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CITOTOXICIDADE, GENOTOXICIDADE E EXPRESSÃO GÊNICA EM CÉLULAS-TRONCO MESENQUIMAIS EXPOSTAS A MATERIAIS ENDODÔNTICOS

Faculdade de Odontologia
Universidade Federal de Minas Gerais
Belo Horizonte
2021

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Tese apresentada ao Colegiado de Pós-Graduação em Odontologia da Faculdade de Odontologia da Universidade Federal de Minas Gerais, como requisito parcial à obtenção do grau de Doutor em Odontologia - área de concentração em Endodontia.

Orientador: Prof. Dr. Antônio Paulino R. Sobrinho

Belo Horizonte
2021

Ficha Catalográfica

048c
2021
T

Oliveira, Patrícia Yanne de.
Citotoxicidade, genotoxicidade e expressão gênica em células-tronco mesenquimais expostas a materiais endodônticos / Patrícia Yanne de Oliveira. -- 2021.

114 f. : il.

Orientador: Antônio Paulino Ribeiro Sobrinho.

Tese (Doutorado) -- Universidade Federal de Minas Gerais, Faculdade de Odontologia.

1. Células-tronco. 2. Genotoxidade. 3. Expressão gênica. 4. Teste de materiais. I. Ribeiro Sobrinho, Antônio Paulino. II. Universidade Federal de Minas Gerais. Faculdade de Odontologia. III. Título.

BLACK - D047

Elaborada por Miriam Cândida de Jesus - CRB 6/2727.

UNIVERSIDADE FEDERAL DE MINAS GERAIS

PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA

COLEGIAD DE PÓS-GRADUAÇÃO EM ODONTOLOGIA

FOLHA DE APROVAÇÃO

Citotoxicidade, genotoxicidade e expressão gênica em células-tronco mesenquimais expostas a materiais endodônticos

PATRÍCIA YANNE DE OLIVEIRA

Tese submetida à Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em Odontologia, como requisito para obtenção do grau de Doutor, área de concentração Endodontia.

Aprovada em 30 de julho de 2021, pela banca constituída pelos membros:

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Belo Horizonte, 30 de julho de 2021.

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Dedico esta conquista aos meus pais, Marcos e Jean por toda ajuda, incentivo e apoio. Aos meus irmãos Fernanda e Felippe que me incentivam e torcem sempre por mim e ao Júnior, meu companheiro e incentivador incondicional.

AGRADECIMENTOS

Agradeço a Deus, pela oportunidade, por olhar sempre por mim e por ter me dado uma família que nunca mediu esforços para que esta etapa fosse concluída.

Aos meus pais Marcos e Jean que sempre me apoiaram incondicionalmente, sempre me ensinaram que cultura e educação vêm sempre em primeiro lugar e que nunca mediram esforços para que a minha carreira acadêmica acontecesse. Nada disso seria possível sem vocês.

Aos meus irmãos Fernanda e Felippe que são meu porto seguro, me apoiam e fazem a vida ser mais leve. Obrigada por me receberem de portas abertas em Belo Horizonte e por estarem sempre junto comigo nesta longa caminhada da vida.

Ao meu companheiro de vida Júnior Velloso, por todo apoio, amizade, incentivo e amor. Você certamente faz a vida ser mais leve e divertida. Obrigada por me acalmar nos momentos difíceis e por estar sempre ao meu lado na Valsa e no Vapor.

A minha tia querida Marlene, por todo carinho, pela presença e pelo amor incondicional. Essa etapa também é mérito seu.

A minha avó Maria Eugênia (em memória) e ao meu querido tio Márcio César de Oliveira (em memória) sempre carinhosos e repletos de palavras de amor. Obrigada por me ensinarem sobre bondade e sobre família. A perda de vocês fez com que o ano de 2020/2021 perdesse um pouco de cor. As batalhas lutadas na pandemia para a conclusão deste trabalho não foram fáceis.

Ao meu orientador, Professor Doutor Antônio Paulino Ribeiro Sobrinho, por ter me recebido de portas abertas dentro da UFMG, por ter sido sempre muito solicito e pelos ensinamentos, dedicação, compreensão e paciência. Não estaria aqui se não fosse por você. Todos os agradecimentos são poucos.

Ao professor e amigo Carlos Magno da Costa Maranduba, que me ensina muito sobre a vida prática, teórica e laboratorial. Muito obrigada por ter me recebido de portas abertas na UFJF. Para mim, você é um grande exemplo de excelência na vida acadêmica, profissional e pessoal. Um exemplo a ser seguido e uma grande referência.

A querida professora Francine Benetti, por todo o suporte, disponibilidade, paciência e carinho. Obrigada pela parceria nesta tese. Você foi parte essencial para a conclusão do meu doutorado.

Às colegas da UFMG, Sâmila Barra, Paula Ribeiro, Layara Tavares, Aline Cruz e Aline Maria, por toda a ajuda e apoio que recebi de vocês nos momentos em que passamos juntas. Vocês fizeram o Doutorado ser mais leve.

Aos colegas e mestres da vida acadêmica Mariane Lacerda, Leandro Rezende, Antônio Márcio Resende do Carmo que em muitos momentos da vida acadêmica me abraçaram.

Agradeço imensamente ao João Rettore, por ter disponibilizado seu tempo para me ajudar e me ensinar muito sobre RT-PCR.

Agradeço ao Professor Humberto de Mello Brandão, que gentilmente me cedeu equipamentos e materiais quando tivemos problemas técnicos nos nossos equipamentos.

A querida amiga Camila Cabral Mazini por todo carinho e suporte tecnológico.

A todas as minhas amigas queridas e plenas, por estarem sempre ao meu lado me apoiando e divertindo. Vocês são incríveis.

A minha família Oliveira: vocês são meu porto seguro.

A toda família Velloso, obrigada por me receberem de portas abertas.

A Universidade Federal de Minas Gerais e a Faculdade de Odontologia da UFMG, com seus professores e funcionários que me receberam e investiram de alguma maneira na minha carreira acadêmica.

Finalmente agradeço a Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) pelo apoio financeiro.

“O correr da vida embrulha tudo.
A vida é assim: esquenta e esfria,
Aperta e daí afrouxa,
Sossega e depois desinquieta
O que ela quer da gente é coragem”.
Guimarães Rosa

RESUMO

No mercado encontramos uma grande variedade de materiais endodônticos disponibilizados para uso clínico, mas diversos estudos mostram divergências de opiniões com relação ao comportamento biológico dos diferentes materiais. Este trabalho teve como objetivos investigar a viabilidade celular, a expressão de genes envolvidos na plasticidade celular e a diferenciação celular em culturas de células-tronco recuperadas de polpa dentária humana (*hDPSCs*) quando em contato com quatro materiais endodônticos (*Endofill, Pulp Canal Sealer, Sealer 26, MTA*) rotineiramente utilizados na clínica odontológica. Objetivou também, por meio de uma revisão sistemática, analisar a biocompatibilidade de cimentos de uso endodôntico sobre células tronco de origem dental. Para isto, o metabolismo celular das *hDPSCs*, quando em contato com os capilares contendo ou não os cimentos, foi avaliado pelo ensaio de *MTT* (24 e 48 horas) e a viabilidade celular foi analisada pelo ensaio de exclusão do azul de tripan (48 horas). A plasticidade celular, na presença dos capilares contendo ou não os cimentos, foi avaliada pela expressão gênica dos marcadores *CD34, CD45, Nestin, CD105, Nanog* e *OCT-4* por *PCR*. Finalmente, a diferenciação celular frente aos cimentos endodônticos foi verificada pela expressão dos genes *RUNX2, ALP, OC/BGLAP* e *DMP1* por *RT-PCR*. Os dados foram analisados pelo teste ANOVA com correção de Bonferroni ($p<0.05$). Observou-se que os cimentos *Pulp Canal Sealer* e o *Endofill* reduziram significativamente a viabilidade e o metabolismo celular quando comparados ao controle após 48 horas ($p<0.001$). O *MTA* e o *Sealer 26* não interferiram na viabilidade celular em ambos os períodos de avaliação ($p>0.05$). As *hDPSCs*, quando cultivadas na presença do *MTA* e *Sealer 26*, expressaram os marcadores *Nestin, CD105, NANOG* e *OCT-4*, e não expressaram *CD34* e *CD45*. Por sua vez, o *MTA* e o *Sealer 26* interferiram positivamente ou negativamente na expressão gênica de *DMP1, OC/BGLAP* e *RUNX2* em relação ao grupo controle ($p<0.05$), mas não houve diferença significativa em relação à expressão gênica de *ALP* ($p>0.05$). Portanto, *MTA* e *Sealer 26* demonstram boa compatibilidade biológica quando na presença das *hDPSCs.* A revisão sistemática demonstrou que a maioria dos materiais, apresentam boa compatibilidade quando em contato com as células tronco, estando aptos a serem utilizados na prática clínica.

Palavras-chave: Células-tronco mesenquimais. Citotoxicidade. Agregado de trióxido mineral. Genotoxicidade. Células-tronco da polpa dentária.

ABSTRACT

Cytotoxicity, genotoxicity and gene expression in mesenchymal stem cells exposed to endodontic cements

On the market, we found a wide variety of endodontics cements available for clinical use, but several studies show divergences of opinion regarding the biological behavior of these different materials. This work aimed to investigate cell viability and metabolism, an expression of genes involved in cell plasticity and cell differentiation in stem cell cultures recovered from human dental pulp (hDPSCs) when in contact with four endodontic cements (Endofill, MTA, Pulp Canal Sealer, Sealer 26) routinely used in endodontic clinic. It also aimed, through a systematic review, to analyze the biocompatibility of endodontic materials on dental stem cells. For this, the viability and metabolism of hDPSCs, when it comes into contact with capillaries that included or not cements, was assessed by MTT assay (24 and 48 hours) and exclusion of trypan blue assay (48 hours). Cellular plasticity, with the presence of capillaries containing or not sealers, was evaluated by the genetic expression of the markers CD34, CD45, Nestin, CD105, Nanog and OCT-4 by PCR. Finally, cell differentiation from endodontics sealers was verified by the expression of the RUNX2, ALP, OC/BGLAP and DMP1 genes by RT-PCR. The data were analyzed using the ANOVA test with Bonferroni correction ($p<0.05$). We note that Pulp Canal Sealer and Endofill sealers decrease cell viability and cellular metabolism when compared to control after 48 hours ($p<0.001$). MTA and Sealer 26 did not interfere with cell viability in the two evaluation periods ($p>0.05$). hDPSCs, when grown in the presence of MTA and Sealer 26, express the Nestin, CD105, NANOG and OCT-4 markers, and do not express CD34 and CD45. In turn, MTA and Sealer 26 interfered in the gene expression of DMP1, OC/BGLAP and RUNX2 in relation to the control group ($p<0.05$), but did not find a significant difference in relation to the ALP gene expression ($p>0.05$). Therefore, MTA and Sealer 26 demonstrate good biological compatibility when in the presence of hDPSCs. The systematic review showed that almost all materials have good compatibility when in contact with stem cells, being able to be used in clinical practice.

Keywords: Mesenchymal stem cells. Cytotoxicity. Mineral trioxide aggregate. Genotoxicity. Dental pulp stem cells.

LISTA DE ILUSTRAÇÕES

LISTA DE TABELAS

LISTA DE ABREVIATURAS E SIGLAS

ACTB	Beta Actina, Do Inglês *Actin Beta*
AHP	*Ah Plus*
ALP	Fosfatase Alcalina, Do Inglês, *Alkaline Phosphatase*
AR	Do Inglês, *Alizarin Red Assay*
BECS	Do Inglês, *Bioactive Cements*
BMSCs	Células-Tronco Mesenquimais Da Medula Óssea, Do Inglês, *Bone Marrow Mesenchymal Stem Cells*
BR	*Bioroot*
BR-BC	*Bioroot BC Sealer*
BR-RCS	*Bioroot RCS Sealer*
BSP	Do Inglês, *Bone Sialoprotein*
CD34	Do Inglês, *Cluster Of Differentiation 34*
CD45	Do Inglês, *Cluster Of Differentiation 45*
CD105	Do Inglês, *Cluster Of Differentiation 105*
COEP	Comitê De Ética Em Pesquisa
CS	*Ceraseal*
CT	Do Inglês, *Threshold Cycle*
DAPI	4',6'-Diamino-2-Fenil-Indol
DMP1	Proteína Da Matriz De Dentina, Do Inglês, *Dentin Matrix Protein-1*
D-MEM	Do Inglês, *Dulbecco's Modified Eagle Medium*
DEPC	Dietil Pirocarbonato
DNTP	Desoxirribonucleotídeos Fosfatados
DSPP	Do Inglês, *Dentin Sialophosphoprotein*
EDTA	Ácido Etilenodiamino Tetra-Acético
EDX	Análise De Energia Dispersiva Por Raio X, Do Inglês, Energy-Dispersive X-Ray Analysis
ELISA	Do Inglês, *Enzyme-Linked Immunosorbent Assay*
ESMTA	*Endoseal MTA*
ESQ	*Endosequence*
ESQ-BC	*Endosequence BC*
ES-TCS	*Endoseal TCS*

FBS	Soro Fetal Bovino, Do Inglês, *Fetal Bovine Serum*
GAPDH	Do Inglês, *Glyceraldehyde 3-Phosphate Dehydrogenase*
GF-2	*Guttaflow 2*
GF-BS	*Guttaflow Bioseal*
hDPSCs	Células-Tronco Da Polpa Dentária Humana, Do Inglês, *Human Dental Pulp Stem Cells*
hDFSCs	Células-Tronco Do Folículo Dentário, Do Inglês, Dental Follicle Stem Cells
hPLSCs	Células-Tronco Do Ligamento Periodontal, Do Inglês, *Human Periodontal Ligament Stem Cells*
HPRT1	Do Inglês, *Hypoxanthine Phosphoribosyltransferase 1*
hTGSCs	Células-Tronco Do Germe Dentário, Do Inglês, *Human Tooth Germ Stem Cells*
IL-1	Interleucina 1, Do Inglês, *Interleukin 1*
IL-6	Interleucina 6, Do Inglês, *Interleukin 6*
IL-8	Interleucina 8, Do Inglês, *Interleukin 8*
IL-10	Interleucina 10, Do Inglês, *Interleukin 10*
MTA	Do Inglês, *Mineral Trioxide Aggregate*
MTAA	*Mta Angelus*
MTAF	*Mta Fillapex*
MSCs	Células-Tronco Mesenquimais, Do Inglês, *Mesenchymal Stem Cells*
MTT	Ensaio De 3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyl Tetrazolium Bromide
MTS	Ensaio De 3-(4,5-Dimethyl-Thiazol-2-Yl)-5-(3-Carboxy-Methoxy-Phenyl)-2-(4-Sulpho-Phenyl)-2H Tetrazolium
N.A.	Do Inglês, *Not Applicable*
NANOG	Do Inglês, *Homeobox NANOG Protein*
NESTIN	Do Inglês, *Neuroectodermal Stem Cell Marker*
OC/BGLAP	Proteína Óssea Gamacarboxyglutamato[Gla]/Osteocalcina
OCN	Osteocalcina
OCT4	Do Inglês, *Octamer-Binding Transcription Factor 4*
PBS	Do Inglês, *Phosphate-Buffered Saline*
PKH26	Do Inglês, *PKH26 Red Fluorescent Cell Linker Kit For General Cell Membrane Labeling*

PR	*Proroot*
PR-ES	*Proroot ES*
RPMI	*Roswell Park Memorial Institute 1640 Medium*
RTPCR	Do Inglês, *Reverse Transcription Polymerase Chain Reaction*
RUNX2	Fator De Transcrição Relacionado Com *Runt 2*
SC	Células-Tronco, Do Inglês, *Stem Cells*
SCR	Sistemas De Canais Radiculares
SEM	Microscopia Eletrônica De Varredura, Do Inglês, *Scanning Electron Microscopy*
SFB	Soro Fetal Bovino
SRB	Do Inglês, *Sulforhodamine B Assay*
TF	*Totalfill Sealer*
TF-BC	*Totalfill BC Sealer*
TNF	Fator De Necrose Tumoral, Do Inglês, *Tumor Necrosis Factor*
UFJF	Universidade Federal De Juiz De Fora
UFMG	Universidade Federal De Minas Gerais

SUMÁRIO

1 CONSIDERAÇÕES INICIAIS

A obturação do sistema de canais radiculares (SCR), considerada uma importante etapa do tratamento endodôntico, tem como objetivo o vedamento hermético e tridimensional dos espaços dos canais, proporcionando condições para o reparo dos tecidos perirradiculares, bem como impedindo a recontaminação bacteriana (SILVA et al., 2016). Atingir tais objetivos, entretanto, ainda é um desafio, principalmente no que tange o comportamento biológico dos materiais obturadores, que além de não serem citotóxicos (ao menos a médio e longo prazo), devem apresentar biocompatibilidade, permitindo o processo de reparação dos tecidos apicais (RABELO, 2012).

Dessa forma, a Odontologia vem, ao longo dos anos, buscando materiais que sejam não apenas tolerados pelos tecidos apicais, mas também, capazes de favorecer um bom prognóstico após o tratamento endodôntico, seja por efeitos antimicrobianos, seja promovendo a cicatrização (BRACKETT *et al.,* 2012; CAMARGO *et al.,* 2014; DIMITROVA- NAKOV *et al.,* 2015; KAUR *et al.,* 2015; SILVA *et al.,* 2015; SILVA *et al.,* 2016).

Dentre esses materiais endodônticos, pode-se citar o *Endofill* (*Dentsply, Maillefer*, Chile) e *Pulp Canal Sealer* (*Kerr, Sybron Endo USA*), que têm sido utilizados na endodontia devido ao sucesso a longo prazo. No entanto, por terem na sua composição o óxido de zinco e eugenol (ZOE), podem induzir irritação periapical (MARTINS *et al.,* 2013).

A fim de contornar tais limitações, surgem os cimentos à base de hidróxido de cálcio, tais como o *Sealer 26* (*Dentsply*, Petrópolis, Brasil), devido às propriedades antimicrobiana, anti-inflamatória, ação alcalinizante, excelente biocompatibilidade e neutralização de endotoxinas presentes na parede celular de bactérias gram negativas (SOARES *et al.,* 2013).

Introduzido na Odontologia em meados dos anos 90, o agregado de trióxido mineral, amplamente difundido na literatura como MTA, tem como precursor o cimento de *Portland*, descoberto por Joseph Aspadim, em 1824. Tem como característica excelente composição físico-química e propriedades biológicas. Promove selamento eficaz, reparação e regeneração do ligamento periodontal, recuperação óssea e formação do cemento. Permite utilização em

ambiente úmido sem perda de propriedades e promove a biocompatibilidade tecidual. Devido a essas características, tem sido amplamente utilizado como padrão-ouro de biocompatibilidade (KIM *et al.,* 2016).

Estes cimentos, quando utilizados, entram em contato direto com as células-tronco mesenquimais humanas (*hMSCs*), que podem ser obtidas a partir de vários tecidos, tais como polpa de dente, ligamento periodontal, ossos, cordão umbilical medula óssea, músculo esquelético, tecido adiposo, pulmão, fígado, entre outros (GRONTHOS *et al.,* 2000).

MSCs são células-tronco adultas multipotentes com propriedades fisiológicas homólogas as do tecido primário, sendo, portanto, um ótimo modelo experimental para se estudar as propriedades biológicas dos materiais (LÓPEZ-GARCÍA *et al.,* 2019; VICTORIA-ESCANDELL *et al.,* 2017). Dentre as células-tronco, as da polpa dentária humana (*hDPSC*) se tornam ainda mais atraentes para as pesquisas na área odontológica, pois apresentam o mesmo fenótipo das células-tronco mesenquimais da medula óssea (*BMSCs*). Inclusive, expressam marcadores moleculares de *MSCs*, apresentam potencial de imunorregulação, capacidade de se diferenciar em células osteogênicas e odontogênicas. Ademais, as *hDPSCs* são facilmente obtidas de dentes humanos. Como modelo de estudo, são muito acessíveis e fornecem um número suficiente de células nos estudos laboratoriais, graças às suas altas taxas de proliferação e expressão de telomerase, que mantêm a sua estabilidade genética (LIU *et al.,* 2015).

Apesar das vantagens de se trabalhar com as *hDPSCs*, poucos estudos se ativeram sobre tais parâmetros (ALSUBAIT et al., 2018; COLLADO-GONZÁLES et al., 2016; DIMITROVA-NAKOV et al., 2015; GUVEN et al., 2013; JAVID et al., 2020; RODRÍGUEZ-LOZANO et al., 2017; RODRÍGUEZ-LOZANO et al., 2019; VICTORIA-ESCANDELL et al., 2017). Portanto, o objetivo deste estudo foi investigar os efeitos causados por materiais endodônticos (*Endofill, MTA, Pulp Canal Sealer, Sealer 26*) quando em contato com culturas de *hDPSCs*. Para tal, avaliou-se a viabilidade celular, a expressão de genes envolvidos na plasticidade celular (*CD34, CD45, Nestin e CD105, OCT4 e NANOG*), e na diferenciação celular sendo fosfatase alcalina (*ALP)*, fator de transcrição relacionado com runt 2 (*RUNX2)*, proteína da matriz de

dentina (*DMP1)* e proteína óssea gamacarboxyglutamato [gla]/osteocalcina (*OC/BGLAP)*.

Além disso, uma revisão sistemática foi realizada, procurando conhecer estudos que utilizaram do modelo aqui proposto, qual seja, a utilização de células-tronco de origem dental como objeto de avaliação da viabilidade celular, citocixicidade, indução de mineralização e morfologia celular de cimentos biocerâmicos utilizados em endodontia.

2 OBJETIVOS

2.1 Objetivo geral

O presente trabalho teve como objetivo investigar os efeitos causados pelo contato dos materiais *Endofill, MTA, Pulp Canal Sealer* e *Sealer 26,* às *hDPSCs* e realizar uma revisão sistematica a respeito dos efeitos de materiais endodônticos biocerâmicos sobre as células-tronco de origem dentária.

2.2 Objetivos específicos

– Analisar, através do ensaio de *3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT),* e do ensaio de exclusão do *Trypan Blue,* a citotoxicidade e viabilidade dos cimentos endodônticos.

– Analisar e traçar o perfil molecular das *hDPSCs*, através da expressão de genes que agem como marcadores de células-tronco hematopoiéticas, mesenquimais e embrionárias. Os marcadores para células-tronco hematopoiéticas testados foram *CD34* e *CD45*; para *MSCs*, *Nestin* e *CD105*; e para células-tronco embrionárias, *Nanog* e *OCT4*. Como controle interno, utilizou-se a expressão da *beta-actina* e do *GAPDH*.

– Caracterizar, por reação em cadeia da Polimerase em Tempo Real (*RT-PCR real time)*, a expressão de alguns genes envolvidos na diferenciação celular, sendo eles: *ALP* – fosfatase alcalina; *RUNX2* - fator de transcrição relacionado com *runt 2*; *DMP1* - proteína da matriz de dentina; *OC/BGLAP* - proteína óssea gamacarboxyglutamato [gla]/osteocalcina. Como controle interno, utilizou-se a expressão da *beta-actina* e o *HPRT1*.

– Verificar através de uma revisão sistemática da literatura, quais são os efeitos biológicos dos cimentos endodônticos biocerâmicos sobre as células-tronco mesenquimais de origem dentária, quanto aos seguintes parâmetros: citotoxicidade, migração, morfologia, adesão celular e atividade da *ALP*.

3 METODOLOGIA EXPANDIDA

3.1 Artigo Um - *The response of Mesenchymal Stem Cells to endodontic materials*

3.1.1 Células-Tronco

Neste estudo, utilizaram-se *hDPSCs* recuperadas de dentes extraídos nas Clínicas da Faculdade de Odontologia da Universidade Federal de Juiz de Fora (UFJF) e, gentilmente cedidas pelo Laboratório Genetec da Faculdade de Ciências Biológicas da UFJF. O estudo foi submetido e aprovado pelo Comitê de Ética em Pesquisa (COEP-UFMG/ CAAE- 87712218.9.0000.5149).

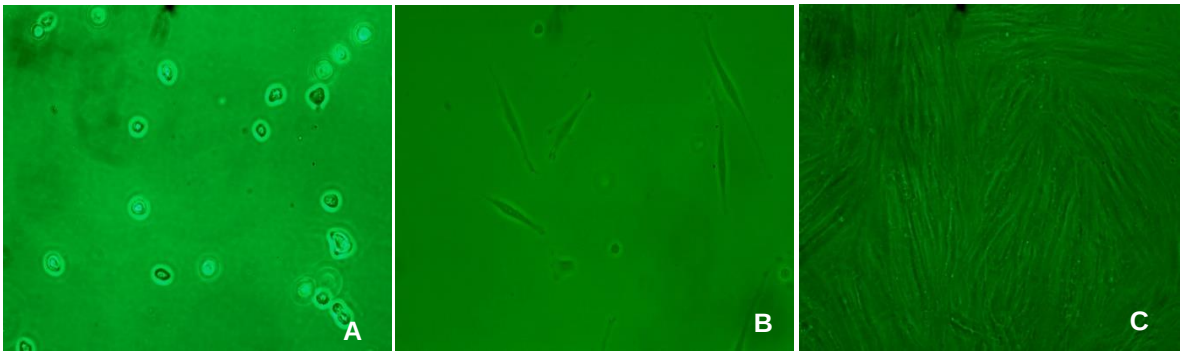
Com intuito de não inferir na estabilidade cromossômica e consequente potencial neoplásico, foram isoladas células em passagem 5, que foram caracterizadas através da análise do seu perfil molecular, analisando-se genes que agem como marcadores de células-tronco hematopoiéticas, mesenquimais e embrionárias.

3.1.2 Cultivo Celular

As *hDPSCs* foram descongeladas e cultivadas em placas em meio D-MEM F12 (*Dulbecco's Modified Eagle Medium, Sigma Chemical Co*., CA, USA) contendo 10% (v/v) de SFB (*Nutricell*, Campinas, SP, Brasil), 2 mM de L-glutamina, 100 unidades mL^{-1} de penicilina e 100 µg mL^{-1} de estreptomicina, em estufa com atmosfera umidificada a 5% de CO_2, a 37°C. As células ficaram incubadas por um período de 24 horas para a adesão ao fundo da placa de cultura. O meio de cultura foi trocado em intervalos de 2 a 3 dias, até que as células atingissem 80 a 95% de confluência. A Figura 1.a demonstra as *hDPSCs* após o descongelamento, com formato circular nesta fase, em decorrência da não aderência das mesmas. A Figura 1.b demonstra as *hDPSCs* em formato fusiforme semelhantes a fibroblastos e já aderidas ao

fundo da placa. A Figura 1.c demonstra essas células já completamente aderidas e confluentes na placa.

Fig 1. (a, b, c): imagens que demonstram as *hDPSCs* após o descongelamento (a), aderidas (b) e em confluência (c).



3.1.3 Preparo dos Materiais Endodônticos

Amostras contendo os materiais endodônticos (Tabela 1) *Endofill* (*Dentsply, Maillefer*, Chile), *Pulp Canal Sealer (Kerr, Sybron Endo, USA*), *Sealer 26* (*Dentsply*, Petrópolis, Brasil) e *MTA* (*Angelus*, Paraná, Brasil), foram preparados sob condições assépticas, em capela de fluxo laminar, com luz ultravioleta foi ligada por 30 minutos antes de todos os procedimentos e manipulados de acordo com as instruções de seus respectivos fabricantes.

Logo após a preparação, todos os materiais foram inseridos em tubos capilares de polietileno com diâmetro interno de 1 mm esterilizados, previamente seccionadosem 2 mm, com o auxílio de uma broca de corte, de modo que seu contato com a suspensão celular fosse padronizado. Tubos capilares vazios foram utilizados como controle. Todos os materiais foram deixados em estufa por 24 horas antes de todas as análises realizadas para que pudessem tomar presa.

Cimentos Endodônticos	**Composição**
Endofill (*Dentsply, Maillefer*, Chile)	Pó: Óxido de Zinco, Resina Hidrogenada, Subcarbonato de Bismuto, Sulfato de Bário e Borato de Sódio. Líquido: Eugenol, Óleo de Amêndoas

	e BHT.
Pulp Canal Sealer (Kerr, Sybron Endo, USA),	Pó: Óxido de zinco, Prata precipitada, Subcarbonato de bismuto, Sulfato de bário. Líquido: Óleo de cravo, Bálsamo do Canadá, Eugenol.
Sealer 26 (Dentsply, Petrópolis, Brasil),	Pó: Trióxido de Bismuto, Hidróxido de Cálcio, Urotropina e Dióxido de Titânio. Resina: Epóxi.
MTA Branco *(Angelus, Paraná, Brasil),*	Silicato tricálcico, Silicato Dicálcico, Aluminato Tricálcico, Óxido de cálcio e Óxido de bismuto. Líquido: água destilada.

Tabela 1: Composição dos materiais endodônticos utilizados.

3.1.4 Viabilidade Celular

Os tubos capilares contendo ou não os materiais endodônticos foram adicionados às culturas de células e o metabolismo celular e a viabilidade foram testados utilizando-se, respectivamente, o ensaio de *3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT)* (*Sigma-Aldrich, USA)* e o ensaio de exclusão do *Trypan Blue*. Os testes de citotoxicidade foram realizados em triplicata e de acordo com a ISO 10993-12: 2012 (E).

3.1.4.1 Ensaio *MTT*

O metabolismo celular foi determinado utilizando-se o ensaio de *3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT)* (*Sigma-Aldrich, St Louis, MO, USA).* Este método determina a atividade da enzima desidrogenase succínica produzida pelas mitocôndrias presentes nas células. Em meio

contendo 100 µL de RPMI 1640 (meio para crescimento celular), suplementado com 10% de SFB, penicilina e estreptomicina, adicionaram-se $1x10^6$ células/poço e os tubos capilares contendo ou não os cimentos, em placas de 96 poços (*TPP, Trasadigen*, Suíça). As placas foram incubadas em estufa (*Thermo Fisher Scientific Inc*, *USA*), durante 24 e 48 horas, a 37ºC. Após os períodos experimentais, o meio de cultura foi removido e as células foram cuidadosamente lavadas com solução salina, tamponada com fosfato (*PBS*). Um volume de 100 mL de solução de succinato de *MTT* (1 mg mL^{-1}) foi adicionado a cada poço e as células foram incubadas durante 4 horas.

Os cristais de formazan resultantes foram dissolvidos quando se removeu o meio de cultura e adicionou-se 100 mL de solvente de sulfóxido de dimetilo (*Sigma-Aldrich, USA*) a cada poço. A absorbância foi medida a 570 nm em leitor de microplacas (*Bio-Tek PowerWave HT EUA, USA*). As análises foram realizadas por três vezes.

3.1.4.2 Ensaio da exclusão do *Trypan Blue*

O *Trypan Blue* foi utilizado para analisar a viabilidade celular, por meio da integridade da membrana celular, bem como realizar a contagem direta das células vivas e mortas. Neste experimento, o corante não penetra em células vivas, mas passa através das membranas de células mortas, por meio da bomba de sódio e potássio.

A viabilidade celular pelo ensaio de exclusão do azul de tripan *(GIBCO, Brasil)* foi realizada em placas de Petri. Foram incubadas $1x10^5$ células/mL, na presença ou ausência dos materiais endodônticos, como acima descrito, após atingirem confluência de 80% e desagregadas com auxílio de tripsina 0,25% e centrifugadas a 1500 RPM. O sobrenadante foi descartado e o pellet foi ressuspendido em 1 mL de meio. Foram misturados 50 µL de suspensão de células a um volume equivalente de corante do *Trypan Blue,* transferidas para placas de Newbauer e incubadas por 3 minutos, a 37ºC. A seguir as células foram examinadas em microscópio invertido. Pelo menos 300 células foram contadas por cultura (realizadas em triplicata) e os resultados foram expressos em porcentagem de viabilidade. Os experimentos foram repetidos três vezes (DE OLIVEIRA MENDES *et al.,* 2003; HUSSEIN & MOHSIN 2019; REZENDE

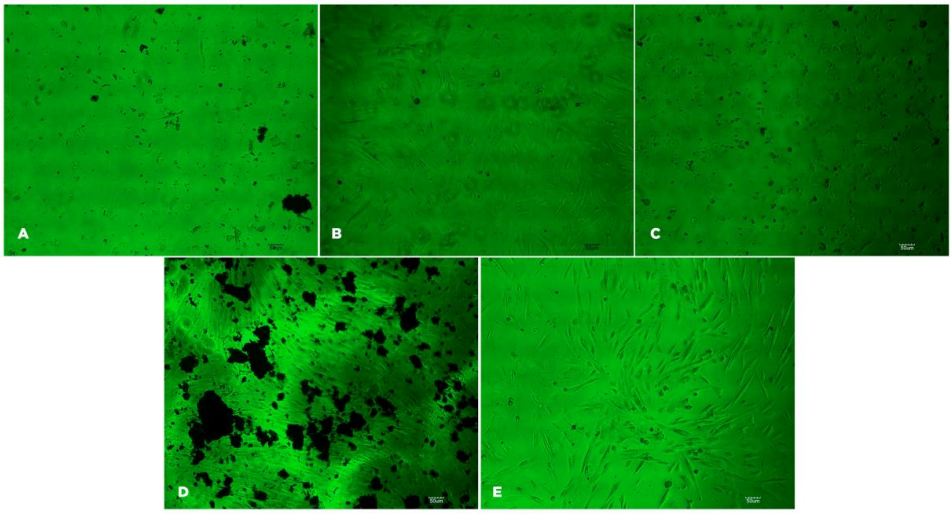
et al., 2007). Cada uma das imagens foi analisada, em duplo cedo, por pesquisadores previamente calibrados.

3.1.5 Plasticidade Celular

Para traçar o perfil molecular das *hDPSCs*, analisaram-se a expressão de genes que agem como marcadores de células-tronco hematopoiéticas, mesenquimais e embrionárias. Os marcadores para células-tronco hematopoiéticas foram *CD34* e *CD45*; para *MSCs*, *Nestin* e *CD105*; e para células-tronco embrionárias, *Nanog* e *OCT4*. Como controle interno, utilizou-se a expressão da *beta-actina* e de *GAPDH*.

Nesta análise, os grupos experimentais, cujos cimentos interferiram negativamente na viabilidade celular (*Endofill* e *Pulp Canal Sealer*), e, consequentemente, apresentaram células insuficientes para que se procedesse à extração do RNA foram descartados (Figura 2).

Fig 2. *hDPSCs* após cultivo celular e contato com os materiais endodônticos. A (cimento *Endofill*); B (controle); C (cimento *Pulp Canal Sealer*); D (cimento *MTA* Branco); E (cimento *Sealer 26*). Podemos observar através das imagens microscópicas que na presença dos cimentos *Endofill* e *Pulp Canal Sealer* as *hDPSCs* não conseguem se manter viáveis em quantidades suficientes para a extração do RNA e análise da plasticidade celular.



3.1.5.1 Extração do RNA

O RNA total foi extraído utilizando-se reagente a base de fenol e do isotiocianato de guadina (*Brazol*, *LGC Biotecnologia*, Brasil) e resuspendido em 50 µL de água tratada com dietilpyrocarbonato (DEPC) (*Sigma Chemical Co., USA*) contend 1 mM de EDTA.

Após a extração, o RNA total foi quantificado por espectrofotometria (*NanoDrop™1000, Thermo Fisher Scientific, USA*) e normalizado para uma concentração de RNA de 2µg/µL. A partir do RNA total sintetizou-se o cDNA, utilizando-se o kit *High Capacity cDNA Reverse Transcription Kit* (*Thermo Fisher Scientific, USA*), conforme recomendações do fabricante *Thermo Fisher Scientific*, *USA*. O cDNA foi diluído em 87,5 µL de água estéril e utilizado na reação de *PCR*.

3.1.5.2 PCR

A mistura da reação consistiu de 2 µL de cada amostra e dos seguintes tampões: 0,8 µL de dNTPs (2,5 mM), 10 mM de TRIS-HCL pH 8.3, 50 mM KCL, 1,5 mM $MgCl_2$, 0,6 µL de cada primer, 0.05 µL (0,5 U) de *Taq polymerase* (*GoTaq® Flexi DNA Polymerase, Promega Corporation, USA*), e 6,65 µL de água Mili-Q estéril. A reação da cadeia de polimerase foi realizada sob condições padrão, como se segue: desnaturação a 95°C (2 minutos), anelamento de 40 ciclos a 60°C (30 segundos) seguido por 72°C (30 segundos), e 72°C (5 minutos). A sequência dos primers utilizados na analise do *PCR* dos genes *OCT4, NANOG, CD34, CD45, Nestin* e *CD105* se encontram na Tabela 2. *Beta-actina* e *GAPDH* foram utilizados como *housekeeping genes* para normalização.

Marcador	***Primer* F**	***Primer* R**	***Amplicon (pb)***	**Tm (°C)**
Embrionário				
OCT4	ACTTCACTGCAC TGTACTCCTCAG	AGGTTCTCTTTCC CTAGCTCCTC	158	60
NANOG	CTACCCCAGCCT TTACTCTTCCTAC	CTCTCCACAGTTA TAGAAGGGACTG	217	60
Controle				

B actina	ATTAAGGAGAAG CTGTGCTACGTC	GATGGAGTTGAA GGTAGTTTCGTG	213	60
GAPDH	GAGTCAACGGAT TTGGTCGT	TGGGATTTCCATT GATGACA	201	60
Hematopoiético				
CD34	AACACCTAGTAC CCTTGGAAGTAC C	AACACTGTGCTG ATTACAGAGGTC	177	60
CD45	GGACACAGAAGT ATTTGTGACAGG	GAGAAGTTGTGG TCTCTGAGAAGT C	176	60
Mesenquimal				
Nestin	GGACCCTCCTAG AGGCTGAG	GTGAGGAGAGGG GAGTAGGG	168	60
CD105	TGCCACTGGACA CAGGATAA	CCTTCGAGACCT GGCTAGTG	205	60

Fonte: **http://www.ncbi.nlm.nih.gov/sites/entrez**

Tabela 2 – Sequência dos *primers* para análise da caracterização celular.

3.1.6 Diferenciação Celular

Para se analisar a diferenciação celular frente aos materiais endodônticos verificou-se a expressão dos genes envolvidos no processo de diferenciação celular, *RUNX2* (osteoclastos), *ALP, OC (BGLAP)* e *DMP1* (osteoblastos) por *RT-PCR real time*. Como controle interno, utilizou-se a expressão do *HPRT1* e *Beta-actin*. Neste experimento, os grupos experimentais foram aqueles que não interferiram na viabilidade celular, quais sejam, os grupos compostos pelos materiais *MTA* e *Sealer 26.*

3.1.6.1 Real Time PCR

A Expressão dos genes *RUNX2, ALP, OC (BGLAP)* e *DMP1* (Tabela 3), foi verificada pela reação em cadeia da polimerase em tempo real (*RT-PCR real time)*. O *RT-PCR real time* foi realizado sob as condições que se seguem: desnaturação a 95°C (10 minutos), anelamento de 40 ciclos a 95°C (15 segundos) seguido por 60°C (1 minuto), e 95°C (15 segundos). A sequência dos primers

utilizados na analise do PCR dos genes *RUNX2, ALP, BGLAP (OC)* e *DMP1* se encontra na Tabela 3. Os *primers* foram obtidos utilizando-se o *software PRIMEREXPRESS (Applied Biosystems, Foster City, CA)* baseado na sequencia de nucleotídeos disponíveis no *Pubmed/Entrez* (http://www.ncbi.nlm.nih.gov/sites/entrez). O *PCR-real time* foi realizado utilizando-se o *Step One Real-time PCR Systems (Applied Biosystems).* O *Fast SYBR® Green Master Mix (Thermo Fisher Scientific, EUA)* foi utilizado na amplificação. O *HPRT1* e *Beta-Actin* foram utilizados como *housekeeping genes* para a normalização. Todas as amostras foram analisadas em duplicata. As reações foram realizadas em um volume de 25 µL, contendo 1 µg de cDNA. O *Sequence Detection Software* versão v 2.0 *(Applied Biosystems)* foi utilizado para analisar os dados após a amplificação. Os resultados foram obtidos com *threshold cycle values*. Os valores foram calculados utilizando-se o método *comparative threshold cycle (CT)* de Schmittgen e Livak (2008). Os níveis de expressão foram calculados como a média dos valores das duplicatas de cada amostra, e os níveis de expressão do mRNA em todas as amostras foram definidos como a razão de cada primer específico em relação a expressão dos *housekeeping genes (HPRT1* e *Beta-Actin*).

Marcador	***Primer* F e R**	**Temperatura De *Melting* (ºc)**	**Tamanho Do Produto**	**Referência Fasta Pubmed**
HPRT1 ID3251	F 5’ TGCTCGAGATGTGATGAAGG 3’ R 5’ TCCCCTGTTGACTGGTCATT 3’	54,5 56,1	192	NM_00019 4.2
ACTB ID 60	F 5’AAACTGGAACGGTGAAGGTG 3’ R 5’GTGGACTTGGGAGAGGACTG 3’	55,4 57,1	206	NM_00110 1.3
* ALP ID 249	F 5’CCACGTCTTCACATTTGGTG 3’ R 5’AGACTGCGCCTGGTAGTTGT 3’	54,2 58,8	196	NM_00047 8.4
OC/ BGLAP ID 632	F 5’ GGCAGCGAGGTAGTGAAGAG 3’ R 5’ AGCAGAGCGACACCCTAGAC 3’	57,5 58,8	194	NM_19917 3.4
RUNX 2 ID 860	F 5’GAACTGGGCCCTTTTTCAGA 3’ R 5’CACTCTGGCTTTGGGAAGAG 3’	55,3 55,6	208	NM_00434 8.3
* DMP1 ID 1758	F 5’CAGGAGCACAGGAAAAGGAG 3’ R 5’CTGGTGGTATCTTGGGCACT 3’	55,6 56,9	213	NM_00440 7.3

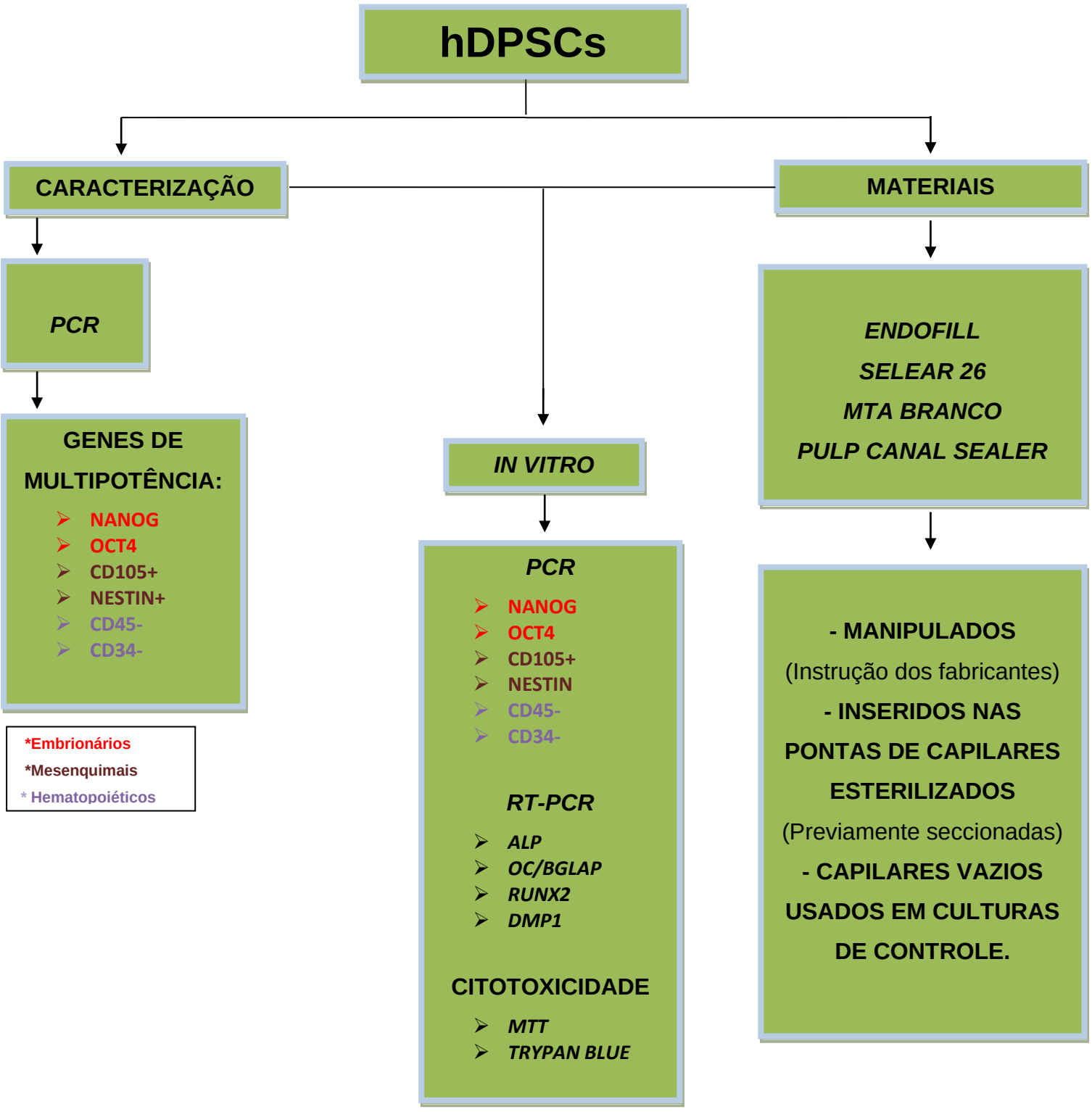
*Fonte: Galler *et al*., 2006

Tabela 3 – Sequência dos *primers* para análise da diferenciação celular.

3.1.7 Análise Estatística

A análise dos dados foi realizada utilizando-se o *software GraphPad Prisma (versão 7; GraphPad Software, Inc., EUA)*. O teste de Kolmogorov-Smirnov foi utilizado para verificar a normalidade e o teste Levene a homogeneidade da variância. O teste ANOVA com correção de Bonferroni foi utilizado para verificar a diferença estatística, seguido do teste Tukey para verificar a diferença entre os diferentes cimentos. O nível de significância foi de 5%.

3.1.8. Desenho Experimental



3.2 Artigo Dois - *Biocompatibility of Bioceramic Root Canal Sealers on Dental Stem Cells: A Systematic Review*

O presente estudo é relatado de acordo com a lista de verificação de itens de relatório preferidos para revisões sistemáticas e meta-análises (PRISMA) (MOHER *et al.,* 2010). Além disso, este estudo foi registrado no registro *Open Science Framework (OSF)* https://osf.io/6dyg2/.

3.2.1 Critérios de Elegibilidade

O critério de inclusão foram estudos que avaliaram os efeitos causados por diferentes cimentos endodônticos biocerâmicos em *hMSCs* de origem dentária. Foram excluídos deste estudo, trabalhos realizados com células-tronco animais, estudos *in vivo* e estudos em que o desfecho não fosse análises biológicas, bem como a falta de caracterização das *hMSCs* explicada nos artigos.

A abordagem de população, intervenção, comparação, resultado (PICO) foi usada para abordar a seguinte questão: “Quais são os efeitos biológicos dos cimentos endodônticos biocerâmicos nas células-tronco mesenquimais de origem dentária?” A população do estudo foi composta por células-tronco mesenquimais humanas de origem dentária. A intervenção explorada foi o contato com cimentos endodônticos biocerâmicos ou seus extratos; a comparação utilizada foram células-tronco sem contato com cimentos endodônticos biocerâmicos. O desfecho avaliado foi o efeito de diferentes cimentos biocerâmicos sobre a citotoxicidade. Os resultados secundários foram o efeito desses materiais na migração celular, morfologia celular, adesão celular e atividade de fosfatase alcalina (*ALP*).

3.2.2 Estratégia de Pesquisa e Fontes de Informação

Pesquisas eletrônicas foram conduzidas nos bancos de dados *PubMed/MEDLINE, Scopus* e *Web of Science* sem restrições de datas com fechamento até fevereiro de 2021. A estratégia de pesquisa foi a seguinte: *“((root canal sealer OR endodontic sealer) AND (stem cell OR stem cells OR*

adult stem cell OR mesenchymal stem cell OR periodontal ligament stem cells OR dental pulp stem cells OR stem cells from human exfoliated deciduous teeth OR dental follicle stem cells OR tooth germ progenitor cells OR stem cells from the apical papilla OR oral mucosa-derived stem cells OR human gingiva-derived stem cells OR dental pulp cells OR periodontal ligament cells))".

Também foram realizadas buscas manuais nas listas de referências dos artigos incluídos e em periódicos específicos, como o *Journal of Endodontics* e *International Endodontic Journal.*

3.2.3 Seleção de Estudos

A seleção do estudo foi realizada por dois autores independentes (P.Y.O. e P.R.G.) em um processo de duas etapas. Na Etapa 1, ambos os autores avaliaram os títulos e resumos dos estudos recuperados das buscas. Foram incluídos estudos com títulos e resumos que atendessem aos critérios de elegibilidade. Para estudos com títulos e resumos que forneceram informações insuficientes para se chegar a uma decisão, os textos completos foram baixados. Na Etapa 2, os dois autores avaliaram os textos completos. Também foram incluídos estudos em que o texto completo atendia aos critérios de elegibilidade. As divergências entre os dois autores foram resolvidas por meio de discussão e, quando necessário, um terceiro autor (F.B.) foi consultado.

3.2.4 Coleta de Dados e Análises

Um autor (P.Y.O.) coletou os seguintes dados dos estudos incluídos: sobrenome do primeiro autor, ano de publicação, materiais usados, manuseio do material, métodos, extratos usados, preparação de extratos ou discos, origem das células, passagem das células, caracterização das células-tronco e períodos de análise. Também foram coletados dados da análise como método de avaliação e principais achados. Posteriormente, um segundo autor (F.B.) revisou os dados coletados pelo autor anterior. Cada um dos estudos incluídos foi analisado em termos de semelhanças para que uma meta-análise pudesse ser realizada. No entanto, após a avaliação, foi encontrada heterogeneidade

considerável; portanto, uma meta-análise não foi realizada. Por haver uma grande diversidade metodológica nos estudos, foi apresentada uma análise descritiva dos resultados.

3.2.5 Avaliação de Risco de Viés

Dois investigadores (P.Y.O. e P.R.G.) avaliaram independentemente a qualidade metodológica dos estudos selecionados, com base nos níveis de evidência propostos pela Lista de Verificação de Avaliação Crítica do Instituto Joanna Briggs para Estudos Experimentais (AMINOSHARIAE & KULILD 2015; DOS REIS-PRADO *et al.,* 2021), com algumas modificações. Os itens da lista de verificação foram os seguintes: objetivo claramente identificado, justificativa do tamanho da amostra (os autores descreveram o tamanho da amostra e os métodos de cálculo do poder ou justificaram o tamanho da amostra utilizada no estudo), randomização da amostra, possibilidade de comparação entre os grupos controle e tratamento, equivalência de linha de base de grupos de controle e de tratamento, descrição clara do protocolo utilizado, método de medição, padronização de medição e análise estatística adequada. Cada item foi pontuado por meio de uma escala de 2 pontos: 0, não relatado ou relatado inadequadamente, e 1, relatado e adequado. Dúvidas e discrepâncias entre os investigadores foram discutidas para se chegar a um consenso e, se não fossem resolvidas, um terceiro examinador (F.B.) era consultado.

4 ARTIGOS CIENTÍFICOS

4.1 Artigo Um – Submetido à *Brazilian Dental Journal*

The response of Mesenchymal Stem Cells to endodontic materials

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Abstract:
An endodontic material must be minimally harmful to stem cells since they are essential, thanks to their capacity for cell proliferation, self-renewal, and differentiation. For this reason, the cell viability and the expression of genes involved in cell plasticity and differentiation were investigated in stem cells recovered from human dental pulp (hDPSCs) that were in contact with four endodontic materials (Endofill, MTA, Pulp Canal Sealer, and Sealer 26). The viability of hDPSCs was assessed by MTT and trypan blue exclusion assays. PCR evaluated cellular plasticity by determining the CD34, CD45, Nestin, CD105, Nanog, and OCT4 expressions. The effect on cell differentiation was determined by RT-PCR's expression of the RUNX2, ALP, OC/BGLAP, and DMP1 genes. The data were analyzed using ANOVA with Bonferroni correction ($p < 0.05$). Pulp Canal Sealer and Endofill decreased cell viability after 48 hours ($p < 0.001$). MTA and Sealer 26 did not disrupt cell viability ($p > 0.05$). When cultivated in the presence of MTA and Sealer 26, hDPSCs expressed Nestin, CD105, NANOG, and OCT-4 and did not express CD34 and CD45. MTA and Sealer 26 interfered with DMP1, OC/BGLAP and RUNX2 expressions ($p < 0.05$) but did not change ALP gene expression ($p > 0.05$). MTA and Sealer 26 showed biological compatibility in the presence of hDPSCs.

Keywords: mesenchymal stem cells, cytotoxicity, mineral trioxide aggregate, genotoxicity, dental pulp stem cells.

1. Introduction

The endodontic treatment culminates with the complete filling of root canal systems which avoid microbial infection. Although sealers are expected to be confined within the root canal space, extrusion may occur. When endodontic sealers contact periodontal tissue, they may interfere in periapical responses resulting in delayed wound healing (1). Hence, Endodontics has always required the use of materials well tolerated by apical tissues, presenting antimicrobial effects and promoting healing (2). Endodontic sealers based on zinc oxide and eugenol (ZOE) have been standard in endodontics since their development, based on their long-term success (3). During decades, Endofill (Dentsply, Maillefer, Chile) and Pulp Canal Sealer (Kerr, Sybron Endo USA) prevailed in the Brazilian and USA market, respectively (3). Moreover, seeking antimicrobial effects and stimulation of periapical tissues healing, root canal sealers based on calcium hydroxide became available in the late 1980s (4).

Another vital material incorporated in the endodontic arsenal was the Mineral Trioxide Aggregate (MTA), used as a dental root repair material and developed by Mahmoud Torabinejad in 1993 (5). MTA is indicated in pathological or iatrogenic root perforations (5) as well as in root-end fillings (5), but it has also been employed in pulp covering or pulpotomy (6). MTA is a powder composed of tricalcium silicate, bismuth oxide, dicalcium silicate, tricalcium aluminate, tetra calcium aluminoferrite, and calcium sulfate dihydrate (7). Due to its outstanding properties, nowadays, MTA is a gold standard in studies that compare the biological properties of dental materials (7).

Several parameters for testing endodontic sealers have been created (8,9). Studies have examined biological properties in many cells such as macrophages, fibroblasts, and endothelial cells (7,10,11,12). Moreover, analyses have demonstrated the importance of stem cells as a model to examine endodontic sealers' properties (1, 2, 8, 9, 11, 12).

Stem cells (SCs) have been identified in many organs and tissues, and each type presents specific physiological properties. Hence, stem cells recovered from dental pulp (HDPSCs) have become a great experimental model for studying the biological properties of dental materials since they are homologous to the cells that materials will be in contact with (1,8). However, few studies have examined their interactions (1,9). The ability to differentiate into odontoblasts and osteoblasts makes SCs extremely important for dentistry (1,8,9). Therefore, it is expected that endodontic materials do not interfere with cell signaling mediated by these cells (1,8, 9).

This study aimed to investigate the effects of endodontic materials (Endofill, MTA, Pulp Canal Sealer, and Sealer 26) when in contact with HDPSCs. For this purpose, cell viability, the expression of genes involved in cell plasticity, and cell differentiation were analyzed. The null hypothesis was that MTA would not present any significant cytotoxic effects among all tested biomaterials.

2. Materials and methods

2.1 Stem Cells

The Genetic Laboratory of the Biological Sciences College of UFJF kindly provided HDPSCs. The Research Ethics Committee of the Federal University of Minas Gerais approved this study (CAAE-87712218.9.0000.5149). As described elsewhere, these cells were generated and characterized by gene expression that acts as hematopoietic markers, mesenchymal, and embryonic stem cells (13).

2.2 Cell Culture

hDPSCs ($1x10^6$) were cultured in 5 mL of medium (D-MEM F12 medium; Sigma Chemical Co.), containing 10% (v/v) FBS (Nutricell, Campinas, SP, Brazil), 2 mM L-glutamine, 100 mL units -1 penicillin, and 100 µg mL-1 streptomycin; cells were placed in an incubator with a humidified atmosphere containing 5% CO_2 at 37°C for 24 hours to allow the cells to adhere to the bottom of the culture plate (13). The culture medium was changed at frequent intervals of 2 to 3 days until the cells reached 80 to 95% confluence, and the cells were used in passage 5 (Fig. 1).

2.3 Endodontic Materials

All materials (Endofill, Dentsply, Maillefer, Chile; Sealer 26, Dentsply, Petrópolis, Brazil; MTA, Angelus, Paraná, Brazil; and Pulp Canal Sealer, Kerr, Sybron Endo, USA) were prepared following manufacturers' instructions in sterile conditions. Soon after preparation, sealers were inserted into the tips of previously sectioned sterilized capillary tubes (test group) so that their contact with the cell suspension could be standardized (Ø = 1.2 mm; length = 10 mm/area = $2.26mm^2$) (7). Empty capillary tubes were used in control cultures. Capillaries were sterilized by exposure to 25 kGray Gamma-ray irradiation (CDTN, Belo Horizonte, MG Brazil).

2.4 Cell Viability

HDPSCs were cultured for 48 hours in 96-well plates. Cell viability was determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and trypan blue exclusion assays, as described elsewhere (9,10,14). The cytotoxicity tests were performed in triplicate and according to the ISO 10993-12:2012 (E).

Growth curves were obtained through the MTT assay to establish the proliferation of cells. For analysis of the proliferation pattern, 1000 cells per well were seeded (day 0) in 96-well plates. The plates were divided into control and test groups so that the control group was cultivated in the absence of biomaterials, while the test groups were influenced by Endofill, Sealer 26, MTA, and Pulp Canal Sealer. For each group, 12-plicates were performed. After 24 and 48, the culture medium was removed, and a new culture medium was added with 10% of a previously prepared solution (5mg/ml) of the MTT reagent (Thiazole Blue Tetrazolium, Sigma, code M2128). Afterward, the plates were incubated in an oven at 37°C with 5% CO2 for 4 hours. The MTT medium was removed, and 200µL of the isopropanol-0.04M HCl acid solubilizer was added. The plates were incubated for one hour. The wells were read in the spectrophotometer (ELx800; Bio-Tek Instruments, Winooski, VT, USA) at 570nm using as white three wells with 200µL of the isopropanol-acid.

The integrity of the cell membrane and the direct count of the living and dead cells was evaluated by Trypan Blue (7). This dye does not enter living cells but passes through the membranes of dead cells. The medium was removed from the wells, and cells were washed with 200µL of PBS. Cells were separated by the addition of 100µl of trypsin /EDTA 0.5%. RPMI-1640 supplemented with 10% FBS (50µL) and 0.5% trypan blue (50 µL) (Merck, Germany) were added additionally to each well, and the plates were incubated for 5 minutes. Subsequently, a 20µL aliquot was removed and placed in a

Neubauer Hemocytometer. The number of viable and non-viable cells was finally counted under the microscope.

2.5 Cell Plasticity

PCR analysis was used to assess genes that act as stem cell markers: CD34 and CD45 genes for hematopoietic cells; Nestin and CD105 for mesenchymal cells; and Nanog and OCT4 for embryonic stem cells. Beta-actin and GAPDH were used as the internal control. This investigation was restricted to HDPSCs cultures in which materials did not interfere with cell viability (Sealers 26 and MTA) so that a sufficient amount of RNA could be isolated for PCR analyses (Fig. 2).

2.5.1 Gene expression analysis

Total RNA from cultured cells was extracted using a reagent based on phenol and guanidine isothiocyanate (Brazil, LGC Biotecnologia, Brazil); then, the RNA was resuspended in 50 μL of water treated with diethylpyrocarbonate (DEPC) (Sigma Chemical Co., Louis, MO) containing 1 mM EDTA.

After extraction, total RNA was quantified by spectrophotometry (NanoDropTM 1000, Thermo Fisher Scientific, Wilmington, Delaware, USA), and all samples were diluted to a concentration of 2 μg/μL. According to the manufacturer's recommendations, cDNA was synthesized from total RNA using a High Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Carlsbad, USA). The cDNA products were diluted in 87.5 μL of sterile water and used in PCR amplification experiments. The PCR mixture consisted of 2 μL of each sample and the following solutions: 0.8 μL of dNTPs (2.5 mM), 10 mM of TRIS-HCL pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.6 μL of each primer, 0.05 μL (0.5 U) of Taq polymerase (GoTaq® Flexi DNA Polymerase, Promega Corporation, USA), and 6.65 μL of sterile Milli-Q water. The polymerase chain reaction was carried out under standard conditions as follows: denaturation at 95°C (2 minutes), annealing for 40 cycles at 60°C (30 seconds) followed by 72°C (30 seconds), and 72°C (5 minutes). The sequences of the primers used in the PCR analysis of OCT4, NANOG, CD34, CD45, Nestin, and CD105 are shown in Table 1.

2.6 Cell Differentiation

RT-PCR was used to evaluate the genes involved in the cell differentiation process, RUNX2 (osteoclasts), ALP, BGLAP (OC), and DMP1 (osteoblasts). RNA extraction and cDNA synthesis were performed as described above. The expression of HPRT1 and beta-actin was used as an internal control. RT-PCR was performed under the following conditions: denaturation at 95°C (10 minutes), annealing for 40 cycles at 95°C (15 seconds) followed by 60°C (1 minute) and 95°C (15 seconds). Table 2 shows the sequences of the primers used in this analysis. Sequences were designed using PRIMER EXPRESS software (Applied Biosystems, Foster City, CA), and they were based on nucleotide sequences available in the PubMed/Entrez database (.ncbi.nlm.nih.gov/sites/entrez). Real-time PCR was performed using Step One Real-time PCR Systems (Applied Biosystems). A Fast SYBR® Green Master Mix detection system (Thermo Fisher Scientific, Foster City, CA, USA) was used to assay primer amplification. HPRT1 and beta-actin were used as housekeeping genes for expression normalization. All the samples were run in duplicate. Reactions were performed in a volume of 25 μL, and each contained 1 μg of cDNA. Sequence

Detection Software version v 2.0 (Applied Biosystems) was used to analyze the data after amplification. Expression levels were then calculated using the comparative threshold cycle (TC) method (15). Values were calculated as the mean of the duplicated sample, and the mRNA expression samples were defined as the ratio of each specific primer to HPRT1 and Beta-actin expression.

2.7 Statistical analysis

Data analysis was performed using GraphPad Prisma software (version 7; GraphPad Software, Inc., USA). The Kolmogorov-Smirnov test was used to verify normality and the Levene test for homogeneity of variance. The ANOVA test with Bonferroni correction was used to verify the statistical difference, followed by the Tukey test to verify the difference between the different materials. The significance level was 5%.

3. Results

3.1 Cell Viability

Cell viability was assessed by MTT assay and showed that, compared to the control groups, no materials were cytotoxic after 24 h. However, at 48 h, Pulp Canal Sealer and Endofill decreased cell viability significantly compared to the control group ($p < 0.001$). MTA and Sealer 26 did not affect cell viability in any of the conditions tested, indicating they were similar to the control groups (Fig. 3). Trypan blue assay findings confirmed these results, showing that both Pulp Canal Sealer and Endofill decreased cell viability by 98.1% ($p < 0.001$) and 31.7% ($p > 0.05$), respectively, at 48 h (data not shown). According to the ISO 10993-5:1999 (E) recommendations, biomaterials that promote a reduction in cell viability by more than 30% are considered cytotoxic.

3.2 Cell Plasticity

Cell plasticity was analyzed in the groups treated with MTA and Sealer 26 for the reasons explained above. The gene expression levels of MSC markers Nestin and CD105 and the embryonic markers NANOG and OCT-4 were detected (Fig. 4). However, gene expression of hematopoietic markers CD34 and CD45 was not detected in either culture group (data not shown).

3.3 Cell Differentiation

MTA and Sealer 26 significantly decreased the expression of DMP1, OC/BGLAP, and RUNX2 compared to their levels in the control group ($p < 0.05$). Nevertheless, neither sealer interfered with ALP gene expression ($p > 0.05$) (Fig. 5).

4. Discussion

In this study, we used HDPSCs to assess the effects on viability and differentiation as well as the genotoxicity of four commercially available endodontic materials: Endofill (Dentsply, Maillefer, Chile), Sealer 26, (Dentsply, Petrópolis, Brazil), MTA (Angelus, Paraná, Brazil) and Pulp Canal Sealer (Kerr,

Sybron Endo, USA). HDPSCs are appropriate for this kind of experiment since they differentiate into odontoblasts and osteoblasts cells, which endodontic materials will contact during clinical application (1). Furthermore, HDPSCs are easily obtained from human teeth (1,13).

The first strategy of this study was to evaluate the viability of HDPSCs when they were cultured in the presence of the materials. It was shown that Endofill and the Pulp Canal Sealer decreased cell viability compared to that of control cells ($p < 0.001$ and $p < 0.0001$). Accordingly, similar results concerning Endofill in macrophage cultures and osteo-1 cells have been previously demonstrated (12, 16). Therefore, Pulp Canal Sealer impaired the viability of primary human cells recovered from periapical tissues and the animal lineage of fibroblasts and type I collagen (2,11). Conversely, in M1 and M2 macrophage cultures, Endofill and Pulp Canal Sealer impaired cell adherence and phagocytosis but did not interfere with cell viability (14).

On the other hand, Sealer 26 and MTA did not affect cell viability, similar to the control groups. In the literature, encouraging results concerning MTA have been reported regarding tissue tolerance and stimulation of mineralization (7,17). Conversely, it was observed that another type of MTA, the MTA Fillapex, reduced macrophage viability, adhesion, and phagocytic activity (18). Sealer 26 did not interfere with HDPSCs viability but reduced osteoblast and macrophage viability (10,16).

Following the cell viability tests, the second strategy of this study was to analyze the differentiation and genotoxic effects of the materials on HDPSCs, explicitly focusing on the materials that did not impair their viability, namely, Sealer 26 and MTA.

To be considered HDPSCs, cells must be isolated from human dental tissues, exhibit adherence capability, fusiform morphology when adhered, contain the potential for differentiation into other cell types, and possess self-renewal ability (13). These cells positively expressed CD27, CD29, CD44, CD73, CD90, CD105, CD146, CD166, CD271, STRO-1, Nestin and Vimentin. In contrast, HDPSCs do not express CD34, CD45, CD14, or CD19, but they sometimes express embryonic cell markers, such as Oct-4, Nanog, and Sox-2 (19). Here, MTA and Sealer 26 did not impair the expression of typical MSC markers, Nestin and CD105, or the embryonic markers NANOG and OCT-4. Moreover, neither material interfered with CD34 and CD45 gene expression (hematopoietic markers). Such findings demonstrate that neither material interfered with HDPSCs' cellular plasticity, validating them as excellent clinical materials. To our knowledge, such findings are unprecedented in the literature.

The genes involved in the cell differentiation process, RUNX2, ALP, OC (BGLAP), and DMP1, were evaluated in cells grown in the presence of materials. MTA and Sealer 26 negatively interfered with the gene expression of DMP1, OC/BGLAP, and RUNX2 ($p < 0.05$). As DMP1 induces the differentiation of immature dental pulp cells into odontoblasts (20), these data suggest that both sealers interfere with this crucial function.

RUNX2 is a transcription factor that controls the bone differentiation and maturation process by modifying the expression of several genes, such as OPN (SPP1) and OC (BGLAP) (21). During osteoblast differentiation, RUNX2 drives pluripotent mesenchymal cells into the odontoblastic lineage (22). When RUNX2 expression is increased, osteoblast maturation is inhibited, OC expression is reduced, and OPN expression is increased (25). Here, RUNX2 expression was diminished by MTA and Sealer 26, which favors the maturation of cells involved in tissue repair. Moreover, RUNX2 belongs to the Runt

domain family, is described as one of the most significant osteogenic transcription factors, and is currently used as a marker of early osteogenic differentiation (22).

ALP analysis is an essential tool for molecular biology and genetic engineering. ALP is responsible for cell proliferation and cell renewal in bone tissue and acts on odontoblasts to stimulate the proliferative process (24). MTA and Sealer 26 did not negatively interfere with ALP gene expression, reinforcing the excellent biocompatibility of these materials.

Osteocalcin (OC) is a crucial component of bone, and it plays a role in bone mineralization and calcium homeostasis, being a significant indicator for the differentiation of osteoblast progenitor cells (23). It was observed that significant downregulation of OC in adipose-derived stem cells (ADSCs) drives the differentiation of osteoblasts (23). As MTA and Sealer 26 negatively interfere with OC expression, both materials contribute to the regenerative processes. ALP, OC, and RUNX2 act in the processes of transformation or proliferation, maturation, and mineralization of the extracellular matrix (24), and here, it is observed that both sealers contribute positively to these processes.

Human dental pulp stem cells (HDPSCs) are of particular relevance in future repair dental therapies. These outcomes showed that Endofill and Pulp Canal Sealer negatively impacted HDPSCs viability, but, conversely, MTA and Sealer 26 did not interfere with the cell viability and the expression of markers involved in cell plasticity and cell differentiation. Hence, the contact of HDPSCs with root canal sealers during the periapical inflammation or healing process is a matter of debate.

Resumo (PT)

Um material endodôntico deve ser minimamente prejudicial às células-tronco, uma vez que essas células são extremamente importantes, devido à sua capacidade de proliferação, autorrenovação e diferenciação celular. Por esse motivo, a viabilidade celular e a expressão de genes envolvidos na plasticidade e diferenciação celular foram investigadas em células-tronco recuperadas de polpa dentária humana (hDPSCs) que estiveram em contato com quatro materiais endodônticos (Endofill, MTA, Pulp Canal Sealer e Sealer 26). A viabilidade das hDPSCs foi avaliada pelos ensaios MTT e de exclusão de azul de tripano. A plasticidade celular foi avaliada pela determinação das expressões dos genes CD34, CD45, Nestin, CD105, Nanog e OCT4 por PCR. O efeito na diferenciação celular foi determinado pela expressão dos genes RUNX2, ALP, OC/BGLAP e DMP1 por RT-PCR. Os dados foram analisados por ANOVA com correção de Bonferroni ($p < 0,05$). Em comparação com o controle, Pulp Canal Sealer e Endofill diminuíram a viabilidade celular após 48 horas ($p < 0,001$). MTA e Sealer 26 não interromperam a viabilidade celular ($p > 0,05$). Quando cultivado na presença de MTA e Sealer 26, as hDPSCs expressaram Nestin, CD105, NANOG e OCT-4 e não expressaram CD34 e CD45. MTA e Sealer 26 interferiram nas expressões de DMP1, OC / BGLAP e RUNX2 ($p < 0,05$), mas não alteraram a expressão do gene ALP ($p > 0,05$). Sendo assim, MTA e Sealer 26 demonstraram compatibilidade biológica na presença de hDPSCs.

Acknowledgements: This work was supported by Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Pesquisa e Desenvolvimento Tecnológico (CNPq) and Pró-Reitoria de Pesquisa da UFMG

(PRPq). APRS and LQV are CNPq fellows. The authors deny any conflicts of interest related to this study.

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Figure Captions

Fig 1 a) hDPSCs after thawing have a circular shape, as a result of their non-adherence; b) hDPSCs with a fusiform shape similar to that of fibroblasts indicate that they are adhered to the bottom of the plate; c)

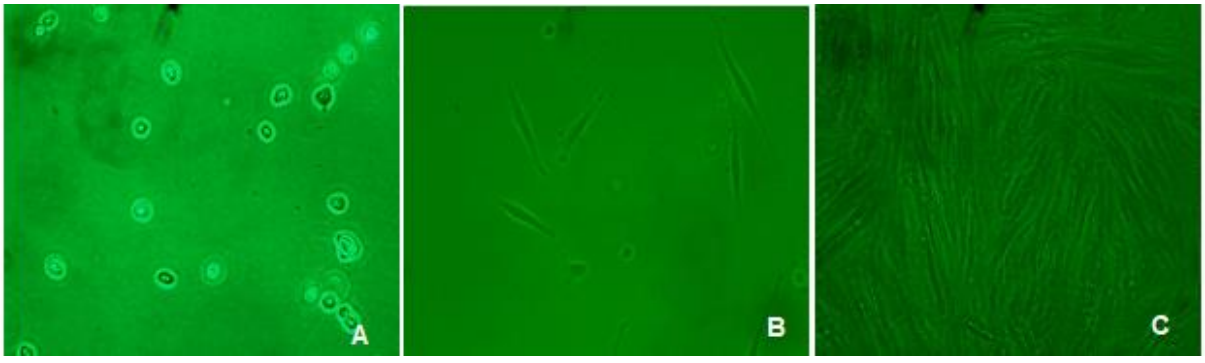
hDPSCs that are fully adhered and confluent. Microscopic magnification of 10x (Nikon TS100F). The green color of the walls is related to the microscope filter selected.

Fig 2 hDPSCs after culture and contact with endodontic materials. A (Endofill); B (Control); C (Pulp Canal Sealer); D (MTA); and E (Sealer 26). We can observe through microscopic images that in the presence of Endofill and Pulp Canal Sealer, hDPSCs cannot remain viable in sufficient quantities for RNA extraction and analysis of cellular plasticity. Bi-refractive structures are found in the cultures, suggesting that sealers might be dissipated from capillaries.

Fig 3 MTT assay of hDPSCs cultures at 24 and 48 h. Bars represent the average of the experiments; lines represent the standard error of the means. Values of $p < 0.05$ are indicated by (*); p values <0.01 are indicated by (**); p values <0.001 are indicated by (***); and p values <0.0001 are indicated by (****)

Fig 4 PCR amplification products were separated by electrophoresis on 6% (p/v) polyacrylamide gels and then were visualized as bands by silver staining. The tested markers are indicated. MTA and Sealer 26 positively expressed Nestin, CD105, NANOG and OCT-4. PCR analysis of each sample was performed three times

Fig 5 Gene expression of RUNX2, OC (BGLAP), DMP1 and ALP in hDPSCs. The Y-axis shows the values of $2^{-\Delta\Delta Ct}$ relative to the expression of the endogenous controls. Values of $p < 0.05$ are indicated by (*); p values <0.01 are indicated by (**); p values <0.001 are indicated by (***); and p values <0.0001 are indicated by (****)



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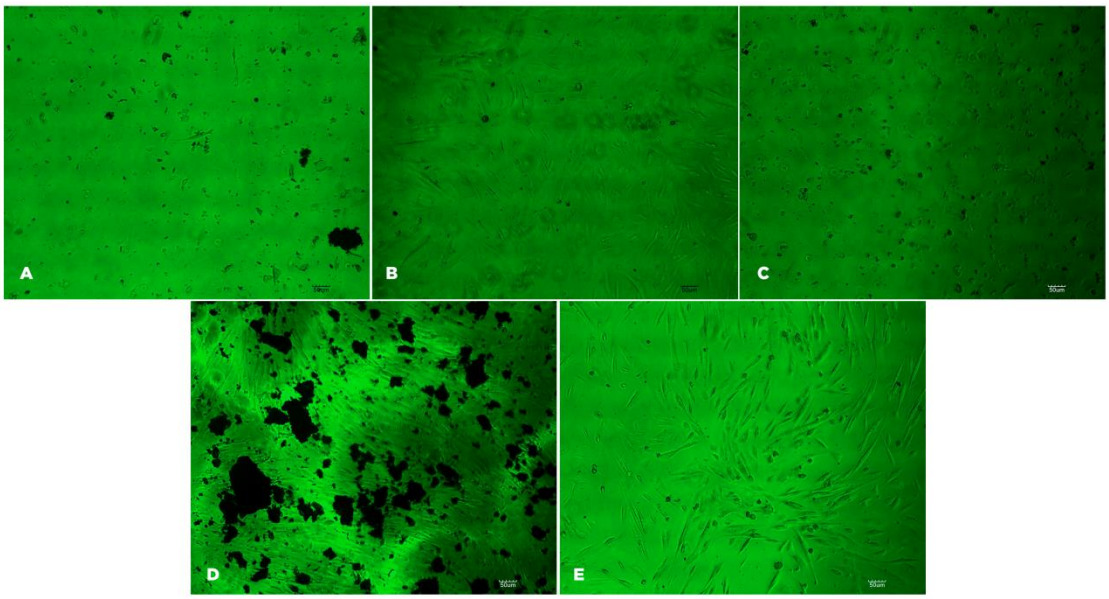

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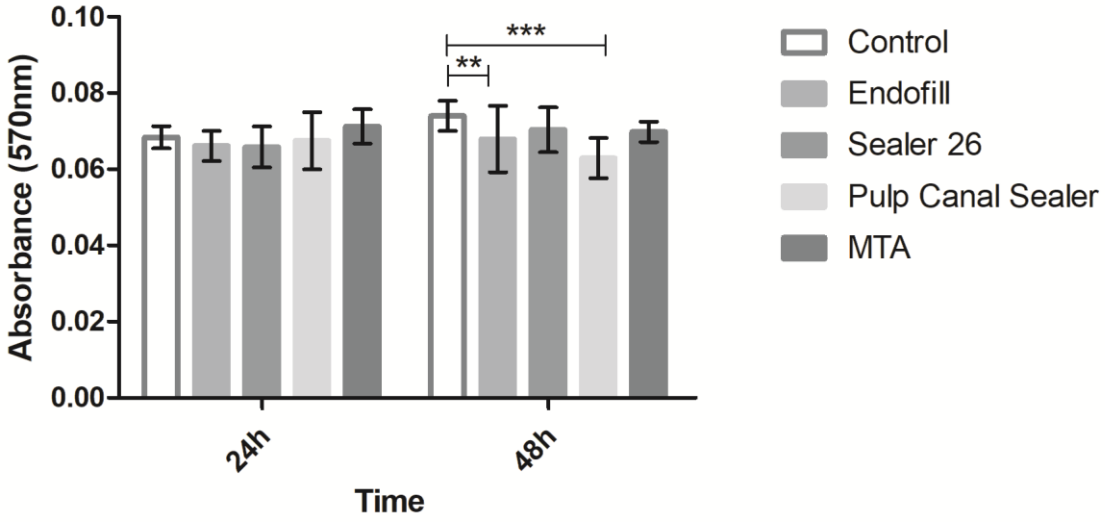

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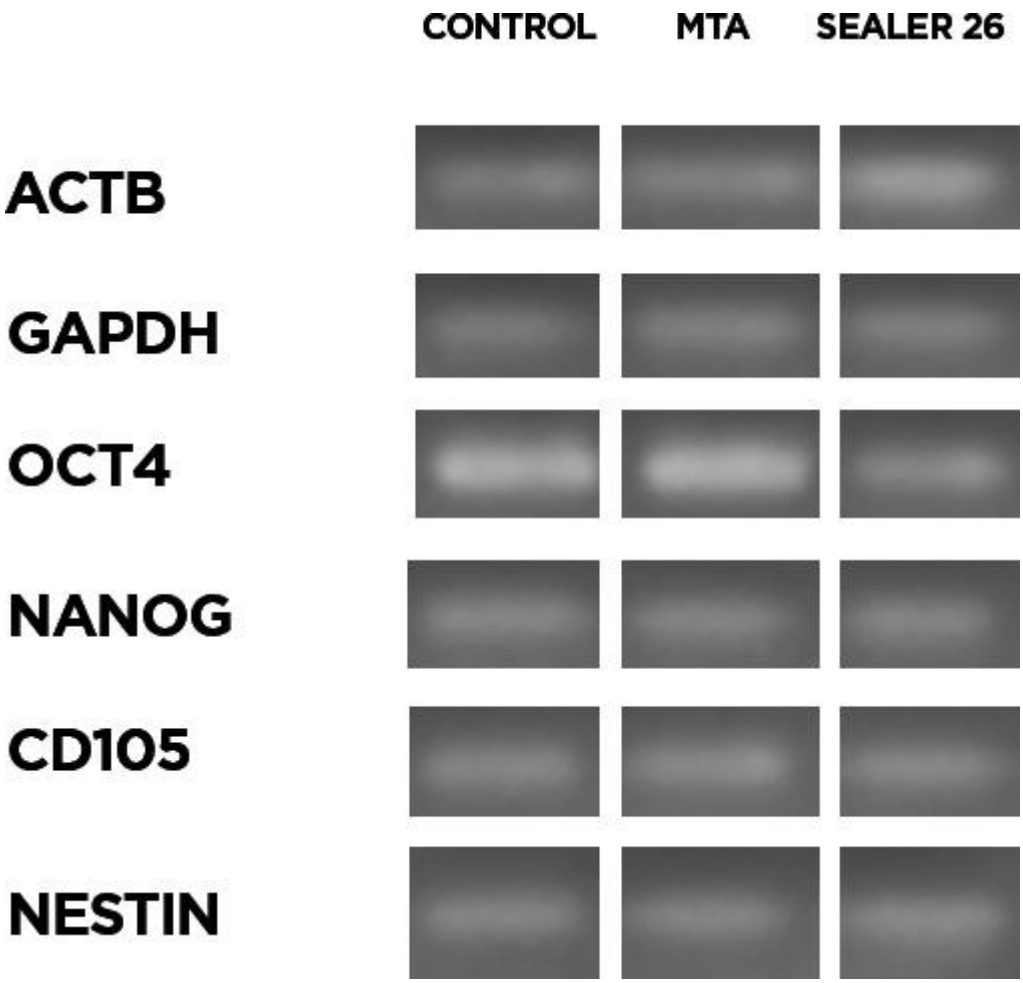

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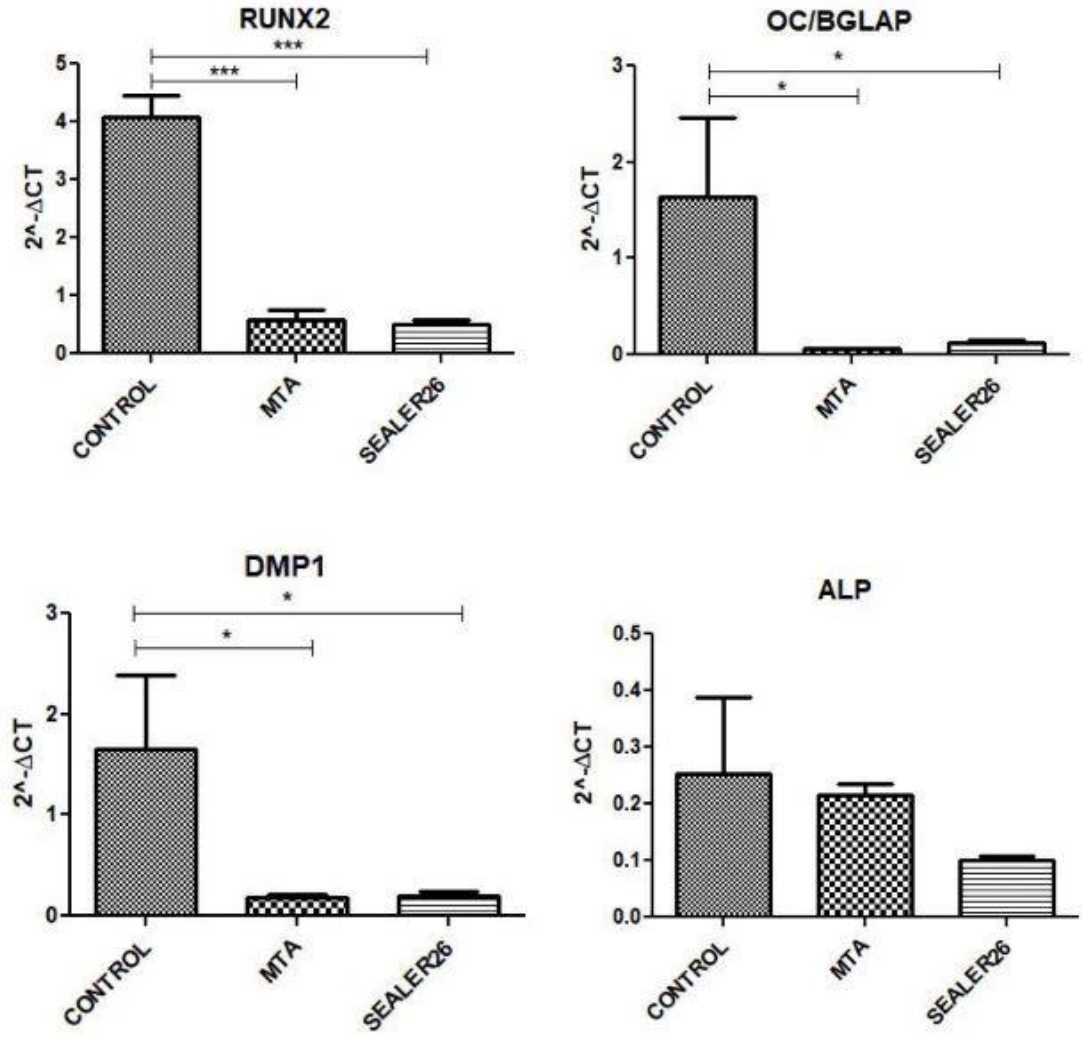

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Table 1 - Sequence of primers for analysis of cell characterization.

Markers	***Primer* F**	***Primer* R**	***Amplicon (pb)***	**Tm (°C)**
Embryonic				
OCT4	ACTTCACTGCACT GTACTCCTCAG	AGGTTCTCTTTCC CTAGCTCCTC	158	60
NANOG	CTACCCCAGCCTT TACTCTTCCTAC	CTCTCCACAGTTA TAGAAGGGACTG	217	60
Control				
Beta actin	ATTAAGGAGAAG CTGTGCTACGTC	GATGGAGTTGAA GGTAGTTTCGTG	213	60
Hematopoietic				
CD34	AACACCTAGTAC CCTTGGAAGTAC C	AACACTGTGCTG ATTACAGAGGTC	177	60
CD45	GGACACAGAAGT ATTTGTGACAGG	GAGAAGTTGTGG TCTCTGAGAAGTC	176	60
Mesenchymal				
Nestin	GGACCCTCCTAG AGGCTGAG	GTGAGGAGAGGG GAGTAGGG	168	60
CD105	TGCCACTGGACA CAGGATAA	CCTTCGAGACCTG GCTAGTG	205	60

Table 2 - Sequence of primers for analysis of cell diferentiation.

Markers	***Primer* F e R**	***Melting* Temperature (°c)**	**Product Size**	**Fasta Pubmed Reference**
HPRT1 ID3251	F 5' TGCTCGAGATGTGATGAAGG 3' R 5' TCCCCTGTTGACTGGTCATT 3'	54,5 56,1	192	NM_000194 .2
ACTB ID 60	F 5'AAACTGGAACGGTGAAGGTG 3' R 5'GTGGACTTGGGAGAGGACTG 3'	55,4 57,1	206	NM_001101 .3
* ALP ID 249	F 5'CCACGTCTTCACATTTGGTG 3' R 5'AGACTGCGCCTGGTAGTTGT 3'	54,2 58,8	196	NM_000478 .4
OC/ BGLAP ID 632	F 5' GGCAGCGAGGTAGTGAAGAG 3' R 5' AGCAGAGCGACACCCTAGAC 3'	57,5 58,8	194	NM_199173 .4
RUNX 2 ID 860	F 5'GAACTGGGCCCTTTTTCAGA 3' R 5'CACTCTGGCTTTGGGAAGAG 3'	55,3 55,6	208	NM_004348 .3
* DMP1 ID 1758	F 5'CAGGAGCACAGGAAAAGGAG 3' R 5'CTGGTGGTATCTTGGGCACT 3'	55,6 56,9	213	NM_004407 .3

* Reference: Galler et al., 2006

4.2 Artigo Dois – Formatado dentro dos padrões do *International Endodontic Journal (IEJ)*

Biocompatibility of Bioceramic Root Canal Sealers on Dental Stem Cells: A Systematic Review

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Abstract

Background Biomaterials have already been studied in several cell types such as macrophages and fibroblasts. In addition, new studies have been carried out testing sealers in stem cells recovered from dental tissues. For this reason, new review studies are needed.

Objectives This systematic review was conducted to investigate the bioactivity of endodontic materials, more specifically bioceramic materials used routinely in dentistry concerning stem cells recovered from dental tissues.

Methods Only *in vitro* studies evaluating the biological effects of bioceramic sealers on stem cells recovered from dental tissues were included with no language restriction. Two authors conducted a systematic search (PubMed/MEDLINE, Scopus, Web of Science, and other databases until February-2021) and undertook data extraction. The quality of the included studies was appraised according to Joanna Briggs Institute Clinical Appraisal Checklist. The search resulted in 307 studies, and 17 were considered.

Results: Biological effects of bioceramic sealers on stem cells from dental tissues were analyzed. Of the seventeen studies, six studies were performed with MTA Fillapex (the sealer most tested). Materials have been handled following manufactures instructions, and almost all studies used extracts and disks from the materials in contact with the stem cells, and regarding the cell types, those from the periodontal ligament were the most used. In this systematic review, almost all studies analyzed the cytotoxicity of dental materials to stem cells, and various studies analyzed cell migration, cellular death, morphology, and some staining assay.

Discussion: High-quality evidence was found. Few studies analyzed the bioceramic sealers on stem cells recovered from dental tissues.

Conclusion: Most bioceramic sealers are suitable materials to be used in clinical practice. However, more studies are needed to determine the impacts of these materials on stem cells, especially MTA Fillapex that showed a tendency for direct cytotoxicity.

Funding: This work was supported by Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Pesquisa e Desenvolvimento Tecnológico (CNPq) and Pró-Reitoria de Pesquisa da UFMG (PRPq).

Introduction

In need of endodontic treatment, filling the root canal system (RCS) well performed at all stages becomes extremely important, as this will seal the main and accessory canals, favoring the periapical repair process. In addition, the root filling will then tridimensional hermetically seal the entire system, thus preventing bacterial invasion. From this perspective, the correct choice of materials to be used in patients from a physical and biological perspective is essential (Reis-Prado et al. 2021, Fonseca et al., 2019).

The ideal endodontic material has been searched for years and must present several characteristics such as being radiopaque, biocompatible, not irritating to tissues, non-staining and having a good working time, among other various qualities and properties (Grossman, 1988). Currently, there is a new concern around stem cells in the dental region, cells responsible for essential tasks such as cell proliferation, differentiation, and self-renewal (Gronthos et al. 2000, Fischbach & Fischbach 2004, Victoria-Escandell et al. al. 2017).

Moreover, new parameters for testing supplied endodontic sealers are being created, and some biomaterials have not yet met the parameters created by Grossman in 1988. New cells can also be tested, such as stem cells from dental tissues (Gronthos et al., 2000). Many studies have already analyzed biological properties in cells such as macrophages, fibroblasts, and endothelial cells. On the other hand, previous studies have demonstrated the importance of biologically analyzing endodontic sealers in stem cells (Moura et al. 2012, Martins et al. 2013 , Scelza et al. 2012, Silva et al. 2016, de Oliveira Mendes et al. 2003, Guven et al. 2013a, Dimitrova-Nakov et al. 2015, Collado-Gonzáles et al. 2016, Rodríguez-Lozano et al. 2017, Victoria-Escandell et al. 2017, Alsubait et al. 2018, Javid et al. 2020).

Stem cells of dental origin are essential for differentiating, for example, into osteogenic and odontogenic cells. Furthermore, these are cells of great interest, thanks to their capacity and potential to promote regeneration of damaged dentin, pulp, resorbed root, periodontal regeneration, and perforation repair (Bansal & Jain, 2015). Finally, as stem cells have homologous physiological properties such as those of the primary tissue where the endodontic materials come into contact, they are a great experimental model to study their biological properties (Victoria-Escandell et al. 2017, López-García et al. 2019a).

For this reason, the present study aimed to carry out a systematic review to investigate the bioactivity of endodontic materials, more specifically bioceramic materials used routinely in dentistry, analyzed in cultures of mesenchymal stem cells recovered from dental tissues.

Materials and Methods

The present study is reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist (Moher et al. 2010). Moreover, this study was registered in the Open Science Framework (OSF) https://osf.io/6dyg2/.

Eligibility Criteria

The inclusion criterion was papers that evaluated the effects caused by different bioceramic endodontic sealers in mesenchymal stem cells of dental origin. Studies carried out on animal stem cells, *in vivo* studies and studies in which the outcome was not biological analyzes were excluded from this study, as well as the lack of characterization of mesenchymal stem cells explained in the articles.

To address the following question: "What are the biological effects of bioceramic endodontic sealers on mesenchymal stem cells of dental origin?" parameters were analyzed, such as the population, intervention, comparison and, outcome (PICO) approach. The study population comprised human mesenchymal stem cells of dental origin; the explored intervention was the cells' contact with bioceramic endodontic sealers or their extracts; the comparison was stem cells in the absence of bioceramic endodontic sealers and those in the presence of sealers. The primary evaluated outcome was the effect of different bioceramic sealers on cell cytotoxicity and, the secondary outcomes were the effect of these materials on cell migration, cell morphology, cell attachment, and phosphatase alkaline (ALP) activity.

Search Strategy and Information Sources

Electronic searches were conducted in PubMed/MEDLINE, Scopus, and Web of Science databases with no date restrictions closing until February 2021. The search strategy was as follows: “((root canal sealer OR endodontic sealer) AND (stem cell OR stem cells OR adult stem cell OR mesenchymal stem cell OR periodontal ligament stem cells OR dental pulp stem cells OR stem cells from human exfoliated deciduous teeth OR dental follicle stem cells OR tooth germ progenitor cells OR stem cells from the apical papilla OR oral mucosa-derived stem cells OR human gingiva-derived stem cells OR dental pulp cells OR periodontal ligament cells))."

Manual searches were also performed in the reference lists of the included articles and specific journals, such as the *Journal of Endodontics* and *the International Endodontic Journal.*

Study selection

In a two-step process, study selections were performed by two independent authors (P.Y.O. and P.R.G.). In Step 1, both authors assessed the titles and abstracts of the studies retrieved from the searches. Studies with titles and abstracts that met the eligibility criteria were included. The full texts were downloaded for studies with titles and abstracts that provided insufficient information to decide. In Step 2, both authors assessed the full texts. Studies in which the full text fulfilled the eligibility criteria were also included. Any disagreements between the two authors were resolved through discussion, and when necessary, a third author (F.B.) was consulted.

Data Collection and Analyses

One author (P.Y.O.) collected the following data from the included studies: first authors' last name, year of publication, materials used, material handling, methods, extracts used, preparation of extracts or disks, cells origin, cells passage, stem cell characterization, and analysis periods. In addition, data of the analysis as evaluations method and main findings were also collected. Subsequently, a second author (F.B.) reviewed the data collected by the previous author. Each of the included studies was analyzed in terms of similarities so that a meta-analysis could be performed. However, after evaluation, considerable heterogeneity was found; therefore, a meta-analysis was not performed. In addition, a wide range of methodological diversity was presented in the studies, being a necessary descriptive analysis of the results was presented instead.

Risk of Bias Assessment

Two investigators (P.Y.O. and P.R.G.) independently assessed the methodological quality of the selected studies based on the levels of evidence as proposed by the Joanna Briggs Institute Critical Appraisal Checklist for Experimental Studies (Aminoshariae & Kulild 2015, dos Reis-Prado et al. 2021), with some modifications. The items on the checklist were as follows: aim clearly stated, sample size justification (description of the sample size and power calculation methods or justification of the sample size used in the study), sample randomization, the possibility of comparison between control and treatment groups, baseline equivalence of control and treatment groups, clear protocol description, measurement method, measurement standardization, and adequate statistical analysis. Each item was scored using a 2-point scale: 0, not reported or reported inadequately; and 1, reported and adequate. Doubts and discrepancies between the investigators were discussed to reach a consensus, and if not resolved, a third examiner (F.B.) was consulted.

Results

Selected Studies

The selection process of the articles is presented in Figure 1. A total of 190 articles were found on searching in the previously cited databases. After the first screening, consisting of a title and abstract evaluations, 26 studies were selected. These studies were then subjected to full-text evaluation that resulted in the exclusion of 9 studies. The reasons for the exclusion of these articles are shown in Figure 1. Finally, 17 studies met the inclusion criteria and were included in this review (Güven et al. 2013a, Güven et al. 2013b, Suciu et al. 2016, Collado-Gonzalez et al. 2017a, Collado-Gonzalez et al. 2017b, Rodriguez-Lozano et al. 2017, Victoria-Escandell et al. 2017, Deog-Gyu et al. 2019, Lee et al. 2019, López-García et al. 2019a, López-García et al. 2019b, Rodriguez-Lozano et al. 2019, López-García et al. 2019c, Gaudin & Peters 2020, Jing et al. 2020, Oh et al. 2020, Rodriguez-Lozano et al. 2020).

The assessed Cohen kappa coefficient value for an inter-investigator agreement was equal to 1 for PubMed, 1 for Scopus, 1 for the Web of Science, 1 for Journal of Endodontics, and 1 for International Endodontic Journal. These values indicated a perfect agreement among reviewers on selecting studies according to the scale of Landis and Koch (1977).

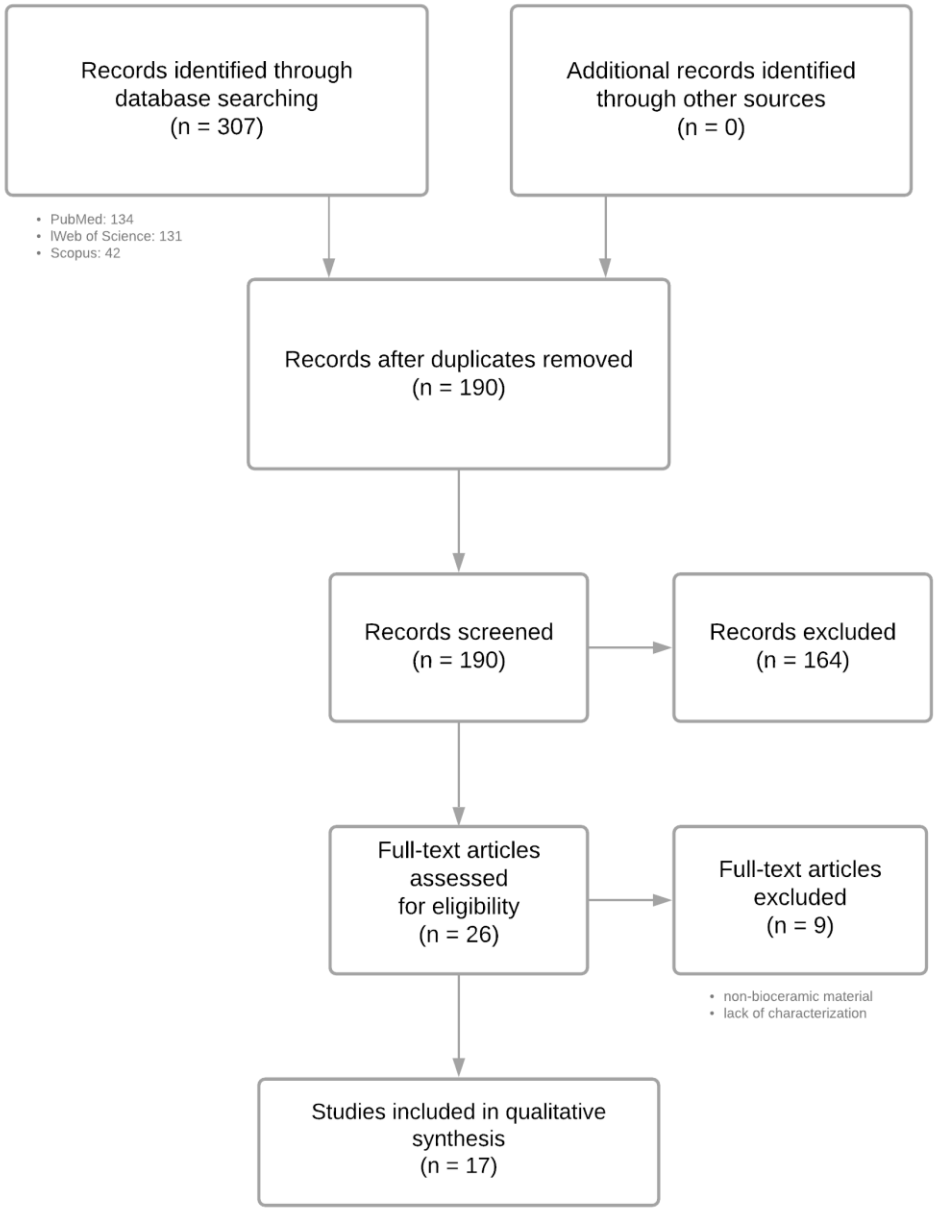

Figure 1. Systematic flow-chart representing study inclusion.

Characteristics of the Included Studies

The characteristics of studies that were eligible for this systematic review are described in Table 1. The analyzes were based on studies carried out with stem cells of dental origin. Of these, three studies were performed using Endosequence BC (Deog-Gyu et al. 2019, Rodriguez-Lozano et al. 2020, López-García et al. 2019c), three using BioRoot RCS (Deog-Gyu et al. 2019, Gaudin & Peters, 2020, Jing et al. 2020), one using BioRoot BC Sealer (Collado-Gonzalez et al. 2017a), two using Nano-Ceramic Sealer (Collado-Gonzalez et al. 2017a, Lee et al. 2019), one with Acroseal (Suciu et al. 2016), one using Bio-C Repair (López-García et al. 2019a) and two with Bio-C Sealer (López-García et al. 2019a, López-García

et al. 2019b). Six studies used MTA Fillapex (Victoria-Escandell et al. 2017, Gaudin & Peters, 2020, Collado-Gonzalez et al. 2017, Collado-Gonzalez et al. 2017, Rodriguez-Lozano et al. 2017, Güven et al. 2013a), two were testing TotalFill BC Sealer (López-García et al. 2019b, Rodriguez-Lozano et al. 2017), two were using CeraSeal (Oh et al. 2020, López-García et al. 2019c), five were using EndoSeal (Deog-Gyu et al. 2019, Collado-Gonzalez et al. 2017a, Oh et al. 2020, López-García et al. 2019c, Lee et al. 2019), one study was performed with ProRoot ES (Gaudin & Peters, 2020), two with GuttaFlow (Collado-Gonzalez et al. 2017b, Rodriguez-Lozano et al. 2019a), two were testing iRoot SP (Güven et al. 2013a, Güven et al. 2013b), one AD Seal (Lee et al. 2019), one WellRoot ST (Lee et al. 2019) and finally one with CRoot, an experimental material (Jing et al. 2020).

All materials have been handled under the instructions of their respective manufacturers, some of which were pre-mixed materials. Almost all the studies used extracts from the materials in contact with the stem cells, except for Deog-Gyu et al. (2019), Suciu et al. (2016), Güven et al. (2013a), Güven et al. (2013b) and Jing et al. (2020). Studies that used extracts were performed at different dilutions, and the most used were 1:1, 1:2 and 1:4 (Collado-Gonzalez et al. 2017a, Collado-Gonzalez et al. 2017b, Rodriguez-Lozano et al. 2017, Victoria-Escandell et al. 2017, Lee et al. 2019, López-García et al. 2019a, López-García et al. 2019b, López-García et al. 2019c, Rodriguez-Lozano et al. 2019b, Gaudin & Peters 2020, Rodriguez-Lozano et al. 2020).

The majority of the included studies reported the preparation of disks that were fabricated with a 5 mm diameter and a 2 mm height (Collado-Gonzalez et al. 2017a, Collado-Gonzalez et al. 201 7b, Lee et al. 2019, López-García et al. 2019a, López-García et al. 2019b, López-García et al. 2019c, Rodriguez-Lozano et al. 2019b, Jing et al. 2020, Oh et al. 2020, Rodriguez-Lozano et al. 2020). Regarding setting time, almost all materials were stored at 37 °C for 24 or 48 hours, except for Victoria-Escandell et al. (2017) and Suciu et al. (2016) studies. However, these authors do not mention anything about the time and temperature where the materials were kept to setting and, Deog-Gyu et al. (2019) stored their materials for 72 hours.

Most studies (twelve of them) used human stem cells from the periodontal ligament (hPLSCs), while two studies chose human dental pulp stem cells (hDPSCs) (Collado-Gonzalez et al. 2017a, Collado-Gonzalez et al. 2017b, Rodriguez-Lozano et al. 2017, Victoria-Escandell et al. 2017, Deog-Gyu et al. 2019. Lee et al. 2019, López-García et al. 2019a, López-García et al. 2019b, López-García et al. 2019c, Rodriguez-Lozano et al. 2019b, Gaudin & Peters 2020, Jing et al. 2020, Oh et al. 2020, Rodriguez-Lozano et al. 2020). Suciu et al. (2016) conducted their study with dental follicle stem cells (hDFSCs) and, Güven et al. (2013a) and, Güven et al. (2013b) used tooth germ stem cells (hTGSCs).

All studies had their respective stem cells characterized by flow cytometry, analyzing embryonic, hematopoietic, and mesenchymal markers. Deog-Gyu et al. (2019) was the only study that did not detail this analysis.

In this systematic review, almost all studies analyzed the cytotoxicity of dental materials to stem cells by cell viability assay. MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was the most used, among Annexin-V/7-AAD staining, Trypan Blue assay and Alamar Blue tests (Deog-Gyu et al. 2019, Collado-Gonzalez et al. 2017a, Suciu et al. 2016, López-García et al. 2019a, Victoria-Escandell et al. 2017, Rodriguez-Lozano et al. 2020, López-García et al. 2019b, Oh et al. 2020, López-

García et al. 2019c, Gaudin & Peters 2020, Collado-Gonzalez et al. 2017b, Rodriguez-Lozano et al. 2017, Rodriguez-Lozano et al. 2019b, Güven et al. 2013a, Lee et al. 2019, Güven et al. 2013b, Jing et al. 2020). Moreover, cell migration was also investigated by the several studies of this systematic review (Deog-Gyu et al. 2019, Collado-Gonzalez et al. 2017a, López-García et al. 2019a, Rodriguez-Lozano et al. 2020, López-García et al. 2019b, López-García et al. 2019c, Rodriguez-Lozano et al. 2017 and, Rodriguez-Lozano et al. 2019). Cellular death was examined by others (Collado-Gonzalez et al. 2017a, Suciu et al. 2016, López-García et al. 2019a, Victoria-Escandell et al. 2017 and, Gaudin & Peters 2020).

Staining analysis was performed in several studies, being alizarin red and alkaline phosphatase staining the most used (Deog-Gyu et al. 2019, Rodriguez-Lozano et al. 2020, López-García et al. 2019b, Oh et al. 2020, López-García et al. 2019c, Jing et al. 2020, Lee et al. 2019, Güven et al. 2013b).

Several studies analyzed the cell morphology (Deog-Gyu et al. 2019, Collado-Gonzalez et al. 2017a, López-García et al. 2019a, Rodriguez-Lozano et al. 2020, López-García et al. 2019b, Oh et al. 2020, López-García et al. 2019c, Collado-Gonzalez et al. 2017b, Rodriguez-Lozano et al. 2017, Rodriguez-Lozano et al. 2019b, Güven et al. 2013a, Lee et al. 2019), while only two studies performed the flow cytometry of cells after the sealers contact (Collado-Gonzalez et al. 2017, Lee et al. 2019),

Finally, the rt-PCR was performed to analyze genes involved in osteoblastic or odontogenic differentiation processes, such as ALP, OCN, DMP1(Victoria-Escandell et al. 2017, Rodriguez-Lozano et al. 2020, Oh et al. 2020, López-García et al. 2019c, Rodriguez-Lozano et al. 2019b, Lee et al. 2019, Güven et al. 2013b and, Jing et al. 2020). Analyzes of cytokines, proteins, and chemokines were performed by Oh et al. (2020), Gaudin & Peters (2020), Rodriguez-Lozano et al. (2019b), Rodriguez-Lozano et al. (2019a), Lee et al. (2019), Güven et al. (2013b), Jing et al. (2020) and Victoria-Escandell et al. (2017). IL-6, IL-8, IL-10 levels were the most analyzed.

Table 2 summarizes the analysis performed in each selected study of this systematic review. The cytotoxicity, morphology and attachment, cell migration and proliferation, mineralization assays, cytokine analysis, and other protein analysis, and a general outcome, were reported in Table 2. In addition, the cytotoxicity data were described below.

Cytotoxicity

Of the 17 studies analyzed, it was reported that bioceramic sealers were biocompatible in almost ones, except for the MTA Fillapex or EndoSeal MTA (Güven et al. 2013a, Victoria-Escandell et al. 2017, Rodríguez-Lozano et al. 2019, Gaudin & Peters 2020, Rodríguez-Lozano et al. 2017b, Suciu et al. 2016). Additionally, most of the studies measured metabolic activity, and the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay prevails, with tests such as the SRB assay and the Alamar Blue being also used on a smaller scale (Victoria-Escandell et al. 2017, Suciu et al. 2016). Of the 17 studies, 14 used a direct contact testing method with sealers prepared either as fresh sample, disc, layer or cylindrical specimens (Güven et al. 2013a, Güven et al. 2013b, Collado-Gonzalez et al. 2017a, Collado-Gonzalez et al. 2017b, Victoria-Escandell et al. 2017, Deog-Gyu et al. 2019, Lee et al. 2019, López-García et al. 2019a, López-García et al. 2019b, López-García et al. 2019c, Rodriguez-Lozano et al. 2019, Gaudin & Peters 2020, Oh et al. 2020, Rodriguez-Lozano et al. 2020). Moreover, other research used

human root model (Jing et al. 2020). Rodrigues-Lozano et al. (2017) and Suciu et al. (2016) did not specify the method used.

Subgroup analysis

Table 3 shows the main bioceramic sealers evaluated. The EndoSeal MTA, MTA Fillapex, EndoSequence BC, BioRoot RCS, Nano-ceramic sealer, Bio-C Sealer and iRoot SP sealers were the most researched. MTA Fillapex sealer was the evaluated material in 6 different studies (Güven et al. 2013a, Victoria-Escandell et al. 2017, Rodríguez-Lozano et al. 2019, Gaudin & Peters 2020, Rodríguez-Lozano et al. 2017b, Suciu et al. 2016), while EndoSeal MTA was analyzed in 4 studies (Collado-González et al. 2017a, Deog-Gyu et al. 2019, Lee et al. 2019, López-García et al. 2019c), EndoSequence BC Sealer in 3 (Deog-Gyu et al. 2019, López-García et al. 2019c, Rodríguez-Lozano et al. 2020), prevailing among the other materials. BioRoot was analyzed in 3 studies (Deog-Gyu et al. 2019, Gaudin & Peters 2020, Jing et al. 2020), Nano-ceramic Sealer, Bio-C Sealer and iRoot were the object of two different studies for each one (Collado-González et al. 2017a, Lee et al. 2019, López-García et al. 2019a, López-García et al. 2019b, Guyen et al. 2013a, Guyen et al. 2013b).

Cytotoxicity results demonstrate that MTA Fillapex was cytotoxic in all studies that it was analyzed (Güven et al. 2013a, Victoria-Escandell et al. 2017, Rodríguez-Lozano et al. 2019, Gaudin & Peters 2020, Rodríguez-Lozano et al. 2017b, Suciu et al. 2016). The EndoSeal MTA sealer was cytotoxic in 2 of 4 studies performed (Collado-González et al. 2017a, López-García et al. 2019c). Additionally, any reviewed study has not examined AR assay, ALP activity and mineralization in MTA Fillapex cultures because cells did not keep on viable (Deog-Gyu et al. 2019, López-García et al. 2019c, Rodríguez-Lozano et al. 2020, Jing et al. 2020, López-García et al. 2019b). Other materials, such as EndoSequence BC Sealer, BioRoot RCS, Nano-ceramic sealer, Bio-C Sealer, and iRoot SP, present similar biocompatible when compared to the control (Deog-Gyu et al. 2019, Rodríguez-Lozano et al. 2020, López-García et al. 2019c, Jing et al. 2020, Gaudin & Peters, 2020, Lee et al. 2019, Guven et al. 2013a, Guven et al. 2013b). None of the materials evaluated were able to reduce ALP expression (Guven et al. 2013b, Lee et al. 2019, Jing et al. 2020, López-García et al. 2019c).

Risk of bias

Table 4 showed the assessment of the risk of bias. Nine studies reported the highest possible score (REFs), justifying the sample size and correctly indicating their protocols. In addition, all the included articles showed a *clearly stated aim, randomization, and correct comparison between control and treatment groups, baseline equivalence of control and treatment groups, measurement method, measurement standardization,* and *adequate statistical analysis*. Low risk of bias was also found in the *sample size justification.* Nevertheless, a high risk of bias was observed for a specific item, such as *the accurate description of the protocols.*

Table 1. Methodological characteristics of the studies included in the review

Author	Material	Material handling	Methods	Extracts used	Preparation of extracts or disks	Cells origin, passage	Stem cell characterization	Analysis period
Güven et al. 2013a	iRoot SP; MTA Fillapex; AH Plus.	The sealers were mixed according to the manufacturers' instructions.	Proliferation: Disks in contact with SCs. SEM: coverslips were used.	1:1	Proliferation: Disks of each sealer were fabricated with a 4 mm diameter and a 3 mm height; stored in a humidity, 37ºC, 24 h, to setting. All sealers were submerged in media with the addition of 1.5 mL of medium to each well. SEM: 0.8 g of each was applied to the surface of sterile coverslips (20 mm×20 mm) forming 400 mm^2 plugs with a thickness of 2 mm, stored in a humidity, 37ºC, 24 h, to setting. The cells were seeded onto the coverslips at a concentration of 25.000 cells/well in 2 mL of growth media.	hTGSCs - Passage 2.	Flow cytometry analysis of the expression of MSCs markers: CD14, CD29, CD34, CD45, CD90, CD105, CD133, CD166 and CD73	MTS Assay: 1, 3, 7, and 14 days. SEM: 1, 3, 7, and 14 days.

Güven et al. 2013b	MTA ProRoot; iRoot SP; Dycal.	The sealers were mixed according to the manufacturers' instructions.	Disks in contact with SCs.	1:1	Disks of each sealer were fabricated with a 4 mm diameter and a 3 mm height; stored in a 95% of humidity, 37ºC, 24 h, to setting. The materials were covered with Mylar sheets to form cylindrical specimens of each material under aseptic conditions.	hTGSCs - passage 3.	Flow cytometry analysis of the expression of MSCs markers: CD14, CD29, CD34, CD45, CD90, CD105, CD133, CD166 and CD73.	MTS assay: 3 and 7 days. Odontogenic differentiation, immunocytochemistry assay, qRT-PCR, ALP activity, and von Kossa staining: 14 days.
Suciu et al. 2016	Acroseal; AH Plus; MTA Fillapex.	The sealers were mixed according to the manufacturers' instructions.	The materials were placed as a thin layer with the help of cell scrappers onto the surface of 2-well chamber slides.	n.a.	n.a.	hDFSCs - passage (?).	Flow cytometry analysis of the expression of MSCs markers: SSEA-4, OCT3/4, NANOG, CD44, CD90, CD73, CD105, CD49E, CD45, CD117, CD34, CXCR4, SCF, CBF beta, THY-1, TIE-2, VIMETIN, HLA-ABC, hTERT, SOX2, c-kit and HLA-DRalfa.	Alamar Blue: 48 h and 5, 9 and 14 days. PKH26 Red Fluorescent Cell Linker Kits: 45 min, 24 h, 5 and 8 days.

Collado-Gonzalez et al. 2017a	BioRoot BC Sealer; Endoseal MTA; Nano-ceramic Sealer.	The sealers were mixed according to the manufacturers' instructions.	Extracts of the materials in contact with SCs.	1:1, 1:2, and 1:4	Disks of each sealer were fabricated with a 5 mm diameter and a 2 mm height; stored at 37°C, 48 h to setting. The ratio between the surface of the sample and the volume of the medium was 6 cm^2 mL^{-1}. The extraction media were then collected at the end of this period and passed through a 0.22 μ m filter. Various dilutions were prepared.	hPLSCs - passage 2 to 4.	Flow cytometry analysis of the expression of MSCs markers: CD73, CD105, CD90, CD45 and CD34.	MTT: 24, 48 and 72 h. Flow cytometry: 72 days. Cellular death: 72 h. Cell migration: 0, 24 and 48 h. SEM: 72 h.
Collado-González et al. 2017b	GuttaFlow Bioseal; GuttaFlow2; MTA Fillapex; AH Plus.	The sealers were mixed according to the manufacturers' instructions.	Extracts of the materials in contact with SCs.	1:1, 1:2, 1:4	Disks of each sealer were fabricated with a 5mm diameter and a 2 mm height; stored in a humidity, 37ºC, 48 h, to setting. Sample disks were stored in the culture medium DMEM for 24 h at 37 ºC, 5% CO_2 and a humid atmosphere. The ratio between the surface of the sample and the volume of the medium was 6 cm^2/mL. The extraction media were collected at the end of this period and passed through a 0.22 μm filter. Subsequently, various dilutions were prepared.	hPLSCs - passage 4 and on.	Flow cytometry analysis of the expression of MSCs markers: CD90, CD73, and CD105.	MTT: 24, 48, 72 h, and 7 days. SEM: 7 days.

Rodríguez-Lozano et al. 2017	MTA Fillapex; TotalFill BC Sealer; AH Plus.	The sealers were mixed according to the manufacturers' instructions.	Extracts of the materials in contact with SCs.	1:1, 1:2, 1:4, and 1:8	The materials were stored at 37°C for 48 h to setting. The ratio between the surface of the sample and the volume of the medium was 6 cm^2 mL^{-1}. The extraction media were filtered and various dilutions were prepared.	hPLSCs - passage (?).	Flow cytometry analysis of the expression of MSCs markers: CD73, CD90, CD105, CD14, CD20, CD34 and CD45.	MTT: 24, 48 and 72 h. Cell Migration: 0, 24 h and 48 h. Morphology analyses: 24, 48, 72 h. Cell Adhesion: 24, 48 and 72 h. SEM: 96h.
Victoria-Escandell et al. 2017	MTA-Angelus; AH-Plus; MTA Fillapex.	The sealers were mixed according to the manufacturers' instructions.	Extracts of the materials in contact with SCs.	1:1, 1:2	100 mg of each freshly mixed RCS (AH-Plus and MTA-Fillapex) and 100 mg of MTA-Angelus powder were immersed in 1 mL of DMEM. These samples were incubated for different time periods comprising 24 h, 48 h, 7 days, 15 days, and 30 days in an incubator at 37°C. The obtained extracts were filtered and stored until their use.	hDPSCs - passage (?).	Analysis of the expression of MSCs markers: STRO1, OCT1, CD133, CD34, nestin and CD45.	Cytotoxicity SRB Assay and Cellular Death: 24, 48 h, 7, 15 and 30 days. Oxidized Protein Analysis: 24 h and 7 days. Enzyme Expression by Western Blot: 24 h. RT-qPCR: 24 and 48h.
Deog-Gyu et al. 2019	AH Plus; EndoSequence BC Sealer; BioRoot RCS; Endoseal MTA.	Endosequence BC and Endoseal MTA: premixed sealers. BioRoot RCS: one spoon of powder was mixed with five drops of a liquid. AH Plus: 1:1 ratio paste, in accordance with the manufacturer.	Disks in contact with SCs.	n.a.	Disks of each material 6 mm in diameter and 3 mm in height were made using Teflon molds; stored in a 100% humidity, 37ºC, 72 h, to setting.	hDPSCs - passage 3.	The cell lines were developed anonymously.	MTT: 0, 24, 48, 72 and 120 h. Cell Migration: 0, 24, 48, 72, and 96 h. SEM: 72 h. AR assay: 15 days.

Lee et al. 2019	AH Plus; AD Seal; EndoSeal MTA; Nano-ceramic Sealer; WellRoot ST.	The sealers were mixed according to the manufacturers' instructions.	Extracts of the materials in contact with SCs.	1:4	Disks of each sealer were prepared with a 5 mm diameter and a 2 mm height; stored in a humidity, 37ºC, 48 h, to setting. All experimental sealers were mixed with DMEM at a concentration of 20 mg/mL to make material extraction medium, incubated for 24 h in 5% CO^2 at 37ºC. The supernatant of material extraction medium was filtered with a 0,22 µm filter, and one dilution was prepared.	hPLSCs - passages 4 to 6.	Flow cytometry analysis of the expression of MSCs markers: CD73, CD90, CD105, CD11b, CD19, CD34, CD45, and HLA-DR.	MTT: 1,3, and 7 days. SEM: 72 h. ELISA: 24 h. Flowcytometry Analysis: 72 h. qPCR: 3, 6, and 9 days. ALP staining: 3,6 and 9 days.
López-García et al. 2019a	Bio-C Repair; Bio-C Sealer.	Ready-to-use materials.	Extracts of the materials in contact with SCs.	1:1, 1:2 and 1:4	Disks of each sealer were fabricated with a 5 mm diameter and a 2 mm height; stored in a humidity, 37ºC, 24 h, to setting. The ratio of material surface area and extraction vehicle volume was calculated as 1.5 cm^2/ mL. The extraction medium was filtered. Undiluted extract media, and two dilutions were prepared.	hPLSCs - passage 3.	Flow cytometry analysis of the expression of MSCs markers: CD73, CD105, CD90, CD34, CD45, CD14 and CD20.	MTT: 24, 48 or 72 h. Cell Migration: 0, 24, 48, and 72 h. Cellular Death: 72 h. SEM 72 h.

López-García et al. 2019b	Bio-C Sealer; TotalFill BC Sealer; AH Plus.	The sealers were mixed according to the manufacturers' instructions.	Extracts of the materials in contact with SCs.	1:1, 1:2 and 1:4	Disks of each sealer were fabricated with a 5 mm diameter and a 2 mm height; stored in a humidity, 37ºC, 48 h, to setting. After this period, sample disks were stored in the culture medium DMEM for 24 h at 37ºC, 5% CO_2 and a humid atmosphere. The ratio of the specimen surface area was 1.5 cm^2/mL. Sealer extracts were prepared, filtered and diluted. Three different dilutions were used.	hPLSCs - passage 3 to 5.	Flow cytometry analysis of the expression of MSCs markers: CD73, CD90, CD105, CD14, CD20, CD34 and CD45.	MTT: 24, 48 and 72 h. Cell Migration: 0, 24, 48 and 72 h. Cell Morphology: 72 h. Cell Attachment and SEM: 72 h. AR assay: 21 days.
López-García et al. 2019c	EndoSequence BC Sealer; CeraSeal; EndoSeal MTA.	The sealers were mixed according to the manufacturers' instructions.	Extracts of the materials in contact with SCs.	1:1, 1:2, 1:4	Disks of each sealer were fabricated with a 5 mm diameter and a 2 mm height; stored in a 100% of humidity, 37ºC, 48 h, to setting. The ratio of the specimen surface area was 1.5 cm^2/mL. The extracts obtained were filtered and diluted.	hPLSCs - passage 2.	Flow cytometry analysis of the expression of MSCs markers: CD90, CD105, CD73, CD34, CD45, CD14, and CD20.	MTT: 24, 48, 72 h. Cell Migration: 0, 24, 48, 72 h. SEM: 72 h. RT-qPCR: 3, 7, 14, and 21 days. AR assay: 21 days.

Rodríguez-Lozano et al. 2019	GuttaFlow Bioseal; GuttaFlow2; MTA Fillapex; AH Plus.	The sealers were mixed according to the manufacturers' instructions.	Extracts of the materials in contact with SCs.	1:1, 1:2, 1:4	Disks of each sealer were fabricated with a 5 mm diameter and a 2 mm height; stored in a humidity, 37ºC, 48 h, to setting. The materials were stored in the culture medium for 24 h at 37◦C in a humid atmosphere containing 5% CO2. The ratio of material surface area to medium volume was set at approximately 1.5 cm^2/mL. The extraction medium was filtered with sterile filters of 0.22 μm diameter pores, and several dilutions were prepared.	hPLSCs - passage 4 and on.	Flow cytometry analysis of the expression of MSCs markers: CD73, CD90, CD105, CD14, CD20, CD34 and CD45.	Cell Viability Assay: 72 h. Cell Migration: 0, 24 and 48 h. Cell morphology and Phalloidin: 72 h. SEM: 72 h. qPCR: 7 days. Immunocytofluorescence: 7 days.
Gaudin & Peters 2020	BioRoot RCS; ProRoot ES; MTA Fillapex; AH Plus.	The sealers were mixed according to the manufacturers' instructions.	Extracts of the materials in contact with SCs.	1:1, 1:2, 1:4, and 1:8	Disks of each sealer were fabricated with a 7 mm diameter and a 3 mm height; stored in a humidity, 37ºC, 48 h, to setting. The extraction of different materials' eluates was performed in general accordance with ISO 10993-12. The extraction was made in complete cell culture medium, and various dilutions were prepared.	hPLSCs - passage 4.	Flow cytometry analysis of the expression of MSCs markers: CD45, CD90, CD105, CD146 and STRO-1.	Trypan Blue: 24. 48 and 72 h. MTT: 24 h. Analysis of Cytokines and Chemokines: 24 h.

Jing et al. 2020	AH Plus JetTM Syringe; Bio-Root RCS; C-Root (experimental material).	AH Plus and Bio-Root were prepared according to the manufacturers' instructions. C-Root powder was mixed with polyethylene glycol-based gel and homogenized for 15 minutes.	A rubber O-ring was used to adjust the position of the tooth and the extent of immersion into the cell culture.	n.a.	A root canal-filling model apparatus was fabricated. The lower 3 to 5 mm proportion of polypropylene Eppendorf tubes was cut to allow 5 mm of the apical third of the root to protrude into a 6-well cell culture plate. A rubber O-ring was used to adjust the position of the tooth and the extent of immersion into the cell culture. Stored in a 100% of humidity, 37ºC, 24 h, to setting.	hPLSCs - passage 3 to 7	Flow cytometry analysis of the expression of MSCs markers: STRO-1, CD45, CD73, CD90 and CD105.	AR and ALP assay: 14 days. Proliferation CCK-8 assay: 1, 3, 5, and 7 days. qRT-PCR: days 7 and 14 days. ELISA: 1, 3, 7 and 14 days.

Oh et al. 2020	CeraSeal; EndoSeal TCS; AH Plus.	The sealers were mixed according to the manufacturers' instructions.	Extracts of the materials in contact with SCs.	1:1	Disks of each sealer were fabricated with a 5 mm diameter and a 2 mm height; stored in a 100% of humidity, 37ºC, 48 h, to setting. To prepare a fresh medium, 200 mg of sealers was placed in the 50 mL conical tube and 10 mL culture medium was added to each tube. The tubes were incubated at 37ºC in 5% CO_2 for 24 h. Afterwards, the supernatant of the fresh material extraction medium was filtered with a 0.2 μm pore-size filter for use. To prepare the setting material extraction medium, each set sample disc was stored in 10 mL culture medium at 37ºC in 5% CO_2 for 24 h. Afterwards, the extracts were filtered using 0.2 μm pore-size filter for use.	hPLSCs - passages 4 to 6.	Flow cytometry analysis of the expression of MSCs markers: CD90, CD105, CD73, CD11b, CD19, CD34, CD45, and HLA-DR.	Proliferation CCK-8 Assay: 1, 3, and 7 days. ELISA: 24h. RT-qPCR: 3 and 7 days. AR assay: 14 days. Cell attachment: 72 h. Material surface morphology: 48 h.

Rodriguez-Lozano et al. 2020	EndoSequence BC Sealer HiFlow; EndoSequence BC Sealer; AH Plus.	The sealers were mixed according to the manufacturers' instructions.	Extracts of the materials in contact with SCs.	1:1, 1:2 and 1:4	Disks of each sealer were fabricated with a 5 mm diameter and a 2 mm height; stored in a 95% of humidity,37ºC, 48 h, to setting. The ratio of material surface area and extraction vehicle volume was calculated as 1.5 cm^2/mL. The extraction medium was filtered. Undiluted extract media, and two dilutions were prepared.	hPLSCs - passage 2 to 4	Flow cytometry analysis of the expression of MSCs markers: CD90, CD105, CD73, CD34, CD45, CD20 and CD14.	SEM and Energy-dispersive X-ray Analysis: 24 h. MTT: 24, 48, 72 h. Cell migration: 0, 24, 48 and 72 h. SEM: 72 h. AR assay: 21 days. RT-qPCR: 7 days.

n.a.: not applicable; SCs: Stem Cells; hDPSCs: human dental pulp stem cells; hPLSCs: human periodontal ligament stem cells; hDFSCs: human dental follicle stem stem; hTGSCS: human tooth germ stem cells; MSCs: mesechymal stem cells; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; SEM: scanning electron microscope; PKH: PKH26 Red Fluorescent Cell Linker Kits; SRB: sulforhodamine B assay; rtPCR: Reverse transcription polymerase chain reaction; DAPI: 4',6'-diamino-2-fenil-indol (marcador fluorescente); AR: alizarin red assay; CCK: cell counting kit-8; ELISA: Enzyme-Linked Immunosorbent Assay; ALP: alkaline phosphatase; ICP-MS: inductively coupled plasma mass spectrometry; MTS: MTS:3-(4,5-dimethyl-thiazol-2-yl)-5-(3-carboxy-methoxy-phenyl)-2-(4-sulpho-phenyl)-2H tetrazolium assay; EDX: energy-dispersive x-ray analysis; PC: odontogenic differentiation médium only; NC: hTGSCs alone.

Table 2. Main results of primary and secondary outcomes of all studies

Author	Cytotoxicity	Morphology and cells attachment	Cell migration and proliferation	Mineralization Assay	Cytokine and other protein analysis	Outcomes

Güven et al. 2013a	MTAF showed a toxic effect compared with control* starting on day 1. On days 3,7 and 14 MTAF showed significant toxic effects compared with iRoot SP*. On day 3, iRoot SP and AHP enxerted different results on hTGSCs*. Only on day 7, a significant reduction in cell viability was observed with iRoot SP compared with control group*.	The highest levels of cell attachment were observed for iRoot SP and the control group over the entire period. On day 1, cells on the coverslips coated with MTAF and AHP displayed a rounded shape, rather than adopting a spreading configuration. After 24 h, MTAF exerted its cytotoxic effects, reducing the number of cells attached to the surface.	n.a.	n.a.	n.a.	MTAF showed a toxic effect and interfered with cell morphology and adhesion, while iRoot SP has a similar cytocompatibility, cell morphology and adhesion than the control.
Güven et al. 2013b	MTA and iRoot SP showed no cytotoxicity for up to 7 days after culturing. The presence of Dycal significantly decreased cell viability*.	n.a.	n.a.	Von Kossa Stainning: Ca_2+ deposit formation was observed by cells that were treated with odontogenic differentiation. ALP activity in odontogenic differentiation medium: All groups showed increased ALP activity* compared with noninduced hTGSCs. However, the group that was treated with iRoot SP had significantly decreased ALP activity* compared with the positive control rtPCR: All differentiated	n.a.	The bioceramic sealer was cytocompatible compared to the control. In addition, mineralization was induced.

				groups displayed significantly increased mRNA expression levels of DSPP compared with the negative control*. iRoot SP showed a significantly lower expression level than the positive control*. Immunocytochemistry assay: DSPP levels were observed for the MTA, iRoot SP and positive control groups but undifferentiated hTGSCs (negative control) showed no expression of either protein.		
Suciu et al. 2016	Alamar Blue: Acroseal showed a higher biocompatibility by comparison with control, MTAF and AHP. At 5 days, Acroseal was similar to control; at 9 and 14 days the cultures exhibited an increase of proliferation compared with control. MTAF showed the greatest cytotoxicity.	PKH26 KIT: After cell seeding was observed that the adhesion was not complete. After 24 h, the adhesion was completed for cells cultivated on plastic surface. The materials do not allow the cells to adhere to their surfaces. This aspect was maintained also after 5 and 8 days in the case of AHP and MTAF.	n.a.	n.a.	n.a.	Acroseal showed a good biocompatibility by comparison with control. MTAF was not cytocompatible. There was no cell adhesion to Acroseal or MTAF.

Collado-Gonzalez et al. 2017a	MTT: ES-MTA decreased cell proliferation compared to the control *. Cell proliferation in the presence of BR-BC (1:1, 1:2 and 1:4 extracts) was higher than ES-MTA and Nano-ceramic Sealer at 72 h*. Flow Cytometry: BR-BC revealed more than 87% of viable cells after exposure. Nano-ceramic Sealer exhibited high rates of cell viability in 1:2 and 1:4 extracts and ES-MTA exhibited low rates of cell viability at concentrations of 1:1 and 1:2.	SEM: The morphology of the cells seeded on BR-BC and Nano-ceramic Sealer had similar characteristics. However, using ES-MTA, cell attachment was limited. At 24 h, microscopic observation showed that, in the presence of ES-MTA eluates had a significantly lower number of attached cells than the control, whereas in the presence of Nano-ceramic Sealer and BR-BC, moderate and high levels of attached cells were observed, respectively.	Scratch wound healing assay: Treatment with the different dilutions of BR-BC eluates for 24 h promoted a cell migration level similar to control. Extracts of BR-BC promoted wound closure in a concentration-dependent manner, comparable to that observed in control extracts*. After 48 h of incubation with the more concentrated dilutions of Nano-ceramic Sealer (1:1 and 1:2), cell migration was lower than the control*.	n.a.	n.a.	Bioceramic sealers showed good cytocompatibility and allowed cell proliferation, adhesion and migration when compared to the control.
Collado-González et al. 2017b	At 168 h, cell viability in the presence of GF-BS at concentrations of 1:1, 1:2, and 1:4 was significantly higher than it was with GF-2, AHP, MTAF, or the control*, whereas MTAF eluates significantly reduced cell viability compared with the control at 24, 48, 72, and 168 h*. GF-2 eluates led to similar	The morphology of the cells seeded on MTAF and AHP was similar, and both resulted in a low rate of cell attachment. By using GF-2 disks, cell attachment was modest, with abundant round cells appearing on the surface of the material. Finally, GF-BS exhibited well-adhered cells with a high degree of cell spreading and production of extracellular matrix.	n.a.	n.a.	n.a.	Bioceramics sealers showed a good cytocompatibility that was similar to control (except for MTAF, which was cytotoxic). They also allowed a good cell spreading and adhesion (except for MTAF).

	cell viability results as GF-BS except at 168 h.					
Rodríguez-Lozano et al. 2017	MTAF in any dilution revealed an impaired proliferation up to 72 h of incubation, whilst TF-BC or AHP allowed a level of proliferation compared with MTAF*. The level of hPDLSC proliferation in the presence of TF-BC eluates was similar to that obtained using control and was also higher than using AHP from 48 h of incubation*. As a positive control, hPLSCs treated with SPS revealed a significantly higher apoptotic cell percentage compared with controls*. hPLSCs exposed to different dilutions of MTAF underwent a time-	The morphology of the cells seeded on TF-BC and AHP had similar characteristics, with substantial cell spreading and production of extracellular matrix. However, using MTAF, cell attachment was limited, with only a few round cells appearing on the surface of the material.	After 48 h of incubation with the more concentrated dilutions of AHP (undiluted, 1:1 and 1:2, cell migration was significantly lower than the control*. hPLSCs incubated with TF-BC eluates had a significantly higher migration compared with the control up to 24 h*, obtaining a complete wound closure after 48 h of culture. In the presence of MTAF extracts, hPLSCs revealed a discrete adhesion to the substrate, whereas treatment with TF-BC and AH Plus displayed a significantly higher adhesion compared with MTAF*. hPDLSC adhesion levels in the presence of TF-BC extracts were comparable to control and also higher compared with AHP*.	n.a.	n.a.	Bioceramics sealers showed a good cytocompatibility that was similar to control (except for MTAF, which was cytotoxic). They also allowed a good cell spreading and adhesion (except for MTAF). hPDLSC adhesion levels in the presence of TF-BC extracts were comparable to control

	dependent and significant decrease in cell viability compared with control*					
Victoria-Escandell et al. 2017	SRB Assay: AHP was most cytotoxic at 24 h compared to the control, MTAA and MTAF.* For longer periods of treatment (48 h and 7, 15, and 30 days), cytometry results revealed that MTAF became the most cytotoxic with higher averages of apoptotic cells.* For all conditions assayed, MTAA was the least cytotoxic material. Flow Cytometry: cell apoptosis was increased in presence of AHP and MTAF.	n.a.	n.a.	n.a.	n.a.	MTAF was cytotoxic with higher averages of apoptotic cells compared to control.

Deog-Gyu et al. 2019	MTT: There are no difference between bioceramic sealers and control. Only AHP showed significant cytotoxicity*	SEM: hDPSCs in direct contact with ESQ-BC, ES-MTA, and BR showed superior spreading compared to the AHP. ESQ-BC and BR groups showed large amounts of small sealer particles on the disk surfaces but exhibited superior cell attachment compared to the AHP group.	Scratch wound healing assay: There are no difference between ESQ-BC and ES-MTA sealers and control. BR exhibited slower cell migration than ESQ-BC and ES-MTA did up to 72 h*. Wound healing was not observed in the AHP group at 24 h, and it was less than the other sealers at 48, 72 h, and 96 h*	AR assay: hDPSCs exposed to ESQ-BC, BR, ES-MTA extracts showed an increase in calcium nodule formation compared to the AHP 15 days and control*	n.a.	Bioceramics sealers were cytocompatible, and allow cell spreading, adhesion and migration similar to control. In addition, induced mineralization.
Lee et al. 2019	AHP showed the lowest cell viability through all experimental periods among all of the tested sealers. The cell viability of AHP, Wellroot ST, and ES-MTA were significantly decreased by time*. In the 72 h extraction media, the cell viability of Wellroot ST was the highest at 3 days, and that of Nano-ceramic Sealer was increased for 7 days*.	Set surface of AHP and AD Seal discs did not show any cell attached due to the cell death. Wellroot ST showed the highest level of cell adhesion and the morphology of attached cells with well-spread and flattened.	n.a.	ALP activity showed increasing time-dependent deep-purple coloration in the three calcium silicate-based sealers. rtPCR: ALP mRNA expression showed a significant increase in time-dependent manner on all of three calcium silicate-based sealers*.	IL-1 and IL-10, and TNF alfa were not expressed in all experimental groups (AHP, ADSeal, ES-MTA, Nano-Ceramic Sealer and WellRoot-ST). AHP was the only to presented a higher expression of IL-6 and IL-8 than the other sealers*.	ES-MTA, Nano-ceramic Sealer and Wellroot ST appear to be less cytotoxic than epoxy resin-based sealers. Pro-inflammatory cytokines were not expressed in all experimental groups (AHP, ADSeal, ES-MTA, Nano-Ceramic Sealer and WellRoot-ST). Mineralization activity was time-dependent on all calcium-silicate-based sealers.

López-García et al. 2019a	MTT: 1:1 and 1:2 dilution of Bio-C Sealer produced a reduction in cellular viability after 72 h*. Flow Cytometry: The percentages of cell survival with undiluted extracts of BECs was similar to the control.	SEM: After 72 h, cells were well individualized, flattened, and spindle-like in shape, with multiple prolongations in the case of Bio-C Repair. However, in the Bio-C Sealer group, less elongated and spindle-shaped cells were found on the surface.	1:1 and 1:2 dilutions of Bio-C Sealer induced a significantly lower cell migration rate than observed in control*. However, Bio-C Repair promoted wound closure after 72 h, comparable to control.	n.a.	n.a.	Bio-C Sealer was cytocompatible, but allowed cell spreading, adhesion and migration in small levels than the control.
López-García et al. 2019b	Significant differences in cell viability were observed after incubation with the 1:2 of all materials compared to the control at 48 h. At 72 h, cell viability was higher in the dilutions of 1:2 and 1:4 of TF-BC compared to control*. Bio-C Sealer at 1:4 dilution showed cell viability similar to the control. Both TF-BC and Bio-C Sealer were less cytotoxic than AHP in all dilutions*.	SEM: hPLSCs seeded on TF-BC disks were well spread, with a predominant fibroblastic shape with multiple cytoplasmic extensions. With Bio-C Sealer, less elongated and spindle-shaped cells were found on the surface, and with AHP, there was reduction in density and spreading*. Bio-C Sealer showed irregular crystalline structures on the surface, whereas fewer particles were detected in TF-BC. The surface of AHP was homogeneous with few particles.	Cell migration rates in undiluted Bio-C Sealer and TF-BC were slightly lower in comparison to the control *. In the undiluted TF-BC, only at 24 h and undiluted group, were significant differences not found. Less cell migration were revealed in the 1:2 and 1:4 dilutions when compared with the control wound closure. At all times and dilutions, the AHP had less migration* and were unable to heal the wound when compared with the control wound closure rates.	AR Assay: TF-BC and Bio-C Sealer exhibited higher mineralized matrix formation than negative control*. With AHP group, no mineralization was detected	n.a.	Bioceramic sealers showed good cytocompatibility and allowed cell propagation, adhesion and migration when compared to the control; they showed higher mineralization than the control.

López-García et al. 2019c	Extracts of CS and ESQ-BC exhibited similar rates to the control. At 72 h, a increase in viability was observed with undiluted CS and ESQ-BC Sealer 1:2*, whereas all dilutions of ESQ-BC produced a reduction in cell viability after 24, 48, and 72 h of incubation*.	Cells adhering to all the material surfaces were detected after 72 h of culture. There was flattened cells with multiple prolongations proliferated on the surface of CS and ESQ-BC, whereas only few and rounded cells were detected on ESQ-BC surfaces.	After 24 and 48 h, wound healing had progressed in all the experimental conditions, except for a pronounced deceleration in cell migration in the case of ESQ-BC*. Treatments with 1:1 and 1:2 dilutions of ESQ-BC induced a higher cell migration at 72 h than the control *.	ESQ-BC, CS and OsteoDiff groups exhibited a higher level AR staining than the control after only after 21 days *. The highest mineralization was observed with the ESQ-BC compared with CS, OsteoDiff, and ESQ-BC*. No mineralization was detected in the ES group. RT-qPCR assays showed an early overexpression of ALP at 3 and 7 days in the presence of ESQ-BC and CS*.	n.a.	Bioceramics sealers were cytocompatible, and allow cell spreading, adhesion and migration similar to control. In addition, favored hPDLSC differentiation and mineralization.
Rodríguez-Lozano et al. 2019	Similar to the control, more than 90% of viable cells were obtained using undiluted extracts of GF-BS and GF-2 after 72 h. AHP and MTAF induced a significant decrease of cell viability compared to control (28.84% and 21.43% of living cells, respectively).	Phalloidin (red fluorescence) and DAPI: hPLSCs showed a gradual increase in growth over time, an extended morphology and a high content of Factin, reaching confluence after 72 h of culture. The cells treated with the undiluted extracts of GF-BS and GF-2 exhibited a similar organized and stretched stress fiber assembly. However, the cells treated with extracts of MTAF and AHP showed a reduction in cell numbers and a very low stretched stress fiber assembly. SEM: After 72 h of culture,	Different dilutions of GF-2 and GF-BS eluates for 24 h promoted a cell migration level similar to that observed in control. Treatment with extracts of GF-2 and GF-BS promoted wound closure in a concentration-dependent manner, comparable to that observed in control extracts*. However, after 48 h of incubation with AHP and MTAF, cell migration was significantly lower than in the control*.	MTAF and AHP were not analyzed. The expression of ALP was seen to be significantly upregulated when hPLSCs were exposed to Osteodiff medium (positive control)*. Finally, BSP expression was upregulated* when hPLSCs were exposed to GF-BS.	n.a.	Bioceramics sealers were cytocompatible, and allow cell spreading, adhesion and migration similar to control (except for MTAF, which was cytotoxic). In addition, induced mineralization (MTAF was not analyzed).

		hPLSCs showed a suitable degree of attachment and merged with each other to reach subconfluence in GF-BS and a moderate level of the same in GF-2. In contrast, AHP and MTAF cell attachment was limited, with abundant round cells appearing on the surface of the material.				
Gaudin & Peters 2020	In 2D cultures, the increase in the cell viability with BR-RCS was observed*. MTAF was strongly cytotoxic even at the lowest extract dilutions (1:1, 1:2, and 1:4), but the cytotoxic effect was reduced compared with the negative control at 1:8 extract. In 3D cell encapsulation with 0.25% PuraMatrix, BR-RCS and PR-ES showed an increase in the viability of hPLSCs at the lowest dilutions (1:1 and 1:2 and 1:1, respectively)*.	n.a.	hPLSCs proliferated in PuraMatrix when seeded at a density of 0.25–0.5 x10^6 cells/mL. In contrast, at the excessively high cell density (1x10^6 cells/mL), hPLSCs did not proliferate, and, instead, the cell population fell to around the same as the maximum 72 h data for the 0.5x10^6 cells/mL.	n.a.	Both MTAF and AHP caused a up-regulation of IL-6 and IL-8 compared with the negative control group*. BR-RCS greatly stimulated the release of IL-10 and, to a lesser degree, IL-4 by hPLSCs when compared with the negative control group*. PR-ES and MTAF exhibited a mild increase in IL-10 production, whereas AHP had no effect on IL-10 production by hPLSCs.	Bioceramics sealers showed a good cytocompatibility that were similar to control (except for MTAF, which was cytotoxic). BR-RCS and PR-ES did not induce proinflammatory cytokines and promoted anti-inflammatory cytokine secretion by PDLSCs
Jing et al. 2020	There were significant differences in cell proliferation among the groups from days 1 to 7*.	n.a.	n.a.	AR assay**:** There were significant differences among the groups*. Compared with negative control, all groups	Days 1 and 3: There were no significant differences for IL-6 secretion among the groups. Days 7 and 14: IL-6 levels were	The bioceramic sealer was cytocompatible compared to the control and mineralization was

	Cells exposed to BR-RCS showed the highest proliferation rate from days 1 to 5*, whereas cells exposed to AHP showed the lowest proliferation rate* from days 3 to 7. The proliferation of cells exposed to BR-RCS was comparable to that in the negative control group before day 5, but was significantly lower at day 7*.			showed more mineralized nodules*. AHP exposed cells showed more mineralized nodules than the BR-RCS and C Root groups*. ALP activity differed significantly among the groups* and was higher in the BR-RCS and C Root groups compared with the AHP*. rtPCR: mRNA expression levels of ALP, OCN, and DMP1 in hPLSCs were different among the groups after 7 days of osteogenic induction*. Day 7: BR-RCS-exposed cells exhibited the highest expression of OCN*. Compared with BR-RCS, C-Root-exposed cells displayed higher ALP and DMP1 mRNA levels*.	highest in the AHP group at day 7 and in the C Root group at day 14*. IL-8 levels also differed among the groups at days 7 and 14, with the highest levels in the BR-RCS group*.	induced showing a comparable osteogenic differentiation capacity. IL-6 and IL-8 levels were similar between control and BR-RCS.
Oh et al. 2020	In fresh media, AHP showed the lowest cell viability in all periods*. At day 7, cell viability of CS increased compared to control and ES-TCS*.	No cell adhesion was observed on the surface of AHP and several dead cells were observed. The surface of CS and ES-TCS showed well-adhered hPLSCs with the production of extracellular matrix and a high cell proliferation. By looking at the material surface next	n.a.	In ALP staining on day 3, all materials were not stained enough to evaluate the difference. On day 7, ALP staining and AR staining on day 14 showed that AHP was less stained than calcium silicate-based sealers. CS and	The expression of IL-6 and IL-8 were higher in AHP than in other sealers*, except for IL-6 in the setting media.	Bioceramic sealers showed good cytocompatibility and allowed cell propagation and adhesion. CS showed less cytotoxicity than the other materials before setting, and the ES-TCS showed

		to the cells in CS characteristic cubic particles can be identified. On the surfaces of CS and ES-TCS, honeycomb morphology or acicular spherule morphology was observed.		ES-TCS showed similar ALP and AR staining intensity to that of the positive control. On day 7, ALP expression was lower in AHP than in other materials*. In OCN expression on day 7, ES-TCS showed higher levels than those of other materials, and AHP showed a lower level than that of the control*. In RUNX2 expression on day 7, the ES-TCS showed a higher level than that of the control*.		better osteogenic potential than the other materials. The pro-inflammatory cytokine levels of bioceramic sealers was similar to the control.
Rodriguez-Lozano et al. 2020	The undiluted extracts of ESQ-BC-HiFlow and ESQ-BC increased cell viability than control at 24 h* whereas the AHP decreased cell viability *. At 48 and 72 h, AHP decreased cell proliferation *. With 1:2 dilution, ESQ-BC-HiFlow and ESQ-BC S increased cell viability at 24 h *. AHP decreased cell viability rates at all periods in this dilution. With dilution 1:4, no significant differences were found with the control	High degree of cells bonded and spreading through the surface of both ESQ-BC-HiFlow and ESQ-BC. The morphology of cells in contact with these materials suggested an active adhesion since multiple prolongations and a flattened morphology was observed. No cells attached to the surface of AHP were found.	In the ESQ-BC-HiFlow, only at 24 h in the undiluted group, significant differences were found*.	AR Assay: ESQ-BC-HiFlow, ES-BC and Osteodiff groups, produced more calcium deposits than the control only after 21 days *. The greatest mineralization was seen with the ESQ-BC compared with ESQ-BC-HiFlow and Osteodiff groups*. rtPCR: At day 7, ALP, and RUNX2 expression were higher in ESQ-BC Sealer and ESQ-BC Sealer HiFlow groups when compared to Osteodiff and control group*.	n.a.	Bioceramics sealers were cytocompatible, and allow cell spreading, adhesion and migration similar to control. In addition, they induced mineralization and had higher expression of RUNX2 and ALP than control after 7 days.

			and both the BC Sealers; AHP showed the same as for the previous dilutions.		

n.a.: not applicable; AHP: AH Plus; ALP: Phophatase Alcaline; AR Assay: Alizarin Red Assay; BECS: bioactive cements; BR: Bio Root; BR-BC: BioRoot BC; BR-RCS: Bio Root RCS; BSP: bone sialoprotein; CS: Ceraseal; DAPI: 4',6'-diamino-2-fenil-indol (fluorescent marker); DMP1: dentine matrix protein 1; DSPP: dentin sialophosphoprotein; ES-MTA: EndoSeal MTA; ES-TCS: Endoseal TCS; ESQ: EndoSequence; ESQ-BC: EndoSequence BC; GF-2: GuttaFlow 2; GF-BS: GuttaFlow Bioseal; hDFSC: human dental follicle stem cells; hDPSCs: human dental pulp stem cells; hPLSCs: human periodontal ligament stem cells; hTGSCS: human tooth germ stem cells; IL-1: interleukin 1; IL-4: interleukin 4; IL-6: interleukin 6; IL-8: interleukin 8; IL-10: interleukin 10; MTA: mineral trioxide aggregate; MTAA: MTA Angelus; MTAF: MTA Fillapex; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; OCN: Osteocalcin; PR: ProRoot; PR-ES: ProRoot ES; PKH26 KIT: PKH26 Red Fluorescent Cell Linker Kit for General Cell Membrane Labeling; rtPCR: Reverse transcription polymerase chain reaction; RUNX2: Runt-related transcription factor 2; SEM: scanning electron microscope; SRB Assay: Sulforhodamine B; TF: Total Fill; TF-BC: TotalFill BC; TNF: tumor necrosis factor.

* indicates statistical significance

Table 3. Results of cytotoxicity, ALP induction and mineralization nodules of the most evaluated bioceramic endodontic sealers

Material	Author	Methods	Results cytotoxicity (MTT or SRB or Alamar Blue) compared to control	Results ALP assay compared to control	Results AR assay compared to control
EndoSeal MTA	Collado-González et al. 2017a Deog-Gyu et al. 2019 Lee et al. 2019 López-García et al. 2019c	Extracts of the materials in contact with SCs: Collado-Gonzalez et al. 2017 (1:1, 1:2, 1:4), Lee et al. 2019 (1:4), López-García et al. 2019c (1:1, 1:2, 1:4) Disks in contact with SCs: Deog-Gyu et al. 2019	- Cytotoxic: Collado-González et al. 2017a, López-García et al. 2019c - Similar: Deog-Gyu et al. 2019, Lee et al. 2019 - Cytocompatible: none	- Induced: Lee et al. 2019 - Similar: none - Reduced: none - Not evaluated: Deog-Gyu et al. 2019, Collado-González et al. 2017a, López-García et al. 2019c	- Induced: none - Similar: Deog-Gyu et al. 2019, López-García et al. 2019c - Reduced: none - Not evaluated: Collado-González et al. 2017a, Lee et al. 2019
MTA Fillapex	Güven et al. 2013a Victoria-Escandell et al. 2017 Rodríguez-Lozano et al.	Extracts of the materials in contact with SCs: Victoria-Escandell et al. 2017 (1:1, 1:2), Rodríguez-Lozano et al. 2019 (1:1, 1:2, 1:4), Gaudin & Peters	- Cytotoxic: Gaudin & Peters 2020, Rodríguez-Lozano et al. 2019, Güven et al. 2013a, Rodríguez-Lozano et al. 2017b, Victoria-Escandell et al. 2017,	n.a.	n.a.

	2019 Gaudin & Peters 2020 Rodríguez-Lozano et al. 2017b Suciu et al. 2016	2020 (1:1, 1:2, 1:4, and 1:8), Rodríguez-Lozano et al. 2017b (1:1, 1:2, 1:4) Disks in contact with SCs: Güven et al. 2013a 2-well chamber slides: Suciu et al. 2016	Suciu et al. 2016 - Similar: none - Cytocompatible: none		
EndoSequence BC Sealer	Deog-Gyu et al. 2019 López-García et al. 2019c Rodríguez-Lozano et al. 2020	Extracts of the materials in contact with SCs: López-García et al. 2019c (1:1, 1:2, 1:4), Rodríguez-Lozano et al. 2020 (1:1, 1:2, 1:4) Disks in contact with SCs: Deog-Gyu et al. 2019	- Cytotoxic: none - Similar: Deog-Gyu et al. 2019, Rodríguez-Lozano et al. 2020, López-García et al. 2019c - Cytocompatible: none	- Induced: Rodríguez-Lozano et al. 2020 - Similar: López-García et al. 2019c - Reduced: none - Not evaluated: Deog-Gyu et al. 2019	- Induced: Rodríguez-Lozano et al. 2020, López-García et al. 2019c - Similar: Deog-Gyu et al. 2019 - Reduced: none
BioRoot RCS	Deog-Gyu et al. 2019 Gaudin & Peters 2020 Jing et al. 2020	Extracts of the materials in contact with SCs: Gaudin & Peters 2020 (1:1, 1:2, 1:4, and 1:8) Disks in contact with SCs: Deog-Gyu et al. 2019 Tooth model immersion into the cell culture: Jing et al. 2020	- Cytotoxic: none - Similar: Deog-Gyu et al. 2019, Jing et al. 2020 - Cytocompatible: Gaudin & Peters 2020	- Induced: Jing et al. 2020 - Similar: none - Reduced: none - Not evaluated: Deog-Gyu et al. 2019, Gaudin & Peters 2020	- Induced: Jing et al. 2020 - Similar: Deog-Gyu et al. 2019 - Reduced: none - Not evaluated: Gaudin & Peters 2020
Nano-ceramic sealer	Collado-González et al. 2017 Lee et al. 2019	Extracts of the materials in contact with SCs: Collado-Gonzalez et al. 2017 (1:1, 1:2, 1:4), Lee et al. 2019 (1:4)	- Cytotoxic: Collado-González et al. 2017 - Similar: none - Cytocompatible: Lee et al. 2019	- Induced: Lee et al. 2019 - Similar: none - Reduced: none - Not evaluated: Collado-González et	n.a.

				al. 2017	
Bio-C Sealer.	López-García et al. 2019a López-García et al. 2019b	Extracts of the materials in contact with SCs: López-García et al. 2019a, 2019b (1:1, 1:2, 1:4)	- Cytotoxic: López-García et al. 2019a - Similar: López-García et al. 2019b - Cytocompatible: none	n.a.	- Induced: López-García et al. 2019b - Similar: none - Reduced: none - Not evaluated: López-García et al. 2019a
iRoot SP	Guyen et al. 2013a Guyen et al. 2013b	Disks in contact with SCs: Güven et al. 2013a, 2013b (1:1)	- Cytotoxic: none - Similar: Guyen et al. 2013a, Guyen et al. 2013b - Cytocompatible: none	- Induced: Guyen et al. 2013b - Similar: none - Reduced: none - Not evaluated: Guyen et al. 2013a	n.a.

n.a.: not applicable; SC: stem cells; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; SRB Assay: Sulforhodamine B; ALP: Phophatase Alcaline; AR Assay: Alizarin Red Assay.

Table 4. Quality assessment of included *in vitro* studies

Quality criteria	Was the aim of the study clearly stated?	Was the sample size justified?	Was the assignment to treatment groups truly random?	Were control and treatment groups comparable at entry?	Were groups treated identically other than for the named interventions?	Were treatments/intervention protocols clearly described?	Were outcomes measured in the same way for all groups?	Were outcomes measured in a reliable way?	Was appropriate statistical analysis used?	Total score
Güven et al. 2013a	1	1	1	1	1	0	1	1	1	8
Güven et al. 2013b	1	0	1	1	1	0	1	1	1	7
Suciu et al. 2016	1	0	1	1	1	0	1	1	1	7
Collado-Gonzalez et al. 2017a	1	1	1	1	1	1	1	1	1	9
Collado-González et al. 2017b	1	1	1	1	1	1	1	1	1	9
Rodríguez-Lozano et al. 2017	1	1	1	1	1	0	1	1	1	8
Victoria-Escandell et al. 2017	1	0	1	1	1	0	1	1	1	7
Deog-Gyu et al. 2019	1	1	1	1	1	0	1	1	1	8
Lee et al. 2019	1	1	1	1	1	1	1	1	1	9
López-García et al. 2019a	1	1	1	1	1	1	1	1	1	9
López-García et al. 2019b	1	1	1	1	1	1	1	1	1	9
López-García et al. 2019c	1	1	1	1	1	1	1	1	1	9
Rodríguez-Lozano et al. 2019	1	1	1	1	1	1	1	1	1	9
Gaudin & Peters 2020	1	0	1	1	1	0	1	1	1	7
	1	1	1	1	1	0	1	1	1	9

Jing et al. 2020										
Oh et al. 2020	1	1	1	1	1	0	1	1	1	8
Rodriguez-Lozano et al. 2020	1	1	1	1	1	1	1	1	1	9

0, not reported or reported but inadequate; 1, reported and adequate

Discussion

Endodontic has been seeking for materials that present a range of characteristics, such as biocompatibility, antimicrobial effect, good apical sealing and that favor an improvement of the inflammatory response, disrupting infectious processes and finally, inducing periodontal healing (De Oliveira Mendes et al. 2003, Silva et al. 2016). Most of those biological researches over dental materials have used a variety of cell types, such as primary cell cultures from fibroblasts, odontoblasts, macrophages, L929 cells, V79 cells, among other cell types (De Oliveira Mendes et al. 2003, Galler et al. 2011, Lessa et al. 2010).

During the root therapy, materials may contact periapical tissues and, obviously, with periodontal stem cells (Johnson, Kulild & Tay, 2016, Victoria-Escandell et al., 2017). Therefore, this systematic review searched for the studies that analyzed the effects of endodontic materials on dental stem cells. In addition, changes in the mineralization effects and toxicity of materials were searched in the selected studies that describe these phenomena after stem cells were in contact with endodontic materials (Johnson, Kulild & Tay, 2016, Victoria-Escandell et al. 2017). Thus, this systematic review of pertinent literature shows a helpful tool to integrate such concepts and data.

More specifically, the focus of this review was on the effects of bioceramic materials on stem cells. It is observed that previous systematic reviews have focused on the properties of conventional sealers in various cell types (Fonseca et al. 2019, Oliveira et al. 2018, Silva et al. 2017, Donnermeye et al. 2018). However, this study only searches for bioceramic sealers and their effects on stem cells of dental origin.

The bioceramic technology has generated a number of biocompatible ceramic materials specifically designed for dental use (Güven et al. 2013a, Güven et al. 2013b, Suciu et al. 2016, Collado-Gonzalez et al. 2017a, Collado-Gonzalez et al. 2017b, Rodriguez-Lozano et al. 2017, Victoria-Escandell et al. 2017, Deog-Gyu et al. 2019, Lee et al. 2019, López-García et al. 2019a, López-García et al. 2019b, Rodriguez-Lozano et al. 2019, López-García et al. 2019c, Gaudin & Peters 2020, Jing et al. 2020, Oh et al. 2020, Rodriguez-Lozano et al. 2020). Several studies have shown the use of bioceramic sealers allow more conservative endodontic preparations, resulting in a significant decrease of inflammatory response, even if when material extravasation occurs during the filling process or root repair (López-García et al. 2019a, López-García et al. 2019b, Rodriguez-Lozano et al. 2019, López-García et al. 2019c, Gaudin & Peters 2020, Jing et al. 2020, Oh et al. 2020). Currently, some of the important bioceramic material properties are good interaction with dentin, biocompatibility, antibacterial activity and biomineralization.

In this systematic review, the studies included evaluated the cytotoxicity and biocompatibility of multiple bioceramic sealers. Among the human stem cells *in vitro* researches, the most studied sealers were the MTA Fillapex followed by EndoSeal MTA, BioRoot RCS, Bio-C Sealer, EndoSequence BC Sealer, Nano-ceramic Sealer, and iRoot SP.

This review unanimously suggested that MTA Fillapex is not a good sealer under in vitro cytotoxicity analyzes, presenting high cytotoxic potential (Güven et al. 2013a, Victoria-Escandell et al. 2017, Rodríguez-Lozano et al. 2019, Gaudin & Peters 2020, Rodríguez-Lozano et al. 2017b, Suciu et al. 2016). In agreement, the study that tested the effects of MTA Fillapex on macrophages (Braga et al. 2015) demonstrated similar findings. Moreover, this review is in line with previous systematic reviews (Silva et al. 2017, Donnermeye 2018, Oliveira

et al. 2018, Fonseca et al. 2019) concerning MTA Fillapex cytotoxicity. This systematic review also shows that the *in vitro* cytotoxicity method used by most of the selected studies to estimate cell survival was MTT assay.

The EndoSeal was a sealer analyzed in 4 of the 17 selected studies of this review. Controversial results were observed since, in two studies, it had cytotoxicity similar to the control (Deog-Gyu et al. 2019, Lee et. al2019), but in another study, it was considered cytotoxic (Collado-González et al. 2017a, López-García et al. 2019c). In general, the other sealers that were analyzed under the same parameters, EndoSequence BC Sealer, BioRoot RCS, iRoot SP, showed good biocompatibility (Güven et al. 2013a, Güven et al. 2013b, Deog-Gyu et al. 2019, López-García et al. 2019c, Gaudin & Peters 2020, Jing et al. 2020, Rodriguez-Lozano et al. 2020).

Cell viability was assayed in the reviewed studies, and some of them also evaluated factors such as favoring mineralization and induction or not of markers such as ALP. ALP is responsible for cell proliferation and cell renewal in bone tissue and acts on odontoblast cells to stimulate the proliferative process (Lopez-Garcia et al. 2019c). ALP expression also regulates cell viability, differentiation, deposition rate, composition, and morphology of the hydroxyapatite crystals formed by these cells (Lopez-Garcia et al. 2019c, Rodriguez-Lozano et al. 2019). It was observed that EndoSeal MTA, EndoSequence BC Sealer, BioRoot RCS, iRoot SP, and Nano-Ceramic Sealer increased ALP expression, as demonstrated in selected studies (Lee et al. 2019, Rodriguez-Lozano et al. 2020, Guven et al. 2013b, Jing et al. 2020). This increased expression could therefore favor cell differentiation and proliferative process.

It was shown that BioRoot RCS, EndoSequence BC Sealer, and Bio-C Sealer induced mineralization processes compared to the control cells without stimuli (Rodriguez-Lozano et al. 2020, Jing et al. 2020, Lópes-García et al. 2019b). In addition, Giacomino et al. (2019) has shown that EndoSequence BC can promote the deposition of mineralized tissue by osteoblast precursor cells.

Finally, it is essential to point out that this systematic review is a pioneer in analyzing the biological behavior of bioceramic endodontic materials in dental stem cells. Several studies have already compared the effects of endodontic materials over the most diverse types of cells, but none of them focus on bioceramics consequence over dental stem cells (Fonseca et al. 2019, Donnermeye et al. 2018, Oliveira et al. 2018, Silva et al. 2017).

Conclusion

From this systematic review, it is possible to infer that most of the bioceramic sealers analyzed in the selected studies are biocompatible and may be used satisfactorily in clinical practice. However, more studies should be performed to determine the impacts of these materials on stem cells. Furthermore, standardization of methodologies could reduce several factors that influenced the analyzes of the biocompatibility of the studied materials.

Acknowledgments

This work was supported by Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Pesquisa e Desenvolvimento Tecnológico CNPq) and Pró-Reitoria de Pesquisa da UFMG (PRPq). APRS and LQV are CNPq fellows. The authors deny any conflicts of interest related to this study.

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5 CONSIDERAÇÕES FINAIS

No presente estudo pôde-se observar que:

1) Quanto à citotoxicidade dos materiais *Endofill, Sealer 26, Pulp Canal Sealer* e *MTA:*

- Os cimentos *Endofill* e *Pulp Canal Sealer* se mostraram citotóxicos após 48 horas de cultivo. Este achado é de extrema importância para as células-tronco, pois é o momento em que as mesmas passam pelo período de duplicação da população celular (do inglês, *cell doubling*).
- Os cimentos *MTA* e *Sealer 26* não se mostraram citotóxicos nos tempos avaliados.

2) Quanto à plasticidade celular dos cimentos *Endofill, Sealer 26, Pulp Canal Sealer* e *MTA:*

- Todos os marcadores testados positivamente para os cimentos *Sealer 26* e *MTA* (*OCT4, NANOG, Nestin* e *CD105*) são expressos normalmente nas células-tronco mesenquimais;
- Todos os marcadores testados negativamente para os cimentos *Sealer 26* e *MTA* (*CD45 e CD34*) não são expressos normalmente nas células-tronco mesenquimais;
- Os cimentos *Endofill* e *Pulp Canal Sealer* não foram testados nessa etapa devido à ausência de volume celular suficiente para a extração do RNA durante o cultivo celular.

3) Quanto ao potencial de diferenciação celular dos cimentos *Endofill, Sealer 26, Pulp Canal Sealer* e *MTA*:

- Os cimentos *MTA* e *Sealer 26* se expressaram de maneira semelhante. Eles reduziram a expressão dos marcadores *DMP1, OC/BGLAP* e *RUNX2,* mas nao interferiram na expressão do gene *ALP*.

- Mais uma vez, os cimentos *Endofill* e *Pulp Canal Sealer* não foram testados devido à ausência de volume celular suficiente para a extração do RNA durante o cultivo celular.

4) Quanto ao comportamento biológico de cimentos biocerâmicos em células-tronco de origem dentária:

- Do ponto de vista biológico, a maioria dos cimentos biocerâmicos são materiais adequados para uso na prática clínica. Porém, mais estudos são necessários para determinar os impactos desses materiais nas células-tronco, principalmente no MTA Fillapex, que apresentou tendência à citotoxicidade direta.

O sucesso de um tratamento endodôntico envolve diversas etapas e a escolha correta de um cimento pode fazer toda diferença no prognóstico do mesmo. Um cimento ideal, requer diversas características, dentre elas ser biocompatível e não ser danoso aos componentes do organismo em que se mantém em contato.

Os resultados deste estudo sugerem ser de importância adequar a escolha do cimento aos seus parâmetros de biocompatibilidade, visto que, alguns cimentos demonstraram ser danosos as células-tronco. Como demonstrado, as céulas-tronco de origem dentária se mantém em contato com estes materiais e, graças a sua capacidade e ao seu potencial de auto renovação, proliferação e diferenciação celular serão fundamentais na cicatrização apical. Além disso, os resultados do presente trabalho demonstraram que estudos utilizando as células-tronco de

origem dentária na presença de materiais são uma ferramenta potente e eficaz na condução de trabalhos de citotoxidade, genotoxicidade e expressão gênica. Assim, deve-se enconrajar novos estudos com essa metodologia, pois foram observados diferentes comportamentos quando estes materiais foram testados em culturas de outros tipos celulares.

Finalmente, pode-se concluir que os cimentos *Endofill* e *Pulp Canal Sealer* impactaram negativamente a viabilidade das hDPSCs. Já os cimentos *MTA* e *Sealer 26* não interferiram na viabilidade celular e na expressão de marcadores envolvidos na plasticidade e diferenciação celular, exceto a expressão de *DMP1*, que está envolvida na diferenciação dos odontoblastos. Os resultados deste estudo demonstram que os cimentos *MTA* e *Sealer 26* são excelentes escolhas clínicas.

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ANEXO A – Aprovação do COEP-UFMG

UNIVERSIDADE FEDERAL DE MINAS GERAIS

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: CITOTOXICIDADE, GENOTOXICIDADE E EXPRESSÃO GÊNICA EM CÉLULAS-TRONCO DE POLPA DENTAL DE DENTES DECÍDUOS EXPOSTAS A CIMENTOS ENDODÔNTICOS

Pesquisador: Antônio Paulino Ribeiro Sobrinho

Área Temática:

Versão: 1

CAAE: 87712218.9.0000.5149

Instituição Proponente: UNIVERSIDADE FEDERAL DE MINAS GERAIS

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.690.495

Apresentação do Projeto:

Segundo os autores do presente Projeto "A odontologia vem, ao longo dos anos, na busca de materiais que sejam bem tolerados pelos tecidos apicais, capazes de favorecer um bom prognóstico após um tratamento e que apresentem efeito antimicrobiano (BRACKETT et al. 2012; CAMARGO et al. 2014; DIMITROVA-NAKOV et al. 2015; KAUR et al. 2015; SILVA et al. 2015; SILVA et al. 2016). Na endodontia, a obturação do canal radicular é de extrema importância para o sucesso de todo o tratamento já realizado, pois esta é responsável pelo selamento dos canais principais e acessórios, favorecendo o processo de reparo apical e periapical. O objetivo da obturação dos sistemas de canais radiculares é, portanto, tornar impermeável ou à prova de sistemas bacterianos de uma forma mais consistente possível o espaço produzido pelo preparo biomecânico. Assim, um cimento endodôntico, além de não citotóxico (ao menos a médio e longo prazo), deve ser biocompatível, permitindo o processo de reparação dos tecidos apicais circunvizinhos ao órgão dentário, favorecendo a eliminação bacteriana local e até mesmo estimulando a reparação tecidual (RABELO, 2012)".

"Alguns dos cimentos endodônticos mais utilizados na atualidade são o Endofill (Dentsply, Maillefer, Chile), um cimento à base de óxido de zinco e eugenol, Sealer 26 (Dentsply, Petrópolis, Brasil), à base de hidróxido de cálcio e óxido de bismuto, o MTA (Angelus, Paraná, Brasil), um

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cimento à base de MTA e o Pulp Canal Sealer (Kerr, Sybron Endo, USA), um cimento à base de óxido de zinco, óleo de cravo, eugenol e bálsamo do Canadá".

"Boa parte dos trabalhos que avaliam a citotoxicidade de cimentos endodônticos se baseia apenas em ensaios colorimétricos como o MTT, que sozinho gera risco de viés, pois se baseia na capacidade das células reduzirem metabolicamente o sal. Além disso, poucos são os estudos realizados hoje em células-tronco caracterizadas. Este estudo tem como objetivo investigar os efeitos causados pelo contato de alguns cimentos endodônticos (Endofill, Sealer 26, MTA e Pul Canal Sealer) no cultivo de células-tronco de polpa dentária provenientes de dentes decíduos esfoliados (SHED). Esta investigação será então, baseada em análise da citotoxicidade e viabilidade celular pela utilização dos ensaios de MTT e Trypan Blue. Estes ensaios serão associados à análise da expressão de alguns genes envolvidos nos processos de diferenciação de osteoblastos, mineralização óssea e osteogênese sendo eles ALP, RUNX2, BMP2, SPARC, COL1A1, DMP1 e DSPP por RT-PCR. Também será feito RT-PCR para analisar os genes associados à multipotência das células-tronco (NANOG, OCT4, CD105, CD90, CD73, CD45, CD34) e por fim, uma analise cromossômica também será executada para buscar possíveis alterações cromossômicas nas SHED após o contato com os cimentos endodônticos".

Objetivo da Pesquisa:

Uma das hipóteses testadas neste trabalho é que o MTA obterá os melhores resultados em relação à citotoxicidade, devido a suas excelentes características e baseado também em estudos prévios da literatura.

Objetivo Primário:

Este estudo tem por objetivo investigar os efeitos causados pelo contato dos cimentos Endofill, Sealer 26, MTA e Pulp Canal Sealer às células-tronco de polpa dental de dentes decíduos (SHED).

Objetivos Secundários:

-Análise da citotoxicidade e viabilidade celular pela utilização dos ensaios de MTT e Trypan Blue;

-Análise da expressão de alguns genes envolvidos nos processos de diferenciação de

osteoblastos, mineralização óssea e osteogênese sendo eles ALP, RUNX2, BMP2, SPARC, COL1A1, DMP1 e DSPP por RT-PCR;

-Analise por RT-PCR da expressão de genes associados à multipotência (NANOG, OCT4, CD105, CD90, CD73, CD45, CD34);

-Os biomateriais serão testados também por Citogenética, buscando possíveis alterações cromossômicas após o contato com os cimentos endodônticos.

Avaliação dos Riscos e Benefícios:
Em relação aos Riscos envolvidos no desenvolvimento da pesquisa, os proponentes do estudo declaram: "Para esta pesquisa, não teremos riscos".

Quanto aos Benefícios da pesquisa os autores enfatizam: "A influência do cimento obturador no resultado final do tratamento endodôntico é de fundamental importância e a busca por um material obturador ideal promove o surgimento de uma gama de cimentos obturadores no mercado. Porém, é de extrema importância a adequação desses materiais quanto ao seu potencial citotóxico, pois este é um fenômeno in vivo complexo que pode desencadear um amplo espectro de efeitos como a morte celular ou alterações funcionais. Além disso, analisar se estes cimentos causam alterações cromossômicas ou modificações na expressão de genes também é essencial para que estes possam ser preconizados".

Comentários e Considerações sobre a Pesquisa:
Pesquisa muito interessante e bastante pertinente para a área da Odontologia e Saúde Coletiva.

Este estudo tem por objetivo investigar os efeitos causados pelo contato dos cimentos endodônticos Endofill, Sealer 26, MTA e Pulp Canal Sealer às células tronco de polpa dental de dentes decíduos (SHED).

As células-tronco de polpa dental de dentes decíduos serão isoladas e caracterizadas em colaboração com o Biobanco Genetec do Departamento de Biologia (ICB) da Universidade Federal de Juiz de Fora, após aprovação do Comitê de Ética e Pesquisa (COEP-UFMG). Essas células serão descongeladas e cultivadas em placas contendo meio D-MEM F12 suplementado com 10% (v/v) de

SFB em atmosfera úmida, com 5% CO2 a 37°C.

Para a caracterização destas células, será realizado o RT-PCR para avaliar possíveis alterações nos genes de multipotência (NANOG, OCT4, CD105, CD90, CD73, CD45 e CD34) e a citogenética, para verificar se o número cromossômico está estável e normal, comprovando que a linhagem celular pode ser utilizada.

Após esta caracterização, os biomateriais endodônticos Endofill, Sealer 26, MTA e o Pulp Canal Sealer serão manipulados de acordo com os fabricantes e serão inseridos nas pontas de tubos capilares esterilizados previamente seccionados (grupo teste), de modo que seu contato com a suspensão celular possa ser padronizado. Tubos capilares vazios serão utilizados em culturas de controle.

Após o período de contato das SHED com os biomateriais, serão feitos dois ensaios para analisar viabilidade e proliferação celular. Após estes testes, o RT-PCR irá novamente analisar os genes de multipotência e avaliará também alguns genes envolvidos nos processos de diferenciação de osteoblastos, mineralização óssea e osteogênese sendo eles ALP, RUNX2, BMP2, SPARC, COL1A1, DMP1 e DSPP. Por fim, o ensaio citogenético será novamente realizado.

Considerações sobre os Termos de apresentação obrigatória:

Foram apresentados os seguintes documentos: Protocolo (projeto) de Pesquisa da Plataforma Brasil (PB), Folha de Rosto do projeto na PB, Projeto de Pesquisa Completo, Parecer Consubstanciado aprovado pelo Colegiado do PPG em Odontologia da Faculdade de Odontologia (FO) da UFMG em 15/12/2017, Parecer aprovado pela Câmara do Departamento de Odontologia Restauradora da FO da UFMG em 29/01/2018, documento assinado pelo Coordenador do estudo que apresenta a justificativa para dispensa do TCLE e Parecer emitido pelo CONEP/CNS (PARECER CONEP (BIOBANCO) Nº. 022/2015) em 02 de Junho de 2015, que aprova a Constituição do Biobanco GENÉTICA HUMANA E TERAPIA CELULAR (GENETEC) do Departamento de Biologia do Instituto de Ciências Biológicas da Universidade Federal de Juiz de Fora (UFJF).

1) Aspectos éticos envolvendo a utilização de células - tronco:

O presente estudo e' baseado na utilização de células-tronco de polpa dental de dentes decíduos (SHED) que serão isoladas e caracterizadas em colaboração com o Biobanco Genetec do

Departamento de Biologia da Universidade Federal de Juiz de Fora, após aprovação do Comitê de Ética e Pesquisa (COEP-UFMG).

De acordo com a Metodologia apresentada, ensaios in-vitro (MTT e coloração por Tripan Blue) e moleculares (analise por RT-PCR, da expressão de genes de multipotência e de reparo tecidual) serão realizados para analisar um possível efeito de citotoxidade e genotoxidade causado pelo contato dos cimentos endodônticos Endofill, Sealer 26, MTA e Pulp Canal Sealer nas células tronco de polpa-dental. Considera-se então, que na seção da Metodologia do projeto estão descritas de forma clara, as etapas e os objetivos de utilização das referidas células tronco, puramente para fins de pesquisa cientifica, e apenas para a realização do PRESENTE projeto de pesquisa.

Como mencionado acima, um dos documentos anexados à Plataforma Brasil e' o Parecer emitido pelo CONEP/CNS (PARECER CONEP (BIOBANCO) N°. 022/2015) em 02 de Junho de 2015, que aprova a Constituição do Biobanco GENÉTICA HUMANA E TERAPIA CELULAR (GENETEC) do Departamento de Biologia do Instituto de Ciências Biológicas da Universidade Federal de Juiz de Fora (UFJF) para utilização em pesquisas científicas.

Considera-se, dessa forma, que os aspectos éticos envolvendo o uso das células tronco isoladas de polpa dental de dentes decíduos (SHED), foram devidamente considerados pelos proponentes, e estão resguardados no desenvolvimento no presente estudo.

2) Solicitação de Dispensa do TCLE

Os proponentes do presente estudo solicitam por meio de documento anexado à Plataforma Brasil a dispensa de Aplicação do TCLE (Termo de Consentimento Livre e Esclarecido) em documento assinado pelo Coordenador do projeto em 15 de Maio de 2018. Nesse documento o Coordenador do estudo reporta sobre a impossibilidade de obtenção do TCLE dos sujeitos envolvidos no estudo, nesse caso, dos doadores de amostras biológicas relativas `as células-tronco de polpa dental de dentes decíduos, isoladas e mantidas no Biobanco Genetec do Instituto de Ciências Biológicas da UFJF. No mesmo documento o Coordenador do estudo declara por meio da assinatura do termo, o seu compromisso em "salvaguardar os direitos" dos referidos sujeitos (doadores).

Além disso, segundo o documento apresentado no item anterior (PARECER CONEP (BIOBANCO) N°. 022/2015) em 02 de Junho de 2015, que aprova a Constituição do Biobanco GENÉTICA HUMANA E

TERAPIA CELULAR (GENETEC), já haviam sido consideradas e analisadas todas as questões referentes aos TCLEs e TALEs relativos à coleta de matéria humano para a constituição do Biobanco em GENÉTICA HUMANA e TERAPIA CELULAR.

Portanto, após analise detalhada de todos os documentos do Projeto, considero suficiente a Justificativa apresentada para a dispensa do TCLE.

Recomendações:

Sou a favor, S.M.J., pela aprovação do presente Projeto de Pesquisa.

Conclusões ou Pendências e Lista de Inadequações:

Sou a favor, S.M.J., pela aprovação do presente Projeto de Pesquisa.

Considerações Finais a critério do CEP:

Tendo em vista a legislação vigente (Resolução CNS 466/12), o COEP-UFMG recomenda aos Pesquisadores: comunicar toda e qualquer alteração do projeto e do termo de consentimento via emenda na Plataforma Brasil, informar imediatamente qualquer evento adverso ocorrido durante o desenvolvimento da pesquisa (via documental encaminhada em papel), apresentar na forma de notificação relatórios parciais do andamento do mesmo a cada 06 (seis) meses e ao término da pesquisa encaminhar a este Comitê um sumário dos resultados do projeto (relatório final).

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Outros	87712218parecer.pdf	04/06/2018 16:21:29	Vivian Resende	Aceito
Outros	87712218parecer.pdf	04/06/2018 16:21:29	Vivian Resende	Aceito
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1093516.pdf	11/04/2018 07:55:05		Aceito
Outros	parecer.pdf	11/04/2018 07:53:30	Antônio Paulino Ribeiro Sobrinho	Aceito
Folha de Rosto	folhaderostoass.pdf	19/03/2018 15:59:26	Antônio Paulino Ribeiro Sobrinho	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	dispensatcle.pdf	15/03/2018 13:54:03	Antônio Paulino Ribeiro Sobrinho	Aceito

UNIVERSIDADE FEDERAL DE MINAS GERAIS

Continuação do Parecer: 2.690.495

Projeto Detalhado / Brochura Investigador	projeto.docx	13/03/2018 19:32:41	Antônio Paulino Ribeiro Sobrinho	Aceito
Declaração de Manuseio Material Biológico / Biorepositório / Biobanco	PARECERBIOBANCOGENETEC.pdf	13/03/2018 19:31:13	Antônio Paulino Ribeiro Sobrinho	Aceito
Outros	aprovacao.pdf	13/03/2018 19:28:44	Antônio Paulino Ribeiro Sobrinho	Aceito
Declaração de Instituição e Infraestrutura	aprovacaoufmg.pdf	13/03/2018 19:27:37	Antônio Paulino Ribeiro Sobrinho	Aceito
Orçamento	orcamento.xlsx	13/03/2018 19:24:30	Antônio Paulino Ribeiro Sobrinho	Aceito
Cronograma	cronograma.docx	13/03/2018 19:18:50	Antônio Paulino Ribeiro Sobrinho	Aceito
Outros	87712218aprovacao.pdf	04/06/2018 16:52:09	Vivian Resende	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

BELO HORIZONTE, 04 de Junho de 2018

Assinado por:
Vivian Resende
(Coordenador)

Endereço: Av. Presidente Antônio Carlos,6627 2º Ad SI 2005
Bairro: Unidade Administrativa II **CEP:** 31.270-901
UF: MG **Município:** BELO HORIZONTE
Telefone: (31)3409-4592 **E-mail:** coep@prpq.ufmg.br

ATIVIDADES DESENVOLVIDAS 2017-2021

ATIVIDADES DESENVOLVIDAS:

APRESENTAÇÃO DE TRABALHOS:

1. **OLIVEIRA, P. Y.**; LACERDA, M. F. L. ; GIRELLI, C. ; **OLIVEIRA, P. Y.** ; RIBEIRO SOBRINHO, A. P. . Citotoxicidade de cimentos endodônticos sobre célulastronco de polpa dental. 2020

2. **OLIVEIRA, P. Y.**; RESENDE, L. M. ; LACERDA, M. F. L. ; CARMO, A. M. R. ; MARANDUBA, C. M. C. . Proliferação de Células-Tronco da Polpa Dental com uso do Laser de Baixa Potência: um estudo *in vitro*. 2019

3. **OLIVEIRA, P. Y.**; RIBEIRO SOBRINHO, A. P. . Análise epidemiológica e avaliação da necessidade de tratamento endodôntico entre indígenas das etnias *Sataré-Mawé* e *Tikuna*. 2018.

4. **OLIVEIRA, P. Y.**; LACERDA, M. F. L. ; LIMA, C. O. ; GIRELLI, C. ; RIBEIRO SOBRINHO, A. P. . Resistência à fratura de restaurações cerâmicas indiretas cimentadas com diferentes cimentos. 2018.

5. **OLIVEIRA, P. Y.**; LACERDA, M. F. L. ; LIMA, C. O. ; GIRELLI, C. ; RIBEIRO SOBRINHO, A. P. . Estudo da anatomia interna em incisivos inferiores pelas técnicas radiográficas e diafanização. 2018. (Apresentação de Trabalho/Outra).

6. **OLIVEIRA, P. Y.**; CARMO, A. M. R. ; RESENDE, L. M. ; LACERDA, M. F. L. ; MARANDUBA, C. M. C. . Células-tronco Mesenquimais nos Tecidos Dentários:Perspectivas para Regeneração de Tecidos. 2017.

7. **OLIVEIRA, P. Y.**; LACERDA, M. F. L. . Estudo comparativo *in vitro* da localização do ápice radiográfico e do forame apical na raiz distal do primeiro molar inferior permanente. 2017.

8. **OLIVEIRA, P. Y.**; LACERDA, M. F. L. . Efetividade da terapia fotodinâmica na eliminação de *Enterococcus faecalis* no preparo de canais radiculares: uma revisão integrativa. 2017.

9. **OLIVEIRA, P. Y.**; LACERDA, M. F. L. . Presença do complexo vermelho em lesões perirradiculares: uma revisão integrativa. 2017.

RESUMOS PUBLICADOS EM ANAIS DE CONGRESSOS:

11.

1. **OLIVEIRA, P. Y.**; MARANDUBA, C. M. C. ; RIBEIRO SOBRINHO, A. P. . ESTUDO COMPARATIVO DA CITOTOXICIDADE DE CIMENTOS ENDODÔNTICOS SOBRE CÉLULAS-TRONCO DE POLPA DENTAL DE DENTES DECÍDUOS. In: 36ª Sociedade Brasileira de Pesquisa Odontológica, 2019, Campinas. 36ª Sociedade Brasileira de Pesquisa Odontológica, 2019.

2. OLIVEIRA, P. Y.; RIBEIRO SOBRINHO, A. P. . Análise epidemiológica e avaliação da necessidade de tratamento endodôntico entre indígenas das etnias Sataré-Mawé e Tikuna. In: 35ª Reunião Anual da Sociedade Brasileira de Pesquisa Odontológica - SBPqO, 2018, Campinas. Análise epidemiológica e avaliação da necessidade de tratamento endodôntico entre indígenas das etnias Sataré-Mawé e Tikuna, 2018.

3. OLIVEIRA, P. Y.; LACERDA, M. F. L. ; GIRELLI, C. ; COUTO, A. M. . Estudo comparativo in vitro da localização do ápice radiográfico e do forame apical na raiz distal do primeiro molar inferior permantente. In: VI semana da integração, ensino, pesquisa e extensão, 2018, Diamantina. 2018.

4. GUSMAO, D. F. T. ; **OLIVEIRA, P. Y.** ; LACERDA, M. F. L. . Revascularização Pulpar: Estudo de Caso Utilizando Tratamento Terapêutico Alternativo. In: Congresso Internacional de Odontologia do Rio de Janeiro, 2017, Rio de Janeiro. 2017.

5. OLIVEIRA, P. Y.; CARMO, A. M. R. ; RESENDE, L. M. ; LACERDA, M. F. L. ; MARANDUBA, C. M. C. . Células-tronco Mesenquimais nos Tecidos Dentários:Perspectivas para Regeneração de Tecidos. In: Congresso Internacional de Odontologia do Rio de Janeiro, 2017, Rio de Janeiro. 2017.

6. FARAGE, I. ; RESENDE, L. M. ; CARMO, A. M. R. ; **OLIVEIRA, P. Y.** . Proliferação de células-tronco mediante aplicação de laser de baixa intensidade. In: 34ª Reunião Anual da Sociedade Brasileira de Pesquisa Odontológica - SBPqO, 2017, Campinas. 2017.

7. OLIVEIRA, P. Y.; LACERDA, M. F. L. ; GIRELLI, C. . Estudo comparativo *in vitro* da localização do ápice radiográfico e do forame apical na raiz distal do primeiro molar inferior permanente. In: 20ª JORNADA ODONTOLÓGICA E 8 a ENCONTRO DE PESQUISA DA PUC MINAS, 2017, belo horizonte. 2017.

8. OLIVEIRA, P. Y.; LACERDA, M. F. L. . Presença do complexo vermelho em lesões perirradiculares. In: 20ª JORNADA ODONTOLÓGICA E 8º ENCONTRO DE PESQUISA DA PUC MINAS, 2017, Belo Horizonte. 2017.

9. OLIVEIRA, P. Y.; LACERDA, M. F. L. . Efetividade da terapia fotodinâmica na eliminação de Enterococcus faecalis durante o preparo biomecânico de canais radiculares. In: 20 a JORNADA ODONTOLÓGICA E 8º ENCONTRO DE PESQUISA DA PUC ? MINAS, 2017, Belo Horizonte. ANAIS DA 20ª JORNADA ODONTOLÓGICA E 8º ENCONTRO DE PESQUISA DA PUC MINAS ODONTOLOGIA: Um retrato da atualidade, 2017.

ARTIGOS PUBLICADOS:

1. OLIVEIRA, P. Y.; LACERDA, M. F. L. ; RESENDE, L. M. ; LIMA, C. O. ; MARANDUBA, C. M. C.; CARMO, A. M. R. Evaluation of the effects of low intensity laser in proliferation of dental pulp stem cells. Brazilian Journal of Development, v. 5, p. 33248-33260, 2019.

2. MOREIRA, V.; **OLIVEIRA, P. Y.** ; LIMA, C. O. ; LACERDA, M. F. L.; GIRELLI, C. Use of platelet rich plasma for endodontic revascularization. Brazilian Journal of Health Review, v. 1, p. 70-80, 2018.

3. SILVA, M. H. C. E. ; **OLIVEIRA, P. Y.** ; LACERDA, M. F. L. ; LIMA, C. O. ; GIRELLI, C. A importância da localização de canais radiculares durante o tratamento endodôntico. Brazilian Journal of Health Review, v. 2, p. 154-161, 2018.

4. GIRELLI, C. F. M.; SANTOS, L. L. L.; SANTOS, T. C.; CUNHA, L. A.; **OLIVEIRA, P. Y.;** LACERDA, M. F. L. S. Type iiib *dens in dente* endodontic retreatment: a case report. RSBO, 2020.

5. LIMA, C. O.; MAGALHÃES, L. T. A.; MARCELIANO-ALVES, M. F.; **OLIVEIRA, P. Y.;** LACERDA, M. F. L. S. Internal lower incisors morphology revealed by computer microtomography. ACTA Odontológica Latinoamericana, 2020.

LIVROS PUBLICADOS:

1. OLIVEIRA, P. Y.; LACERDA, M. F. L. ; GIRELLI, C. . A Localização Do Ápice Radiográfico E Do Forame Apical Em Molares. 1a. ed. Novas Edições Acadêmicas, 2018. 64p.

FORMAÇÃO COMPLEMENTAR:

1. OLIVEIRA, P. Y. Avaliadora "Ad Hoc" dos trabalhos submetidos e apresentados durante a VI Semana da Integração do Ensino, Pesquisa e Extensão da UFVJM.. 2018. Orientação de outra natureza - Universidade Federal dos Vales do Jequitinhonha e Mucuri - Campus JK.

2. Curso de Francês. Aliança Francesa. (Carga horária: 650h). 2009-2020.

3. Revisão Sistemática e Meta-análise. (Carga horária: 40h). Universidade Estadual de Campinas, UNICAMP, Brasil. 2020.

4. Odontologia para Pacientes com Necessidades Especiais. (Carga horária: 25h). Universidade Federal do Rio Grande do Sul, UFRGS, Brasil. 2019.

5. CURSO DE QUALIFICAÇÃO DO PROCESSO DE TRABALHO E GESTÃO DA ATENÇÃO PRIMÁRIA. (Carga horária: 60h). Universidade Federal do Rio Grande do Sul, UFRGS, Brasil. 2018.

6. CURSO DE EAD EM ESTOMATOLOGIA PARA CIRURGIÕES-DENTISTAS DA REDE PÚBLICA DE. (Carga horária: 60h). Universidade Federal do Rio Grande do Sul, UFRGS, Brasil. 2018.

7. Distúrbios alimentares, distúrbios do movimento e qualidade do sono. (Carga horária: 4h). Universidade Federal de Minas Gerais, UFMG, Brasil. 2018.

8. Odontologia baseada em evidências e pesquisa translacional. (Carga horária: 4h). Universidade Federal de Minas Gerais, UFMG, Brasil. 2018.

9. Introdução ao Texto Acadêmico. (Carga horária: 20h). Universidade Federal do Rio Grande do Sul, UFRGS, Brasil. 2018.

10. Iniquidades em Saúde. (Carga horária: 4h). Universidade Federal de Minas Gerais, UFMG, Brasil. 2018.

11. Cambridge English Level 1 Certificate in ESOL internacional (FCE). Cambridge Assessment English, CAE, Inglaterra. 2017.

12. Emergências medicas no consultório Odontológico. (Carga horária: 4h). ABO Juiz de Fora, ABO-JF, Brasil. 2017.

13. Cuidados Básicos com a Saúde Bucal de Pessoas Idosas. (Carga horária: 20h). Universidade Federal do Rio Grande do Sul, UFRGS, Brasil. 2017.

14. Módulo de Endodontia. (Carga horária: 30h). Congresso Internacional de Odontologia do Rio de Janeiro, CIORJ, Brasil. 2017.

15. Controle de Infecções em Serviços de Saúde. (Carga horária: 55h). The Internacional E-learning Association, IELA, Brasil. 2017.

16. Congresso Internacional de Endodontia CANAL 2017. (Carga horária: 30h). Congresso Internacional de Endodontia CANAL 2017, CANAL2017, Brasil. 2017.

17. Odontologia na Estratégia da Saúde da Família. (Carga horária: 20h). Instituto Politécnico de Ensino a Distância, iPED, Brasil. 2017.

18. 20ª Jornada Odontológica PUC Minas. (Carga horária: 28h). Pontifícia Universidade Católica de Minas Gerais, PUC Minas, Brasil. 2017.

19. Curso de Biossegurança na Prática Odontológica no Contexto da Pandemia. (Carga horária: 20h). Universidade Federal de Minas Gerais, UFMG, Brasil. 2020.

20. Como Organizar o Orçamento. (Carga horária: 12h). Fundação Getúlio Vargas, FGV, Brasil. 2020.

21. Neurociência Integrativa - Dor. (Carga horária: 5h). Universidade Federal do Rio Grande do Sul, UFRGS, Brasil 2020.

22. Neurociência Integrativa - Sinapses. (Carga horária: 8h). Universidade Federal do Rio Grande do Sul, UFRGS, Brasil. 2020.

23. Coronavírus e Iniquidades em saúde. (Carga horária: 30h). Universidade Federal do Rio Grande do Sul, UFRGS, Brasil. 2020.

24. Condução e Gabinete de Crises em Saúde. (Carga horária: 5h). Universidade Federal do Rio Grande do Sul, UFRGS, Brasil. 2020.

25. O papel dos dentistas no combate às Fake News sobre fluoretos e cárie den. (Carga horária: 2h). Universidade do Estado do Rio de Janeiro, UERJ, Brasil. 2020.

26. Diagnóstico e tratamento das lesões associadas a dentes inclusos. (Carga horária: 2h). Universidade do Estado do Rio de Janeiro, UERJ, Brasil. 2020.

27. Construindo uma marca pessoal autêntica. (Carga horária: 2h). Universidade do Estado do Rio de Janeiro, UERJ, Brasil. 2020.

28. Fundamentos estético-funcionais e possibilidades de tratamento com Harmoniz. (Carga horária: 2h). Universidade do Estado do Rio de Janeiro, UERJ, Brasil. 2020

29. É possível ser minimamente invasivo e maximamente efetivo em Endodontia?. (Carga horária: 2h). Universidade do Estado do Rio de Janeiro, UERJ, Brasil. 2020.

30. Fluoretos no controle da cárie em Odontopediatria. (Carga horária: 2h). Universidade do Estado do Rio de Janeiro, UERJ, Brasil. 2020.

31. I Jornada de Laser na Odontologia do Vale do Rio Doce. (Carga horária: 9h). Universidade Federal de Juiz de Fora, UFJF, Brasil. 2020.

32. Recobrimento Radicular. (Carga horária: 2h). Universidade do Estado do Rio de Janeiro, UERJ, Brasil. 2020.

33. Políticas de Equidade em Saúde e o Enfrentamento das Violências. (Carga horária: 75h). Universidade Federal do Rio Grande do Sul, UFRGS, Brasil. 2020.

34. Encontro Científico Virtual do Programa de Pós Graduação Stricto-Sensu da F. (Carga horária: 8h). Universidade do Estado do Rio de Janeiro, UERJ, Brasil. 2020.

35. Revisão Sistemática e Meta-análise. (Carga horária: 40h). Universidade Estadual de Campinas, UNICAMP, Brasil. 2020.

36. Cuidado em Saúde: Desenvolvendo Competências Relacionais para o Atendimento. (Carga horária: 16h). Universidade Federal do Rio Grande do Sul, UFRGS, Brasil. 2020.

37. O cuidado em saúde mental na Atenção Básica e a COVID-19. (Carga horária: 30h). Universidade Federal do Rio Grande do Sul, UFRGS, Brasil. 2020.

38. Cuidado em Saúde: Desenvolvendo Competências Relacionais para o Atendimento. (Carga horária: 16h). Universidade Federal do Rio Grande do Sul, UFRGS, Brasil. 2020.

39. Sorriso gengival: opções ortodônticas e cirúrgicas para tratamento. (Carga horária: 2h). Universidade do Estado do Rio de Janeiro, UERJ, Brasil. 2020.

40. O que prescrever para controlar a dor e edema em pós operatório de cirurgia. (Carga horária: 2h). Universidade do Estado do Rio de Janeiro, UERJ, Brasil. 2020.

41. MINI CURSO DE CÂNCER DE BOCA. (Carga horária: 2h). Universidade do Estado do Rio de Janeiro, UERJ, Brasil. 2020.

42. Gestão de Clínicas e Consultórios: o que você precisa saber. (Carga horária: 2h). Universidade do Estado do Rio de Janeiro, UERJ, Brasil. 2020.

43. Promoção, proteção e apoio à amamentação: contribuições do dentista. (Carga horária: 2h). Universidade do Estado do Rio de Janeiro, UERJ, Brasil. 2020.

44. L-PRF: Quais as indicações na Odontologia?. (Carga horária: 2h). Universidade do Estado do Rio de Janeiro, UERJ, Brasil. 2020.

45. O cuidado em saúde mental na Atenção Básica e a COVID-19. (Carga horária: 30h). Universidade Federal do Rio Grande do Sul, UFRGS, Brasil. 2020.

46. Novos conceitos em clareamento em dentes desvitalizados. (Carga horária: 2h). Universidade do Estado do Rio de Janeiro, UERJ, Brasil. 2020.

47. Neurociência Integrativa - Sistema Nervoso Motor. (Carga horária: 28h). Universidade Federal do Rio Grande do Sul, UFRGS, Brasil. 2021.

48. Saúde Mental, Direitos Humanos e Sistema Penal. (Carga horária: 24h). Universidade Federal do Rio Grande do Sul, UFRGS, Brasil. 2021.

49. HIV/AIDS e Zero Discriminação. (Carga horária: 30h). Universidade Federal do Rio Grande do Sul, UFRGS, Brasil. 2021.

50. Política Nacional de Saúde Integral de Lésbicas, Gays, Bissexuais, Travesti. (Carga horária: 30h). Universidade Federal do Rio Grande do Sul, UFRGS, Brasil. 2021.

51. Neurociência Integrativa - Reflexos Medulares. (Carga horária: 28h). Universidade Federal do Rio Grande do Sul, UFRGS, Brasil. 2021.

52. Itinerários terapêuticos, cuidado e cultura. (Carga horária: 20h). Universidade Federal do Rio Grande do Sul, UFRGS, Brasil. 2021.

53. Doenças Genéticas Raras na Atenção Primária à Saúde. (Carga horária: 60h). Universidade Federal do Rio Grande do Sul, UFRGS, Brasil. 2021.

54. Cuidados Paliativos, Saúde Bucal e Temas Associados. (Carga horária: 20h). Universidade Federal do Rio Grande do Sul, UFRGS, Brasil. 2021.