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RESEARCH ARTICLE

# Biocompatibility of Styrene-butadiene Copolymer-modified Calcium Phosphate and Mineral Trioxide Aggregate : A Comparative Histological Study

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## ABSTRACT

The biocompatibility of root canal filling material is one of the basic conditions for a successful endodontic treatment and healing of the periodontium. This study was aimed to evaluate the biocompatibility of calcium phosphate cement modified with the styrene-butadiene copolymer-modified calcium phosphate (mCPC) by its implantation in the subcutaneous tissue of rabbit. Fifteen female rabbits of comparable weight were used in this study, each one had received three different tubes; one containing mCPC, the other with mineral trioxide aggregate Fillapex, and an empty control tube on the subcutaneous tissue of thighs. After a definite time (3, 7, and 14 days), the tissues around the tubes were collected, fixed, and processed for histologic evaluation. A histopathological specialist measured the intensity of inflammation. Kruskal–Wallis test was used to analyze the data. The results showed a significant difference with mCPC group in different periods, there was a high intensity of inflammation at the beginning, then it fell, and sustained as mild inflammation. One can conclude that the new formulation of CPC considered biocompatible, which rises the success rate of endodontic treatment.

**Keywords:** Butadiene; Calcium phosphate; Inflammation; Root canal filling materials; Subcutaneous tissue

## INTRODUCTION

Biocompatibility of the endodontic filling materials is one of the basic conditions for a successful endodontic treatment and healing of the periapical area (Konjhodzic-Prcic et al., 2015). Since these materials are frequently placed in intimate contact with the periodontium (Torabinejad and Parirokh, 2010), they should not irritate periradicular tissues or affect the tooth structure (Grossman, 2014). Furthermore, the material should enhance the healing process of periapical lesion (Ghanaati et al., 2010). It is well known that the chemical nature of endodontic filling materials influences the apical healing as a result of mineralized tissue deposition. However, the formation of this tissue may be affected by the inflammatory response that is elicited by the filling material (Silva-Herzog et al., 2011).

Hydroxyapatite (HA) has the chemical and crystallographic similarity to that of apatite mineral found in mammalian hard tissues. This made HA the most attractive material for replacing human bones and teeth (Boehm et al., 2018). HA can be formed by an acid-base reaction of two calcium phosphates; tetracalcium phosphate (TTCP) (basic), and dicalcium phosphate anhydrous (DCPA) (slightly acidic) (Patel, 2011). To enhance calcium phosphate cement (CPC) performance and improve some properties relevant for their clinical use, such as injectability, cohesion, or setting time, polymers were incorporated into its formulation, either as a second solid phase or dissolved in the liquid phase (Khashaba et al., 2011). Many studies incorporated different types of polymers into CPC (Bigi et al., 2004; Khashaba et al., 2011; Perez et al., 2012). However, this is the first study in which a synthetic polymer widely used in rubber industry was incorporated into the liquid phase of CPC, which is the styrene-butadiene emulsion. This study aimed to evaluate the biocompatibility of this modified calcium phosphate (mCPC) cement though implantation in subcutaneous tissue of rabbit.

## MATERIALS AND METHODS

### Calcium Phosphate Cement Preparation

The powder phase consists of an equivalent molar ratio of TTCP (Applichem, GmbH, USA) and DCPA (SIGMA-ALDRICH, USA), 5% wt bismuth oxide

(ChemicalPoint, Germany) as a radiopaque agent, and 10% wt calcium chloride (MERCK, Germany). The liquid phase consists of 50% styrene-butadiene rubber emulsion (Sika L.L.C., Switzerland) in distilled water. To form the cement, one spoon of powder was mixed with two drops of liquid on a glass slap using cement mixing spatula.

### Biocompatibility Study

Biocompatibility study was performed to assess the biocompatibility and tissue response of the animal to mCPC and to compare it with that of a familiar root canal paste material; mineral trioxide aggregate (MTA) Fillapex (Angelus/Londrina, PR, Brazil), using empty silicon tube as control. Fifteen female rabbits of comparable weight, not more than 2 kg, were purchased from Directorate of Duhok Veterinary, Duhok/Iraq. The animals were housed in an animal house and maintained in standard condition according to the laboratory animal care guide prepared by the scientific committee in the college of dentistry.

An 8 mm long sterilized silicon tube was used to carry the tested materials into the animal`s subcutaneous tissue. The animals were divided into three groups for three time intervals (3, 7, and 14 days), each group with five rabbits and each rabbit received three tubes as follows:

Tube 1: Empty silicon tube as control.
Tube 2: Silicon tube filled with MTA; mixed according to the manufacturing instructions.
Tube 3: Silicon tube filled with mCPC.

An intramuscular injection of ketamine hydrochloride with xylazine solution was used to anesthetize the animal. The procedure was done under the aseptic conditions to prevent any contamination. All the instruments and the tools that had been used were sterile. The anesthetized rabbit was laid on a sterile towel. The hair on the thigh area of the skin was shaved and asepsis with povidone-iodine solution and sterile gauze. Three small longitudinal incisions (about 5 mm each) were made; two on the left thigh and one on the right thigh. The incisions were made through epidermis and dermis layers of the skin reaching to hypodermis, which is loosely attached to the dermis. Using blunt dissection, a pocket of more than 10 mm deep was created in each incision in the subcutaneous layer, by opening the attachment between dermis and hypodermis, to accommodate the tube. Each pocket received one silicone tube; those on the left side, one had received the MTA tube and the other had received an empty tube, for the right side pocket, it had received the tube with mCPC [Figure 1]. Before that, the materials were prepared and mixed freshly and inserted into the tubes with the aid of endodontic plugger. Care was taken to prevent smearing of the tested material on the lateral sides of the tube.

Finally, each pocket was sutured with 3/0 silk and cleaned with povidone-iodine impregnated gauze. After a definite time (3, 7, and 14 days), the animal was sacrificed by the administration of an overdose of ketamine anesthetic solution with xylazine. The implanted tube with the surrounded tissue was removed and placed in 10% formalin solution for 48 h [Figure 2].

Then, the tube was removed from the sample, and each sample was cut along the long axis of the tube into two pieces, including the area around the tube. The tissue samples were subjected to histopathological processes, including dehydration, clearing, and embedding in paraffin wax. The paraffin blocks undergo a series of cutting along the long axis of the tube 4 μm thick section using microtome. The specimens were examined by a histopathologic specialist blinded to the materials and period of time.

Kruskal–Wallis test was used to analyze the data.

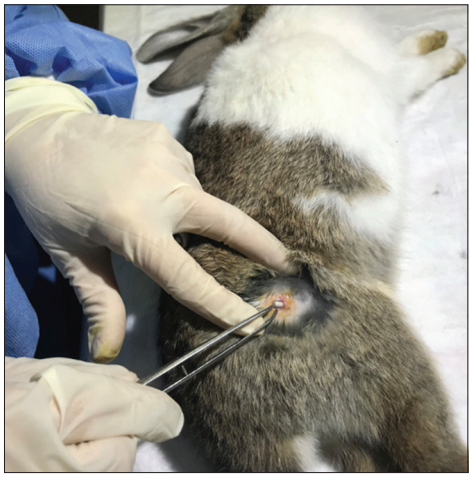

**Figure 1: The pocket receiving silicone tube containing modified calcium phosphate**

**Figure 2: The implanted tube with the surrounded tissue**

## RESULTS

The result of biocompatibility test showed that there was a significant difference between the three different materials, regardless of the period of time [Table 1].

After 3 days of implantation, the mCPC group showed severe inflammatory reaction with dense acute inflammatory cells [Figure 3].

After 7 days, the inflammatory reaction related to mCPC group subsided with mixed acute and chronic inflammatory cells infiltration and an ill-defined fibrous capsule [Figure 4].

After 14 days, the mCPC group showed moderate-to-mild chronic inflammation with well-defined fibrous capsule [Figure 5].

The groups with empty silicon tube (the control group) and MTA had shown a non-significant difference in relation with different period of time. Meanwhile, the group with mCPC had shown a significant difference in different period of time. The intensity of inflammation was high after 3 days, and it falls down after 7 days. Then, it became high again after 14 days [Table 2].

*t*-test was used to compare the intensity of inflammation between two different periods for mCPC. It showed a highly significant difference between the intensity of inflammation in the 3rd and 7th days, but a non-significant difference between the 3rd and 14th days, and between the 7th and 14th days as well [Table 3].

**Table 1: The difference between the three different materials regardless of the period of time**

| Materials | No. of samples | Mean rank | Decision |
|---|---|---|---|
| Empty | 15 | 15.87 | Significant |
| MTA | 15 | 24.83 | |
| mCPC | 15 | 28.30 | |
| Total of | 45 | | |
| Kruskal–Wallis test | | 8.069 | |
| *P*-value of Kruskal–Wallis test | | 0.018* | |

*$P$=0.05

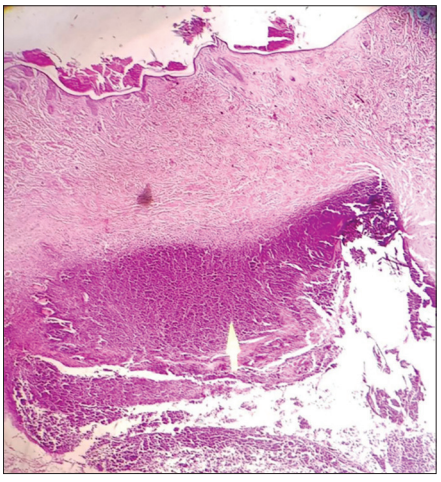

**Figure 3: Modified calcium phosphate group showed a severe inflammatory reaction after 3 days, ×2 (HNE)**

## DISCUSSION

Understanding the inflammatory response associated with endodontic filling materials is essential for their clinical success, since it might predict potential complications associated with over extrusion of the material into the periapical tissue. Biocompatibility is defined as the ability of a material to perform an appropriate host response within a specific application (Ghanaati et al., 2010).

The histological response to endodontic materials implanted in the subcutaneous connective tissue of the rat has been widely accepted as an important secondary test to access the biocompatibility of endodontic filling material (Moura et al., 2014; Silveira et al., 2011; Zmener

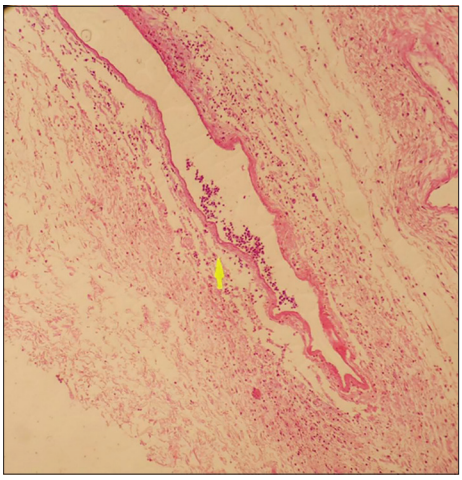

**Figure 4: Modified calcium phosphate group showed a moderate-to-mild inflammatory reaction after 7 days, ×10 (HNE)**

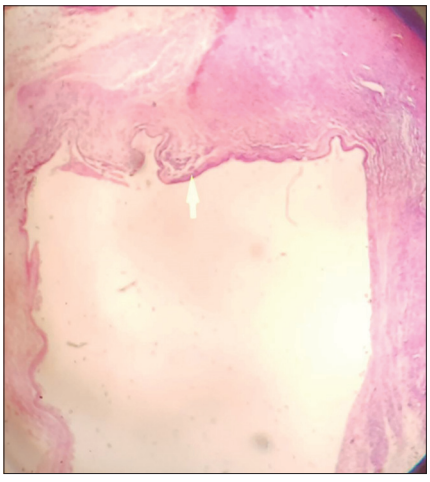

**Figure 5: Modified calcium phosphate group showed moderate-to-mild chronic inflammation after 14 days, ×2 (HNE)**

et al., 1988). However, the periapical tissues in humans are structurally different from the subcutaneous connective tissue of the rat (Zmener, 2004). Furthermore, it was revealed that the inflammatory response patterns induced by the sealing materials were different *in vitro* and *in vivo* (Ghanaati et al., 2010).

Rabbits were used in the present study because they are easily obtainable, and they are an accepted model for determining tissue biocompatibility (Browne and Friend, 1968).

Silicon tubes were used to carry the tested material (Zmener, 2004; Zmener et al., 1988), because studies found that the tissues in contact with polyethylene or Teflon tubes have the tendency to adhere to the tube surfaces, therefore, the interface adjacent to these materials is frequently destroyed during the laboratory procedures, while in the case of silicone tubes, it was surrounded by a thin fibrous connective capsule without signs of adherence to the material surface. In addition, there was no evidence of the presence of foreign body giant cells in contact with the lateral walls or at the ends of the solid silicone rods. These cells were occasionally seen in contact with polyethylene or Teflon (Zmener et al., 1988).

On the 3rd day, considerable levels of inflammatory response were seen in all the groups, including the control empty group. This can be attributed to surgical trauma rather than the response to the materials' toxicity. However, it allowed evaluating the behavior of the materials along the experimental time and during the natural skin healing process as the initial period (Gomes-Filho et al., 2007). Moreover, some researchers found that these substantial levels of inflammatory response were seen in the 1st week after grafting of both control and test groups. This phenomenon could be already observed 24 h after grafting. The operatory trauma caused by the operation itself could have influenced the results within the first 7 days (Scelza et al., 2016). Therefore, the 14 days can be considered as the most relevant of the different time intervals, as its results permitted the actual determination of the irritating potential of the material (Silva-Herzog et al., 2011).

Studies had shown that a material was considered biocompatible if the severity of the connective tissue reaction decreased with time (Silveira et al., 2011). In addition, sealers had shown a comparable pattern of irritation, which was more severe in the beginning and milder with time, in such a way that all sealers showed a persistent mild reaction (Gomes-Filho et al., 2007). Furthermore, early capsule formation is a sign of favorable biocompatibility of the material since inflammation is not severe enough to prevent fibroblasts forming a capsule (Derakhshan et al., 2009).

**Table 2: The difference among the same material in different period of time**

| Duration | No. of samples | Mean rank | Decision |
|---|---|---|---|
| Empty silicon tube (the control group) | | | |
| After 3 days | 5 | 9.70 | Non-significant |
| After 7 days | 5 | 5.40 | |
| After 14 days | 5 | 8.90 | |
| Total of | 15 | | |
| Kruskal–Wallis test | | 3.726 | |
| *P*-value of Kruskal–Wallis test | | 0.155* | |
| MTA group | | | |
| After 3 days | 5 | 9.50 | Non-significant |
| After 7 days | 5 | 6.10 | |
| After 14 days | 5 | 8.40 | |
| Total of | 15 | | |
| Kruskal–Wallis test | | 1.824 | |
| *P*-value of Kruskal–Wallis test | | 0.402* | |
| mCPC group | | | |
| After 3 days | 5 | 12.00 | Significant |
| After 7 days | 5 | 4.10 | |
| After 14 days | 5 | 7.90 | |
| Total of | 15 | | |
| Kruskal–Wallis test | | 9.031 | |
| *P*-value of Kruskal–Wallis test | | 0.011* | |

**P*=0.05

**Table 3: The difference in the intensity of inflammation related to mCPC between different times**

| | Period of time | No. of samples | Mean | SD† | SD‡ Mean | Decision |
|---|---|---|---|---|---|---|
| After 3 and 7 days | | | | | | |
| | 3 days | 5 | 3.00 | 0.000 | 0.000 | High significant |
| | 7 days | 5 | 1.40 | 0.548 | 0.245 | |
| *t*-test *P*-value | | | 0.003* | | | |
| After 3 and 14 days | | | | | | |
| | 3 days | 5 | 3.00 | 0.000 | 0.000 | Non-significant |
| | 14 days | 5 | 2.20 | 0.837 | 0.374 | |
| *t*-test *P*-value | | | 0.099* | | | |
| After 7 and 14 days | | | | | | |
| | 7 days | 5 | 1.40 | 0.548 | 0.245 | Non-significant |
| | 14 days | 5 | 2.20 | 0.837 | 0.374 | |
| *t*-test *P*-value | | | 0.117* | | | |

†Standard deviation. ‡Standard error. **P*=0.05

Groups with empty silicon tube (the control group) and the MTA group had shown a non-significant difference in relation with different period of time. Meanwhile, the group with mCPC had shown a significant difference in different period of time. The intensity of inflammation was high after 3 days, and it fell down after 7 days with a highly significant difference between the two periods, then it became high again after 14 days but with a non-significant difference.

Agreed with this result, Silva et al. found that MTA Fillapex exhibited good biological behavior when the provoked inflammatory intensity and the tissue repairing evolution were evaluated. The material induced a discreet inflammatory infiltrate at the initial periods (7 days), which decreased overtime, and significant fibroblastic and collagen fiber proliferation at the final periods (15 days) (Silva et al., 2013). On the other hand, Scelza et al. found that MTA Fillapex showed high levels of inflammatory response 28 days after grafting when their biocompatibility was tested through an *in vivo* murine bone defect grafting model, and it was demonstrated that MTA Fillapex strongly affects primary human cell viability up to 7 days after setting (Scelza et al., 2016).

Moreover, it was found that MTA Fillapex showed severe cytotoxicity when cells were exposed to fresh elute of the material, and this toxicity did not decrease over the 4 weeks tested periods (Silva et al., 2013). In the same way, MTA Fillapex displayed higher cytotoxic levels to human periodontal ligament fibroblasts *in vitro* in comparison to its chemical precursor, MTA (Yoshino et al., 2013). This may be due to the presence of toxic components such as salicylate resin, diluting resin, and silica in MTA Fillapex composition (Silva et al., 2013; Yoshino et al., 2013), or due to its alkaline pH (Silva et al., 2013). Agreed with this, Scelza et al. found that MTA Fillapex contains salicylate in their composition and evidence indicating potential toxicity for salicylate-containing materials (Scelza et al., 2016).

Others evaluated the biocompatibility and bioactivity of MTA Fillapex in primary culture of human dental pulp cells; it showed initial cytotoxicity, which considered moderate to low and concentration dependent after 24 h setting time. After the initial period of cytotoxicity, MTA Fillapex sealer can promote bioactivity, stimulating the deposition of mineralized nodules and increasing alkaline phosphatase activity (Mestieri et al., 2015).

In this study, mCPC group showed a higher intensity of inflammation than MTA group.

Biocompatibility of CPC is among its wonderful properties (Boehm et al., 2018). However, many factors such as changes in size, porosity, shape, and chemical composition of the material may induce different inflammatory tissue reactions (Ghanaati et al., 2010).

Bismuth oxide ($Bi_2O_3$) considered as one of the common radiopaque agents incorporated into root filling material (Guerreiro-Tanomaru et al., 2009), because of its high radiopacity (Min et al., 2007). Among the heavy metals, bismuth is unusual in that its toxicity is considerably lower than that of its neighbors in the periodic table. However, studies showed that the $Bi_2O_3$ used in MTA is not inert material (Camilleri, 2008), and it is not biocompatible (Camilleri et al., 2004), also $Bi_2O_3$-containing Portland cement-induced toxicity in human dental pulp cells (Min et al., 2007). However, the lack of biocompatibility of $Bi_2O_3$ did not affect the biocompatibility of the MTA, presumably because of the presence of calcium hydroxide (Camilleri et al., 2004).

It was found that gloves made of styrene-butadiene synthetic rubber materials exhibited non-toxic in MTT, slightly toxic in filter diffusion, moderately in agar overlay (Lönnroth, 2005). On the other hand, the epoxidized styrene-butadiene-styrene block copolymer semipermeable membranes are used as wound dressing material and they are biocompatible and can be considered for the application as a biomaterial (Yang and Tsai, 2010).

There is some controversy regarding using freshly mixed or set states of root canal sealers for assessing tissue reaction to the materials in subcutaneous implantation studies. Some emphasized to use freshly mixed sealer as it is similar to the clinical condition, where material may extrude to the periapical areas. A study showed a significant difference between the severity of inflammation in a set and freshly mixed sealer in a certain time interval; the set condition showed a milder response (Derakhshan et al., 2009).

When a CPC with long setting time (30–60 min) was implanted subcutaneously in rats immediately after mixing, it failed to set and this resulted in a severe inflammatory response. Besides, CPC is not converted to HA until its setting reaction is complete (Miyamoto et al., 1999).

Crumbling of CPC paste may be another factor affecting the tissue response. When CPC keeps its shape, TTCP and DCPA stay in close proximity. The dissolution of TTCP increases the pH, whereas the dissolution of DCPA decreases it. As a result of both reactions, the pH of the CPC could be kept within the relatively neutral region provided that the TTCP and DCPA particles stay close together. On the other hand, the TTCP could increase the pH (inducing an inflammatory reaction) in the surrounding micro area, and the DCPA could decrease the pH (also

inducing an inflammatory reaction in other micro areas) if the TTCP and DCPA particles are far enough apart (Miyamoto et al., 1999). Another possible source of the inflammatory reaction caused by the crumble of the CPC may be the size of the HA particles. When the shape of CPC is not retained, small particles or powder HA will form rather than a HA mass. The tissue response is dependent on the kind, form, or surface character of a biomaterial. It has been reported that the tissue reaction to materials also differs, depending on their particle size. A study showed that sealers in large continuous mass seem acceptable to the subcutaneous tissues, while severe reaction produced in relation to fine particulate form (Tagger and Tagger, 1986).

A study determined the biocompatibility of α-tricalcium phosphate-based Sankin Apatite Type I, Type II, and Type III root canal sealers (powder consists of different concentration of α-tricalcium phosphate and HA and liquid polyacrylic acid and water). Results showed that the severity of tissue reaction among the tested materials decreased with time and at the end of the observation period, both Sankin Apatite Type II and Type III, which contain iodoform, were found more biocompatible than Type I (Bilginer et al., 1997).

## CONCLUSION

Within the limitation of this study, one can conclude that the new formulation of CPC considered biocompatible, and this may raise the success rate of endodontic treatment. However, other tests showed be done on this material before the final decision to be used as a root canal obturation material.

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