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PHYSICAL PROPERTIES OF DIFFERENT PULP CAPPING MATERIALS AND HISTOLOGICAL ANALYSIS OF THEIR EFFECT ON DOGS'DENTAL PULP TISSUE HEALING

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ABSTRACT

Aim : This study aim to evaluate the physicochemical properties (pH, calcium ion release, solubility and setting time) of different pulp capping materials [Bioaggregate (BA), Nano Bioactive glass (NBAG), Nano MTA& MTA] in vitro, and to observe the histopathological pulp response following direct pulp capping of dog's teeth.

Material &Methods : for the weight loss, pH changes and calcium ions release, the tested specimens were immersed in distilled water. The solubility tests recorded weight loss of the test materials after immersion in distilled water. The evaluations were performed at 1h, 24h and 7 days. For the histological evaluation twenty adult dogs were used in this study, (8 teeth for each dog), classified into 4 experimental groups as follows :**Group 1:** BA. **Group 2:** NBAG. **Group 3:** MTA. **Group 4:** NMTA, the pulpal tissue response was assessed at 1 month and 3 months time intervals.

Results: Bioaggregate showed the highest mean values for ph, ca ions release and solubility tests, while MTA showed the highest setting time mean value. On the other hand, Nanobioactive glass was the only material which recorded negative solubility. Histological results showed that dental pulpal tissues of teeth capped with MTA revealed absence of inflammatoion in addition to tubular pattern dentin bridge formation at site of exposure, BA showed dentin bridge formation and minimal inflammatory reaction, NMTA and NBAG enhanced the dentin bridge thickness, but of inferior quality than MTA and BA.

Conclusion: based on histological analysis MTA and Bioaggregate are favorable materials for direct pulp capping, however BA revealed the superior physicochemical properties.

KEYWORD: Pulp capping materials – histological evaluation – physical properties – MTA- Nano MTA- Bioaggregate- Bioactiveglass

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INTRODUCTION

Dental pulp vitality is of great importance to teeth, not only for providing nutrition but also as a biological sensor to detect the outside stimuli[1]. Direct pulp capping is a well-established method of vital pulp therapy in which the exposed dental pulp is covered directly with a material that protects the pulp troduction from additional injury and permits healing and repair. A final goal of the application of capping materials is to induce the formation of dentin by pulp cells [2].

An ideal pulp capping material should have the ability of stimulating formation of reparative dentin, maintaining pulp vitality, providing effective bactericidal and/or bacteriostatic action and pulp sealing [3].

Despite Calcium Hydroxide (CH) being considered the gold standard for vital pulp therapy, considerable confusion of its use persist. Recent attempts to develop different pulp capping materials have resulted in the development of Mineral Trioxide Aggregate (MTA) which was first proposed for pulp capping in 1993[4]. when MTA was used for direct pulp capping, it showed better interaction with dental pulp tissue than did CH or acid-etched dentin bonding [5]. It has been shown to induce hard tissue formation with limited inflammation and increases dentin regeneration [6]. However, MTA has a few disadvantages such as long setting time, poor handling characteristics, discoloration and high cost[7].

The advancement of nanotechnology improved the properties and the sealability of endodontic materials [8]. Nano MTA (NMTA) is a new bioactive material with osteoinductive properties used for pulp capping and perforation repair. Also, Nano Bioactive glass (NBAG) is a kind of highly biocompatible, osteoinductive and osteoconductive calcium silicate-based biomaterial [9]. In addition, Bioaggregate (BA) is a white nanoparticle ceramic cement, composed primarily of calcium silicate, calcium hydroxide, and hydroxyapatite [10].

The physical properties of pulp capping materials as solubility, pH and calcium release can affect the pulp tissue healing. The high pH of a material provides bactericidal activity and encourages tissue repair [11]. The ability of a material to release calcium is a key factor for successful pulp capping therapies because of the action of calcium on mineralizing cells differentiation and hard tissue mineralization [12].

Therefore, this study was conducted to test different capping materials physically and examine histologically the effect of these materials on the pulp tissue of dogs' teeth.

MATERIALS AND METHODS

I- Evaluation of pulp capping materials in vitro:

a- PH test: Measurement of pH was performed with a pH meter previously calibrated with buffer solutions at pH 4.0 and 7.0 at 29ºC [13].

b- Calcium ion release test: was performed with atomic absorption spectrophotometer[14].

c- Solubility test: the samples'change in weight was recorded after different time intervals by calculatingThe difference between the original weight of each sample and its final weight [15].

d- Setting time: was measured with a vicat apparatus and recorded the time elapsed between the end of mixing when the needle failed to make a complete circular indentation on the tested material[16].

II- Histological evaluation of pulp tissue reaction to pulp capping materials:

Surgical procdure & sample selection: Twenty adult dogs apparently healthy were used in this study, with an average weight 15-18 kg body weight, and of ages ranging from 12 – 18 months.

The animals were quarantined in separate cages in the department of Veterinary surgery, Anesthesiology and Radiology Faculty of Veterinary Medicine, Suez Canal University.

Food was with held 6-8 hrs prior to surgery. Fifteen minutes before the induction of general anesthesia, each dog was premedicated with I/M injection of chloropromazine hydrochloride (Neurazine) in a dose of 1 mg/ kg [17]. General anesthesia was accomplished by I/V injection of thiopental sodium 2.5% solution until the main reflexes were abolished. The animal, except the site of operation, was draped with sterile towels. Cefotaxime was injected 30 minutes before the operation as well as wound lavage with isotonic solution in a dose of 10 ml/kg/hr (Soduim Chloride 0.9%) containing water soluble antibiotics was administered during surgery[18].

A Total of 160 incisor teeth were used in this study (8 teeth of each dog). The utilized teeth of each dog (8 teeth) were classified into 4 experimental groups as follows :

Group 1: BA, **Group 2:** NBAG, **Group 3:** MTA, **Group 4:** NMTA and the remaining tooth was used as control. To standardize the conditions, all groups were presented in each arch of each dog. Dogs were randomly subdivided into two subgroups (10 dogs each), according to the observation period tested: 1 month and 3 months.

Cavity preparation & teeth restoration: The animal was fixed on the operating table in a supine position and its mouth was opened using mouth gag with tilted head to provide an unblocked airway. Prior to operative procedure, teeth were cleansed and polished with pumice paste to remove plaque and calculus. Animal was covered using sterile towels, exposing only the operative field. On the facial surfaces of the upper and lower incisors (4/arch), class V cavities were prepared in a standardized protocol approximately 1 mm coronal to the gingival margin (approximately 3× 5 mm).The cavity was prepared using sterile carbide inverted cone bur under copious sterile water coolant and at rotational speed ranging between 25000-40000rpm. The pulpal floor of the prepared cavities were finished as close as possible to the pulps until the pink shadow of the pulp became apparent, leaving a thin dentin barrier. A sterile explorer was used to create pulp exposure in the center of the cavity floor. Bleeding of the pulp was controlled using sterile cotton pellets moistened with sterile saline until physiologic haemostasis occurred, and then dried with cotton pellets. After irrigation of the exposure sites, they were dried and capped directly with one of the tested materials. All teeth were sealed with glass ionomer filling (Riva, SDI, Australia). After the operation, dogs were kept in separate cages and were fed with a special soft diet. For pain and infection control, all dogs were given cefotaxime sodium at a dose of 10 mg /kg and diclofenac sodium at a dose of 1.1 mg kg once/day for 5 days after surgery [19].

After 1 and 3 months, the animals were sacrified with thiopental sodium overdose.

The teeth were extracted, fixed in 10% neutral buffered formalin solution and decalcified. The specimens were embedded in paraffin, sectioned at an average thickness of 6 µm, and stained with hematoxyline- eosin [20]. The histopathological changes of pulp tissues were interpreted according to the criteria that were established by **Mercedes et al.** [21] and **Faraco Junior and Holland** [22].

Statistical analysis was carried out using SPSS for windows (SPSS, 20) [23]. Results are considered significant at exact probability values less than 0.05 ($P \leq 0.05$).

RESULTS

I-Evaluation of pulp capping materials in vitro:

1- **PH:** Regarding comparison between the tested materials, Bioaggregate revealed the statistically significant highest mean values of pH through all time intervals. It was followed by NMTA then MTA, while NBAG revealed the lowest values through all time intervals (**Fig. 1a**)

2- **Calcium ion release**: BA showed the highest mean values followed by NMTA and NBG with non significant statistically differences between them then MTA (**Fig. 1b**).

3- **Solubility**: BA and NMTA revealed the statistically highest mean values of solubility respectively followed by MTA then NBAG (negative solubility) (**Fig. 1c).**

4- **Setting time**: MTA recorded the highest mean value of the setting time followed by NBAG, while NMTA and BA showed the lowest one with statistically non significant differences between them **(Fig. 1d)**

II- Histological Evaluation of pulp tissue reaction to capping materials:

1-Hard tissue dentin bridge (**Fig 2**)

A) Thickness of dentin bridge formation

NMTA showed the highest mean values of thickness of dentin bridge, followed by MTA and BA respectively with statistically non significant differences between them then NBAG recorded the lowest value during the first month. After 3 months, NMTA recorded the highest mean value, followed by MTA, NBAG and BA with non significant differences between them statistically. Regarding comparison between time intervals of each material, there were statistically significant differences between all groups that the mean thickness values increased with time (Table 1).

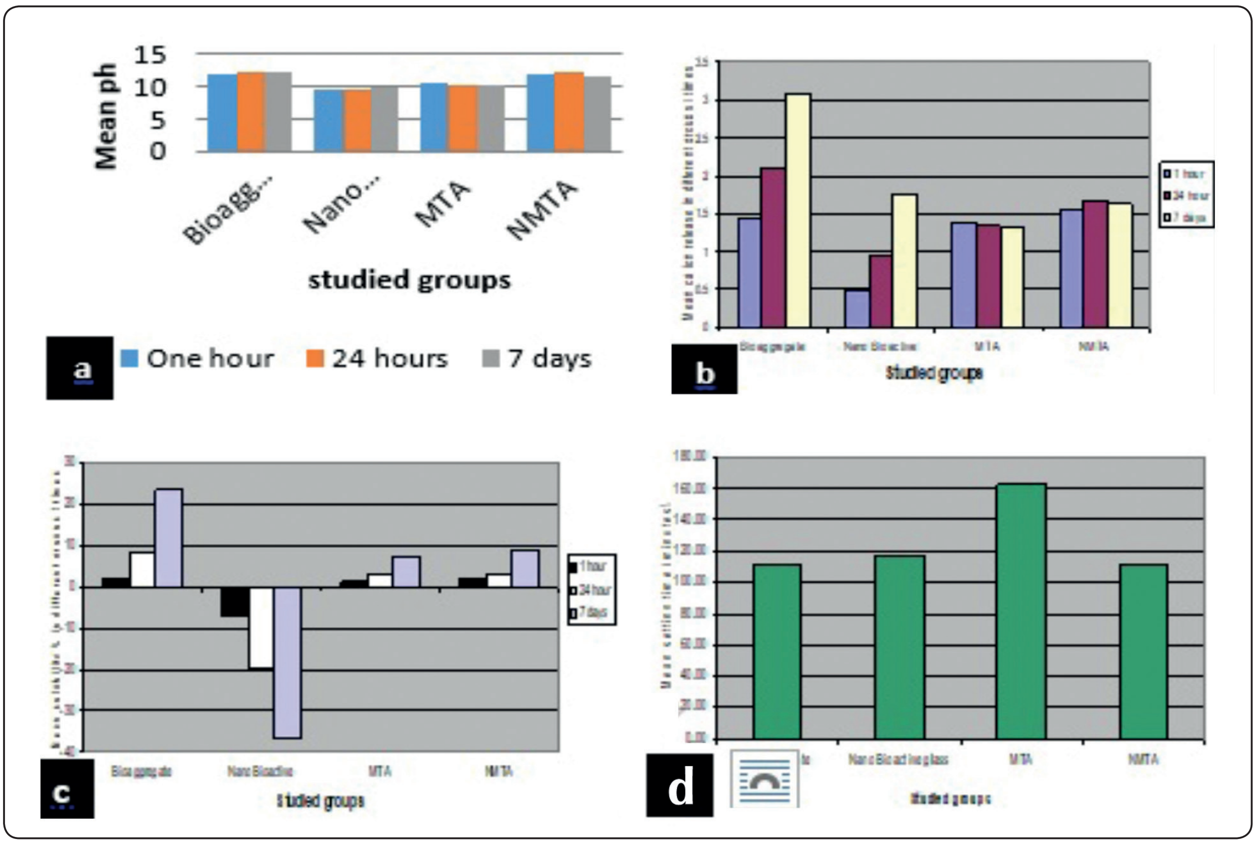

Fig. (1) Bar chart of different groups representing distribution of : a) pH - b) Ca 2+ release- c) Solubility d) Setting time

B) Morphology of dentin bridge

After 1 month; there was a statistically significant difference between the groups (P-value < 0.05). NBAG group showed the highest prevalence of no tubular dentin. NMTA, NBAG and BA groups showed the highest prevalence of irregular dentinal tubules pattern. MTA group showed the highest prevalence of regular dentinal tubules pattern. After 3 months; there was a statistically significant difference between the groups (P-value < 0.05). The results for all groups were the same at one month. As regards the changes by time within each group; there was no statistically significant change in dentin bridge morphology after 3 months in all groups (Table 2).

2-Dental pulp (Fig 3)

A) Intensity of inflammatory reaction:

Regarding comparison between the tested materials, after 1 month, NBAG showed the highest mean inflammatory values followed by NMTA, then BA and MTA recorded the lowest values. While after 3 months, NBAG and NMTA revealed the highest values with non significant statistically differences between them followed by MTA and BA. On the other hand, there were statistically significant differences between time intervals in each material (**Table 3**).

B) Extension of the inflammatory reaction

After 1 month; there was a statistically

TABLE (1) The mean, standard deviation values and results of repeated measures ANOVA test for comparison between dentin bridge thickness in different groups as well as the changes by time.

Material / Time	Bioaggregate		Nano bioactive		MTA		NMTA		P-value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
After one month	147.80[b]	9.36	95.50[c]	8.69	145.32[b]	6.01	188.27[a]	10.24	< 0.001*
After 3 months	235.0[b]	31.71	228.0[b]	37.76	239.40[b]	34.20	276.5[a]	18.93	< 0.001*
P-value	< 0.001*		< 0.001*		< 0.001*		< 0.001*		

means with the different capital letters within each column are significantly different (p < 0.05), for different times.

**** =highly significant***

TABLE (2) The percentages and results of Fisher's Exact test for comparison between morphology of dentin bridges in the different groups after 1 month and 3 months.

Dentin bridge / Time	Bioaggregate	Nano bioactive	MTA	NMTA	P - value
	%	%	%	%	
(1 month)					< 0.001*
No tubule	0	30	0	0	
Irregular pattern	80	70	5	80	
Regular pattern	20	0	95	20	
(3 months)					< 0.001*
No tubule	35	100	0	30	
Irregular pattern	65	0	10	70	
Regular pattern	0	0	90	0	

*: *Significant at $P \leq 0.05$*

significant difference between the groups. MTA group and BA showed the highest prevalence of no inflammatory reaction while NMTA group showed the highest prevalence of moderate inflammatory reaction. NBAG group was the only group to show severe inflammation. After 3 months; there was a statistically significant difference between the groups. MTA group and BA showed the highest prevalence of no inflammation. NMTA group showed the highest prevalence of mild inflammation. NBAG group was the only group to show severe inflammation and tissue necrosis. As regards the changes by time within each group; MTA and BA groups, there was a statistically significant decrease in extension of inflammation. While for NBAG group, there was a statistically significant increase in extension of inflammation from moderate and severe to severe and tissue necrosis respectively. For NMTA group, there was a statistically significant increase in extension of inflammation from minimal and moderate to moderate and severe (**Table 4**).

TABLE (3) The mean, standard deviation values and results of repeated measures ANOVA test for comparison between inflammatory scores in different groups as well as the changes by time.

Time \ Material	Bioaggregate		Nano bioactive		MTA		NMTA		P – value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
After one month	11.0[c]	6.37	35.0[a]	13.22	7.0[d]	3.89	26.0[b]	10.52	< 0.001*
After 3 months	2.70[b]	3.44	13.40[a]	4.78	1.40[b]	1.99	12.0[a]	5.15	< 0.001*
P-value	< 0.001*		< 0.001*		< 0.001*		< 0.001*		

Means with the different superscripts small letters within each row are significantly different p ≤0.05 for different materials.

**** = significant***

TABLE (4) Percentages & results of Fishers Exact TEST for comparison between extension of imflammatory reaction in the different groups after 1 and 3 months.

Time \ Material	Bioaggregate	Nano bioactive	MTA	NMTA	P - value
	%	%	%	%	
(1 month)					
No	15	0	10	0	< 0.001*
minimal	85	0	90	20	
Moderate	0	25	0	80	
Severe	0	75	0	0	
Necrosis	0	0	0	0	
(3 months)					
No	90	0	100	0	
minimal	10	0	0	0	< 0.001*
Moderate	0	0	0	25	
Severe	0	10	0	75	
Necrosis	0	90	0	0	

****= Significant at P ≤ 0.05***

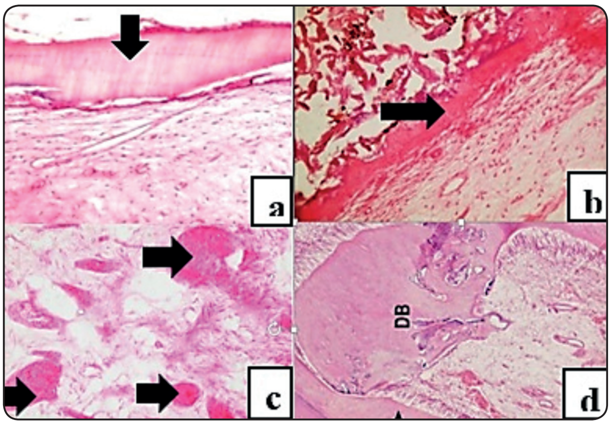

Fig. (2) A photomicrograph showing the dentin bridge formation of different groups after 3 month a) f MTA treated group, showing formation of tubular dentin bridge at the exposure site (arrow), (b) Bioaggregate treated group, showing formation of dentin bridge at the exposure site (arrow), c) pulp of nanobioactive glass treated group, showing discrete multiple pulp stone (arrows), d) pulp of nano MTA - treated group showing formation of thick dentin bridge with cellular inclusion (DB) a,b, d (H&E. Mag x200), c (H&E. Mag x400).

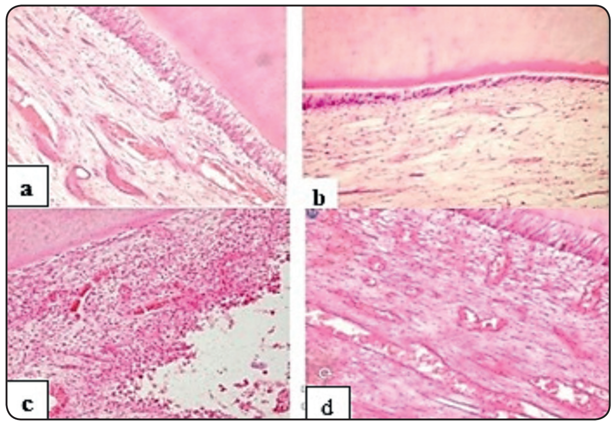

Fig. (3) A photomicrograph showing a) (MTA - treated group) revealed almost normal appearance of pulp tissues, with blood vessles diltation, b) bioaggregate treated group, showing mild inflammation, c) Nanobioactive glass treated group showing diffuse inflammation and loss of odontoblastic layer, d) pulp of nanoMTA treated group, showing marked fibrosis. (H&E. Mag x200)

DISCUSSION

In the present study, all tested materials showed a high pH throughout the time intervals. Bioaggregate showed the highest pH mean values than the other tested materials throughout all time intervals. This might be attributed to the hydration of Bioaggregate resulted in the formation of calcium hydroxide which increased pH by time. This result was in accordance with several studies[23,24,25]. Both MTA and NMTA revealed high pH values in this study. The setting reaction of MTA is a complicated process depending on the exact proportions of mineral phases, their purity and temperature of the mix. On hydration calcium silicates present in MTA undergoes hydrolysis and produce calcium silicate hydrate and calcium hydroxide. About one third of hydration products constituted by calcium hydroxide to render MTA highly alkaline [26].

The higher pH values of NMTA than MTA during all time intervals tested might be due to its higher reactivity, the more rapid hydration process of nano-sized particles over conventional particles and the increased surface area of the reactive molecules is another reason. This was in accordance with saghiri et al. [27].

In the current study, NBAG although recorded high pH values throughout different time intervals, they were lower than other tested materials. When activated with water, BAG releases the ions of its composition which form a mineral matrix equivalent to that of natural hydroxy apatite. The mineral enrichment efficacy leads to an immediate increase of the pH level and then insignificantly increased by time[28] and this was in consonance with Carvalho et al [29] who reported that BAG presented an alkaline pH immediately in the first 10 min, and over the course of 7 days tended to neutralize but from the 14th day on, the values began to rise again.

Regarding Ca 2+ release, BA recorded the highest values which increased by time. This might be inferred to the hydration reaction of calcium silicate particles that triggers the dissolution of their surface with the formation of a calcium silicate hydrate gel and calcium hydroxide [30,31,32].

On the other hand, NBAG showed the lowest mean value of Ca 2+ release during all time intervals. This could be attributed to the content of NBAG of calcium and phosphate. As those when activated with water are capable of generating a carbonated hydroxyapatite layer equivalent chemically and structurally to the mineral of bone. An amorphous silicon oxide is also formed which reduced the levels of calcium hydroxide produced on hydration[33].

MTA displayed the highest prevalence of absence of inflammation in the two evaluating periods followed by BA. This might be due to its high alkalinity which causes denaturation of adjacent cells, tissue proteins and a few bacteria that might be present in the exposed area. As the materials set, the pH changes and the cell injuries subside with an improvement of inflammatory condition[34,35]. In addition, MTA and BA have the ability to release calcium ions during setting, with the subsequent binding of calcium with phosphorus to form hydroxyapatite crystals [36]. These biomaterials are more likely to cause alterations in cellular enzymatic activity than to change the permeability, which facilitates healing [37]. This result was in agreement with other studies[38,39].

It is important to note that shorter setting time promotes the least contact time of the material with the contaminants, which would reduce washout and facilitate the placement of a second restorative material on top [40].

In the present study, MTA showed the highest setting time value followed by NBAG with statistically significant differences between them. While NMTA and BA showed the lowest setting time with no statistically significant difference between them and this result was in compliance with the result of Torabinejad et al[41]. In accordance with Saghiri et al[27], NMTA showed faster setting time than the conventional form. The greater surface area of nanoparticles helps it to react more rapidly with water thus prevent washout of the cement plug before final setting. This was also in agreement with Lee [42] who confirmed that surface area of powder is related directly to the setting time of a cement base material. In addition, Asgary et al. [43] proved that smaller particles provide larger surface area available for hydration and speed up setting.

Furthermore, the decreased setting time of NBAG and BA than MTA might be inferred to the accelerator setting effect of their nanoparticles contents, which act as a seed (site/point), stimulating growth (nucleation) of calcium silicate hydrate[44]. In this study, all tested materials showed some degree of solubility except NBAG which gained weight rather than lose weight which increased by time. This could be due to precipitation of calcium carbonate on its surface. When NBAG are brought into contact with the body fluids a rapid leach of Na+ and congruent dissolution of Ca +2, Po 4 -3 and Si +4 takes place at the glass surface. A polycondensated silica- ric4h (Sigel) layer is formed on the glass bulk, which then serves as a template for the formation of a calcium phosphate layer at its outer surface. Eventually, calcium phosphate crystallizes into hydroxyapatite[45,46]. This was confirmed by other studies[47,48,49] which revealed negative solubility of BAG and NBAG. On the other hand, all other tested materials showed some degree of solubility which increased by time. BA and NMTA showed higher mean value of solubility than MTA during all time intervals as nanoparticles with larger surface area resulted in increase in chemical reactivity.

The histo-morphological data generated by this study regarding the formed dentin bridge showed that NMTA induced thick dentin bridge with irregular dentin pattern in 1 and 3 months evaluation time period while MTA and BA induced thinner dentin bridge with regular dentin pattern. Recent studies have concluded that nano-modification of MTA increased the surface area of the powder and the surface reaction resulting in better hydration of particles and more calcium hydroxide formation that increases its chemical reactivity. Furthermore, the nanomodification of MTA increased the release of calcium ions in post setting time, which can significantly influence the induction of reparative dentin formation of human dental pulp cells. This means that a given mass of material in a nanoparticles form is much more reactive than the same mass of material in its conventional form. These properties may explain the formation of thick dentin bridge with irregular dentinal tubules pattern in NMTA. In addition, MTA was able to stimulate dentinal bridge formation as it induced a high expression of fibronectin/tenascin, two major components of the matrix of reparative dentine bridge, when it used for direct pulp capping.

In the present study, both NBAG and NMTA showed the highest mean inflammatory cell counts at both evaluations time periods. This finding may be attributed to when our bodies exposed to several different types of nanoparticles molecules; it may induce unexpected tissue inflammation. NBG recorded the highest inflammatory cell counts in the two evaluating periods. This might be due to the pivotal role of CaP debris with surrounding tissue. As it was indicated that when biomaterial particles that have been generated from bulk materials interact with immune cells (PMNs, monocytes...), it leads to cell activation and the release of inflammatory mediators. In addition, MTA and Bioaggregate showed the lowest inflammatory cell counts in the two evaluating periods. In a similar study Menezes et al [50] evaluated the histological effects of these four materials (gray and white MTA, gray and white PC) on dog teeth (incisors, canines and premolars) when used as a wound dressing in pulpotomy. They reported that the tissue reaction patterns and cell reactions were identical for ProRoot MTA, MTA Angelus, Portland cement and white Portland cement. They attributed the ability of MTA and Portland cement to support the formation of a dentin bridge to high sealing ability and fast setting. This prevents diffusion of the material into surrounding tissues and reduces microleakage during the healing period.

In this study, we did not observe any acute inflammation or necrosis in the BioAggregate specimens. However, when compared with MTA, BioAggregate differed significantly with respect to forming a dentin bridge. Because BioAggregate differs from MTA in containing tantalum oxide instead of bismuth oxide as a radiopacifier, it may be important to further evaluate the differences between bismuth oxide and tantalum oxide in forming dentin bridges.

CONCLUSION

1- Nanomodifications of the dental materials affect its physicochemical properties. Test of physical properties showed that Bioaggregate was the best regarding ph and ca ions release & Nanobioactive glass was the best in solubility.

2- The histological assessment revealed that, MTA and Bioaggregate are favorable materials for direct pulp capping. NMTA and NBAG enhanced the dentin bridge thickness, but of inferior quality than MTA and BA.

3- BA revealed the superior physicochemical properties, with favorable pulpal tissue response.

REFERENCES

1- Bergenholtz G. Advances since the paper by Zander and Glass (1949) on the pursuit of healing methods for pulpal exposures: historical perspectives. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005; 100: S102-S108.

2- Pashley DH. Dynamics of the pulpo-dentin complex. Crit Rev Oral Biol Med 1996; 7: 104-133.

3- Modena KC, Casas-Apayco LC, Atta MT, Costa CA, Hebling J, Sipert CR, et al. Cytotoxicity and biocompatibility of direct and indirect pulp capping materials. J Appl Oral Sci. 2009;17:544-554.

4- Hilton TJ. Keys to clinical success with pulp capping: A review of the literature. Oper Dent 2009; 34: 615-625.

5- Bogen G, Kim JS, Bakland LK. Direct pulp capping with mineral trioxide aggregate: an observational study. J Am Dent Assoc 2008; 139: 305-315.

6- Utneja S, Nawal RR, Talwar S, Verma M. Current perspectives of bio-ceramic technology in endodontics: calcium enriched mixture cement–review of its composition, properties and applications. Restor Dent Endod 2015;40:1-13.

7- Silva GF, Duarte MAH, Grizzo LT and Buzalaf MAR. PH, Calcium ion release, and setting time of an experimental Mineral Trioxide Aggregate–based root canal sealer. J Endod 2011 ;37:844–846.

8- Tschoppe P, Zandim DL, Martus P, Kielbassa AM. Enamel and dentine remineralization by nanohydroxyapatite toothpastes. J Dent 2011; 39: 430–437.

9- Hench LL. “Bioactive materials for gene control,” new materials and technologies for healthcare, eds L. L. Hench, J. R. Jones and M. B. Fenn. World Scientific, Singapore, 2011, pp 25–48.

10- Garcia F, Asgar K and Lotfi M. Effect of pH on compressive strength of some modification of Mineral Trioxide Aggregate. Biomate Bioeng Dent 2013;3:417-422.

11- Nekoofar MH, Adusei G, Sheykhrezae MS, Hayes SJ, Bryant ST and Dummer PM. The effect of condensation pressure on selected physical properties of Mineral Trioxide Aggregate. Int Endod J 2009;42:453-461.

12- Kogan P, He J, Glickman GN and Watanabe I. The effects of various additives on setting properties of MTA. J Endod. 2006;32:569–572.

13- Zhang S, Yang X and Fan M. BioAggregate and iRoot BP Plus optimize the proliferation and mineralization ability of human dental pulp cells. Int Endod J 2013;46:923–929.

14- K. Peycheva. Pulp-capping with Mineral Trioxide Aggregate. Acta Medica Bulgarica 2015; 26: 192-198.

15- Goldberg M, Smith AJ. Cells and extracellular matrices of dentin and pulp: A biological basis for repair and tissue engineering. Crit Rev Oral Biol Med 2004; 15: 13-27.

16- Massi S, Tanomaru-Filho M, Silva GF, Duarte MAH, Grizzo LT and Buzalaf MA.pH, Calcium ion release, and setting time of an experimental mineral trioxide aggregate–based root canal sealer. Journal of Endodontics. 2011 ;37(6):844–846.

17- Sandra R. Mestrener, Roberto Holland, Eloi De. Influence of age on the behavior of dental pulp of dog teeth after capping with an adhesive system or calcium hydroxide. Dent Traumatol 2003; 255–261.

18- Murray P, Kitasako Y, Tagami J, Windsor L and Smith AJ. Hierarchy of variables correlated to odontoblast-like cell numbers following pulp capping. J Endod 2001;27:297-304.

19- Aeinehchi M, Eslami B and Ghanbariha M. Mineral Trioxide Aggregate (MTA) and Calcium Hydroxide as pulp-capping agents in human teeth: a preliminary report. Int Endod J 2003 ;36: 225–231.

20- Sabeti MA, Nekofar M, Motahhary P, Ghandi M, Simon JH. Healing of apical periodontitis after endodontic treatment with and without obturation in dogs. J Endod 2006;32:628–633.

21- Mercedes S. Dominguez, David E. Witherspoon, James L. Gutmann and Lynne A. Histological and Scanning Electron Microscopy assessment of various vital pulp-therapy materials. J Endod 2003;29: 324-333.

22-Faraco JM and Holland R. Response of the pulp of dogs to capping with Mineral Trioxide Aggregate or a Calcium Hydroxide cement. Dent Traumatol 2003;17:163-166.

23- Aguilar P and Linsuwanont P. Vital pulp therapy in vital permanent teeth with cariously exposed pulp: a systematic review. J Endod 2011; 37: 581-587.

24- Zhang S, Yang X and Fan M. BioAggregate and iRoot BP Plus optimize the proliferation and mineralization ability of human dental pulp cells. Int Endod J 2013;46:923–929.

25- Seux N, Septier D, Chaussain-Miller C, Blacher R, DenBesten P and Goldberg M. Dentonin, a MEPE fragment, initiates pulp healing response to injury. J Dent Res 2007; 86: 780-785.

26- Queiroz AM, Assed S, Leonardo MR, Nelson-Filho P and Silva LA. MTA and Calcium Hydroxide for pulp capping. Appl Oral Sci. 2005;13:126-130.

27- Saghiri MA, Asgar K, Lotfi M and Garcia F. Effect of pH on compressive strength of some modification of Mineral Trioxide Aggregate. Biomate Bioeng Dent 2015;3:417-422.

28- Garcia F and Tanomaru F. Effect of silver nanoparticles on physicochemical and antibacterial properties of Calcium Silicate Cements. Braz Dent J 2016;27:5- 14.

29- Carvalho C, Freire L, Carvalho A, Duarte M, Bauer J, Gavini G. Ions release and pH of Calcium Hydroxide, Chlorhexidine and Bioactive Glass based endodontic medicaments. Braz Dent J 2016; 27: 325-331.

30- Duarte MA, Demarchi AC and Yamashita JC. pH and calcium ion release of two root-end filling materials. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003;95:345-347.

31- Santos AD, Moraes JC, Araujo EB, Yukimitu K and Filho WV. Physico-chemical properties of MTA and a novel experimental cement. Int Endod J 2006; 39: 443-447.

32- Kum KY, Kim EC, Yoo YJ, Zhu Q, Safavi K, Bae KS and Chang SW. Trace metal contents of three tricalcium silicate materials: MTA Angelus, Micro Mega MTA and Bioaggregate. Int Endod J 2015; 48: 704-710.

33- Khvostenkoa D, Hiltonb TJ, Ferracaneb JL, Mitchellc JC, Kruzica JJ. Bioactive glass fillers reduce bacterial penetration into marginal gaps for composite restorations. Dent Mater 2016;32: 73–81.

34- Mozayeni MA, Milani AS, Marvasti LA, Asgary S. Cytotoxicity of calcium enriched mixture cement compared with mineral trioxide aggregate and intermediate restorative material. Aust Endod J 2012;38:70-75.

35- Hilton TJ. Keys to clinical success with pulp capping: A review of the literature. Oper Dent 2009; 34: 615-625.

36- Valles M, Mercade M, Duran-Sindreu F, Bourdelande JL Roig M. Influence of light and oxygen on the color stability of five calcium silicate-based materials. J Endod 2013; 39: 525-528.

37- Lee YL, Lee BS, Lin FH, Yun Lin A, Lan WH, Lin CP. Effects of physiological environments on the hydration behavior of mineral trioxide aggregate. Biomaterials 2004; 25: 787-793.

38- Han LA, Kodama AS. Evaluation of calcium-releasing and apatite-forming abilities of fast-setting calcium silicate-based endodontic materials. Int Endod J 2015;48:124-30.

39- Machado DF, Bertassoni LE, Souza EM, Almeida JB, Rached RN. Effect of additives on the compressive strength and setting time of a Portland cement. Braz Oral Res 2010;24:158-64.

40- Yamamoto SM, Han LP. Noiri YT. Evaluation of the Ca ion release, pH and surface apatite formation of a prototype tricalcium silicate cement. Int Endod J 2013;10:1111-22.

41- Torabinejad M, Hong CU, McDonald F and Pitt Ford TR. Physical and chemical properties of a new root-end filling material. J Endod 2007;33:349–353.

42- Lee YL, Lee BS, Lin FH, Yun Lin A, Lan WH, Lin CP. Effects of physiological environments on the hydration behavior of mineral trioxide aggregate. Biomaterials 2014; 25: 787-793.

43- Asgary S, Eghbal MJ and Parirokh M. A comparative study of histologic response to different pulp capping materials and a novel endodontic cement. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008 ;106: 609–614.

44- Baron L, Gombault A, Fanny M, Villeret B, Savigny F, Guillou N, Panek C, Le Bert M, Lagente V, Rassendren F, Riteau N, Couillin I. The NLRP3 inflammasome is activated by nanoparticles through ATP, ADP and adenosine. Cell Death Dis. 2015; 6:1629- 1637.

45- Jiang, P., Lin, H., Xing, R., Jiang, J., Qu, F.: Synthesis of\7 multifunctional macroporous-mesoporous TiO2-bioglasses for bone tissue engineering. J. Sol-Gel. Sci. Technol. 2012;61: 421–428.

46- Hallab NJ, Jacobs JJ. Biologic effects of implant debris. Bull Hosp Jt Dis 2009;67:182–188.

47- Jones, J.R., Lin, S., Yue, S., Lee, P.D., Hanna, J.V., Smith, M.E., Newport, R.J.: Bioactive glass scaffolds for bone regeneration and their hierarchical characterisation. Proc. Inst. Mech. Eng. Part H J. Eng. Med. 2010; 224: 1373–1387.

48- Xavier JR, Desai P, Varanasi VG, Al-Hashimi I, Gaharwar AK. Advanced Nanomaterials: Promises for improved dental tissue regeneration. In: Anil, editor. Nanotechnology in Endodontics: Current and potential Clinical Applications. 1st ed. Switzerland: Springer International Publishing; 2015. p.5-22.

49- Soo-Kyung Jun, Jung-Hwan Lee, and Hae-Hyoung Lee. The Biomineralization of a Bioactive Glass-Incorporated Light-Curable Pulp Capping Material Using Human Dental Pulp Stem Cells. BioMed Research International 2017.

50-Menezes R, Bramante CM, Letra A, Carvalho VG, Garcia RB. Histologic evaluation of pulpotomies in dog using two types of mineral trioxide aggregate and regular and white Portland cements as wound dressings. Oral Surg Oral Med Oral Pathol Oral Radiol Endod.2004:98:376-3383.