UNIVERSIDAD AUTÓNOMA DE SAN LUIS POTOSÍ

FACULTAD DE ESTOMATOLOGÍA DOCTORADO EN CIENCIAS ODONTOLÓGICAS

EFECTIVIDAD DE NANOPARTÍCULAS DE PLATA Y PERÓXIDO DE ZINC USADOS COMO MEDICACIÓN INTRACONDUCTO EN ENDODONCIA.

TESIS QUE PRESENTA:

IVAN OLIVARES ACOSTA

PARA OBTENER EL GRADO DE:

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DIRECTORA DE TESIS:

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CO-DIRECTOR:

DR. MARCO FELIPE SALAS OROZCO

SAN LUIS POTOSÍ, S.L.P. A 05 DE NOVIEMBRE DE 2024

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DR. FACUNDO RUIZ

DEDICATORIA

Al gran creador por permitirme llegar hasta aquí, a mi esposa y a mi hija, por su amor, apoyo y comprensión y a mis padres por siempre creer en mi.

Efectividad de nanopartículas de plata y peróxido de zinc en gel, usados como medicación intraconducto en endodoncia

Resumen

Introducción: El propósito de este estudio fue evaluar la actividad antimicrobiana del hidróxido de calcio, las nanopartículas de plata y el *peróxido* de zinc en gel mediante el uso de modelos de *dilución*, difusión y *microscopía* láser confocal.

Métodos: Se sintetizaron nanopartículas de plata mediante el método de reducción química, usando como agente aportador de plata nitrato de plata y como reductor citrato trisódico. Por su parte, las nanopartículas de *óxido* de zinc se sintetizaron usando la técnica *sol-gel*, empleando como agente aportador de zinc acetato de zinc y como reductor peróxido de hidrógeno. Se convirtieron ambas nanopartículas en geles utilizando *hidroxietilcelulosa*. Mediante un modelo de difusión usando *CromoAgar Orientador*, un modelo de dilución usando agar cerebro-corazón y un modelo de *microscopía* láser confocal, se observó la inhibición del crecimiento del *Enterococcus faecalis* ATCC 29212.

Resultados: En este estudio encontramos que las nanopartículas de *óxido* de zinc son más efectivas como medicación intraconducto que el hidróxido de calcio y las nanopartículas de plata como antimicrobianos en los modelos de difusión, *dilución* y *microscopía* láser confocal. Entre los grupos de hidróxido de calcio y nanopartículas de plata, las nanopartículas de plata resultaron ser las menos eficientes.

Conclusiones: Las nanopartículas de *óxido* de zinc fueron considerablemente más eficientes que el hidróxido de calcio y las nanopartículas de plata, por lo tanto, pueden ser consideradas como una opción de medicación intraconducto. Además de su capacidad bactericida, la forma en que se almacenan es ideal para su uso en endodoncia y su estabilidad con el paso del tiempo es excelente.

PRODUCCIÓN ACADEMICA

Asistencia a cursos y talleres				
Otorga	**Nombre del curso**	**Fecha**	**Estado/Pais**	**Semestre**
Duke University	Nanotechnology: a maker’s course	ago-20	Estados Unidos	Primer
Technical university of denmarck	Antimicrobial resistance-theory and methods	sep-20	Dinamarca	Primer
Universidad de Barcelona	Técnicas microscópicas de caracterización	oct-20	Barcelona	Primer
Universidad Autónoma de San Luis Potosí	Curso de Bioética	feb-21	San luis Potosí	Segundo
Instituto Politécnico de Paris	How to write and publish a Scientific Paper (Project-Centered Course)	feb-21	París, Francia	Segundo
Johns Hopkins University	Summary Statistics in Public Health	jun-21	Estados Unidos	Segundo
Asociación Mexicana de Microscopìa, A.C	Microscopía Electrónica en Ciencias Odontológicas	feb-21	Baja California	Segundo

Congresos nacionales e internacionales con y sin memoria							
Otorgo	**Titulo**	**Autores**	**Nombre**	**Fecha**	**Clave**	**Lugar /Pais**	**Semestre**
Universidad autónoma de Sinaloa	Inhibicion in vitro de E. Faecalis empleando cementos en endodoncia	Ofelia Candolfi Arballo , Ivan Olivares Acosta, Josue Armenta Molina	XIV encuentro internacional de cuerpos academicos y grupos de investigación en odontologia	abr-22		Sinaloa	Cuarto
Universidad Nacional Autónoma de México	Efectividad de nanopartículas de peróxido de zinc usadas como medicación intraconducto en endodoncia	Ivan Olivares Acosta, Lilian Beatriz Romero Sánchez, Gabriel Alejandro Martínez Castañón, Nuria Patiño Marín, Nereyda Niño Martínez, Jesús Ramón Castillo Hernández, Ofelia Candolfi Arballo	XXX Encuentro Nacional y XXI Iberoamericáno de Investigación en Odontología	nov-22		Ciudad de mexico	Quinto
SRM institute of science and technology	Antimicrobial activity of zinc peroxide nanoparticles in endodotics	Ivan Olivares Acosta	7th International Conference on Nanoscience and Nanotechnology (ICONN-2023)	mar-23		India	Sexto
Universidad Nacional Autónoma de México	Manejo integral de la osteonecrosis maxilar asociada a medicamentos. revisión bibliográfica.	Bryan Josue Rodriguez Osuna, Ivan Olivares Acosta	Congreso nacional e internacional UNAM AMIC	may-24		Ciudad de mexico	Octavo

Asistencia a congresos sin ponencia				
Otorga	**Nombre del congreso**	**Fecha**	**Lugar/país**	**Semes**
FEMFEO	Primer ciclo de conferencias de zona sur	ago-21	Ciudad de Mexico	Tercer
Universidad autónoma de Tlaxcala	XXXII congreso internacional de odontología	sep-21	Tlaxcala	Tercer
REDBIO	8vo congreso internacional de la REDBIO	oct-21	Queretaro	Tercer

Ponencia en curso				
Dirigido a	**Título de la ponencia**	**Fecha**	**Lugar/ País**	**Semestre**
Alumnos de 6to semestre	Inmunología aplicada a la odontología	nov-20	Baja California	Primer
Endodoncistas	Sistema rotarorio Protaper Next	ene-21	Baja California	Segundo
Odontopediatras	Sistemas rotatorios en odontopediatria	may-21	Baja California	Segundo
Odontopediatras	Sistemas rotatorios en odontopediatria	jul-21	Ciudad de México	Tercer
Cirujanos dentistas	Diagnóstico en endodoncia	may-22	Baja California	Cuarto
Endodoncistas	Sistema reciprocante wave one	nov-23	Baja California	Septimo

Distinciones o premios						
Otorga	**Autores**	**Título de la ponencia**	**Nombre del congreso**	**Lugar/ país**	**Lugar (premiación)**	**semestre**
Universidad autónoma de Sinaloa	Ofelia Candolfi Arballo, Ivan Olivares Acosta, Josue Armenta Molina	Inhibición in vitro de E. Faecalis empleando cementos en endodoncia	XIV encuentro internacional de cuerpos academicos y grupos de investigación en odontología	Sinaloa	Segundo lugar investigación basica	Cuarto

Aceptación de publicación de artículos científicos				
Nombre de la publicación	**Revista**	**Manuscrip ID**	**Lugar/pais**	**Semestre**
Inhibición in vitro del crecimiento de enterococcus faecalis empleando cementos para endodoncia	REVMEDUAS		Sinaloa	Quinto
Depression and opinion of dental students regarding the hybrid learning model during the COVID-19 pandemic	BMD Psychology	doi.org/10.1186/s40359-023-01157-8	Reino Unido	Sexto
Antibacterial Effect of Silver Nanoparticles on Polymicrobial Biofilms in Endodontics: Systematic Review and Meta Analysis	journal of nanotechnology	doi.org/10.1155/2024/6186520	Estados Unidos	Octavo

INDICE

1. INTRODUCCIÓN

El objetivo principal de la endodoncia **es** curar o prevenir la patología periapical (1). Las bacterias y sus bioproductos son el principal factor etiológico de la periodontitis apical (2,3), por lo tanto, los procesos de desinfección son primordiales para el éxito del tratamiento de conductos (4–6). Diversos materiales, sistemas y técnicas han sido desarrollados con el objetivo de lograr una óptima desinfección del sistema de conductos radiculares (SCR) (6–8).

La desinfección del SCR clásicamente se realiza con el uso de irrigantes como: NaOCl (5,8,9), Clorhexidina (4,6) y EDTA (4,10). Estos irrigantes suelen usarse en asociación con medicaciones intraconducto: $Ca(OH)_2$ (11–13), pastas triantibióticas (14,15) y pastas iodoformadas (16).

El $Ca(OH)_2$ ha sido usado durante mucho tiempo como la medicación intraconducto de elección para el tratamiento de conductos. Debido a su baja solubilidad, libera lentamente iones de calcio e hidroxilo, proporcionando un ambiente alcalino (pH 12) que le permite inhibir el crecimiento bacteriano (17).

Sin embargo, existen algunas preocupaciones con respecto al uso de $Ca(OH)_2$. El manejo y la colocación adecuada de $Ca(OH)_2$ presentan un desafío para cualquier odontólogo. Además, la eliminación de $Ca(OH)_2$ con frecuencia es incompleta, resultando en un residuo que cubre del 20% al 45% de las superficies de la pared del SCR. Incluso después de una irrigación activada sónica o ultrasónicamente con NaOCl y EDTA, el $Ca(OH)_2$ residual puede interferir con el proceso de obturación y comprometer la calidad del tratamiento (18). Una preocupación adicional es que el $Ca(OH)_2$ no es totalmente efectivo contra varios patógenos endodónticos, incluidos *E. faecalis* y *Candida albicans* (19). La capacidad del $Ca(OH)_2$ para erradicar completamente las bacterias del sistema de conductos radiculares ha sido cuestionada por varios estudios, y un estudio clínico ha demostrado que el número de conductos con bacterias positivas en realidad aumentó después de la medicación con $Ca(OH)_2$ (20). Otros estudios también han indicado que el $Ca(OH)_2$ no podía eliminar bacterias de forma predecible o que los cultivos cambiaban de negativos a positivos después de la colocación de $Ca(OH)_2$ (21).

Debido a todas estas inquietudes, se ha explorado la posibilidad del uso de diversos materiales como pastas triantibióticas, iodoformadas y, recientemente, geles de nanopartículas. La nanotecnología ha incursionado en las diferentes áreas de la odontología. Las aplicaciones clínicas han tenido un impacto considerable: resinas (22–24), acrílicos para prótesis (25,26), recubrimiento de implantes (27,28) e injertos óseos (29) son solo algunos ejemplos. En el área de endodoncia, Kishen et al. (30) en el 2008 fue el primero en proponer el uso de nanopartículas catódicas, específicamente de quitosano y óxido de zinc, como agentes antibacterianos. Las nanopartículas han sido aplicadas en selladores (31–47), irrigantes (48–62) y medicación intraconducto (43,63–73).

Nanopartículas de plata (36,39,41,55,57,59,61–66,70,74–82), quitosano (33,38,43,44,46,48,49,56,67–69,83–90), óxido de zinc (31,35,59,72,91,92), óxido de magnesio (60), óxido de hierro (48,56), vanadato de plata (36,93), zinc (34), fosfato de calcio amorfo (32), silicato de calcio mesoporoso (34) y nanopartículas poliméricas (71,94–96) han sido usadas en endodoncia por el potencial bactericida que presentan. Algunas de ellas han sido cargadas (31), recubiertas (78) y fotoactivadas (90,97,98) con el objetivo de hacer más eficiente su actividad antimicrobiana.

1.1 Nanopartículas de plata (AgNP)

Las partículas metálicas nanométricas son únicas y pueden cambiar considerablemente las características físicas, químicas y biológicas debido a su relación superficie-volumen. Debido a estas propiedades particulares, se han utilizado para varias aplicaciones, incluidas agentes antibacterianos, recubrimientos de dispositivos médicos, sensores ópticos y fármacos (99), y recientemente en el área de la odontología. Las nanopartículas de plata fueron las primeras en ser usadas como irrigantes en endodoncia en el año 2010 (50), y a partir de ahí han sido las más estudiadas y aplicadas en procesos de desinfección (59,100) e inhibición de crecimiento bacteriano (36,66).

1.1.1 Mecanismos de Acción (Figura 1)

Los mecanismos de acción de las AgNP sobre los microorganismos no han sido totalmente dilucidados, sin embargo, se consideran estos 3 mecanismos como los más aceptados:

1. **Interacción electrostática que conduce a la alteración de la membrana celular.** Se produce una atracción entre las cargas positivas y negativas de las nanopartículas y las superficies de las bacterias, respectivamente. Las AgNP se acumulan en la superficie de las bacterias, y esto les permite unirse de forma muy eficiente a la membrana, lo que produce una alteración de la integridad de la pared bacteriana, aumentando así la permeabilidad de esta y produciendo lisis (101).

2. **Alteración de proteínas, ADN y enzimas.** Las AgNP tienen una interacción con los grupos sulfúricos y fósforo de proteínas y ADN, alterando su estructura y funciones. Además, forman carbonilos unidos a proteínas por la presencia de AgNP, lo que cataliza el proceso oxidativo de la cadena de aminoácidos, degradando la proteína e inactivando enzimas (102).

3. **Liberación de iones.** La liberación de iones es dependiente del ritmo de disociación y se regula por la agregación, reacciones de oxidación-reducción, adsorción de biomacromoléculas y transformaciones mediadas biológicamente (54).

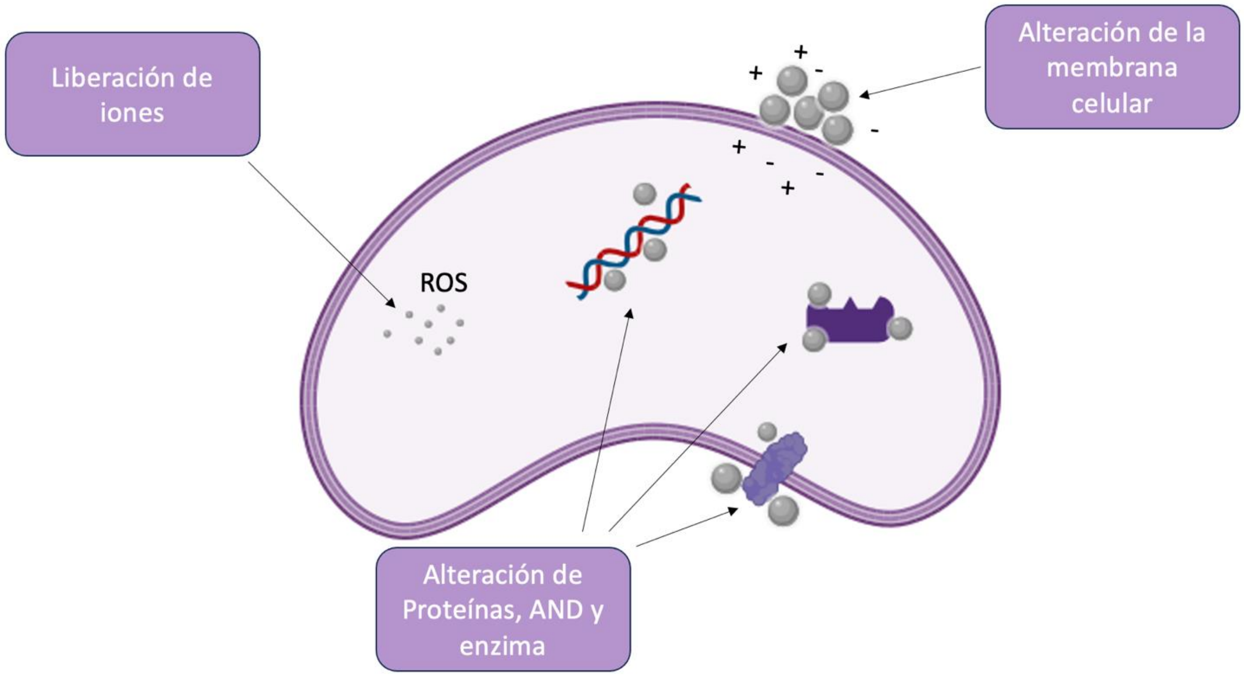


Figura 1. Mecanismos de acción de las nanopartículas de plata

1.2 Nanopartículas de Peróxido de Zinc (ZnO_2NP)

Las nanopartículas de peróxido de zinc poseen buena estabilidad química, baja toxicidad para las células humanas y actividad antimicrobiana, liberando derivados del oxígeno como peróxido de hidrógeno, radicales hidroxilo o radicales de oxígeno, conocidos como especies reactivas de oxígeno (ROS) (103).

Las nanopartículas de peróxido de zinc **hasta** la fecha no han sido probadas como antimicrobianos en endodoncia.

1.2.1 Mecanismos de acción (Figura 2)

1. Alteración de aminoácidos bacterianos
2. Modificación del transporte de sustrato metabólico
3. Inhibición de las actividades enzimáticas bacterianas al oxidar los grupos sulfhidrilo de los aminoácidos
4. Bloqueo de las transcripciones de ARN y la síntesis de ADN (104).

2. METODOLOGÍA

2.1. Síntesis de AgNP

Las AgNP se sintetizaron mediante el método de reducción química descrito por Mariam et al. (105). Cien mL de $AgNO_3$ (7761-88-8, Sigma-Aldrich) 0.1 M se calentaron a 80 °C y 150 rpm, mientras se añadían gota a gota 10 mL de $Na_3C_6H_5O_7 \cdot 2H_2O$ (6132-04-3, Merck) al 1 % m/v, hasta que la solución, inicialmente incolora, adquirió un tono amarillo.

2.2. Síntesis de ZnO_2NP

Para la síntesis de ZnO_2NP se utilizó la metodología sol-gel propuesta por De León-Ramírez et al. (106). Se diluyeron 5 mL de H_2O_2 (7722-84-10, Merck) al 30 % en 50 mL de H_2O destilada y se mantuvo en agitación mientras se agregaba 1 g de $Zn(CH_3COO)_2 \cdot 2H_2O$ (5970-45-6, Sigma-Aldrich). Se obtuvo un sol homogéneo y transparente, el cual se transfirió a una caja Petri para su secado a 37 °C durante 48 h.

2.3. Caracterización de las nanopartículas

Los espectros infrarrojos por transformada de Fourier (FT-IR) se recolectaron en una configuración de reflectancia total atenuada (ATR) en el rango de 4000 a 400 cm^{-1}, utilizando un espectrofotómetro DR-5000. Los espectros de ultravioleta-visible (UV-Vis) se realizaron en un espectrofotómetro DR-5000TM Hach en un intervalo de 200 a 800 nm. Se utilizó un instrumento Zetasizer Nano S DTS1060 Malvern para determinar el tamaño y la carga superficial de las nanopartículas sintetizadas. La microscopía electrónica de transmisión (TEM) se realizó en un microscopio Hitachi H7500 con filamento de LaB_6 operado a 80 kV.

2.4. Elaboración de geles

Siguiendo el protocolo de Bruneira et al. (66), se prepararon geles al 1.5 % m/v de hidroxietilcelulosa $(C_2H_6O_2)n$ (9904-62-0, Cellosize). El gel de AgNP se preparó mezclando 0.75 g de hidroxietilcelulosa con 50 mL de la solución de AgNP y se mezcló vigorosamente hasta obtener un gel homogéneo y fluido. Se obtuvo una solución homogénea. El gel de ZnO_2NP se preparó disolviendo 2 mg de nanopartículas en 50 mL de agua destilada, a la cual se le añadieron 0.75 g de hidroxietilcelulosa.

2.5. Pruebas de susceptibilidad antimicrobiana

Se llevaron a cabo dos pruebas para evaluar la actividad antibacteriana de las nanopartículas. La primera prueba consistió en el método de difusión en discos de agar, donde la zona de inhibición determina el grado de actividad antibacteriana. Para la segunda prueba se utilizó la técnica de dilución, la cual se sigue a través de la absorbancia.

2.5.1. Microorganismo usado

Se utilizó la cepa bacteriana *Enterococcus faecalis* (ATCC® 29212™), cultivada en cajas de Petri en agar soya tripticasa (Becton Dickinson) a 37 °C por 24 h en incubadora (VWR international model 1545). Se seleccionaron 5 colonias y se suspendieron en caldo Muller-Hinton (Becton Dickinson), para obtener una suspensión bacteriana inicial de 0.5 McFarland. Se evaluaron las propiedades del gel de AgNP y de ZnO_2NP. Se utilizó como control positivo una pasta comercial de $Ca(OH)_2$ al 35 % (Ultracal xs, Ultradent).

2.5.2. Difusión en disco

Las pruebas se realizaron por el método de difusión en disco en agar cromo orientador (CHROMagar) con la técnica de Kirby-Bauer (33–35). En el ensayo se utilizaron discos de papel filtro de 6 mm impregnados en (1) gel de AgNP, (2) gel de ZnO_2NP y (3) pasta de $Ca(OH)_2$. Los discos fueron impregnados justo antes del experimento. Después de la impregnación, se dejaron secar los discos durante 5 min. Utilizando pinzas estériles, se depositaron 6 discos equidistantes, asegurando que estuvieran en contacto con la superficie del agar. Posteriormente, las placas se colocaron en incubación de forma invertida a 37 °C en atmósfera aeróbica por 24 h. Finalmente, se determinó la zona de completa inhibición del crecimiento bacteriano utilizando un Vernier. La determinación se realizó colocando una superficie oscura bajo luz reflejada sobre el respaldo de la caja de Petri, sin remover la tapa. El punto final de inhibición completa del crecimiento se estimó a simple vista.

2.5.3. Dilución

Las pruebas de dilución se realizaron en tubos de vidrio estériles de 10 mL, a los cuales se les agregaron 3 mL de caldo Muller-Hinton (Becton Dickinson), 4 µL de la solución bacteriana y 75 mg de (1) gel de AgNP, (2) gel de ZnO_2NP y (3) pasta de $Ca(OH)_2$. Se incubaron por 7 días en agitación constante a 150 rpm a 37 °C en atmósfera aeróbica. Se midió la absorbancia a 650 nm con el equipo Thermospectronic Genesys 5. El control negativo fue caldo de cultivo, y el control positivo fue caldo de cultivo más solución bacteriana.

2.6. Experimentos ex vivo

En este estudio se emplearon un total de 15 dientes extraídos unirradiculares como material de investigación. Este estudio recibió la aprobación del comité de ética de investigación y evaluación de posgrado (CEIP) de la Universidad Autónoma de Baja California (UABC) - Facultad de Ciencias de la Salud (Oficio No. 072/2023-1). Cada diente fue seccionado horizontalmente a 1 mm por debajo de la unión amelocementaria y 2 mm del ápice, utilizando una fresa TF-13 (Manni) con el fin de obtener bloques de dentina de 4 mm. Cada bloque de dentina se dividió en dos mitades semicilíndricas. Posteriormente, cada muestra fue sometida a un tratamiento secuencial, primero con NaOCl (Cloralex) al 5.25% durante 4 min, seguido de EDTA (Zeyco) al 17% durante un período adicional de 4 min. Este procedimiento se llevó a cabo utilizando un baño ultrasónico con el objetivo de eliminar la capa de barro dentinario. Seguidamente, las muestras fueron enjuagadas en agua esterilizada durante 10 min, asegurando la eliminación de cualquier residuo químico. Finalmente, las muestras fueron sometidas a un proceso de esterilización en un autoclave (Lorna) a una temperatura de 121 °C durante 20 min.

Para la infección de los cubos de dentina se utilizó la cepa bacteriana *Enterococcus faecalis* (ATCC® 29212™). Primero se preparó y ajustó una solución bacteriana inicial de 0.5 McFarland en infusión cerebro-corazón (BHI). Posteriormente, se mezclaron 2 mL de BHI, 500 µL de la suspensión bacteriana y el cubo de dentina, utilizando una placa Olympus de 24 pocillos (Genesee Scientific) con capacidad de 3.5 mL. Las muestras se incubaron a 37 °C durante 4 semanas, cambiando el medio cada 48 h para asegurar la viabilidad bacteriana. Finalmente, se tomaron dos muestras y se analizaron con microscopía electrónica de barrido (SEM) para evaluar la formación de biopelículas y la infección bacteriana de los túbulos dentinarios. Las imágenes SEM se adquirieron con el microscopio electrónico de barrido (SEM) Hitachi SU300 a 7.00 kV.

Las biopelículas formadas en los cubos de dentina se tiñeron utilizando un kit de viabilidad bacteriana LIVE/DEAD (Biotium). Este kit proporciona tinción fluorescente de dos colores, tanto de bacterias vivas (verde) como de bacterias muertas (rojo), utilizando dos tintes de ADN, DMAO y EthD-III, respectivamente. Después de la tinción, las muestras se lavaron durante 1 min con *phosphate buffered saline* (PBS, ABI). Se tomaron imágenes de los cubos de dentina teñidos utilizando un microscopio multifotónico Olympus FV 1000. Cada muestra fue examinada y escaneada en áreas aleatorias. Se evaluaron 4 muestras para cada grupo. Se recogieron 20 imágenes en ubicaciones aleatorias de cada disco, lo que produjo 80 imágenes por grupo. El análisis de imagen y la creación de imágenes bidimensionales/tridimensionales se realizaron con el software ImageJ, National Institutes of Health.

3. ANÁLISIS ESTADÍSTICO

Las variables categóricas se informaron como frecuencias y porcentajes, mientras que las variables continuas se informaron como medias, desviaciones estándar (media ± desviaciones estándar) y límites. Se realizaron pruebas de Shapiro-Wilk y Brown-Forsythe para determinar la distribución de las variables. Se utilizó la prueba de Kruskal-Wallis y la prueba U de Mann-Whitney para establecer las diferencias entre grupos. Los datos se analizaron utilizando el software estadístico JMP ver. 15 (SAS Institute, Cary, Carolina del Norte). El nivel de significancia se fijó en $P<0.05$.

4. RESULTADOS

4.1 Caracterización de nanopartículas de plata

Las nanopartículas de plata preparadas en este trabajo tienen una morfología esférica y un tamaño de alrededor de 80 nm, con una distribución de tamaño estrecha, determinada mediante microscopía de transmisión (Figura 3A) y DLS (Figura 3B). La resonancia del plasmón superficial está ubicada a 420 nm (Figura 3C). La carga de la partícula fue de -8.08, dada por el potencial Z (Figura 3D), y los espectros FT-IR mostraron picos característicos a 714 cm⁻¹, 1643 cm⁻¹, 2098 cm⁻¹ y 3331 cm⁻¹ (Figura 3F).

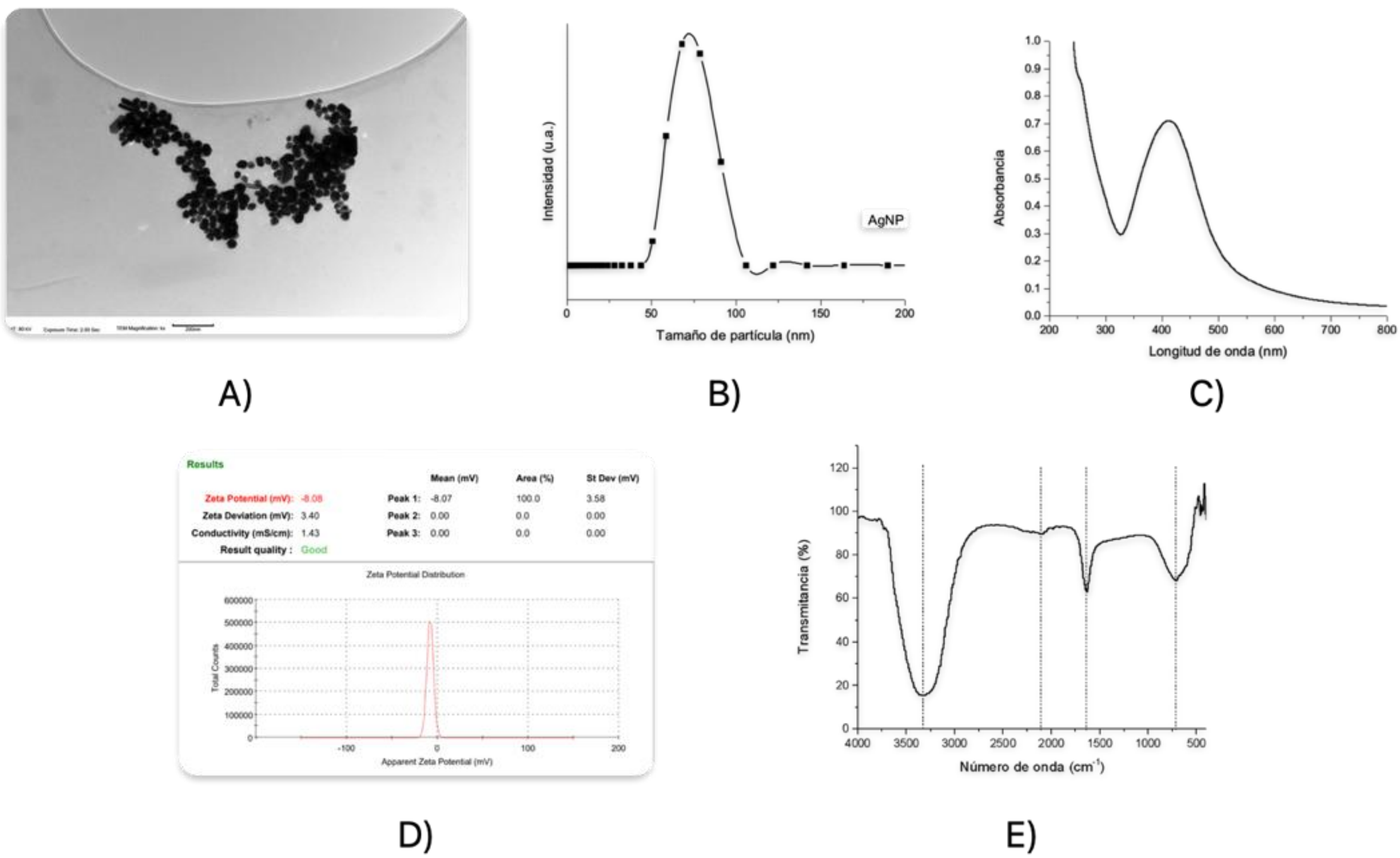


Figura 3. A) Imagen de microscopía electrónica de transmisión, B) DLS, C) Plasmón superficial, D) Potencial Z, E) FT-IR.

4.2 Caracterización de nanopartículas de peróxido de zinc

Las nanopartículas de peróxido de zinc preparadas en este trabajo tienen una morfología esférica y un tamaño de alrededor de 100 nm, con una distribución de tamaño estrecha, determinada mediante microscopía de transmisión (Figura 4A) y DLS (Figura 4B). La resonancia del plasmón superficial está ubicada a 210 nm (Figura 4C). La carga de la partícula fue de 18.4, determinada por el potencial Z (Figura 4D), y los espectros FT-IR mostraron picos característicos a 667 cm^{-1}, 1022 cm^{-1}, 1405 cm^{-1} y 1565 cm^{-1} (Figura 4E).

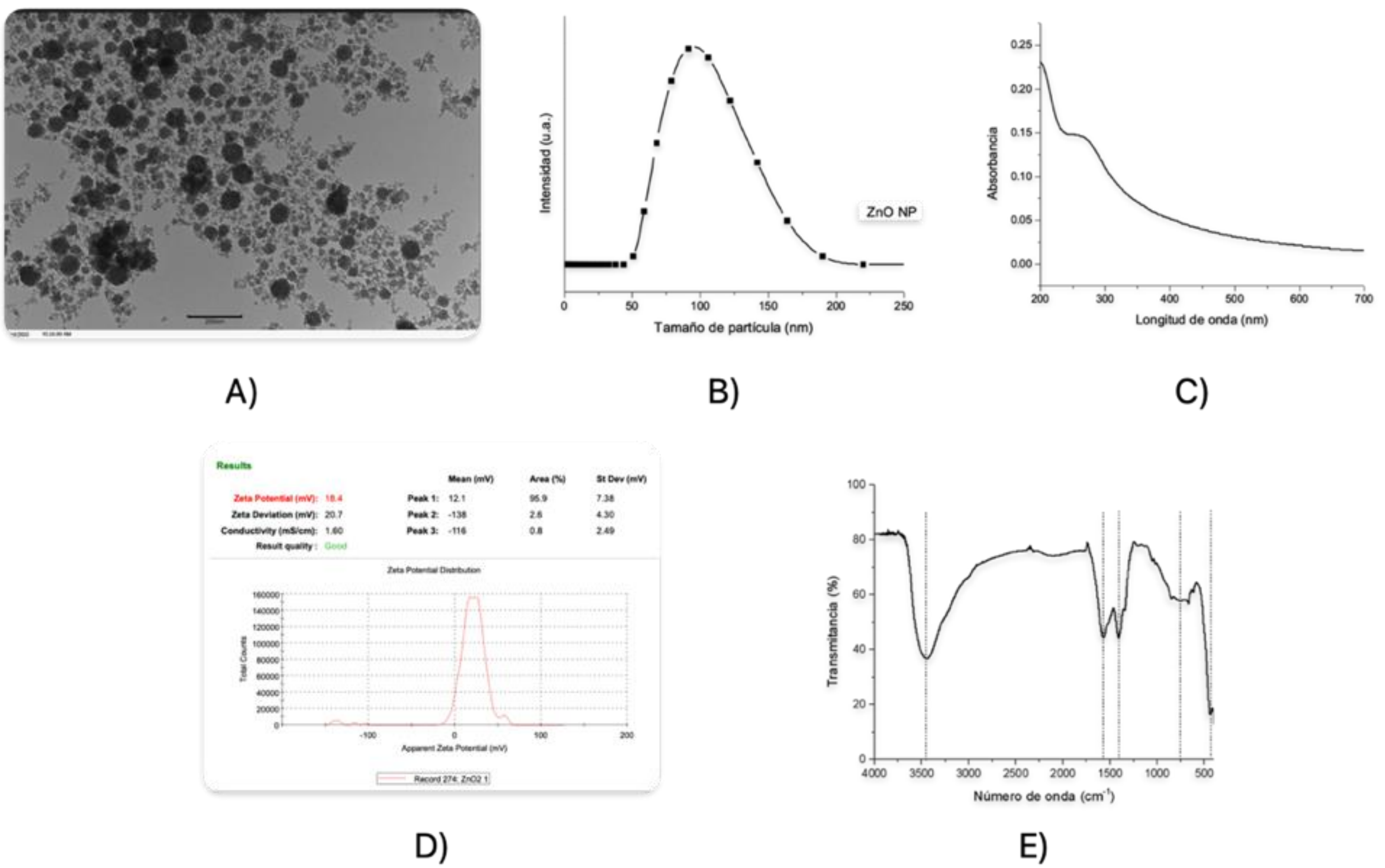


Figura 4. A) Imagen de microscopía electrónica de transmisión, B) DLS, C) Plasmón superficial, D) Potencial Z, E) FT-IR.

4.3 Prueba de ensayo antibacteriano en agar cromo-orientador

Se emplearon 3 medicaciones intraconducto: gel de AgNP, gel de ZnO_2NP y $Ca(OH)_2$. Se replicaron ensayos con un total de 28 repeticiones para cada uno de ellos, donde se midieron los halos de inhibición del crecimiento de la bacteria *Enterococcus faecalis* (ATCC® 29212™). La medición incluyó el diámetro del disco y el halo de inhibición que se observó desde el margen externo del disco que contenía el cemento, teniendo como punto final de medición la aparición de colonias de bacterias.

El gel de ZnO_2NP demostró un halo de inhibición del crecimiento bacteriano mayor, seguido por el hidróxido de calcio, mientras que el gel de AgNP mostró la menor inhibición del crecimiento bacteriano.

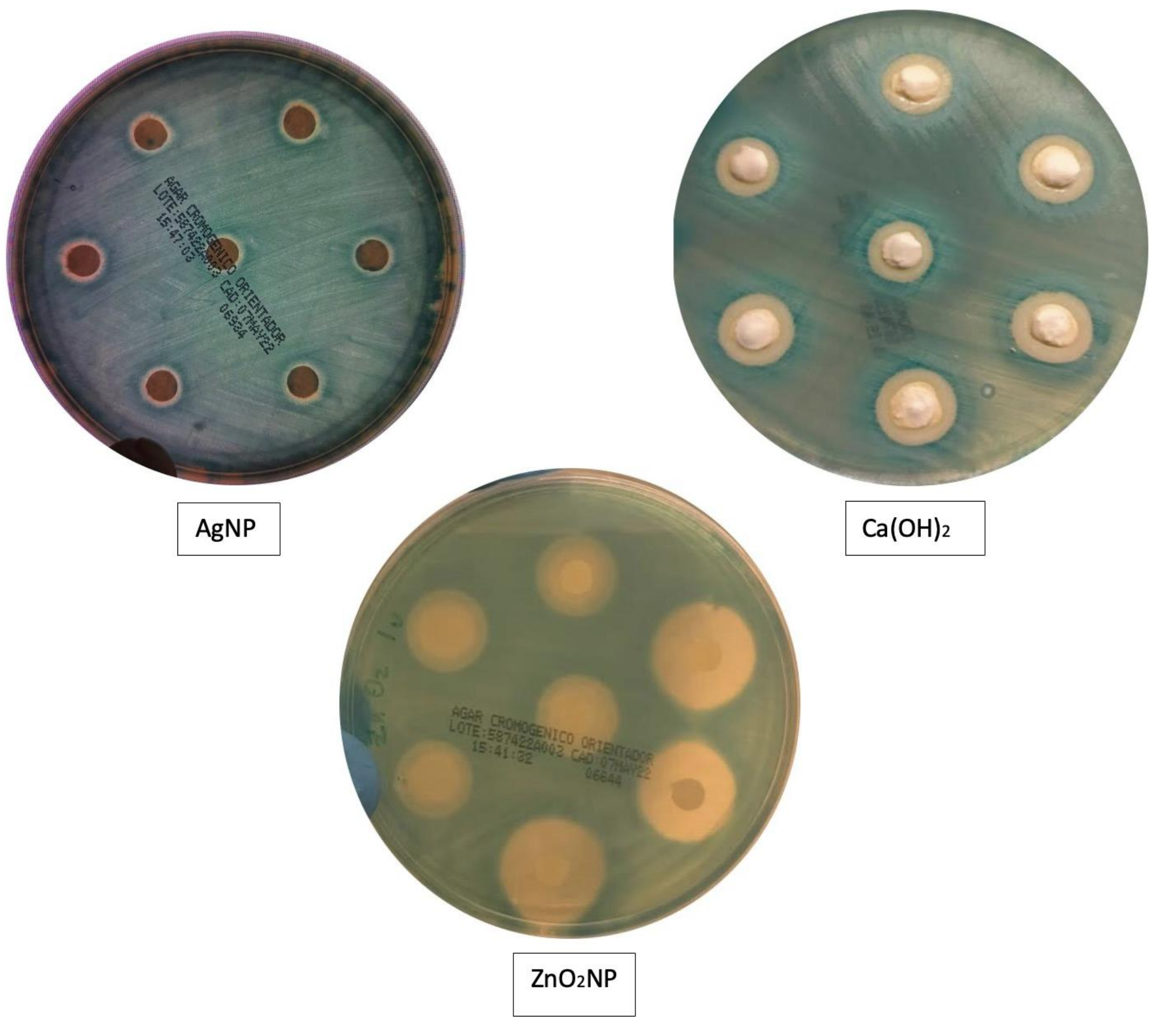
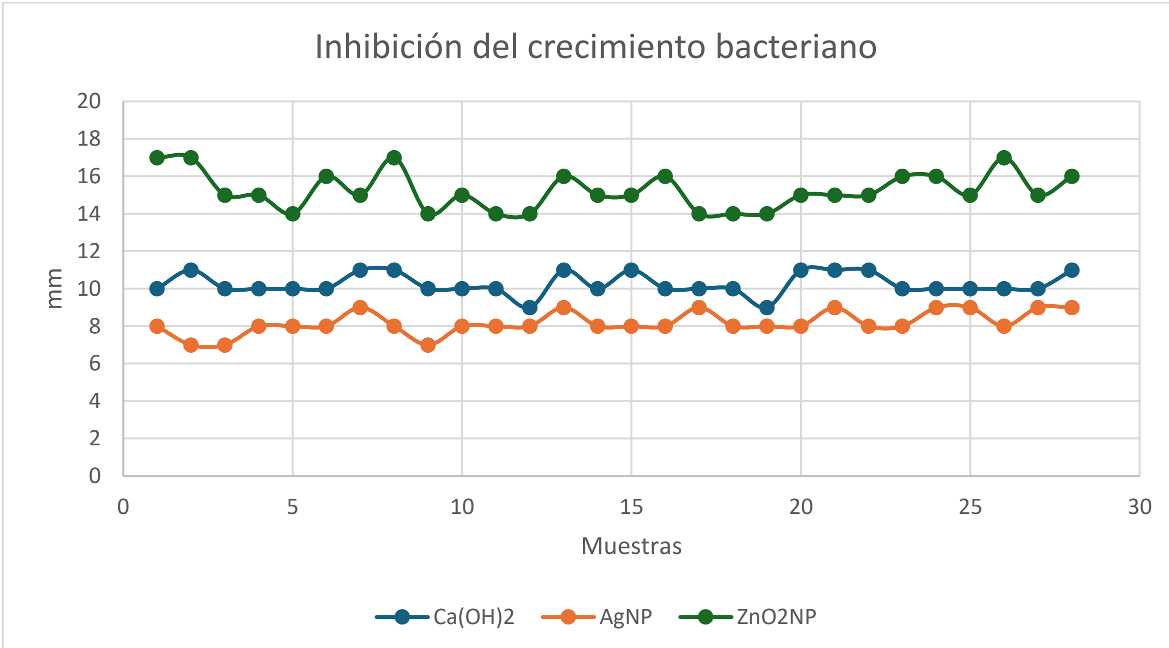


Figura 5. Inhibición del crecimiento bacteriano de AgNP, $Ca(OH)_2$ y ZnO_2NP en el modelo de agar cromo-orientador.



Gráfica 1. Inhibición del crecimiento bacteriano expresado en mm de $Ca(OH)_2$, AgNP y ZnO_2NP.

Tabla 1. Resultados de la prueba de difusión en disco.

Grupo	media (mm)	Desviación estandar (mm)	p-value
ZnO2NP	15.2143	1.0313	<0.001
$Ca(OH)_2$	10.2857	0.5998	
AgNP	8.1786	0.6118	

Comparación	p-value
AgNP vs $CaOH_2$	<0.001
AgNP vs ZnO_2NP	<0.001
$CaOH_2$ vs ZnO_2NP	<0.001

4.4 Prueba de ensayo antibacteriano en dilución

Se emplearon 3 medicaciones intraconducto: gel de AgNP, gel de ZnO_2NP y $Ca(OH)_2$. Se replicaron ensayos con un total de 20 repeticiones para cada uno de ellos, donde se midió la absorbancia de cada uno de los grupos y se determinó, de acuerdo a esta, el grado de inhibición del crecimiento bacteriano. Como control positivo se utilizó *E. faecalis* en medio agar soya tripticasa, y como control negativo se usó agar soya tripticasa. El gel de ZnO_2NP mostró una menor absorbancia, lo que se traduce en una mayor capacidad antibacteriana, seguido por el hidróxido de calcio. El gel de AgNP fue el menos eficiente. Además, se observó que la mayor concentración otorga mayor inhibición al material.

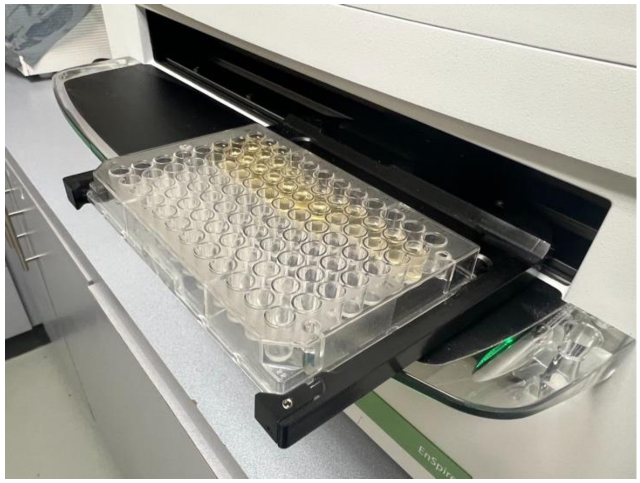
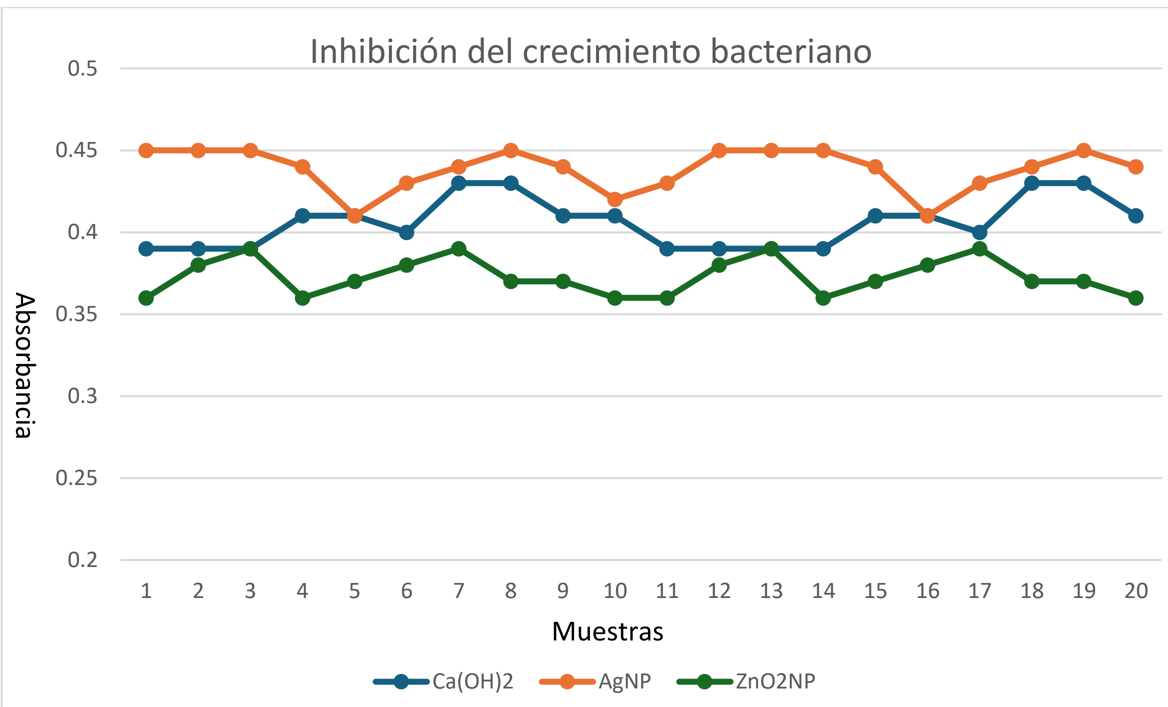


Figura 6. Lectura de absorbancia del modelo de difusión para determinar la inhibición del crecimiento bacteriano de *E. faecalis* en presencia de $Ca(OH)_2$, AgNP y ZnO_2NP.



Gráfica 2. Inhibición del crecimiento bacteriano medido en absorbancia de $Ca(OH)_2$, AgNP y ZnO_2NP.

Tabla 2. Resultados de la prueba de dilución.

Grupo	media (absorbancia)	Desviacion standar	p-value
ZnO2NP	0.37	0.01	<0.001
$Ca(OH)_2$	0.41	0.01	
AgNP	0.44	0.01	
Comparación		**p-value**	
AgNP vs $CaOH_2$		<0.001	
AgNP vs ZnO_2NP		<0.001	
$CaOH_2$ vs ZnO_2NP		<0.001	

Se emplearon 3 medicaciones intraconducto: gel de AgNP, gel de ZnO_2NP y $Ca(OH)_2$. Para el tratamiento con ZnO_2NP, la fluorescencia roja es prominente (después del tratamiento), lo que indica una gran cantidad de células muertas. Esto sugiere que estas nanopartículas matan eficazmente las bacterias de la biopelícula. En el caso del tratamiento con $Ca(OH)_2$, la fluorescencia roja también es significativa, lo que indica un número sustancial de células muertas. Esto demuestra que el hidróxido de calcio tiene efectos antimicrobianos notables, pero parece ser menos eficaz que las nanopartículas de peróxido de zinc. Por último, en el tratamiento con AgNP, la fluorescencia roja está presente, pero no es tan dominante, lo que sugiere que los AgNP tienen un efecto antimicrobiano menor en comparación con las nanopartículas de peróxido de zinc y el hidróxido de calcio. En resumen, los resultados de la tinción vivo/muerto resaltan que las nanopartículas de peróxido de zinc exhiben la actividad antimicrobiana más significativa, seguidas por el hidróxido de calcio, mientras que las AgNP muestran la menor efectividad antimicrobiana entre los tres tratamientos.

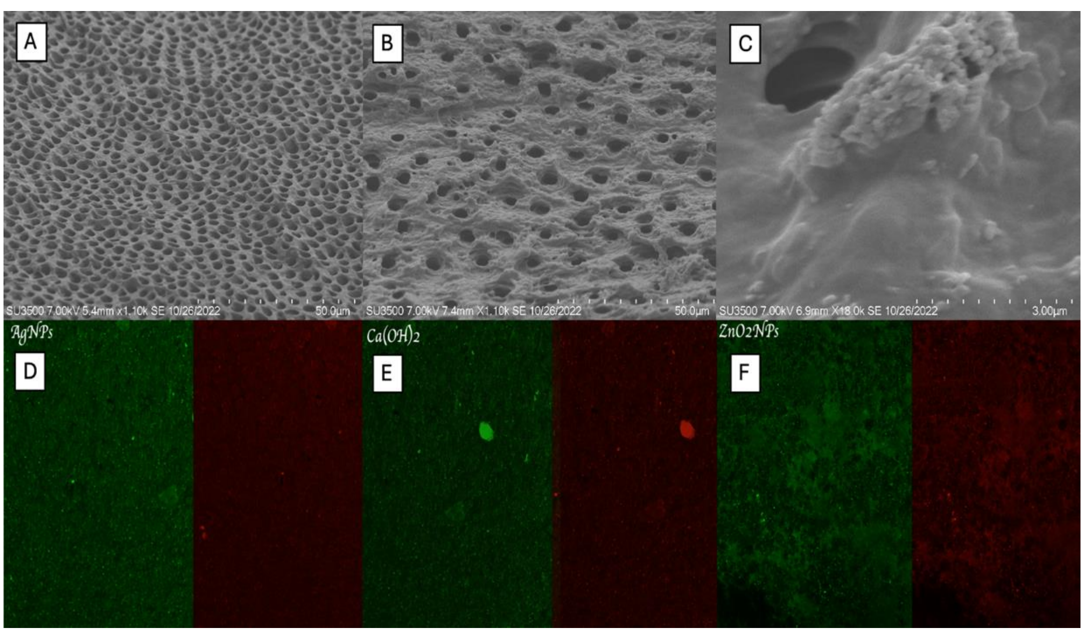


Figura 7. Caracterización y evaluación antimicrobiana de muestras tratadas. (A-C) Imágenes de microscopía electrónica de barrido (SEM) que muestran la morfología de la superficie de muestras de dentina. (A) Muestra de dentina limpia que presenta una estructura porosa. (B) Muestra de dentina con biopelícula de *E. faecalis* que muestra colonización bacteriana dentro de los túbulos. (C) Mayor aumento de la superficie de la dentina que revela la estructura de la biopelícula en los túbulos dentinarios. (D-F) Resultados de tinción vivo/muerto de muestras tratadas con biopelículas visualizadas mediante microscopía de fluorescencia.

5. **DISCUSIÓN**

En endodoncia, se han utilizado diversos materiales como medicación intraconducto con el fin de lograr una desinfección eficaz. Sin embargo, ninguno ha demostrado tener todas las propiedades ideales, tales como baja toxicidad, alta efectividad contra microorganismos resistentes, y facilidad para ser colocado y retirado del sistema de conductos radiculares (105). Esta búsqueda constante de materiales con estas características ha impulsado el uso de tecnologías avanzadas, entre ellas la nanotecnología, que incluye ZnO_2NP, ChNP, AgNP y nuestra propuesta de ZnO_2NP. Aunque el $Ca(OH)_2$ sigue siendo el tratamiento de referencia en endodoncia, en este estudio hemos comparado los efectos de geles de nanopartículas de peróxido de zinc y nanopartículas de plata con el hidróxido de calcio (106).

Los resultados mostraron que las nanopartículas de peróxido de zinc en gel son más eficaces como medicación intraconducto que el hidróxido de calcio y las nanopartículas de plata en los modelos de difusión, dilución y microscopía láser confocal. De los tres grupos, las nanopartículas de plata demostraron ser las menos eficientes. Este resultado podría estar relacionado con el tamaño de las nanopartículas, ya que algunos estudios sugieren que los tamaños más pequeños tienden a ser más efectivos como agentes bactericidas (en nuestro caso, las nanopartículas de plata tenían un tamaño de 80 nm) (107). Además, nuestras nanopartículas de plata tenían una carga negativa, y estudios previos han indicado que las nanopartículas de plata con carga positiva tienen mayor actividad antimicrobiana contra bacterias endodónticas (108).

Estas diferencias en los resultados no solo se deben al tamaño de las nanopartículas, sino también a factores como el método de síntesis, la carga y el tiempo de almacenamiento, ya que las nanopartículas de plata tienden a aglomerarse con el tiempo, lo que altera sus propiedades fisicoquímicas y, por ende, su eficacia antibacteriana (109). Otro aspecto importante es que los modelos experimentales no están estandarizados en el ámbito de la endodoncia, lo que lleva a que los investigadores definan de manera subjetiva las condiciones de los ensayos, haciendo difícil su replicación y comparación (110).

Una posible razón por la que el gel de nanopartículas de peróxido de zinc mostró una mayor actividad antimicrobiana que el gel de nanopartículas de plata y el hidróxido de calcio podría estar relacionada con su capacidad para generar especies reactivas de oxígeno (111). Las bacterias suelen tener dificultades para desarrollar resistencia a estos niveles elevados de especies reactivas, ya que atacan múltiples estructuras celulares (112). De hecho, en la actualidad se están considerando estrategias que se basan en la generación de especies reactivas de oxígeno para tratar infecciones resistentes. Por ejemplo, se ha observado que la inhibición de los sistemas antioxidantes bacterianos las hace más vulnerables a estas especies

(113). Otro mecanismo de acción es la depleción de iones metálicos como el manganeso (Mn^{2+}) y el zinc (Zn^{2+}), que son esenciales para el funcionamiento de las enzimas antioxidantes bacterianas (114). Asimismo, la inhibición de la producción de pigmentos protectores, como la estafiloxantina en *S. aureus*, podría reducir la resistencia a las especies reactivas de oxígeno (115).

En cuanto al gel de nanopartículas de plata, creemos que su principal mecanismo de acción fue la liberación de iones de plata, dado que la mayor parte de las nanopartículas permanecieron confinadas dentro del gel (116). Los iones de plata interactúan con diferentes estructuras celulares, incluyendo la membrana, proteínas, ADN y vías de señalización, generando incluso especies reactivas de oxígeno que causan la muerte de la bacteria (117). No obstante, se ha informado que ciertas bacterias han desarrollado mecanismos para neutralizar estos iones de plata (118). Por ejemplo, se ha descubierto que *Microcystis aeruginosa* puede producir una matriz extracelular que atrapa los iones de plata (119). Se ha reportado un fenómeno similar en *E. faecalis* resistente a nanopartículas de plata (120).

También se ha documentado que las bombas de eflujo de metales pesados contribuyen a expulsar los iones de plata fuera de la célula bacteriana, evitando así que causen daños internos. Recientemente, se ha informado que *E. faecalis* resistente a nanopartículas de plata posee bombas de eflujo como la *Copper Transporter CopA* y la *Heavy Metal Translocating P-type ATPase* (120). Asimismo, se ha observado una alta prevalencia de genes que codifican bombas de eflujo de plata en bacterias aisladas de infecciones endodónticas secundarias (121). Además, las cepas de *E. faecalis* resistentes a nanopartículas de plata han mostrado un aumento en la expresión de genes y proteínas que inactivan las especies reactivas de oxígeno (120). A pesar de esto, es probable que las nanopartículas de peróxido de zinc tengan una mayor capacidad para generar especies reactivas de oxígeno que las nanopartículas de plata, lo cual podría explicar por qué superaron las defensas de *E. faecalis* (ATCC® 29212™) (122). La capacidad de las nanopartículas de peróxido de zinc para producir estas especies ha sido bien documentada (123). Aunque los mecanismos de resistencia bacteriana a nanopartículas han sido objeto de estudio recientemente, su investigación en bacterias endodónticas aún es limitada. Cabe destacar que las bacterias anaerobias obligadas, facultativas y microaerofílicas, que constituyen gran parte de la microbiota endodóntica, presentan pocos mecanismos de defensa contra las especies reactivas de oxígeno (124).

Una ventaja adicional de las nanopartículas de peróxido de zinc en comparación con las de plata es su naturaleza "anfótera". Esto significa que el zinc puede interactuar tanto con ácidos como con bases, formando sales de zinc o complejos de zincato, lo que podría aumentar su efectividad antibacteriana al dañar una mayor variedad de estructuras bacterianas (125). Por ejemplo, Toledano et al. evaluaron nanopartículas poliméricas complementadas con doxiciclina, calcio y zinc, y observaron que las más efectivas eran aquellas que contenían doxiciclina, seguidas por las de zinc y calcio. Los autores también señalaron que la doxiciclina tiene propiedades anfóteras (126).

Otra ventaja del zinc es que, al ser un elemento esencial para los humanos, tanto el óxido de zinc como sus nanopartículas, junto con las nanopartículas de manganeso y dióxido de titanio, son consideradas seguras por la *Food and Drug Administration* (FDA) según el *US Code of Federal Regulations* (Title 21-CFR 182.8991)(127) . Esto no ocurre con las nanopartículas de plata, que pueden causar toxicidad debido a su acumulación en el cuerpo y en el medio ambiente (128).

Finalmente, aunque existen pocos estudios sobre la síntesis de geles con nanopartículas como medicamentos intraconducto, Berrio et al. reportaron recientemente la creación de geles con nanopartículas de plata y cobre, donde los geles con nanopartículas de plata a concentraciones de 0.3 y 0.5 molar mostraron la mayor actividad antimicrobiana. Las características que contribuyeron a esta efectividad fueron su alta disolución, viscosidad adecuada, baja rugosidad superficial y baja porosidad (129). Por otro lado, Roig et al. desarrollaron un gel cargado con nanopartículas de hidróxido de calcio, que es termosensible, presentando una fase líquida y una fase elástica según la temperatura. Este gel demostró una excelente penetración en los túbulos dentinarios y buena biocompatibilidad (130).

Marín-Correa et al. sintetizaron geles con dos concentraciones de nanopartículas de plata (300 y 500 µg/ml) y concluyeron que ambos geles tenían una actividad antimicrobiana similar a la del hidróxido de calcio (131). Samiei et al. desarrollaron geles con nanopartículas de óxido de zinc, además de una combinación de nanopartículas de zinc y plata, comparándolos con una mezcla de hidróxido de calcio y clorhexidina. Sus resultados indicaron que la combinación de hidróxido de calcio y clorhexidina fue más efectiva que los geles con nanopartículas (132).

Una limitación de este estudio es el uso de modelos *in vitro* y *ex vivo*, lo que limita la extrapolación de los resultados a escenarios clínicos, donde las condiciones son más complejas y dinámicas (133).

6. CONCLUSIÓN

El gel complementado con nanopartículas de peróxido de zinc demostró ser más efectivo en términos de actividad antimicrobiana que el gel con nanopartículas de plata y el hidróxido de calcio. Esto podría deberse a la capacidad superior de las nanopartículas de zinc para generar especies reactivas de oxígeno, lo que parece superar las defensas de las bacterias evaluadas.

6. REFERENCIAS BIBLIOGRÁFICAS

1. Hargreaves kenneth M, Stephen C, Berman LH. Cohen's pathways of the pulp. 10th edition. United States: St. Louis, Mo. : Mosby Elsevier, c2011.; 2015. 928 p.
2. Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surgery, Oral Medicine, Oral Pathology. 1965 Sep;20(3):340–9.
3. Fabricius L, Dahlén G, Sundqvist G, Happonen RP, Möller ÅJR. Influence of residual bacteria on periapical tissue healing after chemomechanical treatment and root filling of experimentally infected monkey teeth: Residual bacteria and apical periodontitis. European Journal of Oral Sciences. 2006 Jul 25;114(4):278–85.
4. Ruksakiet K, Hanák L, Farkas N, Hegyi P, Sadaeng W, Czumbel LM, et al. Antimicrobial Efficacy of Chlorhexidine and Sodium Hypochlorite in Root Canal Disinfection: A Systematic Review and Meta-analysis of Randomized Controlled Trials. Journal of Endodontics. 2020 Aug;46(8):1032-1041.e7.
5. Gazzaneo I, Vieira GCS, Pérez AR, Alves FRF, Gonçalves LS, Mdala I, et al. Root Canal Disinfection by Single- and Multiple-instrument Systems: Effects of Sodium Hypochlorite Volume, Concentration, and Retention Time. Journal of Endodontics. 2019 Jun;45(6):736–41.
6. Gonçalves LS, Rodrigues RCV, Andrade Junior CV, Soares RG, Vettore MV. The Effect of Sodium Hypochlorite and Chlorhexidine as Irrigant Solutions for Root Canal Disinfection: A Systematic Review of Clinical Trials. Journal of Endodontics. 2016 Apr;42(4):527–32.
7. Swimberghe RCD, De Clercq A, De Moor RJG, Meire MA. Efficacy of sonically, ultrasonically and laser-activated irrigation in removing a biofilm-mimicking hydrogel from an isthmus model. Int Endod J. 2019 Apr;52(4):515–23.
8. Zandi H, Kristoffersen AK, Ørstavik D, Rôças IN, Siqueira JF, Enersen M. Microbial Analysis of Endodontic Infections in Root-filled Teeth with Apical Periodontitis before and after Irrigation Using Pyrosequencing. Journal of Endodontics. 2018 Mar;44(3):372–8.
9. Verma N, Sangwan P, Tewari S, Duhan J. Effect of Different Concentrations of Sodium Hypochlorite on Outcome of Primary Root Canal Treatment: A Randomized Controlled Trial. Journal of Endodontics. 2019 Apr;45(4):357–63.
10. Matos F de S, Khoury RD, Carvalho CAT, Martinho FC, Bresciani E, Valera MC. Effect of EDTA and QMIX Ultrasonic Activation on the Reduction of Microorganisms and Endotoxins in Ex Vivo Human Root Canals. Braz Dent J. 2019 Jun;30(3):220–6.
11. Arruda MEF, Neves MAS, Diogenes A, Mdala I, Guilherme BPS, Siqueira JF, et al. Infection Control in Teeth with Apical Periodontitis Using a Triple Antibiotic Solution or Calcium Hydroxide with Chlorhexidine: A Randomized Clinical Trial. Journal of Endodontics. 2018 Oct;44(10):1474–9.
12. Bedran NR, Nadelman P, Magno MB, de Almeida Neves A, Ferreira DM, Braga Pintor AV, et al. Does Calcium Hydroxide Reduce Endotoxins in Infected Root Canals? Systematic Review and Meta-analysis. Journal of Endodontics. 2020 Nov;46(11):1545–58.
13. Pereira TC, da Silva Munhoz Vasconcelos LR, Graeff MSZ, Ribeiro MCM, Duarte MAH, de Andrade FB. Intratubular decontamination ability and physicochemical properties of calcium hydroxide pastes. Clin Oral Invest. 2019 Mar;23(3):1253–62.

14. Pereira TC, Vasconcelos LRSMD, Graeff MSZ, Duarte MAH, Bramante CM, Andrade FBD. Intratubular disinfection with tri-antibiotic and calcium hydroxide pastes. Acta Odontologica Scandinavica. 2017 Feb 17;75(2):87–93.
15. Ordinola-Zapata R, Bramante CM, Minotti PG, Cavenago BC, Garcia RB, Bernardineli N, et al. Antimicrobial Activity of Triantibiotic Paste, 2% Chlorhexidine Gel, and Calcium Hydroxide on an Intraoral-infected Dentin Biofilm Model. Journal of Endodontics. 2013 Jan;39(1):115–8.
16. Cassol DV, Duarte ML, Pintor AVB, Barcelos R, Primo LG. Iodoform Vs Calcium Hydroxide/Zinc Oxide based pastes: 12-month findings of a Randomized Controlled Trial. Braz oral res. 2019;33:1–10.
17. Ørstavik D, Haapasalo M. Disinfection by endodontic irrigants and dressings of experimentally infected dentinal tubules. Dental Traumatology. 1990 Aug;6(4):142–9.
18. Lambrianidis T, Margelos J, Beltes P. Removal Efficiency of Calcium Hydroxide Dressing from the Root Canal. Journal of Endodontics.
19. Waltimo TMT, Ørstavik D, Sirén EK, Haapasalo MPP. In vitro susceptibility of Candida albicans to four disinfectants and their combinations. Int Endodontic J. 1999 Nov;32(6):421–9.
20. Peters LB, van Winkelhoff A-J, Buijs JF, Wesselink PR. Effects of instrumentation, irrigation and dressing with calcium hydroxide on infection in pulpless teeth with periapical bone lesions. International Endodontic Journal. 2002;
21. Waltimo T, Trope M, Haapasalo M, Ørstavik D. Clinical Efficacy of Treatment Procedures in Endodontic Infection Control and One Year Follow-Up of Periapical Healing. Clinical Research. 2005;31(12).
22. Ai M, Du Z, Zhu S, Geng H, Zhang X, Cai Q, et al. Composite resin reinforced with silver nanoparticles–laden hydroxyapatite nanowires for dental application. Dental Materials. 2017 Jan;33(1):12–22.
23. Bacali C, Baldea I, Moldovan M, Carpa R, Olteanu DE, Filip GA, et al. Flexural strength, biocompatibility, and antimicrobial activity of a polymethyl methacrylate denture resin enhanced with graphene and silver nanoparticles. Clin Oral Invest. 2020 Aug;24(8):2713–25.
24. Cheng L, Zhang K, Zhang N, Melo MAS, Weir MD, Zhou XD, et al. Developing a New Generation of Antimicrobial and Bioactive Dental Resins. J Dent Res. 2017 Jul;96(8):855–63.
25. de Castro DT, Valente ML da C, Aires CP, Alves OL, dos Reis AC. Elemental ion release and cytotoxicity of antimicrobial acrylic resins incorporated with nanomaterial. Gerodontology. 2017 Sep;34(3):320–5.
26. Köroğlu A, ŞahiN O, Kürkçüoğlu I, Dede DÖ, ÖzdemiR T, Hazer B, et al. Silver nanoparticle incorporation effect on mechanical and thermal properties of denture base acrylic resins. J Appl Oral Sci. 2016 Dec;24(6):590–6.
27. Salaie RN, Besinis A, Le H, Tredwin C, Handy RD. The biocompatibility of silver and nanohydroxyapatite coatings on titanium dental implants with human primary osteoblast cells. Materials Science and Engineering: C. 2020 Feb;107:110210.
28. Yazdani J, Ahmadian E, Sharifi S, Shahi S, Maleki Dizaj S. A short view on nanohydroxyapatite as coating of dental implants. Biomedicine & Pharmacotherapy. 2018 Sep;105:553–7.

29. Belouka SM, Strietzel F. Sinus Floor Elevation and Augmentation Using Synthetic Nanocrystalline and Nanoporous Hydroxyapatite Bone Substitute Materials: Preliminary Histologic Results. Int J Oral Maxillofac Implants. 2016 Nov;31(6):1281–91.
30. Kishen A, Shi Z, Shrestha A, Neoh KG. An Investigation on the Antibacterial and Antibiofilm Efficacy of Cationic Nanoparticulates for Root Canal Disinfection. Journal of Endodontics. 2008 Dec;34(12):1515–20.
31. Abdel Raheem IA, Abdul Razek A, Elgendy AA, Saleh NM, Shaaban MI, Abd El-Hady FK. Design, Evaluation And Antimicrobial Activity Of Egyptian Propolis-Loaded Nanoparticles: Intrinsic Role As A Novel And Naturally Based Root Canal Nanosealer. IJN. 2019 Oct;Volume 14:8379–98.
32. Baras BH, Sun J, Melo MAS, Tay FR, Oates TW, Zhang K, et al. Novel root canal sealer with dimethylaminohexadecyl methacrylate, nano-silver and nano-calcium phosphate to kill bacteria inside root dentin and increase dentin hardness. Dental Materials. 2019 Oct;35(10):1479–89.
33. DaSilva L, Finer Y, Friedman S, Basrani B, Kishen A. Biofilm Formation within the Interface of Bovine Root Dentin Treated with Conjugated Chitosan and Sealer Containing Chitosan Nanoparticles. Journal of Endodontics. 2013 Feb;39(2):249–53.
34. Leng D, Li Y, Zhu J, Liang R, Zhang C, Zhou Y, et al. The Antibiofilm Activity and Mechanism of Nanosilver- and Nanozinc-Incorporated Mesoporous Calcium-Silicate Nanoparticles. IJN. 2020 Jun;Volume 15:3921–36.
35. Naghavi N, Mortazavi M, Nejat A, Javidi M, Zarei M. Zinc oxide nano-particles as sealer in endodontics and its sealing ability. Contemp Clin Dent. 2014;5(1):20.
36. Teixeira ABV, de Castro DT, Schiavon MA, dos Reis AC. Cytotoxicity and release ions of endodontic sealers incorporated with a silver and vanadium base nanomaterial. Odontology. 2020 Oct;108(4):661–8.
37. Wang L, Xie X, Li C, Liu H, Zhang K, Zhou Y, et al. Novel bioactive root canal sealer to inhibit endodontic multispecies biofilms with remineralizing calcium phosphate ions. Journal of Dentistry. 2017 May;60:25–35.
38. Aydın ZU, Özyürek T, Keskin B, Baran T. Effect of chitosan nanoparticle, QMix, and EDTA on TotalFill BC sealers' dentinal tubule penetration: a confocal laser scanning microscopy study. Odontology. 2019 Jan;107(1):64–71.
39. Baras BH, Melo MAS, Sun J, Oates TW, Weir MD, Xie X, et al. Novel endodontic sealer with dual strategies of dimethylaminohexadecyl methacrylate and nanoparticles of silver to inhibit root canal biofilms. Dental Materials. 2019 Aug;35(8):1117–29.
40. Barros J, Costa-Rodrigues J, Lopes MA, Pina-Vaz I, Fernandes MH. Response of Human Osteoblastic and Osteoclastic Cells to AH Plus and Pulp Canal Sealer Containing Quaternary Ammonium Polyethylenimine Nanoparticles. Journal of Endodontics. 2014 Aug;40(8):1149–55.
41. Duque-Aristizabal JC, Isaza-Areiza LM, Tobon-Calle D, Londoño Lopez me. Actividad antibacteriana de nanopartículas de plata inmovilizadas en cemento de óxido de zinc-eugenol contra el enterococcus faecalis: estudio in vitro. Rev Fac Odontol Univ Antioq 2019 Oct 21
42. Kesler Shvero D, Zaltsman N, Weiss EI, Polak D, Hazan R, Beyth N. Lethal bacterial trap: Cationic surface for endodontic sealing: LETHAL BACTERIAL TRAP. J Biomed Mater Res. 2016 Feb;104(2):427–34.
43. Loyola-Rodríguez JP, Torres-Méndez F, Espinosa-Cristobal LF, García-Cortes JO, Loyola-Leyva A, González FJ, et al. Antimicrobial activity of endodontic sealers and

medications containing chitosan and silver nanoparticles against *Enterococcus faecalis*. Journal of Applied Biomaterials & Functional Materials. 2019 Jul;17(3):228080001985177.
44. Nair N, James B, Jacob J. Comparative Evaluation of Antibiofilm Efficacy of Chitosan Nanoparticle- and Zinc Oxide Nanoparticle-Incorporated Calcium Hydroxide-Based Sealer: An In vitro Study. :1.
45. Omidi S, Javidi M. Subcutaneous Connective Tissue Reaction to a New Nano Zinc-Oxide Eugenol Sealer in Rat Model. Iranian Endodontic Journal [Internet]. 2017 Jan 2 [cited 2020 Dec 17]; Available from: https://doi.org/10.22037/iej.2017.14
46. Pattanaik S, Jena A, Shashirekha G. In vitro comparative evaluation of antifungal efficacy of three endodontic sealers with and without incorporation of chitosan nanoparticles against Candida albicans. J Conserv Dent. 2019;22(6):564.
47. Suzuki TYU, Gallego J, Assunção WG, Briso ALF, dos Santos PH. Influence of silver nanoparticle solution on the mechanical properties of resin cements and intrarradicular dentin. Lin CP, editor. PLoS ONE. 2019 Jun 26;14(6):e0217750.
48. Bukhari S, Kim D, Liu Y, Karabucak B, Koo H. Novel Endodontic Disinfection Approach Using Catalytic Nanoparticles. Journal of Endodontics. 2018 May;44(5):806–12.
49. del Carpio-Perochena A, Bramante CM, Duarte MAH, de Moura MR, Aouada FA, Kishen A. Chelating and antibacterial properties of chitosan nanoparticles on dentin. Restor Dent Endod. 2015;40(3):195.
50. Gomes-Filho JE, Silva FO, Watanabe S, Angelo Cintra LT, Tendoro KV, Dalto LG, et al. Tissue Reaction to Silver Nanoparticles Dispersion as an Alternative Irrigating Solution. Journal of Endodontics. 2010 Oct;36(10):1698–702.
51. Jowkar Z, Hamidi SA, Shafiei F, Ghahramani Y. The Effect of Silver, Zinc Oxide, and Titanium Dioxide Nanoparticles Used as Final Irrigation Solutions on the Fracture Resistance of Root-Filled Teeth. CCIDE. 2020 Apr;Volume 12:141–8.
52. Rodrigues CT, de Andrade FB, de Vasconcelos LRSM, Midena RZ, Pereira TC, Kuga MC, et al. Antibacterial properties of silver nanoparticles as a root canal irrigant against *Enterococcus faecalis* biofilm and infected dentinal tubules. Int Endod J. 2018 Aug;51(8):901–11.
53. Wu D, Fan W, Kishen A, Gutmann JL, Fan B. Evaluation of the Antibacterial Efficacy of Silver Nanoparticles against Enterococcus faecalis Biofilm. Journal of Endodontics [Internet]. 2014 Feb [cited 2020 Dec 17];40(2):285–90. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0099239913007322
54. Abbaszadegan A, Nabavizadeh M, Gholami A, Aleyasin ZS, Dorostkar S, Saliminasab M, et al. Positively charged imidazolium-based ionic liquid-protected silver nanoparticles: a promising disinfectant in root canal treatment. Int Endod J. 2015 Aug;48(8):790–800.
55. Afkhami F, Akbari S, Chiniforush N. Entrococcus faecalis Elimination in Root Canals Using Silver Nanoparticles, Photodynamic Therapy, Diode Laser, or Laser-activated Nanoparticles: An In Vitro Study. Journal of Endodontics. 2017 Feb;43(2):279–82.
56. Al-Badr RJ, Al-Huwaizi HF. Antimicrobial Evaluation for Novel Solution of Iron Oxide Nanoparticles Functionalized with Glycine and Coated by Chitosan as Root Canal Final Irrigation. 2020;11(6):10.
57. Aydın H, Er K, Kuştarcı A, Akarsu M, Gençer G, Er H, et al. Antibacterial activity of silver nanoparticles activated by photodynamic therapy in infected root canals. Dent Med Probl. 2020 Dec 31;57(4):393–400.

58. Chávez-Andrade GM, Tanomaru-Filho M, Rodrigues EM, Gomes-Cornélio AL, Faria G, Bernardi MIB, et al. Cytotoxicity, genotoxicity and antibacterial activity of poly(vinyl alcohol)-coated silver nanoparticles and farnesol as irrigating solutions. Archives of Oral Biology. 2017 Dec;84:89–93.
59. de Almeida J, Cechella B, Bernardi A, de Lima Pimenta A, Felippe W. Effectiveness of nanoparticles solutions and conventional endodontic irrigants against Enterococcus faecalis biofilm. Indian J Dent Res. 2018;29(3):347.
60. Monzavi A, Eshraghi S, Hashemian R, Momen-Heravi F. In vitro and ex vivo antimicrobial efficacy of nano-MgO in the elimination of endodontic pathogens. Clin Oral Invest. 2015 Mar;19(2):349–56.
61. Nabavizadeh M, Ghahramani Y, Abbaszadegan A, Jamshidzadeh A, Jenabi P, Makarempour A. In Vivo Biocompatibility of an Ionic Liquid-protected Silver Nanoparticle Solution as Root Canal Irrigant. Iranian Endodontic Journal [Internet]. 2018 Jul 10 [cited 2021 Feb 9];13(3). Available from: https://doi.org/10.22037/iej.v13i3.17386
62. Zheng T, Huang X, Chen J, Feng D, Mei L, Huang Y, et al. A liquid crystalline precursor incorporating chlorhexidine acetate and silver nanoparticles for root canal disinfection. Biomater Sci. 2018;6(3):596–603.
63. Afkhami F, Pourhashemi SJ, Sadegh M, Salehi Y, Fard MJK. Antibiofilm efficacy of silver nanoparticles as a vehicle for calcium hydroxide medicament against Enterococcus faecalis. Journal of Dentistry. 2015 Dec;43(12):1573–9.
64. Balto H, Bukhary S, Al-Omran O, BaHammam A, Al-Mutairi B. Combined Effect of a Mixture of Silver Nanoparticles and Calcium Hydroxide against Enterococcus faecalis Biofilm. Journal of Endodontics. 2020 Nov;46:1689–94.
65. Bruniera JFB, Gabriel-Silva L, Goulart RS, Silva-Sousa YTC, Lara MG, Pitondo-Silva A, et al. Green Synthesis, Characterization and Antimicrobial Evaluation of Silver Nanoparticles for an Intracanal Dressing. Braz Dent J. 2020 Sep;31(5):485–92.
66. Bruniera JFB, Silva-Sousa YTC, Lara MG, Pitondo-Silva A, Marcaccini AM, Miranda CES. Development of Intracanal Formulation Containing Silver Nanoparticles. Braz Dent J. 2014;25(4):302–6.
67. del Carpio-Perochena A, Kishen A, Felitti R, Bhagirath AY, Medapati MR, Lai C, et al. Antibacterial Properties of Chitosan Nanoparticles and Propolis Associated with Calcium Hydroxide against Single- and Multispecies Biofilms: An In Vitro and In Situ Study. Journal of Endodontics. 2017 Aug;43(8):1332–6.
68. Elshinawy MI, Al-Madboly LA, Ghoneim WM, El-Deeb NM. Synergistic Effect of Newly Introduced Root Canal Medicaments; Ozonated Olive Oil and Chitosan Nanoparticles, Against Persistent Endodontic Pathogens. Front Microbiol. 2018 Jul 3;9:1371.
69. Farhadian N, Godiny M, Moradi S, Hemati Azandaryani A, Shahlaei M. Chitosan/gelatin as a new nano-carrier system for calcium hydroxide delivery in endodontic applications: Development, characterization and process optimization. Materials Science and Engineering: C. 2018 Nov;92:540–6.
70. Jhamb S, Singla R, Kaur A, Sharma J, Bhushan J. An in vitro determination of antibacterial effect of silver nanoparticles gel as an intracanal medicament in combination with other medicaments against Enterococcus fecalis. J Conserv Dent. 2019;22(5):479.
71. Makkar H. Fabrication and Appraisal of Poly (Lactic-Co-Glycolic Acid) – Moxifloxacin Nanoparticles using Vitamin E-TPGS: A Potential Intracanal Drug Delivery Agent. JCDR. 2017 57

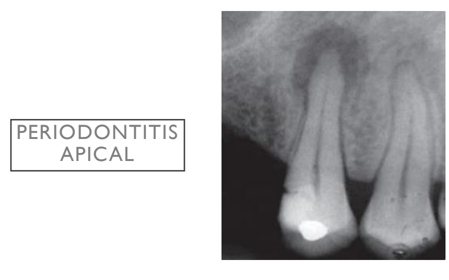
72. Samiei M, Torab A, Hosseini O, Abbasi T, Abdollahi AA, Divband B. Antibacterial Effect of Two Nano Zinc Oxide Gel Preparations Compared to Calcium Hydroxide and Chlorhexidine Mixture. Iranian Endodontic Journal [Internet]. 2018 Jul 10 [cited 2020 Oct 7];13(3). Available from: https://doi.org/10.22037/iej.v13i3.19866
73. Siqueira JF, de Uzeda M. Intracanal medicaments: Evaluation of the antibacterial effects of chlorhexidine, metronidazole, and calcium hydroxide associated with three vehicles. Journal of Endodontics. 1997 Mar;23(3):167–9.
74. Takamiya AS, Monteiro DR, Bernabé DG, Gorup LF, Camargo ER, Gomes-Filho JE, et al. In Vitro and In Vivo Toxicity Evaluation of Colloidal Silver Nanoparticles Used in Endodontic Treatments. Journal of Endodontics. 2016 Jun;42(6):953–60.
75. Abbaszadegan A, Gholami A, Abbaszadegan S, Aleyasin ZS, Dorostkar S, Hemmateenejad B, et al. The Effects of Different Ionic Liquid Coatings and the Length of Alkyl Chain on Antimicrobial and Cytotoxic Properties of Silver Nanoparticles. :1.
76. Charannya S, Duraivel D, Padminee K, Poorni S, Nishanthine C, Srinivasan M. Comparative evaluation of antimicrobial efficacy of silver nanoparticles and 2% chlorhexidine gluconate when used alone and in combination assessed using agar diffusion method: An In vitro study. Contemp Clin Dent. 2018;9(6):204.
77. Chávez-Andrade GM, Tanomaru-Filho M, Basso Bernardi MI, de Toledo Leonardo R, Faria G, Guerreiro-Tanomaru JM. Antimicrobial and biofilm anti-adhesion activities of silver nanoparticles and farnesol against endodontic microorganisms for possible application in root canal treatment. Archives of Oral Biology. 2019 Nov;107:104481.
78. Ertem E, Gutt B, Zuber F, Allegri S, Le Ouay B, Mefti S, et al. Core–Shell Silver Nanoparticles in Endodontic Disinfection Solutions Enable Long-Term Antimicrobial Effect on Oral Biofilms. ACS Appl Mater Interfaces. 2017 Oct 11;9(40):34762–72.
79. Fan B, Fan W, Wu D, Tay F, Ma T, Wu Y. Effects of adsorbed and templated nanosilver in mesoporous calcium-silicate nanoparticles on inhibition of bacteria colonization of dentin. IJN. 2014 Nov;5217.
80. Halkai KR, Mudda JA, Halkai R. Antibacterial E!cacy of Biosynthesized Silver Nanoparticles against Enterococcus faecalis Biofilm: An in vitro Study. :1.
81. Ioannidis K, Niazi S, Mylonas P, Mannocci F, Deb S. The synthesis of nano silver-graphene oxide system and its efficacy against endodontic biofilms using a novel tooth model. Dental Materials. 2019 Nov;35(11):1614–29.
82. Kushwaha V, Yadav R, Tikku A, Chandra A, Verma P, Gupta P, et al. Comparative evaluation of antibacterial effect of nanoparticles and lasers against Endodontic Microbiota: An in vitro study. J Clin Exp Dent. 2018;e1155–60.
83. Araujo HC, da Silva ACG, Paião LI, Magario MKW, Frasnelli SCT, Oliveira SHP, et al. Antimicrobial, antibiofilm and cytotoxic effects of a colloidal nanocarrier composed by chitosan-coated iron oxide nanoparticles loaded with chlorhexidine. Journal of Dentistry. 2020 Oct;101:103453.
84. Barreras US, Méndez FT, Martínez REM, Valencia CS, Rodríguez PRM, Rodríguez JPL. Chitosan nanoparticles enhance the antibacterial activity of chlorhexidine in collagen membranes used for periapical guided tissue regeneration. Materials Science and Engineering: C. 2016 Jan;58:1182–7.
85. Hussein H, Kishen A. Antibiofilm and Immune Response of Engineered Bioactive Nanoparticles for Endodontic Disinfection. JCM. 2020 Mar 9;9(3):730.

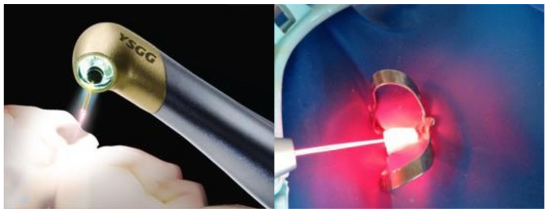
86. Ionescu A, Harris D, Selvaganapathy PR, Kishen A. Electrokinetic transport and distribution of antibacterial nanoparticles for endodontic disinfection. Int Endod J. 2020 Aug;53(8):1120–30.
87. Kishen A, Shi Z, Shrestha A, Neoh KG. An Investigation on the Antibacterial and Antibiofilm Efficacy of Cationic Nanoparticulates for Root Canal Disinfection. Journal of Endodontics. 2008 Dec;34(12):1515–20.
88. Mohan A, Dipallini S, Lata S, Mohanty S, Pradhan PK, Patel P, et al. Oxidative stress induced antimicrobial efficacy of chitosan and silver nanoparticles coated Gutta-percha for endodontic applications. Materials Today Chemistry. 2020 Sep;17:100299.
89. Parolia A, Kumar H, Ramamurthy S, Davamani F, Pau A. Effectiveness of chitosan-propolis nanoparticle against Enterococcus faecalis biofilms in the root canal. BMC Oral Health. 2020 Dec;20(1):339.
90. Shrestha A, Cordova M, Kishen A. Photoactivated Polycationic Bioactive Chitosan Nanoparticles Inactivate Bacterial Endotoxins. Journal of Endodontics. 2015 May;41(5):686–91.
91. Souza GL de, Moura CCG, Silva ACA, Marinho JZ, Silva TR, Dantas NO, et al. Effects of zinc oxide and calcium–doped zinc oxide nanocrystals on cytotoxicity and reactive oxygen species production in different cell culture models. Restor Dent Endod. 2020;45(4):e54.
92. Yavari H, Ghasemi N, Divband B, Rezaei Y, Jabbari G, Payahoo S. The effect of photodynamic therapy and polymer solution containing nano-particles of Ag /ZnO on push-out bond strength of the sealers AH-Plus and MTA Fillapex. J Clin Exp Dent. 2017;9:109–14.
93. Vilela Teixeira AB, de Carvalho Honorato Silva C, Alves OL, Cândido dos Reis A. Endodontic Sealers Modified with Silver Vanadate: Antibacterial, Compositional, and Setting Time Evaluation. BioMed Research International [Internet]. 2019 May 9 [cited 2020 Dec 17];2019:1–9. Available from: https://www.hindawi.com/journals/bmri/2019/4676354/
94. Arias-Moliz MT, Baca P, Solana C, Toledano M, Medina-Castillo AL, Toledano-Osorio M, et al. Doxycycline-functionalized polymeric nanoparticles inhibit *Enterococcus faecalis* biofilm formation on dentine. Int Endod J. 2020 Nov 22;54:413–26.
95. Barros J, Silva MG, Rodrigues MA, Alves FRF, Lopes MA, Pina-Vaz I, et al. Antibacterial, physicochemical and mechanical properties of endodontic sealers containing quaternary ammonium polyethylenimine nanoparticles. Int Endod J. 2014 Aug;47(8):725–34.
96. Toledano M, Osorio E, Aguilera FS, Muñoz-Soto E, Toledano-Osorio M, López-López MT, et al. Polymeric nanoparticles for endodontic therapy. Journal of the Mechanical Behavior of Biomedical Materials. 2020 Mar;103:1–12.
97. Shrestha A, Friedman S, Torneck CD, Kishen A. Bioactivity of Photoactivated Functionalized Nanoparticles Assessed in Lipopolysaccharide-contaminated Root Canals In Vivo. Journal of Endodontics. 2018 Jan;44(1):104–10.
98. Yavari H, Ghasemi N, Divband B, Rezaei Y, Jabbari G, Payahoo S. The effect of photodynamic therapy and polymer solution containing nano-particles of Ag /ZnO on push-out bond strength of the sealers AH-Plus and MTA Fillapex. J Clin Exp Dent. 2017
99.Zhang XF, Liu ZG, Shen W, Gurunathan S. Silver Nanoparticles: Synthesis, Characterization, Properties, Applications, and Therapeutic Approaches. IJMS. 2016 Sep 13;17(9):1534.

100. Wu D, Fan W, Kishen A, Gutmann JL, Fan B. Evaluation of the Antibacterial Efficacy of Silver Nanoparticles against Enterococcus faecalis Biofilm. Journal of Endodontics [Internet]. 2014 Feb [cited 2020 Oct 7];40(2):285–90.
101. Raura N, Garg A, Arora A, Roma M. Nanoparticle technology and its implications in endodontics: a review. Biomater Res. 2020 Dec;24:1–8.
102. Bruna T, Maldonado-Bravo F, Jara P, Caro N. Silver Nanoparticles and Their Antibacterial Applications. IJMS. 2021 Jul 4;22(13):7202.
103. Padmavathy N, Vijayaraghavan R. Enhanced bioactivity of ZnO nanoparticles—an antimicrobial study. Science and Technology of Advanced Materials. 2008 Jul;9(3):035004.
104. Bergs C, Brück L, Rosencrantz RR, Conrads G, Elling L, Pich A. Biofunctionalized zinc peroxide (ZnO_2) nanoparticles as active oxygen sources and antibacterial agents. RSC Adv. 2017;7(62):38998–9010.
105. Prada I, Mico-Munoz P, Giner-Lluesma T, Mico-Martinez P, Muwaquet-Rodriguez S, Albero-Monteagudo A. Update of the therapeutic planning of irrigation and intracanal medication in root canal treatment. A literature review. J Clin Exp Dent. 2019;e185–93.
106. Zou X, Zheng X, Liang Y, Zhang C, Fan B, Liang J, et al. Expert consensus on irrigation and intracanal medication in root canal therapy. Int J Oral Sci. 2024 Mar 1;16(1):23.
107. Menichetti A, Mavridi-Printezi A, Mordini D, Montalti M. Effect of Size, Shape and Surface Functionalization on the Antibacterial Activity of Silver Nanoparticles. JFB. 2023 Apr 26;14(5):244.
108. Abbaszadegan A, Ghahramani Y, Gholami A, Hemmateenejad B, Dorostkar S, Nabavizadeh M, et al. The Effect of Charge at the Surface of Silver Nanoparticles on Antimicrobial Activity against Gram-Positive and Gram-Negative Bacteria: A Preliminary Study. Hazan R, editor. Journal of Nanomaterials. 2015 Jan;2015(1):720654.
109. Britto Hurtado R, Cortez-Valadez M, Flores-Lopez NS, Flores-Acosta M. Agglomerates of Au-Pt bimetallic nanoparticles: synthesis and antibacterial activity. Gold Bull. 2020 Jun;53(2):93–100.
110. Cavassin ED, De Figueiredo LFP, Otoch JP, Seckler MM, De Oliveira RA, Franco FF, et al. Comparison of methods to detect the in vitro activity of silver nanoparticles (AgNP) against multidrug resistant bacteria. J Nanobiotechnol. 2015 Dec;13(1):64.
111. Al-Momani H, Massadeh MI, Almasri M, Al Balawi D, Aolymat I, Hamed S, et al. Anti-Bacterial Activity of Green Synthesised Silver and Zinc Oxide Nanoparticles against Propionibacterium acnes. Pharmaceuticals. 2024 Feb 16;17(2):255.
112. Dharmaraja AT. Role of Reactive Oxygen Species (ROS) in Therapeutics and Drug Resistance in Cancer and Bacteria. J Med Chem. 2017 Apr 27;60(8):3221–40.
113. Carlioz A, Touati D. Isolation of superoxide dismutase mutants in Escherichia coli: is superoxide dismutase necessary for aerobic life? The EMBO Journal. 1986 Mar;5(3):623–30.
114. Corbin BD, Seeley EH, Raab A, Feldmann J, Miller MR, Torres VJ, et al. Metal Chelation and Inhibition of Bacterial Growth in Tissue Abscesses. Science. 2008 Feb 15;319(5865):962–5.
115. Elmesseri RA, Saleh SE, Elsherif HM, Yahia IS, Aboshanab KM. Staphyloxanthin as a Potential Novel Target for Deciphering Promising Anti-Staphylococcus aureus Agents. Antibiotics. 2022 Feb 23;11(3):298.
116. Dakal TC, Kumar A, Majumdar RS, Yadav V. Mechanistic Basis of Antimicrobial Actions of Silver Nanoparticles. Front Microbiol [Internet]. 2016 Nov 16 [cited 2024 Oct 14];7. Available from: http://journal.frontiersin.org/article/10.3389/fmicb.2016.01831/full

117. Rizzello L, Pompa PP. Nanosilver-based antibacterial drugs and devices: Mechanisms, methodological drawbacks, and guidelines. Chem Soc Rev. 2014;43(5):1501–18.
118. Stabryla LM, Johnston KA, Diemler NA, Cooper VS, Millstone JE, Haig SJ, et al. Role of bacterial motility in differential resistance mechanisms of silver nanoparticles and silver ions. Nat Nanotechnol. 2021 Sep;16(9):996–1003.
119. Chen S, Jin Y, Lavoie M, Lu H, Zhu K, Fu Z, et al. A new extracellular von Willebrand A domain-containing protein is involved in silver uptake in Microcystis aeruginosa exposed to silver nanoparticles. Appl Microbiol Biotechnol. 2016 Oct;100(20):8955–63.
120. Salas-Orozco MF, Niño-Martínez N, Martínez-Castañón G, Méndez FT, Morán GMM, Bendaña-Piñeiro AE, et al. Proteomic analysis of an *Enterococcus faecalis* mutant generated against the exposure to silver nanoparticles. J of Applied Microbiology. 2022 Jan;132(1):244–55.
121. Salas-Orozco MF, Martínez NN, Martínez-Castañón GA, Méndez FT, Patiño-Marín N, Ruiz F. Detection of Genes Related to Resistance to Silver Nanoparticles in Bacteria from Secondary Endodontic Infections. Journal of Nanomaterials. 2019 Sep 12;2019:1–7.
122. Ivask A, Bondarenko O, Jepihhina N, Kahru A. Profiling of the reactive oxygen species-related ecotoxicity of CuO, ZnO, TiO2, silver and fullerene nanoparticles using a set of recombinant luminescent Escherichia coli strains: differentiating the impact of particles and solubilised metals. Anal Bioanal Chem. 2010 Sep;398(2):701–16.
123. Wolanov Y, Prikhodchenko PV, Medvedev AG, Pedahzur R, Lev O. Zinc Dioxide Nanoparticulates: A Hydrogen Peroxide Source at Moderate pH. Environ Sci Technol. 2013 Aug 6;47(15):8769–74.
124. Sundqvist G, Figdor D. Life as an endodontic pathogen: Ecological differences between the untreated and root-filled root canals. Endodontic Topics. 2003 Nov;6(1):3–28.
125. Wang J, Teong SP, Riduan SN, Armugam A, Lu H, Gao S, et al. Redox Active Zn@MOFs as Spontaneous Reactive Oxygen Species Releasing Antimicrobials.
126. Toledano-Osorio M, Babu JP, Osorio R, Medina-Castillo AL, García-Godoy F, Toledano M. Modified Polymeric Nanoparticles Exert In Vitro Antimicrobial Activity Against Oral Bacteria. Materials. 2018 Jun 14;11(6):1013.
127. Part F. 182 substances generally recognized as safe. 2006;
128. Jaswal T, Gupta J. A review on the toxicity of silver nanoparticles on human health. Materials Today: Proceedings. 2023;81:859–63.
129. Berrio ME, Jerez-Olate C, Ramírez JA, Saireddy S, González-Rocha G, Ponce A, et al. Novel antibacterial and biocompatible nanostructured gels based on one-step synthesis as a potential disinfectant for endodontic infection control. J Endod. 2024;50(1):74-84.
130. Roig X, Halbaut L, Elmsmari F, Pareja R, Arrien A, Duran-Sindreu F, et al. Calcium hydroxide-loaded nanoparticles dispersed in thermosensitive gel as a novel intracanal medicament. Int Endod J. 2024;
131. Marín-Correa BM, Guzmán-Martínez N, Gómez-Ramírez M, Pless RC, Mundo JR, García-Ramos JC, et al. Nanosilver gel as an endodontic alternative against Enterococcus faecalis in an in vitro root canal system in Mexican dental specimens.
132.Samiei M, Torab A, Hosseini O, Abbasi T, Abdollahi AA, Divband B. Antibacterial effect of two nano zinc oxide gel preparations compared to calcium hydroxide and chlorhexidine mixture. Iran Endod J. 2018;13(3):305.
133. Castelo-Branco DDSCM, Amando BR, Ocadaque CJ, Aguiar LD, Paiva DDDQ, Diógenes EM, et al. Mini-review: from *in vitro* to *ex vivo* studies: an overview of alternative methods for the study of medical biofilms. Biofouling. 2020 Dec 21;1–21.

7. DIAPOSITIVAS DE PRESENTACIÓN





NUEVAS ALTERNATIVAS

MEDICACIÓN INTRACONDUCTO

Peters LB, van Winkelhoff A-J, Buijs JF, Wesselink PR. Effects of instrumentation, irrigation and dressing with calcium hydroxide on infection in pulpless teeth with periapical bone lesions. Int Endod J. 2002 Jan;35(1):13–21.

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HIDRÓXIDO DE CALCIO

$Ca(OH)_2$

Clinical Research

Clinical Efficacy of Treatment Procedures in Endodontic Infection Control and One Year Follow-Up of Periapical Healing

Micro-computed tomography evaluation of the removal of calcium hydroxide medicament from C-shaped root canals of mandibular second molars

Conclusions A considerable proportion of the apical canal space remained filled with $Ca(OH)_2$ in the C-shaped root canals after instrumentation and conventional needle irrigation. Although combining rotary instrumentation and irrigation with sonic or ultrasonic agitation reduced the amount of residual $Ca(OH)_2$ in the C-shaped root canals, the large amount of calcium hydroxide in the critical apical area remains a concern. Alternative strategies should be considered in medication of the apical canal in C-shaped teeth.

Effects of instrumentation, irrigation and dressing with calcium hydroxide on infection in pulpless teeth with periapical bone lesions

L. B. Peters[1], A.-J. van Winkelhoff[2], J. F. Buijs[1] & P. R. Wesselink[1]

Conclusions Although a calcium hydroxide paste was placed in the prepared canals, the number of positive canals had increased in the period between visits. However, the number of microorganisms had only increased to 0.93% of the original number of CFU (sample 1). It is concluded that a calcium hydroxide and sterile saline slurry limits but does not totally prevent regrowth of endodontic bacteria.

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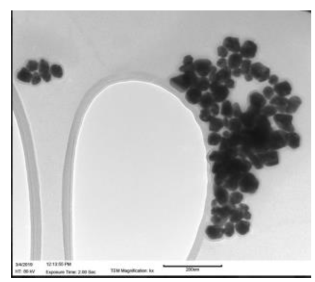
SILICATO DE CALCIO

ANTECEDENTES

NANOPARTÍCULAS



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NANOPARTÍCULAS EN ENDODONCIA

An Investigation on the Antibacterial and Antibiofilm Efficacy of Cationic Nanoparticulates for Root Canal Disinfection

Anil Kishen, BDS, MDS, PhD, Zhilong Shi, PhD,† Annie Shrestha, BDS,* and Koon Gee Neoh, PhD†*

Tissue Reaction to Silver Nanoparticles Dispersion as an Alternative Irrigating Solution

João Eduardo Gomes-Filho, PhD, Fernando Oliveira Silva, DDS, Simone Watanabe, MSc, Luciano Tavares Angelo Cintra, PhD, Karina Vanessa Tendoro, DDS, Luana Godoy Dalto, DDS, Sara Vieira Pacanaro, DDS, Carolina Simonetti Lodi, MSc, and Fernanda Fragoso Ferreira de Melo, DDS

- Óxido de Zinc
- Quitosán
- Óxido de Magnesio
- Óxido de Hierro
- Vanadato de plata
- Zinc
- Fosfato de calcio amorfo
- Silicato de calcio amorfo
- Poliméricas
- Plata

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NANOPARTÍCULAS DE PLATA

In Vitro and *In Vivo* Toxicity Evaluation of Colloidal Silver Nanoparticles Used in Endodontic Treatments

Aline Satie Takamiya, DDS, PhD, Douglas Roberto Monteiro, DDS, PhD, Daniel Galera Bernabé, DDS, PhD, Luiz Fernando Gorup, PhD, Emerson Rodrigues Camargo, PhD, João Eduardo Gomes-Filho, DDS, PhD, Sandra Helena Penha Oliveira, PhD, Debora Barros Barbosa, DDS, PhD

Evaluation of the Antibacterial Efficacy of Silver Nanoparticles against *Enterococcus faecalis* Biofilm

Daming Wu, DDS, Wei Fan, DDS, PhD, Anil Kishen, BDS, MDS, PhD, James L. Gutmann, DDS, PhD, FACD, FICD, FADI, Bing Fan, DDS, MSc, PhD

NANOPARTÍCULAS DE PLATA

Review Article
Mechanisms of Resistance to Silver Nanoparticles in Endodontic Bacteria: A Literature Review

Marco Salas-Orozco,[1] Nereyda Niño-Martínez,[2] Gabriel-Alejandro Martinez-Castañón,[1] Fernando Torres Méndez,[1] Martha Eugenia Compean Jasso,[2] and Facundo Ruiz[2]

NANOPARTÍCULAS DE PERÓXIDO DE ZINC

PAPER

Biofunctionalized zinc peroxide (ZnO_2) nanoparticles as active oxygen sources and antibacterial agents†

Christian Bergs, Lisa Brück, Ruben R. Rosencrantz, Georg Conrads, Lothar Elling and Andrij Pich

ORIGINAL RESEARCH

Synthesized zinc peroxide nanoparticles (ZnO_2-NPs): a novel antimicrobial, anti-elastase, anti-keratinase, and anti-inflammatory approach toward polymicrobial burn wounds

Anaerobes in human infections (including dental/oral infections)

Biofunctionalized zinc peroxide nanoparticles inhibit peri-implantitis associated anaerobes and *Aggregatibacter actinomycetemcomitans* pH-dependent

Katrin Fröber, Christian Bergs, Andrij Pich, Georg Conrads

ENTEROCOCCUS FAECALIS

- Gram positiva
  Anaerobia facultativa
- Pocos nutrientes
  pH de hasta 11.5
- Monocultivo en conductos tratados
- 90% de los casos de fracaso en endodoncia

Alghamdi F, Shakir M. The Influence of Enterococcus faecalis as a Dental Root Canal Pathogen on Endodontic Treatment: A Systematic Review. Cureus. 2020 Mar 13;12(3):e7257

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PLANTEAMIENTO DEL PROBLEMA

La medicación intraconducto es indispensable en los tratamientos endodónticos, previene la proliferación de bacterias entre citas, crea una barrera fisiomecánica y acelera la recuperación de tejidos periapicales.

En este sentido, es necesario el desarrollo de nuevos medicamentos intraconductos que aseguren su efectividad bactericida y faciliten su manejo y aplicación en la práctica odontológica.

20

OBJETIVO GENERAL

Evaluar la capacidad antibacteriana del gel de NPAg y NPZnO_2 con Enteroccocus faecalis para su potencial uso como medicación intraconducto.

21

OBJETIVOS ESPECÍFICOS

- Síntesis de NPAg y NPZnO_2.
- Caracterización de NPAg y NPZnO_2 mediante: Espectroscopia Óptica, Potencial Z, DLS, Microscopia electrónica de transmisión.
- Elaboración de geles de NPAg y NPZnO_2.
- Observar la inhibición bacteriana de los geles de NPAg, NPZnO_2, $Ca(OH)_2$ en antibiograma de agar cromo-orientador de *Enteroccocus Faecalis*.
- Infectar cubos de dentina con *E. faecalis*.
- Observar en microscopia láser confocal con tinción vivo y muerto la inhibición de NPAg, NPZnO_2, $Ca(OH)_2$

22

HIPÓTESIS

Hipótesis 0

- No hay diferencia entre el efecto antibacteriano de los geles de NPAg y NPZnO_2 en comparación con $Ca(OH)_2$.

Hipótesis 1

- Sí hay diferencia entre entre el efecto antibacteriano de los geles de NPAg y NPZnO_2 en comparación con $Ca(OH)_2$.

23

METODOLOGÍA

24

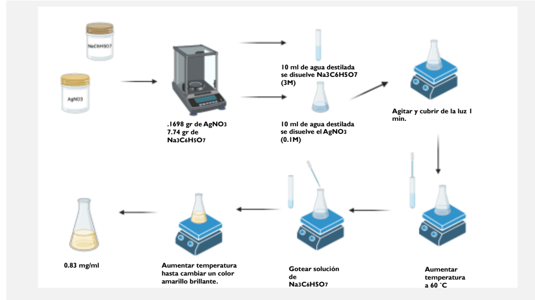
SÍNTESIS DE NANOPARTÍCULAS

ORIGINAL PAPER

Study of Interaction of Silver Nanoparticles with Bovine Serum Albumin Using Fluorescence Spectroscopy

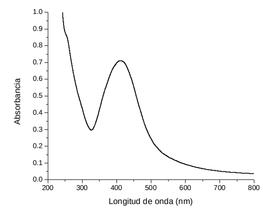
Jessy Mariam · P. M. Dongre · D. C. Kothari

25



SÍNTESIS NPAg

UV- VISIBLE



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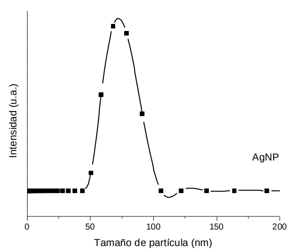
DLS



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POTENCIAL Z

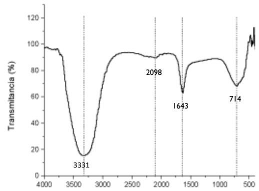
Results

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	18.4	Peak 1:	12.1	95.9	7.38
Zeta Deviation (mV):	20.7	Peak 2:	-138	2.6	4.30
Conductivity (mS/cm):	1.60	Peak 3:	-116	0.8	2.49

30

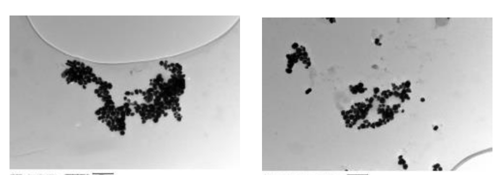
3331 cm-1 O-H
2098 cm-1 C-H
1643 cm-1 N-H
714 cm-1. C-O

INFRARROJO



31

MICROSCOPÍA DE TRANSMISIÓN



32

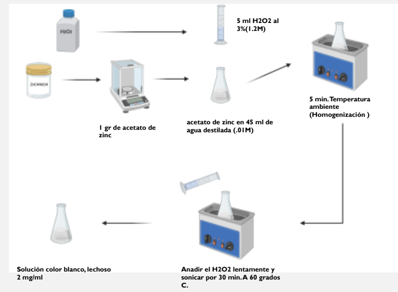
SÍNTESIS DE NANOPARTÍCULAS DE NPZnO2

catalysts MDPI

Article

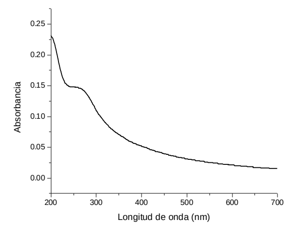
Synthesis and Characterization of Zinc Peroxide Nanoparticles for the Photodegradation of Nitrobenzene Assisted by UV-Light

Jesús Isaías De León Ramírez [1], Víctor Alfredo Reyes Villegas [1], Sergio Pérez Sicairos [2], Esteban Hernández Guevara [1], Mirna Del Carmen Brito Perea [1] and Bertha Landeros Sánchez [1,*]



SÍNTESIS NPZnO2

UV- VISIBLE



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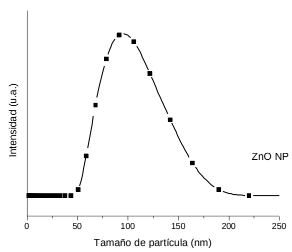
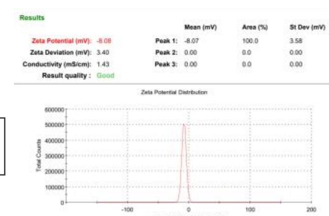
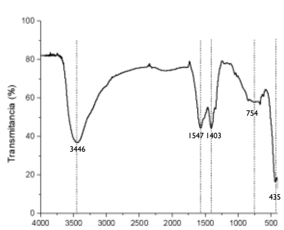


POTENCIAL Z

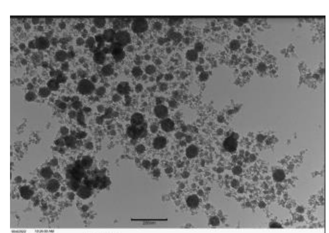


3446 cm-1 H_2O
1574 cm-1 C-O
1403 cm-1 O-O
754 cm-1 O-O
435 cm-1 Zn-O

INFRARROJO



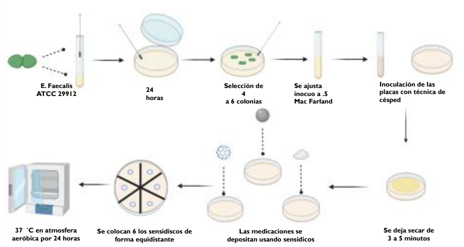
MICROSCOPIA DE TRANSMISIÓN



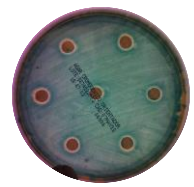
ELABORACIÓN DE GELES

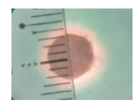
Hidroxietilcelulosa ($C_2H_6O_6$)

ANTIBIOGRAMAS AGAR CROMO-ORIENTADOR

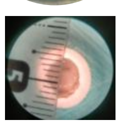


ANTIBIOGRAMAS NPAg

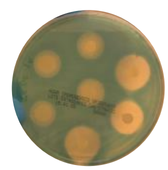


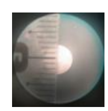


ANTIBIOGRAMAS Ca(OH)2

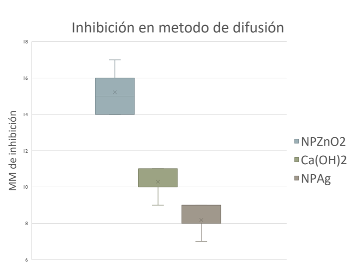


ANTIBIOGRAMAS NPZnO2





INHIBICIÓN EN MM



ANÁLISIS ESTADÍSTICO

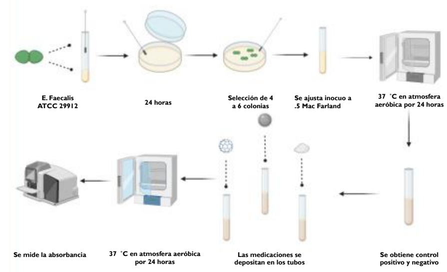
Results of the Disk Diffusion Test

Group	Mean (mm)	Standard Deviation (mm)	p-value
ZnO2NP	15.2143	1.0313	
Ca(OH)2	10.2857	0.5998	<0.001
AgNP	8.1786	0.6118	

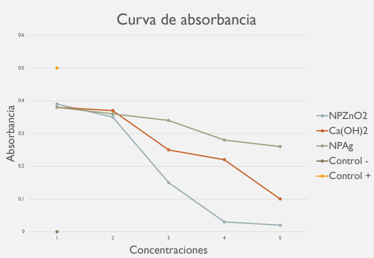
Comparison	p-value
AgNP vs CaOH2	<0.001
AgNP vs ZnO2NP	<0.001
CaOH2 vs ZnO2NP	<0.001

De acuerdo con el resultado del análisis estadístico, se rechaza la hipótesis nula y puede afirmarse que el tipo de material tiene una influencia estadísticamente significativa en el halo de inhibición.

DILUCIÓN AGAR CEREBRO-CORAZÓN



ABSORBANCIA



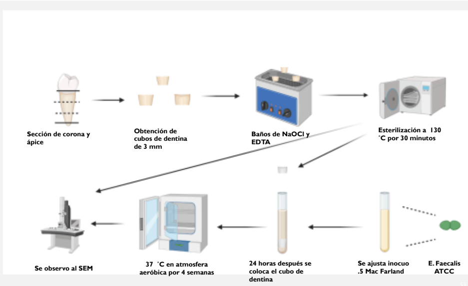
ANÁLISIS ESTADÍSTICO

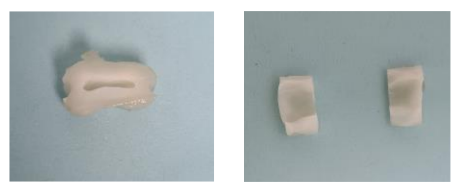
Results of the Absorbance Test

Group	Mean (Absorbance)	Standard Deviation	p-value
ZnO_2NP	0.37	0.01	
$Ca(OH)_2$	0.41	0.00	<0.001
AgNP	0.44	0.01	

Comparison	p-value
ZnO2NP vs CaOH2	<0.001
ZnO2NP vs AgNP	<0.001
CaOH2 vs AgNP	<0.001

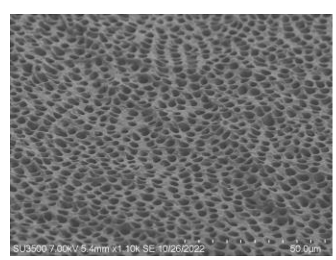
De acuerdo con el resultado del análisis estadístico, se rechaza la hipótesis nula y puede afirmarse que el tipo de material tiene una influencia estadísticamente significativa en la absorbancia.





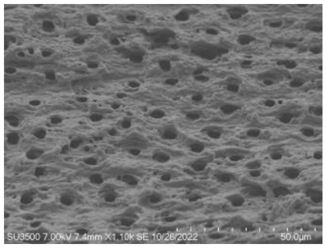
4 semanas en ambiente aerobio a 37° C

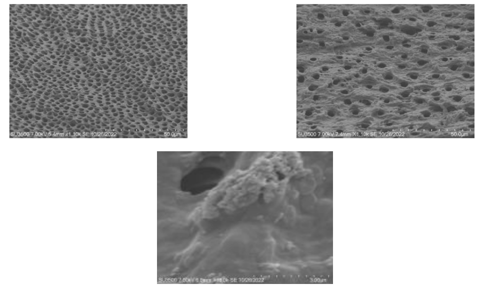
MUESTRA ESTÉRIL



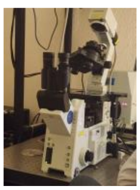
MUESTRA

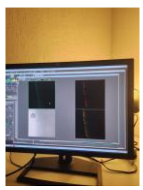
INFECTADA

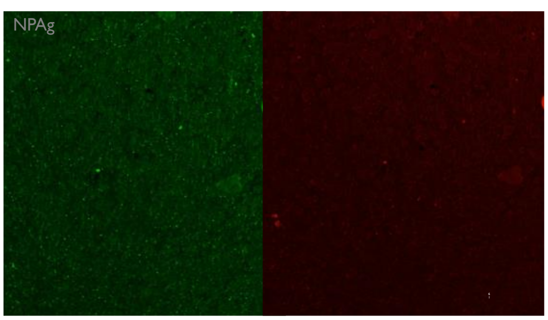


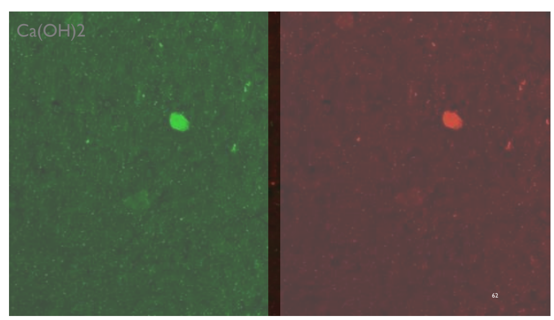


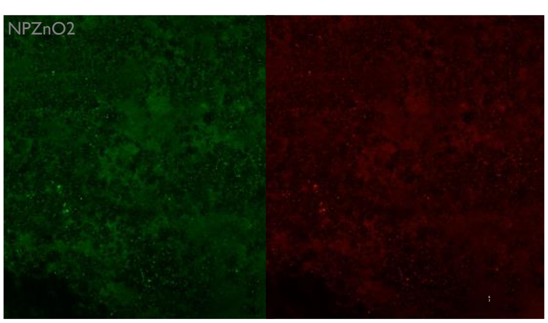
MICROSCOPIA LÁSER CONFOCAL TINCIÓN VIVO MUERTO



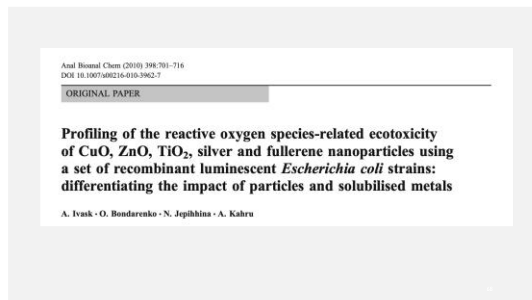


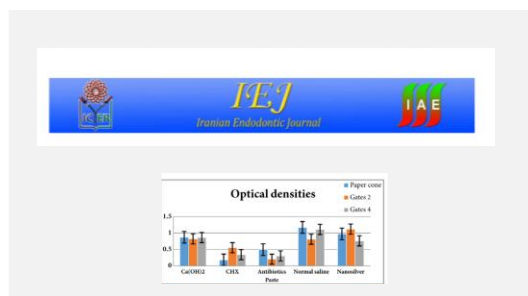


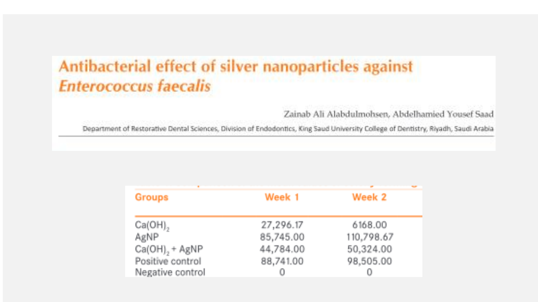




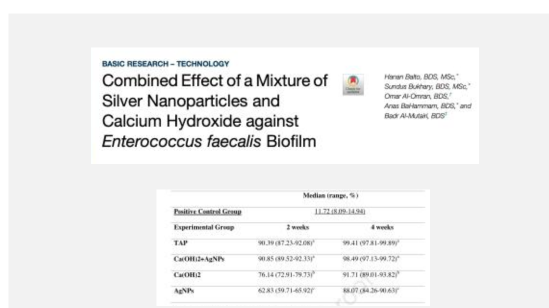
DISCUSIÓN







Groups	Week 1	Week 2
$Ca(OH)_2$	27,296.17	6168.00
AgNP	85,745.00	110,798.67
$Ca(OH)_2$ + AgNP	44,784.00	50,324.00
Positive control	88,741.00	98,505.00
Negative control	0	0



	Median (range, %)	
Positive Control Group	11.72 (8.09-14.94)	
Experimental Group	2 weeks	4 weeks
TAP	90.39 (87.23-92.08)[a]	99.41 (97.81-99.89)[a]
Ca(OH)2+AgNPs	90.85 (89.52-92.33)[a]	98.49 (97.13-99.72)[a]
Ca(OH)2	76.14 (72.91-79.73)[b]	91.71 (89.01-93.82)[b]
AgNPs	62.83 (59.71-65.92)[c]	88.07 (84.26-90.63)[c]

Evaluation of the Antibacterial Efficacy of Silver Nanoparticles against *Enterococcus faecalis* Biofilm

Daming Wu, DDS, Wei Fan, DDS, PhD,* Anil Kishen, BDS, MDS, PhD,† James L. Gutmann, DDS, PhD, FACD, FICD, FADI,‡ and Bing Fan, DDS, MSc, PhD**

Despite the antibacterial effectiveness of AgNPs in dentistry, the possible adverse effects such as cytotoxicity to host cells and dentin staining made it a controversial agent for *in vivo* application (30). Although previous studies have confirmed that the cytoxicity of AgNPs is concentration-dependent (19), further studies are required to optimize the use of AgNPs for root canal disinfection. In summary, the findings

The effect of a combined nanoparticulate/calcium hydroxide medication on the biofilm of *Enterococcus faecalis* in starvation phase

ZHANG Fu-hua, LI Mao, WEI Zhi-jun, ZHAO Bing

Abstract

Keyword: Calcium hydroxide; Silver nanoparticle; Biofilm; *Enterococcus faecalis*

Table 1 Result of bioactivity of biofilm and biomass in different grou

	$Ca(OH)_2$		AgNP		$Ca(OH)_2$+AgNP	
	1 d	7 d	1 d	7 d	1 d	7 d
LgCFU/mL	3.78± 0.69	2.83± 0.48	2.39± 0.23	2.24± 0.29	2.06± 0.22	1.91± 0.28
减少率（%）	73.81± 4.44	86.38± 7.93	91.66± 3.85	92.07± 3.35	96.64± 1.56	96.99± 1.95

CONCLUSIÓN

- El gel complementado con nanopartículas de peróxido de zinc demostró una mayor actividad antimicrobiana en comparación con el gel complementado con nanopartículas de plata y el hidróxido de calcio.
- Esto se podría explicar a la alta capacidad de las nanopartículas de peróxido de zinc a generar especies reactivas del oxígeno que podrían estar superando las defensas de las bacterias evaluadas.

BIBLIOGRAFÍA

Hargreaves kenneth M, Stephen C, Berman LH. Cohen's pathways of the pulp. 10th edition. United States: St. Louis, Mo. : Mosby Elsevier, c2011.; 2015. 928 p.

Siqueira JF, de Uzeda M. Intracanal medicaments: Evaluation of the antibacterial effects of chlorhexidine, metronidazole, and calcium hydroxide associated with three vehicles. J Endod. 1997 Mar;23(3):167–9.

Siqueira JF, Rôças IN, Paiva SSM, Guimarães-Pinto T, Magalhães KM, Lima KC. Bacteriologic investigation of the effects of sodium hypochlorite and chlorhexidine during the endodontic treatment of teeth with apical periodontitis. Oral Surg Oral Med Oral Pathol Oral Radiol Endodontology. 2007 Jul;104(1):122–30.

Basrani B, Ghanem A, Tjaderhane L. Physical and Chemical Properties of Chlorhexidine and Calcium Hydroxide-Containing Medications. J Endod. 2004 Jun;30(6):413–7.

Estrela C, Estrela CRA, Barbin EL, Spanó JCE, Marchesan MA, Pécora JD. Mechanism of action of sodium hypochlorite. Braz Dent J. 2002;13(2):113–7.

SJöGREN U, Figdor D, Spångberg L, Sundqvist G. The antimicrobial effect of calcium hydroxide as a short-term intracanal dressing. Int Endod J. 1991 May;24(3):119–25.

Bacterial Diversity of Endodontic Microbiota by Cloning and Sequencing 16S rRNA. J Endod. 2011 Jul;37(7):922–6.

Waltimo TMT, Orstavik D, Siren EK, Haapasalo MPP. In vitro susceptibility of Candida albicans to four disinfectants and their combinations. Int Endod J. 1999 Nov;32(6):421–9.

Ma J, Wang Z, Shen Y, Haapasalo M. A New Noninvasive Model to Study the Effectiveness of Dentin Disinfection by Using Confocal Laser Scanning Microscopy. J Endod. 2011 Oct;37(10):1380–5.

Peters LB, van Winkelhoff A-J, Buijs JF, Wesselink PR. Effects of instrumentation, irrigation and dressing with calcium hydroxide on infection in pulpless teeth with periapical bone lesions. Int Endod J. 2002 Jan;35(1):13–21.

Siqueirajr J, Rocas I. Exploiting Molecular Methods to Explore Endodontic Infections: Part 1—Current Molecular Technologies for Microbiological Diagnosis. J Endod. 2005 Jun;31(6):411–23.

Siqueirajr J, Rocas I. Exploiting Molecular Methods to Explore Endodontic Infections: Part 2—Redefining the Endodontic Microbiota. J Endod. 2005 Jul;31(7):488–98.

Sundqvist G. Associations between microbial species in dental root canal infections. Oral Microbiol Immunol. 1992 Oct;7(5):257–62.

Fabricius L, Dahlén G, Sundqvist G, Happonen R-P, Möller ÅJR. Influence of residual bacteria on periapical tissue healing after chemomechanical treatment and root filling of experimentally infected monkey teeth: Residual bacteria and apical periodontitis. Eur J Oral Sci. 2006 Jul 25;114(4):278–85.

Ribeiro AC, Matarazzo F, Faveri M, Zezell DM, Mayer MPA. Exploring Bacterial Diversity of Endodontic Microbiota by Cloning and Sequencing 16S rRNA. J Endod. 2011 Jul;37(7):922–6.

Vickerman MM, Brossard KA, Funk DB, Jesionowski AM, Gill SR. Phylogenetic analysis of bacterial and archaeal species in symptomatic and asymptomatic endodontic infections. J Med Microbiol. 2007 Jan 1;56(1):110–8.

CONGRESOS (6)

PONENCIAS(10)

CURSOS (8)


ARTÍCULOS (3)
WILEY
Depression and opinion of dental students regarding the hybrid learning model during the COVID-19 pandemic
BMC


DISTINCIÓN (1)
OTORGA EL PRESENTE
RECONOCIMIENTO

8. ARTICULOS PUBLICADOS

Wiley
Journal of Nanotechnology
Volume 2024, Article ID 6186520, 13 pages
https://doi.org/10.1155/2024/6186520

WILEY

Review Article

Antibacterial Effect of Silver Nanoparticles on Polymicrobial Biofilms in Endodontics: Systematic Review and Meta Analysis

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[1]*Autonomous University of Baja California, Faculty of Health Sciences, Tijuana, Baja California, Mexico*
[2]*Autonomous University of Baja California, Faculty of Chemical Sciences and Engineering, Tijuana, Baja California, Mexico*
[3]*Faculty of Stomatology, Clinical Research Laboratory, Autonomous University of San Luis Potosí, San Luis Potosi, Mexico*

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Academic Editor: Amir Elzwawy

This systematic review investigates the antibacterial effects of silver nanoparticles (AgNPs) on polymicrobial biofilms in endodontics, emphasizing their potential enhancement of traditional treatment methods. Recognizing the limitations of current endodontic disinfectants in fully eradicating complex biofilms, this study focuses on the synergistic potential of AgNPs to improve antimicrobial efficacy. Utilizing a systematic approach based on PRISMA guidelines, the review draws on studies from databases like PubMed, Web of Science, and SCOPUS up to 2024. The included research specifically examines the role of AgNPs in various endodontic applications against resilient biofilms, adhering to strict inclusion criteria that demand clear methodology and measurable outcomes. The results suggest that AgNPs can significantly augment the biofilm disruption capabilities of conventional antimicrobials, potentially due to their ability to modify the biofilm matrix and enhance agent penetration. However, the effectiveness varies depending on the nanoparticle concentration and application technique, indicating a need for further optimized studies. In conclusion, while AgNPs show promise in enhancing endodontic antimicrobial protocols, additional *in vivo* research is necessary to establish standardized, effective, and safe clinical applications. The review highlights the complex interactions between nanoparticle properties and biofilm biology, underscoring the importance of further investigation in this area.

1. Introduction

The primary objective of endodontics is to cure or prevent periapical pathology [1]. Bacteria and their byproducts are the main etiological factors of apical periodontitis [2, 3], making disinfection processes crucial to the success of root canal treatments [4–6]. Various materials, systems, and techniques have been developed to achieve optimal disinfection of the root canal system (RCS) [6–8].

Disinfection of the RCS is traditionally performed using irrigants such as sodium hypochlorite (NaOCl) [5, 8, 9], chlorhexidine (CHX) [4, 6], and ethylenediaminetetraacetic acid (EDTA) [4, 10], often used in association with intracanal medications like calcium hydroxide ($Ca(OH)_2$) [11–13], triple antibiotic pastes [14, 15], and iodoform pastes [16]. These processes have not demonstrated complete elimination of the microbiota present within infected RCS, and bacteria have been detected even after exhaustive chemical and mechanical disinfection [17]. It is well known that primary endodontic infections are polymicrobial, with strict anaerobic bacteria notably dominating. In contrast, persistent infections often involve aerobic and facultative Gram-positive microorganisms, notably *Enterococcus faecalis* [18], a bacterial species predominantly tested in bacteriological studies to determine the effectiveness of endodontic materials [19, 20]. This bacterium is unique in being identified in cases of endodontic failure. It is considered one of the most difficult to eradicate due to its ability to withstand extreme conditions, form biofilms, penetrate dentinal tubules, and tolerate high pH levels [21]. However, these monocultures of *E. faecalis* do not represent the real conditions faced by clinicians, as this species is not the sole perpetrator of apical periodontitis but only part of a mixed microbial community with complex interactions that influence the progression and outcome of periapical pathology [22].

Nanotechnology has rapidly developed and serves as an exceptional tool for various biomedical applications, including tissue regeneration, biosensors, drug delivery, and antimicrobial uses, to name a few [23]. Nanomaterials offer unique physicochemical properties due to their larger surface-to-volume ratio and a higher number of atoms present near the surface. These advantages can be leveraged to design specific materials capable of interacting with tissues to achieve maximum antimicrobial efficacy with minimal toxicity possible [24]. Nanoparticles, such as zinc oxide (ZnO NPs), copper (CuNPs), titanium dioxide (TiO2 NPs), and gold (AuNPs), have demonstrated significant antimicrobial properties. For instance, ZnO NPs are known to generate reactive oxygen species (ROS) that damage bacterial cell membranes and disrupt metabolic processes. Similarly, CuNPs release copper ions that interfere with cell membranes and cause DNA damage, making them effective against bacteria, viruses, and fungi. TiO2 NPs, activated by UV irradiation, also generate ROS that can destroy microorganisms, while AuNPs, though more commonly recognized for their applications in therapy and diagnostics, possess antimicrobial properties through membrane disruption and ROS generation [25]. Silver nanoparticles (AgNPs) have been the most studied and are the best understood among nanoparticles. Their efficacy is attributed to the release of silver ions (Ag^{+}) that interact with bacterial cell membranes, causing permeability and integrity disruptions, as well as damage to DNA and enzymatic functions. Additionally, AgNPs induce ROS generation, contributing to oxidative stress and cell death. These properties, combined with their potential biocompatibility at low concentrations, have positioned AgNPs as key components in the disinfection of surfaces, medical devices, and in dental applications, where their use in root canal disinfection and antimicrobial filling materials is under investigation. Thus, antimicrobial nanoparticles represent a robust field of research with significant potential to enhance infection control strategies across various health sectors [26].

In the field of endodontics, they have been applied as irrigants [27], intracanal medications [19], in sealers [28], and combined with gutta-percha [29] to harness their antimicrobial activity. It has been demonstrated that the ultrasonic activation of AgNPs as irrigants in endodontics enhances their antimicrobial activity, killing up to 90% of *E. faecalis* cultures [30, 31]. This is due to the removal of the smear layer and the opening of dentinal tubules, which facilitates the penetration of the irrigant and its contact with the bacteria present in the root canal [32]. However, most of these studies have been conducted on monobiofilms, and the efficacy of nanoparticles on polymicrobial biofilms has been less investigated.

With this background, the present systematic review summarizes the results regarding the effectiveness of AgNPs' antimicrobial activity against polymicrobial biofilms *in vitro* in endodontics.

2. Materials and Methods

The International Prospective Register of Systematic Reviews (PROSPERO) database was searched for registered protocols on a similar topic. There was no registered protocol for the study of the use of AgNPs and antibacterial activity on polymicrobial biofilms. However, this study could not be registered because the new inclusion criteria of PROSPERO do not allow the registration of systematic reviews of *in vitro* studies. The systematic review followed PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. The selection criteria were as follows.

2.1. Inclusion Criteria

(1) In vitro studies investigating the effectiveness of AgNPs used in irrigants, sealing cements, or as intracanal medication on polymicrobial biofilms.

(2) Publications up to the year 2024.

(3) Studies published in scientific journals with an impact factor.

(4) Research utilizing clearly defined methodologies to measure antimicrobial effectiveness.

(5) Articles not in English.

2.2. Exclusion Criteria

(1) Studies not focused on endodontic-related polymicrobial biofilms.

(2) Narrative and systematic reviews, case reports, letters to the editor, experimental/exploratory studies, opinions, and abstracts.

(3) Articles not in the English language.

The research question was "Are AgNPs effective antimicrobial agents against polymicrobial biofilms in endodontics in *in vitro* studies?"

(1) Population (P): *In vitro* polymicrobial biofilms relevant to endodontic infections.

(2) Intervention (I): Use of silver nanoparticles as endodontic disinfectants.

(3) Comparison (C): Conventional endodontic disinfectants.

(4) Outcome (O): Improvement in microbial eradication or antimicrobial efficacy.

The search strategy was conducted on PubMed, Web of Science, and SCOPUS up to April 2024, with no time restrictions and considering only articles published in English. Gray literature was not considered because it often lacks peer review, compromising its quality and reliability.

Additionally, it is subject to publication bias by including negative or inconclusive results. The search terms were as follows:

(1) "Silver nanoparticles" and "biofilms" and "endodontics".

(2) "Silver nanoparticles" and "polymicrobial biofilms" and "endodontics".

Two reviewers independently selected studies according to the established criteria for inclusion in the systematic review. The selection process involved an initial screening of titles and abstracts to identify potentially relevant studies and the manual removal of duplicates, followed by a review of full texts to determine those meeting the selection criteria. Only studies deemed eligible by both reviewers were included in the review. Additionally, a manual search of the references from the included studies was conducted to find other potentially relevant studies. Two reviewers performed data extraction. Data extracted for analysis included the type of study, sample size, concentration of AgNPs, alternative agents used, and study outcomes. Assessment of Risk of Bias: a customized criterion for risk of bias was developed based on previous systematic reviews [33, 34]. The reasons for the exclusion of articles are described in Table 1.

A meta-analysis was conducted to determine the effectiveness of different types of nanoparticles against polymicrobial biofilms in endodontics. In the meta-analysis, the results of the groups using nanoparticles were compared with the groups that used NaOCl. Two of the three studies included in the meta-analysis reported their results in the form of graphs, so the WebPlotDigitizer application was used to extract the data from the graphs. However, due to the format of the graphs, there were study groups for which the data could not be extracted accurately. The chi-square test and I2 statistic were used to assess heterogeneity. The random-effects model was used to assess the average distribution for studies with substantial unexplained heterogeneity.

3. Results

In this systematic review, we initiated the search for studies through three databases. A total of 106 records were identified and distributed among PubMed (71), Scopus (19), and Web of Science (16). After removing 13 duplicate records, 93 records remained for screening. During the screening phase, 81 records were excluded based on initial review criteria, leaving 12 reports for detailed retrieval. All 12 reports were successfully retrieved, and of these, 6 were assessed for eligibility. The main exclusion criterion applied at this stage was the nonuse of polymicrobial biofilms, leading to the exclusion of 3 reports (Figure 1). Table 1 summarizes the characteristics of the studies excluded for various reasons. Ultimately, 3 studies met all inclusion criteria and were included in the systematic review. These studies contribute to the existing body of knowledge on the use of polymicrobial biofilms in scientific research. The rigorous selection process ensured the inclusion of highly relevant studies, which were then analyzed to synthesize the findings of this review.

The three included studies utilized human teeth as samples. Each study detailed the types of materials used, including AgNPs, CHX, and graphene oxide matrices. Control groups were established for comparative data, and the sample sizes varied across studies. Specific details on sample size, materials, and control groups are summarized in Table 2.

In the study of Tülü et al., scanning electron microscopy (SEM) images showed that after one week, a dense biofilm made up of various bacterial species covered the entire dentine surface. No bacteria grew in the negative control samples. Before treatment, the samples had about 7.47 log colony-forming units (CFU) of bacteria. The least reduction in bacteria was seen in the saline-treated samples for both 1-day and 7-day treatments. Adding AgNPs to $Ca(OH)_2$ significantly improved its antibacterial effect at both 1-day and 7-day applications. The 7-day treatment was more effective than the 1-day treatment. Confocal laser scanning microscopy (CLSM) images confirmed that the saline group had mostly live bacteria, while the mixture of AgNPs with CHX killed significantly more bacteria than other treatments. $Ca(OH)_2$ mixed with AgNPs was more effective than $Ca(OH)_2$ alone. However, live bacteria were still found in deeper layers of the biofilm [38]. In the study of Ioannidis et al., the authors found that NaOCl 2.5% was the most effective, significantly reducing viable bacteria compared to other irrigants. Silver nanoparticles in a graphene oxide matrix (Ag-GO), NaOCl 1%, and CHX 2% also showed higher efficacy than saline and EDTA 17%. The negative control group had no detectable bacteria. NaOCl 2.5% and Ag-GO were most effective in the middle lateral canals, while NaOCl 2.5% was the most effective in the apical lateral canals. Ag-GO showed significant biofilm disruption, especially with ultrasonic agitation irrigation (UAI), which enhanced its effectiveness. NaOCl 2.5% also showed maximum biofilm disruption with no viable biovolume detected. EDTA 17% and NaOCl 1% improved biofilm disruption with UAI, though EDTA 17% still had a high percentage of live biofilm [39]. In the study of Ertem et al., for smear-layer removal, sodium phytate (SP) and ethyleneglycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA) were effective when combined with silver nanoparticles in a silica shell (AgNPs@SiO2) and NaOCl, successfully cleaning the smear layer and opening dentinal tubes. The samples treated with AgNPs@SiO2 + Tris + NaOCl did not remove the smear layer. Antimicrobial tests on biofilms of *E. faecalis* and a five-species mixture showed significant reductions in viable cells. Solutions with AgNPs@SiO2 prevented biofilm regrowth for up to 168 hours, demonstrating long-term effectiveness. SEM images confirmed effective biofilm removal with AgNPs@SiO2 and SP. The release of Ag+ from AgNPs@SiO2 was sufficient to be lethal to bacteria over an extended period. Cytotoxicity tests on human gingival fibroblasts showed that the new solutions were less toxic than conventional ones, making them safer for prolonged contact [40].

Table 1: List of articles excluded from the systematic review and their reasons.

Authors	Year	Title	Reason
Lara et al. [35]	2022	Inhibition of mixed biofilms of *Candida* albicans and methicillin-resistant *Staphylococcus aureus* by positively charged silver nanoparticles and functionalized silicone elastomers	Uses biofilms composed of bacteria and fungi
Bruneira et al. [36]	2020	Green synthesis, characterization and antimicrobial evaluation of silver nanoparticles for an intracanal dressing	Does not use polymicrobial biofilms
Linares et al. [37]	2016	Efficacy of antimicrobial solutions against polymicrobial root canal biofilm	Does not include silver nanoparticles in the antimicrobial solutions

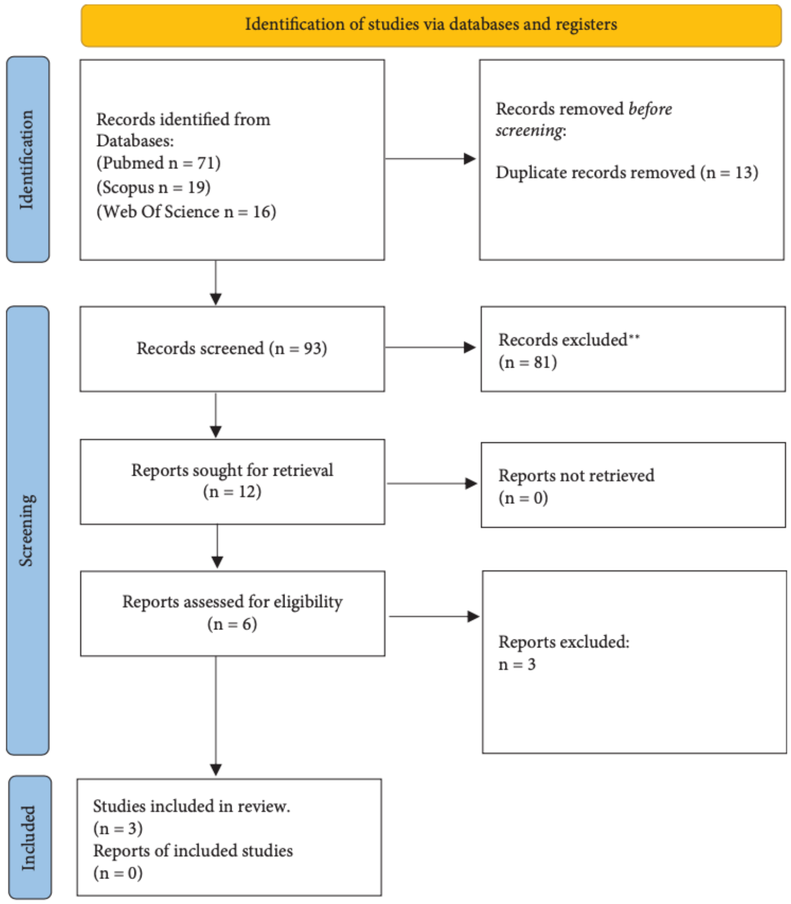

Figure 1: Flowchart illustrating the systematic review process used to identify, screen, and select studies focusing on polymicrobial biofilms.

The meta-analysis revealed significant heterogeneity among the individual studies, indicating that the results varied more than would be expected by chance alone (Figure 2). The overall combined effect of nanoparticle treatments on polymicrobial biofilms in endodontics was not statistically significant, with a t^2 value of −1.15 and a p value of 0.26. This implies that, collectively, the treatments did not show a consistent and significant effect based on the available data. The heterogeneity analysis showed a chi^2 value of 92.24 with a p value <0.001 and an I^2 value of 66%, suggesting that 66% of the variability among the studies was due to differences in study design, populations, outcomes measured, and other factors, rather than random chance. The prediction interval ranged from −4.17 to 2.88, further indicating variability in the treatment effects observed across the studies. The funnel plot analysis, depicted in Figure 3, shows the relationship between the standard error and the standardized mean difference for the included studies. The distribution of the points around the line of no effect (vertical red line) indicates some asymmetry, suggesting the potential presence of publication bias.

Table 3 summarizes the methodological quality of the three studies included in the systematic review. Each study was evaluated based on several criteria of methodological rigor, such as the use of human teeth, statistical calculation of sample size, verification of bacterial inoculation, mention of particle size, presence of a control group, prior preparation of the teeth, consistency in irrigation time, and blinding of the observer. The risk of bias analysis of the three studies showed that Ioannidis et al. and Ertem et al. had a low risk of bias, while Tülü et al. had a medium risk of bias. Ioannidis et al. and Ertem et al. used human teeth and verified particle size and the presence of a control group, although only Ioannidis et al. statistically calculated the sample size. Tülü et al. did not report the use of human teeth or the calculation of sample size but did verify bacterial inoculation. All studies ensured consistency in irrigation time and reported no conflicts of interest, although none reported on observer blinding.

Table 2: Summary of data extracted from studies.

Author/year/country	Type of study	Specimens used	Sample size	Bacterial inoculation was done with	Experimental group/group of interest	Control group	Method of detection	Type of nanoparticle	Size of nanoparticle (nm)	Nanoparticle concentration	Results
Tülü et al. 2021 Turkey [38]	*in Vitro*	Dentine blocks	*Five samples were used per group*	*Enterococcus faecalis, Streptococcus mutans, Lactobacillus acidophilus,* and *Actinomyces naeslundii*	Silver nanoparticles (AgNPs) combined with calcium hydroxide (Ca(OH)2) or chlorhexidine (CHX)	Saline solution NaOCl	Culture-based analysis and confocal laser scanning microscopy (CLSM)	Spherical AgNPs	2.5	210 ppm	The study found that AgNPs enhanced the antibacterial effectiveness of both Ca(OH)2 and CHX against the biofilm, with a combination of CHX and AgNPs showing significant bacterial reduction
Ioannidis et al. 2019 UK [39]	*in Vitro*	Single-rooted teeth, prepared root canals	*Six samples were used per group*	*Propionibacterium acnes, Actinomyces radicidentis, Staphylococcus epidermidis, Streptococcus mitis, Enterococcus faecalis*	Ag-GO (silver nanoparticles in a graphene oxide matrix)	Saline solution NaOCl	SEM/EDS, STEM and CLSM for biofilm analysis	Silver nanoparticles in a graphene oxide (Ag-GO) matrix	20–50	0.25% Ag-GO suspension	Ag-GO showed enhanced antibacterial effectiveness against the biofilm, especially when ultrasonic activation was used. 2.5% NaOCl was superior in microbial killing compared to other treatments. Significant reduction in biofilm volume with Ag-GO
Ertem et al. 2017 Switzerland [40]	*in Vitro*	Single-rooted teeth prepared root canals	*Not reported*	*Propionibacterium acnes, Actinomyces radicidentis, Staphylococcus epidermidis, Streptococcus mitis, Enterococcus faecalis*	AgNPs@SiO2 (silver nanoparticles in a silica shell) combined with sodium phytate (SP) or EGTA	Saline solution NaOCl	SEM/EDS, STEM, CLSM for biofilm analysis	Core-shell silver nanoparticles (AgNPs @ SiO2)	119 ± 29	AgNPs@ SiO2) suspension	AgNPs @ SiO2 based irrigation solutions showed excellent antimicrobial activities for at least 7 days, significantly reducing biofilm volume compared to controls

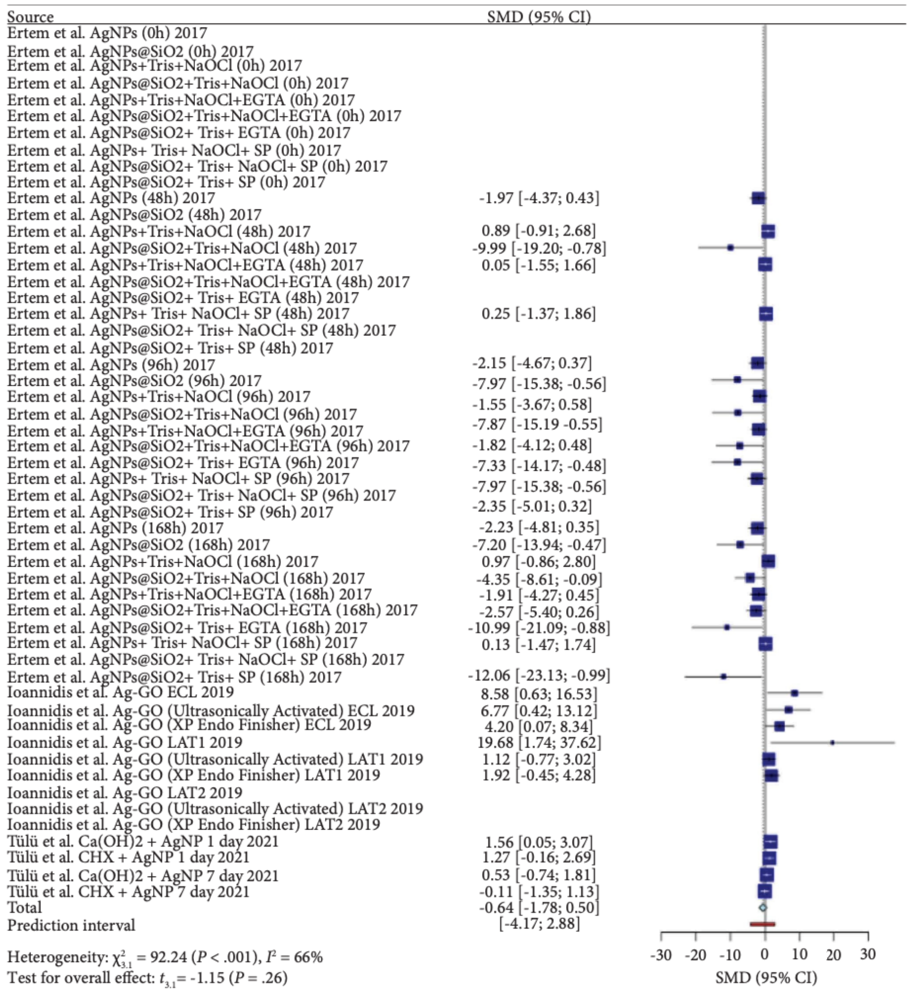

Figure 2: Forest plot of standardized mean differences (SMD) with 95% confidence intervals (CI) for various study groups evaluating the effects of different nanoparticle treatments. The horizontal lines represent the 95% CI for each group, and the boxes represent the SMD. The diamond at the bottom represents the overall effect size. Heterogeneity was assessed using chi^2 ($Q = 92.24$, $P < 0.001$) and I^2 (66%). The test for the overall effect shows $t^2 = -1.15$ ($P = 0.26$).

4. Discussion

This systematic review critically evaluated the antibacterial effects of AgNPs against polymicrobial biofilms in endodontics. The results across the included studies consistently highlighted AgNPs' potent antimicrobial activity, which can be attributed to their ability to penetrate microbial cell membranes and disrupt essential cellular functions, ultimately leading to cell death [41]. Notably, AgNPs alone do not disrupt biofilms effectively, which underscores the necessity of using these nanoparticles in conjunction with other antimicrobial agents to maximize their therapeutic potential [42].

The interaction of AgNPs with multispecies biofilms in endodontics underscores the synergistic potential of the nanoparticles with other antimicrobial agents. This suggests that combinations of AgNPs with conventional antimicrobials or endodontic irrigants could offer improved therapeutic strategies. This synergy could potentially allow for the use of lower concentrations of AgNPs in the future, reducing the risk of toxicity while maintaining or enhancing antimicrobial efficacy. However, a crucial finding from this review is the variability in the concentration and size of the AgNPs used in the studies, which directly affects their efficacy and biocompatibility. Higher concentrations of

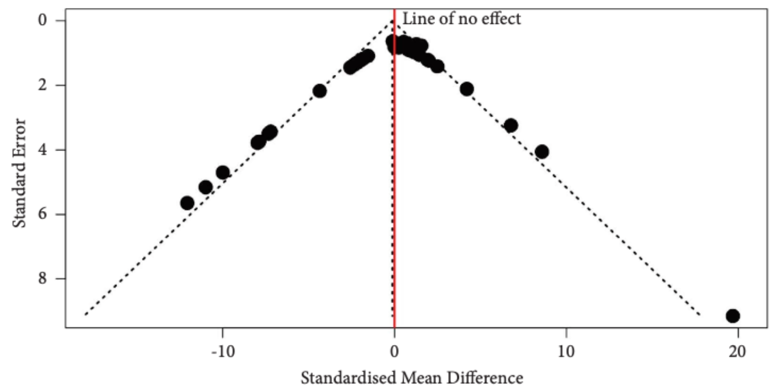

Figure 3: Funnel plot showing the relationship between standard error and standardized mean difference for the included studies. The vertical red line represents the line of no effect. The observed asymmetry may suggest the presence of publication bias.

AgNPs tend to be more effective against biofilms but also pose greater risks of cytotoxicity, highlighting the need to optimize dosing to maximize antimicrobial efficacy while minimizing adverse effects [38–40].

CLSM has become essential in evaluating the efficacy of AgNPs as disinfectants in endodontic mixed biofilms due to its ability to provide detailed, three-dimensional images of these structures [43, 44]. CLSM allows the observation of specific cross-sections within biofilms, crucial for analyzing how nanoparticles interact with and penetrate biofilm layers, enhancing antimicrobial activity [45]. Unlike invasive microscopy techniques, CLSM maintains the biofilm's intact structure during visualization, accurately simulating the root canal environment [46]. It differentiates between living and dead cells using fluorescent markers, essential for assessing the real-time effectiveness of AgNPs and understanding antimicrobial dynamics [47]. The optical pinhole in CLSM reduces image artifacts, resulting in clearer, more defined images for precise observation of nanoparticle effects on biofilms [48]. Additionally, CLSM's ability to use multiple fluorescent dyes simultaneously makes it ideal for studying mixed biofilms, allowing exploration of complex bacterial interactions and development of effective disinfection strategies in endodontics [49].

In the study by Tülü et al., AgNPs showed greater antimicrobial activity when mixed with CHX rather than with $Ca(OH)_2$ [38]. This can be attributed to the synergy between their mechanisms of action, where CHX damages cell membranes and facilitates the penetration of AgNPs, while AgNPs release silver ions that cause DNA and enzymatic damage. These results are consistent with previous studies [50].

In the study by Ioannidis et al., the authors found that NaOCl 2.5% was more effective than silver nanoparticles in a graphene oxide matrix [39]. This may be due to the size of the nanoparticles, which ranged from 20 to 50 nm, potentially hindering their penetration into bacteria and diffusion within the biofilm. It has also been reported that nanoparticles smaller than 10 nm exhibit the highest antimicrobial activity [51].

Finally, in the study by Ertem et al., the solutions with AgNPs@SiO2 and SP or EGTA were effective in removing the smear layer, eliminating the biofilm, and preventing its regrowth for up to 168 hours, demonstrating long-term effectiveness. According to the authors, these characteristics are due to the efficient removal of the smear layer, allowing the opening of dentinal tubules, which facilitates the penetration and action of antimicrobial agents. Additionally, the nanoparticles were able to modify the biofilm matrix and enhance agent penetration, while the slow and continuous release of silver ions (Ag^+) from the nanoparticles prolonged their long-term antimicrobial effect [40].

Antimicrobial resistance is a growing concern in endodontics, especially with the increase in infections by multispecies biofilms resistant to conventional treatments [52, 53]. Generally, biofilms composed of various species show greater resistance to disinfectants compared to biofilms formed by a single type of microorganism [54]. An example of this is the increased resistance of *E. coli* and *E. faecalis* to disinfecting agents when these bacteria were cultured together in biofilms, and it was also observed that *E. faecalis* increased its survival when surrounded by colonies of *E. coli* [55]. Furthermore, additional research has revealed that *S. aureus* tends to have a higher number of adhered cells in environments of mixed-species biofilms compared to those formed by a single species, indicating that some microorganisms can provide a protective effect on others in mixed-composition biofilms [56].

Furthermore, it has recently been reported that some of the resistance mechanisms of biofilms to antibiotics and disinfectants are also related to resistance to nanoparticles. However, little has been studied about this in the area of endodontics and dentistry in general [57]. For example, the extracellular polymeric substances (EPS), primarily composed of polysaccharides and proteins, along with nucleic acids and phospholipids, serve as a defense mechanism against the antimicrobial activity of various nanoparticles, especially AgNPs [58]. It has been reported that bacteria produce EPS in response to nanoparticles, notably *B. subtilis*, which produces extracellular polysaccharides and poly

Table 3: Assessment of risk of bias.

Author/year/country	Were human teeth used as specimens?	Was the sample size statistically calculated?	Was bacterial inoculation verified?	Was particle size mentioned?	Was a control group present?	Were the teeth cleaned and shaped before irrigation?	Was the irrigation time the same for experimental and control groups?	Was the observer/Evaluator blind to the groups?	Was there any conflict of interest?	Risk of bias
Tülü et al./2021/Turkey [38]	N/A	Not reported	Yes	Yes	Yes	N/A	Yes	Not reported	None	Medium
Ioannidis et al./2019/UK [39]	Yes	Yes	No	Yes	Yes	Yes	Yes	Not reported	None	Low
Ertem et al./2017/Switzerland [40]	Yes	Not reported	Yes	Yes	Yes	Yes	Yes	Not reported	None	Low

gamma-glutamate (PGA) as a defense capable of sequestering nanoparticles and metals, neutralizing their bactericidal activity [59]. Additionally, it was reported that *E. faecalis* produces a specialized EPS for the inactivation of AgNPs, containing proteins with the von Willebrand factor A (VWA) domain, which has a high affinity for sequestering Ag+ released by the AgNPs [60].

As mentioned earlier, the high mutation rates in biofilms have also been linked to bacterial resistance to nanoparticles. For example, the Dbs protein, involved in DNA-binding stress response, is key in DNA repair mechanisms in *P. aeruginosa* PAO1, which is resistant to carboxyl-group quantum dots [61]. Similarly, *E. faecalis* resistant to AgNPs expresses several proteins dedicated to DNA damage repair. Among these proteins, the most relevant are RecJ, an exonuclease that works with single-stranded DNA, NAD-dependent DNA ligase, the MutS DNA mismatch repair protein, and the RecA recombinase. RecJ is involved in homologous recombination and mismatch correction, while MutS, MutS2, and MutL participate in the correction of pairing errors. RecA recombinase is involved in the repair of double-strand breaks in DNA. Finally, NAD-dependent DNA ligase is involved in the repair of single- and double-strand breaks in DNA [60]. These DNA repair mechanisms increase the mutation rates of bacteria, fostering the emergence of resistant strains [62].

Nanoparticles exert their antimicrobial action through the excessive production of reactive oxygen species (ROS). ROS kill bacteria by causing oxidative damage to essential cellular components, including membranes, proteins, and nucleic acids. ROS disrupt bacterial cell membranes through lipid peroxidation, increasing membrane permeability and leading to the loss of vital cellular contents. They also oxidize proteins, particularly enzymes, causing inactivation and impairing metabolic processes. Additionally, ROS induce DNA and RNA damage, resulting in mutations and replication errors that hinder bacterial growth and reproduction. The combined damage to these critical structures overwhelms the bacteria's antioxidant defenses, leading to irreversible cell injury and death, making ROS an effective antimicrobial agent with a reduced risk of resistance development [63]. Bacterial cells have antioxidant systems, such as glutathione, the principal antioxidant in bacteria, to regulate these reducing conditions and counteract oxidative stress [64]. Specifically, it has been reported that bacteria such as *P. aeruginosa*, *E. coli*, *B. subtilis*, *P. mirabilis*, *E. faecalis*, and *E. cloacae* demonstrate the ability to manage elevated levels of ROS induced by nanoparticles, thanks to enzymes like superoxide dismutase (SOD) [65].

Efflux pumps represent a crucial defensive strategy of bacteria against nanoparticles [66]. These pumps belong to five main families: resistance-nodulation-division (RND), ATP-binding cassette (ABC), multidrug and toxic compound extrusion (MATE), small multidrug resistance (SMR), and major facilitator superfamily (MFS), which differ in their localization, structure, and mechanism of action. RND pumps are typical in Gram-negative bacteria and are larger, while ABC pumps use adenosine triphosphate (ATP) to function and can be either homodimers or heterodimers. MATE pumps rely on H+ or Na+ gradients to expel substances, and SMR pumps specialize in resistance against certain cations and heavy metals. The MFS family is found in Gram-positive bacteria and also assists in the expulsion of drugs and heavy metals [67].

A specific resistance mechanism to AgNPs is the SilCBA efflux pump, located in the sil operon, which was discovered in *S. typhimurium* and has been found in bacteria from persistent endodontic infections [68]. Mutations in genes related to resistance to silver and copper, as well as in outer membrane proteins, can confer resistance to AgNPs in bacteria such as *K. pneumoniae* [69]. Additionally, the CusCFBA system and the CzcABC efflux system show the ability to transport Ag and Cu ions out of the cell, contributing to tolerance against these nanoparticles [70]. Lastly, the CopA protein, part of the cop operon, plays a role in resistance to AgNPs by participating in the expulsion of Cu, demonstrating the complexity of bacterial mechanisms to counteract the effects of NPs [71]. This systematic review underscores the urgent need for focused research to explore the mechanisms of biofilm resistance to nanoparticle penetration and to develop strategies that enhance the diffusion of AgNPs within the biofilm matrix. The emerging knowledge of microbial defenses against nanoparticles should inform the future design of nanoparticle-based endodontic therapies.

The limited number of studies included in this review poses a significant limitation to the robustness of the conclusions that can be drawn. The meta-analysis detected significant heterogeneity among the individual studies, indicating that the study results varied more than would be expected by chance alone. Although some individual studies showed effects, the overall combined effect was not statistically significant. This implied that, collectively, nanoparticle treatments did not show a consistent and significant effect based on the available data. The detected heterogeneity suggested inconsistent effects in magnitude and/or direction across the studies, with 66% of the variability among studies arising from heterogeneity rather than random chance.

The significant heterogeneity observed in the meta-analysis could be attributed to several factors, including variations in study design (such as different methodologies, sample sizes, and experimental protocols), differences in the populations studied (different combinations of bacteria used), and variability in the specific outcomes measured or definitions used. Additionally, inconsistencies in statistical methods and reporting practices, publication bias and differences in the quality of the included studies (methodological quality and risk of bias) likely contributed to the heterogeneity. Moreover, the reliance on *in vitro* studies is a notable constraint, as these do not fully replicate the complex biological and microenvironmental conditions encountered in clinical scenarios [72].

5. Conclusion

In conclusion, AgNPs represent a promising antimicrobial strategy against multispecies biofilms in endodontics. However, further studies are essential to optimize their formulation and application, understand the potential risks of resistance and cytotoxicity, and establish clinical protocols that integrate AgNPs safely and effectively into endodontic practice. Multidisciplinary collaboration among nanotechnologists, microbiologists, and clinicians will be key to translating the potential of AgNPs into successful long-term clinical applications in endodontics.

Data Availability

The data supporting the findings of this study are available within the article and its supplementary materials. Additional datasets generated and/or analyzed during the current study are not publicly available due to (reason, e.g., privacy restrictions and data use agreements) but are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest related to the content of this manuscript.

References

[1] kenneth M. Hargreaves, C. Stephen, and L. H. Berman, *Cohen's Pathways of the Pulp*, Mosby Elsevier, St. Louis, MO, USA, 2015.

[2] S. Kakehashi, H. R. Stanley, and R. J. Fitzgerald, "The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats," *Oral Surgery, Oral Medicine, Oral Pathology*, vol. 20, no. 3, pp. 340–349, 1965.

[3] L. Fabricius, G. Dahlén, G. Sundqvist, R.-P. Happonen, and Å. J. R. Möller, "Influence of residual bacteria on periapical tissue healing after chemomechanical treatment and root filling of experimentally infected monkey teeth: residual bacteria and apical periodontitis," *European Journal of Oral Sciences*, vol. 114, no. 4, pp. 278–285, 2006.

[4] K. Ruksakiet, L. Hanák, N. Farkas et al., "Antimicrobial efficacy of chlorhexidine and sodium hypochlorite in root canal disinfection: a systematic review and meta-analysis of randomized controlled trials," *Journal of Endodontics*, vol. 46, no. 8, pp. 1032–1041.e7, 2020.

[5] I. Gazzaneo, G. C. S. Vieira, A. R. Pérez et al., "Root canal disinfection by single- and multiple-instrument systems: effects of sodium hypochlorite volume, concentration, and retention time," *Journal of Endodontics*, vol. 45, no. 6, pp. 736–741, 2019.

[6] L. S. Gonçalves, R. C. V. Rodrigues, C. V. Andrade Junior, R. G. Soares, and M. V. Vettore, "The effect of sodium hypochlorite and chlorhexidine as irrigant solutions for root canal disinfection: a systematic review of clinical trials," *Journal of Endodontics*, vol. 42, no. 4, pp. 527–532, 2016.

[7] R. C. D. Swimberghe, A. De Clercq, R. J. G. De Moor, and M. A. Meire, "Efficacy of sonically, ultrasonically and laser-activated irrigation in removing a biofilm-mimicking hydrogel from an isthmus model," *International Endodontic Journal*, vol. 52, no. 4, pp. 515–523, 2019.

[8] H. Zandi, A. K. Kristoffersen, D. Ørstavik, I. N. Rôças, J. F. Siqueira, and M. Enersen, "Microbial analysis of endodontic infections in root-filled teeth with apical periodontitis before and after irrigation using pyrosequencing," *Journal of Endodontics*, vol. 44, no. 3, pp. 372–378, 2018.

[9] N. Verma, P. Sangwan, S. Tewari, and J. Duhan, "Effect of different concentrations of sodium hypochlorite on outcome of primary root canal treatment: a randomized controlled trial," *Journal of Endodontics*, vol. 45, no. 4, pp. 357–363, 2019.

[10] F. d. S. Matos, R. D. Khoury, C. A. T. Carvalho, F. C. Martinho, E. Bresciani, and M. C. Valera, "Effect of EDTA and QMIX ultrasonic activation on the reduction of microorganisms and endotoxins in ex vivo human root canals," *Brazilian Dental Journal*, vol. 30, no. 3, pp. 220–226, 2019.

[11] M. E. F. Arruda, M. A. S. Neves, A. Diogenes et al., "Infection control in teeth with apical periodontitis using a triple antibiotic solution or calcium hydroxide with chlorhexidine: a randomized clinical trial," *Journal of Endodontics*, vol. 44, no. 10, pp. 1474–1479, 2018.

[12] N. R. Bedran, P. Nadelman, M. B. Magno et al., "Does calcium hydroxide reduce endotoxins in infected root canals? Systematic review and meta-analysis," *Journal of Endodontics*, vol. 46, no. 11, pp. 1545–1558, 2020.

[13] T. C. Pereira, L. R. da Silva Munhoz Vasconcelos, M. S. Z. Graeff, M. C. M. Ribeiro, M. A. H. Duarte, and F. B. de Andrade, "Intratubular decontamination ability and physicochemical properties of calcium hydroxide pastes," *Clinical Oral Investigations*, vol. 23, no. 3, pp. 1253–1262, 2019.

[14] T. C. Pereira, L. R. S. M. D. Vasconcelos, M. S. Z. Graeff, M. A. H. Duarte, C. M. Bramante, and F. B. D. Andrade, "Intratubular disinfection with tri-antibiotic and calcium hydroxide pastes," *Acta Odontologica Scandinavica*, vol. 75, no. 2, pp. 87–93, 2017.

[15] R. Ordinola-Zapata, C. M. Bramante, P. G. Minotti et al., "Antimicrobial activity of triantibiotic paste, 2% chlorhexidine gel, and calcium hydroxide on an intraoral-infected dentin biofilm model," *Journal of Endodontics*, vol. 39, no. 1, pp. 115–118, 2013.

[16] D. V. Cassol, M. L. Duarte, A. V. B. Pintor, R. Barcelos, and L. G. Primo, "Iodoform vs calcium hydroxide/zinc oxide based pastes: 12-month findings of a randomized controlled trial," *Brazilian Oral Research*, vol. 33, pp. e002–e010, 2019.

[17] A. Byström and G. Sundqvist, "Bacteriologic evaluation of the effect of 0.5 percent sodium hypochlorite in endodontic therapy," *Oral Surgery, Oral Medicine, Oral Pathology*, vol. 55, no. 3, pp. 307–312, 1983.

[18] Ll. Narayanan and C. Vaishnavi, "Endodontic microbiology," *Journal of Conservative Dentistry*, vol. 13, no. 4, p. 233, 2010.

[19] F. Afkhami, S. Akbari, and N. Chiniforush, "Entrococcus faecalis elimination in root canals using silver nanoparticles, photodynamic therapy, diode laser, or laser-activated nanoparticles: an in vitro study," *Journal of Endodontics*, vol. 43, no. 2, pp. 279–282, 2017.

[20] A. Saad and Z. Alabdulmohsen, "Antibacterial effect of silver nanoparticles against *Enterococcus faecalis*," *Saudi Endodontic Journal*, vol. 7, no. 1, p. 29, 2017.

[21] S. E.-D. Mohamed Saber and S. A. El-Hady, "Development of an intracanal mature Enterococcus faecalis biofilm and its susceptibility to some antimicrobial intracanal medications; an in vitro study," *European Journal of Dermatology*, vol. 06, no. 01, pp. 043–050, 2012.

[22] G. O. Zoletti, E. M. Pereira, R. P. Schuenck, L. M. Teixeira, J. F. Siqueira, and K. R. N. Dos Santos, "Characterization of virulence factors and clonal diversity of Enterococcus faecalis isolates from treated dental root canals," *Research in Microbiology*, vol. 162, no. 2, pp. 151–158, 2011.

[23] S. Aggarwal, "Nanotechnology in endodontics: current and potential clinical applications," *Endodontology*, vol. 28, no. 1, p. 78, 2016.
[24] A. Shrestha and A. Kishen, "Antibacterial nanoparticles in endodontics: a review," *Journal of Endodontics*, vol. 42, no. 10, pp. 1417–1426, 2016.
[25] L. Luo, W. Huang, J. Zhang, Y. Yu, and T. Sun, "Metal-based nanoparticles as antimicrobial agents: a review," *ACS Applied Nano Materials*, vol. 7, no. 3, pp. 2529–2545, 2024.
[26] N. Temur, N. S. Gundes, B. Korkmaz, Z. A. Ozkaya, and I. Ocsoy, "Silver nanoparticles in dentistry," in *Silver Nanoparticles for Drug Delivery*, pp. 265–288, Elsevier, Amsterdam, Netherlands, 2024.
[27] A. Shrestha and A. Kishen, "Antibacterial efficacy of photosensitizer functionalized biopolymeric nanoparticles in the presence of tissue inhibitors in root canal," *Journal of Endodontics*, vol. 40, no. 4, pp. 566–570, 2014.
[28] A. Kishen, Z. Shi, A. Shrestha, and K. G. Neoh, "An investigation on the antibacterial and antibiofilm efficacy of cationic nanoparticulates for root canal disinfection," *Journal of Endodontics*, vol. 34, no. 12, pp. 1515–1520, 2008.
[29] D.-K. Lee, S. V. Kim, A. N. Limansubroto et al., "Nanodiamond–gutta percha composite biomaterials for root canal therapy," *ACS Nano*, vol. 9, no. 11, pp. 11490–11501, 2015.
[30] F. Afkhami, P. Ahmadi, N. Chiniforush, and A. Sooratgar, "Effect of different activations of silver nanoparticle irrigants on the elimination of Enterococcus faecalis," *Clinical Oral Investigations*, vol. 25, no. 12, pp. 6893–6899, 2021.
[31] E. Pedullà, C. Genovese, R. Messina et al., "Antimicrobial efficacy of cordless sonic or ultrasonic devices on Enterococcus faecalis-infected root canals," *Journal of Investigative and Clinical Dentistry*, vol. 10, no. 4, p. e12434, 2019.
[32] R. M. Nair, S. Jayasree, E. S. Poornima, and M. Ashique, "Comparative evaluation of antimicrobial efficacy on Enterococcus faecalis and smear layer removal in curved canals by different irrigation techniques: an: in vitro: study," *Journal of Conservative Dentistry*, vol. 25, no. 4, pp. 409–414, 2022.
[33] E. AlShwaimi, D. Bogari, R. Ajaj, S. Al-Shahrani, K. Almas, and A. Majeed, "In vitro antimicrobial effectiveness of root canal sealers against Enterococcus faecalis: a systematic review," *Journal of Endodontics*, vol. 42, no. 11, pp. 1588–1597, 2016.
[34] R. Sarkis-Onofre, J. Skupien, M. Cenci, R. Moraes, and T. Pereira-Cenci, "The role of resin cement on bond strength of glass-fiber posts luted into root canals: a systematic review and meta-analysis of in vitro studies," *Operative Dentistry*, vol. 39, no. 1, pp. E31–E44, 2014.
[35] H. H. Lara and J. L. Lopez-Ribot, "Inhibition of mixed biofilms of Candida albicans and methicillin-resistant Staphylococcus aureus by positively charged silver nanoparticles and functionalized silicone elastomers," *Pathogens*, vol. 9, no. 10, p. 784, 2020.
[36] J. F. B. Bruniera, L. Gabriel-Silva, R. S. Goulart et al., "Green synthesis, characterization and antimicrobial evaluation of silver nanoparticles for an intracanal dressing," *Brazilian Dental Journal*, vol. 31, no. 5, pp. 485–492, 2020.
[37] M. Ruiz-Linares, B. Aguado-Pérez, P. Baca, M. Arias-Moliz, and C. Ferrer-Luque, "Efficacy of antimicrobial solutions against polymicrobial root canal biofilm," *International Endodontic Journal*, vol. 50, no. 1, pp. 77–83, 2017.
[38] G. Tülü, B. Ü. Kaya, E. S. Çetin, and M. Köle, "Antibacterial effect of silver nanoparticles mixed with calcium hydroxide or chlorhexidine on multispecies biofilms," *Odontology*, vol. 109, no. 4, pp. 802–811, 2021.
[39] K. Ioannidis, S. Niazi, P. Mylonas, F. Mannocci, and S. Deb, "The synthesis of nano silver-graphene oxide system and its efficacy against endodontic biofilms using a novel tooth model," *Dental Materials*, vol. 35, no. 11, pp. 1614–1629, 2019.
[40] E. Ertem, B. Gutt, F. Zuber et al., "Core–shell silver nanoparticles in endodontic disinfection solutions enable long-term antimicrobial effect on oral biofilms," *ACS Applied Materials and Interfaces*, vol. 9, no. 40, pp. 34762–34772, 2017.
[41] F. Afkhami, P. Forghan, J. L. Gutmann, and A. Kishen, "Silver nanoparticles and their therapeutic applications in endodontics: a narrative review," *Pharmaceutics*, vol. 15, no. 3, p. 715, 2023.
[42] F. Fahad, R. A. Al-Hashimi, and Z. R. Hussien, "The antibacterial efficacy and cytotoxicity of incorporating nanoparticles into sodium hypochlorite as irrigant solution," *Bio Nano Science*, vol. 12, 2023.
[43] D. Wu, W. Fan, A. Kishen, J. L. Gutmann, and B. Fan, "Evaluation of the antibacterial efficacy of silver nanoparticles against Enterococcus faecalis biofilm," *Journal of Endodontics*, vol. 40, no. 2, pp. 285–290, 2014.
[44] J. Xu, Y. Li, H. Wang, M. Zhu, W. Feng, and G. Liang, "Enhanced antibacterial and anti-biofilm activities of antimicrobial peptides modified silver nanoparticles," *International Journal of Nanomedicine*, vol. 16, pp. 4831–4846, 2021.
[45] T. R. Neu and J. R. Lawrence, "Investigation of microbial biofilm structure by laser scanning microscopy," *Advances in Biochemical Engineering*, vol. 146, pp. 1–51, 2014.
[46] C. Hope, D. Clements, and M. Wilson, "Determining the spatial distribution of viable and nonviable bacteria in hydrated microcosm dental plaques by viability profiling," *Journal of Applied Microbiology*, vol. 93, no. 3, pp. 448–455, 2002.
[47] N. d. M. Fiallos, D. Cecchin, C. O. de Lima, R. Hirata, E. J. N. L. Silva, and L. M. Sassone, "Antimicrobial effectiveness of grape seed extract against Enterococcus faecalis biofilm: a confocal laser scanning microscopy analysis," *Australian Endodontic Journal*, vol. 46, no. 2, pp. 191–196, 2020.
[48] A. Keleş, C. Keskin, M. Kalkan et al., "Visualization and characterization of Enterococcus faecalis biofilm structure in bovine dentin using 2D and 3D microscopic techniques," *Archives of Microbiology*, vol. 203, no. 1, pp. 269–277, 2021.
[49] A. Bridier, F. Dubois-Brissonnet, A. Boubetra, V. Thomas, and R. Briandet, "The biofilm architecture of sixty opportunistic pathogens deciphered using a high throughput CLSM method," *Journal of Microbiological Methods*, vol. 82, no. 1, pp. 64–70, 2010.
[50] A. Oncu, B. Celikten, B. Aydın et al., "Antibacterial efficacy of silver nanoparticles, sodium hypochlorite, chlorhexidine, and hypochlorous acid on dentinal surfaces infected with Enterococcus faecalis," *Microscopy Research and Technique*, vol. 87, no. 9, pp. 2094–2102, 2024.
[51] A. Menichetti, A. Mavridi-Printezi, D. Mordini, and M. Montalti, "Effect of size, shape and surface functionalization on the antibacterial activity of silver nanoparticles," *Journal of Functional Biomaterials*, vol. 14, no. 5, p. 244, 2023.
[52] B. Crumpton, S. McClanahan, and U. Dc, "Antibiotic resistance and antibiotics in endodontics," *Clinical Update*, vol. 25, no. 12, pp. 23–25, 2003.
[53] M. Barbosa-Ribeiro, B. P. Gomes, R. Arruda-Vasconcelos, I. d. A. Monteiro, M. J. F. Costa, and P. H. Sette-de-Souza, "Antibiotic resistance profile of clinical strains of enterococci from secondary/persistent endodontic infections: what do we

know? A systematic review of clinical studies," *Journal of Endodontics*, vol. 50, no. 3, pp. 299–309, 2024.

[54] P. Sanchez-Vizuete, B. Orgaz, S. Aymerich, D. Le Coq, and R. Briandet, "Pathogens protection against the action of disinfectants in multispecies biofilms," *Frontiers in Microbiology*, vol. 6, 2015.

[55] L. Laganenka and V. Sourjik, "Autoinducer 2-dependent Escherichia coli biofilm formation is enhanced in a dual-species coculture," *Applied and Environmental Microbiology*, vol. 84, no. 5, 2018.

[56] V. Oxaran, K. K. Dittmann, S. H. Lee et al., "Behavior of foodborne pathogens Listeria monocytogenes and Staphylococcus aureus in mixed-species biofilms exposed to biocides," *Applied and Environmental Microbiology*, vol. 84, no. 24, 2018.

[57] M. F. Salas-Orozco, A. C. Lorenzo-Leal, I. de Alba Montero, N. P. Marín, M. A. C. Santana, and H. Bach, "Mechanism of escape from the antibacterial activity of metal-based nanoparticles in clinically relevant bacteria: a systematic review," *Nanomedicine: Nanotechnology, Biology and Medicine*, vol. 55, 2024.

[58] S. Fulaz, S. Vitale, L. Quinn, and E. Casey, "Nanoparticle–biofilm interactions: the role of the EPS matrix," *Trends in Microbiology*, vol. 27, no. 11, pp. 915–926, 2019.

[59] R. Deol, A. Louis, H. L. Glazer, W. Hosseinion, A. Bagley, and P. Chandrangsu, "Poly-gamma-glutamic acid secretion protects Bacillus subtilis from zinc and copper intoxication," *Microbiology Spectrum*, vol. 10, no. 2, pp. e0132921–21, 2022.

[60] M. F. Salas-Orozco, N. Niño-Martínez, G. Martínez-Castañón et al., "Proteomic analysis of an Enterococcus faecalis mutant generated against the exposure to silver nanoparticles," *Journal of Applied Microbiology*, vol. 132, no. 1, pp. 244–255, 2022.

[61] Y. Yang, J. M. Mathieu, S. Chattopadhyay et al., "Defense mechanisms of Pseudomonas aeruginosa PAO1 against quantum dots and their released heavy metals," *ACS Nano*, vol. 6, no. 7, pp. 6091–6098, 2012.

[62] K. Wu, H. Li, X. Cui et al., "Mutagenesis and resistance development of bacteria challenged by silver nanoparticles," *Antimicrobial Agents and Chemotherapy*, vol. 66, no. 10, pp. e0062822–22, 2022.

[63] B. Das, S. K. Dash, D. Mandal et al., "Green synthesized silver nanoparticles destroy multidrug resistant bacteria via reactive oxygen species mediated membrane damage," *Arabian Journal of Chemistry*, vol. 10, no. 6, pp. 862–876, 2017.

[64] A. S. Gort and J. A. Imlay, "Balance between endogenous superoxide stress and antioxidant defenses," *Journal of Bacteriology*, vol. 180, no. 6, pp. 1402–1410, 1998.

[65] S. Kamat and M. Kumari, "Emergence of microbial resistance against nanoparticles: mechanisms and strategies," *Frontiers in Microbiology*, vol. 14, 2023.

[66] S. Silver and L. T. Phung, "A bacterial view of the periodic table: genes and proteins for toxic inorganic ions," *Journal of Industrial Microbiology and Biotechnology*, vol. 32, no. 11-12, pp. 587–605, 2005.

[67] P. Blanco, S. Hernando-Amado, J. A. Reales-Calderon et al., "Bacterial multidrug efflux pumps: much more than antibiotic resistance determinants," *Micro Organisms*, vol. 4, no. 1, p. 14, 2016.

[68] H. Wang, J. Li, C. Min et al., "Characterization of silver resistance and coexistence of sil operon with antibiotic resistance genes among gram-negative pathogens isolated from wound samples by using whole-genome sequencing," *Infection and Drug Resistance*, vol. 15, pp. 1425–1437, 2022.

[69] C. A. Woolley, J. M. Sutton, and M. E. Wand, "Mutations in SilS and CusS/OmpC represent different routes to achieve high level silver ion tolerance in Klebsiella pneumoniae," *BMC Microbiology*, vol. 22, no. 1, p. 113, 2022.

[70] S. A. Gudipaty, A. S. Larsen, C. Rensing, and M. M. McEvoy, "Regulation of Cu (I)/Ag (I) efflux genes in Escherichia coli by the sensor kinase CusS," *FEMS Microbiology Letters*, vol. 330, no. 1, pp. 30–37, 2012.

[71] S. Saeb, P. Steinmann, and A. Javili, "Bounds on size-dependent behaviour of composites," *Philosophical Magazine*, vol. 98, no. 6, pp. 437–463, 2018.

[72] A. E. Roberts, K. N. Kragh, T. Bjarnsholt, and S. P. Diggle, "The limitations of in vitro experimentation in understanding biofilms and chronic infection," *Journal of Molecular Biology*, vol. 427, no. 23, pp. 3646–3661, 2015.

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Depression and opinion of dental students regarding the hybrid learning model during the COVID-19 pandemic

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Abstract

Background The global spread of COVID-19 forced schools at all educational levels to close, which was repeated in more than 60 countries. In addition, the COVID-19 pandemic has affected the mental health of dental students world wide. This study hypothesizes that the prevalence of depression in dental students from El Salvador is higher than that reported in studies from Europe, Asia, and North America.

Methods This study was an online cross-sectional survey performed at the Faculty of Dentistry of the University of Salvador. The PHQ-9 questionnaire was applied to know the level of depression of the students, and a questionnaire focused on learning the opinion of the students on the hybrid teaching model adopted. Approximately 450 students participated in both questionnaires.

Results Regarding the levels of depression present in the students, 14% had minimal depression, 29% had medium depression, 23% had moderate depression and, 34% had severe depression. The students had an excellent opinion regarding the hybrid learning model.

Conclusions The prevalence of depression in dental students in El Salvador seems to be higher than that reported in studies in non-Latin American countries. Therefore, universities must generate care plans for mental health to avoid these harmful effects on students during future contingencies.

Keywords COVID-19, Depression, Dental students, Pandemics, Prevalence, Epidemiology

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Introduction

The disease that caused the most recent pandemic that affected the world was initially identified in December 2019 in Wuhan, China. This respiratory viral disease was named coronavirus disease 2019 (COVID-19). Approximately three months later, on March 11, 2020, COVID-19 was declared a global pandemic by the World Health Organization. Four months after the COVID-19 virus was identified, the first patient diagnosed with COVID-19 in El Salvador was confirmed on March 18, 2020 [1]. According to the global trends of the COVID-19 pandemic [2], experts estimated that 20% of the total population of El Salvador could contract the virus and require hospitalization. Likewise, between 4 and 9% of those infected would require care in intensive care units, which could cause the collapse of the country's health services [3]. The COVID-19 pandemic has been active in El Salvador for approximately two and a half years (from January 3, 2020, to August 2, 2022). During this period, about 191,000 positive cases of COVID-19 have been reported, of which 4,200 incidents have caused death. The fight against the pandemic in El Salvador has consisted of administering approximately 11 million doses of vaccines [1].

The rapid spread of the COVID-19 pandemic worldwide meant that vulnerable populations had to be contained at home. Students are among these vulnerable populations, so classes must be suspended at many different educational levels worldwide. This suspension, in turn, interrupted the student's study plans and activities for a long time. Therefore, online teaching had to be used to try to compensate for the deficiencies in education caused by COVID-19 [4].

However, some university courses, such as dentistry, require theoretical learning and constant clinical practice. Therefore, the clinical practice was the most challenging aspect to compensate for due to the high risk of transmission of COVID-19 and because dental schools had to suspend them entirely. At the same time, they developed strategies to allow students to return to clinical practice safely [5]. Dental education is based on three parts. The first part is the theory, which can easily be carried out through online classes. The second is practical training in simulation labs; virtual reality simulations; however not all faculties worldwide (especially in Latin America) have virtual reality simulators for this purpose. Finally, the third component is clinical practice, which can hardly be replaced. Therefore, it is vital to know the students' opinions on the measures taken during the pandemic to implement hybrid learning models, especially in the Latin American context. In the final months of the pandemic, many dental schools opted for a hybrid education model. The hybrid learning model combines theoretical online teaching with clinical practices to carry out education safely. Mainly, in the final months of the pandemic, the administration of vaccines to the general populous kept the pandemic in control [6, 7].

Likewise, the prolonged confinement during the COVID-19 pandemic also caused a deterioration in the population's mental health within the central psychological affections are stress, anxiety, and depression. In addition, it has been previously reported that catastrophic events (such as pandemics) that have economic and social consequences increase the prevalence of mental illnesses in the population [8]. This prevalence compounds students' psychological problems due to the tremendous cognitive demand and economic issues they present during their university career development, even under normal conditions [9]. Many articles have been published on general depression during the COVID-19 pandemic. However, according to the literature, few studies have been carried out in populations of dental students, and even fewer meet quality criteria. Of these, only one has been carried out in a Latin American population such as Brazil. Therefore, we consider that this article contributes to the study of the prevalence of depression in Latin American dental students. The prevalence in this population can be very different from the others due to specific social and economic factors present in Latin America (for example, gender inequalities, lower economic income, less access to technology and less access to psychological care in Latin American countries) that are very different to those present in first world European, Asian or North American countries [10–12].

This study hypothesizes that the prevalence of depression in dental students from El Salvador is higher than that reported in studies from Europe, Asia, and North America. This study aims to know the different degrees of depression among dental students from the University of El Salvador Faculty of Dentistry and their opinion on the effectiveness of the hybrid model of learning implemented during the final months of the COVID-19 pandemic. Therefore, the first objective of this study is to know the different degrees of depression among dental students at the Faculty of Dentistry of the University of Salvador. The study's second objective is to know the opinion on the effectiveness of the hybrid learning model that the University has implemented during the final months of the pandemic.

Materials and methods

Study type

This was an observational, descriptive, and analytical study. The questionnaires used in this study were distributed to dental students at the Faculty of Dentistry of the University of Salvador between October, November, and December 2021 (Tables 1 and 5). The questionnaires were applied individually through the google forms platform.

Table 1 Questionnaire to determine levels of depression of dental students (PHQ-9).

Over the last four weeks, how often have you been bothered by any of the following problems?	**Not at all**	**Several days**	**More than half the days**	**Nearly every day**
1. Little interest or pleasure in doing things?	0	1	2	3
2. Feeling down, depressed or hopeless	0	1	2	3
3. Trouble falling asleep, staying asleep, or sleeping too much	0	1	2	3
4. Feeling tired or having little energy	0	1	2	3
5. Poor appetite or overeating	0	1	2	3
6. Feeling bad about yourself - or that you're a failure or have let yourself or your family down	0	1	2	3
7. Trouble concentrating on things, such as reading the newspaper or watching television	0	1	2	3
8. Moving or speaking so slowly that other people could have noticed. Or, the opposite - being so fidgety or restless that you have been moving around a lot more than usual	0	1	2	3
9. Thoughts that you would be better off dead or hurting yourself in some way	0	1	2	3

Table 2 Demographic characteristics from the questionnaire about the hybrid learning model

1. Age (median, range)	21 (17–36)
2. Gender	Frequency (%)
Male	116 (25)
Female	347 (75)
3. Semesters	Frequency (%)
II	105 (22)
IV	138 (30)
VI	82 (18)
VIII	25 (5)
X	31 (6)
XII	37 (9)
XIV	45 (10)

** The results are reported by frequencies and percentages*

Inclusion and exclusion criteria

Inclusion criteria were enrolled students, students of both genders, and students of any age. The exclusion criteria were: students dropped out during the period of application of the questionnaires.

Table 3 Association between levels of depression with age group, gender, and semester of dental students

Factors	**No depression**	**With depression**	**Total**	**Chi-square**	**p-Value**
Age groups					
<21	121	150	271	1,053	0.3048
>21	95	97	192		
Gender					
Female	151	196	347	5,474	0.0193[a]
Male	65	51	116		
Semesters					
Initial	147	178	325	0,8853	0.3468
Advanced	69	69	138		

[a]p < 0.05 indicates a significant association with depression.

Table 4 Demographic characteristics from the questionnaire about the hybrid learning model

1. Age (median, range)	21 (17–36)
2. Gender	Frequency (%)
Male	105 (23)
Female	347 (77)
3. Semesters	Frequency (%)
II	101 (22.3)
IV	127 (28)
VI	75 (16.6)
VIII	32 (7)
X	39 (8.6)
XII	35 (8)
XIV	43 (9.5)

** The results are reported by frequencies and percentages*

Ethical approval

The approval of the ethics committee of the University of El Salvador was obtained.

Questionnaire to determine levels of depression of dental students

The questionnaire used to determine the levels of depression of dental students was the Patient Health Questionnaire-9 (PHQ-9). This questionnaire consisted of two parts: the first included nine questions, and the second included a single question [13]. The PHQ-9 is a questionnaire that evaluates the presence of depression symptoms in the last four weeks. The questionnaire classified the symptoms into 4 degrees of depression, which were:

- • Minimal/no depression (score: 0–4).
- • Mild depression (score: 5–9).
- • Moderate depression (score:10–14).
- • Severe depression (score: 15–27).

In a recent study, the PHQ-9 showed good sensitivity (0.88), specificity (0.85), and 95% confidence interval

Table 5 Questionnaire to evaluate the attitude of students towards the hybrid learning model

Questions	Answers	Frequency	%	95% confidence interval
4. I am satisfied with the effectiveness of learning the online courses:	Strongly agree	29	6.4	0.044–0.091
	In agreement	90	19.9	0.163–0.239
	Neutral	227	50.2	0.455–0.549
	In disagreement	83	18.4	0.149–0.223
	Strongly disagree	23	5.1	0.033–0.076
5. The learning effectiveness of online courses is better than that of face-to-face courses:	Strongly agree	14	3.1	0.017–0.052
	In agreement	22	4.9	0.031–0.073
	Neutral	135	29.9	0.257–0.343
	In disagreement	157	34.7	0.303–0.393
	Strongly disagree	124	27.4	0.234–0.318
6. I think that professional dental lab format courses could change to online courses:	Strongly agree	18	4.0	0.024–0.063
	In agreement	35	7.7	0.055–0.107
	Neutral	97	21.5	0.178–0.255
	In disagreement	156	34.5	0.301–0.391
	Strongly disagree	146	32.3	0.280–0.368
7. Are you worried that covid-19 will create financial pressure for your school studies?	Strongly agree	144	31.9	0.276–0.364
	In agreement	161	35.6	0.312–0.402
	Neutral	114	25.2	0.213–0.295
	In disagreement	28	6.2	0.042–0.089
	Strongly disagree	5	1.1	0.004–0.027
8. Are you worried that the pandemic will affect your learning?	Strongly agree	236	52.2	0.474–0.568
	In agreement	139	30.8	0.265–0.352
	Neutral	66	14.6	0.115–0.182
	In disagreement	8	1.8	0.008–0.035
	Strongly disagree	3	0.7	0.001–0.020
9. My institution quickly adapted to hybrid learning:	Strongly agree	48	10.6	0.080–0.139
	In agreement	147	32.5	0.282–0.370
	Neutral	191	42.3	0.376–0.469
	In disagreement	48	10.6	0.080–0.139
	Strongly disagree	29	6.4	0.044–0.091
10. My institution has organized e-learning appropriately:	Strongly agree	44	9.7	0.072–0.129
	In agreement	150	33.2	0.288–0.377
	Neutral	179	39.6	0.350–0.442
	In disagreement	56	12.4	0.095–0.158
	Strongly disagree	23	5.1	0.033–0.076
11. My institution has provided students with training on teaching tools and software used for distance learning:	Strongly agree	37	8.2	0.059–0.112
	In agreement	131	29.0	0.248–0.334
	Neutral	165	36.5	0.320–0.411
	In disagreement	93	20.6	0.170–0.246
	Strongly disagree	26	5.8	0.038–0.084
12. For the online classes, I mainly used the following equipment:	Laptop/desktop pc	356	78.8	0.746–0.823
	Smartphone	90	19.9	0.163–0.239
	Tablet	5	1.1	0.004–0.027
	Computers in an institution outside of the University (for example, public library, internet cafe)	1	0.2	0.000–0.014
13. For the online classes, I mainly used the following network:	Own network	381	84.3	0.805–0.874
	Mobile data	68	15.0	0.119–0.187
	Public access point	1	0.2	0.000–0.014
	Network in an institution outside of the University (for example, public library, internet cafe)	2	0.4	0.000–0.017

Table 5 Questionnaire to evaluate the attitude of students towards the hybrid learning model

Questions	Answers	Frequency	%	95% confidence interval
4. I am satisfied with the effectiveness of learning the online courses:	Strongly agree	29	6.4	0.044–0.091
	In agreement	90	19.9	0.163–0.239
	Neutral	227	50.2	0.455–0.549
	In disagreement	83	18.4	0.149–0.223
	Strongly disagree	23	5.1	0.033–0.076
5. The learning effectiveness of online courses is better than that of face-to-face courses:	Strongly agree	14	3.1	0.017–0.052
	In agreement	22	4.9	0.031–0.073
	Neutral	135	29.9	0.257–0.343
	In disagreement	157	34.7	0.303–0.393
	Strongly disagree	124	27.4	0.234–0.318
6. I think that professional dental lab format courses could change to online courses:	Strongly agree	18	4.0	0.024–0.063
	In agreement	35	7.7	0.055–0.107
	Neutral	97	21.5	0.178–0.255
	In disagreement	156	34.5	0.301–0.391
	Strongly disagree	146	32.3	0.280–0.368
7. Are you worried that covid-19 will create financial pressure for your school studies?	Strongly agree	144	31.9	0.276–0.364
	In agreement	161	35.6	0.312–0.402
	Neutral	114	25.2	0.213–0.295
	In disagreement	28	6.2	0.042–0.089
	Strongly disagree	5	1.1	0.004–0.027
8. Are you worried that the pandemic will affect your learning?	Strongly agree	236	52.2	0.474–0.568
	In agreement	139	30.8	0.265–0.352
	Neutral	66	14.6	0.115–0.182
	In disagreement	8	1.8	0.008–0.035
	Strongly disagree	3	0.7	0.001–0.020
9. My institution quickly adapted to hybrid learning:	Strongly agree	48	10.6	0.080–0.139
	In agreement	147	32.5	0.282–0.370
	Neutral	191	42.3	0.376–0.469
	In disagreement	48	10.6	0.080–0.139
	Strongly disagree	29	6.4	0.044–0.091
10. My institution has organized e-learning appropriately:	Strongly agree	44	9.7	0.072–0.129
	In agreement	150	33.2	0.288–0.377
	Neutral	179	39.6	0.350–0.442
	In disagreement	56	12.4	0.095–0.158
	Strongly disagree	23	5.1	0.033–0.076
11. My institution has provided students with training on teaching tools and software used for distance learning:	Strongly agree	37	8.2	0.059–0.112
	In agreement	131	29.0	0.248–0.334
	Neutral	165	36.5	0.320–0.411
	In disagreement	93	20.6	0.170–0.246
	Strongly disagree	26	5.8	0.038–0.084
12. For the online classes, I mainly used the following equipment:	Laptop/desktop pc	356	78.8	0.746–0.823
	Smartphone	90	19.9	0.163–0.239
	Tablet	5	1.1	0.004–0.027
	Computers in an institution outside of the University (for example, public library, internet cafe)	1	0.2	0.000–0.014
13. For the online classes, I mainly used the following network:	Own network	381	84.3	0.805–0.874
	Mobile data	68	15.0	0.119–0.187
	Public access point	1	0.2	0.000–0.014
	Network in an institution outside of the University (for example, public library, internet cafe)	2	0.4	0.000–0.017

Table 5 (continued)

Questions	Answers	Frequency	%	95% confidence interval
14. The instructions given by most teachers (exam modes, task solving, etc.) are adapted to distance learning:	Strongly agree	39	8.6	0.062–0.117
	In agreement	150	33.2	0.288–0.377
	Neutral	185	40.9	0.363–0.456
	In disagreement	54	11.9	0.091–0.153
	Strongly disagree	24	5.3	0.035–0.079
15. Most teachers are making an effort to facilitate distance learning:	Strongly agree	88	19.5	0.159–0.234
	In agreement	191	42.3	0.376–0.469
	Neutral	125	27.7	0.236–0.320
	In disagreement	36	8.0	0.057–0.109
	Strongly disagree	12	2.7	0.014–0.047
16. Generally, the teaching materials are adequate for the technical demands of online learning:	Strongly agree	31	6.9	0.047–0.096
	In agreement	164	36.3	0.318–0.409
	Neutral	188	41.6	0.370–0.463
	In disagreement	56	12.4	0.095–0.158
	Strongly disagree	13	2.9	0.016–0.049
17. Teachers have generally organized and adapted to online learning:	Strongly agree	57	12.6	0.097–0.161
	In agreement	170	37.6	0.331–0.422
	Neutral	176	38.9	0.344–0.436
	In disagreement	38	8.4	0.060–0.114
	Strongly disagree	11	2.4	0.012–0.044
18. Which of the following was the most used methodology to teach?	Online classes in zoom	63	13.9	0.109–0.175
	Online classes in teams	24	5.3	0.035–0.079
	Online classes in google classroom	171	37.8	0.333–0.424
	Online classes on the university platform	181	40.0	0.355–0.447
	Whatsapp groups	2	0.4	0.000–0.017
	Daily or weekly tasks	11	2.4	0.012–0.044
19. I am concerned about the lack of practical education:	Strongly agree	280	61.9	0.572–0.664
	In agreement	125	27.7	0.236–0.320
	Neutral	44	9.7	0.072–0.129
	In disagreement	1	0.2	0.000–0.014
	Strongly disagree	2	0.4	0.000–0.017
20. I am afraid that it will not be possible to make up for the lack of practical education during my studies:	Strongly agree	171	37.8	0.333–0.424
	In agreement	149	33.0	0.286–0.375
	Neutral	104	23.0	0.192–0.272
	In disagreement	23	5.1	0.033–0.076
	Strongly disagree	5	1.1	0.004–0.027
21. I feel safe with the measures adopted by my institution to continue with clinical and laboratory practice:	Strongly agree	74	16.4	0.131–0.201
	In agreement	140	31.0	0.267–0.354
	Neutral	169	37.4	0.329–0.420
	In disagreement	43	9.5	0.070–0.126
	Strongly disagree	26	5.8	0.038–0.084
22. I feel confident in serving patients in clinical practices:	Strongly agree	47	10.4	0.078–0.136
	In agreement	95	21.0	0.174–0.251
	Neutral	167	36.9	0.325–0.416
	In disagreement	90	19.9	0.163–0.239
	Strongly disagree	53	11.7	0.089–0.151

Table 5 (continued)

Questions	Answers	Frequency	%	95% confidence interval
23. The pandemic has affected my manual dexterity, and this is reflected in the quality of the treatments I perform:	Strongly agree	74	16.4	0.131–0.201
	In agreement	118	26.1	0.221–0.304
	Neutral	196	43.4	0.387–0.480
	In disagreement	49	10.8	0.082–0.141
	Strongly disagree	15	3.3	0.019–0.055
24. I feel confident caring for patients who have recovered from COVID-19:	Strongly agree	71	15.7	0.125–0.194
	In agreement	123	27.2	0.232–0.316
	Neutral	175	38.7	0.342–0.433
	In disagreement	55	12.2	0.093–0.156
	Strongly disagree	28	6.2	0.042–0.089

* *The results are reported by frequencies and percentages*

(0.82 to 0.88). This study employed the Spanish version of the PHQ-9 questionnaire. The Spanish version previously reported a specificity of 88%, a sensitivity of 87%, and an accuracy of 88% [14]. The cut-off point used to determine clinically essential levels of depression (moderate to severe depression) was a value equal to or greater than 10 points [15, 16]. In addition, the questionnaire included three questions about the primary demographic data of the participants. Those three questions were about age, gender, and the year of the degree that the participant is studying [17] (Table 1).

Questionnaire to evaluate the opinion of students towards the hybrid learning model

The questionnaire to evaluate the students' opinions towards hybrid learning consisted of 24 questions. The questionnaire was developed based on questions asked in previous studies that have already been published and validated [18–20]. The wording of the questions reported in Table 5 was an English translation from the Spanish version. The first three questions were about the primary demographic data of the participants (Table 3). Questions 4–8 were about the effectiveness of online classes and factors that can affect student performance. The following three questions were about the mechanisms applied by the University to carry out online learning. Questions 12 and 13 were about the students' tools to access online classes. Questions 14–17 were about the performance of professors during online courses. Questions 18 through 24 were about students' clinical practice during the pandemic (Table 4).

Statistical Analyses

The Netquest (GfK group, Núremberg, Germany) online application was used to obtain the study's sample size. A population of 463 students, a heterogeneity of 50%, and a confidence level of 95% were used to calculate the minimum sample size required. The minimum sample size was 211 students. The data analysis was carried out with the software GraphPad Prism version 8.3.1. (Graph Pad Software Inc, California, USA). To obtain the level of depression of each student surveyed, we added the score of each question to get the total points. Finally, the levels of depression were divided into two categories, no depression (below 10) and depression (10 and above), by taking a recommended cut-off score of 10 [21] according to the cut-off point with a score of 10, determined in a previous study. Likewise, the different semesters reported by the students were grouped into two categories, beginning semesters (from semesters 2 to 8) and advanced semesters (from semesters 10 to 14). In both questionnaires, ages were reported as medians and ranges, and gender and semester studied were reported as frequencies and percentages. The analysis of factors associated with depression was performed using the Chi-square test. Cronbach's alpha was calculated for the 21 questions that comprise the questionnaire to assess the students' opinion on the hybrid learning model and the nine questions of the PHQ-9 questionnaire. The study used Cronbach's alpha calculation in RStudio version 2021.09.1+372 "Ghost Orchid" Release (RStudio Team (2021). RStudio: Integrated Development Environment for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com/.) and used the "alpha ()" function from the "psych" package.

Results

Sample Characteristics

The total number of students who answered the mental health survey was 463. 75% of respondents were women, and 25% were men. The median age of the participants was 21 years, with a range of 17 to 36 years of age (Table 2). The total number of dentistry students who answered the questionnaire on the effectiveness of the hybrid learning model was 452. 23% were men, and 77% were women. The median age of the participants was 21 years, with a range of 17 to 36 years of age (Table 4).

Depression levels of dentistry students in El Salvador

Cronbach's alpha value for the PHQ-9 questionnaire was 0.86, with a 95% confidence interval of 0.76 to 0.92. According to the methodology of the PHQ-9 questionnaire, surveyed students' levels of depression were classified into four groups, shown in Fig. 1. The entire study population answered the questionnaire (463 students). 43% of the students reported severe depression, 23% of the students reported moderate depression, and 29% of the students had medium depression. Finally, only 14% of the students did not present depression, or it was minimal (Fig. 1). Regarding the association of the variables of age group (<21,>21), gender, and semester studied (initial or advanced) with the different levels of depression, only gender showed a significant association.

Hybrid Learning Model Assessment

Regarding the questionnaire on the attitude and effectiveness of the hybrid model during the pandemic, Cronbach's alpha value was 0.74 (acceptable) with a 95% confidence interval of 0.64–0.82.

Effectiveness of online classes and factors that can affect student performance (Questions 4–8)

Most students were neutral about the effectiveness of online learning, followed by disagreement with online learning (about 60%). Likewise, most students disagreed that the clinical practice laboratories could be taken online (about 67%), and most agreed that the epidemic would affect their learning and cause economic problems (about 66%).

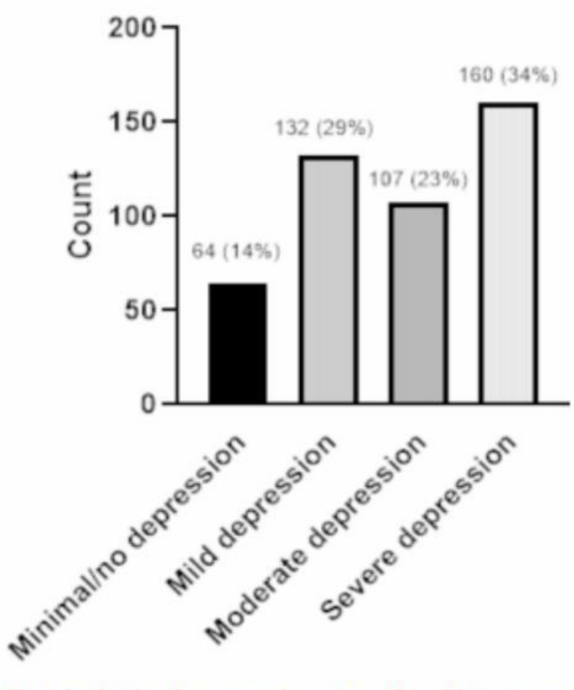

Fig. 1 Results of the frequency and percentage of the different degrees of depression

Mechanisms applied by the University to carry out online learning (Questions 9–11)

42% of students considered that the University quickly adapted to the hybrid model, and the other 42% had a neutral opinion (question 9). Likewise, approximately 43% of students considered that the University organized online learning adequately, while the other 40% had a neutral opinion (question 10). Finally, 37% of the students considered that the institution provided adequate tools and training for online learning, while 36.5% had a neutral opinion (question 11).

Students' devices to access online classes (Questions 12 and 13)

Around 80% of the students had their own laptop/desktop pc and internet network.

Professors' performance during online classes (Questions 14–17)

Approximately 40% of the students agreed that the instructions given by the professors were well adapted to distance learning, while the other 60% had a neutral opinion (question 14). 60% of the students considered that the professors made an effort to facilitate distance learning for their students (question 15). Forty-three-point-2% of the students felt that the teaching materials during online learning were adequate, and about 40% had a neutral opinion (question 16). Approximately 40% of the students consider that the teachers have adapted to distance learning, while the other 60% had a neutral opinion (question 17).

Platforms used for online learning (question 18)

The three leading platforms used for online learning were the university platform, google classroom, and zoom.

Students' clinical practice during the pandemic (Questions 19–24)

90% of students were concerned about the lack of clinical practice (question 19). 70% of the students considered that they could not recover the clinical and laboratory course during the rest of their studies (question 20). Around 40% of the students feel safe with the measures taken by the University to continue with clinical practices and laboratories, while the other 60% had a neutral opinion (question 21). Only 30% of students felt safe when treating patients, 37% had an impartial idea, and the remaining 23% did not feel safe (question 22). Approximately 53% of the students did not consider that they had lost manual dexterity during the pandemic (question 23). Finally, about 42% of the students felt safe when treating patients who recovered from COVID-19, while approximately 40% had a neutral opinion (question 24).

Discussion

The study's objectives were to know the degrees of depression of dental students during the contingency due to the COVID-19 pandemic and dental students' opinions of hybrid learning implemented by the University of El Salvador Faculty of Dentistry during the COVID-19 pandemic. As far as we know, no studies have been carried out in Latin American countries where the presence of depression in dental students was investigated and where the opinion of students regarding the hybrid learning model was analyzed.

The PHQ-9 was the questionnaire used in this study to detect levels of depression in dental students [22]. This questionnaire has been widely used in previous similar studies [22, 23]. Other widely used questionnaires for the same purpose are the DASS-21 and HADS questionnaire [10, 24]. The total number of students who answered the PHQ-9 questionnaire was 463. Our study has the second largest sample size, only after the survey by Siddiqui & Qian (2021), in which the sample size was 655 students. Likewise, this study has the first place in sample size (463 students) in a Latin American country [25]. The second place is occupied by the study by Medeiros et al. in Brazil, with a sample size of 113 students [10].

The median age of the students who answered the two questionnaires was 21. This data coincides with similar studies in which a mean age of 21 was reported [10]. However, there are studies where the average age reaches 25 [24]. In this study, the percentage of women and men was 75% and 25%, respectively. These percentages are similar to those reported by previous studies. For example, in the survey by Medeiros et al., the authors noted that of the sample studied, 77% were women and, 23% were men [10]. In the German study by Mekhemar et al., the authors reported a percentage of women of 74% and men of 26% [26]. Two studies conducted in Malaysia reported 79% women and 21% men [25, 27]. Shailaja et al. reported 82% of women and 18% of men [28]. On the other hand, Hakami et al. reported more balanced percentages of men and women. The authors reported 55% women and 45% men [29]. The differences in the average age and the ratios of men and women between this study and previous studies are mainly due to the different populations studied. The differences in the number of respondents between the two questionnaires are because the questionnaires were applied independently.

Before the COVID-19 pandemic, depression in dental and medical students was approximately 28% in the US [30, 31]. Previous studies on the prevalence of depression during the general population pandemic report range from 12 to 31% [32, 33]. Deep et al. surveyed the pandemic in which they reported a 9% prevalence of depression in 199 dental students; in this study, the authors used the PHQ-9 questionnaire [34]. Knipe et al. also used the PHQ-9 questionnaire during the pandemic to report the prevalence of depression in dental students. The authors found a prevalence of depression of 35.4% in 344 dental students [35].

This study's prevalence of moderate and severe depression (>10) was 57%. The increased prevalence of depression may be due to the COVID-19 pandemic, which exerts more psychological stress on dental students than they experience under normal conditions. This percentage coincides with similar studies also carried out during the covid-19 pandemic. For example, Medeiros et al. reported with the PHQ-9 a prevalence of depression of 39.4% in 113 dental students in Brazil during the COVID-19 pandemic [10]. Chi et al. also registered with the PHQ-9 a prevalence of depression of 14.4% in 14 US dental students. However, the author's sample size was meager, invalidating the results [23]. Kwaik et al. reported a 70% prevalence of depression in 305 Palestinian dental students. However, the questionnaire used for screening for depression was not the PHQ-9; the authors used the DASS-21 questionnaire, which could explain the high percentage of depression reported [36]. Hakami et al. used the DASS-21 questionnaire to register a prevalence of depression of 60.7% in 422 Saudi Arabian students [29]. Gas et al. used the DASS-21 questionnaire to report a prevalence of depression of 27.2% in 190 dental students from Turkey [37]. It is crucial to consider that the studies mentioned above were carried out during the initial and intermediate stages of the development of the pandemic. In contrast, our research was carried out in the final step. This difference in methodology could explain the considerable variation in the reported percentages of depression questionnaires used to detect depression and the different sample sizes. Finally, our study found a positive association between the degree of depression and female gender, coinciding with the report by Medeiros et al. [10]. However, other studies do not find an association between gender and levels of depression [25]. In general, this study's prevalence of depression in dental students (57%) is higher than that reported in previous studies in Europe, Asia, and North America. For example, in a study that analyzed the mental health of medical science students (including dental students) in 9 countries, an overall prevalence of depression of 40% was found. This study included the countries of Mexico, Colombia, Venezuela, Chile, Brazil, Spain, Germany, Italy, and Japan [38].

A study in the USA reported a prevalence of depression of 14.4% [23]. Two studies conducted in India registered a prevalence of depression of 53.5% and 20% [22, 28]. Alfadley et al. reported a prevalence of depression of 10.9% [24]. Likewise, two studies in Malaysia reported depression in dental students at 24% and 33.6% [25, 27]. In addition, some studies report that COVID-19 infection in relatives of dental students multiplies by three the probability that they will develop symptoms of depression [39]. The above analyses were conducted during the COVID-19 pandemic and in dental students.

Regarding dental students' attitudes towards the hybrid learning, questions 4 through 8 assess the effectiveness of online classes. Most dental students were neutral (50%) or disagreed (55%) on the efficacy of online learning, which coincides with similar studies reporting that 45% of dental students surveyed indicate that online learning needs to improve to be more effective [19]. In questions 9, 10, and 11 were about the mechanisms applied by the University to carry out online learning, 40% of the students had a neutral opinion, and another 40% agreed that the faculty had adequately adapted to the hybrid model and provided the appropriate tools for online learning. In a study in Jordan, students reported feeling comfortable (54%) with how the faculty implemented online teaching [20]. So, the hybrid model applied in the faculty of El Salvador has a degree of acceptance similar to those used in other parts of the world. Likewise, in this study (questions 12 and 13), 80% of the students had the necessary tools to take classes online. Access to online courses is similar to other studies; for example, in a survey conducted in India, 86.1% of students reported accessing online classes regularly [40]. In questions 14–17 (professors' performance during online courses), 40 and 60% of the students consider that the teachers adapted excellently to online teaching. A similar study affirms this data in Italy, where dental students indicated that 70% of teachers had successfully adapted to online instruction [41]. In this study, the most used platforms to take classes online were the university platform, google classroom, and zoom. These data are very similar to a study in Brazil, where the leading platforms were virtual meetings (Zoom/Skype), the educational platform Moodle and the University system [42]. In questions 19–24 (students' clinical practice during the pandemic), 90% of dental students are concerned about the lack of clinical practice. Several similar studies during the COVID-19 pandemic are consistent with these findings. For example, Etajuri et al. report that more than 50% of dental students do not feel satisfied with the clinical practice received during the pandemic [43]. Hattar et al. said 87% of dental students indicated their clinical practices were affected during the pandemic [20]. Finally, in this study, less than half of the students reported feeling safe when treating patients or with the protection measures adopted by the faculty. This trend has been reported in previous studies [44]. The general result of the questionnaire on the hybrid learning model indicates that the students were not affected by this learning model, which seems to contradict the depression levels obtained in this study and the results of similar studies. For example, a study conducted at a Lebanese University reported that online learning is associated with increased levels of depression in students [45]. A survey of students from public and private universities in Malaysia reported similar results [46]. The different results between the studies mentioned above and ours could be due to other diagnostic methods for depression and the diverse populations of students and university courses.

Likewise, each region's economic, social, and personality factors can affect the prevalence of depression in students [47]. Latin American countries face aspects of their socioeconomic conditions that can affect mental health—for example, the lack of food in various areas of difficult access [48]. Alfayumi-Zeadna et al. reported that some economic and social factors that increased depression in Israeli students during the pandemic were: low income, job loss, region of residence, marital status, whether they own their home or not, and income level [49]. Yin et al. reported that medical students with low social support were more likely to have high levels of depression [50]. Browning et al. conducted a study in seven states in the United States where they analyzed the social and economic factors that affected students' mental health during the COVID-19 pandemic. The main factors that influenced the students' mental health were: not being in good health, spending little time outdoors, having low income, spending much time in front of the computer, and being a woman. The latter coincides with previous studies that have reported a higher prevalence of depression in women due to different factors such as hormones, interpersonal violence after childhood, body shame and dissatisfaction [51].

Gębska et al. analyzed the relationship between the appearance of physical symptoms (Stomatognathic System Disorders) and the stress generated during the COVID-19 pandemic in physiotherapy students. The authors found a connection between physical symptoms and students with type D personality ('distressed personality') [52]. Type D personality is a type of personality with the characteristic of being more susceptible and generating higher stress levels in complicated situations such as the COVID-19 pandemic. Due to the above, people with this personality type are also more vulnerable to developing moderate or severe levels of depression [53, 54]. With the presence of psychological disorders such as depression, not only did the frequency of temporomandibular disorders increase in students but also increased bruxism associated with depression in dental students during the pandemic [55]. Shailaja et al. reported that cyberchondria (when the excessive search for information about a disease on the internet increases the concern about the said disease) is also associated with high stress, anxiety, and depression levels in dental students during the COVID-19 pandemic [28].

Other studies have reported the co-occurrence of psychological disorders and alcohol abuse [56]. For example, the study by Fernandez et al. reported a relationship between alcohol abuse and moderate or severe anxiety levels in dental students in various regions of Brazil during the COVID-19 pandemic [57]. In addition, alcohol abuse by college students during the COVID-19 pandemic was associated with increased suicidal behavior [58–60]. The study by Chang et al. reported that students from rural areas and non-medical majors had fewer psychological symptoms (most had anxiety) compared with students from the suburbs and in medical majors (most had depression) [61]. As reported by Sanabria-Mazo et al., perhaps one of the main factors influencing the development of depression in Latin American students is social inequities (such as income level, employment status, education level, ethnic group, area of residence, and religion) [62]. Likewise, one way to reduce the psychological impact of COVID-19 on Latin American students is through self-employment and entrepreneurship, which helped reduce economic and social inequalities during the pandemic [63].

One of this study's strengths is that the sample size was more extensive than most studies in similar populations. In addition, it was possible to analyze practically the entire population of interest in this study. Regarding the limitations, the questionnaires were applied individually, so we could not determine associations between the variables. The questionnaires were only used in one University, so it is difficult to extrapolate the results to the population of dental students throughout the country.

Conclusions

According to the results of this study, 57% of the students presented moderate or severe levels of depression, which makes them candidates for receiving psychological attention. Therefore, this article contributes to a better understanding of this problem in this type of population [12]. Regardless of the levels of depression, the opinion of the students towards the hybrid learning model turns out to be quite good.

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Author's Contribution
Conceptualization, Marco Felipe Orozco, Wendy Yesenia Escobar de González, and Miguel Angel Santana; Data curation, Marco Felipe Orozco, Wendy Yesenia Escobar de González, and Ricardo Martinez Rider and Nuria Patiño Marín; Formal analysis, Nuria Patiño Marín and Ricardo Martinez Rider; Investigation, Juan Carlos Hernandez Cabanillas, Ivan Acosta, Ricardo Martinez Rider and Miguel Angel Santana; Methodology, Marco Felipe Orozco, Wendy Yesenia Escobar de González, and Jesus Ramón Castillo-Hernandez; Supervision, Jesus Ramón Castillo-Hernandez, Juan Carlos Hernandez Cabanillas, Ricardo Martinez Rider and Ivan Acosta; Writing – original draft, Marco Felipe Orozco and Miguel Angel Santana; Writing – review & editing, Nuria Patiño Marín, Jesus Ramón Castillo-Hernandez, Juan Carlos Hernandez Cabanillas, Ivan Acosta, and Miguel Angel Santana.

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Data Availability
The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
The protocol was approved by the ethics committee of the University of El Salvador; all participants signed informed consent before their participation. All methods were performed in accordance with the relevant guidelines and regulations.

Consent for publication
Not applicable.

Competing interests
I declare that the authors have no competing interests as defined by BMC or other interests that might be perceived to influence the results and discussion reported in this paper.

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References

1. WHO, Global. El Salvador [Internet]. WHO. Global: El Salvador. [citado 22 de julio de 2022]. Disponible en: https://covid19.who.int/region/amro/country/sv
2. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The lancet. 2020;395(10223):497–506.
3. Miller L, Luković E, Wagener G. Guiding airway management and personal protective equipment for COVID-19 intubation teams. Br J Anaesth. 2020;125(3):e288–90.
4. Daniel SJ. Education and the COVID-19 pandemic. Prospects. 2020;49(1):91–6.
5. Jum'ah AA, Elsalem L, Loch C, Schwass D, Brunton PA. Perception of health and educational risks amongst dental students and educators in the era of COVID-19. Eur J Dent Educ. 2021;25(3):506–15.
6. Alsharif AT, Alsharif B, Alsharif L, Althagafi N, Natto ZS, Kassim S. Effectiveness of WhatsApp as a part of a hybrid learning environment: an opportunity for post-COVID-19 pandemic pedagogy. J Contemp Dent Pr. 2020;21:1331–6.
7. Elkhatat AM, Al-Muhtaseb SA. Hybrid online-flipped learning pedagogy for teaching laboratory courses to mitigate the pandemic COVID-19 confinement and enable effective, sustainable delivery: investigation of attaining course learning outcome. SN Soc Sci. 2021;1(5):1–16.
8. Goldmann E, Galea S. Mental health consequences of disasters. Annu Rev Public Health. 2014;35(1):169–83.
9. Saleh D, Camart N, Romo L. Predictors of stress in college students. Front Psychol. 2017;8:19.
10. Medeiros RAD, Vieira DL, Silva EVFD, Rezende LVMDL, Santos RWD, Tabata LF. Prevalence of symptoms of temporomandibular disorders, oral behaviors, anxiety, and depression in Dentistry students during social isolation due to COVID-19.J Appl Oral Sci. 2020;28.
11. Santabarbara J, Idoiaga N, Ozamiz-Etxebarria N, Bueno-Notivol J. Prevalence of anxiety in Dental students during the COVID-19 outbreak: a Meta-analysis. Int J Environ Res Public Health. 2021;18(20):10978.

12. Santabárbara J, Ozamiz-Etxebarria N, Idoiaga N, Olaya B, Bueno-Novitol J. Meta-analysis of the prevalence of depression in dental students during COVID-19 pandemic. Med (Mex). 2021;57(11):1278.
13. Spitzer RL, Kroenke K, Williams JB, Patient Health Questionnaire Primary Care Study Group, Patient Health Questionnaire Primary Care Study Group. Validation and utility of a self-report version of PRIME-MD: the PHQ primary care study. JAMA. 1999;282(18):1737–44.
14. Diez-Quevedo C, Rangil T, Sanchez-Planell L, Kroenke K, Spitzer RL. Validation and utility of the patient health questionnaire in diagnosing mental disorders in 1003 general hospital spanish inpatients. Psychosom Med. 2001;63(4):679–86.
15. Manea L, Gilbody S, McMillan D. A diagnostic meta-analysis of the Patient Health Questionnaire-9 (PHQ-9) algorithm scoring method as a screen for depression. Gen Hosp Psychiatry. 2015;37(1):67–75.
16. Kroenke K, Spitzer RL, Williams JB. The PHQ-9: validity of a brief depression severity measure. J Gen Intern Med. 2001;16(9):606–13.
17. Levis B, Benedetti A, Thombs BD. Accuracy of Patient Health Questionnaire-9 (PHQ-9) for screening to detect major depression: individual participant data meta-analysis.bmj. 2019;365.
18. Cheng HC, Lu SL, Yen YC, Siewchaisakul P, Yen AMF, Chen SLS. Dental education changed by COVID-19: Student's perceptions and attitudes. BMC Med Educ. 2021;21(1):1–9.
19. Puljak L, Čivljak M, Haramina A, Mališa S, Čavić D, Klinec D, et al. Attitudes and concerns of undergraduate university health sciences students in Croatia regarding complete switch to e-learning during COVID-19 pandemic: a survey. BMC Med Educ. 2020;20(1):1–11.
20. Hattar S, AlHadidi A, Sawair FA, Alraheam IA, El-Ma'aita A, Wahab FK. Impact of COVID-19 pandemic on dental education: online experience and practice expectations among dental students at the University of Jordan. BMC Med Educ. 2021;21(1):1–10.
21. Manea L, Gilbody S, McMillan D. Optimal cut-off score for diagnosing depression with the Patient Health Questionnaire (PHQ-9): a meta-analysis. CMAJ. 2012;184(3):E191–6.
22. Chakraborty T, Subbiah GK, Damade Y. Psychological distress during COVID-19 lockdown among dental students and practitioners in India: a cross-sectional survey. Eur J Dent. 2020;14(S 01):70–8.
23. Chi DL, Randall CL, Hill CM. Dental trainees' mental health and intention to leave their programs during the COVID-19 pandemic. J Am Dent Assoc. 2021;152(7):526–34.
24. Khanagar SB, Alfadley A. Psychological impact of the COVID-19 pandemic on dental interns in Riyadh, Saudi Arabia: a cross-sectional survey. Int J Clin Pediatr Dent. 2020;13(5):508.
25. Siddiqui FS, Qian G. Psychological impact of self-quarantine on Malaysian dental students during COVID-19 pandemic.Med J Dr DY Patil Vidyapeeth. 2021
26. Mekhemar M, Attia S, Dörfer C, Conrad J. Dental students in Germany throughout the COVID-19 pandemic: a psychological assessment and cross-sectional survey. Biology. 2021;10(7):611.
27. Samsudin ADB, Jaafar AB, Idaham NIB, Jali MHBM. The Effect of COVID-19 Pandemic on Psychological Wellness among Dental Students of a Malaysian Public University.Ulum Islam Malays J Islam Sci. 2021
28. Shailaja B, Shetty V, Chaudhury S, Thyloth M. Exploring cyberchondria and its associations in dental students amid COVID-19 infodemic. Ind Psychiatry J. 2020;29(2):257.
29. Hakami Z, Khanagar SB, Vishwanathaiah S, Hakami A, Bokhari AM, Jabali AH, et al. Psychological impact of the coronavirus disease 2019 (COVID-19) pandemic on dental students: a nationwide study. J Dent Educ. 2021;85(4):494–503.
30. Rotenstein LS, Ramos MA, Torre M, Segal JB, Peluso MJ, Guille C, et al. Prevalence of depression, depressive symptoms, and suicidal ideation among medical students: a systematic review and meta-analysis. JAMA. 2016;316(21):2214–36.
31. Lerman AR, Yamamoto KK, Taylor GW, Saeed SG. High depressive symptom prevalence in dental students associated with lifestyle and well-being characteristics. J Dent Educ. 2020;84(7):771–80.
32. Batra K, Sharma M, Batra R, Singh TP, Schvaneveldt N. Assessing the psychological impact of COVID-19 among college students: an evidence of 15 countries. En MDPI; 2021. p. 222.
33. Wang ZH, Yang HL, Yang YQ, Liu D, Li ZH, Zhang XR, et al. Prevalence of anxiety and depression symptom, and the demands for psychological knowledge and interventions in college students during COVID-19 epidemic: a large cross-sectional study. J Affect Disord. 2020;275:188–93.
34. Deeb GR, Braun S, Carrico C, Kinser P, Laskin D, Golob Deeb J. Burnout, depression and suicidal ideation in dental and dental hygiene students. Eur J Dent Educ. 2018;22(1):e70–4.
35. Knipe D, Maughan C, Gilbert J, Dymock D, Moran P, Gunnell D. Mental health in medical, dentistry and veterinary students: cross-sectional online survey. BJPsych Open. 2018;4(6):441–6.
36. Abu Kwaik A, Saleh R, Danadneh M, Kateeb E. Stress, anxiety and depression among dental students in times of covid-19 lockdown. 2021
37. Gaş S, Ekşi Özsoy H, Cesur Aydın K. The association between sleep quality, depression, anxiety and stress levels, and temporomandibular joint disorders among Turkish dental students during the COVID-19 pandemic.CRANIO®. 2021;1–6.
38. Michaeli D, Keough G, Perez-Dominguez F, Polanco-Ilabaca F, Pinto-Toledo F, Michaeli J, et al. Medical education and mental health during COVID-19: a survey across 9 countries. Int J Med Educ. 2022;13:35.
39. Morales-Montoya M, Córdova-Limaylla N, Briceño-Vergel G, Ladera-Castañeda M, Garcia-Luna G, Cachay-Criado H, et al. Psychological impact on dental students and professionals in a Lima population during COVID-19s wave: a study with predictive models. Sci Rep. 2022;12(1):1–12.
40. Shrivastava KJ, Nahar R, Parlani S, Murthy VJ. A cross-sectional virtual survey to evaluate the outcome of online dental education system among undergraduate dental students across India amid COVID - 19 pandemic. Eur J Dent Educ. 2022;26(1):123–30.
41. Varvara G, Bernardi S, Bianchi S, Sinjari B, Piattelli M. Dental education challenges during the COVID-19 pandemic period in Italy: undergraduate student feedback, future perspectives, and the needs of teaching strategies for professional development. En MDPI; 2021. p. 454.
42. Silva PG, de B CAL, Borges MMF, Moreira DM, Alencar PNB, Avelar RL, et al. Distance learning during social seclusion by COVID-19: improving the quality of life of undergraduate dentistry students. Eur J Dent Educ. 2021;25(1):124–34.
43. Etajuri EA, Mohd NR, Naimie Z, Ahmad NA. Undergraduate dental students' perspective of online learning and their physical and mental health during COVID-19 pandemic. PLoS ONE. 2022;17(6):e0270091.
44. Almulhim B, Alassaf A, Alghamdi S, Alroomy R, Aldhuwayhi S, Aljabr A, et al. Dentistry amidst the COVID-19 pandemic: knowledge, attitude, and practices among the saudi arabian dental students. Front Med. 2021;8:654524.
45. Fawaz M, Samaha A. E-learning: Depression, anxiety, and stress symptomatology among lebanese university students during COVID - 19 quarantine. En Wiley Online Library; 2021. pp. 52–7.
46. Moy FM, Ng YH. Perception towards E-learning and COVID-19 on the mental health status of university students in Malaysia. Sci Prog. 2021;104(3):00368504211029812.
47. Langer ÁI, Crockett MA, Bravo-Contreras M, Carrillo-Naipayan C, Chaura-Marió M, Gómez-Curumilla B et al. Social and Economic Factors Associated With Subthreshold and Major Depressive Episode in University Students During the COVID-19 Pandemic.Front Public Health. 2022;10.
48. Zila-Velasque JP, Grados-Espinoza P, Quispe-Chura K, Valdiviezo-Morales CG, Diaz-Vélez C, Valladares-Garrido MJ. Prevalence and factors associated with food insecurity in eight high-altitude cities in Peru during the second wave of the COVID-19 pandemic: a retrospective, cross-sectional study. BMC Public Health. 2022;22(1):1–13.
49. Alfayumi-Zeadna S, Gnaim-Abu Touma L, Weinreich M, O'Rourke N. COVID-19 and Mental Health of Minority Arab Higher-Education students in Israel: Social, Economic, and academic factors. Int J Environ Res Public Health. 2022;19(20):13466.
50. Yin Y, Yang X, Gao L, Zhang S, Qi M, Zhang L et al. The association between social support, COVID-19 exposure, and medical students' mental health. Front Psychiatry. 2021;622.
51. Browning MH, Larson LR, Sharaievska I, Rigolon A, McAnirlin O, Mullenbach L, et al. Psychological impacts from COVID-19 among university students: risk factors across seven states in the United States. PLoS ONE. 2021;16(1):e0245327.
52. Gębska M, Dalewski B, Pałka Ł, Kołodziej Ł, Sobolewska E. Type D personality and Stomatognathic System Disorders in Physiotherapy students during the COVID-19 pandemic. J Clin Med. 2021;10(21):4892.
53. Gębska M, Kołodziej Ł, Dalewski B, Pałka Ł, Sobolewska E. The influence of the COVID-19 pandemic on the stress levels and occurrence of Stomatoghnatic System Disorders (SSDs) among Physiotherapy students in Poland. J Clin Med. 2021;10(17):3872.
54. Gębska M, Dalewski B, Pałka Ł, Kołodziej Ł, Sobolewska E. The importance of type d personality in the development of temporomandibular disorders

(TMDs) and depression in students during the COVID-19 pandemic. Brain Sci. 2021;12(1):28.

55. Kolak V, Pavlovic M, Aleksic E, Biocanin V, Gajic M, Nikitovic A, et al. Probable bruxism and psychological issues among Dental students in Serbia during the COVID-19 pandemic. Int J Environ Res Public Health. 2022;19(13):7729.
56. Lai HMX, Cleary M, Sitharthan T, Hunt GE. Prevalence of comorbid substance use, anxiety and mood disorders in epidemiological surveys, 1990–2014: a systematic review and meta-analysis. Drug Alcohol Depend. 2015;154:1–13.
57. Fernandez M, Vieira S, Silva IS, Cardoso NRJ, de Bielavski T, Rakovski CH et al. C,. Anxiety symptoms and alcohol abuse during the COVID-19 pandemic: A cross - sectional study with Brazilian dental undergraduate students. J Dent Educ. 2021;85(11):1739-48.
58. Xu Y, Su S, Jiang Z, Guo S, Lu Q, Liu L et al. Prevalence and risk factors of mental health symptoms and suicidal behavior among university students in Wuhan, China during the COVID-19 pandemic.Front Psychiatry. 2021;12.
59. Pramukti I, Strong C, Sitthimongkol Y, Setiawan A, Pandin MGR, Yen CF, et al. Anxiety and suicidal thoughts during the COVID-19 pandemic: cross-country comparative study among indonesian, taiwanese, and Thai University students. J Med Internet Res. 2020;22(12):e24487.
60. Demenech LM, Neiva-Silva L, Brignol SMS, Marcon SR, Lemos SM, Tassitano RM et al. Suicide risk among undergraduate students in Brazil in the periods before and during the COVID-19 pandemic: results of the SABES-Grad national survey.Psychol Med. 2022;1–13.
61. Chang J, Yuan Y, Wang D. Mental health status and its influencing factors among college students during the epidemic of COVID-19. Nan Fang Yi Ke Da Xue Xue Bao. 2020;40(2):171–6.
62. Sanabria-Mazo JP, Useche-Aldana B, Ochoa PP, Rojas-Gualdrón DF, Mateo-Canedo C, Carmona-Cervelló M, et al. Social inequities in the impact of COVID-19 lockdown measures on the mental health of a large sample of the colombian population (PSY-COVID study). J Clin Med. 2021;10(22):5297.
63. Hernández-Sánchez BR, Cardella GM, Sánchez-García JC. Psychological factors that lessen the impact of covid-19 on the self-employment intention of business administration and economics students from Latin America. Int J Environ Res Public Health. 2020;17(15):5293.

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Inhibición in vitro del crecimiento de enterococcus faecalis empleando cementos para endodoncia

In vitro inhibition of the growth of enterococcus faecalis using endodontic sealers

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RESUMEN
Objetivo: Evaluar la inhibición del crecimiento de la bacteria *Enterococcus faecalis* (ATCC® 29212™) en presencia de cementos selladores (CS) utilizados en tratamientos endodónticos mediante experimentación in vitro. **Materiales y Métodos:** Se empleó un diseño metodológico de tipo experimental basado en ensayos de laboratorio. Se utilizó el método de difusión en agar para analizar la actividad antibacteriana de tres CS: EndoSequence (BC Sealer), AHPlus (Dentsply De Trey) y Sealapex (Kerr). Se organizaron los datos y se graficaron los resultados. **Resultados:** Se obtuvo una mayor inhibición del crecimiento bacteriano con el cemento AHPlus (1.50 mm), seguido de EndoSequence (1.10 mm), el CS Sealapex mostró muy poca actividad inhibitoria de *E. faecalis* (0.2 mm). **Conclusión:** La inhibición del crecimiento de *E. faecalis* por la presencia de los CS utilizados en endodoncia fue distinta entre sí, existe variabilidad importante en la capacidad antibacteriana de los CS analizados, los hallazgos de este estudio confirman los datos reportados en otros estudios, mientras que para algunos cementos fueron contrastantes.
. **Palabras clave:** *Enterococcus faecalis*, inhibición bacteriana, cementos endodónticos.

ABSTRACT
Objective: To evaluate the inhibition of the growth of *Enterococcus faecalis* bacteria (ATCC® 29212™) in the presence of sealer cements (CS) used in endodontic treatments by means of in vitro experimentation. **Materials and Methods:** An experimental methodological design based on laboratory tests was used. The agar diffusion method was used to analyze the antibacterial activity of three CS: Endosequence (BC Sealer), AHPlus (Dentsply De Trey) and Sealapex (Kerr). The data were organized and the results were plotted. **Results:** The highest inhibition of bacterial growth was obtained with AHPlus cement (1.50 mm), followed by Endosequence (1.10 mm), the CS Sealapex showed very little inhibitory activity of *E. faecalis* (0.2 mm). **Conclusion:** The inhibition of the growth of *E. faecalis* by the presence of the CS used in endodontics was different from each other, there is significant variability in the antibacterial capacity of the CS analyzed, the findings of this study confirm the data reported in other studies, while for some cements were contrasting.
Keywords: *Enterococcus faecalis*, bacterial inhibition, endodontic cements.

INTRODUCCIÓN

El objetivo primario del tratamiento de conductos radiculares en endodoncia es preservar la integridad de las estructuras periapicales y, en caso de no existir, dar los medios propicios para devolver el estado de salud[1].

Las bacterias y sus bioproductos son el principal factor etiológico en la patología pulpar y periapical, por lo tanto, la desinfección de forma eficiente del sistema de conductos radiculares es determinante en el éxito endodóntico[1,2].

El tratamiento de conductos comprende varios pasos operatorios que juntos forman una cadena tan fuerte como su eslabón más débil, esos pasos operatorios son: la conformación, que implica dar una forma geométrica específica al sistema de conductos radiculares, la desinfección mediante agentes irrigantes bactericidas y la obturación, la principal función de este último paso de obturación es impedir la filtración y proliferación de bacterias mediante el sellado de los conductos radiculares previamente conformados[3], este proceso se realiza con materiales que promueven la reparación apical y eviten propagación de las bacterias que pudieran encontrarse en zonas inaccesibles como tubulillos dentinarios, conductos laterales y conductos accesorios; mediante un sellado estable y tridimensional que no interfiera con el proceso de reparación[4].

Se han recomendado un gran número de materiales para la obturación del sistema de conductos radiculares. La mayoría de las técnicas emplean un material sólido como la gutapercha y un cemento sellador. Independientemente del material sólido, un cemento es esencial para cada técnica y ayuda a lograr un sellado eficiente[5].

Los cementos selladores son una interfaz entre el material de obturación sólido y la pared de dentina. Los selladores también ocupan espacios e irregularidades en el conducto radicular, los conductos laterales y accesorios, además de los espacios entre los conos de gutapercha accesorios utilizados en la técnica de compactación lateral. Además de servir como lubricantes durante el proceso de obturación[6].

De acuerdo con los postulados de Grossman, definidos por primera vez en 1988, los requisitos ideales de un cemento sellador son: la habilidad para adherirse a la superficie radicular tridimensionalmente, no citotóxico y bien tolerado por los tejidos periradiculares, buena estabilidad dimensional, no reabsorbible, insoluble en tejidos orales, soluble con disolventes, fácil de manipular, buena radiopacidad, que no produzca corrosión, proveer un sellado hermético, no pigmentar la estructura, y ser bacteriostático o bactericida[7].

La periodontitis apical es producto de la interacción entre los diferentes microorganismos presentes en las infecciones intraradiculares. Una de las principales causas de fracaso en endodoncia es la presencia de especies bacterianas, un claro ejemplo de este grupo es *Enterococcus faecalis*[6], la cual es una bacteria gram positiva, comensal en el tracto gastrointestinal de los seres humanos. Se ha encontrado con frecuencia en los dientes con fracaso endodóntico en valores de prevalencia que van del 30 al 90%, además de tener nueve veces más probabilidades de encontrarse en este tipo de casos que en los casos de infecciones primarias[8].

Una característica notable de *E. faecalis* la constituye su capacidad para sobrevivir y crecer en microambientes que pudieran ser inadecuados para un gran número de bacterias, esta capacidad de resistencia por parte de *E. faecalis* en microambientes complejos está relacionada con la supervivencia en los sistemas de conductos radiculares de dientes que han sido sometidos a tratamiento endodóntico y en los cuales los nutrientes son limitados[8], éste es el motivo por el cual esta especie bacteriana es excelente para observar la capacidad bactericida o bacteriostática de diferentes materiales de uso endodóntico.

Los cementos de más uso en el área de endodoncia son los formulados a partir de óxido de zinc y eugenol, hidróxido de calcio, selladores a base de resina y los nuevos selladores biocerámicos[9]. A pesar de las afirmaciones de los fabricantes sobre las ventajas de cada clase de selladores y artículos científicos que los respaldan[10–14], es importante generar más evidencia científica que demuestre la superioridad de un cemento sobre otro en cuanto a sus características utilidad se refiere, especialmente su capacidad antibacteriana.

Cementos a base de hidróxido de calcio.

Se crearon con la intención de incorporar las buenas propiedades biológicas del hidróxido de calcio a los selladores, evitando al mismo tiempo su rápida reabsorción en el periápice y en el interior del conducto. Se sugiere que el efecto antimicrobiano del hidróxido de calcio se produce por su capacidad de liberar iones Hidroxilo y por tener un pH alto. Lamentablemente estas acciones no se han demostrado. Se requiere solubilidad para la liberación de hidróxido de calcio y que su actividad sea constante, lo anterior es inconsistente con el propósito de un sellador[3,15–19].

Cementos selladores a base de resina.

Fueron introducidos en la práctica endodóntica por sus características favorables, como la adhesión a la estructura dentaria, largo tiempo de trabajo, facilidad de manipulación y buen sellado[20].

Cementos selladores biocerámicos.

Son biocompatibles, no producen respuesta inmunológica periapical, son estables en ambientes biológicos y no sufren contracción. Poseen la capacidad de producir hidroxiapatita durante su proceso de fraguado, generando un enlace químico entre la dentina y el material de obturación. Son fáciles de usar, ya que poseen un tamaño de partícula menor a 2 µm, lo que permite ser usados en una jeringa pre mezclada, presentan además un pH muy alcalino durante las primeras 24 horas lo que se traduce a una elevada actividad antimicrobiana[21–23].

Se ha estudiado con anterioridad la actividad antimicrobiana de cementos selladores frente a distintas bacterias[10,11,13,15,19,24], específicamente frente a *E. faecalis*[25–32], sin embargo, es importante probar la actividad antimicrobiana de cementos novedosos empleados en la actualidad y comparar su efectividad.

El objetivo de este estudio fue evaluar la inhibición del crecimiento de la bacteria *Enterococcus faecalis* (ATCC® 29212™) en presencia de cementos selladores utilizados en tratamientos endodónticos mediante experimentación in vitro.

MATERIALES Y MÉTODOS

Se empleó un diseño metodológico de tipo experimental basado en ensayos de laboratorio. Se utilizó el método de Difusión en agar (Kirby Bauer)[33–35], para analizar la actividad antibacteriana de tres cementos selladores de diferente composición: Endosequence (BC Sealer), AHPlus (Dentsply De Trey) y Sealapex (Kerr) (Cuadro 1). A continuación, se describen las principales etapas del trabajo de laboratorio.

Cuadro 1. Características de los cementos selladores endodónticos utilizados en el estudio.

Característica	**AH Plus**	**Sealapex Root Canal Sealer**	**Endosequence**
Composición	*Pasta A:* resina epóxica, tungstato de calcio, óxido de circonio, sílice, pigmentos de óxido de hierro. *Pasta B:* aminas, tungstato de calcio, óxido de circonio, sílice, aceite de silicona	Hidróxido de calcio polimérico sin eugenol	Óxido de circonio, silicatos de calcio, fosfato de calcio monobásico, hidróxido de calcio, agentes de relleno y espesantes
Empresa	Dentsply De Trey	Kerr	BC Sealer
Lote	1810000794	7081108	19001SP

Preparación del medio de cultivo.

Se empleó el medio de cultivo Agar Muller-Hinton (Becton Dickinson®), se preparó con las instrucciones que indica el fabricante; se mantuvo a temperatura ambiente una hora antes de utilizarlo.

Preparación del inóculo.

Se utilizó el método de suspensión directa de colonias, a partir de una placa de cultivo de 24 horas de la bacteria *Enterococcus faecalis* (ATCC® 29212™) se seleccionaron primeramente de 4 a 6 colonias y se ajustó el inóculo a una turbidez equivalente al 0.5 de la escala de MacFarland, se utilizó vórtex durante 15-20 segundos para homogenizar.

Inoculación de las placas.

Durante los primeros 15 minutos posteriores al ajuste del inóculo, se introdujo un asa bacteriológica dentro de la suspensión y se recolectó una cantidad con la cual se inocularon las placas de agar previamente preparadas con la técnica de inoculación en césped, sin dejar ninguna zona libre del agar. Se dejaron secar de 3 a 5 minutos para posteriormente depositar los discos.

Colocación de los discos con cementos en el medio de cultivo.

Los discos de papel filtro se impregnaron con cada cemento, preparados según las indicaciones del fabricante, con pinzas estériles se colocaron manualmente de 5 a 6 discos equidistantes por placa de agar, presionando ligeramente la parte superior del disco asegurando que hicieran contacto perfectamente con la superficie del agar. Posteriormente, las placas se colocaron en incubación de forma invertida a 37 grados centígrados, en atmósfera aeróbica por 24 horas.

Lectura de los resultados.

Se midieron las zonas de completa inhibición del crecimiento bacteriano con un vernier o regla, contra una superficie oscura bajo luz reflejada sobre el respaldo de la caja de Petri sin remover la tapa. El punto final de inhibición completa del crecimiento se estimó a simple vista.

Los tres cementos que se utilizaron para esta investigación se encuentran descritos en el cuadro 1 donde se indica la composición, el lote y la marca de la empresa que los desarrolla.

RESULTADOS

Se emplearon 3 cementos selladores: Endosequence (BC Sealer), AHPlus (Dentsply De Trey) y Sealapex (Kerr) (Cuadro 1), se replicaron ensayos con un total de 20 repeticiones para cada uno de ellos (n=60), donde se midieron los halos de inhibición de crecimiento de la bacteria *Enterococcus faecalis* (ATCC® 29212™) la medición incluyó el diámetro del disco y el halo de inhibición que se observó desde el margen externo del disco que contenía el cemento, teniendo como punto final de medición la aparición de las colonias de bacterias.

El cemento sellador que mostró un halo de inhibición de crecimiento bacteriano mayor fue AH Plus (Figura 1), seguido por EndoSequence (Figura 2), que, aunque tuvo variaciones menores en los resultados, mostró inhibición de manera constante.

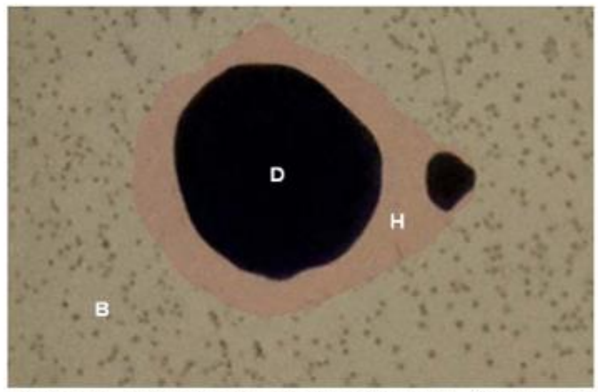

Figura 1. Inhibición de crecimiento de *E. faecalis* por parte del cemento sellador AH Plus, se observa una zona bien delimitada alrededor del disco impregnado con el sellador (se resaltó en color claro la zona donde no existió crecimiento bacteriano). D: Disco, H: Halo, B: Unidades formadoras de colonias de *E. faecalis*.

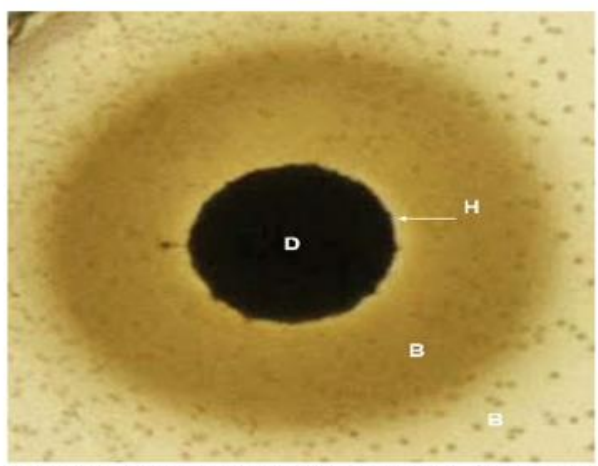

Figura 2. Inhibición de crecimiento de *E. faecalis* por parte del cemento sellador Endosequence, se observa una zona libre de bacterias (puntos) alrededor del disco impregnado con el sellador. D: Disco, H: Halo (flecha), B: Unidades formadoras de colonias de la bacteria

El cemento sellador cuya inhibición del crecimiento de *E. faecalis* fue prácticamente nula fue Sealapex, ya que solo en una medición resultó tener 1 mm de inhibición del margen del disco a la aparición de las unidades formadoras de colonia de las bacterias, solo en ese caso se consideró el diámetro del disco (7 mm en total), mientras que en todas las demás mediciones la inhibición fue prácticamente inexistente (1.4 mm en promedio de todas las mediciones) (Figura 3 y 4).

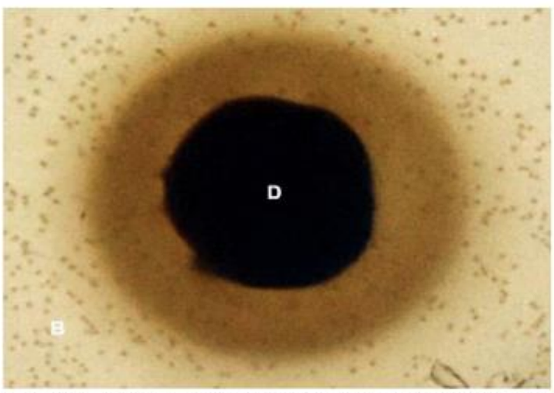

Figura 3. Inhibición de crecimiento de *E. faecalis* prácticamente nulo por parte del cemento sellador Sealapex, se observan unidades formadoras de colonias (puntos) alrededor del disco impregnado con sellador. D: Disco. B: Unidades formadoras de colonias de la bacteria

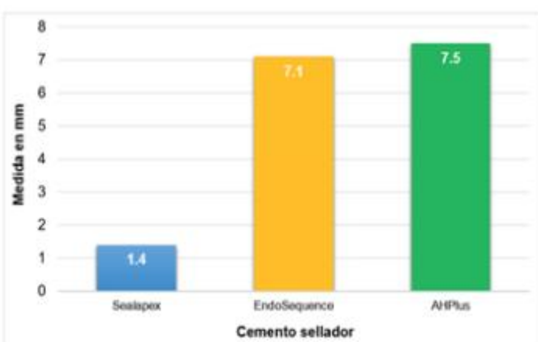

Figura 4. Promedio de las medidas de los halos de inhibición de crecimiento de *E. faecalis* que mostraron los cementos selladores analizados (medidas en mm).

DISCUSIÓN

La mayoría de los fracasos en un tratamiento endodóntico resultan ser por la ineficiencia en el proceso de desinfección de los conductos, así como la complejidad de realizar el proceso, sobre todo en los conductos accesorios[18]. Cada vez se realizan mejoras en los protocolos químico mecánicos para aumentar la efectividad en la desinfección, siendo los cementos una parte muy importante ya que con el sellado se busca inhibir el crecimiento de bacterias que pueden ocasionar fracasos en el tratamiento y comprometer la salud del paciente[30].

En esta investigación se analizó, mediante experimentación in vitro, la inhibición del crecimiento bacteriano ocasionada por cementos utilizados en tratamientos endodónticos, dentro de los principales hallazgos se demostró que el cemento AHPlus tiene una buena actividad antibacteriana, no así en el caso del cemento Sealapex, posiblemente se requieran hacer más pruebas con diferentes concentraciones para poder evaluar la eficiencia en su uso.

Los resultados fueron constantes para los tres cementos (n=20) en todos los ensayos.

Resultados similares fueron descritos por Poggio y colaboradores (2017), donde el cemento AHPlus presentó una importante inhibición de crecimiento bacteriano (1.2mm), en comparación con Sealapex (0.2mm)[30]. Para el caso del cemento EndoSequence, Singh y colaboradores (2019) resaltaron que la medida promedio de la inhibición fue de 14.4mm[10], significativamente mayor a la mostrada en éste ensayo donde se obtuvieron medidas menores y el promedio resultó de 7.1 mm, se conserva la capacidad de inhibición bacteriana pero sugiere continuar con el análisis de este material en próximas investigaciones.

Un estudio que contrasta los resultados que se presentan es el de Barbosa y colaboradores (2020), ya que, aunque muestra resultados midiendo parámetros distintos a los de este estudio (UFC/ml vs inhibición de UFC en medio sólido), resultó mayor crecimiento de células bacterianas en presencia del cemento AHPlus (9.16 UFC/ml), por su parte, EndoSequence mostró menos crecimiento bacteriano (8.82 UFC/ml) y Sealapex arrojó el menor número de bacterias (8.75 UFC/ml), lo cual indica que la mayor inhibición la presentó Sealapex[18], contrario a lo observado en éste estudio.

Los hallazgos de Heredia *et al.* (2017) muestran resultados relativamente diferentes, ya que observaron un halo de inhibición de 6 mm con el cemento Sealapex[36], mientras que en los resultados de este estudio fue prácticamente nula la inhibición, en este caso es importante hacer notar la naturaleza del cemento, porque aunque se observó un halo de 6 mm en el cemento a base de hidróxido de calcio (Sealapex), se sigue conservando un efecto inhibitorio mayor en cementos a base de resina como Topseal (7.7 mm)[36] o AHplus (7.5 mm en este estudio) lo que sugiere que la naturaleza y del cemento podría ser clave para inferir su capacidad inhibitoria, independientemente de la empresa que lo elabora[36].

Los tres cementos que se evaluaron son los más utilizados en el medio académico y profesional donde se desarrolló la presente investigación, siendo ésta una de las razones principales por las cuales se eligió evaluarlos, sin embargo, existe gran variabilidad de cementos que se podrían analizar proponiendo nuevas preguntas a responder para investigaciones posteriores, adicionalmente, será importante utilizar diferentes métodos y otros microorganismos para observar y evaluar la inhibición bacteriana en otros modelos.

El beneficio de contar con el mejor agente antibacteriano para sellar los conductos endodónticos es muy importante en el resultado final del tratamiento. Medir la inhibición del crecimiento bacteriano permite al profesionista elegir la mejor opción y utilizarla en la práctica clínica, tanto en el medio privado como en los tratamientos realizados en la comunidad universitaria, favoreciendo la disminución casos de fracaso endodóntico por contaminación.

CONCLUSIÓN

La inhibición del crecimiento de *E. faecalis* por la presencia de los cementos selladores utilizados en endodoncia fue distinta entre sí, demostrando que existe variabilidad importante en la capacidad antibacteriana de los cementos selladores analizados. Los hallazgos de este estudio confirman los datos reportados en otros estudios con los cementos AHPlus y Endo-Sequence, mientras que para Sealapex algunos de los resultados fueron contrastantes generando oportunidades de análisis en investigaciones posteriores.

Referencias

1. Sundqvist G. Bacteriological studies of necrotic dental pulps [Internet]. Vol. 7. Umea, Sweden; 1976. Disponible en: http://www.diva-portal.org/smash/get/diva2:719968/FULLTEXT02
2. Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surg Oral Med Oral Pathol. 1965;20:340–9.
3. Ng Y-L, Mann V, Rahbaran S, Lewsey J, Gulabivala K. Outcome of primary root canal treatment: systematic review of the literature. Part 2. Influence of clinical factors. Int Endod J. 2008;41(1):6–31.
4. Hugh CL, Walton RE, Facer SR. Evaluation of intracanal sealer distribution with 5 different obturation techniques. Quintessence Int. 2005;36(9):721–9.
5. American Association of Endodontists. Guide to Clinical Endodontics [Internet]. 2013. Disponible en: https://www.aae.org/specialty/clinical-resources/guide-clinical-endodontics/
6. Hargreaves KM, Berman LH. Cohen´s Pathways of the pulp. 11a ed. St Louis, Vasa: Elsevier; 2016.
7. Gopikrishna V, Grossman LI, Chandra BS. Grossman's endodontic practice. [Internet]. 13va. Canada; 2020 [citado el 21 de enero de 2022]. 526 p. Disponible en: https://dokumen.pub/grossmans-endodontic-practice-9789389859928.html
8. Sjögren U, Figdor D, Spångberg L, Sundqvist G. The antimicrobial effect of calcium hydroxide as a short-term intracanal dressing. Int Endod J. 1991;24(3):119–25.
9. Komabayashi T, Colmenar D, Cvach N, Bhat A, Primus C, Imai Y. Comprehensive review of current endodontic sealers. Dent Mater J. 2020;39(5):703–20.
10. Singh G, Gupta I, Elshamy FMM, Boreak N, Homeida HE. In vitro comparison of antibacterial properties of bioceramic-based sealer, resin-based sealer and zinc oxide eugenol based sealer and two mineral trioxide aggregates. Eur J Dent. 2016;10(3):366–9.
11. Hasheminia M, Razavian H, Mosleh H, Shakerian B. In vitro evaluation of the antibacterial activity of five sealers used in root canal therapy. Dent Res J. 2017;14:62.
12. Colombo M, Poggio C, Dagna A, Meravini M-V, Riva P, Trovati F, et al. Biological and physico-chemical properties of new root canal sealers. J Clin Exp Dent. 2018;10(2):e120–6.
13. Shin J-H, Lee D-Y, Lee S-H. Comparison of antimicrobial activity of traditional and new developed root sealers against pathogens

related root canal. J Dent Sci. 2018;13(1):54–9.

14. Kharouf N, Arntz Y, Eid A, Zghal J, Sauro S, Haikel Y, et al. Physicochemical and Antibacterial Properties of Novel, Premixed Calcium Silicate-Based Sealer Compared to Powder-Liquid Bioceramic Sealer. J Clin Med. 2020;9(10):E3096.

15. Nirupama DN, Nainan MT, Ramaswamy R, Muralidharan S, Usha HHL, Sharma R, et al. In Vitro Evaluation of the Antimicrobial Efficacy of Four Endodontic Biomaterials against Enterococcus faecalis, Candida albicans, and Staphylococcus aureus. Int J Biomater. 2014;2014:383756.

16. Donnermeyer D, Bürklein S, Dammaschke T, Schäfer E. Endodontic sealers based on calcium silicates: a systematic review. Odontol. 2019;107(4):421–36.

17. Lim M, Jung C, Shin D-H, Cho Y, Song M. Calcium silicate-based root canal sealers: a literature review. Restor Dent Endod. 2020;45(3):e35.

18. Barbosa VM, Pitondo-Silva A, Oliveira-Silva M, Martorano AS, Rizzi-Maia C de C, Silva-Sousa YTC, et al. Antibacterial Activity of a New Ready-To-Use Calcium Silicate-Based Sealer. Braz Dent J. 2020;31(6):611–6.

19. Bose R, Ioannidis K, Foschi F, Bakhsh A, Kelly RD, Deb S, et al. Antimicrobial Effectiveness of Calcium Silicate Sealers against a Nutrient-Stressed Multispecies Biofilm. J Clin Med. 2020;9(9):2722.

20. Azar NG, Heidari M, Bahrami ZS, Shokri F. In vitro cytotoxicity of a new epoxy resin root canal sealer. J Endod. agosto de 2000;26(8):462–5.

21. Zhang W, Li Z, Peng B. Ex vivo cytotoxicity of a new calcium silicate-based canal filling material. Int Endod J. 2010;43(9):769–74.

22. Raghavendra SS, Jadhav GR, Gathani KM, Kotadia P. Bioceramics in endodontics – a review. J Istanb Univ Fac Dent. 2017;51(3 Suppl 1):S128–37.

23. Šimundić Munitić M, Budimir A, Jakovljević S, Anić I, Bago I. Short-Term Antibacterial Efficacy of Three Bioceramic Root Canal Sealers Against Enterococcus Faecalis Biofilms. Acta Stomatol Croat. 2020;54(1):3–9.

24. Huang Y, Li X, Mandal P, Wu Y, Liu L, Gui H, et al. The in vitro antimicrobial activities of four endodontic sealers. BMC Oral Health. 2019;19.

25. Du T, Wang Z, Shen Y, Ma J, Cao Y, Haapasalo M. Combined Antibacterial Effect of Sodium Hypochlorite and Root Canal Sealers against Enterococcus faecalis Biofilms in Dentin Canals. J Endod. 2015;41(8):1294–8.

26. Wang Z, Shen Y, Haapasalo M. Dentin extends the antibacterial effect of endodontic sealers against Enterococcus faecalis biofilms. J Endod. 2014;40(4):505–8.

27. Bukhari S, Karabucak B. The Antimicrobial Effect of Bioceramic Sealer on an 8-week Matured Enterococcus faecalis Biofilm Attached to Root Canal Dentinal Surface. J Endod. 2019;45(8):1047–52.

28. AlShwaimi E, Bogari D, Ajaj R, Al-Shahrani S, Almas K, Majeed A. In Vitro Antimicrobial Effectiveness of Root Canal Sealers against Enterococcus faecalis: A Systematic Review. J Endod. 2016;42(11):1588–97.

29. Alsubait S, Albader S, Alajlan N, Alkhunaini N, Niazy A, Almahdy A. Comparison of the antibacterial activity of calcium silicate- and epoxy resin-based endodontic sealers against Enterococcus faecalis biofilms: a confocal laser-scanning microscopy analysis. Odontol. 2019;107(4):513–20.

30. Poggio C, Trovati F, Ceci M, Colombo M, Pietrocola G. Antibacterial activity of different root canal sealers against Enterococcus faecalis. J Clin Exp Dent. 2017;9(6):e743–8.

31. Šimundić Munitić M, Poklepović Peričić T, Utrobičić A, Bago I, Puljak L. Antimicrobial efficacy of commercially available endodontic bioceramic root canal sealers: A systematic review. PloS One. 2019;14(10):e0223575.

32. Prestegaard H, Portenier I, Ørstavik D, Kayaoglu G, Haapasalo M, Endal U. Antibacterial activity of various root canal sealers and root-end filling materials in dentin blocks infected ex vivo with Enterococcus faecalis. Acta Odontol Scand. 2014;72(8):970–6.

33. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 1966;45(4):493–6.

34. García-Rodríguez JA, Cantón R, García-Sánchez JE, Gomez-Lus ML, Martínez Martínez L, Rodríguez-Avial C, et al. Procedimientos en Microbiología Clínica. Métodos básicos para el estudio de la sensibilidad a los antimicrobianos. [Internet]. Picazo JJ, editor. 2000. Disponible en: https://www.seimc.org/contenidos/documentoscientificos/procedimientosmicrobiologia/seimc-procedimientomicrobiologia11.pdf

35. Cona E. Condiciones para un buen estudio de susceptibilidad mediante test de difusión en agar. Rev Chil Infectol [Internet]. 2002 [citado el 27 de marzo de 2022];19. Disponible en: http://www.scielo.cl/scielo.php?script=sci_arttext&pid=S0716-10182002019200001&lng=en&nrm=iso&tlng=en

36. Heredia-Veloz D, Abad-Coronel D, Villavicencio-Caparó E. Eficacia antibacteriana de tres selladores endodónticos frente al Enterococcus faecalis. Rev Estomatol Hered. 2017;27(3):132.

9. CARTA COMITÉ ETICA

Universidad Autónoma de Baja California

FACULTAD DE CIENCIAS DE LA SALUD

"2023, año de la concienciación sobre las personas con trastorno del espectro autista"

Tijuana, B.C; marzo 03 de 2023

Oficio No. 168/2023-1

ASUNTO: Respuesta a la Solicitud de Evaluación de Proyecto

DR. IVÁN OLIVARES ACOSTA
PROFESOR INVESTIGADOR
P R E S E N T E

Por medio de la presente le envío un cordial saludo a la vez que me permito informarle que su proyecto de investigación titulado *"Efectividad de nanopartículas de plata y peróxido de Zinc en gel, usados como medicación intraconducto en endodoncia"* y sometido al Comité de Evaluación en Investigación y Posgrado (CEIP) de la Facultad, registrado con el Folio P-01-2023-1, ha sido dictaminado como **APROBADO**, así mismo se le indica que el comité ha emitido algunas sugerencias que se enviarán adjunto a éste documento y que se solicita se realicen antes de iniciar con el protocolo, esperando que los resultados de dicho proyecto sean en beneficio de la comunidad universitaria y la sociedad en general.

Sin más por el momento, me despido poniéndome a sus apreciables órdenes.

ATENTAMENTE
"POR LA REALIZACIÓN PLENA DEL SER"

DCS ANA GABRIELA MAGALLANES RODRÍGUEZ
PRESIDENTA DEL CEIP

Universidad Autónoma de Baja California
FACULTAD DE CIENCIAS DE LA SALUD

C.c.p. Mtra. Ofelia Candolfi Arballo, Coordinadora de Investigación y Posgrado, FACISALUD.

10. ACTA DE REGISTRO DE TRABAJO TERMINAL

UNIVERSIDAD AUTÓNOMA DE SAN LUIS POTOSÍ
FACULTAD DE ESTOMATOLOGÍA
DOCTORADO EN CIENCIAS ODONTOLÓGICAS

ARS ET SCIENTIA AT SALUTEM
FACULTAD DE ESTOMATOLOGÍA UASLP

DOCTORADO U.A.S.L.P. Facultad de Estomatología Ciencias Odontológicas

ACTA DE DEL REGISTRO DE TRABAJO TERMINAL

En la ciudad de San Luis Potosí. S. L. P., el día primero del mes de diciembre del año dos mil veintiuno, en acuerdo previo con el estudiante, con la Directora del Trabajo Terminal y con el Comité Académico del programa se registró en las oficinas del programa de Doctorado en Ciencias Odontológicas el nombre del estudiante: Iván Olivares Acosta, el nombre de la Directora del Trabajo Terminal. Lilian Beatriz Romero Sánchez y el Titulo del Trabajo Terminal: "EFECTIVIDAD DE NANOPARTÍCULAS DE PLATA Y PERÓXIDO DE ZINC EN GEL USADOS COMO MEDICACIÓN INTRACONDUCTO EN ENDODONCIA" en cumplimiento del artículo 73, 74 y 75 del Reglamento General de Estudios de Posgrado de la Universidad Autónoma de San Luis Potosí.

Para constancia se levanta la presente acta que firman el Coordinador y la Directora del trabajo Terminal del programa de Doctorado en Ciencias Odontológicas.

Dra. Nuria Patiño Marín
Coordinadora del programa de Doctorado en Ciencias Odontológicas

Dra. Lilian Beatriz Romero Sánchez
Directora del Trabajo Terminal

11. AGRADECIMIENTOS

Al gran creador por siempre iluminar mi camino, a mi hija por todo el amor que me da, a mi esposa por su amor y comprensión, a mis padres por siempre creer en mi, a mis hermanos por su apoyo, a la Dra. Lilian Beatriz Romero Sanchez, mi directora, por su paciencia y disposicion, a mis amigos Jesus y Victor por adentrarme al mundo de la nanotecnologia, a Lore por toda la ayuda que tuvimos, sin ti no lo hubieramos logrado , a Juan Carlos, la verdad me hiciste batallar mas de lo que me ayudaste, pero siempre es un placer caminar contigo en cualquier proyecto, al Dr. Marco Felipe Salas Orozco por su ayuda desinteresada y a la Dra Nuria Patiño Marín por su objetividad, la cual nos permitio lograr nuestra metra.