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Biocompatibility of mineral trioxide aggregate and three new endodontic cements: An animal study

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ABSTRACT

Background: Introducing new endodontic cements should await comprehensive investigations and new formulations have to be tested *in vivo* before applying in human beings. So, the purpose of this study was to compare the biocompatibility of new endodontic cements, calcium aluminate α-aluminate cement (CAAC), calcium aluminate α-aluminate plus cement (CAAC plus), and a mixture of wollastonite and CAAC cement (WOLCA) and mineral trioxide aggregate (MTA), in subcutaneous connective tissue of rats.

Materials and Methods: Twenty-seven Wistar rats were divided into three groups of 7, 14, and 30 experimental days. Sterile polyethylene tubes were filled with MTA, CAAC, CAAC Plus, and WOLCA cement and implanted subcutaneously. Empty tubes were implanted as negative control. After the experimental periods, animals were sacrificed by anesthetic overdosing. The occurrence of inflammatory responses was scored according to the previously established scores. Data were statistically analyzed using Friedman, Wilcoxon, Kruskal-Wallis, and Mann-Whitney tests. The level of significance was 5% ($P<0.05$).

Results: There was a statistically significant difference between experimental and negative control sites in each group ($P<0.05$). CAAC Plus showed the highest mean scores of inflammation, compared with MTA, CAAC, and WOLCA cement sits at the end of all periods ($P<0.05$). There were no statistically significant differences between inflammatory scores of each site in different experimental groups, except CAAC plus sites, in which inflammation increased significantly with time ($P<0.05$).

Conclusion: According to the results of the current study, biocompatibility of CAAC and WOLCA cement were comparable with that of MTA, but CAAC Plus induced an inflammatory response higher than MTA, therefore is not biocompatible.

Key Words: Biocompatibility, mineral trioxide aggregate, new endodontic cement, sodium hexametaphosphate

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INTRODUCTION

The goal of endodontics is to prevent or treat apical periodontitis[1] and most of the endodontic failures occur as the result of leakage of irritants through improperly sealed root end fillings into periradicular tissues.[2-5] An ideal orthograde or retrograde root canal filling material should seal the pathways of communication between root canal system and its surrounding tissues.[6] The materials used in root canal therapy, particularly root end filling, are frequently in direct contact with soft and hard periodontal tissues; therefore, a root filling material is necessary to be highly biocompatible and nontoxic.[7] In 1990s, mineral trioxide aggregate (MTA), a new root-ending endodontic material, was developed at the university of Loma Linda.[8] MTA is now used extensively in endodontics for pulp capping, pulpotomy, repair of root perforations, root end filling, root canal filling, and apical barrier formation in teeth with necrotic pulps and open apices.[9,10]

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Despite all the benefits listed for MTA, it has also some disadvantages. The main drawbacks of MTA include the potential for tooth discoloration, presence of some toxic elements in the material composition, high cost, long setting time, difficult handling, and difficulty in its removal after setting.[11,12]

Efforts have been made to overcome these shortcomings. Introducing new substitutes for MTA should await comprehensive investigations, and new formulations have to be tested *in vitro* as well as *in vivo* before applying in human beings.[13]

Recently, investigators of Torabinejad Dental Research Center at Isfahan University of Medical Sciences (IUMS), Isfahan, Iran, have formulated new cements to be used in endodontics. These materials include calcium aluminate α-aluminate cement (CAAC), calcium aluminate α-aluminate plus cement (CAAC Plus), and a mixture of 1 to 1 wollastonite and CAAC cement (WOLCA). CAAC contains calcium aluminates (60-70% CA, 10-15% CA2, and 0-5% C12A7) and alpha aluminate (α-AL_2O_3 5-15%). CAAC plus is a mixture of CAAC and 5% by weight Sodium Hexametaphosphate (Na-HMP) to improve physical properties of CAAC.

Wollastonite is a naturally occurring calcium silicate ($CaSio_3$) with a theoretical composition of 48.3% CaO and 51.7% SiO_2.[14]

Although there is an extensive knowledge on the biocompatibility of MTA,[15-17] the biological properties of these new cements (CAAC, CAAC Plus, and WOLCA cement) have not been evaluated. Thus, the purpose of this study was to compare the biocompatibility of these cements with each other and MTA in subcutaneous connective tissue of rats.

MATERIALS AND METHODS

The protocol of this study was approved ethically by research council of the IUMS. In this experimental animal study, 27 healthy male Wistar rats weighing 250 to 300 g were used. Animals were divided into three groups of nine with respect to experimental duration. Rats were anesthetized with an intramuscular injection of Ketamine (60 mg/kg, Alfasan, Woerden-Holland), Acepromazine (2.5 mg/kg, Alfasan, Woerden-Holland), and Atropine (0.04 mg/kg, Alfasan, Woerden-Holland). The dorsal skin was shaved and disinfected with povidone-iodine solution (10%) (Daroupakhsh, Tehran, Iran). Five 15 mm long incisions were made through the skin using a no. 15 scalpel blade and pockets were prepared in one direction by undermining the incisions longitudinally by blunt dissection for 20 mm.

Sterile polyethylene tubes (1.5 mm of inner diameter and 7 mm in length) were filled with MTA (Dentsply Tulsa Dental, Tulsa, OK) CAAC, CAAC Plus, and WOLCA cements which have been prepared according to manufacturer's instructions, under aseptic conditions; each tube were implanted in each subcutaneous pocket of rats. Empty tubes were implanted as negative control. Wounds were sutured for 7 days. At the end of experimental periods of 7, 14, and 30 days, animals of the respective group were sacrificed by anesthetic overdosing. After histological processing, tissue samples were serially sectioned longitudinally to a thickness of 4 μm and stained with hematoxylin and eosin. Sections were evaluated under a light microscope (OLYMPUS CH30 RF200, Olympus Optical Co., Ltd. Japan) equipped with a digital camera (Sony ExwaveHAD, Tokyo, Japan) using 10× and 40× objective lenses by two independent examiners in a blind manner. The occurrence of inflammatory response were scored according to previously established scores[16] 0 (no reaction) for absence of inflammatory cells; 1+ (mild reaction) for presence of mild chronic inflammatory infiltrate, or few eosinophilic or giant cells; 2+ (moderate reaction) for presence of moderate chronic inflammatory infiltrate, or some eosinophilic or giant cells, or 3 + (severe reaction) for presence of an intense chronic inflammatory infiltrate, large number of eosinophilic or giant cells.

Differences between the inflammatory responses of sites were statistically analyzed using Friedman test while Wilcoxon test was used to compare individual pairs of groups. Differences between the three sets of data were statistically analyzed by Kruskal-Wallis and Mann-Whitney tests. The level of significance was set at α=0.05.

RESULTS

Table 1 presents the mean values of histological scores in different groups. Statistically significant differences were found between sites on 7th, 14th, and 30th days following implantation (P=0.018, P<0.001, and P<0.001, respectively). There were also statistically significant differences between experimental and negative control sites on all three experimental periods following implantation [Table 2].

Table 1: Mean scores and standard deviation of inflammatory response after 7, 14, and 30 days following implantation

Cement	Mean±SD		
	7 days	14 days	30 days
ProRoot MTA	1.55±0.52[b]	1.44±0.52[b]	1.11±0.33[b]
CAAC	1.66±0.50[b]	1.66±0.50[b]	1.44±0.52[b]
CAAC plus	1.88±0.33[b*]	2.33±0.50[c*]	2.55±0.52[c†]
WOLCA cement	1.55±0.52[b]	1.55±0.52[b]	1.11±0.33[b]
Negative control	0.55±0.88[a]	0.33±0.50[a]	0.22±0.44[a]

Different letters show statistical difference in each column (P<0.05), †*Shows statistical difference between time periods in each group (P<0.05), MTA: Mineral trioxide aggregate, CAAC: calcium aluminate α-aluminate cement, WOLCA: wollastonite and CAAC cement

Table 2: *P* values for comparisons of test groups with negative control group (Wilcoxon test)

Cement	*P* values		
	7 days	14 days	30 days
MTA	0.024*	0.008*	0.011*
CAAC	0.026*	0.014*	0.009*
CAAC plus	0.014*	0.007*	0.007*
WOLCA	0.043*	0.015*	0.005*

*Statistically significant difference with negative control group, MTA: Mineral trioxide aggregate, CAAC: calcium aluminate α-aluminate cement, WOLCA: wollastonite and CAAC cement

The CAAC plus group showed the highest mean scores of inflammation and was significantly different from MTA, CAAC, and WOLCA cement sites on 14th (P=0.038, P=0.034, and P=0.038, respectively) and 30th days (P=0.006, P=0.008, and P=0.009, respectively) following implantation. There were no statistically significant differences between mean inflammatory scores of other experimental sites (P>0.05).

There were no statistically significant differences between mean inflammatory scores of matched sites in different experimental periods, except CAAC plus group, in which inflammation increased with time (P=0.024).

Photomicrographs of different inflammatory reactions are presented in Figures 1 to 4.

DISCUSSION

The term biocompatibility is often described as the ability of a material to perform with an appropriate host response in a specific application.[18] Because of continuous introduction of new dental materials, evaluation of their biologic potential is necessary.[19] According to ISO-6876 and 10993-5 standards,[20] tissue implantation of different materials in the body of laboratory animals has been proposed. Although data from laboratory animals could not be extended to human beings, it is considered as a valuable method to evaluate their biological properties.[21,22] Rat subcutaneous implantation studies are acceptable experimental models for this assessment[23] and the inert nature of polyethylene tubes makes them suitable for implantation studies, so implantation of polyethylene or silicon tubes filled with endodontic materials into subcutaneous connective tissue of rats simulates *in situ* conditions of the materials.[24]

Numerous studies have used MTA to seal the natural, pathological, and iatrogenic communications between root canal system and periapical tissues.[16,25] Biocompatibility of MTA has been reported in many *in vitro* and *in vivo* studies.[5,10,15-16,26]

Also, biocompatibility of new materials should be evaluated to ensure that a new material does not cause irritation, unwanted reactions, or tissue necrosis compared with control groups. For this reason, histological investigations evaluate the inflammatory response adjacent to the materials.[26]

The present study evaluated histological inflammatory response adjacent to test materials. The severity of inflammation against CAAC Plus cement was higher than other test materials at all periods and the inflammatory response against this material increased with time. At 7 days after implantation of materials, no statistically significant difference was observed between experimental groups, and at 14 and 30 days, severity of inflammation against CAAC Plus cement was significantly more than other groups.

In this study, empty polyethylene tubes as negative control group revealed no inflammation to mild inflammatory response, which is similar to previous studies.[10,27] Initial inflammatory response to empty tubes is probably the result of surgical process of tube implantation.[27,28]

At 7 days, the MTA group displayed a mild-to-moderate inflammatory response which was reduced to a mild reaction after 30 days. This has been reported before by several studies showing the biocompatibility of MTA.[10,17,26,29,30]

CAAC and WOLCA cement groups also revealed a similar response to MTA which was not significantly different.

According to these results, CAAC is a biocompatible material which is consistent to previous *in vitro* studies on biocompatibility of similar calcium aluminate cements.[31,32] WOLCA cement is a

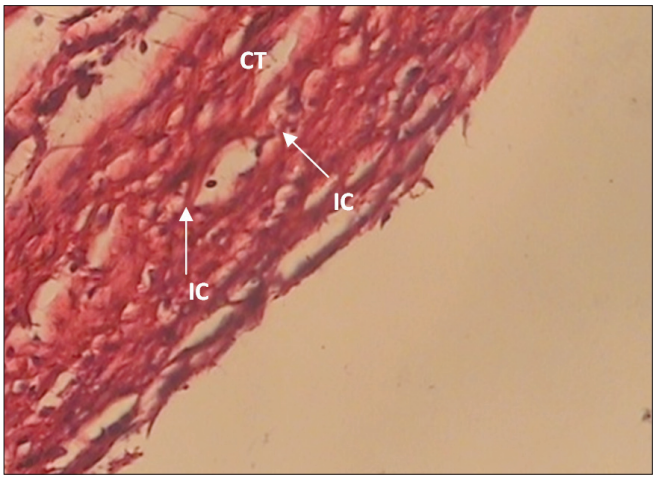

Figure 1: The 30-day MTA specimen with grade + inflammation (×400 Mag) Mild inflammatory cells (IC) are infiltrated in surrounding connective tissue (CT)

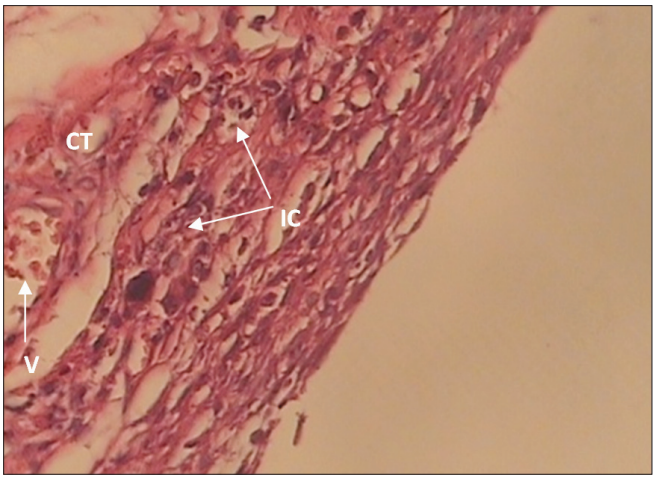

Figure 2: The 30-day WOLCA specimen with grade ++ inflammation (×400 Mag) Moderate infiltration of inflammatory cells (IC) can be seen in connective tissue (CT) and around the Capillaries (V)

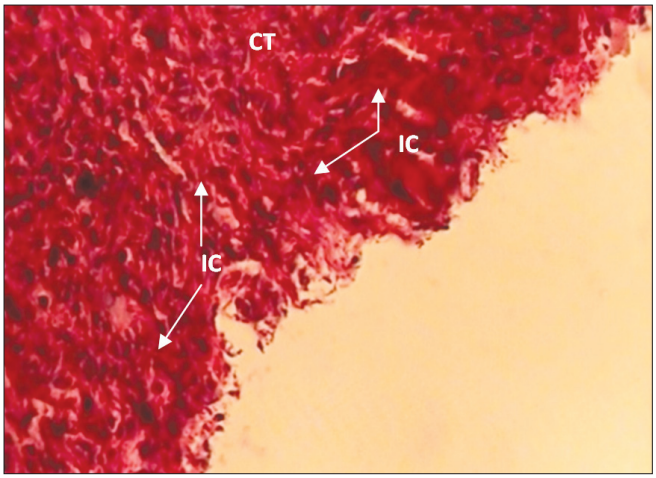

Figure 3: The 30-day CAAC Plus specimen with grade +++ inflammation (×400 Mag) Severe inflammatory cells (IC) are infiltrated in connective tissue

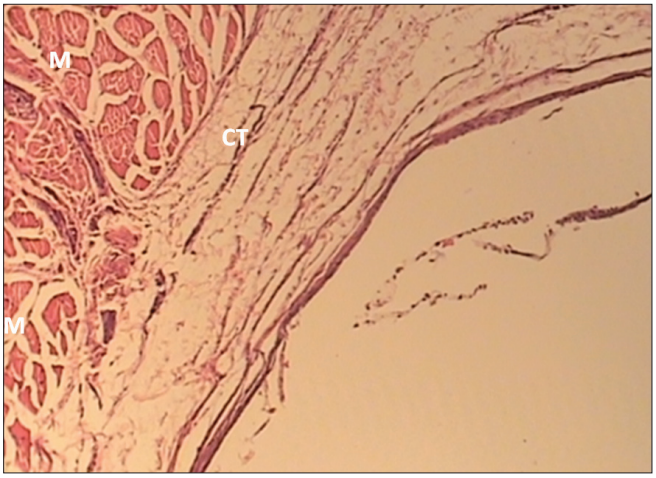

Figure 4: The 30-day Negative control specimen with grade 0 inflammation (×100 Mag) Inflammatory cells around the connective tissues (CT) and muscles (M) are absent

mixture of CAAC and Wollastonite in a ratio of 1 : 1. Wollastonite is a naturally occurring calcium silicate, the composition of which is similar to MTA. Previously, the biocompatibility of MTA and other calcium silicate cements has been proved.[5,15,16,26] It seems that the new material made from mixing two biocompatible cements (CAAC and calcium silicate) is still biocompatible.

The effect of aqueous surface chemistry is very important for small particles. Typically, this occurs in slurries. Weak inter-particle bonds in slurry of flocculated particles make the slurry more viscous than slurry of dispersed particles. In other words, if slurry contains highly dispersed particles, it will have a low viscosity. Since the internal structure of dispersed slurry approaches that of liquid and dispersed particles could be packed more firmly than flocculated particles, it leads to better handling. If particles in slurry are not sufficiently dispersed, the particle charge can be increased by adding a polyelectrolyte[33] such as Na-HMP.

CAAC Plus contains 5% by weight Na-HMP as a dispersant to get these advantages, but the severity of tissue inflammation against CAAC Plus increased by the time. It seems that adding this additive to CAAC reduced its biocompatibility.

Hesaraki *et al.* evaluated the effects of adding Na-HMP on basic properties of calcium phosphate cement and mentioned that although Na-HMP made

this cement more stable and improved its injectability properties, it weakened other basic properties of this cement-like compressive strength and increased its setting time.[34]

According to these findings, it seems that severe inflammatory response to CAAC Plus cement can be due to its prolonged setting time. This is consistent with other investigations which have shown that delay in curing reaction of a substance causes an acute inflammatory response.[35,36]

Previously, the ability of Na-HMP to change the electrical charge of the materials has been demonstrated[34] which alters the proteins and cells absorbed to the material's surface.[37] This issue can also explain the difference between the biocompatibility of this cement and CAAC.

CONCLUSION

According to the results of the current study, biocompatibility of CAAC and WOLCA cements were comparable with that of MTA, but CAAC Plus showed higher inflammatory response than MTA and is not biocompatible. CAAC and WOLCA cements can be two alternatives for MTA. However, more studies are deemed necessary to evaluate the biocompatibility of these cements.

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