Biocompatibility Evaluation of Biodentine in Subcutaneous Tissue of Rats

Graziela Garrido Mori, PhD, Ligia Moraes Teixeira, MSc,† Danilo Louzada de Oliveira, MSc,‡ Larissa Menegucci Jacomini,‡ and Sindinéia Rodrigues da Silva, DDS‡*

Abstract

Introduction: Biodentine (Septodont, St-Maur-des-Fossés, France) is a new material suitable for various clinical situations in endodontics, such as perforation repair, retrograde filling, pulp capping, and others. Because it is a new material, its properties should be analyzed before routine clinical use. Thus, this study evaluated the biocompatibility of Biodentine in the subcutaneous tissue of rats. **Methods:** This study was conducted on 15 male rats. Two incisions were made on the dorsal region of each animal for the introduction of 4 tubes. One tube was empty, 1 was filled with zinc oxide–eugenol cement, 1 was filled with mineral trioxide aggregate, and the last tube was filled with Biodentine. After 7, 14, and 30 days, the animals were sacrificed, and the specimens were submitted to histotechnical preparation. The histologic sections were stained with hematoxylin-eosin and analyzed using light microscopy. Scores were established according to the inflammatory process and were statistically compared using the Kruskal-Wallis test ($P < .05$). **Results:** The analysis of the histologic sections evidenced a nonsignificant or mild presence of inflammatory reaction in the connective tissue in contact with the empty tube and the tube containing MTA, which was different from the tube containing zinc oxide eugenol. The connective tissue was moderately inflamed at 7 days when in contact with Biodentine; however, at 14 and 30 days, the inflammatory process was mild or nonsignificant. **Conclusions:** Biodentine was biocompatible with tissue after the 14th day. *(J Endod 2014;40:1485–1488)*

Key Words

Biocompatibility, Biodentine, MTA

From the Disciplines of *Endodontics and †Surgery, and ‡Dental School of Presidente Prudente, UNOESTE, São Paulo, Brazil.

Address requests for reprints to Dr Graziela Garrido Mori, Faculdade de Odontologia de Presidente Prudente, UNOESTE, Rua José Bongiovani, 700 Presidente Prudente, SP, Brazil CEP 19050-920. E-mail address: grazielagm@hotmail.com

0099-2399/$ - see front matter

http://dx.doi.org/10.1016/j.joen.2014.02.027

One of the great challenges of endodontic treatment is the repair process of pulp tissue when it is unnaturally violated as in cases of traumatic pulp exposure and root perforation (1). The search for materials that can replace the loss of tooth structure and contribute to the repair process is critical for successful treatment in these clinical situations (2).

Because of its ability to induce the formation of mineralized tissue, mineral trioxide aggregate (MTA) has become 1 of the standard materials for the treatment of perforations, pulp capping, and retrograde filling (3, 4). MTA has hydrophilic properties (3, 5), good radiopacity, low solubility, high pH (6, 7), and expansion capacity after setting (7). MTA is also biocompatible with tissue (8, 9), and it has antimicrobial activity (10). Furthermore, it increases the expression of type 1 collagen and osteocalcin in osteoblasts, and it stimulates the production of bone morphogenetic protein 2 (2) and the expression of alkaline phosphatase (3, 10).

However, MTA has undesirable physical and chemical properties, such as solubilization, especially when in contact with saliva (7). MTA's handing difficulties when mixed with water (6, 8), its extended setting time, and the possible discoloration of tooth structures with its use are other disadvantages (1, 11).

Biodentine (Septodont, St-Maur-des-Fossés, France) is a tricalcium silicate cement (Ca_3SiO_5) developed based on the composition of Portland cement with the manufacturer attempting to improve on the physicochemical and biological properties (1, 11–13). The material is indicated as a replacement for damaged dentin, and it can be used in cases of pulp capping, perforation repair, retrograde filling, apexification, and temporary coronal sealing (1, 14, 15).

Despite Biodentine being a promising material, there have been few studies evaluating the characteristics of this material, especially its biocompatibility with tissues. Thus, the present study evaluated the biocompatibility of Biodentine in the subcutaneous tissue of rats.

Materials and Methods

This study was conducted on 15 male rats (*Rattus norvegicus albinus*, Wistar) weighing 180–200 g. The study was approved by the Institutional Review Board on Animal Experimentation of UNOESTE (protocol: 1168). During the study, the animals were kept in cages identified according to the group and study period. The cages were cleaned daily, and the animals were fed solid food and water. However, for 12 hours preoperatively, the animals were given water only because they were thirsty.

For surgical interventions, the animals were anesthetized with a combination of ketamine hydrochloride (Dopalen; Sespo Indústria e Comércio Ltda, São Paulo, Brazil) and xylazine hydrochloride (Anasedan–Agribrands do Brasil Ltda, São Paulo, Brazil) by intramuscular injection at a dose of 0.05 mL/100 g of weight for each substance. Anesthesia was delivered using a disposable insulin syringe.

Thereafter, 60 sterile polyethylene tubes 1.3 mm in internal diameter and 10 mm in length were selected and divided into 4 groups:

1. Group 1 (negative control, $n = 15$): Empty polyethylene tubes
2. Group 2 (positive control, $n = 15$): Polyethylene tubes filled with zinc oxide and eugenol (Biodinâmica, Lobato, PR, Brazil)
3. Group 3 (MTA, $n = 15$): Polyethylene tubes filled with MTA (Angelus, Londrina, PR, Brazil)

4. Group 4 (Biodentine, $n = 15$): Polyethylene tubes filled with Biodentine

Before insertion of the tubes into the subcutaneous tissue of the animals, the rats were submitted to trichotomy, and antisepsis was performed with gauze moistened with 0.12% chlorhexidine (Periogard; Pfizer Ltda, São Paulo, Brazil). Thereafter, the dorsal region was cleaned with gauze moistened with saline solution to remove any chlorhexidine residue.

Two incisions were performed on the median dorsal region (upper and lower dorsal regions) of each rat using a No. 15 blade (Embramac Exportação e Importação, São Paulo, Brazil). Lateral to the incisions, the cutaneous tissue was pinched, and the tissue was dissected using blunt-ended scissors.

The materials were prepared following the manufacturers' instructions. Kerr files #15 (Dentsply Maillefer, Ballaigues, Switzerland) were used to place the materials inside the tubes, and pediatric excavators (SS White Duflex, Rio de Janeiro, Brazil) were used for material condensation. The tubes were completely filled with the materials analyzed. The tubes were then introduced into the subcutaneous tissue immediately after filling with the materials.

Each animal received 4 tubes: 2 in the upper dorsal region (group 1 to the left of the incision and group 4 to the right) and 2 tubes in the lower dorsal region (group 2 to the left of the incision and group 3 to the right). Then, the incisions were closed with Nylon 5-0 sutures (Ethicon, Johnson & Johnson, São José dos Campos, São Paulo, Brazil).

After 7, 14, and 30 days, 5 animals were killed by administering an anesthetic overdose. The tissues containing the tubes were removed, and the samples were fixed in 10% neutral formalin for 48 hours. The tubes were then removed from the tissue, and the specimens were embedded in paraffin. Longitudinal sections were made that were 5 μm thick, and sections were obtained every 50 μm, which yielded up to 12 sections per specimen. The sections were stained with hematoxylin-eosin and were analyzed by light microscopy.

The histologic sections were analyzed with attention paid to the presence and type of inflammatory process, the proliferation of connective tissue, and the occurrence of destructive processes, such as abscess or tissue necrosis. The intensity of inflammation was classified by the following established scores, which varied according to the intensity of the inflammatory process:

Score 1 = Nonsignificant
Score 2 = Mild
Score 3 = Moderate
Score 4 = Severe

These criteria were described in the work of Mori et al (16).

The scores were assigned by an experienced blinded examiner, and they were organized on specific sheets. The data were statistically analyzed using the Kruskal-Wallis test at a significance level of 5% ($P < .05$).

Results

Group 1: All Empty

The analysis of the histologic control sections confirmed the biocompatibility of the tubes with the connective tissue. At 7 days, there were poorly organized collagen fibers and young fibroblasts (Fig. 1*A*). Small areas exhibited neutrophils and clotting regions. At 14 days, the connective tissue was well organized, with collagen fibers, fibroblasts, and few blood vessels (Fig. 1*B*). At 30 days, the dense connective tissue became organized, with an absence of inflammatory cells (Fig. 1*C*). In several sections, there was great proliferation of dense connective tissue toward the inner part of the tube. In all the study periods, the inflammatory process was nonsignificant or mild. The analysis of scores did not show statistically significant differences regarding the inflammatory process between study periods (Table 1).

Group 2: Zinc Oxide Eugenol

Microscopic analysis of the histologic sections confirmed the irritability of zinc oxide eugenol to the connective tissue. At 7 days, there were noticeable blood clots and inflammatory cells, especially neutrophils. Some sections exhibited poorly organized collagen fibers and young fibroblasts, and other sections presented abscess formation (Fig. 1*D*). At 14 and 30 days, the connective tissue displayed few collagen fibers, fibroblasts, or blood vessels. Great quantities of neutrophils and macrophages were observed (Fig. 1*E* and *F*), indicating a moderate to severe inflammatory process. There were no statistically significant differences between the study periods (Table 1).

Group 3: MTA

The histologic sections were characterized by mild or nonsignificant inflammation. At 7 days, immature collagen fibers, fibroblasts, and proliferating blood vessels were present (Fig. 1*G*). At 14 and 30 days, the connective tissue was better organized, with many fibroblasts and collagen fibers (Fig. 1*H* and *I*). Few inflammatory cells were observed in any of the study periods. The analysis of the scores did not show statistically significant differences regarding the inflammatory process between study periods (Table 1).

Group 4: Biodentine

At 7 days, the histologic sections were characterized by few collagen fibers or fibroblasts. Clot areas, proliferating blood vessels, and inflammatory cells were observed in several sections (Fig. 1*J*). The inflammatory process was moderate to severe during the study period. At 14 and 30 days, the inflammatory process was reduced. Several sections exhibited lymphocytes and macrophages, characterizing moderate inflammation; however, in most of the specimens, the inflammation was mild. The onset of connective tissue formation, with collagen fibers, fibroblasts, and blood vessels, was also observed (Fig. 1*K* and *L*). Comparison of the scores in this group revealed statistically significant differences between the 7-day period and other study periods ($P < .05$), thus confirming a reduction in the inflammatory process (Table 1).

Comparison among Experimental Groups

Comparison between groups 1 and 2 revealed statistically significant difference between the 2 groups in all the study periods ($P < .05$), indicating differences in the tissue response to the materials that were used (Table 1). Group 3 was different from group 2 in all the study periods ($P < .05$); however, the scores of group 3 did not show statistically significant differences regarding inflammation between the study periods for group 1.

At 7 days, group 4 was significantly different from groups 1 and 3 in all the study periods ($P < .05$). At 14 and 30 days, group 4 showed a statistically significant difference compared with group 2 in all periods ($P < .05$), and there were no differences compared with the other groups (Table 1).

Discussion

The biocompatibility of dental materials is fundamental for avoiding significant inflammatory reactions and for allowing repair. Several tests can be used to evaluate the reactions of cells and tissues to the