CARLOS ROBERTO EMERENCIANO BUENO

AVALIAÇÃO DA RESPOSTA TECIDUAL E CAPACIDADE DE MINERALIZAÇÃO DE CIMENTOS QUE CONTÉM COMPOSTOS BIOCERÂMICOS, RESINOSOS E COM HIDRÓXIDO DE CÁLCIO.

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Resumo

Bueno, CRE. Avaliação da Resposta Tecidual e Capacidade De Mineralização de Cimentos que Contém Compostos Biocerâmicos, Resinosos e Com Hidróxido De Cálcio, 2015. 56p. Dissertação (Mestrado em Endodontia) – Faculdade de Odontologia, Campus de Araçatuba, Universidade Estadual Paulista “Júlio de Mesquita Filho”.

Resumo

A obturação ideal é uma combinação de um cimento com um material sólido, geralmente guta percha, que corrobora com o escoamento do cimento fluido, espalhando-o e preenchendo possíveis espaços vazios. Em virtude da possibilidade de contato direto com os tecidos periapicais, estes cimentos devem ser biocompatíveis e, se possível, estimular a mineralização para proporcionar selamento apical. Com o objetivo de avaliar, *in vivo*, a resposta tecidual e a capacidade de mineralização dos cimentos endodônticos Smartpaste® Bio, Sealapex® e Acroseal®, foi realizado implante subcutâneo em 40 ratos *Wistar* e adotados os períodos experimentais de 7, 15, 30 e 60 dias (10 animais por período de tempo). Cada animal recebeu quatro implantes, três tubos de polietileno com os cimentos a serem testados e um tubo vazio como controle. Após cada período pós-operatório, os animais foram eutanasiados e os tubos de polietileno, juntamente com o tecido circunjacente foram removidos e fixados. Para a análise histológica da espessura da cápsula fibrosa, infiltrado inflamatório e mineralização as peças foram incluídas em historresina, e coradas em HE, Von Kossa ou permaneceram sem coloração para a luz polarizada. Os resultados foram submetidos ao teste de Kruskal Wallis e Dunn ($p<0,05$). **Resultados:** Todos os cimentos produziram reação inflamatória moderada nos períodos iniciais. O Smartpaste Bio® apresentou a menor reação inflamatória aos 15 dias ($p<0,05$). O Sealapex® induziu maior mineralização, seguido do Smartpaste Bio®. O Acroseal® não apresentou indução de mineralização. **Conclusão**: Ao final do experimento, todos os cimentos testados apresentaram compatibilidade tecidual. Com exceção do Acroseal, todos induziram mineralização.

Palavras-chave: Teste de materiais, Inflamação, Cimentos dentários, Hidróxido de cálcio.

Abstract

Bueno, CRE. Biocompatibility and biomineralization assessment of bioceramic, epoxy- resin based and calcium hydroxide root canal sealers. Araçatuba, 2015. 56p. Dissertation (Master's Degree in Endodontics) – Dental School of Araçatuba, São Paulo State University "Júlio de Mesquita Filho".

Abstract

The cleaning and shaping of root canals is essential to achieve biological and mechanical goals in the endodontic treatment, providing the appropriate conical shape for subsequent obturation. The optimal obturation is a combination of a sealer with a central core, usually gutta percha, spreading and filling possible gaps. Once there is a direct contact with periapical tissue, the sealer should be biocompatible and, if possible, stimulate mineralization to perform an apical sealing. In order to evaluate *in vivo* biological response and tissue mineralization capacity of the endodontic sealers Smartpaste Bio® Sealapex® and Acroseal®, subcutaneous implants in 40 Wistar rats were performed. Analisys were at 7, 15, 30 and 60 days experimental periods (10 animals for each time period). Each animal received four implants, three polyethylene tubes with the sealers in test and one empty tube as control. After each post-operative period animals were euthanized and the polyethylene tubes, along with surrounding tissue were removed and fixed. In order to histologically analysis fibrous capsule thickness, inflammatory infiltrate and mineralization, the pieces were included in historesin and stained in HE, Von Kossa or remained without staining for observation under polarized light. The results were statistically analyzed by Kruskal-Wallis and Dunn ($p<0,05$). **Results**: All sealers promoted moderate inflammatory reaction at initial periods. Smartpaste Bio® presented the lowest inflammatory reaction at 15 days period ($p<0.05$). Sealapex® induced higher mineralization, followed by Smartpaste Bio®. Acroseal® showed no mineralization areas. **Conclusion**: At the end of the experiment, all tested sealers presented biocompatibility. With exception of Acroseal, all induced biomineralization.

Keywords: Materials testing, inflammation, dental cements, calcium hydroxide.

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ARTIGO

Biocompatibility and biomineralization assessment of bioceramic, epoxy-resin based and calcium hydroxide root canal sealers.

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Keywords: Acroseal, Biocompatibility, Mineralization, Sealapex, Smartpaste Bio.

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

Abstract

Aim: To evaluate the tissue response and mineralization ability of endodontic sealers in the rat subcutaneous tissue to implanted polyethylene tubes filled with Smartpaste Bio®, Acroseal® and Sealapex®.

Methodology: Forty Wistar rats were assigned to one of four groups according to periods of time (10 animals/group) and received subcutaneous implants containing the sealers to be tested and empty tube as controls. After 7, 15, 30 and 60 days the animals were euthanized and the polyethylene tubes removed with the surrounding tissues. Inflammatory infiltrate and thickness of fibrous capsule were histologically evaluated. Mineralization was analysed with Von Kossa staining and polarized light. Data were tabulated and subject to Kruskal-Wallis and Dunn's test ($P<0.05$).

Results: All tested materials induced moderate inflammatory reaction in the initial periods. Smartpaste Bio® induced the mildest inflammatory reactions in 15 days ($P<0.05$). No difference was observed among groups in 30 and 60 days. Von Kossa positive and birefringent structures to polarized light revealed a larger mineralization area in Sealapex® followed by Smartpaste Bio®. Acroseal® induced mild tissue reaction but it did not present signs of mineralization.

Conclusions: At the end of the experiment, all tested sealers presented biocompatibility. With exception of Acroseal, all induced biomineralization.

Keywords: Acroseal, Biocompatibility, Mineralization, Sealapex, Smartpaste Bio.

Introduction

Efficient cleaning and shaping of the root canal system is essential to achieve the biological and mechanical objectives of endodontic treatment, involving the removal of the pulp tissue or remains, microorganisms and their by-products, while providing the appropriate conical shape for subsequent root canal filling, reaching the desired three-dimensional obturation (Schilder 2006). Gutta-percha alone as filling material is not sufficient to provide adequate root canal system sealing, requiring its association with an endodontic sealer to fill gaps between cone and root canal walls, spreading the fluid sealer (Weis *et al*. 2004, Rahimi *et al*. 2009). As the sealer reaches the apical foramen, becomes in direct contact with periapical tissue, therefore should be biocompatible. Although the contact area is small, there is always concern about adverse reactions the sealer could cause on the tissues (Branstetter & Fraunhofer 1982, Orstavik 2005, Chhabra *et al*. 2011).

The endodontic sealers are divided into different groups according to its main component such as zinc oxide and eugenol, resin-based sealers and sealers containing calcium hydroxide (Kim *et al.* 2010). Depending on these main component, local adverse effects is possible, such as delaying or hindering repair (Schmalz *et al*. 2000, Geurtsen 2001).

The bioceramic sealers are being introduced in the market with a composition of tricalcium silicate, dicalcium silicate, calcium phosphates, calcium hydroxide and zirconium oxide as radiopacifier, all components applicable for biomedical and dental use, besides hydrophilic characteristics (Koch & Brave 2009, Zhang *et al*. 2009). The Smart Seal® obturation system (CRD Ltd, Stamford, UK) consists of a bioceramic sealer (Smartpaste Bio®) claimed as hydroxyapatite-based and a cone polymer with an external layer of an expandable hydrophilic hydrogel, the Smartpoint® (Kim *et al*. 2010, Economides *et al*. 2012). The manufacturer affirms that Smartpaste Bio® produces hydroxyapatite and calcium hydroxide as a byproduct of setting reaction, besides alkaline pH, antibacterial activity, radiopacity, and biocompatibility (ProSmart Product Information 2009, Loushine *et al.* 2011).

Acroseal® (Specialites-Septodont, Saint Maur-des-Fosses, France) is an endodontic sealer containing 28% calcium hydroxide in its composition, along with radiopaque excipient and a resin compound (epoxy resin). Previous studies demonstrated their antimicrobial activity against *Enterococcus faecalis,* low toxicity and suitable physicochemical properties (Eldeniz *et al*. 2007, Pinheiro *et al*. 2009, Marciano *et al.* 2011). According to the manufacturer, the sealer formulation has recently been modified with a reduction of calcium hydroxide concentration and an increase in its resinous compound diglycidylether of bisphenol A (DGEBA).

Sealapex® (SybronEndo, Glendora, CA) is a sealer that contains calcium oxide in its composition and forms calcium hydroxide after being hydrous by contact with tissue fluid. Sealapex® is characterized by biocompatibility, and osteoinductive ability to stimulate the deposition of mineralized tissue inducing apical sealing after endodontic treatment (Holland & Souza 1985, Gomes-Filho *et al.* 2012). This sealer was submitted to a reformulation, which presents 2-year shelf life instead of previous 1-year shelf life. One of the major alterations in Sealapex was the replacement of the radiopacifier from barium sulfate to bismuth trioxide (Leonardo *et al.* 2007).

Limited data concerning Smart Seal® obturation system are currently available and there is a lack of scientific studies about the biocompatibility of Smartpaste Bio® and its mineralization ability. Also, the reformulation in Acroseal® and Sealapex® components needs a complete research.

Therefore, the aim of this study was to analyze the biocompatibility (inflammation response) and mineralization ability of the endodontic sealers Smartpaste Bio®, Acroseal® and Sealapex®. The null hypothesis tested was that there is no biocompatibility and no mineralization induction by Smartpaste Bio®, Acroseal® and Sealapex®.

Materials and methods

Forty male 4- to 6-month-old Wistar rats, weighing 250–280 g, were used in the study. The animals were housed in controlled temperature rooms and received water and food *ad libitum*. Animal care was performed according to the

Araçatuba School of Dentistry-UNESP Ethical Committee, which approved the experimental project.

One hundred and twenty 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. Acroseal® and Sealapex® were prepared according to the manufacturers' recommendations and inserted into the tubes with a lentulo spiral (Dentsply Maillefer, Tulsa, OK, USA). Smartpaste Bio® is conditioned in a ready-to-use syringe and was directly inserted into the polyethylene tubes. Forty extra polyethylene empty tubes were used as control, totaling one hundred and sixty tubes in the experiment.

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

At 7, 15, 30 and 60 days after implantation, the animals were euthanized by anesthetic overdose. Polyethylene tubes with the surrounding tissues were removed and fixed in 10% buffered formalin at pH 7.0 (Gomes-Filho *et al.* 2009). The specimens were processed for glycol methacrylate embedding (Gomes-Filho *et al.* 2001), serially sectioned into 3 µm cuts, and stained with hematoxylin-eosin (HE). The 10 µm cuts were stained according to the Von Kossa technique or remained no stained.

The Polarized Light (PL) was used to observe birefrigent structures and Von Kossa (VK) technique was used to observe biomineralization, once mineralized structures stain darkly (Holland *et al.* 1999; Cintra *et al.* 2013).

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; 3, 125 or more inflammatory cells and severe reaction (400x magnification). Fibrous capsules were considered thin when <150 µm and thick when >150 µm. Calcification was recorded as positive or negative to Von Kossa stain and present or absent to polarized light (100x magnification).

Data were statistically analyzed by Kruskal-Wallis and Dunn; $P<0.05$ was considered significant.

Results

Control group

A moderate chronic inflammatory reaction (median score 2) was observed at the first two time periods (Table 1), on days 7 and 15 (Fig 1 - A,B). The inflammatory cell infiltration, consisting of lymphocytes and macrophages, was present in the fibrous capsule, which was thick. On the following periods, 30 and 60 days, the fibrous capsule surrounding the tube was thin, with few inflammatory cells (median score 1). The control group was negative to Von Kossa stain and no birefringent structures under polarized light were observed in all periods (Table 2).

Smartpaste Bio®

Only on day 7 a moderate inflammatory cell infiltration consisting of lymphocytes and macrophages was present in a thick fibrous capsule. On days 15, 30 and 60 the intensity of the inflammation was reduced (median score 1) and the fibrous capsule was thin, similar to the control group (Fig 1). Granulations birefringent to polarized light were present mostly on day 7. From day 15 to 60 the birefringent granulations started to become absent (Table 2). Von Kossa positive was observed in all time periods.

Acroseal®

On days 7 and 15 a moderate inflammatory cell infiltration (median score 2) consisting of lymphocytes and macrophages was present in a thick fibrous capsule (Table 1). On days 30 and 60, the inflammation intensity reduced and fibrous capsule was thin, similar to the control group (Fig 1 K,L). Birefringent granulations to polarized light were absent and Von Kossa was negative in all time periods (Fig 2 I-P)

Sealapex®

On days 7 and 15, a moderate inflammatory reaction (median score 2) consisting mainly of lymphocytes and macrophages was present in the thick fibrous capsule (Table 1). The intensity of the inflammation reduced from the 30th day on, until day 60, remaining macrophages phagocyting extravasated sealer (median score 1). The fibrous capsule near the tube opening was thin (Fig 1 G, H) and granulations birefringent to polarized light and Von Kossa positive staining were observed near the tube opening in all time periods (Fig 2 A-H).

Comparison Among Groups

The data were compared for each time period as shown in Table 1 and 2. After 7 days, HE staining revealed a similar tissue response of the inflammatory reaction, and there were no statistically significant differences among the inflammation scores of the experimental groups. On day 15, there was a statistically difference between inflammatory cell numbers of the Smartpaste Bio® and the other groups, once its inflammatory score was lower than the others ($P<0.05$) also presented a thin fibrous capsule. On days 30 and 60 there was no statistically significant difference among the scores of the different groups regarding the inflammation tissue response.

The polarized light and Von Kossa analysis revealed that Acroseal® had no mineralization induction in any time periods, unlike Sealapex®, which presented birefrigent structures under polarized light and Von Kossa positive in all time periods (Table 2). The Smartpaste bio® presented these birefrigents

structures and Von Kossa positive in 100% specimens until day 15 and decreased over time, differentiating from each time period along the experiment, but maintained the already mineralized structures (Table 2).

Discussion

The null hypothesis was rejected, once tested sealers presented biocompatibility and, all sealers but Acroseal® promoted mineralization. At initial experimental periods, all groups evoked mild-to-moderate inflammatory reactions that decreased and the fibrous capsule became thinner over time.

The use of subcutaneous implants started with Torneck (1966; 1967). His pioneering research aimed to evaluate the reaction of the subcutaneous connective tissue of rats to implanted polyethylene tubes and became one commonly used method to preliminary evaluate the biocompatibility (Zmener *et al*. 1990, Kaplan *et al.* 2003, Parirokh & Torabinejad 2010). In the present study, the reactions to empty tubes were similar to the results previously reported (Olson *et al.* 1981, Gomes-Filho *et al*. 2009).

According to Gomes Filho et al (2001), the Glicol Metacrilate technique is an excellent alternative for the evaluation of the biocompatibility of endodontic sealers, once is easy to execute and reproducible, providing a better definition of the degree of the inflammatory process. The specimens were sectioned into 3 µm cuts, and stained with HE to analyze inflammatory infiltrate. The scores used to analyze inflammatory reaction were according to previous studies (Gomes-Filho *et al.* 2009, Gomes-Filho *et al.*2012, Cintra *et al.* 2013). The 10 µm cuts were stained according to the Von Kossa technique, to observe mineralized structures, or remained without staining to allow the observation under polarized light of birefringent structures related to calcium carbonate crystals (Holland *et al.* 1999, Gomes-Filho *et al.* 2008a).

The reaction of calcium ions from $Ca(OH)_2$ ionic dissociation with carbon dioxide from tissues, forms calcite crystals, birefrigentes to the polarized light, which induced the formation of calcified areas, moreover, the calcium utilization reduces the carbon dioxide used by bacteria for anaerobic respiration (Holland *et al*. 2002, Saif *et al*. 2008) while the high pH from provided by the hydroxyl,

favors the tissue restoration process and antimicrobial properties (Tagger *et al.* 1988, Estrela *et al*. 1995, Desai & Chandler 2009).

Regarding the Smartpaste Bio®, the use of a pre-mixed sealer eliminates the potential of heterogeneous consistency during on-site mixing. Studies involving other bioceramic endodontic sealers already revealed hard tissue deposition (Güven *et al.* 2013) and favorable tissue response to partial pulpotomy (Azimi *et al.* 2014). Monobasic calcium phosphates are included in the sealer to facilitate reaction with calcium hydroxide to produce water and hydroxyapatite upon activation of the sealer by water (Yang 2002). Chang *et al.* (2014) conducted a study with a bioceramic sealer and observed lower inflammatory mediators and better osteoblast expression, which matches the previous study of Bosio *et al.* (2014), where they classified the bioceramic as biocompatible. These earlier biocompatibility findings of bioceramic sealers also match the results of this study.

It is interesting to observe that birefrigent granulations 100% present at initial periods decreased over time in Smartpaste Bio®, probably related to a reduction in calcium release after 30 days, but remained mineralized structures stained by Von Kossa, present in all time periods.

Even with reformulation, Acroseal® maintained biocompatibility, inducing low inflammatory response, similar to results reported by Khashaba *et al.* (2011). In all analyzed periods no mineralized areas were observed, similar occurrence were reported by Gomes-Filho *et al.* (2008b) and Neto *et al.* (2010).

Eldeniz *et al.* (2007) conduced a study concerning pH level and calcium ion release evidencing the lower Acroseal®'s calcium and Hydroxyl release. This may be justified by the low solubility of components, once contains epoxy compounds (diglycidyl ether of bisphenol A and methenamine), resulting in a less ion release (Siqueira *et al.* 1995) which explains the absence of mineralization observed in this study.

Duarte *et al.* (2000) performed a calcium release study where Sealapex® presented high calcium and hydroxyl release, especially after longer time intervals, which could be verified in this *in vivo* study by the presence of

birefrigent granulations under polarized light from 7 to 60 days, and biomineralization in all time periods.

Previous studies with Sealapex® original formula presented mild inflammation in initial periods, reducing over time (Mittal *et al.* 1995). Analyzing Sealapex® new formulation, inflammatory infiltrate increase at 14 days (Silva-Herzog *et al.* 2011) and at 90-day experimental period (Leonardo *et al.* 2007), suggesting the alterations might have affected tissue compatibility. The results in this research reveal a moderate initial inflammatory response, decreasing after 30 days.

Conclusion

At the end of the experiment, all tested sealers presented biocompatibility, with a statistical difference of Smartpaste Bio® in 15 days. With exception of Acroseal®, all induced biomineralization. Further study is necessary to better analyze the behavior of this biocerâmic material and its physicochemical properties and to confirm the findings of the present study.

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The authors deny any conflict of interest.

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Table 1. Percentage of samples in each group categorized according to the inflammatory score and the rating thickness of fibrous capsule.

	Score (%)				Capsule
7 Days	0	1	2	3	
Control	0	0	100	0	Thick
Sealapex	0	0	90	10	Thick
Acroseal	0	0	90	10	Thick
Smartpaste Bio	0	0	100	0	Thick
15 days					
Control	0	0	100	0	Thick
Sealapex	0	0	90	10	Thick
Acroseal	0	0	100	0	Thick
Smartpaste Bio	0	80*	20	0	Thin
30 days					
Control	0	100	0	0	Thin
Sealapex	0	90	10	0	Thin
Acroseal	0	90	10	0	Thin
Smartpaste Bio	10	90	0	0	Thin
60 Days					
Control	0	100	0	0	Thin
Sealapex	0	60	40	0	Thin
Acroseal	10	80	10	0	Thin
Smartpaste Bio	10	80	10	0	Thin

* statistical difference

Table 2. Percentage of samples in each group categorized according to Von Kossa positive to mineralization and presence of birefrigents crystals under polarized light.

7 days	Von Kossa (%)	Polarized Light (%)
Control	0	0
Sealapex	100	100
Acroseal	0	0
Smartpaste Bio	100	100
15 days		
Control	0	0
Sealapex	100	100
Acroseal	0	0
Smartpaste Bio	100	50
30 days		
Control	0	0
Sealapex	100	100
Acroseal	0	0
Smartpaste Bio	100	50
60 Days		
Control	0	0
Sealapex	100	100
Acroseal	0	0
Smartpaste Bio	100	30

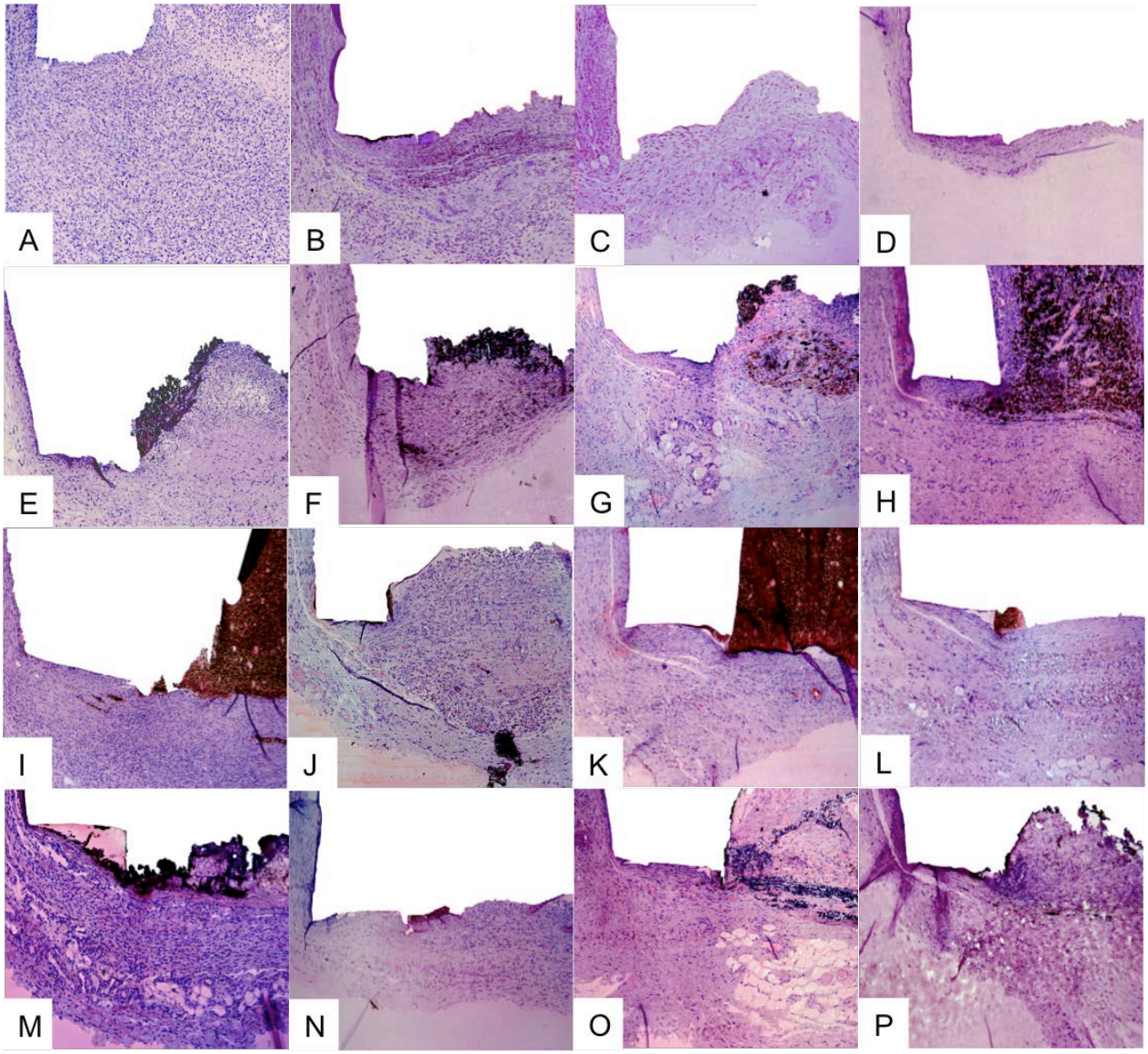

Figure 1 Subcutaneous tissue reactions in the experimental groups. Control group: (A, B) thick fibrous capsule and moderate inflammatory reaction (7 and 15 days HE, 100x); (C) reduction in the thickness of fibrous capsule and mild inflammatory reaction (30 days HE, 100x); (D) thin fibrous capsule and mild inflammatory reaction (60 days HE, 10x). Sealapex: (*E, F*) thick fibrous capsule formation and moderate inflammatory cell infiltration (7 and 15 days HE, 100x); (*G*) reduction in the thickness of fibrous capsule formation and mild inflammatory cell infiltration, consisting of macrophages (30 days HE, 100x); and (*H*) thin fibrous capsule formation and mild inflammatory cell infiltration, with macrophages phagocyting sealer (60 days HE, 100x). Acroseal: (I,J) thick fibrous capsule and moderate inflammatory cell infiltration (7 and 15 days HE, 100x); (K,L) the fibrous capsule surrounding the tube was thin with few chronic inflammatory cells (30 and 60 days HE, 100x); Smarpaste Bio: (M) thick fibrous capsule and moderate inflammatory reaction (7 days HE, 100x); (N-P) thin fibrous capsule and mild inflammatory reaction (15, 30 and 60 days respectively HE, 100x);

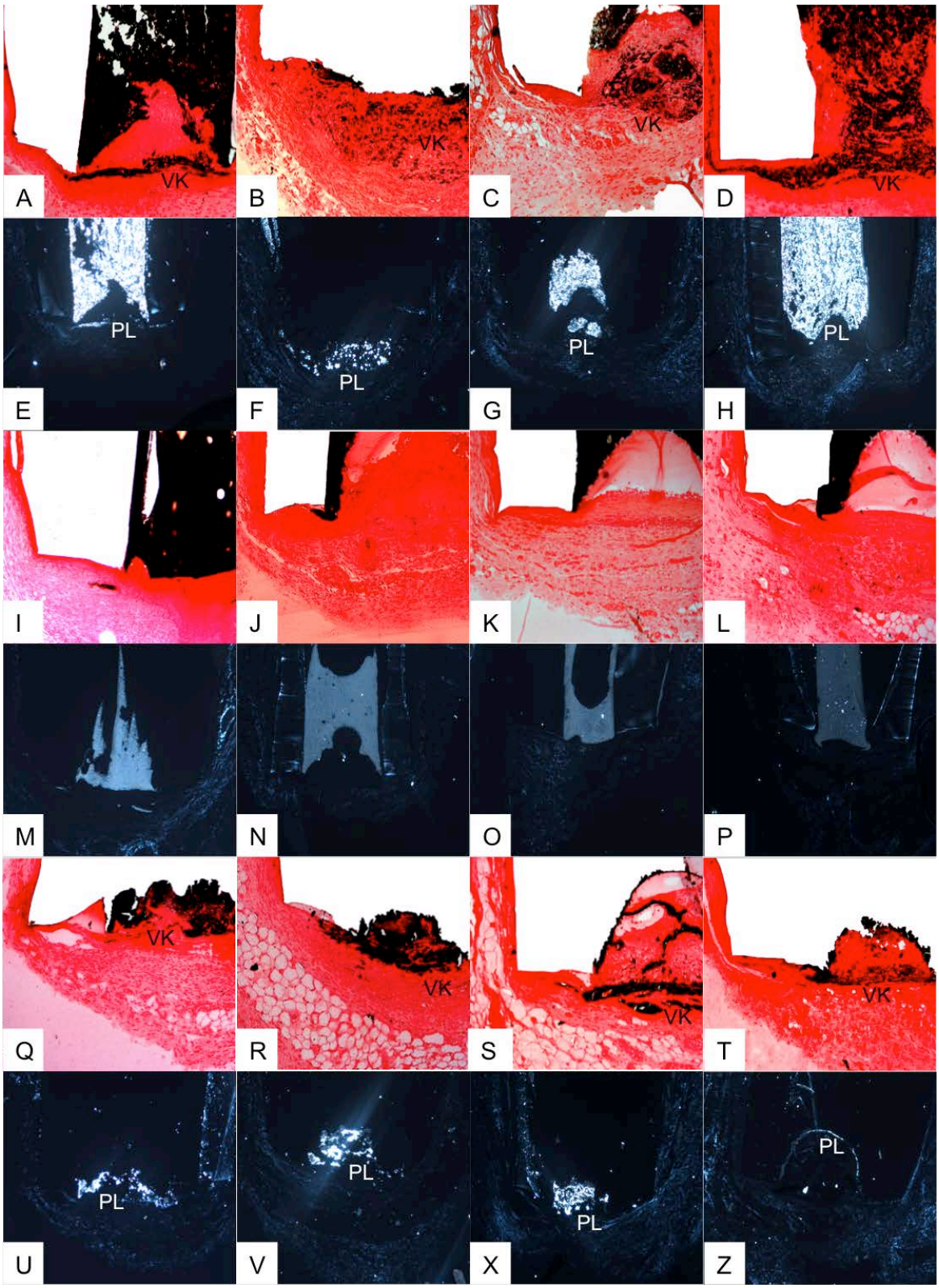

Figure 2 Biomineralization in the experimental groups. Sealapex: (A-D) the presence of dystrophic calcification on the tube opening (7, 15, 30, 60 days respectively; Von Kossa, 10x) and (E-H) the presence of birefringent structures to polarized light (7, 15, 30, 60 days respectively, polarized light 5x); Acroseal: (I-L) the absence of dystrophic (7, 15, 30, 60 days, respectively; Von Kossa, 10x); (M-P) the absence of birefringent structures to polarized light (7, 15, 30 and 60 days, respectively; polarized light, 5x); Smartpaste Bio: (Q-T) the presence of dystrophic calcification on the tube opening (7, 15, 30 and 60 days, respectively; Von Kossa, 10x) and (U-Z) the presence of birefringent structures to polarized light, slightly disappearing through the time periods (7, 15, 30 and 60 days, respectively; polarized light, 5x).

Anexos

Anexo A

International Endodontic Journal

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Author Guidelines

Content of Author Guidelines: 1. General, 2. Ethical Guidelines, 3. Manuscript Submission Procedure, 4. Manuscript Types Accepted, 5. Manuscript Format and Structure, 6. After Acceptance

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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 (http://www.consort- statement.org). A CONSORT checklist (http://www.consort-statement.org/mod_product/uploads/CONSORT%202001%20checklist.doc) 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 (http://www.wma.net/en/20activities/10ethics/10helsinki/index.html) (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 (http://www.endnote.com) or Reference Manager (http://ww.refman.com) for reference management and formatting. The EndNote reference style can be obtained upon request to the editorial office (iejeditor@cardiff.ac.uk (mailto:iejeditor@cardiff.ac.uk)). Reference Manager reference styles can be searched for here: www.refman.com/support/rmstyles.asp
(http://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 LegendsTables: 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.
(http://authorservices.wiley.com/bauthor/illustration.asp)

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

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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.

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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.

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The corresponding author will receive an email alert containing a link to a web site. A working email address must therefore be provided for the corresponding author. The proof can be downloaded as a PDF (portable document format) file

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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 (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/ (http://www.ncbi.nlm.nih.gov/geo/)) or ArrayExpress (http://www.ebi.ac.uk/microarray/submissions_overview.html (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 Relatório Final do trabalho intitulado **"Avaliação da compatibilidade biológica de cimentos endodônticos"**, Processo FOA nº 2012-02397, sob responsabilidade de Eloi Dezan Júnior e colaboração de Diego Valentim, Loiane Massunari, Gustavo Arcos Lopes e Camilla Pires Bruno foi aprovado pela CEUA em 12 de março de 2014.

CERTIFICATE

We certify that the study entitled **"Evaluation of biological compatibility of sealers"**, Protocol FOA nº 2012-02397, under the supervision of Eloi Dezan Júnior and collaboration of Diego Valentim, Loiane Massunari, Gustavo Arcos Lopes and Camilla Pires Bruno had its the Final Report approved by the CEUA on March 12, 2014.

Prof. Dr. Edilson Ervolino
Coordenador da CEUA
CEUA Coordinator

CEUA - Comissão de Ética no Uso de Animais
Faculdade de Odontologia de Araçatuba
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Anexo C

unesp UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de Araçatuba

INSTRUÇÃO NORMATIVA N.º 014-CPPGCO, de 14 de março de 2013.

Dispõe sobre as Normas para Redação de Dissertações e Teses do Programa de Pós-Graduação em CIÊNCIA ODONTOLÓGICA, de acordo com a Resolução UNESP-24, DE 24/02/2012.

O Coordenador do Programa de Pós-Graduação em Ciência Odontológica desta Faculdade, considerando a decisão do Conselho do Programa, por ocasião de sua reunião, levada a efeito em 08/03/2013, baixa a seguinte instrução normativa:

Artigo 1º - A redação do trabalho de Dissertação ou Tese do aluno do Programa de Pós-Graduação em Ciência Odontológica poderá ser realizada pela forma tradicional ou em formato de artigo.
Artigo 2º - O trabalho em formato de artigo deverá conter os seguintes elementos em sua estrutura:
I – Trabalho resultando em somente um artigo
1. Capa;
2. Folha de Rosto;
3. Ficha catalográfica (no verso da folha de rosto);
4. Dados Curriculares (facultativo);
5. Dedicatória (facultativo);
6. Agradecimentos (facultativo);
7. Epígrafe (facultativo);
8. Título e Resumo em Português;
9. Título e Resumo em Inglês (Abstract);
10. Lista de figuras (facultativo);
11. Lista de tabelas (facultativo);
12. Lista de abreviaturas (facultativo);
13. Sumário;
14. Artigo na íntegra (redigido nas normas do periódico escolhido)
15. Anexos

II – Trabalho resultando em dois artigos ou mais
1.Capa;
2. Folha de Rosto;
3. Ficha catalográfica (no verso da folha de rosto);
4. Dados Curriculares (facultativo);
5. Dedicatória (facultativo);
6. Agradecimentos (facultativo);
7. Epígrafe (facultativo);
8. Título e Resumo Geral em Português;
9. Título e Resumo Geral em Inglês (Abstract);
10. Lista de figuras (facultativo);
11. Lista de tabelas (facultativo);
12. Lista de abreviaturas (facultativo);
13. Sumário;
14. Introdução Geral (com referências inseridas como notas de rodapé);
15. Artigos na íntegra (redigido nas normas do periódico escolhido)
16. Anexos

Parágrafo único – Os anexos deverão obrigatoriamente conter as normas dos periódicos nos quais os artigos foram redigidos. Além disso, toda a informação que vier a complementar o trabalho e que não constar no texto do artigo deverá ser colocada sob a forma de anexos. Estes poderão incluir, mas não se limitam a, imagens, fluxogramas, protocolos laboratoriais, tabelas, etc.
Artigo 3º - Esta instrução normativa entra em vigor na data de sua publicação.

Conselho do Programa, 14 de março de 2013.

Prof. Adj. ALBERTO CARLOS BOTAZZO DELBEM
Coordenador do Programa