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# Functionalization of filling core's material for improved endodontic treatment

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TESE DE DOUTORAMENTO

# FUNCTIONALIZATION OF FILLING CORE'S MATERIAL FOR IMPROVED ENDODONTIC TREATMENT

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**PROGRAMA DE DOUTORAMENTO EN CIENCIAS ODONTOLÓXICAS**

SANTIAGO DE COMPOSTELA

CONFLICTS OF INTEREST DECLARATION

INÊS RIBEIRO VALENTE LUCAS FERREIRA, DECLARE NO CONFLICTS OF INTEREST RELATED TO THE PRESENT DOCTORAL THESIS

FUNCTIONALIZATION OF FILLING CORE'S MATERIAL FOR IMPROVED ENDODONTIC TREATMENT

“O laboratório da perseverança

Perseverar é suportar, manter firme a orientação

porque se tem diante dos olhos uma meta a alcançar.

Perseverar é acreditar que o presente mantém uma aliança com o futuro”

José Tolentino de Mendonça, in O Pequeno Caminho das Grandes Perguntas

“Sê humilde para evitar o orgulho,

mas voa alto para alcançar a sabedoria”

Santo Agostinho

“I am among those who think that science has great beauty”

Marie Curie

To my Grandmother,

Mariazinha

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To God, for always being by my side and guiding me on my journey…

# RESUMEN

**Introducción y objetivos:** El objetivo principal del tratamiento endodóncico (TE) es lograr un sellado tridimensional del sistema de conductos radiculares, al mismo tiempo que se previene la filtración coronal y apical. La obturación de conductos radiculares ha sido reconocida como un procedimiento endodóncico con un gran impacto en el resultado del tratamiento endodóncico. A pesar del reconocido desarrollo en materiales y dispositivos tecnológicos en Endodoncia, el pronóstico de la periodontitis apical (PA) después del TE aún no es predecible. La PA es una enfermedad oral altamente prevalente en todo el mundo. Aunque la endodoncia es la primera opción de tratamiento para dientes con lesiones periapicales, en la última década se ha informado un aumento en la prevalencia de PA en dientes obturados. De hecho, un tratamiento inadecuado es más propenso a estar asociado a PA persistente. Sin embargo, los dientes bien tratados aún fallan. El conocimiento de la etiología microbiana de la PA es crucial, ya sea para los clínicos o los investigadores para desarrollar e implementar la mejor práctica clínica, basada en evidencia científica sólida. En el caso de dientes necróticos con PA, el clínico debe hacer frente a diversos factores, como los diferentes tipos de infecciones, primarias, secundarias/persistentes, con las respectivas particularidades en lo que se refiere al momento de la infección y al tipo de microbiota involucrada, así como a la respuesta inmune del huésped. Debido a la localización de la infección, la mayoría de las veces dentro del sistema de conductos radiculares, esta debe ser eliminada, clínicamente, mediante procedimientos mecánicos y químicos. Además, para mantener la desinfección proporcionada por la instrumentación mecánica y la irrigación, es de suma importancia lograr un sellado tridimensional del sistema de conductos radiculares, al mismo tiempo que se previene la filtración coronal y apical. Los materiales de relleno son generalmente una combinación de un material de núcleo rígido o semirrígido y un sellador. La gutapercha (GP), un isómero trans de poliisopreno (1, 4, trans–poliisopreno), es el material de núcleo más común. La mayoría de la GP disponible comercialmente está compuesta de componentes orgánicos (polímero GP (19-22%) y cera/resinas (1-4%)) y componentes inorgánicos, como óxido de zinc (60-75%). También se incluyen opacificantes, como el sulfato de bario. Si bien se reconoce ampliamente que la GP tiene muchas ventajas, como una gran plasticidad, una toxicidad mínima, radiopacidad, manipulación simple y facilidad de eliminación con calor o solventes, aún existen algunas desventajas importantes. Entre ellas, se ha destacado la falta de propiedades autoadhesivas, ya que la GP no se adhiere realmente a las superficies del sellador o de la dentina. Este aspecto puede comprometer el sellado hermético debido a una posible filtración apical o coronal. Además, a pesar de su contenido de óxido de zinc (ZnO), no previene su contaminación por biofilm. En cuanto a la desinfección en la consulta clínica, no existe un protocolo consensuado. Los selladores endodóncicos se pueden dividir en diferentes grupos según su composición química: ZnO-eugenol, hidróxido de calcio, resina epoxi, silicona, ionómero de vidrio y más recientemente selladores a base de silicato de calcio. Los selladores de resina epoxi se consideran un "gold standard" en endodoncia. Sin embargo, los materiales a base de silicato de calcio han ganado popularidad. Además, con respecto a la capacidad de resistencia de adhesión a la dentina, los estudios son contradictorios. A principios de la década de 2000, se introdujo el concepto de "monobloque" en el que la GP, el sellador y la dentina generaban una sola unidad. Según las interfases generadas, podría considerarse un "monobloque" primario, secundario o

terciario. Este último puede crearse utilizando sistemas de obturación como EndoREZ (Ultradent Products Inc., South Jordan, UT, EE. UU.), Activ GP (Brasseler USA, Savannah, GA, EE. UU.) o más recientemente el sistema de obturación TotalFill BC (FKG DENTAIRE SA, La Chaux-de-Fonds, Suiza). Sin embargo, ningún material de relleno disponible actualmente puede satisfacer las propiedades ideales, logrando un mejor sellado hermético a largo plazo entre el núcleo de GP y el sellador. En este sentido, cualquier contribución en la capacidad de sellado de la obturación del conducto radicular sería valiosa para mejorar el resultado del TE. Se han explorado nuevos enfoques para mejorar las deficiencias de los materiales endodóncicos comúnmente utilizados o se han introducido otros nuevos. La funcionalización es un camino cada vez más prometedor para la mejora de las propiedades de los materiales dentales. Puede definirse como "el proceso de añadir nuevas funciones, características, capacidades o propiedades a un material modificando la química de la superficie del material, con el objetivo de inducir una biorrespuesta deseada o inhibir una reacción potencialmente adversa". La GP recubierta de resina (EndoREZ GP Points, Ultradent, South Jordan, UT) tenía como objetivo principal mejorar la capacidad de adhesión a un sellador a base de resina metacrílica (EndoREZ sealer, Ultradent, South Jordan, UT). Sin embargo, se informó de una mala resistencia de adhesión de este sistema de relleno a la dentina, debido a la contracción de polimerización del sellador. También han surgido GP impregnada y recubierta con ionómero de vidrio (ActiV GP, Brasseler USA, Savannah, GA, USA.). No obstante, la resistencia de adhesión y la penetración del sellador no fueron superiores a las del sellador a base de resina utilizado con GP convencional. Se ha probado la incorporación de materiales bioactivos, como el biovidrio de fosfato de niobio, sin una mejora real de las propiedades biológicas. Por otra parte, la funcionalización de GP con nanopartículas de plata proporcionó propiedades antibacterianas contra microorganismos endodóncicos refractarios asegurando al mismo tiempo la biocompatibilidad. Recientemente, se comenzó a comercializar una GP recubierta con nanopartículas biocerámicas (TotalFill BC Points) y un sellador a base de silicato de calcio (TotalFill BC Sealer). Sin embargo, los pocos estudios disponibles no encontraron una adhesión superior del sellador de silicato de calcio a los conos de GP impregnados, en comparación con el sellador a base de resina epoxi adherido al GP convencional. La tecnología de plasma es un enfoque reciente para la modificación/funcionalización de superficies de sustratos endodóncicos. Los plasmas se clasifican generalmente como térmicos y no térmicos, o plasmas fríos, según las temperaturas relativas de las diferentes especies de plasma (electrones, iones y neutros). El tratamiento con plasma no térmico (TPN) puede generarse en condiciones de vacio o atmosféricas, utilizando gases inertes como argón (Ar) o helio (He), gases reactivos como oxígeno (O2) o nitrógeno (N2), o una mezcla de gases. La gran ventaja de esta tecnología es que proporciona un enfoque efectivo y limpio para la funcionalización de superficies sin alterar la estructura original de los materiales, y es compatible con los tejidos humanos. Se pueden mejorar o incluso habilitar una variedad de interacciones basadas en reacciones químicas con materiales de superficie mediante el tratamiento de plasma (TP), incluida la limpieza, la activación, el grabado o la ablación y la deposición de películas delgadas (recubrimiento). En endodoncia, la aplicación de TPN en la superficie de la dentina intrarradicular parece ser beneficiosa para la desinfección del conducto radicular. En cuanto a la adhesión de la restauración, se informó que esta tecnología mejoró la humectabilidad y la hidrofilicidad de la dentina, aumentando la fuerza de unión entre el adhesivo y la dentina. Con relación a la interfaz intrarradicular del sellador endodóncico, aún no está del todo claro el efecto de este enfoque potencial para mejorar la adhesión. Además, en lo que respecta al efecto sobre la GP, los tratamientos con plasma han sido escasamente investigados. Otra estrategia propuesta es la deposición de una película delgada de ZnO sobre da GP. El ZnO es un material multipropósito con una amplia gama de aplicaciones en diversos campos debido a su síntesis fácil y respetuosa con el medio ambiente, biocompatibilidad, propiedades antimicrobianas y antifúngicas y alta estabilidad química. A escala nanométrica, las películas delgadas de ZnO tienen muchas características notables debido a su considerable fuerza de unión, buena calidad óptica y excelentes propiedades piezoeléctricas, antibacterianas y antifúngicas, ofreciendo muchas aplicaciones potenciales en varios campos. La presente tesis destaca el efecto de la funcionalización de superficies dentinarias y de GP, a través del TPN y recubrimiento de una película delgada de ZnO, sobre la adhesividad entre interfaces GP/selladores/dentina, con vistas a un mejor sellado de la obturación. La hipótesis de esta tesis asume que los sustratos funcionalizados endodóncicos podrían tener un impacto positivo en la resistencia de unión entre materiales de núcleo, como GP, y selladores endodóncicos, así como en la interfaz dentina/sellador. El objetivo principal fue investigar el efecto de la funcionalización en sustratos endodóncicos, como dentina intrarradicular y superficies de GP, evaluando la resistencia de unión de las interfaces. Como objetivos específicos se consideraron los siguientes: analizar, a través de una revisión sistemática, el efecto del TPN en la adhesión dentina/selladores intrarradiculares (Estudio 1); evaluar el efecto del PNT sobre superficies de GP convencionales y biocerámicas, evaluando su rugosidad, energía libre superficial, estructura química y humectabilidad del sellador (Estudio 2); explorar la influencia de la funcionalización de la superficie da GP con una película delgada de ZnO nanoestructurada en su adhesividad a los selladores endodóncicos (Estudio 3).

**Material y métodos:** La metodología constó de tres fases. Para el primer estudio se realizó una revisión sistemática "Efecto del tratamiento con plasma en la adhesión de los selladores de conductos radiculares a la dentina intrarradicular". Se siguieron las recomendaciones de la guía 2020 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). Este estudio se realizó para responder a la pregunta PICO "¿Afecta el tratamiento con TPN la adhesión de los selladores a la dentina en comparación con ningún tratamiento?". Se realizó una búsqueda bibliográfica sin límites de tiempo ni de idioma, hasta mayo de 2023, en PubMed–MEDLINE, Scopus, Web of Science, OpenGrey y tres revistas de endodoncia (Journal of Endodontics, International Endodontic Journal y Australian Endodontic Journal). Además, se realizó una búsqueda adicional utilizando las listas de referencias de todos los artículos incluidos. Solo se incluyeron estudios in vitro que trataron la dentina con tecnología de plasma y evaluaron los efectos de los tratamientos con TPN en la adhesión de los selladores de conductos radiculares a la dentina. Se excluyeron los estudios que no utilizaron un grupo control (sin TP). Los estudios incluidos se sometieron a una evaluación de calidad y extracción de datos. Dos autores evaluaron de forma independiente el riesgo de sesgo en cada estudio seleccionado. Se consideraron los siguientes parámetros: (1) aleatorización, (2) cegamiento, (3) estandarización de la selección de muestras, (4) preparación estandarizada (operador único) y (5) informe de datos. En el segundo estudio "Funcionalización de superficies de gutapercha con tratamientos de plasma de argón y oxígeno para mejorar la adhesividad", se produjeron discos redondos de muestras de GP a partir de pellets de GP. Las muestras se produjeron creando moldes apropiados y luego plastificando la GP en un horno de calentamiento en seco de laboratorio a 80 °C, seguido de un proceso de enfriamiento a temperatura ambiente. Se probaron dos tipos de GP: convencional y biocerámica. Se empleó un procedimiento metalográfico estandarizado con papeles abrasivos de carburo de silicio grueso (hasta grano 600) para producir discos de GP con rugosidad superficial similar en ambas superficies. Se utilizó un sistema de plasma de baja presión de Diener Electronic (modelo Zepto) para la activación de la superficie de GP. Los tratamientos de plasma se ejecutaron considerando tres parámetros principales: (i) gas de trabajo (Ar u O2), (ii) tiempo de tratamiento (30 s, 60 s, 120 s o 180 s) y (iii) potencia (25 W o 50 W). La presión de trabajo fue constante para todos los tratamientos (~80 Pa), mientras que la presión base fue siempre inferior a 20 Pa. Se evaluó la topografía superficial de las muestras de GP antes y después de la activación con tratamientos de plasma, midiendo la rugosidad superficial con un perfilómetro óptico. Se evaluó el análisis topográfico superficial, la energía libre superficial y el ángulo de contacto. La energía libre superficial se calculó midiendo el ángulo de contacto entre las soluciones (agua, glicerol y 1-bromonaftaleno) y las superficies de GP, aplicando el método de Owens y Wendt. Por último, se evaluó el análisis químico a través de la espectroscopia infrarroja por transformada de Fourier (FT-IR) y la humectabilidad de los selladores (Endoresin y AH Plus Bioceramics). En el análisis estadístico se utilizaron las pruebas de Kruskal-Wallis y t de Student. Para el tercer estudio "Película delgada de ZnO nanoestructurada para mejorar la adhesión de gutapercha a selladores endodóncicos", las muestras de GP convencionales se dividieron aleatoriamente en tres grupos: a) GP sin tratar (control); b) GP tratado con plasma de argón (TP); c) GP funcionalizado (TP seguido de deposición de película delgada de ZnO). La funcionalización de la superficie da GP abarcó un proceso de varios pasos. Primero, se aplicó un TP de argón a baja presión para modificar las superficies de GP, seguido de una deposición de película delgada de ZnO por deposición física de vapor utilizando pulverización catódica con magnetrón reactivo. En este proceso, un material diana normalmente un metal, se bombardea con iones Ar+ dentro de una cámara de vacuo. La colisión de iones Ar+ con el material diana produce una transferencia de impulso y energía. Como consecuencia, los átomos del material diana se desplazan de sus ubicaciones originales y se expulsan al vacuo circundante interactuando con el gas reactivo O2, y luego se depositan sobre la superficie de la GP. La morfología de la superficie de las muestras de GP se evaluó cualitativamente mediante microscopía electrónica de barrido MEB. El ángulo de contacto con el agua se midió mediante la técnica de gota sésil. Otras pruebas exhaustivas incluyeron la evaluación de la resistencia de unión a la tracción, evaluando la adhesión de los selladores Endoresin y AH Plus Bioceramic al GP. Con respecto al análisis estadístico, los resultados del ángulo de contacto con el agua se evaluaron utilizando medidas repetidas de ANOVA (3 niveles: control, TP y TP + ZnO). Los resultados de la resistencia de unión a la tracción se analizaron con un ANOVA de dos vías seguido de pruebas post-hoc de Bonferroni.

**Resultados:** En cuanto a la revisión sistemática, de 188 artículos iniciales, se incluyeron 4 estudios. Todos los estudios tuvieron un alto riesgo de sesgo con respecto al cegamiento, proceso de aleatorización y preparación estandarizada de la muestra (operador único), porque estos parámetros no fueron mencionados. Todos los estudios informaron todos los resultados y realizaron la estandarización de la muestra, por lo que se consideraron con bajo riesgo de sesgo en estos parámetros. Ninguno de los estudios incluidos tuvo un bajo riesgo de sesgo en todos los parámetros evaluados, por lo que el riesgo general de sesgo de los estudios seleccionados fue alto. Tres de estos estudios basaron la capacidad de adhesión en la prueba de empuje en dientes humanos extraídos, mientras que el otro utilizó muestras de dentina bovina para medir el ángulo de contacto con el sellador (humectabilidad). Los tratamientos de plasma se realizaron en condiciones de vacío utilizando un reactor de vidrio en dos de los estudios, mientras que los otros dos utilizaron plasma a presión atmosférica. Dos estudios utilizaron plasma Ar, mientras que los otros dos aplicaron una mezcla de gases. El tiempo de aplicación varió entre 30 s y 1 min. No hubo consenso sobre el efecto del TPN en la adhesión del sellador AH Plus a la dentina radicular. La fuerza de unión de BioRoot RCS y Endosequence BC pareció verse influenciada positivamente por el TPN. En el caso de MTA Fillapex, la fuerza de unión disminuyó con el PNT. Los principales hallazgos del segundo estudio fueron los siguientes. Independientemente del tipo de GP (convencional o biocerámica), los tratamientos de plasma realizados en atmósferas de Ar u O2 mostraron diferentes comportamientos, dependiendo de la potencia y la duración. En comparación con el control (29,40 nm), para GP convencional, los valores de rugosidad más altos se registraron con una atmósfera de Ar a 50 W durante 120 s (32,04 nm; $p = 0{,}002$) y una atmósfera de O2 a 25 W durante 120 s (31,29 nm; $p = 0{,}005$). En comparación con el control (Ra = 26,50 nm) para GP biocerámica, los valores de rugosidad más altos se lograron para los tratamientos realizados en una atmósfera de Ar a 50 W durante 60 s (33,94 nm; $p < 0{,}001$) y en una atmósfera de O2 a 25 W durante 30 s (29,87 nm; $p < 0{,}001$). Los tratamientos con plasma aumentaron la energía libre superficial de las muestras en relación con el control, independientemente del tipo de GP ($p < 0{,}001$). En comparación con el control (41,02 mJ/m2), para el GP convencional, los valores más altos de energía libre superficial se registraron con una atmósfera de Ar a 50 W durante 60 s (55,23 mJ/m2; $p < 0{,}001$) o a 25 W durante 30 s (54,64 mJ/m2; $p < 0{,}001$) y una atmósfera de O2 a 50 W durante 180 s (57,87 mJ/m2; $p < 0{,}001$). A su vez, en comparación con el control (31,41 mJ/m2) sobre GP biocerámica, los mayores valores de energía libre superficial se lograron con una atmósfera de Ar, a 25 W, para una duración de tratamiento de 30 s (59,13 mJ/m2; $p < 0{,}001$) y con una atmósfera de O2, a 25 W, durante 120 s (65,70 mJ/m2; $p < 0{,}001$). El análisis por espectroscopia infrarroja por transformada de Fourier (FT-IR) mostró que, aunque se detectaron cambios químicos, se mantuvo la estructura molecular básica de la GP evaluada. Para el análisis de mojabilidad de los selladores, los parámetros aplicados para cada tipo de GP, considerando un buen equilibrio entre potencia, tiempo e impacto respectivo en la rugosidad y energía libre superficial fueron: Ar a 50 W durante 60 s y O2 a 25 W durante 120 s. Para el sellador Endoresin en GP convencional, hubo diferencias significativas entre el control (superficies de GP no tratadas) y GP tratadas con plasma Ar ($p = 0{,}002$). En GP biocerámica, ambos tratamientos de plasma con los parámetros seleccionados mejoraron la humectabilidad del sellador en comparación con el grupo control (Ar: $p = 0{,}037$; O2: $p < 0{,}001$). Con respecto al sellador AH Plus Biocerámico, ambas atmósferas (Ar y O2) produjeron diferencias significativas, en GP convencional y biocerámica, con valores aumentados, en comparación con el control (Ar: $p < 0{,}001$; O2: $p < 0{,}001$). Los principales hallazgos del tercer estudio se presentan a continuación. El análisis de la morfología de la superficie por SEM reveló que la GP sin tratar (control) mostró cristales de ZnO incrustados en la matriz de GP, cubiertos por una capa orgánica constituida por componentes de cera/resina. TP con Ar provocó la eliminación de la capa superficial de cera/resina, exponiendo los cristales de ZnO incrustados en la matriz de GP y descubriendo la porosidad de la superficie promovida por los límites de los granos de ZnO. Las muestras de GP sometidas a una deposición delgada de película de ZnO, aumentaron el área de superficie a cubrir con ZnO, beneficiándose de sus propiedades adicionales. El GP funcionalizada con ZnO disminuyó el ángulo de contacto del agua en comparación con el control ($p < 0{,}001$). El valor medio de la resistencia de unión en el grupo Endoresin fue significativamente mayor que en el grupo de AH Plus Biocerâmico, para todas las condiciones probadas (control, TP y TP + ZnO) ($p < 0{,}001$). Se observó una diferencia estadísticamente significativa entre el control y el GP funcionalizada con ZnO ($p=0{,}006$), siendo este último el que presentó el valor medio de resistencia de unión más alto. No se observó una diferencia significativa en los valores de resistencia de unión entre TP y TP+ZnO ($p=0{,}553$), ni entre TP y control ($p=0{,}203$).

**Conclusiones:** No hubo consenso sobre el efecto del TPN en la adhesión del sellador AH Plus a la dentina radicular. Sin embargo, sí pareció que el TPN podría tener un efecto positivo en la adhesión de BioRoot RCS y Endosequence BC. Los parámetros del TPN deberían optimizarse para obtener una base de evidencia más sólida, en endodoncia, sobre su papel como herramienta adyuvante para aumentar la adhesión de los selladores a la dentina. Estos hallazgos deben interpretarse con cautela debido a la escasez de estudios sobre el tema. La funcionalización de superficies de GP con tratamientos de plasma con Ar u O2 puede aumentar la rugosidad, la energía libre superficial y la humectabilidad de Endoresin y AH Plus Bioceramic, lo que podría mejorar sus propiedades adhesivas en comparación con GP no tratada. La funcionalización de GP con una película delgada de ZnO nanoestructurada favorece la capacidad de resistencia de unión con selladores de diferentes composiciones químicas e induce un cambio hacia la hidrofilicidad. La deposición de una película delgada de ZnO preservó la morfología de la superficie de la GP modificada por TP, mejorando la fuerza de adhesión a los selladores Endoresin y AH Plus Bioceramico, en comparación con la GP sin tratamiento. Teniendo en cuenta que este tema ha sido poco discutido en la literatura endodóncica actual, los datos aquí presentados pueden aportar una perspectiva novedosa. Sin embargo, debe destacarse que el comportamiento de los materiales de relleno y la dentina en condiciones in vitro puede no reflejar verdaderamente la configuración clínica. Se necesitan más estudios para confirmar la relevancia clínica de los presentes hallazgos

# RESUMO

**Introdución e obxectivos:** O obxectivo principal do tratamento endodóncico (TE) é conseguir un selado tridimensional do sistema de conductos radiculares evitando infiltración coronal e apical. A obturación do conducto radicular foi recoñecido como un procedemento de endodoncia cun gran impacto no resultado do tratamento endodóncico. A pesar do recoñecido desenvolvemento en materiais e dispositivos tecnolóxicos en Endodoncia, o prognóstico da periodontite apical (PA) posterior ao TE aínda non é previsible. A PA é unha enfermidade bucal de alta prevalencia en todo o mundo. Aínda que el TE sexa primeira opción de tratamento para os dentes con lesións periapicais, na última década informouse dun aumento da prevalencia de PA nos dentes tratados endodóncicamente. De feito, un tratamento inadecuado é máis propenso a asociarse a PA persistente. Con todo, os dentes ben tratados aínda fracasan. O coñecemento da etioloxía microbiana da PA é fundamental, xa sexa para que os médicos ou investigadores poidan desenvolver e implementar a mellor práctica clínica, baseada en evidencia científica sólida. Para os dentes necróticos con PA, o médico debe tratar varios factores, como os diferentes tipos de infeccións, primarias, secundarias/persistentes coas particularidades respectivas no que se refire ao momento da infección e ao tipo de microbiota implicada así como a resposta inmune do hóspede. Debido á localización da infección, a maioría das veces dentro do sistema de conductos radiculares, debe ser eliminada, clinicamente, mediante procedementos mecánicos e químicos. Ademais, para manter a desinfección proporcionada pola instrumentación mecánica e pola irrigacion, é de máxima importancia conseguir un selado tridimensional do sistema de conductos radiculares, evitando ao mesmo tempo infiltración coronal e apical. Os materiais obturadores son xeralmente unha combinación dun material de núcleo ríxido ou semiríxido e un selante. A gutapercha (GP), un isómero trans do poliisopreno (1, 4, trans-poliisopreno), é o material do núcleo máis común. A maior parte da GP dispoñible comercialmente está composto por compoñentes orgánicos (polímero GP (19-22%) e cera/resinas (1-4%)) e compoñentes inorgánicos, como óxido de cinc (60-75%). Tamén se inclúen opacificantes, como o sulfato de bario. Aínda que é amplamente recoñecido que a GP ten moitas vantaxes como unha gran plasticidade, unha mínima toxicidade, radiopacidade, manipulación sinxela e facilidade de eliminación con calor ou disolventes, tamén están presentes algunhas desvantaxes importantes. Entre estas, destacouse a falta de propiedades autoadhesivas, xa que la GP non se adhiere verdadeiramente ás superficies da dentina e selante. Este aspecto pode comprometer o selado hermético debido a unha posible infiltración apical ou coronal. Ademais, o seu contido en óxido de cinc (ZnO), non impide a contaminación por biofilm. En canto á desinfección da GP, non existe un protocolo consensuado. Os selantes de endodoncia pódense dividir en diferentes grupos segundo a súa composición química: ZnO-eugenol, hidróxido de calcio, resina epoxi, silicona, ionómero de vidro e, máis recentemente, selantes a base de silicato de calcio. Os selantes de resina epoxi considéranse un "gold standard" en endodoncia. Non obstante, os materiais a base de silicato de calcio medraron en popularidade. Non obstante, no que se refire á capacidade de adhesión á dentina, os estudos son conflitivos. A principios dos anos 2000 introduciuse o concepto "monoblock" no que GP, o selante e a dentina xeraban unha única unidade. Segundo as interfaces xeradas podería considerarse un "monobloque" primario, secundario ou terciario. Este último pódese crear utilizando sistemas de obturación como EndoREZ (Ultradent Products Inc., South Jordan, UT,

USA), Activ GP (Brasseler USA, Savannah, GA, EUA) ou máis recentemente o sistema TotalFill BC (FKG DENTAIRE SA, La Chaux-de-Fonds, Suíza). Aínda que, ningún material obturador dispoñible actualmente pode satisfacer as propiedades ideais, logrando un mellor selado estanco a longo prazo entre o núcleo GP e o selante. Neste sentido, calquera contribución na capacidade de selado da obturación do conducto radicular sería valiosa para mellorar o resultado do TE. Ten sido explorados novos enfoques para mellorar as deficiencias dos materiais de endodoncia de uso habitual ou introducíronse outros novos. A funcionalización é un enfoque cada vez máis prometedor para a mellora das propiedades dos materiais dentais. Pódese definir como "o proceso de engadir novas funcións, características, capacidades ou propiedades a un material cambiando a química da superficie do material, co obxectivo de inducir unha bioresposta desexada ou inhibir unha reacción potencialmente adversa". A GP revestida de resina (EndoREZ GP Points, Ultradent, South Jordan, UT) tiña o obxectivo principal de mellorar a capacidade de adhesión a un selante a base de resina metacrílica (EndoREZ, Ultradent, South Jordan, UT). Non obstante, informouse dunha pouca forza de adhesión á dentina, debido á contracción da polimerización do selante. Tamén xurdiron GP impregnadas e revestidas con ionómero de vidro (ActiV GP, Brasseler USA, Savannah, GA, USA). Con todo, a forza de unión e a penetración do selante non foron superiores ao selante a base de resina usado con GP convencional. Probáronse a incorporación de materiais bioactivos, como o biovidrio de fosfato de niobio, sen unha mellora real das propiedades biolóxicas. Por outra banda, a funcionalización de GP con nanopartículas de prata proporcionou propiedades antibacterianas contra microorganismos endodóncicos refractarios ao tempo que garantiu a biocompatibilidade. Recentemente, comezaron a comercializarse GP revestida con nanoparticulas biocerámicas (TotalFill BC Points) e un selante a base de silicato de calcio (TotalFill BC Sealer). Con todo, os poucos estudos dispoñibles non atoparon unha unión superior do selante de silicato de calcio aos conos de GP impregnados, en comparación co selante a base de resina epoxi e GP convencional. A tecnoloxía do plasma é un enfoque recente para a modificación/funcionalización de superficies de substratos endodóncicos. Os plasmas clasifícanse xeralmente en térmicos e non térmicos, ou plasmas fríos, en función das temperaturas relativas das diferentes especies plasmáticas (electróns, ións e neutros). O tratamento con plasma non-térmico (TPN) podense xenerar en condicións de vacuo ou atmosféricas, utilizando gases inertes como argón (Ar) ou helio (He), gases reactivos como osíxeno ($O_2$) ou nitróxeno ($N_2$), ou unha mestura de gases. A gran vantaxe desta tecnoloxía é que proporciona un enfoque eficaz e limpo para a funcionalización da superficie sen alterar a estrutura orixinal dos materiais, e é compatible cos tecidos humanos. Unha variedade de interaccións baseadas en reaccións químicas cos materiais superficiais pódense mellorar ou incluso activar mediante o tratamento con plasma (TP), incluíndo limpeza, activación, gravado ou ablación e deposición de película fina (revestimento). En endodoncia, a aplicación de TPN na superficie intrarradicular da dentina parece ser beneficiosa para a desinfección do conduto radicular. En canto á adhesión da restauración, esta tecnoloxía mellorou a humectabilidade e a hidrofilia da dentina, aumentando a forza da unión adhesivo-dentina. Respecto da interface intrarradicular- selante endodóncico, aínda non está do todo claro o efecto deste enfoque potencial para mellorar a adhesión. Ademais, no que se refire ao efecto na GP, os tratamentos con plasma foron pouco investigados. Outra estratexia para funcionalizar a GP é a deposición dunha fina película de revestimento de ZnO. O ZnO é un material multiusos cunha ampla gama de aplicacións en diversos campos debido á súa síntese sinxela e respectuosa co medio ambiente, á súa biocompatibilidade, ás propiedades antimicrobianas e antifúnxicas e á súa elevada estabilidade química. A escala nanométrica, as películas finas de ZnO teñen moitas características notables debido á súa considerable forza de enlace, boa calidade óptica e excelentes propiedades piezoeléctricas, antibacterianas e antifúnxicas, que ofrecen moitas aplicacións potenciais en diversos campos. A presente tese destaca o efecto da funcionalización das superficies de dentina e GP, a través do TP e de recubrimento de una película fina de ZnO, avaliando a adhesividade entre as interfaces GP/selante/dentina, en vista dun mellor selado de obturación. A hipótese desta tese supón que os substratos funcionalizados endodóncicos poden ter un impacto positivo na forza de unión entre os materiais do núcleo, como GP, e os selantes endodóncicos, así como na interface dentina/selante. O obxectivo principal foi investigar o efecto da funcionalización sobre substratos endodóncicos, como a dentina intraradicular e a GP, avaliando a forza de unión das interfaces. Como obxectivos específicos consideráronse os seguintes: analizar, mediante unha revisión sistemática, o efecto do TPN na adhesión intraradicular de dentina/selantes (Estudo 1); avaliar o efecto do TPN en superficies de GP convencionais e biocerámicas, avaliando a súa rugosidade, enerxía libre superficial, estrutura química e humectabilidade do selante (Estudo 2); explorar a influencia da funcionalización da superficie da GP cunha película fina de ZnO nanoestruturada na súa adherencia aos selantes endodóncicos (Estudo 3).

**Material e métodos:** A metodoloxía constaba de tres fases. Para o primeiro estudo, realizouse unha revisión sistemática "Efecto do tratamento con plasma na adhesión dos selantes endodonticos á dentina intraradicular". Seguíronse as recomendacións das directrices PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Análises) de 2020. Este estudo realizouse para responder á pregunta PICO "¿O tratamento con plasma non-térmico afecta a adhesión dos selantes á dentina en comparación con ningún tratamento?". Ata maio de 2023 realizouse unha busca bibliográfica sen límites de tempo ou idioma en PubMed–MEDLINE, Scopus, Web of Science, OpenGrey e tres revistas de endodoncia (Journal of Endodontics, International Endodontic Journal e Australian Endodontic Journal). Ademais, realizouse unha busca adicional utilizando as listas de referencias de todos os artigos incluídos. Só se incluíron estudos in vitro que trataron a dentina con tecnoloxía de plasma e avaliaron os efectos do TP sobre a adhesión dos selantes a dentina. Excluíronse os estudos que non utilizaron un grupo control (sen TP). Os estudos incluídos foron sometidos a avaliación de calidade e extracción de datos. Dous autores avaliaron de forma independente o risco de sesgo en cada estudo seleccionado. Consideráronse os seguintes parámetros: (1) aleatorización, (2) cegamento, (3) estandarización da selección da mostra, (4) preparación estandarizada (operador único) e (5) presentación de datos. No segundo estudo "Funcionalización de superficies de gutapercha con tratamentos con plasma de argón e osíxeno para mellorar a adherencia", producíronse discos redondos de GP a partir de pellets. As mostras producíronse creando moldes axeitados e plastificando GP nun forno de laboratorio de quecemento en seco a 80 °C, seguido dun proceso de arrefriamento a temperatura ambiente. Probáronse dous tipos de GP: convencional e biocerámica. Empregouse un procedemento metalográfico estandarizado con papeis abrasivos de carburo de silicio (ata grano 600) para producir discos de GP cunha rugosidade superficial similar en ambas superfíces. Utilizouse un sistema de plasma de baixa presión de Diener Electronic (modelo Zepto) para a activación da superficie da GP. Os tratamentos con plasma executáronse tendo en conta tres parámetros principais: (i) gas de traballo (Ar ou O2), (ii) tempo de tratamento (30 s, 60 s, 120 s ou 180 s) e (iii) potencia (25 W ou 50 s). W). A presión de traballo foi constante para todos os tratamentos (~ 80 Pa), mentres que a presión base foi sempre inferior a 20 Pa. A topografía superficial das mostras de GP avaliouse antes e despois da activación con tratamentos con plasma, medindo a rugosidade da superficie cun perfilómetro óptico. Valorouse a análise topográfica da superficie, a enerxía libre superficial e o ángulo de contacto. Calculouse a enerxía libre superficial medindo o ángulo de contacto entre diferentes solucions (auga, glicerol e 1-bromonaftaleno) e as superficies de GP, aplicando o método de Owens e Wendt. Por último, avaliouse a análise química a través da espectroscopia infravermella de transformada de Fourier (FT-IR) e a moxabilidade de dos selantes (Endoresin e AH Plus Bioceramic). Na análise estatística utilizáronse a proba de Kruskal-Wallis e t-Student. Para o terceiro estudo "Fina película de ZnO nanoestructurada para mellorar a adhesión da gutapercha aos selantes endodóncicos" as mostras de GP convencionais dividíronse aleatoriamente en tres grupos: a) GP sen tratamento (control); b) GP tratada con plasma de argón (TP); c) GP funcionalizada (TP seguido de deposición de película fina de ZnO). A funcionalización da superficie da GP abarcou un proceso de varios pasos. En primeiro lugar, aplicouse un TP con argón a baixa presión para modificar a superficie da GP, seguido dunha deposición de película fina de ZnO a través de a través da deposición física de vapor mediante pulverización catódica con magnetrón reactivo. Neste proceso, un material diana normalmente un metal, é bombardeado con ións Ar+ dentro dunha cámara de vacuo. A colisión dos ións Ar+ co material diana resulta nunha transferencia de enerxía. Como consecuencia, os átomos son desprazados dos seus lugares orixinais e expulsados ao vacuo circundante interactuando co gas reactivo O2, e despois depositados na GP. A morfoloxía superficial das mostras de GP avaliaronse cualitativamente mediante microscopía electrónica de varrido (MEV). O ángulo de contacto da auga foi medido pola técnica de gota sésil. Outras probas incluíron a avaliación da forza de unión á tracción, avaliación da adhesión dos selantes Endoresin e AH Plus Bioceramic a GP. En canto á análise estatística, avaliáronse os resultados do ángulo de contacto da auga mediante medidas repetidas de ANOVA (3 niveis: control, TP e TP + ZnO). Os resultados da forza de unión á tracción analizáronse cun ANOVA bidireccional seguido de probas post-hoc de Bonferroni.

**Resultados:** En canto á revisión sistemática, dos 188 artigos iniciais incluíronse 4 estudos. Todos os estudos tiñan un alto risco de sesgo con respecto ao cegamento, o proceso de aleatorización e a preparación estandarizada da mostra (operador único), porque estes parámetros non foron mencionados. Todos os estudos mostraron a totalidade dos resultados e realizaron a estandarización da mostra, polo que se considerou que tiñan un risco baixo de sesgo nestes parámetros. Ningún dos estudos incluídos tivo un risco baixo de sesgo en todos os parámetros avaliados, polo que o risco global de sesgo dos estudos seleccionados foi alto. Tres destes estudos basearon a capacidade de adhesión na proba de “pus-out” en dentes extraídos humanos, mentres que o outro utilizou mostras de dentina bovina para medir o ángulo de contacto con selante (humectabilidade). Os tratamentos de plasma realizáronse en condicións de vacuo mediante un reactor de vidro en dous dos estudos, mentres que os outros dous utilizaron plasma a presión atmosférica. Dous estudos utilizaron plasma de Ar, mentres que os outros dous aplicaron unha mestura de gases. O tempo de aplicación variou entre 30 s e 1 min. Non houbo consenso sobre o efecto do tratamento de plasma sobre a adhesión do selante AH Plus á dentina radicular. A forza de enlace de BioRoot RCS e Endosequence BC parecía estar influenciada positivamente polo NTP. Para MTA Fillapex, a forza de unión disminuiu con o tratamento de plasma. Os principais resultados do segundo estudo foron os seguintes: independentemente do tipo de GP (convencional ou biocerámica), os tratamentos con plasma realizados en atmosferas de Ar ou O2 mostraron comportamentos diferentes, segundo a potencia e a duración. En comparación co control (29,40 nm), para a GP convencional, os valores de rugosidade máis altos rexistráronse cunha atmosfera de Ar a 50 W durante 120 s (32,04 nm; p = 0,002) e unha atmosfera de O2 a 25 W durante 120 s (31,29 nm). ; p = 0,005).

En comparación co control (Ra = 26,50 nm) para a GP biocerámica, os valores máis altos de rugosidade conseguíronse para os tratamentos realizados en atmosfera de Ar a 50 W durante 60 s (33,94 nm; p < 0,001) e en atmosfera de O2 a 25 s. W durante 30 s (29,87 nm; p < 0,001). Os tratamentos con plasma aumentaron a enerxía libre da superficie das mostras en relación ao control, independentemente do tipo de GP (p < 0,001). En comparación co control (41,02 mJ/m2), para a GP convencional, os valores máis altos de enerxía libre de superficie rexistráronse cunha atmosfera de Ar a 50 W durante 60 s (55,23 mJ/m2; p < 0,001) ou a 25 W durante 30 segundos. s (54,64 mJ/m2; p < 0,001) e unha atmosfera de O2 a 50 W durante 180 s (57,87 mJ/m2; p < 0,001). Pola súa banda, en comparación co control (31,41 mJ/m2) en GP biocerámica, os valores máis altos de enerxía libre de superficie alcanzáronse cunha atmosfera de Ar, a 25 W, para unha duración de tratamento de 30 s (59,13 mJ/m2; p < 0,001) e cunha atmosfera de O2, a 25 W, durante 120 s (65,70 mJ/m2; p < 0,001). A análise por espectroscopia infravermella da transformada de Fourier (FT-IR) mostrou que detectaronse cambios químicos, mantívose a estrutura molecular básica da GP. Para a análise de humectabilidade dos selantes os parámetros aplicados para cada tipo de GP, considerando un bo equilibrio entre potencia, tempo e respectivo impacto na rugosidade e enerxía libre superficial foron: Ar a 50 W durante 60 s e O2 a 25 W durante 120 s. Para o selante Endoresin, en GP convencional, houbo diferenzas significativas entre o control (superficies de GP non tratadas) e GP tratada con plasma de Ar (p = 0,002). En GP biocerámica, ambos tratamentos con plasma cos parámetros seleccionados melloraron a humectabilidade do selante Endoresin en comparación co grupo control (Ar: p = 0,037; O2: p < 0,001). No que se refire ao selante AH Plus Biocerámico, ambas as atmosferas (Ar e O2) produciron diferenzas significativas, na GP convencional e biocerámica, con valores aumentados, en comparación co control (Ar: p < 0,001; O2: p < 0,001). A continuación preséntanse os principais resultados do terceiro estudo. A análise da morfoloxía superficial mediante SEM revelou que a GP non tratada (control) mostraba cristais de ZnO incrustados na matriz de GP, cubertos por unha capa orgánica constituída por compoñentes de cera/resina. TP con Ar provocou a eliminación da capa superficial de cera/resina, expoñendo os cristais de ZnO incrustados na matriz da GP e descubrindo a porosidade superficial promovida polos límites dos grans de ZnO. As mostras de GP sometidas a deposición fina de película de ZnO, aumentaron a superficie a cubrir con ZnO, beneficiando as súas propiedades adicionais. A GP funcionalizada con ZnO diminuíu o ángulo de contacto coa auga en comparación co control (p<0,001). O valor medio da forza de unión no grupo Endoresin foi significativamente superior ao do grupo do AH Plus biocerámico, para todas as condicións probadas (control, TP e TP + ZnO) (p<0,001). Houbo unha diferenza estatisticamente significativa entre o control e a GP funcionalizada con ZnO (p=0,006), presentando esta última o valor medio de forza de enlace máis alto. Non houbo diferenzas significativas nos valores de forza de unión entre TP e TP + ZnO (p=0,553), nin entre TP e control (p=0,203).

**Conclusións:** Non houbo consenso sobre o efecto do TPN sobre a adhesión do selante AH Plus á dentina radicular. Non obstante, pareceu que o TPN podería ter un efecto positivo na adhesión dos selantes BioRoot RCS e Endosequence BC. Os parámetros TPN deberían optimizarse para obter unha base de evidencia máis sólida, en endodoncia, sobre o seu papel como ferramenta auxiliar para aumentar a adhesión dos selantes á dentina. Estes achados deben ser interpretados con cautela debido á escaseza de estudos sobre o tema. A funcionalización das superficies de GP con tratamentos de plasma con Ar ou O2 aumentou a rugosidade, a enerxía libre da superficie e a moxabilidade dos selantes Endoresin e AH Plus Bioceramico, o que pode mellorar as súas propiedades adhesivas en comparación co a GP non tratada. A funcionalización da GP cunha película fina de ZnO nanoestruturada favorece a capacidade de adhesión con selantes de

diferentes composicións químicas. A deposición da película fina de ZnO preservou a morfoloxía da superficie de GP modificada por TP, mellorando a forza de unión dos selantes Endoresin e AH Plus biocerâmico, en comparación co a GP non tratada. Tendo en conta que este tema apenas é recente, os datos aquí presentados poden engadir unha visión novedosa. Non obstante, hai que subliñar que o comportamento dos materiais obturadores e da dentina en condicións in vitro pode non reflectir verdadeiramente a configuración clínica. Son necesarios máis estudos para confirmar a relevancia clínica dos presentes achados.

# ABSTRACT

**Introduction and objectives:** The main goal of the endodontic treatment (ET) is to achieve a tridimensional sealing of the root canal system while preventing coronal and apical leakage. Root canal filling has been recognized as an endodontic procedure with a great impact in the outcome of the endodontic treatment. Despite the recognized development in materials and technological devices in Endodontics, the prognosis of apical periodontitis (AP) after ET is not still predictable. AP is a highly prevalent oral disease, worldwide. Even though the ET is the first treatment option for teeth with periapical lesions, an increased prevalence of AP in root filled teeth has been reported in the last decade. In fact, an inadequate treatment is more prone to be associated to persistent AP. Yet, well-treated teeth still fail. The knowledge of the microbial etiology of AP is crucial, either for clinicians or researchers to develop and implement the best clinical practice, based on solid scientific evidence. For necrotic teeth with AP, the clinician must deal with several factors, such as the different types of infections, primary, secondary/persistent with the respective particularities in what concerns the moment of the infection and the type of microbiota involved as well as the host immune response. Due to the location of the infection, most often inside the root canal system, it must be eliminated, clinically, by mechanical and chemical procedures. Additionally, to maintain the disinfection provided by the mechanical instrumentation and irrigation, it is of utmost importance to achieve a tridimensional sealing of the root canal system, while preventing coronal and apical leakage. Filling materials are generally a combination of a rigid or semi-rigid core material and a sealer. Gutta-percha (GP), a trans-isomer of polyisoprene (1, 4, trans–poly isoprene), is the most common core material. Most of the commercially available GP is composed of organic components, (GP polymer (19-22%) and wax/resins (1-4%)) and inorganic components, such as zinc oxide (60-75%). Opacifiers, such as barium sulphate are also included. While it is widely recognized that GP has many advantages such as, a great plasticity, a minimal toxicity, radiopacity, simple manipulation, and ease of removal with heat or solvents, some important disadvantages are still present. Amongst these the lack of self-adhesive properties has been stressed, as GP does not truly bond to sealer or dentin surfaces. This aspect can compromise the hermetic seal due to potential apical or coronal leakage. Besides that, although its zinc oxide (ZnO) content, it does not prevent biofilm contamination of GP. Concerning chair-side disinfection, there is not a consensual protocol. Endodontic sealers can be divided into different groups according to their chemical composition: ZnO-eugenol, calcium hydroxide, epoxy resin, silicone, glass ionomer and more recently calcium silicate-based based sealers. Epoxy-resin sealers are considered a "gold standard" in Endodontics. Nevertheless, calcium silicate-based materials have grown in popularity. However, regarding push-out bond strength ability to dentin, the studies are conflicting. In the early 2000s, the "monoblock" concept has been introduced in which GP, the sealer and the dentin generated a single unit. According to the interfaces generated it could be considered a primary, secondary or tertiary "monoblock". The latter can be created by using obturation systems such as EndoREZ (Ultradent Products Inc., South Jordan, UT, USA), Activ GP (Brasseler USA, Savannah, GA, USA) or more recently TotalFill BC filling system (FKG DENTAIRE SA, La Chaux-de-Fonds, Switzerland). Even though, no currently available filling material can satisfy the ideal properties, achieving a better long-term fluid-tight seal between the GP core and the sealer. In this sense, any contribution in

the sealing ability of the root canal obturation would be valuable to improve endodontic treatment's outcome. Newer approaches have been explored to improve the shortcomings of commonly used endodontic materials or introduced new ones. Functionalization is an increasingly promising approach for property improvement of dental materials. It can be defined as "the process of adding new functions, features, capabilities or properties to a material by changing the surface chemistry of the material, with the aim of inducing a desired bioresponse or inhibit a potentially adverse reaction". The resin coated GP (EndoREZ GP Points, Ultradent, South Jordan, UT) had the main purpose of improving the adhesion capacity to a methacrylic resin-based sealer (EndoREZ sealer, Ultradent, South Jordan, UT). However, a poor bond strength of this filling system to dentin was reported, due to the polymerization shrinkage of the sealer. Glass ionomer-impregnated and coated GP have also emerged (ActiV GP, Brasseler USA, Savannah, GA, USA). However, the bond strength and sealer penetration were not superior to the resin-based sealer used with conventional GP. Incorporation of bioactive materials, such as, niobium phosphate bioglass have been tested, without a real improvement of the biological properties. On the other hand, the functionalization of GP with silver nanoparticles provided antibacterial properties against refractory endodontic microorganisms while assuring biocompatibility. Recently, a bioceramic nanoparticulated coated GP (TotalFill BC Points) and a calcium silicate-based sealer (TotalFill BC Sealer) began to be commercialized. Hence, the few studies available did not find a superior bond of the calcium silicate sealer to impregnated GP cones, compared to the epoxy resin-based sealer bonded to conventional GP. Plasma technology is recent approach to surface modification/functionalizing of endodontic substrates. Plasmas are generally classified as thermal and non-thermal, or cold plasmas, based on the relative temperatures of the different plasma species (electrons, ions, and neutrals). Non-thermal plasma (NTP) is artificially made of, and can be generated under vacuum or atmospheric conditions, using inert gases like argon (Ar) or helium (He), reactive gases such as oxygen (O2) or nitrogen (N2), or a mixture of gases. It is compatible with human tissues. The great advantage of this technology is that it provides an effective and clean approach to surface functionalization without altering the original structure of the materials. A variety of chemical-reaction based interactions with surface materials can be enhanced or even enabled by plasma treatment (PT), including cleaning, activation, etching or ablation, and film thin deposition (coating). In Endodontics, the application of NTP on intrarradicular dentin surface seems to be beneficial for root canal disinfection. Regarding restoration adhesion this technology was reported to improved dentin wettability and hydrophilicity, increasing the adhesive-dentin bond strength. Concerning endodontic sealer-intrarradicular interface, it is still not entirely clear the effect of this potential approach to improve adhesion. Furthermore, in what concerns GP's effect, plasma treatments have been scarcely researched. Another reported functionalization strategy is the deposition of a thin coating film, such as a ZnO film. ZnO is a multipurpose material with an extensive range of applications in diverse fields due to its easy and environmentally friendly synthesis, biocompatibility, antimicrobial and antifungal properties, and high chemical stability. At the nanometric scale, ZnO thin films have many remarkable features due to their considerable bond strength, good optical quality, and excellent piezoelectric, antibacterial, and antifungal properties, offering many potential applications in various fields.

The present thesis highlights the effect of dentin and GP surfaces' functionalization, through PT and ZnO thin film coating, on the adhesiveness between GP/sealers/dentin interfaces, in view of a better obturation seal. The hypothesis of this thesis assumes that endodontic functionalized substrates might have a positive impact in the bond strength between core materials, such as GP, and endodontic sealers, as well as at the interface dentin/sealer. The main

objective was to investigate the effect of functionalization on endodontic substrates, such as intraradicular dentine and GP surfaces, assessing interfaces' bond strength. The following specific objectives were considered: to analyze, through a systematic review, the effect of NTP on intraradicular dentin/sealers' adhesion (Study 1); to evaluate the effect of NTP on conventional and bioceramic GP surfaces, assessing their roughness, surface free energy, chemical structure, and sealer wettability (Study 2); to explore the influence of GP surface's functionalization with a nanostructured ZnO thin film on its adhesiveness to endodontic sealers (Study 3).

**Material and methods:** The methodology consisted of three phases. For the first study, a systematic review "Effect of plasma treatment on root canal sealers' adhesion to intraradicular dentin" was performed. The recommendations of the 2020 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were followed. This study was conducted to answer the PICO question "Does the NTP treatment affect sealers' adhesion to dentin compared to no treatment?". A literature search was undertaken without limits on time or language, until May 2023, in PubMed–MEDLINE, Scopus, Web of Science, OpenGrey, and three endodontic journals (Journal of Endodontics, International Endodontic Journal, and Australian Endodontic Journal). Moreover, an additional search was conducted using the reference lists of all included papers. Only in-vitro studies that treated dentin with plasma technology and assessed the effects of NTP treatments on root canal sealers' adhesion to dentin were included. Studies that did not use a control group (without PT) were excluded. The included studies underwent quality assessment and data extraction. Two authors independently evaluated the risk of bias in each selected study. The following parameters were considered: (1) randomization, (2) blinding, (3) standardization of specimen selection, (4) standardized preparation (single operator), and (5) reporting of data. In the second study "Functionalization of gutta-percha surfaces with argon and oxygen plasma treatments to enhance adhesiveness", round discs of GP samples were produced from GP pellets. The samples were produced by creating appropriate molds and then plasticizing GP in a laboratory dry-heating oven at 80 °C, followed by a cooling process at room temperature. Two type of GP were tested: conventional and bioceramic. A standardized metallographic procedure was employed with coarse silicon carbide abrasive papers (until 600 grit) to produce GP discs with similar surface roughness in both faces. A Low-Pressure Plasma Cleaner by Diener Electronic (Zepto Model) was used for the GP surface activation. Plasma treatments were executed considering three main parameters: (i) working gas (Ar or O2), (ii) treatment time (30 s, 60 s, 120 s, or 180 s), and (iii) power (25 W or 50 W). The work pressure was constant for all the treatments (~ 80 Pa), while the base pressure was always lower than 20 Pa. The surface topography of the GP specimens was evaluated before and after activation with plasma treatments, measuring the surface roughness with an optical profilometer. The surface topographical analysis, the surface free energy and the contact angle were assessed. The surface free energy was calculated by measuring the contact angle between the solutions (water, glycerol and 1-bromonaphthalene) and the GP surfaces, applying the Owens and Wendt method. Lastly, the chemical analysis through the Fourier transform-infrared spectroscopy (FT-IR) and sealers' wettability (Endoresin and AH Plus Bioceramics) were assessed. Kruskal-Wallis and Student's t-test were used in statistical analysis. For the third study "Nanostructured ZnO thin film to enhance gutta-percha's adhesion to endodontic sealers" conventional GP samples were divided randomly into three groups: a) Untreated GP (control); b) GP treated with argon plasma (PT); c) Functionalized GP (PT followed by ZnO thin film deposition). GP' surface functionalization encompassed a multi-step process. First, a low-pressure argon PT was applied to modify the GP surfaces, followed by a

ZnO thin film deposition by physical vapor deposition using reactive magnetron sputtering. In this process, a target material, typically a metal or compound, is bombarded with Ar+ ions inside a vacuum chamber. The collision of Ar+ ions with the target result in a momentum and energy transference. As a consequence, the atoms of the target material are displaced from their original locations and ejected into the surrounding vacuum interacting with the O2 reactive gas, and then deposited on the GP substrate. The surface morphology of the GP samples was assessed qualitatively by scanning electron microscopy (SEM). The water contact angle was measured by the sessile drop technique. Further comprehensive testing included the tensile bond strength assessment, evaluating Endoresin and AH Plus Bioceramic sealers' adhesion to GP. Regarding statistical analysis, water contact angle results were evaluated using ANOVA repeated measures (3 levels: control, PT, and PT + ZnO). Tensile bond strength results were analyzed with a two-way ANOVA followed by Bonferroni post-hoc tests.

**Results:** Regarding to the systematic review, out of an initial 188 articles, 4 studies were included. All studies had a high risk of bias with respect to blinding, randomization process, and standardized sample preparation (single operator), because these parameters were not mentioned. All of the studies reported all results and performed sample standardization, so they were considered to have a low risk of bias in these parameters. None of the included studies had a low risk of bias in all parameters evaluated, so the overall risk of bias of the selected studies was high. Three of these studies based the adhesion ability on the push-out test in human extracted teeth, while the other used bovine dentin samples to measure the contact angle with the sealer (wettability). NTP treatments were performed under vacuum conditions using a glass reactor in two of the studies, while the two other used an atmospheric-pressure plasma jet. Two studies used Ar plasma, while the other two applied a mixture of gases. The application time varied between 30 s and 1 min. There was no consensus about the effect of NTP on the AH Plus sealer's adhesion to radicular dentin. The bond strength of BioRoot RCS and Endosequence BC seemed to be positively influenced by NTP. For MTA Fillapex, the bond strength decreased with NTP. The principal findings of the second study were the following. Independently of the GP type (conventional or bioceramic), plasma treatments carried out in Ar or O2 atmospheres showed different behaviors, depending on the power and duration. Comparing to the control (29.40 nm), for conventional GP, the highest roughness values were registered with an Ar atmosphere at 50 W for 120 s (32.04 nm; $p = 0.002$) and an O2 atmosphere at 25 W for 120 s (31.29 nm; $p = 0.005$). Comparing to the control (Ra = 26.50 nm) for bioceramic GP, the highest values of roughness were achieved for the treatments performed in an Ar atmosphere at 50 W for 60 s (33.94 nm; $p < 0.001$) and in an O2 atmosphere at 25 W for 30 s (29.87 nm; $p < 0.001$). Plasma treatments increased samples' surface free energy relative to the control, independently of GP type ($p < 0.001$). Comparing to the control (41.02 mJ/m2), for the conventional GP, the highest surface free energy values were registered with an Ar atmosphere at 50 W for 60 s (55.23 mJ/m2; $p < 0.001$) or at 25 W for 30 s (54.64 mJ/m2; $p < 0.001$) and an O2 atmosphere at 50 W for 180 s (57.87 mJ/m2; $p < 0.001$). In turn, comparing to the control (31.41 mJ/m2) on bioceramic GP, the highest values of surface free energy were achieved with an Ar atmosphere, at 25 W, for a treatment duration of 30 s (59.13 mJ/m2; $p < 0.001$) and with an O2 atmosphere, at 25 W, for 120 s (65.70 mJ/m2; $p < 0.001$). The Fourier Transform-infrared Spectroscopy (FT-IR) analysis showed that although chemical changes were detected, the basic molecular structure of the assessed GPs was maintained. For sealers' wettability analysis the parameters applied for each GP type, considering a good balance between power, time and respective impact on roughness and surface free energy were: Ar at 50 W during 60 s and O2 at 25 W during 120 s. For Endoresin sealer in conventional GP, there

were significant differences between the control (not treated GP surfaces) and Ar plasma treated GP ($p = 0.002$). In bioceramic GP both plasma treatments with the selected parameters improved the sealer's wettability when compared with the control group (Ar: $p = 0.037$; O2: $p < 0.001$). Regarding AH Plus Bioceramic sealer, both atmospheres (Ar and O2) produced significant differences, in conventional and bioceramic GPs, with increased values, compared with the control (Ar: $p < 0.001$; O2: $p < 0.001$). The main findings of the third study are below presented. Surface morphology analysis by SEM revealed that untreated GP (control) showed ZnO crystals encrusted on the GP matrix, covered by an organic layer constituted by wax/resin components. PT with Ar caused the removal of the wax/resin surface layer, exposing the ZnO crystals embedded in the GP matrix and uncovering the surface porosity promoted by the ZnO grains boundaries. The GP samples submitted ZnO film thin deposition, increased the surface area to be covered with ZnO, benefiting from its additional properties. ZnO-functionalized GP decreased the water contact angle compared to the control ($p<0.001$). The mean bond strength value in Endoresin group was significantly higher than that in AH Plus bioceramic group, for all tested conditions (control, PT and PT + ZnO) ($p<0.001$). There was a statistically significant difference between the control and the ZnO-functionalized GP ($p=0.006$), with the latter presenting the highest mean bond strength value. There was no significant difference in bond strength values between PT and PT+ZnO ($p=0.553$), nor between PT and control ($p=0.203$).

**Conclusions:** There was no consensus about the effect of NTP on the AH Plus sealer's adhesion to radicular dentin. However, it did appear that NTP could have a positive effect on the adhesion of BioRoot RCS and Endosequence BC. The NTP parameters should be optimized to obtain a stronger evidence base, in endodontics, on its role as an adjuvant tool to increase sealers' adhesion to dentin. These findings should be interpreted cautiously due to the scarcity of studies on the topic. The functionalization of GP surfaces with Ar or $O_2$ plasma treatments can increase roughness, surface free energy and wettability of the Endoresin and AH Plus Bioceramic, which might improve its adhesive properties when compared to non-treated GP. Functionalization of GP with a nanostructured ZnO thin film enhances the bond strength with sealers of different chemical composition and induced a shift towards hydrophilicity. The ZnO thin film deposition preserved GP's surface morphology modified by PT, enhancing the bond strength to both Endoresin and AH Plus Bioceramic sealers, compared to untreated GP. Considering that this topic has been scarcely discussed in the current endodontic literature the data herein reported may add a novel insight. However, it must be stressed that the behaviour of filling materials and dentin in in-vitro conditions, may not truly reflect the clinical set-up. Further studies are needed to confirm the clinical relevance of the present findings.

# ABBREVIATIONS

AP - Apical periodontitis

Ar - Argon

CSS - Calcium silicate-based based sealers

ET - Endodontic treatment

FT-IR - Fourier transform-infrared spectroscopy

GP - Gutta-percha

He - Helium

mL – milliliters

mm – millimeters

min - minute

NPs - Nanoparticles

NDGP - Nanodiamond gutta-percha composite

NTP - Non-thermal plasma

$N_2$ – Nitrogen

$O_2$ - Oxygen

PT – Plasma treatment

POBS – Push-out bond strength

SEM - Scanning electron microscopy

µm - micrometer

XRD - X-ray diffraction analysis

ZnO - Zinc oxide

# Index

# 1
# Introduction

# 1. INTRODUCTION

Endodontic treatment (ET), also designated by root canal treatment, is the most frequent conservative therapy to manage severe inflammatory or infectious lesions of the root canal system. The aim of ET is either to avoid the development of apical periodontitis (AP) or, in cases where the disease is already present, to create the appropriate conditions for periradicular tissue healing (Siqueira *et al.* 2008).

AP is a highly prevalent oral disease worldwide with half of the adult population having at least one tooth involved with this pathology (Tibúrcio-Machado *et al.* 2021). It is worth to emphasize that although ET is still the first treatment option for AP, an increased prevalence of AP has been reported in the last decade, both in untreated as well as in filled teeth (Jakovljevic *et al.* 2020). Besides the fact that most of the data was collected from cross-sectional studies, which may include AP in healing process, or in immunocompromised individuals, the quality of ET was proved to influence the outcome.  Inadequate ET are more prone to be associated to persistent AP.

Nevertheless, well-treated teeth still fail (Siqueira 2001). Root canal treatment failure will result from the inability to eradicate AP, even when the ET is according to the state of art. The knowledge of the microbial etiology of AP is crucial, either for clinicians or researchers to develop and implement the best clinical practice, based on solid scientific evidence. Intra-radicular infections are characterized as sessile multispecies communities of selected microorganisms (biofilms), adhered to the dentinal walls. They are particularly relevant in the apical root canal section challenging the actual endodontic disinfection protocols (Siqueira Jr. *et al.* 2022).

For the maximum success it is crucial to recognize the different clinical conditions and provide adequate therapeutic strategies. In vital teeth the actual endodontic procedures are highly predictable, with an increased success rate over the last decade. However, for necrotic teeth with AP, the clinician must deal with several factors, such as the different types of infections, primary, secondary/persistent with the respective particularities in what concerns the moment of the infection and the type of microbiota involved as well as the host immune response (Siqueira 2001, Siqueira *et al.* 2008). ET's positive outcome in infected teeth results, thus, from the reduction or eradication of the intra-radicular microorganisms from the root canal system to a certain burden, compatible to the host response (Siqueira *et al.* 2008). This would enable the affected tooth to remain in the oral cavity, functional, with healthy perirradicular tissues and an asymptomatic patient.

Endodontic infections are not susceptible to spontaneous healing, through the defense mechanisms of the host, neither through systemic antibiotic therapy (Siqueira *et al.* 2008). Due to the location of the infection, most often inside the root canal system, it must be eliminated, clinically, by mechanical and chemical procedures. A proper access, an adequate chemo-mechanical preparation and hermetic obturation, are the main disinfection steps of ET (Siqueira

Jr. *et al.* 2022). In this sense, it is of utmost importance to achieve a three-dimensional sealing of the root canal system, while preventing coronal and apical leakage (Siqueira Jr. *et al.* 2022). Root canal filling has been most widely reported as a combination of a rigid or semi-rigid core material and a sealer. A variety of core materials have been used, including silver cones, Resilon and gutta-percha (GP) (Berman *et al.* 2020). However, the most widely accepted method of obturation, still involves GP as a core material. Bowman was the first to use GP as a filling material, in 1867 (Prakash *et al.* 2005). GP is a trans-isomer of polyisoprene (1, 4, trans–poly isoprene), being harder, more brittle and less elastic than rubber (Prakash *et al.* 2005). It is obtained from the latex coagulation of Malaysian trees and can, chemically, present two distinct crystalline forms: alpha and beta. Natural GP, taken directly from the tree, is in the alpha form (lower viscosity, runny and sticky), whereas commercial GP is in the beta form (higher viscosity, solid, compactible and elongatible). During the process of manufacturing, if the cooling is done rapidly, beta form results. On the other hand, if it is cooled slowly, alpha form will be present (Prakash *et al.* 2005). Most of the commercially available GP is composed of organic components (GP polymer (19-22%) and wax/resins (1-4%)) and inorganic components, such as zinc oxide (ZnO) (60-75%). Opacifiers, such as barium sulphate are also included (1-17%) (Maniglia-Ferreira C *et al.* 2005). GP is a viscoelastic and thermoplastic material that is temperature sensitive. At room temperature, GP is presented in a solid and stiff state. It becomes soft at 60°C and melts at 95°C-100°C. The physical properties such as tensile strength, stiffness, brittleness and radiopacity are dependent on the organic and inorganic components (Vishwanath V *et al.* 2019). The major advantages of GP are its minimal toxicity, plasticity, simple manipulation, radiopacity and ease of removal with solvents or heat (Vishwanath V *et al.* 2019). Disadvantages include no self-adhesive properties, as it does not truly bond to sealer or dentin surfaces, which can compromise the hermetic seal due to potential apical or coronal leakage (Vishwanath V *et al.* 2019). Besides that, although GP is commercialized in a sterilized packaging it can be easily contaminated by handling (Subha *et al.* 2013). Its ZnO content does not prevent biofilm colonization (Vishwanath V *et al.* 2019). Additionally, the chair-side disinfection protocol with sodium hypochlorite, often recommended, has not reached a consensus (Salvia *et al.* 2011, Subha *et al.* 2013, Makade *et al.* 2017).

The endodontic sealer act as a bonding agent between the GP core and the dentinal walls. A wide variety of sealers are commercially available. They can be classified according to their chemical composition: ZnO-eugenol, calcium hydroxide, epoxy resin, silicone, glass ionomer and more recently calcium silicate-based based sealers (CSS). Despite claims by the manufacturers on the advantages of each class, there is no evidence-based data, demonstrating the superiority of one sealer over another (Berman *et al.* 2020). Epoxy resin-based root canal sealers has been considered a “gold standard” in endodontics due to their good properties, which include long-term dimensional stability, apical sealability, low toxicity and reduced solubility (Flores *et al.* 2011). Furthermore, it has been pointed out that these sealers have good retention to root dentin, exhibiting higher values of push-out bond strength when compared with other endodontic sealers (Sagsen *et al.* 2011, Carvalho *et al.* 2015). Over the last two decades, calcium silicate-based materials have become increasingly popular. As root canal sealers, these formulations have been extensively studied and compared with conventional ones (Silva *et al.* 2019, Sfeir *et al.* 2021). In-vitro studies have shown their promising properties, particularly their biocompatibility, antimicrobial properties, and bioactivity (Sfeir *et al.* 2021). Nevertheless, the potential impact of their higher solubility is a matter of debate and needs to be clarified as it may affect their long-term sealing ability (Silva *et al.* 2017). Contrary to the traditional ones, those sealers are hydraulic, and their setting is conditioned by the presence of moisture (Sfeir *et al.* 2021). Regarding push-out bond strength ability to dentin, the studies are conflicting (Sfeir *et al.* 2021), presenting better (Wanees Amin *et al.* 2012) and poorer (Delong *et al.* 2015) performances.

In the early 2000s, the "monoblock" concept has been introduced in which GP, the sealer and the dentin generated a single unit (Tay *et al.* 2007). Monoblock is described by the number of interfaces between the filling material and the dentinal wall. Using only 1 filling material, like mineral trioxide aggregate (MTA), a single interface between dentin and this material will be created - primary monoblock (Tay *et al.* 2007). The use of a sealer associated with a solid filling material, like GP, creates two interfaces, one between the sealer and the filling core, the other between the dentin and the sealer - secondary monoblock (Tay *et al.* 2007). Tertiary monoblock is considered when a third circumferential interface is created between the bonding substrate and the abutment material (Tay *et al.* 2007). In this case, the use of a cone with a coating layer is advocated. It can be created by using obturation systems such as EndoREZ (Ultradent Products Inc., South Jordan, UT, USA), Activ GP (Brasseler USA, Savannah, GA, USA) or more recently TotalFill BC filling system (FKG DENTAIRE SA, La Chaux-de-Fonds, Switzerland). Indeed, as Grossman proposed in 1978, the ideal properties of a root filling material remain: easy handling and ample working time; dimensionally stable; sealing ability; conforming to the complex internal anatomy; non-irritant; not staining the tooth structure; antimicrobial properties; impervious and non-porous; unchanged by tissue fluid; radiopaque; easily removed (Berman *et al.* 2020). Notwithstanding the great technological developments in endodontic materials and all the efforts of the researchers, no currently available filling material can satisfy all these requirements. Thus, there is still a gap in achieving a better long-term fluid-tight seal between the sealer and the GP core, improving ET's outcome in infected teeth.

Materials science has made significant advances, impacting the tools and materials used in endodontic practice (Chan *et al.* 2023). Newer approaches have been explored to improve the shortcomings of commonly used endodontic materials or introduced new ones (Zakrzewski *et al.* 2021, Chan *et al.* 2023). Functionalization is an increasingly promising approach for property improvement of dental materials. It can be described as "the process of adding new functions, features, capabilities or properties to a material by changing the surface chemistry of the material, with the aim of inducing a desired bioresponse or inhibit a potentially adverse reaction" (Chan *et al.* 2023). Regarding endodontic field, several surface treatment and coating options have been studied in recent years to achieve certain beneficial effects (Vishwanath V *et al.* 2019).

In 2005, the resin coated GP (EndoREZ GP Points, Ultradent, South Jordan, UT) was introduced with main purpose of improving the adhesion capacity of GP to a methacrylic resin-based sealer (EndoREZ sealer, Ultradent, South Jordan, UT). Resin coated GP is covered with a polybutadiene diisocyanate-methacrylate adhesive (Kim *et al.* 2010). This adhesive includes a hydrophobic portion, chemically compatible with GP polyisoprene and another hydrophilic portion, chemically compatible with the hydrophilic methacrylic resin-based sealers (Kikly *et al.* 2020). This resin-coated GP cones have been recommended in association to methacrylate resin-based sealers for the formation of tertiary monoblock – EndoREZ system. A poor bond strength of this filling system to dentin was reported, due to the polymerization shrinkage of its sealer (Bergmans *et al.* 2005) . Furthermore, gaps and silver leakage were recognized between the EndoREZ sealer and the GP resin coating (Tay *et al.* 2005). Around the same time glass ionomer-impregnated and coated GP emerged (ActiV GP, Brasseler USA, Savannah, GA, USA). ActiV GP system consists of glass–ionomer-coated GP cones using with a glass–ionomer sealer. Glass ionomer coated GP has a 2-µm coating of glass ionomer particles on its surface, as well as incorporated into the body of the cone. The bond to both the core and dentin through the sealer is mentioned to as a "tertiary monoblock" (Fransen *et al.* 2008). ActiV GP system was related to a higher percentage of voids (Başer Can *et al.* 2017). Also, the bond strength and sealer penetration was not superior to the resin-based sealer used with conventional GP (Deniz Sungur *et al.* 2016). Regarding sealing ability there has been conflicting data. Monticelli *et al.* (2007) showed that there was no difference in leakage between teeth obturated with GP/AH Plus sealer and ActiV GP system using a fluid filtration system. Nevertheless, using a bacterial leakage model, it was reported that ActiV GP system resulted in significantly more leakage compared with GP/AH Plus sealer (Monticelli *et al.* 2007).

Incorporation of bioactive materials have been also tested. GP incorporating niobium phosphate bioglass has presented promising results for antimicrobial activity (Carvalho *et al.* 2016), bioactivity (Sampaio *et al.* 2023) and self-adhesiveness to dentin (Carvalho *et al.* 2015). However, it did not appear to improve the biological properties of GP, as it affected the viability of human periodontal ligament fibroblasts and partially disrupted physiological cell function (Meneses *et al.* 2020).

Due to the unique properties of nanoparticles (NPs), which include their nano size, high surface area-to-volume ratio, enhanced solubility, ability to functionalize diverse substrates and increased reactivity and antibacterial activity, their use in endodontics has attracted considerable interest (Zakrzewski *et al.* 2021). The functionalization of GP with silver NPs provide antibacterial properties against *Enterococcus.faecalis* (Mohan *et al.* 2020) and *E.coli* (Monisha *et al.* 2023). Furthermore, Mozayeni *et al.* (2017) in a biocompatibility study on a rat model, showed that GP-coated with silver NPs was biocompatible. Likewise, functionalization GP with chitosan NPs presented higher antimicrobial activity than the conventional ones and improved its mechanical properties (i.e., higher values for tensile strength and elongation) providing enhanced resistance to fracture (Cardelle-Cobas *et al.* 2019). Recently, the TotalFill BC filling system began to be commercialized in Europe. It consists of bioceramic NPs coated GP (TotalFill BC Points) and a calcium silicate-based sealer (TotalFill BC Sealer). It was suggested that a gap-free seal is created when this obturation system is used with the single cone technique (Trope *et al.* 2015). Therefore, the scarce studies available did not find a superior bond of the CSS to impregnated GP cones, compared to the epoxy resin-based sealer bonded to conventional GP (Banphakarn *et al.* 2019). Eltair M *et al.* (2018) showed that a significantly fewer interfacial gaps were found between conventional GP and the sealer (epoxy resin-based or CSS) compared to the bioceramic coated GP, independently of the obturation technique (lateral compaction or single cone).

Ongoing clinical trials are testing nanodiamond-embedded GP with promising results (Lee *et al.* 2017). Lee *et al.* (2015) improved the mechanical properties of a nanodiamond GP composite (NDGP) functionalized with amoxicillin (ND-AMC) to reduce the probability of root canal reinfection. NDGP has been shown to increase treatment efficacy and can be used in conjunction with conventional endodontic therapy procedures.

Plasma technology is another approach to surface modification/functionalizing of endodontic substrates. Plasma was discovered in 1879 by the physicist Sir William Crookes (Lata S *et al.* 2022). It is not clear when it was first used in the field of dentistry, but it is believed that the first application was in 1991 for implant surface treatments (Smith *et al.* 1991). Plasma is considered the fourth state of matter, is abundant in nature (stars, auroras, lightning, etc.) and involves a combination of neutral particles, electrons and ions. It can be produced by heating a gas or subjecting it to a strong electromagnetic field until the gas particles are ionized (Borges Ac *et al.* 2021). Plasmas are usually categorized as thermal and non-thermal plasmas (NTP), or cold plasma, based on the relative temperatures of the diverse plasma species (electrons, ions, and neutrals) (Borges Ac *et al.* 2021). In thermal plasmas (natural phenomena), electrons and heavy particles are in thermal equilibrium, while in NTP electrons are hotter than ions, and neutrons are at room temperature. NTP is artificially made of, and can be produced under vacuum or atmospheric conditions, using inert gases like argon (Ar) or helium (He), reactive gases such as oxygen ($O_2$) or nitrogen ($N_2$), or a mixture of gases (Ritts Ac *et al.* 2010, Prado M. *et al.* 2016). The great advantage of this technology is that it provides an efficient and clean approach to surface functionalization without altering the original structure of the materials (Chen M *et al.* 2013). NTP has acquired considerable attention for its potential to enhance adhesion in various dental applications, including composites, fiber posts, and acrylic resin dentures (Yavirach P *et al.* 2009, Ritts Ac *et al.* 2010, Zhang H *et al.* 2010)**.** In Endodontics, the application of NTP on intrarradicular dentin surface seems to be beneficial for root canal disinfection and therefore a lot of data has been published within the last decade (Jungbauer *et al.* 2021). Plasma produces a high concentration of charged particles and reactive oxygen species that interact with microorganisms, causing damage to cell structure through etching and oxidation, and disrupting the conductivity of the cell surface, resulting in death (Jungbauer *et al.* 2021). A recent systematic review evaluated the effects of NTP on dental restoration adhesion and concluded that this technology improved dentin wettability and hydrophilicity, increasing the adhesive-dentin bond strength (Stasic *et al.* 2021). Regarding endodontic sealer-intrarradicular interface, it is still not entirely clear the effect of this approach (Stasic *et al.* 2021). On GP, plasma treatments have been scarcely researched (Prado M. *et al.* 2016, Alves Mj *et al.* 2018), lacking studies about the several parameters that might influence its performance.

In 2018, Alves Mj *et al.* (2018) proposed a functionalized GP cone with a nanostructured ZnO thin film. GP coated cones presented a higher antibacterial activity over refractory endodontic microorganisms, such as *Enterococcus faecalis.* Additionally, an appropriate cytocompatibility was reported. Thin coating film is defined as a layer of material between nanometers and micrometers' thick that is deposited on a substratum with the aim of altering its surface characteristics (Qadir *et al.* 2019). At the nanometric scale, ZnO thin films have many remarkable properties due to their large bond strength, good optical quality, and excellent piezoelectric, antibacterial, and antifungal properties, therefore presenting many potential applications in various fields (Carvalho *et al.* 2014, Costa D *et al.* 2019). ZnO is a multipurpose material with an extensive range of applications in diverse fields due to its easy and environmentally friendly synthesis, biocompatibility, and high chemical stability (Sharma *et al.* 2022). Until now, no studies have investigated the performance of GP functionalized with ZnO thin films concerning sealers' adhesion.

The present thesis highlights the effect of dentin and GP surfaces' functionalization, through PT and thin film coating, on the adhesiveness between GP/sealers/dentin interfaces, in view of a better obturation seal.

# 2
# HYPOTHESIS AND OBJECTIVES

# 2. HYPOTHESIS AND OBJECTIVES

## 2.1 HYPOTHESIS

The hypothesis of this thesis assumes that functionalized substrates, such as root dentin or GP surfaces, might have a positive impact in the bond strength between core materials and endodontic sealers, as well as at the interface dentin/sealer. This hypothesis is justified by the findings emerging in the scientific literature, reporting that the potential of functionalization through PT would enhance wettability and surface energy of the substrate, improving surface's reactivity. This would result in an increased adhesion performance, while maintaining the core chemical structure of the substrates and assuring cytocompatibility. Furthermore, the functionalization with an antimicrobial thin nanoparticle-based film would enhance bonding and hinder microbial colonization.

## 2.2 OBJECTIVES

**Main Objective:** To investigate the effect of functionalization on endodontic substrates, such as intraradicular dentine and gutta-percha surfaces, assessing interfaces' bond strength, in view of a better sealing ability of root canal filling.

**Specific objectives:|**
- To analyze, through a systematic review, the effect of NTP on intraradicular dentin/sealers' adhesion (Paper 1)
- To evaluate the effect of NTP on conventional and bioceramic GP surfaces, assessing their roughness, surface free energy, chemical structure, and sealer wettability (Paper 2)
- To explore the influence of GP surface's functionalization with a nanostructured ZnO thin film on its adhesiveness to endodontic sealers (Paper 3)

# 3
# METHODOLOGY

# 3. METHODOLOGY

## 3.1 Effect of Plasma Treatment on Root Canal Sealers' Adhesion to Dentin

This systematic review was conducted in accordance with the 2020 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. (Page *et al.* 2021).

### 3.1.1 Eligibility Criteria

This study was carried out in order to answer the PICO question "Does the NTP treatment affect sealers' adhesion to dentin compared to no treatment?" (Figure 1).

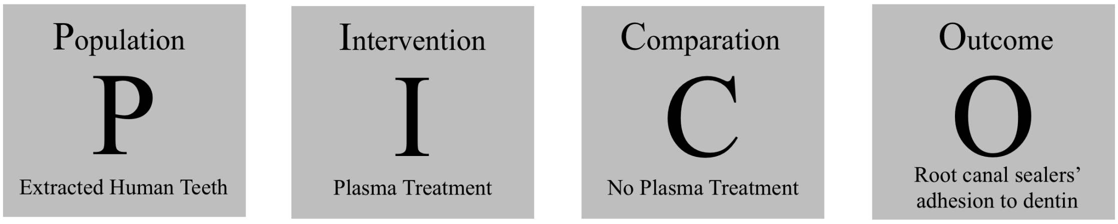


**Figure 1. The PICO strategy for the research question construction. (Source: own elaboration)**

3.1.1.1 Included and excluded criteria

Studies that did not include a control group (without plasma treatment) were excluded. In vitro studies that treated dentin with plasma technology and evaluated the effects of NTP treatments on the adhesion of root canal sealers to dentin were included.

### 3.1.2 Search Strategy

The literature search was conducted until May 2023 without language or publication date restrictions. An electronic search was performed on PubMed (Medline), Scopus, and Web of Science. The search strategy combined Medical Subject Heading (MeSH) terms, text words (tw), and truncation terms. The Boolean operators "AND" and "OR" were applied to create the search strategy (Table 1).

In addition, the grey literature was searched using OpenGrey, and a manual search of the Journal of Endodontics, International Endodontic Journal and Australian Endodontic Journal was conducted to identify additional papers. An additional search was performed using the reference lists of all included papers. References from various databases were imported into EndNote X9 software (Thomson Reuters, New York, NY, USA), which automatically removed duplicate records.

Table 1. Search strategy in various databases. (Source: https://www.mdpi.com/2076-3417/13/15/8655# )

| Database | Search Strategy | Findings |
|---|---|---|
| PubMed | **#1** ((non-thermal plasma[Title/Abstract]) or (nonthermal plasma[Title/Abstract]) or (Plasma Gases[Title/Abstract]) or (plasma treatment[Title/Abstract]) or plasma[Title/Abstract] or (Plasma Gases[MeSH Terms]) or plasma[MeSH Terms]) | |
| | **#2** ((dental cements[MeSH Terms]) or (root canal sealants[MeSH Terms]) or (dental cement *[Title/Abstract]) or (root canal seal *[Title/Abstract]) or (endodontic seal *[Title/Abstract]) or (root canal fill *[Title/Abstract]) or (seal*[Title/Abstract])) | |
| | **#3** ((endodontic *[Title/Abstract]) or (root canal[Title/Abstract]) or (endodontic treatment[Title/Abstract]) or (root canal treatment[Title/Abstract]) or (Root Canal Therapy[Title/Abstract]) or (Root Canal Therapy[MeSH Terms]) or (Endodontics[MeSH Terms]) | |
| | **#1 and #2 and #3** | 96 |
| ELSEVIER Scopus | **#1** TITLE-ABS-KEY("non-thermal plasma" or "nonthermal plasma" or "Plasma Gases" or "plasma treatment" or plasma) | |
| | **#2** TITLE-ABS-KEY("dental cements" or "root canal sealants" or "dental cement *" or "root canal seal *" or "endodontic seal *" or "root canal fill *" or "seal *") | |
| | **#3** TITLE-ABS-KEY("endodontic *" or "root canal" or "endodontic treatment" or "root canal treatment" or "Root Canal Therapy") | |
| | **#1 and #2 and #3** | 140 |
| Clarivate Web of Science | #1 TS = ("non-thermal plasma" or "nonthermal plasma" or "Plasma Gases" or "plasma treatment" or plasma) | |
| | **#2** TS = ("dental cements" or "root canal sealants" or "dental cement *" or "root canal seal*" or "endodontic seal *" OR "root canal fill *" or "seal *") | |
| | **#3** TS = ("endodontic *" or "root canal" or "endodontic treatment" or "root canal treatment" or "Root Canal Therapy") | |
| | **#1 and #2 and #3** | 83 |

### 3.1.3 Selection of the Studies

Two reviewers independently evaluated the searched titles and abstracts and discarded ineligible articles. If the title and abstract were insufficient to confirm or exclude a specific study, they read the full text. In case of disagreement, a third author determined whether the article should be included.

### 3.1.4 Data Extraction

The following details were obtained independently by two reviewers from each included study: tooth type, non-thermal treatment (i.e., gas/application time, plasma mode, device used, distance, pressure, and power applied), methodology for testing adhesion ability (push-out testing parameters (i.e., filling materials used, storage, canal segments analyzed, slice thickness, plunger diameter, and plunger loading direction) and contact angle analysis), and principal results.

### 3.1.5 Risk-of-Bias Assessment

Two independent authors assessed the quality of the included studies. For each study selected, the risk of bias was assessed.

The method used to assess the risk of bias was adapted from a previously published systematic review (Augusto *et al.* 2022). The parameters under consideration were the following: (1) randomization, (2) blinding, (3) standardization of specimen selection, (4) standardized preparation (single operator), and (5) reporting of data. If the above parameters were mentioned, the risk of bias was recorded as low; if the parameters were not mentioned, it was recorded as high; if their mention was not clear, it was recorded as unclear. Disagreements between authors were solved by discussion with a third author.

## 3.2 FUNCTIONALIZATION OF GP SURFACES WITH PLASMA TREATMENTS

### 3.2.1 Specimen preparation and standardization

Round discs of GP specimens (10 mm diameter and 2 mm thickness) were made from GP pellets: conventional GP (DiaDent Gutta-Percha Pellets; Choongchong Buk Do, Republic of Korea) and bioceramic GP (TotalFill Bioceramic Gutta-Percha Pellets; FKG Dentaire, La-Chaux-de-fonds, Switzerland). As another study (De-Deus G *et al.* 2021), these GP discs were prepared by making appropriate molds and then plasticizing GP in a laboratory dry-heating oven at 80 °C, followed by a cooling process at room temperature. A standardized metallographic approach using coarse silicon carbide abrasive paper (up to 600 grit) was used to produce GP discs with equivalent surface roughness on both sides (Figure 2). The surface roughness of the conventional GP samples (Ra Zscore: n = 135, t = -2.5 × $10^{-13}$, p ≅ 1.0) or the bioceramic samples (Ra Zscore: n = 135, t = 9.45 × $10^{-15}$, p ≅ 1.0) were not statistically significantly different. An online computer-generated number was used to randomly assign samples to the different groups (www.randomizer.org).

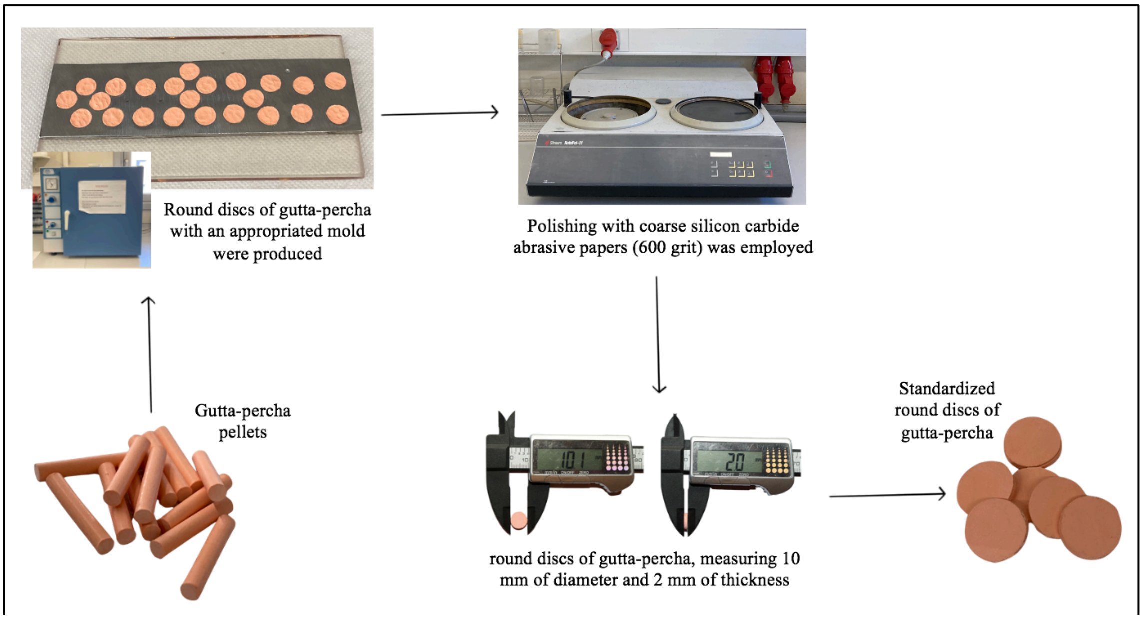


**Figure 2. Samples preparation and standardization. (Source: own elaboration)**

### 3.2.2 Characterization of the GP specimens

X-ray diffraction (XRD) analysis was executed using a Siemens D 5000 diffractometer (D8 Discover; Bruker AXS, Karlsruhe, Germany) with Cu-Ka radiation ($\lambda$ = 1.5418 A) and was performd with a scan range of 5° – 90° ($2\theta$) using a $\theta/2\theta$ configuration and a step time of 2 seconds. Crystalline phases were recognized using the Inorganic Crystal Structure Database (ICSD).

### 3.2.3 Functionalization of GP surfaces

A Zepto laboratory-sized plasma system (Diener Electronic; Ebhausen, Germany), equipped with a 13.56 MHz generator, was employed for the GP surface activation (Figure 3). Three main parameters were considered in the plasma treatments: (i) working gas (Ar or $O_2$), (ii) treatment time (30 s, 60 s, 120 s, or 180 s), and (iii) power (25 W or 50 W). The work pressure was constant for all the treatments (~ 80 Pa), while the base pressure was always less than 20 Pa. Figure 4 shows a schematic illustration of the effect of the different plasma treatment (Ar and $O_2$) applied to GP surfaces.

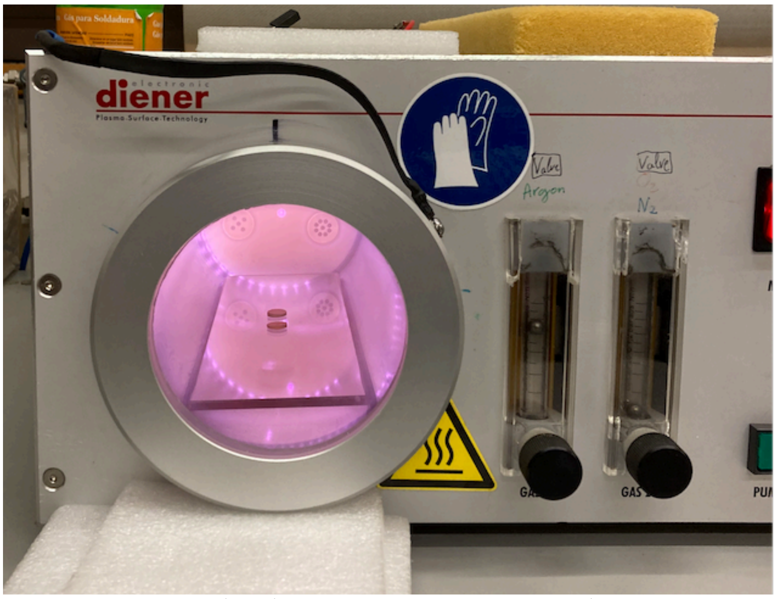


**Figure 3. Gutta-percha plasma surface treatment with a Low-Pressure Plasma equipment by Diener Electronic, Zepto Model. (Source: own elaboration)**

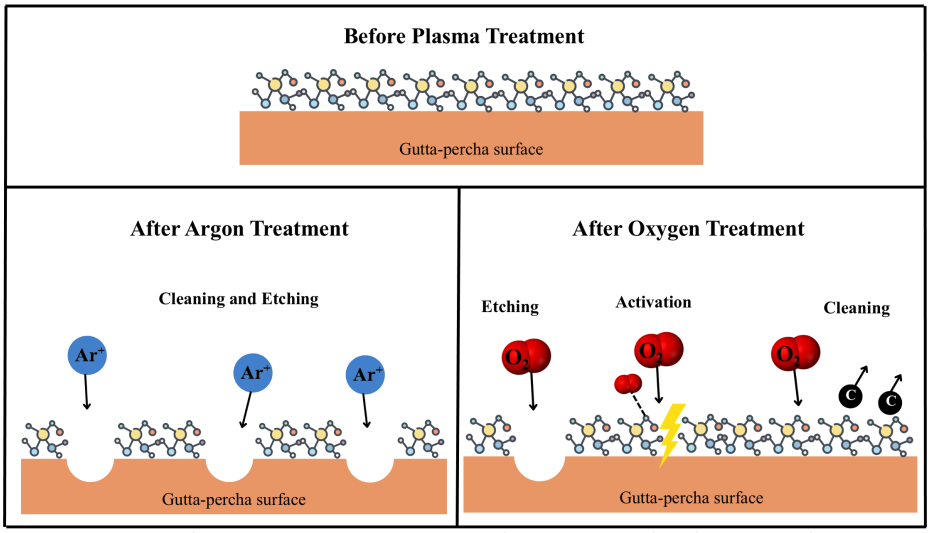


**Figure 4. Schematic representation of the effect of the different plasma treatment (Argon and Oxygen) applied to gutta-percha surfaces. (Source: https://www.nature.com/articles/s41598-023-37372-x )**

### 3.2.4 Topographical analysis

An optical profilometer was used to examine the surface of the samples by measuring the surface roughness (Profilm 3D; Filmetrics, San Diego, CA, USA) (Figure 5). For each specimen, three different scans were taken at different locations on the surface using composite white light interferometry and phase shift interferometry to ensure greater sensitivity to different amplitudes of surface irregularities. Each treatment was tested on conventional and bioceramic GP specimens (n = 10). The average and standard deviation of the surface texture parameters, such as the arithmetical mean height (Ra) and the root-mean-square height (Rq), were estimated. Samples not treated with plasma were included in the control group.

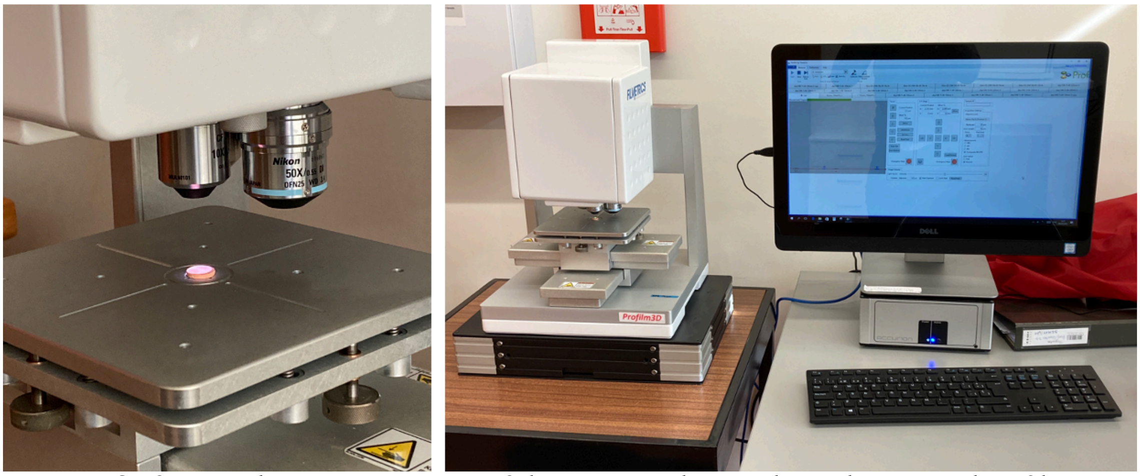


**Figure 5. Surface roughness measurement of the gutta-percha samples with an optical profilometer (Profilm 3D; Filmetrics, San Diego, CA, USA). (Source: own elaboration)**

### 3.2.5 Surface free energy analysis

Immediately after the plasma treatments, the contact angle among the solutions (water, glycerol, and 1-bromonaphthalene) and the GP surfaces was calculated using an optical contact angle (OCA 20; DataPhysics Instruments GmbH, Filderstadt, Germany) at room temperature (Figure 6). The drop volume was 0.5 mL for water and 1-bromonaphthalene, and 3 mL for glycerol. Liquids were released from the tip of the syringe by placing it above the surface of the GP and allowing it to rise to the interface between the GP and the liquid. Samples not treated with plasma were included in the control group. Using the sessile drop technique, five drops were added to each solution (n = 5). The surface free energy was measured based on the results collected by using the Owens *et al.* (1969) method, explained by equation (1):

$$\frac{\sigma_l\,(coscos\,\theta+1)}{2(\sqrt{\sigma_l{}^D}} = \left(\sqrt{\sigma_s{}^P}\right)\frac{\sqrt{\sigma_l{}^P}}{\sqrt{\sigma_l{}^D}} + \sqrt{\sigma_s{}^D} \qquad \text{Equation (1)}$$

where $\sigma_l{}^D$ and $\sigma_l{}^P$ are, respectively, the dispersive and polar components of the surface tension of the liquid used, and θ is the contact angle of the corresponding liquid with the GP disc/sample. From these three parameters, the GP surface energy's dispersive and polar components ($\sigma_s{}^D$ and $\sigma_s{}^P$, respectively) were determined through a linear fit of the data obtained using the three liquids. The total surface energy $\sigma_s$ was the sum of both $\sigma_s{}^D$ and $\sigma_s{}^P$ components.

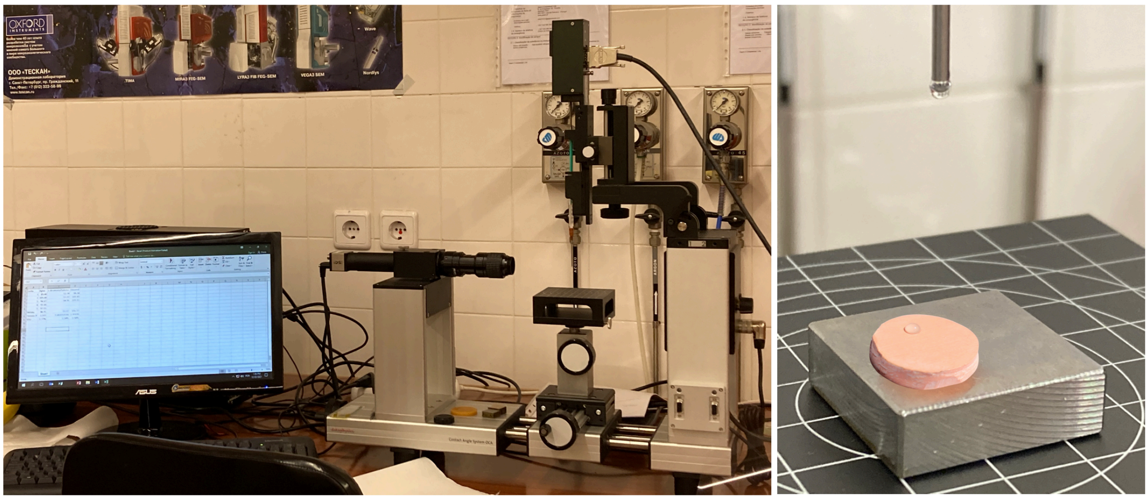

**Figure 6. Equipment for measuring the contact angle between the solutions (water, glycerol, and 1-bromonaphthalene) and the GP surfaces (Optical contact angle OCA 20; DataPhysics Instruments GmbH, Filderstadt, Germany). (Source: own elaboration)**

### 3.2.6 Chemical analysis

Fourier transform-infrared spectroscopy (FT-IR) was conducted to study the chemical modifications in the attenuated total reflectance mode using a Jasco FT/IR 4100 system (Jasco International; Hachioji, Tokyo, Japan) with a wavelength range of 600 – 4000 cm-1 and a resolution of 4 cm-1 (Figure 7). Five measurements were executed for each experimental condition (n = 5).

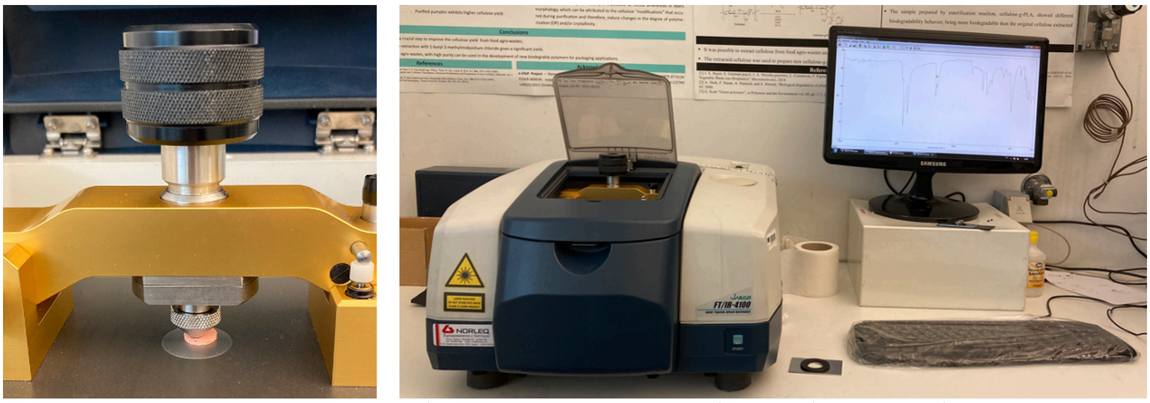

**Figure 7. Fourier transform-infrared spectroscopy (FT-IR) analysis of the GP samples, using a Jasco FT/IR 4100 system (Jasco International; Hachioji, Tokyo, Japan). (Source: own elaboration)**

### 3.2.7 Sealers' wettability assessment

The contact angle among GP surfaces and the sealers was calculated using the same optical contact angle equipment (OCA 20; DataPhysics Instruments GmbH, Filderstadt, Germany) at room temperature (Figure 8). An epoxy resin-based sealer (Endoresin cement; Endogal, Sarria, Lugo, Spain) and a bioceramic sealer (AH Plus Bioceramic; Dentsply Sirona, Ballaigues, Switzerland) were tested on conventional and bioceramic GP surfaces according to the manufacturer's instructions. For each PT gas, a set of parameters (time and power) that could be related to better adhesion, such as roughness and surface free energy, are selected for experimental assay.

One drop of sealer (0.1 mL) was applied to the GP surfaces using a 0.5 mL BD ultrafine syringe. Ten drops of the same sealer were assessed for each PT (n = 10), and the control group (n = 10) included samples that were not subjected to PT.

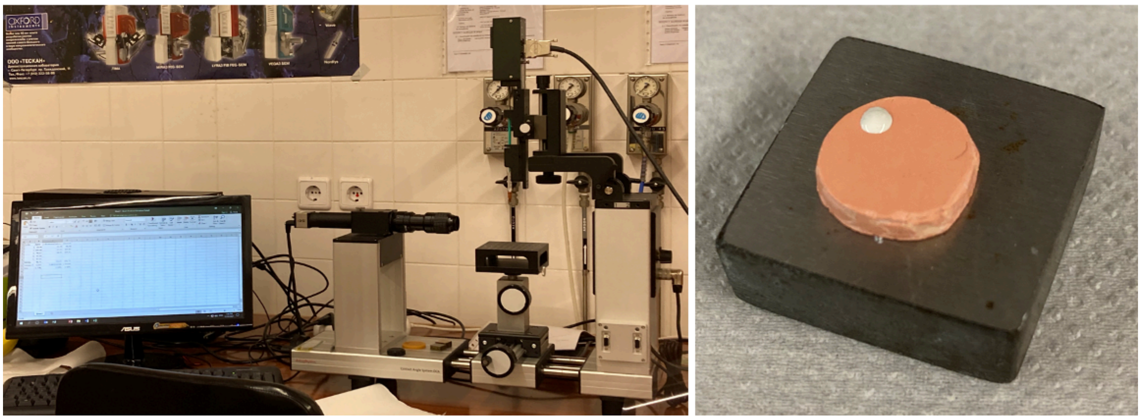

**Figure 8. Equipment for measuring the contact angle between the sealers and the GP surfaces (Optical contact angle OCA 20; DataPhysics Instruments GmbH, Filderstadt, Germany). (Source: own elaboration)**

The wettability of the sealer was monitored for 1 minute. The following equation (2) was used to calculate the sealer wettability (SW) (Prado M. *et al.* 2016):

$$SW (\%) = \frac{(\text{initial angle - final angle})}{\text{initial angle}} \times 100 \qquad \text{Equation (2)}$$

### 3.2.8 Statistical analysis

The IBM SPSS Statistics software (version 28.0; IBM, Armonk, NY, USA) was used for the statistical analysis. The significance level was set at 5% ($p < 0.05$). All assumptions were verified (normality: Kolmogorov-Smirnov test and PP-plot; homoscedasticity of variance: Levene' s test).

Student's t-test was applied to confirm the similarity of surface roughness (Ra values) among all samples (sample standardization). Pearson correlation was used to assess the linear association among Ra and Rq roughness parameters. Student's t-test for independent samples was performed to analyze significant differences between control and experimental groups. Sealers' wettability was calculated through Kruskal-Wallis test with multiple comparisons when significant differences were identified.

## 3.3 FUNCTIONALIZATION OF GP SURFACES WITH A NANOSTRUCTURED ZNO THIN FILM

### 3.3.1 Materials used

*Gutta-percha (GP)*

Round disks of GP samples (10-mm diameter and 2-mm thickness) were produced from GP pellets (Gutta-percha Bal Plus Pellets; Meta Biomed Co, Ltd; Korea) by creating appropriate molds and then plasticizing GP in a laboratory dry-heating oven at 80 °C, followed by a cooling process at room temperature. A standardized metallographic procedure was used to produce specimens of similar surface roughness by polishing the specimens with coarse silicon carbide paper (180 to 600 grit). The GP round disks were used to surface morphology and contact angle analysis. Additionally, GP pellets submitted to the same standardized metallographic procedure, on its two flat sides, were used for the tensile bond strength testing with the sealers.

Sealers

- AH Plus Bioceramic (calcium silicate-based sealer; manufactured by Maruchi; distributed by Dentsply DeTrey GmbH)

Composition: zirconium dioxide, tricalcium silicate, dimethyl sulfoxide, lithium carbonate, and a thickening agent

- Endoresin (epoxy resin-based sealer; manufactured by Meta Biomed. Ltd, Chungcheongbuk-do, Korea; distributed by Galician Endodontics Company S.L., Lugo, Spain)

Composition: Base of epoxy resin oligomer, hydroxyethyl salicylate, bismuth III carbonate; Catalyst of polybutane diol amino benzoate, calcium phosphate, bismuth III carbonate

### 3.3.2 Functionalization of GP

*Plasma treatment (PT)*

GP was submitted to PT in an Ar atmosphere using a low-pressure plasma cleaner (Plasma System Zepto; Diener electronic) powered at 50 W for 60 s. The work pressure was constant for all the treatments (~ 80 Pa), while the base pressure was always less than 20 Pa. These parameters were derived from the second research work (3.2)

*Deposition of ZnO thin film*

The ZnO thin film was deposited onto GP surfaces by reactive magnetron sputtering at a working pressure of 5×10−1 Pa, while keeping the flow of Ar (30 sccm) and O2 (20 sccm) constant using a reactive chamber with a volume of 50 dm3 (Figure 9). A metallic zinc target with 99.96% purity was used for the depositions, with a 50.6-mm diameter and 6-mm thickness. The base pressure was kept lower than 4×10−4 Pa, and the Zn target was linked to a DC source, setting the target potential at -378 V.

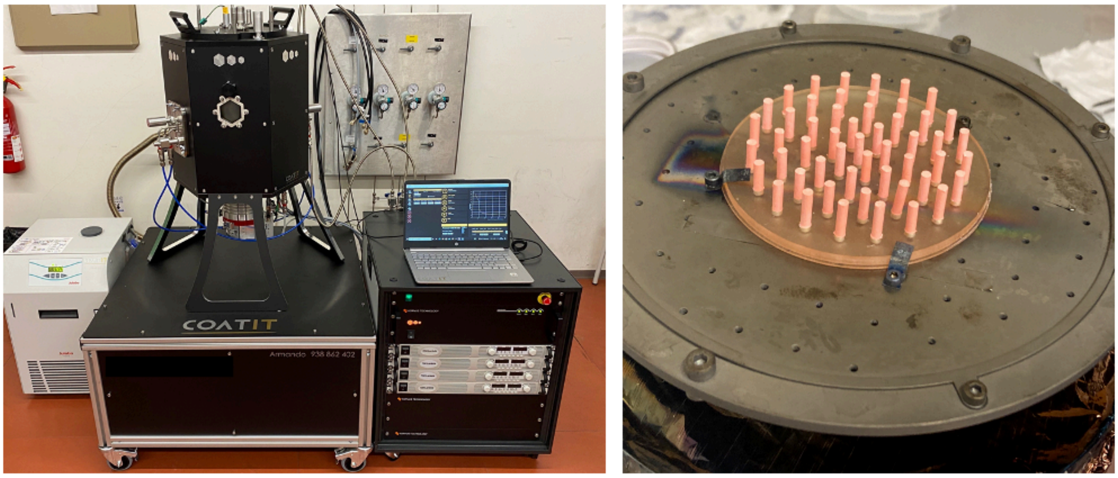


**Figure 9. Custom-made system used for the deposition of ZnO thin film on GP surfaces. (Source: own elaboration)**

The process of physical vapor deposition using reactive magnetron sputtering is described in Figure 10. In this technique, a target material, typically a metal or compound, is bombarded with $Ar^{+}$ ions inside a vacuum chamber. The collision of $Ar^{+}$ ions with the target result in a momentum and energy transference. As a result, the atoms of the target material are moved from their original locations and ejected into the surrounding vacuum where they interact with the reactive gas $O_2$ and are then deposited onto the GP substrate.

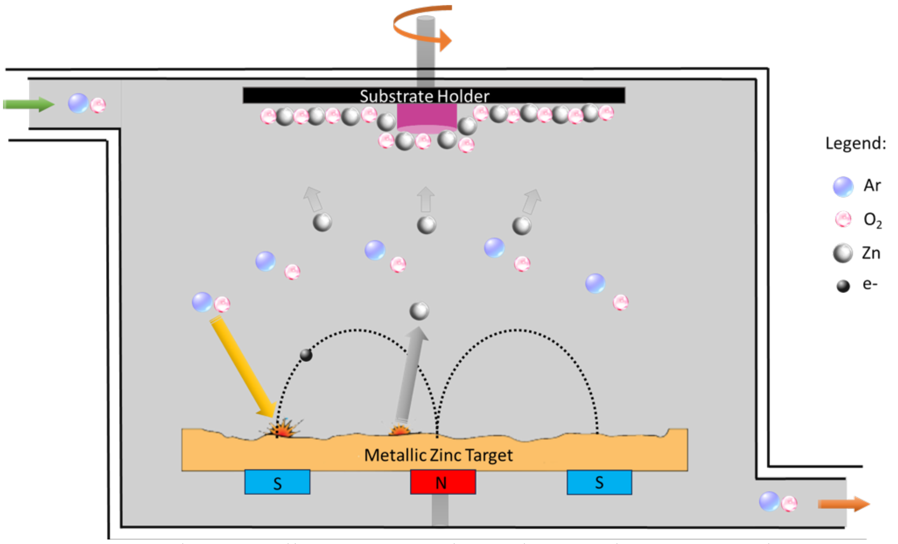


**Figure 10. Schematic illustration of physical vapor deposition with reactive magnetron sputtering used in the production of ZnO thin films.**
**(Source: https://bmcoralhealth.biomedcentral.com/articles/10.1186/s12903-024-04496-z )**

### 3.3.3 Surface morphology analysis

The surface morphology of untreated (control) and treated GP surfaces (PT and PT followed by ZnO thin film deposition) was assessed qualitatively by scanning electron microscopy (SEM) (n=3). GP round disks surfaces were sputter-coated with a thin conductive film of Au-Pd alloy and then evaluated using an FEI Quanta 400 FEG/ESEM microscope in a vacuum at 15-kV accelerating voltage. The presence of morphological changes was qualitatively assessed from the images obtained.

### 3.3.4 Contact angle analysis

The G*Power v3.1.9.6 program was used to establish an a priori sample size. The procedure applied was an analysis of variance (ANOVA) with repeated measures, within factors, using an alpha-type error of 0.05 with a power (1-ß) of 0.90, with an effect size of 0.4. Fifteen specimens per group were determined as the ideal size. GP round disks were allocated randomly into three groups: a) Untreated GP (control); b) GP treated with argon PT; c) Functionalized GP (Ar PT followed by ZnO thin film deposition). An online computer-generated number was used to randomly assign samples to the different groups (www.randomizer.org).

The contact angle was calculated by the sessile drop technique. For each sample, a drop of 0.5 mL of distilled water was applied to the surface of the GP round discs using a micro-syringe, and images were taken at room temperature using optical contact angle equipment. (OCA 20; DataPhysics Instruments GmbH, Filderstadt, Germany). After the water was applied on the GP surface for 5 s, the angle of contact was recorded (Chen M *et al.* 2013). Contact angle measures (n=15) were performed on the untreated (control), activated (PT), and functionalized (PT + ZnO thin film) GP surfaces.

### 3.3.5 Tensile bond strength

The G*Power v3.1.9.6 program was used to determine an a priori sample size. The procedure applied was ANOVA with fixed effects, main effects, and interactions, using an alpha-type error of 0.05 with a power (1-ß) of 0.80 and six groups, with an effect size of 0.4. Ten specimens (each specimen refers to 2 pellets) per group were determined as the ideal size. Samples were allocated randomly into two groups, according to the sealer: Endoresin and AH Plus Bioceramic. Each group was subdivided into three sub-groups: a) Untreated GP (control); b) GP treated with Ar PT; c) Functionalized GP (Ar PT followed by ZnO thin film deposition). An online computer-generated number was used to randomly assign samples to the different groups (www.randomizer.org).

Using an automated micropipette, a 0.01-mL droplet of each sealer investigated was precisely applied to the central region of a flat-surfaced GP pellet. An identical pellet was then carefully aligned and fixed against the initial pellet in a specially designed apparatus (n=10; 1 sample refers to 2 pellets) (Figure 11A). A dental microbrush applicator tip was used to carefully remove any excess extruded material. The prepared specimens were then kept at 37°C for 7 days in contact with gauze moistened with phosphate-buffered saline (pH 7.2). Any non-standard samples were immediately replaced. The bond strength between the GP surfaces and the sealer was assessed using a custom-designed apparatus, illustrated in Figure 11B. Once the samples were stabilized, the moving part of the container was attached to the tensile machine. Measurements were made using a universal testing machine from Shimadzu (model AG-IS) equipped with a 50 N load cell at a speed of 0.5 mm/min, while the GP pellets were subjected to a tensile force. The tensile bond strength test was carried out in a random order by an operator who was blinded to the specific sealer being tested. Bond strength was calculated by a real-time computer software program that plotted a load/time curve during the test. The tensile force required to separate the GP pellets was recorded in Newtons (N), and the tensile bond strength in Mega Pascals (MPa), taking into account the GP pellets sectional area.

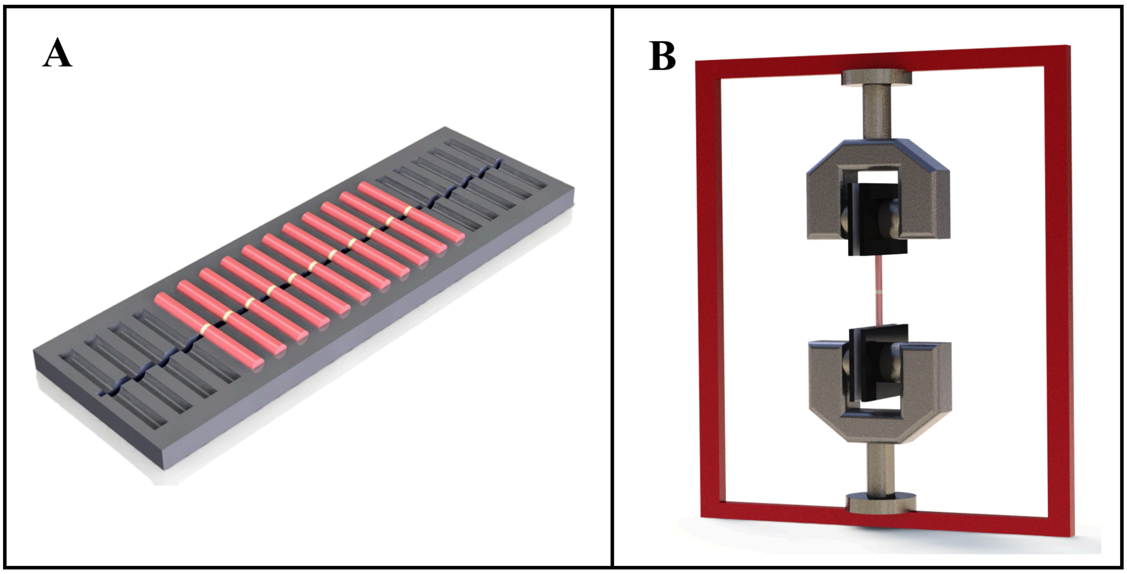


**Figure 11. Schematic representation of the custom-designed apparatus for bonding the GP surfaces and the sealer (A), and the tensile bond strength test apparatus (B).**
**(Source: https://bmcoralhealth.biomedcentral.com/articles/10.1186/s12903-024-04496-z )**

### 3.3.6 Statistical analysis

The statistical analysis was done using the IBM SPSS Statistic 28.0. software (SPSS Inc, Chicago, Illinois, EUA). The significance level was set at 5% ($p<0.05$). The results were confirmed with the Kolmogorov-Smirnov test for the normality of the distribution and the Levene test for the homogeneity of variances. Water contact angles' data were assessed using ANOVA repeated measures (3 levels: control, PT, and PT+ZnO). Tensile bond strength results were evaluated using two-way ANOVA followed by Bonferroni post-hoc tests. All the conditions for the application of the ANOVA procedure were evaluated based on the residuals (normality, zero mean, homogeneity of variance, and independence).

# 4
# DISCUSSION

# 4. DISCUSSION

The long-term clinical success of ET depends, essentially, on cleaning and shaping of the root canal system followed by a three-dimensional obturation (Gulabivala *et al.* 2023). The main objective of root canal obturation is to prevent coronal and apical leakage and entomb the remaining bacteria that may persist after treatment (Siqueira *et al.* 2008). The obturation is performed by using a core filling material, generally GP, and a root canal sealer. The improvement of dentin sealer's adhesion means that the sealer will have the ability to promote a better union between filing materials and dentin. Distinct filling techniques (e.g., single cone, lateral compaction, or thermoplastic obturation) and endodontic sealers can influence filling materials' penetration into dentinal tubules, impacting sealers' adhesion (Abdellatif *et al.* 2021). An improved adhesion aims to decrease gap-containing regions which allow fluid infiltration through the different interfaces, either between sealer-dentin or sealer core material (Schwartz 2006). In this sense, it would also prevent obturation to dislodge during treatment procedures, improving ET success (Schwartz 2006). Sealing ability is widely recognized as crucial to successful root canal treatments (Schwartz 2006).

Despite the great development in endodontic materials, studies still show the presence of interfacial gaps in root canal fillings, independent of sealers' chemical composition, GP type or filling techniques (Zhang *et al.* 2009, Eltair M *et al.* 2018, Quaresma *et al.* 2023). Minimizing gaps is clinically relevant, preventing bacteria and their by-products to colonize and degrade filling materials (Siqueira *et al.* 2008). On the other hand, the use of filling materials with improved antibacterial properties is recommended to prevent microbial re-infection (Alves Mj *et al.* 2018, Cardelle-Cobas *et al.* 2019, Mohan *et al.* 2020, Monisha *et al.* 2023).

The main objective of this thesis was to investigate the effect of functionalization of endodontic substrates, such as dentine and GP surfaces, assessing interfaces' bonding, in view of a better sealing ability of root canal filling. The field of endodontics has been at the vanguard of embracing technological advancements to improve treatment outcomes (Lee *et al.* 2015, Chan *et al.* 2023). Surface functionalization is an increasingly promising approach for property enhancement of dental materials (Chan *et al.* 2023). Application of plasma technology has been studied in various areas for the treatment of surfaces, materials or devices to realize specific qualities (Kim *et al.* 2014, Lata S *et al.* 2022). PT provides an effective and clean approach for surface functionalization without changing the materials' original structure (Chen M *et al.* 2013). It can induce several significant phenomena, including: i) eliminating superficial contaminants (cleaning); ii) altering surface morphology and topography through etching/ablation; iii) activating the surface by generating new reactive species, resulting in the formation of novel chemical groups, crosslinking, and chain scission (Von Woedtke *et al.* 2013). It has been shown to increase the wettability and hydrophilicity of different surfaces, such as dentin and enamel, making it perform better molecular interactions improving their adhesive features (Chen M *et al.* 2013). Dentin hydrophilicity is mainly due to surface etching promoted by NTP. In consequence of surface etching, there is an increased exposition of the inorganic phase which is more hydrophilic. Following NTP treatment of surfaces like dentin, there is, also, an increased polar oxygen-containing reactive groups of dentine surface (Liu *et al.* 2016, Stasic *et al.* 2021).

In this thesis a systematic review was included to assess the effect of NTP treatments on root canal sealer's adhesion to dentin, compared to no treatment. Only *in-vitro* studies, presenting a control group were included. After the comprehensive reading of the selected studies, four papers fulfilled the eligibility criteria. All included studies presented a high risk of bias with respect to blinding, randomization process, and standardized sample preparation (single operator), because these parameters were not declared. All of the studies reported all results and performed sample standardization, so they were considered to have a low risk of bias in these parameters. None of the included studies had a low risk of bias in all parameters evaluated, so the overall risk of bias of the selected studies was high. Three of the included studies based the adhesion ability on the push-out test in human extracted teeth (Menezes *et al.* 2017, Yeter *et al.* 2020, Garlapati *et al.* 2021), while the other used bovine dentin samples to measure the contact angle with the sealer (wettability)(Prado *et al.* 2016). Silva *et al.* (2019) assessed the reliability of using bovine teeth as an alternative to human teeth for testing the push-out bond strength of sealers to dentin and concluded that the dentin substrate did not influence the sealers' bond strength (Silva *et al.* 2019).

Our systematic review highlighted the need to optimize NTP parameters. Concerning the gases studied, Prado *et al.* (2016) and Yeter *et al.* (2020), reported the use of Ar and the two others used a mixture of gases (He and Ar). The exposure period varied from 30 s to 1 minute. In the studies reviewed, Prado *et al.* (2016) and Garlapati *et al.* (2021) conducted NTP treatments under vacuum conditions using a glass reactor, while Yeter *et al.* (2020) and Menezes *et al.* (2017) employed an atmospheric-pressure plasma jet. Low-pressure plasmas are generated and sustained within vacuum chambers, where the mean free path of particles (atoms, ions, and electrons) is relatively long, allowing them to travel greater distances between collisions. Compared to atmospheric-pressure conditions (typically close to 1 atmosphere, where the particles' mean free path is much shorter), low thermal plasmas are much more controllable and reproducible. This advantage of low-pressure plasma compensates (at least partially) the need for expensive vacuum equipment (Šimončicová *et al.* 2019, Borges Ac *et al.* 2021, Lata S *et al.* 2022). Additionally, although atmospheric-pressure plasmas have recognized advantages, such as being generated in an open environment and be easily implemented, they tend to become thermal (Kim *et al.* 2014). Thus, particular precautions need to be implemented, as hot plasmas can damage heat-sensitive materials and burn living tissues (Borges Ac *et al.* 2021).
Disparities about adhesion tests prevented a clear comparison of the results. Root canal filling materials' adhesion ability to dentin can be tested using the push out bond strength test, or through the contact angle calculation. The push-out test was the methodology used by all except for Prado's group, that assessed adhesion through the measure of the contact angle between the treated dentin surface and the sealer (wettability) (Prado *et al.* 2016). In the 3 selected studies that applied the push-out methodology, the root canals were filled with sealer and GP (Menezes *et al.* 2017, Yeter *et al.* 2020, Garlapati *et al.* 2021). However, according to Neelakantan *et al.* (2011), filling the canals only with sealer ensures that there are no confounding factors and that the adhesion strength tested is that of the sealer.

Regarding to the type of endodontic sealers investigated, all the studies included the standard resin-epoxy-based AH Plus. The epoxy-resin-based sealers interact with dentin through the dentin tags formation into dentinal tubules and have been associated to a higher bonding ability to root canal dentin (Neelakantan *et al.* 2015, Silva *et al.* 2019). Its better performance concerning bond strength to dentin has been justified by the ability of this type of sealers to form a covalent bond, with open epoxide rings to exposed amino groups of the dentin collagen.

Analyzing the results of the included studies, the effect of the PT on dentin shows conflicting results regarding AH Plus sealer's adhesion. Prado *et al.* (2016) conducted a study to assess the effects of Ar NTP treatment on the surface properties of bovine dentin. The study demonstrated a significant increase in surface free energy, which was correlated with improved wettability of the dentin when treated with Ar NTP for 30 seconds, compared to the untreated control. The chemical modifications induced by Ar NTP were evaluated using Fourier Transform Infrared Spectroscopy (FT-IR). The results indicated a reduction in organic compounds of the dentin, specifically in the amide I and II bands, and an increase in the inorganic component, particularly the carbonate band. These changes were attributed to the etching capability of the Ar NTP, although no associated topographical alterations were observed on the dentin surface. Wettability in this study was quantified through the measurement of the contact angle between the dentin surfaces and the AH Plus sealer. There is an inverse relationship between contact angle and surface free energy: a lower contact angle indicates higher surface free energy, which suggests better adhesion potential. Prado *et al.* (2016) found that Ar NTP treatment resulted in a reduced contact angle, implying enhanced surface free energy and, consequently, improved adhesion of the sealer to the dentin surfaces. Based on these findings, the authors concluded that Ar NTP treatment promotes better bonding of the AH Plus sealer to dentin surfaces. It is important to note that this study exclusively evaluated the resinous AH Plus sealer. Further research may be necessary to determine if similar benefits are observed with other types of sealers. Additionally, Yeter *et al.* (2020) reported that Ar PT did not influence the bond strength of AH Plus to dentin. Menezes *et al.* (2017) showed that when a mixture of He and $O_2$ (98% He and 2% $O_2$) was applied for 60 seconds, the bond strength of AH Plus to dentin was similar in the plasma treated samples and control groups. Lastly, (Garlapati *et al.* 2021) reported that a mixture of He and Ar enhanced the bond strength of AH Plus to dentin. The other sealers tested in the included investigations were MTA Fillapex (Menezes *et al.* 2017), BioRoot RCS (Garlapati *et al.* 2021) and Endosequence BC (Yeter *et al.* 2020). With the limitations of the present systematic review, it was highlighted that the bond strengths of BioRoot RCS and Endosequence BC were positively influenced by PT (Yeter *et al.* 2020, Garlapati *et al.* 2021), contrarily to the effect on MTA Fillapex (Menezes *et al.* 2017). MTA Fillapex is composed of a salicylate–resin matrix filled with MTA, natural resin, bismuth oxide, and silica (Silva *et al.* 2013). This sealer's composition is primarily resin, which raises doubts concerning its classification as a true CSS or MTA-based sealer (Komabayashi *et al.* 2020). The low bond strength of MTA Fillapex may be due to the low adhesive capacity of forming an interfacial layer with tag-like structures on dentin (Baechtoldi *et al.* 2014). Garlapati *et al.* (2021) found that CSS, such as BioRoot RCS, exhibited the highest POBS values, followed by AH Plus sealer, after applying NTP with He and Ar atmospheres on dentin surfaces. Additionally, dentin treated with NTP showed more than twice the bond strength compared to non-plasma-treated dentin (control), regardless of the sealer used. The superior bond strength of the bioceramic sealer following NTP treatment, as compared to the resinous AH Plus, was further supported by other studies emphasizing its strong performance, particularly in the middle region of the root canal (Yeter *et al.* 2020). The increased POBS values can be attributed to the chemical properties of bioceramic sealers, including their fluidity, ability to spread easily over dentin walls due to low contact angle, and enhanced dentin wettability after NTP treatment (Chen M *et al.* 2013, Gade *et al.* 2015, Yeter *et al.* 2020). The modification of the dentin surface after NTP treatment, such as increased wettability and chemical interaction, may enhance dentinal tubule penetration and bond strength of bioceramic sealers(Yeter *et al.* 2020). While the authors did not clarify the lesser influence of NTP on the push-out bond strength of AH Plus (Yeter *et al.* 2020), it is possible that chemical and structural changes in dentin surfaces caused by various irrigating solutions could have influenced the results (De Assis *et al.* 2011). NTP has been shown to penetrate deeply into dentinal tubules, potentially reaching as far as or further than bacteria, producing reactive oxygen species, and eliminating remaining microorganisms in addition to cleaning and etching (Lehmann *et al.* 2013). This may enhance mechanical retention and adhesion. This rationale was supported by findings from Menezes *et al.* (2017), Prado *et al.* (2016), and Garlapati *et al.* (2021), who reported improved adhesion on NTP-treated surfaces. However, these findings should be interpreted cautiously due to the heterogeneity of study designs, including variations in plasma treatment devices, plasma parameters (such as power, frequency, gas type, and application time), adhesion methodologies, and types of sealers used, making quantitative analysis challenging.

Nevertheless, the findings of the systematic review herein presented pointed out the promising effect that PT might have on filling adhesion, justifying exploring its effects in other endodontic substrates, like GP. So, recognizing the poor adhesion of GP to sealers, new approaches have emerged in the last years, such as GP functionalization/modification in various ways, in order to increase its adhesiveness (Vishwanath V *et al.* 2019). GP cones coated with methacrylate resin, glass ionomer, apatite calcium phosphate, and more recently with bioceramic nanoparticles have been suggested as a way of increasing GP adhesion to specific sealers (Vishwanath V *et al.* 2019). Plasma technology has been scarcely assessed on GP surfaces as a method of enhancing adhesiveness, and in that sense the second specific objective of the present thesis was to evaluate the effect of low-pressure Ar and $O_2$ plasma atmospheres on conventional and bioceramic GP standardized smooth discs, assessing their roughness, surface free energy, chemical structure and sealer wettability. Considering that, depending on the different parameters used for plasma application (like pressure, time, working gas composition, and the nature of the substrate) a diversity of interactions was enhanced or even enabled. Different gases (Ar or $O_2$), powers (25W, or 50W) and exposure times (30s, 60s, 120s, or 180s) were tested in control and experimental groups of GP surfaces, optimizing parameters. The surface treatment for all specimens was conducted under low-pressure, in a plasma chamber (Plasma System Zepto, Diener electronic), equipped with a 40 kHz/100W generator and a rotary pump to get a primary vacuum (base pressure of 20 Pa and working pressure nearly 80 Pa).

Like in other studies, a set of standardized GP discs were manufactured for the present study, avoiding the commercially available GP cones for clinical use (Prado M. *et al.* 2016, De-Deus G *et al.* 2021). Two brands of GP, conventional and bioceramic, were tested. A bioceramic GP is a modification of the conventional one by impregnating and coating its surface with bioceramic calcium-silicate nanoparticles (Osiri *et al.* 2018). Although not mandatory, bioceramic GP has been recommended to be used with a CSS. Theoretically, a gap-free seal would be created when this obturation system is used with the single cone technique (Trope *et al.* 2015), creating a tertiary monoblock with three adhesive interfaces (Tay *et al.* 2007). It is expected that the bioceramic GP/CSS interface will result in a chemical union between the calcium silicate nanoparticles of the cone and the sealer. However, this mechanism remains unclear. In fact, there have been few studies on bioceramic GP. Eltair M *et al.* (2018) assessed the adaptation of a CSS with either bioceramic GP or conventional GP, compared with AH Plus sealer through scanning electron microscopy (SEM). The authors reported that fewer gaps were found between conventional GP and the sealers compared to those observed when using the bioceramic GP. Osiri *et al.* (2018) indicated that root reinforcement may be achieved after obturation using bioceramic GP/CSS, but without differences, comparing to the obturation with epoxy resin-based sealer/conventional GP. Lastly, Banphakarn *et al.* (2019) using hemisectioned roots compared the shear bond strength of a CSS to dentin and bioceramic GP, with that of an epoxy resin-based sealer. They concluded that the bond of the CSS to bioceramic GP was not superior to the epoxy resin-based sealer and conventional GP.

PT has proven to be a promising technology for modifying the surfaces of polymeric materials as it is environmentally friendly, allowing a wide variety of modifications on the substrate surfaces, without affecting their bulk characteristics (Řezníčková *et al.* 2011). The characteristics of the plasma medium are highly dependent on the specific settings used during its generation. These settings include gas composition, pressure, power, and duration of application (Strazzi-Sahyon *et al.* 2021). Under varying conditions, the plasma can produce a medium abundant in free electrons, excited ions, atoms, molecules, radicals, and UV/visible radiation (Strazzi-Sahyon *et al.* 2021, Lata S *et al.* 2022). In this study Ar was selected. Besides being an inert gas, it was reported to influence the surface topography of GP surfaces uncovering some surface porosity (Prado M. *et al.* 2016, Alves Mj *et al.* 2018), increasing the surface free energy and favoring the wettability of sealers (AH Plus and Pulp Canal Sealer) to GP surfaces (Prado M. *et al.* 2016). In turn, $O_2$ plasma reacts chemically with the GP to form highly reactive oxygen species, increasing roughness and surface free energy, apart from positively influencing the sealers' wettability (Prado M. *et al.* 2016). Corroborating the results of another study (Prado M. *et al.* 2016), we also confirmed the positive effect of PT on GP, regardless of the gas used.

XRD analysis was performed to analyze the crystalline structure of the two types of GPs, suggesting a predominance of ZnO crystals inside both types of GP matrixes. However, the diffraction patterns also showed differences between them, with zirconium oxide ($ZrO_2$) being only detected in the bioceramic GP. Roughness, surface free energy, chemical structure and sealer wettability were assessed before and after PT. Roughness surface was measured with an optical profilometer, and independently of the GP type, plasma treatments carried out in Ar or $O_2$ atmospheres showed different behaviors, depending on the power and duration. Surface free energy was calculated through the contact angle analysis between the GP surface and the solutions (water, glycerol, and 1-bromonaphthalene). An increase in surface energy means an improvement in the wettability and consequently in the adhesion ability (Prado M. *et al.* 2016). This study investigates the effects of Ar and $O_2$ plasma treatments on the surface free energy of conventional and bioceramic GP. Both plasma treatments significantly increased the surface free energy of the GP samples compared to their respective control groups. These findings are consistent with those of Prado M. *et al.* (2016), who reported similar effects for conventional GP. Enhanced surface free energy is associated with lower contact angles and increased wettability, as demonstrated in this study. FT-IR was used to analyze the chemical structure of GP surfaces before and after plasma treatments. The chemical analysis revealed oxidation of the polyisoprene matrix, indicated by an intensified stretching signal for the C=O bond in both types of GP. This observation aligns with the findings of Prado M. *et al.* (2016), who also noted an increase in C=O stretching for conventional GP treated in a reactive O2 atmosphere, suggesting the formation of new active sites. In contrast to Prado M. *et al.* (2016) study that reported a reduction in O-H stretching, our study detected a slight increase in the O-H stretching shoulder at 3320 $cm^{-1}$ in the plasma-treated bioceramic GP, albeit in minor traces. This increase may be related to the generation of free radicals and/or polymer chain scission in the bioceramic GP, which contains more $ZrO_2$ crystals than conventional GP. This composition likely enhances reactivity with the environment, leading to the formation of new intermolecular bonds and potential hydrogen bonding networks. The chemical modifications observed on the GP surfaces, reflected in wavelength variations between conventional and bioceramic GP spectra, indicate differences in chemical composition, which were also confirmed by XRD analysis. Despite these modifications, FT-IR spectra of plasma-treated and non-treated GP showed only slight differences in peak intensity, particularly at 3300–3450 $cm^{-1}$ (O-H stretching), ~1730 $cm^{-1}$ (C=O stretching), and ~1600 $cm^{-1}$ (C=C stretching). This finding supports the assertion by Prado M. *et al.* (2016) that the main molecular structure of GP remains largely preserved after PT. The chemical modifications and surface etching induced by plasma treatments have been shown to promote interatomic bonding in various substrates, including dentin, enamel, and composites, thereby enhancing their adhesive properties (Chen M *et al.* 2013, Dong *et al.* 2013, Strazzi-Sahyon *et al.* 2021). Sealer´s wettability was measured by calculating the contact angle between GP surfaces and the endodontic sealers Endoresin and AH Plus Bioceramic. Similar to other authors, contact angle measurement was considered a useful indicator of the wettability of a liquid, which, in the present case were the two canal sealers studied (Ballal *et al.* 2013). AH Plus Bioceramic is a recent premixed calcium silicate-based sealer comprising zirconium dioxide, tricalcium silicate, dimethyl sulfoxide, lithium carbonate, and a thickening agent (Sanz *et al.* 2022). While it exhibits favorable physical properties and antibacterial activity due to its high pH, its solubility may impact the obturation quality (Souza *et al.* 2023). Recent studies have highlighted its cytocompatibility and bioactive potential, surpassing the epoxy resin-based AH Plus and rivaling EndoSequence BC Sealer (Sanz *et al.* 2022). Epoxy resin-based sealers, like Endoresin, considered the gold standard, have been continuously used in comparative studies due their good properties (Flores *et al.* 2011).

The selected plasma parameters for each type of GP were optimized to achieve a balance between power, duration, and the resultant effects on surface roughness and free energy. The parameters tested for sealer wettability analysis included Ar plasma at 50 W for 60 seconds and O2 plasma at 25 W for 120 seconds. In the case of Endoresin sealer on conventional GP, significant differences were observed between the control group (untreated GP surfaces) and GP treated with Ar plasma. For bioceramic GP, both plasma treatments with the selected parameters significantly improved the sealer's wettability compared to the control group. Regarding AH Plus Bioceramic sealer, both Ar and O2 plasma treatments produced significant increases in wettability for both conventional and bioceramic GP, compared to the control. Overall, both plasma treatments enhanced the wetting properties of conventional and bioceramic GP when used with Endoresin and AH Plus Bioceramic sealers. Given these findings, plasma technology can be considered an environmentally friendly process. During plasma activation, the interaction of energetic particles with GP surfaces results in several surface modifications, including cleaning, etching to remove contaminants, promoting surface roughness, and activating the surface by forming new functional groups and inducing chain scission (which creates free radicals that act as anchorage points) (Pedrosa *et al.* 2016, Alves Mj *et al.* 2018). These combined effects modify both the physical (roughness) and chemical (crosslinking bonds) characteristics of the GP surface, creating interlocking points and active polar groups.

Surface activation is indicated by increased surface roughness and free surface energy, which enhances adhesion at the GP/sealer interface, resulting in better wettability (Prado M. *et al.* 2016). These results are consistent with other studies that have shown plasma treatments can influence the physicochemical properties of materials or substrates, such as roughness and surface free energy, revealing new capabilities of these conventional substrates (Prado M. *et al.* 2016). The different sets of parameters studied, including the type of plasma atmosphere, power, and exposure time, significantly influenced the effects of PT on GP surfaces, providing new insights not previously described.

Recalling the above, although GP is still the core material of choice for root canal filling due to the great physico-chemical properties that possesses, aside with a high cytocompatibility, it presents some drawbacks (Vishwanath V *et al.* 2019). The poor antimicrobial/antibiofilm ability presented as well as the lack of adhesion to endodontic sealers are some of its limitations. Attempting to improve its antimicrobial properties Alves Mj *et al.* (2018) proposed a new strategy for GP functionalization. They tested a novel approach to increase the antibiofilm efficacy of GP, modifying its surface by using Ar PT, followed by the deposition of a ZnO thin film. However, no studies have investigated its performance concerning sealers' adhesion. Therefore, the third specific aim of the present thesis assessed the influence of a nanostructured ZnO thin film deposition over GP surface on the adhesion to an epoxy resin-based (Endoresin) and a calcium silicate-based endodontic sealer (AH Plus Bioceramic).

Thin film deposition is the process of creating and depositing thin film coatings onto a substrate material. Over the past years, a diversity of physical and chemical deposition techniques has been used to generate nanostructured thin films (Qadir *et al.* 2019). Physical vapor deposition is a thin film deposition technique which implies vaporizing a solid material in a vacuum, and then depositing that material onto a substrate. Coatings created through this technique are highly durable, and resistant to corrosion (Qadir *et al.* 2019, Ichou *et al.* 2023). Sputtering is a vacuum coating process that falls under the category of physical vapor deposition. The sputtering process consists of bombarding ions, coming from an inert gas (for example, argon), on the solid target, causing their atoms to be ejected towards the substrate (Qadir *et al.* 2019). It has great advantages such as reproducibility, thickness control, high mechanical durability, good adhesion to the substrate, as well as easy control of the structure and composition of the film (Qadir *et al.* 2019). It presents limitations in terms of low deposition rates, plasma ionization efficiency and substrate heating (Qadir *et al.* 2019). These limitations were overcome with the introduction of magnetrons. The magnetron sputtering is an assisted plasma technology which proposes the creation of a magnetic field, increasing the probability of collision between electrons and gas atoms, which causes greater ionization, resulting in a greater spraying rate, and therefore a greater deposition rate on substrate (Kelly *et al.* 2000, Qadir *et al.* 2019). Reactive magnetron sputtering, used for the deposition of ZnO thin films, showed antibacterial and antifungal properties (Carvalho *et al.* 2014, Costa D *et al.* 2019). Similar to another study (Alves Mj *et al.* 2018), in our work the ZnO thin films were deposited on the GP surface, using physical vapor deposition technique by reactive magnetron sputtering.

The main findings of the third study are presented below. Through SEM analysis we were able to report the effect of functionalization with Ar PT and ZnO deposition, compared to the control (non-treated GP). Surface morphology analysis showed ZnO crystals encrusted on the GP matrix, covered by an organic layer constituted by wax/resin components. PT with Ar caused the removal of the wax/resin surface layer, exposing the ZnO crystals embedded in the GP matrix and uncovering the surface porosity promoted by the ZnO grains boundaries. The GP samples submitted ZnO film thin deposition, increased the surface area to be covered with ZnO, benefiting from its additional antimicrobial properties. As main results, the mean water contact angle of functionalized GP (PT following ZnO thin film deposition - PT + ZnO) was lower compared to the control presenting a statistically significant difference. The mean tensile bond strength in Endoresin group was significantly higher than that in AH Plus bioceramic group, for all tested conditions (control, PT and PT + ZnO) At last, the mean bond strength value between functionalized GP (PT + ZnO) surfaces and the distinct sealers (Endoresin and AH Plus Bioceramics) was statistically different from the control, with higher values for functionalized GP

The investigations included in the present thesis, reflected in the 3 published papers, evaluated the impact of functionalization of different endodontic substrates in sealers' adhesion. Intraradicular dentin treated with NTP seems to be promising, being stressed the need to optimize parameters, concerning the gas or gases applied, power and exposure time. Additionally, the effect can vary with the different chemical composition of the sealers. Regarding GP, the different studies enabled to emphasize the potential of NTP and of the nanostructured antimicrobial ZnO thin film, to improve surface properties, such as an increased hydrophilicity and sealer adhesion ability. In this sense, our findings can show a path for further research, with a promising outcome, improving endodontic treatment success. The primary etiology of pulp and periapical infections is mediated by microorganisms in the form of biofilms (Siqueira *et al.* 2008). These complex multispecies structures can resist the conventional strategy of cleaning and shaping, and intra-canal dressings (Siqueira 2001). The potential of NTP in Endodontics emerged after the positive reports in the medical field (Lata S *et al.* 2022). ET success is dependent on the infection source elimination (Siqueira *et al.* 2008). Although the inflammatory burden for periapical healing is dependent on other factors besides intraradicular infection, it is widely recognized the correlation between the presence of microorganisms in the time of filling and post-treatment disease (Siqueira Jr. *et al.* 2022). NTP properties, allowing microbial deactivation and improving adhesion, can act as an adjuvant tool in root canal treatment (Jungbauer *et al.* 2021). As there is no technique or obturation that can predictably ensure microleakage, root canal filling has the crucial aim of entombing the remaining microorganisms that persist after chemo-mechanical preparation (Siqueira Jr. *et al.* 2022). In this sense surface functionalization, reported in the present investigation, would be of great value, improving filling materials adhesion and preventing bacteria to reach the periapical tissues. Hence, functionalization can add important properties to the endodontic substrates and materials, enabling a conservative preparation to reach the requirements of a hermetic filling, as well as retaining as much as possible cervical and radicular dentine thick. More than an alternative, functionalization should be seen as an adjuvant step, improving interfaces characteristics, such as, favoring adhesion. Although preparation errors or other procedural mishaps may not, by themselves, questioned the root canal treatment outcome, those root canal areas not touched by the endodontic instruments or irrigating solution increase the risk of ET failure. Preventing microleakage trough a bonded obturation might be promising in endodontic treatment success. The range of different gases and parameters of power and treatment periods that can be applied is still very wide, showing the great potential of NTP.

Considering that this topic has been scarcely discussed in the endodontic literature, the data here presented can make a significant contribution to highlight the potential of endodontic material functionalization. However, more studies are needed to evaluate the adhesion capacity of functionalized dentin/GP and other substrates as endodontic instruments in clinical endodontic outcome. In-vitro and ex-vivo laboratory investigations, as well as controlled randomized studies need to be effectively planned and designed to establish clear comparations between studies and confirm the clinical relevance of the present findings.

# 5
# CONCLUSIONS

# 5. CONCLUSIONS

1. There is no consensus about the effect of PT on the AH Plus sealer's adhesion to radicular dentin, even though, PT seems to have a positive effect on the adhesion of CCS (as BioRoot RCS and Endosequence BC). These findings should be interpreted cautiously due to the scarcity of studies on the topic.

2. The functionalization of GP surfaces with Ar or $O_2$ plasma treatments can increase roughness, surface free energy and wettability of the Endoresin and AH Plus Bioceramic, which might improve its adhesive properties when compared to non-treated GP.

3. Functionalization of GP with a nanostructured ZnO thin film enhances the bond strength with sealers of different chemical composition and induced a shift towards hydrophilicity. The ZnO thin film deposition preserved GP's surface morphology modified by PT, enhancing the bond strength to both Endoresin and AH Plus Bioceramic sealers, compared to untreated GP.

# 6
# REFERENCES

# 6. REFERENCES


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# 7
# FULL COPY OF THE PUBLICATIONS

# 7. FULL COPY OF THE PUBLICATIONS

## Paper 1

FERREIRA I, LOPES C, FERREIRA A, VAZ F, PINA-VAZ I, MARTÍN-BIEDMA B. *EFFECT OF PLASMA TREATMENT ON ROOT CANAL SEALERS' ADHESION TO INTRARADICULAR DENTIN - A SYSTEMATIC REVIEW.* APPLIED SCIENCES. 2023; JUL 27; 13:8655.

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applied sciences

MDPI

*Systematic Review*

# Effect of Plasma Treatment on Root Canal Sealers' Adhesion to Intraradicular Dentin—A Systematic Review

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**Abstract:** This investigation aimed to assess, through a systematic review, the effect of non-thermal plasma treatments on root canal sealers' adhesion to dentin. This study followed the 2020 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. A literature search was undertaken without limits on time or language, until May 2023, in PubMed–MEDLINE, Scopus, Web of Science, OpenGrey, and three endodontic journals. The included studies underwent quality assessment and data extraction. Out of an initial 188 articles, 4 studies were included. Three of these studies based the adhesion ability on the push-out test in human extracted teeth, while the other used bovine dentin samples to measure the contact angle with the sealer (wettability). While there was no consensus about the effect of non-thermal plasma (NTP) on the AH Plus sealer's adhesion to radicular dentin, NTP seemed to positively influence the adhesion ability of BioRoot RCS and Endosequence BC. The findings of the present review should be interpreted cautiously due to the scarcity of studies on the topic. The NTP parameters should be optimized to obtain a stronger evidence base in endodontics on its role as an adjuvant tool to increase sealers' adhesion to dentin.

**Keywords:** adhesion; gutta-percha; plasma treatments; root canal filling; root canal obturation; root canal therapy; root canal sealers

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## 1. Introduction

Endodontic therapy, generally focused on root canal treatment, has a major goal directed to the cure or prevention of periradicular periodontitis. Orthograde endodontic treatment includes cleaning and shaping of the root canal system, preparing it for the subsequent filling, with the aim of maintaining disinfection and preventing reinfection. The latter is achieved by a core—most often gutta-percha—and an endodontic sealer, under well-defined criteria of length and density. The treatment is completed by an adequate coronal restoration [1].

A dentin sealer's adhesion is its ability to adhere to the root canal's walls and promote the union of the filling materials to dentin. Different sealers and filling techniques (e.g., single cone, lateral compaction, or thermoplastic obturation) have been reported to produce an impact in the penetration of the sealer into dentinal tubules, thereby influencing dentin sealers' adhesion [2]. Optimal adhesion of the root filling to the intraradicular dentin leads to fewer gap-containing regions, which would allow fluid infiltration within either sealer–dentin or sealer–core–filling material interfaces [3]. Consequently, it also avoids sealer dislodgment during operative procedures, increasing endodontic treatment success rates [3]. It is widely accepted that sealing ability is of utmost importance to successful outcomes of root canal treatments [3].

A great variety of endodontic sealers are available commercially. They are divided into different groups according to their chemical composition, properties, or therapeutic additives, which influence their performance [4]. Studies have also shown that the sealers' bond strength to dentin may be affected by the pretreatment of canal walls and the type of sealer used [5].

The physicochemical properties of sealers interfere with their ability to adhere to dentin [6]. The epoxy-resin-based sealer AH Plus (Dentsply DeTrey GmbH, Konstanz, Germany) is the gold-standard sealer due to its extensively studied physical properties, such as its high bond strength to dentin [6]. This advantage has been justified by epoxy-resin-based sealers' chemical bonding to exposed collagen and their great capacity to form smooth and compact tags inside dentinal tubules [6,7]. MTA Fillapex is composed of a salicylate–resin matrix filled with MTA, natural resin, bismuth oxide, and silica [8]. This sealer's composition is primarily resin, which raises doubts concerning its classification as a true calcium-silicate-based sealer or MTA-based sealer [4]. Nevertheless, it is reported that the set sealer releases calcium and hydroxyl ions. When the material comes into contact with phosphate-containing fluids, these ions cause the formation of apatite, which may deposit within collagen fibrils, promoting controlled mineral nucleation on dentin, seen as the formation of an interfacial layer with tag-like structures [7,8]. MTA Fillapex's low bond strength could be due to the low adhesion capacity of these tag-like structures [9].

Calcium-silicate-based sealers, such as BioRoot RCS and Endosequence BC, have become popular in endodontics, mainly due to their biocompatibility and bioactivity [10]. These sealers have the potential to adhere chemically to dentin through the production of hydroxyapatite during setting [6]. Although they have undergone great development to improve their performance, there is still a lack of consensus regarding their bond strength to intraradicular dentin [6,11].

Plasma, considered to be the fourth state of matter, is an electrically conductive medium that responds to electric and magnetic fields and is also a source of large quantities of highly reactive species such as electrons, ions, electronically excited neutrons, and free radicals [12]. Plasmas are generally classified as thermal and non-thermal (or cold plasma), based on the relative temperatures and energy of the different plasma species (electrons, ions, and neutrons) [12]. In thermal plasmas, electrons and heavy particles are in thermal equilibrium, while in non-thermal plasma (NTP), electrons are hotter than ions and neutrons are at room temperature [12]. NTP can be used under vacuum or atmospheric conditions, using inert gases like argon (Ar) or helium (He), reactive gases such as oxygen ($O_2$) or nitrogen ($N_2$), or a mixture of two or more gases [13,14]. Plasma treatments provide an effective and clean technology for surface activation without changing the materials' original structure and functional properties [15]. Previous studies have shown that NTP is efficient for cleaning/decontaminating and sterilizing instruments [16] and tooth whitening [17], and it seems to be a promising tool in combating dental biofilms [18]. Moreover, this technology has been shown to increase the wettability and hydrophilicity of different surfaces, such as dentin, enamel, and composites, improving their adhesive features or etching dentinal tubules, ensuring higher mechanical retention of root canal sealers [19].

To the best of our knowledge, to date, no systematic review has evaluated the influence of NTP on the adhesion between endodontic sealers and intraradicular dentin. Thus, this work aimed to assess, through a systematic review, the effect of NTP treatments on root canal sealers' adhesion to dentin.

## 2. Materials and Methods

This systematic review followed the recommendations of the 2020 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [20].

### 2.1. Eligibility Criteria

This study was conducted to answer the PICO question "Does the NTP treatment affect sealers' adhesion to dentin compared to no treatment?", with the following parameters: extracted teeth as the participants, plasma treatment as the intervention, no treatment as the comparison, and evaluating root canal sealers' adhesion to dentin as the outcome.

Only in vitro studies that treated dentin with plasma technology and assessed the effects of NTP treatments on root canal sealers' adhesion to dentin were included. Studies that did not use a control group (without plasma treatment) were excluded.

### 2.2. Search Strategy

The search was carried out in May 2023 on PubMed (Medline), Scopus, and Web of Science. The electronic search combined Medical Subject Heading (MeSH) terms, text words (tw), and truncation terms. The Boolean operators "AND" and "OR" were used to create the search strategy (Table 1). No language or publication date restrictions were applied. Additionally, gray literature was investigated through OpenGrey, and a manual search of the *Journal of Endodontics*, *International Endodontic Journal*, and *Australian Endodontic Journal* was performed to find any additional papers. Moreover, an additional search was conducted using the reference lists of all included papers. References from different databases were imported into the EndNote X9 software (Thomson Reuters, New York, NY, USA), which automatically removed duplicate records.

**Table 1.** Search strategy in different databases.

| Database | Search Strategy | Findings |
|---|---|---|
| PubMed | #1 ((non-thermal plasma[Title/Abstract]) or (nonthermal plasma[Title/Abstract]) or (Plasma Gases[Title/Abstract]) or (plasma treatment[Title/Abstract]) or plasma[Title/Abstract] or (Plasma Gases[MeSH Terms]) or plasma[MeSH Terms]) | |
| | #2 ((dental cements[MeSH Terms]) or (root canal sealants[MeSH Terms]) or (dental cement *[Title/Abstract]) or (root canal seal *[Title/Abstract]) or (endodontic seal *[Title/Abstract]) or (root canal fill *[Title/Abstract]) or (seal*[Title/Abstract])) | |
| | #3 ((endodontic *[Title/Abstract]) or (root canal[Title/Abstract]) or (endodontic treatment[Title/Abstract]) or (root canal treatment[Title/Abstract]) or (Root Canal Therapy[Title/Abstract]) or (Root Canal Therapy[MeSH Terms]) or (Endodontics[MeSH Terms]) | |
| | #1 and #2 and #3 | 96 |
| Scopus | #1 TITLE-ABS-KEY("non-thermal plasma" or "nonthermal plasma" or "Plasma Gases" or "plasma treatment" or plasma) | |
| | #2 TITLE-ABS-KEY("dental cements" or "root canal sealants" or "dental cement *" or "root canal seal *" or "endodontic seal *" or "root canal fill *" or "seal *") | |
| | #3 TITLE-ABS-KEY("endodontic *" or "root canal" or "endodontic treatment" or "root canal treatment" or "Root Canal Therapy") | |
| | #1 and #2 and #3 | 140 |
| Web of Science | #1 TS = ("non-thermal plasma" or "nonthermal plasma" or "Plasma Gases" or "plasma treatment" or plasma) | |
| | #2 TS = ("dental cements" or "root canal sealants" or "dental cement *" or "root canal seal*" or "endodontic seal *" OR "root canal fill *" or "seal *") | |
| | #3 TS = ("endodontic *" or "root canal" or "endodontic treatment" or "root canal treatment" or "Root Canal Therapy") | |
| | #1 and #2 and #3 | 83 |

### 2.3. Selection of the Studies

Two reviewers independently assessed the searched titles and abstracts and discarded the non-eligible papers. When the title and abstract were insufficient to confirm or exclude a particular study, they read the full text. In case of divergence, a third author decided whether the paper should be included.

### 2.4. Data Extraction

The following information was extracted and recorded from each included study: tooth type, non-thermal treatment (i.e., gas/application time, plasma mode, device used, distance, pressure, and power applied), methodology for testing adhesion ability (push-out testing parameters (i.e., filling materials used, storage, canal segments analyzed, slice thickness, plunger diameter, and plunger loading direction) and contact angle analysis), and main results.

### 2.5. Risk-of-Bias Assessment

Two authors independently evaluated the risk of bias in each selected study. The risk of bias assessment method was adapted from a previously published systematic review [21]. The following parameters were considered: (1) randomization, (2) blinding, (3) standardization of specimen selection, (4) standardized preparation (single operator), and (5) reporting of data. If the above parameters were mentioned, the risk of bias was recorded as low; if the parameters were not mentioned, it was recorded as high; if their mention was not clear, it was recorded as unclear. Disagreements among authors were resolved through discussion with a third author.

## 3. Results

Figure 1 shows the flow diagram of the search strategy. After duplicates were removed, the search generated 188 studies. After the analysis of titles and abstracts, five were selected. After comprehensive reading of these studies, one was excluded due to not treating dentin with plasma technology [22]. Therefore, four studies fulfilled the eligibility criteria and were included in this systematic review.

Table 2 shows the results of the included papers' risk of bias. All studies had a high risk of bias with respect to blinding, randomization process, and standardized sample preparation (single operator), because these parameters were not mentioned. All of the studies reported all results and performed sample standardization, so they were considered to have a low risk of bias in these parameters. None of the included studies had a low risk of bias in all parameters evaluated, so the overall risk of bias of the selected studies was high. Table 3 summarizes the included studies.

**Table 2.** Quality assessment of the included studies [13,23–25].

| Author (Year) | Randomization | Blinding | Standardization of Sample Selection | Standardization Preparation (Single Operator) | Reporting of Data |
|---|---|---|---|---|---|
| Prado et al., 2016 | High | High | Low | High | Low |
| Menezes et al., 2017 | High | High | Low | High | Low |
| Yeter et al., 2020 | High | High | Low | High | Low |
| Garlapati et al., 2021 | High | High | Low | High | Low |

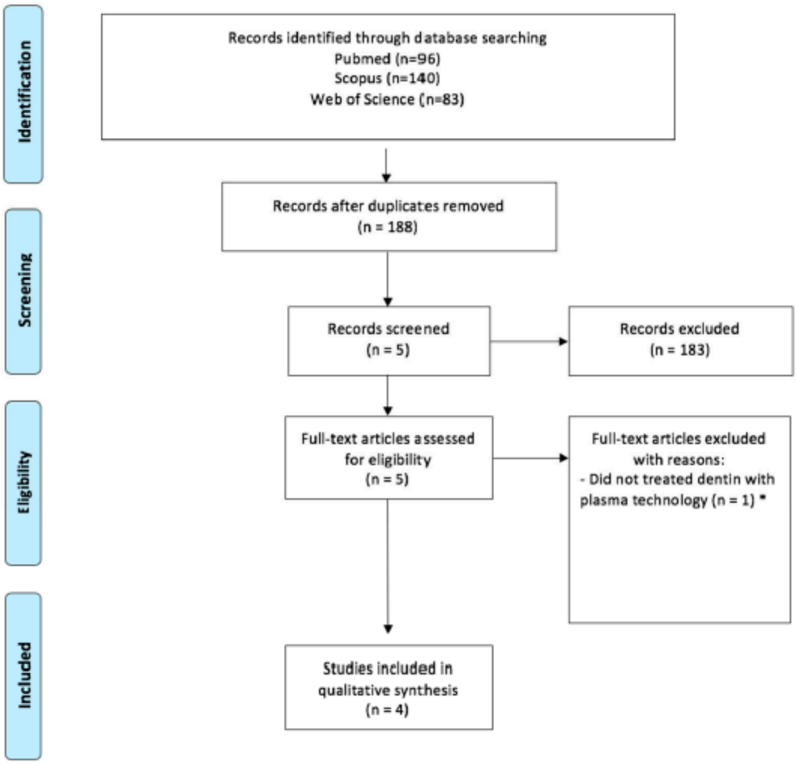


**Figure 1.** Flow diagram of the search strategy. * Record excluded [22].

### 3.1. *NTP Treatment Methodology*

Three studies used human single-rooted extracted teeth [23–25]. Only one used bovine teeth [13]. Prado, et al. [13] and Garlapati, et al. [25] applied NTP treatments under vacuum conditions using a glass reactor, while Yeter, et al. [23] and Menezes, et al. [24] used an atmospheric-pressure plasma jet. Under vacuum conditions, a power of 60 W was applied to generate the plasma, with a working pressure of 10 Pa and a base pressure of 2 Pa. For plasma application through a plasma jet mode, the gas pressure was kept at 6 bar and 2.5 bar; the distance between the tip of the plasma jet and the dentin was approximately 5 mm. Two studies used argon plasma [13,23], while the other two applied a mixture of gases [24,25]. The application time was 30 s, except in the study of Menezes, et al. [24], where it was 1 min.

**Table 3.** Characteristics of the included studies (GP: gutta-percha; Ar: argon; $O_2$: oxygen; He: helium; P: pressure; SFE: surface free energy, SE: sealer wettability) [13,23–25].

| Author | Tooth Type | Non-Thermal Treatment | | | | | | Methodology for Testing Dentin-Sealer Adhesion Capacity | | | | | | | | | Main Results |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Bond Strength Analysis—Push-Out Test | | | | | | | | Contact Angle Analysis | |
| | | Gas | Plasma Mode | Application Time | Distance | Power | Pressure | Filling Material | Storage and Duration | Canal Segments | Slice Thickness | Plunger Diameter | Crosshead Speed | Plunger Loading Direction | | | |
| Prado et al., 2016 | Bovine incisors | Argon | Vacuum | 30 s | - | 60 W | $P_{base}$ = 2 Pa $P_{work}$ = 10 Pa | AH Plus | - | - | - | - | - | - | | Wettability—contact angle between the dentin and the AH Plus sealer | Argon plasma increased the wettability of AH Plus, favoring its bonding to dentin |
| Menezes et al., 2017 | Human single-rooted premolars | Mixture of 98% He and 2% $O_2$ | Jet | 1 min | 5 mm | - | 6 bars | GP + AH Plus GP + MTA Fillapex | 100% humidity for 2 days | Coronal; middle; apical | 1 mm | 0.76 mm coronal; 0.60 mm middle; 0.40 mm apical | 0.5 mm/min | Unclear | | - | Regarding AH Plus, bond strength was similar in the plasma and control groups. For MTA Fillapex, the bond strength decreased with plasma treatment |
| Yeter et al., 2020 | Human single-rooted mandibular premolars | Argon | Jet | 30 s | 5 mm | - | 2.5 bars | GP + AH Plus GP + Endosequence BC | 100% humidity for 7 days | Coronal; middle | Unclear | Unclear | 0.5 mm/min | Apical–coronal | | - | Argon plasma did not influence the bond strength of AH Plus to dentin. The Endosequence BC showed a better bond strength than the AH Plus after argon plasma treatment |
| Garlapati et al., 2021 | Human single-rooted mandibular premolar | Mixture of He and Ar | Vacuum | 30 s | - | 60 W | $P_{base}$ = 2 Pa $P_{work}$ = 10 Pa | GP + AH Plus GP + BioRoot RCS | Not mentioned | Middle | 2 mm | 1 mm | 1 mm/min | Unclear | | - | Plasma treatment enhanced the bond strength of BioRoot RCS and AH Plus |

### 3.2. Dentin Sealers' Adhesion Assessment

- Push-out test [23–25]
  (a) Filling material and sample storage: Three studies filled the canal with gutta-percha and sealer [23–25]. The storage time ranged from 2 to 7 days, and the specimens were kept in an incubator at 37 °C and 100% humidity. Garlapati, et al. [25] did not mention the storage conditions and time.
  (b) Slice thickness and canal segments: The thickness of the slices varied between 1 and 2 mm. Yeter, et al. [23] did not clearly describe the slice thickness. Menezes, et al. [24] used apical, middle, and coronal thirds, Garlapati, et al. [25] used only the middle third, and Yeter, et al. [23] used the coronal and middle thirds.
  (c) Plunger diameter, speed, and direction: Menezes, et al. [24] used three plunger sizes to equal the diameter of each root third, Garlapati, et al. [25] used a plunger of 1 mm, and Yeter, et al. [23] did not mention the plunger diameter used. The plunger's loading direction was unclear in two studies. Yeter, et al. [23] applied an apical–coronal direction. The crosshead speed varied between 0.5 mm/min and 1 mm/min.
- Contact angle analysis [13].

In one of the studies, adhesion was assessed based on the wettability of the resin-based sealer AH Plus. It was calculated through the contact angle between the dentin surfaces and the sealer [13].

### 3.3. Influence of NTP on Dentin Sealers' Adhesion

The epoxy-resin-based sealer AH Plus was tested in all of the included studies. The other sealers tested were MTA Fillapex [24], BioRoot RCS [25], and Endosequence BC [23]. In two studies, plasma treatment did not influence the bond strength of AH Plus to dentin [23,24]. On the other hand, Garlapati, et al. [25] and Prado, et al. [13] concluded that plasma treatment improved the AH Plus–dentin adhesion. The bond strength of BioRoot RCS and Endosequence BC was positively influenced by plasma treatment. For MTA Fillapex, the bond strength decreased with plasma treatment.

## 4. Discussion

Root canal filling materials' adhesion to dentin has been widely tested using the push-out bond strength (POBS) test, also called dislodgement resistance [26]. Well-controlled experiments are challenging when using biological samples, due to the substantial effects of the inherent biological, physical, and chemical variances imposed by natural samples. A recent study investigated the reliability of using bovine teeth as an alternative to human teeth for testing the POBS of sealers to dentin and concluded that the dentin substrate did not influence the sealers' bond strength [27]. Only one of the studies included in our review used bovine teeth [13]. The variations in push-out methodology are a concern because they prevent the comparison of results from different researchers [26]. Generally, studies have followed two philosophies: the root canals are either filled with the sealer alone or combined with gutta-percha with the filling techniques of cold lateral compaction, single-cone filling, or specific obturation systems such as Resilon/Epiphany [28,29]. Three studies included in this review filled the samples with gutta-percha and sealer [23–25]. According to some authors, filling the canals only with sealer ensures that there are no confounding factors and that the adhesion strength tested is that of the sealer [28].

The adhesion processes are mostly influenced by the relative surface free energy, which determines the predisposition of the material's surface for establishing new interactions/bonds with the surrounding medium. In the same way, wettability is influenced by the interfacial tensions and, in turn, by the surface free energy [30]. Thus, contact angle evaluation has been widely used to measure the surface wettability of different materials [22]. The contact angle has an inverse relationship with the surface free energy (wettability), i.e., the lower the contact angle, the greater the surface free energy and, hence, likely greater adhesion [13]. Prado, et al. [13] observed increased surface free energy, correlated with the higher wettability of bovine dentin with AH Plus after 30 s of argon NTP, compared to the control (i.e., without NTP). They also reported a chemical change based on FTIR results. Argon plasma treatment reduced the organic compounds of dentin (amide I and II bands) and increased the inorganic component (the carbonade band), due to its ability to etch dentin surfaces; no associated topographical changes occurred. Based on these findings, the authors concluded that argon treatment favored the bonding of the sealer to dentin surfaces [13]. However, only the resinous AH Plus sealer was evaluated.

There is no consensus or sufficient data about NTP's effect on radicular dentin in terms of endodontic sealers' adhesion. In the present investigation, a systematic review was conducted to answer the following PICO question: Does the NTP treatment affect sealers' adhesion to dentin compared to no treatment? A few ex-vivo studies met the selection criteria, even though some disparity in the materials and methodologies was registered, which prevented a meta-analysis from being performed. Our findings indicate that NTP on dentin root walls might positively impact sealers' adhesion, considering the increased POBS or surface energy and wettability values reported in the selected literature.

The bioceramic sealer (BioRoot RCS) showed the highest POBS values, followed by the epoxy-resin-based sealer (AH Plus), after mixing helium and argon atmospheres on dentin surfaces [25]. Moreover, the NTP dentin groups showed an increase in bond strength more than two times higher than the non-plasma-treated dentin (control groups), independent of the sealer [25]. The better bond strength of the bioceramic sealer after NTP, compared to the resinous AH Plus [25], was also corroborated by other authors who stressed its good performance, particularly in the middle region of the root canal [23]. Albeit with different plasma applications, both studies included two recently developed calcium-silicate-based sealers: BioRoot RCS [25] and Endosequence [23], reported to have adhesive characteristics and bioactivity. A recent review of current sealers points out that tricalcium silicate sealers are associated with the lowest relative microleakage compared to the standard AH Plus [4]. The higher POBS values obtained can derive from the chemical nature of bioceramic sealers, affecting properties such as fluidity, their easy spread over the dentin walls due to their low contact angle, or an increase in dentin wettability after NTP [15,23,31]. It was reported that the dentin surface modification after NTP, such as enhanced wettability and chemical interaction, could favor dentinal tubule penetration and the bioceramic sealer's bond strength [23]. Although the authors did not explain the minor influence of NTP on the POBS evaluation of AH Plus [23], other factors, such as the chemical and structural alterations that different irrigating solutions can produce in dentin surfaces, might have affected the results [32].

There are other endodontic procedures aimed to open plasma treatments that can be created under low pressure or atmospheric pressure and increase wettability, such as the standard chelating agent EDTA [33]. However, the additional NTP generally increased these properties, acting as an adjuvant procedure, as shown by the higher POBS values or wettability observed after EDTA exposure [13,25]. Conversely, in the study of Yeter, et al. [23], the final flush was performed with NaOCl. NaOCl may have caused a deproteinization, causing a hydrophilic surface that did not favor the resinous sealer's hydrophilicity [32]. With a similar irrigating solution sequence (EDTA followed by NaOCl), Menezes, et al. [24] found similar bonding values to the control for the NTP groups with either AH Plus or MTA Fillapex. The type of sealer might also have influenced the results.

NTP has been reported to reach deep into the dentinal tubules, similar to or further than bacteria, creating reactive oxygen species and damaging the remaining microorganisms, in addition to cleaning/etching [19]. Thus, it seems to ensure higher mechanical retention and adhesion. This rationale was corroborated by Menezes, et al. [24], Prado, et al. [13], and Garlapati, et al. [25], who reported improved adhesion of NTP surfaces.

Plasma treatments can be carried out at low pressure or atmospheric pressure. The main difference lies in the pressure at which they operate, which affects the plasma density, confinement, and particle behavior [34]. Low-pressure plasmas are generated and sustained in a vacuum or low-pressure environment using vacuum chambers. The benefit of low-pressure plasmas is that the mean free path of the particles (i.e., atoms, ions, and electrons) is relatively long, meaning that they can travel greater distances between collisions. The conditions are much more controllable and reproducible compared to atmospheric-pressure conditions (typically close to 1 atmosphere, where the particles' mean free path is much shorter than in low-pressure plasmas due to the higher gas density), which could compensate (at least partially) for the practical drawbacks of using low-pressure plasma—especially the need for expensive vacuum equipment [35,36]. On the other hand, atmospheric-pressure plasmas have become very attractive because they are generated in an open environment and can be easily implemented [37]. Nevertheless, if particular precautions are not taken, they tend to become thermal, i.e., hot plasmas that can damage heat-sensitive materials or burn living tissues [38]. In the included studies, Prado, et al. [13] and Garlapati, et al. [25] applied NTP treatments under vacuum conditions using a glass reactor, while Yeter, et al. [23] and Menezes, et al. [24] used an atmospheric-pressure plasma jet.

Using low-pressure plasma in dental applications offers several advantages, such as enhanced control over the plasma parameters (e.g., gas composition, pressure, and power), deeper penetration into complex dental structures, access to confined spaces, and uniform treatments due to the better diffusion of the reactive species [22,39–41]. Furthermore, the reduced heat and controlled plasma conditions make low-pressure plasma treatments suitable for treating delicate dental components, such as resin-based composites, polymer-based materials, or dental implants [22,39–41]. However, the potential drawbacks of plasma treatments must be carefully considered. Excessive exposure or high-energy plasma can damage the dentin structure, limiting the treatment's effectiveness and durability. Moreover, implementing plasma treatments requires specialized equipment and expertise, which can increase the cost and complexity of dental procedures. Despite these considerations, plasma treatments offer advantages such as enhanced bonding, improved biocompatibility, effective sterilization, and reduced dentin hypersensitivity [34]. Dental professionals should understand the potential benefits and challenges so as to make informed decisions about incorporating plasma treatments into their practice.

Plasma treatments offer a promising avenue for enhancing dentin surfaces, and the choice of gas composition (e.g., low-pressure processes) plays a crucial role in determining the treatment outcomes [42]. Gas mixing can lead to synergistic effects, creating chemical reactions or interactions that are more effective than using each gas individually [43]. Also, mixing gases expands the range of possible low-pressure plasma treatments and allows for selective treatments [43]. One common gas mixture used in low-pressure plasma treatments is argon (Ar) and oxygen ($O_2$), which provides several benefits [43]. Ar, like helium (He), is an inert gas with low thermal conductivity, which helps minimize the thermal effects on dentin during plasma treatment. It also acts as a carrier gas, facilitating the transport and interaction of reactive species within the plasma [42]. Oxygen, on the other hand, introduces additional reactive species, enabling more effective cleaning, surface modification, or chemical reactions with the dentin surface [42]. The Ar + $O_2$ or He + $O_2$ combination is also an effective sterilization method [18]. The reactive species generated in plasma, such as oxygen radicals, have antimicrobial properties, enabling them to eliminate bacteria, viruses, and other pathogens [18]. This sterilization capability is particularly valuable in infection control during dental procedures, reducing the risk of post-treatment infections [18]. If gases are carefully selected and mixed, the plasma parameters can be controlled, allowing for fine-tuning of the treatment process. Thus, dental professionals can optimize the treatment conditions, ensuring efficient and effective results while minimizing potential risks and adverse effects.

Compared to untreated dentin, i.e., not subjected to plasma treatment, the studies included in this systematic review suggest—albeit with low-certainty evidence—that plasma treatment may be a promising tool for improving the adhesion of endodontic sealers to dentin. Some of the parameters used, such as the time of NTP application, were based on investigations of dental composites' adhesion, because there are insufficient data on root canal dentin–sealer adhesion [14,23]. The type of plasma atmosphere, exposure time, and assessment tools might need to be unified to optimize NTP. However, its high cost has been highlighted.

The findings of the present review should be interpreted cautiously, due to the scarcity of studies on the topic. Moreover, a quantitative analysis was not feasible due to the heterogeneity of the study designs in terms of the plasma treatment (i.e., type of devices used; plasma parameters like power, frequency, gas type, and application time), adhesion methodology, and type of sealers used. Although a total of 188 studies were obtained from the electronic search, only 4 were included after applying the eligibility criteria. Nonetheless, the overall risk of bias of the included studies was high. However, the strict selection of the studies enabled an overview of this contemporary topic, highlighting its potential.

## 5. Conclusions

The studies included in this systematic review suggest that plasma treatment may be a promising tool for improving endodontic sealers' adhesion to dentin. There is a need to optimize NTP's parameters to develop a stronger evidence base in endodontics on its role as an adjuvant tool to increase sealers' adhesion to dentin. This optimization could help improve the outcomes of root canal treatments.

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# Paper 2

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# Functionalization of gutta-percha surfaces with argon and oxygen plasma treatments to enhance adhesiveness

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**Gutta-percha's lack of adhesion has been presented as a drawback to avoid gaps at sealer/gutta-percha interface. Plasma treatments have been scarcely assessed on gutta-percha surfaces as a method of enhancing adhesiveness. This study aimed to evaluate the effect of low-pressure Argon and Oxygen plasma atmospheres on conventional and bioceramic gutta-percha standardized smooth discs, assessing their roughness, surface free energy, chemical structure, and sealer wettability. A Low-Pressure Plasma Cleaner by Diener Electronic (Zepto Model) was used. Different gases (Argon or Oxygen), powers (25 W, or 50 W), and exposure times (30 s, 60 s, 120 s, or 180 s) were tested in control and experimental groups. Kruskal–Wallis and Student's t-test were used in data analysis. Statistically significant differences were detected when *P*<0.05. Both gases showed different behaviors according to the parameters selected. Even though chemical changes were detected, the basic molecular structure was maintained. Argon or Oxygen plasma treatments favoured the wetting of conventional and bioceramic gutta-perchas by Endoresin and AH Plus Bioceramic sealers (*P*<0.001). Overall, the functionalization of gutta-percha surfaces with Argon or Oxygen plasma treatments can increase roughness, surface free energy and wettability, which might improve its adhesive properties when compared to non-treated gutta-percha.**

Plasma treatments have been disseminated in several fields of Dentistry as a surface treatment to improve adhesion, etching (e.g., dentin), or simply cleaning (tooth bleaching)[1]. More recently, they have been successfully used to functionalize biomaterials by either increasing cell adhesion (osteointegration) or improving their antimicrobial/antibiofilm characteristics[2,3]. Generally, Argon (Ar) atmospheres are responsible for the physical activation mechanism (cleaning and etching), while the Oxygen ($O_2$) reactive atmosphere has a main role in promoting chemical reactions/modifications at the surface of the treated samples, although it can also act as an etching agent[4]. The power or the duration used influences the energy of the particles constituting the plasma (positive ions, electrons, neutral gas atoms or molecules, and ultraviolet (UV) light) resulting in different types of interactions with the gutta-percha (GP) surface.

Conventional GP is still the gold-standard core-filling endodontic material[5] It consists of a trans-isomer of polyisoprene matrix (1, 4, trans–polyisoprene) mixed with organic and inorganic components, such as zinc oxide, waxes, resins, and barium sulfate[6]. The physical and thermomechanical properties, such as tensile strength, stiffness, radiopacity, and viscoelasticity, hinder its proper adhesion to dentin and sealers[5,7,8]. Ideally, adhesion of GP to both dentin walls and sealers would prevent leakage or loss of the seal. This drawback preventing to avoid gaps at sealer/gutta-percha interface, can influence the filling quality, strongly correlated with the therapeutic's

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outcome[9]. The main goal of the endodontic treatment (ET) is to achieve a tridimensional sealing of the root canal system while preventing coronal and apical leakage. The recognized lack of a true adhesion of root canal sealers to dentin has been leading to investigations about the impact of root dentine conditioning on the sealing ability of the fillings[10]. Studies indicate that the surface modification through the irrigating protocols appear to influence the adhesion of sealers to root dentine. Additionally, a strong correlation between sealing ability and bond strength was also emphasized[11].

In the last years, GP cones coated with methacrylate resin, glass ionomer, apatite calcium phosphate, and more recently with bioceramic nanoparticles have been suggested as a way of increasing GP adhesion to specific sealers[5]. The introduction of polymer-based cones, such as Resilon, matching a recommended resin-based sealer (Epiphany), re-introduced the concept of "monoblock", challenging the traditional gutta-percha/resin sealer obturation[7]. However, the lack of information about its real impact on the sealing ability precluded their wide use. Despite the great technological advancements in endodontic materials, there is still a gap in achieving a better long-term fluid-tight seal between the gutta-percha core and the sealer[12].

The emergence of novel endodontic proposals, such as calcium silicate-based root canal sealers (CSS), has changed the concept of "hermetic seal" to chemical bonding and activity[13]. Some manufacturers advise its use in combination with calcium silicate-coated/impregnated GP cones (CSGP)[14]. Hence, the few studies available did not find a superior bond of the CSS to impregnated gutta-percha cones, compared to the epoxy resin-based sealer, bonded to conventional GP[15]. On the other hand, besides root canal sealer's ability to adhere to the core material is a desirable characteristic, the methodology usually applied has been recently questioned[12]. One of the main limitations encountered is the fact that bond strength has essentially been evaluated considering the bond between sealers and the dentin walls, namely by push-out bond strength resistance tests[12,16]. Besides, the findings based on heterogenous protocols, are contradictory[8,15,16]. Thus, there is limited information about the adhesion ability between the solid core, usually GP cone, and the sealer. Amongst other properties, an adequate flow and wetting of the substrate seem to be relevant to the sealers' performance[8]. Recently, an innovative and reproducible way of testing bonding between GP and various types of root canal sealers was suggested[12]. Despite some limitations, such as evaluating GP discs instead of the clinically available GP cones and the fact that only conventional GP has been included, both CSSs studied presented a weaker bond to conventional GP, compared to the epoxy resin-based sealer (AH Plus)[12]. The authors suggested future research in the topic, including different brands of GP, matched with the respective sealers[12].

Due to their polymeric-like matrix, GP cones are heat-sensitive materials, thus requiring low-temperature surface modification, which non-thermal gas plasmas can provide at low or atmospheric pressure[17]. Depending on the plasma settings (gas composition, pressure, power, duration), a medium rich in free electrons, excited ions, atoms, or molecules, radicals, and UV/visible radiation is created. This physical environment can modify the surface of the substrate, both physically and chemically, improving its surface energy without damping the main core properties of the material's matrix[3,18]. Cold plasma treatments performed at low-pressure plasma systems are described as environmentally clean procedures suitable to almost all substrates, such as dentin[18] or GP[17]. Hence, there are few reports specifically concerning modified/functionalized GP surfaces by plasma treatments in Dentistry, reinforcing the importance of the present investigation and the potentialities of improving GP's adhesiveness and thus ET's success[2,17]. For that purpose, smooth discs of conventional and bioceramic GP were functionalized. The topographic changes (roughness) and surface free energy, as well as chemical changes, and sealers' wettability were analyzed in view of a better GP/sealer adhesion ability. The present study aimed to assess the influence of two distinct plasma atmospheres (Ar or $O_2$) for different periods (30 s, 60 s, 120 s or 180 s) and powers (25 W or 50 W) on conventional and bioceramic GP types, evaluating surface and chemical features. We tested the null hypotheses that none of the GP's type surfaces would show topographic, surface free energy, chemical or wettability changes, independently of the atmospheres or parameters used in the plasma treatment.

## Methods

### Specimen preparation and standardization

Round discs of GP samples/specimens (10 mm diameter and 2 mm thickness) were produced from GP pellets: conventional GP (DiaDent Gutta-Percha Pellets; Choongchong Buk Do, Republic of Korea) and bioceramic GP (TotalFill Bioceramic Gutta-Percha Pellets; FKG Dentaire, La-Chaux-de-fonds, Switzerland). Similar to another study[12], these GP discs were produced by creating appropriate molds and then plasticizing GP in a laboratory dry-heating oven at 80 °C, followed by a cooling process at room temperature. A standardized metallographic procedure was employed with coarse silicon carbide abrasive papers (until 600 grit) to produce GP discs with similar surface roughness in both faces. There were no statistically significant differences in the surface roughness of the conventional GP samples (Ra Zscore: $n = 135$, $t = -2.5 \times 10^{-13}$, $P \cong 1.0$) or the bioceramic samples (Ra Zscore: $n = 135$, $t = 9.45 \times 10^{-15}$, $P \cong 1.0$). Samples were randomly allocated to the different groups using an online computer-generated number (www.randomizer.org).

### Characterization of the GP specimen

*X-ray diffraction (XRD) analysis*

XRD analysis was performed using a Siemens D 5000 diffractometer (D8 Discover; Bruker AXS, Karlsruhe, Germany) with Cu-Ka radiation ($\lambda = 1.5418$ A) and was conducted with a scan range of 5°–90° (2θ) using a θ/2θ configuration and a step time of 2 s. Crystalline phases were identified using the Inorganic Crystal Structure Database (ICSD).

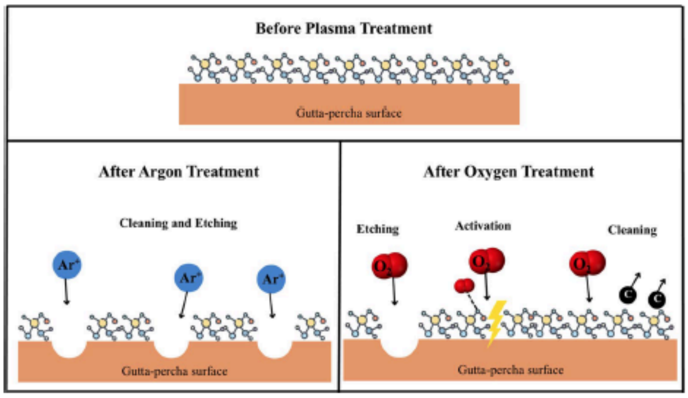


**Figure 1.** Schematic representation of the effect of the different plasma treatment (Argon and Oxygen) applied to gutta-percha surfaces.

## Surface activation of GP surfaces

A Zepto laboratory-sized plasma system (Diener Electronic; Ebhausen, Germany), equipped with a 13.56 MHz generator, was used for the GP surface activation. Plasma treatments were executed considering three main parameters: (i) working gas (Ar or $O_2$), (ii) treatment time (30 s, 60 s, 120 s, or 180 s), and (iii) power (25 W or 50 W). The work pressure was constant for all the treatments (~ 80 Pa), while the base pressure was always lower than 20 Pa. Figure 1 shows a schematic representation of the effect of the different plasma treatment (Ar and $O_2$) applied to GP surfaces.

## Topographical analysis

The surface of the specimens was evaluated topographically by measuring the surface roughness with an optical profilometer (Profilm 3D; Filmetrics, San Diego, CA, USA). For each sample, three different scans were taken at distinct surface sites using composite white-light interferometry and phase-shifting interferometry to ensure greater sensitivity to different amplitudes of the surface irregularities. Each treatment was tested in different conventional and bioceramic GP samples (n = 10). The average and standard deviation of the surface texture parameters, such as the arithmetical mean height (Ra) and the root-mean-square height (Rq), were calculated. The control group included samples not submitted to plasma treatment.

## Surface free energy analysis

Immediately after the activation treatments, the contact angle between the solutions (water, glycerol, and 1-bromonaphthalene) and the GP surfaces was measured using an optical contact angle (OCA 20; DataPhysics Instruments GmbH, Filderstadt, Germany) at room temperature. The drop volume was 0.5 mL for water and 1-bromonaphthalene, and 3 mL for glycerol. Liquids were released from the syringe tip by positioning it above the GP surface and allowing it to rise to the GP/liquid interface. The control group included samples not subjected to plasma treatment. Five drops were added to each solution using the sessile drop technique (n = 5). The surface free energy was calculated based on the data collected by applying the Owens and Wendt[19] method, described by Eq. (1):

$$\frac{\sigma_l(\cos\cos\theta + 1)}{2(\sqrt{\sigma_l^D}} = \left(\sqrt{\sigma_s^P}\right)\frac{\sqrt{\sigma_l^P}}{\sqrt{\sigma_l^D}} + \sqrt{\sigma_s^D} \quad (1)$$

where $\sigma_l^D$ and $\sigma_l^P$ are, respectively, the dispersive and polar components of the surface tension of the liquid used, and θ is the contact angle of the corresponding liquid with the GP disc/sample. From these three parameters, the GP surface energy's dispersive and polar components ($\sigma_s^D$ and $\sigma_s^P$, respectively) were determined through a linear fit of the data obtained using the three liquids. The total surface energy $\sigma_s$ was the sum of both $\sigma_s^D$ and $\sigma_s^P$ components.

## Chemical analysis

Fourier transform-infrared spectroscopy (FT-IR) was performed to study the chemical modifications in the attenuated total reflectance mode using a Jasco FT/IR 4100 system (Jasco International; Hachioji, Tokyo, Japan) with a wavelength range of 600–4000 $cm^{-1}$ and a resolution of 4 $cm^{-1}$. Five measurements were performed for each experimental condition (n = 5).

### Sealers wettability assessment

The contact angle between GP surfaces and the sealers was measured using the same optical contact angle (OCA 20; DataPhysics Instruments GmbH, Filderstadt, Germany) at room temperature. Following the manufacturer's instructions, an epoxy resin-based sealer (Endoresin cement; Endogal, Sarria, Lugo, Spain) and a bioceramic sealer (AH Plus Bioceramic; Dentsply Sirona, Ballaigues, Switzerland), were tested in conventional and bioceramic GP surfaces. One set of parameters (time and power) for each plasma treatment gas, that might be related to a better adhesion, such as roughness and surface free energy, will be chosen to the experimental assay.

After one drop of sealer (0.1 mL) was deposited on the GP surfaces with a 0.5 mL BD ultrafine syringe. Ten drops of the same sealer were evaluated for each plasma treatment (n = 10), being that the control group (n = 10) included samples not subjected to plasma treatment.

The sealer wettability was followed up for 1 min, using the next Eq. (2) to evaluate the sealer wettability (SW)[17]:

$$SW(\%) = \frac{(\text{initial angle} - \text{final angle})}{\text{initial angle}} \times 100 \quad (2)$$

### Statistical analysis

The IBM SPSS Statistics software (version 28.0; IBM, Armonk, NY, USA) was used for the statistical analysis. The level of significance was set at 5% ($P < 0.05$). All applicability conditions were verified (normality: Kolmogorov–Smirnov test and PP-plot; homoscedasticity of variance: Levene' s test).

Student's t-test was used to confirm the similarities in surface roughness (Ra values) between all samples (sample standardization). Pearson correlation was performed to evaluate the linear association between Ra and Rq roughness parameters. Student's t-test for independent samples was used to evaluate significant differences among control and experimental groups. Sealer wettability was evaluated using Kruskal–Wallis test with multiple comparisons when significant differences were detected.

## Results

### Characterization of the GP specimens

Both types of GP were analyzed in terms of crystalline structure. The XRD analysis suggests a predominance of zinc oxide (ZnO) crystals inside both types of GP matrixes, evidenced by the narrower and more intense peaks, represented in Fig. 2 by the symbol (+), according to the ICSD #01-082-9745 card. Nevertheless, the diffraction patterns evidence differences between the two GPs. The bioceramic GP is richer in zirconium oxide ($ZrO_2$) crystalline compounds (ICSD #01-077-5342), as shown by the double peak at 2θ ~ 28.2° with a mix of $ZrO_2$ and barium sulfate ($BaSO_4$) phases or even the peaks at 2θ between 50° and 55°. In turn, $BaSO_4$ crystals (ICSD #01-083-2053) prevailed over $ZrO_2$ in the conventional GP, evidenced by the triplet between 24.8° and 28.6° or the weak double peak at 42.6°. $BaSO_4$ crystals (ICSD #01-083-2053), although in minor traces, were also noticed in the diffractogram of the bioceramic GP (Fig. 2.

### Topographical analysis

The surface topography of the GP discs was analyzed before and after activation with plasma treatments. The analysis was performed based only on the Ra parameter since a strong positive and statistically significant association (r = 0.981, $P < 0.001$) detected between the Ra and Rq parameters confirmed a similar behavior.

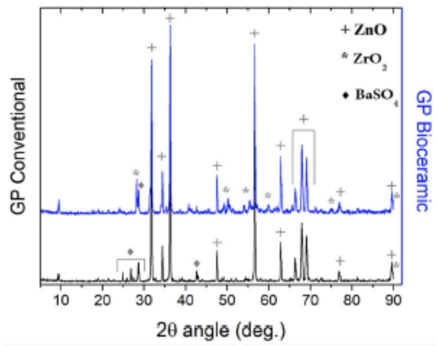


**Figure 2.** X-ray diffractograms of the conventional and bioceramic gutta-perchas before being submitted to plasma treatments (ZnO: zinc oxide; $ZrO_2$: zirconium oxide; $BaSO_4$: barium sulfate).

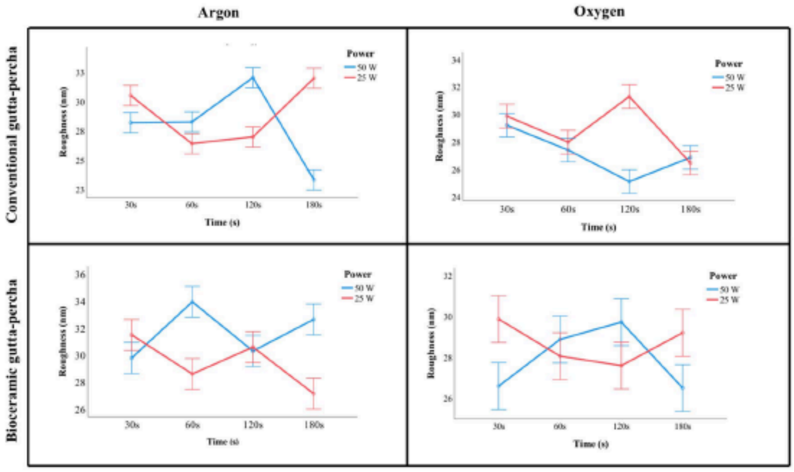


**Figure 3.** Roughness mean values of conventional and bioceramic gutta-percha tested with different gases (Argon and Oxygen), powers (25 W and 50 W), and times (30 s, 60 s, 120 s and 180 s).

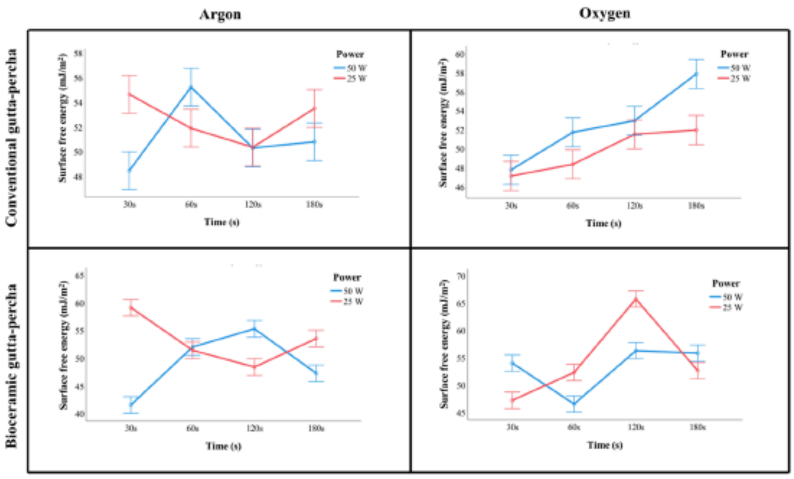


**Figure 4.** Surface free energy mean values (mJ/m²) of conventional and bioceramic gutta-percha tested with different gases (Argon and Oxygen), powers (25 W and 50 W), and times (30 s, 60 s, 120 s and 180 s).

Independently of the GP type, plasma treatments carried out in Ar or $O_2$ atmospheres showed different behaviors, depending on the power and duration (Fig. 3). Comparing to the control (29.40 nm) for conventional GP, the highest roughness values were registered with an Ar atmosphere at 50 W for 120 s (32.04 nm; $P=0.002$) and an $O_2$ atmosphere at 25 W for 120 s (31.29 nm; $P=0.005$). Comparing to the control (Ra = 26.50 nm) for bioceramic GP, the highest values of roughness were achieved for the treatments performed in an Ar atmosphere at 50 W for 60 s (33.94 nm; $P<0.001$) and in an $O_2$ atmosphere at 25 W for 30 s (29.87 nm; $P<0.001$).

## Surface free energy analysis

Plasma treatments increased samples' surface free energy relative to the control, independently of GP type ($P<0.001$). Figure 4 shows the values of surface free energy for the different experimental groups. Comparing to the control (41.02 mJ/m$^2$), for the conventional GP the highest surface free energy values were registered with an Ar atmosphere at 50 W for 60 s (55.23 mJ/m$^2$; $P<0.001$) or at 25 W for 30 s (54.64 mJ/m$^2$; $P<0.001$) and an $O_2$ atmosphere at 50 W for 180 s (57.87 mJ/m$^2$; $P<0.001$). In turn, comparing to the control (31.41 mJ/m$^2$) on bioceramic GP, the highest values of surface free energy were achieved with an Ar atmosphere, at 25 W, for a treatment duration of 30 s (59.13 mJ/m$^2$; $P<0.001$) and with an $O_2$ atmosphere, at 25 W, for 120 s (65.70 mJ/m$^2$; $P<0.001$).

## Chemical analysis

The FT-IR analysis showed wavelength variations between conventional and bioceramic GP spectra, which may result from their different chemical compositions. After plasma treatments the main peaks observed in both GP spectra remained, namely the peaks at 2850–2950 cm$^{-1}$ which correspond to –C–H stretching vibration; at 1400–1500 cm$^{-1}$ the peaks bending vibration of C–H in the = $CH_2$ and ~ 1150 cm$^{-1}$ the peak assigned to the stretching vibration of C–C. This result confirms that the basic molecular structure of the material was maintained in both Ar and $O_2$ treatments (Supplementary files: Figs. 1 and 2). However, a detailed analysis of Fig. 5, shows slight differences in the GP spectra after being submitted to a plasma atmosphere. The conventional GP's 1735, 1480, and 1177 cm$^{-1}$ peaks (corresponding to the CO stretching) increased due to the polyisoprene matrix oxidation with the plasma treatment varied out, especially into an oxygen atmosphere[20]. Similarly, the bioceramic GP spectra showed an increase of 1741, 1460, and 1170 cm$^{-1}$ peaks compared to the control. Moreover, the smooth shoulder at ~ 3320 cm$^{-1}$ (corresponding to the O–H stretching) confirms the bioceramic GP oxidation in both Ar and $O_2$ plasma treatment.

## Sealers' Wettability

The parameters selected and applied for each GP type, considering a good balance between power, time and respective impact on roughness and surface free energy were: Ar at 50 W during 60 s and $O_2$ at 25 W during 120 s.

For Endoresin sealer in conventional GP, there were significant differences between the control (not treated GP surfaces) and Ar plasma treated GP ($P=0.002$). In bioceramic GP both plasma treatments with the selected parameters improved the sealer's wettability when compared with the control group (Ar: $P=0.037$; $O_2$: $P<0.001$). Regarding AH Plus Bioceramic sealer, both atmospheres (Ar and $O_2$) produced significant differences, in conventional and bioceramic GPs, with increased values, compared with the control (Ar: $P<0.001$; $O_2$: $P<0.001$). All these results can be observed in Fig. 6.

## Discussion

The present investigation provided some additional findings about GP plasma treated surfaces, not thoroughly investigated so far. Ar and $O_2$ plasma treatments produced an impact in GP surface features reflected in topographic, surface free energy, chemical or wettability changes, which might improve the adhesiveness of distinct GP types to sealers. In that sense the null hypothesis was rejected.

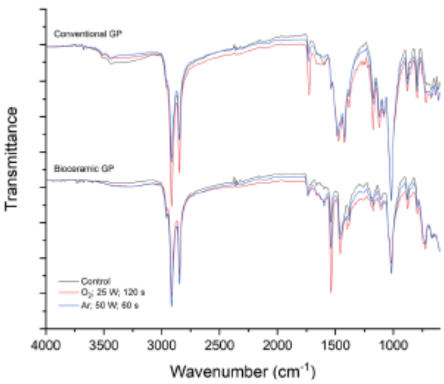


**Figure 5.** Representative Fourier transform-infrared spectroscopy's spectra of the parameters selected for each gutta-percha (GP) type (conventional and bioceramic) considering a good balance between power, time and respective impact on roughness and surface free energy (Ar at 50 W during 60 s and $O_2$ at 25 W during 120 s), compared to the control (without plasma treatment).

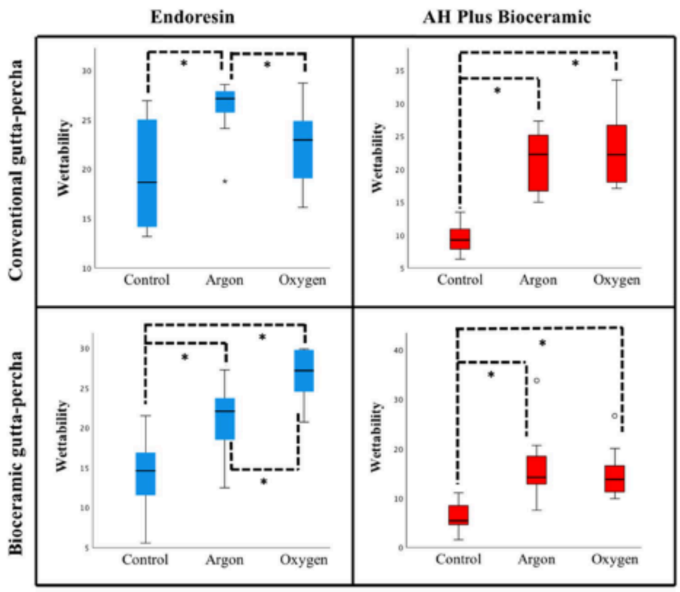


**Figure 6.** Sealers' wettability (%) on conventional and bioceramic gutta-percha surfaces with different plasma treatment (Ar at 50 W during 60 s and $O_2$ at 25 W during 120 s), compared to the control (without plasma treatment) (*significant at $P<0.05$).

The scientific literature points out a lack of adhesive characteristics of the GP filling material, preventing a tight seal between the root canal filling materials, namely sealer/GP core[17]. In the present study, one of the focuses of plasma treatment's applications in Endodontics was the surface modification of GP's solid core, aiming to enhance its adhesion to endodontic sealers. Because there are several commercially available GP brands and little information on their adhesion properties, novel brands of conventional and bioceramic GPs were selected. Generally, a bioceramic GP is a modification of the conventional one by impregnating and coating its surface with bioceramic calcium-silicate nanoparticles[14]. Like in other studies, a set of standardized GP discs were manufactured for the present study, instead of using the commercially available GP cones for clinical use[12,17]. Conventional and bioceramic GP samples were produced from pellets, able to be used in thermoplastic techniques. There is no relevant information available about possible drawbacks on bioceramic GP heating.

The effects of activating GP surfaces with Ar and $O_2$ plasma treatments were assessed for different periods and powers, based on topographic modifications (roughness) and surface free energy of the samples. Not plasma treated GP surfaces were used as respective, conventional and bioceramic GP controls. Chemical surface features and wettability evaluation with two distinct endodontic sealers were also investigated.

The present findings are in accordance with other investigation supporting the fact that physicochemical properties of materials or substrates, such as roughness and surface free energy, might be influenced by plasma treatments, allowing to discover new capabilities of these conventional substrates[17]. The different set of parameters studied, such as the type of plasma atmosphere, the power, or exposure time influenced the plasma treatment effects on GP surfaces, not previously described.

Surface free energy represents a measure of adhesion strength due to quantifying the intermolecular attraction/bonding that occurs when a surface is modified. An increase in surface energy means an improvement in the molecular adhesion of the solid surface caused by stronger interatomic attractive forces[17]. In the present study, both Ar and $O_2$ plasma treatments significantly increased the surface free energy of conventional and bioceramic GP, compared to the respective control group. These findings were corroborated by another investigation in conventional GP[17]. A surface that has a lower contact angle and consequently a high surface free energy, is likely to present greater wettability, as shown in the present study. Similar to other authors contact angle measurement was considered a useful indicator of the wettability of a liquid, which, in the present case was the two canal sealers studied[21]. For sealers' wettability assessment the parameters selected and applied for each GP type, presenting a good balance between power, period and respective impact on roughness and surface free energy, were: (i) Ar at 50 W during 60 s and (ii) $O_2$ at 25 W during 120 s. Ar was selected because besides being an inert gas it showed to have the biggest influence on the physical activation of the surfaces increasing the roughness mean values of both type of GP, associated with specific powers. However, for periods longer than 60 s, the activation achieved by the energetic $Ar^+$ ions begin to fade by the consequent collisions that are now removing the topography effects initially created. On the other hand, the plasma treatments carried out in an $O_2$ atmosphere also promoted great increments on the roughness values of both conventional and bioceramic GP and a great reactivity (surface energy) of the bioceramic GP. The reactive nature of the $O_2$ plasma plays a determinant role in the formation of oxygen-containing species groups (increment of C=O and O–H stretching, (Fig. 5)) that due their reactivity is able to link and create new components with the sealers[17]. Both sealers' wettability was clearly improved, in these conditions, in both GP types. Although not mandatory, some manufacturers advise using CSGP with a CSS through the single-cone technique, potentially increasing bonding, and tooth's fracture resistance[14]. As studies on GP/sealers adhesion present contradictory results[12], these primary findings are promising as they may reflect an improvement in distinct GP/sealer type adhesion, independent of its matching. Further studies exploring the complexities of the substrates (GP/sealers) as well as the possible correlations of this variables with the clinical success are needed.

Concerning specific endodontic sealers with different chemical compositions, such as Endoresin and AH Plus Bioceramic it was found that both Ar and $O_2$ plasma treatments favoured sealers' wettability, promoting an easier spread of the sealer drop on the GP-treated surfaces, compared to the control (non-treated GP surfaces). Benefits of plasma treatments such as increasing surface free energy of GP samples and favoring sealers' wettability were corroborated by other study[17], referred to as likely to enhance adhesion.

The chemical analysis on treated GP surfaces revealed polyisoprene matrix oxidation, intensifying the stretching signal for the C=O bond on both types of GP. Similar results were found by other authors[17], who noticed an increase in the C=O stretching for conventional GP samples treated in a reactive $O_2$ atmosphere, promoting the formation of new active sites[17]. Conversely, while other authors reported a reduction in the O–H stretching, in our study a smooth shoulder at 3320 $cm^{-1}$ evidenced its increase in the bioceramic GP activated, albeit in minor traces. This undeniable evidence might be closely related to the free radical's generation and/or polymer chain scission in a GP richer in $ZrO_2$ crystals than the conventional GP (Fig. 2), thus favoring the reactivity with the environment and the formation of new intermolecular bonds, probably creating hydrogen bonding networks. The chemical modifications on the samples' surface, such as the wavelength variation between conventional and bioceramic GP spectra, reflect the different chemical compositions also confirmed in the XRD analysis. Nevertheless, FT-IR peaks of plasma-treated and non-treated GP presented slight differences in peak the intensity, more specifically at 3300–3450 $cm^{-1}$ associated with O–H stretching, at ~ 1730 $cm^{-1}$ related to C–O stretching and at ~ 1600 $cm^{-1}$, attributed to C–C stretching. This finding agrees with other study who reported that the "same main peaks" of GP samples were still present after plasma treatment, indicating the preservation of most of its molecular structure[17]. Chemical modifications and surface etching produced by plasma treatments have been further described as promoting interatomic bonding in different substrates (dentin, enamel, and composites), thus favoring their adhesive characteristics[1,22–24].

Among several techniques used to modify the properties of a material's surface, the plasma treatment is often used to enhance the wettability and surface energy of polymers in very short periods, an added value solution able to overcome the well-known polymers adhesion problems, without changing their main characteristics[2,25]. Moreover, plasma treatments are green (environmentally friendly) processes. During the plasma activation, the interaction of the energetic particles with the GP results in several surface effects, such as cleaning and etching to remove contaminants and promote surface roughness, plus activation by the formation of new functional groups and chain scission (formation of free radicals acting as anchorage points)[2,25]. The occurrence of these combined effects modifies both the physical (roughness) and chemical (crosslinking bonds) characteristics of the GP surface, allowing the creation of interlocking points and the presence of active polar groups. The activation of the surface can be noticed by an increase in the surface roughness, and free surface energy, which enhances the adhesion at the interface GP/sealer, expressed as better wettability[17].

One of the major strengths of the present investigation was to optimize a set of treatment plasma parameters to be investigated in distinct GP types, quantifying topographic modifications (roughness) and surface free energy of the samples, compared to the respective control. Apart from this, as the topic has been scarcely discussed in the current endodontic literature it can add novel data. This is one of the few reports about the effects of non-thermal treatment plasma, assessing different parameters, on bioceramic and conventional GP filling core material, in view of a better adhesion ability. As limitation, it must be stressed that the behaviour of GP discs in an in-vitro condition, may not certainly reflect the clinical set-up. However, pursuing recent guidelines, the reproducibility of the experiment can overcome some of the constraints.

## Conclusions

In conclusion, the present findings highlight the positive impact of plasma treatment in the GP surface features, independently of its composition, conventional or bioceramic, or the gas, Ar or $O_2$. The assessed outcome of roughness, surface free energy and wettability to endodontic sealers might contribute to improve gutta-percha adhesive characteristics to endodontic sealers. However, the selection of the adequate parameters, such as power and time exposure, within each of the atmospheres (Ar and $O_2$) seemed to play a role in the desired outcome.

## Data availability

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

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## Competing interests

The authors declare no competing interests.

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# Paper 3

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BMC Oral Health

RESEARCH Open Access

# Nanostructured ZnO thin film to enhance gutta-percha's adhesion to endodontic sealers

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**Abstract**

**Background** Gutta-percha (GP) combined with an endodontic sealer is still the core material most widely used for tridimensional obturation. The sealer acts as a bonding agent between the GP and the root dentinal walls. However, one of the main drawbacks of GP core material is the lack of adhesiveness to the sealer. ZnO thin films have many remarkable features due to their considerable bond strength, good optical quality, and excellent piezoelectric, antibacterial, and antifungal properties, offering many potential applications in various fields. This study aimed to explore the influence of GP surface's functionalization with a nanostructured ZnO thin film on its adhesiveness to endodontic sealers.

**Methods** Conventional GP samples were divided randomly into three groups: (a) Untreated GP (control); (b) GP treated with argon plasma (PT); (c) Functionalized GP (PT followed by ZnO thin film deposition). GP's surface functionalization encompassed a multi-step process. First, a low-pressure argon PT was applied to modify the GP surface, followed by a ZnO thin film deposition via magnetron sputtering. The surface morphology was assessed using SEM and water contact angle analysis. Further comprehensive testing included tensile bond strength assessment evaluating Endoresin and AH Plus Bioceramic sealers' adhesion to GP. ANOVA procedures were used for data statistical analysis.

**Results** The ZnO thin film reproduced the underlying surface topography produced by PT. ZnO thin film deposition decreased the water contact angle compared to the control ($p < 0.001$). Endoresin showed a statistically higher mean bond strength value than AH Plus Bioceramic ($p < 0.001$). There was a statistically significant difference between the control and the ZnO-functionalized GP ($p = 0.006$), with the latter presenting the highest mean bond strength value.

**Conclusions** The deposition of a nanostructured ZnO thin film on GP surface induced a shift towards hydrophilicity and an increased GP's adhesion to Endoresin and AH Bioceramic sealers.

**Keywords** Adhesiveness, Calcium silicate-based sealer, Epoxy resin-based root canal sealer, Gutta-percha, Plasma treatment, ZnO thin film

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## Introduction

The success of endodontic treatment depends on canal shaping, cleaning, disinfection protocols, and the hermetic filling of the root canal system. The main goal of root canal obturation is to prevent coronal and apical leakage and entomb the remaining bacteria that may persist after treatment [1]. Gutta-percha (GP) combined with an endodontic sealer is still the core material most widely used for tridimensional obturation. The sealer acts as a bonding agent between the GP and the root dentinal walls. Commercially available endodontic sealers vary in composition, leading to different interactions with dentin and GP [2–4]. Epoxy resin-based sealers are widely recommended for root canal filling due to their good physical properties [5]. Calcium silicate-based sealers (CSS) have gained prominence due to their high biocompatibility, bioactive feature, and antimicrobial action [6]. The bond strength of root canal sealers has been tested mainly to the dentinal walls, whilst there is still a lack of knowledge regarding their adhesion ability to core-filling materials, which might influence sealing ability and filling resistance to dislodgement [4, 7]. In this study, two sealers were tested, an epoxy resin-based sealer – Endoresin, and a calcium silicate-based sealer – AH Plus Bioceramic. AH Plus Bioceramic is a recent premixed calcium silicate-based sealer comprising zirconium dioxide, tricalcium silicate, dimethyl sulfoxide, lithium carbonate, and a thickening agent [8]. While it exhibits favorable physical properties and antibacterial activity due to its high pH, its solubility may impact the obturation quality [9]. Recent studies have highlighted its cytocompatibility and bioactive potential, surpassing the epoxy resin-based AH Plus and rivaling EndoSequence BC Sealer [8]. Epoxy resin-based sealers, like Endoresin, has been used as the gold standard material due their physicochemical properties [10]. There is a lack of knowledge regarding the performance of AH Plus Bioceramic and Endoresin in terms of bond strength to GP.

Until now, none have supplanted GP, which continue to be universally accepted as the 'gold standard' core filling in root canal treatment [11]. The commercially denominated GP is a polymer that contains approximately 20% of GP (matrix), 66% of zinc oxide (filler), 11% of heavy metal sulfates (radiopacifier) and 3% of waxes and/or resins (plasticizer) [12]. One of the main drawbacks of GP core material is the lack of adhesiveness to the sealer. Its hydrophobic nature tends to pull the sealer away during setting [13]. In the last few years, studies have been developed to find a way to improve the characteristics of GP [11, 14–18]. The concept of coating the GP by methacrylate resin, glass ionomer, apatite calcium phosphate and nanoparticles (chitosan, silver and bioceramic) has emerged, to enhance its antimicrobial characteristic and adhesion ability [11]. However, there is still a gap in achieving a better long-term fluid-tight seal between the GP core and the sealer [16].

Zinc oxide (ZnO) constitutes a significant portion of GP's elemental composition [19]. Classified as safe by the US Food and Drug Administration, ZnO is a low-cost material that is easy to process, abundant in nature, biosafe, biocompatible, and non-toxic, presenting antimicrobial activity [14, 20]. At the nanometric scale, ZnO thin films have many remarkable features due to their considerable bond strength, good optical quality, and excellent piezoelectric, antibacterial, and antifungal properties, offering many potential applications in various fields [21–23]. A previous study proposed a novel approach to increase the antibiofilm efficacy of GP, modifying its surface by using Argon (Ar) plasma treatment (PT), followed by the deposition of a ZnO thin film [14]. PT has several applications, and one of the most used is directly related to the modification and functionalization of the materials' surfaces [15, 24, 25], in a controlled, reproducible, and homogeneous way without changing the main properties of the bulk material [25–28].

The interaction of plasma with surfaces is greatly affected by the energy input, pressure, working gas composition and the nature of the substrate [26, 31]. Depending on these factors, a diversity of chemical-reaction based interactions with surface materials can be enhanced or even enabled by plasma application, including cleaning, activation, etching or ablation, and film thin deposition (coating) [25].

Although the functionalized GP with ZnO thin film has shown promising results in terms of biocompatibility and antibacterial properties [14], to our knowledge, no studies have investigated its performance concerning sealers' adhesion. Therefore, the present investigation focused on the influence of GP surface's functionalization with a nanostructured ZnO thin film deposition on the adhesion to an epoxy resin-based and a calcium silicate-based endodontic sealer. The null hypothesis was that GP surface's functionalization will not have impact on sealer adhesion.

## Materials and methods

### Materials used

#### *Gutta-Percha (GP)*

Round disks of GP samples (10-mm diameter and 2-mm thickness) were produced from GP pellets (Gutta-percha Bal Plus Pellets; Meta Biomed Co, Ltd; Korea) by creating appropriate molds and then plasticizing GP in a laboratory dry-heating oven at 80 °C, followed by a cooling process at room temperature. A standardized metallographic procedure was employed to produce samples with similar surface roughness, for that the specimens were polished with coarse silicon carbide abrasive papers (180 to 600 grit). The GP round disks were used to surface morphology and contact angle analysis. Additionally, GP pellets submitted to the same standardized metallographic procedure, on its two flat sides, were used for the tensile bond strength testing with the sealers.

#### *Sealers*

- AH Plus Bioceramic (calcium silicate-based sealer; manufactured by Maruchi; distributed by Dentsply DeTrey GmbH).
- Endoresin (epoxy resin-based sealer; manufactured by Meta Biomed. Ltd, Chungcheongbuk-do, Korea; distributed by Galician Endodontics Company S.L., Lugo, Spain).

### Functionalization of GP

#### *Plasma treatment (PT)*

GP was submitted to PT in an Ar atmosphere using a low-pressure plasma cleaner (Plasma System Zepto; Diener electronic) powered at 50 W for 60 s. The working pressure never exceeded 80 Pa, while the base was kept at 20 Pa for all treatments. These parameters were applied based on a previous study [27].

### Deposition of ZnO thin film

The ZnO thin film was deposited onto GP by reactive magnetron sputtering at a working pressure of $5\times10^{-1}$ Pa, while keeping the flow of Ar (30 sccm) and $O_2$ (20 sccm) constant using a reactive chamber with a volume of 50 $dm^3$. A metallic zinc target with 99.96% purity was used for the depositions, with a 50.6-mm diameter and 6-mm thickness. The base pressure was kept lower than $4\times10^{-4}$ Pa, and the Zn target was connected to a DC source, setting the target potential at -378 V.

The process of physical vapor deposition using reactive magnetron sputtering is depicted in Fig. 1. In this process, a target material, typically a metal or compound, is bombarded with $Ar^+$ ions inside a vacuum chamber. The collision of $Ar^+$ ions with the target result in a momentum and energy transference. As a consequence, the atoms of the target material are displaced from their original locations and ejected into the surrounding vacuum interacting with the $O_2$ reactive gas, and then deposited on the GP substrate.

### Surface morphology analysis

The surface morphology of untreated (control) and treated GP surfaces (PT and PT followed by ZnO thin film deposition) was evaluated qualitatively by scanning electron microscopy (SEM) ($n=3$). GP round disks surfaces were sputter-coated with a thin conductive film of Au-Pd alloy and then analyzed using an FEI Quanta 400 FEG/ESEM microscope in a vacuum at 15-kV accelerating voltage. The images obtained were qualitatively evaluated regarding the presence of morphological changes.

### Contact angle analysis

The G*Power v3.1.9.6 program was used to determine an a priori sample size. The procedure used was an analysis of variance (ANOVA) with repeated measures, within factors, using an alpha-type error of 0.05 with a power (1-ß) of 0.90, with an effect size of 0.4. Fifteen specimens per group were established as the ideal size. GP

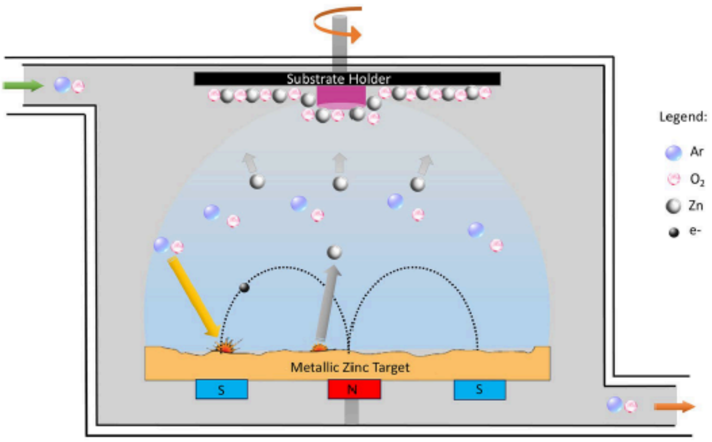


**Fig. 1** Schematic illustration of physical vapor deposition with reactive magnetron sputtering used in the production of ZnO thin films

round disks were divided randomly into three groups: (a) Untreated GP (control); (b) GP treated with argon plasma (PT); (c) Functionalized GP (PT followed by ZnO thin film deposition). The samples were randomly allocated using an online computer-generated number (www.randomizer.org).

The contact angle was measured by the sessile drop technique. One drop per each sample of 0.5 mL of distilled water was dispensed on the surface of the GP round disks using a micro-syringe, and images were captured at room temperature using the optical contact angle equipment (OCA 20; DataPhysics Instruments GmbH, Filderstadt, Germany). After the water was applied on the GP surface for 5 s, the angle of contact was recorded [28]. Contact angle measures ($n=15$) were conducted on the untreated (control), treated with Ar plasma (PT), and functionalized (PT + ZnO thin film) GP surfaces.

**Tensile bond strength**

The G*Power v3.1.9.6 program was used to determine an a priori sample size. The procedure used was ANOVA with fixed effects, main effects, and interactions, using an alpha-type error of 0.05 with a power (1-ß) of 0.80 and six groups, with an effect size of 0.4. Ten specimens (each specimen refers to 2 pellets) per group were established as the ideal size. Samples were divided randomly into two groups, according to the sealer: Endoresin and AH Plus Bioceramic. Each group was subdivided into three sub-groups: (a) Untreated GP (control); (b) GP treated with argon plasma (PT); (c) Functionalized GP (PT followed by ZnO thin film deposition). The samples were randomly allocated using an online computer-generated number (www.randomizer.org).

A 0.01-mL droplet of each sealer tested was precisely dispensed onto the central region of a flat-surfaced GP pellet using an automatic micropipette. Subsequently, an identical pellet was carefully aligned and affixed against the initial one ($n=10$; 1 sample refers to 2 pellets), in a specially developed mold Fig. 2A. Any excess extruded material was meticulously removed with a dental microbrush applicator tip. The prepared samples were then stored at 37 °C, in contact with gauze moistened with a phosphate-buffered saline solution (pH 7.2) for 7 days. Any nonstandard sample was promptly replaced. The bond strength between the GP surface and the sealer was evaluated using a custom-designed apparatus, illustrated in Fig. 2B. After ensuring the samples' stabilization, the moving part of the container was attached to the tensile machine. The measurements were conducted while subjecting the GP pellets to a tensile force, using a universal testing machine from Shimadzu (model AG-IS) equipped with a 50-N load cell at a 0.5-mm/min speed. The tensile bond strength test was performed in a random order by an operator blinded to the specific sealer under test. Bond strength was determined by a real-time computer software program that plotted a load/time curve during the test. The tensile force required to separate the GP pellets was recorded in Newtons (N), and the tensile bond strength in Mega Pascals (MPa), considering the GP pellets sectional area.

**Statistical analysis**

The statistical analysis was performed using the IBM SPSS Statistic 28.0. software (SPSS Inc, Chicago, Illinois, EUA). The significance level was set at 5% ($p<0.05$). The data were verified with the Kolmogorov-Smirnov test for the normality of the distribution and the Levene test for the homogeneity of variances. Water contact angle results were evaluated using ANOVA repeated measures (3 levels: control, PT, and PT + ZnO). Tensile bond strength results were analyzed with a two-way ANOVA followed by Bonferroni post-hoc tests. All the conditions

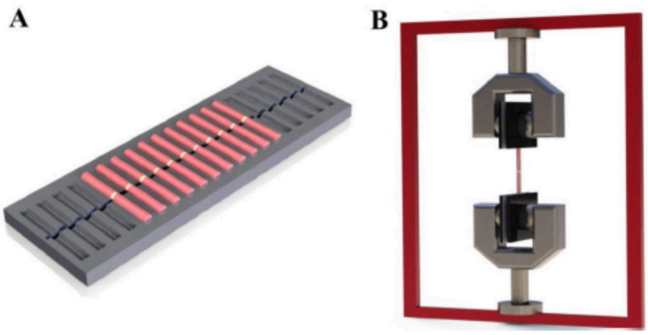


**Fig. 2** Schematic representation of the custom-designed apparatus for bonding the gutta-percha surface and the sealer (**A**), and the tensile bond strength test apparatus (**B**)

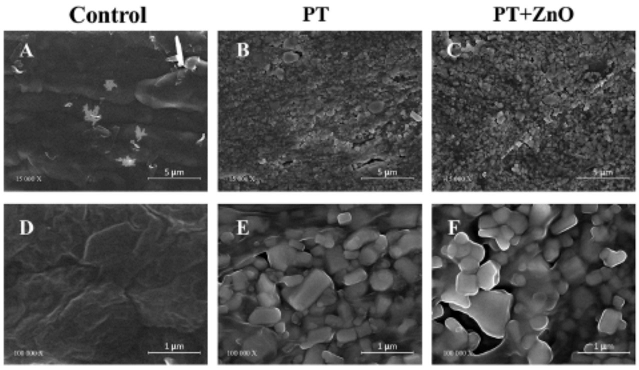


**Fig. 3** Representative scanning electron microscopy images of untreated (control; **A** and **D**), treated with argon plasma (plasma treatment - PT; **B** and **E**), and functionalized gutta-percha surfaces (plasma treatment following ZnO thin film deposition – PT + ZnO; **C** and **F**)

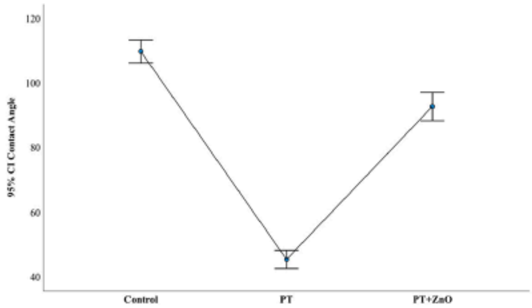


**Fig. 4** Mean distribution water contact angles in the distinct gutta-percha surfaces ($n=15$): untreated (control), treated with argon plasma (PT), and functionalized gutta-percha surfaces (plasma treatment followed by ZnO thin film deposition – PT + ZnO)

for applying the ANOVA procedure were evaluated based on the residuals (normality, zero mean, homogeneity of variance, and independence).

## Results

### Surface morphology analysis

Figure 3 shows the SEM surface morphology analysis of the distinct GP surfaces: untreated (control), treated with Ar plasma (PT), and functionalized (PT + ZnO thin film). Untreated GP (control) showed ZnO crystals encrusted on the GP matrix, covered by an organic layer constituted by wax/resin components (Fig. 3. A and D). PT with Ar caused the removal of the wax/resin surface layer, exposing the ZnO crystals embedded in the GP matrix and uncovering the surface porosity promoted by the ZnO grains boundaries (Fig. 3. B and E). The GP samples submitted to PT + ZnO increased the surface area to be covered with ZnO, benefiting from its additional properties (Fig. 3. C and F).

### Contact angle analysis

Figure 4 illustrates water contact angles evolution of GP surfaces after plasma treatment (PT) and functionalization (PT + ZnO) from their pristine form (control). The water contact angles were significantly reduced after PT ($p<0.001$) and functionalization with PT + ZnO ($p<0.001$), comparing to the control. The difference between the mean water contact angle of PT and PT + ZnO was also statistically significant ($p<0.001$). The mean and standard deviation of the water contact angle values were observed for distinct conditions: control (109.78°±6.51), PT (45.28°±5.19), and PT + ZnO (92.68°±7.90).

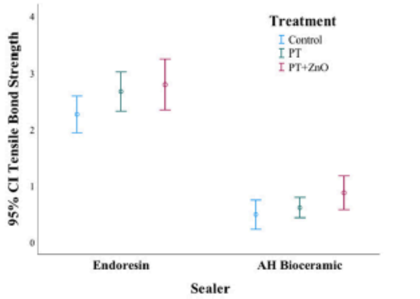


**Fig. 5** Mean tensile bond strength values (MPa, $n=10$) of untreated (control), treated with Ar plasma (PT), and functionalized gutta-percha surfaces (plasma treatment followed by ZnO thin film deposition – PT+ZnO) to endodontic sealers

**Table 1** ANOVA of tensile bond strength (df: degrees of Freedom, Sig: P value)

| Source | Sum of type III squares | f | Mean square | F | Sig. |
|---|---|---|---|---|---|
| Sealer | 55.738 | 1 | 55.738 | 274.691 | <0.001 |
| Treatment | 2.133 | 2 | 1.066 | 5.255 | 0.008 |
| Sealer * Treatment | 0.203 | 2 | 0.102 | 0.500 | 0.609 |
| Error | 10.957 | 54 | 0.203 | | |
| Total | 69.032 | 59 | | | |

Dependent Variable: Tensile Bond strength

a. R Squared=0.841 (Adjusted R Squared=0.827)

### Tensile bond strength

Figure 5 shows the mean tensile bond strength values (MPa) of untreated (control), treated with Ar plasma (PT), and functionalized (PT+ZnO) GP surfaces. Both sealers had some degree of adhesiveness to non-treated GP (control) but with a significant difference between them ($F(1,54)=274.7$; $p<0.001$). The mean bond strength value in Endoresin group was significantly higher than that in AH Plus bioceramic group, for all GP conditions (control, PT and PT+ZnO). There were significant differences concerning treatments ($p=0.008$) (Table 1).

Bonferroni multiple comparisons test (Fig. 6) showed a statistically significant difference between the mean bond strength values of the control and the functionalized (PT+ZnO thin film) GP ($p=0.006$), with the latter presenting the highest bond strength value. There was no significant difference in bond strength values between PT and PT+ZnO ($p=0.553$), nor between PT and control ($p=0.203$).

### Discussion

Achieving a tridimensional sealing of the root canal system while preventing coronal and apical leakage is crucial for the root canal treatment outcome [29]. Despite the great development in endodontic materials, studies still show the presence of interfacial gaps in root canal fillings, independent of sealers' chemical composition, GP type or filling techniques [16, 30, 31]. Minimizing gaps is clinically relevant, preventing bacteria and their by-products to colonize and degrade filling materials. On the other hand, the use of filling materials with improved antibacterial properties is recommended to prevent microbial re-infection [14].

The present investigation evaluated the impact of the GP surfaces modified by plasma treatment and functionalized with ZnO thin film deposition on its adhesiveness to sealers with different chemical compositions: epoxy resin-based (Endoresin) and calcium silicate-based (AH Plus Bioceramic). PT was used to etch, clean, and activate the GP surfaces resulting in the exposure of the ZnO crystals and simultaneously enhancing the adhesion of the ZnO thin film [14, 27]. GP surfaces' morphology, water contact angle and tensile bond strength were

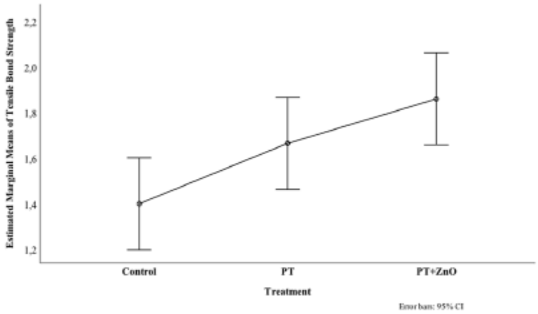


**Fig. 6** Comparation of estimated marginal means for tensile bond strength of untreated (control), treated with Ar plasma (PT), and functionalized gutta-percha surfaces (plasma treatment followed by ZnO thin film deposition – PT+ZnO) (Bonferroni multiple comparisons test)

analyzed by comparing distinct GP samples: non-treated (control), treated with Ar plasma (PT), and functionalized by depositing a ZnO thin film (PT+ZnO). The main findings to be stressed are that: (i) the epoxy resin-based sealer (Endoresin) showed a statistically higher mean bond strength value than AH Plus Bioceramic, in all conditions studied, (ii) functionalized GP (PT+ZnO) presented an increased hydrophilicity, compared to the control (not treated GP), and (iii) functionalized GP (PT+ZnO) seems to improve sealer's adhesion. The present sample data was inconsistent with the null hypothesis, stating that GP surface's functionalization will not have impact on sealer adhesion. So, the null hypothesis was rejected. With the limitations of the present study, the alternative hypothesis should be considered, i.e., the deposition of a ZnO thin film on GP surface might increase GP's adhesion to filling materials such as Endoresin and AH Plus Bioceramic sealers.

To evaluate the wettability, the water contact angle was measured on the distinct GP surfaces (untreated and modified). The water contact angle serves as a measure of how a liquid interacts with a solid surface [32]. A lower contact angle implies that the liquid spreads more on the surface, indicating better wettability [32]. This wettability is directly associated with an increase in the surface free energy, leading to a higher affinity for establishing new bonds, which typically results in improved adhesion [32]. The results revealed that the untreated GP is hydrophobic (mean water contact angle of 109.78°), while the modified GP exhibits significantly improved wettability. Yet, it was observed that the mean water contact angle is lower for the GP treated with PT (45.28°) compared to the GP functionalized with the ZnO thin film (92.68°). The observed difference is primarily attributed to the exposure of ZnO crystals, following the treatment into the Ar plasma atmosphere. The ZnO thin film deposition may have masked the surface characteristics induced by PT, increasing the water contact angle of functionalized GP (PT+ZnO) compared to PT.

PT can induce several significant phenomena, including: (i) eliminating superficial contaminants (cleaning); (ii) altering surface morphology and topography through etching; (iii) activating the surface by generating new reactive species, resulting in the formation of novel chemical groups, crosslinking, and chain scission [25]. GP comprises an organic polymeric matrix (GP polymer, resins, and waxes) with embedded inorganic components (zinc oxide and barium sulfate) [11]. Our findings confirm that the PT, promoted into an Ar atmosphere, with the selected parameters (50 W; 60s) [27] was responsible for the remotion of the organic layer on the GP surface (Fig. 3). The PT effectively promoted the surface etching (cleaning superficial contaminants) and activation, exposing the ZnO crystals and increasing the surface's porosity [15, 27]. These effects result in the creation of new chemical anchoring points, which may favor adhesion. Additionally, the deposition of the ZnO thin film on Ar pre-treated GP, reproduced the main features of the GP surface topography, covering the whole surface. Moreover, the addition of ZnO thin films alters the microstructure of the GP (Fig. 3). The reduced size of the ZnO particles, compared to the PT treatment, would also contribute to an improved adhesion. Smaller particles possess a larger surface area relative to their volume, providing more opportunities for bonding and creating a more interlocked or mechanically entangled structure that enhances adhesion. A previous study [14] stressed the homogeneous layer of ZnO thin film deposited on Ar plasma treated GP surfaces. Furthermore, the significant enhancement on the antimicrobial/antibiofilm ability of ZnO deposition after PT, compared to the film deposited on the native organic layer of the control (not treated GP) has been highlighted. This coating seems to be responsible for reducing the surface porosity in comparison with untreated PT cones. Contrarily, the immersion of GP cones in sodium hypochlorite caused significant surface irregularity which might favor biofilm adhesion [14].

In the last few years, several approaches have been proposed to enhance the adhesive characteristics of GP [11, 15, 16, 27]. The use of coated GP cones incorporated with bioceramic nanoparticles have been suggested, with contradictory findings. Eltair et al. [16] through SEM analysis, concluded that the interface between bioceramic GP cones and CSS was not satisfactory, independently of the obturation technique (lateral compaction or single cone). Bankantan et al. [33] did not find a superior shear bond strength of the CSS. Recently, Quaresma et al. [31] reported that the push-out bond strength between obturated teeth with CSS sealers and bioceramic GP cones showed higher bond strength values, compared to conventional GP and epoxy resin-based sealer. However, different methodologies can influence the bond strength analysis. In the present investigation, Endoresin displayed a stronger bond strength to conventional (non-treated) GP samples than the calcium silicate-based sealer AH Plus Bioceramic, corroborating other studies [31, 34, 35], which indicate varying adhesion levels with sealers of different chemical compositions. Furthermore, it should be noted that bond strength of both sealers was substantially enhanced after functionalization of GP surfaces (PT+ZnO thin film deposition), compared to the control.

There are few studies exploring the sealer adhesion ability to GP [3, 34–36]. Shear, tensile and push-out bond strength tests have been used and contradictory results have been stated due the heterogeneity of the experimental methodologies [3, 34, 36, 37]. As limitations of the current study, it is important to highlight that commercially available GP may vary in its composition depending on the manufacturer. It is conceivable that variations in bond strength values may occur if an alternative brand of GP is used as the substrate or a different assessment methodology is considered. In addition, the tensile bond strength test is a sensitive technique because small changes in the sample or in stress distribution during the load application can affect the results [39]. Further studies with different brands of gutta-percha and sealers are needed to confirm the clinical relevance of the present findings.

## Conclusion

The present findings suggest that the functionalization of GP with a nanostructured ZnO thin film favors the bond strength ability with sealers of different chemical composition. The ZnO thin film deposition preserved GP's surface morphology modified by PT, enhancing the bond strength to both Endoresin and AH Plus Bioceramic sealers, compared to untreated GP. Further investigation is required to obtain enough scientific evidence to suggest it as a valid alternative to the conventional GP core filling material in root canal treatment.

### Author contributions

Inês Ferreira: Conceptualization; Methodology, Investigation; Formal analysis; Writing - Original Draft, Writing - Review & Editing; Claudia Lopes: Methodology, Formal analysis, Investigation, Writing - Original Draft; Armando Ferreira: Methodology, Formal analysis, Investigation, Writing - Original Draft; Ana Cristina Braga: Formal analysis; Software, Writing - Review & Editing; Filipe Vaz: Resources, Writing - Review & Editing; Irene Pina-Vaz: Conceptualization, Supervision, Resources, Writing - Review & Editing; Benjamin Martin-Bedma: Conceptualization, Supervision, Writing - Review & Editing.

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### Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors declare no competing interests.

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Functionalization is a promising approach for property improvement of endodontic substrates. The present thesis highlights the effect of dentin and gutta-percha surfaces' functionalization on the adhesiveness between gutta-percha/sealers/dentin interfaces, in view of a better obturation seal. We concluded that, despite no consensus being found about the effect of plasma treated dentin on the adhesion of resinous sealers it increased the adhesion of calcium silicate-based sealers. Functionalization of gutta-percha surfaces with Ar or $O_2$ plasma treatments increased roughness, surface free energy and wettability of the Endoresin and AH Plus Bioceramic, which might improve its adhesive properties. Plasma treated gutta-percha, coated with a nanostructured ZnO thin film enhanced the bond strength with endodontic sealers.