 4, 566–572 www.ijdmsrjournal.com.

Attash, I.M., AL-Ashou W.M., 2022. Push-out Bond Strength Evaluation for Different Endodontic Sealers (A Comparative Study). RDENTJ. 22, 301-312. https:// doi: 10.33899/rdenj.2022.130209.1105.

Benetti, F., de Azevedo Queiroz Í.O., Oliveira C, Conti L.C, Azuma, M., Oliveira, P., Cintra, A., 2019. Cytotoxicity and biocompatibility of a new bioceramic endodontic sealer containing calcium hydroxide. Braz Oral Res. 33, e042. https://doi: 10.1590/1807-3107bor-2019.vol33.0042. Epub 2019 May 16. PMID: 31508725.

Braga, J.M., Oliveira, R.R., de Castro Martins, R., Vieira, L.Q., Sobrinho, A.P., 2015. Assessment of the cytotoxicity of a mineral trioxide aggregate-based sealer with respect to macrophage activity. Dent. Traumatol. 3, 390–395. https://doi.org/10.1111/edt.12190.

Camargo, R.V., Silva-Sousa, Y.T., Mazzi-Chaves, J.F., Lopes, F.C., Steier, L., Sousa-Neto, M.D., 2017. Evaluation of the physicochemical properties of silicone-and epoxy resin based root canal sealers. Braz. Oral Res. 31, 72–80. https://doi.org/10.1590/1807-3107BOR-.

Camilleri, J., 2015. Mineral trioxide aggregate: present and future developments. Endod. Top. 32, 31–46. https://doi.org/10.1111/etp.12073.

Cintra, L.A., Benetti, F., de Azevedo Queiroz, Í.O., de Araújo Lopes, J.M., Penha de Oliveira, S.H., Sivieri Araújo, G., Gomes-Filho, J. E., 2017. Cytotoxicity, biocompatibility, and biomineralization of the new high-plasticity MTA material. J. Endod. 43, 774–778. https://doi.org/10.1016/j.joen.2016.12.018. Epub 2017 Mar 18. PMID: 28320539.

Collado-Gonzalez, M., Tomas-Catala, C.J., Onate-Sanchez, R.E., Moraleda, J.M., Rodriguez-Lozano, F.J., 2017. Cytotoxicity of GuttaFlow Bioseal, GuttaFlow2, MTA Fillapex, and AH Plus on human periodontal ligament stem cells. J. Endod. 43, 816–822. https://doi.org/10.1016/j.joen.2017.01.001.

Cosme-Silva, L., Gomes-Filho JE, Benetti F, Dal-Fabbro R, Sakai, VT., Cintra, L.T.A., Ervolino, E., Viola, N.V., 2019. Biocompatibility and immunohistochemical evaluation of a new calcium silicate-based cement, Bio-C Pulpo. Int Endod J. 52, 689-700. https://doi: 10.1111/iej.13052. Epub 2019 Jan 3. PMID: 30515845.

Delfino, M.M., Guerreiro-Tanomaru, J.M., Tanomaru-Filho, M., Sasso-Cerri, E., Cerri, PS., 2020. Immunoinflammatory response and bioactive potential of GuttaFlow bioseal and MTA Fillapex in the rat subcutaneous tissue. Sci Rep. 28, 7173. https:// doi: 10.1038/s41598-020-64041-0. PMID: 32346066; PMCID: PMC7188821.

Dimitrova-Nakov, S., Uzunoglu, E., Ardila-Osorio, H., Baudry, A., Richard, G., Kellermann, O., Goldberg, M., 2015. In vitro bioactivity of Bioroot™ RCS, via A4 mouse pulpal stem cells. Dent. Mater. 31, 1290–1297. https://doi.org/10.1016/j.dental.2015.08.163.

Eid, A., Mancino, D., Joudi, F., Rekab, M.S., Layous, K., Hamadah, O., Haikel, Y., Kharouf, N., 2021. Comparative evaluation of the apical sealing ability of BioRoot and AH Plus sealers: An in vitro study. Int. J. Dent. Oral Sci. 8, 2309–2313 http://scidoc.org/IJDOS.php.

Gandolfi, M.G., Siboni, F., Prati, C., 2016. Properties of a novel polysiloxane-guttapercha calcium silicate-bioglass containing root canal sealer. Dent. Mater. 32, e113–e126. https://doi.org/10.1016/j.dental.2016.03.001.

Gaudin, A., Tolar, M., Peters, O.A., 2020. Cytokine production and cytotoxicity of calcium silicate–based sealers in 2-and 3-dimensional cell culture models. JOE 46, 818–826. https://doi.org/10.1016/j.joen.2020.03.011.

Ha, W.N., Nicholson, T., Kahler, B., Walsh, L.J., 2017. Mineral trioxide aggregate-A review of properties and testing methodologies. Materials (Basel) 2, 1261. https://doi.org/10.3390/ma10111261.

Haji, T.H., Selivany, B.J., Suliman, A.A., 2022. Sealing ability in vitro study and biocompatibility in vivo animal study of different bioceramic based sealers. Clin. Exp. Dent. Res. 8, 1582–1590. https://doi.org/10.1002/cre2.652.

Hoshino, R.A., Silva, D., Delfino, M.M., Guerreiro-Tanomaru, J.M., Tanomaru-Filho, M., Sasso-Cerri, E., Filho, I.B., Cerri, P.S., 2020. Physical Properties, Antimicrobial Activity and In Vivo Tissue Response to Apexit Plus. Materials (Basel). 5, 1171. https:// doi: 10.3390/ma13051171. PMID: 32151089; PMCID: PMC7085033.

Jung, C., Kim, S., Sun, T., Cho, YB., Song, M., 2019. Pulp-dentin regeneration: current approaches and challenges. J Tissue Eng. 29, 2041731418819263. https:// doi: 10.1177/2041731418819263. PMID: 30728935; PMCID: PMC6351713.

Jung, S., Sielker, S., Hanisch, M.R., Libricht, V., Schäfer, E., Dammaschke, T., 2018. Cytotoxic effects of four different root canal sealers on human osteoblasts. PLoS One 26, e0194467. https://doi.org/10.1371/journal.pone.0194467. PMID: 29579090; PMCID: PMC5868789.

Lodiene, G., Morisbak, E., Bruzell, E., Ørstavik, D., 2008. Toxicity evaluation of root canal sealers in vitro. Int Endod J. 41, 72-79. https:// doi: 10.1111/j.1365-2591.2007.01321.x. Epub 2007 Oct 10. PMID: 17931390.

Moretton, T.R., Brown, C.E., Legan JJ, Kafrawy A.H., 2000.Tissue reactions after subcutaneous and intraosseous implantation of mineral trioxide aggregate and ethoxybenzoic acid cement. J Biomed Mater Res. 5, 528-533. https:// doi: 10.1002/1097-4636(20001205)52:3 < 528::aid-jbm11 > 3.0.co;2-9.

Orstavik, D., 2005. Materials used for root canal obturation: technical, biological and clinical testing. Endod. Topics 12, 25–38. https://doi.org/10.1111/j.1601-1546.2005.00197.

Pawińska, M., Łuczaj-Cepowicz, E., Kierklo, A., Marczuk-Kolada, G., Hołownia, A., 2015. Assessment of cytotoxic potential of root canal sealers after hardening - an ex vivo study. Postepy Hig Med Dosw (Online). 22, 69:503-9. https:// doi: 10.5604/17322693.1150134.

Poggio, C., Riva, P., Chiesa, M., Colombo, M., Pietrocola, G., 2017. Comparative cytotoxicity evaluation of eight root canal sealers. J Clin Exp Dent. 1, e574-e578. https:// doi: 10.4317/jced.53724. PMID: 28469826; PMCID: PMC5410681.

Prado, M.C., Carvalho, N.K., Vitti, R.P., Ogliari, F.A., Sassone, L. M., Silva, E.L., 2018. Bond strength of experimental root canal sealers based on MTA and butyl ethylene glycol disalicylate. Braz. Dent. J. 29, 195–201. https://doi.org/10.1590/0103-6440201801833. PMID: 29898068.

Quintana, R.M., Jardine, A.P., Grechi, T.R., Grazziotin-Soares, R., Ardenghi, D.M., Scarparo, RK., Grecca FS., Kopper, PMP., 2019. Bone tissue reaction, setting time, solubility, and pH of root repair

materials. Clin Oral Investig. 23, 1359-1366. https:// doi: 10.1007/s00784-018-2564-1. Epub 2018 Jul 18. PMID: 30022271.

Raghavendra, S.S., Jadhav, G.R., Gathani, K.M., Kotadia, P., 2017. Bioceramics in endodontics - a review. J. Istanb. Univ. Fac. Dent. 2, S128–S137. https://doi.org/10.17096/jiufd.63659. PMID: 29354316; PMCID: PMC5750835.

Reszka, P., Nowicka, A., Dura, W., Marek, E., Lipski, M., 2019. SEM and EDS study of TotalFill BC Sealer and GuttaFlow Bioseal root canal sealers. Dent. Med. Probl. 56, 167–172. https://doi.org/10.17219/dmp/105561. PMID: 31140764.

Saghiri, M.A., Orangi, J., Tanideh, N., Asatourian, A., Janghorban, K., Garcia-Godoy, F., Sheibani, N., 2015. Repair of bone defect by nano-modified white mineral trioxide aggregates in rabbit: A histopathological study. Med. Oral Patol. Oral Cir. Bucal 1, e525–e531. https://doi.org/10.4317/medoral.20290. PMID: 26034924; PMCID: PMC4598919.

Santos. J.M., Pereira, S., Sequeira, DB., Messias, A.L., Martins, J.B., Cunha, H., Palma, PJ., Santos, AC., 2019. Biocompatibility of a bioceramic silicone-based sealer in subcutaneous tissue. J Oral Sci. 61, 171-177. https:// doi: 10.2334/josnusd.18-0145. PMID: 30918214.

Saraiva, J.A., da Fonseca, T.S., da Silva, G.F., Sasso-Cerri, E., Guerreiro-Tanomaru, J.M., Tanomaru-Filho, M., Cerri, P.S., 2018. Reduced interleukin-6 immunoexpression and birefringent collagen formation indicate that MTA Plus and MTA Fillapex are biocompatible. Biomed. Mater. 20,. https://doi.org/10.1088/1748-605X/aaa1f5. PMID: 29242419 035002.

Saygili, G., Saygili, S., Tuglu, I., Davut Capar, I., 2017. In vitro cytotoxicity of GuttaFlow Bioseal, GuttaFlow 2, AH-plus and MTA fillapex. Iran Endod J. 12, 354–359. https://doi.org/10.22037/iej.v12i3.15415. PMID: 28808465; PMCID: PMC5527214.

Seo, D.G., Lee, D., Kim, Y.M., Song, D., Kim, S.Y., 2019. Biocompatibility and mineralization activity of three calcium silicate-based root canal sealers compared to conventional resin-based sealer in human dental pulp stem cells. Materials (Basel) 12, 2482. https://doi.org/10.3390/ma12152482.

Sfeir, G., Zogheib, C., Patel, S., Giraud, T., Nagendrababu, V., Bukiet, F., 2021. Calcium silicate-based root canal sealers: A narrative review and clinical perspectives. Materials (Basel) 15, 3965. https://doi.org/10.3390/ma14143965. PMID: 34300886; PMCID: PMC8306764.

Stanford, J.W., 1980. Recommended standard practices for biological evaluation of dental materials. Fédération Dentaire International, Commission of Dental Materials, Instruments, Equipment and Therapeutics. Int Dent J. 30, 140-88. PMID: 6931825.

Tassery, H., Remusat, M., Koubi, G., Pertot, J., 1997. Comparison of the intraosseous biocompatibility of Vitremer and super EBA by implantation into the mandible of rabbits. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 83, 602-8. https:// doi: 10.1016/s1079-2104(97)90127-x. PMID: 9159822.

Troiano, G., Perrone, D., Dioguardi, M., Buonavoglia, A., Ardito, L. o., Muzio, L., 2018. In vitro evaluation of the cytotoxic activity of three epoxy resin-based endodontic sealers. Dent. Mater. J. 37, 374–378. https://doi.org/10.4012/dmj.2017-148.

Zhang, W., Peng, B., 2015. Tissue reactions after subcutaneous and intraosseous implantation of iRoot SP, MTA and AH Plus. Dent Mater J. 34,774-80. https:// doi: 10.4012/dmj.2014-271. PMID: 26632225.