Biocompatibility of a Self-adhesive Gutta-percha–based Material in Subcutaneous Tissue of Mice

Felipe Gonçalves Belladonna, DDS, MSc, [*] *Mônica Diuana Calasans-Maia, DDS, MSc, PhD,* [†] *Adriana Terezinha Neves Novellino Alves, DDS, MSc,* [†] *Rodrigo Figueiredo de Brito Resende, DDS, MSc,* [†] *Erick Miranda Souza, DDS, MSc, PhD,* [‡] *Emmanuel João Nogueira Leal Silva, DDS, MSc, PhD,* [§] *Sandra Rivera Fidel, DDS, MSc, PhD,* [*] *and Gustavo De-Deus, DDS, MSc, PhD* [†]

Abstract

Introduction: The purpose of this study was to evaluate the biocompatibility of a self-adhesive gutta-percha material and compare it with that of conventional gutta-percha. **Methods:** Standard quantities of bioactive gutta-percha and conventional gutta-percha were directly inserted subcutaneously into the dorsal connective tissue of 30 BALB/c mice according to ISO 10993-6. After 7, 21, and 63 days each, 10 animals were euthanized, and the materials and surrounding tissue were removed. Tissue samples were subjected to histological processing resulting in 5-μm-thick slices stained with hematoxylin-eosin and Gomori trichrome stain. A grade ranging from I–IV was used to classify the inflammatory reaction. The Mann-Whitney *U* test with Bonferroni correction was used to compare the grade of inflammation induced by the materials at each time point. Qualitative evaluation of biocompatibility over time was also performed. **Results:** Bioactive gutta-percha was more biocompatible than conventional gutta-percha at each time interval ($P < .05$). Tissue exposed to bioactive gutta-percha reached "no inflammation" (grade I) at the 21-day interval, whereas it took 63 days for the conventional gutta-percha to reach the "slight inflammation" level (grade II). **Conclusions:** Bioactive gutta-percha presented good tissue reaction at all time points. It may serve as an alternative to gutta-percha in terms of biocompatibility. *(J Endod 2014;40:1869–1873)*

Key Words

Bioactive gutta-percha, biocompatibility, dental materials, gutta-percha, root canal filling

From the *Department of Endodontics, Rio de Janeiro State University, Rio de Janeiro, Brazil; †Federal Fluminense University, Rio de Janeiro, Brazil; ‡Department of Dentistry II, Faculty of Dentistry, Federal University of Maranhão, Brazil; and §Department of Endodontics, Grande Rio University (UNIGRANRIO), Duque de Caxias, Brazil.

Address requests for reprints to Prof Gustavo De-Deus Av Henrique Dodsworth 85, apto 808, Lagoa, Rio de Janeiro, RJ 22061-030. E-mail address: endogus@gmail.com

0099-2399/$ - see front matter

http://dx.doi.org/10.1016/j.joen.2014.07.013

Since its introduction in endodontics by Bowman in 1867, gutta-percha has been the most frequently applied core root filling material (1). It is well established that gutta-percha by itself cannot fully obturate the root canal space because of its poor adaptability to the dentinal wall. Hence, the use of a sealer is always recommended in order to achieve an improved root filling (2). However, sealers may adhere to gutta-percha and the root canal wall with varying strength, resulting in 2 potential weak links inside the root filling that might lead to microleakage (3). Consequently, the root canal system should be ideally filled with 1 single material forming a monoblock with the root canal wall.

Recently, an initial report on a newly developed root filling material (Smartodont LLC, Zurich, Switzerland) suggested some interesting properties. The material could work without sealer because of the incorporated ultrafine bioactive particles (4). This novel composite material consists of nanometric, alkaline, radiopaque particles incorporated into polyisoprene, the matrix polymer of gutta-percha. It showed good immediate sealing properties when heated and applied into simulated root canals in resin blocks (5) and a high pH, suggesting antimicrobial activity (4, 6).

Although root filling materials are designed to be contained within the canal spaces (7), these materials might be inadvertently pushed into the periradicular tissues as a result of procedural errors or with the use of certain filling techniques, triggering apical tissue reactions that might delay healing and influence the outcome of treatment (8, 9). Therefore, before advocating clinical use, an initial screening on tissue interaction and response to any new filling materials are of paramount importance. Several methods have been used to evaluate tissue responses to new endodontic materials introduced on the market. One of the most practical and widely used methods is the implantation of these materials in the subcutaneous connective tissue of laboratory animals (10–14). The irritative effect of endodontic materials is evaluated by the histopathological analyses of the tissue reaction around the implants (10).

To date, no study assessed the biological properties of this self-adhesive gutta-percha–based material. Therefore, the aim of this study was to histopathologically evaluate the biocompatibility of bioactive gutta-percha by implantation into the subcutaneous connective tissue of mice. Conventional gutta-percha was used as the reference material for comparison. The null hypothesis tested was that bioactive gutta-percha is not as biocompatible as conventional gutta-percha.

Materials and Methods

Animal Model

Thirty male BALB/c mice weighing 20–30 g were used in this study. The 50-day-old animals were kept in temperature-controlled rooms and received water and food ad libitum. They were randomly assigned to 2 groups according to the material subcutaneously implanted (bioactive gutta-percha and conventional gutta-percha) and followed for 3 evaluation periods (7, 21, and 63 days), resulting in 5 animals per group per evaluation period. The care of the animals was performed according to the Fluminense Federal University Ethical Commission on Teaching and Research Animals, which approved the project before the beginning of the experiment, under the protocol no. 235.

Tested Materials

Conventional gutta-percha (Dentsply, Petrópolis, Brazil) was used as the control. The tested material, a prototype sample of bioactive gutta-percha, contained a proprietary mixture of trans-1.4-polyisoprene, zinc oxide, radiopaque nanometric bioactive particles, and softener (5). Both materials were cut into standardized sizes of $1 \times 1 \times 0.3$ cm.

Surgical Procedures

The animals were anesthetized by an intramuscular injection of ketamine chlorhydrate (0.1 mg/mL) associated with xylazine (0.05 mg/mL) (Veltbrands, São Paulo, Brazil). The surgical site on the dorsal skin was shaved and disinfected with 5% iodine solution (Vansil, Rio de Janeiro, Brazil), and an incision of approximately 2-cm long was made in a head-tail orientation using a no. 15 blade (Solidor, Santa Catarina, Brazil). Subsequently, the subcutaneous tissue was dissected to insert the materials. Each animal received 1 sample of either bioactive gutta-percha or conventional gutta-percha. After material implantation, the incisions were closed by means of a 5-0 mononylon suture (Ethicon, São Paulo, Brazil).

After 7, 21, and 63 days of surgical implantation, 10 animals (5 per material group) were anesthetized and euthanized with an overdose of the same anesthetic agent used for surgical implantation, and an excisional biopsy of the implanted materials and surrounding tissues was performed.

Histologic Analysis

The samples were fixed in 10% buffered formalin solution for 24 hours and processed for conventional histopathological examination. The connective tissue adjacent to the material was sectioned at a microtome setting of 5 μm. Five sections from each specimen were selected and stained with hematoxylin-eosin and Gomori trichrome stain. Histopathological evaluations were made under a light microscope (Carl Zeiss, Oberkachen, Germany) at 40, 100, 200, and 400× magnifications on the basis of the tissue responses stimulated by the bioactive gutta-percha and conventional gutta-percha materials. The observer was blinded to the tested materials.

The following histological events were assessed: inflammatory infiltrate (polymorphonuclear cells and mononuclear cells), capacity of cellularity and vascularization (fibroblasts and blood vessels), and macrophagic activity (macrophage cells). A score was used to quantify the presence or absence of these events as follows: (−) absent, (+) slight, (++) moderate, and (+++) intense. Depending on these features, the inflammatory reaction of the connective tissue was classified as follows: grade I, scattered chronic inflammatory cells (no inflammation); grade II, infiltration of inflammatory cells and wavy collagen fiber deposits and fibrosis (slight inflammation); grade III, dense infiltration of inflammatory cells, limited areas of tissue edema, and vascular congestion (moderate inflammation); and grade IV, very dense infiltration of acute and chronic inflammatory cells, widespread edematous areas, and vascular congestion along with fibrin deposits (severe inflammation) according to the criteria described by Shahi et al (15).

Statistical and Qualitative Analysis

A qualitative description of the results of the biocompatibility test was a priori provided and, the grade of inflammation induced by the materials was further statistically compared (SPSS 17.0; SPSS Inc, Chicago, IL) at each time point by using a Mann-Whitney *U* procedure with

A

	7 Days		21 Days		63 Days	
Histopathologic event	BGP	CGP	BGP	CGP	BGP	CGP
Polymorphonuclear	++	+++	-	++	-	++
Mononuclear	+	+++	+	+++	-	++
Fibroblasts	+	++	+	++	+	+
Blood vessels	+	++	+	+	+	+
Macrophage	+	++	-	++	-	++

BGP, Bioactive gutta-percha; CGP, conventional gutta-percha.

Score: (-) absent; (+) slight; (++) moderate; (+++) intense.

B

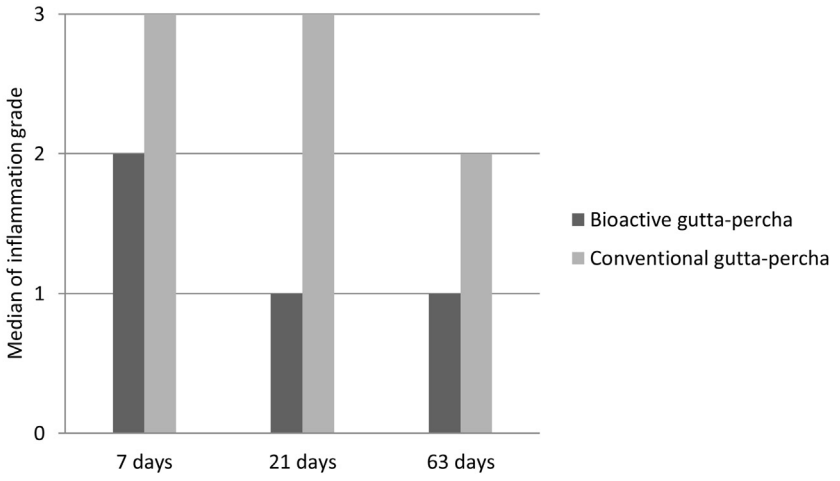

Figure 1. (*A*) Summary of data obtained after histopathological events observed in each group in different periods of study. (*B*) The median inflammation grades in tested periods and materials.