Biocompatibility of the different portions of the content of AH Plus® sealer tubes through subcutaneous implantation

Josete Veras Viana **PORTELA**[1]
Rielson José Alves **CARDOSO**[2]
Cássio José Alves de **SOUSA**[3]
Huang Huai **YING**[4]

ABSTRACT

Objective: Following the ISO/FDI and ANSI/ADA criteria, this study evaluated tissue response to the resinous sealer AH Plus®, analyzing its initial, middle and final tube segments as well as the total mixture of the two pastes that comprises it. This methodology was based on the clinical observation of the differences in consistency, homogeneity and fluidity of this sealer according to which part of the tube is used. **Methods:** Two subcutaneous implants were carried out in the dorsal region of 5 guinea pigs (Cavia porcellus) for each portion of the tested sealer and total mixture. The observation periods were 30 and 90 days. The animals were sacrificed and the implants were removed and histologically processed to obtain serial sections which were stained using hematoxylin and eosin. **Results:** The histological evaluation using an optical microscope at 20x, 100x, 200x, 400x and 1000x magnifications showed that the sealer induced moderate to severe inflammatory response at 30 days with expressive inflammatory infiltrate, which decreased to moderate to mild response at 90 days, with mild or moderate inflammatory infiltrate. There was no significant difference between the segments of the tube. **Conclusion:** This evaluation led to the conclusion that the studied sealer does not present conditions of biocompatibility within the parameters and the experimental conditions adopted and there is no biological difference between the initial, medium and final segments or complete mixture of the two pastes.

Keywords: Biocompatible materials. Subcutaneous tissue. Endodontics.

How to cite this article: Portela JVV, Cardoso RJA, Sousa CJA, Ying HH. Biocompatibility of the different portions of the content of AH Plus® sealer tubes through subcutaneous implantation. Dental Press Endod. 2011 Oct-Dec;1(3):56-64.

» The authors report no commercial, proprietary, or financial interest in the products or companies described in this article.

[1]Professor of Endodontics, NOVAFAPI.

[2]PhD in Endodontics, University of São Paulo. Full Professor, Center of Dental Research São Leopoldo Mandic.

[3]PhD in Oral Rehabilitation, University of São Paulo. Associate Professor, Federal University of Uberlândia.

[4]MSc in Endodontics, Center of Dental Research São Leopoldo Mandic..

Received: October 20, 2011 / Accepted: November 5, 2011.

Contact address: Josete Veras Viana Portela
Rua Angélica, 1331, Ap. 900 Ed. Grand Monde – 64.048-260 – Teresina / PI – Brazil
E-mail: josete_veras@uol.com.br

Introduction

Endodontic therapy is characterized by an interconnected series of operative steps. Obturation requires special attention because substances and materials are introduced into the root canals and they may be in permanent contact with apical and periapical tissues.

An endodontic filling material must have physicochemical properties required for sealing and biological compatibility with the apical and periapical tissues. It must be inert or capable of inducing apical mineralization, known as biological sealing. When these conditions are met, the root canal treatment is considered to be successful.

Various materials have been proposed for endodontic obturation. The chosen material must not be cytotoxic, otherwise it might negatively interfere with the repair process of the tissue with which it is in contact.

Recent studies have shown that gutta-percha is the best root canal filling material, in spite of the slight irritation caused by the presence of zinc oxide in its composition.

The constant search for new root canal sealers has encouraged the study of the properties of existing materials as well as research to develop new materials with desirable physicochemical and biological properties.

The biological evaluation of root canal sealers using specific tests was carried out in line with standards set by the International Organization for Standardization (ISO), and document ANSI/ADA No. 41, of 1982.[1] The use of standardized methodologies facilitates the comparison of results from studies that use identical materials.

Among the obturation materials used for root canals, the cement-based plastic resins have become increasingly popular. AH Plus® sealer is an epoxy/amine based cement, in the form of two 4 ml tubes of paste, and equal amounts of paste A and paste B are used to prepare it. It has a working time of 4 hours at 23 °C, and setting time of 8 hours at 37 °C, according to the manufacturer. One drawback of the sealer is the difference in consistency, homogeneity and fluidity that is easily observed according to which section of the cement inside the tube is being used. The separation of the components that occurs in AH Plus® may cause chemical changes in different segments of the tube, leading to changes in the biological behavior of this material. This evaluation of the sealer's biocompatability was motivated by the fact that there were no studies in the literature that assess this property.

Material And Methods

Manipulation of AH Plus® sealer

An analytical scale (Gehaka, model AG 200) was used to weigh each segment of material. The scale has a minimum capacity of 0.002 g and maximum of 210 g. The content of two tubes of the cement was distributed onto glassime weighing paper; the weight of each tube was 8.64 g (Fig 1). This was considered the standard weight for the divisions of all tested cement tubes. Thus, each of the three

Figure 1. Net weight obtained for the contents of each tube of cement.

segments of each tube was calculated to be 2.88 g. The 2.88 g portions were stored in aluminum tubes with an internal layer of varnish immediately after weighing and kept at room temperature (Fig 2). Five sets of AH Plus® sealer were used for the experiment: one set (lot 045000181) was used to check the weight, two sets (lot 04000181) were used for the division into segments and two sets (lot 0403001599) for the total mixture.

Surgical Procedures (Subcutaneous implantation)

Forty guinea pigs weighing approximately 800 g each were used to study the subcutaneous response to materials. Medication with atropine sulfate at a dose of 0.044 mg / kg (SC) was applied ten minutes before anesthesia to prevent cardiac arrhythmia in animals. The animals received an intraperitoneal injection of 0.6 ml of ketamine (100 mg / ml) mixed with acepromazine (0.5 mg / ml) as anesthetic. After anesthesia, trichotomy and skin disinfection with iodine alcohol solution at 5% were carried out to maintain the aseptic chain.

The vehicles that contained the material (specimens) were Teflon® tubes with an internal diameter of 1.3 mm and an external diameter of 1.6 mm. One of the ends of the tube was filled with a small amount of paraffin to prevent leakage and consequent contamination of the side walls, which were used as a control of the technique.

After trichotomy (Fig 3) and skin disinfection with 5% iodine alcohol solution, two small incisions were made (Figs 4 and 5) on the animals' backs for the introduction of needles. The methodology for the introduction of Teflon carriers, containing the material to be tested and using prepared needles, was proposed by Safavi et al.[9] After manipulation according to the manufacturers instructions, the cement was placed into the Teflon carriers with the aid of a stereoscopic magnification lens. The needles were introduced with their respective piston in position into the subcutaneous connective tissue of the animal parallel to the outer surface of the skin, up to about 2 cm deep (Fig 6). The original piston was removed, the Teflon tube was placed, with the end containing the material facing forward, and another plunger, without bevel, was introduced into the needle to gently insert the Teflon tube into the subcutaneous tissue. Each animal received two implants containing the same material (the initial, middle, final portion or the total mixture of the two pastes). A total of 10 implants for each portion, for each observation period were carried out.

Laboratorial processing

The experimental criteria were carried out

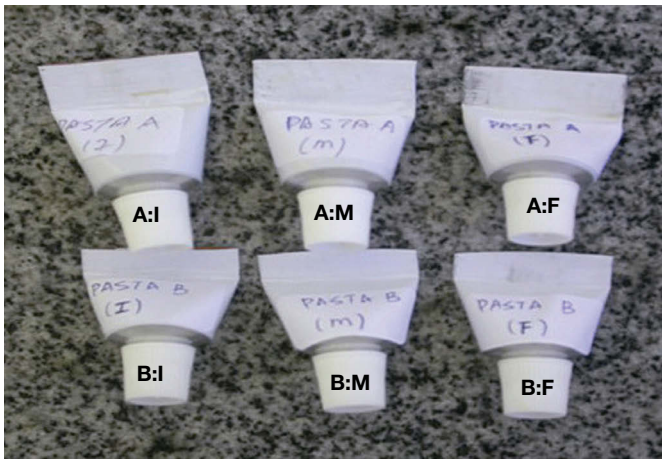

Figure 2. The portions of each paste properly stored: (A:I) initial portion of Paste A, (A:M) middle portion of Paste A, (A:F) final portion of Paste A, (B:I) initial portion of Paste B, (B:M) middle portion of Paste B, (B:F) final portion of Paste B.

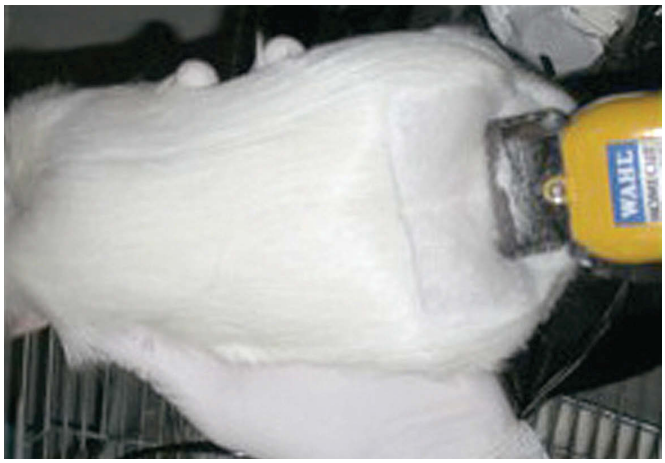

Figure 3. Trichotomy of the animal's back.

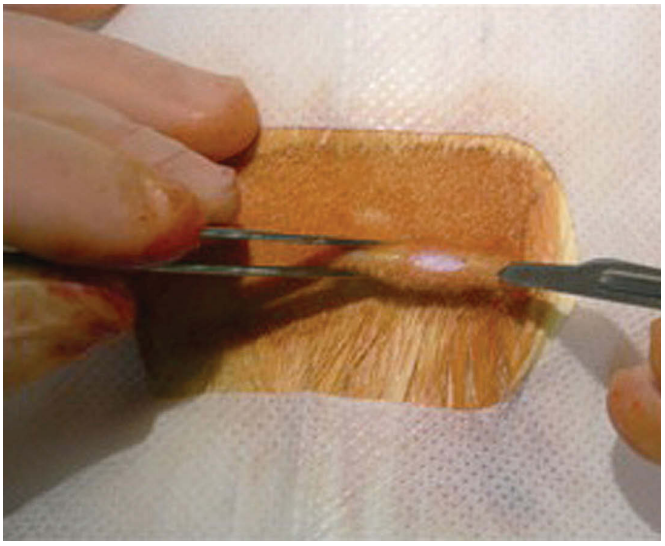

Figure 4. Making the incision in the animal's back.

Figure 5. After incisions.

according to the methodology defined by the Fédération Dentaire Internationale, Technical Report No. 9, page 173, item 4.11.

The observation times were 30 and 90 days, after which the animals were submitted to ortothanasia in a carbon dioxide chamber, the skin of the back was dissected and the tubes removed, with about 1 cm of surrounding tissue. The specimens were fixed for at least 48 hours in a 10% buffered formalin solution, pH 7.4.

After rinsing in running water for 12 hours, the specimens were dehydrated in increasing concentrations of ethanol solutions (70% to 100%), two baths of xylol and embedded in paraffin for histological processing.

Twenty-four slides were prepared, each with six sections, with approximately 144 semi-serial sections with the microtome set at 5 μm, in a plane parallel to the direction of the tube entry, in order to obtain the material / conjunctive tissue contact interface. The hematoxylin and eosin staining technique was used. After routine processing, slides were evaluated under an optical microscope.

Evaluation

The severity of the inflammatory response determined the acceptability (or not) of the materials. The classification of severity of response was obtained by recording the findings according to criteria established by the FDI.

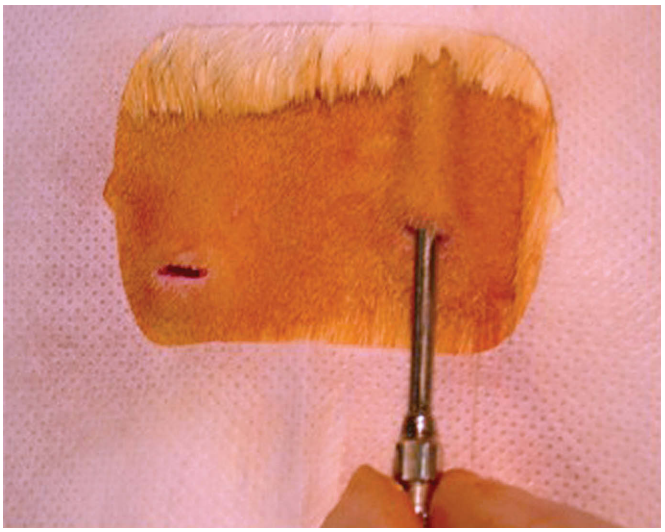

Figure 6. Introduction of the needles and their pistons.

Results

Control

As described in the methodology, the areas defined as control (absence or minimal degree of inflammation) were the connective tissue interfaces with the side walls of the Teflon® tube, as shown in (Fig 7). The formation of a fibrous capsule without the presence of cells that indicate a significant inflammatory process can be observed, showing the slight reactivity to Teflon.

Materials tested:

Table 1 shows the observation periods and distribution of the number of implants studied. Eighty implants were used in total, 10 for each observation time, totaling 20 implants for each portion of the tube and the total mixture of the material. It also illustrates the general aspects of the inflammatory responses of these portions and the intensity of the inflammation seen in each portion implanted, according to the criteria of FDI (1980)[3] and ADA/ANSI (1982)[1].

At 30 days, the portions of the assessed AH Plus® had similar inflammatory reactions, ranging from moderate to severe. This response is not acceptable from the standpoint of biocompatibility, according to the established criteria.

At 90 days of observation, there was a decrease in inflammatory response, which ranged from moderate to mild. The accumulation of inflammatory cells could be observed in many situations with a dispersion of the material in the connective tissue, promoting the perpetuation of an inflammatory response (chronic type). This demonstrates the low-intensity toxicity of the material tested.

The formation of thick fibrous capsule at 30 days, with a large focal accumulation of inflammatory cells, was a constant finding. At 90 days there was a significant decline and reduction of this infiltration.

Inflammatory responses did not differ significantly between the different segments of the AH Plus® sealer. On the sides of the tube, used as control, the formation of fibrous capsule occurred, always thinner than in the specimen opening region where the tested material was in contact with the tissue.

The tissue responses observed had the same magnitude and histological characteristics for all segments tested for each experimental period (Fig 8 and Fig 9).

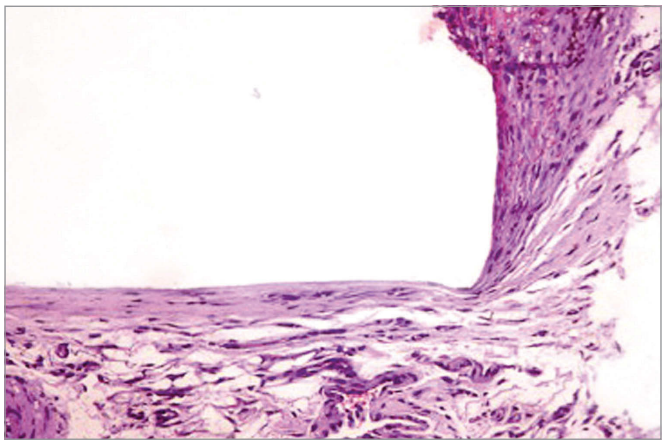

Figure 7. Histological figure that represents control areas.

Discussion

The biocompatibility of endodontic materials is characterized by several parameters such as genotoxicity, mutagenicity, carcinogenicity, cytotoxicity,

Table 1. List of subcutaneous implants and quality of inflammatory responses.

	AH Plus®							
	Initial Portion		Middle Portion		Final Portion		total	
Experimental Period (days)	30	90	30	90	30	90	30	90
Total of Implants	10	10	10	10	10	10	10	10
Slight inflammation	- (0%)	3 (30%)	- (0%)	6 (60%)	- (0%)	6 (60%)	- (0%)	4 (40%)
Moderate inflammation	6 (60%)	7 (70%)	3 (30%)	4 (40%)	5 (50%)	4 (40%)	6 (60%)	6 (60%)
Severe inflammation	4 (40%)	- (0%)	7 (70%)	- (0%)	5 (5%)	- (50%)	4 (40%)	- (0%)

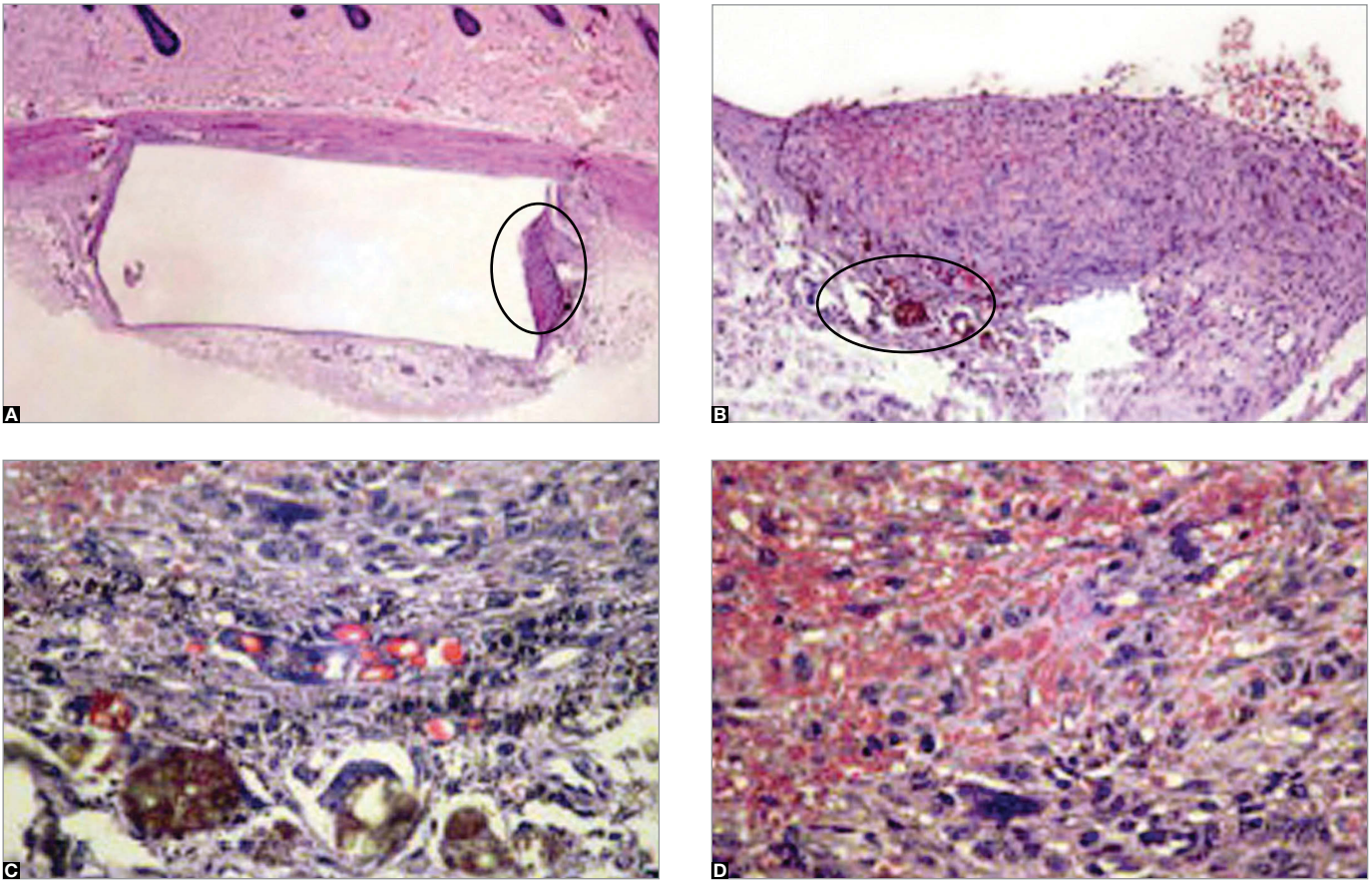

Figure 8. A) AH Plus® subcutaneous implant at 30 days. Overview of the region occupied by the Teflon tube/ **B**) Magnification of the demarcated area of **A**. Presence of extensive inflammatory infiltrate. **C** and **D**) Details of the demarcated area of **B** showing the focal accumulationof inflammatory cells with presence of giant cells and hyperemic areas.

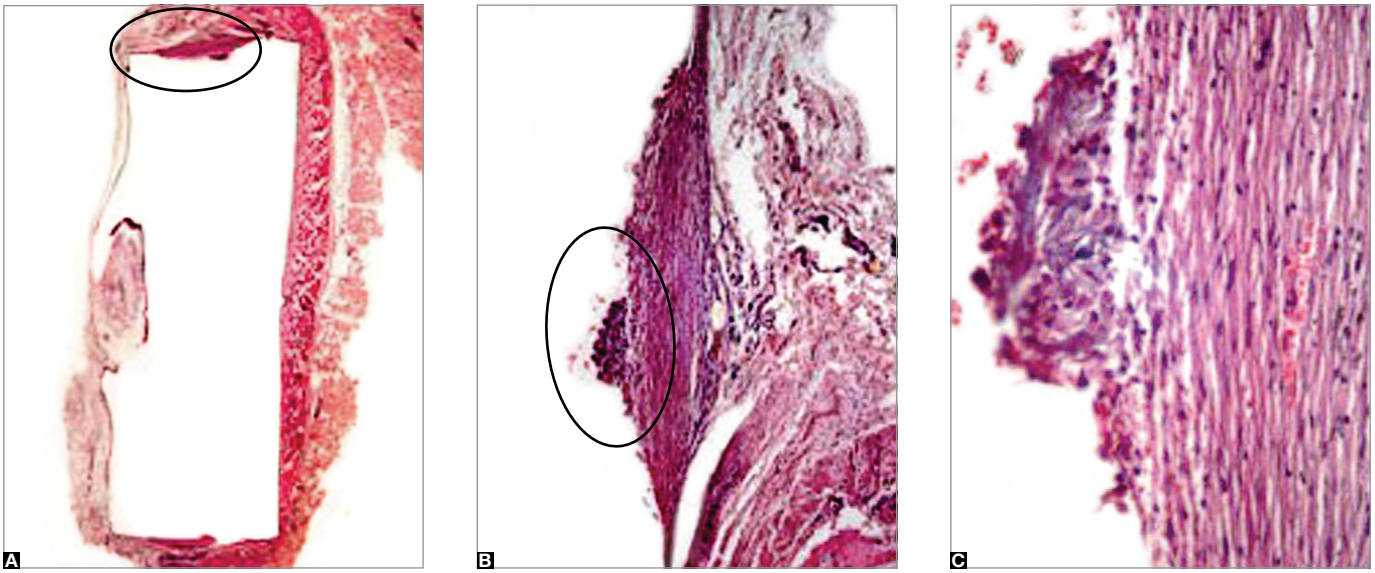

Figure 9. A) Overview of subcutaneous implantation. Note the formation of fibrous capsule at the interface with the cement **B**) Magnification of A showing the contact area of cement/tissue **C**) Detail of the fibrous capsule of **B**, Note the thick fibrous capsule and moderate inflammatory process.

histocompatibility or microbial effects. It is biologically impossible to characterize a material as biocompatible or non-biocompatible after using just one methodological test. Its properties need to be investigated using several *in vitro* and *in vivo* tests.

Many tests have been suggested to evaluate the biocompatibility of endodontic sealers, in order to reproduce as closely as possible the clinical use of these materials. When considering the biological properties of materials used in root canal filling, several features must be observed, depending on the aim of the study.

The results of any investigation are influenced by the methods used. According to Paffenbarger[8] (American Dental Association), the "technique used for any material is as important as the material itself, because an inferior technique can ruin or damage a superior material." Thus, Spangberg,[10] Olsson et al,[6,7] Langeland et al[4] agreed that the studied materials should be handled and applied in laboratory tests exactly as recommended by manufacturers and as they are used in everyday practice. The sealers for root canal filling should be tested in their paste form because in a clinical situation the setting of the material is only complete after it has been introduced.[11]

For many decades, ISO/FDI, ADA, COMIET and other governmental or non-governmental organizations tried to regulate and standardize the various research methodologies recommended to evaluate the biocompatibility of materials used in clinical procedures. Thus, a sequence of tests was divided into initial tests, secondary tests and application tests, the latter described as pre-clinical tests.

The test of implantation of materials in subcutaneous tissue is the most widely used of the recommended secondary tests to assess biocompatibility of filling materials. The technique is standardized, and can be more accurately controlled because it has fewer variables. It enables one to determine the degree of irritability of various portions of the material studied.

New materials that have no acceptable scientific basis to justify their use are frequently introduced on the market. Therefore it is important to prove whether the main biological aspects of these materials meet those recommended by the organizations that seek the uniformity and standardization of tests, so that these materials can be widely accepted by the scientific community.

The secondary biocompatibility tests of dental materials are carried out using small animals. The advantage of implantation in subcutaneous tissue is that it shows the reaction of the connective tissue that occurs in the area of contact between material and tissue. An analysis of the methodology used in these studies shows that this procedure involves the careful evaluation of results, because inflammatory reactions are cumulative due to the initial surgery and may mask the true tissue response to the material. Seeking to circumvent this problem, Safavi et al.[9] developed a needle and plunger system, both having a beveled edge that completely blocks the needle. This methodology is now commonly used and was combined with the methodology recommended by the FDI for this study (Fig 6).

Several studies have shown that the evaluation of biocompatibility of subcutaneous implants of specimens of endodontic materials is a reproducible and acceptable methodology. However, the differences between animal species, implantation sites, methods, observation times and the criteria used to evaluate results makes it difficult to compare the results. The results produced by this methodology sometimes differ from those obtained by several authors who use different methodologies, and result in the definition of inflamatory response patterns according to particular observation criteria. In order to create reproducible results that can be compared with other researchers, this study used the methodology defined by the international scientific community (FDI[3], ADA/ANSI[1]).

AH Plus® sealer was launched in the 90s and has been widely studied. Some studies have evaluated the profile of the biological behavior of this material. The results of this study with subcutaneous implants (Table 1) show that, at 30 days, the inflammatory response for all portions of the material was moderate to severe (Fig 8), with obvious presence of chronic inflammatory cells and foreign body type giant cells in direct contact with the material. At 90 days, the response was mild to moderate, which shows that the trend is for a reduction of the inflammatory response, with significant regression of the inflammatory process and formation of thick fibrous capsule with the material dispersed at a distance, without

the intense inflammatory phenomena observed in the first experimental period (Fig 9).

These results are consistent with those found by Chita,[2] who used the same methodology to compare AH Plus® cement with Endo Rez and Konne. The former had a lower inflammatory response at 90 days, although the chronic inflammatory profile continued. The formation of fibrous capsule between the implanted material and the tissue, without significant inflammatory infiltrate, has been considered as a criterion of acceptability of the material (FDI, 19803; Olsson et al.[6,7]), but the presence of chronic inflammation cells adjacent to the material at any observation period demonstrates the toxic nature of the cement. These results lead to the conclusion that the tested material is not biocompatible according to the defined parameters.

Montes[4] evaluated Epiphany® cement, a dual setting resin material with AH Plus® using intraosseous implants in guinea pigs, and found favorable tissue responses with Epiphany, unlike AH Plus,® which presented a severe reaction at 30 days and a mild to moderate reaction at 90 days.

In this study, the plane of the histological section passes through the opening of the Teflon® tube, including the entire interface between the connective tissue and the side walls of the tubes, which served as an excellent negative control. The areas that were examined in the histological sections were generally free of inflammation, indicating that the responses at the entrance of the tubes were related to the toxicity of the materials and demonstrated the compatibility of Teflon® (Fig 7). These areas are used as control, because of the excellent biocompatibility of Teflon when implanted in subcutaneous or intraosseous tissue.

Although the AH Plus® sealer is not biocompatible according to the FDI criteria, it has been accepted as a filling material because it has suitable physical characteristics such as good working time, good radiopacity and low solubility. It is because of these properties that the cement is one of the most frequently used by professionals. The biological aspect has proven capable of more research at all levels, so you can reach a conclusion. The simple fact that it has been shown to be more biocompatible than its predecessor, does not make it biocompatible by itself.

AH Plus Jet is a new form of AH Plus®. According to the manufacturer, it maintains the same chemical properties but with modified packaging. The material is mixed in a syringe with a cannula through which it is dispensed ready for use directly within the canal. There were no studies in the literature that use the new format of this material.

In this investigation, it was possible to define this cement as not acceptable according to the biocompatibility parameters initially established, despite showing a significant reduction in its potential for irritation at 90 days of observation. This is an incentive to continue using other tests over a longer observation period. There was also no significant difference in biological response when evaluating the various portions of the tubes or when a total homogenization was carried out.

Conclusion

The results show that the inflammatory response did not differ significantly between the the various segments of the tube or with the complete homogenization of AH Plus® cement.

The evaluation of the biocompatibility of AH Plus® cement does not enable it to be classified as biologically compatible within the established parameters and experimental conditions.

References

1. American National Standards Institute, American Dental Association. Specification N. 41 for Biological Evaluation of Materials. Approved 1982 Jan 4.
2. Chita JJ. Avaliação da resposta inflamatória em tecido subcutâneo de cobaias “guinea pig” a cimentos endodônticos [dissertação]. Campo Grande (MS): Universidade Federal de Mato Grosso do Sul; 2004.
3. Federation Dental International. Technical Report #9. In: Langeland K, Cotton WR. Recommended standard practices for biological evaluation of dental materials. [s.l.]:[s.n.]; 1980.
4. Pascon EA. Projeto biocompatibilidade dos materiais endodônticos: biocompatibilidade de resina poliuretana derivada da mamona [tese]. Ribeirão Preto (SP): Universidade de São Paulo;1999.
5. Montes CRM. Avaliação histológica da resposta inflamatória aos cimentos endodônticos AH Plus e Epiphany, através de implantes intraósseos, segundo critério estabelecidos pela Federação Dentária Internacional [monografia]. Uberlândia (MG): Associação Brasileira de Odontologia; 2005.
6. Olsson B, Sliwkowski A, Langeland K. Intraosseous implantation for biological evaluation of endodontic materials. J Endod. 1981;7(6):253-65.
7. Olsson B, Sliwkowski A, Langeland K. Subcutaneous implantation for the biological evaluation of endodontic materials. J Endod. 1981;7(8):355-67.
8. Paffenbarger GC. List of certified dental materials. In: American Dental Association specifications for dental materials. 4ª ed. Chicago: ADA; 1961. p. 69.
9. Safavi KE, Pascon EA, Langeland K. Evaluation of tissue reaction to endodontic materials. J Endod. 1983;9(10):421-9.
10. Spangberg L. Biological effects of root canal filling materials. 1. Media for the investigation of the toxic effect of water-soluble substances on human cells in vitro. Odontol Revy. 1969;20(2):123-32.
11. Pascon EA, Sousa CJA, Langeland K. Biocompatibility of endodontic materials: guinea pig bony tissue response to polyurethane resin derived from castor bean oil [abstract 869]. J Dent Res. 2001;80:144.