



Evaluation of the Biocompatibility of a Recent Bioceramic Root Canal Sealer (BioRoot™ RCS): *In-vivo* Study

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Abstract

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BACKGROUND: Recently, new calcium silicate bioceramic sealers were introduced to the market. The selection of root canal sealers should not only be based on the different physical parameters but also on local biocompatibility and tissue tolerance.

AIM: This study aimed to evaluate and compare the *in-vivo* biocompatibility of a BioRoot RCS in parallel to MTA Fillapex and AH Plus sealers.

METHODS: Polyethylene tubes containing the freshly mixed test materials were implanted in the subcutaneous tissue of 32 Wistar rats. Empty tubes served as negative controls. After 7, 14, 30, and 60 days, the animals were sacrificed, and the implants with surrounding tissues were processed for routine histological analysis. Histological sections were analyzed under light microscopy. The tissue response was determined by the inflammatory cell infiltration intensity and the fibrous capsule thickness.

RESULTS: Results revealed a statistically significant decrease of the inflammation intensity by time within each group for all tested sealers and control. A well-defined thin capsule was observed for all tested sealers at 60 days.

CONCLUSION: BioRoot RCS exhibited rapid recovery of inflammation similar to controls. Thus, within the limitations of this study, it can be considered a biocompatible sealer with acceptable tissue tolerance.

Introduction

The endodontics face is constantly changing due to the technical and material advancements. Bioceramics are the most distinguished recently introduced materials in endodontics, due to their superior physical properties, biocompatibility and bioactivity by deposition of hydroxyapatite which greatly increase the seal at the dentin-sealer interface [1], [2], [3].

Recently, new tri-calcium silicate bioceramic sealers were introduced to the market for dental use. Selection of root canal sealers should not be based only on the different physical parameters, but also on local biocompatibility and tissue tolerance [4], [5]. Calcium silicate-based materials are acknowledged as bioactive materials because they are proved to simulate the deposition of mineralized tissues and repair [3], [6], [7].

Biocompatibility is one of the most essential prerequisites of root canal filling materials. Endodontic sealers can get in direct contact with periapical tissues and may trigger adverse reactions affecting periapical repair and the final outcome of endodontic treatment [8], [9]. All

sealers available in practices exhibit a degree of toxicity, thus extrusion of sealers into periapical area should be avoided [10]. Root canal sealers should have acceptable tissue tolerance to preclude or heal apical periodontitis. Up-to-date, no sealer fulfilled all Grossman's [11] criteria of an ideal endodontic sealer [9].

BioRoot™ RCS (Septodont, France) is a recently introduced tri-calcium silicate-based bioceramic sealer. It utilizes the "Active Biosilicate Technology" which is resin and eugenol free, providing exceptional biological and bioactive properties [12].

Several studies investigated the physical properties of BioRoot [13], corroborating its bioactive properties and its relatively lower cytotoxic [14], [15], [16], [17], [18] and genotoxic [19] profile compared to other sealers.

MTA Fillapex (Angelus, Brazil) is a salicylate resin bioceramic sealer, with enhanced physical properties but has been shown to be relatively cytotoxic and genotoxic [15], [19], [20].

AH Plus (Dentsply, Germany) a resin epoxy-based sealer is considered the gold standard

of endodontic sealers because of its excellent physicochemical properties [21], [22].

Biocompatibility evaluation of newly introduced materials involves initial *in-vitro* assessment of the cytotoxicity, followed by preliminary *in-vivo* studies in laboratory animals and clinical studies [23], [24], [25].

Subcutaneous implantation is an *in-vivo* method simulating the clinical conditions, used to evaluate the tissue response of newly introduced dental materials [7], [26].

In previous *in-vitro* cytotoxicity assays, BioRoot RCS sealer showed superior biocompatibility [17], [19] on pulpal stem cells [16] and periodontal ligament cells [18].

Nevertheless, although BioRoot-RCS seems to be a promising material, to the best of our knowledge, this is the first *in-vivo* study to evaluate its biological safety. Thus, our study was conducted to evaluate the *in-vivo* biocompatibility of the recently introduced calcium silicate bio-ceramic sealer (BioRoot RCS) compared to MTA Fillapex and AH Plus.

Materials and Methods

The present study was approved by the Ethical Committee of the Faculty of Dentistry, Minia University. (No: 260/2018). All experimental procedures conformed to the international guiding principles for biomedical research involving animals and relevant guidelines [27].

Selection of the animal model

Thirty-two male Wistar rats, with an average weight of (280–300 g) and average age of (4–5) months, were selected. The sample size was established based on previous research [7], [28], [29]. Animals were housed in a climatized room, with a 12 h day-night cycle at a temperature of $24 \pm 2^\circ\text{C}$. All animals were fed with a semi-purified diet and water ad libitum. Animals were then given water only 12 h before surgery. The animals were divided into four equal groups (8 rats each) according to the observation periods (7 days, 14 days, 30 days, and 60 days) post-implantation.

Surgical implantation procedures

The surgical procedures were performed following previous studies [7], [29], [30]. The tested materials were freshly prepared according to the manufacturers' instructions and placed in sterile polyethylene tubes.

Animals were anesthetized by intraperitoneal administration of 10% ketamine hydrochloride (80 mg/kg) associated with xylazine hydrochloride (10 mg/kg). After

shaving and disinfection of surgical sites, four incisions (2 cm) were performed. Then, two scapular and two caudal pockets were created using a blunt scissor, to accommodate the polyethylene tubes.

Four sterile polyethylene tubes were implanted in the dorsal region of each rat ($n = 4$), one empty tube as a control and the others filled with the tested sealers [7], [30]. All surgical incisions were closed with #3–0 black silk sutures Figure 1.

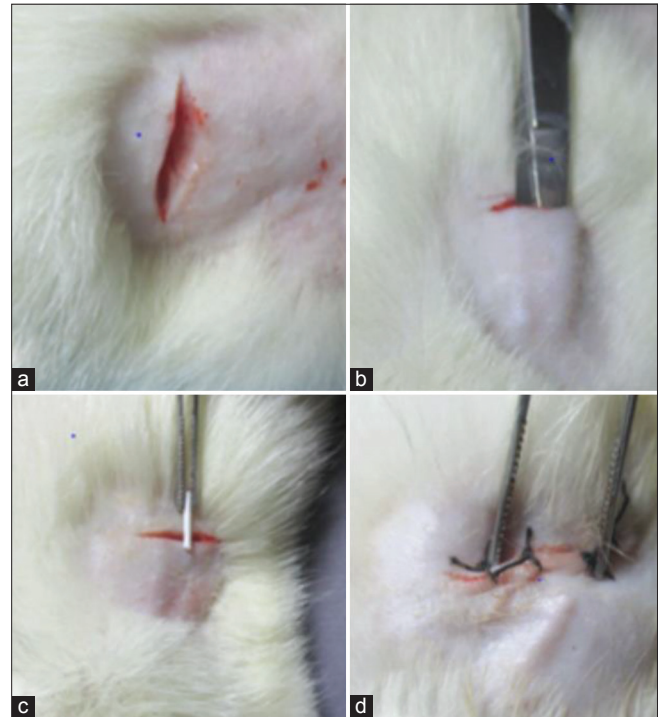


Figure 1: (a-d) Surgical implantation of polyethylene tubes

After surgery, animals of each group were individually housed and maintained on semi-purified diet and water. At each observation period, eight animals were sacrificed by anesthetic overdose and the tubes were excised with the surrounding tissues.

Specimens preparation and histopathological evaluation

The specimens were fixed in 10% formalin for 48 h and coded according to the tested material and the observation time. The polyethylene tubes were carefully removed through a small incision [31].

Specimens were then embedded in paraffin blocks. Serial sections of $4 \mu\text{m}$ thickness were prepared by a rotary microtome for further staining with hematoxylin and eosin stain (H&E).

Histological sections were analyzed at different magnifications, under a light microscope (Olympus CX31, Ireland) by an experienced pathologist blinded to materials type and implantation intervals. The tissue response was determined by the inflammatory cell infiltration intensity and the fibrous capsule thickness. The inflammatory events were scored based on previous studies [9], [32], [33] as

follows: (1) Few or absent; (2) mild; (3) moderate, and (4) severe reaction. Fibrous capsules were considered thin when $<150\ \mu\text{m}$ and thick when $\geq 150\ \mu\text{m}$.

Statistical analysis

Data management and statistical analysis were performed using the Statistical Package for the Social Sciences (SPSS) version 18. Data were explored for normality by checking the data distribution and using Kolmogorov–Smirnov and Shapiro–Wilk tests. Categorical data were summarized as count and percentages. Non-normally distributed numeric variables were compared between groups by Kruskal–Wallis, and pairwise comparison was performed using Mann–Whitney U test. Comparisons over time regarding

numeric variables were done by Friedman test. For categorical variables, differences were analyzed with Chi-square test. Adjustments of p value were done using the Bonferroni method for multiple testing. $p \leq 0.05$ were considered statistically significant.

Results

Representative images of the subcutaneous tissue reactions (between host tissue and implanted tube opening) in the control and sealer groups are shown in Figure 2 (A-D) and the histological analysis is summarized in Tables 1 and 2 and Figures 3-5.

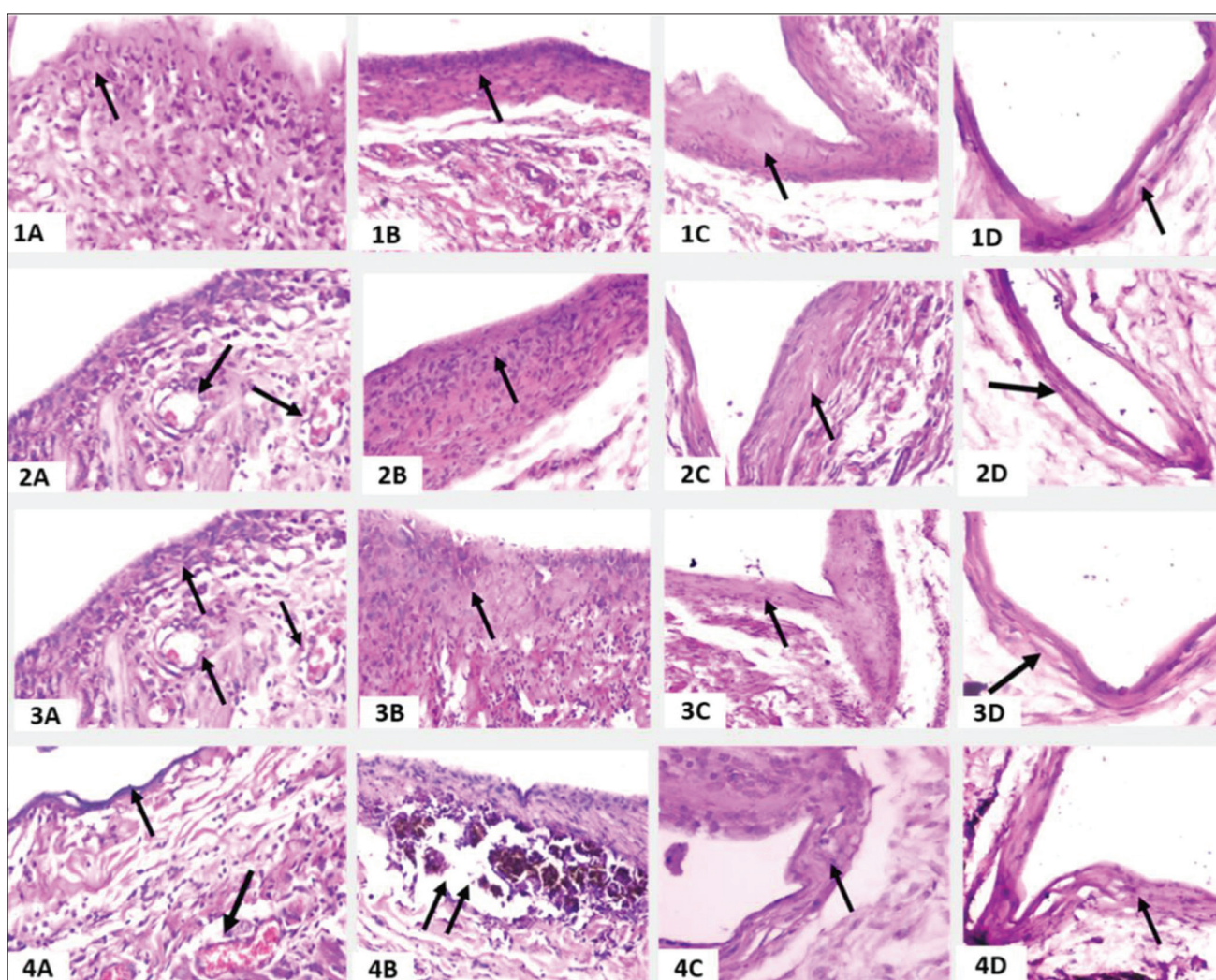


Figure 2: Representative images of the subcutaneous tissue reactions. Control Group 1: (1A) Moderate inflammatory response (arrow), (1B) mild inflammatory reaction and thick fibrotic capsule (arrow), (1C and 1D) absence of inflammation and thin mature fibrous capsule (arrows). BioRoot RCS Group 2: (2A) Severe inflammatory cells infiltration with marked angiogenesis (arrows), (2B) mild inflammation with thick fibrotic capsule (arrow), (2C) well organized fibrous capsule with mild reaction (arrow), (2D) no inflammation with thin mature fibrous capsule (arrow). AH Plus Group 3: (3A) Tissue necrosis on the tubular orifice and severe inflammatory cell infiltration (arrow). (3B) Thick fibrous capsule, moderate infiltration (arrow), (3C) mild inflammation with clear tube orifice without necrotic tissues (arrow), (3D) thin mature fibrous capsule with the absence of inflammation (arrow). MTA Fillapex Group 4: (4A) Extensive tissue necrosis and severe inflammatory reaction (arrow). (4B) Thin layer of necrotic tissue with black brownish-leaked deposits of the tested material (arrows). (4C) Mild reaction with clear tube orifice without necrotic tissues (arrow). (4D) Thin mature fibrous capsule with non-significant inflammation (arrow). A, B, C, and D conform to post-implantation observation periods 7, 14, 30, and 60 days, respectively (H&E $\times 400$)

Table 1: Histological analysis scores for all groups

Time/n	Materials	Inflammation scores				Median	Capsule thickness	
		1	2	3	4		Thick	Thin
7 days n=8	Control	0	2	5	1	3 ^a	8	0
	BioRoot RCS	0	1	2	5	4 ^a	8	0
	AH Plus	0	1	3	4	3,5 ^a	7	1
	MTA Fillapex	0	1	1	6	4 ^a	7	1
	p value=0.136 ns						p=0.54 ns	
14 days n=8	Control	0	5	3	0	2 ^a	4	4
	BioRoot RCS	0	4	3	1	2,5 ^a	5	3
	AH Plus	0	2	4	2	3 ^a	8	0
	MTA Fillapex	0	1	4	3	3 ^a	7	1
	p value=0.084 ns						p=0.083 ns	
30 days n=8	Control	7	1	0	0	1 ^b	0	8
	BioRoot RCS	2	6	0	0	2 ^a	1	7
	AH Plus	0	6	1	1	2 ^a	3	5
	MTA Fillapex	3	4	1	0	2 ^a	6	2
	p value=0.004 ns						p=0.006 ns	
60 days n=8	Control	8	0	0	0	1 ^a	0	8
	BioRoot RCS	7	1	0	0	1 ^a	0	8
	AH Plus	7	1	0	0	1 ^a	0	8
	MTA Fillapex	6	2	0	0	1 ^a	1	7
	p value=0.529 ns						p=0.38 ns	

Significance level p<0.05, *significant, ns=non-significant.

At 7 days, histological analysis revealed that all tested sealers showed severe inflammatory cell infiltration in a thick poorly organized fibrous capsule,

Table 2: Effect of time on the inflammatory intensity and capsule thickness within the same group

Variable	Inflammatory scores	Capsule thickness
Control	0.00*	p=0.0002*
BioRoot RCS	0.00*	p=0.0002*
AH plus	0.00*	p=0.0014*
MTA Fillapex	0.00*	p=0.0002*

Significance level p<0.05, *significant

while the controls showed moderate inflammation at the tube orifice. Marked angiogenesis (arrows) was observed in all test groups (Figure 2, A1–4). However, a significant difference was not observed among the groups (p > 0.05).

At 14 days, BioRoot RCS showed rapid recovery and mild inflammatory response similar to control. AH Plus and MTA Fillapex exhibited greater inflammation than controls, while three MTA Fillapex specimens exhibited a severe median score. The fibrous capsule was thick in all specimens of each group in this period (Figure 2, B1–4). There were no statistically significant differences between the study periods.

At 30 days, the inflammatory reaction significantly declined over time for all tested groups at (p = 0.04). Most of the specimens had mild inflammatory cell infiltration, while control specimens had no inflammation (median score: 1). A significant difference was observed between BioRoot RCS, MTA Fillapex, and AH Plus when compared to control. Most specimens showed well-defined capsules, except for MTA Fillapex, where most specimens retained a thick fibrous capsule (Figure 2, C1–4)

At 60 days, complete subsidence of inflammation occurred and almost all specimens showed negligible inflammatory cell infiltration. Nevertheless, a significant difference was absent between the groups. A thin mature fibrous capsule was observed around the implanted tubes of all specimens (Figure 2, D1–4).

Friedman test revealed a statistically significant decrease of the inflammation intensity and capsule thickness over time, within the same group of all tested sealers and controls.

The same letters indicate the absence of statistical differences among the groups in each analysis period.

Discussion

The evaluation of biocompatibility of any dental material intended for clinical use necessitate a planned assessment in sequential stages, including *in-vitro* cell line cultures, tissue reaction in animals, and clinical trials, to protect the patient from possible hazards [25], [34].

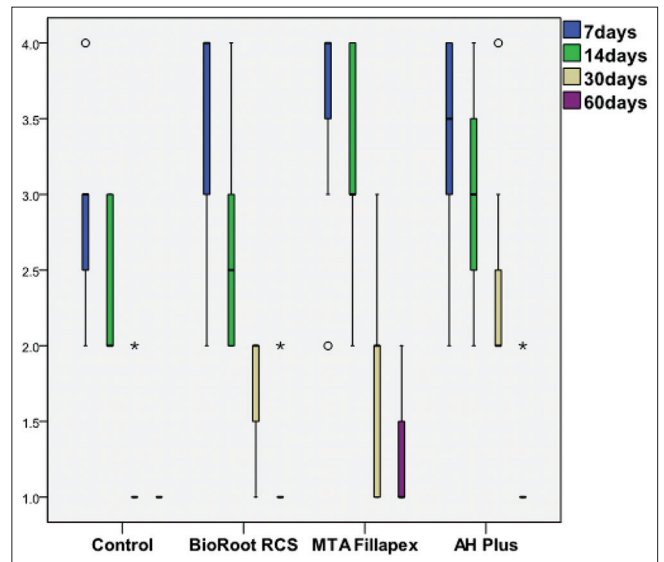


Figure 3: Box plot showing median inflammatory scores in different groups

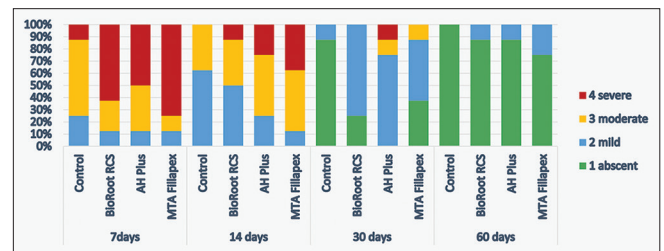


Figure 4: Bar chart illustrating frequency of different scores of inflammation intensity

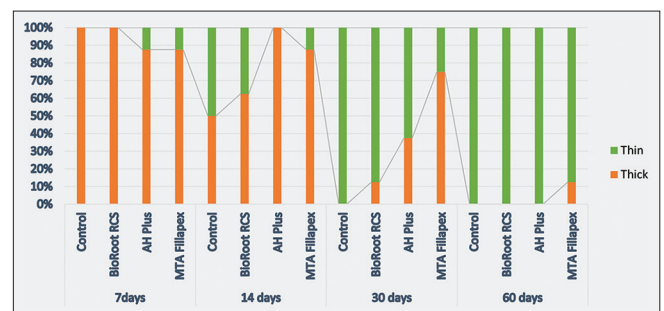


Figure 5: Bar chart illustrating frequency of different capsule thickness scores

Our study was conducted to evaluate the *in-vivo* biocompatibility of the recent BioRoot in parallel to MTA Fillapex and compared to AH Plus sealers.

The severity of the inflammatory response of the tested materials and the fibrous capsule thickness was investigated along four observation time intervals. Rat subcutaneous implantation was used, as it is the most common, reliable, and standardized method for testing the biocompatibility of materials [35]. This conformed to recommendations of the ISO of using different time intervals to evaluate both short and long-term inflammatory response [7], [9], [25], [30].

Most sealers are toxic when freshly mixed, so we tested the freshly mixed state to simulate clinical conditions [33], [36]. The implanted polyethylene tubes were removed from tissue specimens after formalin fixation, to retain the tube space for easier histologic interpretation and also to facilitate sectioning of the paraffin blocks [31].

Regarding histological analysis results, inflammatory reactions were noticed in both experimental and control (empty tubes) groups at the 1st week observation time. However, the experimental groups initially presented a severe inflammatory reaction relative to the moderate reaction of the control group. This was in agreement with Zmener *et al.* [24].

The initial inflammatory reaction adjacent to controls could be attributed to the incisions' surgical trauma and the physical presence of the tubes. This is in contradiction with Silva *et al.* [37], who stated that empty polyethylene tubes caused no inflammatory reaction.

Regarding BioRoot RCS, a severe inflammatory response with marked angiogenesis was observed at 7 days, similar to other tested groups; however, the inflammation declined over time.

Calcium silicate-based materials are known to release calcium ions upon interaction with tissue fluids. Thus, the initially severe inflammatory response (7 days) could be attributed to the ascending alkaline pH upon setting. Furthermore, the heat generated during setting reaction promotes inflammatory cell recruitment releasing cytokines [38], [39], [40]. *In-vitro* study on BioRoot by Camps *et al.* [18] showed that calcium hydroxide released during setting caused chronic inflammatory reactions in viable cells.

There is a substantial correlation between the development of the fibrous capsule and material biocompatibility, as it is considered the immune response rendering foreign bodies well tolerated by the tissues [20], [25], [36].

In the present study, the formation of granulation tissue followed by early deposition of collagen fibers occurred at the 1st week, in all experimental and control (empty tube) groups, indicating favorable interaction with the adjacent living tissues.

Concerning the long-term tissue response, a significant reduction in the inflammation intensity, subsequently, more organization of the collagenous capsule around the tubes occurred over time. A thin well-defined fibrous capsule was observed in all tested sealers similar to controls, particularly at the end of 60 days, denoting tissue tolerance and biocompatibility of BioRoot RCS, MTA Fillapex, and AH Plus. This was corroborated by Bueno *et al.* [30] and Shahi *et al.* [41] who reported subsidence of inflammation over time with thinning of the fibrous capsule.

MTA Fillapex showed a significantly severe initial inflammatory reaction, this was corroborated by Bueno *et al.* [30] and Silveira *et al.* [42] who found it a normal initial finding when using MTA containing cement. This could be attributed to its salicylate resin matrix and to its high alkalinity. Moreover, its high flow and setting time may lead to the prolonged dissolution of toxic leachates into tissues [43]. Furthermore, a lot of MTA Fillapex specimens demonstrated a thick capsule even at 30 days' period, which could be related to its high solubility [20].

Nevertheless, the variance in the inflammatory reaction may be attributed to different radiopacifiers, zirconium oxide as a radiopacifier in BioRoot is deliberated to be more biocompatible than bismuth oxide of MTA Fillapex [25]. In a study by Slompo *et al.* [44], zirconia oxide radiopacifier preserved the viability of fibroblasts.

Since the *in-vivo* tissue reaction to BioRoot was not studied yet, so we could not relate our results to previous studies. However, in a study by Mori *et al.* [33] who tested tissue reaction of biodentine, a root repair material having a similar composition to BioRoot, they found an early intense reaction which decreased by 14 and 30 days. Furthermore, our results were consistent with Talabani *et al.* [25] and Simsek *et al.* [45], who assessed the rat subcutaneous reaction of biodentine and concluded it is biocompatible. Furthermore, another study by Chakar *et al.* [46] found minimal cytotoxicity with BioMM sealer, a sealer similar in constituents to BioRoot.

The biocompatibility of calcium silicate cement is mainly related to the amount of calcium release. Thus, the enhanced biocompatibility of BioRoot may be related to the Ca²⁺ release and high solubility rendering it more alkaline than MTA Fillapex [13], [25], [43].

Moreover, tri-calcium silicate which is the main constituent of BioRoot was shown to increase cell proliferation and promote osteogenic differentiation in a previous study [47].

Conclusion

BioRoot RCS presented a rapid recovery of inflammation similar to controls. Thus, within

the limitations of this study, it can be considered a biocompatible sealer with acceptable tissue tolerance. All tested sealers demonstrated a significant reduction of inflammatory reaction throughout the experimental periods and complete healing occurred with thinning of the fibrous capsule. Therefore, BioRoot-RCS, MTA Fillapex, and AH Plus are considered biocompatible.

To the best of our knowledge, up-to-date, there is no *in-vivo* study evaluating the tissue response to tricalcium silicate bioceramic sealer (BioRoot RCS). This research is deliberated in the first study comparing the tissue response to BioRoot RCS in the subcutaneous tissue of a rat model. Thus, further *in-vivo* studies are recommended to corroborate our findings.

References

- Nasseh A. The rise of bioceramics. *Endod Prac.* 2009;2:17-22.
- Jain PR, Ranjan MA. The rise of bioceramics in endodontics: A review. *Int J Pharm Bio Sci.* 2015;6(1):416-22.
- Parírokh M, Torabinejad M, Dummer PM. Mineral trioxide aggregate and other bioactive endodontic cements: An updated overview-Part I: Vital pulp therapy. *Int Endod J* 2018;51(2):177-205. <https://doi.org/10.1111/iej.12841>
PMid:28836288
- Bratel J, Jontell M, Dahlgren U, Bergenholtz G. Effects of root canal sealers on immunocompetent cells *in vitro* and *in vivo*. *Int Endod J.* 1998;31(3):178-88. <https://doi.org/10.1046/j.1365-2591.1998.00148.x>
PMid:10321164
- Bernath M, Szabo J. Tissue reaction initiated by different sealers. *Int Endod J.* 2003;36(4):256-61. <https://doi.org/10.1046/j.1365-2591.2003.00662.x>
PMid:12702119
- Bueno CR, Valentim D, Marques VA, Gomes-Filho JE, Cintra LT, Jacinto RC, *et al.* Biocompatibility and biomineralization assessment of bioceramic-, epoxy-, and calcium hydroxide-based sealers. *Braz Oral Res.* 2016;30(1):81-9. <https://doi.org/10.1590/1807-3107bor-2016.vol30.0081>
PMid:27305513
- Cintra LT, Benetti F, de Azevedo Queiroz ÍO, de Araújo Lopes JM, de Oliveira SH, Araújo GS, *et al.* Cytotoxicity, biocompatibility, and biomineralization of the new high-plasticity MTA material. *J Endod.* 2017;43(5):774-8. <https://doi.org/10.1016/j.joen.2016.12.018>
PMid:28320539
- Al-Haddad A, Ab Aziz C, Zeti A. Bioceramic-based root canal sealers: A review. *Int J Biomater.* 2016;2016:9753210. <https://doi.org/10.1155/2016/9753210>
PMid:27242904
- Santos JM, Pereira S, Sequeira DB, Messias AL, Martins JB, Cunha H, *et al.* Biocompatibility of a bioceramic silicone-based sealer in subcutaneous tissue. *J Oral Sci.* 2019;61(1):171-7. <https://doi.org/10.2334/josnurd.18-0145>
PMid:30918214
- Ricucci D, Langeland K. Apical limit of root canal instrumentation and obturation, Part 2. A histological study. *Int Endod J.* 1998;31(6):394-409. <https://doi.org/10.1046/j.1365-2591.1998.00183.x>
PMid:15551607
- Grossman L, Oliet S, del Rio C. *Endodontic Practice.* Vol. 11. Philadelphia, PA, USA: Auflage, Lea & Febiger; 1988. p. 234-41.
- Septodont. BioRoot RCS; 2019. Available from: <https://www.oraverse.com/bio/img/bioroot-scientificfile.pdf>. [Last accessed on 2019Aug 26].
- Siboni F, Taddei P, Zamparini F, Prati C, Gandolfi MG. Properties of BioRoot RCS, a tricalcium silicate endodontic sealer modified with povidone and polycarboxylate. *Int Endod J.* 2017;50(2):e120-36. <https://doi.org/10.1111/iej.12856>
PMid:28881478
- Vouzara T, Dimosiari G, Koulaouzidou EA, Economides N. Cytotoxicity of a new calcium silicate endodontic sealer. *J Endod.* 2018;44(5):849-52. <https://doi.org/10.1016/j.joen.2018.01.015>
PMid:29550005
- Poggio C, Riva P, Chiesa M, Colombo M, Pietrocola G. Comparative cytotoxicity evaluation of eight root canal sealers. *J Clin Exp Dent.* 2017;9(4):e574-8. <https://doi.org/10.4317/jced.53724>
PMid:28469826
- Dimitrova-Nakov S, Uzunoglu E, Ardila-Osorio H, Baudry A, Richard G, Kellermann O, *et al.* *In vitro* bioactivity of Bioroot™ RCS, via A4 mouse pulpal stem cells. *Dent Mater.* 2015;31(11):1290-7. <https://doi.org/10.1016/j.dental.2015.08.163>
PMid:26364144
- Collado-González M, García-Bernal D, Oñate-Sánchez RE, Ortolani-Seltenerich PS, Lozano A, Forner L, *et al.* Biocompatibility of three new calcium silicate-based endodontic sealers on human periodontal ligament stem cells. *Int Endod J.* 2017;50(9):875-84. <https://doi.org/10.1111/iej.12703>
PMid:27666949
- Camps J, Jeanneau C, El Ayachi I, Laurent P, About I. Bioactivity of a calcium silicate-based endodontic cement (BioRoot RCS): Interactions with human periodontal ligament cells *in vitro*. *J Endod.* 2015;41(9):1469-73. <https://doi.org/10.1016/j.joen.2015.04.011>
PMid:26001857
- Eldeniz AU, Shehata M, Högg C, Reichl FX. DNA double-strand breaks caused by new and contemporary endodontic sealers. *Int Endod J.* 2016;49(12):1141-51. <https://doi.org/10.1111/iej.12577>
PMid:26574345
- Benetti F, de Azevedo Queiroz ÍO, Oliveira PH, Conti LC, Azuma MM, Oliveira SH, *et al.* Cytotoxicity and biocompatibility of a new bioceramic endodontic sealer containing calcium hydroxide. *Braz Oral Res.* 2019;33:e042. <https://doi.org/10.1590/1807-3107bor-2019.vol33.0042>
PMid:31508725
- Zhang W, Peng B. Tissue reactions after subcutaneous and intraosseous implantation of iRoot SP, MTA and AH Plus. *Dent Mater J.* 2015;34(6):774-80. <https://doi.org/10.4012/dmj.2014-271>
PMid:26632225
- Simsek N, Akinci L, Gecor O, Alan H, Ahmetoglu F, Taslidere E. Biocompatibility of a new epoxy resin-based root canal sealer in subcutaneous tissue of rat. *Eur J Dent.* 2015;9(1):31-5. <https://doi.org/10.4103/1305-7456.149635>
PMid:25713481
- Cosme-Silva L, Gomes-Filho JE, Benetti F, Dal-Fabbro R, Sakai VT, Cintra LT, *et al.* Biocompatibility and immunohistochemical evaluation of a new calcium silicate-based cement, Bio-C pulpo. *Int Endod J.* 2019;52(5):689-700. <https://doi.org/10.1111/iej.13052>
PMid:30515845

24. Zmener O, Pameijer CH, Kokubu GA, Grana DR. Subcutaneous connective tissue reaction to methacrylate resin-based and zinc oxide and eugenol sealers. *J Endod.* 2010;36(9):1574-9. <https://doi.org/10.1016/j.joen.2010.06.019>
PMid:20728730
25. Talabani RM, Garib BT, Masaeli R. Biocompatibility of three calcium silicate based materials implanted in rat subcutaneous tissue. *Biomed Res.* 2019;30(4):591-9. <https://doi.org/10.35841/biomedicalresearch.30-19-264>
26. Browne RM. Animal tests for biocompatibility of dental materials-relevance, advantages and limitations. *J Dent.* 1994;22:S21-4. [https://doi.org/10.1016/0300-5712\(94\)90035-3](https://doi.org/10.1016/0300-5712(94)90035-3)
PMid:7844271
27. Kilkenny C, Browne W, Cuthill IC, Emerson M, Altman DG. Animal research: Reporting *in vivo* experiments: The ARRIVE guidelines. *Br J Pharmacol.* 2010;160(7):1577-9. <https://doi.org/10.1111/j.1476-5381.2010.00872.x>
PMid:20649561
28. Cintra LT, Benetti F, de Azevedo Queiroz ÍO, Ferreira LL, Massunari L, Bueno CR, *et al.* Evaluation of the cytotoxicity and biocompatibility of new resin epoxy-based endodontic sealer containing calcium hydroxide. *J Endod.* 2017;43(12):2088-92. <https://doi.org/10.1016/j.joen.2017.07.016>
PMid:29032822
29. Benetti F, Queiroz ÍO, Cosme-Silva L, Conti LC, Oliveira SH, Cintra LT. Cytotoxicity, biocompatibility and biomineralization of a new ready-for-use bioceramic repair material. *Braz Dent J.* 2019;30(4):325-32. <https://doi.org/10.1590/0103-6440201902457>
30. Bueno CR, Vasques AM, Cury MT, Sivieri-Araújo G, Jacinto RC, Gomes-Filho JE, *et al.* Biocompatibility and biomineralization assessment of mineral trioxide aggregate flow. *Clin Oral Investig.* 2019;23(1):169-77. <https://doi.org/10.1007/s00784-018-2423-0>
PMid:29572687
31. Karanth P, Manjunath MK, Kuriakose ES. Reaction of rat subcutaneous tissue to mineral trioxide aggregate and Portland cement: A secondary level biocompatibility test. *J Indian Soc Pedod Prev Dent.* 2013;31(2):74-81. <https://doi.org/10.4103/0970-4388.115698>
32. Parirokh M, Mirsoltani B, Raoof M, Tabrizchi H, Haghdoost AA. Comparative study of subcutaneous tissue responses to a novel root-end filling material and white and grey mineral trioxide aggregate. *Int Endod J.* 2011;44(4):283-9. <https://doi.org/10.1111/j.1365-2591.2010.01808.x>
PMid:21091493
33. Mori GG, Teixeira LM, de Oliveira DL, Jacomini LM, da Silva SR. Biocompatibility evaluation of biodentine in subcutaneous tissue of rats. *J Endod.* 2014;40(9):1485-8. <https://doi.org/10.1016/j.joen.2014.02.027>
PMid:25146039
34. Murray PE, Godoy CG, Godoy FG. How is the biocompatibility of dental biomaterials evaluated? *Med Oral Patol Oral Cir Bucal.* 2007;12(3):258-66.
PMid:17468726
35. International Organization for Standardization. Biological Evaluation of Medical Devices. Part 6: Tests for Local Effects after Implantation. Geneva, Switzerland: International Organization for Standardization; 2016.
36. Derakhshan S, Adl A, Parirokh M, MashadiAbbas F, Haghdoost AA. Comparing subcutaneous tissue responses to freshly mixed and set root canal sealers. *Iran Endod J.* 2009;4(4):152-7.
PMid:24019838
37. Silva LA, Azevedo LU, Consolaro A, Barnett F, Xu Y, Battagliano RA, *et al.* Novel endodontic sealers induce cell cytotoxicity and apoptosis in a dose-dependent behavior and favorable response in mice subcutaneous tissue. *Clin Oral Investig.* 2017;21(9):2851-61. <https://doi.org/10.1007/s00784-017-2087-1>
PMid:28281012
38. Pinheiro LS, Iglesias JE, Boijink D, Mestieri LB, Kopper PM, de Poli Figueiredo JA, *et al.* Cell viability and tissue reaction of NeoMTA plus: An *in vitro* and *in vivo* study. *J Endod.* 2018;44(7):1140-5. <https://doi.org/10.1016/j.joen.2018.03.007>
PMid:29866406
39. Zaki DY, Zaazou MH, Khallaf ME, Hamdy TM. *In vivo* comparative evaluation of periapical healing in response to a calcium silicate and calcium hydroxide based endodontic sealers. *Open Access Maced J Med Sci.* 2018;6(8):1475-9. <https://doi.org/10.3889/oamjms.2018.293>
PMid:30159080
40. Gandolfi MG, Siboni F, Primus CM, Prati C. Ion release, porosity, solubility, and bioactivity of MTA plus tricalcium silicate. *J Endod.* 2014;40(10):1632-7. <https://doi.org/10.1016/j.joen.2014.03.025>
PMid:25260736
41. Shahi S, Rahimi S, Lotfi M, Yavari H, Gaderian A. A comparative study of the biocompatibility of three root-end filling materials in rat connective tissue. *J Endod.* 2006;32(8):776-80. <https://doi.org/10.1016/j.joen.2006.01.014>
PMid:16861081
42. Silveira CM, Pinto SC, Zedebinski RD, Santos FA, Pilatti GL. Biocompatibility of four root canal sealers: A histopathological evaluation in rat subcutaneous connective tissue. *Braz Dent J.* 2011;22(1):21-7. <https://doi.org/10.1590/s0103-64402011000100003>
PMid:21519643
43. Khalil I, Naaman A, Camilleri J. Properties of tricalcium silicate sealers. *J Endod.* 2016;42(10):1529-35. <https://doi.org/10.1016/j.joen.2016.06.002>
PMid:27523906
44. Slompo C, Peres-Buzalaf C, Gasque KC, Damante CA, Ordinola-Zapata R, Duarte MA, *et al.* Experimental calcium silicate-based cement with and without zirconium oxide modulates fibroblasts viability. *Braz Dent J.* 2015;26(6):587-91. <https://doi.org/10.1590/0103-6440201300316>
PMid:26963200
45. Simsek N, Alan H, Ahmetoglu F, Taslidere E, Bulut ET, Keles A. Assessment of the biocompatibility of mineral trioxide aggregate, bioaggregate, and biodentine in the subcutaneous tissue of rats. *Niger J Clin Pract.* 2015;18(6):739-43. <https://doi.org/10.4103/1119-3077.154219>
PMid:26289510
46. Chakar S, Changotade S, Osta N, Khalil I. Cytotoxic evaluation of a new ceramic-based root canal sealer on human fibroblasts. *Eur J Dent.* 2017;11(02):141-8. https://doi.org/10.4103/ejd.ejd_2_17
PMid:28729783
47. Bjerre L, Bünger CE, Kassem M, Mygind T. Flow perfusion culture of human mesenchymal stem cells on silicate-substituted tricalcium phosphate scaffolds. *Biomaterials.* 2008;29(17):2616-27. <https://doi.org/10.1016/j.biomaterials.2008.03.003>
PMid:18374976