MARIA ESTER FRANÇA DE MELO

AVALIAÇÃO INTEGRADA DAS PROPRIEDADES ANTIMICROBIANAS, QUÍMICAS E BIOLÓGICAS DO BIO-C TEMP E ULTRACAL XS EM CÉLULAS HUMANAS DO LIGAMENTO PERIODONTAL E SANGUE PERIFÉRICO *IN VITRO*

BRASÍLIA, 2026.

UNIVERSIDADE DE BRASÍLIA

FACULDADE DE CIÊNCIAS DA SAÚDE

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE

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Dissertação apresentada como requisito parcial para a obtenção do Título de Mestre em Ciências da Saúde pelo Programa de Pós-Graduação em Ciências da Saúde da Universidade de Brasília.

Orientadora: Profa. Dra. Taia Maria Berto Rezende

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Aprovado em 9 de fevereiro de 2026

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Dedico este trabalho a Deus, origem de toda ciência e conhecimento, por ser a luz que orienta e dá propósito à minha vida.

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“A ciência nunca resolve um problema sem criar outros dez.”

George Bernard Shaw

RESUMO

A medicação intracanal é utilizada entre as fases do tratamento endodôntico com o objetivo de exercer uma barreira físico-química, desinfetar, controlar microrganismos, reduzir inflamação e auxiliar na cicatrização. O hidróxido de cálcio é a medicação padrão ouro, se destacando por sua alcalinidade, no entanto, novas medicações intracanais tem surgido no mercado, como medicações intracanais biocerâmicas. Nesse contexto, este trabalho foi dividido em dois capítulos, com o objetivo de investigar de forma integrada as propriedades físico-químicas, antimicrobianas e os efeitos biológicos de medicamentos intracanais à base de hidróxido de cálcio e biocerâmica. No Capítulo 1, foi realizada uma avaliação comparativa das propriedades físico-químicas, atividade antimicrobiana e respostas biológicas dos medicamentos intracanais Bio-C Temp, à base de silicato de cálcio, e UltraCal XS, à base de hidróxido de cálcio. A composição elementar foi determinada por fluorescência de raios X, enquanto a liberação de íons cálcio e a variação de pH foram avaliadas ao longo de 14 dias. A atividade antimicrobiana foi investigada frente a *Enterococcus faecalis*, *Staphylococcus aureus* e *Candida albicans*. Os efeitos biológicos foram analisados em células mononucleares do sangue periférico humano (PBMCs), por meio da avaliação da viabilidade celular, migração e produção de óxido nítrico sob condições basais e inflamatórias. A composição elementar revelou elevadas porcentagens de agentes radiopacificadores em ambos os materiais, bem como a presença de elementos dopantes, especialmente no Bio-C Temp, que apresentou composição mais heterogênea. O UltraCal XS exibiu maior liberação inicial de íons cálcio e pH mais elevados nos períodos iniciais, enquanto o Bio-C Temp apresentou liberação mais sustentada ao longo do tempo. Ambos mostraram atividade antimicrobiana limitada, predominantemente bacteriostática. Biologicamente, o UltraCal XS manteve elevada viabilidade celular e promoveu supressão consistente da produção de óxido nítrico em condições inflamatórias, enquanto o Bio-C Temp apresentou efeitos dependentes da concentração, com redução da viabilidade e migração celular em altas concentrações, mas recuperação em maiores diluições. O Capítulo 2 teve como objetivo avaliar o comportamento biológico do medicamento intracanal biocerâmico Bio-C Temp em células do ligamento periodontal humano (hPDLSCs), comparativamente ao UltraCal XS. Foram analisados os efeitos dos extratos dos materiais sobre a viabilidade celular, morfologia, migração, proliferação e expressão gênica de citocinas inflamatórias e anti-inflamatórias, sob condições basais e inflamatórias. Os resultados evidenciaram que o Bio-C Temp apresentou comportamento biológico dependente da concentração, com alterações morfológicas, redução da viabilidade e da migração celular em concentrações mais elevadas, enquanto diluições mais altas favoreceram respostas celulares compatíveis com citocompatibilidade. Esses achados reforçam que a interação entre medicamentos intracanais biocerâmicos e células periodontais é influenciada pelas condições de exposição e pelo microambiente inflamatório. De forma geral, os resultados deste trabalho demonstram que medicamentos intracanais à base de hidróxido de cálcio e biocerâmica apresentam comportamentos físico-químicos e biológicos distintos. Enquanto o hidróxido de cálcio se destaca pela rápida alcalinização e estabilidade citobiológica, os materiais biocerâmicos oferecem liberação iônica mais sustentada e respostas biológicas moduladas pela concentração. Esses achados reforçam a importância da seleção criteriosa do medicamento intracanal, considerando o tempo de permanência da medicação no canal, a condição inflamatória periapical e o potencial impacto sobre as células

envolvidas no reparo tecidual, contribuindo para uma abordagem mais biológica e personalizada na terapia endodôntica.

Palavras-chave: Medicamentos intracanais; Hidróxido de cálcio; Biocerâmica; Bio-C Temp; UltraCal XS; Resposta biológica.

ABSTRACT

Intracanal medication is used between the stages of endodontic treatment with the aim of providing a physicochemical barrier, promoting disinfection, controlling microorganisms, reducing inflammation, and assisting tissue healing. Calcium hydroxide is considered the gold standard intracanal medicament due to its alkalinity; however, new intracanal formulations have emerged on the market, including bioceramic-based intracanal medications. In this context, the present work was divided into two chapters and aimed to integratively investigate the physicochemical properties, antimicrobial activity, and biological effects of calcium hydroxide- and bioceramic-based intracanal medications. In Chapter 1, a comparative evaluation of the physicochemical properties, antimicrobial activity, and biological responses of the intracanal medications Bio-C Temp, a calcium silicate–based material, and UltraCal XS, a calcium hydroxide–based material, was performed. Elemental composition was determined by X-ray fluorescence, while calcium ion release and pH variation were evaluated over a 14-day period. Antimicrobial activity was assessed against *Enterococcus faecalis*, *Staphylococcus aureus*, and *Candida albicans*. Biological effects were analyzed in human peripheral blood mononuclear cells (PBMCs) by assessing cell viability, migration, and nitric oxide production under basal and inflammatory conditions. Elemental analysis revealed high percentages of radiopacifying agents in both materials, as well as the presence of dopant elements, particularly in Bio-C Temp, which exhibited a more heterogeneous composition. UltraCal XS showed higher initial calcium ion release and higher pH values at early time points, whereas Bio-C Temp demonstrated a more sustained release over time. Both materials exhibited limited antimicrobial activity, predominantly bacteriostatic. Biologically, UltraCal XS maintained high cell viability and promoted consistent suppression of nitric oxide production under inflammatory conditions, whereas Bio-C Temp exhibited concentration-dependent effects, with reduced cell viability and migration at higher concentrations and recovery at greater dilutions. Chapter 2 aimed to evaluate the biological behavior of the bioceramic intracanal medication Bio-C Temp in human periodontal ligament stem cells (hPDLSCs), in comparison with UltraCal XS. The effects of material extracts on cell viability, morphology, migration, proliferation, and gene expression of pro- and anti-inflammatory cytokines were analyzed under basal and inflammatory conditions. The results demonstrated that Bio-C Temp exhibited concentration-dependent biological behavior, with morphological alterations and reduced cell viability and migration at higher concentrations, while higher dilutions promoted cellular responses compatible with cytocompatibility. These findings reinforce that the interaction between bioceramic intracanal medications and periodontal cells is influenced by exposure conditions and the inflammatory microenvironment. Overall, the results of this study demonstrate that calcium hydroxide- and bioceramic-based intracanal medications present distinct physicochemical and biological behaviors. While calcium hydroxide stands out for its rapid alkalinization and cytobiological stability, bioceramic materials offer more sustained ionic release and biological responses modulated by concentration. These findings highlight the importance of careful selection of intracanal medication, considering the duration of material placement within the root canal, the periapical inflammatory condition, and the potential impact on cells involved in tissue repair, contributing to a more biological and personalized approach to endodontic therapy.

Keywords: Intracanal medications; Calcium hydroxide; Bioceramics; Bio-C Temp; UltraCal XS; Biological response.

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CAPÍTULO 1

Figure 1. Calcium ion release (ppm) and pH variation over time for different dilutions of the intracanal medications UltraCal XS and Bio-C Temp. Calcium release profiles are shown for UltraCal XS (A) and Bio-C Temp (B), with direct comparison between materials at the 1:1 dilution (C). pH variation over time is shown for UltraCal XS (D) and Bio-C Temp (E), with direct comparison between materials at the 1:1 dilution (F). Graphs represent mean ± standard deviation from three independent biological replicates performed in technical triplicates. Calcium levels were measured using a portable calcium ion meter (LaquaTwin Ca^{2+}), and pH measurements were performed using a digital pH meter (Prolab). *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001 represent statistical differences; ns indicates not significant. Statistical analyses were performed using Two-Way ANOVA followed by Dunnett's multiple comparisons test for comparisons within materials (A, B, D, and E) relative to the 1:1 dilution, and Sidak's multiple comparisons test for direct comparisons between materials (C and F).

Figure 2. PBMCs were exposed to extracts of UltraCal XS and Bio-C Temp at different dilutions (1:1, 1:2, 1:4, and 1:16). (A–C) Cytotoxicity assessment by the trypan blue exclusion assay after 24 h of exposure, showing the number of viable cells and cell viability percentage relative to the control (unstimulated PBMCs). (D–F) Cell migration evaluated using a conventional Transwell assay after 4 h of incubation at 37 °C in the presence or absence of material extracts. (C and F) Direct comparison of PBMC viability and migration between UltraCal XS and Bio-C Temp at the 1:1 dilution. Bars represent mean and standard deviation from three biological replicates performed in technical triplicates. **p < 0.01, ***p < 0.001, and ****p < 0.0001 indicate statistical differences by one-way ANOVA followed by Dunnett's multiple comparisons test.

Figure 3. Nitrite production (μM) by PBMCs exposed to intracanal medications. (A) Control group without exposure to intracanal medications in basal, LPS (1 $\mu g.mL^{-1}$), and LPS+IFN-γ (1 $\mu g.mL^{-1}$) conditions. PBMCs were exposed to extracts of UltraCal XS (B-D) and Bio-C Temp (E–G) at different dilutions (1:1, 1:2, 1:4, and 1:16) in different conditions: basal (B and E) stimulated with LPS (C and F) (1 $\mu g.mL^{-1}$) LPS plus IFN-γ (D and G) (1 $\mu g.mL^{-1}$), after 48 h of incubation. Nitrite levels (μM) in the culture supernatants were quantified by the Griess reaction as an indirect indicator of

nitric oxide (NO) production. Graphs represent the mean and standard deviation of three independent biological replicates performed in technical triplicates. *p < 0.05, **p < 0.01, and ****p < 0.0001 represent statistical differences by one-way ANOVA followed by Dunnett's multiple comparisons test.

Supplementary Figure 1. MALDI-ToF (matrix-assisted laser desorption ionization–time of flight) spectra of intracanal medication extracts. (A) Matrix; (B–E) UltraCal XS extracts at dilutions (B) 1:1, (C) 1:2, (D) 1:4, (E) 1:16; (F–I) Bio-C Temp extracts at dilutions (F) 1:1, (G) 1:2, (H) 1:4, (I) 1:16.

CAPÍTULO 2

Figure 1. hPDLSC viability by MTT assay. hPDLSC viability after 24h of incubation with UltraCal XS (A), Bio-C Temp (B) extracts (1:1, 1:2, 1:4 and 1:16). Graphs represent the mean and standard deviation of the absorbance and percentage of cell viability of three biological replicates performed in technical triplicates. *p < 0.05; **p=0.002; ***p=0.0001 and ****p<0.0001, represent statistical difference by one-way ANOVA and Tukey post-test.

Figure 2. Morphological characterization of hPDLSCs exposed to intracanal medications. SEM images show hPDLSCs after exposure to extracts of UltraCal XS (A) and Bio-C Temp (B) at dilutions of 1:1, 1:2, 1:4, and 1:16. Upper rows present lower-magnification views of general cell distribution, and bottom rows show higher-magnification details of membrane projections and cell attachment. Quantitative analysis includes cell size (C, E) and number of cell extensions (D, F). Data represent three biological replicates in technical triplicates. *p < 0.05, **p < 0.01, ***p < 0.001; one-way ANOVA with Dunnett's post hoc test.

Figure 3. Migratory behavior and proliferation of hPDLSCs exposed to UltraCal XS and Bio-C Temp at 1:1, 1:2, 1:4, and 1:16 dilutions, after 24h and 48h. (A) Migration and proliferation of hPDLSCs exposed to UltraCal XS at 1:1, 1:2, 1:4, and 1:16 dilutions. *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001. (B) Migration and proliferation of hPDLSCs exposed to Bio-C Temp at 1:1, 1:2, 1:4, and 1:16 dilutions. **p < 0.01 and ****p < 0.0001. (C) Migration and proliferation of hPDLSCs exposed to UltraCal XS and Bio-C Temp at the 1:1 dilution. **p < 0.0 and ****p < 0.0001 indicate statistical differences by one-way or two-way ANOVA with Tukey post-test. (D) Representative scratch-assay images of control and experimental groups in all tested dilutions at 0h, 24h, and 4 h. Grids indicate the cell-lost area.

Figure 4. *TNF-α, IL-1β, IL-6* and *IL-10* genes expression by hPDLSC. Cells when exposed to UltraCal XS (1:2), and Bio-C Temp (1:2) in different conditions: basal (A, D, G and J), stimulated with LPS (B, E, H and K) (1 μg.mL^{-1}) and LPS plus IFN-γ (C, F, I and L) (1 μg.mL^{-1}), after 24 h of incubation. Graphs represent mean and standard deviation of three biological replicates in technical triplicate. For analysis of *TNF-α* and *IL-1β* expression, the CT of samples that did not show amplification was set at 40. *p<0.05, **p < 0.01, ***p < 0.001 and ****p<0.0001 represent statistical difference verified by two-way ANOVA and Bonferroni post-test.

Supplementary Figure 1. MALDI-ToF (matrix-assisted laser desorption ionization–time of flight) spectra of intracanal medication extracts. (A) Matrix; (B–E) UltraCal XS extracts at dilutions (B) 1:1, (C) 1:2, (D) 1:4, (E) 1:16; (F–I) Bio-C Temp extracts at dilutions (F) 1:1, (G) 1:2, (H) 1:4, (I) 1:16.

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Anexo 1. Parecer consubstanciado do Conselho de Ética em Pesquisa, Faculdade de Ciências da Saúde da Universidade de Brasília - UnB e da Universidade Católica de Brasília – UCB, referente a células mononucleares do sangue periférico (PBMCs).

Anexo 2. Parecer consubstanciado do Conselho de Ética em Pesquisa, Faculdade de Ciências da Saúde da Universidade de Brasília - UnB e da Universidade Católica de Brasília – UCB, referente a células do ligamento periodontal (hPDLSCs).

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Anexo 4. Trabalhos em fase de submissão, elaborados durante o período de mestrado.

LISTA DE ABREVIATURAS E SIGLAS

ANOVA – *analysis of variance* (análise de variância)

ATCC – *American Type Culture Collection*

CAAE – certificado de apresentação para apreciação ética

CBM – concentração bactericida mínima

CFM – concentração fungicida mínima

CHCA – *α-cyano-4-hydroxycinnamic acid* (α-ciano-4-hidroxicinâmico)

CIM – concentração inibitória mínima

CO_2 – dióxido de carbono

Ca – Cálcio

W – Tungstênio

Ti – Titânio

Ba – Bário

Sr – Estrôncio

K – Potássio

Dy – Disprósio

Ge – Germânio

S – Enxofre

Ct – *cycle threshold* (ciclo limiar)

DMEM – *Dulbecco's Modified Eagle Medium* (meio Eagle modificado por Dulbecco)

DMSO – *dimethyl sulfoxide* (dimetilsulfóxido)

DNA – *deoxyribonucleic acid* (ácido desoxirribonucleico)

EDTA – *ethylenediaminetetraacetic acid* (ácido etilenodiamino tetraacético)

GAPDH – *glyceraldehyde-3-phosphate dehydrogenase* (gliceraldeído-3-fosfato desidrogenase)

IFN-γ – *interferon-gamma* (interferon-gama)

IL – *interleukin* (interleucina)

LPS – *lipopolysaccharide* (lipopolissacarídeo)

MALDI – *matrix-assisted laser desorption/ionization* (dessorção/ionização a laser assistida por matriz)

MEV – microscopia eletrônica de varredura

MOPS – *3-(N-morpholino)propanesulfonic acid*

MTT – *3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide* (3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazólio brometo)

NO – *nitric oxide* (óxido nítrico)

PBS – *phosphate-buffered saline* (solução salina tamponada com fosfato)

PBMCs – *peripheral blood mononuclear cells* (células mononucleares do sangue periférico)

PCR – *polymerase chain reaction* (reação em cadeia da polimerase)

qPCR – *quantitative polymerase chain reaction* (reação em cadeia da polimerase quantitativa)

RNA – *ribonucleic acid* (ácido ribonucleico)

ROS – *reactive oxygen species* (espécies reativas de oxigênio)

RPMI – *Roswell Park Memorial Institute medium*

SFB – soro fetal bovino

TCLE – termo de consentimento livre e esclarecido

TNF – *tumor necrosis factor* (fator de necrose tumoral)

TRIzol – *thiocyanate–phenol–chloroform reagent*

UFC – unidades formadoras de colônia

XRF – *X-ray fluorescence* (fluorescência de raios X)

SUMÁRIO

PREFÁCIO

A dissertação de mestrado aqui apresentada dá continuidade os estudos do nosso grupo sobre os materiais biocerâmicos. Já foram publicados, pelo nosso grupo de pesquisa, trabalhos sobre materiais biocerâmicos de reparo e de obturação, os quais apresentaram resposta celular relevante. Com a recente introdução da medicação intracanais biocerâmica na prática endodôntica, decidimos investigar as propriedades físico-químicas e biológicas desses materiais no meu trabalho de mestrado.

Durante minha iniciação científica, tive a oportunidade de participar duas vezes do Programa Institucional de Bolsas de Iniciação em Desenvolvimento Tecnológico e Inovação (PIBITI) como bolsista CNPq. Sob a orientação da Profa. Taia Rezende, na Universidade Católica de Brasília, iniciei meu trabalho no Biodonto com os cimentos biocerâmicos de obturação, analisando a atividade antimicrobiana e a viabilidade celular nos cimentos AH Plus, Bio-C Sealer e Bio-C Sealer Íon+. Foi nesse período que tive meu primeiro contato com laboratório, cultivo celular e com a pesquisa científica. A familiaridade adquirida com os biomateriais ao longo dessa trajetória motivou-me a seguir na linha dos biocerâmicos também no mestrado. No segundo semestre de 2023, concluí minha graduação em Odontologia pela UCB e, no primeiro semestre de 2024, ingressei no Programa de Pós-Graduação em Ciências da Saúde, como bolsista CAPES.

Assim, o conteúdo desta dissertação foi estruturado em consonância com meu aprendizado sobre o tema e as demandas atuais de pesquisa na área. Sua escrita seguiu o formato descrito no documento de Normas para a redação de teses e dissertações do Programa de Pós-graduação em Ciências da Saúde – Universidade de Brasília, sendo escolhido o formato em capítulos, para a apresentação dos elementos textuais. No Capítulo 1, apresento a fase inicial do meu projeto de dissertação, conduzido com células mononucleares do sangue periférico (PBMCs), que foi extremamente desafiador, devido à baixa aderência e sensibilidade celular. Antes do início da coleta sanguínea, o trabalho passou por apreciação ética na Universidade de Brasília e Universidade Católica de Brasília. Nesse capítulo, investiguei a medicação intracanal biocerâmica Bio-C Temp em comparação ao

hidróxido de cálcio UltraCal XS. Inicialmente, realizei os ensaios de atividade antimicrobiana, conduzidos contra duas bactérias e um fungo. Em seguida, realizei os testes de pH ao longo do tempo. Posteriormente, foram realizados os testes de liberação de íons cálcio, os quais contaram com o apoio da Profa. Loise Pedrosa Salles e de sua aluna Luíza Otávio. Por fim, conduzi os ensaios biológicos com PBMCs, avaliando viabilidade celular, migração e produção de óxido nítrico (NO) sob condições basais e inflamatórias. A partir desses experimentos, observei que o Bio-C Temp apresentou baixa viabilidade celular na concentração 1:1. Esse achado despertou a necessidade de compreender melhor a composição do material e suas reações biológicas com outros tipos celulares. Dessa forma, com o apoio da Central Analítica do Instituto de Química da Universidade de Brasília, foi realizada a análise elementar por fluorescência de raios x, aprofundando a caracterização do material. Portanto, a fluorescência de raios x foi conduzida após as análises biológicas com PBMCs e foi diretamente motivada pelos resultados de baixa viabilidade celular. Até aquele momento, a medicação intracanal não havia sido investigada em células do ligamento periodontal e, como nosso grupo já trabalhava com esse tipo celular e eu já possuía experiência com essas células na iniciação científica, desenvolvemos o Capítulo 2.

Assim, o Capítulo 2 foi direcionado às células do ligamento periodontal humano, o que precisou passar novamente pela apreciação no comitê de ética da UnB e UCB. Nesse capítulo, avaliei os efeitos do Bio-C Temp e do UltraCal XS sobre essas células, analisando viabilidade celular, morfologia, migração, proliferação e expressão gênica por qPCR, sob condições basais e inflamatórias. O primeiro experimento foi a avaliação da viabilidade celular, na qual contei com o apoio de colegas do grupo que já trabalhavam com células do ligamento periodontal. Observei maior viabilidade celular em comparação às PBMCs; no entanto, ainda com viabilidade reduzida. Em conversas com a Profa. Taia, decidimos analisar essas células por microscopia eletrônica de varredura para observar seu estado morfológico. Em seguida, os experimentos de proliferação e migração mostraram uma resposta celular diminuída; nas imagens do ensaio de migração, verificou-se aumento da área da lesão (risco), o que me levou a realizar a contagem de área e a análise numérica. Por fim, com o apoio do Prof. Felipe Saldanha e de sua aluna Elizabete, finalizamos o capítulo com o experimento de qPCR.

Diante disso, a realização deste trabalho só foi possível graças ao apoio entre diferentes instituições e colaboradores, cuja contribuição foi fundamental para o desenvolvimento mais integrado e aprofundado deste trabalho. Espero que estes capítulos representem um acréscimo relevante à literatura sobre materiais endodônticos, especialmente no que se refere às medicações intracanais biocerâmicas, contribuindo para uma melhor compreensão de suas interações biológicas e propriedades físico-químicas. Além disso, que os achados aqui apresentados possam auxiliar na tomada de decisão clínica quanto à escolha desses materiais, além de servir de base para o aprimoramento e o desenvolvimento de novas formulações.

INTRODUÇÃO

O ambiente perirradicular constitui um microambiente biológico altamente complexo e dinâmico, formado por diferentes tecidos e populações celulares intimamente relacionadas ao sistema de canais radiculares (MÁRTON; KISS, 2001). A comunicação contínua entre o canal radicular e os tecidos perirradiculares ocorre por meio do forame apical, canais laterais e ramificações acessórias, permitindo que microrganismos e seus subprodutos oriundos de infecções intracanais influenciem diretamente a homeostase perirradicular (NAIR, 2004). Ao romper a homeostase perirradicular, uma resposta imune inflamatória complexa é estabelecida, com a participação coordenada de diferentes tipos celulares da imunidade inata e adaptativa (NAIR, 2004). Leucócitos polimorfonucleares, macrófagos, linfócitos e células mononucleares do sangue periférico (PBMCs) são recrutados para o local da infecção, onde passam a produzir uma ampla gama de mediadores inflamatórios e anti-inflamatórios (KARAMIFAR; TONDRI; SAGHIRI, 2025). O balanço entre esses mediadores é determinante para o direcionamento da resposta biológica, podendo resultar na manutenção da inflamação crônica, com desenvolvimento e progressão das lesões perirradiculares, ou na modulação da resposta inflamatória, favorecendo a resolução do processo infeccioso, a cicatrização e o restabelecimento da integridade dos tecidos locais (NAIR, 2004). Assim, a dinâmica celular e molecular do ambiente perirradicular, estabelecida pelo controle ou não do processo infeccioso, exerce papel central tanto na destruição tecidual, quanto nos processos reparativos e regenerativos.

O controle do processo infeccioso associado às alterações perirradiculares é realizado, primordialmente, por meio do tratamento endodôntico, cujo objetivo é a eliminação ou redução significativa da carga microbiana presente no sistema de canais radiculares (YUE; WANG, 2022). Esse controle é alcançado por meio da limpeza e formatação dos canais, associadas à limpeza químico-mecânica, que visa remover tecidos infectados, biofilmes bacterianos e subprodutos microbianos (CHONG; PITT FORD, 1992). Entretanto, a complexidade anatômica do sistema de canais radiculares e as limitações inerentes aos instrumentos endodônticos dificultam a completa desinfecção do sistema (ARIAS; PETERS, 2022; YOUNG; PARASHOS; MESSER, 2007; ZOU et al., 2024). Nesse contexto, as medicações intracanais atuam

como coadjuvantes importantes, complementando a ação da instrumentação e da irrigação ao manter atividade antimicrobiana residual, neutralizar subprodutos bacterianos e contribuir para a modulação do ambiente inflamatório perirradicular (CHONG; PITT FORD, 1992). Para que sejam clinicamente eficazes, essas medicações devem apresentar propriedades essenciais, como estabilidade físico-química, liberação controlada de íons, ação antimicrobiana adequada e, sobretudo, biocompatibilidade com os tecidos perirradiculares (AHMAD et al., 2022). O equilíbrio entre a eficácia antimicrobiana e a preservação dos tecidos perirradiculares é fundamental para evitar efeitos adversos que possam comprometer os processos de reparo tecidual (SILVEIRA; BUENO; SCHREIBER, 2024).

Neste cenário, o hidróxido de cálcio permanece como padrão-ouro entre as medicações intracanais, sendo o material mais utilizado em abordagens de múltiplas visitas para controle infeccioso do espaço do canal radicular (MANFREDI; FIGINI; GAGLIANI; LODI, 2016). Recomendado pela primeira vez como agente de capeamento pulpar direto por Hermann em 1920, ao longo de um século, o hidróxido de cálcio foi incorporado a diversos materiais odontológicos e formulações antissépticas, incluindo agentes seladores temporários para o canal radicular (USLU et al., 2021). Essa medicação é amplamente utilizada devido à sua biocompatibilidade, baixa solubilidade e difusão controlada, além da capacidade de promover reparo por meio da liberação de íons hidroxila (SIQUEIRA et al., 2009). Essa liberação gera um pH altamente alcalino (cerca de 12,5), capaz de desnaturar proteínas, inativar enzimas bacterianas e danificar membranas celulares microbianas. No entanto, sua eficácia pode ser limitada na presença de certos microrganismos, como o *Enterococcus faecalis* (LIMA et al., 2020), além de limitações físico-químicas, incluindo baixa fluidez dependendo do veículo utilizado e radiopacidade variável (FAVA & SAUNDERS, 1999; TANOMARU-FILHO et al., 2007).

Com o objetivo de superar algumas limitações do hidróxido de cálcio, surgiram novas medicações intracanais, com base biocerâmica, em decorrência dos resultados positivos encontrados com cimentos de reparo e obturadores com esta mesma base. O primeiro material biocerâmico introduzido no mercado foi o agregado de trióxido mineral (MTA), um material de reparo hidráulico à base de silicato de cálcio, que demonstrou excelentes propriedades biológicas, apesar do tempo prolongado de presa e características de manipulação complexas (TORABINEJAD; WHITE, 1995).

Em sequência, cimentos obturadores à base de MTA, como o BioRoot RCS, foram desenvolvidos (CAMPS et al., 2015), que oferecem propriedades físico-químicas aprimoradas e maior bioatividade. Assim, mais recentemente, a base biocerâmica também passou a ser utilizada para desenho de medicações intracanais. Produtos como Bio-C Temp (Angelus, Londrina, Brasil), iRoot FM (Innovative BioCeramix Inc., Vancouver, Canadá) e EndoSequence BC Temp (Brasseler USA, Savannah, EUA) são medicações intracanais à base de silicato de cálcio desenvolvidos para promover maior bioatividade no sistema de canais radiculares.

Em virtude da recente inserção no mercado, ainda são limitados os estudos que avaliam o comportamento biológico das medicações intracanais biocerâmicas, especialmente considerando os limitados testes pré-clínicos e a necessidade de análises pós-mercado. Estudos *in vitro* demonstraram que a citotoxicidade do Bio-C Temp (Angelus) é dependente da dose e do tempo de exposição, com concentrações mais altas apresentando efeitos moderados a fortes, enquanto concentrações menores apresentam citotoxicidade leve em linhagens celulares VERO (VILLA et al., 2020). Ensaios com células osteoblásticas Saos-2 indicaram que, na diluição 1:2, o Bio-C Temp (Angelus) apresentou viabilidade celular reduzida. Entretanto, o material foi capaz de aumentar a atividade de fosfatase alcalina e a formação de nódulos mineralizados ao longo do tempo, indicando potencial efeito bioativo mesmo na presença de citotoxicidade inicial (GUERREIRO et al., 2021). Em consonância com esses achados, estudos conduzidos com células da polpa dentária humana (hDPCs) evidenciaram que, em concentrações menos diluídas (1:2), o Bio-C Temp (Angelus) exibiu viabilidade celular significativamente inferior à dos materiais a base de hidróxido de cálcio, além de uma redução temporal na viabilidade entre 24 e 72 horas (BENETTI et al., 2024). Por outro lado, o iRoot FM (Innovative Bioceramix Inc.) apresentou efeito positivo na proliferação de células-tronco da papila apical (SCAP), com aumento significativo em baixas concentrações e desempenho superior ao hidróxido de cálcio e à pasta tripla antibiótica (BI et al., 2018). Estudos *in vivo* em tecido subcutâneo de ratos demonstraram que o Bio-C Temp (Angelus) induziu uma resposta inflamatória inicial, com presença de macrófagos e linfócitos, e áreas necróticas reduzidas ao longo do tempo, sugerindo capacidade de regeneração tecidual superior à pasta de hidróxido de cálcio (LOPES et al., 2022a; LOPES et al., 2024b). Comparações com outros materiais, como Calcipex Plane II e Vitapex, indicaram formação de

precipitados calcíticos e presença de cálcio e fósforo nos tecidos adjacentes, embora as áreas calcificadas fossem menores na presença do Bio-C Temp (Angelus) (EDANAMI et al., 2023).

Apesar das medicações intracanais serem aplicadas no interior do sistema de canais radiculares, a presença de canais laterais, ramificações apicais e foraminas acessórias favorece a difusão desses materiais para os tecidos perirradiculares (ARIAS; PETERS,2022; YOUNG; PARASHOS; MESSER, 2007; ZOU et al., 2024). Além disso, o extravasamento, intencional ou não, durante a aplicação clínica pode resultar no contato direto dessas substâncias com o ligamento periodontal e o osso periapical (DANIEL, 2025; THANVI et al., 2025), podendo impactar diretamente nos processos de cicatrização da lesão perirradicular. Diante desse contexto, esta dissertação de mestrado teve como objetivo geral avaliar, de forma integrada, as propriedades físico-químicas, a atividade antimicrobiana e as respostas biológicas de medicações intracanais utilizadas no tratamento endodôntico. A pesquisa foi estruturada em dois artigos científicos complementares, nos quais um material à base de hidróxido de cálcio (Ultracal XS, Ultradent, South Jordan, EUA) e outro a base de biocerâmico (Bio-C Temp, Angelus) foram comparados quanto ao seu comportamento frente a microrganismos endodônticos e a células envolvidas nos processos inflamatórios e reparativos do ambiente perirradicular, utilizando modelos *in vitro* que simulam condições clinicamente relevantes.

OBJETIVOS

Objetivo Geral

Avaliar as propriedades físico-químicas, atividade antimicrobiana e o comportamento biológico da medicação intracanal biocerâmica Bio-C Temp (Angelus), comparando-a com a medicação a base de hidróxido de cálcio, Ultracal XS (Ultradent), na presença de células mononucleares do sangue periférico e do ligamento periodontal humano.

Objetivos específicos

1. Analisar a composição elementar das medicações intracanais Bio-C Temp (Angelus) e Ultracal XS (Ultradent) por fluorescência de raios-X (XRF).
2. Avaliar a liberação de íons cálcio e o pH das medicações intracanais Bio-C Temp (Angelus) e Ultracal XS (Ultradent), em diferentes períodos de exposição.
3. Estabelecer a concentração inibitória mínima (CIM) do Bio-C Temp e UltraCal XS ou percentual de inibição do crescimento contra as bactérias *Enterococcus faecalis, Staphylococcus aureus* e o fungo *Candida albicans.*
4. Investigar a viabilidade de PBMCs em contato com as medicações intracanais Bio-C Temp (Angelus) e Ultracal XS (Ultradent), pelo ensaio de exclusão do Azul de Tripan.
5. Avaliar a migração das PBMCs após exposição às medicações intracanais Bio-C Temp (Angelus) e Ultracal XS (Ultradent), utilizando placas tipo *Transwell*.
6. Determinar a produção de óxido nítrico (NO) pelas PBMCs na presença das medicações intracanais Bio-C Temp (Angelus) e Ultracal XS (Ultradent), pelo ensaio de Griess, utilizando diferentes estímulos (LPS e LPS em conjunto com IFN-γ).

7. Avaliar a viabilidade de células-tronco do ligamento periodontal (hPDLSCs) em contato com as medicações intracanais Bio-C Temp (Angelus) e Ultracal XS (Ultradent), por meio do ensaio MTT.
8. Analisar a morfologia das hPDLSCs em contato com as medicações intracanais Bio-C Temp (Angelus) e Ultracal XS (Ultradent), por meio da microscopia eletrônica de varredura (MEV).
9. Avaliar a migração das hPDLSCs após exposição às medicações intracanais Bio-C Temp (Angelus) e Ultracal XS (Ultradent), utilizando o ensaio de *Scratch/*Arranhão.
10. Avaliar a proliferação das hPDLSCs após exposição às medicações intracanais Bio-C Temp (Angelus) e Ultracal XS (Ultradent), pela contagem celular com corante Azul de Tripan, em diferentes períodos.
11. Determinar a expressão gênica das hPDLSCs por qPCR em resposta às medicações intracanais Bio-C Temp (Angelus) e Ultracal XS (Ultradent), utilizando diferentes estímulos (LPS e LPS em conjunto com IFN-γ).

MATERIAIS E MÉTODOS

Delineamento Experimental

O presente estudo foi estruturado em dois capítulos. O primeiro capítulo envolveu a caracterização físico-química e análise biológica em células mononucleares do sangue periférico humano (PBMCs), dos biomateriais intracanais Bio-C Temp (Angelus) e UltraCal XS (Ultradent). Inicialmente, foi realizada a análise por fluorescência de raios X (XRF) para a determinação da composição elementar dos materiais. Em seguida, foram conduzidos os ensaios de liberação de íons cálcio e de avaliação do pH de extratos preparados com as medicações intracanais, seguida da análise antimicrobiana destes extratos. Após essa etapa, foram realizadas as análises biológicas utilizando PBMCs, nas quais foram avaliadas a viabilidade celular, migração e a produção de óxido nítrico (NO) por meio do ensaio de Griess, sob condições basais e inflamatórias induzidas. O segundo capítulo consistiu na avaliação dos efeitos biológicos das medicações intracanais em células do ligamento periodontal humano. Foram avaliadas a viabilidade celular, a capacidade proliferativa, a migração celular (ensaio de cicatrização de ferida) e a expressão gênica por meio da reação em cadeia da polimerase (PCR), envolvendo marcadores associados à inflamação e ao processo de regeneração periodontal. Esses dois capítulos permitiram investigar de forma integrada o impacto das medicações intracanais sobre células diretamente envolvidas na cicatrização periapical, no contexto de extravasamento das medicações intracanais (Figura 1). As análises estatísticas foram comuns aos dois trabalhos e assim, descritas ao final desta seção.

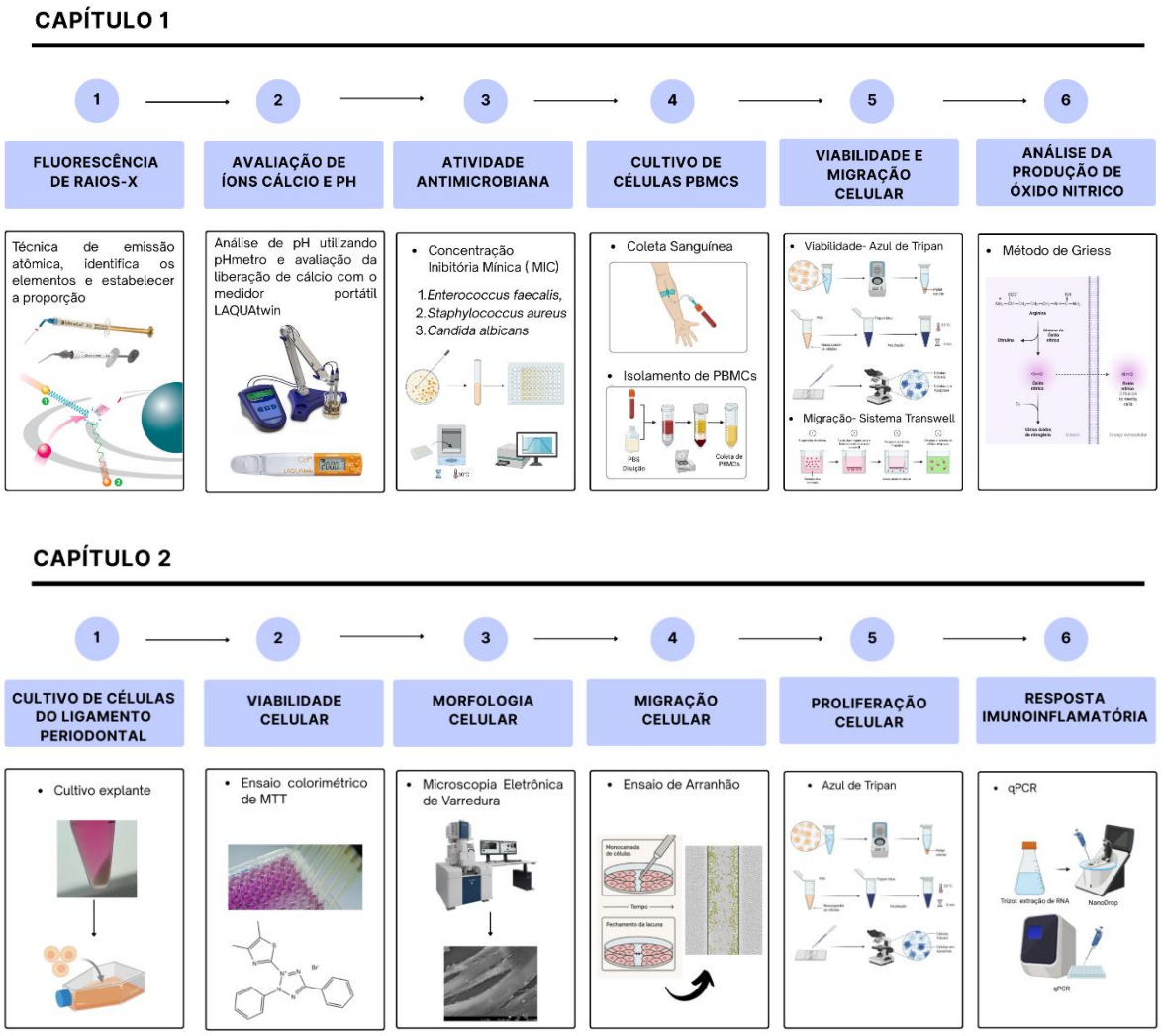

Figura 1. Fluxograma representativo dos dois capítulos desta dissertação.

Capítulo 1

Análise por Fluorescência de Raios X (XRF)

A composição elementar do Bio-C Temp (Angelus) e do UltraCal XS (Ultradent) foi analisada por fluorescência de raios X (XRF). Amostras de cada material foram colocadas no suporte de amostras para obtenção de uma superfície plana e uniforme antes das medições. As análises de XRF foram realizadas em um espectrômetro de bancada EDX-720 (Shimadzu Corporation, Kyoto, Japão), operado em atmosfera de ar ambiente, com colimador de 5 mm. Não foi aplicada rotação das amostras. Foram utilizadas duas condições de excitação, conforme o protocolo padrão do equipamento:

(1) ânodo de ródio, 50 kV, 75 μA (auto), faixa de aquisição de 0–40 keV, para elementos de médio a alto peso atômico; e (2) ânodo de ródio, 15 kV, 604 μA (auto), faixa de aquisição de 0–20 keV, para elementos de baixo peso atômico (Na–Sc). Cada condição foi adquirida por 99 segundos de tempo real, mantendo o tempo morto do detector em aproximadamente 39%. Os dados espectrais foram processados com o *software* EDX-Navi (Shimadzu Corporation) para identificação dos picos de emissão característicos e determinação da composição elementar relativa de cada material. As análises foram realizadas em amostras preparadas sobre filme de polipropileno (espessura de 5 μm) (Preoteasa e Ciortea, 2002).

Extratos das medicações intracanais

Os extratos de Bio-C Temp (Angelus) e UltraCal XS (Ultradent) foram preparados de acordo com as recomendações da ISO 10993-5 (ISO 10993-5, 2009) e utilizados nas metodologias a seguir. De cada material, 40 mg foram pesados e transferidos para tubos cônicos estéreis (Prolab, São Paulo, Brasil), aos quais foram adicionados 10 mL de meio de cultura DMEM (Gibco, Grand Island, EUA). As suspensões foram imediatamente homogeneizadas e, em seguida, filtradas por filtros de membrana de 0,22 μm (Sigma-Aldrich, St. Louis, EUA) para remoção de resíduos particulados. Após a filtração, foram preparadas diluições seriadas de cada extrato – 1:1, 1:2, 1:4 e 1:16 – utilizadas em todos os experimentos. Para verificar qualitativamente a presença de componentes derivados dos materiais nos extratos empregados nos ensaios antimicrobianos e biológicos, foi realizada espectrometria de massas por dessorção/ionização a laser assistida por matriz – tempo de voo (MALDI-TOF MS) como controle analítico. Alíquotas de cada extrato foram misturadas com solução saturada de matriz α-ciano-4-hidroxicinâmico (CHCA) preparada em 50% acetonitrila (Sigma) e 0,1% ácido trifluoroacético (Sigma), e aplicadas em placa-alvo de aço inoxidável. Os espectros foram adquiridos em espectrômetro de massas MALDI-TOF (Microflex LT, Bruker Daltonics, Bremen, Alemanha). Essa análise foi realizada para confirmar a presença de componentes solúveis liberados pelas medicações intracanais nos extratos antes dos ensaios subsequentes (Figura Suplementar 1 – apresentada nos capítulos 1 e 2 da dissertação).

Liberação de íons cálcio (Ca^{2+}) e avaliação de pH

A liberação de íons cálcio e o pH foram avaliados nas mesmas placas experimentais. A concentração de cálcio (ppm) foi mensurada com um medidor portátil de íons cálcio (LAQUAtwin Ca-11, HORIBA, São Paulo, Brasil), utilizando alíquotas de 500 µL dos extratos de Bio-C Temp (Angelus) e UltraCal XS (Ultradent) nas diluições 1:1, 1:2, 1:4 e 1:16, nos períodos de 0, 1, 3 e 24 h e aos 3, 4, 7 e 14 dias, conforme instruções do fabricante. O pH foi determinado nos mesmos tempos experimentais utilizando um pHmetro digital (PH-009(I)A, Mold & Bacteria Laboratories Store, Mississauga, Canadá).

Atividade antimicrobiana

A atividade antimicrobiana dos extratos de Bio-C Temp (Angelus) e UltraCal XS (Ultradent) foi avaliada por ensaio de inibição de crescimento (Concentração Inibitória Mínima – CIM), baseado no protocolo do *Clinical and Laboratory Standards Institute*, com adaptações (Wiegand et al., 2008). Os extratos de ambas as medicações intracanais foram preparados nas diluições 1:1, 1:2, 1:4 e 1:16 e testados contra *E. faecalis* (ATCC 19433), *S. aureus* (ATCC 25923) e *C. albicans* (ATCC 10231). Para *E. faecalis* e *S. aureus*, os bioensaios foram realizados em meio Mueller Hinton (Himedia, Mumbai, Índia), utilizando inóculo de 5×10^5 UFC/mL na fase logarítmica de crescimento. As placas foram incubadas por 12 h a 37 °C sob agitação moderada. O ensaio antifúngico para *C. albicans* foi realizado em meio RPMI 1640 (câmara de Neubauer) suplementado com MOPS (Sigma - 0,165 $mol \cdot L^{-1}$), utilizando inóculo inicial de $2,5 \times 10^3$ UFC/mL na fase logarítmica. As placas foram incubadas por 48 h a 30 °C. A atividade antibacteriana e antifúngica foi determinada por inibição total ou percentual de crescimento em comparação com os controles experimentais. Nos casos de inibição de 100%, os efeitos bactericidas ou fungicidas foram confirmados por semeadura de todas as concentrações em meios sólidos (determinação de CBM/CFM). Para os grupos que não atingiram a CIM, o percentual de inibição foi calculado tomando o controle positivo como referência de 100% de crescimento, permitindo estimar o efeito relativo de cada concentração testada.

Isolamento de células mononucleares de sangue periférico humano

O sangue venoso foi coletado por punção venosa a vácuo, após aprovação dos Comitês de Ética em Pesquisa (CAAE: 79508624.5.3001.0029; 79508624.5.0000.0030, anexo 1). As PBMCs humanas foram isoladas por centrifugação em gradiente de densidade utilizando Histopaque-1077 (Sigma). Resumidamente, 16 mL de sangue total foram depositados sobre 16 mL de Histopaque-1077 (Sigma) à temperatura ambiente e centrifugados (400 g, 30 min, 20 °C, sem freio). A camada superior de plasma foi descartada, permanecendo somente a interface contendo linfócitos, monócitos e plaquetas, que foi coletada e transferida para novo tubo cônico. Em seguida, as células foram diluídas 1:4 em PBS e novamente centrifugadas (400 g, 30 min, 20 °C, sem freio). O sobrenadante foi descartado e as células lavadas com 10 mL de PBS, seguidas de centrifugação (250 g, 10 min, 20 °C, sem freio). Finalmente, o *pellet* celular foi ressuspendido em 10 mL de meio DMEM (Gibco) suplementado com 10% de soro fetal bovino (Gibco), 0,5% de aminoácidos MEM (Sigma), 0,05% gentamicina (Sigma), 0,5% L-glutamina (Prado Laboratórios, Curitiba, Brasil) e 0,5% penicilina/estreptomicina (Prado Laboratórios) (1000 $U \cdot mL^{-1}$) (Ulmer et al., 1984).

Viabilidade de células PBMCs

A viabilidade celular foi avaliada após 24 h de incubação das placas (Prolab) contendo células ($1x10^4$ células/mL) expostas aos extratos de Bio-C Temp (Angelus) e UltraCal XS (Ultradent) nas diluições 1:1, 1:2, 1:4 e 1:16, sob 5% de CO_2, 37 °C e 95% de umidade. O controle positivo consistiu em poços em triplicata contendo células apenas em meio de cultura (100% de viabilidade), enquanto o controle negativo consistiu em poços em triplicata incubados em solução de lise - 10 mM Tris (Sigma), 1 mM EDTA (SCQ Soluções Laboratoriais, São Paulo, Brasil), 0,1% Triton X-100 (Sigma), pH 7,4 - representando 0% de viabilidade. Após o período de incubação, as células foram ressuspendidas e adicionou-se solução de azul de tripan a 0,4% (Sigma) por 1 minuto. Células vivas e mortas foram contadas imediatamente após o período experimental de 24 h (Crowley et al., 2016).

Migração de células PBMCs

O ensaio de migração celular foi realizado utilizando placas *Transwell* de 24 poços com membranas de poros de 5 µm (Sigma). Os extratos de Bio-C Temp (Angelus) e UltraCal XS (Ultradent) foram testados nas diluições 1:1, 1:2, 1:4 e 1:16. Uma suspensão celular contendo 1×10^6 células/mL foi preparada, e 100 µL dessa suspensão foram cuidadosamente pipetados sobre a membrana de cada inserto. As placas foram incubadas a 37 °C e 5% de CO_2 por 10 minutos para estabilização celular. Em seguida, 600 µL de cada extrato foram adicionados ao compartimento inferior de cada poço. As placas foram novamente incubadas a 37 °C e 5% de CO_2 por 4 h, permitindo a migração das células através da membrana. Após a incubação, os insertos foram removidos, e os extratos presentes no compartimento inferior foram coletados para quantificação. As células migradas foram quantificadas por contagem direta em câmara de Neubauer (Prolab) sob microscópio óptico, sendo o número total expresso em células/mL (Justus et al., 2014).

Produção de óxido nítrico

A produção de NO foi mensurada pelo método descrito por Green et al. (1982), com modificações. As PBMCs foram expostas aos extratos de UltraCal XS (Ultradent) e Bio-C Temp (Angelus) nas diluições 1:1, 1:2, 1:4 e 1:16 em três condições experimentais: basal, estimulada com LPS (Sigma, 1 $\mu g \cdot mL^{-1}$) e estimulada com LPS mais IFN-γ (Sigma, 1 $\mu g \cdot mL^{-1}$). Após 48 h de incubação, 100 µL do sobrenadante de cultura de cada poço foram coletados e transferidos para placas de 96 poços (Techno Plastic Products, Trasadingen, Suíça), e 100 µL de DMEM (Gibco) foram adicionados aos poços utilizados para a curva padrão de nitrito de sódio. Em seguida, foram adicionados 100 µL de uma solução composta por 1% de sulfanilamida em ácido fosfórico 2,5% (Vetec, Rio de Janeiro, Brasil) e 1% de naftiletilenodiamina em ácido fosfórico 2,5% (razão 1:1) (Sigma). Após 10 minutos de incubação, a absorbância foi medida a 490 nm em leitor de microplacas (BioTek Instruments, Winooski, EUA). A concentração de nitrito foi determinada com base na equação derivada da curva padrão de nitrito de sódio (1,5625 µM a 200 µM) (Green et al., 1982).

Capítulo 2

Cultura primária de células do ligamento periodontal

Foram utilizados terceiros molares doados após exodontia, com aprovação do Comitê de Ética da Universidade de Brasília e Universidade Católica de Brasília (CAAE: 91049325.6.0000.0030 e 91049325.6.3001.0029 – anexo 2). Após a extração, os dentes foram armazenados em 10 mL de DMEM (Gibco) contendo 200 µL de anfotericina B (Sigma) e 10 µL de gentamicina (Sigma) (Huang et al., 2006). As células do ligamento periodontal humano (hPDLSCs) foram obtidas por raspagem radicular do dente extraído. Em seguida, o ligamento periodontal foi transferido para placas de 6 poços (Techno Plastic Products) e cultivado em meio DMEM (Gibco), suplementado com 50 $U.mL^{-1}$ de penicilina (Gibco), 50 $\mu g.mL^{-1}$ de estreptomicina (Gibco) e 20% de soro fetal bovino (SFB) (Cultilab, Campinas, São Paulo, Brasil). As culturas foram incubadas em atmosfera umidificada com 5% de CO_2 (Pedrosa et al., 2022). Após confluência, a monocamada celular foi recuperada com 300 µL de solução de tripsina 0,5% (p/v) (Sigma) e 10 mM de EDTA (Sigma), por 2 minutos, a 37 °C. Em seguida, as células foram centrifugadas a 1.500 rpm por 5 minutos e transferidas para frascos menores contendo DMEM (Gibco) suplementado com 2 nM de glutamina (Gibco), 50 $U.mL^{-1}$ penicilina (Gibco), 50 $\mu g.mL^{-1}$ estreptomicina (Gibco) e 10% SFB (Cultilab) (Al-Habib et al., 2019). O preparo dos extratos das medicações intracanais utilizados neste capítulo seguiu o mesmo procedimento descrito no Capítulo 1.

Viabilidade de células hPDLSCs

A citotoxicidade dos cimentos foi avaliada em cultura de hPDLSCs. Inicialmente, hPDLSCs (1×10^4 células.mL^{-1}) foram cultivadas em placas de 96 poços (Techno Plastic Products), por 24 h em incubadora de CO_2 a 37 °C. Após esse período, 200 µL do sobrenadante celular foram substituídos pelos extratos dos materiais e as placas foram novamente incubadas a 37 °C por 24 h. Após a incubação, foi realizado o ensaio MTT (3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazólio brometo), e as placas foram lidas em 570 nm (Loosdrecht et al., 1991).

Morfologia celular de hPDLSCs

As células (2 × 10^5 células por poço) foram expostas aos extratos das medicações intracanais Bio-C Temp e UltraCal XS nas diluições 1:1, 1:2, 1:4 e 1:16. As células foram semeadas em placas de cultura de 6 poços (Kasvi) contendo meio DMEM (Gibco). Lâminas de cobertura de vidro redondas (13 mm × 13 mm; Fisher Scientific, Suwanee, Georgia, EUA) foram posicionadas no fundo dos poços antes da semeadura celular. Após 24 horas de exposição, as lâminas foram fixadas em solução de Karnovsky 0,1 M (2% glutaraldeído e paraformaldeído) por 24 horas e lavadas duas vezes com tampão cacodilato de sódio 0,1 M. Em seguida, as células foram pós-fixadas em tetróxido de ósmio a 1% (Sigma) por 30 minutos e lavadas duas vezes com água destilada. A desidratação foi realizada com soluções de acetona a 50%, 70%, 90% e duas vezes a 100%. Após a secagem ao ponto crítico, as lâminas foram metalizadas com ouro e analisadas em microscópio eletrônico de varredura (JSM 7001F, Jeol). As imagens foram obtidas com ampliações de 300×, 1500× e 5000×. Para a análise quantitativa, o número de células aderidas foi contado em imagens adquiridas a 300×, em três campos distintos para cada condição experimental, garantindo adequada confiabilidade estatística. O *software ImageJ* (NIH, Bethesda, MD) foi utilizado para a contagem celular.

Migração e proliferação de hPDLSCs

A avaliação da migração celular foi realizada utilizando o método de *scratch* (He et al., 2022). As células (2,5 × 10^5 células por poço) foram cultivadas em placas de 6 poços (Techno Plastic Products) contendo DMEM (Gibco) suplementado com 100 $\mu g.mL^{-1}$ penicilina (Gibco), 100 $\mu g.mL^{-1}$ estreptomicina (Gibco) e 10% SFB (Cultilab) até formação da monocamada celular confluente. Em seguida, uma área de desaderência celular artificial foi produzida na superfície com ponteira de micropipeta de 1000 µL. As células remanescentes foram lavadas e adicionou-se novo meio DMEM (Gibco) contendo os extratos de Bio-C Temp e UltraCal XS (1:1, 1:2, 1:4 e 1:16). As culturas foram monitoradas até 48 h, com imagens registradas em 0, 24 e 48 h. As imagens foram processadas e as células presentes na área de ferida foram contadas com auxílio do *software ImageJ* (NIH, Bethesda, MD). O potencial proliferativo das hPDLSCs foi avaliado pela técnica de exclusão com Azul de Tripan

(MP Biomedicals, Irvine, EUA) (Cheng et al., 2017). As células (1 × 10^4) foram incubadas com os extratos por 24 e 48 h; em seguida, foram ressuspensas, e adicionou-se solução de Azul de Tripan (MP Biomedicals) 0,4% por 1 minuto. A contagem celular foi realizada imediatamente e comparada ao número inicial de células.

Expressão gênica de citocinas em hPDLSCs

A expressão de *TNF-α, IL-1β, IL-6* e *IL-10* por hPDLSCs incubadas com os extratos das medicações intracanais foi avaliada por PCR em tempo real. Para estabelecer as diferentes condições de cultura *in vitro*, estímulos adicionais foram utilizados: LPS (1 μg.mL^{-1}) (lipopolissacarídeos de *Escherichia coli*, Sigma), IFN-γ (1 μg.mL^{-1}) (Interferon-gama, Sigma) e a combinação de LPS mais IFN-γ por 24 h. Após a incubação celular, o RNA total foi extraído utilizando o método TRIzol™ (ThermoFisher Scientific, Califórnia, EUA) (Xu et al., 2021). A quantificação do RNA foi realizada no equipamento *NanoDrop One*® (ThermoFisher Scientific). O DNA complementar (cDNA) foi sintetizado utilizando o *High-Capacity Reverse Transcription Kit*, seguindo as instruções do fabricante (Thermo SCD 500 Scientific, Califórnia, EUA). A análise da expressão gênica foi então realizada por PCR em tempo real utilizando o sistema *StepOne Plus™ Real-Time PCR System* (ThermoFisher Scientific), com o reagente *SYBR™ Green PCR Master Mix* (ThermoFisher Scientific) para avaliação de genes envolvidos em respostas inflamatórias e anti-inflamatórias. Especificamente, os níveis de expressão de *TNF-α, IL-1β, IL-6* e *IL-10* foram analisados utilizando o gene *GAPDH* como controle endógeno. As sequências dos primers estão apresentadas na Tabela 1. Todas as reações foram realizadas em duplicata, e o valor de variação relativa de expressão foi obtido pelo método $2^{-\Delta\Delta Ct}$ (Pfaffl, 2001). Para normalizar a carga das amostras, as diferenças nos ciclos limiares (ΔCt) foram obtidas subtraindo-se o valor de Ct do gene endógeno (GAPDH) dos valores de Ct dos genes avaliados. A mediana dos valores de Ct das amostras controle foi utilizada como referência.

GENE	SEQUÊNCIA DO *PRIMER (5'–3')*
TNF-α	Direto: CACAGTGAAGTGCTGGAAAC
	Reverso: GATCAAAGCTGTAGGCCCA
IL-1β	Direto: GCACACAGGCTCCGAGATGAA
	Reverso: GTGCTTGGTCCGTCTTTCT
IL-6	Direto: CCCACAGGAACGTGAAAAGTCA
	Reverso: TGCAAAGTCTCAGGATCTCT
IL-10	Direto: TAAGGGTTTACTTGGGTTGCCA
	Reverso: TCTGGGCCATGGTGGTTTCC
GAPDH	Direto: AGTGGCAAAGTGGAGATT
	Reverso: GTGGAGTCATACTGGAACA

Tabela 1. Sequência dos *primers* para cada gene utilizado no ensaio de PCR.

Análises estatísticas

Todos os experimentos realizados nos capítulos 1 e 2 foram realizados em três réplicas técnicas e repetidos em três experimentos biológicos independentes, totalizando n=9/grupo. A normalidade da distribuição dos dados foi verificada pelo teste de Shapiro–Wilk. Os resultados foram expressos como média e desvio-padrão. Para comparações entre os grupos, aplicou-se ANOVA de uma via ou de duas vias, de acordo com o delineamento experimental. Quando foram detectadas diferenças estatisticamente significativas, realizou-se o pós-teste de comparações múltiplas de Tukey (para ANOVA de uma via) e o pós-teste de Dunnett (para ANOVA de duas vias). Um nível de significância de $p < 0,05$ foi adotado para todas as análises. O mesmo modelo de análise estatística foi empregado em todos os experimentos deste estudo.

CAPÍTULO 1 (Intended submission: Journal of Endodontics; IF=3,6)

Physicochemical Properties and Biological Responses to Bioceramic and Calcium Hydroxide–Based Intracanal Medicaments

ABSTRACT

Introduction: Intracanal medicaments support chemomechanical preparation by controlling microbes and modulating periapical inflammation. While calcium hydroxide–based formulations remain common, calcium silicate–based bioceramics provide sustained alkalinity, calcium release, and bioactivity. This study compared the physicochemical, antimicrobial, and biological properties of Bio-C Temp (bioceramic) and UltraCal XS (calcium hydroxide). **Methodology:** Elemental composition was analyzed by X-ray fluorescence. Calcium release and pH were measured over 14 days. Antimicrobial activity against *Enterococcus faecalis*, *Staphylococcus aureus*, and *Candida albicans* was assessed via minimum inhibitory, bactericidal, and fungicidal concentration assays. Biological effects on human peripheral blood mononuclear cells were evaluated through cell viability, migration, and nitric oxide (NO) production under basal and inflammatory conditions (lipopolysaccharide and lipopolysaccharide plus interferon-γ). **Results:** Both materials presented a high calcium content, but Bio-C Temp exhibited a more heterogeneous formulation with dopant radiopacifier elements. UltraCal XS released calcium faster and reached higher initial alkalinity, whereas Bio-C Temp showed a more sustained release profile. Antimicrobial activity was limited and mainly bacteriostatic. UltraCal XS maintained cell viability across all dilutions, while Bio-C Temp reduced viability and migration at higher concentrations, with recovery at lower dilutions. UltraCal XS consistently reduced nitrite production under LPS and LPS plus IFN-γ stimulation, whereas Bio-C Temp showed a variable, concentration-dependent modulation of nitric oxide levels. **Conclusions:** Bio-C Temp and UltraCal XS exhibit distinct physicochemical and biological behaviors. Both maintain alkalinity and limited antimicrobial effects, but their NO production differs in a concentration-dependent manner, supporting complementary use as intracanal medicaments.

Keywords: Intracanal medicaments; Calcium hydroxide; Bioceramic material; Bio-C Temp; UltraCal XS; Peripheral blood mononuclear cells.

INTRODUCTION

Intracanal medicaments are widely used as adjuncts to chemomechanical preparation to enhance the disinfection of the root canal system and to modulate the periapical inflammatory environment (1). Among these, calcium hydroxide has long been considered the intracanal medicament of choice due to its antimicrobial properties, high alkalinity, and ability to stimulate mineralized tissue formation (2,3,4). However, the antimicrobial effectiveness of calcium hydroxide may be limited by dentin buffering, low solubility, and interactions with organic tissue remnants and microbial by-products within the root canal system (5).

In recent years, the development of calcium silicate–based bioceramic materials has expanded the range of intracanal medications available in endodontics (6). These materials have been designed to provide sustained alkaline conditions, controlled calcium ion release, and bioactive behavior, potentially influencing not only microbial control but also host tissue responses (6,7). Because intracanal medications may diffuse through accessory canals or be inadvertently extruded beyond the apical foramen, their physicochemical characteristics and biological effects on host cells are clinically relevant (7). While previous studies have evaluated the antimicrobial activity and cytocompatibility of both calcium hydroxide–based and bioceramic intracanal medications, available evidence remains fragmented, often focusing on isolated biological endpoints or single material classes (7). Moreover, limited information is available regarding the immunomodulatory effects of these materials on human immune cells under inflammatory conditions, despite the central role of immune-mediated responses in periapical pathogenesis and healing.

In this context, Bio-C Temp (Angelus, Londrina, Brazil) is a calcium silicate–based bioceramic intracanal medicament that has gained clinical popularity due to its handling properties and reported alkalinity (8,5). However, comprehensive data integrating its elemental composition, antimicrobial behavior against endodontically relevant microorganisms, and immunobiological effects on human immune cells remain scarce. Therefore, the aim of this study was to comparatively evaluate the physicochemical properties, antimicrobial activity, and biological effects of the bioceramic intracanal medicament Bio-C Temp (Angelus) and a calcium hydroxide–based formulation (UltraCal XS) (Ultradent Products Inc., South Jordan, USA).

Elemental composition, calcium ion release, and pH variation were analyzed; antimicrobial activity was assessed against microorganisms associated with persistent endodontic infections; and biological responses were investigated using human peripheral blood mononuclear cells (PBMCs), focusing on cell viability, migration, and nitric oxide production under basal and inflammatory conditions.

MATERIALS AND METHODS

X-ray Fluorescence (XRF) Analysis

The elemental composition of Bio-C Temp (Angelus) and UltraCal XS (Ultradent) was analyzed by X-ray fluorescence (XRF). Samples of each material were placed on the sample holder to obtain a flat and uniform surface prior to measurement. XRF analyses were performed using an EDX-720 benchtop spectrometer (Shimadzu), operated under ambient air atmosphere with a 5 mm collimator. No sample spinning was applied. Two excitation conditions were used according to the instrument's standard measurement protocol: (1) Rh anode, 50 kV, 75 μA (auto), acquisition range 0–40 keV, for medium-to-high atomic weight elements; and (2) Rh anode, 15 kV, 604 μA (auto), acquisition range 0–20 keV, for low atomic weight elements (Na–Sc). Each condition was acquired for 99 seconds of live time, with detector dead time maintained at approximately 39%. Spectral data were processed using the manufacturer's proprietary software to identify the characteristic emission peaks and determine the relative elemental composition of each material. Analyses were performed on samples prepared on polypropylene film (5 μm thickness) (9).

Extracts of intracanal medications

Extracts of Bio-C Temp (Angelus) and UltraCal XS (Ultradent) were prepared according to ISO 10993-5 recommendations (ISO 10993-5, 2009). For each material, 40 mg was weighed and transferred to sterile tubes, followed by the addition of 10 mL of DMEM culture medium (Gibco, USA). The suspensions were immediately mixed and then filtered using 0.22 μm membrane filters to remove particulate residues. After filtration, serial dilutions of each extract were prepared - 1:1, 1:2, 1:4, and 1:16 - which

were used in all experiments. To qualitatively verify the presence of material-derived components in the extracts used for antimicrobial and biological assays, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was performed as an analytical control. Aliquots of each extract were mixed with a saturated solution of α-cyano-4-hydroxycinnamic acid (CHCA) matrix prepared in 50% acetonitrile and 0.1% trifluoroacetic acid, and spotted onto a stainless-steel target plate. Spectra were acquired using a MALDI-TOF mass spectrometer (Microflex LT, Bruker Daltonics, Bremen, Germany). This analysis was conducted to confirm the presence of soluble components released from the intracanal medications into the extracts prior to subsequent assays (Supplementary Figure 1).

Calcium Ion (Ca^{2+}) Release and pH Evaluation

Calcium ion release and pH were assessed using the same experimental plates. Calcium concentration (ppm) was measured with a portable calcium ion meter (LAQUAtwin Ca-11, HORIBA, São Paulo, Brazil) using 500 µL aliquots of Bio-C Temp (Angelus) and UltraCal XS (Ultradent) extracts (1:1, 1:2, 1:4, 1:16) at 0, 1, 3 and 24 h, and at 3, 4, 7 and 14 days, according to the manufacturer's instructions. pH was determined at the same time points using a digital pH meter (PH-009(I)A, Mold & Bacteria Laboratories Store, Mississauga, Canada). All procedures were performed in triplicate and independently repeated (n = 9/group).

Antimicrobial activity against *Enterococcus faecalis*, *Staphylococcus aureus* and *Candida albicans*

The antimicrobial activity of Bio-C Temp (Angelus) and UltraCal XS(Ultradent) extracts was evaluated using a growth inhibition assay (Minimum Inhibitory Concentration - MIC) based on the Clinical and Laboratory Standards Institute protocol, with adaptations (Wiegand et al., 2008). Extracts of both intracanal medications were prepared at dilutions of 1:1, 1:2, 1:4, and 1:16 and tested against *E. faecalis* (ATCC 19433), *S. aureus* (ATCC 25923), and *C. albicans* (ATCC 10231). For *E. faecalis* and *S. aureus*, bioassays were performed in Mueller Hinton medium (Himedia), using an inoculum of 5×10^5 CFU/mL in logarithmic growth phase. Plates

were incubated for 12 h at 37 °C under moderate shaking. The antifungal assay for *C. albicans* was carried out in RPMI 1640 medium (Sigma) supplemented with MOPS (0.165 mol.L^{-1}), using an initial inoculum of 2.5 × 10^3 CFU/mL in logarithmic phase. Plates were incubated for 48h at 30 °C. Antibacterial and antifungal activity was determined by total or percentage growth inhibition compared with experimental controls. In cases where 100% inhibition was observed, the bactericidal or fungicidal effects were confirmed by plating all concentrations onto solid media (Minimum Bactericidal/Fungicidal Concentration, MBC/MFC determination).

Isolation of Human Peripheral Blood Mononuclear Cells (PBMCs)

Venous blood was collected using the vacuum venipuncture technique, after the approval of the Research Ethics Committees (CAAE: 79508624.5.3001.0029; 79508624.5.0000.0030). Human PBMCs were isolated by density gradient centrifugation using Histopaque-1077 (Sigma). Briefly, 16 mL of whole blood was layered over 16 mL of Histopaque-1077 at room temperature and centrifuged (400 g, 30 min, 20 °C, no brake). The upper plasma layer was discarded, leaving only the interface containing lymphocytes, monocytes, and platelets, which was collected and transferred to a new conical tube. Then, cells were diluted 1:4 in PBS and centrifuged again (400 g, 30 min, 20 °C, no brake). The supernatant was discarded, and cells were washed with 10 mL of PBS, followed by centrifugation (250 g, 10 min, 20 °C, no brake). Finally, the cell pellet was resuspended in 10 mL of DMEM medium supplemented with 10% fetal bovine serum, 0.5% MEM amino acids, 0.05% gentamicin, 0.5% L-glutamine, and 0.5% penicillin/streptomycin (1000 U.mL^{-1}) (10).

Cell Viability Assessment

Cell viability was assessed after 24h of incubation of plates containing cells exposed to Bio-C Temp (Angelus) and UltraCal XS (Ultradent) extracts at 1:1, 1:2, 1:4, and 1:16 dilutions, under 5% CO_2, 37 °C, and 95% humidity. The positive control consisted of triplicate wells containing cells in culture medium (100% viability), while the negative control consisted of triplicate wells incubated in lysis solution (10 mM Tris, 1 mM EDTA, 0.1% Triton X-100, pH 7.4), representing 0% viability. After the incubation

period, cells were resuspended, and a 0.4% trypan blue solution (Sigma Aldrich) was added for 1 minute. Live and dead cells were counted immediately after the 24h experimental period (11).

Cell Migration Assay

A cell migration assay was performed using 24-Transwell inserts with 5-µm pore membranes. Extracts of the materials Bio-C Temp (Angelus) and UltraCal XS (Ultradent) were tested at 1:1, 1:2, 1:4, and 1:16 dilutions. A cell suspension containing 1×10^6 cells/mL was prepared, and 100 µL of this suspension was carefully pipetted onto the membrane of each insert. Plates were incubated at 37 °C and 5% CO_2 for 10 minutes to allow cell equilibration. Subsequently, 600 µL of each extract were added to the lower chamber of each well. Plates were then incubated again at 37 °C and 5% CO_2 for 4h, allowing cells to migrate through the membrane. After incubation, inserts were removed, and extracts present in the lower chamber were carefully collected for quantification. Migrated cells were quantified by direct counting using a Neubauer chamber under an optical microscope. The total number of migrated cells was calculated and expressed as cells/mL (12).

Nitric Oxide (NO) Production

Nitric oxide production was measured using the method described by Green et al. (1982), with modifications. PBMCs were exposed to UltraCal XS (Ultradent) and Bio-C Temp (Angelus) extracts at 1:1, 1:2, 1:4, and 1:16 dilutions under three experimental conditions: basal, stimulated with LPS (1 $\mu g.mL^{-1}$), and stimulated with LPS plus IFN-γ (1 $\mu g.mL^{-1}$). After 48h of incubation, 100 µL of culture supernatant from each well was collected and transferred to 96-well plates (TPP, USA), and 100 µL of DMEM (Gibco, USA) was added to wells used for the sodium nitrite standard curve. Then, 100 µL of a solution composed of 1% sulfanilamide in 2.5% phosphoric acid and 1% naphthylethylenediamine in 2.5% phosphoric acid (1:1 ratio) was added. After a 10-minute incubation, absorbance was measured at 490 nm using a microplate reader (Bio-Tek PowerWave HT, USA). Nitrite concentration was determined based on the equation derived from the sodium nitrite standard curve (1.5625 µM to 200 µM) (13).

Statistical analyses

All experiments were performed in three technical replicates and repeated in three independent biological experiments. Normality of the data distribution was verified using the Shapiro–Wilk test. Results were expressed as mean and standard deviation. For comparisons among groups, one-way or two-way ANOVA was applied according to the experimental design. When statistically significant differences were detected, Tukey's multiple-comparison post-test (one-way ANOVA) or Dunnett's and Sidak's post-tests (two-way ANOVA) were performed. A significance level of $p < 0.05$ was considered for all analyses.

RESULTS

X-ray Fluorescence (XRF) Analysis

UltraCal XS (Ultradent) and Bio-C Temp (Angelus) were analyzed using X-ray fluorescence to assess their elemental chemical composition (Table 1). UltraCal XS (Ultradent) was predominantly composed of calcium (48.416%) and barium (43.792%), with smaller amounts of sulfur (7.638%) and trace levels of strontium (0.154%). In contrast, Bio-C Temp (Angelus) exhibited a more heterogeneous composition, with calcium (46.935%) and tungsten (46.246%) as major constituents. Titanium (3.839%) and lower concentrations of barium (1.418%), strontium (0.403%), potassium (0.398%), dysprosium (0.290%), germanium (0.277%), and sulfur (0.195%) were also detected. These findings indicate that although both materials contain calcium as a major component, radiopacifying agents represent a substantial portion of their compositions. UltraCal XS (Ultradent) presents a high barium content, whereas Bio-C Temp (Angelus) contains significant tungsten along with a wider range of minor elements that also act as radiopacifying dopants.

Element	UltraCal XS (Ultradent) (%)	Bio-C Temp (Angelus) (%)
Ca	48.416	46.935
W	-	46.246
Ti	-	3.839
Ba	43.792	1.418
Sr	0.154	0.403
K	-	0.398
Dy	-	0.290
Ge	-	0.277
S	7.638	0.195

Table 1. Comparison of the elemental composition of the intracanal medications UltraCal XS (Ultradent) and Bio-C Temp (Angelus) analyzed by X-ray fluorescence (XRF).

Calcium Ion (Ca^{2+}) Release and pH Evaluation

Calcium ion release and pH variation of UltraCal XS (Ultradent) and Bio-C Temp (Angelus) extracts at different dilutions were evaluated over time (Figure 1). Both materials initially exhibited high alkaline pH and elevated calcium ion release, followed by a progressive reduction over time at all tested concentrations. UltraCal XS (Ultradent) showed the highest Ca^{2+} release in the 1:1 dilution at early time points, with a gradual decrease up to 336h. Lower calcium release was observed with increasing dilutions; however, a transient increase occurred at 168h in the 1:2 and 1:4 dilutions (Figure 1A). Bio-C Temp (Angelus) exhibited a more stable calcium release profile, with a marked increase at 168h, particularly in the 1:2 dilution (Figure 1B). Direct comparison of the 1:1 extract demonstrated higher Ca^{2+} release for UltraCal XS (Ultradent) during the first 72h, whereas Bio-C Temp (Angelus) showed lower but more sustained release after 7 days ($p < 0.0001$) (Figure 1C). At the longest experimental period (14 days), Bio-C Temp (Angelus) exhibited calcium ion release comparable to or higher than that of UltraCal XS (Ultradent) at the 1:4 and 1:16 dilutions (data not shown).

All extracts maintained an alkaline pH throughout the experimental period. UltraCal XS (Ultradent) exhibited higher initial pH values, particularly in the 1:1 and 1:2

dilutions, followed by a gradual decrease over time (Figure 1D). Bio-C Temp (Angelus) showed lower initial pH values, with a pronounced reduction at 72h and subsequent stabilization up to 14 days (Figure 1E). Direct comparison of the 1:1 extract indicated higher pH values for UltraCal XS (Ultradent) during the first 7 days, whereas Bio-C Temp (Angelus) demonstrated partial recovery at 14 days ($p < 0.05$, Figure 1F). At the longest experimental period (14 days), Bio-C Temp (Angelus) exhibited higher pH values compared with UltraCal XS (Ultradent) at the 1:4 and 1:16 dilutions (data not shown).

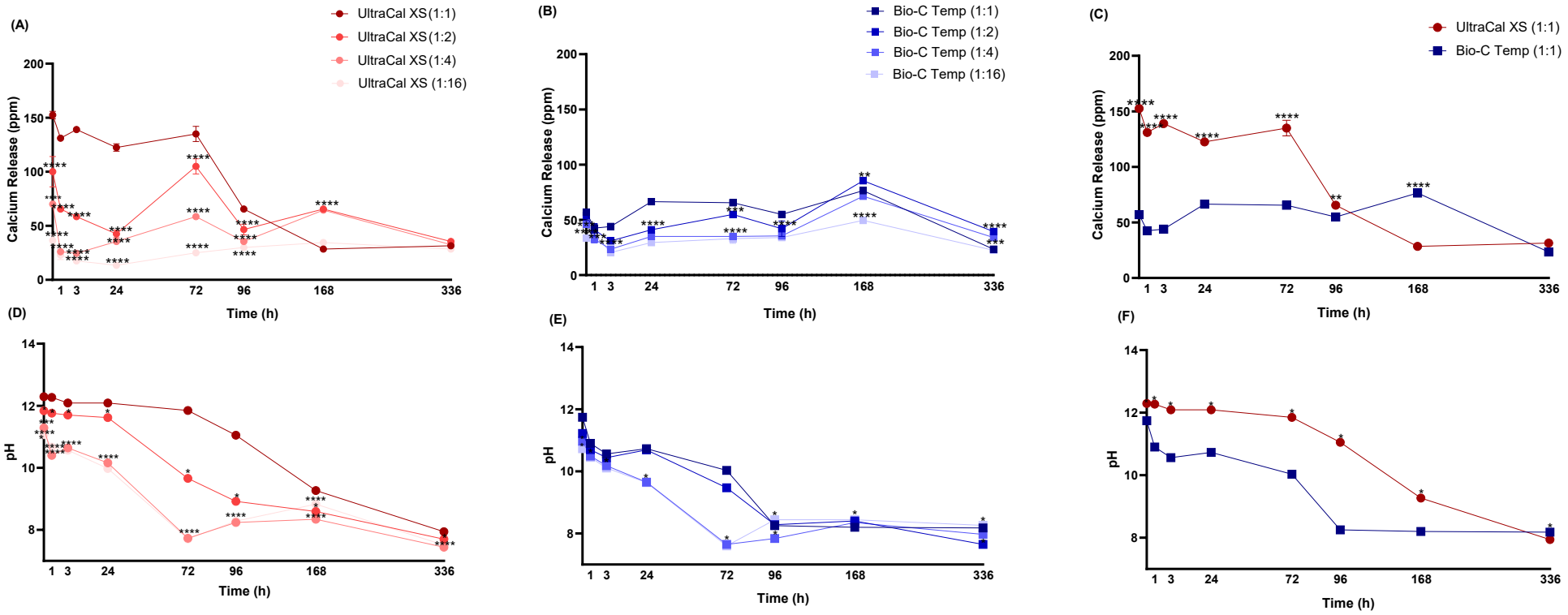

Figure 1. Calcium ion release (ppm) and pH variation over time for different dilutions of the intracanal medications UltraCal XS (Ultradent) and Bio-C Temp (Angelus). Calcium release profiles are shown for UltraCal XS (Ultradent) (A) and Bio-C Temp (Angelus) (B), with direct comparison between materials at the 1:1 dilution (C). pH variation over time is shown for UltraCal XS (Ultradent) (D) and Bio-C Temp (Angelus) (E), with direct comparison between materials at the 1:1 dilution (F). Graphs represent mean ± standard deviation from three independent biological replicates performed in technical triplicates. Calcium levels were measured using a portable calcium ion meter (LaquaTwin Ca^{2+}), and pH measurements were performed using a digital pH meter (Prolab). *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001 represent statistical differences; ns indicates not significant. Statistical analyses were performed using Two-Way ANOVA followed by Dunnett's multiple comparisons test for comparisons within materials (A, B, D, and E) relative to the 1:1 dilution, and Sidak's multiple comparisons test for direct comparisons between materials (C and F).

Antimicrobial activity against *E. faecalis*, *S. aureus* and *C. albicans*

Antimicrobial activity of UltraCal XS (Ultradent) and Bio-C Temp (Angelus) is summarized in Table 2. Both materials showed detectable MIC values against *E. faecalis* across all dilutions, indicating growth inhibition; however, no MBC values were observed, demonstrating the absence of bactericidal activity under the tested conditions. Against *S. aureus*, neither material exhibited detectable MIC or MBC values, although all dilutions produced measurable percentages of bacterial inhibition, with the highest inhibition observed for UltraCal XS (Ultradent) 1:1 (88%) and Bio-C Temp (Angelus)1:1 (71%). For *C. albicans*, inhibitory activity was detected only for UltraCal XS (Ultradent) at the 1:1 and 1:2 dilutions, and fungicidal activity occurred exclusively at the 1:1 dilution. Bio-C Temp (Angelus) did not exhibit antifungal or fungicidal activity**.**

TESTED MATERIAL	MIC *E. faecalis*	MBC *E. faecalis*	MIC *S. aureus*	MBC *S. aureus*	MIC *C. albicans*	MFC *C. albicans*
UltraCal XS 1: 1	D	ND	ND (88%)	ND	D	D
UltraCal XS 1:2	D	ND	ND (78%)	ND	D	ND
UltraCal XS 1:4	D	ND	ND (70%)	ND	ND	ND
UltraCal XS 1:16	D	ND	ND (68%)	ND	ND	ND
Bio-C Temp 1:1	D	ND	ND (71%)	ND	ND	ND
Bio-C Temp 1:2	D	ND	ND (68%)	ND	ND	ND
Bio-C Temp 1:4	D	ND	ND (64%)	ND	ND	ND
Bio-C Temp 1:16	D	ND	ND (66%)	ND	ND	ND

Table 2. Growth inhibition of *E. faecalis* (ATCC 194330)*, S. aureus* (ATCC 25923) and *C. albicans* (ATCC 10231) in the presence of UltraCal XS (Ultradent) and Bio-C Temp (Angelus). Positive control was represented by Ampicillin 20 $\mu g.mL^{-1}$, Gentamicin 20 $ug.mL^{-1}$ and Amphotericin B 10 $\mu g.mL^{-1}$. Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) are represented by Detected (D) and Not Detected ND. Non-detected MIC was followed by percentage of microorganism inhibition between parentheses when it was detected.

Assessment of cell viability and migration

PBMC viability and migratory responses following exposure to UltraCal XS (Ultradent) and Bio-C Temp (Angelus) extracts at different dilutions were evaluated (Figure 2). UltraCal XS (Ultradent) extracts preserved PBMC viability at all tested dilutions, with cell metabolic activity consistently above 97% and no significant

differences relative to the control group ($p > 0.05$), indicating the absence of cytotoxic effects under the experimental conditions (Figure 2A). In contrast, Bio-C Temp (Angelus) extracts induced a concentration-dependent reduction in PBMC viability (Figure 2B). Exposure to the 1:1 dilution resulted in a pronounced decrease in cell viability, whereas the 1:2 dilution reduced viability to 78% ($p < 0.0001$). At higher dilutions, PBMC viability increased markedly, reaching 97% at 1:4 and 99% at 1:16, with no significant differences compared with the control ($p > 0.05$). Direct comparison between materials demonstrated that UltraCal XS (Ultradent) remained non-cytotoxic across all concentrations, whereas Bio-C Temp (Angelus) exhibited cytotoxic effects only at higher extract concentrations (data not shown).

PBMC migratory activity was strongly modulated by extract concentration for both materials (Figure 2D–F). UltraCal XS (Ultradent) significantly impaired PBMC migration at the 1:1 dilution ($p < 0.001$); however, this effect was attenuated as the extracts were diluted, with progressive restoration and enhancement of the migratory response, and the 1:16 dilution promoting migration beyond control levels (Figure 2D). Similarly, Bio-C Temp (Angelus) nearly abolished PBMC migration at the 1:1 dilution ($p < 0.0001$), whereas increasing dilutions progressively restored migratory activity, with reduced migration at the 1:2 ($p < 0.0001$) and 1:4 ($p < 0.01$) dilutions and no difference from the control at 1:16 (Figure 2E). Direct comparison of undiluted extracts demonstrated significantly greater PBMC migration in the presence of UltraCal XS (Ultradent) than Bio-C Temp (Angelus) ($p < 0.01$), although both materials induced migratory responses significantly lower than the control ($p < 0.0001$) (Figure 2F). Collectively, these findings indicate that PBMC migratory behavior is highly concentration-dependent, with lower extract concentrations favoring enhanced immune cell migration for both intracanal medications.

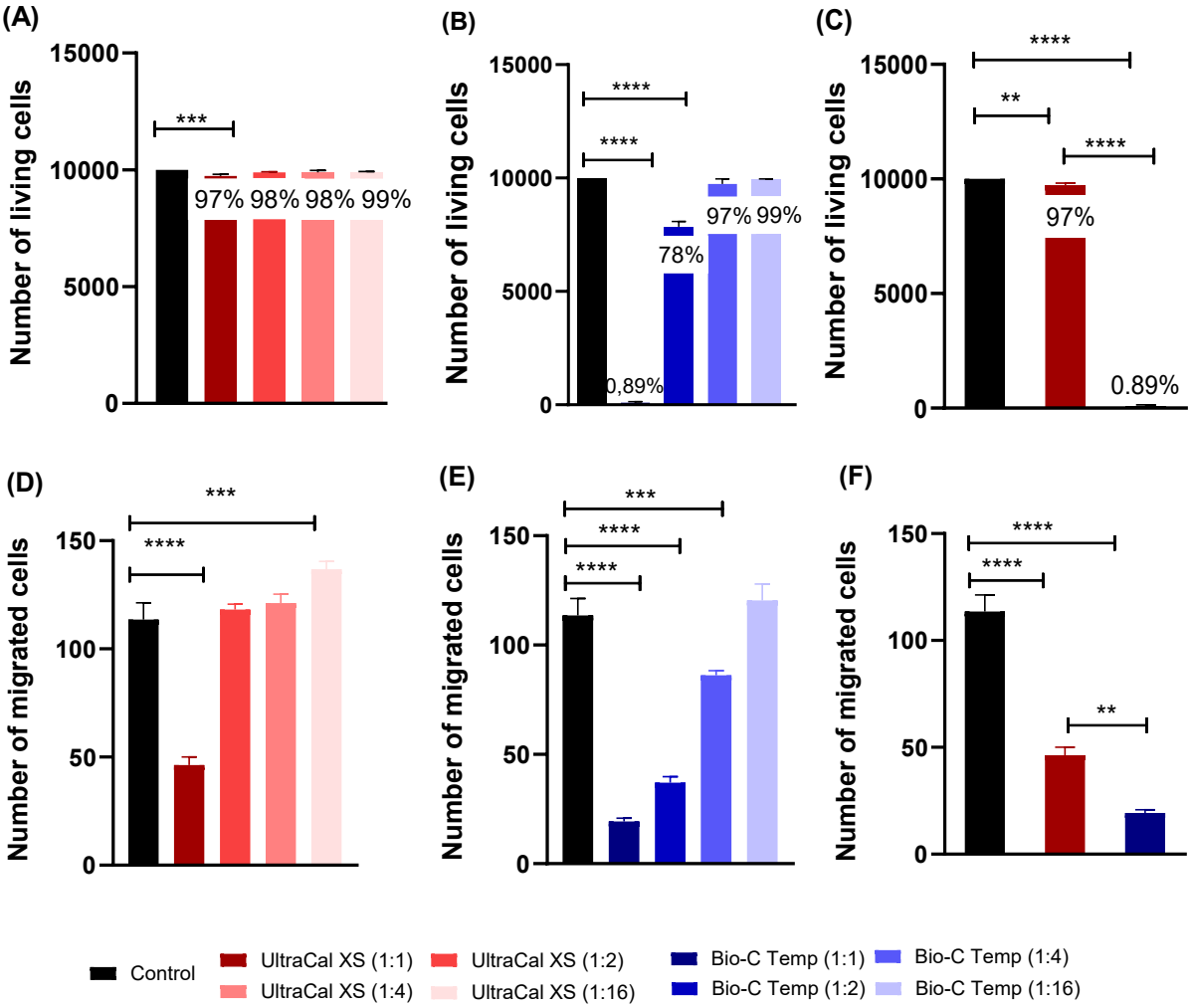

Figure 2. PBMCs were exposed to extracts of UltraCal XS (Ultradent) and Bio-C Temp (Angelus) at different dilutions (1:1, 1:2, 1:4, and 1:16). (A–C) Cytotoxicity assessment by the trypan blue exclusion assay after 24 h of exposure, showing the number of viable cells and cell viability percentage relative to the control (unstimulated PBMCs). (D–F) Cell migration evaluated using a conventional Transwell assay after 4 h of incubation at 37 °C in the presence or absence of material extracts. (C and F) Direct comparison of PBMC viability and migration between UltraCal XS (Ultradent) and Bio-C Temp (Angelus) at the 1:1 dilution. Bars represent mean and standard deviation from three biological replicates performed in technical triplicates. **p < 0.01, ***p < 0.001, and ****p < 0.0001 indicate statistical differences by one-way ANOVA followed by Dunnett's multiple comparisons test.

Nitric Oxide Production

Nitrite production by PBMCs exposed to the intracanal medications was assessed under basal, LPS-stimulated, and LPS plus IFN-γ-stimulated conditions. As expected, the control group showed a stepwise increase across these conditions, confirming progressive inflammatory activation. Under LPS stimulation, UltraCal XS (Ultradent) significantly reduced nitrite levels at the 1:1, 1:2, and 1:4 dilutions ($p < 0.0001$) (Figure 3C). Moreover, Bio-C Temp (Angelus) produced a more limited effect, with a significant reduction only at the 1:4 dilution ($p < 0.05$) (Figure 3F). In the LPS plus IFN-γ condition, UltraCal XS (Ultradent) consistently decreased nitrite production at all dilutions ($p < 0.05$ and $p < 0.0001$) (Figure 3D), with the strongest suppression at 1:1 and significant reductions across the remaining concentrations (Figure 3D). Bio-C Temp (Angelus) showed a dilution-dependent response, with the lowest nitrite levels at 1:1 and progressive increases up to 1:16 ($p < 0.05$, $p < 0.01$) (Figure 3G).

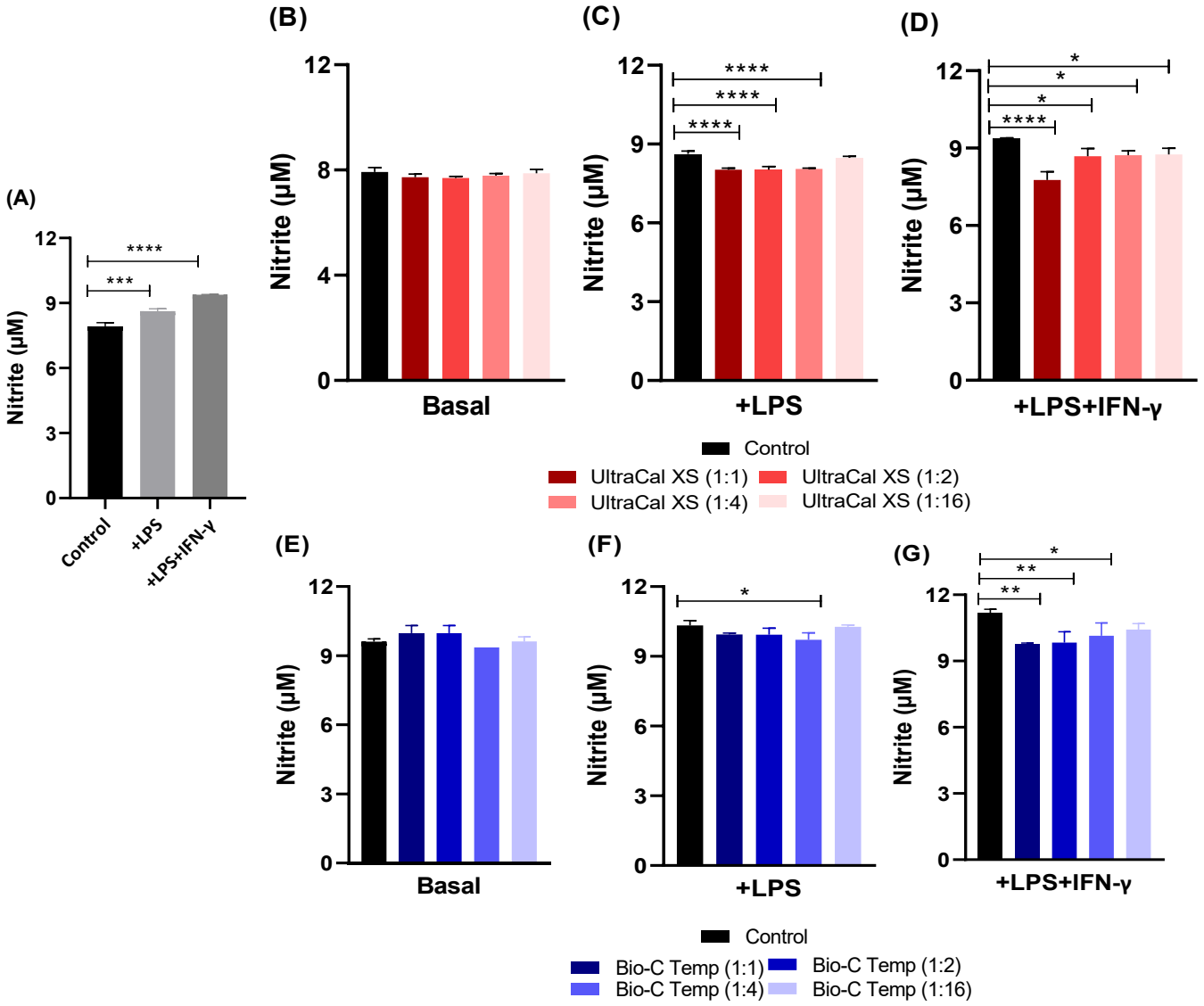

Figure 3. Nitrite production (µM) by PBMCs exposed to intracanal medications. (A) Control group without exposure to intracanal medications in basal, LPS (1 µg.mL^{-1}), and LPS plus IFN-γ (1 µg.mL^{-1}) conditions. PBMCs were exposed to extracts of UltraCal XS (Ultradent) (B-D) and Bio-C Temp (Angelus) (E–G) at different dilutions (1:1, 1:2, 1:4, and 1:16) in different conditions: basal (B and E) stimulated with LPS (C and F) (1 µg.mL $^{-1}$) LPS plus IFN-γ (D and G) (1 µg.mL^{-1}), after 48 h of incubation. Nitrite levels (µM) in the culture supernatants were quantified by the Griess reaction as an indirect indicator of nitric oxide (NO) production. Graphs represent the mean and standard deviation of three independent biological replicates performed in technical triplicates. *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001 represent statistical differences by one-way ANOVA followed by Dunnett's multiple comparisons test.

DISCUSSION

This *in vitro* study evaluated the physicochemical, antimicrobial, and biological behavior of two intracanal medications with different formulations: UltraCal XS (Ultradent), a calcium hydroxide–based material, and Bio-C Temp (Angelus), a calcium silicate–based bioceramic. Although both materials rely on calcium-related bioactivity, they showed distinct patterns of ion release, cellular response, and immunomodulation. UltraCal XS (Ultradent) was associated with rapid calcium release, higher early alkalinity, stable cytocompatibility, and consistent suppression of nitric oxide under inflammatory stimulation. Bio-C Temp (Angelus) showed a more sustained alkaline profile and calcium release over time, with biological effects that varied according to concentration. Both materials demonstrated limited antimicrobial activity, mainly bacteriostatic.

Intracanal medications are used as adjuncts to chemomechanical preparation to control residual infection and modulate inflammation (14). Calcium hydroxide remains the most used dressing because of its high pH, antimicrobial potential, and established clinical performance. Bioceramic intracanal medications have been introduced to provide sustained ion release and bioactivity while improving handling characteristics (15). Comparing these materials allows assessment of whether newer formulations offer different biological responses that may be clinically relevant. X-ray fluorescence analysis confirmed calcium as a major constituent in both materials. Differences were observed in the radiopacifying agents. UltraCal XS (Ultradent) contained predominantly barium-based compounds, whereas Bio-C Temp (Angelus) incorporated tungsten as the main radiopacifier, with smaller amounts of titanium and radiopacifying dopant elements. These compositional differences help explain the distinct physicochemical and biological behaviors observed. The higher elemental heterogeneity of Bio-C Temp (Angelus) may contribute to concentration-dependent cellular responses, highlighting the importance of dilution and ion diffusion when interpreting *in vitro* findings (8).

Classically, calcium hydroxide–based intracanal medications exert their effects through the dissociation of calcium and hydroxyl ions, resulting in environmental

alkalinization and biologically relevant calcium ion availability (3). In this study, UltraCal XS (Ultradent) produced an early and pronounced increase in calcium release and alkalinity, consistent with calcium hydroxide formulations. This behavior is favorable for rapid neutralization of acidic environments and early microbial suppression (16,17). However, Bio-C Temp (Angelus) exhibited a slower but sustained calcium release, maintaining alkaline conditions at later time points. It has been previously demonstrated that Bio-C Temp (Angelus) maintains a stable alkaline profile over time, without significant pH reduction, while calcium hydroxide-based formulations, such as UltraCal XS (Ultradent), exhibit higher initial alkalinity followed by a significant decrease in later periods (16). This profile may be relevant in clinical situations requiring longer intracanal medication periods.

The antimicrobial activity of intracanal medications is closely linked to elevated pH values, generated by hydroxyl ion dissociation, which interfere with microbial metabolism by inhibiting enzymatic activity and compromising cell membrane integrity (18). In the present study, both materials maintained alkaline conditions over time, which is consistent with the growth inhibition observed against *E. faecalis, corroborating previous findings against the same microorganism (16,4).* However, despite sustained alkalinity and calcium ion availability, antimicrobial effects were predominantly bacteriostatic, with no detectable bactericidal activity across most concentrations. These findings support the concept that intracanal medications complement but do not replace effective irrigation, biofilm disruption, and canal shaping during endodontic treatment.

In clinical practice, these materials may also inadvertently extrude beyond the apical foramen or diffuse through accessory canals, leading to direct contact with periradicular tissues. Under such conditions, intracanal medications are no longer confined to the root canal space and may interact with different cell populations involved in periapical inflammation and repair, and in different concentrations. Then, PBMCs, including monocytes and lymphocytes, are key components of this environment, as they are actively recruited to periapical lesions and regulate inflammatory responses, cytokine release, and tissue healing (19). Consequently, assessing the effects of intracanal medications on PBMC viability and behavior is essential for understanding how these materials may influence periradicular repair in

situations of material diffusion or inadvertent extrusion. To this end, cell viability assays showed stable cytocompatibility for UltraCal XS (Ultradent) across all dilutions. Bio-C Temp (Angelus) reduced cell viability at the highest concentration, with recovery observed after dilution. This concentration-dependent response suggests that the biological behavior of bioceramic materials is sensitive to local exposure conditions. In VERO cells, moderate to severe cytotoxicity was observed at concentrations of 100–25 mg/mL of Bio-C Temp (Angelus), while lower concentrations produced only mild effects (20). Similar patterns were described in Saos-2 cells and human dental pulp cells, in which Bio-C Temp (Angelus) reduced cell viability at lower dilutions (1:1 and 1:2) compared to UltraCal XS (Ultradent) but exhibited biological behavior comparable to calcium hydroxide-based materials at higher dilutions (≥1:4 or ≥1:16) (4,21). *In vivo*, buffering and diffusion of tissue fluid may reduce the impact of higher concentrations of the material.

Cell migration followed a pattern similar to cell viability. Both materials reduced migration at higher concentrations, whereas dilution restored migratory capacity. Since immune cell migration is involved in periapical defense and repair (19), these findings indicate that excessive local concentrations of intracanal medications may temporarily affect cellular recruitment. Under diluted conditions, Bio-C Temp (Angelus) supported migration comparable to UltraCal XS (Ultradent). In accordance with these findings, previous studies using human dental pulp cells showed that Bio-C Temp (Angelus) did not promote cell migration or wound closure after 48 hours (8).

Nitric oxide is an important mediator in the periradicular environment, contributing to tissue homeostasis under basal conditions and to host defense during immune-inflammatory responses. At low levels, NO participates in vascular regulation and cellular signaling, whereas increased production by activated immune cells occurs in response to microbial challenge and tissue injury, influencing antimicrobial activity, cytokine modulation, and inflammatory cell function (22). While controlled NO production may support periradicular defense, excessive or sustained NO levels have been associated with tissue damage and delayed healing (22). In this context, the modulation of NO production by intracanal medications becomes biologically relevant. Accordingly, nitrite production by PBMCs exposed to UltraCal XS (Ultradent) and Bio-C Temp (Angelus) was evaluated under basal and inflammatory stimulation conditions

(23). UltraCal XS (Ultradent) consistently reduced nitrite levels following LPS and LPS plus IFN-γ stimulation, indicating a sustained modulatory effect under pro-inflammatory conditions. In contrast, Bio-C Temp (Angelus) demonstrated a more variable, concentration-dependent modulation of NO production. Given the role of nitric oxide in inflammation and tissue damage, these differences may influence periapical healing dynamics. Classically, calcium hydroxide–based intracanal medications may indirectly influence NO production by modulating the local inflammatory environment through sustained alkalinization, which affects immune cell activation and mediator release (24). Overall, the data indicate that calcium hydroxide–based and bioceramic intracanal medications exhibit distinct but potentially complementary biological behaviors that depend on concentration and exposure conditions.

Within the limitations of this *in vitro* study, UltraCal XS (Ultradent) and Bio-C Temp (Angelus) demonstrated distinct physicochemical and biological profiles consistent with their different formulations. UltraCal XS (Ultradent) was associated with rapid calcium ion release, higher early alkalinity, favorable cytocompatibility, and consistent modulation of inflammatory nitric oxide production. Bio-C Temp (Angelus) showed a more sustained calcium release and alkaline behavior over time, with biological responses that were dependent on extract concentration. Both materials exhibited limited antimicrobial activity, predominantly bacteriostatic, supporting their use as adjuncts to chemomechanical preparation rather than as sole antimicrobial strategies. Taken together, these findings suggest that calcium hydroxide–based and bioceramic intracanal medications may present complementary characteristics, with clinical behavior influenced by concentration, diffusion, and exposure conditions.

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to this study.

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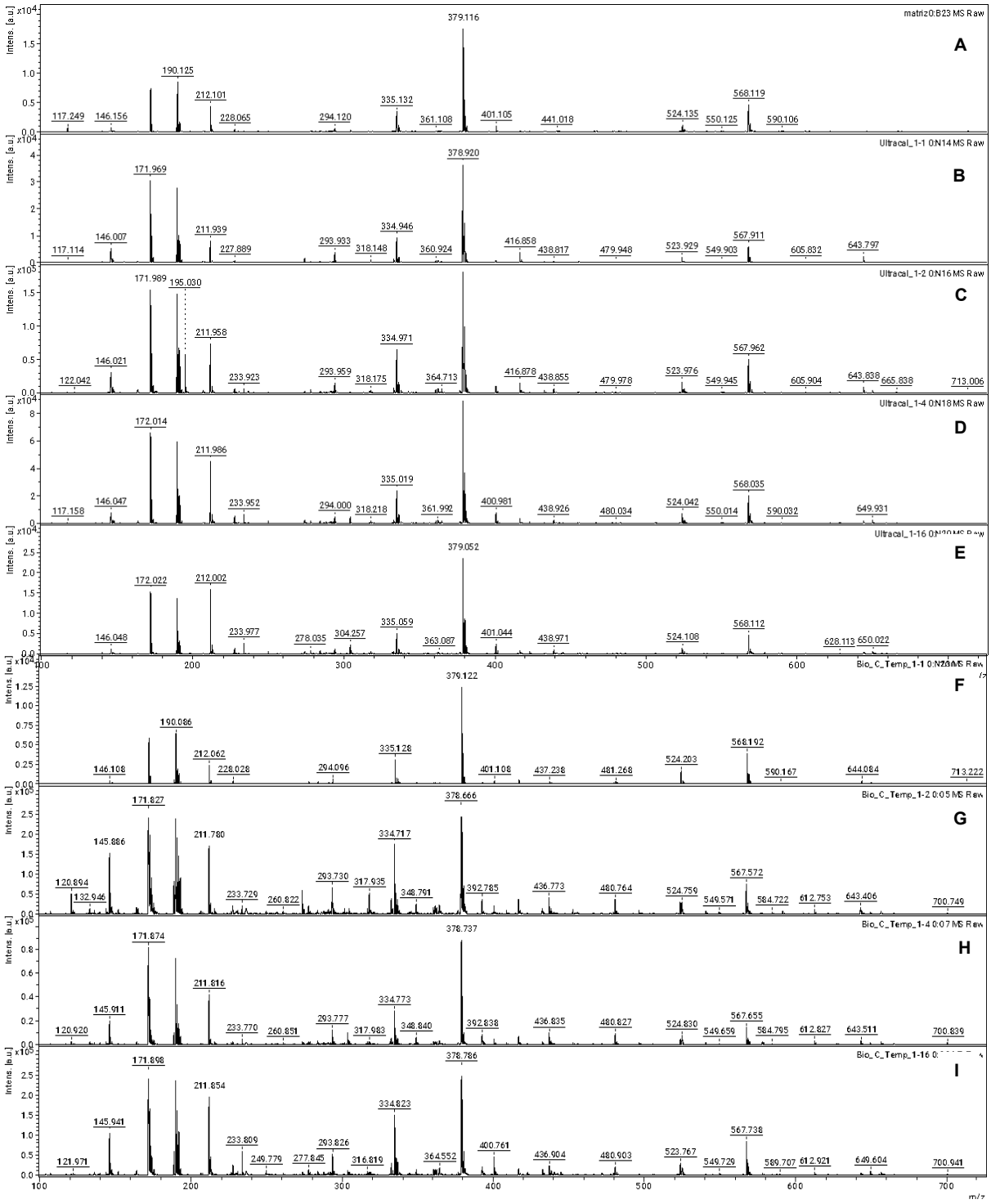
SUPPLEMENTARY FIGURES:



Supplementary Figure 1. MALDI-ToF (matrix-assisted laser desorption ionization–time of flight) spectra of intracanal medication extracts. (A) Matrix; (B–E) UltraCal XS extracts at dilutions (B) 1:1, (C) 1:2, (D) 1:4, (E) 1:16; (F–I) Bio-C Temp extracts at dilutions (F) 1:1, (G) 1:2, (H) 1:4, (I) 1:16.

CAPÍTULO 2 (Intended submission: International Endodontic Journal; IF=7,1)

IN VITRO EVALUATION OF THE BIOLOGICAL BEHAVIOR OF BIOCERAMIC BIO-C TEMP IN HUMAN PERIODONTAL LIGAMENT CELLS

ABSTRACT

Aim: To analyze the biological effects of a bioceramic intracanal medicament (Bio-C Temp) compared to calcium hydroxide–based formulation (UltraCal XS) on human periodontal ligament stem cells (hPDLSCs), focusing on cell viability, morphology, migration, proliferation, and cytokine gene expression under basal and inflammatory conditions.

Methodology: Extracts of Bio-C Temp and UltraCal XS were prepared according to ISO 10993-5 and evaluated at dilutions of 1:1, 1:2, 1:4, and 1:16. hPDLSCs were exposed to the extracts, and cell viability was assessed by MTT assay. Cell morphology and adhesion were analyzed by scanning electron microscopy, migration by scratch assay, and proliferation by Trypan Blue exclusion. Cytokine expression of *TNF-α, IL-1β, IL-6,* and *IL-10* was quantified by real-time PCR under basal conditions and after stimulation with LPS, IFN-γ, or their combination.

Results: Both intracanal medications exhibited concentration-dependent biological effects. At the highest concentration (1:1), Bio-C Temp significantly reduced cell viability, altered cellular morphology, and impaired migration and proliferation, whereas these effects were attenuated at higher dilutions. UltraCal XS showed moderate cytotoxicity but consistently promoted migration and proliferation across dilutions. Morphological analysis revealed reduced cell spreading and fewer membrane extensions at higher concentrations. Under inflammatory conditions, UltraCal XS markedly upregulated cytokine expression following LPS stimulation, while Bio-C Temp induced more pronounced cytokine modulation under combined LPS and IFN-γ stimulation.

Conclusions: Bio-C Temp and UltraCal XS elicit distinct, concentration--dependent biological responses in hPDLSCs. Reduced material exposure, as occurs with diffusion and tissue buffering, enhances biocompatibility and cellular responses, underscoring

the importance of dilution-dependent biocompatibility when intracanal medicaments interact with periapical tissues.

INTRODUCTION

Periodontitis is a common and widespread disease that leads to the destruction of periodontal tissues, including alveolar bone, periodontal ligament, and cementum (Helgeland et al., 2018). Intracanal medication acts as an adjuvant during endodontic treatment by reducing the microbial load and creating favorable conditions for periradicular tissue repair (Ordinola-Zapata et al., 2022). In this context, the use of intracanal materials with good biocompatibility, which interact adequately with surrounding tissues without causing adverse reactions, is fundamental (Lemons, 1996). Furthermore, bioactive materials are able to induce the formation of apatite layers and promote biomineralization, fundamental processes for tissue regeneration (Hench & Wilson, 1984).

Although calcium hydroxide remains the gold standard for intracanal medication, its limitations in eliminating resistant microorganisms and in consistently promoting periapical repair have encouraged the investigation of alternative materials. Achieving an appropriate balance between antibacterial efficacy and cytocompatibility continues to represent a major challenge in endodontic therapy (Mohammadi & Dummer, 2011; Hussein & Kishen, 2022). More recently, calcium silicate-based bioceramic materials have been adapted for use as intracanal medications, with the aim of extending the bioceramic bioactive potential to the root canal environment. In this context, bioceramic intracanal medicaments have emerged as promising alternatives, offering biological properties that may be more closely aligned with contemporary clinical demands. Products such as Bio-C Temp (Angelus, Londrina, PR, Brazil), iRoot FM (Innovative Bioceramix Inc., Vancouver, BC, Canada), and EndoSequence BC Temp (Brasseler, Savannah, USA) are calcium silicate–based intracanal medicaments developed to provide enhanced bioactivity within the root canal system. Bio-C Temp (Angelus) consists of tricalcium silicate, dicalcium silicate, tricalcium aluminate, calcium oxide, a resin-based vehicle, calcium tungstate, polyethylene glycol, and titanium dioxide (Guerreiro et al., 2021). Previous studies have shown that Bio-C Temp (Angelus) exhibits antimicrobial activity, particularly against *Enterococcus faecalis*; however, its antimicrobial capacity is significantly lower than that of other calcium hydroxide-based medications, such

as UltraCal XS (Ultradent, South Jordan, UT, USA) (Guerreiro et al., 2021; Park et al., 2015). Regarding cellular metabolic activity, studies using a human osteosarcoma cell line (Saos-2) demonstrated good cell viability with increasing dilutions of the material (Guerreiro et al., 2021; Silveira et al., 2024). Similarly, *in vitro* studies using mesenchymal stem cells derived from the human periodontal ligament have demonstrated that Bio-C Temp (Angelus) maintains cell viability, supporting its biocompatibility (Braido et al., 2025). In addition, the inclusion of calcium tungstate in the material formulation may be associated with changes in tooth color (de Campos et al., 2023).

Intracanal medicaments may diffuse through dentinal tubules or be inadvertently extruded beyond the apical foramen, resulting in contact with periodontal ligament tissues. Under these conditions, the biological effects of intracanal medicaments appear to depend on their concentration and proximity to periodontal ligament cells, with higher concentrations associated with inhibitory effects and lower concentrations potentially inducing more favorable cellular responses (Shahravan et al., 2012; Thong et al., 2008). Despite encouraging *in vitro* evidence indicating that bioceramic intracanal medicaments may promote mineralization, tissue repair (Bi et al., 2018) and acceptable cell viability (Oliveira et al., 2020), the biological mechanisms underlying their interaction with periodontal ligament cells and inflammatory environments remain incompletely elucidated. Limited information is available regarding how different intracanal medicaments modulate key cellular functions of human periodontal ligament stem cells (hPDLSCs). This fact is critical for periodontal and periapical healing, given that hPDLSCs play a central role in tissue regeneration, extracellular matrix organization, and immune modulation in the periapical region (Queiroz et al., 2021).

Therefore, the present study aimed to investigate and compare the concentration-dependent effects of a bioceramic intracanal medicament (Bio-C Temp) and a calcium hydroxide–based formulation (UltraCal XS) on hPDLSC viability, morphology, migratory and proliferative behavior, as well as cytokine gene expression under basal and inflammatory conditions. By exploring cellular responses under different inflammatory stimuli, this study seeks to provide mechanistic insight into how these materials interact with periodontal ligament

cells, thereby contributing to a better clinical understanding of their biological behavior and supporting a more informed selection of intracanal medications in endodontic practice.

MATERIALS AND METHODS

The manuscript of this laboratory study has been written following the Preferred Reporting Items for Laboratory Studies in Endodontology (PRILE) 2021 guidelines (Nagendrababu et al., 2021). The flowchart is presented in Figure 1.

Figure 1. Flowchart for Preferred Reporting Items for Laboratory Studies in Endodontology (PRILE) 2021 guidelines

Preparation of intracanal medications

The intracanal medications Bio-C Temp (Angelus) and UltraCal XS (Ultradent) were prepared for extract formation according to ISO 10993-5 recommendations (ISO 10993-5:2009). For each material, 40 mg was weighed and transferred to sterile tubes, followed by the addition of 10 mL of DMEM culture medium (Dulbecco's Modified Eagle Medium) (Gibco, St. Louis, USA). Suspensions were immediately mixed and then filtered using 0.22 µm membrane to remove particulate residues. After filtration, serial dilutions of each extract were prepared, generating the concentrations 1:1, 1:2, 1:4, and 1:16, which were used in all experimental procedures. Extracts of intracanal medications were analyzed by matrix-assisted laser desorption ionization (Supplementary Figure 1).

Primary culture of periodontal ligament cells

Initially, third molars donated after extraction were used in this study, which was approved by the ethics committee. After teeth had been extracted, they were stored in 10 mL of DMEM (Gibco) with 200 µL of amphotericin B (Sigma, St. Louis, USA) and 10 µL of gentamicin (Sigma) (Huang et al., 2006). Human periodontal ligament cells (hPDLSCs) were obtained by root scraping of the extracted tooth. Then, periodontal ligament was transferred to a 6-well plate and cultured in DMEM medium (Gibco), supplemented with 50 $U.mL^{-1}$ of penicillin (Gibco), 50 $\mu g.mL^{-1}$ of streptomycin (Gibco), and 20% Fetal Bovine Serum (FBS) (Cultilab, Campinas, São Paulo, Brazil). The culture was incubated in a humidified atmosphere with 5% CO_2 (Pedrosa et al., 2022). After confluence, the cell monolayer was recovered with 300 µL of 0.5% (w/v) trypsin solution (Sigma) and 10 mM ethylenediamine tetra acetic acid (EDTA) (Sigma), for 2 min, at 37 °C. Then, cells were centrifuged at 1,500 rpm for 5 min and added to small culture flasks containing DMEM (Gibco), supplemented with 2 nM glutamine (Gibco), 50 $U.mL^{-1}$ penicillin (Gibco), 50 $\mu g.mL^{-1}$ streptomycin (Gibco) and 10% FBS (Cultilab) (AL-HABIB et al., 2019).

Effect of Bio-C Temp and UltraCal XS on hPDLSC viability

The toxicity of sealers was evaluated in hPDLSC culture. Initially, hPDLSC ($1x10^4$ cells.mL^{-1}) were cultured in 96-well plates for 24h in a CO_2 incubator at 37 °C. After this period, 200 µL of cell supernatant was exchanged for sealer extracts, and plates were again incubated at 37 °C for 24h. After the incubation period, the MTT (3-(4,5- dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide) assay was performed and plates were read at 570 nm (Loosdrecht et al., 1991).

Effect of Bio-C Temp and UltraCal XS Extracts on hPDLSC Morphology by Scanning Electron Microscopy

The morphological changes of hPDLSCs were analyzed using Scanning Electron Microscopy (SEM). Cells (2 × 10^5 cells per well) were exposed to intracanal medication extracts of Bio-C Temp and UltraCal XS at the dilutions 1:1, 1:2, 1:4, and 1:16. Cells were seeded in 6-well culture plates (Kasvi) containing DMEM medium (Gibco). Round glass coverslips (13 mm × 13 mm; Fisher Scientific, Suwanee, Georgia, USA) were placed at the bottom of the wells prior to cell seeding. After 24h of exposure, the coverslips were fixed in 0.1 M Karnovsky's solution (2% glutaraldehyde and paraformaldehyde) for 24h and washed twice with 0.1 M sodium cacodylate buffer. Subsequently, cells were post fixed in 1% osmium tetroxide (Sigma) for 30 min and washed twice with distilled water. Dehydration was performed using acetone solutions at 50%, 70%, 90%, and 2 x 100%. After drying at critical point, the coverslips were gold metallized and analyzed using a scanning electron microscope (JSM 7001F, Jeol). Images were obtained at magnifications of 300×, 1500×, and 5000×. For quantitative analysis, the number of adhered cells was counted in images acquired at 300× magnification, in three distinct fields for each extract condition, ensuring adequate statistical reliability. ImageJ software (NIH, Bethesda, MD) was used for cell counting.

Effect of Bio-C Temp and UltraCal XS on hPDLSC migration and proliferation

Cell migration assessment was performed using the scratch method (He et al., 2022). In this assay, cells (2.5 × 10^5 cells per well) were seeded in 6-well culture plates (Prolab) in DMEM medium (Gibco), supplemented with 100 $\mu g.mL^{-1}$ penicillin (Gibco) and 100 $\mu g.mL^{-1}$ streptomycin (Gibco) and 10% FBS (Cultilab), maintained until a confluent monolayer was formed. Then, an artificial wound was reproduced on well surfaces with a 1000 µL plastic micropipette tip. Remaining cells were washed, and new DMEM medium (Gibco), supplemented with 100 $\mu g.mL^{-1}$ penicillin (Gibco) and 100 $\mu g.mL^{-1}$ streptomycin (Gibco), was added together with the Bio-C Temp and UltraCal XS intracanal medication extracts (1:1, 1:2, 1:4 and 1:16). Cultures were incubated and monitored for up to 48h. Photographs with the aid of microscopy were taken at 0, 24 and 48h. Images were processed, and cells located in the wound region were counted with the aid of ImageJ software (NIH, Bethesda, MD). The proliferative potential of hPDLSC was performed using the Trypan Blue exclusion technique (Cheng et al., 2017). hPDLSC (1 × 10^4 cells) were incubated with Bio-C Temp and UltraCal XS extracts (1:1, 1:2, 1:4 and 1:16) for 24 and 48h. Subsequently, cells were resuspended, and a 0.4% Trypan Blue dye solution (Sigma) was added for 1 minute. Cell numbers were immediately counted and compared with the initial cell number.

Effect of Bio-C Temp and UltraCal XS on cytokine gene expression

The expression of *TNF-α, IL-1β, IL-6*, and *IL-10* in hPDLSCs exposed to intracanal medication extracts was evaluated by real-time PCR. To establish distinct *in vitro* inflammatory conditions, cells were additionally stimulated with LPS (1 $\mu g.mL^{-1}$) (*Escherichia coli lipopolysaccharides*, Sigma), IFN-γ (1 $\mu g.mL^{-1}$) (Interferon-gamma, Sigma), or a combination of LPS plus IFN-γ for 24h. After cell incubation, total RNA was extracted using the TRIzol™ reagent (ThermoFisher Scientific, California, USA) (Xu et al., 2021). RNA quantification was performed using the NanoDrop One® (ThermoFisher Scientific, California, USA). The complementary DNA (cDNA) was synthesized using the High-Capacity Reverse Transcription Kit, following the manufacturer's instructions (ThermoFisher). Gene expression analysis was performed using the StepOnePlus™ Real-Time PCR

System (ThermoFisher) to assess the transcriptional levels of inflammatory and anti-inflammatory markers. The primer sequences used for RT-qPCR are listed in Table 1, and GAPDH was used as the constitutive gene.

All reactions were performed in duplicates, and the relative fold change value was obtained by the $2^{-\Delta\Delta Ct}$ method (Pfaffl, 2001) To normalize sample loading, the differences in threshold cycles (ΔCt) were obtained by subtracting the Ct value for the endogenous reference (*GAPDH*) from the Ct values of the evaluated genes. The median Ct values of the samples from control were used as a reference.

GENE	**PRIMER SEQUENCE *(5'–3')***
TNF-α	Forward: CACAGTGAAGTGCTGGAAAC
	Reverse: GATCAAAGCTGTAGGCCCA
IL-1β	Forward: GCACACAGGCTCCGAGATGAA
	Reverse: GTGCTTGGTCCGTCTTTCT
IL-6	Forward: CCCACAGGAACGTGAAAAGTCA
	Reverse: TGCAAAGTCTCAGGATCTCT
IL-10	Forward: TAAGGGTTTACTTGGGTTGCCA
	Reverse: TCTGGGCCATGGTGGTTTCC
GAPDH	Forward: AGTGGCAAAGTGGAGATT
	Reverse: GTGGAGTCATACTGGAACA

Table 1. Sequence of primers for each gene used in the PCR assay.

Statistical analyses

All experiments were performed in three technical replicates and repeated in three independent biological experiments. Normality of the data distribution was verified using the Shapiro–Wilk test. Results were expressed as mean and standard deviation. For comparisons between groups, one-way or two-way ANOVA was applied according to the experimental design. When statistically significant differences were detected, Tukey's multiple-comparison post-test (for

one-way ANOVA) and Dunnett's post-test (for two-way ANOVA) were performed. A significance level of $p < 0.05$ was considered for all analyses.

RESULTS

Effect of Bio-C Temp and UltraCal XS on hPDLSC viability

Cell cultures incubated with UltraCal XS exhibited the greatest cytotoxicity when exposed to the undiluted extract (1:1), with cell viability reduced to 79% compared with the control (1.31 ± 0.08 vs. 1.66 ± 0.07; $p < 0.001$; Figure 2A). As the extract was progressively diluted, a gradual recovery in cell viability was observed. Specifically, the 1:2 and 1:4 dilutions resulted in partial recovery, with viabilities of 79% and 84%, respectively; however, these values remained significantly lower than those of the control group (1.32 ± 0.03 and 1.40 ± 0.07 vs. 1.66 ± 0.07; $p < 0.001$ and $p < 0.01$; Figure 2A). In contrast, at the highest dilution (1:16), UltraCal XS did not exhibit cytotoxic effects, as cell viability reached 90% of the control. A similar concentration-dependent response was observed for Bio-C Temp (Figure 2B). Exposure to the undiluted extract (1:1) led to a marked reduction in cell viability 56% compared with the control; (0.93 ± 0.03 vs. 1.66 ± 0.07; $p < 0.0001$, Figure 2B). Progressive recovery was evident with increasing dilutions, with viabilities of 92%, 97% and 97% for the 1:2, 1:4 and 1:16 dilutions, respectively. At these higher dilutions, no statistically significant differences were detected relative to the control group, indicating low cytotoxicity of Bio-C Temp at reduced concentrations.

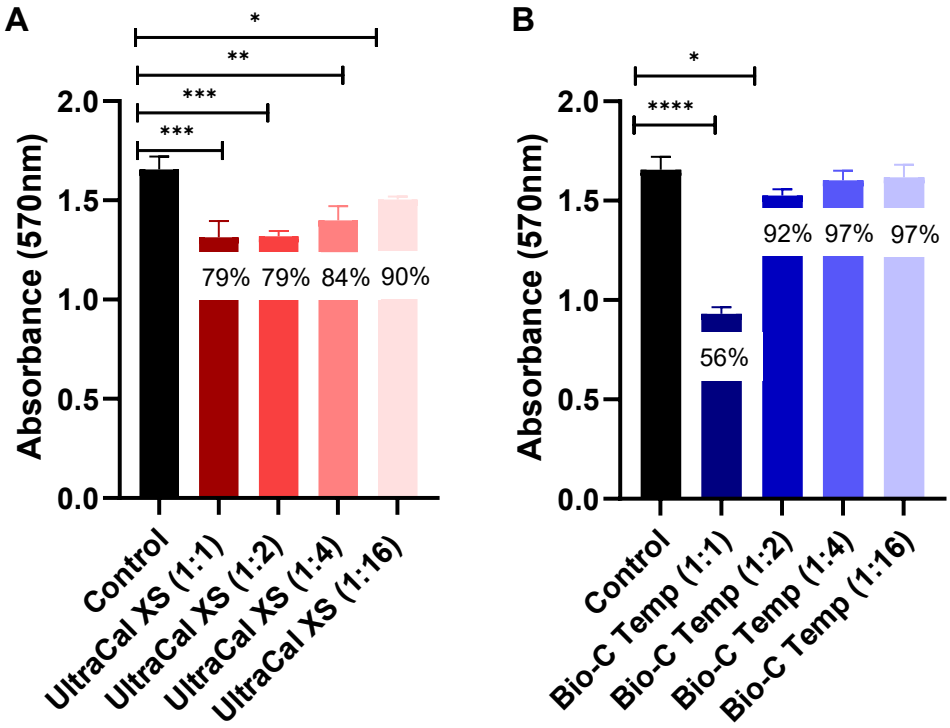


Figure 2. hPDLSC viability by MTT assay. hPDLSC viability after 24h of incubation with UltraCal XS (A), Bio-C Temp (B) extracts (1:1, 1:2, 1:4 and 1:16). Graphs represent the mean and standard deviation of the absorbance and percentage of cell viability of three biological replicates performed in technical triplicates. *p < 0.05; **p=0.002; ***p=0.0001 and ****p<0.0001, represent statistical difference by one-way ANOVA and Tukey post-test.

Effect of Bio-C Temp and UltraCal XS Extracts on hPDLSC Morphology by Scanning Electron Microscopy

Microphotographs obtained at 300× and 5000× magnifications (Figure 3A and 3B) by SEM were analyzed after exposure to UltraCal XS and Bio-C Temp extracts at different dilutions, considering two main parameters: cell size and the number of cellular extensions. SEM images of cells treated with the 1:1 extract showed rounded and retracted cell bodies, along with altered membrane projections, indicating marked morphological alterations compatible with cell damage. At the highest concentrations (1:1 and 1:2), both materials induced marked alterations in overall cellular architecture, including reduced cell spreading. These qualitative observations were supported by quantitative analyses, which demonstrated a significant reduction in the number of cellular

extensions for Bio-C Temp at the 1:1 (3.33 ± 0.58 vs. 6.00 ± 1.00; $p < 0.05$, Figure 3F) and 1:2 (3.67 ± 1.15 vs. 6.00 ± 1.00; $p < 0.01$; Figure 3F) dilutions. For UltraCal XS, the number of cellular extensions also decreased at the 1:1 and 1:2 concentrations compared with the control; however, an increase was observed at the 1:4 dilution (8.33 ± 0.58 vs. 6.00 ± 1.00; $p < 0.05$, Figure 3D). In contrast, Bio-C Temp did not significantly alter average cell size but consistently reduced the number of cellular extensions, corroborating the loss of membrane projections detected in the SEM images.

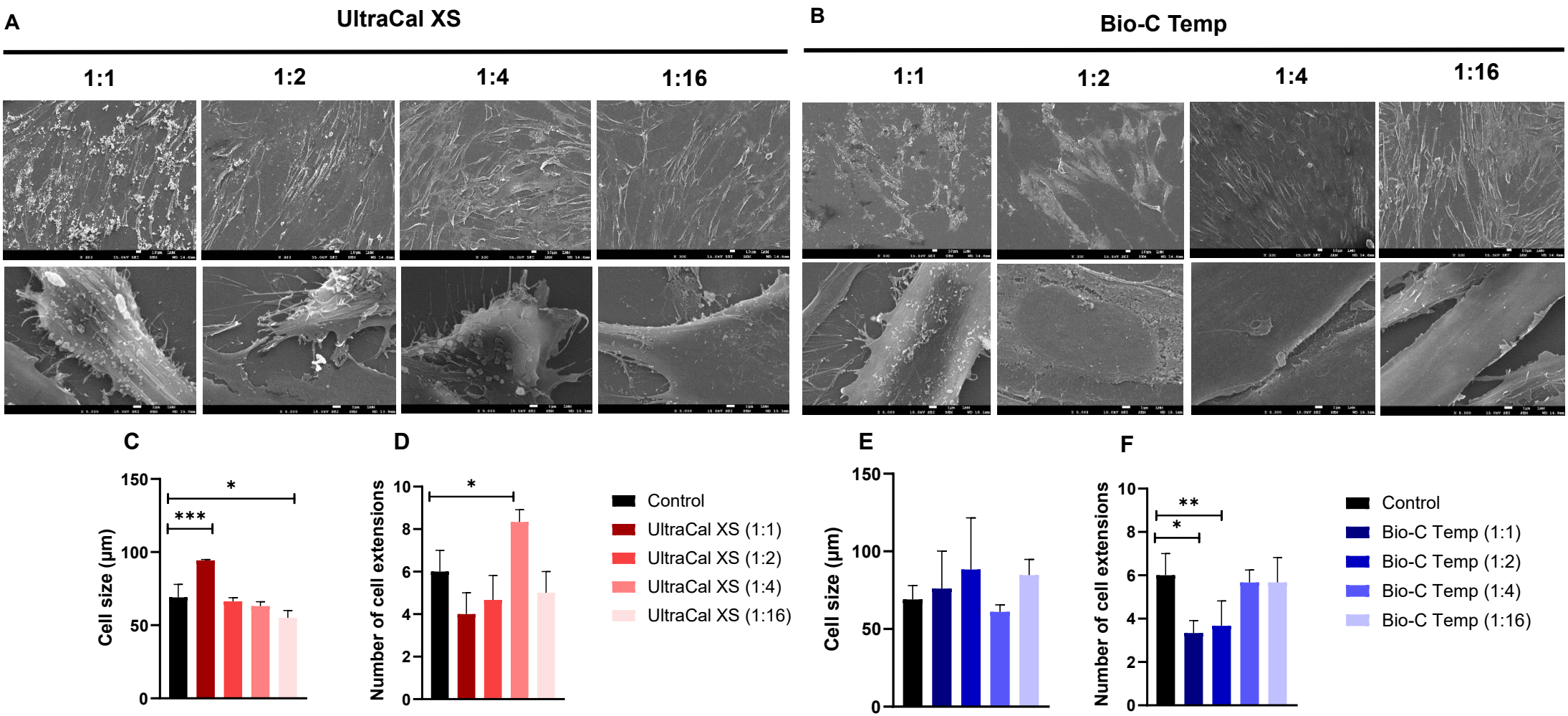


Figure 3. Morphological characterization of hPDLSCs exposed to intracanal medications. SEM images show hPDLSCs after exposure to extracts of UltraCal XS (A) and Bio-C Temp (B) at dilutions of 1:1, 1:2, 1:4, and 1:16. Upper rows present lower-magnification views of general cell distribution, and bottom rows show higher-magnification details of membrane projections and cell attachment. Quantitative analysis includes cell size (C, E) and number of cell extensions (D, F). Data represent three biological replicates in technical triplicates. *p < 0.05, **p < 0.01, ***p < 0.001; one-way ANOVA with Dunnett's post hoc test.

Effect of of Bio-C Temp and UltraCal XS on hPDLSC migration and proliferation

It was observed that UltraCal XS and Bio-C Temp resulted in distinct and dose-dependent effects on cell migration and proliferation (Figure 4). UltraCal XS consistently increased migratory activity and proliferation between 24h and 48h. In contrast, Bio-C Temp markedly reduced both parameters at the highest concentrations (1:1 and 1:2). At 48h, migratory activity was significantly reduced for Bio-C Temp at 1:1 (59.3 ± 3.1 vs. 427.3 ± 8.5, $p < 0.0001$, Figure 4B) and 1:2 (70.7 ± 7.8 vs. 427.3 ± 8.5; $p < 0.0001$, Figure 4B). This reduction in migratory activity was accompanied by an increase in wound area in the Bio-C Temp groups. In the Bio-C Temp 1:1 group, wound areas measured 89.4 mm² at 24h and 97.7 mm² at 48h, while in the 1:2 group wound areas of 36.4 mm² (24h) and 70.9 mm² (48h) were observed, indicating persistence of unclosed wound regions at higher concentrations. This loss of migratory capacity and increased wound area were not observed at the lower dilutions (1:4 and 1:16), in which cell migration was detected. Due to the marked cytotoxicity of Bio-C Temp at the 1:1 dilution, comparative analyses between materials were primarily performed at 1:2. At this concentration, UltraCal XS significantly increased migratory activity and proliferation between 24h and 48h, whereas Bio-C Temp markedly reduced both parameters. Similar patterns were observed across the other tested dilutions, with UltraCal XS consistently promoting higher migration and proliferation than Bio-C Temp (data not shown). Representative images from the cell migration assay corroborated the quantitative data, showing complete or near-complete closure in the control and UltraCal XS groups, and persistent unclosed wound areas in the Bio-C Temp 1:1 and 1:2 groups.

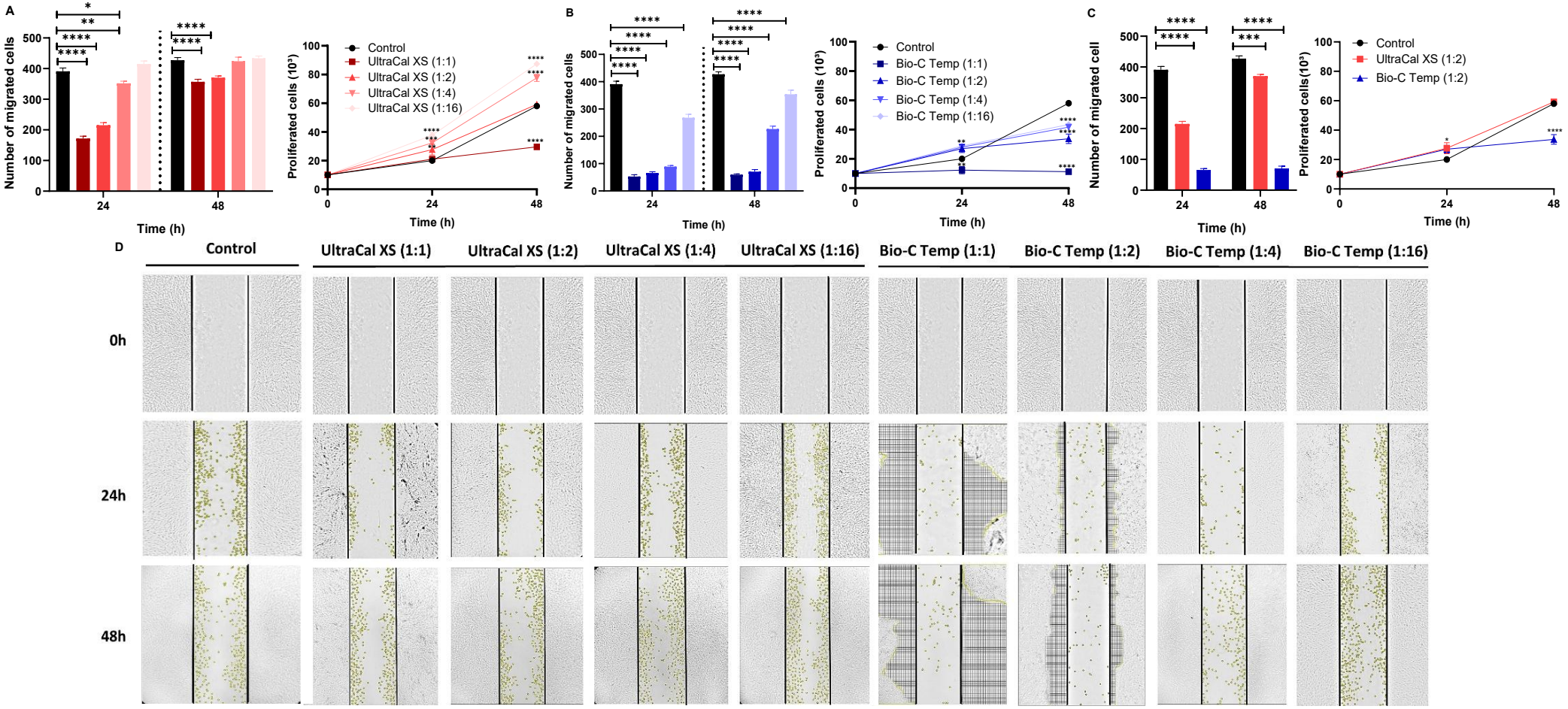


Figure 4. Migratory behavior and proliferation of hPDLSCs exposed to UltraCal XS and Bio-C Temp at 1:1, 1:2, 1:4, and 1:16 dilutions, after 24h and 48h. (A) Migration and proliferation of hPDLSCs exposed to UltraCal XS at 1:1, 1:2, 1:4, and 1:16 dilutions. *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001. (B) Migration and proliferation of hPDLSCs exposed to Bio-C Temp at 1:1, 1:2, 1:4, and 1:16 dilutions. **p < 0.01 and ****p < 0.0001. (C) Migration and proliferation of hPDLSCs exposed to UltraCal XS and Bio-C Temp at the 1:1 dilution. *p < 0.05, ***p < 0.001 and ****p < 0.0001 indicate statistical differences by one-way or two-way ANOVA with Tukey post-test. (D) Representative scratch-assay images of control and experimental groups in all tested dilutions at 0h, 24h, and 4 h. Grids indicate the cell-lost area.

Effect of Bio-C Temp and Ultracal XS on cytokine gene expression

The expression of the analyzed cytokines was differentially modulated depending on the stimulus applied. Under basal conditions, only modest changes were observed, with *TNF-α* expression significantly reduced following exposure to Bio-C Temp compared with the control (0.32 ± 0.01 vs. 1.01 ± 0.13; $p<0.01$, Figure 5A). In contrast, *IL-6* expression was significantly increased by Bio-C Temp compared with both the control and UltraCal XS (2.32 ± 0.19 vs. 1.01 ± 0.21; $p < 0.001$ and 2.32 ± 0.19 vs. 0.82 ± 0.27; $p < 0.001$, respectively; Figure 5G), whereas no significant differences were detected for *IL-1β*. Following LPS stimulation, UltraCal XS markedly upregulated *TNF-α* (6.75 ± 0.77 vs. 1.01 ± 0.20 and 0.84 ± 0.05; $p<0.0001$, Figure 5B), *IL-1β* (55.68 ± 8.54 vs. 1.04 ± 0.36 and 0.34 ± 0.00; $p<0.0001$, Figure 5E), *IL-6* (90.70 ± 19.25 vs. 1.01 ± 0.17 and 2.72 ± 0.69; $p < 0.001$, Figure 5H), and *IL-10* (9.29 ± 3.42 vs. 1.36 ± 0.98 and 1.98 ± 0.89; $p<0.05$, $p < 0.01$, Figure 5K) compared with control and Bio-C Temp groups. Collectively, these findings indicate that UltraCal XS exerts a more pronounced immunomodulatory effect under LPS-induced inflammatory conditions. Conversely, under combined LPS and IFN-γ stimulation, Bio-C Temp significantly increased the expression of *IL-1β* (0.76 ± 0.17 vs. 1.03 ± 0.31 and 0.08 ± 0.03; $p<0.05$, $p<0.01$, Figure 5F), *IL-6* (4.05 ± 0.41 vs. 1.06 ± 0.46 and 0.53 ± 0.12; $p < 0.001$ and $p<0.0001$, Figure 5I), and *IL-10* (6.68 ± 1.36 vs. 1.09 ± 0.53 and 1.19 ± 0.36; $p< 0.001$ Figure 5L), whereas UltraCal XS showed limited modulation under these conditions.

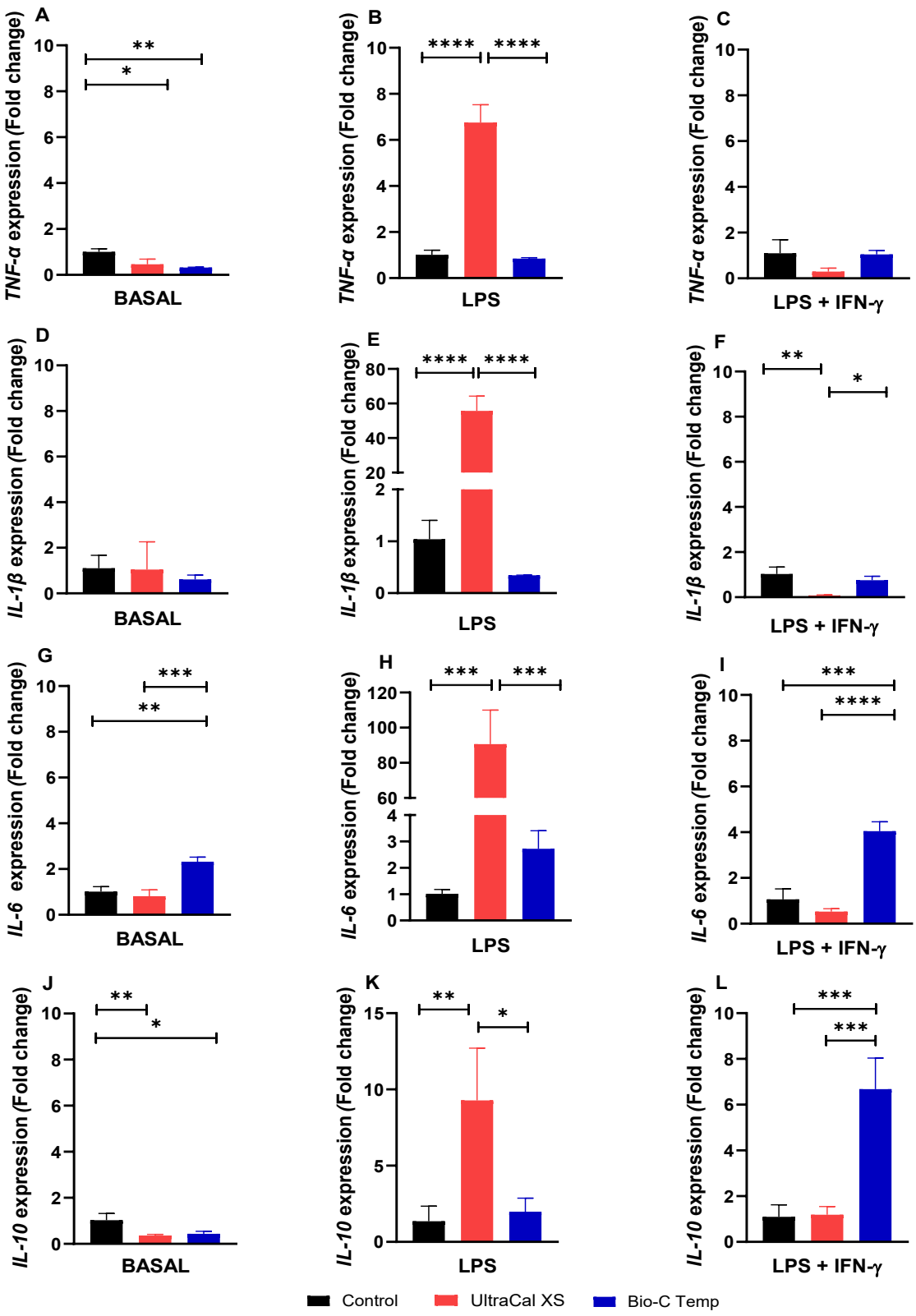


Figure 5. *TNF-α, IL-1β, IL-6* and *IL-10* genes expression by hPDLSC. Cells when exposed to UltraCal XS (1:2), and Bio-C Temp (1:2) in different conditions: basal (A, D, G and J), stimulated with LPS (B, E, H and K) (1 μg.mL^{-1}) and LPS plus IFN-γ (C, F, I and L) (1 μg.mL^{-1}), after 24 h of incubation. Graphs represent mean and standard deviation of three biological replicates in technical triplicate. For analysis of *TNF-α* and *IL-1β* expression, the CT of samples that did not show amplification was set at 40. *p<0.05, **p < 0.01, ***p < 0.001 and ****p<0.0001 represent statistical difference verified by two-way ANOVA and Bonferroni post-test.

DISCUSSION

Intracanal medications are routinely used as adjuncts to chemomechanical preparation to enhance microbial control and support the resolution of periapical inflammation, thereby promoting periradicular repair (Ordinola-Zapata et al., 2022). Beyond antimicrobial efficacy, their interaction with host cells represents a critical factor influencing tissue response, particularly when material components come into close contact with periapical tissues (Hussein & Kishen, 2022). Among intracanal medications, the use of calcium hydroxide continues to be the preferred antiseptic medication in the multi-visit approach for disinfecting the root canal space (Manfredi et al., 2016); however, the most recent advance in endodontics is the incorporation of bioceramics into intracanal medications, such as Bio-C Temp (Angelus). Within this conceptual framework, the present findings indicate that Bio-C Temp (Angelus) and UltraCal XS (Ultradent) elicit concentration-dependent cellular responses, which may be interpreted as reflecting different degrees of cellular exposure to soluble material components rather than discrete clinical scenarios.

In vitro biocompatibility studies commonly employ material extracts at different concentrations to explore concentration-dependent cellular responses. Although simplified, this approach may help distinguish between conditions of higher and lower cellular exposure to soluble components released from intracanal medications. From a conceptual perspective, undiluted extracts may approximate situations of more direct cellular exposure to higher concentrations of released substances, whereas diluted extracts may reflect indirect exposure resulting from diffusion, buffering, and dilution within surrounding tissues (Silva et al., 2021; Ramos et al., 2025). Such an approach may provide insight into how intracanal medications interact with periapical cells under different exposure conditions.

The viability data demonstrated that both materials induced concentration-dependent effects on hPDLSCs. Bio-C Temp (Angelus) exhibited a marked reduction in cell viability at the highest concentration, whereas UltraCal XS (Ultradent) showed a more moderate effect under the same condition.

Progressive dilution of both extracts resulted in partial or complete recovery of cell viability. This pattern is consistent with previous *in vitro* studies reporting increased cytotoxicity of intracanal medications at lower dilutions and improved biocompatibility as material concentration decreases (Guerreiro et al., 2021; Villa et al., 2020; Oliveira et al., 2020). Collectively, these findings suggest that the cytotoxic effects observed are closely related to the degree of cellular exposure rather than to irreversible toxic properties of both materials.

In parallel with the viability results, morphological analysis revealed that higher concentrations of both materials were associated with pronounced alterations in cell architecture, including reduced spreading and loss of cytoplasmic extensions. These features have been described as indicators of cellular stress and impaired adhesion in material–cell interaction studies (Guerreiro et al., 2021; Villa et al., 2020; Oliveira et al., 2020). Quantitative SEM analysis corroborated these observations, particularly for Bio-C Temp (Angelus), which consistently reduced the number of membrane projections without significantly affecting cell size. Similarly, previous studies demonstrated that Bio-C Temp (Angelus) exposure induced morphological alterations in primary human dental pulp cells, characterized by reduced cytoskeletal organization and less elongated fibroblast-like features compared with well-organized cellular patterns (Oliveira et al., 2021). The correspondence between reduced viability and altered morphology suggests that structural changes may reflect functional impairment under conditions of higher material exposure.

Extending these observations, the migration and proliferation assays provided functional evidence of concentration-dependent cellular responses. UltraCal XS (Ultradent) was associated with increased migratory and proliferative activity over time, whereas Bio-C Temp (Angelus) significantly reduced both parameters at higher concentrations, with attenuation of these effects at lower dilutions. Rather than indicating an inherent lack of biocompatibility, these results suggest that Bio-C Temp (Angelus) may exert a transient inhibitory influence on cell dynamics when present at higher concentrations. This finding agrees with previous reports showing that exposure of dental pulp cells to Bio-C Temp (Angelus) is associated with impaired migratory behavior, cytoskeletal disorganization, and reduced cell elongation. Given that actin stress filaments

generate the forces required for cell motility, the reduced migratory capacity observed in the bioceramic group in the wound-healing assay may be associated with less well-organized F-actin stress fibers (Oliveira et al., 2021; Li et al., 2015). The convergence of reduced viability, altered morphology, and impaired migration under these conditions reinforces the interpretation that cellular responses are modulated by exposure level.

Given the central role of inflammation in periapical disease, cytokine expression analysis was performed to further characterize the immunobiological behavior of the materials under different inflammatory stimuli. Under basal conditions, both materials induced minimal changes in cytokine expression, indicating limited disruption of cellular homeostasis in the absence of an inflammatory challenge. Under LPS stimulation, UltraCal XS (Ultradent) induced a pronounced upregulation of both pro-inflammatory and anti-inflammatory cytokines, indicating active modulation of inflammatory signaling in this experimental context.

In contrast, Bio-C Temp (Angelus) demonstrated more substantial cytokine modulation under combined LPS and IFN-γ stimulation, suggesting a differential interaction with cells exposed to a more complex inflammatory environment. These findings are consistent with previous *in vivo* studies reporting that Bio-C Temp (Angelus) exhibits bioactive behavior, characterized by transient increases in alkaline phosphatase activity, reduced inflammatory infiltrate, increased fibroblast density, collagen deposition, and induction of osteocalcin- and osteopontin-positive mineralized structures (Lopes et al., 2024). An *in vivo* rat subcutaneous implantation study showed that Bio-C Temp induces hydroxyapatite and calcite formation, although in lower amounts than calcium hydroxide pastes such as Vitapex, indicating quantitative differences in mineralization capacity (EDANAMI et al., 2023).

Taken together, these findings suggest that UltraCal XS (Ultradent) may be more suitable for clinical scenarios requiring rapid cellular activation and early inflammatory modulation, whereas Bio-C Temp (Angelus) may be better adapted to situations involving sustained inflammation or prolonged intracanal medication. These distinctions may assist clinicians in tailoring intracanal medication selection to the biological demands of each case. However, it is essential to

emphasize that this analogy represents a conceptual model rather than a direct clinical equivalence. *In vivo*, periapical tissues are subject to dynamic biological processes, including vascular clearance, immune cell recruitment, and extracellular matrix interactions, which cannot be fully replicated *in vitro*. Therefore, while the present findings provide a mechanistic insight into how different exposure levels may influence cellular behavior, extrapolation to clinical outcomes should be made with caution.

CONCLUSION

This study demonstrated that both intracanal medications induced concentration-dependent cellular responses, indicating that biological effects are strongly influenced by the level of exposure. UltraCal XS (Ultradent) showed a more stimulatory and immunomodulatory profile, while Bio-C Temp (Angelus) exhibited transient inhibitory effects at higher concentrations, with recovery after dilution. These findings highlight the importance of considering material diffusion and local concentration when interpreting *in vitro* biocompatibility data and when selecting intracanal dressings for different biological contexts.

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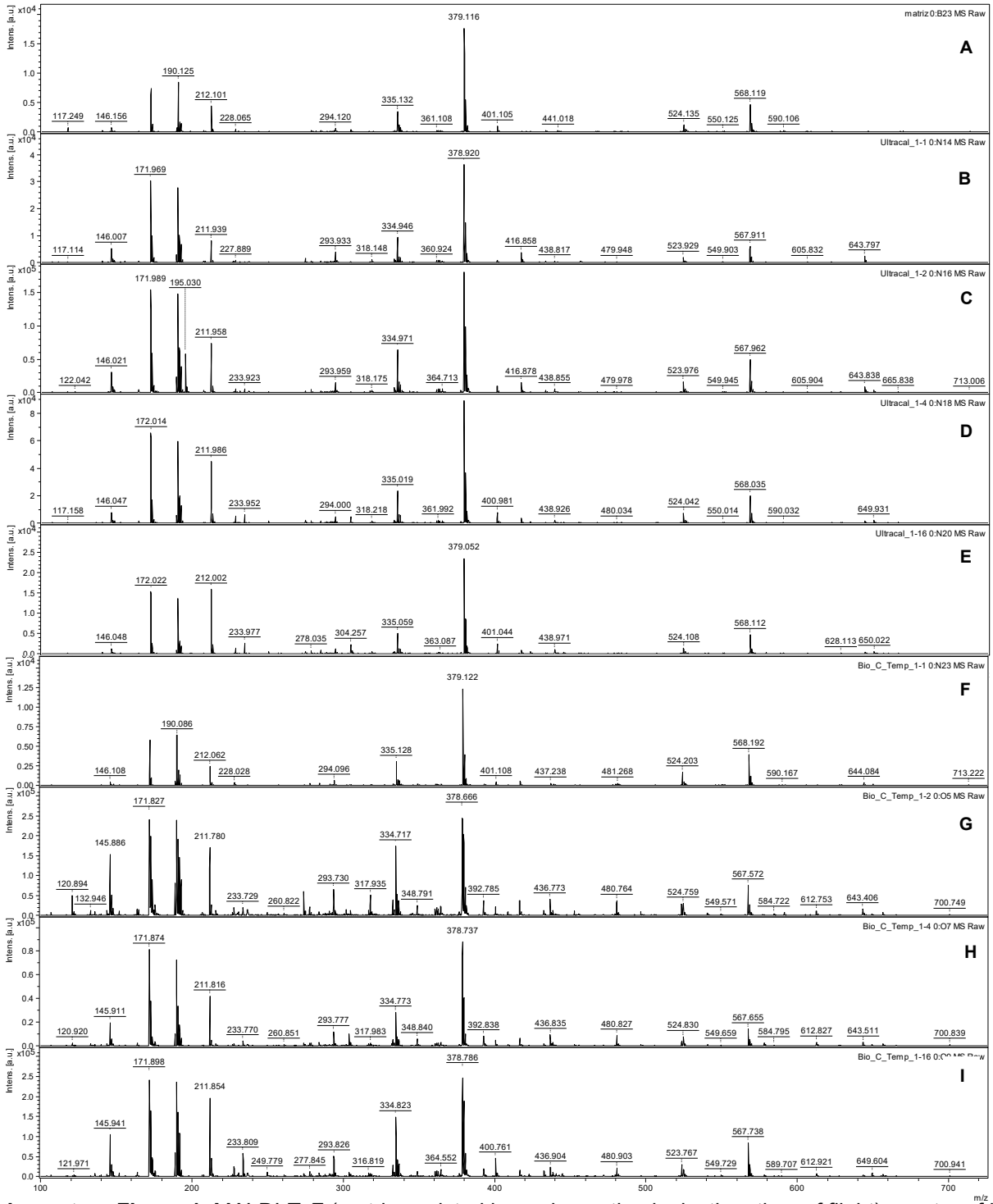
SUPPLEMENTARY FIGURES:



Supplementary Figure 1. MALDI-ToF (matrix-assisted laser desorption ionization–time of flight) spectra of intracanal medication extracts. (A) Matrix; (B–E) UltraCal XS extracts at dilutions (B) 1:1, (C) 1:2, (D) 1:4, (E) 1:16; (F–I) Bio-C Temp extracts at dilutions (F) 1:1, (G) 1:2, (H) 1:4, (I) 1:16.

CONSIDERAÇÕES FINAIS

Os dois capítulos apresentados nesta dissertação contribuem de forma significativa para o avanço das pesquisas sobre materiais endodônticos, especialmente os biocerâmicos. Eles apresentam resultados relacionados a uma nova medicação intracanal, abordando sua atividade antimicrobiana e as respostas biológicas em dois tipos celulares distintos. Além disso, incluem análises físico-químicas do material, ampliando a compreensão de suas propriedades e potenciais aplicações clínicas.

No Capítulo 1, foram realizadas análises físico-químicas e biológicas das medicações intracanais UltraCal XS (Ultradent) e Bio-C Temp (Angelus). A atividade antimicrobiana foi avaliada contra três microrganismos, apresentando efeito predominantemente bacteriostático. A análise celular em PBMCs indicou que o UltraCal XS (Ultradent) manteve boa viabilidade, permitiu migração e modulou a produção de óxido nítrico, reduzindo significativamente os níveis de nitrito em todas as diluições. Em contraste, o Bio-C Temp (Angelus) apresentou citotoxicidade dose-dependente, baixa migração e efeito limitado sobre a produção de óxido nítrico, com resposta dose-dependente. Estes achados evidenciam que, apesar de uma atividade antimicrobiana semelhante entre as duas medicações intracanais, as respostas biológicas diferem significativamente, reforçando a importância de integrar análises físico-químicas e celulares para compreender o potencial terapêutico das medicações intracanais e suas implicações no reparo tecidual. Apesar da inexistência de literatura prévia utilizando este tipo celular, os achados se mostraram consistentes com resultados obtidos em outros tipos celulares relevantes, além de apresentarem metodologias e análises inéditas que contribuem para ampliar a literatura na área.

No Capítulo 2, foi realizada uma avaliação biológica das medicações intracanais UltraCal XS (Ultradent) e Bio-C Temp (Angelus) voltada para as células do ligamento periodontal, na qual foram obtidas respostas em termos de citotoxicidade, migração, proliferação, morfologia e expressão gênica. O Bio-C Temp (Angelus) apresentou alta toxicidade nos extratos mais concentrados, com sinais de recuperação à medida que as diluições aumentaram, resultados semelhantes ao encontrado no capítulo 1, com células PBMCs. Esse comportamento foi confirmado

nos ensaios de migração e proliferação, nos quais o Bio-C Temp (Angelus) mostrou incapacidade de fechamento da ferida no ensaio de *scratch*, indicando impacto negativo na capacidade regenerativa das células, nos períodos analisados. Em contraste, o UltraCal XS (Ultradent) manteve viabilidade celular consistente em todas as diluições, promovendo migração eficaz e proliferação adequada, quase completando a cicatrização da área desnuda *in vitro*. A análise morfológica por microscopia eletrônica de varredura corroborou esses achados, mostrando morte celular em altas concentrações de Bio-C Temp (Angelus), recuperação com o passar das diluições e preservação estrutural nas células expostas ao UltraCal XS (Ultradent). A expressão gênica indicou que ambos os materiais responderam aos estímulos inflamatórios, UltraCal XS (Ultradent) induziu aumento significativo tanto de citocinas pró-inflamatórias, quanto anti-inflamatórias, evidenciando modulação ativa da resposta inflamatória. Em contraste, o Bio-C Temp (Angelus) apresentou maior efeito modulador de citocinas quando as células foram expostas à ambientes inflamatórios mais complexos. Estes resultados reforçam que a biocompatibilidade e o efeito modulador das medicações intracanais dependem não apenas da composição química, mas também da concentração e do tipo celular exposto. O UltraCal XS (Ultradent) demonstrou perfil mais favorável para promover viabilidade, migração e proliferação das células do ligamento periodontal, enquanto o Bio-C Temp (Angelus), apesar de efeitos tóxicos em concentrações elevadas, demonstrou capacidade de recuperação em diluições menores e potencial modulador em situações inflamatórias complexas, destacando aspectos que podem ser explorados em estudos futuros.

Em conclusão, a integração das evidências apresentadas nestes dois artigos fornece uma visão integral do comportamento físico-químico, antimicrobiano e biológico da medicação intracanal biocerâmica Bio-C Temp (Angelus) e do UltraCal XS (Ultradent), contribuindo para o avanço das pesquisas na área de materiais endodônticos. Os achados destacam diferenças importantes entre formulações, evidenciando como alterações na composição e na presença de elementos distintos podem impactar a atividade antimicrobiana, a citotoxicidade e a capacidade regenerativa celular, oferecendo lacunas para futuras modificações e desenvolvimento de novas formulações. Além disso, reforçam que uma medicação intracanal ideal deve equilibrar atividade antimicrobiana efetiva com boa biocompatibilidade, garantindo não apenas o controle microbiano, mas também a

preservação e modulação das células periapicais, fundamentais para a reparação tecidual e o sucesso clínico.

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ANEXOS

Anexo 1. Parecer consubstanciado de aprovação do projeto no CEP/UnB e CEP/UCB referente as células PBMCs (primeira e última página do parecer).

FACULDADE DE CIÊNCIAS DA SAÚDE DA UNIVERSIDADE DE BRASÍLIA - UNB

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Bio-C Temp: análise antimicrobiana, imunológica e osteoclastogênica in vitro.

Pesquisador: Maria Ester França de Melo
Área Temática:
Versão: 2
CAAE: 79508624.5.0000.0030
Instituição Proponente: Programa de Pós-graduação em Ciências da Saúde
Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 7.048.361

Apresentação do Projeto:
Conforme documento "PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_2321477.pdf", postado em 21/07/2024:

concordância		14:35:42	de Melo	
Outros	curriculopesquisadora.pdf	27/04/2024 14:34:44	Maria Ester França de Melo	Aceito
Outros	Curriculoorientadora.pdf	27/04/2024 14:34:07	Maria Ester França de Melo	Aceito
Outros	clinicaucb.pdf	27/04/2024 14:32:48	Maria Ester França de Melo	Aceito
Outros	biotec.pdf	27/04/2024 14:32:22	Maria Ester França de Melo	Aceito
Folha de Rosto	folhaderosto.pdf	27/04/2024 14:29:52	Maria Ester França de Melo	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

BRASILIA, 02 de Setembro de 2024

Assinado por:
Cristiane Tomaz Rocha
(Coordenador(a))

Endereço: Faculdade de Ciências da Saúde, Universidade de Brasília - Campus Darcy Ribeiro
Bairro: Asa Norte **CEP:** 70.910-900
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Página 15 de 15

UNIVERSIDADE CATÓLICA DE BRASÍLIA - UCB

PARECER CONSUBSTANCIADO DO CEP

Elaborado pela Instituição Coparticipante

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Bio-C Temp: análise antimicrobiana, imunológica e osteoclastogênica in vitro.

Pesquisador: Maria Ester França de Melo
Área Temática:
Versão: 3
CAAE: 79508624.5.3001.0029
Instituição Proponente: Universidade Católica de Brasília - UCB
Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 7.395.333

Apresentação do Projeto:
Trata-se de um projeto de pesquisa do Programa de Pós-graduação em Ciências da Saúde da Universidade de Brasília, tendo o Curso de Odontologia da Universidade Católica de Brasília como co-participantes, a ser desenvolvido pela aluna de mestrado Maria Ester França de Melo, orientada pela Profa. Dra. Taia Maria Berto Rezende.

		19:41:43	de Melo	
Outros	orcamento.pdf	27/04/2024 14:38:19	Maria Ester França de Melo	Aceito
Outros	responsabilidade.pdf	27/04/2024 14:36:22	Maria Ester França de Melo	Aceito
Outros	curriculopesquisadora.pdf	27/04/2024 14:34:44	Maria Ester França de Melo	Aceito
Outros	Curriculoorientadora.pdf	27/04/2024 14:34:07	Maria Ester França de Melo	Aceito
Outros	clinicaucb.pdf	27/04/2024 14:32:48	Maria Ester França de Melo	Aceito
Outros	biotec.pdf	27/04/2024 14:32:22	Maria Ester França de Melo	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

BRASILIA, 19 de Fevereiro de 2025

Assinado por:
MARCELO HENRIQUE SOLLER RAMADA
(Coordenador(a))

Endereço: QS 07 ¿ Lote 01 ¿ EPCT ¿ Águas Claras, Bloco C, 2° Andar, Sala C204-A.
Bairro: Taguatinga **CEP:** 71.966-700
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Anexo 2. Parecer consubstanciado de aprovação do projeto no CEP/UnB e CEP/UCB referente a células hPDLSCs. (primeira e última página do parecer).

FACULDADE DE CIÊNCIAS DA SAÚDE DA UNIVERSIDADE DE BRASÍLIA - UNB

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Bio-C Temp: análise da viabilidade, migração, proliferação e expressão gênica em células do ligamento periodontal humano

Pesquisador: Maria Ester França de Melo

Área Temática:

Versão: 1

CAAE: 91049325.6.0000.0030

Instituição Proponente: Programa de Pós-graduação em Ciências da Saúde

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 7.843.391

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Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

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FACULDADE DE CIÊNCIAS DA SAÚDE DA UNIVERSIDADE DE BRASÍLIA - UNB

BRASILIA, 18 de Setembro de 2025

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(Coordenador(a))

UNIVERSIDADE CATÓLICA DE BRASÍLIA - UCB

PARECER CONSUBSTANCIADO DO CEP

Elaborado pela Instituição Coparticipante

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Bio-C Temp: análise da viabilidade, migração, proliferação e expressão gênica em células do ligamento periodontal humano

Pesquisador: Maria Ester França de Melo

Área Temática:

Versão: 2

CAAE: 91049325.6.3001.0029

Instituição Proponente: Universidade Católica de Brasília - UCB

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 7.977.349

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Situação do Parecer:
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Necessita Apreciação da CONEP:
Não

BRASILIA, 13 de Novembro de 2025

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Anexo 3. Trabalhos em colaboração publicados durante o período de mestrado.

Artigo 1. Antimicrobial, toxicity, and cellular interactions of bioceramic sealers in periodontal ligament: in vitro insights. Clinical Oral Investigations (IF: 3.1, A1 CAPES)

Clinical Oral Investigations (2025) 29:505
https://doi.org/10.1007/s00784-025-06591-z

RESEARCH

Antimicrobial, toxicity, and cellular interactions of bioceramic sealers in periodontal ligament: in vitro insights

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Abstract

Introduction This study aimed to evaluate the antimicrobial, cytotoxic, proliferative, migratory, and immunomodulatory potential of Bio-C Sealer and Bio-C Sealer ion+extracts compared to AH Plus, using human periodontal ligament stem cells (hPDLSCs).

Materials and methods Initially, the minimum concentrations of sealer extracts required to inhibit the growth of *Enterococcus faecalis* and *Candida albicans* were determined. hPDLSCs were then obtained from extracted third molars of young donors and cultured under standard conditions. Cells were characterized by flow cytometry using mesenchymal stem cell markers (CD44, CD73, CD90, CD105) and a negative cocktail of hematopoietic markers, following the minimal criteria of the International Society for Cellular Therapy. Subsequently, the cytotoxic, proliferative, and migratory effects on hPDLSCs were assessed using MTT, Trypan Blue exclusion and scratch assays. Finally, the gene expression profile of hPDLSCs exposed to sealer extracts was analyzed by qPCR. Statistical differences were evaluated using one-way and two-way ANOVA followed by Tukey's post hoc tests.

Results AH Plus and Bio-C Sealer ion+inhibited 100% of *E. faecalis* growth at extract dilutions of 1:1, 1:2, and 1:4. How-

Artigo 2. Impact of Cellular Senescence on the Immune-Inflammatory Response and Regenerative Capacity of Human Dental Pulp Cells. Journal of Endodontics (IF: 3.6, A1 CAPES)

BASIC RESEARCH – BIOLOGY

Impact of Cellular Senescence on the Immune-Inflammatory Response and Regenerative Capacity of Human Dental Pulp Cells

Johnny Carvalho da Silva, MD, DDS,[*] Raquel Figuerêdo Ramos, MD, DDS,[*] Ana Angélica Soares Vieira da Silva, DDS,[†] Maria Ester França de Melo, DDS,[*] Mayara Alves de Oliveira, MD, DDS,[*] Larissa Barbosa de Sousa, MD, DDS,[*] Elizabete Cristina Iseke Bispo, MD,[‡] Ingrid Gracielle Martins da Silva, MD,[§] Maurício Gonçalves da Costa Sousa, PhD, MD, DDS,[‖¶#] José Raimundo Corrêa, PhD, MD,[§] Felipe Saldanha-Araujo, PhD, MD,[‡] and Taia Maria Berto Rezende, PhD, MD, DDS[†**††]*

SIGNIFICANCE

This study demonstrates that cellular senescence impairs the immune inflammatory balance and regenerative capacity of dental pulp cells, which may compromise the success of conservative endodontic treatments. Targeting

ABSTRACT

Introduction: Cellular senescence is a state of irreversible cell cycle arrest that can compromise tissue homeostasis through the secretion of inflammatory mediators and disruption of the cellular microenvironment. In dental pulp, senescence may impair reparative functions and increase susceptibility to inflammation and degeneration. However, the specific effects of senescence on the immune behavior of pulp cells remain poorly understood. This study aimed to investigate how senescence influences the viability, morphology, migration, proliferation, and immune response of human dental pulp cells. **Methods:** Cellular senescence was induced with 500 μM doxorubicin and confirmed by β-galactosidase staining. To simulate immunoinflammatory conditions, cells were stimulated with lipopolysaccharide, either alone or in combination with interferon-gamma. Morphologic

Anexo 4. Trabalho em colaboração elaborado durante o período de mestrado, submetido e em fase de apreciação.

Artigo 1: Inflammation-Driven Senescence Reduces the Regenerative Capacity of apical papilla stem cell in vitro: Implications for regenerative endodontic therapy. Submetido na revista International Endodontic Journal (IF: 7.1, A1 CAPES)

TITLE

Inflammation-Driven Senescence Reduces the Regenerative Capacity of apical papilla stem cell in vitro: Implications for regenerative endodontic therapy.

RUNNING TITLE

Apical papilla stem cell senescence

KEYWORDS

Regenerative Endodontics, Cellular Senescence, Mesenchymal Stem Cells, Dental Papilla, Senescence-Associated Secretory Phenotype

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