UNIVERSIDADE ESTADUAL PAULISTA
"Júlio de Mesquita Filho"

Vanessa Abreu Sanches Marques

DISSERTAÇÃO

AVALIAÇÃO DA RESPOSTA TECIDUAL E DA CAPACIDADE DE MINERALIZAÇÃO DE CIMENTOS ENDODÔNTICOS RESINOSOS

Araçatuba – SP

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AVALIAÇÃO DA RESPOSTA TECIDUAL E DA CAPACIDADE DE MINERALIZAÇÃO DE CIMENTOS ENDODÔNTICOS RESINOSOS

Dissertação apresentada à Faculdade de Odontologia de Araçatuba, Universidade Estadual Paulista "Júlio de Mesquita Filho" - UNESP como parte dos requisitos para obtenção do título de Mestre em Ciência Odontológica, área de concentração em Endodontia.

Orientador: Prof. Adj. Eloi Dezan-Junior

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Epígrafe

"Não é sobre ter
Todas as pessoas do mundo pra si
É sobre saber que em algum lugar
Alguém zela por ti
É sobre cantar e poder escutar
Mais do que a própria voz
É sobre dançar na chuva de vida
Que cai sobre nós
É saber se sentir infinito
Num universo tão vasto e bonito
É saber sonhar
E então fazer valer a pena cada verso
Daquele poema sobre acreditar
Não é sobre chegar no topo do mundo
Saber que venceu
É sobre escalar e sentir
Que o caminho te fortaleceu
É sobre ser abrigo
E também ter morada em outros corações
E assim ter amigos contigo
Em todas as situações
A gente não pode ter tudo
Qual seria a graça do mundo se fosse assim
Por isso eu prefiro sorrisos
E os presentes que a vida trouxe
Pra perto de mim
Não é sobre tudo que o seu dinheiro
É capaz de comprar
E sim sobre cada momento
Sorriso a se compartilhar
Também não é sobre correr
Contra o tempo pra ter sempre mais
Porque quando menos se espera
A vida já ficou pra trás
Segura teu filho no colo
Sorria e abraça teus pais
Enquanto estão aqui
Que a vida é trem-bala parceiro
E a gente é só passageiro prestes a partir"

Trem Bala – Ana Vilela

Marques VAS. Avaliação da resposta tecidual e da capacidade de mineralização de cimentos endodônticos resinosos, 2017. 61p. Dissertação (Mestrado em Endodontia) – Universidade Estadual Paulista (Unesp), Faculdade de Odontologia, Araçatuba.

Resumo

Objetivo: Avaliar a resposta tecidual e a capacidade de biomineralização dos materiais endodônticos SK Seal Root Canal Sealer (SK Seal), Sealer 26® e AH plus® em tecido subcutâneo de ratos. **Material e Métodos:** Vinte e quatro ratos Wistar (n=6) receberam implantes subcutâneo contendo os cimentos e um tubo vazio como controle. Após 7, 15, 30 e 60 dias, os animais foram eutanasiados e os tubos de polietileno foram removidos junto com o tecido circunjacente. Em seguida, os espécimes foram processados para análise em Hematoxilina-Eosina, von Kossa, luz polarizada e imunoistoquímica para fibronectina (FN) e tenascina (TN). Os dados foram tabulados e analisados através do teste de Kruskal-Wallis e Dunn ($p<0,05$). **Resultados:** Todos os materiais testados induziram uma reação inflamatória moderada aos 7 e 15 dias ($p> 0,05$). Não foram observadas diferenças entre os grupos após 30 ou 60 dias ($p> 0,05$). A cápsula fibrosa foi considerada espessa aos 7 dias, tornando-se fina no final do experimento. Todos os grupos apresentaram marcadores positivos para FN e TN em todos os tempos de análise, com maior imunomarcação para os cimentos em comparação ao grupo controle ($p <0,05$). Os cimentos não apresentaram von Kossa positiva ou estruturas birrefringentes à luz polarizada. **Conclusão:** Todos os cimentos testados apresentaram biocompatibilidade, porém não estimularam a mineralização.

Palavras-chave: Inflamação, Calcificação Fisiológica, Obturação do Canal Radicular, Receptores de Fibronectina, Tenascina.

Marques VAS. Biocompatibility and biomineralization assessment of resinous root canal sealers, 2016. 61p. Dissertation (Master's Degree in Endodontics) – São Paulo State University (Unesp), School of Dentistry, Araçatuba.

Abstract

Aim: The aim of this study was to evaluate biocompatibility and biomineralization of the endodontic materials SK Seal Root Canal Sealer (SK Seal), Sealer 26® and AH plus® in subcutaneous tissue of rats. **Methodology:** Twenty-four Wistar rats (n=6) received subcutaneous implants containing the test sealers, and an empty tube as control. After 7, 15, 30 and 60 days, the animals were killed and polyethylene tubes were removed with the surrounding tissues. The pieces were processed for Hematoxylin-Eosin, von Kossa, polarized light and immunohistochemical analysis for fibronectin (FN) and tenascin (TN). Data were tabulated and analyzed via Kruskal-Wallis and Dunn's test ($p<0,05$). **Results:** All tested materials induced a moderate infammatory reaction after 7 and 15 days. ($p>0,05$). No difference was observed among groups after days 30 or 60 days ($p>0.05$). The fibrous capsule was considered thick on the 7th day, and classified as thin at the end of the experiment. All groups presented positive markers for FN and TN in all analyzed time, with higher immunolabeling to sealers in comparison with the control group ($p<0,05$). The sealers did not present von Kossa positive or birefringent structures to polarized light. **Conclusion:** All tested sealers demonstrated biocompatibility, but did not stimulate the mineralization.

Keywords: Inflammation, Calcification Physiologic, Root Canal Obturation, Receptors Fibronectin, Tenascin.

Sumário

Biocompatibility and biomineralization assessment of resinous root canal sealers

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Keywords: Inflammation, Calcification Physiologic, Root Canal Obturation, Receptors Fibronectin, Tenascin.

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The manuscript is according to the guidelines for authors of International Endodontic Journal (Anexo A).

Biocompatibility and biomineralization assessment of resinous root canal sealers

Abstract

Aim: The aim of this study was to evaluate biocompatibility and biomineralization of the endodontic materials SK Seal Root Canal Sealer (SK Seal), Sealer 26® and AH plus® in subcutaneous tissue of rats.

Methodology: Twenty-four Wistar rats (n=6) received subcutaneous implants containing the test sealers, and an empty tube as control. After 7, 15, 30 and 60 days, the animals were killed and polyethylene tubes were removed with the surrounding tissues. The pieces were processed for Hematoxylin-Eosin, von Kossa, polarized light and immunohistochemical analysis for fibronectin (FN) and tenascin (TN). Data were tabulated and analyzed via Kruskal-Wallis and Dunn's test ($p<0,05$).

Results: All tested materials induced a moderate infammatory reaction after 7 and 15 days. ($p>0,05$). No difference was observed among groups after days 30 or 60 days ($p>0.05$). The fibrous capsule was considered thick on the 7th day, and classified as thin at the end of the experiment. All groups presented positive markers for FN and TN in all analyzed time, with higher immunolabeling to sealers in comparison with the control group ($p<0,05$). The sealers did not present von Kossa positive or birefringent structures to polarized light.

Conclusion: All tested sealers demonstrated biocompatibility, but did not stimulate the mineralization.

Keywords: Inflammation, Calcification Physiologic, Root Canal Obturation, Receptors Fibronectin, Tenascin.

Introduction

The success of endodontic therapy is directly related to infection control by cleaning, shaping and obturating the root canal system (Sousa *et al.* 2004, Schilder 2006). The obturation completes the treatment and must be performed as hermetic as possible, with gutta-percha points and an endodontic sealer, in order to reduce gaps between the obturation and the root canal walls, preventing and minimizing infiltration or sealing microorganisms and, preferably, filling in difficult access areas of the root canal system (Estrela *et al.* 2007, Anusavice *et al.* 2013).

Among the several characteristics of an ideal endodontic material, the biocompatibility is a desirable property, since there will be direct contact with the periapical tissues. In addition, when the material induces the biomineralization, the repair process is also improved (Grosmann 1958, Holland *et al.* 1985). This improvement occurs from the participation of two glycoproteins present in the extracellular matrix: fibronectin (FN) and tenascin (TN). Tenascin has antiadhesive activity, which along with the fibronectin adhesive and scattering activity, allows the cellular movement (Sage & Bornstein 1991, Aukhil *et al*. 1996), as well as assists in the healing (Chiquet-Ehrismamm 1990, Willems *et al.* 1996). Fibronectin regulates cell adhesion, migration, cell differentiation and repair (Mohri *et al.* 1997), and facilitates platelet aggregation through its deposition on collagen and / or fibrin (Grinnel, 1984), also contributing to healing (Aukhil *et al.* 1996; Willems *et al.* 1996). In addition, both matrix components are involved in the pulp repair process through cell differentiation, stimulating the formation of dentin barrier (Mizuno & Banzai 2008; Piva *et al.* 2006).

Currently, there are a wide diversity of endodontic sealers available and the principal difference is related to its main component such as zinc oxide and eugenol, glass ionomer, epoxy resins, calcium hydroxide, among others (Orstavik 2005). Depending on the type of these major components, local adverse effects may occur, delaying or preventing repair (Geurtsen 2001).

Resinous endodontic sealers were introduced by Schröeder and their wide use is related to the excellent adhesion to root canal walls and marginal sealing ability, reducing apical and coronary infiltration (Leonardo & Leal 2008, Silveira *et al.* 2011).

The Sealer 26® (Dentsply Indústria e Comércio Ltda., Petrópolis, RJ) is an endodontic sealer containing 37% of calcium hydroxide in the powder composition, while

the liquid has epoxy bisphenol resin. Previous studies have shown that this sealer presents low toxicity (Barbosa *et al.* 1993), ability to induce repair through the deposition of mineralized tissue (Holland *et al.* 2002, Barbosa *et al.* 2003), preventing bacterial infiltration (Siqueira *et al.* 1999, Siqueira *et al.* 2001, Barbosa *et al.* 2003).

AH Plus® (Dentsply, DeTrey) has been considered satisfactory for its physico-chemical properties (Duarte *et al.* 2010, Marciano *et al.* 2011), sealing ability (Santos *et al.* 2010) and antimicrobial activity (Zhang *et al.* 2009, Rezende *et al.* 2016). However, many studies report its cytotoxicity (Al-Hiyasat *et al.* 2010, Ashraf *et al.* 2012), explained by the release of diglycidil ether from bisphenol A, identified as mutagenic (Heil *et al.* 1996). Another factor that may contribute to the cytotoxicity of AH Plus® is the release of small amounts of formaldehyde or through the amine and epoxy components of the resin (Cohen *et al.* 1998, Athanassiadis *et al.* 2015).

The SK Seal Root Canal Sealer (Skada Limited, Marlborough Hill, Harrow, UK) consists of a resin sealer avaliable on the market in double syringe. The base consists of epoxy oligomer, ethylene glycol, bismuth subcarbonate, calcium, zirconium phosphate and calcium oxide. The catalyst contains polyaminobenzoate, trietatolamine, calcium phosphate, bismuth subcarbonate, zirconium oxide and calcium oxide. The syringe automatically provides the two components (base and catalyst) in the ratio of 2: 1, facilitating the manipulation. According to the manufacturer, this sealer complies the requirements of ISO6876:1986 (E) for root canals sealing and in combination with gutta-percha has the following properties: sealing capacity, not staining dental structure, insoluble in liquids, excellent biocompatibility, as well as excellent radiopacity [http://www.skadadental.com/p/81/sk-seal].

Until now, there are no scientific studies on the physical-chemical and biological properties of SK Seal Root Canal Sealer (SK Seal). Therefore, the tissue response and the biomineralization capacity of SK Seal Root Canal Sealer, Sealer 26® and AH Plus® should be evaluated by subcutaneous rat implants when compared to the control group. The null hypothesis was that biocompatibility and mineralization were not induced by SK seal, Sealer 26® or AH Plus®.

Methodology

Twenty-four male 4–6-month-old Wistar rats (250–280 g) were used in the study. The animals were housed in temperature-controlled rooms and provided water and food

ad libitum. Animal care was performed according to *Araçatuba School of Dentstry*, UNESP, Ethical Committee, which approved the experimental project (CEUA 2015-00452).

Seventy-two polyethylene tubes (Abbott Laboratories of Brazil, Sao Paulo, SP, Brazil) with a 1.0 mm internal diameter, 1.6 mm external diameter, and 10.0 mm length were filled with the tested sealers, spatulated according to manufacturers' recommendations and inserted into the tubes with the syringe assistance. Twenty-four empty tubes were used as control.

After administration of intramuscular anesthesia with xylazine (10 mg/kg Rhobifarma Indústria Farmacêutica Ltda, Hortolândia, Brazil) and ketamine (25 mg/kg União Química Farmacêutica Nacional S/A, São Paulo, Brazil), the dorse of the animals were shaved, antisepsis was obtained with 5% iodine solution, and a 2.0 cm incision was performed in a head-tail orientation with #15 Bard-Parker blade (BD, Franklin Lakes, USA), creating two pockets on each side of the incision. Three polyethylene tubes, containing the sealers, and an empty tube, were implanted in each animal in opposite directions (upper right, upper left, lower right, and lower left), and the skin was closed with a 4/0 silk suture (Johnson & Johnson Produtos Profissionais Ltda, São José dos Campos, Brazil).

After the experimental periods of 7, 15, 30, and 60 days, the animals were euthanized by an anesthetic overdose. The polyethylene tubes, were removed with the surrounding tissue and fixed in 10% buffered formalin at pH 7.0 (Cintra *et al.* 2013). The specimens were embedded in paraffin (Cintra *et al.* 2013), serially cut into 5 μm sections, and stained with Hematoxylin-Eosin (HE) or submitted to immunohistochemistry by using an indirect immunoperoxidase technique (Garcia *et al.* 2013) for fibronectin (primary antibody rabbit, SC-9068, Santa Cruz Biotechnology, CA, USA) and tenascin - C (primary antibody rabbit, SC-20932, Santa Cruz Biotechnology, CA, USA). The specimens were submitted to the previously described procedures suppressing the use of primary antibodies to negative control. The 10 μm sections were stained according to the Von Kossa (VK) technique or not stained, to be analyzed by polarized light (PL).

Tissue reactions at the open end of the tubes were scored according to previous studies (Yaltirik et al. 2004, Gomes-Filho *et al*. 2012, Cintra *et al*. 2013) as follows: 0, few inflammatory cells or no reaction; 1, less than 25 cells and mild reaction; 2, between 25 and 125 inflammatory cells and moderate reaction; and 3, 125 or more inflammatory cells and severe reaction (400 × magnification). Fibrous capsules were considered thin

when <150μm and thick when ≥150μm. Calcification was classified as positive or negative by Von Kossa staining and present or absent under PL (100 × magnification).

Immunolabeling for fibronectin and tenascin was defined as the presence of brownish color in the extracellular matrix. The criteria for establishing the adopted scores were: 0 = absence of immunolabeling; 1 = low immunolabeling standard; 2 = moderate immunolabeling standard; 3 = high immunolabeling standard (1000 × magnification).

Data were statistically analyzed by Kruskal–Wallis and Dunn's test; $p < 0.05$ was considered significant.

Results

Group control

Moderate chronic inflammatory reaction (score 2) was observed after 7 and 15 days (Table 1; Figure 1, Aa e Bb). Inflammatory cells, such as lymphocytes and macrophages, were present in the fibrous capsule, classified as thick. On days 30 and 60, a mild inflammatory infiltrate was observed in a thin fibrous capsule. A few samples at the end of the experiment showed insignificant inflammatory cells (score 0). The control group was negative for VK staining (Figure 2, A-D) and no birefringent structures under PL (Figure 2, a-d) were observed throughout all analyzed periods.

In the immunohistochemical analysis for fibronectin and tenascin, the control group presented a low immunolabeling pattern in all analyzed periods (Table 2).

AH Plus

On days 7, 15 and 30, a moderate inflammatory reaction (score 2) was observed, with presence of macrophages and lymphocytes (Table 1), reducing until day 60 (score 1). The fibrous capsule was considered thick in the first two experimental periods and thin by the end of the experiment (Figure 1, Ee - Hh). VK staining was negative and there were no birefringent structures for PL in all analyzed periods (Figure 2, Ee - Hh).

In immunohistochemical analysis, a moderate standard (score 2) of immunolabeling was observed at all time points for both markers (Table 2).

Sealer 26

Moderate inflammatory reaction (score 2) was observed on days 7, 15 and 30, exhibiting lymphocytes and macrophages. On days 7 and 15, the fibrous capsule was

considered thick (Table 1). On day 60, the inflammatory reaction became mild and the capsule was thin (Figure 1 - Ll). Sealer 26® was negative for VK staining in all periods and did not show birefringent structures for PL (Figure 2 - Ii-Ll).

The immunohistochemical analysis, on days 7, 30 and 60, presented a moderate standard of fibronectin immunolabeling. On days 15, the sealer presented moderate to high immunolabeling standard (Figure 3, I-L). For tenascin, a moderate standard of immunolabeling was present throughout all experimental periods (Table 2).

SK Seal Root Canal Sealer

After 7 days, a mild inflammatory reaction (score 1) was present in half of the specimens analyzed. The other samples presented moderate to severe inflammatory reaction. On day 15, a moderate inflammatory reaction (score 2) was present, with a predominance of lymphocytes and macrophages. The fibrous capsule was considered thick only on the first time period. On days 30 and 60 (Table 1), there was a reduction of the inflammatory reaction (score 1) and the thickness the fibrous capsule, classified as thin (Figure 1, Mm - Pp). VK staining was negative and birefringent granulations were absent at PL (Figure 2, m-p) in all analyzed periods. (Figure 2, M-P).

In the immunohistochemical analysis, on days 7, 30 and 60, there was a moderate immunolabeling for FN. On day 15, moderate to high immunolabeling were detected. (Table 2). This response was similar for tenascin (score 2-3) on day 7. On day 15, there was a moderate (score 2) immunolabeling for this protein (Figure 4, N), decreasing at the end of the experiment (score 1).

Comparison among groups

Data were compared for each time period as shown in Tables 1 and 2. On the 7th day, the SK Seal presented a slight inflammation (score 1) when compared to other groups, with no significant statistical difference ($p > 0.05$). On days 15, 30 and 60, there was mild to moderate response in tissues (score 1-2), with no significant statistical difference between them ($p > 0.05$). The fibrous capsule was considered thick on the 7, becoming thin at the end of the experiment.

Analysis of VK and PL of SK Seal, Sealer 26® and AH Plus® revealed a lack of mineralization in all analyzed experimental periods (Table 1).

In the immunohistochemical analysis (Table 2) for fibronectin, on days 7, all experimental sealers presented a moderate standard, with significant statistical difference for AH Plus® when compared to the control group ($p < 0.05$). On day 15, when compared to the control group, Sealer 26® presented statistical difference ($p<0,05$) with a high standard of immunolabeling. On days 30, all the sealers presented moderate immunolabeling and SK Seal presented statistical difference when compared to the control group ($p<0,05$). Sealer 26® and AH Plus® presented statistical difference, in relation to the control group, on days 60 ($p<0,05$).

For tenascin (Table 2), AH Plus® presented moderate immunolabeling at all times, with statistical difference on days 15, 30 and 60 when compared to the control group ($p <0.05$). Sealer 26® maintained moderate marking in all periods of analysis ($p> 0.05$). SK Seal presented statistical difference when compared to the control group on days 7 and 15 ($p <0.05$), and low immunolabeling in the final periods.

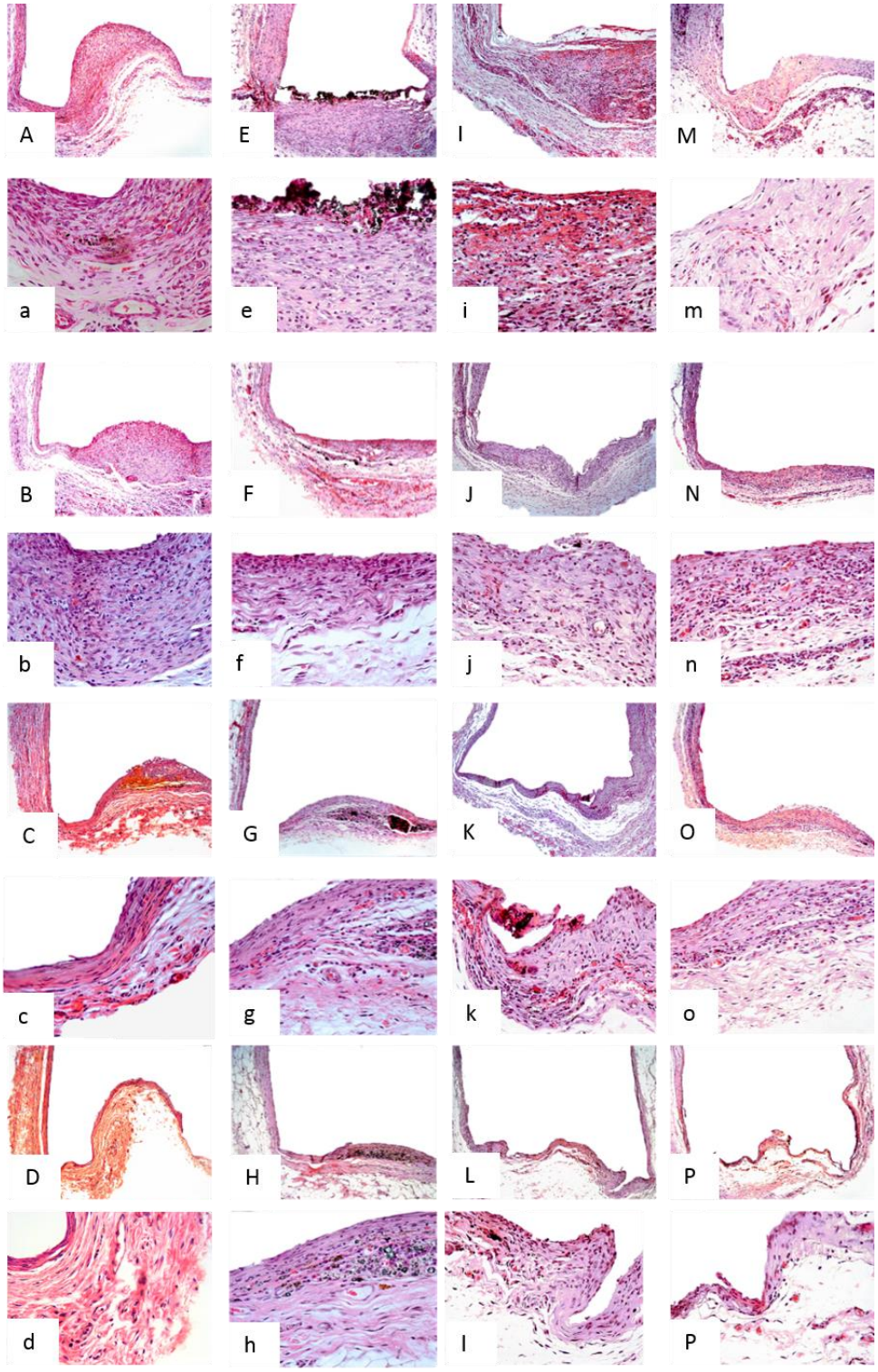

Figure 1 – Tissue reaction on experimental groups: Control Group: A- D (days 7, 15, 30 and 60, HE, 100×) and a-d (days 7, 15, 30 and 60, HE, 400×); AH Plus: E-H (days 7, 15, 30 and 60, HE, 100×) and e-h (days 7, 15, 30 and 60, HE, 400×); Sealer 26: I-L (days 7, 15, 30 and 60, HE, 100×) and i-l (days 7, 15, 30 and 60, HE, 400×); SK Seal Root Canal Sealer: M-P (days 7, 15, 30 and 60, HE, 100×) and m-p (days 7, 15, 30 and 60, HE, 400×).

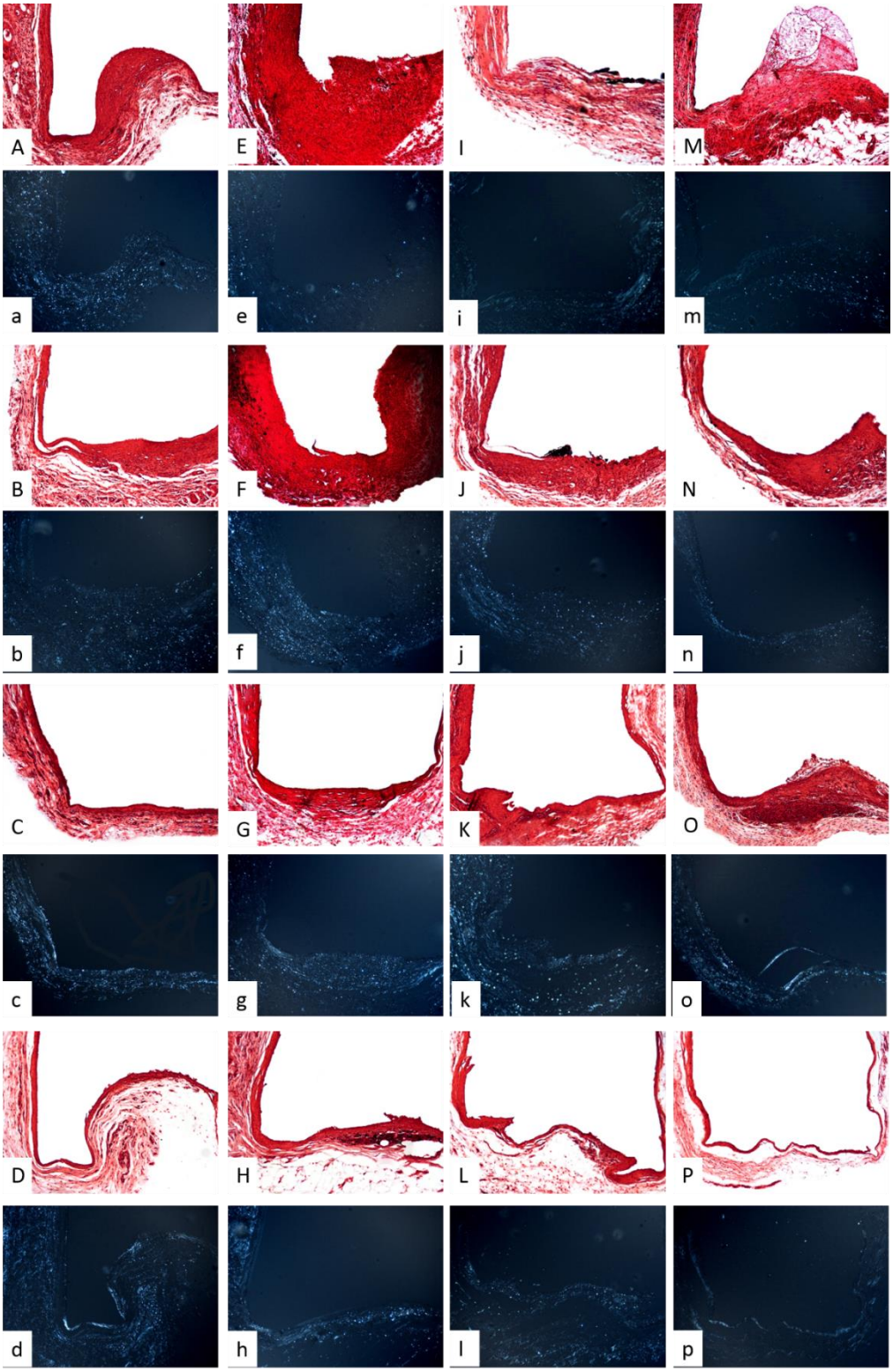

Figure 2 - Mineralization on experimental groups: Control group: A- D (days 7, 15, 30 and 60; Von Kossa, 100×) and a-d (days 7, 15, 30 and 60, polarized light, 100×); AH Plus: E-H (days 7, 15, 30 and 60; Von Kossa, 100×) and e-h (days 7, 15, 30 and 60; polarized light, 100×); Sealer 26: I-L (days 7, 15, 30 and 60; Von Kossa, 100×) and i-l (days 7, 15, 30 and 60; polarized light, 100×); SK Seal Root Canal Sealer: M-P (days 7, 15, 30 and 60; Von Kossa, 100×) and m-p (days 7, 15, 30 and 60, polarized light, 100×).

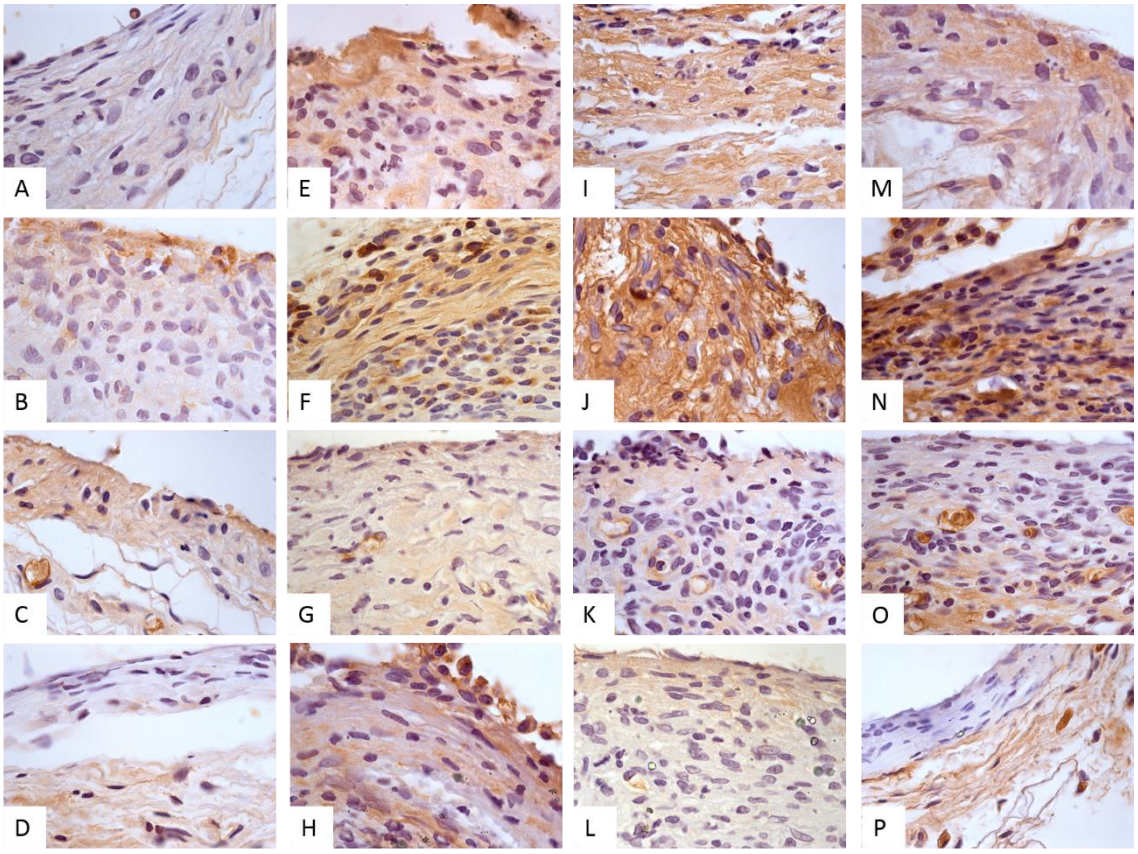

Figure 3 – Immunohistochemical staining for fibronectin. Control Group: A - D (days 7, 15, 30 and 60); AH Plus: E - H (days 7, 15, 30 and 60); Sealer 26: I - L (days 7, 15, 30 and 60); SK Seal Root Canal Sealer: M - P (days 7, 15, 30 and 60). [1000x].

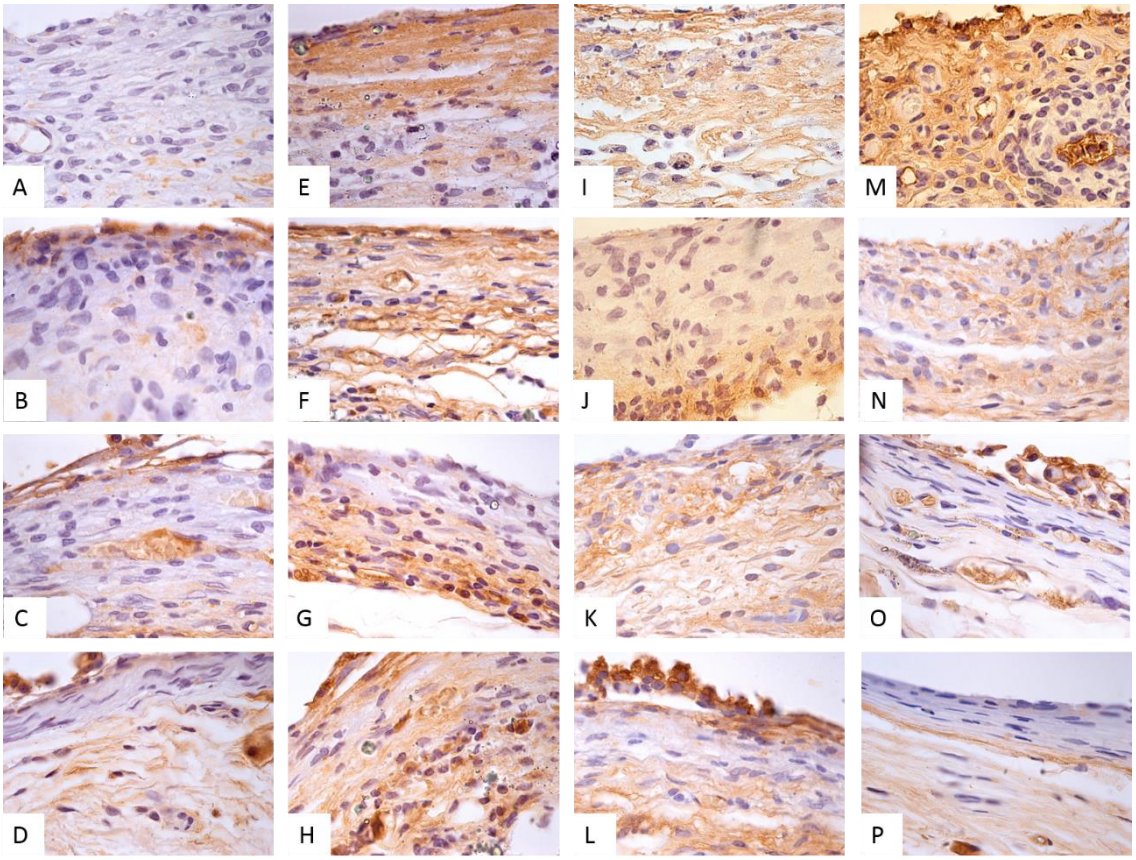

Figure 4 - Immunohistochemical staining for tenascin. Control Group: A - D (days 7, 15, 30 and 60); AH Plus: E - H (days 7, 15, 30 and 60); Sealer 26: I - L (days 7, 15, 30 and 60); SK Seal Root Canal Sealer: M - P (days 7, 15, 30 and 60). [1000x].

Table 1 – Percentage of samples in each group categorized according to the inflammatory score, fibrous capsule thickness, Von Kossa and Polarized light.

Group	Score(%)				Med	Fibrous Capsule	Von Kossa (%)	Polarized Light (%)
	0	1	2	3				
7 days								
Control	0	0	100	0	2ª	Thick	0	0
AH Plus	0	0	100	0	2ª	Thick	0	0
Sealer 26	0	20	80	0	2ª	Thick	0	0
SK Seal	0	50	33	17	1ª	Thick	0	0
15 days								
Control	0	17	83	0	2ª	Thick	0	0
AH Plus	0	40	60	0	2ª	Thin	0	0
Sealer 26	0	0	100	0	2ª	Thick	0	0
SK Seal	0	0	100	0	2ª	Thin	0	0
30 days								
Control	0	100	0	0	1ª	Thin	0	0
AH Plus	0	33	50	17	2ª	Thin	0	0
Sealer 26	0	33	50	17	2ª	Thin	0	0
SK Seal	0	60	40	0	1ª	Thin	0	0
60 days								
Control	33	67	0	0	1ª	Thin	0	0
AH Plus	0	68	16	16	1ª	Thin	0	0
Sealer 26	0	67	33	0	1ª	Thin	0	0
SK Seal	20	80	0	0	1ª	Thin	0	0

*Same letters indicate that there was no statistical difference between the groups ($p > 0.05$).

Table 2 - Percentage of scores attributed to fibronectin and tenascin staining in all groups.

Group	Score Fibronectin (%)					Score Tenascin (%)				
	0	1	2	3	Med	0	1	2	3	Med
7 days										
Control	0	83	17	0	1^{a}	0	67	33	0	1^{a}
AH Plus	0	0	83	17	2^{b}	0	0	83	17	2^{ab}
Sealer 26	0	0	100	0	2^{ab}	0	0	100	0	2^{ab}
SK Seal	0	0	100	0	2^{ab}	0	0	50	50	3^{b}
15 days										
Control	0	83	17	0	1^{a}	0	83	17	0	1^{a}
AH Plus	0	0	67	33	2^{ab}	0	0	67	33	2^{b}
Sealer 26	0	0	50	50	3^{b}	0	33	67	0	2^{ab}
SK Seal	0	0	50	50	3^{ab}	0	0	60	40	2^{b}
30 days										
Control	0	83	17	0	1^{a}	0	100	0	0	1^{a}
AH Plus	0	0	100	0	2^{ab}	0	0	67	33	2^{b}
Sealer 26	0	0	83	17	2^{ab}	0	17	83	0	2^{ab}
SK Seal	0	0	67	33	2^{b}	0	67	33	0	1^{ab}
60 days										
Control	0	100	0	0	1^{a}	0	83	17	0	1^{a}
AH Plus	0	0	67	33	2^{b}	0	0	67	33	2^{b}
Sealer 26	0	0	83	17	2^{b}	0	17	83	0	2^{ab}
SK Seal	0	0	100	0	2^{ab}	0	60	40	0	1^{ab}

*Different letters indicate that there is statistical difference between the groups ($p < 0.05$).

Discussion

This study evaluated the biocompatibility and mineralization ability of resinous endodontic sealers. Based off of the results, the hypothesis tested was partially rejected, since all tested sealers demonstrated biocompatibility, but none induced biomineralization. The mild-to-moderate inflammatory reactions presented in all groups during the initial experimental periods subsequently decreased and the fibrous capsule became thinner. The empty tubes used as control presented similar reactions to results already reported in literature (Gomes-Filho *et al.* 2009, Bueno *et al.* 2016).

The AH Plus® and Sealer 26® demonstrated biocompatibility, corroborating previous studies (Veloso *et al.* 2006, Gomes-Filho *et al.* 2007, Farhad *et al.* 2011, Mutoh *et al.* 2013). A moderate inflammatory reaction was present in the initial periods of both groups, decreasing over time, along with the capsule around the tube.

The SK Seal was biocompatible when compared to the other sealers and the control group, since it showed a slight to moderate response in the subcutaneous tissue in the first analyzed time periods and this reaction reduced until the end of the experiment. The fibrous capsule was thick only on day 7, and became thin from the 15th day onwards. This is possibly one of the first work evaluating the biocompatibility of SK Seal, hindering comparisons.

Throught all the experiment, von Kossa were negative and birefringent structures were absent under polarized light in all tested sealers. Although Sealer 26® contains 37% calcium hydroxide in its composition, the three materials studied are considered resinous sealers and the organic matrix of these compounds contains bisphenol (BISGMA). It has already been shown that this compound may interfere with the biomineralization promoted by MTA based sealers (Gomes-Filho *et al.* 2008). Several studies reported the presence of unpolymerized monomers on the surface of these materials due to the presence of oxygen or water (Rathbun *et al.* 1991).

Bueno *et al*. (2016) evaluating a sealer containing calcium hydroxide with a resinous epoxy matrix (Acroseal) also did not observe induction of mineralization in all the experimental periods and correlates this result to the relative insolubility of the epoxy base. It is also worth mentioning the need for sealers with calcium hydroxide to have a more soluble matrix, in order to contribute to the biomineralization of these sealers. We attribute this explanation to the results of our experiment, since Sealer 26® has calcium hydroxide and a matrix based on epoxy resin which also did not induce mineralization.

According to the manufacturer, the spatulation of Sealer 26® requires, two to three parts of the powder for a drop of liquid. Since there is no standardized tool for this procedure, is clinically difficult to achieve a correct dosage, which may interfere in the final result.

As the dynamics of the extracellular matrix in the repair of subcutaneous tissue of rats, the glycoproteins fibronectin and tenascin were immunohistochemistry evaluated during the experimental periods. It's possible to observe that the tested sealers showed a higher fibronectin marking in relation to the control group at the analyzed periods and at 30 days, this immunolabeling was moderate to high for the SK Seal. For tenascin, at 7 and 15 days, SK Seal showed a high immunolabeling in relation to the other sealers, and only at 60 days, its marking was equal to the control group. The other sealers showed a moderate immunolabeling for this glycoprotein in all time periods.

Previous studies reported the adhesive properties of these glycoproteins with direct participation in tissue repair processes (Chiquet-Ehrismamm 1990). Martinez *et al.* (2000) identified, through immunolabeling technique, the distribution of TN, FN and type III collagen in human pulps, and observed a strong labeling for TN and FN. Piva *et al.* (2006) studied the expression of FN and TN during pulp repair induced by $Ca(OH)_2$ and verified that both extracellular matrix glycoproteins were expressed during the healing process of the human pulp, after mechanical procedure and capping with calcium hydroxide. In addition, TN was evidenced in advanced stages of dentin barrier formation. Vita *et al.* (2008) analyzed the tissue repair in subcutaneous of rats by FN labeling, using the immunoflorescence technique, and reported its presence in endodontic retrofilling materials (MTA and Consistent Sealapex), however statistical differences were not observed in immunolabeling of glycoprotein, when compared between groups and between the periods of observation. The same glycoprotein was evaluated by Fayaze *et al.* (2011), in response to fibroblasts of the human periodontal ligament with retrofilling materials (ProRoot MTA, Portland cement, and amalgam) and it was observed that after 1 week, Portland cement and MTA groups showed higher expression of fibronectin, but there was no significant difference between these two groups. All studies emphasized the importance of understanding the cells and elements of the extracellular matrix participation in the repair of the involved tissues, when in contact with endodontic materials.

Biocompatibility studies on rat subcutaneous tissue of obturation materials have been limited to the histological aspects of the tissue reactions caused by the implanted materials components. A few researches on standard staining of extra cellular matrix

proteins during tissue repair in contact with endodontic sealers has been published, which hinders to compare this results on the dynamics of the extracellular conjunctive matrix, through the immunohistochemical technique. Thus, more studies are required to better understand the reflexes caused by the obturation materials when in contact with the extracellular matrix.

Conclusion

At the end of the experiment, the sealers SK Seal, Sealer 26® and AH PLus® were biocompatible, but did not induce biomineralization.

Acknowledgments

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ANEXO A

Guidelines for Publishing Papers in the Internation Endodontic Journal

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1. GENERAL

International Endodontic Journal publishes original scientific articles, reviews, clinical articles and case reports in the field of Endodontology; the branch of dental sciences dealing with health, injuries to and diseases of the pulp and periradicular region, and their relationship with systemic well-being and health. Original scientific articles are published in the areas of biomedical science, applied materials science, bioengineering, epidemiology and social science relevant to endodontic disease and its management, and to the restoration of root-treated teeth. In addition, review articles, reports of clinical cases, book reviews, summaries and abstracts of scientific meetings and news items are accepted.

Please read the instructions below carefully for details on the submission of manuscripts, the journal's requirements and standards as well as information concerning the procedure after a manuscript has been accepted for publication in International Endodontic Journal. Authors are encouraged to visit Wiley Author Services for further information on the preparation and submission of articles and figures.

2. ETHICAL GUIDELINES

International Endodontic Journal adheres to the below ethical guidelines for publication and research.

2.1. Authorship and Acknowledgements

Authors submitting a paper do so on the understanding that the manuscript has been read and approved by all authors and that all authors agree to the submission of the manuscript to the Journal.

International Endodontic Journal adheres to the definition of authorship set up by The International Committee of Medical Journal Editors (ICMJE). According to the ICMJE, authorship criteria should be based on 1) substantial contributions to conception and design of, or acquisiation of data or analysis and interpretation of data, 2) drafting the article or revising it critically for important intellectual content and 3) final approval of the version to be published. Authors should meet conditions 1, 2 and 3.

Acknowledgements: Under acknowledgements please specify contributors to the article other than the authors accredited. Please also include specifications of the source of funding for the study and any potential conflict of interests if appropriate. Please find more information on the conflict of interest form in section 2.6.

2.2. Ethical Approvals

Experimentation involving human subjects will only be published if such research has been conducted in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki (version 2008) and the additional requirements, if any, of the country where the research has been carried out. Manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and written consent of each subject and according to the above mentioned principles. A statement regarding the fact that the study has been independently reviewed and approved by an ethical board should also be included. Editors reserve the right to reject papers if there are doubts as to whether appropriate procedures have been used.

When experimental animals are used the methods section must clearly indicate that adequate measures were taken to minimize pain or discomfort. Experiments should be carried out in accordance with the Guidelines laid down by the National Institute of Health (NIH) in the USA regarding the care and use of animals for experimental procedures or with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and in accordance with local laws and regulations.

All studies using human or animal subjects should include an explicit statement in the Material and Methods section identifying the review and ethics committee approval for each study. The authors MUST upload a copy of the ethical approval letter when submitting their manuscript. Editors reserve the right to reject papers if there is doubt as to whether appropriate procedures have been used.

2.3 Clinical Trials

2.3.1 Randomised control clinical trials

Randomised control clinical trials should be reported using the guidelines available at www.consort-statement.org. A CONSORT checklist and flow diagram (as a Figure) should also be included in the submission material. The International Endodontic Journal asks that authors submitting manuscripts reporting from a clinical trial to register the trials in any of the following public clinical trials registries: www.clinicaltrials.gov, https://www.clinicaltrialsregister.eu/, http://isrctn.org/. Other primary registries if named in the WHO network will also be considered acceptable. The clinical trial registration number and name of the trial register should be included in the Acknowledgements at the submission stage.

2.3.2 Epidemiological observational trials

Submitting authors of epidemiological human observations studies are required to review and submit a 'strengthening the reporting of observational studies in Epidemiology' (STROBE) checklist and statement. Compliance with this should be detailed in the materials and methods section. (www.strobe-statement.org)

2.4 Systematic Reviews

Systematic reviews should be reported using the PRISMA guidelines available at http://prisma-statement.org/. A PRISMA checklist and flow diagram (as a Figure) should also be included in the submission material.

2.5 DNA Sequences and Crystallographic Structure Determinations

Papers reporting protein or DNA sequences and crystallographic structure determinations will not be accepted without a Genbank or Brookhaven accession number, respectively. Other supporting data sets must be made available on the publication date from the authors directly.

2.6 Conflict of Interest and Source of Funding

International Endodontic Journal requires that all authors (both the corresponding author and co-authors) disclose any potential sources of conflict of interest. Any interest or relationship, financial or otherwise that might be perceived as influencing an author’s

objectivity is considered a potential source of conflict of interest. These must be disclosed when directly relevant or indirectly related to the work that the authors describe in their manuscript. Potential sources of conflict of interest include but are not limited to patent or stock ownership, membership of a company board of directors, membership of an advisory board or committee for a company, and consultancy for or receipt of speaker's fees from a company. If authors are unsure whether a past or present affiliation or relationship should be disclosed in the manuscript, please contact the editorial office at iejeditor@cardiff.ac.uk. The existence of a conflict of interest does not preclude publication in this journal.

The above policies are in accordance with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals produced by the International Committee of Medical Journal Editors (http://www.icmje.org/).

It is the responsibility of the corresponding author to have all authors of a manuscript fill out a conflict of interest disclosure form, and to upload all forms together with the manuscript on submission. The disclosure statement should be included under Acknowledgements. Please find the form below:

Conflict of Interest Disclosure Form

2.7 Appeal of Decision

The decision on a paper is final and cannot be appealed.

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3. OnlineOpen

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3.1 MANUSCRIPT SUBMISSION PROCEDURE

Manuscripts should be submitted electronically via the online submission site http://mc.manuscriptcentral.com/iej. The use of an online submission and peer review site enables immediate distribution of manuscripts and consequentially speeds up the review process. It also allows authors to track the status of their own manuscripts. Complete

instructions for submitting a paper is available online and below. Further assistance can be obtained from iejeditor@cardiff.ac.uk.

3.2. Getting Started

• Launch your web browser (supported browsers include Internet Explorer 5.5 or higher, Safari 1.2.4, or Firefox 1.0.4 or higher) and go to the journal's online Submission Site: http://mc.manuscriptcentral.com/iej

• Log-in, or if you are a new user, click on 'register here'.

• If you are registering as a new user.

- After clicking on 'register here', enter your name and e-mail information and click 'Next'. Your e-mail information is very important.

- Enter your institution and address information as appropriate, and then click 'Next.'

- Enter a user ID and password of your choice (we recommend using your e-mail address as your user ID), and then select your areas of expertise. Click 'Finish'.

• If you are registered, but have forgotten your log in details, please enter your e-mail address under 'Password Help'. The system will send you an automatic user ID and a new temporary password.

• Log-in and select 'Author Centre '

3.3. Submitting Your Manuscript

• After you have logged into your 'Author Centre', submit your manuscript by clicking on the submission link under 'Author Resources'.

• Enter data and answer questions as appropriate. You may copy and paste directly from your manuscript and you may upload your pre-prepared covering letter.

• Click the 'Next' button on each screen to save your work and advance to the next screen.

• You are required to upload your files.

- Click on the 'Browse' button and locate the file on your computer.

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- When you have selected all files you wish to upload, click the 'Upload Files' button.

• Review your submission (in HTML and PDF format) before completing your submission by sending it to the Journal. Click the 'Submit' button when you are finished reviewing.

3.4. Manuscript Files Accepted

Manuscripts should be uploaded as Word (.doc) or Rich Text Format (.rft) files (not write-protected) plus separate figure files. GIF, JPEG, PICT or Bitmap files are acceptable for submission, but only high-resolution TIF or EPS files are suitable for printing. The files

will be automatically converted to HTML and PDF on upload and will be used for the review process. The text file must contain the abstract, main text, references, tables, and figure legends, but no embedded figures or Title page. The Title page should be uploaded as a separate file. In the main text, please reference figures as for instance 'Figure 1', 'Figure 2' etc to match the tag name you choose for the individual figure files uploaded. Manuscripts should be formatted as described in the Author Guidelines below.

3.5. Blinded Review

Manuscript that do not conform to the general aims and scope of the journal will be returned immediately without review. All other manuscripts will be reviewed by experts in the field (generally two referees). International Endodontic Journal aims to forward referees´ comments and to inform the corresponding author of the result of the review process. Manuscripts will be considered for fast-track publication under special circumstances after consultation with the Editor.

International Endodontic Journal uses double blinded review. The names of the reviewers will thus not be disclosed to the author submitting a paper and the name(s) of the author(s) will not be disclosed to the reviewers.

To allow double blinded review, please submit (upload) your main manuscript and title page as separate files.

Please upload:

• Your manuscript without title page under the file designation 'main document'

• Figure files under the file designation 'figures'

• The title page and Acknowledgements where applicable, should be uploaded under the file designation 'title page'

All documents uploaded under the file designation 'title page' will not be viewable in the html and pdf format you are asked to review in the end of the submission process. The files viewable in the html and pdf format are the files available to the reviewer in the review process.

3.6. Suspension of Submission Mid-way in the Submission Process

You may suspend a submission at any phase before clicking the 'Submit' button and save it to submit later. The manuscript can then be located under 'Unsubmitted Manuscripts' and you can click on 'Continue Submission' to continue your submission when you choose to.

3.7. E-mail Confirmation of Submission

After submission you will receive an e-mail to confirm receipt of your manuscript. If you do not receive the confirmation e-mail after 24 hours, please check your e-mail address carefully in the system. If the e-mail address is correct please contact your IT department. The error may be caused by some sort of spam filtering on your e-mail server. Also, the e-mails should be received if the IT department adds our e-mail server (uranus.scholarone.com) to their whitelist.

3.8. Manuscript Status

You can access ScholarOne Manuscripts any time to check your 'Author Centre' for the status of your manuscript. The Journal will inform you by e-mail once a decision has been made.

3.9. Submission of Revised Manuscripts

To submit a revised manuscript, locate your manuscript under 'Manuscripts with Decisions' and click on 'Submit a Revision'. Please remember to delete any old files uploaded when you upload your revised manuscript.

4. MANUSCRIPT TYPES ACCEPTED

Original Scientific Articles: must describe significant and original experimental observations and provide sufficient detail so that the observations can be critically evaluated and, if necessary, repeated. Original Scientific Articles must conform to the highest international standards in the field.

Review Articles: are accepted for their broad general interest; all are refereed by experts in the field who are asked to comment on issues such as timeliness, general interest and balanced treatment of controversies, as well as on scientific accuracy. Reviews should generally include a clearly defined search strategy and take a broad view of the field rather than merely summarizing the authors´ own previous work. Extensive or unbalanced citation of the authors´ own publications is discouraged.

Mini Review Articles: are accepted to address current evidence on well-defined clinical, research or methodological topics. All are refereed by experts in the field who are asked to comment on timeliness, general interest, balanced treatment of controversies, and scientific rigor. A clear research question, search strategy and balanced synthesis of the evidence is expected. Manuscripts are limited in terms of word-length and number of figures.

Clinical Articles: are suited to describe significant improvements in clinical practice such as the report of a novel technique, a breakthrough in technology or practical approaches to recognised clinical challenges. They should conform to the highest scientific and clinical practice standards.

Case Reports: illustrating unusual and clinically relevant observations are acceptable but they must be of sufficiently high quality to be considered worthy of publication in the Journal. On rare occasions, completed cases displaying non-obvious solutions to significant clinical challenges will be considered. Illustrative material must be of the highest quality and healing outcomes, if appropriate, should be demonstrated.

Supporting Information: International Endodontic Journal encourages submission of adjuncts to printed papers via the supporting information website (see submission of supporting information below). It is encouraged that authors wishing to describe novel procedures or illustrate cases more fully with figures and/or video may wish to utilise this facility.

Letters to the Editor: are also acceptable.

Meeting Reports: are also acceptable.

5. MANUSCRIPT FORMAT AND STRUCTURE

5.1. Format

Language: The language of publication is English. It is preferred that manuscript is professionally edited. A list of independent suppliers of editing services can be found at http://authorservices.wiley.com/bauthor/english_language.asp. All services are paid for and arranged by the author, and use of one of these services does not guarantee acceptance or preference for publication

Presentation: Authors should pay special attention to the presentation of their research findings or clinical reports so that they may be communicated clearly. Technical jargon should be avoided as much as possible and clearly explained where its use is unavoidable. Abbreviations should also be kept to a minimum, particularly those that are not standard. The background and hypotheses underlying the study, as well as its main conclusions, should be clearly explained. Titles and abstracts especially should be written in language that will be readily intelligible to any scientist.

Abbreviations: International Endodontic Journal adheres to the conventions outlined in Units, Symbols and Abbreviations: A Guide for Medical and Scientific Editors and Authors. When non-standard terms appearing 3 or more times in the manuscript are to be

abbreviated, they should be written out completely in the text when first used with the abbreviation in parenthesis.

5.2. Structure

All manuscripts submitted to International Endodontic Journal should include Title Page, Abstract, Main Text, References and Acknowledgements, Tables, Figures and Figure Legends as appropriate

Title Page: The title page should bear: (i) Title, which should be concise as well as descriptive; (ii) Initial(s) and last (family) name of each author; (iii) Name and address of department, hospital or institution to which work should be attributed; (iv) Running title (no more than 30 letters and spaces); (v) No more than six keywords (in alphabetical order); (vi) Name, full postal address, telephone, fax number and e-mail address of author responsible for correspondence.

Abstract for Original Scientific Articles should be no more than 250 words giving details of what was done using the following structure:

• **Aim:** Give a clear statement of the main aim of the study and the main hypothesis tested, if any.

• **Methodology:** Describe the methods adopted including, as appropriate, the design of the study, the setting, entry requirements for subjects, use of materials, outcome measures and statistical tests.

• **Results:** Give the main results of the study, including the outcome of any statistical analysis.

• **Conclusions:** State the primary conclusions of the study and their implications. Suggest areas for further research, if appropriate.

Abstract for Review Articles should be non-structured of no more than 250 words giving details of what was done including the literature search strategy.

Abstract for Mini Review Articles should be non-structured of no more than 250 words, including a clear research question, details of the literature search strategy and clear conclusions.

Abstract for Case Reports should be no more than 250 words using the following structure:

• **Aim:** Give a clear statement of the main aim of the report and the clinical problem which is addressed.

• **Summary:** Describe the methods adopted including, as appropriate, the design of the study, the setting, entry requirements for subjects, use of materials, outcome measures and analysis if any.

• **Key learning points**: Provide up to 5 short, bullet-pointed statements to highlight the key messages of the report. All points must be fully justified by material presented in the report.

Abstract for Clinical Articles should be no more than 250 words using the following structure:

• **Aim:** Give a clear statement of the main aim of the report and the clinical problem which is addressed.

• **Methodology:** Describe the methods adopted.

• **Results:** Give the main results of the study.

• **Conclusions:** State the primary conclusions of the study.

Main Text of Original Scientific Article should include Introduction, Materials and Methods, Results, Discussion and Conclusion

Introduction: should be focused, outlining the historical or logical origins of the study and gaps in knowledge. Exhaustive literature reviews are not appropriate. It should close with the explicit statement of the specific aims of the investigation, or hypothesis to be tested.

Material and Methods: must contain sufficient detail such that, in combination with the references cited, all clinical trials and experiments reported can be fully reproduced.

(i) Clinical Trials should be reported using the CONSORT guidelines available at www.consort-statement.org. A CONSORT checklist and flow diagram (as a Figure) should also be included in the submission material.

(ii) Experimental Subjects: experimentation involving human subjects will only be published if such research has been conducted in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki (version 2008) and the additional requirements, if any, of the country where the research has been carried out. Manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and written consent of each subject and according to the above mentioned principles. A statement regarding the fact that the study has been independently reviewed and approved by an ethical board should also be included. Editors reserve the right to reject papers if there are doubts as to whether appropriate procedures have been used.

When experimental animals are used the methods section must clearly indicate that adequate measures were taken to minimize pain or discomfort. Experiments should be carried out in accordance with the Guidelines laid down by the National Institute of Health (NIH) in the USA regarding the care and use of animals for experimental procedures or with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and in accordance with local laws and regulations.

All studies using human or animal subjects should include an explicit statement in the Material and Methods section identifying the review and ethics committee approval for each study, if applicable. Editors reserve the right to reject papers if there is doubt as to whether appropriate procedures have been used.

(iii) Suppliers: Suppliers of materials should be named and their location (Company, town/city, state, country) included.

Results: should present the observations with minimal reference to earlier literature or to possible interpretations. Data should not be duplicated in Tables and Figures.

Discussion: may usefully start with a brief summary of the major findings, but repetition of parts of the abstract or of the results section should be avoided. The Discussion section should progress with a review of the methodology before discussing the results in light of previous work in the field. The Discussion should end with a brief conclusion and a comment on the potential clinical relevance of the findings. Statements and interpretation of the data should be appropriately supported by original references.

Conclusion: should contain a summary of the findings.

Main Text of Review Articles should be divided into Introduction, Review and Conclusions. The Introduction section should be focused to place the subject matter in context and to justify the need for the review. The Review section should be divided into logical sub-sections in order to improve readability and enhance understanding. Search strategies must be described and the use of state-of-the-art evidence-based systematic approaches is expected. The use of tabulated and illustrative material is encouraged. The Conclusion section should reach clear conclusions and/or recommendations on the basis of the evidence presented.

Main Text of Mini Review Articles should be divided into Introduction, Review and Conclusions. The Introduction section should briefly introduce the subject matter and justify the need and timeliness of the literature review. The Review section should be divided into logical sub-sections to enhance readability and understanding and may be supported by up to 5 tables and figures. Search strategies must be described and the use

of state-of-the-art evidence-based systematic approaches is expected. The Conclusions section should present clear statements/recommendations and suggestions for further work. The manuscript, including references and figure legends should not normally exceed 4000 words.

Main Text of Clinical Reports and Clinical Articles should be divided into Introduction, Report, Discussion and Conclusion. They should be well illustrated with clinical images, radiographs, diagrams and, where appropriate, supporting tables and graphs. However, all illustrations must be of the highest quality

Acknowledgements: International Endodontic Journal requires that all sources of institutional, private and corporate financial support for the work within the manuscript must be fully acknowledged, and any potential conflicts of interest noted. Grant or contribution numbers may be acknowledged, and principal grant holders should be listed. Acknowledgments should be brief and should not include thanks to anonymous referees and editors. See also above under Ethical Guidelines.

5.3. References

It is the policy of the Journal to encourage reference to the original papers rather than to literature reviews. Authors should therefore keep citations of reviews to the absolute minimum.

We recommend the use of a tool such as EndNote or Reference Manager for reference management and formatting. The EndNote reference style can be obtained upon request to the editorial office (iejeditor@cardiff.ac.uk). Reference Manager reference styles can be searched for here: www.refman.com/support/rmstyles.asp

In the text: single or double authors should be acknowledged together with the year of publication, e.g. (Pitt Ford & Roberts 1990). If more than two authors the first author followed by et al. is sufficient, e.g. (Tobias et al. 1991). If more than 1 paper is cited the references should be in year order and separated by "," e.g. (Pitt Ford & Roberts 1990, Tobias et al. 1991).

Reference list: All references should be brought together at the end of the paper in alphabetical order and should be in the following form.

(i) Names and initials of up to six authors. When there are seven or more, list the first three and add et al.

(ii)Year of publication in parentheses

(iii) Full title of paper followed by a full stop (.)

(iv) Title of journal in full (in italics)

(v) Volume number (bold) followed by a comma (,)

(vi) First and last pages

Examples of correct forms of reference follow:

Standard journal article

Bergenholtz G, Nagaoka S, Jontell M (1991) Class II antigen-expressing cells in experimentally induced pulpitis. International Endodontic Journal 24, 8-14.

Corporate author

British Endodontic Society (1983) Guidelines for root canal treatment. International Endodontic Journal 16, 192-5.

Journal supplement

Frumin AM, Nussbaum J, Esposito M (1979) Functional asplenia: demonstration of splenic activity by bone marrow scan (Abstract). Blood 54 (Suppl. 1), 26a.

Books and other monographs

Personal author(s)

Gutmann J, Harrison JW (1991) Surgical Endodontics, 1st edn Boston, MA, USA: Blackwell Scientific Publications.

Chapter in a book

Wesselink P (1990) Conventional root-canal therapy III: root filling. In: Harty FJ, ed. Endodontics in Clinical Practice, 3rd edn; pp. 186-223. London, UK: Butterworth.

Published proceedings paper

DuPont B (1974) Bone marrow transplantation in severe combined immunodeficiency with an unrelated MLC compatible donor. In: White HJ, Smith R, eds. Proceedings of the Third Annual Meeting of the International Society for Experimental Rematology; pp. 44-46. Houston, TX, USA: International Society for Experimental Hematology.

Agency publication

Ranofsky AL (1978) Surgical Operations in Short-Stay Hospitals: United States-1975. DHEW publication no. (PHS) 78-1785 (Vital and Health Statistics; Series 13; no. 34.) Hyattsville, MD, USA: National Centre for Health Statistics.8

Dissertation or thesis

Saunders EM (1988) In vitro and in vivo investigations into root-canal obturation using thermally softened gutta-percha techniques (PhD Thesis). Dundee, UK: University of Dundee.

URLs

Full reference details must be given along with the URL, i.e. authorship, year, title of document/report and URL. If this information is not available, the reference should be removed and only the web address cited in the text.

Smith A (1999) Select committee report into social care in the community [WWW document]. URL http://www.dhss.gov.uk/reports/report015285.html

[accessed on 7 November 2003]

5.4. Tables, Figures and Figure Legends

Tables: Tables should be double-spaced with no vertical rulings, with a single bold ruling beneath the column titles. Units of measurements must be included in the column title.

Figures: All figures should be planned to fit within either 1 column width (8.0 cm), 1.5 column widths (13.0 cm) or 2 column widths (17.0 cm), and must be suitable for photocopy reproduction from the printed version of the manuscript. Lettering on figures should be in a clear, sans serif typeface (e.g. Helvetica); if possible, the same typeface should be used for all figures in a paper. After reduction for publication, upper-case text and numbers should be at least 1.5-2.0 mm high (10 point Helvetica). After reduction, symbols should be at least 2.0-3.0 mm high (10 point). All half-tone photographs should be submitted at final reproduction size. In general, multi-part figures should be arranged as they would appear in the final version. Reduction to the scale that will be used on the page is not necessary, but any special requirements (such as the separation distance of stereo pairs) should be clearly specified.

Unnecessary figures and parts (panels) of figures should be avoided: data presented in small tables or histograms, for instance, can generally be stated briefly in the text instead. Figures should not contain more than one panel unless the parts are logically connected; each panel of a multipart figure should be sized so that the whole figure can be reduced by the same amount and reproduced on the printed page at the smallest size at which essential details are visible.

Figures should be on a white background, and should avoid excessive boxing, unnecessary colour, shading and/or decorative effects (e.g. 3-dimensional skyscraper histograms) and highly pixelated computer drawings. The vertical axis of histograms should not be truncated to exaggerate small differences. The line spacing should be wide enough to remain clear on reduction to the minimum acceptable printed size.

Figures divided into parts should be labelled with a lower-case, boldface, roman letter, a, b, and so on, in the same typesize as used elsewhere in the figure. Lettering in figures

should be in lower-case type, with the first letter capitalized. Units should have a single space between the number and the unit, and follow SI nomenclature or the nomenclature common to a particular field. Thousands should be separated by a thin space (1 000). Unusual units or abbreviations should be spelled out in full or defined in the legend. Scale bars should be used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. In general, visual cues (on the figures themselves) are preferred to verbal explanations in the legend (e.g. broken line, open red triangles etc.)

Figure legends: Figure legends should begin with a brief title for the whole figure and continue with a short description of each panel and the symbols used; they should not contain any details of methods.

Permissions: If all or part of previously published illustrations are to be used, permission must be obtained from the copyright holder concerned. This is the responsibilty of the authors before submission.

Preparation of Electronic Figures for Publication: Although low quality images are adequate for review purposes, print publication requires high quality images to prevent the final product being blurred or fuzzy. Submit EPS (lineart) or TIFF (halftone/photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Do not use pixel-oriented programmes. Scans (TIFF only) should have a resolution of 300 dpi (halftone) or 600 to 1200 dpi (line drawings) in relation to the reproduction size (see below). EPS files should be saved with fonts embedded (and with a TIFF preview if possible). For scanned images, the scanning resolution (at final image size) should be as follows to ensure good reproduction: lineart: >600 dpi; half-tones (including gel photographs): >300 dpi; figures containing both halftone and line images: >600 dpi.

Further information can be obtained at Wiley Blackwell's guidelines for figures: http:/authorservices.wiley.com/bauthor/illustration.asp.

Check your electronic artwork before submitting it: http://authorservices.wiley.com/bauthor/eachecklist.asp.

5.5. Supporting Information

Publication in electronic formats has created opportunities for adding details or whole sections in the electronic version only. Authors need to work closely with the editors in developing or using such new publication formats.

Supporting information, such as data sets or additional figures or tables, that will not be published in the print edition of the journal, but which will be viewable via the online edition, can be submitted. It should be clearly stated at the time of submission that the supporting information is intended to be made available through the online edition. If the size or format of the supporting information is such that it cannot be accommodated on the journal's website, the author agrees to make the supporting information available free of charge on a permanent Web site, to which links will be set up from the journal's website. The author must advise Wiley Blackwell if the URL of the website where the supporting information is located changes. The content of the supporting information must not be altered after the paper has been accepted for publication.

The availability of supporting information should be indicated in the main manuscript by a paragraph, to appear after the References, headed 'Supporting Information' and providing titles of figures, tables, etc. In order to protect reviewer anonymity, material posted on the authors Web site cannot be reviewed. The supporting information is an integral part of the article and will be reviewed accordingly.

Preparation of Supporting Information: Although provision of content through the web in any format is straightforward, supporting information is best provided either in web-ready form or in a form that can be conveniently converted into one of the standard web publishing formats:

• Simple word-processing files (.doc or .rtf) for text.

• PDF for more complex, layout-dependent text or page-based material. Acrobat files can be distilled from Postscript by the Publisher, if necessary.

• GIF or JPEG for still graphics. Graphics supplied as EPS or TIFF are also acceptable.

• MPEG or AVI for moving graphics.

Subsequent requests for changes are generally unacceptable, as for printed papers. A charge may be levied for this service.

Video Imaging: For the on-line version of the Journal the submission of illustrative video is encouraged. Authors proposing the use such media should consult with the Editor during manuscript preparation.

6. AFTER ACCEPTANCE

Upon acceptance of a paper for publication, the manuscript will be forwarded to the Production Editor who is responsible for the production of the journal.

6.1. Figures

Hard copies of all figures and tables are required when the manuscript is ready for publication. These will be requested by the Editor when required. Each Figure copy should be marked on the reverse with the figure number and the corresponding author's name.

6.2 Proof Corrections

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7 Guidelines for reporting of DNA microarray data

The International Endodontic Journal gives authors notice that, with effect from 1st January 2011, submission to the International Endodontic Journal requires the reporting of microarray data to conform to the MIAME guidelines. After this date, submissions will be assessed according to MIAME standards. The complete current guidelines are available at http://www.mged.org/Workgroups/MIAME/miame_2.0.html. Also, manuscripts will be published only after the complete data has been submitted into the public repositories, such as GEO (http://www.ncbi.nlm.nih.gov/geo/) or ArrayExpress (http://www.ebi.ac.uk/microarray/submissions_overview.html), in MIAME compliant format, with the data accession number (the identification number of the data set in the database) quoted in the manuscript. Both databases are committed to keeping the data private until the associated manuscript is published, if requested.

Prospective authors are also encouraged to search for previously published microarray data with relevance to their own data, and to report whether such data exists. Furthermore, they are encouraged to use the previously published data for qualitative and/or quantitative comparison with their own data, whenever suitable. To fully acknowledge the original work, an appropriate reference should be given not only to the database in question, but also to the original article in which the data was first published. This open approach will increase the availability and use of these large-scale data sets and improve the reporting and interpretation of the findings, and in increasing the comprehensive understanding of the physiology and pathology of endodontically related tissues and diseases, result eventually in better patient care.

ANEXO B

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"

CAMPUS ARAÇATUBA
FACULDADE DE ODONTOLOGIA
FACULDADE DE MEDICINA VETERINÁRIA

CEUA - Comissão de Ética no Uso de Animais
CEUA - Ethics Committee on the Use of Animals

CERTIFICADO

Certificamos que o Projeto de Pesquisa intitulado **"Avaliação da resposta tecidual e da capacidade de mineralização de cimentos endodônticos resinosos"**, Processo FOA nº 2015-00452, sob responsabilidade de Elói Dezan Júnior apresenta um protocolo experimental de acordo com os Princípios Éticos da Experimentação Animal e sua execução foi aprovada pela CEUA em 24 de Junho de 2015.

VALIDADE DESTE CERTIFICADO: 01 de Maio de 2017.
DATA DA SUBMISSÃO DO RELATÓRIO FINAL: até 01 de Junho de 2017.

CERTIFICATE

We certify that the study entitled **"Biocompatibility and mineralization assessment of resinous root canal sealers"**, Protocol FOA nº 2015-00452, under the supervision of Elói Dezan Júnior presents an experimental protocol in accordance with the Ethical Principles of Animal Experimentation and its implementation was approved by CEUA on June 24, 2015.

VALIDITY OF THIS CERTIFICATE: May 01, 2017.
DATE OF SUBMISSION OF THE FINAL REPORT: June 01, 2017.

Profa. Adj. Maria Cristina Rosifini Alves Rezende
Vice-Coordenadora da CEUA
CEUA Vice-Coordinator

CEUA - Comissão de Ética no Uso de Animais
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