Al-Rafidain Dental Journal

rden.mosuljournals.com

Biocompatibility of Newly Prepared Nanocalcium Oxide Based Root Canal Sealer (In Vivo study)

Raghad A. Al-Askary [1], Sawsan H. Al - Jubori [2]

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

Article information

Received: December 16, 2021
Accepted: January 30, 2022
Available online: September 5, 2023

Keywords
Bioceramic Sealer, Biocompatibility, Subcutaneous Implantation, Nanoparticles.

***Correspondence e-mail:**
Raghadadnan_77@uomosul.edu.iq

Abstract

Aims: To evaluate in vivo the biocompatibility of newly prepared calcium oxide based nanosealer. **Materials and Methods:** Twenty albino healthy male rabbits were used, each received three polyethylene tubes; one filled with BioRoot sealer (+ve control), one received the newly prepared nanosealer, and the last left empty served as (-ve control) were implanted in the corresponding skin pockets made at the dorsal skin of the anesthetized rabbits. Then the rabbits divided into four equal groups according to the observation periods. Tissue biopsies were collected at (3, 7, 14, and 28) day after the implantation. The specimens were processed and stained with hematoxylin and eosin and examined microscopically. Statistical analysis was performed by Kruskal Wallis and Mann-Whitney tests for analyses the inflammatory tissues response of each group at observation times. **Results:** Histopathologically; at 3 days (-ve) and (+ve) control groups revealed sever inflammatory reaction, while the experimental group represented moderate tissues inflammation. These inflammatory tissues reactions were reduced over times for all group until subsided completely at 28 days but still faster for experimental sealer. All groups revealed thin full organized fibrous capsule at 28 days representing the tissues tolerance of implanted materials. Statistically the experimental nanosealer represented the least inflammatory tissue reaction among groups. There were no statistically significance differences in fibrous capsule thickness among the groups. **Conclusions:** The prepared nanosealer represented high biocompatibility than other groups.

الخلاصة

الأهداف: تهدف هذه الدراسة إلى تقييم رد فعل النسيج الضام لمادة السداده النانوية المانعه للتسرب المحضرة والمكونة من أكسيد الكالسيوم. **المواد وطرائق العمل:** تم استخدام عشرين أرنباً من الذكور الأصحاء ، تلقى كل منهم ثلاثة أنابيب من البولي إيثيلين. واحد مملوء بمادة السداده المانعة للتسرب BioRoot بمثابة مجموعة سيطرة ايجابية ، والاخرمملوء بمادة السداده المانعه للتسرب النانوية المحضرة (مجموعة التجربة) ، وتم ترك الاخر فارغ بمثابة مجموعة سيطرة سلبية، وقد تم زرعهم في جيوب صنعت في النسيج الضام على ظهور الأرانب المخدرة. ثم قسمت الأرانب إلى أربع مجموعات متساوية حسب فترات المراقبة. تم جمع خزعات الأنسجة ل (3 ، 7 ، 14 ، 28) يومًا بعد الزرع. تمت معالجة العينات وتلوينها باستخدام الهيماتوكسيلين والأيوسين وفحصها مجهريًا. تم إجراء التحليل الإحصائي بواسطة اختبارات Kruskal Wallis و Mann-Whitney لتحليل استجابة الأنسجة الضامة لكل مجموعة لجميع أوقات المراقبة. **النتائج:** نسيجيا في مدة 3 أيام بعد الزرع مجموعة المراقبة السلبية والايجابية أظهرت تفاعل التهابي شديد، بينما المجموعة التجريبية اظهرت التهاب الأنسجة المتوسط. تم قلت التفاعلات النسيجية الالتهابية هذه مع مرور الوقت لجميع المجموعات حتى اختفت تمامًا في اليوم 28 ولكنها لا تزال أسرع بالنسبة إلى السداد التجريبي. اظهرت جميع المجموعات عن تكون كبسولة ليفية رقيقة كاملة وبشكل منظم في اليوم 28 تمثل تأقلم الأنسجة الضامة للمواد المزروعة. **أما** إحصائياً، اظهر مانع التسرب النانوي التجريبي أقل تفاعل التهابي للنسيج الضام بين المجموعات ولم تكن هناك فروق ذات دلالة إحصائية في سمك الكبسولة الليفية بين المجموعات. **الاستنتاجات:** إن مانع التسرب النانوي المحضر اظهر توافقًا حيويًا عاليًا مقارنة بالمجموعات الأخرى.

DOI: 10.33899/rdenj.2022.132515.1149

INTRODUCTION

Biocompatibility is an essential requirement of any root canal sealer; as the root filling material constitutes a true implant coming into direct contact with the vital tissue at the apical and lateral foramina of the root [1,2]. The sealer is said to be biocompatible, when it comes into contact with the periapical tissue; fails to trigger an adverse reaction such as toxicity, irritation, inflammation or necrosis and it can be permitted or induced periapical tissues healing and repair [3,4].

The chemical composition of the endodontic sealer may influence positively or negatively on the final result of the endodontic therapy. Therefore, it must be nonirritating and biocompatible with the living connective tissues [5,6].

Bioceramic sealers have been brought into the scope of endodontic sealers due to the following properties: high pH, excellent seal (bond chemically and micromechanically to dentin), biocompatible, bioactive, and permit healing of the surrounding periapical tissue [7,8].

Many researchers reported that the production of sealers with nanosize level can improve their physicochemical characteristics, increasing the biocompatibility and biomineralization abilities, enhancing their antibacterial property, and provide good sealing ability [9,10].

Biocompatibility of endodontic materials is usually evaluated by in vitro cytotoxicity using cell culturing methods or in vivo implantation (subcutaneous and/or intraosseous) using laboratory animals. However, although the cell culturing methods give some valuable information about the response of specific cells to the tested material, they do not provide the full picture of how a tissue reacts to the material and cannot reflect the healing reactions of living tissue under in vivo conditions [11,12].

The purpose of this study was to evaluate the reaction of the subcutaneous connective tissue to a newly prepared sealer.

MATERIALS AND METHODS

Preparation the Root Canal Nanosealer

The sealer consists of powder part and liquid part; after several pilot studies, the final formula for the prepared sealer that gave the best clinical sealer consistency and properties within the limits of ANSI/ADA Specification No.57/2012[13] was as the following. The powder part consists mainly of nano CaO (44%) in addition to Glutamic amino acid, zirconium oxide (20nm), and silica oxide (15-20)nm, while the liquid part consists mainly from distilled water (82%) in addition to propylene glycol. The powder/liquid ratio was 1g powder part to 0.3ml liquid part and the mixing time was 38 second.

Biocompatibility Test

The biocompatibilty study was conducted according to the ISO 10993-6[14] (Biological evaluation of medical devices: Tests for local effects after implantation).

Twenty healthy male albino rabbits with approximately (1.5±0.25kg) in weight and 4-6 months of age were used in this study. The use of the rabbits was approved by the research ethics committee with REC reference No. (UoM.Dent/A.L.24/21) in (16/03/2021), College of Dentistry/University of Mosul/Iraq. The animals were divided randomly into four equal groups according to the biopsies collection after implantation periods (3, 7, 14, and 28) days.

The rabbits were housed in private veterinarian clinic throughout the study time and under supervision of veterinary physician for preserving their health and habitual situation.

Each rabbit was generally anesthetized using intramuscular administration of a rodent anesthesia. The dorsal skin of the rabbits was shaved and disinfected. Subcutaneous trichotomy 10 mm lengths were made with a sterile blade No. 10 on the back of rabbits along the spine in a head-tail orientation. Two incisions were made on the left half and one on the right half 2 cm from the spine and 4 cm apart from each other.

Each animal received three polyethylene tubes (1.5 mm inner diameter, 10 mm length); one tube filled with BioRoot sealer (+ve control) was implanted on the left side near the head, and one tube filled with the experimental sealer was implanted on the right side near the head. The tube filled with standard sealer mass and inserted immediately to each corresponding incised pocket. The third incision received an empty tube as a (-ve) control implanted on the left side near the tail.

After that the margins of the wounds were closed with 3/0 black silk suture, and disinfected with Oxytetracyclin solution.

At the end of each implanted periods five animals were sacrificed by anesthetic overdose. The tubes were removed together with the surrounding tissues as a block section (20 x 20 mm), and fixed in 10% formaldehyde solution for 24hours.The tissue samples were processed for paraffin embedding and subjected to 5 μm thickness longitudinal serial sections using microtome, and then the resultant slides were stained with hematoxylin and eosin.

Histological analysis for all slides was performed by experienced oral pathologist blinded to materials type and implantation intervals using Olympus light microscopy at 40X magnification. The intensity and the degree of inflammatory reaction were evaluated at the connective tissue adjacent to the open end of tube. The inflammatory events were scored based on the following FDI criteria [15]: Grade 0: No inflammatory cells or presence 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 (more than 125) inflammatory cells.

While for fibrous capsule; thickness was evaluated using micrometer lens at 40X and considered as thin when it was < 150 µm and thick when it was > 150 µm.

Kruskal-Wallis test was used to evaluate the scores of inflammatory tissues reaction and Mann-Whitney test were done at 0.05 significant levels to comparison the difference in tissues response among groups at different implantation period.

RESULTS

Groups Comparison Histopathologically

Histopathological representative images of testing material for the implantation periods (3,7,14, and 28) days are illustrate in figure (1).

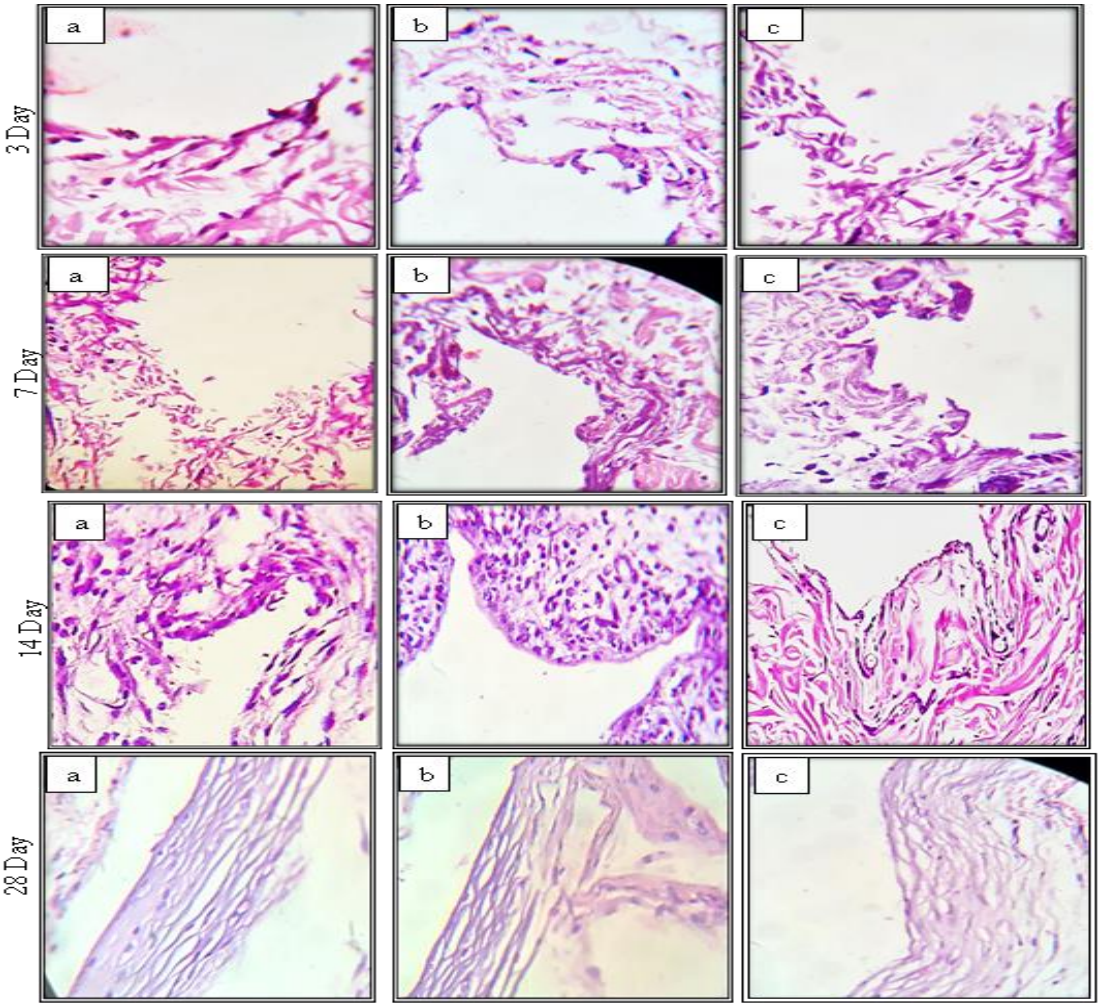

Figure (1): Microscopical Image Representing the Inflammatory Tissues Reaction in (-ve) Control Group/Empty Tube (a), (+ve) Control Group/BioRoot Sealer (b), and Experimental Sealer Group (c) at 3,7,14, and 28 Days after Surgical Implantation at (40X) magnification.

In three day, observation period; both of (-ve) control and BioRoot sealer groups showed sever to moderate tissues reaction, slight granulation tissue formation and some fibroblasts with not well defined fibrous capsule. While the experimental sealer group showed equal slides (50:50) with mild and moderate tissues reaction with slight granulation tissue and sufficient fibroblast number with not well-defined fibrous capsule.

In seven day, observation period; the (-ve) control group revealed moderated to mild inflammatory cell infiltration with few granulation tissue formation and fibroblast number and thick fibrous capsule.

The BioRoot group showed moderated inflammatory tissues reaction with moderate less organized granulation tissue formation and sufficient fibroblast number and thick fibrous capsule. While in the experimental sealer group there is mild to moderate inflammatory cell infiltration with more organized granulation tissue full about half the field of the slide and thick fibrous capsule.

In fourteen-day, observation time; The (-ve) control and BioRoot sealer groups represented mild to moderate inflammatory cell infiltration with moderate organized granulation tissue formation and sufficient fibroblast number and thick fibrous capsule. While the experimental group showed mild to absence inflammatory cell infiltration and tissue reaction with profound more organized granulation tissue and thick fibrous capsule.

In twenty-eight-day, observation time; all the groups gave the same histological picture by complete resolution of the inflammatory tissue's reaction. The granulation tissues began to the remodeling with more organized collagen fiber arrangements and new blood vessels formation, and there is a uniform regular thin fibrous capsule forms around the implanted tube which is surrounded by mature connective tissue.

Groups Comparison Statistically

The mean values and the frequency of the inflammatory cells scores and the proportion of fibrous capsule thickness for the (-ve) control group (empty tube), (+ve) control group (BioRoot sealer), and experimental sealer group at different observation times were represented in table (1).

Table (1): Mean and Standard deviation of the Inflammatory Tissues Response for Testing Groups at different Observation Periods

Times Periods	N	Mean ± Standard Deviation	Scores of Inflammatory Cells (% of Frequency)				Proportion of Fibrous Capsule Thickness
			0	1	2	3	
(-ve) Control/Empty Tube							
3 day	10	2.40 ± 0.51			60%	40%	100% (1)
7 day	10	1.60 ± 0.51		40%	60%		100% (2)
14 day	10	1.20 ± 0.42		80%	20%		100% (2)
28 day	10	0.00 ± 0.00	100%				100% (1)
(+ve) Control/ BioRoot Sealer							
3 day	10	2.60 ± 0.51			40%	60%	100% (1)
7 day	10	2.00 ± 0.00			100%		100% (2)
14 day	10	1.40 ± 0.51		60%	40%		100% (2)
28 day	10	0.00 ± 0.00	100%				100% (1)
Experimental Sealer							
3 day	10	1.50 ± 0.52		50%	50%		100% (1)
7 day	10	1.30 ± 0.48		70%	30%		100% (2)
14 day	10	0.6 ± 0.51	40%	60%			100% (2)
28 day	10	0.00 ± 0.00	100%				100% (1)

* Statistically Significant Differences; NS Not Significant
SD: Standard Deviation

The groups showed different tissues inflammatory response. However, all the groups showed decreased in the inflammatory tissues response over the times after implantation until no tissue inflammatory reactions remained at 28 day as represent in figure (2).

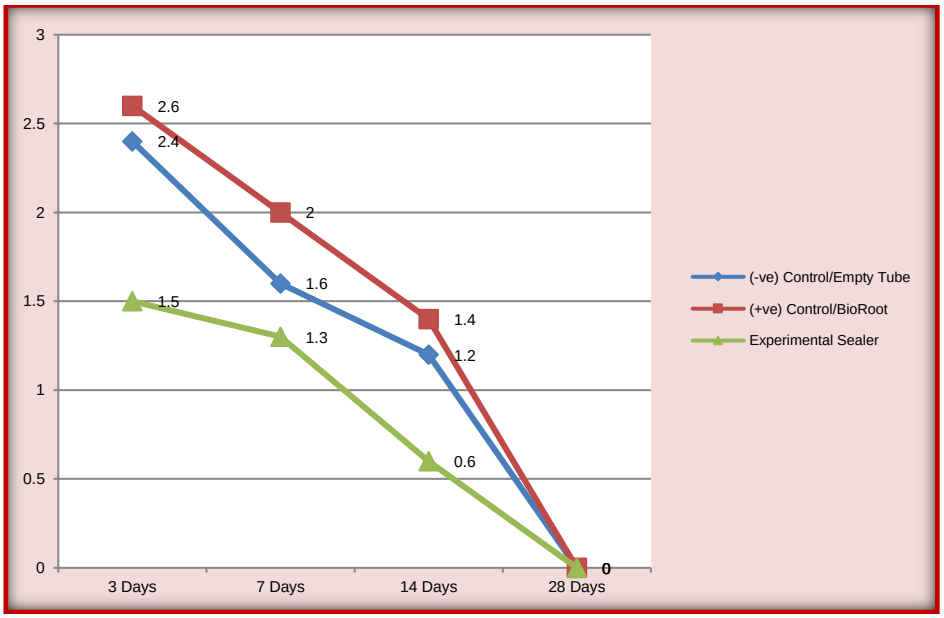

Figure (2): Histogram Representing the Evanescence of Inflammatory Tissues Response over Observation times.

The thickness of fibrous capsule increased at the (7 and 14) day after implantation for all groups then decrease over time to be thin organized capsule

around the material with no statistical differences in the fibrous capsule thickness scores among the groups.

Kruskal-Wallis test was done at 0.05 significant to compare the effect of each material on the severity of tissues reaction. Kruskal Wallis test revealed statistically significant differences among the material's effects on the inflammatory tissue reaction at (3, 7, and 14) day as shown in table (2). Mann-Whitney test was used at 0.05 significant levels to see which pairs of groups differ significantly as shown in table (3).

Table (2): Kruskal-Wallis Test for Comparison the Mean for Severity of Inflammation of Different Materials after Implantation Periods

Materials Groups	N	Mean ± SD	Chi-Square	P-value
3 Day				
(-ve) Control/Empty Tube	10	2.40 ± 0.51		
(+ve) Control/BioRoot	10	2.60 ± 0.51	13.533	0.001*
Experimental Sealer	10	1.50 ± 0.52		
7 Day				
(-ve) Control/Empty Tube	10	1.60 ± 0.52		
(+ve) Control/BioRoot	10	2.00 ± 0.00	10.268	0.006*
Experimental Sealer	10	1.30 ± 0.48		
14 Day				
(-ve) Control/Empty Tube	10	1.20 ± 0.42		
(+ve) Control/BioRoot	10	1.40 ± 0.51	10.009	0.007*
Experimental Sealer	10	0.60 ± 0.51		

* Statistically Significant Differences at (P≤0.05)

Table (3): Mann-Whitney test for Revealing the Significantly Difference in Tissues Response for each Pairs of Groups after Implantation Periods

Materials Groups	N	Mean ± SD	Z-value	P-value
3 Day				
(-ve) Control/Empty Tube	10	2.40 ± 0.51	0.872	0.383NS
(+ve) Control/BioRoot	10	2.60 ± 0.51		
(-ve) Control/Empty Tube	10	2.40 ± 0.51	2.936	0.003*
Experimental Sealer	10	1.50 ± 0.52		
(+ve) Control/BioRoot	10	2.60 ± 0.51	3.245	0.001*
Experimental Sealer	10	1.50 ± 0.52		
7 Day				
(-ve) Control/Empty Tube	10	1.60 ± 0.51	2.179	0.029*
(+ve) Control/BioRoot	10	2.00 ± 0.00		
(-ve) Control/Empty Tube	10	1.60 ± 0.51	1.314	0.189NS
Experimental Sealer	10	1.30 ± 0.48		
(+ve) Control/BioRoot	10	2.00 ± 0.00	3.199	0.001*
Experimental Sealer	10	1.30 ± 0.48		
14 Day				
(-ve) Control/Empty Tube	10	1.20 ± 0.42	0.951	0.342NS
(+ve) Control/BioRoot	10	1.40 ± 0.51		
(-ve) Control/Empty Tube	10	1.20 ± 0.42	2.439	0.015*
Experimental Sealer	10	0.60 ± 0.51		
(+ve) Control/BioRoot	10	1.40 ± 0.51	2.757	0.006*
Experimental Sealer	10	0.60 ± 0.51		

NS Not significant; * Statistically Significant Differences at (P≤0.05)

DISCUSSION

When sealer extruded through the apical foramen and even when kept within the canal space, an inflammatory response of varying intensity usually develops in the area where the sealers contact the vital apical and periradicular tissues [16,17].

After three day observation, moderate to severe infiltration of inflammatory cells (neutrophil infiltration) was observed in the (-ve) control and BioRoot sealer groups. These tissues response may be due to the trauma of the surgical procedure for tube implantation as reported in many studies [1,8]. However, the experimental sealer group showed moderate to mild infiltration of inflammatory cells; this less tissues reaction may be associated with the experimental sealer's composition and properties.

Periapical tissue reaction after root canal treatment may be influenced by various factors depending on the chemical nature of the endodontic sealer. Studies shown that most of bioceramic sealers have been found to be biocompatible and this was attributed to their ability to form $Ca(OH)_2$, and $Ca_3(PO_4)_2$ byproducts and this can promote the biomineralization and osseo-conductivity event when the sealer extruded through the apical foramen during root canal filling [2,18,19].

In addition, alkaline pH could neutralize the lactic acid from osteoclasts and prevent dissolution of mineralized components; this will in turn stimulate the deposition of hard tissue and accelerate the healing process [5,12].

From other side, studies showed that the use of scaffold containing extracellular matrix protein that containing acidic amino acid like (Aspartic and Glutamic) will enhance the cell adhesion, proliferation, and differentiation [20,21].

Studies reported that the nanosized sealer's particles may promote the adhesion, differentiation, and proliferation of the cells leading to a faster repair and healing [9,10].

After seven days observation the (-ve) control and experimental sealer groups exhibited a reduction in inflammatory cell infiltration, while the BioRoot sealer group exhibited moderate inflammatory infiltration and this may be due to highly solubility for BioRoot sealer [8,22].

After fourteen-day observation, the (-ve) control and BioRoot sealer groups exhibited more tendencies to mild inflammatory tissues reaction, while the experimental sealer group exhibited mild to absence inflammatory infiltration. However, all groups exhibited large amounts of collagen fibers and new blood vessels indicating normal tissues formation, although the experimental sealer group represented the larger amount of fibroblast cell and the more organized collagen fiber.

The intensity of the reaction was diminished by the day 14, and this reduction continued progressively through

the 28 days indicating the biocombatibilty of the tested materials [11,12].

All tested groups demonstrated a significant reduction in the inflammatory reaction throughout the experimental periods and the healing occurred by surrounding the tested materials with thin fibrous capsule. The presence of thin organized fibrous capsule at the end of tissues healing surrounding the tested material indicated that the material was well tolerated by the tissue [4,18].

Declaration of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

REFERENCES

1. Fonseca DA, Paula AB, Marto CM, Coelho A, Paulo S, Martinho JP, Carrilho E, Ferreira MM. Biocompatibility of Root Canal Sealers: A Systematic Review of In Vitro and In Vivo Studies. *Materials*. 2019; 12(24): 4113-4147.
2. Jafari F, Jafari S. Composition and physicochemical properties of calcium silicate based sealers: A review article. *J Clin Exp Dent*. 2017; 9(10): e1249-e1255.
3. De Oliveira NG, Araújo PR, Da Silveira MT, Sobral AP, Carvalho MV. Comparison of the biocompatibility of calcium silicate-based materials to mineral trioxide aggregate: Systematic review. *Eur J Den*. 2018; 12(2): 317-326.
4. Silveira CM, Pinto SC, Zedebski Rde A, Santos FA, Pilatti GL. Biocompatibility of four root canal sealers: a histopathological evaluation in rat subcutaneous connective tissue. *Braz Dent J*. 2011; 22(1): 21-27.
5. Bueno CR, Valentim D, Marques VA, Gomes-Filho JE, Cintra LT, Jacinto RC, Dezan-Junior E. Biocompatibility and biomineralization assessment of bioceramic, epoxy, and calcium hydroxide-based sealers. *Braz Oral Res*. 2016; 30(1):S1806-S1815.
6. Abu Zeid ST, Saleh AA. Solubility, pH Changes and Releasing Elements of Different Bioceramic and Mineral Trioxide Aggregate Root Canal Sealers Comparative Study. *J Trauma Treat*. 2015; 4(2): 249-252.
7. Donnermeyer D, Bürklein S, Dammaschke T, Schäfer E. Endodontic sealers based on calcium silicates: a systematic review. *Odontology*. 2019; 107(4):421-436.
8. El-Mansy LH, Ali MM, Hassan R, Beshr KA, El Ashry SH. Evaluation of the Biocompatibility of a Recent Bioceramic Root Canal Sealer (BioRoot™ RCS): In-vivo Study. *J Med Sci*. 2020; 8(D):100-106.
9. Alenazy MS, Mosadomi HA, Al-Nazhan S, and Rayyan MR (2018) Clinical considerations of nanobiomaterials in endodontics: A systematic review; *Saudi Endod J*; 8(3):163-169.

10. AlKahtani RN (2018) The implications and applications of nanotechnology in dentistry: A review, *Saudi Dent J*; 30(2): 107-116.
11. Saad AY. Physicochemical, Cytotoxicity, and Biological Properties of Calcium Silicate-Based Root Canal Sealers: A Literature Review. *Saudi Endod J*. 2020; 10(3):173-180.
12. Benetti F, Queiroz I, Cosme-Silva L, Conti L, Oliveira SH, and Cintra LT (2019) Cytotoxicity, Biocompatibility and Biomineralization of a New Ready-for-Use Bioceramic Repair Material, *Braz Dent J*; 30(4): 325-332.
13. American National Standards Institute/American Dental Association (ANSI/ADA). Specification no. 57: *Endodontic sealing materials* (2012). Chicago: 1-16.
14. International Organization for Standardization. (2007). Biological evaluation of medical devices-Part 6: Tests for local effects after implantation. In: International Standard ISO 10993-6. 2nd Edn. ISO: Geneva, Switzerland; Pp. 1-28.
15. Stanford JW (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(2): 141-188.
16. Ricucci D, Rôças IN, Alves FR, Loghin S, Jr JF. Apically extruded sealers: fate and influence on treatment outcome. *J Endod*. 2016; 42(2): 243-249.
17. Khashaba RM and Moussa MM (2016) In Vivo Biocompatibility of Four Endodontic Sealers: A Comparative Study, *Madridge J Dent Oral Surg*; 1(1): 4-12.
18. Monea M, Bereșescu G, Stoica A, Ștefănescu T. Biocompatibility of Calcium Hydroxide – Based Root Canal Sealers: A Histological Study, *Key Engineering Materials*. 2016; 695: 243-246.
19. Sfeir G, Zogheib C, Patel S, Giraud T, Nagendrababu V, Bukiet F. Calcium Silicate-Based Root Canal Sealers: A Narrative Review and Clinical Perspectives. *Materials*. 2021; 14(4): 3965-3986.
20. Wang H, Li Y, Zuo Y, Li J, Ma S, Cheng L. Biocompatibility and osteogenesis of biomimetic nano-hydroxyapatite/polyamide composite scaffolds for bone tissue engineering. *Biomaterials*. 2007; 28(22): 3338–3348.
21. Ouellette RJ, Rawn JD. Amino Acids, Peptides, and Proteins. In: Principles of Organic Chemistry, 1st ed, 2015, Elsevier Inc., Ch:14, pp: 371-393.
22. Siboni F, Taddei P, Zamparini F, Prati C, Gandolfi M. Properties of BioRoot RCS, a Tricalcium Silicate Endodontic Sealer Modified with Povidone and Polycarboxylate. *Int Endod J*. 2017; 50(52): e120–e136.