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Bismuth subnitrate as filler for epoxy-based root canal sealers

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Abstract

Objective: The purpose of the present study was to evaluate flow, film thickness, working time, setting time, dimensional change, sorption, solubility and cytotoxicity of an experimental epoxy-based root canal sealer with addition of bismuth subnitrate in different concentrations.

Methods: Endodontic sealers were prepared with an epoxy resin with bismuth subnitrate additions of 20%, 40%, 60%, 80%, 100% and 120%. Flow, film thickness, working time, setting time, dimensional change, sorption, solubility, and cytotoxicity were studied according to ISO standards. Data were analyzed by one-way ANOVA and *Tukey.*

Results: The flow, working time, water sorption, and solubility significantly decreased with increasing filler particle concentration. The film thickness and dimensional change values significantly increased with higher filler particle addition. There was no difference in the results of setting time and cytotoxicity comparing the groups.

Conclusion: Addition of bismuth subnitrate appears to be a promising filler particle for root canal sealer.

Keywords: Bismuth subnitrate; cytotoxicity; dentistry; epoxy-based endodontic sealer; filler particle

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Subnitrato de Bismuto como partícula para cimentos endodônticos à base de resina epóxica

Resumo

Objetivo: O objetivo deste estudo foi avaliar a adição de subnitrato de bismuto em diferentes concentrações em relação a propriedades reológicas em um cimento endodôntico experimental a base de resina epóxica.

Métodos: Os cimentos endodônticos foram preparados a base de resina epóxica com a adição de subnitrato de bismuto nas proporções de 20%, 40%, 60%, 80%, 100% e 120%. Os testes de escoamento, espessura de película, tempo de trabalho, tempo de presa, alteração dimensional, sorção e solubilidade e citotoxicidade foram realizados de acordo com a norma da ISO. Os dados foram analisados com o teste ANOVA de uma via e Tukey.

Resultados: Os valores do escoamento, tempo de trabalho, sorção e solubilidade diminuíram significantemente com o aumento da concentração da partícula. A espessura de película e a alteração dimensional aumentaram significantemente com o aumento da adição de partícula. Os resultados de tempo de trabalho e citotoxicidade não apresentaram diferença entre os grupos.

Conclusão: A adição de subnitrato de bismuto parece ser uma partícula promissora para ser usada em cimento endodôntico.

Palavras-chave: Subnitrato de bismuto; citotoxidade; odontologia; cimento endodôntico; partícula de carga

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Introduction

Resin-based sealers are among the major sealers to achieve total three-dimensional sealing of root canals due to their possible adhesion to tooth substrate [1]. Rheological properties such as film thickness, flow, and dimensional stability are of paramount importance to a root canal sealer. All sealers present filler particles added in the composition to increase related properties. Barium sulfate, zinc oxide, and bismuth oxide are frequently used as filler particles [2].

Bismuth is a chemical element with atomic number 83. Bismuth salts such as bismuth subnitrate in combination with antibiotics have been used against *Helicobacter pylori* [3-5]. Bismuth oxides have been frequently used as filler particles in Portland sealers due to their radiopacity and rheological properties [2,6]. However, this filler have not been used in resin-based endodontic sealers yet. In addition, the resin-based sealers have been presented better properties than water-based sealers mainly regarding adhesion to dentin [7].

The purpose of the present study was to evaluate flow, film thickness, working time, setting time, dimensional change, sorption, solubility and cytotoxicity of an experimental epoxy-based root canal sealer with addition of bismuth subnitrate in different concentrations.

Methods

Experimental Sealer Formulation

Endodontic sealers were prepared with epoxy resin-based sealer (bisphenol-A and epichlohydrin) at 2:1 (base:catalyst) with bismuth subnitrate ($Bi_5O(OH)_9(NO_3)_4$) additions of 20%, 40%, 60%, 80%, 100% and 120%, in weight. Colloidal silica (particle diameters of 7 nm) was added at 0.05% to adapt the viscosity of these sealers.

The particle size of the bismuth salt was obtained by laser diffraction. The mean diameter particle was 8.33 µm and particle size distribution is shown in Figure 1.

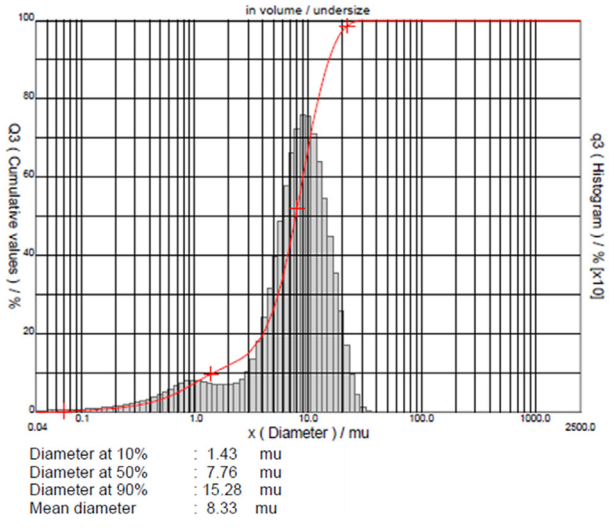

Fig. 1. The particle size distribution of bismuth subnitrate.

Properties of the sealers

The flow, film thickness, working time, setting time, and dimensional change following setting properties were conducted based on ISO 6876. The water sorption, solubility, and cytotoxicity were determined based on the ISO 4049.

A digital caliper (Digimess, São Paulo, Brazil) was used to obtain the flow, film thickness, working time, and water sorption and solubility. Gilmore needles were used for setting time. A micrometer (ausJENA, Jena, Germany) was used for dimensional change following setting, an analytical balance (Shimadzu Corp., Tokyo, Japan) for water sorption and solubility, and an MTT assay for cytotoxicity, as described below.

Flow

A total of 0.05 mL of each experimental sealer was placed on a glass plate (40×40×5 mm) with a graduated 1.5 mL syringe. Another plate with a mass of 20±2 g and a load of 100 g was applied on top of the material. Ten minutes after the start of mixing, the load was removed, and the major and minor diameters of the compressed material were measured using a digital caliper. For each experimental group, the test was conducted three times and the mean value was recorded.

Film thickness

Two glass plates (5×10 mm) were placed together and their combined thickness was measured. A volume of 0.05 mL of experimental sealer was placed at the center of one of the plates, and a second plate was placed on top of the material. At 180±5 seconds after the start of mixing, a load of 150±3 N was applied vertically onto the top glass plate. Ten minutes after the start of mixing, the thickness of the two glass plates and the interposed sealer film was measured using a digital caliper. The film thickness was recorded by the difference between the thickness of the two glass plates with and without sealer. The mean value of three measurements was recorded as the film thickness of material.

Working time

This test had the same sequence as the flow test, but was repeated with longer intervals from mixing until the 150 N load was applied. The working time was determined as the time interval from mixing to the application of the load, when the resulting diameter of the sealer was 10% less than the diameter determined in the flow test. The test was repeated three times and the mean values were recorded.

Setting time

Rings measuring 10 mm in diameter and 1mm in height were filled with material. These specimens were maintained under controlled temperature and humidity conditions, 37±1°C and 95%, respectively. Measurements were conducted using Gilmore needles, weighing 100±0.5 g and having a flat ends of 2.0±0.1 mm diameter. The needle was lowered vertically onto the horizontal surface of each

sample so that it touched every 5 minutes. The setting time was recorded when the needle did not produce any visible indent on the sealer surface.

Dimensional change following setting

Ring molds having dimensions of 6 mm diameter and 12 mm high were filled with sealer for the stability test. These specimens were positioned between 2 glass plates (25×70×1 mm). Five minutes after the start of mixing, the specimens were placed in desiccators at 37ºC and 95% relative humidity. The specimens were removed from the matrixes and the thickness was measured. They were immersed in distilled water at 37ºC for 30 days and then new measurements were made. A micrometer capable of measuring 0.001 mm was used. The difference between before and after storage was calculated. Measurements were made three times and the mean value of these measurements was recorded as the dimensional change of the material.

Water sorption and solubility

Sealers disks were produced in a silicone matrix (10.0 mm diameter, 1 mm thick). Specimens were placed in a desiccator at 37°C for 22 hours, then in a desiccator at 23°C for 2 hours. The disks were repeatedly weighed in an analytical balance until a constant mass (m1) to be obtained (i.e., until the mass loss of each specimen was not more than 0.1 mg in any 24 hours period). Diameter and thickness of each specimen were measured with a digital caliper to calculate the volume (V) of each disk (in mm). Thereafter, the specimens were stored in sealed glass vials with 10 mL of distilled water at 37°C for 7 days. After seven days, the disks were weighed after washing them under running water and gently wiping them with an absorbent paper to obtain a mass (m_2) and then returned them to the desiccator. Next, the specimens were weighed until a constant mass (m_3) to be obtained. Water sorption (WS) and solubility (SL), µg/mm^3, were calculated using the following formulae:

$$WS = \frac{m_2 - m_3}{V} \quad (1)$$

$$SL = \frac{m_1 - m_3}{V} \quad (2)$$

Cytotoxicity

The cell viability was analyzed using mononuclear cells obtained from human peripheral blood. These cells were routinely maintained in Dulbecco's modified Eagle's medium (DMEM) with HEPES-HDMEM, with 10% fetal calf serum. The cells were maintained with endodontic sealers incubated for 72 hours at 37ºC and 5% CO_2. The controls consisted of cells incubated without endodontic sealer. The rate of viable cells was quantified by testing (3-4,5-dimethylthiazol-2-yl)-2,5-diphenol tetrazolium bromide) MTT assay after 24 and 48 hours in contact with the endodontic sealer.

Statistical analysis

Data were analyzed with one-way ANOVA and Tukey multiple comparisons were used with a significance level of 5% for all tests.

Results

The results of rheological properties of experimental sealer are shown in Table 1.

Flow

The flow values significantly decreased with increasing filler particle concentration comparing 20% and the other proportions; 40% compared to 100% and 120%; 60% and 80% compared to 120% proportions (P<0.05).

Film thickness

The film thickness values increased with higher filler particle addition, with a statistically significant difference (P>0.05).

Working time

The working time significantly decreased comparing 20%, 40%, and 60% with higher filler particle concentrations and 80% compared to 120% (P<0.05).

Setting time

There is no differences (P>0.05) between the filler particle proportions.

Table 1. Flow, film thickness, working time, setting time, dimensional change, sorption and solubility of the sealers with bismuth subnitrate in different proportions.

	Flow (mm)	Film thickness (mm)	Working time (min)	Setting time (h)	Dimensional change (%)	Sorption (µg/mm^3)	Solubility (µg/mm^3)
20%	31.55 (1.41)^{a}	13.3 (5.8)^{a}	55.33 (02.08)^{a}	06:58 (00:40)^{a}	-0.14 (0.02)^{a}	35.67 (7.43)^{e}	16.5 (4.37)^{a}
40%	24.78 (1.32)^{b}	23.3 (5.8)^{a,b}	56.67 (04.04)^{a}	06:55 (00:24)^{a}	-0.31 (0.02)^{b,c}	42.02 (5.37)^{d,e}	16.23 (4.75)^{a,b}
60%	23.89 (0.71)^{b,c}	23.3 (5.8)^{b,c}	51.33 (02.89)^{a}	06:51 (00:26)^{a}	-0.34 (0.04)^{c}	64.64 (7.83)^{b,c}	13.09 (6.26)^{a,b}
80%	23.4 (0.41)^{b,c}	40 (10)^{c,d}	40.33 (02.52)^{b}	06:40 (00:25)^{a}	-0.32 (0.05)^{b,c}	81.76 (9.74)^{a}	7.84 (5.49)^{b,c}
100%	22.15 (0.13)^{c,d}	60 (10)^{c,d}	34.67 (01.15)^{b,c}	06:33 (00:20)^{a}	-0.25 (0.07)^{b}	68.05 (2.88)^{b}	3.04 (0.67)^{c}
120%	20.39 (0.03)^{d}	63.3 (5.8)^{d}	32.67 (02.31)^{c}	05:57 (00:15)^{a}	*	53.14 (3.25)^{c,d}	3.21 (1.57)^{c}

Different letters in the same column represent statistically significant differences (P<0.05).
* It was not possible to evaluate because of disintegration of the specimens.

Dimensional change following setting

Specimens of sealers with higher filler particle concentration presented higher dimensional change, compared to 20% and the other filler particle concentrations (P<0.05). There was also statistically significant difference between 60% and 100% filler particle proportions.

Water sorption and solubility

Sorption with 20% and 40% filler particle proportions was significantly lower than with other concentrations. Solubility was lower in the groups with higher filler particle proportions (P<0.05).

Cytotoxicity

There is no differences (P>0.05) between the filler particle proportions regarding cytotoxicity. The results are shown in Figure 2.

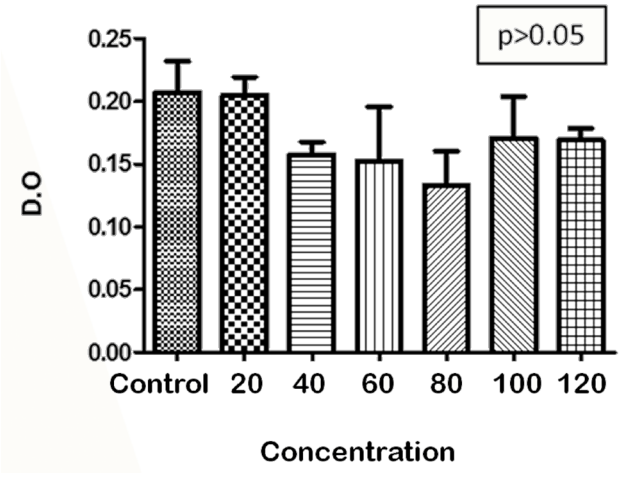

Fig. 2. Cytotoxicity of the sealer with bismuth subnitrate in different proportions.

Discussion

Sealers are intended for use in conjunction with a solid core to fill the root canal. Clinical considerations have led to a set of desirable properties for these materials [8]. These properties include being easy to manipulate, allowing different obturating methods, stability in the oral environment, radiopacity, biocompatible, antimicrobial, presenting low shrink or expansion during polymerization, self-adhesive, forming a stable bond to dentin that does not degrade with time and function and is not affected by oxidizing agents like sodium hypochlorite, strengthens the tooth, and easily removed for post placement or retreatment [9]. The filler particles have an important role in these properties. In the present study, the addition of bismuth subnitrate with different concentrations influenced the properties of the resin-based endodontic sealer.

Flow is important because it reflects the ability of the sealer to penetrate into small irregularities and ramifications of the root canal system and dentinal tubules [10]. According to ISO 6876, the flow should be at least 20 mm. In the present study, all bismuth subnitrate concentrations were appropriate for endodontic sealers regarding the standard of flow. The values were similar to other resin-based sealers, including AHPlus and Epiphany [11-13]. An adequate flow is desirable to provide well-adapted fillings [10,13,14]. Film thickness of the sealer is also related to how well the root canal is sealed [15,16]. According to ISO 6876, the film thickness should be less than 50 µm. The groups with 100% and 120% showed values higher than the standard. However, this standard is for water-based sealers. Other resin-based sealers also presented values approximately 50 µm [17,18].

The working and setting times must be long enough to place and adjust the root filling [18]. There is no exact value stated for working and setting times of endodontic sealers. ISO 6876 requires that a sealer should be at least 90% of the working time stated by the manufacturer and within 10% of the setting time standard in the manufacturer's instructions. In the present study, these times were considered appropriate for endodontic sealers. These values are dependent on the sealer components, particle size, room temperature, and relative humidity. The values for the sealers of the present study were similar to commercial sealers [19].

Water sorption and solubility have influence on the long-term degradation behavior of resin-based filling materials. A plasticizing effect with separation of the polymer chains could occur due to water uptake [20] and could cause a dimensional change of the material. According to ISO 6876, the endodontic sealer cannot shrink more than 1% and swell more than 0.1% to avoid gaps in the root filling or cracking of the tooth by expansion. In the present study, the experimental endodontic sealers presented swelling higher than 0.1%. Other resin-based sealers present higher dimensional change than the values stated by ISO 6876 [11]. Water sorption and solubility depended on the individual composition of the material [21]. The polar nature of a polymer matrix and the presence of their linkages are important for water sorption and hygroscopic expansion of resin-based materials. The filler particle of the composite material can also affect the water sorption characteristics [22].

Water sorption and solubility have a significant influence on mechanical properties and degradation of endodontic sealers [20,23]. In addition to dimensional change, water sorption could lead to unreacted monomers leaching, causing degradation of the root canal seal [20]. According to ISO 4049, the water sorption of the resin-based material cannot be higher than 40 µg/mm^3 and the water solubility must be less than 7.5 µg/mm^3. In the present study, the water sorption for the sealer with bismuth subnitrate in a proportion from 40% to 120% was not in accordance with the requirement. However, this standard is for restorative materials and the resin-based endodontic sealers present higher sorption than restorative filling materials [22,24,25]. Solubility of the sealers with bismuth subnitrate in proportions from 20% to 80% presented values higher than the recommendation. Other resin-based sealers also present higher values of sorption

and solubility than the values stated by ISO 4049 [12]. Increased sealer degradation could lead to monomeric components leaching out, increasing cytotoxicity [20] in the periapical region. A cytotoxicity test is necessary for drugs or products that may come into contact with human tissues to define safety. *In vitro* tests have streamlined this process [26]. In the present study, the cytotoxicity showed values in accordance to ISO 6876 standard.

In the search for an endodontic sealer covering all ideal properties, the epoxy-based sealer with bismuth subnitrate as a filler particle can be an alternative for clinical use. The microbiological properties, bond strength, and radiopacity of this sealer could be tested in future studies.

Conclusion

The addition of bismuth subnitrate with up to the some concentration or with 100% and 120% appears to be a promising filler particle for root canal sealer.

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