

EVALUATION OF BIOCOMPATIBILITY OF TWO BIOACTIVE COMPOSITE RESIN MATERIALS VERSUS MTA: A COMPARATIVE HISTOPATHOLOGICAL STUDY

Fawzy A. Darweesh*, Mohammad S. Elnawawy**
Enas T. Enan*** and Tayseer Mohamed Maaly****

ABSTRACT

Objective: This study was conducted to evaluate the biocompatibility of two bioactive materials, Cention N (CN) and ACTIVA bioactive, then compare the result with that of Mineral Trioxide Aggregate (MTA) as a retrograde filling materials.

Methods: Sixty Albino male rats were used in the study, four polyethylene tubes were subcutaneously implanted into the rats, an empty one was used as a control group, and the other three tubes were filled with ACTIVA, Cention N and MTA-HP. Rats were then divided into three groups (n = 20 rats) following the sacrifice time into three intervals: one, two and three weeks, then, the tissue specimens were analyzed histopathologically. The collected data were subjected to statistical analysis.

Results: ANOVA test showed that the lowest inflammatory reaction was recorded for ACTIVA while a high degree of chronic inflammation was recorded for MTA group, with highly significant differences, $P < 0.001$. ACTIVA bioactive material exhibited observable decline of inflammation and fibrosis in a comparison to the control.

Conclusions: The two bioactive materials showed a higher biocompatibility comparable to MTA. ACTIVA showed the highest degree of biocompatibility related to the decreased intensity of inflammation, with well-formed fibrous connective tissue conversion and improved healing patterns.

KEYWORDS: Biocompatibility, Bioactive, ACTIVA, Cention N.

* Assistant Professor, Oral Biology Dep. Faculty of Dentistry, Zagazig University

** Assistant Professor, Conservative Dentistry Dep. Faculty of Dentistry, Mansoura University

*** Associate Professor, Department of Dental Biomaterials, Faculty of Dentistry, Mansoura University, Mansoura, Egypt.

**** Assistant Professor, Dental Biomaterials Department, Faculty of Dentistry, Zagazig University

INTRODUCTION

Non-surgical root canal treatment is a probable treatment plane in many endodontic cases, although surgery may be selected for teeth with insistent periapical pathogenesis which is not reactive to non-surgical means or once non-surgical retreatment is unsuccessful⁽¹⁾. Surgical root canal remedy often includes resecting a part of apex of the root, preparing and filling a cavity in the root-end. The aim of retrograde filling is to cap the root canal so as to avoid the entrance of microorganisms or their toxins from the space of canal into peri radicular tissues⁽²⁾. The corresponding advance of different instruments and materials, together with good thoughtful of wound healing biology, made surgical managing practicable substitute to extraction and replacement of the tooth other than a treatment of previous option⁽³⁾. Surgical root canal treatment, that might comprise apicectomy in which a consequent contact between adjacent periapical tissues with root-end filling material may happen, producing an exciting state for endodontists⁽⁴⁾.

Root filling and repair materials included in endodontic surgery and root canal therapy are significant for the healing and advance of periapical periodontitis, particularly when this condition is associated with bone defects. Perfect repair and root filling materials would be biocompatible, non-cytotoxic, osteoinductive, simply manipulated, mechanically and chemically constant and radiopaque⁽⁶⁾. Over the last years, a lot of restorative and endodontic supplies have been planned for root-end filling, as zinc oxide eugenol, amalgam, polycarboxylate cement, glass ionomer cement, composite resin, and Cavit^(6,7). Inappropriately, the ideal retrograde filling material is not found yet. Mineral trioxide aggregate (MTA) was recommended primarily as a root-end filling material. Also, it was used in pulpotomy, pulp capping, and repair of root perforations⁽⁸⁾. MTA is the prime type of repair and root-end filling materials and is extensively used as a case to assess current materials⁽⁹⁾. Yet, it has many drawbacks⁽¹⁰⁾, as difficult handling steps, extended setting time, consis-

tency like sand, in addition to staining of the tooth. The mechanical properties of materials affected by the long setting time, complex clinical steps are required and contamination hazard is augmented⁽¹¹⁾. This requires an improvement in physical characteristics to avoid these disadvantages⁽¹²⁾.

Composite resin was recommended for root end filling, though its biocompatibility is influenced by the nature of its leachable constituents⁽¹³⁾. In addition to release of enough amounts of formaldehyde into water producing local allergic reactions⁽¹⁴⁾. Ethylene glycol dimethyl acrylate and diethylene glycol methacrylate have cytotoxic effects and enhancement of bacterial growth leading to an inflammatory response and tissue injury in animal models^(15,16). ACTIVA is the principal bioactive composite resin containing an ionic resin matrix, bioactive fillers and a shock-absorbing resin factor that have the chemical and physical characters of natural dentition. It releases phosphate, calcium, and fluoride ions and also recharges with them⁽¹⁷⁾. ACTIVA cause an accepted reaction that stimulates process of remineralization, and apatite crystal creation that connects the tooth and the restoration with each other, sealing the margins against micro leakage and failure. Hydrophilicity of resin matrix is an important property as it facilitates the diffusion of calcium, phosphate, and fluoride ions, and considered a prerequisite for bioactive materials⁽¹⁸⁾. The long-recognized clinical efficacy of glass ionomer cements in dental field proposes their application for peri radicular surgery due to formation of chemical bond to dentin, slight irritation of the tissues, and bone mineralization due to fluoride discharge⁽¹⁹⁾.

Cention N (CN) was presented in the market as a novel restorative material. It is an "alkasite" restorative. Alkasite is a new type of restorative material, that like ormocer or compomer materials which considered basically a subgroup of the composite resin restorations. The original group pays alkaline filler that able to discharging acid-neutralizing ions.⁽²⁰⁾ Numerous studies with a variety of test materials and methods have been led to evaluate the biocom-

patibility. The principal method is cytotoxicity test on cell or tissue cultures or grafting into bone or subcutaneous connective tissue in trial animals⁽²¹⁾. Despite the fact that ACTIVA and Cention N have a wide range of applications in cavity restoration as bioactive resin composite, a possible application may be as retrograde filling materials. Till now, no data was recorded about the biocompatibility of Cention N. Thus, the present study was conducted to assess the biocompatibility of ACTIVA, Cention N and MTA.

MATERIALS AND METHODS

Materials that were used in this study are shown in **Table 1**.

Animal study and Grouping:

Animal selection: Animals were selected from the Medical Experimental Research Center at Faculty of Medicine, Zagazig University., Egypt. Rats were around 2 months during performing the experiment and were kept in polycarbonate cage with wire lids in ordinary condition with light standard conditions (light: dark, 13h–11h) and libitum. Animal experiments were accomplished following the criteria of examination and ethics committee of the community rules associated with experimental animals. Sixty male rats (*Rattus norvegicus* Albinus, Wistar) that weighed about 180–200g, utilized along with the guidelines of ARRIVE (2013, The Animal Research Reporting an In Vivo Experiments). Steps of the study followed the ISO 10993-1 (1992) and ISO 109932 (1992)

standards^(22, 23). Rats were divided following three different intervals.

Animal grouping: Cleaning of the cages was done every day, allowing entrance of water and food. Water was permitted for animals only 12h prior to the surgery. A sterilized polyethylene tubes (10mm and 1.3mm, length and diameter respectively) were utilized and the test materials were inserted inside them, then tubes were imbedded into the dorsal section beneath skin of every rat, then they were divided into four groups according to the test material (group, n=60 tubes) :

Group 1: Empty polyethylene tubes, act as control group.

Group 2: Polyethylene tubes filled with restorative ACTIVA.

Group 3: Polyethylene tubes filled with alkasite composite.

Group 4: Polyethylene tubes filled with MTA-HP.

The materials were used following manufacturers' instructions, **the first material**, ACTIVA bioactive material is a two-paste system supplied in an automix syringes, it was dispensed into the polyethylene tube using a mix tip placed on top of the syringe then light cure using light-curing unit (LEDition, Ivoclar Vivadent, Germany) with light intensity of 600 mW/cm². **The second material**, CN supplied in the form of powder and liquid, the ratio was 1 scoop of powder to a drop of liquid, parallel to the weight ratio of 4.6–1: powder/liquid, the liquid contains dimethacrylate

TABLE (1): Materials used in the study.

| Material | Constituents | Manufacturer |
|-----------|---|---|
| ACTIVA | Methacrylates and Diurethane, Polyacrylic acid | Pulpdent, Watertown, MA, USA |
| Cention N | Powder: silicate glass filler and calcium fluorosilicate and Liquid contains Urethanedimethacrylate | Ivoclar Vivadent Schaan FL-9494 Liechtenstein |
| MTA-HP | Powder: CaO, K ₂ O, SiO ₂ , Al ₂ O ₃ , SO ₃ Liquid: water and plasticizer | Angelus Co. Londrina, Brazil |

and initiators, while the powder composed of glass fillers. Powder and liquid mixed manually. **The third material**, MTA that prepared by mixing three measures of powder with one measure of water obtaining paste like consistency. Glass slab and plastic spatula were used for mixing. It was inserted into the tubes using a condenser with a fine tip. The tubes were completely occupied by the tested materials then allowed for setting.

Prior to the surgery, intraperitoneal injection of ketamine hydrochloride and xylazine hydrochloride (3, 1 respectively; 0.05mL/100g of weight; I/P) was performed to anesthetize the animals. The dorsal area of rats was sent to trichotomy, and sterility was done by 0.12% chlorhexidine utilizing gauze. Gauze saturated with saline solution was used to swap the dorsal section, to eliminate remnants of antiseptic solution. Four incisions were made on the dorsal area of rats (two anteriorly and two posteriorly). Pinching skin that located adjacent to the incisions, in addition to subcutaneous separation was performed by a scissors with blunt-end. Four tubes per every animal: two tubes into the anterior dorsal area and two tubes in the posterior area. Nylon 3-0 sutures were used to close the incisions. Along with the time period of scarification, the rats were additionally, subdivided into three groups according to time interval: one, two and three weeks. At the end of each week, twenty animals received Intraperitoneal injection of xylazine hydrochloride and ketamine hydrochloride (one-to-one; 0.15mL/100g of weight; I/P). Skin Biopsies and subcutaneous tissues measuring (2× 2cm) including the implants were taken over one-cm safety margins.

Histological steps

The subcutaneous tissues with the tubes were excised, and inserted in 10% neutral formalin for 48h. Then, trimming of the specimens was done parallel to the tube and about 2mm of tissue was left on each side, separated into two identical halves, and the tubes were removed, the tubes were not removed before fixation for two reasons, first

reason is to avoid tearing or damage of the excised tissues during tubes removal, second reason is that tubes considered as a guide during tissue trimming after fixation. The specimens were dehydrated by insertion in an ascending concentration of ethyl alcohol, and then they were cleared in xylene and then inserted in paraffin at 58–62 C°. Samples were moulded parallel to the long axis of the tube to display the required area (tube opening) then serial slices of four µm thickness were set for staining with hematoxylin & eosin stain (H&E).

Statistical methods

Analysis of data was performed by Statistical Package of Social Science software computer program (SPSS, Inc., Chicago, IL, USA) version 26. Data was supplied as mean and standard deviation. ANOVA, One-way Analysis of variance and Tuckey were utilized to compare the quantitative parametric data of more than two groups. Statistical significance between groups when P value<0.05.

RESULTS

Histopathological findings (Fig.1)

After one week, control group (empty tube) showed mild inflammatory reaction associated with minimal necrotic tissue. ACTIVA group revealed mild to moderate inflammatory reaction around the tube, there was mild inflammatory response with mild inflammatory cells infiltration. CN group revealed mild to moderate inflammatory reaction with mild inflammatory cells infiltration. MTA-HP group showed Mild to moderate inflammatory reaction.

After 2 weeks, control group showed mild inflammatory cells infiltration. ACTIVA group demonstrated obvious decrease in inflammation with decrease in number of inflammatory cells. CN group demonstrated marked decrease in inflammation with decrease of inflammatory cells number. MTA- HP group showed similar reaction to the previous group but with a higher degree regarding inflammatory cells.

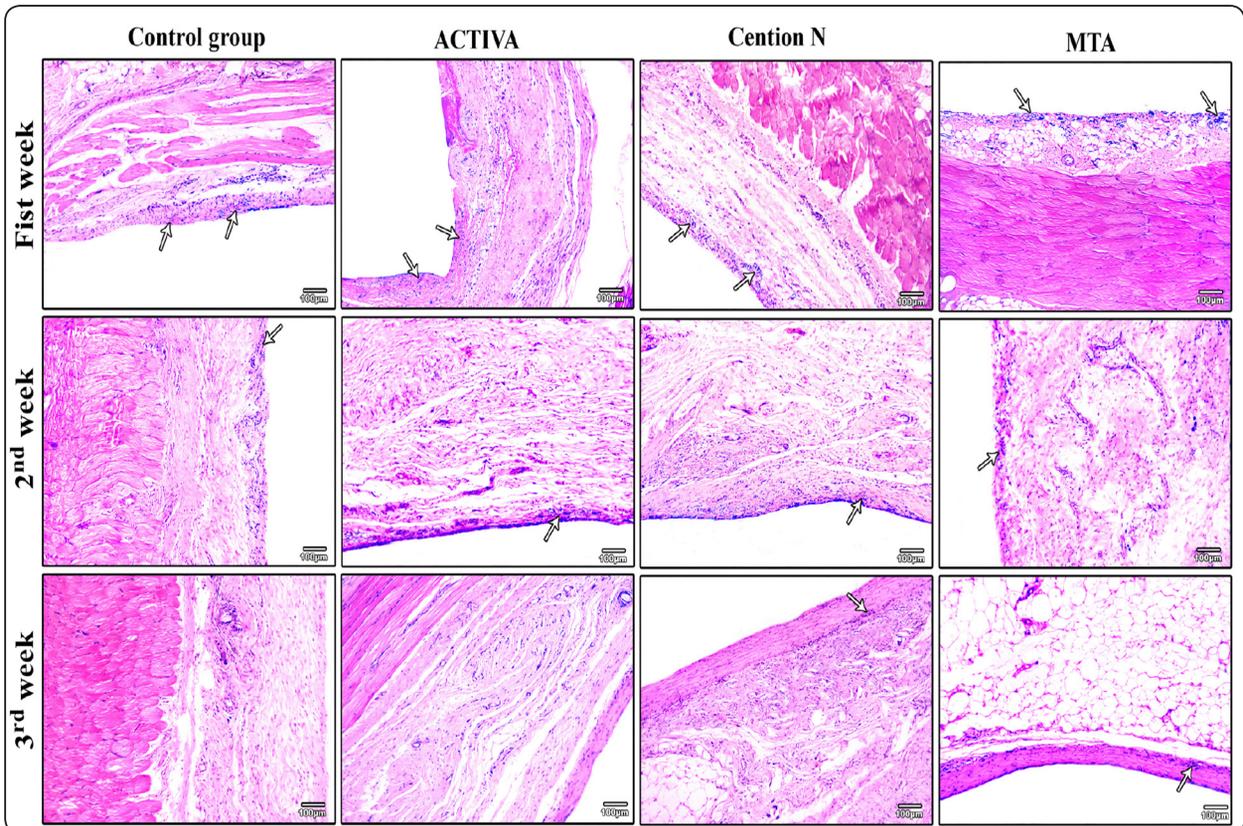


Fig. (1) Photomicrograph of H&E staining showing the tissue surrounding the tube of control, ACTIVA, Cention N and MTA groups after one, two and three weeks . **After one week**, *control tube* showing slight inflammatory response within and around the thin capsule with minimal infiltration with few inflammatory cells (arrow), *ACTIVA tube* show mild to moderate degree of inflammatory response (arrow head), *Cention N tube* show moderate inflammatory reaction (arrow), *MTA tube* show moderate degree of inflammation (arrow). **After two week**, *Control tube* show decrease of the inflammatory response (arrow), *ACTIVA tube* show marked decrease of inflammatory reaction (arrow), *Cention N tube* show intense decrease in inflammatory reaction (arrow), *MTA tube* show relatively thick inflammatory capsule (arrow). **After 3 weeks**, *control tube* show normal connective tissue capsule, *ACTIVA tube* show mild inflammatory response. *Cention N* show moderate inflammatory response (arrow), *MTA tube* showing moderate inflammatory cells infiltration (arrow) (H& E X100).

After 3 weeks, control group showed sparse inflammatory cells with normal connective tissues capsule. ACTIVA group showed few inflammatory cells. CN group showed moderate inflammatory cells infiltration. MTA- HP group showed moderate inflammatory cells infiltration.

Statistical Analysis

Mean of inflammatory response and standard deviation at all intervals among the different materials are shown in **Table 2**. Mean values of inflammatory response were declining by time for all materials, and they were statistically different

in all groups (**Fig.2**). For ACTIVA and MTA, the inflammatory response on the first week after implantation was significantly more than that of the third week post implantation ($p= 0.001, 0.019$). For CN, there were significant differences in the inflammatory response values between the different periods ($p<0.001$). ACTIVA recorded the lowest values at all intervals in comparison to other materials. Tissue reaction between ACTIVA and control group was not significant, but it was significant with MTA and CN, $P<0.001$. The highest values were recorded for MTA at all intervals with highly significant differences with other three groups, $P<0.001$ (fig. 2).

TABLE (2): Mean and standard deviation of inflammatory responses in different intervals of test materials

| | Control | ACTIVA | CN | MTA | P value |
|---------|--------------------------|-----------------------------|----------------------------|---------------------------|---------|
| W1 | 0.8830±0.1766 | 0.7190±0.1438 ^{BC} | 2.185±0.4370 ^{AB} | 5.495±1.099 ^A | <0.001* |
| W2 | 0.491±0.098 ^a | 0.646±0.129 ^{BC} | 1.474±0.295 ^{aAB} | 4.677±0.935 ^A | <0.001* |
| W3 | 0.437±0.087 ^a | 0.510±0.102 ^{aB} | 0.825±0.165 ^{abB} | 3.572±0.714 ^{aA} | <0.001* |
| P value | <0.001* | 0.019* | <0.001* | 0.004* | |

Statistical significant difference among the group with dissimilar superscript letters in the same column. Data expressed as mean±SD, *: significance≤0.05. Significance vs. Control: A, Significance vs. MTA: B, and Significance vs. CN: C. Significance vs. W1: a and Significance vs. W2:b

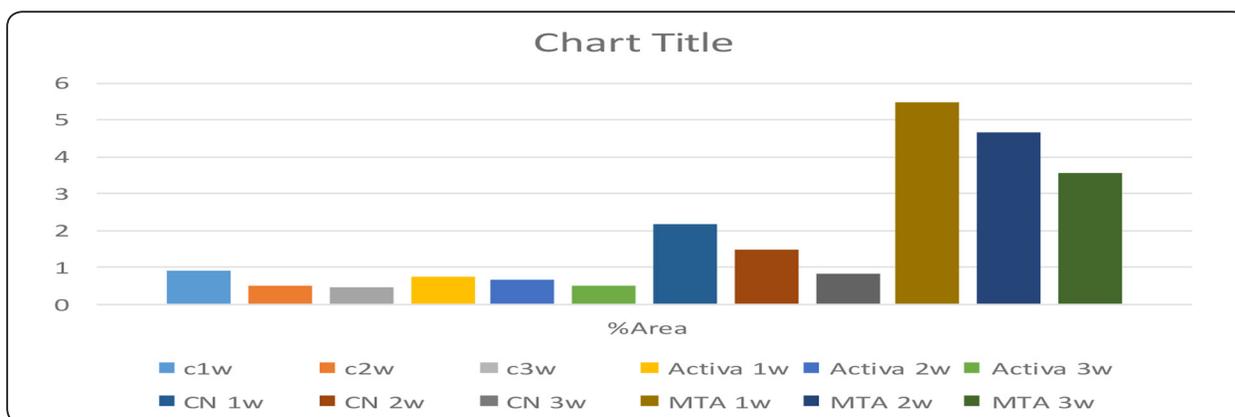


Fig. (2): Mean values of inflammatory response of different materials

DISCUSSION

Biocompatibility is the ability of the material to be used with a suitable host reaction in a certain application⁽²⁴⁾. It is necessary to evaluate the degree and potential of threats of any novel dental material before the clinical use, as presence of the material adjacent to vital tissues may produce irritant effects on these tissues. The biocompatibility can be evaluated by in vitro cytotoxicity method by cell culture, and in vivo method by subcutaneous tissue surgical placement. Subcutaneous tissue implantation of the materials is considered as an ideal method for the evaluation of its biocompatibility and therefore detect the inflammatory reactions⁽²⁵⁾. However, information obtained from laboratory animals could not be similar to that obtained from human beings, it

was considered as a good method to assess the biological properties⁽²⁶⁾. Rat subcutaneous implantation researches are satisfactory experimental methods for this evaluation⁽²⁷⁾ and implantation of the materials in trial animals can mimic in situ environments of the material⁽²⁸⁾.

Mutoh *et al.*⁽²⁹⁾ found that using sterile tubes for implanting the materials prevents the discharge of the substance into surrounding tissue. It was found that these tubes be similar to a root canal of the tooth and they are more valuable than insertion the material in a direct contact on the tissue. These tubes are made of polyethylene which is inert and appropriate for subcutaneous implantation experiments. The released material at the polyethylene tube orifice resembles the conditions of root canal treat-

ment as the interface of subcutaneous tissue-tube produces an inflammatory response and enhancement of tissue fluids and blood into the tube area⁽²⁵⁾. Our study used sterile polyethylene tubes for the implantation process. Biocompatibility of MTA has been reported, in many studies⁽³⁰⁻³²⁾ which used MTA to seal the natural, pathological, and iatrogenic communications between root canal system and periapical tissues. Previous study⁽³³⁾ reported the biocompatibility of MTA and its ability to stimulate periradicular tissues repair (used as root end filling material). MTA enhances hard tissue formation and that calcium hydroxide exhibited a similar effect⁽³³⁾. Numerous trials have been accepted to produce materials with similar bioactive characters to that of calcium silicate-based cements, and resulted in development of two bioactive resin based materials such as ACTIVA and Cention N.

Many of the endodontic materials are likely to be placed in an intimate contact with oral tissues when used for the treatment of perforations, apical barriers and retrograde fillings. Thus, such materials could be assessed accurately for both cytotoxicity and biocompatibility to certify successful treatment⁽³⁴⁾. Previously⁽³⁵⁾, ACTIVA was evaluated compared to MTA-HP regarding its biocompatibility, but according to our knowledge, no studies evaluated a combination of the three repair materials (ACTIVA, MTA and CN) histologically to detect their level of biocompatibility or cytotoxicity.

In the current study, just as shown in previous studies⁽³⁶⁾, subcutaneous implantation of the control empty tubes produced minimal reaction or mild inflammatory response that declined from first week to the third one, and this is explained by the reaction to the surgical process of implantation⁽³⁷⁾.

Following the third week post- subcutaneous implantation of ACTIVA, it was noticed progressive healing and marked decrease of inflammatory cells infiltration, a good fibrous capsule encompassed numerous collagen fibers in parallel form similar to the control group. Composition and nature of

the leachable elements of composite resin materials have great effects on the biocompatibility on the adjacent tissues⁽³⁸⁾. As referred to Table 1, ACTIVA composed of diurethane and methacrylate lacking presence of bisphenol A (BPA) glycidyl dimethacrylate (BisGMA). It is well known that (BPA) and its derivatives have high risk and reasons for irritating effect on living cells⁽³⁹⁾. This composition explained the results obtained from ACTIVA, that were consistent with a previous study which reported an excellent biocompatibility of rat subcutaneous tissues of ACTIVA and high healing capability comparable to calcium silicate- based cements^{(35),(40)}.

At the first week of follow-up interval, CN group revealed slight to moderate inflammatory reaction around the tube, with a significant increase of the inflammatory response compared to ACTIVA. However, after third week, CN group showed progressive healing and a fibrous capsule contained primarily many parallel collagen fiber and it was well formed similar to ACTIVA without any significant differences. The ion releasing property of CN has been related to its composition of alkaline fillers. It releases amounts of fluoride comparable to those of Resin Modified Glass Ionomer Cement (RMGI), this explained by its low monomer conversion and also decreased crosslinking density in polymer complex of CN. It was expected that released fluoride help reduction of dentine demineralization by stimulating development of low soluble fluorohydroxyapatite. In addition to bacterial metabolism prevention thus decreasing bacterial growth⁽⁴¹⁾. Fluoride-releasing materials have cytotoxic effect which associated with the released fluoride concentration as it can cause inflammation and apoptosis.

MTA revealed a slight to adequate inflammatory reaction that diminished by time, at the third week of follow-up interval, a mild inflammatory response only was noticed with limited organization of the fibrous capsule noticed and a higher degree regarding inflammatory cells than ACTIVA and CN⁽⁴²⁾. The results of this study, were in agreement

with many studies conducted on biocompatibility of MTA which concluded that this cement first causes a moderate inflammatory reaction which declines by time, The early inflammatory response following the MTA implantation can be related to the response to pH change, the heat produced during setting, and the formation of inflammatory cytokines like IL1 and IL6 at the start of the method^(43,44). Elimination of bismuth oxide from the ingredients of MTA HP considerably decreased the cytotoxicity influencing osteoblasts and dental pulp cells, mostly when combined with dicalcium silicate⁽⁴⁵⁾.

CONCLUSIONS

1. Following the results of our study, new bioactive resin based materials ACTIVA and CN exhibited excellent biocompatibility and they were comparable with that of MTA.
2. MTA exhibited higher inflammatory response compared to other materials.
3. Biocompatibility was improved by the third week post implantaion and ACTIVA recorded the lowest inflammatory response at all intervals while MTA showed the highest one.

REFERENCE

1. Gutmann JL: Perspectives on root-end resection. *J Hist Dent*.1999; 47(3):135-6.
2. Saxena P, Gupta S, Newaskar V.Biocompatibility of root-end filling materials: recent update.*Restor Dent Endod*. 2013; 38(3): 119–127.
3. Hargreaves KM, Cohen S, Berman LH, editors. *Cohen's Pathways of the pulp*. 10th ed. St Louis: Mosby Elsevier; 2011. p720–76.
4. de Sousa Reis M, Scarparo RK, Steier L, de Figueiredo JAP. Periradicular inflammatory response, bone resorption, and cementum repair after sealing of furcation perforation with mineral trioxide aggregate (MTA Angelus™) or biodentine™. *Clin Oral Investig*; 2019.<https://doi.org/10.1007/s00784-019-02833-z>.
5. Lv F, Zhu L, Zhang J, Yu J, Cheng X, Peng B. Evaluation of the in vitro biocompatibility of a new fast-setting ready-to-use root filling and repair material. *IntEndod J*, 2017;50(6): 540–8.
6. Glickman G, Hartwell G. Endodontic surgery. In: Ingle J, Bakland L, Baumgartner J, *dingle's Endodontics*. 6th ed. Hamilton: BC Decker Inc; 2008. P1261-94.
7. Chong S, Pitt Ford R. Root-end filling materials: rationale and tissue response. *Endod Topics* 2005; 11:114-130.
8. Torabinejad M, Parirokh M. Mineral trioxide aggregate. A comprehensive literature review—part II: leakage and biocompatibility investigations. *J Endod*. 2010;36(2):190–202.
9. Eid AA, Hussein KA, Niu LN. (2014). Effects of tricalciumsilicate cements on osteogenic differentiation of human bone marrow-derived mesenchymal stem cells in vitro. *Acta Biomaterialia* 10(7), 3327–34.
10. Parirokh M, Torabinejad M (2010). Mineral trioxide aggregate: a comprehensive literature review—Part III: clinical applications, drawbacks, and mechanism of action. *Journal of Endodontics* 36(3), 400–13.
11. Kang JY, Lee BN, Son HJ et al. (2013). Biocompatibility of mineral trioxide aggregate mixed with hydration accelerators. *Journal of Endodontics* 39(4), 497–500.
12. Parirokh M, Torabinejad M. Mineral trioxide aggregate: a comprehensive literature review—part I: chemical, physical, and antibacterial properties. *J Endod*. 2010; 36(1):16–27.
13. Geurtsen W. Biocompatibility of resin-modified filling materials. *Crit Rev Oral Biol Med* 2000; 11: 333– 55.
14. Oysaed H, Ruyter IE, Sjovik Kleven IJ. Release of formaldehyde from dental composites. *J Dent Res* 1988; 67: 1289– 94.
15. Spahl W, Budzikiewicz H, Geurtsen W. Determination of leachable components from four commercial dental composites by gas and liquid chromatography/mass spectrometry. *J Dent* 1998; 26: 137– 45.
16. Ozbas H, Yaltirik M, Bilgic B, Issever H. Reactions of connective tissue to compomers, composite and amalgam root-end filling materials. *IntEndod J* 2003; 36: 281– 7.
17. Zmener O, Pameijer CH, Hernandez S. Resistance against bacterial leakage of four luting agents used for cementation of complete cast crowns. *Am J Dent* 2014;27(1):51-55.
18. Activa (Bioactive restorative material) pamphlet, Pulpdent Co., Watertown. <https://www.pulpdent.com/activa-bioactive-white-paper/>. Accessed 5 Nov 2019.

19. Tencer F, Allen L, Woodard L. The effect of local controlled release of sodium fluoride on stimulation of bone growth. *J Biomed Mater Res* 1989;23(6):571-89.
20. Cention N (Alkaside) Ivoclar Vivadent AG/Liechtenstein. <https://www.ivoclarvivadent.in/p/all/cention-n/>. Accessed 10 Nov 2019.
21. O'Brien WJ. Biocompatibility tests. In: WJ O'Brien, ed. *Dental materials and their selection*. 3rd ed. Kimberly Drive, IL: Quintessence Publishing Company; 2002. p. 391–2.
22. Anderson JM. Biological responses to materials. *Annu Rev Mater Res*. 2001; 31(1):81–110. 17.
23. Zmener O, Lalis RM, Pameijer CH, Chaves C, Kokubu G, Grana D. Reaction of rat subcutaneous connective tissue to a mineral trioxide aggregate-based and zinc oxide and eugenol sealer. *J Endod*. 2012; 38(9):1233–8.
24. Sunnegårdh-Grönberg K. Calcium Aluminate Cement as Dental Restorative. Mechanical Properties and Clinical Durability. Umeå, Sweden: Umeå University Odontological Dissertations; 2004.
25. Shahi S, Rahimi S, Lotfi M, Yavari HR, Gaderian AR. A comparative study of the biocompatibility of three root-end filling materials in rat connective tissue. *Journal of Endodontics*. 2006; 32: 776–780.
26. Geneva. 1994. Organization IS. ISO 10993-6(F)-07-15. Biological evaluation of medical devices. Part 6. Tests for local effects after implantation
27. Moretton TR, Brown CE, Jr, Legan JJ, Kafrawy AH. Tissue reactions after subcutaneous and intraosseous implantation of mineral trioxide aggregate and ethoxybenzoic acid cement. *J Biomed Mater Res*. 2000;52:528–33.
28. Ber BS, Hatton JF, Stewart GP. Chemical modification of proroct mta to improve handling characteristics and decrease setting time. *J Endod*. 2007; 33: 1231–1234.
29. Mutoh N, Tani-Ishii N. A biocompatible model for evaluation of the responses of rat periapical tissue to a new zinc oxide-eugenol sealer. *Dent Mater J*. 2011; 30: 176–182.
30. Martinez Lalis R, Esain ML, Kokubu GA, Willis J, Chaves C, Grana DR. Rat subcutaneous tissue response to modified Portland cement, a new mineral trioxide aggregate. *Braz Dent J*. 2009;20:112–7.
31. de Moraes CA, Bernardineli N, Garcia RB, Duarte MA, Guerisoli DM. Evaluation of tissue response to MTA and Portland cement with iodoform. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2006;102:417–21
32. McNamara RP, Henry MA, Schindler WG, Hargreaves KM. Biocompatibility of accelerated mineral trioxide aggregate in a rat model. *J Endod*. 2010;36:1851–5]
33. Economides N, Pantelidou O, Kokkas A, Tziafas D. Short-term periradicular tissue response to mineral trioxide aggregate (MTA) as root-end filling material. *Int Endod J* 2003;36:44-8.
34. Minotti PG, Ordinola-Zapata R, Midenza RZ, Marciano MA, Cavenago BC, Bramante CM, Garcia RB, Duarte MAH, IGd M. Rat subcutaneous tissue response to calcium silicate containing different arsenic concentrations. *J Appl Oral Sci*. 2015;23(1):42–8.
35. Biocompatibility of new bioactive resin composite versus calcium silicate cements: an animal study.
36. Bortoluzzi EA, Broon NJ, Bramante CM, Garcia RB, de Moraes IG, Bernardineli N. Sealing ability of MTA and radiopaque Portland cement with or without calcium chloride for root-end filling. *J Endod*. 2006; 32: 897–900.
37. Aminozarbian MG, Barati M, Salehi I, Mousavi SB. Biocompatibility of mineral trioxide aggregate and three new endodontic cements: An animal study. *Dent Res J (Isfahan)* 2012; 9: 54–59.
38. Geurtsen W. Biocompatibility of resin-modified filling materials. *Crit Rev Oral Biol Med*. 2000;11(3):333–55.
39. Hanks C, Strawn S, Watahai J, Craig R. Cytotoxic effects of resin components on cultured mammalian fibroblasts. *J Dent Res*. 1991;70(11):1450–5.
40. Buonavoglia A, Lauritano D, Perrone D, Ardito F, Troiano G, Dioguardi M, Candotto V, Silvestre F, Lo LM. Evaluation of chemical-physical properties and cytocompatibility of TheraCal LC. *J Biol Regul Homeost Agents*. 2017; 31(2 Suppl 1):1–9.
41. Monomer conversion, dimensional stability, biaxial flexural strength and fluoride release of resin based restorative material containing alkaline filler.
42. Gonçalves, F.; Campos, L.M.P.; Rodrigues-Júnior, E.C.; Costa, F.V.; Marques, P.A.; Francci, C.E.; Braga, R.R.; Boaro, L.C.C. cytotoxic effect of glass ionomer on human dental pulp stem cells correlate with fluoride release. A comparative study of bulk-fill composites: Degree of conversion, post-gel shrinkage and cytotoxicity. *Braz. Oral. Res*. 2018, 32, e17].

43. Yavari H, Shahi SH, Rahimi S, Shakouhi SL. Connective tissue reaction to white and gray MTA mixed with distilled water or chlorhexidine in rats. *Iranian Endodontic Journal*. 2009; 4:25–30.
44. Escobar-García DM, Aguirre-López E, Méndez-González V, Pozos-Guillén A. Cytotoxicity and Initial Biocompatibility of Endodontic Biomaterials (MTA and Biodentine™) Used as Root-End Filling Materials. *Biomed Res Int*. 2016: 7926961.
45. Holland R, de Souza V, Nery MJ, Faraco Júnior IM, Bernabé PF, Otoboni Filho JA, et al. Reaction of rat connective tissue to implanted dentin tube filled with mineral trioxide aggregate, Portland cement or calcium hydroxide. *Braz Dent J*. 2001; 12: 3–8.
46. Chiang T-Y, Ding S-J. Comparative physicochemical and biocompatible properties of radiopaque dicalcium silicate cement and mineral trioxide aggregate. *J Endod*. 2010; 36(10):1683–7