



**UNIVERSIDADE ESTADUAL PAULISTA**  
**"Júlio de Mesquita Filho"**

**CÂMPUS DE ARAÇATUBA – FACULDADE DE ODONTOLOGIA DEPARTAMENTO**  
**DE ODONTOLOGIA RESTAURADORA DISCIPLINA DE ENDODONTIA**

## **TESE DE DOUTORADO**

**PEDRO HENRIQUE CHAVES DE OLIVEIRA**

**POTENCIAL DO BIOVIDRO F18 DOPADO COM COBALTO EM PROCEDIMENTOS**  
**ENDODÔNTICOS**

**ARAÇATUBA-SP**  
**2025**



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**TESE DE DOUTORADO**

Programa de Pós-graduação em Ciências

Área de concentração: Endodontia

**PEDRO HENRIQUE CHAVES DE OLIVEIRA**

**POTENCIAL DO BIOVIDRO F18 DOPADO COM COBALTO EM PROCEDIMENTOS**  
**ENDODÔNTICOS**

Tese de Doutorado apresentada à faculdade de Odontologia de Araçatuba da Universidade Estadual Paulista "Júlio de Mesquita Filho" – UNESP, como parte dos requisitos para obtenção do título de Doutor em Ciências, área de concentração em Endodontia

Aluno: Pedro Henrique Chaves de Oliveira  
Orientador: Prof. Assoc. Luciano Tavares Angelo Cintra

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## Dedicatória

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À Prof<sup>a</sup>. Assoc. Diana Gabriela Soares dos Passos. Professora, é tão incrível

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Epígrafe

*“Pode-se encontrar a felicidade mesmo nas horas mais sombrias, se  
a pessoa se lembrar de acender a luz”*

J.K. Rowling



## Resumo Geral

Oliveira, PHC. **Potencial do biovidro F18 dopado com cobalto em procedimentos endodônticos. 2025. Tese de doutorado** – Faculdade de Odontologia, Universidade Estadual Paulista, Araçatuba, 2025.

## Resumo

A endodontia busca encontrar materiais que, além de biocompatíveis, participem ativamente da formação tecidual, induzindo ou modulando o processo de reparo. Estudos prévios do nosso grupo de pesquisa verificou adequadas propriedades biológicas empregando um material vitro-cerâmico experimental (F18), com potencial para ser usado como material reparador em diferentes condições clínicas. Além disso, estudos recentes mostraram que esse material possui capacidade de induzir a formação de colágenos, tecidos ósseos e vasculares. Dessa forma, este estudo teve como objetivo avaliar o material experimental F18 dopado ou não com cobalto, em procedimento de capeamento pulpar direto e como selador em perfuração de furca, em modelos *in vivo* em ratos Wistar para essas condições clínicas. Foram utilizados 80 ratos, divididos em 2 experimentos de 40 animais (n=10 por grupo em cada experimento). Para avaliarmos o material como capeador, obtivemos os seguintes grupos: Grupo controle - Guta percha (GP), Grupo MTA Angelus (MTA), Grupo F18 em pó (F18), Grupo F18+Co (F18+Co). Para analisarmos os materiais como seladores, os seguintes grupos foram obtidos: Grupo controle - Guta percha (GP), Grupo MTA Angelus (MTA), Grupo F18 Putty (F18), Grupo F18+Co (F18+Co). **Experimento 1:** desgastes dentários de 0,5mm de profundidade e 0,5x1mm de extensão foram realizados na superfície oclusal dos 1<sup>os</sup> e 2<sup>os</sup> para exposição pulpar. Em seguida, foi realizado irrigação com solução salina, controle do sangramento, secagem e capeamento pulpar direto. **Experimento 2:** perfurações de furca de 0,5mm de diâmetro foram realizadas nos 1<sup>os</sup> e 2<sup>os</sup> molares superiores. Em seguida, foi realizado irrigação com solução salina, controle do sangramento, secagem e selamento da perfuração com um dos materiais. Em ambos experimentos os dentes foram selados com ionômero de vidro e restaurados com resina flow. Decorridos 7 e 30 dias os animais foram eutanasiados e as maxilas processadas para análise histológica e imunoistoquímica. A inflamação foi avaliada em cortes teciduais corados em H&E., o colágeno foi quantificado após polarização em cortes corados

com Picrosírios red, e a capacidade biomineralizadora foi avaliada por meio da imunomarcagem de osteopontina e osteocalcina. Testes estatísticos foram aplicados ( $p < 0,05$ ). **Resultados 1:** Aos 7 dias, o grupo GP apresentou inflamação predominantemente leve, enquanto F18 e F18+Co apresentaram inflamação de moderada a severa ( $p < 0,05$ ). O grupo MTA apresentou inflamação de leve a moderada, sem diferença para os demais grupos ( $p > 0,05$ ). Aos 30 dias, houve redução do padrão inflamatório, com semelhança entre GP e MTA ( $p > 0,05$ ) e maior resposta inflamatória para F18 e F18+Co, diferente dos demais grupos ( $p < 0,05$ ). Foi observado maior porcentagem de fibras colágenas imaturas aos 7 dias e 30 dias, sem diferenças entre os grupos ( $p > 0,05$ ). Quanto à imunorreatividade (IR), os grupos MTA, F18 e F18+Co, apresentaram maior IR para OCN comparado ao grupo GP em 7 e 30 dias ( $p < 0,05$ ), assim como para OPN no período de 7 dias ( $p < 0,05$ ). Já aos 30 dias, o grupo MTA apresentou maior IR comparado aos demais grupos ( $p < 0,05$ ). **Resultados 2:** Aos 7 dias, o grupo GP apresentou inflamação leve a moderada, diferente do grupo F18+Co que apresentou inflamação predominantemente severa ( $p < 0,05$ ). O grupo F18 Putty apresentou inflamação moderada sem diferença para os grupos GP e MTA ( $p > 0,05$ ), enquanto MTA apresentou inflamação moderada a severa sem diferença para os demais grupos ( $p > 0,05$ ). Aos 30 dias, todos os grupos apresentaram inflamação leve ( $p > 0,05$ ). Quanto à maturação do colágeno, em 7 dias, o grupo F18 Putty apresentou menor porcentagem de fibras imaturas e maior de maduras, comparado aos demais grupos ( $p < 0,05$ ). Já em 30 dias, o grupo MTA apresentou maior porcentagem de fibras imaturas e menor de maduras, comparado aos demais grupos ( $p < 0,05$ ). Quanto à capacidade biomineralizadora, aos 7 dias os grupos MTA, F18+Co e F18 Putty apresentaram maior IR para OCN e OPN comparado ao grupo GP ( $p > 0,05$ ). Aos 30 dias, os grupos MTA e F18+Co apresentaram maior IR para OCN e OPN comparado ao grupo GP ( $p > 0,05$ ). Já o grupo F18 Putty apresentou IR para OCN semelhante aos demais grupos ( $p > 0,05$ ), e IR para OPN semelhante ao grupo GP ( $p > 0,05$ ). Conclui-se que em ambos os procedimentos, evidenciou-se maior biocompatibilidade para o MTA. Além disso, mesmo não sendo superior ao MTA, foi evidenciado capacidade biomineralizadora para os bividros F18+Co, F18 e F18 Putty.

**Palavras-chave:** Biomineralização; Capeamento da polpa dentária; Materiais biocompatíveis; Endodontia; Defeitos da furca



## General Abstract

Oliveira, PHC. **Potential of F18 bioglass doped with cobalt in endodontic procedures.**

**Tese de Doutorado** - Faculdade de Odontologia, Universidade Estadual Paulista, Araçatuba, São Paulo, 2025.

### Abstract

Endodontics searches to find materials that, in addition to being biocompatible, participating in tissue formation, inducing or modulating the repair process. Previous studies by our research group verified biological properties using an experimental glass-ceramic material (F18), with a potential to be used as bioinductive material in a variety of clinical conditions. Furthermore, recent studies have shown that it can induce collagen, bone and vascular tissue. Therefore, this study aimed to evaluate the experimental material F18, doped or not with cobalt, in direct pulp capping and as a sealer for furcation perforation *in vivo* models in Wistar rats of these clinical procedures to explore tissue response to biomaterials. Eighty rats were divided into two experiments of 40 animals (n=10 per group in each experiment). To evaluate the material as a capping material, we obtained the following groups: Control Group - Gutta-percha (GP), MTA Angelus Group (MTA), F18 Powder Group (F18), and F18+Co Group (F18+Co). To analyze the materials as sealers, the following groups were formed: Control Group - Gutta-percha (GP), MTA Angelus Group (MTA), F18 Putty Group (F18), and F18+Co Group (F18+Co). **Experiment 1:** The teeth were worn down of 0.5 mm in depth and 0.5 x 1 mm in length on the occlusal surface of 1<sup>st</sup> e 2<sup>nd</sup> molars to expose the pulp. Subsequently, saline irrigation, bleeding control, drying, and direct pulp capping were performed. **Experiment 2:** Furcation perforations of 0.5 mm diameter were performed on the 1<sup>st</sup> e 2<sup>nd</sup> maxillary molars. Subsequently, saline irrigation, bleeding control, drying, and sealing with one of the materials were performed. In both experiments, the teeth were sealed with glass-ionomer cement and restored with flowable resin. After 7 and 30 days, the animals were euthanized, and the maxillae submitted for histological and immunohistochemical analysis. Inflammation was assessed in tissue sections stained with H&E, collagen was quantified after polarization in Picrosirius red-stained sections, and biomineralization capacity was assessed by immunohistochemistry of osteopontin and osteocalcin. Statistical tests were applied (p<0.05). **Results 1:** At 7 days, the GP group presented predominantly mild inflammation, while F18 and F18+Co presented moderate to severe inflammation (p<0.05). MTA presented mild to moderate inflammation, with no difference from the other groups (p>0.05). At 30 days,

there was a reduction in the inflammatory pattern, with similarity between GP and MTA ( $p>0.05$ ) and a greater inflammatory response for F18 and F18+Co, different from the other groups ( $p<0.05$ ). A higher percentage of immature collagen fibers was observed at 7 days and 30 days, with no differences among the groups ( $p>0.05$ ). Regarding immunoreactivity (IR), the MTA, F18 and F18+Co groups presented a higher IR for OCN compared to the GP group at 7 and 30 days ( $p<0.05$ ), as well as for OPN in the 7-day period ( $p<0.05$ ). At 30 days, MTA presented a higher IR compared to the other groups ( $p<0.05$ ). **Results 2:** At 7 days, the GP group presented mild to moderate inflammation, different from the F18+Co group, which presented predominantly severe inflammation ( $p<0.05$ ). The F18 Putty group presented moderate inflammation with no difference from GP and MTA ( $p>0.05$ ), while MTA presented moderate to severe inflammation with no difference from the other groups ( $p>0.05$ ). At 30 days, all groups presented mild inflammation ( $p>0.05$ ). Regarding collagen maturation, in 7 days, F18 Putty presented a lower percentage of immature fibers and a higher percentage of mature fibers, compared to the other groups ( $p<0.05$ ). At 30 days, MTA presented a higher percentage of immature fibers and a lower percentage of mature fibers, compared to the other groups ( $p<0.05$ ). Regarding biomineralization, at 7 days, MTA, F18+Co, and F18 Putty presented a higher RI for OCN and OPN compared to GP ( $p>0.05$ ). At 30 days, MTA and F18+Co showed higher RI for OCN and OPN compared GP ( $p>0.05$ ). F18 Putty showed RI for OCN similar to the other groups ( $p>0.05$ ), and RI for OPN similar to GP ( $p>0.05$ ). It was concluded that in both procedures, MTA showed more biocompatibility. Furthermore, although it was not superior to MTA, it was observed biomineralization ability for F18+Co, F18 and F18 Putty bioglass.

**Keywords:** Biomineralization; Pulp capping; Biocompatible materials; Endodontics; Furcation defects

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## **LISTA DE ABREVIACES**

1.  $\text{Ca(OH)}_2$  – Hidrxido de clcio
2. MTA – Agregado trixido mineral
3. GP – Guta percha
4. F18 – Biovidro F18 em p
5. F18 Putty – Biovidro F18 em forma de Putty
6. F18+Co – Biovidro F18 dopado com cobalto
7. EDTA – cido etilenoaminotetractico
8. H&E – Colorao de Hematoxilina e Eosina
9. PSR – Colorao de Picrosirius red
10. OCN – Osteocalcina
11. OPN – Osteopontina
12. TPV - Terapia pulpar vital

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## INTRODUÇÃO GERAL

Estudos da engenharia tecidual indicam que os biovidros são compostos capazes de promoverem indução tecidual, desempenhando papel osteoindutor e osteocondutor, além da capacidade de guiar a formação de tecidos moles (Hench et al. 1991, 2006; Jones et al. 2013; Souza et al. 2017a; Chaves et al. 2024). Os vidros bioativos foram desenvolvidos inicialmente por Hench e colaboradores em 1969, e a composição mais bioativa, denominada Biovidro 45S5, consiste em 46,1%  $\text{SiO}_2$ , 24,4%  $\text{Na}_2\text{O}$ , 26,9%  $\text{CaO}$  e 2,6%  $\text{P}_2\text{O}_5$  (mol%). Este foi o primeiro produto que demonstrou grande capacidade de se ligar a estruturas ósseas (Hench et al. 1991, 2006). Nesse contexto, foi desenvolvido um biovidro ativo, conhecido como F18 que também pertence ao sistema  $\text{SiO}_2\text{--Na}_2\text{O--K}_2\text{O--CaO--MgO--P}_2\text{O}_5$  e que possui as características físico-químicas necessárias para a regeneração tecidual (Souza et al. 2016ab, 2017bc; Gabbai-Armelin et al. 2017; Chaves et al. 2024). Essas características colocaram esse material em evidência despertando o interesse pelo conhecimento de seu comportamento em situações clínicas na endodontia.

O F18 possui grande versatilidade comparado com materiais à base de biovidro, mantendo a bioatividade e desencadeando efeitos antimicrobianos (Souza et al. 2016a, 2017c). Com esse biovidro, foi possível desenvolver vários tipos de produtos, tais como: solução irrigante adjuvante á procedimentos de regeneração pulpar (de Paula et al. 2024), soluções para hipersensibilidade dentinária (Reis et al. 2021), pastas para medicação intracanal (de Araújo Lopes et al. 2020), fibras condutoras para formação de nervos (Souza et al. 2016b), fibras para guiar a regeneração de cartilagens (Souza et al. 2017b), membranas (Hidalgo et al. 2018) e scaffolds para guiar a regeneração tecidual (Marin et al. 2021; Gabbai-Armelin et al. 2017). Suas propriedades fundamentais já foram testadas, tais como citotoxicidade, genotoxicidade, biocompatibilidade, bioatividade, degradação, molhabilidade, permeabilidade e resistência mecânica (Souza 2016ab, 2017bc; Gabbai-Armelin et al. 2017). Uma outra característica é que o mesmo possui propriedades angiogências devido ao aumento do óxido nítrico, o que gera produção de fatores de crescimento vascular (Keshaw et al. 2005; Gorustovich et al. 2010; Stähli et al. 2015; Oishi et al., 2019), podendo favorecer a formação de tecido pulpar (Oishi et al. 2019). Adicionalmente, outros estudos demonstraram seu

potencial antibacteriano (Passos et al. 2021; Oliveira et al. 2020), fato que o torna um material ainda mais interessante para uso em endodontia.

Por outro lado, mesmo a literatura tendo demonstrado o potencial do F18 como um material capaz de induzir a formação de tecidos colagenosos (Souza et al. 2017b), ósseos (Souza et al. 2017a; Gabbai-Armelin et al. 2017), e vasculares (Oishi et al. 2019) novos estudos estão sendo conduzidos associando este material à metais de transição como Cobalto (Co), Cobre (Cu), Prata (Ag) e Zinco (Zn). Esses metais são conhecidos por seus efeitos anabólicos, principalmente no metabolismo ósseo (Nielsen et al. 1990; Saltman et al. 1993). Sabe-se que quando metais de transição entram em contato com os fluidos corporais, induzem a proliferação de células de origens óssea e também vasculares (Hoppe et al. 2011), particularmente por agirem como uma enzima que funciona como um co-fator que influenciará na sinalização de vias envolvidas com a formação desses tipos de tecidos (Sun et al. 1997). Nos estudos de implantodontia, quando há a incorporação de metais de transição na superfície do implante, observa-se que a osteointegração é melhorada devido a troca iônica relacionada com a dopagem desses elementos químicos (Salinas et al. 2011; Goh et al. 2013; Anand et al. 2014). Outros estudos mostraram que ao associar os biovidros com o cobalto e a prata, obteve-se melhores respostas teciduais, como por exemplo, atividades anti-oxidantes, anti-cancerígenas e antimicrobianas (Zhou et al. 2013; Souza et al. 2017a). O cobalto é um metal de transição que tem se destacado por apresentar estímulo à angiogênese, possibilitando a formação de tecidos vasculares (Littmann et al. 2018) e melhorando as características biológicas, magnéticas e ópticas de materiais utilizados na biomedicina geral (Colis et al. 2012). Ainda, estudos conduzidos por equipes distintas, demonstraram a capacidade do F18+Co em induzir a vascularização, por meio de proliferação de células endoteliais (Souza et al. 2017b; Oishi et al. 2019). Os tratamentos endodônticos são um desafio à manutenção da vitalidade do dente ou mesmo à sua permanência na cavidade. Dentre os tratamentos para manutenção da vitalidade temos os procedimentos conservadores, tópico científico extremamente explorado nos dias atuais, como o capeamento pulpar, pulpotomia e procedimentos regenerativos (Duncan 2022; ESE 2019). Esses procedimentos objetivam permitir a manutenção do remanescente pulpar, sem a remoção da polpa pelos procedimentos radicais de pulpectomia,

permitindo que dentes que foram traumatizados, fraturados, ou até aqueles que tiveram suas polpas expostas por lesões cariosas sejam mantidos vitais (Yan et al. 2023; Taha et al. 2020; ESE 2019).

Diferentes materiais já foram propostos como capeadores, entretanto a busca por materiais mais adequados e com melhores propriedades físicas e biológicas permanece. Na endodontia, o  $\text{Ca(OH)}_2$  é um material muito utilizado em procedimentos de exposição pulpar (Le Fournis et al. 2019; Taha 2020). Ao avaliar o  $\text{Ca(OH)}_2$ , F18 e F18+Co como medicação intracanal, utilizando o modelo de biocompatibilidade em tubos de polietileno, foi observado melhor desempenho do F18 e do F18+Co na resposta inflamatória e reparadora, além de ter sido evidenciado atividade antimicrobiana (de Araújo Lopes et al. 2020). Outro material vastamente empregado em procedimentos capeadores é o MTA (Torabinejad et al, 1993; Holland et al. 2001; Camilleri & Pitt Ford et al. 2006). Este material tem sido escolhido pois possui capacidade de induzir o processo de mineralização pela sua dissociação cálcica e também por se apresentar um material bioindutor, sendo capaz de promover a formação de tecido ósseo e dentinário (Lee et al. 1993; Darvell et al. 2011; Parirokh et al. 2010). Além disso, o MTA se mostrou superior ao  $\text{Ca(OH)}_2$  neste tipo de procedimento devido a suas propriedades mecânicas (Okiji & Yoshida 2009; Parirokh et al. 2018; Cushley et al. 2020).

Apesar de apresentar excelentes propriedades biológicas, o MTA possui limitações como as de manipulação, aplicação e, em algumas situações, pode promover alteração cromática dos dentes, devido à presença de óxido de bismuto em sua composição, utilizado como radiopacificador (Darvell et al. 2011; Haapasalo et al. 2015; Marciano et al. 2015). Assim, considerando o potencial dos biovidros para procedimentos endodônticos conservadores, o primeiro objetivo deste estudo é avaliar o material F18, comparando-o com o F18 dopado com cobalto como material capeador em tecido pulpar utilizando o modelo *in vivo* de capeamento pulpar direto em ratos Wistar.

A procura pelo aperfeiçoamento de técnicas e biomateriais para a preservação de tecidos é de extrema relevância. Além da manutenção dos tecidos pulpaes, situações desafiadoras em endodontia como os acidentes trans-operatórios, indicam a necessidade da investigação de condutas terapêuticas que visam o reparo dos tecidos comprometidos. Contudo, em particular, temos as perfurações endodônticas que, dependendo da sua condição e extensão, podem levar a perda

do elemento dentário. Segundo a American Association of Endodontics, as perfurações podem ser definidas como uma comunicação entre a porção externa do dente com os canais radiculares, podendo ser provocadas patologicamente ou mecanicamente (Cardoso et al. 2018; American of Endodontics Association, 2003).

O selamento das perfurações deve ser realizado, sempre que possível, imediatamente à ocorrência, pois essas lesões podem se contaminar causando destruição dos tecidos periodontais e dentários, podendo resultar na perda dentária (Alhadainy et al. 1994; Lagisetti et al. 2018). Além disso, esta ocorrência está relacionada com a maior parte dos insucessos endodônticos (Lagisetti et al. 2018). Seu tratamento exige materiais que tenham um potencial osteoindutor, biocompatibilidade, radiopacidade e estabilidade dimensional (Kakani et al. 2015). Diversos materiais têm sido indicados para o selamento de perfuração de furca, dentre eles o MTA (da Silva et al. 2011) e o biodentine (Perard et al. 2013), além do próprio hidróxido de cálcio (Wu et al. 1993). O MTA é o material mais utilizado, pois além das propriedades semelhantes ao hidróxido de cálcio (Roberts et al. 2018), apresenta propriedades físicas adequadas (Okiji & Yoshiba 2009). Entretanto, como já comentado o MTA apresenta algumas limitações (Marciano et al. 2015; de Sousa Reis et al. 2019). Dessa forma, considerando as dificuldades dos materiais já utilizados para selamento de furca, como a alteração cromática provocada pelo MTA e o valor comercial de outros materiais, como o biodentine, o segundo objetivo deste estudo é avaliar o material vitro-cerâmico experimental F18 dopado com cobalto e o F18 na forma de putty como material selador de perfurações de furca utilizando o modelo *in vivo* para perfurações em ratos Wistar.

Diante do exposto, essa pesquisa tem como finalidade identificar, por meio de análises biológicas *in vivo*, o potencial de produtos que possam contribuir para a terapia endodôntica e que trará para a comunidade científica embasamento para o desenvolvimento de estudos complementares, que culminarão no aprimoramento de biomateriais que possam ser futuramente empregues clinicamente em procedimentos endodônticos críticos.

## 2. OBJETIVOS

**Geral – Experimento 1:** Avaliar a resposta tecidual induzida pelos biovidros quando utilizados como materiais capeadores, comparando-os ao MTA em polpa dentária de ratos Wistar, evidenciando o perfil inflamatório, reparo e a capacidade de induzir o

processo de mineralização.

**Geral – Experimento 2:** Avaliar a resposta tecidual induzida pelos biovidros quando utilizados como seladores em perfuração de furca *in vivo*, evidenciando a resposta tecidual dos materiais experimentais vitrocerâmicos F18 em sua forma de Putty e F18 dopado com cobalto, comparando-o com o MTA, por meio de análises de biocompatibilidade, reparo e biomineralização.

### **Específicos:**

#### **Experimento 1:**

Evidenciar as seguintes respostas induzidas pelos materiais experimentais como capeadores pulpare, comparando-os com o MTA, por meio de análises do:

- Infiltrado inflamatório, pela coloração de H&E, classificando o infiltrado inflamatório;
- Maturidade colagenosa, pela coloração de PSR, evidenciando os níveis de reparo colagenoso induzidos no tecido pulpar;
- Capacidade de induzir o processo de mineralização tecidual, por meio da imunorreatividade de OCN e OPN.

#### **Experimento 2:**

Avaliar as seguintes respostas induzidas pelos materiais experimentais como seladores de perfuração de furca, comparando-os com o MTA, por meio de análises do:

- Infiltrado inflamatório, pela coloração de H&E, classificando o infiltrado inflamatório;
- Maturidade colagenosa, pela coloração de PSR, evidenciando a capacidade de induzir o reparo no tecido dental e periodontal comprometido;
- Capacidade de induzir o processo de mineralização tecidual, pela imunorreatividade de OCN e OPN.

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Capítulo 1

Article 1

International Endodontic Journal

**Inflammatory and biomineralizer profile of F18 and F18+Co bioglass in pulp capping experiment. Study in Wistar rats**

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**Running title:** F18 and pulp capping

**Key words:** Bioglass, MTA, pulp capping, biomaterials, endodontics

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## **Inflammatory and biomineralizer profile of different forms of F18 bioglass in an experimental methodology of pulp capping in Wistar rats**

**Aim:** To evaluate the biocompatibility, collagen maturity, and biomineralization ability of F18 and F18+Co compared to MTA in a pulp capping *in vivo* model.

**Methodology:** Forty animals (n=10/group) were divided into the following groups: Gutta-percha (GP), MTA Angelus (MTA), F18, and F18+Co. The pulp of the 1<sup>st</sup> and 2<sup>nd</sup> molars were exposed with a sphere bar. Subsequently, saline irrigation, bleeding control, drying, and direct pulp capping were performed. After 7 and 30 days, the animals were euthanized, and the maxillae were processed for histological and immunohistochemical analyses. Inflammation was assessed in tissue sections stained with H&E, collagen maturity was determined by PSR, and the biomineralization potential was analyzed by immunohistochemistry for osteocalcin and osteopontin. The data were submitted to specific statistical tests ( $p < 0.05$ )

**Results:** At 7 days, the GP group presented predominantly mild inflammation, while F18 and F18+Co presented moderate to severe inflammation ( $p < 0.05$ ). The MTA group presented mild to moderate inflammation, with no difference from the other groups ( $p > 0.05$ ). At 30 days, there was a reduction in the inflammatory pattern, with similarity between GP and MTA ( $p > 0.05$ ) and a greater inflammation for F18 and F18+Co, different from the other groups ( $p < 0.05$ ). A higher percentage of immature collagen fibers was observed at 7 days and 30 days, with no differences among the groups ( $p > 0.05$ ). Regarding immunoreactivity (IR), MTA, F18 and F18+Co presented a higher IR for OCN compared to the GP at 7 and 30 days ( $p < 0.05$ ), as well as for OPN in 7 days ( $p < 0.05$ ). At 30 days, the MTA group presented a higher IR compared to the other groups ( $p < 0.05$ ).

**Conclusions:** It is concluded that in this *in vivo* pulp capping model, the inflammation reduced for all materials over time and that, although mineralization ability for F18 and F18+Co was detected, MTA presented better tissue mineralization potential.

**Keywords:** Bioglass, MTA, biomaterials, pulp capping, endodontics

## Introduction

Conservative therapies are nowadays being explored extensively (Duncan 2022; ESE 2019). Several situations can lead to dental pulp exposure, including pathological processes, dental restorations, crown preparations and prostheses, fractures, and iatrogenic complications (Mente et al., 2014; Ring et al., 2016). Depending on the size and extent of pulp exposure, this process can be treated through pulp capping, aiming to preserve the pulp (Ding et al., 2013; Ring et al., 2016). Currently, many materials are favored for their quality and predictability in different endodontic treatments, such as pulp capping (Plotino et al., 2007). In clinical practice, in cases of occupational iatrogenesis or when the pulp has been exposed to a carious process, but it is still vital, pulp capping should be performed immediately to avoid contamination and promote better conditions for tissue repair, reaching clinical success rate of up to 97.8% (Aguilar et al. 2011; Weldon et al. 2002; Yong et al. 2021).

This treatment modality is considered conservative and, due to recent bioengineering studies, may be indicated in more complex cases, such as pulpitis without periapical alterations (Yong and Cathro et al., 2021; Taha et al., 2020; Al-Hiyasat et al., 2006; Koncke et al., 1970). The literature shows that the pulpectomy procedure in teeth with vital pulp that had the pulp exposed provides a more reliable result. However, the prognosis for teeth maintenance following radical procedures is not as favorable as that for teeth with preserved dental pulp, since this tissue is responsible for the defense mechanisms against harmful stimuli (Kojima et al. 2004; Ng et al. 2008; Randow et al. 1986; Ou et al. 1986). Furthermore, a systematic review by Nie et al. (2021), which included 40 studies, shows that biomaterials used in pulp capping, such as MTA, not only modulate inflammation but also induce pulp regeneration.

Among the biomaterials, MTA is considered a gold standard pulp capping material for its biocompatibility, bioactivity and ability of inducing the formation of reparative dentin (Camilleri & Pitt Ford et al. 2006; American Association of Endodontics et al. 2016; Taha et al. 2020; Le Fournis et al. 2019; Komabayashi et al. 2016), and also for its dimensional stability and mechanical stress (Parirokh et al. 2018; Cushley et al. 2020). Studies show that MTA can induce the mineralization process below the exposed pulp, promoting the formation of dentin tissue and maintaining its vitality through its calcium-dissociation capacity (Darvell et al., 2011; Parirokh et al.,

2010). On the other hand, MTA has some limitations, including tooth staining and difficulty in handling and application (Haapasalo et al., 2015; Marciano et al., 2015).

Several studies are currently evaluating bioglasses due to their potential to induce tissue formation, playing osteoinductive roles, and forming soft tissues (Hench et al. 1991, 2006; Jones et al. 2013; Souza et al. 2017a). Recently, a bioglass composition known as F18 was formulated and shown to guide bone (Souza et al. 2016ab, 2017bc; Gabbai-Armelin et al. 2017), collagenous (Souza et al. 2017b), and vascular tissue regeneration (Keshaw et al. 2005, Gorustovich et al. 2010, Stähli et al. 2015, Oishi et al. 2019) and, more recently, the literature elucidated the ability of F18 to act on pulp repairment (Chaves et al. 2024). Additionally, F18 has been explored in membranes to guide tissue regeneration (Hidalgo et al., 2018; Marin et al., 2021; Gabbai-Armelin et al., 2017) and to alleviate dentin hypersensitivity (Reis et al., 2021). Alongside all these characteristics, a formulation of F18 doped with cobalt has shown a great ability of vascularization, which is associated with the complex tissue formation, making it of great interest to explore in pulp tissue (Zhou et al. 2013, Souza et al. 2017a).

Given the clinical potential of this material, this study aimed to explore the tissue response to F18 and F18+Co, compared with MTA in an *in vivo* model for pulp capping, evaluating the inflammatory process, collagen maturity, and biomineralization capacity in Wistar rats. The null hypothesis is that F18 and F18+Co won't be able to decrease inflammation, induce repair, and initiate the biomineralization process.

## **Objectives**

This study aimed to analyze the tissue response to MTA, F18, and F18+Co using a modified *in vivo* model for pulp capping in Wistar rats, assessing inflammation, collagen maturity, and biomineralization capacity.

## **Material and Methods**

This study was conducted according to Preferred Reporting Items for Animal Studies in Endodontology (PRIASE) 2021 guidelines (Nagendrababu et al., 2021; Figure 1). All experimental procedures were approved and conducted by the Animal Ethics Committee, nº 0293-2021, and in compliance with the UK Animals (Scientific Procedures) Act 1986.

## **Animals and drugs**

Forty male Wistar rats (*Rattus albinus*, Wistar), with an average of 250g, were collected from the local vivarium. The animals were kept in controlled environments, according to the requirements previously established by the local ethics committee, with a temperature between 22-24°C with a controlled light cycle (12 hours light and 12 hours dark) and in collective cages, containing four rats per cage, fed throughout the experimental period with a solid diet and water “ad libitum”, except in the first 24 hours after surgical interventions. A sedative based on 2% xylazine hydrochloride (Xilazin, Syntec – Barueri, Brazil – 10 mg/kg) and an anesthetic based on 10% ketamine hydrochloride (Ketamin, Syntec – Barueri, Brazil – 25 mg/kg) were administered intramuscularly to anesthetize the animals. The experimental period with each animal did not exceed 15 minutes to avoid the need for supplemental anesthesia. The animals were monitored after the surgical procedure and received a dose of 150 mg/kg of sodium dipyrone for postoperative analgesia. The euthanasia was performed after 7 and 30 days of the procedure by an overdose of sodium thiopental anesthetic (150 mg/kg – Thiopentax, Cristalia Produtos Quimicos Farmaceuticos Ltda).

## **Pulp capping procedure**

The 1st and 2nd maxillary molars of 40 animals underwent direct pulp capping, totaling 80 teeth evaluated in the experiment. Pulp chamber exposures were performed by an experienced and qualified researcher using a surgical microscope (Leica M525 OH4, Leica Microsystems, Inc., 2009) at 40x magnification. Pulp exposures were performed on the occlusal surface of the first and second maxillary molars using a carbon steel bur (Ln Long Neck Bur - Maillefer, Dentsply) with a 0.5 mm diameter sphere at the end. Thus, pulp exposure procedures were standardized for all animals to a diameter of 0.5 mm.

The exposed teeth were isolated with a gingival barrier (TOPDAM, FGM, Produtos Odontológicos, Joinville, Santa Catarina, Brazil, 2023), which controlled the penetration of salivary fluids. After isolation, irrigation with saline solution was performed to control bleeding. The surgical site was dried with sterile cotton balls and absorbent paper cones adapted to the cavity. The pulp capping was then performed using different materials randomly. After capping, the cavities were sealed with

ionomer glass (IonoSeal, VOCO GmbH, Cuxhaven, Alemanha), then etched with 37% phosphoric acid (Condac, FGM, Produtos odontológicos, Joinville, Santa Catarina, Brazil, 2023), and flowable resin (Oppalis flow, FGM, Produtos odontológicos, Joinville, Santa Catarina, Brazil, 2023) was applied. Selective occlusal adjustment was performed to prevent trauma. The groups were divided according to the type of material used for capping, as follows: Gutta Percha (GP) (n=10), MTA Angelus (MTA) (n=10) and F18 Powder (F18) (n=10) and F18 doped with cobalt (F18+Co) (n=10). As the procedure was conducted on two teeth of the experimental animals, each group had 20 sealed teeth. Two experimental periods of 7 and 30 days were carried out.

## PRIASE 2021 Flowchart\*

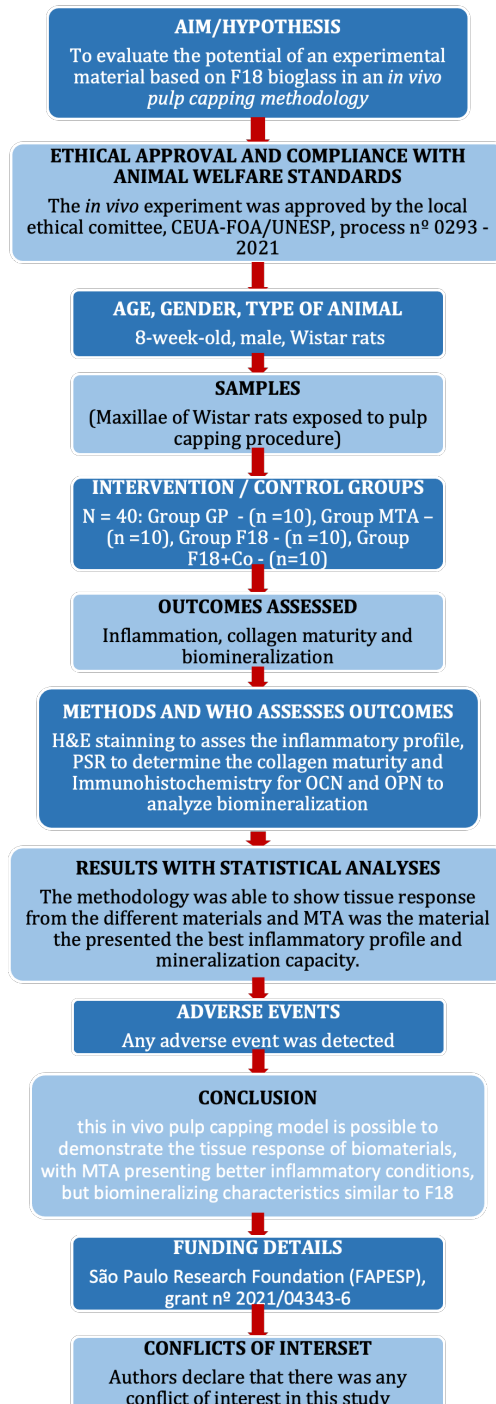


Figure1. PRIASE 2021 Flowchart\*

Samples process

After each time point, five animals from each group were euthanized with an overdose of thiopental (Cintra et al. 2016), and their maxillae were collected, having their right hemimaxilla removed and fixed in 10% formalin solution, buffered at neutral pH for the first 24 hours, and then washed in running water for 12 hours. After fixation, the hemimaxillae were demineralized in 10% EDTA solution, washed in running water, dehydrated in alcohol, diaphanized in xylene, and embedded in paraffin. After inclusion, the samples were sectioned with semi-serial sections, 5µm thick, performed on a microtome (Leica - RM 2045). Slides were obtained for staining with Hematoxylin and Eosin, Picrosirius red, and Immunohistochemistry for OCN and OPN.

For the immunohistochemistry technique, histological sections were deparaffinized in xylene and then hydrated in a series of ethanol concentrations. Antigen retrieval was performed by immersing the histological slides in Diva Decloaker® buffer (Biocare Medical, CA, USA) in a pressurized Decloaking Chamber® (Biocare Medical, CA, USA) at 95°C for 10 minutes. At the end of each step of the immunohistochemical reaction, washes were performed in 0.1 M phosphate-buffered saline (PBS), pH 7.4. Histological sections were immersed in 3% hydrogen peroxide for 1 hour and in 1% bovine serum albumin for 12 hours to block endogenous peroxidase and nonspecific sites, respectively. The histological slides were incubated with their primary antibody diluted in PBS plus 0.1% Triton X-100 (PBS-TX) for 24 hours in a humid chamber. In subsequent steps, the Universal Dako Labeled (HRP) Streptavidin-Biotin Kit® (Dako Laboratories, CA, USA) was used. The histological sections were incubated with the biotinylated secondary antibody for 2 hours and subsequently treated with streptavidin conjugated with horseradish peroxidase (HRP) for 1 hour. 3,3'-diaminobenzidine tetrahydrochloride (DAB chromogen Kit®, Dako Laboratories, CA, USA) was used as the chromogen for development, and counterstaining was performed with Harris hematoxylin. As a negative control, the specimens were subjected to the procedures described above, omitting the use of primary antibodies.

## **Analyses**

The slides containing a representative section of each specimen were subjected to descriptive and qualitative analysis by two blinded and calibrated researchers (L.T.A.C.

and E.E.). The descriptive analyses consisted of describing the histopathological phenomenon, which provided a global characterization in relation to the experimental variables (Cintra et al., 2013).

The qualitative analysis of the inflammatory infiltrate was based on the mean number of inflammatory cells present in different pre-established regions of each specimen, as observed under a microscope at 400x magnification (Leica QWin V3, Leica Microsystems). They were classified into:

- Score 1: absent or negligible number of inflammatory cells;
- Score 2: mild inflammatory infiltrate (less than 10 cells per field);
- Score 3: moderate inflammatory infiltrate (between 10 and 25 cells per field);
- score 4 for intense inflammatory infiltrate (more than 25 cells per field) (Cintra et al. 2013).

Collagen fiber maturation levels were analyzed quantitatively in PSR-stained sections under polarized light microscopy and QWin program (400X magnification, Leica QWin V3, Leica Microsystems), which allowed the determination of Greenish-yellow fibers that were considered immature and thin, while yellowish-red fibers were considered mature and thick.

Immunohistochemical analysis was performed under brightfield illumination under an optical microscope (Optiphot-2, Nikon, Japan). Immunostaining was defined as brownish staining present in the cytoplasm of cells and/or in the extracellular matrix. The semiquantitative analysis, which elucidates the information on the number of immunoreactive cells and the intensity of the immunostaining of the extracellular matrix, was performed using six histological sections from each animal. The scores of the immunostaining pattern were:

- Score 1: <20% of immunoreactive cells and weak staining of the extracellular matrix;
- Score 2: >20% and <40% of immunoreactive cells and weak staining of the extracellular matrix;
- Score 3: >40% and <60% of immunoreactive cells and moderate staining of the extracellular matrix;
- Score 4: >60% and <80% of immunoreactive cells and strong staining of the extracellular matrix;

- Score 5 >80% of immunoreactive cells and strong staining of the extracellular matrix, adapted from Cosme-Silva et al. (2019).

### **Statistical Analyses**

The statistical analyses were performed using the software GraphPad Prism (GraphPad Prism 10.1.2.0) (GraphPad Software, Boston, Massachusetts, USA) and a significance level of 5% ( $p < 0.05$ ). The histological and immunohistochemical analyses were semiquantitative, with scores assigned. The quantitative analysis for PSR was subjected to a normality test; if it had a normal distribution, an ANOVA test was applied. In this study, all data did not have a normal distribution, so the Kruskal-Wallis' test was applied. When a significant difference was observed, the data collected were compared among the groups using the multiple comparison test of Dunn.

### **Results**

#### **Inflammatory infiltrate**

According to the statistics for the H&E staining, the inflammatory infiltrate presented itself as discrete in GP in 7 days and with a mild to moderate intensity in MTA, with no statistical difference ( $p=0.5201$ ). However, F18 and F18+Co exhibited a predominant severe inflammatory infiltrate, characterized by a high number of inflammatory cells, which was statistically different from GP ( $p = 0.0005$ ) and MTA ( $p = 0.0475$ ). At 30 days, a reduction in the inflammatory infiltrate is observed in all groups. In GP and MTA, the inflammation was considered absent to mild, with no statistical difference between them ( $p > 0.9999$ ). For F18 and F18+Co, the inflammatory infiltrate showed a moderate intensity, being statistically different from GP ( $p=0.0120$ ) and MTA ( $p=0.0023$ ) and similar to each other ( $p=0.0004$ ) (Table 1, Figure 2, Graphs 1 and 2).

**Tabela 1.** Scores, median, and p value for the inflammatory infiltrate analysis for all the groups in 7 and 30 days.

| Parameters of analyses  |              | 7 days         |                   |                |                | 30 days          |                |                |                |
|-------------------------|--------------|----------------|-------------------|----------------|----------------|------------------|----------------|----------------|----------------|
|                         |              | GP             | MTA               | F18            | F18+Co         | GP               | MTA            | F18            | F18+Co         |
| Inflammatory infiltrate | 1 - Absent   | 0/10           | 0/10              | 0/10           | 0/10           | 4/10             | 5/10           | 0/10           | 0/10           |
|                         | 2 - Mild     | 7/10           | 4/10              | 0/10           | 0/10           | 4/10             | 4/10           | 2/10           | 3/10           |
|                         | 3 - Moderate | 3/10           | 4/10              | 3/10           | 5/10           | 2/10             | 1/10           | 5/10           | 4/10           |
|                         | 4 - Severe   | 0/10           | 2/10              | 7/10           | 5/10           | 0/10             | 0/10           | 3/10           | 3/10           |
|                         | Median*      | 2 <sup>A</sup> | 2.5 <sup>AB</sup> | 4 <sup>B</sup> | 4 <sup>B</sup> | 1.5 <sup>A</sup> | 1 <sup>A</sup> | 3 <sup>B</sup> | 3 <sup>B</sup> |

\*Different letters in the lines indicate statistically significant differences in each experimental period.

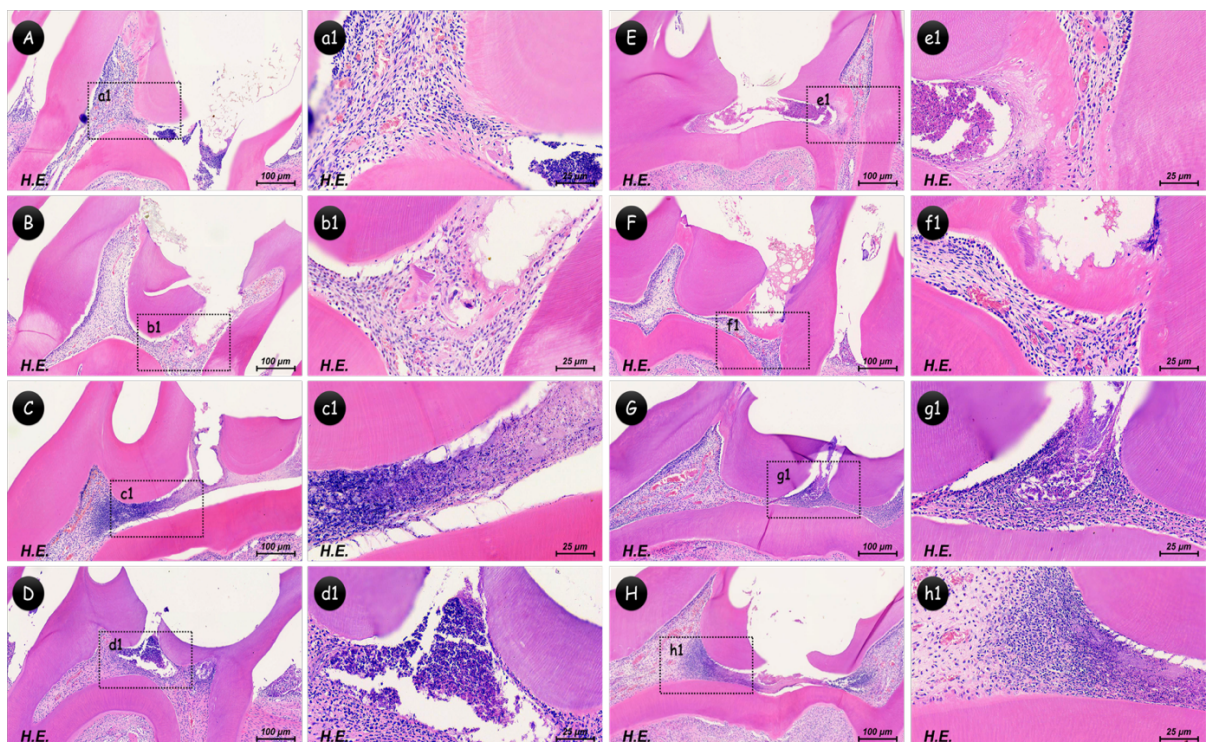
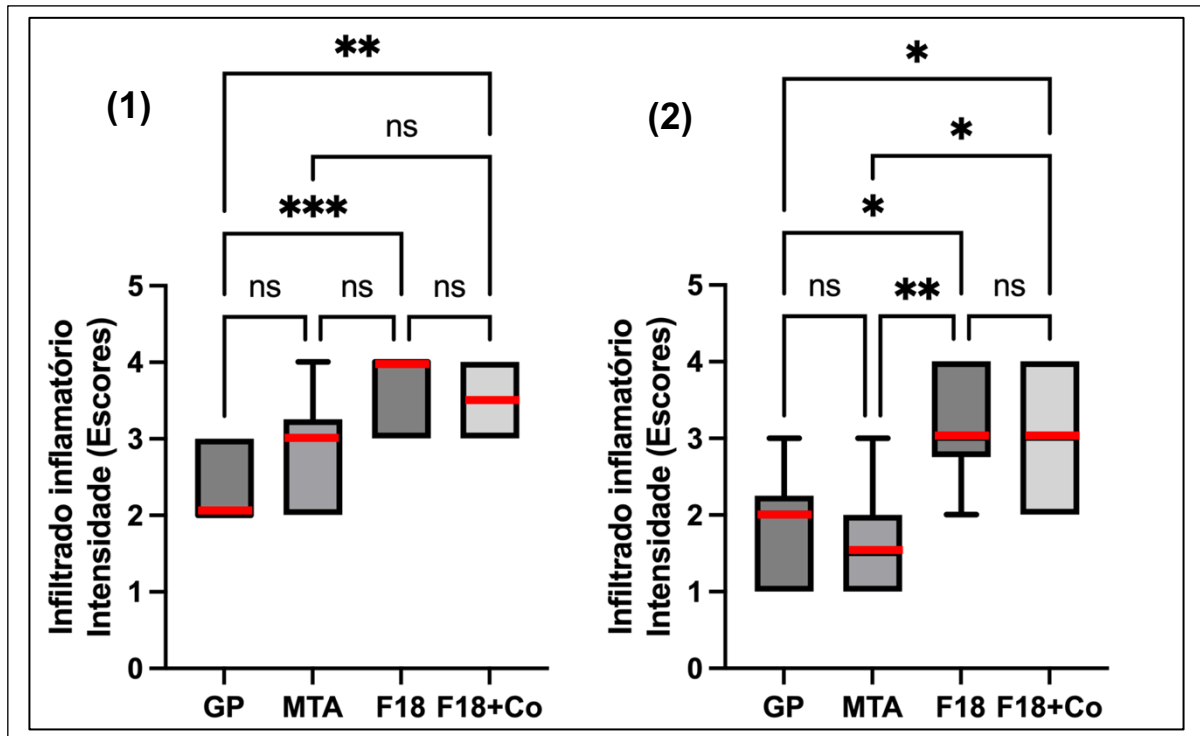


**Figure 2.** Representative images of the inflammatory infiltrate from the different experimental groups and periods of analysis. **(A,a1,E,e1)** GP group; **(B,b1,F,f1)** MTA group; **(C,c1,G,g1)** F18 Group; **(D,d1,H,h1)** F18+Co group. Period of 7 days **(A-D,a1-d1)** and 30 days **(E-H,e1-h1)**. Hematoxylin & Eosin staining, 100x **(A-H)** and 400x magnification **(a1-h1)**.

**Graphs 1 and 2.** Inflammatory intensity is represented by scores for each of the materials analyzed at 7 and 30 days.



\*Red lines in the graphs represent median values for each group in both periods of analysis.

### Collagen maturity

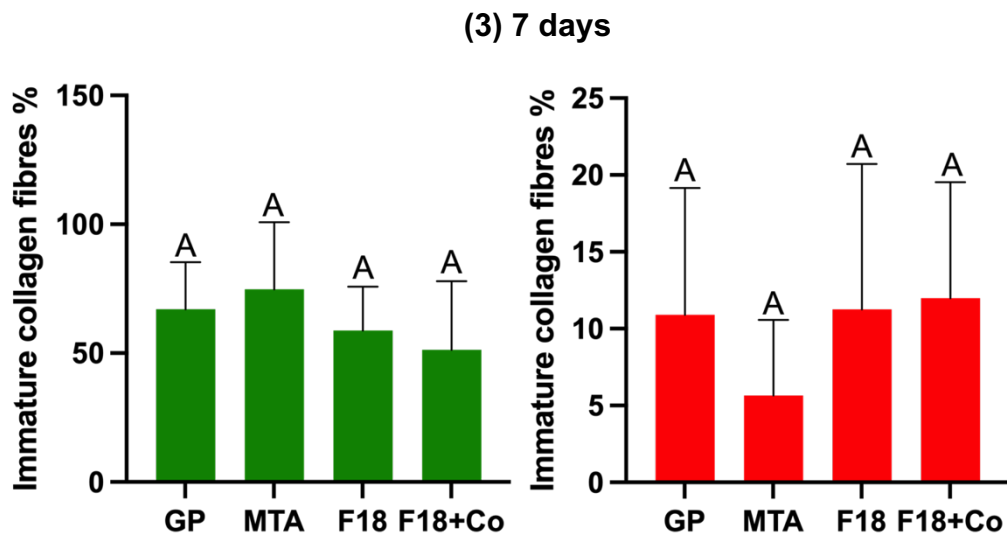
To the analysis of PSR staining, the percentage of immature fibres was higher in 7 and 30 days for all groups, with no statistical difference ( $p=0.3065$ ), ( $p=0.6111$ ).

**Table 2.** Percentage and standard deviation (SD) of collagen fibres in 7 and 30 days according to each experimental group.

| Time    | Material  | Immature collagen<br>Greenish-yellow |          | Mature collagen<br>Yellowish-red |          |
|---------|-----------|--------------------------------------|----------|----------------------------------|----------|
| 7 days  | GP        | 67.08 ± 18.26 <sup>A</sup>           | p=0.3065 | 32.92 ± 18.26 <sup>A</sup>       | p=0.2181 |
|         | MTA       | 74.77 ± 25.98 <sup>A</sup>           |          | 25.23 ± 25.98 <sup>A</sup>       |          |
|         | F18+Co    | 51.36 ± 25.21 <sup>A</sup>           |          | 50.45 ± 25.9 <sup>A</sup>        |          |
|         | F18 Putty | 58.86 ± 16.90 <sup>A</sup>           |          | 41.14 ± 16.90 <sup>A</sup>       |          |
| 30 days | GP        | 69.54 ± 24.78 <sup>A</sup>           | p=0.6111 | 30.46 ± 24.78 <sup>A</sup>       | p=0.4788 |
|         | MTA       | 73.91 ± 17.79 <sup>A</sup>           |          | 26.09 ± 17.79 <sup>A</sup>       |          |
|         | F18+Co    | 75.92 ± 12.18 <sup>A</sup>           |          | 24.08 ± 12.18 <sup>A</sup>       |          |
|         | F18 Putty | 67.65 ± 12.08 <sup>A</sup>           |          | 32.35 ± 12.08 <sup>A</sup>       |          |

\*Different letters in rows indicate significant statistical differences for each experimental period (p<0.05)

**Graphs 3 and 4.** Percentage of mature and immature fibres deposited in the pulp tissue according to each experimental group at 7 and 30 days.



## (4) 30 days

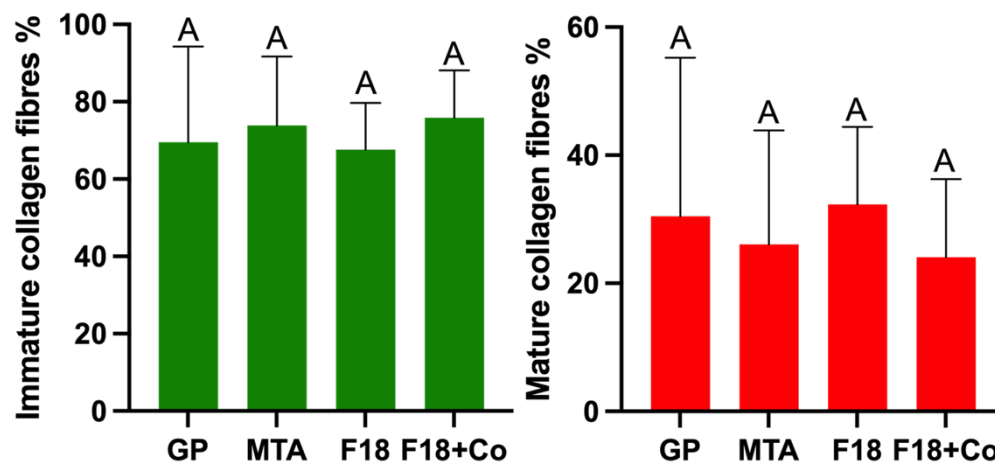
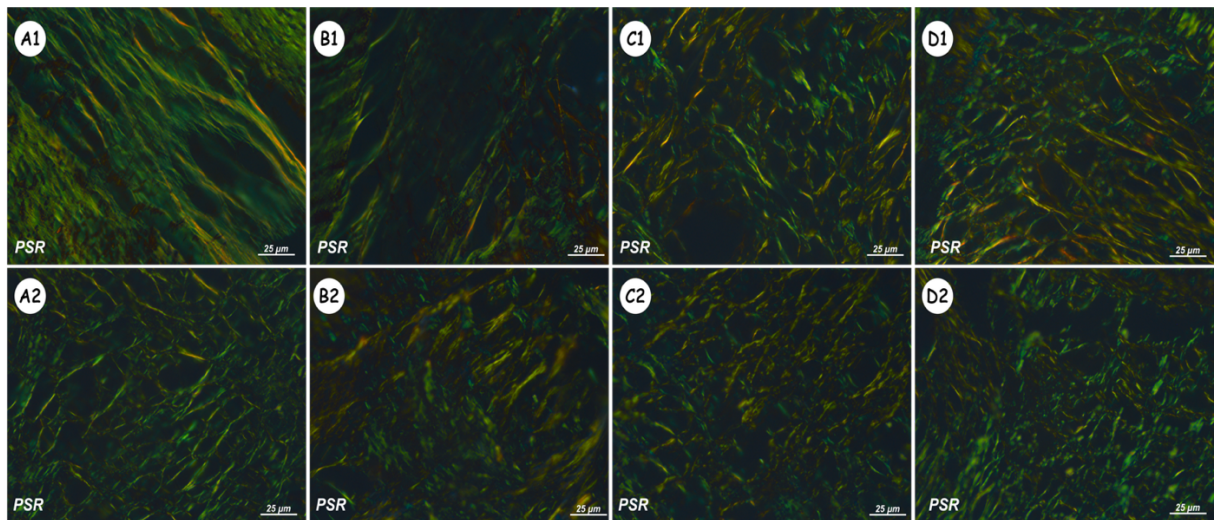


**Figure 3.** Representative images of the collagen fibres from the different experimental groups and periods of analysis. **(A1,A2)** GP group; **(B1,B2)** MTA group; **(C1,C2)** F18 **(D1,D2)** F18+Co group. Period of 7 days **(A1-D1)** and period of 30 days **(A2-D2)**. Picrosirius red staining, 1000x magnification.

## Biom mineralization

### Immunostaining of OCN

In the immunohistochemical analysis for Osteocalcin (OCN), it was possible to detect that, after 7 days, the GP group did not present immunostaining, which differed from the other groups ( $p < 0.0001$ ). The MTA group presented a median of 3, indicating >40% and <60% of immunoreactive cells and moderate staining of the extracellular matrix, while the F18 and F18+Co groups presented a median of 2, representing >20%

and <40% of immunoreactive cells and a low staining pattern of the extracellular matrix; however, MTA, F18 and F18+Co did not present statistical difference ( $p < 0.0001$ ). In the 30 days, an increase in immunostaining was noted, with the GP group having a score of 1, <20% of immunoreactive cells, and low immunostaining of the extracellular matrix, being statistically different from the other groups ( $p < 0.0001$ ). MTA, F18, and F18+Co presented a score of 4, with >60% and <80% immunoreactive cells and strong staining of the extracellular matrix, similar in statistical analysis ( $p < 0.0001$ ) (Table 3 and Figure 3).

**Table 3.** Immunostaining intensity obtained in scores and p value for OCN in 7 and 30 days.

| Parameters of analyses | 7 days  |                |                |                |              | 30 days        |                  |                |                |              |
|------------------------|---------|----------------|----------------|----------------|--------------|----------------|------------------|----------------|----------------|--------------|
|                        | GP      | MTA            | F18            | F18+Co         |              | GP             | MTA              | F18            | F18+Co         |              |
| OCN                    | 0       | 6/10           | 0/10           | 0/10           | $p < 0.0001$ | 3/10           | 0/10             | 0/10           | 0/10           | $p < 0.0001$ |
|                        | 1       | 4/10           | 0/10           | 4/10           |              | 7/10           | 0/10             | 0/10           | 0/10           |              |
|                        | 2       | 0/10           | 4/10           | 4/10           |              | 0/10           | 0/10             | 0/10           | 0/10           |              |
|                        | 3       | 0/10           | 6/10           | 2/10           |              | 0/10           | 0/10             | 5/10           | 5/10           |              |
|                        | 4       | 0/10           | 0/10           | 0/10           |              | 0/10           | 5/10             | 5/10           | 5/10           |              |
|                        | 5       | 0/10           | 0/10           | 0/10           |              | 0/10           | 5/10             | 0/10           | 0/10           |              |
|                        | Median* | 0 <sup>A</sup> | 3 <sup>B</sup> | 2 <sup>B</sup> |              | 1 <sup>A</sup> | 4.5 <sup>B</sup> | 4 <sup>B</sup> | 4 <sup>B</sup> |              |

\*Different letters in rows indicate significant statistical differences for each experimental period ( $p < 0.05$ ).

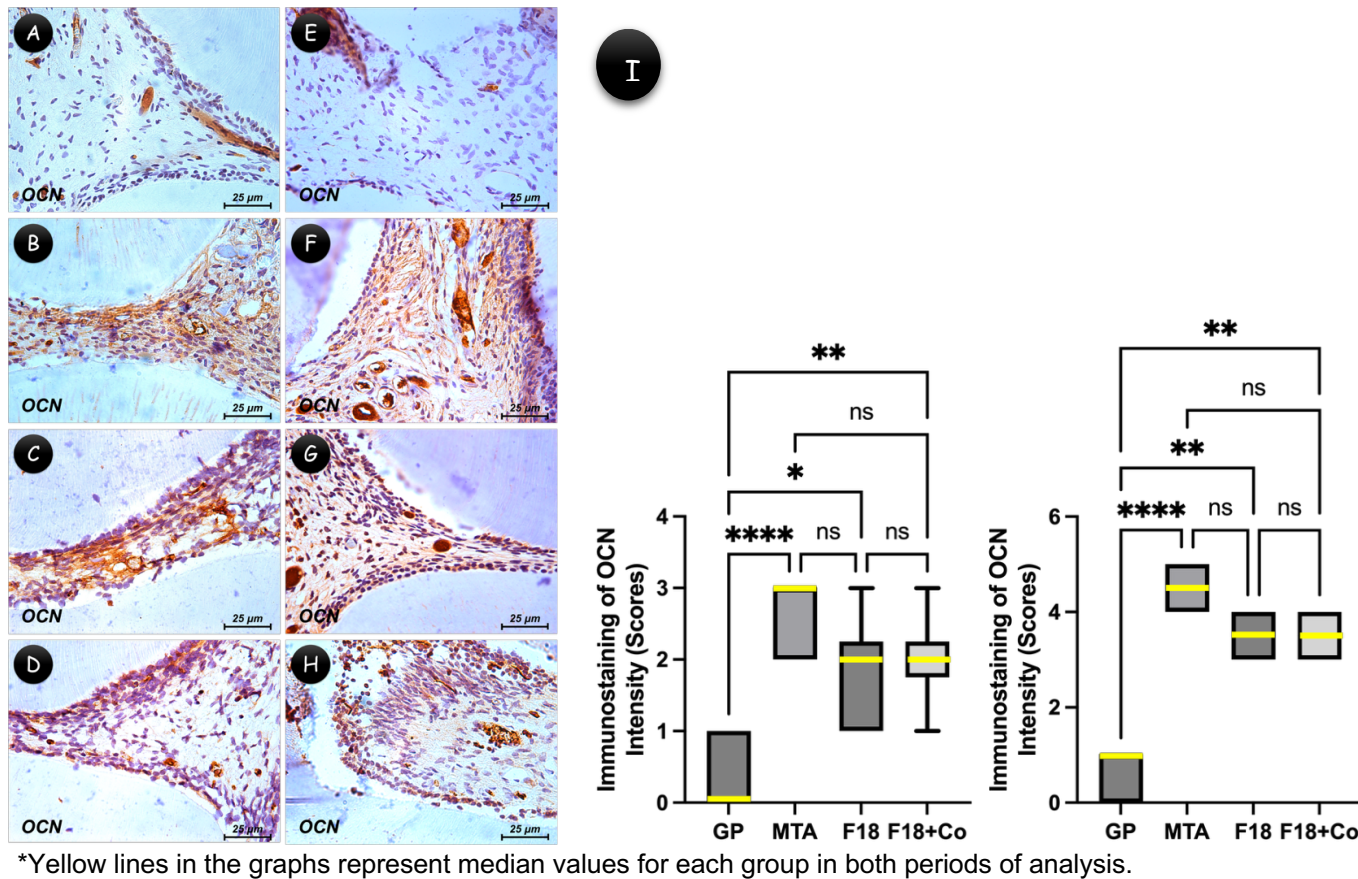


**Figure 4.** Representative images of the immunostaining of OCN for the different experimental groups and periods of analysis. **(A,E)** GP group; **(B,F)** MTA group; **(C,G)** F18 group; **(D,H)** F18+Co group. Period of 7 days **(A-D)**; 30 days **(E-F)**. Immunostaining for OCN, 400x magnification **(A-H)**. **(I)** Graphical projection for the immunostaining of OCN evaluated in scores in the different experimental groups.

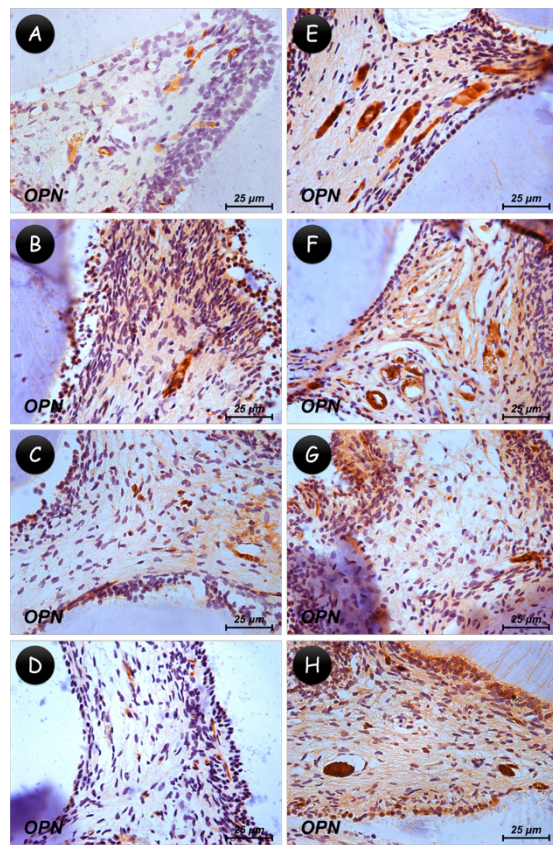
### Immunostaining of OPN

In the analysis of Osteopontin (OPN), a discreet immunostaining was identified for the GP group, differentiating it from MTA, F18, and F18+Co ( $p < 0.0001$ ). The MTA presented a median of 3 but did not differ statistically from the F18 and F18+Co groups at 7 days ( $p < 0.0001$ ). During the 30 days, an increase in the immunostaining pattern was observed, with the GP group presenting a median of 2.5, similar to the F18 and F18+Co groups, which presented a median of 3 ( $p < 0.0001$ ). The MTA group differed from the other groups, demonstrating the highest immunostaining pattern with a median of 4 ( $p < 0.0001$ ) (Table 4 and Figure 4).

**Table 3.** Immunostaining intensity obtained in scores and p value for OPN in 7 and 30 days.

| Parameters of analyses | 7 days  |                |                |                |          | 30 days          |                |                |                |          |
|------------------------|---------|----------------|----------------|----------------|----------|------------------|----------------|----------------|----------------|----------|
|                        | GP      | MTA            | F18            | F18+Co         |          | GP               | MTA            | F18            | F18+Co         |          |
| OPN                    | 0       | 7/10           | 0/10           | 0/10           | p<0.0001 | 0/10             | 0/10           | 0/10           | 0/10           | p<0.0001 |
|                        | 1       | 3/10           | 0/10           | 0/10           |          | 0/10             | 0/10           | 0/10           | 0/10           |          |
|                        | 2       | 0/10           | 2/10           | 6/10           |          | 5/10             | 0/10           | 4/10           | 4/10           |          |
|                        | 3       | 0/10           | 8/10           | 4/10           |          | 5/10             | 0/10           | 6/10           | 6/10           |          |
|                        | 4       | 0/10           | 0/10           | 0/10           |          | 0/10             | 7/10           | 0/10           | 0/10           |          |
|                        | 5       | 0/10           | 0/10           | 0/10           |          | 0/10             | 3/10           | 0/10           | 0/10           |          |
|                        | Median* | 1 <sup>A</sup> | 3 <sup>B</sup> | 2 <sup>B</sup> |          | 2.5 <sup>A</sup> | 4 <sup>B</sup> | 3 <sup>A</sup> | 3 <sup>A</sup> |          |

\*Different letters in rows indicate significant statistical differences for each experimental period (p<0.05)



\*Blue lines in the graphs represent median values for each group in both periods of analysis.

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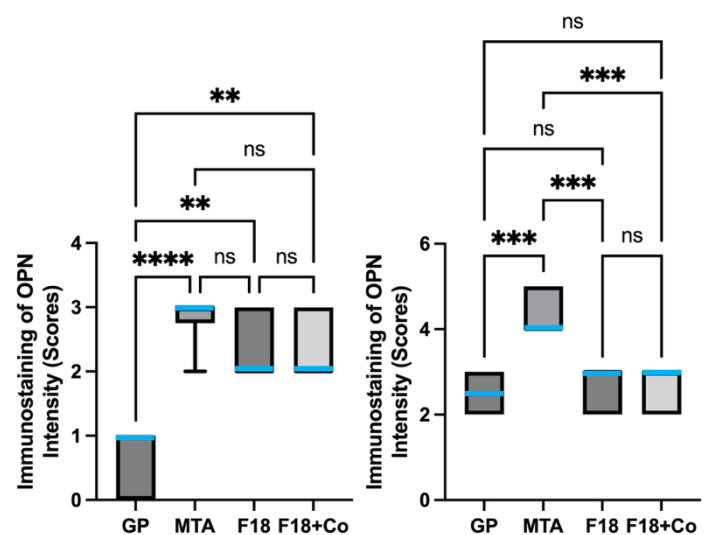


**Figure 5.** Representative images of the immunostaining of OPN for the different experimental groups and periods of analysis. (A,E) GP group; (B,F) MTA group; (C,G) F18 group; (D,H) F18+Co group. Period of 7 days (A-D); 30 days (E-F). Immunostaining for OPN, 400x magnification (A-H). (I) Graphical projection for the immunostaining of

OPN evaluated in scores in the different experimental groups.

## Discussion

Vital Pulp Therapy (VPT) consists of dental interventions that aim to preserve the vitality of the compromised dental pulp, whether by caries, trauma or restorative procedures, maintaining its biological function, regenerative capacity, preserving the tooth against harmful stimuli by its preceptive ability (Kojima et al. 2004; ESE 2019; Duncan 2022; Chaves et al., 2024). The pulp capping procedures are often combined with capping materials, such as MTA, which has been proven to induce both hard and soft tissue formation (Torabinejad et al., 2010; Parirokh et al., 2010; Camilleri et al., 2006). However, with the evolution of tissue engineering, researchers' attention has been drawn to the search for potential pulp capping materials that could maintain pulp preservation (Lin et al. 2021; Shi et al. 2025). F18 is a bioglass with bioactivity, capable of inducing the formation of both hard and soft tissues (Chaves et al., 2024). Nevertheless, this study showed that F18 and F18+Co decreased inflammation over time and stimulated bone marker secretion, but they did not behave like MTA. Despite this result, the collected data encourage further investigations. Additionally, it validated once more the methodological procedure of pulp capping in Wistar rats. Therefore, the null hypothesis was partially rejected, as this study demonstrated a decrease in inflammation and the potential biomineralization ability of the bioglasses; however, it did not evidence their ability to induce repair of the pulp tissue.

The success of VPT depends on multiple factors, including the technique used, the material employed, and the pulp diagnosis made prior to the procedure (Cushley et al., 2021). Regarding the materials used, a systematic review conducted by Cushley et al. (2021) demonstrated the effectiveness of direct pulp capping with various biomaterials, including  $\text{Ca(OH)}_2$ , MTA, and Biodentin. It revealed clinical success rates of  $\text{Ca(OH)}_2$  of 74% at 6 months, 65% at 1 year, 59% between 2 and 3 years, and 56% between 4 and 5 years. For MTA, the rates were higher: 91%, 86%, 84%, and 81%, respectively. Biodentine demonstrated the best results in the first years, with a success rate of 96% at 6 months, 86% at 1 year, and 86% between 2 and 3 years. These findings reinforce that pulp capping is a viable option to preserve the vital pulp, and that the appropriate choice of biomaterial is crucial for the success of the treatment, with promising long-term prospects. Thus, the relevance of exploring materials *in vivo*

is an approach that can contribute to the standardization of surgical techniques, find new pulp capping materials, and comprehend pulp tissue response.

Bioglasses are bioinductive materials with reparative potential, capable of inducing the formation of both soft and hard tissues; therefore, promising for the medical-dental field (Hench et al., 1991, 2006; Jones et al., 2013; Souza et al., 2017a). One bioglass that has stood out for its properties is F18. This bioglass presents tissue biocompatibility and the ability to induce the formation of bone, collagenous, and vascular tissue (Stähli et al., 2003; Keshaw et al., 2005; Gorustovich et al., 2010; Gabbai-Armelin et al., 2017; Souza et al., 2017b; Oishi et al., 2019). F18 has already been explored in dental sciences as a paste for intracanal medicament, as an additional irrigant solution in endodontic treatment (de Araújo Lopes et al. 2020; de Paula et al. 2024) and recently, a study compared the efficacy of this bioglass to  $\text{Ca(OH)}_2$  in pulp capping in mice, showing that it was not superior to  $\text{Ca(OH)}_2$ , but it had biological potential, not only inducing hard, but soft tissue as well, which is very promising for pulp repair (Chaves et al. 2024). On the other hand, this present research compared GP, MTA, F18, and F18+Co. The analysis of pulp inflammation was assessed using H&E staining (de Oliveira et al., 2022; Gomes-Filho et al., 2009, 2011; Cintra et al., 2017). Inflammation was classified from absent to severe, using pre-established scoring systems (Cintra et al., 2013). Despite the bioglasses' proven biocompatibility, when the tissue inflammatory reaction was analyzed in pulp tissue compared to MTA, although F18 and F18+Co could decrease the inflammatory profile, they did not achieve a level lower than that of MTA. Studies point out that MTA when in contact with connective tissues, releases calcium and hydroxyl ions that initially promotes necrosis, which is associated to its alkalinity; however, calcium ions rapidly bind with carbon from the cell's tissue and form calcite crystals, decreasing the inflammation and inducing cell differentiation (Komabayashi et al. 2008); moreover, MTA has mechanical properties that might be associated with pulp tissue stability, facilitating the inflammation to decrease (Parirokh et al. 2018, Cushley et al. 2020). F18 and F18+Co might have a longer period to enable the decrease of the inflammation, since their composition is based on an association of different compounds (Souza et al. 2017a), making tissue response different from the MTA.

In addition to inflammation, it is known that collagen is the primary matrix of pulp tissue (Thant et al., 2023) and that fibroblasts continually induce collagen deposition

during the repair process (Diegelman & Evans, 2004). Therefore, this study evaluated collagen maturity, differentiating between mature and immature collagen. Mature collagen is associated with the repair process, whereas collagen is deposited after the intervention or induction of biomaterials. Alternatively, immature fibers can indicate a delay in the repair process. (Cintra et al. 2017; de Oliveira et al. 2022). Although, it is known that collagen immaturity can also indicate constant and intensive repair in the pulp (Huang et al. 2023). However, the outcomes of this study show that all materials and both periods of analysis revealed that immature fibers were more expressed, with no difference among the groups, which indicates a low capacity to induce collagen repair. Considering the period of analysis and the amount of tissue lost due to pulp exposure, a full maturation of collagen could have been compromised, and observing the amount of collagen deposited after the pulp contact with MTA and comparing it with the bioglasses, it is possible to see more collagen expression when MTA was applied, such finding leads us to explore in a different way of analysis the collagen repair, suggesting the identification of the collagen type, and the difference regarding their expression, independently to their maturation.

A complementary analysis of repair capacity was the evaluation of the mineralization potential of the materials, as pulp needs to induce hard tissue formation (Duncan et al. 2022). Two specific immunomarkers, Osteocalcin (OCN) and Osteopontin (OPN), were used to examine the mineralization process (Cosme-Silva et al., 2019). OCN, a non-collagenous protein present in bone and dentin, plays a fundamental role in regulating biomineralization and calcium ion homeostasis of tissues in contact with sealing materials (Cosme-Silva et al., 2019; Zairi et al., 2012; Tani-Ishii et al., 2007). For OPN, it is considered a key marker in bone remodeling, as it is involved in cell adhesion, mineralization, and the activity of osteoblasts and osteoclasts (Silva et al., 2014). Thus, due to their significance, this study explored their immunoreaction to understand how the biomaterial could interfere with hard tissue formation. GP is a bioinert material, being selected to this research as a control group, explaining the reason for the low immunolabeling of it and understanding its incapability of inducing tissue formation (Vishwanath et al. 2019), which could be well noticed in the analysis of OCN and OPN, having an increase of the score over time, probably because of the immunological system that will always work to repair tissue damages (Van Dyken et al. 2013; Minutti et al. 2017). MTA, F18, and F18+Co were similar in terms of OCN at 7 and 30 days of analysis, which corroborates with the literature that

mentions the guidance of hard tissue by bioglasses (Souza et al., 2016a, 2017 b; Gabbai-Armelin et al., 2017) and affirms the MTA biomineralization properties (Camilleri et al., 2006). Nonetheless, for OPN, the results were different, with similarity among MTA, F18, and F18+Co at 7 days, but different results at 30 days, with higher immunolabeling induced by MTA. This result can be associated with MTA's ability to participate in the remodeling process of hard tissue constantly (Camilleri et al. 2006, Komabayashi et al. 2008), as OPN is a biomarker that evidences the potential of maintaining the stimuli to form and remodel mineralized tissue (Rodriguez et al.; Silva et al., 2014; 2013; Luukkonen et al., 2019). Unlike MTA, which constantly promotes ion interaction, the bioglasses in the pulp did not present the same response. However, these findings demonstrate that both materials have mineralization-inducing properties; however, MTA still yields better results regarding mineralization ability (Rodriguez et al., 2013; Luukkonen et al., 2019). The similar performance of F18 bioglass compared to MTA, especially at 30 days, points to its biological efficacy in promoting events related to bone regeneration.

Apart from the results collected, 30 days, can be a short period of analysis, a limitation to the study that could have allowed to explore F18s capping potential, a fact that stimulate further analysis with extended periods and also evaluate their vascularization abilities (Stähli et al., 2003; Keshaw et al., 2005; Gorustovich et al., 2010, Gabbai-Armelin et al. 2017; Souza et al. 2017b; Oishi et al., 2019), which could bring very interesting results for pulp therapies by the fact that a biomaterial with angiogenic potential can avoid inadequate blood supplies that leads to necrosis or scar tissue formations, resulting in failure of tissue regeneration (Dubey et al. 2023).

Overall, MTA presented the best results, regarding inflammatory response and immunolabeling of OPN. In addition to this, the pulp capping methodology could provide valuable insights and important information for pulp therapy, encouraging further research to understand pulp tissue and capping materials, and facilitating collaboration for pulp maintenance (Duncan et al., 2022). In this context, it is essential to conduct further studies with adapted methods of analysis. Finally, F18 and F18+Co, although not superior to MTA, were able to decrease inflammation and immunoreactive OCN and OPN in this scenario of the study.

## Conclusion

It is concluded that the *in vivo* pulp capping model enabled the observation that MTA had better inflammatory and mineralization abilities compared to F18 and F18+Co in pulp tissue.

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Capítulo 2

Artigo 2

International Endodontic Journal

## **Tissue response of F18 Putty and F18+Co bioglass in an *in vivo* model of sealing of furcation perforation**

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**Running title:** F18 and furcation defect

**Key words:** Bioglass, MTA, furcation defect, biomaterials, endodontics

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**Tissue response and biomineralization ability of different formulations of F18 bioglass in an *in vivo* model of sealment of furcation defects in Wistar rats**

## Abstract

**Aim:** This study evaluated the biocompatibility and biomineralization of different formulations of the bioglass F18 compared to MTA in an *in vivo* model of furcation defects of Wistar rats.

**Methodology:** The furcations of the 1st and 2nd upper right molars of 40 rats were accessed, irrigated with saline solution and, after bleeding control and being dried, they sealed with one of the following materials: Gutta-percha, Mineral Trioxide Aggregate (MTA), F18 in consistent form (F18 Putty) and doped with cobalt (F18+Co).

**Results:** At 7 days, GP presented mild to moderate inflammation, different from the F18+Co, which presented predominantly severe inflammation ( $p < 0.05$ ). F18 Putty presented moderate inflammation with no difference from the GP and MTA ( $p > 0.05$ ), while MTA presented moderate to severe inflammation with no difference from the other groups ( $p > 0.05$ ). At 30 days, all groups presented mild inflammation ( $p > 0.05$ ). Regarding collagen maturation, the F18 Putty presented a lower percentage of immature fibers and a higher percentage of mature fibers, compared to the other groups ( $p < 0.05$ ). At 30 days, the MTA presented a higher percentage of immature fibers and a lower percentage of mature fibers, compared to the other groups ( $p < 0.05$ ). Regarding biomineralization, at 7 days, the MTA, F18+Co, and F18 Putty presented a higher RI for OCN and OPN compared to GP ( $p > 0.05$ ). At 30 days, the MTA and F18+Co showed higher RI for OCN and OPN compared to GP ( $p > 0.05$ ). The F18 Putty showed RI for OCN similar to the other groups ( $p > 0.05$ ), and RI for OPN similar to GP ( $p > 0.05$ ).

**Conclusion:** It is concluded that bioglasses and MTA have biocompatibility and contribute to tissue repair. In addition, MTA and F18+Co have greater stimulation of biomineralization ability compared to F18 Putty.

**Keywords:** Furcation defect, Biocompatibility, Biomineralization, Collagen maturity

## Introduction

Over the years, various technologies and materials have emerged that have improved the quality and predictability of endodontic treatment. On the other hand, it is a highly complex area of dentistry that can lead to common accidents in dental offices (Leonardo et al., 2012; Plotino et al., 2007; Medina-Fernandez et al., 2019). One of the most common iatrogenic events is furcation perforation, which can lead to furcation lesions, one of the conditions with the worst prognosis for tooth retention (Lagiseti et al., 2018). Furcation perforation is an anomalous communication between the root canal system and the external dental surface and multiarticular teeth, connecting the internal part of the teeth with the periodontal region (American Association of Endodontists, Cardoso et al. 2018, Koç et al. 2021). This condition occurs in up to 12% of endodontically treated teeth. It is primarily caused by the professional's lack of expertise in accessing and instrumenting the root canals, followed by external and internal resorption (Farzeneh et al., 2004; Mente et al., 2014; Cardoso, 2018). Independent of the cause, furcation perforations must be sealed as soon as possible, avoiding contamination (Weldon et al. 2002) and inflammatory reaction that could cause irreversible damage to the periodontium, which can cause ligament loss, compromising tooth support and insertion (Ford et al. 1995; Fuss et al. 2000; Singh et al. 2013).

Nowadays, various biomaterials can stimulate tissue formation and are applied in furcation perforation repair (Sinkar et al., 2015; Marques et al., 2016). Studies indicate that most failures in the treatment of furcation perforations are due to the difficulty in achieving a hermetic seal, especially because of the complexity and location of the furcation region, as well as limitations presented by some types of materials available on the market for performing the sealing procedure (Jantararat et al. 1998). Sealing with biomaterials may promote the repair of tissues damaged by perforation (Singh et al. 2013). These materials must present characteristics such as sealing capacity, high biocompatibility and ability to induce tissue formation, antimicrobial properties, ease of handling and insertion, and a cost commensurate with the market reality, MTA has shown over the years all of these characteristics (Estrela et al. 2023), because of that, it is worldwide chosen by clinicians to be used in different endodontic procedures, such as the sealing of furcation perforation (Taesis et al. 2006; Koç 2021).

However, MTA has some limitations, including tooth discoloration, difficulty in handling and application, and a relatively high cost (Darvell et al., 2011; Haapasalo et al., 2015; Marciano et al., 2015). Therefore, it is necessary to search for new materials that present better properties and cost-effectiveness. Studies involving active bioglasses have been increasing and gaining notoriety in the scientific community due to their ability to promote tissue formation, playing osteoinductive and osteoconductive roles, in addition to their ability to induce soft tissue formation (Hench et al. 1991, 2006; Jones et al. 2013; Souza et al. 2017a; Reis et al. 2021). Recently, a bioglass composition, known as F18, doped with cobalt, was formulated and has been shown to exhibit bioactivity (Souza et al., 2016a, 2016 b, 2017a, 2017 b; Gabbai-Armelin et al., 2017). Moreover, it has been shown to induce hard and collagenous tissue, and it has the potential to promote vascular regeneration by increasing nitric oxide and, consequently, vascular growth factors (Keshaw et al., 2005; Gorustovich et al., 2010; Stähli et al., 2015; Oishi et al., 2019). Furthermore, it has antimicrobial activity (de Araújo et al. 2020). This bioglass has been explored in the dental field and has already shown promising results in endodontics (de Araújo et al., 2020; de Paula et al., 2024), prompting our research group to investigate whether it could be used as a sealing material in critical clinical situations, such as cases of furcation perforations.

In addition to F18+Co, a new formulation of F18 has been proposed, in putty form, which is supposedly an easy-to-manipulate and insert biomaterial for endodontic perforation. Therefore, with all their properties highlighted and associated with the importance of studying tooth maintenance, this research has focused on investigating in an *in vivo* model in Wistar rats the tissue response to different compositions of materials that could be clinically used in furcation perforations. The null hypothesis is that the materials will not be able to elucidate the biocompatibility or bioinductiveness in periodontium tissue.

## **Objectives**

The aim of this *in vivo* study was to replicate a specific endodontic condition, the furcation defect, and to elucidate the tissue response of F18+Co and F18 Putty compared to MTA.

## **Material and methods**

This study was conducted according to Preferred Reporting Items for Animal Studies in Endodontology (PRIASE) 2021 guidelines (Nagendrababu et al., 2021; Figure 1). All experimental procedures were approved and conducted by the Animal Ethics Committee, nº 0293-2021, and in compliance with the UK Animals (Scientific Procedures) Act 1986 (Figure 1).

## **Experimental animals and drugs**

Forty male rats (*Rattus albinus*, Wistar), six weeks old, were provided from the local vivarium (São Paulo State University, Araçatuba, Unesp). The animals were kept in controlled environments, with a temperature between 22°C and 24°C, with a controlled light cycle (12 hours light and 12 hours dark) and in collective cages (4 rats/cage), received solid food “ad libitum”, except in the first 24 hours after the experimental procedures. The anesthesia was administered intramuscularly with the injection of a sedative based on 2% Xylazine Hydrochloride (Xylazine, Syntec - Barueri, Brazil - 10mg/kg) and an anesthetic based on 10% Ketamine Hydrochloride (Cetamin, Syntec - Barueri, Brazil - 25mg/kg). The procedures took a maximum of 15 minutes, preventing the animals from coming out of the sedation. The animals were monitored all the time until their complete recovery after the surgical procedure and received a dose of 150 mg/kg of sodium dipyrone to control postoperative analgesia.

The euthanasia was performed after 7 and 30 days of the procedure by an overdose of sodium thiopental anesthetic (150 mg/kg – Thiopentax, Cristalia Produtos Quimicos Farmaceuticos Ltda).

## **Furcation defects and sealment procedure**

Forty rats were used, and furcation defects were performed in the 1<sup>st</sup> and 2<sup>nd</sup> molars of the right maxilla of each animal, totaling 80 teeth in the experiment. The furcation perforations were created by an experienced researcher skilled in the use of a surgical microscope (Leica M525 OH4, Leica Microsystems, Inc., 2009) at 40x magnification. The furcations were exposed in the center of the pulp chamber floor, at a depth of 2 mm in the first and second right upper molars of the animals with a carbon steel drill (Ln Long Neck Drill - Maillefer, Dentsply) equipped with a sphere of 0.5 mm of diameter at the end, at low speed under saline irrigation. The teeth that underwent the procedure received a gingival barrier (Topdam, FGM, Joinville, Santa Catarina, Brazil) to control

the penetration of saliva fluids. Then, saline solution was used to irrigate and remove dentin fragments, as well as the organic content. The surgical site, after being cleaned, was dried with sterile cotton balls and an absorbent paper cone (#40, Tanari Indústria LTDA, Manacapuru, Amazonas, Brazil) until bleeding was absent. The furcation defect was randomly sealed with experimental materials. The final restoration was made with a layer of ionomer glass (IonoSeal, VOCO GmbH, Cuxhaven, Alemanha). After that, the remaining dentin and enamel were conditioned with 37% phosphoric acid (Condac, FGM, Joinville, Santa Catarina, Brazil) and received flowable resin (Opallis Flow, FGM, Joinville, Santa Catarina, Brazil). A selective adjustment in the occlusal surface was made to avoid occlusion trauma.

The groups were divided according to the experimental materials applied: Guta percha group (n=10), MTA (n=10), F18+Co (n=10), F18 Putty (n=10). The experimental periods were of 7 and 30 days.

### **Samples processing**

In each experimental period, five animals from each group were euthanized, and their hemimaxillae were fixed in a 10% formalin solution buffered at neutral pH for the first 24 hours. They were then washed in running water for 12 hours. After fixation, the hemimaxillae were demineralized in 10% EDTA solution, washed in running water, dehydrated in alcohol, diaphanized in xylene, and embedded in paraffin. After inclusion, the samples were sectioned into semi-serial sections, 5 µm thick, using a microtome (Leica RM 2045). Slides were obtained for staining with Hematoxylin and Eosin, Picrosirius red and Immunohistochemistry for OCN and OPN.

Histological sections were obtained for immunohistochemistry and then deparaffinized in xylene and hydrated in ethanol. Antigen retrieval was performed by immersing the histological slides in Diva Decloaker® buffer (Biocare Medical, CA, USA) in a pressurized Decloaking Chamber® (Biocare Medical, CA, USA) at 95°C for 10 minutes. At the end of each step of the immunohistochemical reaction, washes were performed in 0.1 M phosphate-buffered saline (PBS), pH 7.4. Histological sections were immersed in 3% hydrogen peroxide for 1 hour and in 1% bovine serum albumin for 12 hours to block endogenous peroxidase and nonspecific sites, respectively. The histological slides were incubated with their primary antibody diluted in PBS plus 0.1% Triton X-100 (PBS-TX) for 24 hours in a humid chamber. In subsequent steps, the Universal Dako Labeled (HRP) Streptavidin-Biotin Kit® (Dako Laboratories, CA, USA)

was used. The histological sections were incubated with the biotinylated secondary antibody for 2 hours and subsequently treated with streptavidin conjugated with horseradish peroxidase (HRP) for 1 hour. 3,3'-diaminobenzidine tetrahydrochloride (DAB chromogen Kit®, Dako Laboratories, CA, USA) was used as the chromogen for development, and counterstaining was performed with Harris hematoxylin. As a negative control, the specimens were subjected to the procedures described above, omitting the use of primary antibodies.

## **Analyses**

The analyses were performed by two blinded and calibrated researchers (L.T.A.C. and E.E.), who evaluated histological sections at 400x magnification using Leica QWin V3 (Leica Microsystems).

Histological analysis of the hemimaxillae enabled the characterization of the inflammatory profile of the tissue response to the different materials. The results were presented through three analyses: one descriptive, semi-quantitative, and quantitative.

Slides containing representative sections of each specimen were evaluated under optical microscopy and used in the descriptive analysis. The descriptive analysis involved describing the histopathological process, considering the type and number of inflammatory cells, fiber organization, and the presence or absence of necrosis (Cintra et al., 2013c). For the semi-quantitative analysis of the inflammatory process, scores were attributed according to the approximate average number of inflammatory cells present in different fields of the same specimen. The classification of the inflammatory infiltrate was as follows:

- Score 1 - absent or negligible inflammatory cells;
- Score 2 - mild inflammatory infiltrate (fewer than 10 cells per field);
- Score 3 - moderate inflammatory infiltrate (between 10 and 25 cells per field);
- Score 4 for intense inflammatory infiltrate (more than 25 cells per field) (Cintra et al. 2013b; Cintra et al. 2014a; Cintra et al. 2014b).

Collagen fiber maturation levels were analyzed quantitatively, in PSR-stained sections under polarized light microscopy. The QWin program was used (400X magnification, Leica QWin V3, Leica Microsystems), allowing the selection of corresponding colors for each type of collagen fiber in the periodontal tissue. After color selection, the program automatically calculated the stained area. Greenish-yellow

fibers were considered immature and thin, while yellowish-red fibers were considered mature and thick (Junqueira, 1982).

For the Immunohistochemical analysis, it was performed under brightfield illumination under an optical microscope (Optiphot-2, Nikon, Japan). The Immunostaining was defined as brownish staining present in the cell cytoplasm and/or extracellular matrix. Semiquantitative analysis, which provided information on the number of immunoreactive cells and the intensity of immunolabeling of the extracellular matrix. The study was performed using five histological sections from each animal to confirm the accuracy of the data collection.

The immunostaining pattern scores were:

- Score 0 - complete absence of immunoreaction; (score 1) <20% of immunoreactive cells and weak staining of the extracellular matrix;
- Score 2 -> 20% and <40% of immunoreactive cells and weak staining of the extracellular matrix;
- Score 3 -> 40% and <60% of immunoreactive cells and moderate staining of the extracellular matrix;
- Score 4 -> 60% and <80% of immunoreactive cells and strong staining of the extracellular matrix;
- Score 5 -> 80% of immunoreactive cells and strong staining of the extracellular matrix, analysis pattern adapted from (Cosme-Silva et al. 2019).

## PRIASE 2021 Flowchart\*

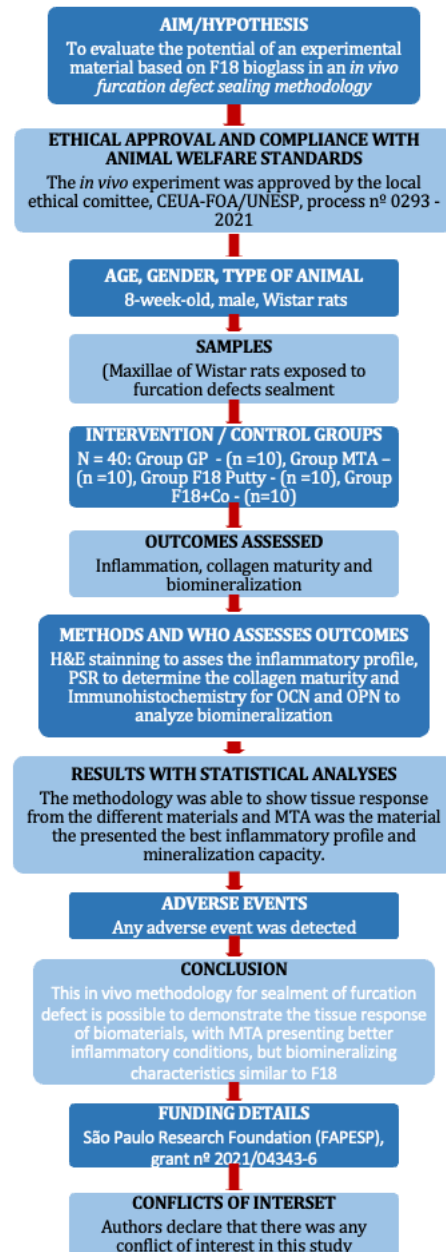


**Figure 1.** PRIASE 2021 Flowchart.

### Statistical Analyses

Statistics was performed by GraphPad Prism (GraphPad Prism 10.1.2.0) (GraphPad Software, Boston, Massachusetts, USA) and the significance level was 5% ( $p < 0.05$ ). The semi-quantitative analysis was subjected to the Kruskal-Wallis' test. If a significant difference was observed, the data collected were compared among the groups using the multiple comparison Dunn's test. For the quantitative data, firstly, the data were

submitted to a normality test. If the data had a normal distribution, an ANOVA test was applied; if not, a Kruskal-Wallis' test was followed by Dunn's test.

## Results

### Inflammatory infiltrate

The Inflammation at 7 days was exacerbated in all groups, characterized by an inflammatory infiltrate rich in inflammatory cells, including macrophages, neutrophils, and lymphocytes, typical of acute inflammation. The GP group presented a moderate level of inflammatory infiltrate and was only different from the F18+Co group ( $p=0.0008$ ). The MTA group presented a moderate to severe inflammatory infiltrate and was not statistically different from any of the specific groups ( $p=0.0008$ ). The F18+Co group had a higher median, indicating a severe inflammatory infiltrate, and was not statistically different from the MTA group ( $p = 0.0008$ ). Finally, the F18 Putty group presents a slight lightness, differing only from the F18+Co group ( $p=0.0008$ ). At 30 days, there was a reduction in the inflammation characteristic for all groups. Only F18 Putty presented a moderate inflammatory infiltrate, while the other groups had a mild inflammatory character. However, there was no statistical difference between them ( $p=0.3031$ ) (Table 1; Graphs 1 and 2).

**Table 1.** Scores of the inflammatory infiltrate, median, and p-value for all experimental groups and periods of analysis.

| Parameters of analysis  |              | 7 days           |                    |                |                 | 30 days        |                |                |                |
|-------------------------|--------------|------------------|--------------------|----------------|-----------------|----------------|----------------|----------------|----------------|
|                         |              | GP               | MTA                | F18+Co         | F18 Putty       | GP             | MTA            | F18+Co         | F18 Putty      |
| Inflammatory infiltrate | 1 – Absent   | 0/10             | 0/10               | 0/10           | 0/10            | 0/10           | 0/10           | 0/10           | 0/10           |
|                         | 2 – Mild     | 5/10             | 2/10               | 0/10           | 4/10            | 8/10           | 7/10           | 6/10           | 4/10           |
|                         | 3 - Moderate | 5/10             | 4/10               | 3/10           | 6/10            | 2/10           | 3/10           | 4/10           | 6/10           |
|                         | 4 – Severe   | 0/10             | 4/10               | 7/10           | 0/10            | 0/10           | 0/10           | 0/10           | 0/10           |
|                         | Median*      | 2.5 <sup>A</sup> | 3.5 <sup>ABC</sup> | 4 <sup>B</sup> | 3 <sup>AC</sup> | 2 <sup>A</sup> | 2 <sup>A</sup> | 2 <sup>A</sup> | 3 <sup>A</sup> |

\*Different letters in rows indicate significant statistical differences for each experimental period ( $p<0.05$ ).

**Graph 1.** Intensity in scores of the inflammatory infiltrate for each experimental material in 7 and 30 days.

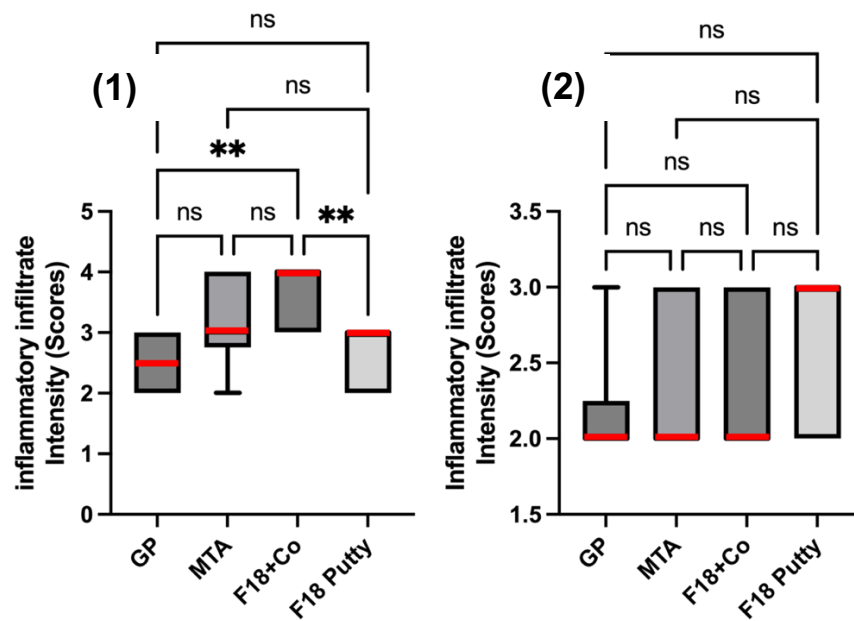
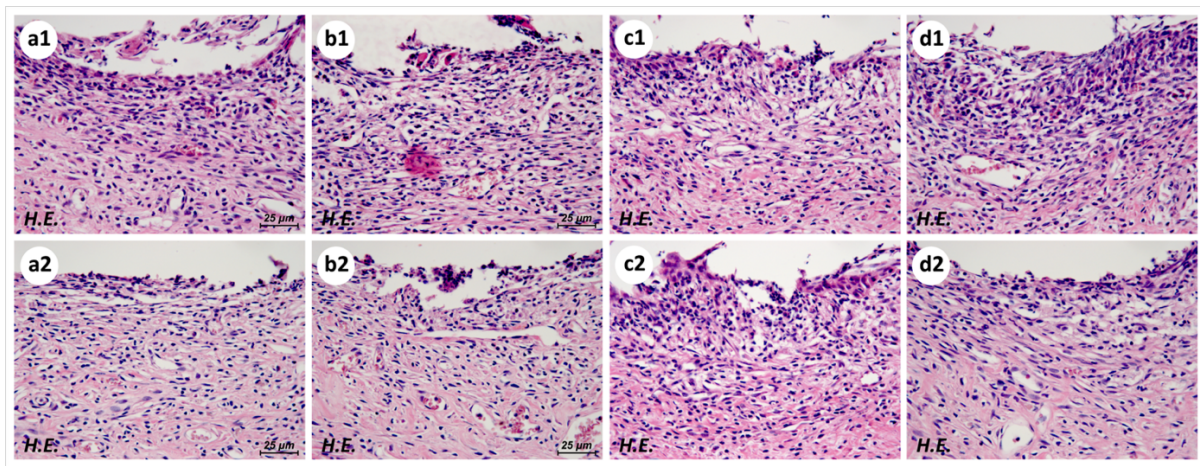


**Figure 2.** Representative images of the inflammatory infiltrate of the experimental groups for each period of analysis. **(a1,a2)** GP group; **(b1,b2)** MTA group; **(c1,c2)** F18 Putty group **(c1,c2)**; F18+Co group **(d1,d2)**. **(a1-d1)** period of 7 days; **(a2-d2)** period of 30 days. Hematoxylin & Eosin, 400x magnification.

### Collagen Maturity

The color presented by collagenous fibers assessed collagen maturity. It is evident that during the 7 days, the MTA Group presented a higher percentage of mature collagen fibers ( $70.89 \pm 6.97$ ), which differed significantly from the GP, F18+Co, and F18 Putty groups, which were similar among themselves ( $p < 0.0001$ ). After 30 days, the MTA group had a higher percentage of immature collagen fibers ( $69.25 \pm 11.67$ ), whereas

the other experimental groups, GP, F18+Co, and F18 Putty, had a higher rate of mature collagen fibers ( $p < 0.0001$ ) (Table 2; Graphs 3 and 4).

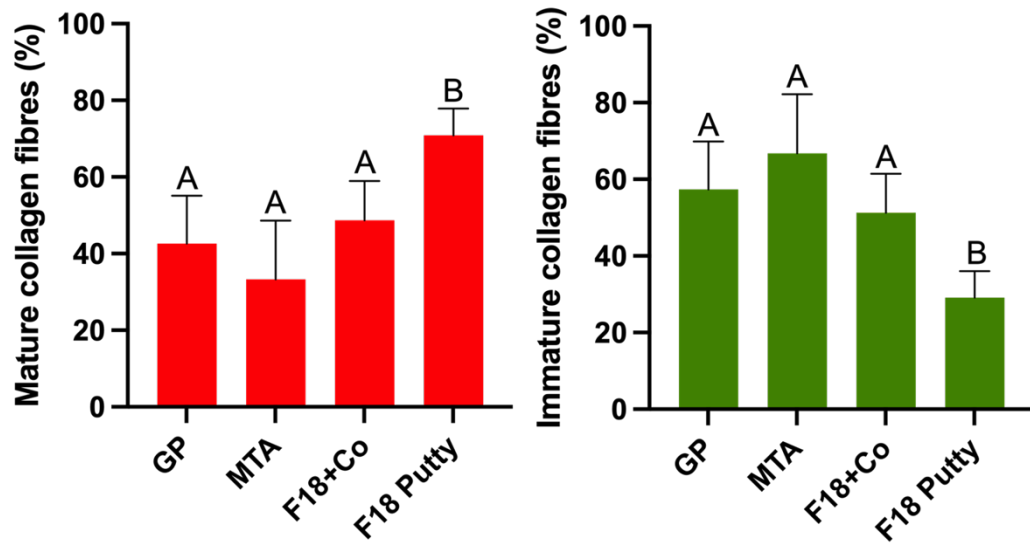
**Table 2.** Percentage of collagen fibers and standard deviation (SD) for all groups and periods of analysis.

| Time    | Material  | Immature collagen<br>Greenish-yellow |              | Mature collagen<br>Yellowish-red |              |
|---------|-----------|--------------------------------------|--------------|----------------------------------|--------------|
| 7 days  | GP        | 57.37 ± 11.89 <sup>A</sup>           | $p < 0.0001$ | 42.63 ± 11.89 <sup>A</sup>       | $p < 0.0001$ |
|         | MTA       | 66.73 ± 14.57 <sup>A</sup>           |              | 33.27 ± 14.57 <sup>A</sup>       |              |
|         | F18+Co    | 51.27 ± 10.19 <sup>A</sup>           |              | 48.73 ± 10.19 <sup>A</sup>       |              |
|         | F18 Putty | 29.11 ± 06.97 <sup>B</sup>           |              | 70.89 ± 06.97 <sup>B</sup>       |              |
| 30 days | GP        | 38.69 ± 09.55 <sup>A</sup>           | $p < 0.0001$ | 61.31 ± 09.55 <sup>A</sup>       | $p < 0.0001$ |
|         | MTA       | 69.25 ± 11.67 <sup>B</sup>           |              | 30.75 ± 11.67 <sup>B</sup>       |              |
|         | F18+Co    | 33.59 ± 07.07 <sup>A</sup>           |              | 66.41 ± 07.07 <sup>A</sup>       |              |
|         | F18 Putty | 25.75 ± 07.95 <sup>A</sup>           |              | 74.25 ± 07.95 <sup>A</sup>       |              |

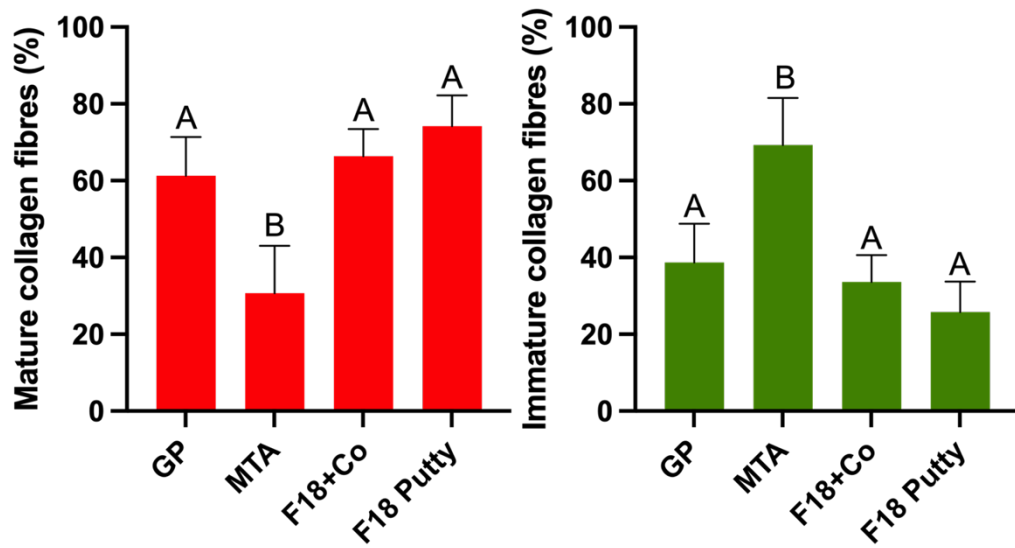
\*Different letters in rows indicate significant statistical differences for each experimental period ( $p < 0.05$ ).

**Graphs 3 and 4.** Percentage of mature and immature fibers deposited in the periodontal tissue according to each experimental group at 7 and 30 days.

(3) 7 days



(4) 30 days



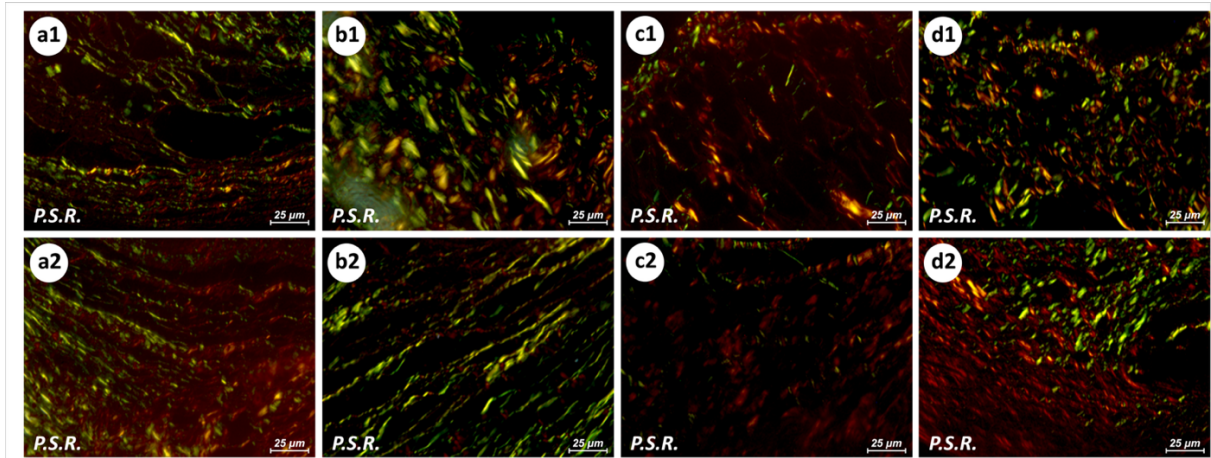


**Figure 3.** Representative images of the collagen maturity of the experimental groups for each period of analysis. **(a1,a2)** GP group; **(b1,b2)** MTA group; **(c1,c2)** F18 Putty group; **(d1,d2)** F18+Co group. **(a1-d1)** period of 7 days; **(a2-d2)** period of 30 days. Picrosirius red staining, 1000x magnification.

### Biom mineralization

The ability to induce the formation of mineralized tissue was evaluated by immunostaining Oxytocin (OCN) and Osteopontin (OPN).

### OCN Immunostaining

The evaluation of OCN in a 7-day period showed that the GP group showed a score of 1, which represents <20% of immunoreactive cells and weak staining of the extracellular matrix, different from the other groups ( $p=0.0004$ ). MTA, F18+Co, and F18 Putty presented a score of 2, indicating that >20% and <40% of immunoreactive cells and weak staining of the extracellular matrix were observed, similar among them and statistically different from the GP group ( $p = 0.0004$ ). In 30 days, the GP Group presented a score of 3, which represents >40% and <60% of immunoreactive cells and moderate staining of the extracellular matrix, being the same as the F18 Putty group, with a score of 4, characterizing in the immunohistochemical analysis >60% and <80% of immunoreactive cells and strong staining of the extracellular matrix ( $p<0.0001$ ). However, F18 Putty was also similar to the other groups ( $p<0.0001$ ). The MTA group presented a score of 5, with more than 80% of immunoreactive cells and strong staining of the extracellular matrix, similar to the F18+Co and F18 Putty groups, which had immunostaining scores of 4 ( $p < 0.0001$ ) (Table 3; Figure 4).

**Table 3.** Scores, median, and p value for the immunostaining of OCN for all groups and period of analysis.

| Analysis Parameters | 7 days         |                |                |                |            | 30 days        |                |                |                 |            |
|---------------------|----------------|----------------|----------------|----------------|------------|----------------|----------------|----------------|-----------------|------------|
|                     | GP             | MTA            | F18+Co         | F18 Putty      |            | GP             | MTA            | F18+Co         | F18 Putty       |            |
| <b>0</b>            | 4/10           | 0/10           | 0/10           | 0/10           |            | 0/10           | 0/10           | 0/10           | 0/10            |            |
| <b>1</b>            | 6/10           | 1/10           | 3/10           | 3/10           |            | 2/10           | 0/10           | 0/10           | 0/10            |            |
| <b>2</b>            | 0/10           | 7/10           | 5/10           | 4/10           |            | 2/10           | 0/10           | 0/10           | 0/10            |            |
| <b>3</b>            | 0/10           | 2/10           | 2/10           | 3/10           | p = 0.0004 | 6/10           | 1/10           | 2/10           | 4/10            | p < 0.0001 |
| <b>4</b>            | 0/10           | 0/10           | 0/10           | 0/10           |            | 0/10           | 3/10           | 7/10           | 6/10            |            |
| <b>5</b>            | 0/10           | 0/10           | 0/10           | 0/10           |            | 0/10           | 6/10           | 1/10           | 0/10            |            |
| <b>Median*</b>      | 1 <sup>A</sup> | 2 <sup>B</sup> | 2 <sup>B</sup> | 2 <sup>B</sup> |            | 3 <sup>A</sup> | 5 <sup>B</sup> | 4 <sup>B</sup> | 4 <sup>AB</sup> |            |

\*Different letters in rows indicate significant statistical differences for each experimental period (p<0.05).

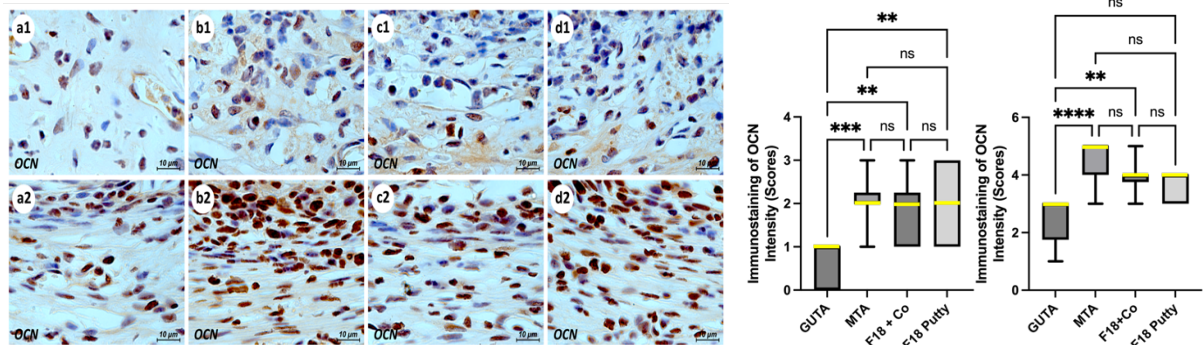


**Figure 4.** Representative images of the immunostaining of OCN of the experimental groups for each period of analysis. **(a1,a2)** GP group; **(b1,b2)** MTA group; **(c1,c2)** F18 Putty group; **(d1,d2)** F18+Co group. **(a1-d1)** period of 7 days; **(a2-d2)** period of 30 days. Immunohistochemistry, 400x magnification. **(e)** Graphical projection for the immunostaining of OCN evaluated in scores in the different experimental groups.

### OPN Immunostaining

In the analysis of OPN, immunolabeling during 7 days revealed that the GP group presented a score of 1.5, which differed from that of the MTA, F18+Co, and F18 Putty groups (p < 0.0001). The MTA group had a score of 3, F18+Co, 2.5, and F18 Putty, 2, with no statistical difference among them (p < 0.0001). After 30 days, there was a similarity in immunostaining between the GP and F18 Putty groups, with a median of 3 (p < 0.0001). There was also statistical similarity between the MTA and F18+Co groups (p < 0.0001) (Table 2; Figure 5).

**Table 4.** Scores, median, and p value for the immunostaining of OCN for all groups and period of analysis.

| Analysis Parameters | 7 days           |                |                  |                |              | 30 days        |                |                |                |              |
|---------------------|------------------|----------------|------------------|----------------|--------------|----------------|----------------|----------------|----------------|--------------|
|                     | GP               | MTA            | F18+Co           | F18 Putty      |              | GP             | MTA            | F18+Co         | F18 Putty      |              |
| <b>0</b>            | 5/10             | 0/10           | 0/10             | 0/10           | $p < 0.0001$ | 0/10           | 0/10           | 0/10           | 0/10           | $p < 0.0001$ |
| <b>1</b>            | 5/10             | 0/10           | 0/10             | 0/10           |              | 1/10           | 0/10           | 0/10           | 0/10           |              |
| <b>2</b>            | 0/10             | 3/10           | 5/10             | 8/10           |              | 2/10           | 0/10           | 0/10           | 3/10           |              |
| <b>OPN 3</b>        | 0/10             | 7/10           | 5/10             | 2/10           |              | 7/10           | 1/10           | 3/10           | 7/10           |              |
| <b>4</b>            | 0/10             | 0/10           | 0/10             | 0/10           |              | 0/10           | 8/10           | 7/10           | 0/10           |              |
| <b>5</b>            | 0/10             | 0/10           | 0/10             | 0/10           |              | 0/10           | 1/10           | 0/10           | 0/10           |              |
| <b>Median*</b>      | 1.5 <sup>A</sup> | 3 <sup>B</sup> | 2.5 <sup>B</sup> | 2 <sup>B</sup> |              | 3 <sup>A</sup> | 4 <sup>B</sup> | 4 <sup>B</sup> | 3 <sup>A</sup> |              |

\*Different letters in rows indicate significant statistical differences for each experimental period ( $p < 0.05$ ).

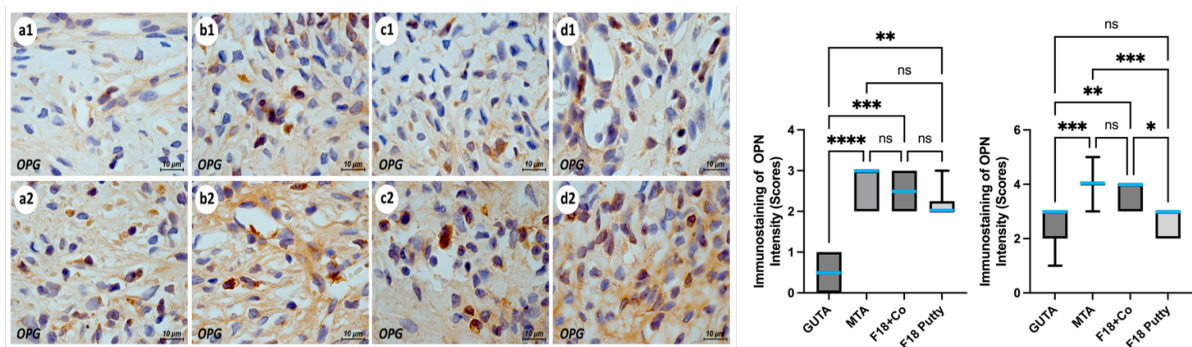


**Figure 5.** Representative images of the immunostaining of OPN of the experimental groups for each period of analysis. **(a1,a2)** GP group; **(b1,b2)** MTA group; **(c1,c2)** F18 Putty group; **(d1,d2)** F18+Co. **(a1-d1)** period of 7 days; **(a2-d2)** period of 30 days. Immunohistochemistry, 400x magnification. **(e)** Graphical projection for the immunostaining of OPN evaluated in scores in the different experimental groups.

## Discussion

F18 bioglass has been shown in the literature to be a biocompatible material capable of inducing the formation of different types of tissues (Souza et al. 2016ab, 2017bc; Gabbai-Armelin et al. 2017; Souza et al. 2017b; Keshaw et al. 2005, Gorustovich et al. 2010, Stähli et al. 2015, Oishi et al., 2019). In this study, the potential of F18 in the form of Putty (F18 Putty) and F18 doped with cobalt (F18+Co) was analyzed, regarding their ability to induce tissue formation and as biomineralizer materials, comparing them to MTA in cases of furcation perforation (Camilleri et al. 2013; da Fonseca et al., 2019). F18, in powder form, has already been studied in dental science, such as in

experimental intracanal pastes, using polyethylene tubes implanted in the subcutaneous tissue of rats (de Araujo Lopes et al. 2020), as an additional irrigant in endodontic treatment (de Paula et al. 2022a) and as a pulp capping material (Chaves et al. 2024). However, scientific evidence is in need, especially for the version doped with cobalt (F18+Co) and its putty form (F18 Putty). The literature brings methodologies that can evidence tissue response to biomaterials in furcation defects, reproducing specific clinical conditions (Silva et al. 2009; da Fonseca et al. 2019). Therefore, this study reproduced the *in vivo* model of furcation defects, modifying the final restoration and indicating reasonable tissue conditions to analyze after periodontium contact with the bioglasses and MTA. The biomaterials, MTA, F18+Co and F18 Putty could decrease the inflammatory process over time and exhibited biomineralization ability in the periodontal tissue, thereby refuting the null hypothesis of this study.

The periodontal tissue was analyzed by assessing the inflammatory infiltrate using H&E staining (de Oliveira et al., 2021; Gomes-Filho et al., 2009, 2011; Cintra et al., 2017a). Inflammation was classified from absent to severe, using pre-established scores (Cintra et al., 2013b; Cintra et al., 2014a; Cintra et al., 2014b). It was observed that both bioglass F18+Co and F18 Putty were not superior biomaterials to MTA. A decrease in the inflammatory infiltrate was observed over time; nevertheless, it presented moderate inflammation at 30 days, not statistically different from MTA ( $p > 0.05$ ). The experimental model may have a limitation, as MTA, which is used in clinical situations such as furcation sealing (Camilleri et al. 2013; da Fonseca et al., 2019), also presented an inflammatory infiltrate similar to that of GP, a bioinert material (Vishwanath et al. 2019), property that indicates that it should not have an interaction with tissues, expecting it to be different in inflammatory analysis, a profile that brings the attention to propose some modification in the methodology, such as, including a decontamination procedure before sealing the defected area.

Additionally, inflammatory conditions were assessed by evaluating tissue repair through the deposition of collagen. This method helps to understand the stimulus exerted by the biomaterial on the tissue, inducing the formation of reparative collagenous tissue (Cintra et al. 2017a). Fibroblasts proliferate in areas undergoing repair (Diegelmann & Evans, 2004). Therefore, in the PSR analysis, mature fibers indicate repair that began long ago, whereas immature fibers are observed in regions undergoing collagen remodeling (Cintra et al., 2017; de Oliveira et al., 2022). However,

some studies indicate that mature collagen fibers can be found in inflamed regions due to the presence of the fibrosis process (de Oliveira et al., 2022).

Nevertheless, in the context of this research, this assertion could not be directly linked to the observed outcomes. F18 Putty presented a lower number of immature fibers at 7 days ( $p = 0.0001$ ); on the other hand, F18+Co showed similar collagen deposition to MTA. This finding may be related to the slow rate of collagen remodeling that F18 Putty could not induce, but for F18+Co, because of the difference of their form and composition, enabling a deeper interaction with the tissue, signaling cells in a faster pattern (Souza et al. 2016ab, 2017bc; Gabbai-Armelin et al. 2017, Souza et al. 2017b). At 30 days, MTA presented a higher number of immature fibers ( $p < 0.0001$ ), indicating a greater reparative potential for MTA compared to F18 Putty, F18+Co, and Gutta-percha. Gabbai-Armelin et al. 2017) evaluated the proliferation of fibroblast cells *in vitro* and elucidated the F18 Putty's ability to induce collagen deposition. Nonetheless, in this comparative *in vivo* model, it did not yield similar results to MTA, maintaining its reputation as a biomaterial with established applicability in endodontics (Parirokh et al., 2010; Silva et al., 2019; Pinheiro et al., 2021).

Besides inflammation and collagen deposition, an additional way to analyze the inductive potential of a biomaterial is to explore its biomineralizing activity (Silva et al. 2014; Cosme-Silva et al. 2019). OCN is a non-collagenous protein found in bone and dentin that participates in the control of biomineralization and calcium ion homeostasis in tissues upon contact with sealing materials (McKee et al. 1996, Orimo et al. 2010; Cosme-Silva et al. 2019; Zairi et al. 2012; Tani-Ishii et al. 2007). Another biomarker is OPN, a secreted non-collagenous bone matrix that induces bone remodeling, produced by pre-osteoblasts, osteoblasts, and osteocytes (Ivanovski et al., 2001), as well as extraosseous cells, such as odontoblasts (Gaballah et al., 2018). These proteins have a mechanism that dictates the success of treatment in cases of furcation perforations (Mohamed et al., 2023). Thus, OCN and OPN analyses helped identify the biomineralizing potential of F18+Co, F18 Putty, and confirmed this property for MTA. The outcomes extracted from the immunohistochemistry analysis showed that, in 7 days, the F18+Co, F18 Putty, and MTA groups presented greater immunostaining for OCN and OPN compared to the GP group ( $p < 0.001$ ). These findings confirm that both materials have mineralization-inducing properties. However, within 30 days, only F18+Co showed a similarity to MTA in the immunolabeling of OPN, which highlights the need to explore different mineralizing mechanisms in the future to comprehend the

bioglasses' mineralization potential. F18 Putty, despite presenting slightly higher immunostaining than the GP control group, showed no statistical difference at 30 days. For the OCN expression in GP, it occurred due to possible repair induced by the organism itself, rather than gutta-percha's biomineralizing activity, which is null (Silva et al., 2019; Vishwanath et al., 2019).

Finally, a sealing material should present physicochemical characteristics such as dimensional stability, color, radiopacity, hermetic sealing, insolubility in contact with fluids, flowability, and easy insertion (Camilleri et al. 2011; Marciano MA et al. 2014; Islam et al. 2005; Cavenago et al. 2014; Duque et al. 2018). Bioglass F18 in Putty form is an aqueous, highly fluid material that has proven difficult to manipulate for use in the perforated furcation region. Although its initial proposal was to be an easy-to-manipulate and insert material, this characteristic was not observed during its application. Besides, it has a transparent coloration that makes it difficult to see when applied. Furthermore, when inserted into the defect, the biomaterial did not adhere to the adjacent cavity walls due to its high degree of fluidity, hindering its adhesion and mechanical strength. This is the opposite of what MTA presents, despite being considered in the literature to lack mechanical rigidity (Duarte et al., 2018). Different from the F18 Putty, the F18+Co, presented in a powder form, could be very easily inserted, and the only negative characteristic was its blue color, which could lead to crown discoloration in long-term analysis. Therefore, one hypothesis that could be attached to F18 Putty's low capacity of decreasing inflammation and induction of maintenance of secretion of OCN and OPN in furcation perforation was its Putty-like form.

In summary, the reproduced *in vivo* methodology of furcation perforation model allowed the demonstration of biological results, proving its applicability. Additionally, it enabled us to confirm that MTA was the material that most induced the formation of reparative collagen and had the most interaction with mineral biomarkers. However, F18s could decrease the inflammation and were identified to have biomineralization ability, especially for F18+Co. Furthermore, a better response can be observed for F18 bioglasses, suggesting their incorporation into polymeric bases, such as hydrogels or scaffolds, which improves the distribution and adaptation of the material in the applied tissue (Pankajakshan et al. 2020; de Souza Araújo et al. 2022).

## Conclusion

It is concluded that bioglass and MTA are biocompatible and contribute to the repair of periodontal tissue in furcation defects. In addition, MTA and F18+Co have greater stimulus to biomineralization compared to F18 Putty.

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## **Anexos**

1. Certificado emitido pelo comitê de ética
2. Figura 1 – Sequência experimental de capeamento pulpar em ratos Wistar.
3. Figura 2 – Sequência experimental de selamento de furca em ratos Wistar.
4. "IJER Journal's guideline"



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CEUA - Ethics Committee on the Use of Animals

### CERTIFICADO

Certificamos que o Projeto de Pesquisa intitulado "**Potencial do biovidro F18 dopado com cobalto em procedimentos endodônticos**", Processo FOA nº 0293-2021, sob responsabilidade de Luciano Tavares Angelo Cintra apresenta um protocolo experimental de acordo com os Princípios Éticos da Experimentação Animal e sua execução foi aprovada pela CEUA em 26 de Novembro de 2021.

**VALIDADE DESTE CERTIFICADO:** 30 de Novembro de 2023.

**DATA DA SUBMISSÃO DO RELATÓRIO FINAL:** até 30 de Dezembro de 2023.

### CERTIFICATE

We certify that the study entitled "**Potential of F18 bioglass doped with cobalt in endodontic procedures**", Protocol FOA nº 0293-2021, under the supervision of Luciano Tavares Angelo Cintra presents an experimental protocol in accordance with the Ethical Principles of Animal Experimentation and its implementation was approved by CEUA on November 26, 2021.

**VALIDITY OF THIS CERTIFICATE:** November 30, 2023.

**DATE OF SUBMISSION OF THE FINAL REPORT:** December 30, 2023.

**Prof. Associado João Carlos Callera**  
Coordenador da CEUA  
CEUA Coordinator

CEUA - Comissão de Ética no Uso de Animais  
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## Sequência experimental

### Capítulo 1 - Capeamento pulpar

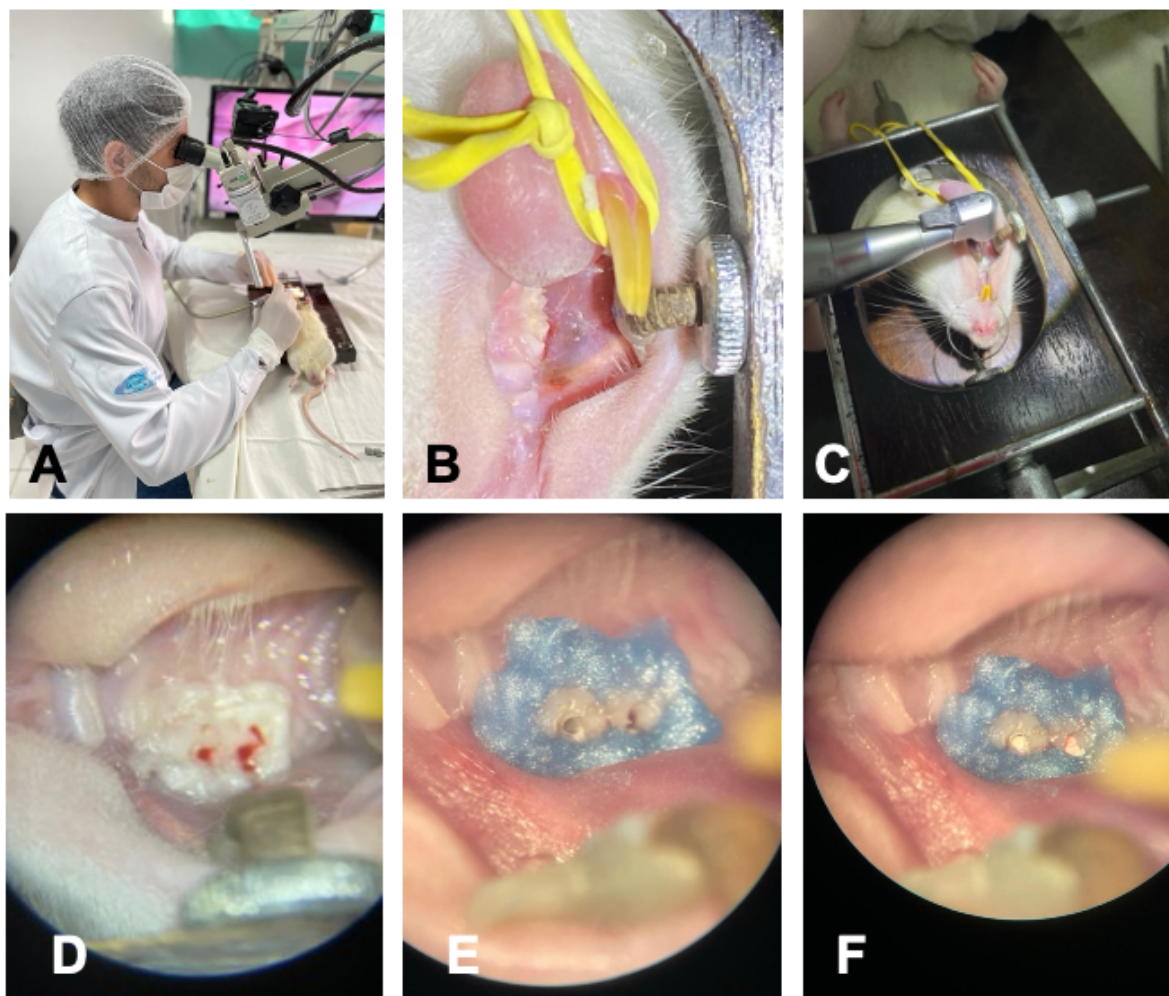


**Figura 3:** **A** - Pesquisador experiente e habilitado realizando o experimento com auxílio de microscópio cirúrgico em um aumento de 40x; **B** - Animal posicionado sobre mesa cirúrgica, anestesiado e sedado para a realização do capeamento pulpar; **C** – Momento da exposição pulpar no 1º e no 2º molar da hemimaxila direita do animal, utilizando uma broca esférica em baixa rotação; **D** - Sangramento provocado após a perfuração da câmara pulpar; **E** - Dentes isolados com barreira gengival para controlar a penetração de fluidos salivares e material capeador já inserido; **F** - Imagem após selamento com ionômero de vidro e resina flow.

## Sequência experimental

### Capítulo 2 – Selamento de furca

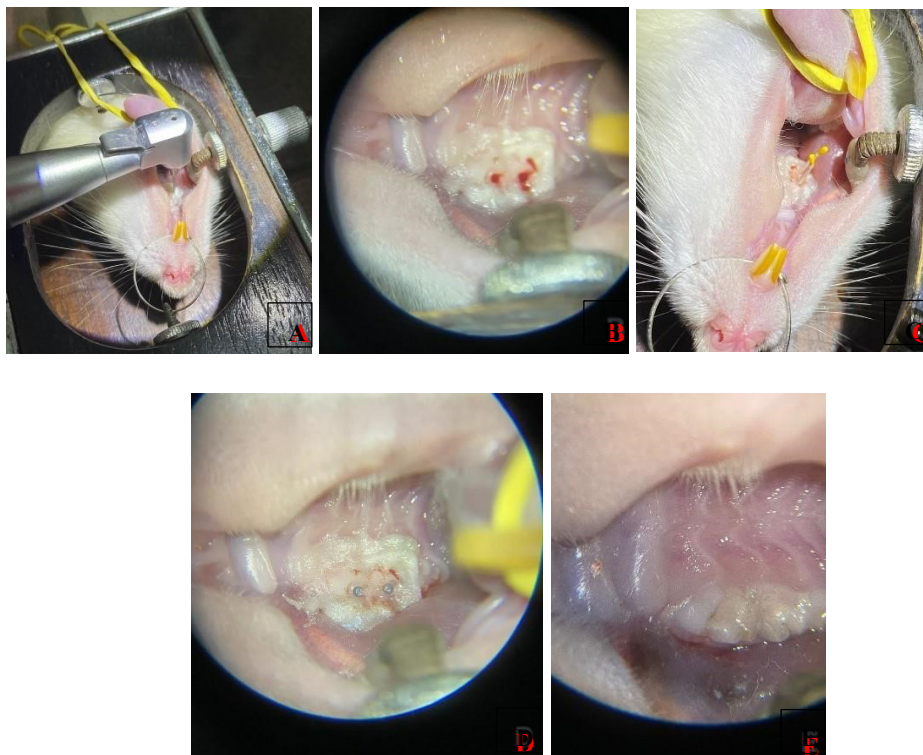


**Figura:** **A** - Animal posicionado sobre mesa cirúrgica, anestesiado e sedado para a realização da perfuração de furca com uma broca esférica em baixa rotação; **B** - Sangramento provocado após a perfuração da furca e barreira gengival, para controlar a penetração de fluidos salivares; **C** - Local cirúrgico sendo seco com cones de papel absorvente para posterior aplicação dos biomateriais; **D**: Biomaterial no interior da perfuração de furca e câmara pulpar; **E** - Imagem após a introdução do biomaterial, ionômero de vidro e resina flow. Imagem feita em visão do microscópio. (Imagens obtidas em microscópio cirúrgico em 40x)

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- v. Acknowledgements.

**Important: the journal operates a double-blind peer review policy. Please anonymise your manuscript and prepare a separate title page containing author details.**

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

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## **2.1 Reporting Guidelines**

### **2.1.1 Case reports/case series**

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When submitting manuscripts that have been written using the PRICE 2020 guidelines, authors should include the following statement in the beginning of “Report” section: “This case report has been written according to Preferred Reporting Items for Case reports in Endodontics (PRICE) 2020 guidelines.

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Nagendrababu V, Chong BS, McCabe P, Shah PK, Priya E, Jayaraman J, Pulikkotil SJ, Setzer FC, Sunde PT, Dummer PMH (2020) PRICE 2020 guidelines for reporting case reports in Endodontics: a consensus-based development. *International Endodontic Journal* 53, 619-26. (<https://www.ncbi.nlm.nih.gov/pubmed/32090342>)

Nagendrababu V, Chong BS, McCabe P, Shah PK, Priya E, Jayaraman J, Pulikkotil SJ, Dummer PMH (2020) PRICE 2020 guidelines for reporting case reports in Endodontics: Explanation and elaboration. *International Endodontic Journal* 53, 922-47. ([/doi/abs/10.1111/iej.13300](https://doi.org/10.1111/iej.13300))

### 2.2.2. Randomised clinical trials

Randomised clinical trials should be reported to comply with the Preferred Reporting Items for Randomised Trials in Endodontics (PRIRATE) 2020 guidelines (Nagendrababu et al. 2020, doi: 10.1111/iej.13294).

When submitting manuscripts that have been written using the PRIRATE 2020 guidelines, authors should include the following statement in the beginning of “Materials and Methods” section: “This randomised clinical trial has been written according to Preferred Reporting Items for RAndomised Trials in Endodontics (PRIRATE) 2020 guidelines.

A PRIRATE 2020 checklist (for editors/referees) and flowchart (as a Figure to be included in the manuscript for readers) should also be completed and included in the submission material. The PRIRATE 2020 checklist and flowchart can be downloaded from: <http://pride-endodonticguidelines.org/prirate/>.

It is recommended that authors consult the following papers when writing manuscripts, which explains the rationale for the PRIRATE 2020 guidelines and their importance:

Nagendrababu V, Duncan HF, Bjørndal L, Kvist T, Priya E, Jayaraman J, Pulikkotil SJ, Pigg M, Rechenberg DK, Vaeth M, Dummer PMH (2020) PRIRATE 2020 guidelines for reporting randomised trials in Endodontics: a consensus-based development. *International Endodontic Journal* 53, 764-73. ([/doi/abs/10.1111/iej.13294](https://doi.org/10.1111/iej.13294))

Nagendrababu V, Duncan HF, Bjørndal L, Kvist T, Priya E, Jayaraman J, Pulikkotil SJ, Dummer PMH (2020) PRIRATE 2020 guidelines for reporting trials in Endodontics: Explanation and elaboration. *International Endodontic Journal* 53, 774-03. ([/doi/abs/10.1111/iej.13304](https://doi.org/10.1111/iej.13304))

### Clinical Trials

The *International Endodontic Journal* asks that authors submitting manuscripts reporting a clinical trial register the trial a priori in any of the following public clinical trials registries:

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### 2.2.3. Observational trials

Observational studies should be reported to comply with the Preferred Reporting items for Observational studies in Endodontics (PROBE) 2023 guidelines (Nagendrababu et al. 2023, doi: 10.1111/iej.13873).

When submitting manuscripts that have been written using the PROBE 2023 guidelines, authors should include the following statement in the beginning of “Materials and Methods” section: “This observational studies has been written according to Preferred Reporting items for Observational studies in Endodontics (PROBE) 2023 guidelines”.

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It is recommended that authors consult the following papers when writing manuscripts, which explains the rationale for the PROBE 2023 guidelines and their importance:

Nagendrababu V, Duncan HF, Fouad AF, Kirkevang LL, Parashos P, Pigg M, Vaeth M, Jayaraman J, Suresh N, Arias A, Wigsten E, Dummer PMH. PROBE 2023 guidelines for reporting observational studies in Endodontics: A consensus-based development study. *Int Endod J.* 2023 Mar;56(3):308-317. [/doi/epdf/10.1111/iej.13873](https://doi.org/10.1111/iej.13873)

Nagendrababu V, Duncan HF, Fouad AF, Kirkevang LL, Parashos P, Pigg M, Vaeth M, Jayaraman J, Suresh N, Jakovljevic A, Dummer PMH. PROBE 2023 guidelines for reporting observational studies in endodontics: explanation and elaboration. *Int Endod J.* 2023 Jun;56(6):652-685. doi: 10.1111/iej.13909. [/doi/10.1111/iej.13909](https://doi.org/10.1111/iej.13909)

#### **2.2.4. Diagnostic accuracy studies**

Diagnostic accuracy studies should be reported to comply with the Preferred Reporting Items for Diagnostic Accuracy Studies in Endodontics (PRIDASE) 2024 guidelines (Nagendrababu et al. 2024, doi: 10.1111/iej.14075).

When submitting manuscripts that have been written using the PRIDASE 2024 guidelines, authors should include the following statement in the beginning of “Materials and Methods” section: “This observational studies has been written according to Preferred Reporting Items for Diagnostic Accuracy Studies in Endodontics (PRIDASE) 2024”.

A PRIDASE 2024 checklist (for editors/referees) and STARD flowchart (as a Figure to be included in the manuscript for readers) should also be completed and included in the submission material. The PRIDASE 2024 checklist can be downloaded from <https://pride-endodonticguidelines.org/pridase/> and STARD flowchart can be downloaded from: <https://www.equator-network.org/reporting-guidelines/stard/>

It is recommended that authors consult the following papers when writing manuscripts, which explains the rationale for the PRIDASE 2024 guidelines and their importance:

Nagendrababu V, Pigg M, Duncan HF, Abbott PV, Fouad AF, Kruse C, Patel S, Rechenberg DK, Setzer FC, Rossi-Fedele G, Dummer PM. PRIDASE 2024 guidelines for reporting diagnostic accuracy studies in endodontics: A consensus-based

development. International Endodontic Journal. 2024 Aug;57(8):996-1005. doi: 10.1111/iej.14075. [/doi/10.1111/iej.14075](https://doi.org/10.1111/iej.14075)

Pigg M, Nagendrababu V, Duncan HF, Abbott PV, Fouad AF, Kruse C, Patel S, Rechenberg DK, Suresh N, Shetty N, Dummer PM. PRIDASE 2024 guidelines for reporting diagnostic accuracy studies in endodontics: Explanation and elaboration. International Endodontic Journal,. 2025 Jan;58(1):6-36. doi: 10.1111/iej.14148 [/doi/10.1111/iej.14148](https://doi.org/10.1111/iej.14148)

### **2.2.5. Animal studies**

Animal studies should be written using the Preferred Reporting Items for Animal Studies in Endodontology (PRIASE) 2021 guidelines (Nagendrababu et al. 2021, doi: 10.1111/iej.13477).

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It is recommended that authors consult the following papers when writing manuscripts, which explain the rationale for the PRIASE 2021 guidelines and their importance:

Nagendrababu V, Kishen A, Murray PE, Nekoofar MH, de Figueiredo JA, Priya E, Jayaraman J, Pulikkotil SJ, Camilleri J, RM S, Dummer PMH (2021) PRIASE 2021 guidelines for reporting animal studies in Endodontology: a consensus-based development. International Endodontic Journal 54, 848-57. ([/doi/10.1111/iej.13477](https://doi.org/10.1111/iej.13477))

Nagendrababu V, Kishen A, Murray PE, Nekoofar MH, de Figueiredo JA, Priya E, Jayaraman J, Pulikkotil SJ, Jakovljevic A, Dummer PMH (2021) PRIASE 2021 guidelines for reporting animal studies in Endodontology: Explanation and Elaboration. International Endodontic Journal 54, 858-86. ([/doi/10.1111/iej.13481](https://doi.org/10.1111/iej.13481))

### **2.2.6. Laboratory studies**

Laboratory studies should be reported using the Preferred Reporting Items for Laboratory studies in Endodontology (PRILE) 2021 guidelines (Nagendrababu et al. 2021, doi: 10.1111/iej.13542).

When submitting manuscripts that have been written using the PRILE 2021 guidelines, authors should include the following statement in the beginning of “Materials and Methods” section: “The manuscript of this laboratory study has been written according to Preferred Reporting Items for Laboratory studies in Endodontology (PRILE) 2021 guidelines.”

A PRILE checklist (for editors/referees) and flowchart (as a Figure to be included in the manuscript for readers) should also be completed and included in the submission material. The PRILE 2021 checklist and flowchart can be downloaded from: <http://pride-endodonticguidelines.org/prile/>

It is recommended that authors consult the following papers when writing manuscripts, which explain the rationale for the PRILE 2021 guidelines and their importance:

Nagendrababu V, Murray PE, Ordinola-Zapata R, OA Peters, IN Rôças, JF Siqueira Jr, E Priya, J Jayaraman, SJ Pulikkotil, J Camilleri, C Boutsoukis, G Rossi-Fedele, PMH Dummer (2021) PRILE 2021 guidelines for reporting laboratory studies in Endodontics: a consensus-based development. *International Endodontic Journal* May 3. doi: 10.1111/iej.13542. ([/doi/abs/10.1111/iej.13542](https://doi.org/10.1111/iej.13542))

Nagendrababu V, Murray PE, Ordinola-Zapata R, OA Peters, IN Rôças, JF Siqueira Jr, E Priya, J Jayaraman, SJ Pulikkotil, N Suresh, PMH Dummer (2021) PRILE 2021 guidelines for reporting laboratory studies in Endodontics: Explanation and elaboration. *International Endodontic Journal* ([/doi/abs/10.1111/iej.13565](https://doi.org/10.1111/iej.13565))

### 2.2.7 Systematic reviews

The abstract and main body of the systematic review should be reported using the PRISMA for Abstract and PRISMA guidelines respectively (<http://www.prisma-statement.org/>). Authors submitting a systematic review must register the protocol in one of the readily-accessible sources/databases at the time of project inception and not retrospectively (e.g. PROSPERO database, OSF registries). The protocol registration number, name of the database or journal reference should be provided at the submission stage in the “Registration” section in the abstract and ‘Methods’ section in the main body of the text.

A PRISMA checklist and flow diagram as a Figure (to be included in the manuscript for readers) should also be included in the submission material. Source of funding (grant number, if available) should be added in the ‘Acknowledgements’ section.

It is recommended that authors consult the following papers, which help in the production of high quality reviews:

Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLOS Medicine* 6, e1000097.

Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JP, Clarke M, Devereaux PJ, Kleijnen J, Moher D (2009) The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *Journal of Clinical Epidemiology* 62, e1-34.

Nagendrababu V, Duncan HF, Tsesis I, Sathorn C, Pulikkotil SJ, Dharmarajan L, Dummer PMH (2019) PRISMA for abstracts: best practice for reporting abstracts of systematic reviews in Endodontology. *International Endodontic Journal* 52, 1096-1107. ([/doi/10.1111/iej.13118](https://doi.org/10.1111/iej.13118))

Nagendrababu V, Dilokthornsakul P, Jinatongthai P, Veettil SK, Pulikkotil SJ, Duncan HF, Dummer PMH (2020) Glossary for systematic reviews and meta-analyses. *International Endodontic Journal* 53, 232-249. ([doi/full/10.1111/iej.13217](https://doi.org/10.1111/iej.13217))

### 2.2.8 Scoping reviews

Reviews should be reported using the PRISMA guidelines. A checklist for scoping reviews should also be included in the submission material - see: <http://www.prisma-statement.org/Extensions/ScopingReviews>.

### 2.2.9 Guidelines for reporting of microarray and next-generation sequencing data

Submission will be assessed according to MIAME and MINSEQE standards. The complete current guidelines are available at

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## Appendix

### Abbreviations:

The 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.

## Additional Guidelines for Cover Pictures, Visual Abstracts, Frontispieces and Table of Contents Graphics

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The content of the video should not display overt product advertising. Educational presentations are encouraged.

Any narration should be in English, if possible. A typed transcript of any speech within the video/audio should be provided. An English translation of any non-English speech should be provided in the transcript.

All embedded rich media will be subject to peer review. Editors reserve the right to request edits to rich media files as a condition of acceptance. Contributors are asked to be succinct, and the Editors reserve the right to require shorter video/audio duration.

The video/audio should be high quality (both in content and visibility/audibility). The video/audio should make a specific point; particularly, it should demonstrate the features described in the text of the manuscript.

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## **Examples of correct forms of reference**

### **Standard journal article**

Jakovljevic, A., Duncan, H.F., Nagendrababu, V., Jacimovic, J., Milasin, J. & Dummer, P.M.H. (2020) Association between cardiovascular diseases and apical periodontitis: an umbrella review. *International Endodontic Journal*, 53, 1374–1386.

Selman, P. (2016) The global decline of intercountry adoption: what lies ahead? *Social Policy and Society*, 11(3), 381–397.

### **Corporate author**

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

Department of Health. (2009) Living well with dementia: a national dementia strategy.

### **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.

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