Biocompatibility of New Calcium Aluminate Cement: Tissue Reaction and Expression of Inflammatory Mediators and Cytokines

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Abstract

Introduction: The aim of this study was to evaluate the biocompatibility of a new calcium aluminate cement (EndoBinder) in subcutaneous tissue of rats in comparison with mineral trioxide aggregate and calcium hydroxide hard-setting cement. **Methods:** Polyethylene tubes (1.5 × 10 mm) containing the dental cements were implanted into dorsal subcutaneous tissue of 30 rats. After experimental periods of 7, 30, and 90 days, biopsies were performed for tissue response analysis under optical light microscope. The mRNA extraction was performed for molecular evaluation of the inflammatory process in the peri-implant tissue, which was submitted to quantitative real-time polymerase chain reaction analysis for inflammatory mediators and cytokines *TNF-α*, *Ptges2*, *Il-1β*, *Il-4*, and *Il-10*. **Results:** On the basis of the score used to grade the tissue reaction (0–3), EndoBinder (0) presented no inflammatory reaction after the 90-day period, a similar result to mineral trioxide aggregate and calcium hydroxide. The thickness of inflammatory capsules (μm) also presented significant decrease during the course of periods ($P < .05$). As regards expression of inflammatory mediators, *Ptges2* and *Il-10* were detected only at 7 and 30 days, with no statistically significant difference among the experimental groups ($P > .05$). **Conclusions:** EndoBinder induced limited inflammatory reaction. It was considered biocompatible when tested in subcutaneous tissue of rats. *(J Endod 2014;40:2024–2029)*

Key Words

Biocompatibility, calcium aluminate cement, calcium hydroxide, mineral trioxide aggregate, tissue reaction

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Several studies have reported that calcium hydroxide continues to be one of the most used materials for pulp/dentin complex protection, against which new products must be evaluated and compared, especially in biological tests involving animals and humans (1, 2). Despite the widespread use of calcium hydroxide, some questions with regard to its performance persist (3). Therefore, the constant development of new materials for pulp therapy application created mineral trioxide aggregate (MTA) (4).

The MTA cement was initially used as material for retrograde filling and root and furca perforation treatments (4); however, because of its good clinical performance, it has also been used for several other clinical applications such as pulpotomy (5), treatment of teeth with incomplete apexification (6), root resorption (7), intracoronal barrier before bleaching, and apical plug (8), root canal filling (4), and pulp capping in conservative treatments (9). Among the advantages of MTA in comparison with calcium hydroxide, its higher mechanical strength and marginal sealing ability are outstanding (10). However, some of its negative properties such as poor handling characteristics (11), low flow ability (12), high rates of tooth structure staining (13), solubility in moist conditions (14), long setting time (15), and presence and release of arsenic (16) above the safe limits proposed by ISO 9917-1 specification (17) must be considered.

The negative features observed for calcium hydroxide and MTA cements justify the development of new materials that incorporate the appropriate biological properties of both materials in a new product with easy handling, application, and adequate mechanical strength (15). Thus, a calcium aluminate-based cement, EndoBinder (Binderware, São Carlos, SP, Brazil), was developed at the Federal University of São Carlos (Brazil-UFSCAR, patent number PI0704502-6).

The laboratory synthesis process of EndoBinder provides several advantages in comparison with MTA such as greater control of impurity levels, especially Fe_2O_3, which promotes tooth staining (7, 13), and free MgO and CaO, responsible for an undesired expansion of the material in contact with moisture (18). The balance between stoichiometric phases rich in Al_2O_3 and $CaCO_3$, responsible for the hydrophilic setting process of EndoBinder, promotes greater compatibility between living tissues and cement, as well as adequate physicochemical properties (12, 19). However, *in vivo* studies that prove the biological compatibility of EndoBinder are necessary before its validation as a material for clinical application.

Thus, the aim of this study was to evaluate the biocompatibility of this new calcium aluminate cement, containing zinc oxide as radiopacifying agent, by means of tissue reaction analysis and expression of inflammatory mediators in rats in comparison with MTA and calcium hydroxide.

Materials and Methods

Selection of Animals

The study was developed in accordance with the determinations of the Research Ethics Committee on the Use of Animals - Araraquara School of Dentistry/UNESP (Process CEUA No. 3/2013), in compliance with the ethical principles for use of laboratory animals at all stages of the experiment. The animals were kept in plastic cages

(40 × 32 × 17 cm) in an acclimatized bioterium with established lighting (12 hours light/12 hours dark at temperature of 21°–25°C), receiving balanced rations (Nuvilab, Colombo, PR, Brazil) and water *ad libitum*.

Subcutaneous Implant

Thirty male rats (*Rattus novergicus*; Wistar Institute, Philadelphia, PA) were selected, with weight ranging between 250 and 300 g, and 10 specimens were used for each of the evaluation time intervals (7, 30, and 90 days).

Polyethylene tubes measuring 1.5 mm in internal diameter and 10 mm long were obtained according to the methodology described by Souza et al (20). The tubes were immersed in 70% alcohol for 120 minutes and abundantly washed with sterilized distilled water, and after this they were autoclaved. The materials tested were as follows: EndoBinder + ZnO (radiopacifying agent, 20% by weight), white MTA (Ângelus, Londrina, PR, Brazil), and calcium hydroxide (Biodinâmica, Ibiporã, PR, Brazil). All the materials were manipulated in accordance with the manufacturers' recommendations, with the proportion of 1 g powder to 0.21 mL distilled water being used for EndoBinder and for MTA 1 dose of powder (0.15 mg) to 1 drop (0.5 mL) of distilled water. For calcium hydroxide P.A., 1 g powder was placed on a glass plate, which was manipulated with 1 mL physiological solution at 0.9% (1:1) until a homogeneous paste was obtained.

After manipulation, the previously sterilized polyethylene tubes were filled with the cements by using sterile lentulo spirals (Dentsply/Maillefer, Ballaigues, Switzerland) compatible with the internal diameter of the tubes. For the surgical procedures, the animals were anesthetized by intraperitoneal administration of a solution composed of 10% ketamine chloride (Ketamina Agener; União Química Farmacêutica Nacional S/A, Embu-Guaçu, SP, Brazil) and xylazine (Dopazer; Laboratórios Calier S/A, Barcelona, Spain) in the proportion of 75 mg/kg and 10 mg/kg, respectively.

After trichotomy and disinfection of the operative field with sterile gauze imbibed in a solution of 5% povidone-iodine, 2 central incisions, one on the left and the other on the right side (5 mm long each), were performed in the dorsum of each animal by using a no. 15 scalpel blade. Lateral divulsion of the subcutaneous tissue was carefully performed starting from each central incision by using a blunt-tipped scissors for this purpose. Thus, 2 surgical recesses with a mean depth of 20 mm were obtained, in which the polyethylene tubes filled with the test materials were implanted longitudinally, remaining parallel to the incisions. Two polyethylene tubes filled with MTA or EndoBinder were implanted in 15 rats. In another 15 rats, tubes without any material (negative control) or filled with calcium hydroxide were implanted. After this, suturing was performed with 3-0 silk thread (Ethicon, São José dos Campos, SP, Brazil).

After the time intervals of 7, 30, and 90 days had elapsed, the animals were killed by anesthetic overdose, and biopsies were obtained with sufficient safety margin around the polyethylene tubes. At one of the extremities, tissue samples were collected for molecular evaluation of the inflammatory process, with mRNA being extracted and submitted to quantitative real-time polymerase chain reaction analysis (qPCR) for inflammatory mediators and cytokines. The histopathological analysis was performed in the connective tissue adjacent to the other extremity of the polyethylene tube. For this purpose, the surgical parts ($n = 5$) were immediately immersed in a 10% formalin solution (Merck, Darmstadt, Germany), in which they remained in a fixation process for the period of 24 hours. After this, they were submitted to routine laboratory processing. Semi-serial 5-μm-thick histologic cuts were obtained and stained with hematoxylin-eosin (Merck).

Histopathological Analysis

Histopathological analysis was performed by using an optical light microscope Axio Star Plus (Carl Zeiss, Oberkochen, Germany) at 32, 64, 125, and 320× magnifications. The histopathological events evaluated were the following: inflammatory infiltrate (polymorphonuclear and mononuclear cells), cellularity (fibroblasts), vascularization (blood vessels), and macrophagic activity (macrophages and multinuclear giant cells independently scored). On the basis of the tissue responses stimulated by the cements and by the control group and in accordance with the standard ISO 7405 (21), a score was used to quantify the absence or presence of these events; the inflammatory reaction was classified as (0) absent, (1) discrete, (2) moderate, and (3) severe. The thickness of the inflammatory capsule (μm) was gauged with the aid of the software program AxioVision 4.6 (Carl Zeiss, http://www.zeiss.de/microscopy).

qPCR

Samples of the tissues submitted to biopsy in the different time intervals of analysis (7, 30, and 90 days) were used for total RNA extraction in accordance with the protocol for the RNAqueous-4PCR kit (Ambion; Life Technologies, Grand Island, NY). For each total RNA sample extracted from the samples, the complementary DNA (cDNA) was synthetized for the qPCR reaction. For this purpose, the protocol of the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) was used.

The qPCR was performed in StepOnePlus Real-Time PCR System equipment (Applied Biosystems) by using cDNA as template, a set of TaqMan primers and probes designed to detect the genes encoding to interleukin 1β (*Il-1*β), tumor necrosis factor-α (*TNF-*α), interleukin-4 (*Il-4*), interleukin 10 (*Il-10*), and prostaglandin E synthase 2 (*Ptges2*), Taqman Universal Master Mix (Applied Biosystems), and nuclease-free water. To control the amount of cDNA input, the expression of all genes was normalized against the gene encoding to the housekeeping gene β-actin (*Act*β).

For evaluation of the inflammatory response, real-time PCR was performed for the proinflammatory cytokines; for evaluation of the resolution of inflammation, the mRNA expression of the anti-inflammatory cytokines *Il-4* and *Il-10* was evaluated.

Statistical Analysis

The normal distribution of data was tested by the Kolmogorov-Smirnov test, and the values obtained were statistically compared (2-way analysis of variance, the Bonferroni test, $P < .05$) with the aid of Graphpad Prism 4.0 Software (GraphPad Software, La Jolla, CA).

Results

Histopathological Analysis

The values obtained for all the histopathological events evaluated in each time interval are presented in Table 1. Generally the tissue reactions diminished during the course of time for all tested materials. Only one severe score was determined for MTA with regard to blood vessels at the 7-day period; all other scores were moderate or lower.

Period of 7 Days

For EndoBinder, at the tubular opening was observed the formation of an ample loose inflammatory capsule, still disorganized, with moderate mixed inflammatory infiltrate, characterized by the presence of neutrophils and lymphocytes. Moderate fibroangioblastic proliferation and discrete local collagenization, associated with the presence of mononuclear phagocytes (macrophages), and multinuclear giant cells