João Miguel Marques dos Santos

Prevenção da Microinfiltração Coronária no Tratamento Endodôntico

Coimbra, 2008

Orientadores:

Professor Doutor António Manuel Silvério Cabrita
Faculdade de Medicina - Universidade de Coimbra

Professor Doutor Shimon Friedman
Faculty of Dentistry - Universidade de Toronto

A elaboração deste trabalho decorreu no Departamento de Medicina Dentária, Estomatologia e Cirurgia Maxilo-Facial, no Instituto de Microbiologia da Faculdade de Medicina da Universidade de Coimbra e no Instituto de Tecnologia Biomédica da Estação Zootécnica Nacional – pólo de Santarém.

III – Resumo

Resumo

O principal factor etiológico para o desenvolvimento de patologia periapical é a infecção intrarradicular por microrganismos da flora oral. Por isso, o tratamento endodôntico tem como objectivo preservar as condições de assépsia ou eliminar os microrganismos do interior dos canais radiculares, tendo em vista promover a manutenção ou o restabelecimento da integridade dos tecidos periapicais.

As condições de assépsia, alcançadas durante a fase de preparação biomecânica, devem ser preservadas com a realização da obturação tridimensional do sistema canalar, seguida da restauração coronária. Com este procedimento, o clínico pretende impedir a entrada ou reentrada dos microrganismos no sistema canalar e a sua posterior progressão até aos tecidos periapicais.

No entanto, a longo prazo, mesmo que todas as etapas terapêuticas anteriormente referidas sejam meticulosamente executadas, os microrganismos podem invadir o canal a partir da cavidade de acesso, dos canais laterais ou dos túbulos dentinários. A microinfiltração coronária resultante da recidiva de cárie, de fractura ou degradação da interface dente/material de restauração, desempenha um papel primordial neste processo. A partir do momento em que os microrganismos chegam ao sistema de canais radiculares podem encontrar condições propícias à sua progressão ao longo da obturação canalar e interagir com os tecidos periapicais. A sua interacção directa ou indirecta através da libertação de toxinas facilitam o desenvolvimento de patologia periapical pós-tratamento, ou seja, influenciam negativamente o prognóstico do tratamento endodôntico.

De um modo genérico podemos afirmar que, utilizando os critérios de Strindberg, as terapêuticas actualmente disponíveis permitem taxas de sucesso, após 4 a 6 anos, de cerca de 95% para situações de biopulpectomia, e de aproximadamente 80% para dentes com periodontite apical.

Por conseguinte, o desenvolvimento de materiais e métodos terapêuticos que permitam impedir a propagação dos microrganismos constitui um desígnio para a optimização do prognóstico do tratamento.

A clorohexidina é um agente antimicrobiano, de largo espectro, efectivo contra a maioria dos microrganismos envolvidos no desenvolvimento da periodontite apical. Reveste-se de particular interesse devido à sua capacidade de eliminar o *Enterococcus faecalis* e a *Candida albicans*, espécies resistentes aos clássicos medicamentos de uso intracanalar e frequentemente associadas aos casos de insucesso do tratamento endodôntico. A sua adsorção à dentina e a posterior libertação gradual, ao longo de várias semanas, em concentrações com eficácia antimicrobiana, conferem-lhe a designada substantividade antimicrobiana. Esta propriedade pode possibilitar uma resistência adicional da obturação canalar à microinfiltração coronária, quer pela existência de um mecanismo farmacológico capaz de compensar falhas adesivas ou coesivas no material de obturação, quer pela melhoria do substrato para a adesão resultante do condicionamento da dentina.

O objectivo deste trabalho foi avaliar, *in vitro* e *in vivo*, a eficácia de um gel de clorohexidina a 2%, aplicado como medicação intracanalar por um período de 7 dias, na prevenção da microinfiltração coronária após a obturação.

No modelo experimental *in vitro* a hipótese foi testada frente a 3 materiais de obturação diferentes: a) um cimento endodôntico extensamente estudado e com alargada utilização clínica, o Pulp Canal Sealer® (Kerr – SybronEndo); b) um cimento endodôntico recentemente introduzido no mercado, o GuttaFlow® (Colténe/Whaladent) e c) um material recém desenvolvido, o Resilon™/Epiphany™ (Pentron Clinical Technologies). Este último material representa um novo paradigma na obturação de canais radiculares, substituindo a utilização de gutapercha por um polímero à base de policaprolactona (Resilon™), que é utilizado como material de núcleo, associado a um cimento endodôntico (Epiphany™), com o qual tem possibilidade de estabelecer ligação química.

Realizou-se a preparação biomecânica de 140 canais de dentes monorradiculares, com morfologia recta, e comprimento padronizado de 16 milímetros. As raizes foram divididas em 12 grupos teste, de 10 espécimes cada, e 4 grupos controlo, de 5 espécimes cada, sendo depois aleatoriamente atribuídos

aos grupos em estudo. Metade dos grupos teste foram previamente medicados com o gel de clorohexidina a 2%, durante uma semana, e a outra metade imediatamente obturada com cada um dos 3 materiais anteriomente referidos. Posteriormente todas as amostras foram submetidas à inoculação coronária de bactérias *(Enterococcus faecalis*) ou fungos (*Candida albicans*), no modelo experimental de dupla câmara desenvolvido por Torabinejad e colaboradores, durante um período de 80 dias.

De acordo com a metodologia utilizada no modelo experimental *in vitro* e os resultados encontrados pode concluir-se que:

1 – A medicação intracanalar com gel de clorohexidina a 2%, durante uma semana, conferiu uma resistência adicional à microinfiltração coronária de Candida albicans (p=0,0396), todavia o mesmo não se verificou para o Enterococcus faecalis (p=0,3554).

2 – A obturação canalar com Epiphany™/Resilon™ ou guta-percha/GuttaFlow®, apresentou uma resistência superior à microinfiltração coronária do que o método clássico de compactação lateral de guta-percha associada ao cimento endodôntico Pulp Canal Sealer®.

3 – Os diferentes perfis de contaminação apresentados pelas amostras submetidas à infiltração de bactérias ou de fungos sugerem que o modelo experimental tem impacto nos resultados obtidos. Não obstante, manteve-se o perfil geral de resistência de todos os grupos estudados frente a ambos os microrganismos.

No estudo experimental *in vivo* a hipótese foi testada frente a um único material de obturação, tendo sido escolhido o Resilon™/Epiphany™. Esta opção prendeu-se sobretudo com a necessidade de manter o tamanho da amostra dos diferentes grupos dentro do nível exigido pelo planeamento estatístico. Além disso optámos por utilizar o material mais recentemente desenvolvido, por representar uma nova abordagem na área da obturação canalar. O modelo permitiu avaliar a biocompatibilidade dos materiais utilizados, bem como a sua eficácia clínica perante a microinfiltração coronária de microrganismos.

Neste estudo foram utilizadas 6 cadelas de raça Beagle, com cerca de dois anos de idade, e o protocolo operatório envolveu a utilização de 10 prémolares birradiculares em cada animal. Os canais em estudo foram alvo de aleatorização

por blocos, tendo em cada cadela sido atribuído um prémolar ao grupo controlo positivo, três aos grupos controlo negativo e seis aos grupos teste. O grupo 1 foi preparado e obturado na mesma sessão com Epiphany™/Resilon™. O grupo 2 foi preparado e medicado com gel de clorohexidina a 2%, durante uma semana, e na segunda sessão obturado com o mesmo material e técnica que o grupo 1.

Relativamente à metodologia e aos resultados obtidos com o modelo experimental *in vivo*, podemos concluir que:

1 – O estudo rejeita a hipótese nula, ou seja, a medicação intracanalar com gel de clorohexidina a 2% não conferiu, neste modelo experimental, uma protecção adicional contra o desenvolvimento de inflamação periapical em raízes obturadas expostas à microinfiltração coronária. De facto, canais sem medicação e obturados numa única sessão apresentaram uma menor incidência de inflamação periapical.

2 – Independentemente da abordagem terapêutica, em sessão única ou com medicação intracanalar, a realização de biopulpectomia e obturação canalar com o sistema Resilon™/Epiphany™, numa sessão única ou com medicação intracanalar durante 1 semana, permite um prognóstico histológico favorável, traduzido pela ausência de inflamação periapical em 66% dos casos tratados.

3 – A obturação canalar com Resilon™/Epiphany™ não impediu o desenvolvimento de inflamação grave, verificada em 8% das raízes expostas ao meio oral, sugerindo que este material pode ser permeável ao ingresso de microrganismos.

4 – A histomorfometria permitiu a introdução de parâmetros de avaliação quantitativa úteis na análise da reacção inflamatória periapical. O aumento da intensidade da inflamação foi directamente proporcional à espessura do ligamento periodontal e à área de cemento secundário. Nos grupos tratados com gel de clorohexidina a 2% ambas as variáveis apresentaram um aumento estatisticamente significativo.

5 – A extrusão periapical de cimento endodôntico Epiphany™ intensificou a resposta inflamatória periapical.

No enquadramento dos modelos experimentais *in vitro* e *in vivo* utilizados não se verificou uma correlação entre os resultados obtidos em ambos os estudos.

Concretamente, o modelo *in vivo* não permitiu confirmar a protecção adicional contra a microinfiltração coronária conferida pela medicação intracanalar, com gel de clorohexidina a 2%, verificada no modelo *in vitro*. Este estudo reforça os argumentos defendidos pelos autores que questionam a validade clínica dos resultados obtidos nos modelos de microinfiltração *in vitro*.

IV – Abstract

Abstract

Introduction

The development and progression of endodontically induced apical periodontitis is caused by invasion of microorganisms into the root canal system. Thus, root canal treatment procedures focus on exclusion of microorganisms from the root canal system, aiming to maintain or reestablish the integrity of the periapical tissues.

The treatment procedure comprises the establishment of an aseptic environment, biomechanical root canal preparation to curtail microorganisms, three-dimensional filling of the root canal system to resist potential microbial ingress, and a coronal restoration to prevent microbial ingress in the long-term. Following this procedure, clinicians aim to obstruct the coronal pathway for microbial reinfection of the root canal system and the consequent disease of the periapical tissues. Nevertheless, even when all of these therapeutic procedures are meticulously applied, in the long term microorganisms can eventually invade the root canal via the coronal access cavity, lateral canals and dentinal tubules. The principal pathway for microbial invasion of root-filled teeth is coronal microleakage, resulting from caries, cracks or deterioration of the coronal restoration. Once the root canal system is invaded, microrganisms can propagate through the filled canal and interact with the host's periapical tissues. This interaction results in the development of post-treatment apical periodontitis, thereby negatively affecting the outcome of endodontic treatment.

It can be generally stated that the conventional root canal treatment procedures support healthy periapical tissues in 95% of teeth undergoing biopulpectomy, and in approximately 80% of teeth with apical periodontitis, when

using Strindberg's criteria for outcome assessment four to five years after root canal treatment. To enhance these outcomes, therapeutic materials and methods must be developed that can inhibit the ingress and proliferation of microorganisms in root-filled canal systems.

Chlorhexidine is a wide spectrum antimicrobial agent effective against most of the oral microorganisms associated with apical periodontitis. Of particular interest is its ability to kill microorganisms such as *Enterococcus faecalis* and *Candida albicans,* that may resist the conventional root canal medications and that are frequently associated with persistence of apical periodontitis after treatment. Chlorhexidine is adsorbed by dentine and subsequently slowly released at substantive antimicrobial levels over a period of several weeks, imparting the so-called "antimicrobial substantivity". If antimicrobial substantivity could be imparted in the root canal system, it would provide extra resistance to coronal microleakage.

Objective

The purpose of this study was to evaluate, *in vitro* and *in vivo,* the efficacy of 2% chlorhexidine gel applied for 7 days as an intracanal medicament, in preventing coronal microleakage and development of apical periodontitis after root canal filling.

***In vitro* Coronal Microleakage Study**

This study evaluated the ability of intracanal medication with 2% chlorhexidine gel, followed by root filling with 3 different materials, to resist microbial ingress of *E. faecalis* and *C. albicans* over a period of 80 days. The double chamber coronal microleakage experimental model developed by Torabinejad *et al.* in 1990 was used.

Material and Methods

One hundred forty freshly extracted human teeth with straight, single roots, fully developed apices and without root caries, were de-coronized to a standardized length of 16 millimetres. The canals were prepared biomechanically with the Hero 642® system. Apical patency was maintained throughout the preparation procedure with a size 25 K-type file. The roots were randomly divided into 12 test groups of 10 specimens each, and 4 control groups of 5 specimens each. The groups were duplicated to allow independent and simultaneous testing with *E. faecalis* and *C. albicans*. Half of the test groups were

immediately root filled (A, B, and C) and the other half had the root canals medicated with 2% chlorhexidine gel for a week (D, E and F). The test groups were root-filled as follows: Groups A and D, with Gutta-percha and Pulp Canal Sealer®; groups B and E, with Gutta-percha and GuttaFlow®; groups C and F, with Resilon™ and Epiphany™. In group P (positive control), the canals were not filled, while in group N (negative control), the canals were filled with gutta-percha alone with the apices sealed with All-Bond 2®.

Group	n	CHX 2%	Root canal filling	Apical patency
N	5	-	Gutta-percha (GP)	Apex sealed
P	5	-	-	Apex patent
A	10	-	GP+Pulp Canal Sealer®	Apex patent
B	10	-	GP+GuttaFlow®	Apex patent
C	10	-	Resilon™+Epiphany™	Apex patent
D	10	+	GP+Pulp Canal Sealer®	Apex patent
E	10	+	GP+GuttaFlow®	Apex patent
F	10	+	Resilon™+Epiphany™	Apex patent

Teeth were coated with nail polish except for the apical 2 mm, and each tooth was sealed in a 10 ml glass vial, with an 18-gauge needle inserted through the vial cover and bonded into the pulp chamber and canal orifice with All-Bond 2®. After sterilization with ethylene oxide at 30ºC temperature, Brain Heart infusion (for inoculation with *E. faecalis*), or Sabouraud Dextrose (for inoculation with *C. albicans*) was injected into each vial. Each inoculum was inserted trough the needle into the root canal every 4 days, and the broth monitored daily for turbidity. When turbidity occurred, the broth was cultured and biochemical identification tests (API-Biomerriux) performed to confirm contamination with the inoculated microorganisms.

Legends to Figures

Figure 15. Sample of the root specimens used in the study, with size standardized to 16 millimetres from the apex.

Figure 16. Chemical structure of methylcellulose.

Table 3. Description and characterization of positive control, negative control and test groups

Figure 17. Experimental model with an Endo-Eze® tip cemented to the coronal part of the root and suspended in the vial trough the silicon septum.
Figure 18. Injection of the culture medium in the inferior chamber until 4 apical millimeters of the root tip are submerged.
Figure 19. Incubation of samples for 48 hours, to confirm sterility conditions before starting the inoculation.
Figure 20. Coronal inoculation of microorganisms with a hypodermic insulin needle.
Figure 21. Inspection of samples to detect signs of contamination indicated by turbidity in the culture medium.
Figure 22. Culture of *E. faecalis* in solid medium to confirm morphological compatibility with the inoculated strain.
Figure 23. Biochemical identification test API 20 (Biomerriux) to recognize *E. faecalis.*

Statistical methods

Statistics were performed using SPSS 14.0. The length of time until turbidity occurred was compared among the different groups using Kaplan-Meier survival analysis. Significant differences in Kaplan Meier curves were evaluated using the log-rank test at $p<0,05$.

Results *In vitro*

As expected, turbidity in the positive control group specimens occurred rapidly and consistently. In contrast, all the negative control group specimens had no turbidity throughout the study period.

Among the test groups inoculated with *E. faecalis,* the better performance in specimens medicated with chlorhexidine gel was not statistically significant ($p=0,3554$) from the immediately root-filled specimens. Disregarding the medication with chlorhexidine, specimens filled either with Resilon™/Epiphany™ or gutta-percha/GuttaFlow® performed better than those filled with gutta-percha/Pulp Canal Sealer® ($p=0,0005$ and $0=0,0270$, respectively).

Among the test groups inoculated with *C. albicans,* the specimens medicated with chlorhexidine gel had a significantly better performance ($p=0,0396$) than the immediately root-filled specimens. The differences among the root filling material combinations were not statistically significant.

Legends to Figures

Figure 24. On the left side of the image, samples from the positive control with turbidity indicating contamination of the inferior chamber (white arrows).

Table 4. Treatment of each experimental group and days on which turbidity occurred in *E. faecalis* coronally inoculated samples.

Graphic 1. Development of contamination in the samples of the different test groups, inoculated with *E. faecalis*, over the experimental period.

Graphic 2. Number of samples showing contamination with *E. faecalis* at the end of the experimental period.

Table 5. Treatment of each experimental group and days on which turbidity occurred in *C. albicans* coronally inoculated samples.

Graphic 3. Development of contamination in the samples of the different test groups, inoculated with *C. albicans*, over the experimental period.

Graphic 4. Number of samples showing contamination with *C. albicans* at the end of the experimental period.

Graphic 5. Global comparison between samples medicated with intracanal chlorhexidine gel (EfCHX and CaCHX) or immediately root filled (Ef and Ca) inoculated with *E. faecalis* (Ef and EfCHX) or with *C. albicans* (Ca and CaCHX).

Conclusions of the *In vitro* Study

Based on the *in vitro* experimental method used and the results obtained it was concluded that:

1 – Intracanal medication with 2% chlorhexidine gel for one week conferred additional resistance to coronal ingress of *C. albicans* ($p=0.0396$), but not to ingress of *E. faecalis* ($p=0.3554$).

2 – Root fillings with the Resilon™/Epiphany™ and with GuttaFlow® better resisted coronal microbial ingress than root fillings with laterally-compacted gutta-percha and Pulp Canal Sealer®.

3 – The different ingress patterns observed with bacterial and fungal inoculation suggested that the experimental model had an impact on the results. Nevertheless, the general resistance pattern to microbial ingress was comparable for both microorganisms.

In vivo Coronal Microleakage Study

The purpose of this study was to assess *in vivo* the efficacy of chlorhexidine applied for 7 days as an intracanal medicament, in preventing periapical inflammation subsequent to coronal exposure and inoculation of root-filled teeth. The animal experimental model developed by Friedman *et al.* in 1997 was used.

In this study we tested the hypothesis using only one type of root canal filling, Resilon™ core and Epiphany™ sealer, in order to maximize the sample size for statistical analysis. The most recently developed root filling material was selected in order to investigate this novel approach to root canal filling. In addition to testing the clinical efficacy of Resilon™/Epiphany™ in preventing coronal microbial ingress and consequent periapical inflammation, this model also allowed the evaluation of the materials' tissue compatibility.

Material and Methods

Canals in the six two-rooted mandibular premolars of six Beagle dogs were prepared with Hero 642® (MicroMega) and assigned to the experimental group. One canal in each tooth was immediately filled with Root Canal Sealant Epiphany™ and Resilon™ core material (Pentron Clinical Technologies), and the access sealed with Photac™ Fil. In the other canal, 2% chlorhexidine gel was placed with a Lentulo spiral, and the access sealed with Photac™ Fil. One week later, the medicated canals were reaccessed, chlorhexidine gel rinsed out and root canals filled as in the one-step group. After three weeks, the pulp chambers of the teeth were exposed and left open to the oral environment for one week, subsequently inoculated with isologous plaque and resealed with Photac™Fil.

Group	Number of roots (n)	Treatment protocol	Exposure and inoculation
Positive control	12	Root canal preparation without filling	Yes
Negative control	18	Group 1 – one session Resilon™/Epiphany™	No
	18	Group 2 – Clorhexidine+ Resilon™/Epiphany™	
Test group	36	Group 1 – one session Resilon™/Epiphany™	Yes
	36	Group 2 – Clorhexidine+ Resilon™/Epiphany™	

Additionally, three two-rooted maxillary premolars in each dog were treated in the same way as the teeth in the experimental group, but the pulp chambers were left sealed to serve as a negative control. A fourth maxillary premolar in each dog was prepared but not root-filled, and exposed to the oral environment to serve as a positive control.

Periapical radiographs were taken before and after treatment, and post-operative controls were done at four and seven months. At seven months, dogs were sacrificed, jaw and maxillary blocks removed, decalcified in Morse's solution (equal parts of 50% formic acid and 20% sodium citrate) and processed for histological evaluation. Serial sections (6 microns thick) were done along the sagittal plane, in a mesio-distal orientation including the entire root canal system and periradicular tissues. Two out of every ten sections were mounted on glass slides and stained with hematoxylin and eosin. Moreover, for each root one slide was stained with Van Giemsa and another with Brown and Brenn. The periradicular tissues were examined under a light microscope and the following histomorphological parameters were evaluated:

Parameters	Scores
Periapical inflammation	(0) None, normal appearance of the root surface and surrounding tissues (1) Mild, localized inflammatory cell infiltrate without bone and root resorption (2) Severe, diffuse infiltrate associated with bone and tooth resorption
Mineralized tissue resorption	(0) Absent (1) Cementum resorption (2) Cementum and dentin resorption
Apical openings sealed with mineralized tissue	(0) Complete sealing (1) Sealing beyhond half of apical area (2) Sealing up to half of apical area
Apical limit of root canal filling	(0) Cemental canal (1) Mild sealer extrusion (2) Extrusion of core filling material
Phagocytic cells	(0) Absent (1) Present
Bacteria	(0) Not found (1) Found
Periodontal ligament thickness	Average of the measurements obtained from 3 predefined points in the apical region of the evaluated slides
Secondary cementum area	Average of the measurements obtained in the defined apical area of the evaluated slides

Legends to Figures

Statistical Methods

Statistics were performed using SPSS 14.0. The degree of periapical inflammation was analyzed with the Chi-square test for a level of significance of 95% ($p<0,05$). Histomorphometric measurements of the periodontal ligament thickness and secondary cementum deposition were studied with analysis of variance (ANOVA) and Student's t- test for a level of significance of 95% ($p<0,05$).

Results *In vivo*

The total sample of the study included 120 roots. Of the total of 108 root canal fillings performed, the great majority (95 roots) were within the limit of cemento-dentinal junction and the minority (13 roots) had sealer extrusion into the periapical tissues. The latter are analyzed separately.

Only the 12 positive control roots revealed radiographic evidence of periapical pathology. Histologically, none of the 29 roots in the negative control group had severe inflammation, while 9/29 roots (31%) had mild inflammation. In the experimental group, mild inflammation was observed in 13/32 roots (41%) and severe inflammation in 2/32 roots (6%) medicated with chlorhexidine gel, while in the one-step group mild inflammation was observed in 5/33 roots (15%) and severe inflammation in 3/33 roots (9%). The lower incidence of periapical inflammation in the immediately-filled roots was statistically significant (Pearson chi square, p = 0,020). The coronal exposure and inoculation in the experimental group resulted in the development of severe inflammation in 5/ 65 roots (8%).

The 13 specimens with Epiphany™ sealer extruded into the periapical tissues showed formation of a fibrous capsule around the material, associated with mononuclear inflammatory infiltrate at the periphery. Nine of these specimens had a mild reaction, and four specimens with large amounts of extruded material had a moderate inflammatory reaction accompanied by the presence of foreign body giant cells.

Histomorphometry evidenced a statistically significant correlation between periodontal ligament thickness and the level of periapical inflammation. The average thickness found in roots without periapical inflammation was 279 µm, in mild inflammation 323 µm and with severe inflammation 496 µm.

Measurements of the apical area of secondary cementum deposition showed a statistically significant increase in roots treated with chlorhexidine gel medication. Statistical analysis evidenced a direct correlation between this parameter and the presence of periapical inflammation.

Legends to Figures, Graphics and Tables

Figure 61. Radiographic control at 3 months. A) 1st quadrant with positive control (arrow); B) 2nd quadrant, negative controls (arrows); C) and D) mandible, test group teeth.

Figure 62. Radiographic control at 6 months after inoculation. A) 1st quadrant, negative controls; B) 2nd quadrant, augmented apical thickness of the *lamina dura* (arrow); C) e D) mandible, test group teeth.

Figures 63. Light microscopy images of the periapical region of a vital pulp dog's premolar. Radicular dentin (1), apical cementum (2), periodontal ligament (black arrow), alveolar bone (3), dental nerve (white arrow) and blood vessels (red arrows).

instrumentation. A and B) large deposition of secondary cementum near the overinstrumented area (black arrow) and Epiphany™ sealer agglomerate (blue arrow) in the periapical tissues; C) area of dense inflammatory infiltrate (yellow arrow); D) fibrous tissue capsule (blue arrow) surrounding Epiphany™ sealer, with inflammatory cells in the proximity; E and F) detail of the capsule (blue arrow), mononuclear inflammatory cells (yellow arrow) and macrophages (red arrow).

Figure 82. Light microscopy images of a serial section of the same specimen, approximately 100 µm distant from the previous figure. A) highlighting the apical cementum destruction induced by overinstrumentation (black arrows) and Epiphany™ sealer forced into the periapical tissues (blue arrow) surrounded by inflammatory infiltrate (yellow arrows); B) Giant foreign body cells (black arrows) at periphery of the extravased Epipahny™ sealer.

Figure 83. Light microscopy images of a serial section of the same specimen, approxilmatelly 100 µm further on from previous section. A) fibrous capsule (black arrow) surrounding extravased material (blue arrow) with inflammatory infiltrate in the proximity (yellow arrow); B) Detail of a fragment of cellular cementum (1) Displaced into the periapical tissues by the overinstrumentation, macrophages (red arrow) and erythocytes (green arrow).

Figure 84. Light microscopy image highlighting mononuclear inflammatory cells (black arrows), macrophages showing signs compatible with Epiphany™ sealer phagocytosis (red arrow), and a blood vessel nearby (green arrow)

Figure 85. Light microscopy image highlighting active resorption of the alveolar bone in the periapical region. Bone *trabeculae*, osteocyte (white arrow) and osteoclast (black arrow).

Figure 86. Light microscopy images of a specimen with extravased Epiphany™ sealer. A) Inflammatory reaction localized arround two agglomerates of Epiphany™ sealer extravased into the periapical tissue (black arrows); B) serial section tangential to the organized fibrous capsule arround extravased material (black arrows), large concentration of mononuclear inflammatory cells (yellow arrows) a foreign body giant cell (green arrow).

Graphic 14. Intensity of tissular reaction to the extruded materials.

Conclusions of the *In vivo* Study

Based on the *in vivo* experimental method used and the results obtained it was concluded that:

1 – The study rejected the null hypothesis, in that intracanal medication with 2% chlorhexidine gel did not confer an additional protection against the development of apical periodontitis in root canals exposed to coronal microbial ingress. In fact, canals filled in one-step without such medication had a lower incidence of periapical inflammation.

2 – Root filling with Resilon™/Epiphany™ did not prevent development of severe periapical inflammation in 8% of the roots, suggesting that it can be permeable to microbial ingress.

3 – Histomorphometry allowed quantitative evaluation of the inflammatory periapical reaction. The increase in inflammation was directly proportional to the thickness of the periodontal ligament and of the secondary cementum. These two parameters were increased in the roots medicated with 2% chlorhexidine gel.

4 – Periapical extrusion of the Epiphany™ sealer intensified the inflammatory response.

No correlation was found between the results of the *in vitro* and *in vivo* studies, as the protection against microbial ingress conferred by intracanal medication with 2% chlorhexidine gel, observed in the *in vitro* study, was not evident in the *in vivo* study. This finding corroborated previous reports on the questionable clinical validity of *in vitro* microbial leakage models.