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Biocompatibility of Portland cement combined with different radiopacifying agents

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(Received September 9, 2013; Accepted January 9, 2014)

Abstract: The aim of this study was to evaluate the response of rat subcutaneous tissue to Portland cement combined with two different radiopacifying agents, iodoform (CHI₃) and zirconium oxide (ZrO₂). These materials were placed in polyethylene tubes and implanted into the dorsal connective tissue of Wistar rats for 7 and 15 days. The specimens were then stained with hematoxylin and eosin, and inflammatory reaction parameters were evaluated by light microscopy. The intensity of the inflammatory response to the sealants was analyzed by two blind calibrated observers throughout the experimental period. Histological analysis showed that all the materials caused a moderated inflammatory reaction at 7 days, which then diminished with time. At 15 days, the inflammatory reaction was almost absent, and fibroblasts and collagen fibers were observed indicating normal tissue healing. The degrees of the inflammatory reaction on different days throughout the experimental period were compared using the non-parametric Kruskal-Wallis test. Statistical analysis

demonstrated no significant differences amongst the groups, and Portland cement associated with radiopacifying agents gave satisfactory results. Therefore, Portland cement used in combination with radiopacifying agents can be considered a biocompatible material. Although our results are very encouraging, further studies are needed in order to establish safe clinical indications for Portland cement combined with radiopacifying agents.

(J Oral Sci 56, 29-34, 2014)

Keywords: biocompatibility; Portland cement; radiopacifying; endodontics; subcutaneous implant; materials testing.

Introduction

Biocompatibility is an important requirement for dental materials, as any toxic components may induce irritation or even degeneration of the surrounding tissues (1,2). Therefore, the biocompatibility of all dental materials that could potentially come in contact with tissues should be investigated (1). This property is of special significance for endodontic materials (3,4) because sealants may cause different reactions in the dental pulp (4-7). Subcutaneous tissue reaction is one of the *in vivo* biocompatibility tests that have been used for evaluating several new materials (2,8-10).

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doi.org/10.2334/josnusd.56.29
DN/JST.JSTAGE/josnusd/56.29

Portland cement is the main component of mineral trioxide aggregate (MTA), but bismuth oxide at a 4:1 ratio is added to MTA to provide radiopacity (11). Portland cement (PC) has emerged as an alternative to MTA because of its relative lower cost, similar chemical composition and biocompatibility with MTA, and several studies have compared both materials (12-16). As PC may potentially be used as an endodontic material (11,17), it does not have sufficient radiopacity to be visualized radiographically, and therefore a radiopacifying agent must be added to it (11).

A number of studies have testing new combinations of PC with different radiopacifying agents, and zirconium oxide in particular has demonstrated excellent results, as it is biocompatible and does not interfere with the properties of PC (11,18,19). Another tested radiopacifying agent is iodoform, which has been used successfully in dentistry and research for many years, showing both antibacterial properties and biocompatibility (20).

Therefore, the aim of this study was to evaluate the response of rat subcutaneous tissue to Portland cement combined with two different radiopacifying agents, iodoform (CHI_3) and zirconium oxide (ZrO_2).

Materials and Methods

The Ethics Committee of Bauru School of Dentistry, University of São Paulo, approved the protocol for this study (#025/2010).

Twelve male rats weighing 200-250 g, from the Central Animal Laboratory of Bauru Dental School, were randomly distributed into three groups: Group I – Portland cement (PC) ($n = 4$); Group II – Portland cement added to iodoform (PC + CHI_3) ($n = 4$); Group III – Portland cement added to zirconium oxide (PC + ZrO_2) ($n = 4$).

Thirty-six polyethylene tubes (Abbott Labs of Brazil, São Paulo, Brazil) with an internal diameter of 1.0 mm, external diameter of 1.6 mm, and length of 10.0 mm were disinfected in 5% sodium hypochlorite for 1 hour, washed in saline solution for 15 min, dried with sterile gauze, then filled with the materials to be tested. Three tubes were used in each animal. All the cements used were based on pure white PC (Votorantim-Cimentos, São Paulo, SP, Brazil) with addition of different radiopacifying agents. A ratio of 20% radiopacifier and 80% white PC by weight was used to fill the tubes (11). The radiopacifying agents and PCs were prepared in sterilized glass plaque, using as the measure parameter the MTA kit spoon for the powder (1 g cement powder) and two drops of distilled water (0.3 mL liquid), obtaining a paste of the material (21). The tubes were filled with the tested material previous to

subcutaneous implantation.

The animals were anesthetized with an intramuscular injection of a mixture of ketamine (Dopalen)/xilazine (Anasedan) at 0.4 mL/0.02 mL/kg body weight. The dorsal skin was shaved, disinfected with 5% tincture of iodine, and two small parallel incisions (anterior and posterior), approximately 1.5 cm long, were made with a scalpel. Two separated pockets were created by blunt dissection, one in the cranial portion, used for housing one tube and, another in the caudal portion, housing two tubes, one on each side, for implantation of the tubes in the subcutaneous tissue. The tubes containing freshly mixed sealants were then placed into the pockets, taking care to prevent any spillage of the material into the tissue. After implantation, the wounds were sutured and the animals did not receive antibiotics and/or analgesics.

Six animals were sacrificed by an overdose of anesthetics at both 7 and 15 days after the operation. The tubes were excised together with the surrounding skin and connective tissue. The samples were immersed for 48 h in buffered 10% formalin (0.1 mol/L solution), and then subjected to histological processing. The specimens were dehydrated in an ascending series of ethanol rinses, the blocks were embedded in paraffin, and tissue sections 5 μm thick were cut and stained with hematoxylin and eosin.

Histologic sections of the sealant-connective tissue interface (at the open ends of the tubes) were analyzed by light microscopy for inflammatory tissue reactions. The connective tissue response adjacent to the outer lateral wall of the tube served as a negative control.

The observers were blinded to the materials used in the specimens. The overall mean value of the inflammatory reaction for each material was determined for each rat at each time point. Evaluation was performed according to a modified version of the Coutinho-Filho et al. (22) criteria. The degree of inflammation was determined according to the type, number and concentration of the predominant cells. The presence or absence of neutrophils, inflammatory infiltrates, giant cells, and macrophages was recorded. For each of these elements, the following numerical scores were assigned: grade 0, none (without inflammatory cells); grade 1, mild (inflammatory cells present in small numbers or in small groups); grade 2, moderate (inflammatory cells in large numbers, yet not predominantly in the microscopic field); grade 3, intense (inflammatory infiltrate, predominantly in the microscopic field); grade 4, necrosis. The Kruskal-Wallis test was used for statistical analysis, and the level of statistical significance was defined as $P < 0.05$.

Table 1 Histological evaluation of tested materials at 7 and 15 days (values as scores)

Specimens	Scores	
	7 days	15 days
PC-1A	1	2
PC-1B	3	1
PC-1C	1	1
PC-2A	2	2
PC-2B	2	1
PC-2C	3	2
PC+CHI ₃ -1A	1	0
PC+CHI ₃ -1B	0	0
PC+CHI ₃ -1C	1	0
PC+CHI ₃ -2A	1	1
PC+CHI ₃ -2B	2	2
PC+CHI ₃ -2C	1	1
PC+ZrO ₂ -1A	2	1
PC+ZrO ₂ -1B	1	1
PC+ZrO ₂ -1C	1	1
PC+ZrO ₂ -2A	1	1
PC+ZrO ₂ -2B	2	1
PC+ZrO ₂ -2C	2	1

Results

Two blinded and previously calibrated observers analyzed the intensity of the inflammatory response in the connective tissue around the sealants during the experimental period (kappa 0.96). The connective tissue response adjacent to the outer lateral wall of the tube was evaluated as a negative control, and showed no inflammatory reaction at any time point. Histological evaluation showed that all the materials caused a moderate inflammatory reaction at 7 days, which had decreased by 15 days (Table 1).

At 7 days, 66.7% of the specimens from the PC group demonstrated grade 2 to 3 inflammation characterized by the presence of large numbers of inflammatory cells. The bulk of the remaining material and giant cells were also observed in most cases (Fig. 1). At 15 days this percentage (66.7%) corresponded to that of specimens demonstrating grade 1 inflammation, in accordance with the tissue repair that had occurred during this period (Fig. 2).

After 7 days, 66.7% of the specimens from the PC + CHI₃ group was classified as having grade 1 inflammation, presenting sporadic inflammatory cells associated with remnants of the material in some cases (Fig. 3). At 15 days, 83.4% of the specimens were graded as 0 to 1, with nearly no tissue inflammation and the presence of collagen fibers, indicating normal tissue healing (Fig. 4).

In the PC + ZrO₂ group, 50% of the specimens demonstrated grade 2 inflammation at 7 days, inflammatory cells

Fig. 1 At 7 days, Portland cement (Group I) was associated with moderate to intense inflammation, with the presence of a large number of inflammatory cells (ii). The bulk of the remaining material (M) and giant cells (GC) are also observed.

Fig. 2 At 15 days, Portland cement (Group I) was associated with fibroblasts and collagen fibers, indicating normal tissue healing.

being associated with the remaining material (Fig. 5). At 15 days, 83.4% of the specimens had grade 1 inflammation, and the presence of fibroblasts and collagen fibers indicated normal tissue healing (Fig. 6).

The Kruskal-Wallis test demonstrated no statistically significant differences among the PC groups and the other tested groups ($P > 0.05$). However, morphological analysis revealed a lower prevalence of inflammation in specimens that included radiopacifying agents.

Discussion

Implantation of new endodontic materials into subcutaneous tissues of rats is one of the most suitable methods for determining their local effects and biocompatibility (2,9,10,22-24). As well as preliminary tests for materials that are utilized for root-end filling, furcal perforation,

Fig. 3 At 7 days, Portland cement mixed with iodoform (Group II) was associated with sporadic inflammatory cells (ii) and remnants of the material (M) in some cases.

Fig. 4 At 15 days, Portland cement mixed with iodoform (Group II) showed nearly no tissue inflammation and the presence of collagen fibers, indicating normal tissue healing.

Fig. 5 At 7 days, Portland cement mixed with zirconium oxide (Group III) showed moderate associated inflammation with the presence of inflammatory cells (ii), adjacent to remaining material (M).

Fig. 6 After 15 days, Portland cement mixed with zirconium oxide (Group III) showed remaining material (M), mild inflammation (ii), and the presence of fibroblasts and collagen fibers, indicating normal tissue healing.

and as apical barriers, such materials must be investigated for their biocompatibility characteristics, as toxic components present in them might induce irritation or even degeneration of the surrounding tissues, especially if they are accidentally extruded into the periradicular tissues (4,25).

Portland cement and MTA have similar major constituents, except for bismuth oxide, which is added to MTA to provide radiopacity (11,26,27). Although this feature does not compromise the execution of pulpotomy procedures with PC and their follow-up assessments, this property is necessary in order to distinguish the material from surrounding anatomical structures such as tooth and bone (11,17,18,28), and to permit the material to achieve the minimum values recommended by the American National Standards Institute / American Dental Association

(ANSI/ADA) standard No 57/2000 (29).

The ideal radiopacifying material should only present the necessary radiopacity for the cement and should be inert, free from any contaminants, non-toxic and added in minimal amounts (11,18). Húngaro-Duarte et al. (11), Coomaraswamy et al. (27) and de Moraes et al. (30) showed that addition of bismuth oxide to PC dramatically changed the material constants; these results have stimulated the search for an alternative radiopacifying agent for use with PC. Addition of other radiopacifying agents such as zirconium oxide and iodoform results in a cement with greater radiopacity than dentin, and both of these agents are present in the formulations of several other endodontic sealants. Recent studies have shown that zirconium oxide does not interfere with the properties of PC such as calcium ion release, solubility and

setting time, thus allowing PC to retain its strength and sealing properties. Addition of these two radiopacifying agents also does not change the pH, thus maintaining the alkalization ability of PC, which is important for maintaining the antimicrobial activity of the cement (21).

The present results are in agreement with those of previous studies, suggesting that these radiopacifying agents do not cause adverse effects (11,20). The iodoform was tested as a radiopacifier for addition to Portland cement because of its satisfactory radiopacity, its widespread availability to clinicians, and earlier reports indicating that it is harmless to pulp and periapical tissues (28,30). The zirconium oxide, a bioinert material with good biocompatibility, has been confirmed to have good biocompatibility and no cytotoxic effects on fibroblasts (20,31). Gomes Cornélio et al. (20) showed that Portland cement associated with zirconium oxide is not cytotoxic to periodontal ligament cells and might be a good alternative as a radiopacifying agent. Therefore, the addition of radiopacifying agents such as zirconia and iodoform as capping materials may be considered, since they allowed satisfactory tissue repair throughout the observation period.

In this investigation, histological evaluation showed that all tested materials had caused an inflammatory reaction at 7 days, and that this then decreased with time. Although there were no significant differences among the groups, morphological analysis revealed that there was less inflammation in specimens that included radiopacifying agents. In general, the cellular events and inflammatory responses observed in the present study were similar in the experimental groups. Therefore, the present results indicate that these radiopacifying agents are biocompatible, and provide preliminary data for their potential use with Portland cement in various dental applications. Although our results are very encouraging, further research *in vitro* and *in vivo* will be needed to assess the biological effects of these radiopacifying agents with Portland cement, including their inflammatory and regenerative properties. Additional high-quality randomized control trials will be needed to analyse and appraise the long-term effects of these agents.

Acknowledgments

The authors would like to acknowledge the financial support of The São Paulo Research Foundation (FAPESP Grants #2009/11284-4 to TMO). The authors would like to thank Daniele Santi Ceolin and Patrícia De Sá Mortágua Gemino for their excellent laboratory assistance.

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