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Tesis doctoral

ANÁLISIS DE LA BIOACTIVIDAD Y ADAPTABILIDAD DE IONÓMEROS DE VIDRIO UTILIZADOS EN ODONTOLOGÍA RESTAURADORA

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A mis padres;
porque todo lo que soy es gracias a vosotros.

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hacen constar que,

la tesis doctoral titulada “Análisis de la bioactividad y adaptabilidad de ionómeros de vidrio utilizados en odontología restauradora”, presentada por el doctorando D. James Ghilotti Rodríguez ha sido realizada bajo nuestra dirección y reúne las condiciones necesarias para su presentación y defensa.

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Publicaciones presentadas

A continuación, se presentan las referencias bibliográficas de las 3 publicaciones que comprenden el compendio de artículos presentado en el presente proyecto de tesis doctoral:

Ghilotti J, Mayorga P, Sanz JL, Forner L, Llena C. Remineralizing Ability of Resin Modified Glass Ionomers (RMGICs): A Systematic Review. J Funct Biomater. 2023 Aug 11;14(8):421. <https://doi.org/10.3390/jfb14080421>

Ghilotti J, Fernández I, Sanz JL, Melo M, Llena C. Remineralization Potential of Three Restorative Glass Ionomer Cements: An In Vitro Study. J Clin Med. 2023; 12(6):2434. <https://doi.org/10.3390/jcm12062434>

Ghilotti J, Alzina-Cendra A, Sanz JL, Forner L, Llena C. Does Silver Diamine Fluoride Affect the Adaptation of High-Viscosity Resin-Modified Glass Ionomer to Dentin? An In Vitro Study. Appl Sci. 2023; 13(2):991. <https://doi.org/10.3390/app13020991>

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Introduction

The approach of minimal intervention dentistry has brought about changes in the treatment of carious lesions. With the application of selective caries removal techniques comes the need to use restorative materials that are capable of remineralizing the preserved dentin.

Dentin is a mineralized structure that constitutes the major part of the tooth's structure and is made up of both a mineral portion (70%) and an organic portion (18%), together with the presence of water. These characteristics make its remineralization more complex than that of enamel.

Dental caries is a multifactorial pathology that has the potential to cause lesions with loss of dental tissue. In enamel, the predominant bacteria are of an acidogenic nature, while at the dentin level proteolytic bacteria predominate. When caries reaches dentin, different zones can be identified. A zone with bacterial invasion, great destruction of the structures at histological level and soft consistency, another more rigid zone with partial demineralization and collagen fibers, and finally a zone with dentin that maintains its healthy characteristics. Nowadays, it is considered an excessive treatment to remove all the demineralized dentin, eliminating only the soft portion.

The remineralization process involves an increase in the mineral fraction of the tissue. This requires materials that can release mineral ions, especially calcium and phosphate. Remineralization in dentin is more complex due to collagen, which acts as a framework for the mineral structure.

Biomaterials science defines bioactivity as the ability of a material to form hydroxyapatite on its surface. These materials allow mineral bonding with dental tissues through ion exchange.

Glass ionomers are materials based on the acid-base reaction between a weak acid and a basic glass. The polymeric acids used are different and in variable proportions. Some are introduced to modify some characteristics such as working time and chelating action. Initially, in the acid-base reaction, there is a release of calcium ions. At this moment, the material has little resistance and can be molded with tools. Subsequently a silica gel is formed and the aluminum causes cross-linking of the polyacrylic acid. Finally, thanks to water, hydration takes place, which causes maturation.

The distinguishing feature of glass ionomers is the exchange of ions with the mineral tissues of the tooth, specifically fluoride ions. They also have the ability to recharge with this ion when it is present in its surroundings.

To improve the physical and mechanical characteristics of glass ionomers, resin-modified glass ionomers have emerged. In addition to the classical components, they also have monomers and a photoinitiator of the chemical reaction. These new materials therefore have a double setting reaction: acid-base and photopolymerization. The presence of monomers can reduce their biocompatibility, especially during the first 24 hours.

To increase the remineralizing potential of resin-modified glass ionomers, bioactive glasses are being added to give the material a bioinductive and regenerative potential. For their use as restorative materials, high-density resin-modified glass ionomers have emerged. They are more viscous due to the content of bioactive glasses, strontium, or zirconium. In this way, the material has a shorter setting and hardening time and a notable improvement in its physicochemical and mechanical properties.

Another material with excellent dentin remineralizing capabilities is silver diamine fluoride. It is used in a 38% solution and has a triple action against dental caries. Thanks to the high concentration of fluoride, it allows greater diffusion in the tissues, restoring their hardness. It also has an important bactericidal effect, and, thanks to the ammonia, it keeps the pH stable. The disadvantage of this product is due to the black spots caused by silver precipitation, which is directly

proportional to the demineralization of the tissue. To reduce this effect, it is proposed to use potassium iodide immediately after the silver diamine fluoride, as it prevents the precipitation of excess silver.

Glass ionomers are chemically bonded to the tooth by adhesion through ionic bonds formed during the chelating reaction between the carboxyl groups of polyacrylic acid and the calcium in the hydroxyapatite crystals of dental tissues. Although not strictly necessary, it is recommended to condition dentin with polyacrylic acid before using a glass ionomer. Resin-modified glass ionomers have better adhesion due to micromechanical retention of resin infiltration into the dentin tubules. In this case, conditioning can also be performed with orthophosphoric acid or self-etching adhesives. There is no change in bond strength to dentin treated with silver diamine fluoride.

Objectives

The main objective of this doctoral thesis project was to evaluate the ability of glass ionomers to remineralize demineralized dentin, as well as their ability to adapt to silver diamine fluoride- and potassium iodide-treated dentin walls to support restorative use after selective caries removal.

In this context, a total of 3 studies are presented. Their specific objectives are as follows:

Study 1: To perform a qualitative synthesis of the available in vitro evidence on dentin remineralization of resin-modified glass ionomers.

Study 2: To evaluate the depth to which a resin-modified glass ionomer (Riva Light Cure HV) and two traditional glass ionomers (Ketac Molar Aplicap and Equia Forte HT) can remineralize previously demineralized dentin sections.

Study 3: To evaluate the adaptability of a resin-modified glass ionomer (Riva Light Cure) on silver diamine fluoride- and potassium iodide-treated dentin walls. Also,

to evaluate how conditioning with polyacrylic acid (PA) or orthophosphoric acid (OPA) affects in this regard.

Materials and methods

Study 1: An advanced search was performed in 4 databases (Medline, Scopus, Web of Science and Lilacs). In vitro studies analyzing the ability of resin-modified glass ionomers to remineralize dentin were accepted. After data extraction, the variables were divided into methodological variables and outcome variables. The methodological variables included the materials used, the tests performed and their duration. The outcome variables included the significant results found for each test, the time they were recorded (duration) and their level of statistical significance as a p value. Quality assessment was performed using a modified CONSORT checklist for the evaluation of in vitro studies of dental materials. A qualitative synthesis of eligible studies was performed.

Study 2: We evaluated to what depth Riva Light Cure HV (RL), Ketac Molar Aplicap (KM) and Equia Forte HT (EF) can remineralize previously artificially demineralized dentin. 1-, 2- and 3-mm thick sections of third molar dentin including the material extracts were prepared for therapeutic purposes. Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR/ATR) and energy dispersive X-ray spectroscopy (EDX) were used to identify changes in dentin structure. In both cases the results were recorded after 1, 7, 14, and 28 days.

Study 3: The adaptability of Riva Light Cure HV on silver diamine fluoride/potassium iodide- (SDF/PI) treated dentin walls was assessed, including the variable of conditioning by PA or OPA. Cavities were created in molars without caries, fissures or structural defects extracted for therapeutic reasons. Scanning electron microscopy was used to examine the adaptability.

Results

Study 1: In general, all resin-modified glass ionomers showed the ability to remineralize dentin. No statistically significant differences are found when comparing conventional glass ionomers with resin-modified glass ionomers. The addition of bioactive glasses in concentrations between 10% and 30% has shown to increase remineralization. The addition of casein phosphopeptide-amorphous calcium phosphate (CCP-ACP) to the composition also showed the same effect, although this was only performed in one study.

Study 2: FTIR/ATR analysis shows peaks indicating the formation of carbonate hydroxyapatite. All materials have the ability to produce this mineral, showing how the effect takes longer in proportion to the increase in thickness. However, EDX reveals that, compared to the positive control, none of the materials in the study improve the calcium/phosphate ratio at 3 mm depth, which they can achieve at a lower thickness. KM shows the best results at 1 mm, while RL and EF at 2 mm.

Study 3: Without the use of dentin conditioning, regardless of whether SDF/PI is applied, there are always gaps between the RL and dentin walls. There are no statistically significant differences with conditioning prior to the use of SDF/PI. The best results were obtained with OPA conditioning without the use of SDF/PI but showed statistical differences with SDF/PI groups only in the coronal wall.

Conclusions

Overall, the results of this doctoral thesis project give rise to 3 contributions:

- The remineralizing potential of resin-modified glass ionomers for restorative purposes on demineralized dentin has been confirmed, thanks to the qualitative synthesis of the available scientific evidence in this regard.*
- It has been shown that the addition of resin to the glass ionomer composition does not affect the remineralizing potential, but there are some differences between products of different brands.*

- It has been shown that the use of silver diamine fluoride and potassium iodide for dentin remineralization do not cause complications in the subsequent adaptation to the walls of glass ionomers modified with high viscosity resin.

ABREVIATURAS

Materiales

ACP	Fosfato de calcio amorfo
IV	Ionómero de vidrio
IVMR	Ionómero de vidrio modificado con resina
HEMA	2-hidroxietil metacrilato
BAGs	Vidrios Bioactivos
FP	Fluoruro de plata
FDP	Fluoruro diamínico de plata
YP	Yoduro de potasio
AP	Ácido poliacrílico
AO	Ácido ortofosfórico
RL	Riva Light Cure HV
KM	Ketac Molar Aplicap
EF	Equia Forte HT
CCP	Fosfopéptido de caseína
SBF	Solución corporal simulada
CSCs	Cementos a base de silicatos cálcicos

Ensayos

FTIR/ATR	Espectroscopia de reflexión total atenuada-infrarroja transformada de Fourier
EDX	Espectroscopía de rayos X de energía dispersa
SEM	Microscopia electrónica de barrido
TMR	Microradiografía transversa
PLM	Microscopia de luz polarizada
Micro-CT	Microtomografía computadorizada

Otros

ISO	<i>Organization for Standardization</i>
SMART	Tratamiento restaurador atraumático modificado con plata
ART	Tratamiento restaurador atraumático
PRISMA	<i>Preferred Reporting Items for Systematic reviews and Meta-Analyses</i>
C+	Control positive
C-	Control negativo

RESUMEN GLOBAL

A continuación, se presenta un resumen global de la temática, de los principales resultados y conclusiones. El resumen se encuentra estructurado en 7 apartados: 1. Introducción; 2. Justificación; 3. Objetivos; 4. Metodología; 5. Resultados; 6. Discusión; 7. Conclusiones.

1. Introducción

La Odontología Restauradora se encarga del tratamiento de los dientes afectados por lesiones de caries u otras patologías dentales. Sin embargo, la caries sigue siendo la patología que con mayor frecuencia afecta al diente. En la actualidad, el abordaje quirúrgico-reparador sigue el criterio de la eliminación selectiva de la dentina cariada en combinación con el uso de materiales de restauración con potencial remineralizante y/o bioinductivo (1).

El objetivo principal de este nuevo enfoque terapéutico, que se considera de mínima intervención, es conservar hasta un 25-50% más de dentina reduciendo el riesgo de provocar una afectación pulpar iatrogénica (2).

1.1 Estructura de la dentina

El complejo dentino pulpar, conforma estructural y funcionalmente una verdadera unidad biológica. debido a que la pulpa mantiene la vitalidad de la dentina y ésta a su vez le proporciona protección. Además, comparten un origen embrionario común, ya que ambas derivan del ectomesénquima que forma la papila del germen dentario (3).

La dentina es el eje estructural del diente y el tejido mineralizado que conforma el mayor volumen del mismo. En la porción coronal se halla recubierta a manera de casquete por el esmalte, mientras que en la región radicular se encuentra tapizada por el cemento(4,5). Forma un tejido único con la pulpa al que se le denomina complejo dentino-pulpar (6).

En cuanto a su composición, está formada en un 70% por minerales, siendo el contenido orgánico un 18% y el 12% restante agua(7). Entre sus componentes orgánicos, el colágeno tipo I es el más abundante, suponiendo un 85%, aunque también puede encontrarse colágeno tipo III y V. La parte no correspondiente al colágeno de la matriz orgánica, está formada por fosfoproteínas, que suponen aproximadamente un 50% (8,9).

La dentina presenta una conformación tubular en cuyo interior se encuentran las prolongaciones de los odontoblastos. Los túbulos dentinarios siguen un trayecto en “S” desde la pulpa hasta la dentina externa y la densidad es mayor en la dentina profunda que en la dentina superficial, al igual que su diámetro (4,10,11). A nivel estructural se pueden diferenciar: la dentina peritubular, que es hipermineralizada y con poco contenido colágeno, y la dentina intertubular con una red de fibras colágenas sobre cuyos ejes principales se disponen los cristales de hidroxiapatita (12).

Durante la dentinogénesis, pueden identificarse tres lugares distintos de mineralización. En la capa más externa, o dentina del manto, la mineralización es impulsada por vesículas derivadas de la matriz celular que salen por la membrana citoplasmática (10). En la mayoría de la dentina la mineralización es mediada por moléculas de la matriz extracelular (13), mientras que en la dentina peritubular los minerales necesarios derivan del suero sanguíneo (10).

Tras la formación dental se puede seguir formando dentina en la interfase dentino-pulpar mediante dentinogénesis terciaria. Esta formación es una respuesta a estímulos nocivos. Cuando estos son leves o moderados y los odontoblastos no se ven afectados la dentina se denomina reactiva. Si el estímulo es lo suficientemente grave, provocando la muerte de los odontoblastos, serán unas células parecidas a estos que derivan de las células de Höehl las que producirán la dentina que en este caso se denomina reparativa (14,15).

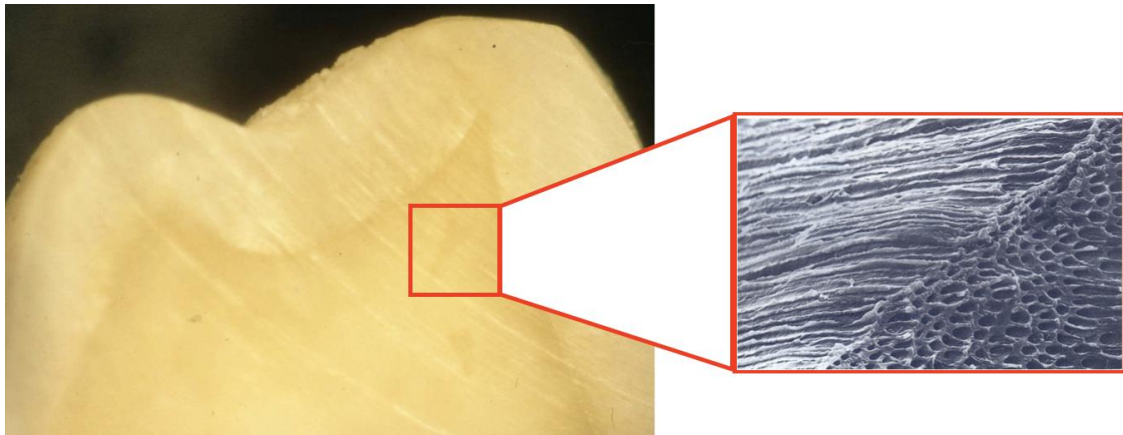


Figura 1.1 Imagen con detalle del complejo dentino-pulpar en el cual se observan los túbulos dentinarios en sección transversal y longitudinal.

1.2 Caries dental

La caries dental es la enfermedad más prevalente de cuantas existen y afecta a la práctica totalidad de la población en algún momento de su vida (16,17). Se trata de una enfermedad dinámica, mediada por biopelículas, modulada por la dieta, multifactorial, no transmisible, que resulta en la pérdida neta de minerales de los tejidos duros dentales (18), siendo la causa más común de destrucción de la dentina (19).

La composición microbiana de las biopelículas cariogénicas no es la misma en el esmalte que en la dentina. En el primero predominan las de naturaleza acidogénica, mientras que las de naturaleza proteolítica predominan en la dentina (20).

Desde un punto de vista patogénico, en condiciones de salud, existe un equilibrio entre el huésped y las comunidades microbianas (biopelícula eubiótica). Sin embargo, bajo ciertas condiciones, las interacciones entre el huésped y estas comunidades microbianas se desequilibran y aparece la enfermedad (biopelícula disbiótica) (21). La matriz de las biopelículas presenta muchos polisacáridos de glucano y fructano, que se consideran importantes factores de virulencia en la patogénesis de la caries dental (22).

La sobreexposición a los carbohidratos de la dieta es un factor que causa este desequilibrio de comunidades microbianas y la transformación de una biopelícula eubiótica en disbiótica. Cuando el pH de la biopelícula cae por debajo del umbral crítico (aproximadamente 5,5), se produce la desmineralización del esmalte. En condiciones saludables, los procesos de desmineralización y la remineralización en el esmalte se alternan en un equilibrio dinámico. Cuando este equilibrio se rompe, se produce un proceso neto de pérdida mineral en el área subsuperficial del esmalte, dando lugar a un esmalte debilitado y poroso que corresponde a la lesión inicial de caries. Si esta situación se puede revertir, aumentando los períodos de remineralización, se conseguirá una ganancia de mineral. En este caso, la lesión de caries se detendrá o incluso se remineralizará, de lo contrario progresará hasta cavitarse y afectará a la dentina (23).

La saliva es un factor protector contra la desmineralización, por su efecto de arrastre, y por su composición mineral y orgánica junto a su capacidad de amortiguar los cambios de pH y favorece la remineralización (24).

En la caries dentinaria, se pueden identificar 3 zonas distintas, lo que permite establecer una correlación entre la remoción en la práctica clínica y la histología. La más externa y próxima al lugar en el cual se inició la lesión se caracteriza por la destrucción de la estructura histológica y la invasión bacteriana, los túbulos dentinarios están desorganizados y su interior está ocupado por bacterias. Debido a la desmineralización que acompaña al proceso de caries, la dentina peritubular desaparece y el diámetro tubular aumenta. Las bacterias van invadiendo la dentina intertubular, en la que se aprecia una desmineralización severa, las fibras de colágeno quedan expuestas total o parcialmente y están desnaturalizadas (25). Esta dentina también se conoce con el nombre de dentina blanda o infectada. Una zona intermedia también conocida como afectada o coriacea. Se caracteriza a nivel histológico por tener dentina peritubular evidente y dentina intertubular parcialmente desmineralizada con cristales de hidroxiapatita más cortos debido a que la desmineralización afecta primero a sus extremos. Destaca una característica importante y es que los túbulos dentinarios están llenos de cristales de whitloquita, un tipo de fosfato cálcico ($\text{Ca}_9\text{Mg}(\text{PO}_3\text{OH})(\text{PO}_4)_6$), los cuales son de gran tamaño y más resistentes al

ataque ácido. En cuanto a los depósitos intratubulares, no se conoce con certeza si son un mecanismo de defensa activo o el resultado de un fenómeno cíclico de disolución-precipitación de los cristales, lo que está claro es que su presencia disminuye la permeabilidad dentinaria y, por tanto, el paso de ácidos, bacterias y productos bacterianos, sirviendo de protección para el tejido pulpar. Las fibras colágenas no están desnaturalizadas y presenta sus bandas características (26). Una zona más profunda de dentina sana y dura cuyas características histológicas se corresponden con una estructura tubular normal, una alta carga de componente mineral y fibras de colágeno.

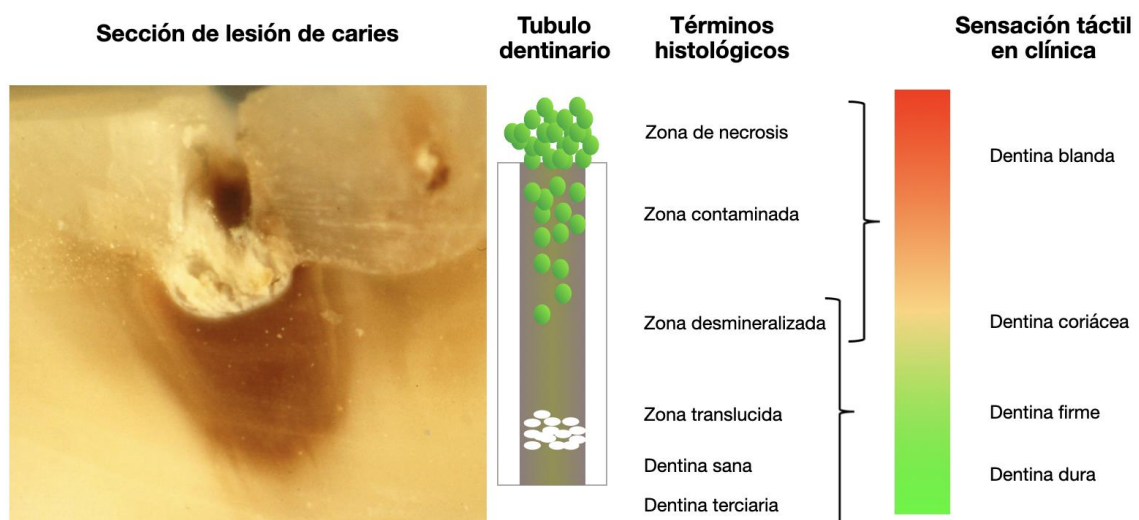


Figura 1.2 Esquema características de una lesión de caries en dentina.

1.3 Tratamiento de la lesión de caries

Tradicionalmente el tratamiento de la lesión de caries de dentina se planteaba como la eliminación de todo el tejido infectado y afectado hasta alcanzar dentina sana(25,27). En la actualidad este enfoque se considera un sobretratamiento.

Hoy en día, se realiza la denominada remoción selectiva de la caries. Esta técnica se basa en la eliminación de la dentina blanda y completamente desestructurada conservando la porción de dentina coriácea y firme (1,28). Esta dentina se corresponde histológicamente a la dentina parcialmente mineralizada, que se ha denominado clásicamente “dentina afectada” (29).

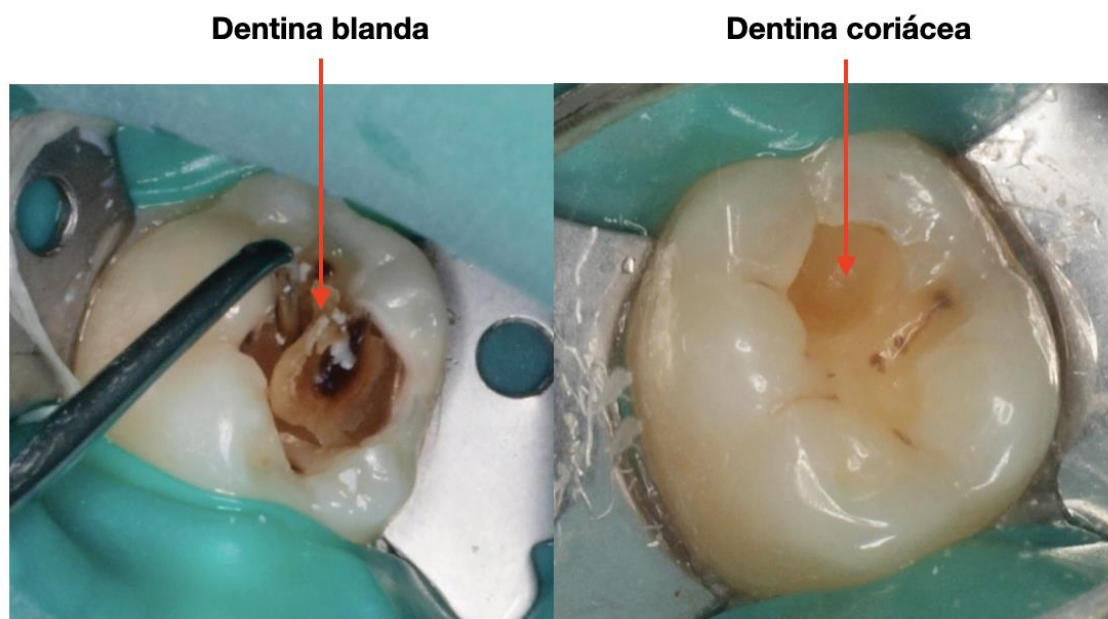


Figura 1.3 Imágenes clínicas de los distintos tipos de dentina en una lesión de caries.

Una remoción no selectiva de la caries implica una sobrepreparación innecesaria de la estructura dental con el daño resultante sobre el complejo dentino-pulpar. La eliminación innecesaria y excesiva de la estructura dental sana tiende a comprometer la integridad mecánica del diente(30) y la vitalidad pulpar(31). La eliminación selectiva de la caries, consigue eliminar el tejido infectado, reducir la carga infectiva y preserva la vitalidad de los odontoblastos y de las células precursoras de la pulpa, favoreciendo la formación de dentina terciaria y reparativa (32).

La aplicación de la remoción selectiva de la caries determina la necesidad de volver a remineralizar la dentina remanente para devolverle la correcta

funcionalidad (33). Debido a esto se han estudiado y desarrollado distintos materiales con capacidad remineralizante (34).

El conjunto de técnicas encaminadas a efectuar la remoción selectiva de caries y preservar la vitalidad pulpar, se incluyen dentro de lo que hoy se conoce como “terapia pulpar vital”, que incluye lo que clásicamente se conocía como recubrimiento pulpar indirecto, recubrimiento pulpar directo y pulpotomía parcial(35).

1.4 Remineralización de la dentina

La remineralización se define como la ganancia neta de material calcificado en la estructura del diente, que reemplaza el que anteriormente se perdió por la desmineralización. La remineralización convencional a menudo implica el uso de soluciones que contienen iones de calcio y fosfato en presencia de diversas concentraciones de fluoruro (36).

La remineralización convencional no se produce por nucleación espontánea del mineral en la matriz orgánica, sino más bien por el crecimiento de los cristales de hidroxiapatita en la dentina parcialmente desmineralizada (37), la estrategia de remineralización convencional, por tanto, depende del crecimiento de cristalitos de apatita existentes. Por lo tanto, el contenido mineral de la capa superficial de la lesión influye en las características de la posterior remineralización, incluyendo la ubicación y la densidad de deposición mineral (38).

El proceso de remineralización del esmalte se basa en una ganancia de componentes minerales (39), mientras que la remineralización de la dentina implica un proceso más complejo de interacción entre ganancia de minerales y su interacción con la matriz de colágeno (40). Ello implica la reconstitución de dos fases diferenciadas: por un lado, el colágeno, orgánico, tipo I y, por el otro, la apatita, inorgánica. Por tanto, la remineralización por sí sola es insuficiente para la recuperación total de la dentina desmineralizada, ya que también es

necesario restaurar la estructura de la matriz de colágeno y que ambas fases se unan de manera específica (41). La recuperación del colágeno desnaturalizado desempeña un papel fundamental en el restablecimiento de las propiedades micromecánicas de la dentina cariada a través de la remineralización intrafibrilar (42,43).

La biomineralización utiliza análogos biomiméticos de las proteínas de la matriz dentinaria para inducir nanoprecursores de fosfato de calcio amorfo (ACP) en los compartimentos internos de fibras de colágeno. Este proceso de remineralización biomimética representa un enfoque basado en la creación de nanocristales que sean lo suficientemente pequeños como para caber en las zonas de brecha entre moléculas de colágeno adyacentes y establecer un orden jerárquico en el colágeno mineralizado (44).

1.5 Materiales con poder remineralizante

En la ciencia de los biomateriales, la bioactividad refleja la capacidad de un material de formar hidroxiapatita en su superficie. En este caso, la bioactividad se traduce en la inducción de la formación de una unión mineral entre el material bioactivo y el sustrato dentinario, mediante un intercambio iónico con los fluidos tisulares del microambiente circundante (45). El tratamiento de las lesiones de caries profundas implica el uso de agentes liberadores de iones para inducir la remineralización de la dentina desmineralizada a través de la formación de apatita (46). Así es como se consigue la biomineralización comentada anteriormente.

Se han propuesto materiales con alta capacidad de remineralización de la dentina, basados en la presencia en su composición de cristales bioactivos, estroncio y flúor debido a sus biocompatibilidad y capacidad de formar hidroxiapatita. A continuación, se describirán las características de algunos de estos materiales.

1.5.1 Ionómeros de vidrio

Los ionómeros de vidrio (IV) pertenecen a un grupo de materiales basados en una reacción de fraguado ácido-base, en el que un ácido débil reacciona con un polvo de vidrio de carácter básico. Esta reacción ocurre en un medio rico en agua (47,48). El nombre que se les ha dado por parte de la *International Organization for Standardization* (ISO), es el de cementos de polialquenoato de vidrio.

Sus tres componentes fundamentales son: un ácido polimérico soluble, un vidrio básico y agua (49).

Los ácidos poliméricos utilizados son: ácido polioalquenoico, homopolímeros (ácido acrílico) y el 2:1 copolímero del ácido poliacrílico. Generalmente en las formulaciones de los IV se hallan presentes todos en diferentes proporciones (50). El ácido maleico y el ácido itacónico pueden estar presentes en concentraciones más bajas para mejorar el manejo y aumentar el tiempo de trabajo (51). El ácido tartárico o el ácido cítrico se pueden incorporar a la solución por su actividad quelante. Su función es prevenir la precipitación de sales de aluminio,(52) manteniéndolo en forma libre, mediante este mecanismo, puede prevenir la formación prematura de enlaces cruzados iónicos que involucran al aluminio (51) .

Respecto al vidrio, es esencial que tenga un carácter básico, su componente esencial es un silicato de flúor y aluminio, también puede contener fosfato, sodio, calcio, o estroncio (47). Es la liberación de los componentes del vidrio en forma de iones lo que le va a otorgar a estos materiales la capacidad de remineralizar el tejido dental (53). Del vidrio que permanece en la estructura tras la reacción de fraguado dependerá la resistencia del IV (49).

Finalmente, el agua, cuya función esencial es servir de solvente de los ácidos poliméricos, permitiéndoles actuar como liberador de protones, siendo éste el carácter esencial de la reacción de fraguado de los IV (54).

El fraguado del IV se realiza mediante una reacción ácido-base que involucra las siguientes etapas:

- Disolución y descomposición: después de mezclar el polvo y el líquido, el ácido disuelve la superficie de las partículas de vidrio para liberar iones SiO_4^{4-} , Ca^{2+} , Na^+ , Sr y F^- .
- Fraguado inicial: el Ca^{2+} liberado en el medio acuoso reacciona con el ácido poliacrílico para formar una estructura tridimensional reticulada. En este momento, el material tiene poca resistencia y se puede tallar con instrumentos afilados.
- Fraguado final: la reacción de fraguado continúa durante las siguientes 24 horas. Durante este tiempo, el SiO_4^{4-} forma el gel de sílice. Los iones Al^{3+} de movimiento lento entran en el medio acuoso y entrecruzan las cadenas de ácido poliacrílico desplazando a los iones Ca^{2+} . Esto aumenta la resistencia final del cemento fraguado.
- Maduración: las cadenas de poliacrilato reticuladas de aluminio y calcio se hidratan con el tiempo absorbiendo agua del medio acuoso. Este proceso se conoce como maduración (55).

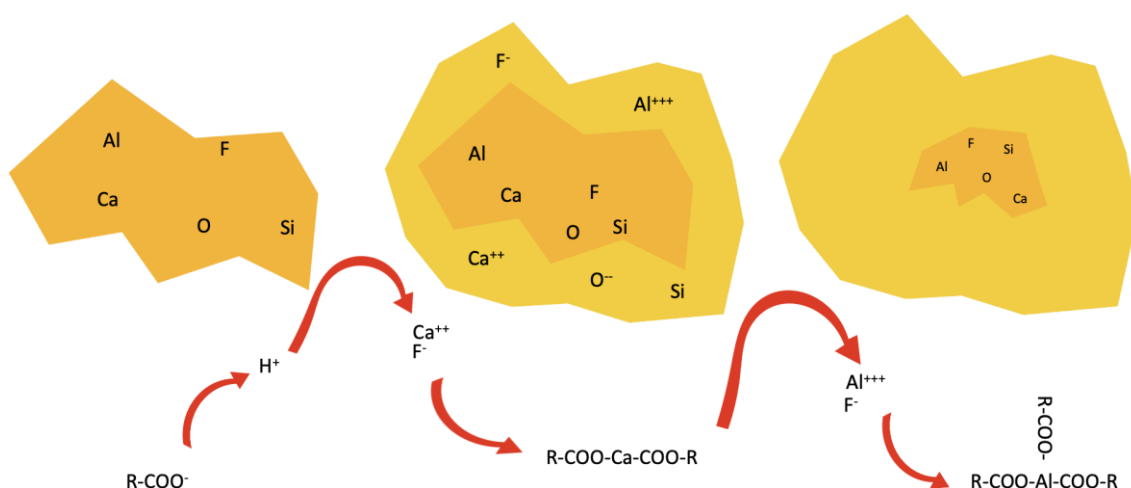


Figura 1.3 Ilustración esquemática del proceso de fraguado del IV. Acción del ácido débil sobre el vidrio. Fraguado inicial con formación del gel de sílice alrededor del vidrio y reticulación gracias al Ca^{2+} . Fraguado final con entrecruzamiento a cargo del aluminio.

Dos o tres minutos después de la mezcla de los componentes, la reacción de fraguado ácido-base inicial ya no permite manejar el material. La bioactividad de IV se produce mediante un intercambio iónico entre el material y el sustrato (53). El ion más importante es el flúor que se libera durante largos periodos de tiempo. Además, los IV pueden recargarse de flúor cuando está presente en el medio y aumenta el pH del entorno (56). Sin embargo, la posibilidad de recargarse va disminuyendo a medida que transcurre el tiempo, habiéndose demostrado que esta capacidad desaparece tras un mes aproximadamente de su colocación (57).

Con el objetivo de mejorar las características físicas y mecánicas surgieron los ionómeros de vidrio modificados con resina (IVMR) (58). Estos materiales se ven menos afectados por la humedad, pero siguen manteniendo la capacidad de liberar flúor (59). Además de los componentes esenciales de los IV clásicos, incorporan monómeros de resina, el más habitual es 2-hidroxietil metacrilato (HEMA), junto a un iniciador de la polimerización que suele ser una canforoquinona (60). Estos nuevos materiales presentan doble fraguado, por un lado el fraguado químico típico de los IV por reacción ácido-base y por el otro la fotopolimerización a cargo de la resina (60,61).

La presencia de monómeros de resina puede afectar a las propiedades biológicas de los IVMR, especialmente en cuanto a su biocompatibilidad (ausencia de respuesta negativa por parte de un tejido vivo) (62,63). Esta propiedad disminuye debido al efecto citotóxico del componente de resina, especialmente durante las primeras 24 horas (64).

Para mejorar el potencial remineralizante de los IVMR, se están incorporando vidrios bioactivos (BAGs) a su composición, que también confieren un potencial bioinductivo y regenerativo al material. La bioinductividad se refiere a la capacidad del material para interactuar positivamente con los tejidos vivos favoreciendo la migración y la diferenciación celular (65).

Para su uso como material de restauración se desarrollaron los IVMR de alta densidad. Son materiales muy viscosos por la incorporación de BAGs, estroncio o incluso zirconio. Esto resulta en una reducción de los tiempos de trabajo y

endurecimiento, y una notable mejora de sus propiedades físico-químicas y mecánicas, junto con una mínima solubilidad. Pueden ser autopolimerizables o fotopolimerizables (66).

1.5.2 Fluoruro de plata

El fluoruro de plata (FP), se presenta en diferentes formulaciones y concentraciones. El más utilizado es el fluoruro diamínico de plata (FDP) se presenta en solución al 38%, siendo un líquido alcalino incoloro (pH 10), compuesto por 24-27% de plata (Ag), 7,5-11% de amoníaco (NH_3) y aproximadamente 5-6% de fluoruro (F) (67). EL FDP una alternativa económica y fácil de usar para la remineralización de la dentina desmineralizada (68). Actualmente existen formulaciones que, en lugar de contener amoníaco como solvente, utilizan agua, cuyo efecto remineralizante parece ser similar al del FDP (69).

El FP tiene una triple acción frente a las lesiones de caries. En primer lugar, debido a las altas concentraciones de fluoruro, se logra una mayor difusión en el esmalte y en la dentina, favoreciendo su remineralización. En segundo lugar, la plata posee un efecto bactericida y permanece en las zonas desmineralizadas e hipomineralizadas, endureciéndolas y previniendo su reinvasión mediante una serie de metabolitos. En tercer lugar, el amoníaco estabiliza la solución y mantiene el pH (70,71). Además, en la dentina, el FP ayuda a mantener la integridad de la matriz de colágeno debido a su capacidad para inhibir las metaloproteinasas bacterianas que juegan un papel importante en la degradación enzimática del colágeno (72).

Sin embargo, los dientes tratados adquieren un color negro como resultado de la precipitación de fosfato de plata, provocando un problema estético (73–75). Cuanto mayor es el grado de desmineralización, más se absorben los iones de plata, aumentando así la discoloración y la distinción entre los tejidos afectados y sanos (76). La mancha permanece con el tiempo y solo se puede eliminar mediante métodos físicos (77). Para disminuir este efecto y aumentar la

aceptación del paciente, se ha sugerido la aplicación de una solución saturada de yoduro de potasio (YP) inmediatamente después del FDP (78,79). Se postula que el YP previene la tinción mediante la precipitación del exceso de iones de plata en forma de yoduro de plata blanco (80), aunque estudios recientes indican que sigue apareciendo un cambio de color apreciable clínicamente (81).

1.6 Adhesión a la dentina de los ionómeros de vidrio

Los IV se unen químicamente con la estructura del diente. La adhesión se basa en la unión iónica de los múltiples grupos carboxílicos del ácido polialquenoico con el calcio abundantemente disponible en el tejido dental duro(82). El mecanismo de adhesión del IV a la estructura inorgánica del diente implica una reacción de quelación entre los grupos carboxilo del ácido poliacrílico y el calcio de los cristales de hidroxiapatita del diente (55). Pese a que los IV no requieren procedimientos adicionales para su adhesión se recomienda el tratamiento previo con un acondicionador de ácido poliacrílico para incrementar la fuerza de adhesión a la dentina (83,84).

La adhesión de los IVMR es mayor que la de los IV, gracias a la fuerza de unión de las resinas (85). Esto se debe a que se produce a través de 2 mecanismos diferentes, el ya comentado para los IV y una retención micromecánica, obtenida por la infiltración de los componentes orgánicos en la superficie dentinaria parcialmente desmineralizada por la característica de autograbado de los IVMR (86). Para algunos de estos materiales, al igual que para los IV se recomienda el ácido poliacrílico como acondicionador, pero además, se ha informado que es posible aumentar la adhesión con el uso de grabado ácido (87) y adhesivos de autograbado (88,89).

Los IVMR en ocasiones deben adherirse a dentina previamente tratada con FDP/YP, esto hizo plantear el posible problema que podría surgir respecto a la adaptación con las paredes y su unión. Una revisión sistemática reciente concluye, no sin destacar la disparidad metodológica entre los estudios incluidos, que la aplicación de FDP/YP no tiene ningún impacto adverso en la fuerza de

unión (72,90–92). Curiosamente, algunos estudios incluso indican que aumentó la fuerza de unión de los IV en dentina tratada con FDP/YP (93) .

2. Justificación

El actual enfoque de mínima intervención en Odontología ha supuesto un cambio de paradigma respecto al tratamiento de la caries en Odontología Restauradora. La remoción selectiva de la dentina cariada ha creado la necesidad de disponer y utilizar materiales con alto potencial remineralizante.

Los IV tradicionales se utilizan desde hace mucho tiempo. Los IVMR son ampliamente utilizados en la actualidad debido a su fácil manejo y propiedades mecánicas. Sin embargo, carecen en algunos aspectos de evidencia suficiente.

Ya existen revisiones sistemáticas que señalan el potencial protector de estos materiales en la reducción de la desmineralización del tejido dental proximal (94–96), pero no del potencial remineralizante sobre la dentina comprobado con estudios *in vitro*.

Los ensayos *in vitro* implican la vía preliminar para el análisis de los materiales dentales de manera previa a su avance con modelos animales o estudios clínicos. En relación a estudios sobre el poder remineralizante de los materiales dentales, los ensayos de composición elemental y estructural de la dentina en contacto con estos materiales puede determinar el efecto de los mismos.

El uso del FP se está utilizando cada día más en el ámbito europeo, encontrando en la Odontología Pediátrica su máximo beneficio, en lo que ha venido en llamarse tratamiento restaurador atraumático modificado con plata (SMART), basado en una modificación de los procedimientos del tratamiento restaurador atraumático (ART), en el que se realiza una fase previa de estabilización de la lesión mediante la aplicación de soluciones de FP, seguidas de restauración con diferentes materiales, fundamentalmente ionómeros. Es por ello que, en la práctica clínica nos encontremos con dentina tratada con FP a la cual sea necesario adherir IV (97).

Teniendo en cuenta los argumentos previamente descritos, se presenta en este proyecto de tesis doctoral, en primer lugar, una revisión sistemática sobre el poder para remineralizar la dentina por parte de los IVMR, valorando también el efecto de distintos aditivos incorporados en la actualidad, como visión actualizada sobre la evidencia disponible en este campo.

Seguidamente, se describe un estudio en el que se analizan las propiedades remineralizantes de tres IV de restauración diferentes. Dos de ellos son IV tradicionales mientras que uno es un IVMR. El análisis se centra en comprobar hasta que profundidad son capaces de remineralizar dentina con el objetivo de suplir la falta de evidencia científica sobre este aspecto.

En último lugar, se presenta un estudio en el que se comprueba la capacidad de adaptación de un IVMR sobre las paredes dentinarias que han sido previamente tratadas con FDP y YP, y con diferentes acondicionadores, con el fin de valorar las repercusiones del uso de este producto remineralizante sobre la adaptación del material restaurador. En este caso también se pretende cubrir una carencia de evidencia científica al respecto.

3. Objetivos

3.1 Objetivo general

Evaluar la capacidad de los IV de remineralizar dentina desmineralizada, así como su capacidad de adaptación a las paredes dentinarias tratadas con FDP y YP con el fin de avalar su empleo restaurador tras la remoción selectiva de la caries.

3.2 Objetivos específicos

3.2.1 Realizar una síntesis cualitativa de la evidencia *in vitro* disponible sobre la remineralización de los IVMR sobre la dentina.

3.2.2 Analizar la profundidad hasta la cual un IVMR (Riva Light Cure HV) y dos IV tradicionales (Ketac Molar Aplicap y Equia Forte HT) tienen capacidad de remineralizar cortes de dentina previamente desmineralizados.

3.2.3 Identificar la capacidad de adaptación de un IVMR (Riva Light Cure) sobre las paredes de dentina tratada con FDP y YP, con acondicionamiento con ácido poliacrílico (AP) o ácido ortofosfórico (AO) o sin ningún acondicionamiento.

4. Metodología

A continuación, se presenta un resumen de la metodología empleada en los estudios del presente proyecto de tesis doctoral, dividido por fases correspondientes a cada estudio y a cada objetivo.

Para la Fase 1, correspondiente a la revisión sistemática, se presenta el resumen de las principales secciones metodológicas del protocolo PRISMA (*Preferred Reporting Items for Systematic reviews and Meta-Analyses*) (98,99) para la presentación de datos de revisiones sistemáticas y metaanálisis. Entre ellos: los criterios de inclusión reflejados en la pregunta PICOS (100), las bases de datos y estrategia de búsqueda, la selección de estudios, la extracción de datos y el análisis de calidad. Para una descripción completa de la metodología de la revisión véase el apartado “*Materials and Methods*” del anexo 1.

Para las Fases 2 y 3 correspondientes a los estudios experimentales, se presenta un resumen de la metodología empleada: materiales utilizados y ensayos realizados. Para una descripción completa de la metodología de los estudios experimentales véase el apartado “*Materials and Methods*” de los anexos 2 y 3.

Los dos estudios experimentales comparten las siguientes características:

- Los materiales de estudio fueron preparados siguiendo las instrucciones de sus respectivos fabricantes.
- Los dientes utilizados eran todos molares retenidos que se extrajeron por motivos médicos y que fueron donados por los/as pacientes de forma voluntaria tras un consentimiento informado. El protocolo fue aprobado por el Comité Ético de la *Universitat de València* (ID:2030332).

4.1 Fase 1 (objetivo 3.2.1): revisión sistemática sobre el poder para remineralizar la dentina por parte de los IVMR.

4.1.1 Protocolo

La presentación de datos de la presente revisión se realizó de acuerdo al protocolo PRISMA 2020 (98).

4.1.2 Pregunta PICOS

Tabla 4.1.1. Distribución de la pregunta PICOS

Población / <i>population</i> (P)	Ionómeros de vidrio modificados con resina
Intervención / <i>intervention</i> (I)	Aplicación sobre dentina desmineralizada
Comparación / <i>control</i> (C)	Otros materiales
Resultado / <i>outcome</i> (O)	Remineralización de dentina
Diseño del estudio / <i>study type</i> (S)	<i>In vitro</i>

4.1.3 Bases de datos consultadas

Se realizó una búsqueda avanzada en 4 bases de datos: MEDLINE, Scopus, Web of Science y Lilacs.

Tabla 4.1.2 Estrategia de búsqueda avanzada

1: (resin-modified glass ionomer cement*)
2: (bioactivity) OR (remineralization)
3: (dentin)
1 AND # 2 AND #n° 3

4.1.4 Selección de estudios

Se utilizó el *software* de gestión de referencias bibliográficas *Mendeley* v2.77.0 (Elsevier, AMS, Países Bajos) para realizar el proceso de selección de estudios.

4.1.5 Extracción de datos

- Características del estudio: autores y año.
- Variables metodológicas: materiales estudiados, tipo de ensayos realizados, duración de los ensayos
- Variables de resultados: tiempo en el que se obtuvieron (duración), resultados significativos obtenidos en cada prueba y su nivel de significación estadística como valor de p.

4.1.6 Análisis de calidad

El análisis del riesgo de calidad de cada uno de los estudios incluidos se realizó utilizando la guía “*Modified CONSORT checklist of items for reporting in vitro studies of dental materials*” (101).

4.2 Fase 2 (Objetivo 3.2.2): estudio *in vitro* sobre el poder remineralizante de 3 ionómeros de Vidrio: Riva Light Cure HV (RL), Ketac Molar Aplicap (KM) y Equia Forte HT (EF), en diferentes grosores de dentina.

Materiales de estudio



Riva Light Cure HV
(SDI®, Victoria, Australia)



Ketac Molar Aplicap
(3M ESPE®, Seefeld, Alemania)



Equia Forte HT
(GC Corp®, Tokio, Japón)

Substrato:

Cortes de dentina de terceros molares incluidos

Figura 4.2.1 Características de la muestra

4.2.1 Ensayos del estudio

- Espectroscopía infrarroja con transformada de Fourier con reflectancia total atenuada (FTIR/ATR)
- Espectroscopía de rayos X de energía dispersa (EDX)

4.3 Fase 3 (Objetivo 3.2.3): estudio *in vitro* sobre la capacidad de adaptación de un ionómero de vidrio modificado con resina: RL con respecto a las paredes de dentina tratada con FDP/YP y acondicionada en formas distintas.

Materiales de estudio



Riva Light Cure HV
(SDI®, Victoria, Australia)



Riva Conditioner
(SDI®, Victoria, Australia)



Gel Etchant
(Kerr®, Bioggio, Suiza)



Riva Star
(SDI®, Victoria, Australia)

Substrato:

Molares extraídos sin caries, fisuras o defectos estructurales

Figura 4.3.1 Características de la muestra

4.3.1 Ensayo utilizado

- Microscopia Electrónica de Barrido (SEM)

5. Resultados

A continuación, se presenta un resumen de los resultados de los distintos estudios que conforman el presente proyecto de tesis doctoral, dividido por fases pertenecientes a cada estudio y objetivo planteado.

Para una descripción detallada de los resultados de la revisión sistemática véase el apartado “*Results*” del anexo 1.

Para una descripción detallada de los resultados de los estudios experimentales véase el apartado “*Results*” de los anexos 2 y 3.

5.1 Estudio 1 (Fase 1, Objetivo 3.2.1)

5.1.1 Datos generales

Tabla 5.1.1 Datos bibliométricos de la publicación correspondiente

Revista	<i>Journal of Functional Biomaterials</i>
Fecha de publicación	11/08/2023
Factor de Impacto (JCR 2022)	4.842
Área	<i>Engineering, Biomedical - SCIE</i>
Posición	32/96 (Q2)

JCR: Journal Citation Reports; SCIE: Science Citation Index Expanded

Título: “*Remineralizing Ability of Resin Modified Glass Ionomers (RMGICs): A Systematic Review.*”

5.1.2 Metodología de los estudios incluidos

Tras realizar la búsqueda avanzada en 4 bases de datos y realizar el proceso de selección de los estudios se incluyeron un total de 8 estudios. En total se estudiaron 6 distintos IVMR, asociándose en 4 ocasiones con un vidrio bioactivo.

El análisis directo del potencial remineralizante de los materiales utilizados en los estudios se midió con diferentes ensayos. Para la observación directa de la interfase dentina-material se utilizó mayoritariamente el SEM (3 estudios), pero también se utilizaron la microradiografía transversa (TMR), la microscopia de luz polarizada (PLM) y la microtomografía computadorizada (Micro-CT). El cambio a nivel estructural de la dentina después de la aplicación de los materiales se comprobó con EDX y FTIR/ATR.

El estudio indirecto de la bioactividad se realizó mediante ensayos de liberación de iones por parte de los materiales utilizados. Los iones estudiados fueron los que conforman el tejido dentinario calcificado y que están involucrados en el proceso de remineralización: Ca^{2+} , PO_4^{3-} , F^- y SiO_4^{4-} .

5.1.3 Resultados de los estudios incluidos

Todos los IVMR mostraron capacidad para remineralizar la dentina.

Los estudios en los cuales se comparaban IV con IVMR no mostraron diferencias estadísticamente significativas entre ellos. La incorporación de porcentajes entre el 10% y el 30% de diferentes BAGs (45S5, NbG, S53P4) ha demostrado incrementar la remineralización. Agregar a la composición fosfopéptido de caseína (CCP) con ACP (CCP-ACP) también mostró el mismo efecto, aunque solo se realizó en un estudio. Los ensayos EDX y FTIR/ATR no mostraron resultados concluyentes.

Véase “*Table 5*” (anexo 1) para los resultados exactos de cada estudio. En “*Table 3*” y “*Table 4*” para la composición y fabricantes de todos los materiales.

5.2 Estudio 2 (Fase 2, Objetivo 3.2.2)

5.2.1 Datos generales

Tabla 5.2.1 Datos bibliométricos de la publicación correspondiente

Revista	<i>Journal of Clinical Medicine</i>
Fecha de publicación	22/03/2023
Factor de Impacto (JCR 2022)	3.904
Área	<i>Medicine, General & Internal - SCIE</i>
Posición	58/167 (Q2)

Título: “*Remineralization Potential of Three Restorative Glass Ionomer Cements: An In Vitro Study.*”

5.2.2 Comprobación de los cambios estructurales con FTIR/ATR

El análisis de las muestras de dentina mediante FTIR/ATR muestra como en KM muestra picos indicativos de grupos CO_3 y PO_4 los cuales sugieren formación de hidroxiapatita carbonatada a 1 mm de grosor ya a las 24 horas, esta diferencia es apreciable también a los 14 días. Igualmente, el EF muestra formación de hidroxiapatita carbonatada, pero en este caso con un comportamiento lineal tanto en el tiempo como en los diferentes grosores, con la única excepción a los 28 días en la muestra de 2 mm donde el pico correspondiente a PO_4 es más marcado. Finalmente, el RL a las 24 horas carece de grupos fosfato (PO_4). Este va apareciendo e incrementándose con el tiempo hasta los 28 días. Los picos que señalan la presencia de COO^- y CO_3 están presentes desde el principio.

Véase el apartado “*Materials and Methods*” del Anexo 2 para la descripción de los picos de interés en el estudio.

Véase el apartado “*Results*” del Anexo 2 para examinar las gráficas de todas las muestras del estudio.

5.2.3 Análisis de la composición elemental con EDX

La relación entre el Calcio y el Fósforo (Ca/P) se utilizó como indicador de la mineralización de las muestras.

Comparando los resultados con el Control Positivo (C+) de dentina no desmineralizada RL y EF consiguieron una mayor remineralización en las muestras de 2 mm a los 7 días, sin diferencias estadísticas entre ellos. Además, en muestras de 3 mm de grosor no mostraron diferencias significativas en ninguno de los tiempos del estudio. Contrariamente, el KM mostró la mayor remineralización a 1 mm a los 7 días, siendo a este grosor siempre su mayor efecto. A 3 mm tampoco mostró cambios con el C+.

En la comparación con el Control Negativo (C-) de dentina desmineralizada RL y EF volvieron a mostrar el mayor efecto a 2 mm de espesor a los 7 días, sin diferencias significativas ($p > 0,05$). Sin embargo, en KM se encontró la mayor remineralización a 1 mm 14 días después de la aplicación, con diferencias significativas respecto a los demás dos materiales ($p < 0,05$).

Véase el apartado “*Results*” del Anexo 2 para examinar las tablas de datos de las muestras del estudio.

5.3 Estudio 3 (Fase 3, Objetivo 3.2.3)

5.3.1 Datos generales

Tabla 5.3.1 Datos bibliométricos de la publicación correspondiente

Revista	<i>Applied Sciences - Basel</i>
Fecha de publicación	11/01/2023
Factor de Impacto (JCR 2022)	2.719
Área	<i>Engineering, Multidisciplinary - SCIE</i>
Posición	58/167 (Q2)

Título: *“Does silver diamine fluoride affect the adaptation of high-viscosity resin-modified glass ionomer to dentin? An in vitro study”*

5.3.2 Valoración de la adaptación de IVMR

En todas las muestras en las que no se utilizó ningún procedimiento de acondicionamiento de dentina, independientemente de la aplicación o no de FDP/YP, se observaron *gaps* entre el RL y la pared de dentina. Cuando se acondicionó la dentina con AP o AO aparecieron menos espacios en la interfase dentina-IVMR si previamente no se había empleado FDP/YP, pero sin diferencias estadísticamente significativas ($p>0,05$).

Tanto en la pared coronal como en la media de todas las paredes de la cavidad, el acondicionamiento mediante AO previo a la colocación del IVMR (AO+IVMR) mostró conseguir los mejores resultados de adaptación frente al resto de grupos estudiados ($p<0,05$). El grupo AO+IVMR también obtuvo mejores resultados en la pared cervical frente al grupo control ($p<0,05$). Finalmente, en la pared axial tanto el acondicionamiento con AO, con AP y el uso de FDP/YP mostraron mejorías en la adaptación en comparación con el grupo control.

6. Discusión

A continuación, se expone una discusión conjunta de la metodología, resultados y limitaciones de los estudios de la presente tesis doctoral. Se incluye también una consideración sobre las perspectivas futuras, así como las líneas de investigación derivadas de dicho proyecto.

En el texto se han introducido nuevas referencias bibliográficas al margen de las presentadas originalmente en los artículos con el fin de mantener un enfoque actualizado del tema.

6.1 Sobre la metodología

6.1.1 Revisión sistemática (Anexo 1)

Hoy en día, la comprobación del potencial remineralizante de los materiales dentales utilizados para el tratamiento de lesiones profundas de caries se realiza con múltiples ensayos diferentes, lo que ocurre porque no existe un protocolo sistematizado para estos estudios (102).

Por un lado, se utilizan ensayos con los que observar directamente la formación de nuevas estructuras minerales, así como la realización de pruebas sobre la dentina tratada para valorar los cambios ocurridos. Y, por otro lado, se realizan ensayos directamente sobre los materiales comprobando las características mediante las cuales pueden desencadenar los fenómenos de remineralización.

Para la comprobación directa de los cambios en dentina la mayoría de los estudios utilizan tejido desmineralizado, con la intención de reproducir las condiciones clínicas en el límite de las posibilidades. La elección de desmineralizar la dentina de manera artificial fue la más escogida (103–106), pero en ocasiones también se utiliza dentina sana (107,108).

Los materiales son aplicados de manera directa sobre la dentina y fraguan en contacto con esta. Esto reproduce la realidad clínica en la cual el fraguado, ácido-básico y fotopolimerización, ocurren tras su aplicación en la cavidad dental (109). Con el objetivo de preservar la estructura normal del tejido dental, los dientes utilizados se conservan en solución de timol al 0,1% para su desinfección, ya que es el desinfectante que menos altera las características de la estructura dental (110), mientras que tras la colocación del material se utiliza solución corporal simulada (SBF).

El SEM se utiliza para una visualización de la superficie de la dentina con el fin de observar la formación de cristales de hidroxiapatita y otras estructuras mineralizadas. El EDX y el FTIT/ATR permiten una valoración de los cambios de

composición molecular y elemental. Sin embargo, las técnicas PLM, TMR y micro-CT ofrecen una comparación entre la situación inicial y final valorándose así la ganancia neta de material mineral. Hay varios artículos que apoyan el uso de EDX para evaluar el efecto remineralizante en asociación a FTIR/ATR (111,112), Micro-Raman (113) y XRD (114–116). Estas técnicas son menos invasivas y permiten el análisis de la misma muestra en distintos momentos ya que no requieren recubrimiento. Por esta razón, el uso de SEM-EDX es muy útil para estos estudios y deben incorporarse al grupo de análisis estándar para evaluar la remineralización.

Para la comprobación indirecta se realizan disco del material fraguado los cuales se almacenan en una solución durante un arco preciso de tiempo. Sucesivamente se realizan mediciones de la solución para examinar la cantidad de iones liberados por el material.

6.1.2 Estudios experimentales (Anexos 2 y 3)

En el primer estudio experimental, se compararon el RL, KM y el EF. Los 3 materiales son utilizados con fines restauradores. Según indican los fabricantes presentan características adecuadas para obturaciones cervicales, oclusales y proximales.

El uso de dientes incluidos extraídos permite una mayor estandarización de la muestra puesto que se asume que no hay cambios en la estructura dentinaria debido a estímulos externos. De la misma forma la elección de obtener la desmineralización a través de una solución quelante (EDTA 18%) persigue la misma finalidad. El motivo de la elección de utilizar cortes de dentina de distinto grosor (1, 2 y 3mm) fue poder valorar hasta qué profundidad son capaces de actuar los materiales del estudio.

El uso del FTIR/ATR permite determinar el potencial remineralizante de un material ya que permite la identificación de diferentes grupos funcionales. La presencia de picos en la zona alrededor de 1020 cm^{-1} indican la presencia de

grupos fosfato, que, en este caso suelen ser grupos PO_4 . La presencia de otros picos en la región de 1420 cm^{-1} indica que hay grupos carbonato como el CO_3 . La asociación de estos dos picos implica la presencia de hidroxiapatita carbonatada, la cual se espera encontrar después del proceso de remineralización de la dentina (112,116–119).

Mediante el EDX es posible conocer los elementos presentes en la muestra y su cantidad relativa. Los elementos de mayor interés son el calcio y el fosfato. El aumento de la relación Ca/P es un indicador importante de remineralización (114), ya que permite establecer si un material es eficaz cuando se aplica sobre dentina desmineralizada de acuerdo con las tendencias actuales de mínima intervención (120).

En el segundo estudio experimental, se comprobó la adaptación a las paredes de dentina del RL. Este IVMR está diseñado para utilizarse como material restaurador (90). Asimismo, la dentina se acondicionó con AP (Riva Conditioner), como recomienda por la casa comercial o con AO, de la manera tradicional, previamente al uso de composites. Igualmente, se valoró el efecto de la aplicación de FDP/YP (Riva Star) como agente remineralizante de manera previa al uso de IVMR.

Se realizaron cavidades cuyo margen oclusal fuera en esmalte y el cervical en dentina para, de esta forma, poder valorar la adaptación sobre distintos tejidos dentales (121). Las muestras fueron sometidas a termociclado para simular las condiciones de la cavidad bucal.

El uso del SEM permite evaluar la adaptación del material en todo el perímetro y medir con precisión la posible presencia de *gaps* (122–124). No obstante, el tratamiento de secado previo necesario, así como el vacío creado en la cámara del SEM, pueden provocar la separación del material debido a la contracción. Existen alternativas para evitar estos inconvenientes, pero es difícil determinar hasta qué punto las estructuras de interés han sido enmascaradas o alteradas por el proceso de preparación (125).

El acondicionamiento previo de la dentina mediante AO o AP ya demostró permitir la formación de *tags* de resina en los túbulos dentinarios, cosa que no ocurre sin previo acondicionamiento (68,121).

El FDP/YP es un material con alta capacidad remineralizante del tejido dental y de promover la formación de dentina terciaria (126). Un estudio reciente demostró que aumenta la densidad mineral de la dentina tratada con FDP (127). Su uso antes de colocar un IVMR está avalado por la literatura científica disponible (128,129).

El IVMR tiene un coeficiente de expansión térmica bastante alto en comparación con la estructura del diente (130). Por lo tanto, después del termociclado, pudieron aparecer ligeros cambios en la adaptación marginal

6.2 Sobre los resultados

6.2.1 Revisión sistemática (Anexo 1)

Todos los IVMR utilizados en los estudios incluido en la revisión sistemática mostraron presentar capacidad para remineralizar dentina. Cabe destacar, que cuando se compararon IVMR con IV tradicionales valorando únicamente la liberación de F los resultados, se muestra una reducción del poder remineralizante (108). Esto probablemente se debe a que la incorporación de resina reduce la cantidad de vidrio básico en la composición, siendo éste el responsable de la liberación de iones (131). Mientras que cuando se valora mediante otras pruebas como Micro-CT, SEM, FTIR, TMR, etc., se demostró que la incorporación de resina no afecta el potencial remineralizante de los materiales, demostrando su eficacia (132,133).

En algunos casos, la incorporación de algunos aditivos como CCP-ACP o BAGs (45S5, NbG o S53P4), mostró resultados prometedores ya que resultó en una promoción de la remineralización (104,106,134). Estos aditivos ya habían

demostrado sus capacidades en otros estudios de forma independiente (135–139).

6.2.2 Estudios experimentales (anexos 2 y 3)

En ambos estudios el objetivo fue valorar si los IVMR son adecuados para la obturación de cavidades tras la remoción selectiva de la caries dental.

En el primero de ellos (anexo 2), donde se comprueba la capacidad de remineralizar dentina, se obtuvieron resultados distintos según el material utilizado.

Todos los materiales estudiados permiten la formación de hidroxiapatita carbonatada demostrando su potencial remineralizante. Pero, KM es el que lo consigue con mayor velocidad (24 horas a 1mm). A mayor tiempo, el efecto progresa hasta mayores profundidades. Este hecho es de esperar sabiendo que la mayor parte de liberación de iones flúor por parte de los IV es los primeros 7 días (140) con un efecto de máximo en las primeras 24 horas (141,142) que, sucesivamente, disminuye de forma lineal, alcanzando una meseta en 10 a 20 días (143). Una revisión sistemática reciente afirma que son necesarios entre 1 y 10 días para la formación de la interfase mineral como resultado de un proceso de difusión de iones químicos entre el IV y la dentina (144).

De los tres IV, el único que incorpora resina en su composición (RL) fue el que tardó más tiempo (14 días) en conseguir formación de hidroxiapatita carbonata. Esto puede deberse al menor contenido de componentes clásicos de los IV. No obstante, RL muestra un aumento de este mineral directamente relacionado con el tiempo de exposición y de manera constante en los tres espesores de dentina.

Estos resultados concuerdan con el de otro estudio (143) en el cual también se observa que los IVMR liberan menor cantidad de fluoruros frente a los IV convencionales.

Los cambios en la relación Ca/P indican de nuevo que KM alcanza su máximo efecto a 1mm, mientras que resulta llamativo que EF y RL lo hagan a 2mm. En este caso el contenido de resina no provoca diferencias. Esto presenta una importante implicación clínica para la elección del material utilizado según el espesor de dentina desmineralizada en la cavidad a tratar. A 3 mm ningún material consigue un aumento de la relación Ca/P, siendo este un aspecto de menor interés puesto que debido al tamaño dental (145,146) no será posible esta situación clínica.

EF y RL integran en su composición estroncio que tiene propiedades químicas y físicas similares a las del calcio, por lo que, teóricamente, es capaz de reemplazarlo en la hidroxiapatita (147). Un estudio que utilizó vidrios bioactivos con estroncio mostró menos desmineralización del esmalte y de la dentina, pero detectó mejoras en la remineralización frente a BAGs sin estroncio (148).

En el tercer estudio (anexo 3), se valora el efecto de FDP/YP sobre la adaptación de un IVMR.

En el presente estudio, la dentina con solo el acondicionamiento con AP aumentó significativamente la adaptación en la pared axial del IVMR ($p < 0,05$) mientras que no encontró diferencias en el resto de paredes ni en la media de todas ellas. Esto concuerda con un estudio que evaluó la misma situación encontrando adaptación similar con y sin acondicionamiento. Sin embargo, pudo identificar hibridación en áreas donde se utilizó AP (123).

Una revisión sistemática y meta-análisis concluyó que la preaplicación de FDP/YP no altera la adhesión de los IV e incluso mejora la adhesión (149), aunque un estudio muestra como demorar la restauración una semana incrementa la fuerza de unión de los IVMR (150). Los resultados obtenidos en este estudio indican que FDP/YP no provocó cambios significativos en la presencia de *gaps* ($p > 0,05$) independientemente del acondicionamiento de la dentina. Incluso en el subgrupo sin acondicionamiento previo se observó una

adaptación significativamente mayor en la pared axial ($p<0,05$). Estos resultados concuerdan con los obtenidos en otros estudios (93,151).

Por último, cabe destacar que se utilizó FDP con una doble capa de YP para reducir la mancha oscura causada por la plata. Según la literatura revisada, YP no parece influir sobre la adaptación de los IV (152).

6.3 Limitaciones

Considerando la naturaleza *in vitro* de las pruebas realizadas en los estudios incluidos en la revisión sistemática, la extrapolación de los resultados al ámbito clínico debe realizarse con cautela. Factores externos como la saliva, los cambios de temperatura o variaciones en el pH podrían modular los efectos producidos sobre la dentina por los IVMR. Las principales limitaciones residen en la gran variabilidad de los ensayos utilizados para evaluar la remineralización de la dentina, así como el proceso para desmineralizar la misma y las condiciones de conservación durante los ensayos (153). Debido a esto, la revisión sistemática solo permitió una comparación cualitativa de los resultados.

Los estudios experimentales presentan las mismas limitaciones respecto al carácter *in vitro*.

Además, en el segundo estudio otra limitación es la carencia de un protocolo establecido para su realización. Por ejemplo, el tamaño de la muestra para los grupos de prueba varía entre los estudios en el campo (154–157). En el presente estudio, se seleccionó un tamaño de muestra de $n=3$ para cada grupo. El bajo tamaño de la muestra, tanto en el presente estudio como en estudios similares, puede reducir la representatividad de los resultados. Por lo tanto, los resultados deben interpretarse como una evaluación preliminar de la capacidad de remineralización de los IV a nivel de laboratorio y debe servir como una base para futuros estudios.

6.4 Perspectivas futuras

El estudio de la remineralización de los IV precisa ser investigado más profundamente mediante estudios *in vitro* que utilicen una batería de pruebas que se complementen, para determinar con exactitud todas las características de estos materiales. Éste es un aspecto fundamental para poder desarrollar una guía clínica precisa de su uso.

La composición de estos materiales se está modificando con la incorporación de aditivos cuya finalidad es potenciar el efecto remineralizador. La caracterización físico-química de estas nuevas composiciones es necesaria para comprender la interacción entre los componentes tradicionales y los nuevos.

En las investigaciones sobre otros materiales utilizados para terapia pulpar vital, como en el caso de los cementos a base de silicato cálcico, ya se están realizando estudios sobre las vías de señalización y los biomarcadores que son capaces modular esos materiales cuando interaccionan con células de la pulpa dental (158,159). De la misma forma es interesante que se apliquen esos ensayos también con los IV, en particular con los IVMR, para valorar su citocompatibilidad y bioactividad.

La investigación actual dirigida a la incorporación de aditivos a los IVMR requiere el establecimiento de protocolos estandarizados que permitan su replicación y comparación entre ellos.

Están despertando un gran interés los BAGs debido a sus amplias aplicaciones (160). Su estudio ha mostrado características adecuadas para el uso en Odontología (161), indicándose como aditivos adecuados para materiales restauradores (162). Por lo tanto, el estudio de la formulación adecuada entre IV y BAG es de gran importancia para conseguir materiales con mayores beneficios en la remineralización.

En la literatura ya existe evidencia de estudios *in vitro* sobre la correcta adhesión de IV a paredes de dentina previamente tratadas con FDP/YP (163). Y ahora, una vez comprobada una adecuada adaptabilidad en esta situación, es necesario progresar hacia estudios en animales y ensayos clínicos para confirmar que su uso es apropiado y eficiente en la práctica clínica.

Dentro del enfoque de mínima intervención, también adquieren importancia nuevos sistemas para la remoción selectiva de la caries. Se trata tanto de instrumentos para la remoción mecánica como productos para la remoción química (164–167). Es necesario realizar estudios que comprueben el efecto de estas técnicas sobre la dentina remanente, ya que sus características pueden favorecer la formación de cristales de hidroxiapatita y mejorar la adhesión del material de restauración.

6.5 Líneas de investigación actuales

En estos momentos, después de haber comprobado la profundidad a la cual llegan a remineralizar los IV y los IVMR y tras observar variaciones en su comportamiento con el tiempo, desde nuestro grupo de investigación estamos llevando a cabo nuevos estudios comparando estos materiales con cementos a base de silicato cálcico (CSCs). Por otro lado, estamos observando la influencia del método de remoción de dentina sobre el sustrato en el cual se utilizan estos materiales

Los ensayos actuales se dividen en tres fases:

- Determinación de la profundidad de remineralización de la dentina por parte de CSCs;
- Determinar el tiempo necesario de IV, IVMR Y CSCs para conseguir remineralización a 1 mm de profundidad;
- Determinar si la eliminación química de la caries dental favorece una sucesiva remineralización del tejido sano remanente.

A corto plazo, esperamos aportar conocimiento sobre el alcance de la acción de estos materiales con el fin de ayudar los profesionales a escoger el material con las características más apropiadas en cada caso clínico.

A medio y largo plazo, esperamos que la evidencia desarrollada permita realizar protocolos precisos por parte del personal investigador y/o de las sociedades científicas que mejoren la predictibilidad de los resultados de los tratamientos remineralizadores de la dentina profunda afectada por caries.

7. Conclusiones

A continuación, se exponen las conclusiones de la presente tesis doctoral, respondiendo a los objetivos específicos previamente planteados.

Los ionómeros de vidrio modificados con resinas utilizados en odontología restauradora muestran una adecuada capacidad para remineralizar la dentina desmineralizada, como se desprende de los resultados obtenidos a partir de los estudios *in vitro* incluidos en la revisión sistemática efectuada.

Los ionómeros de vidrio Ketac Molar Aplicap, Equia Forte HT y el ionómero de vidrio modificado con resinas Riva Light Cure HV muestran un buen efecto remineralizante a 1 mm de profundidad, mientras que a 3 mm no hay evidencias que indiquen remineralización.

La aplicación de fluoruro diamínico de plata y de yoduro de potasio en la dentina de manera previa a la colocación de un ionómero de vidrio de alta densidad no empeora la adaptación, a las paredes, del ionómero. El acondicionamiento después del uso del fluoruro diamínico de plata tampoco produce cambios.

En conjunto, los resultados del presente estudio de tesis doctoral dan lugar a 3 aportaciones:

- Se ha confirmado el poder remineralizante de ionómeros de vidrio modificados con resinas con finalidad restauradora frente a la dentina desmineralizada, gracias a la síntesis cualitativa de la evidencia científica disponible al respecto.
- Se ha demostrado que la incorporación de resina a la composición de ionómeros de vidrio no afecta al poder remineralizante, pero que existen algunas diferencias entre productos de distintas marcas.

- Se ha evidenciado que el uso de fluoruro diamínico de plata y de yoduro de potasio para la remineralización de dentina no provoca complicaciones en la adaptación sucesiva a las paredes por parte de ionómeros de vidrio modificados con resinas de alta viscosidad.

CONCLUSIONS

The conclusions of this doctoral thesis are presented below, in response to the previously stated specific objectives.

The resin-modified glass ionomers used in restorative dentistry show an adequate ability to remineralize demineralized dentin, as can be seen from the results obtained from the in vitro studies included in the systematic review performed.

Glass ionomers Ketac Molar Aplicap and Equia Forte HT, and the resin-modified glass ionomer Riva Light Cure HV show a remineralizing effect at 1 mm depth, while at 3 mm there is no evidence to indicate remineralization.

The application of silver diamine fluoride and potassium iodide to dentin prior to placement of a high-density glass ionomer does not worsen the wall adaptation of the ionomer. Conditioning after the use of silver diamine fluoride also produces no change.

Overall, the results of the present doctoral thesis project give rise to 3 contributions:

- The remineralizing potential of resin-modified glass ionomers for restorative purposes on demineralized dentin has been confirmed, thanks to the qualitative synthesis of the available scientific evidence in this regard.*
- It has been shown that the addition of resin to the glass ionomer composition does not affect the remineralizing potential, but there are some differences between products of different brands.*
- It has been shown that the use of silver diamine fluoride and potassium iodide for dentin remineralization do not cause complications in the subsequent adaptation to the walls of glass ionomers modified with high viscosity resin.*

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ANEXOS

A continuación, se presentan las publicaciones de los 3 estudios que comprenden el presente proyecto de tesis doctoral:

Systematic Review

Remineralizing Ability of Resin Modified Glass Ionomers (RMGICs): A Systematic Review

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Abstract: The selective caries removal approach leads to the need to use materials with the ability to remineralize remaining partially demineralized dentin. Among the materials proposed are resin-modified glass ionomer cements (RMGICs). The aim of this systematic review was to evaluate, based on in vitro experimental studies, whether RMGICs are suitable for remineralizing affected dentin. A systematic literature search was performed in four databases, followed by article selection, data extraction, and quality assessment. Studies assessing the remineralizing potential of RMGICs on dentin were included in our review. Studies which compared such properties between different RMGICs or with other materials were also eligible. The studies report the remineralizing ability of RMGICs, albeit with differences between different commercial products. RMGICs show a similar ability to conventional GICs to remineralize affected dentin, fulfilling the function for which they are designed. Moreover, the incorporation of additives, such as bioactive glass (BAG) or CCP-ACP, improves their remineralizing potential. The results of this review support the use of RMGICs as restorative materials after selective caries removal.

Keywords: resin-modified glass ionomer cements; remineralization; dentin; in vitro



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1. Introduction

Dentin is a highly mineralized organic tissue that, together with the dental pulp, forms the dentin–pulp complex [1]. In terms of its composition, it is majorly constituted of minerals (70%), organic components (20%), and water (10%) [2]. The mineral structure is formed by hydroxyapatite crystals arranged in different ways depending on the location. The organic components include type I collagen and phosphoproteins [2]. At a structural level, a distinction can be made between peritubular dentin, which is hypermineralized and has little collagen content, and intertubular dentin, which is formed by a network of collagen fibers with hydroxyapatite crystals arranged on their main axes [3].

Dental caries is a biofilm-mediated, diet-modulated, multifactorial, non-communicable, dynamic disease resulting in net mineral loss of dental hard tissues [4]. The microbial composition of cariogenic biofilms is not the same in enamel and dentin. Enamel is dominated by those of an acidogenic nature, while those with a proteolytic nature predominate on dentin [5].

In a healthy state, there is an equilibrium between the host and the microbial communities (eubiotic biofilm). However, under certain conditions, the interactions between the host and these microbial communities become unbalanced and the disease appears (dysbiotic biofilm) [6]. Overexposure to dietary carbohydrates is a factor which causes this imbalance of microbial communities and the transformation of a eubiotic biofilm into a dysbiotic biofilm. When the pH of the biofilm drops below the critical threshold (approximately 5.5), demineralization occurs. Under healthy conditions, the processes of demineralization and remineralization in enamel alternate in a dynamic equilibrium. When this equilibrium is broken, a net process of mineral loss occurs in the subsurface area of the enamel, giving rise to a weakened and porous enamel that corresponds to the initial caries lesion. If this

situation can be reversed by increasing the periods of remineralization, mineral gain will occur. In this case, the caries lesion will stop or even remineralize [7]. Saliva is a protective factor against demineralization, primarily due to its dragging and cleansing effects and its mineral and organic composition that buffers pH changes and favors remineralization [8].

In the case of dentin, recovery is more complex than in enamel because it involves the reconstitution of two different phases: on the one hand, organic type I collagen and, on the other, inorganic apatite. This implies that remineralization alone is insufficient for the total recovery of demineralized dentin. In this manner, it is also necessary to restore the structure of the collagen matrix and for both phases to be linked in a specific way [9]. Biomineralization uses biomimetic analogs of dentin matrix proteins to induce amorphous calcium phosphate (ACP) nanoprecursors in the internal compartments of collagen fibers. This biomimetic remineralization process represents an approach based on creating nanocrystals that are small enough to fit into the gap zones between adjacent collagen molecules and establish a hierarchical order in the mineralized collagen [10]. A material capable of performing this process is considered bioactive.

Thus, the remineralization process in enamel is based on a gain of mineral components, whereas dentin remineralization involves a more complex process of interaction between mineral gain and its interaction with the collagen matrix.

Restorative dentistry aims to restore the functionality of dental tissues that have lost part of their structure due to dental caries. The longevity of these restorations is influenced by several factors, such as the considerable differences in the mechanical, physical, adhesive, and handling properties of the various restoration materials and adhesive systems [11]. A meta-analysis shows that posterior resin composite restorations have shown annual failure rates of 1 to 3%. The main causes of replacement are fractures and secondary caries [12]. To reduce the need for replacement due to secondary caries, materials with cariostatic properties have been developed [13].

The current minimally invasive treatment approach involves the removal of infected tissue, but it does include the preservation of affected tissue that is partially demineralized [14]. This requires the use of materials that have the ability to remineralize the preserved tissue. This process consists of restoring minerals through the formation of new inorganic mineral tissue [15]. Among the materials used for this purpose are glass ionomers.

Glass ionomers (GICs) are a group of materials whose polymerization is based on an acid–base reaction between the weak acid and the basic glass of which they are composed [16]. The calcium–fluor–alumino–silicate content in the glass powder is responsible for the remineralizing ability of GICs [17]. These materials are capable of releasing fluoride over long periods of time and even recharging with fluoride if it is present in high concentrations in the medium [18]. Fluoride release is considered beneficial because it promotes the formation of fluorapatite, which is slightly less soluble than hydroxyapatite [19]. When fluoride ions are released, they can saturate the liquid phase in and around the surface of the restorative tooth, resulting in the precipitation of CaF_2 crystals. This reduces the chances of demineralization and accelerates the remineralization process. This process can be considered bioactive [11]. However, their mechanical characteristics are poor for many clinical uses [20].

Resin-modified glass ionomer cements (RMGICs) were developed to improve the physical and mechanical properties of GICs [21]. The most common resin monomer is 2-hydroxyethyl methacrylate (HEMA), to which a photoinitiator, such as camphoroquinone, is added to allow a light-mediated setting. These materials undergo a dual-setting reaction consisting of the typical acid–base reaction of GICs and photopolymerization [22]. However, the presence of resin monomers may affect the biological properties of RMGICs, especially with regard to their biocompatibility. This property decreases due to the cytotoxic effect of the resin component, especially during the first 24 h [23]. In order to improve the remineralizing potential of RMGICs, bioactive glasses are being incorporated into their composition, which also confers a bioinductive and regenerative potential to the material.

Bioinductivity refers to the material's ability to positively interact with living tissues by favoring cell migration and differentiation [24].

Given that the remineralization processes of enamel and dentin are different, as mentioned above, the present systematic review of in vitro studies is proposed with the aim of analyzing, based on the available scientific literature, the remineralizing potential of RMGICs on affected dentin and the possible positive effects of some of the additives they incorporate. These results could support the clinical use of these materials in deep caries lesions.

2. Materials and Methods

2.1. Protocol and Registration

This systematic review was performed following the PRISMA 2020 guidelines [25]. The protocol of this systematic review was previously registered in the Open Science Framework (OSF) registries (<https://doi.org/10.17605/OSF.IO/SQ8VC> (accessed on 3 February 2023)).

2.2. Inclusion and Exclusion Criteria

The research question of our review, based on the PICOS system, aimed to evaluate the current knowledge regarding the remineralizing potential of RMGICs on dentin, compared to a control or to another composition of RMGIC (P: resin-modified glass ionomers; I: application on demineralized dentin; C: comparison with other materials; O: dentin remineralization; S: in vitro studies).

In this way, in vitro studies evaluating the remineralizing potential of one or more RMGICs on dentin were eligible for inclusion in this review. The assessment of ion release from RMGICs was included among the assays on remineralization potential and thus was eligible for review.

Studies that only assessed cell adhesion on the materials were excluded as well as those that analyzed the materials' cytotoxicity alone. Studies that assessed the remineralizing potential of RMGICs on dental enamel were also excluded.

2.3. Search Strategy

An advanced electronic search was performed on MEDLINE, Scopus, Web of Science, and Lilacs in October 2022.

The search strategy included the terms "resin-modified glass ionomer cement*", "bioactivity", "remineralization", and "dentin", combined with the Boolean operators AND and OR (Table 1). The selection of the search strategy was based on previous works within this framework and their most cited terms. In addition, the references of the included studies were manually screened after the selection process to verify additional potentially eligible studies.

Table 1. Search strategy.

Database	Search Strategy	Findings
Medline	n° 1 (resin-modified glass ionomer cement*)	2003
	n° 2 (bioactivity) OR (remineralization)	154,091
	n° 3 (dentin)	42,496
	n° 1 AND n° 2 AND n° 3	54
Scopus	n° 1 (resin-modified glass ionomer cement*)	2142
	n° 2 (bioactivity) OR (remineralization)	88,229
	n° 3 (dentin)	44,380
	n° 1 AND n° 2 AND n° 3	32

Table 1. *Cont.*

Database	Search Strategy	Findings
Web Of Science	n° 1 (resin-modified glass ionomer cement*)	2376
	n° 2 (bioactivity) OR (remineralization)	94,721
	n° 3 (dentin)	52,709
	n° 1 AND n° 2 AND n° 3	54
Lilacs	n° 1 (resin-modified glass ionomer cement*)	277
	n° 2 (bioactivity) OR (remineralization)	626
	n° 3 (dentin)	8662
	n° 1 AND n° 2 AND n° 3	6

The search results were exported from each database to a reference management software (Mendeley, Elsevier, Amsterdam, The Netherlands) to check for duplicates. Once the duplicates were removed, a first screening was performed of the titles and abstracts of the articles using the inclusion and exclusion criteria mentioned above. Studies that met the criteria were subsequently assessed for inclusion in the qualitative synthesis via full-text analysis.

2.4. Data Extraction

The data extraction process was subdivided into three separate categories: study characteristics, methodology, and results. Authors and years of publication were recorded as study characteristics. Methodological variables, in terms of the materials, included the following properties: the type of glass ionomers used and the material it was compared to. The variables related to remineralization assessment consisted of the following: the assay used and its duration. The outcome variables included the significant results found for each test, their significance value, and the time in which they were recorded (duration).

2.5. Quality Assessment

The checklist proposed by Faggion et al. [26] to evaluate the quality or risk of bias of in vitro studies on dental materials was used to assess the quality of the included studies.

3. Results

3.1. Study Selection and Flow Chart

The search identified 146 references related to dentin remineralization by RMGICs, from which 54 were found in MEDLINE, 32 in Scopus, 54 in Web of Science, and 6 in Lilacs. After excluding 65 duplicates, 81 references remained to be examined. After reading the title and abstract, 68 references were excluded because they did not meet the inclusion criteria. Furthermore, after reading the full text of the 13 remaining articles, 8 were eligible for our review. Three were excluded because they assessed dentin microhardness, one was excluded for assessing dentin color changes, and another study was excluded for assessing demineralization (Figure 1). The selection of the articles was carried out in duplicate by two of the study researchers, JG and CL. In cases where there was no coincidence, the article was re-read jointly until a consensus was reached.

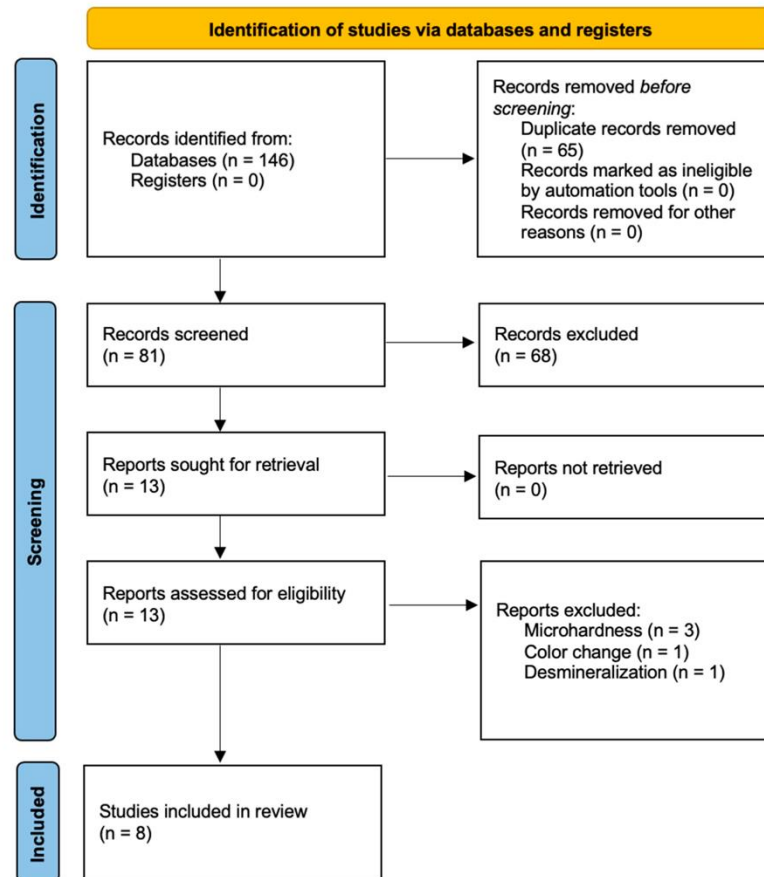


Figure 1. PRISMA flow diagram.

3.2. Quality Assessment

The quality of the included studies was assessed using the list proposed by Fagion [26] for the evaluation of in vitro studies on dental materials (Table 2). All of them had a correctly structured abstract (Item 1), as well as an introduction in which the scientific background, explanation of the rationale, and objectives are stated (Items 2 and 3). With the exception of the study by Xie et al. [27], the remaining studies explained the intervention performed in sufficient detail to allow replication (Item 3). In all cases, the outcome measures, both primary and secondary, were accurately defined, including how and when they were assessed (Item 4). No study stated how the sample size was determined nor was randomization performed (Items 5, 6, 7, 8, 9). The statistical methodologies used allowed for comparison by groups for primary and secondary outcomes (Item 10). Moreover, in six studies [27–32], for each outcome, the effect and precision were estimated with at least a 95% confidence interval (Item 11). The study's limitations were only discussed in two cases [29,32], addressing sources of potential bias and imprecision (Item 12). The source of funding was stated in all but one of the included studies [29] (Item 13). Finally, none of the studies provided access to the full study protocol (Item 14).

Table 2. Results of the assessment of in vitro studies by the use of the modified CONSORT checklist proposed by Faggion [26]. Cells marked with an asterisk “*” represent study fulfilment for the given quality assessment parameter. Cells left blank represent non-fulfilment.

Studies	Modified CONSORT Checklist Proposed by Faggion [17]														
	1	2a	2b	3	4	5	6	7	8	9	10	11	12	13	14
Xie et al. [27]	*	*	*		*						*	*		*	
Moraes et al. [28]	*	*	*	*	*						*	*		*	
Prabhakar et al. [29]	*	*	*	*	*						*	*	*		
Yang et al. [30]	*	*	*	*	*						*	*		*	
Zhang et al. [31]	*	*	*	*	*						*	*		*	
Zhao et al. [32]	*	*	*	*	*						*	*	*	*	
Yli-Urpo et al. [33]	*	*	*	*	*						*			*	
Toledano et al. [34]	*	*	*	*	*						*			*	

3.3. Studied Materials

Different RMGICs and GICs were used in the studies (Table 3). Also, different additives were used with the intention of increasing the remineralizing effect as well as some experimental materials (Table 4).

Table 3. List of RMGICs and GICs studied.

Material	Abbreviation	Composition	Manufacturer	Times Studied
Vitro Fil LC	VFLC	Powder: Fluorine silicate, strontium, aluminum, charge, activators, and iron oxide. Liquid: 2-hydroxyethyl methacrylate, aqueous solution of polyacrylic and tartaric acids, benzoyl peroxide, and camphorquinone.	DFL Indústria e Comércio, Rio de Janeiro, Brazil	1
Resiglass F	RF	Powder: Calcium fluorosilicate, barium, aluminum, polyacrylic acid, and inorganic fillers. Liquid: Dimethacrylate groups, deionized water, and catalysts.	Biodinâmica Química e Farmacêutica, Ibiporã, Paraná, Brazil	1
Fuji II	FII		GC Corporation, Tokyo, Japan	2
Fuji II LC	FLC	Powder: Fluro alumino silicate glass. Liquid: Distilled water, polyacrylic acid, 2-hydroxyethyl methacrylate, urethane dimethacrylate, camphorquinone.	GC Corporation, Tokyo, Japan	5
Ketac-Bond	KB	Powder: Calcium–aluminum–lanthanum–fluorosilica glass, pigments. Liquid: Polycarboxylic acid, tartaric acid, water, conservation agents.	3M Deutschland GmbH, Neuss, Germany	1
Vitrebond Plus	VP	Paste: HEMA, Bis-GMA, water, initiators, and radiopaque FAS (BL7AL). Liquid: Resin-modified polyalkenoic acid, HEMA, water, initiators.	3M Deutschland GmbH, Neuss, Germany	1
Experimental RMGIC	EXP	Powder: (Fluoro) alumino silicate glass. Liquid: light-curable star-shape poly(acrylic acid), water, 0.9% CQ, 1.8% DMAEMA, and 0.05% Hydroquinone.		1
Fuji VII	FVII	Powder: Fluro alumino silicate glass, Polyacrylic acid powder. Liquid: Polyacrylic acid, Polybasic carboxylic acid.	GC Corporation, Tokyo, Japan	1
Fuji VIII	FVIII	Powder: alumino silicate glass. Liquid: 2-HEMA 25–50%; tartaric acid 5–10%; 7,7,9(or 7,9,9)-trimethyl-4,13-dioxo-3,14-dioxo-5,12-diazahexadecane-1,16-diyl bismethacrylate 1–5%; 2-Hydroxy-1,3 dimethacryloxypropane 1–5%.	GC Corporation, Tokyo, Japan	1

Table 4. List of additives and other materials studied.

Material	Abreviation	Composition	Manufacturer	Times Studied
45S5	45S5	SiO ₂ = 45%, CaO = 24.5%, Na ₂ O = 24.5%, P ₂ O ₅ = 6%	Sylc, OSspray Ltd., London, United Kingdom	1
Niobophosphate glass (experimental)	NbG	Nb ₂ O ₅ = 41.8%, P ₂ O ₅ = 32.5%, CaO = 18.8%, Al ₂ O ₃ = 2.7%, Na ₂ O = 1.2%, SrO = 0.04%		1
S53P4 bioactive glass Frit	S53P4	SiO ₂ = 53%, Na ₂ O = 23%, CaO = 20%, and P ₂ O ₅ = 4%	MO-SCI® Health Care, Rolla, MO, USA	1
Bioactive glass S53P4	S53P4	SiO ₂ = 53%, Na ₂ O = 23%, CaO = 20%, and P ₂ O ₅ = 4%	Vivoxid Ltd., Turku, Finland	2
ART Composite (experimental)	AC	Pyromellitic dianhydride glycerol dimethacrylate, Ethoxylated bisphenol A dimethacrylate, TTCP, silicon carbide/DCPA, DCPA, sodium hexafluorosilicate, Benzoyl peroxide, tertiary amine, camphorquinone		2
Casein phosphopeptide-amorphous calcium phosphate	CPP-ACP			1

3.4. Study Methodology and Results

The remineralization assays used by the included studies and their significant results are shown in Table 5.

In two cases [28,33], the assessment of remineralizing potential was performed by comparing the release of F[−], Ca²⁺, and PO₄^{3−} ions between the tested materials. The comparison between pure Vitro Fil LC (VFLC) and Resiglass F (RF) showed no differences, but when incorporating different percentages of bioactive glasses (BAGs) (45S5 and NbG), the release of ions increased in VFLC in all cases except for F[−] in the VFLC + 5% 45S5 group. Contrarily, F[−] release generally decreased upon BAG incorporation in RF, while Ca²⁺ increased and PO₄^{3−} release exhibited a different behavior depending on the percentage of BAGs, i.e., decreasing at lower percentages (5%) and increasing at higher ones (20%). The addition of 30% of another BAG (S53P4) to Fuji II LC (FLC) also increased the release of F[−], Ca²⁺, and SiO₄^{4−} ions.

A wide variety of methods were used among the included studies to assess the remineralization potential of the tested materials. Scanning electron microscopy (SEM) showed a higher remineralization pattern of VFLC versus RF [28] in one study. In another study, it was used to confirm the remineralization ability of an experimental RMGIC [27]. It was also used to confirm that FLC + 30% S53P4 produces more calcified precipitates than without the incorporation of the BAG [33].

Fourier transform infrared spectroscopy (FTIR) was used in one study to assess remineralization potential [28]. A greater remineralization potential was observed from Ketac-Bond (KB) versus Vitrebond Plus (VP). However, in the same study, energy dispersive spectroscopy (EDX) results showed the opposite. Moreover, polarized light microscopy (PLM) showed greater remineralization when incorporating 10% BAG (S53P4) to Fuji II (FII) and FLC [29].

In two studies [30,31], transverse microradiography (TMR) was used to compare an experimental composite (ART Composite; AC) with FLC. At 2 weeks, in both cases, AC exhibited better results, while at 4 weeks, this occurred in only one of the studies. Finally, in one study [32], the analysis was carried out by micro-computed tomography (Micro-CT), and it was observed that by incorporating Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) to VII, greater remineralization was achieved.

Table 5. Summary of the results of included studies showing significant differences between different materials. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. SEM: Scanning electron microscopy; FTIR/ATR: Fourier transform infrared spectroscopy—attenuated total reflectance; PLM: polarized light microscopy; EDX: Energy-dispersive X-ray spectroscopy; FTIR: Fourier transform infrared spectroscopy; TMR: transverse microradiography; Micro-CT: micro computed tomography.

Studies	Materials	Assay	Time	Results			
Xie et al. [27]	EXP2.7	SEM EDX	14 days	SEM: C - < EXP2.7(10), EXP2.7(15), EXP2.5(15) EDX: no difference			
	EXP2.5						
	EXP2.7(10)						
	EXP2.7(15)						
	EXP2.7(20)						
	EXP2.5(10)						
Moraes et al. [28]	EXP2.5(15)	SEM EDX	14 days	SEM: C - < EXP2.7(10), EXP2.7(15), EXP2.5(15) EDX: no difference			
	EXP2.5(20)						
	VFLC				Ion release F ⁻	7 days	F ⁻ : VFLC + 5% 45S5 < VFLC < VFLC + 10% 45S5, VFLC + 20% 45S5, VFLC + 5% NbG, VFLC + 10% NbG, VFLC + 20% NbG *; RF + 5% 45S5, RF + 10% 45S5, RF + 20% 45S5, RF + 5% NbG, RF + 10% NbG < RF < RF + 20% NbG *
	VFLC + 5% 45S5						
	VFLC + 10% 45S5						
	VFLC + 20% 45S5						
	VFLC + 5% NbG	Ion release Ca ²⁺	Ca ²⁺ : VFLC < VFLC + 5% 45S5, VFLC + 10% 45S5, VFLC + 20% 45S5, VFLC + 5% NbG, VFLC + 10% NbG, VFLC + 20% NbG *; RF + 5% 45S5, RF + 5% NbG < RF < RF + 10% 45S5, RF + 10% NbG, RF + 20% 45S5, RF + 20% NbG *				
	VFLC + 10% NbG						
	VFLC + 20% NbG						
	RF						
	RF + 5% 45S5	Ion release PO ₄ ³⁻		PO ₄ ³⁻ : VFLC < VFLC + 5% 45S5, VFLC + 10% 45S5, VFLC + 20% 45S5, VFLC + 5% NbG, VFLC + 10% NbG, VFLC + 20% NbG *; RF + 5% 45S5, RF + 5% NbG < RF, RF + 10% 45S5, RF + 10% NbG < RF + 20% 45S5, RF + 20% NbG *			
	RF + 10% 45S5						
	RF + 20% 45S5						
	RF + 5% NbG						
	RF + 10% NbG	SEM	28 days	SEM: all VFLC < all RF FTIR/ATR: no difference			
	RF + 20% NbG						
Prabhakar et al. [29]	FII	PLM	28 days	FII < FII + 10% S53P4, FLC < FLC + 10% S53P4 **			
	FII + 10% S53P4						
Yang et al. [30]	FLC	TMR	4 weeks	AC > FLC at 4 weeks *			
	AC		8 weeks	AC = FLC at 8 weeks			
Zhang et al. [31]	FLC	TMR	4 weeks	AC > FLC at 4 and 8 weeks *			
	AC		8 weeks				
Zhao et al. [32]	FVII	Micro-CT	28 days	FVII + CPP-ACP > FVII, FVIII ***			
	FVII + CPP-ACP						
	FVIII						
Yli-Urpo et al. [33]	FII	Ion release SiO ₄ ⁴⁻	1, 6, 24, 72, 168, 336 h	SiO ₄ ⁴⁻ : FLC + 30% S53P4 > FII + 30% S53P4, FLC + 10% S53P4 > FII, FII + 10% S53P4, FLC at 72 h, 168 h; FLC + 30% S53P4 > all at 336 h *** F ⁻ : FLC + 30% S53P4 > all at 72 h; FII + 30% S53P4 > all at 336 h Ca ²⁺ : FLC + 30% S53P4 > all at 168 h, 336 h PO ₄ ³⁻ : FII + 30% S53P4 < all at 336 h			
	FII + 10% S53P4						
	FII + 30% S53P4	Ion release F ⁻					
	FLC						
	FLC + 10% S53P4	Ion release PO ₄ ³⁻					
FLC + 30% S53P4							
Toledano et al. [34]	KB	SEM	336 h	FLC + 30% S53P4 has CaP precipitation on surface			
	VP	SEM	336 h	FLC + 30% S53P4 has CaP precipitation on surface			

4. Discussion

Currently, the conservative approach for the treatment of dental caries follows the criteria of the selective removal of carious dentin in combination with the use of materials with remineralizing, restorative, and/or bioinductive potential [35]. The European Society of Endodontology supports this approach and indicates the use of GICs as the indirect pulp-capping materials [14]. The remineralization process causes changes in the mineral structure of the dental hard tissue [36]. The need to place materials with remineralizing potential to recover the functionality of the affected dentin favored the implementation of GICs, and the need to improve their handling and mechanical properties led to the

introduction of RMGICs. In contrast, it has already been demonstrated that the presence of resin in the composition can cause adverse effects on the pulp, such as inflammation. At the z clinical level, however, the results are positive even though they cannot be considered as biocompatible as traditional GICs [37].

There are already systematic reviews that point out the protective potential of RMGICs in the reduction of demineralization of the proximal dental tissue [38–40]. However, it is necessary to assess to what extent these materials can remineralize already affected dentin. Various randomized clinical trials showed that RMGICs have a high clinical efficacy in indirect pulp capping [41,42]. Accordingly, the aim of this study was to perform a systematic review of the available literature to assess dentin remineralization produced by these materials. Since this was a systematic review of in vitro studies, it was not possible to register it in PROSPERO.

Regarding the results, the assays used to assess the remineralization potential of RMGICs were very varied and did not follow a pre-established protocol [43]. This makes it difficult to compare studies in order to establish which materials best achieve this purpose. On several occasions, a traditional GIC was compared with an RMGIC. The most studied RMGIC was the FLC (in specific, five studies). Another aspect studied is the possible improvement of the remineralizing properties due to the incorporation of additives. In some cases, the incorporation of some additives showed promising results, such as CCP-ACP or BAG (45S5, NbG or S53P4), all of which resulted in a promotion of the remineralization. These additives had already demonstrated their capabilities in other studies independently [36,44–46]. The incorporation of these compounds into RMGICs has been shown to increase their remineralization potential [28,29,33].

Overall, the comparison between GICs and modified RMGICs has shown no significant differences, with small variations depending on the studies [34]. This result is of particular interest since, in studies assessing only fluoride release, it was concluded that the RMGICs had less remineralizing power. On evaluating remineralization by means of other more specific technologies, such as Micro-CT, SEM, FTIR, TMR, etc., it was shown that the incorporation of resin does not affect the materials' remineralizing potential [47,48].

There are several articles that support the use of EDX to assess the remineralizing effect of GICs in association with FTIR [49–51], Micro-Raman [52], and XRD [53–55]. These techniques are less invasive and allow the analysis of the same sample at further endpoints as they do not require a coating. For this reason, the use of SEM–EDX is very useful for these studies and should be incorporated into the standard group of analyses performed to evaluated remineralization.

The new experimental compositions being studied should be taken into account for future research due to the positive results obtained. Materials such as EXP2.7(15) or AC have shown promising results with SEM and TMR compared to traditional RMGICs (FLC).

The mechanical properties of the studied materials were shown to be insufficient to withstand high masticatory loads. The incorporation of bioactive glasses may increase their microhardness, as shown in the study by Xie et al. [27]. However, in the study by Moraes et al. [28], no differences were observed. When RMGICs are used in areas of high masticatory load, it is appropriate to use the sandwich technique to reduce the risk of fracture of the restoration and the tooth substrate, showing similar results to those of a composite filling [56]. The higher mechanical strength, together with the fluoride release effect of RMGICs, reduces the possibility of secondary caries by forming fluorapatite, whose critical point of demineralization is lower than that of hydroxyapatite [57].

When assessing the quality of the included studies, a similar structural pattern is observed. Studies reported essential data, such as a sufficient abstract, clear objective(s), detailed description of the methodology, mention of statistical tests used, and relevant conclusions; but the sample size was never justified nor was randomization performed and, most importantly, study limitations were also not addressed in the discussion.

Current research directed towards the incorporation of additives to RMGICs requires the establishment of standardized protocols that allow their replication and a comparison

between them. In this systematic review, only qualitative comparisons could be made due to the differences in the methodology of the included studies. This acts as a limitation of the present work. The main limitations of this study reside in the large variability of assays used to assess dentin remineralization, as well as the process to demineralize dentin, and the preservation conditions during the assays [58]. The use of standardized methodologies could allow for future reviews to perform a meta-analysis or quantitative synthesis. It should also be highlighted that the in vitro nature of the assessed studies' results implies a limited application of the results of the present study to the clinical setting. Instead, the results from this review should be interpreted with caution and treated as preliminary evidence in this regard. Future studies are needed to confirm the reported results.

5. Conclusions

RMGICs show a similar ability to remineralize affected dentin as GICs in vitro, fulfilling the function for which they are designed. The incorporation of additives like BAG and CCP-ACP may potentially improve their remineralizing potential. Therefore, within the limitations of the in vitro nature of the included studies, this review supports the use of RMGICs as restorative materials after the selective removal of dental caries.

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Article

Remineralization Potential of Three Restorative Glass Ionomer Cements: An In Vitro Study

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Abstract: The aim of this in vitro study was to evaluate the remineralizing ability of three glass ionomers on demineralized dentin with different thicknesses and time periods. Fifty third molars were obtained and were sectioned into 1-, 2-, and 3-mm thick slices ($n = 36$ for each thickness). The specimens were demineralized with 18% EDTA for 2 h. From the glass ionomer cements (GICs) under study (Ketac Molar Aplicap, Equia Forte, or Riva Light Cure), 1 mm was placed over each slice, set, and preserved in PBS until observation after 1, 7, 14, and 28 days after placement. For each material, thickness, and time, three samples were prepared. Using Fourier Transform Infrared Spectrometry (FTIR), apatite formation was determined on the side opposite to that on which the material had been placed. By means of Energy Dispersive Spectroscopy (EDX), the changes in the Calcium/Phosphate (Ca/P) ratio were evaluated. These changes were compared between the different materials by means of a two-way ANOVA test, considering time and dentin thickness, for a significance level of $p < 0.05$. Results: FTIR showed a peak at 1420 cm^{-1} , evidencing the presence of carbonated hydroxyapatite in all the materials after 14 days, which indicates that a remineralization process occurred. Riva Light Cure showed the most homogeneous results at all depths at 28 days. The Ca/P ratio was maximum at 7 days in 2 mm of dentin for Riva Light Cure and Equia Forte HT (3.16 and 3.07; respectively) and for Ketac Molar at 14 days in 1 mm (3.67). All materials induced remineralization. Equia Forte achieved the greatest effect at 2 mm and Ketac Molar at 1 mm, whereas Riva Light Cure showed similar results at all depths. In terms of Ca/P ratio, Equia Forte and Riva Light Cure remineralized best at 2 mm, whereas for Ketac Molar, it was 1 mm. Carbonate apatite formation was higher at 24 h and 7 days for Ketac Molar, whereas it decreased at 14 days for Ketac Molar and peaked in Riva Light Cure and Equia Forte.

Keywords: resin-modified glass ionomer cements; remineralization; Fourier Transformed Infrared Spectroscopy; energy dispersive X-ray spectroscopy



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1. Introduction

Dentin is an organic, highly mineralized tissue that, together with the pulp, forms the dentin–pulp complex. It is made up of 70% minerals, with 20% organic content, and the remaining is 10% water [1]. It is formed by a network of collagen fibers on which hydroxyapatite crystals are deposited [2].

Caries is the most common cause of dentin destruction [3]. The existence of a correlation between histopathological findings and the clinical behavior of dentin in caries is elucidated in the research by Fusayama et al., who concluded that the color and hardness of carious dentin does not correspond to its degree of bacterial invasion [4].

The therapeutic approach to the carious lesion, from a minimally invasive perspective, has changed the traditional concept of “non-selective dentin removal”, until hard dentin is reached in the entire cavity, to the concept of “selective dentin removal”. The latter involves the removal of all or part of the soft dentin (depending on the depth of the cavity), leaving the leathery and firm dentin, and treating it appropriately to achieve remineralization [5].

Leathery and firm dentine correspond histologically to partially mineralized dentin, which has been classically called “affected dentin” [6]. In this area, the hydroxyapatite crystals are shorter, and the collagen fibers are not denatured, maintaining their characteristic bands [4].

Remineralization is defined as the net gain of calcified material in tooth structure, which replaces that previously lost through demineralization. The conventional strategy involves the use of solutions containing calcium and phosphate ions in the presence of various concentrations of fluoride. This occurs by the growth of hydroxyapatite crystals in partially demineralized dentin [7]. From a clinical point of view, it is of great importance to know the depth to which the GICs can act in order to be able to assess the thickness of demineralized dentin that can be maintained in the selective removal of caries.

Dentin tissue recovery is complex because it involves the reconstitution of two different phases: on the one hand, organic type I collagen, and on the other hand, inorganic apatite, both linked in a specific spatial relationship. This implies that remineralization alone is insufficient for the total recovery of demineralized dentin, so it is also necessary to restore the structure of the collagen matrix and for both phases to be linked in a specific manner [8].

Glass ionomer (or polyalkenoate) cements (GICs) belong to a group of materials based on an acid–base setting reaction, in which a weak acid reacts with a basic glass powder. They usually contain different proportions of polymeric acids such as polyalkenoic acid, homopolymers (acrylic acid), and a 2:1 copolymer of polyacrylic acid and maleic acid. Regarding glass, it is essential that it has a basic nature. Its essential component is calcium-fluoro-alumino-silicate powder, but it can also contain phosphate, sodium, calcium, or strontium. Water is the third essential element. Its main function is to serve as a solvent for the polymeric acids, allowing them to act as a proton releaser. This is crucial for the setting reaction of GICs [9]. Some GICs contain additional chelators such as tartaric acid or citric acid to prevent the precipitation of aluminum salts by avoiding the premature formation of ionic cross-links [10].

Resin-modified glass ionomer cements (RMGICs) incorporate resin monomers which add a light curing reaction to the material. The proportion of resin in the material modifies its physical, mechanical, and biological characteristics and properties. RMGICs are described to be “dual-cured”, where resin monomers undergo photopolymerization upon light curing, and the GIC component is chemically cured in an acid–base reaction. The polymerized resin acts as a bridge and strengthens the material [11]. Biomineralization uses biomimetic analogs of dentin matrix proteins to induce the formation of amorphous calcium phosphate (ACP) nano-precursors in the internal compartments of collagen fibrils. This biomimetic remineralization process represents an approach based on creating nanocrystals that are small enough to fit into the gap zones between adjacent collagen molecules and establish a hierarchical order in the mineralized collagen [12].

To study the remineralization ability of dental materials, Fourier Transform Infrared Spectroscopy (FTIR), among other methods, can be used. This spectroscopic analysis method allows determining the nature of the mineral component of samples and provides quantitative information on the changes in mineral and matrix composition as mineralization takes place [13,14]. Energy Dispersive Spectroscopy (EDX), on the other hand, is used to analyze the chemical elements found on the surface of dentin once it has been treated with different remineralizing agents [15,16].

The rationale of the present work focuses on evaluating the remineralizing potential of different restorative GICs on different dentin thicknesses, since, in the literature, there is little information on both the remineralizing potential and the depth of remineralization achieved.

The aim of the present *in vitro* study is to evaluate the remineralization potential of three GICs at different depths of artificially demineralized dentin by analyzing their ability to form hydroxyapatite and by means of a chemical element analysis. The null hypothesis is that the remineralizing ability of the studied materials will not differ among them or at different times and dentin thicknesses.

2. Materials and Methods

2.1. Sample Preparation

The study protocol was approved by the Research Ethics Committee of the University of Valencia (ID: 2030322). A total of 50 impacted wisdom teeth extracted surgically for medical reasons were selected. After extraction, the teeth were stored in 0.1% Thymol solution at 4 degrees Celsius for 24 h. Organic debris was removed, and they were preserved in Phosphate Buffer Solution (PBS) until their use in the study, no more than 3 months after extraction.

The teeth were embedded in epoxy resin (Resin Pro[®], Barcelona, Spain) and kept at room temperature for 24 h until complete setting. Subsequently, the resin blocks were cut longitudinally with the Mintron Stuers[®] precision cutting machine (Madrid, Spain) to obtain 1-, 2-, and 3-mm thickness sections with dentin on both sides of the cut, making a total of 114 cuts ($n = 36$ for each of the above-mentioned thicknesses, 6 to be used as controls (3 as positive controls and 3 as negative controls)). The samples for the positive controls were directly preserved in Phosphate Buffered Saline (PBS). The samples for the negative controls were demineralized, as was done with the study samples (described below), and then preserved in PBS.

After obtaining the sample sections, the study samples and the three samples used as negative control were demineralized. The demineralization process was as follows: the samples were immersed in 18% Ethylene Diamine Tetraacetic Acid (EDTA) solution (Clarben, Fuenlabrada, Madrid, Spain) for 2 h, after which, they were washed with distilled water and then dried. The GICs tested in the study, their compositions, and batch numbers are shown in Table 1.

Table 1. Composition, manufacturer, and batch number of the studied GICs.

Material	Composition	Manufacturer	Batch N°
Ketac Molar Aplicap (KM)	Liquid: water, copolymer, polyacrylic acid, maleic and tartaric acid Powder: aluminum-calcium-lanthanum fluorosilicate glass	3M ESPE [®] Seefeld, Germany	56420
Equia Forte HT (EF)	Liquid: polycarboxylic acid, water Powder: strontium fluoro aluminum silicate glass, iron oxide	GC Corp [®] , Tokio, Japan	901584
Riva Light Cure HV (RL)	Liquid: polyacrylic acid, tartaric acid, HEMA Powder: bioactive glass (Ionglass [™]), contains fluoride and strontium ions.	SDI [®] Victoria, Australia	873003

GICs were handled according to the manufacturer's instructions. Ionomer (1 mm) was applied on each of the dentin samples, as shown in Figure 1. After applying the materials, EF and RL were light-cured for 40 seconds with an LED-B lamp (Guilin Woodpecker Medical Instrument, Guilin, Guangxi, China). KM was left 3.30 min to complete its polymerization. All samples were immersed in 2 mL of PBS for preservation until the time of measurement. Three specimens of each sample were prepared. Both the storage and characteristics of the samples were based on the methodology of previous studies [17–19]. The sample preparation is illustrated in Figure 2.

Parallelly, four 1-mm blocks were obtained for each pure material and time period, which were also preserved in PBS.

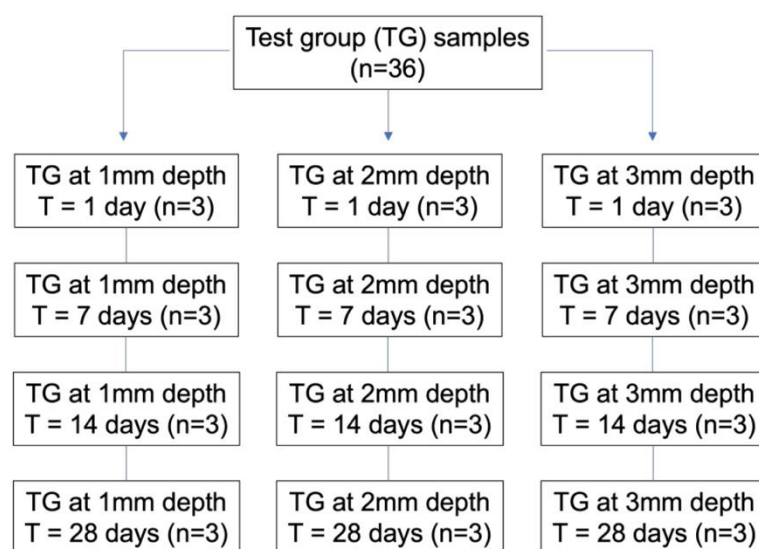


Figure 1. Flowchart representing the different test groups. Three test groups (TG) were assessed (KM, EF, and RL (n = 36 each)). Three different dentin depths were tested (1, 2, and 3 mm). Four different time points were tested (1, 7, 14, and 28 days).

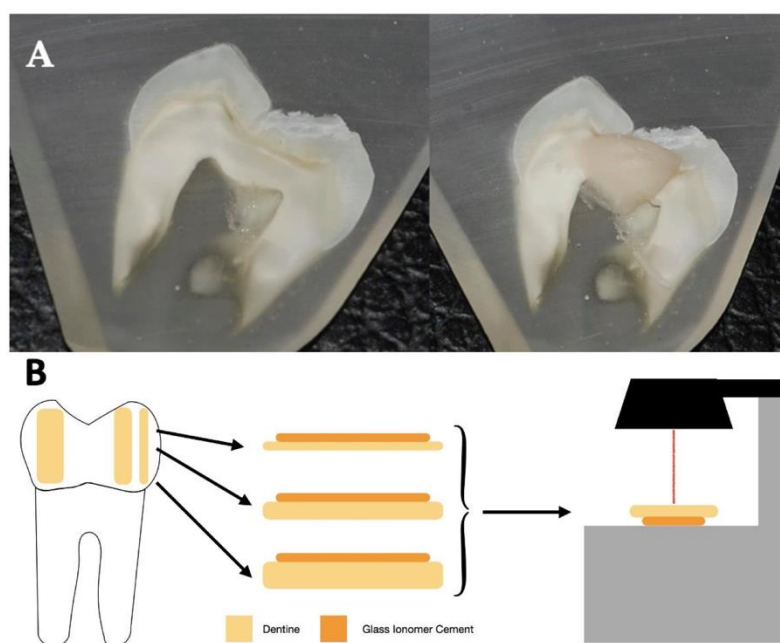


Figure 2. (A) Sample before and after the application of the GIC. (B) Schematic illustration of the sample preparation and analysis.

Subsequently, and after each time period, the changes in the mineral composition of the dentin on the side opposite to that on which the material had been placed were analyzed. This was performed to assess the remineralization potential of the tested GICs at greater depths of dentin and not superficially. This is remarkable because, to our knowledge, no previous study has assessed the remineralization potential in depth.

2.2. Fourier Transform Infrared Spectrometry (FTIR)

This spectroscopic analysis technique is used to understand the structure of individual molecules and the composition of molecular mixtures. In the present study, it was used to determine the changes in the structure and composition of demineralized dentin that occurred on the side opposite to that on which the material was placed. This was done to evaluate the remineralization in depth induced by the different materials in demineralized dentin at different study times. The Cary 630 FTIR equipment (Agilent Technologies® California, CA, USA) was used for this purpose. In this case, it was FTIR with attenuated reflectance (FTIR-ATR) by absorbance.

The analysis of the samples with this technique produced graphs that provide information regarding the degree of light absorbance of the sample (in our case, dentin), corresponding to a particular type of compound. The intervals of interest for our study were [10]:

- 3000–3700 cm^{-1} : Corresponding with OH group.
- 1636 cm^{-1} : Corresponding with COO^- group.
- 1021 cm^{-1} : Corresponding with PO_4 groups.
- 820 and 1420 cm^{-1} : corresponding with CO_3 group.
- 700 cm^{-1} : Corresponding with P_2O_7 group.
- The joint presence of PO_4 and CO_3 together indicates the presence of carbonate hydroxyapatite.

To analyze the composition of the pure material, the blocks of pure material were crushed to facilitate the measurement process. The spectra recorded the absorbance between 650 cm^{-1} and 4000 cm^{-1} , and 64 scans were performed in each measurement with a resolution of 2 cm^{-1} . Between each sample, 2-propanol was used to clean the surface and avoid contamination between samples.

2.3. Energy Dispersive Spectroscopy (EDX)

This is an analytical technique used for qualitative and quantitative elemental analysis. The aim of this technique was to determine the changes in the chemical composition of dentin, which was previously demineralized at a depth of 1, 2, and 3 mm, after contact with the different studied GICs. A Scanning Electron Microscope (SEM) (Hitachi S4800, Hitachi High-Technologies Corporation, Tokyo, Japan) with the XFlash 5030 system (Bruker, MA, USA) for EDX was used. Measurements were performed at 1000× magnification and a voltage of 10 kV.

2.4. Statistical Analysis

From the calcium (Ca) and phosphate (P) values obtained in the samples, the Ca/P ratio was calculated in the positive and negative control dentin samples and in the dentin treated with the different materials, thicknesses, and times. The percentage of remineralization was determined according to the increase in the Ca/P ratio in the treated dentin relative to the positive and negative controls. The Kruskal Wallis test with the Dunn–Bonferroni post hoc method was applied, considering time and dentin thickness for each material. The significance level considered was $p < 0.05$. SPSS 28.0 statistical package (IBM, Chicago, IL, USA) was used.

3. Results

3.1. Fourier Transform Infrared Spectrometry (FTIR)

Figure 3 shows the plots corresponding to the pure material at 24 h. In Ketac Molar, the presence of an initial band around 3462 cm^{-1} stands out. Additionally, peaks were found near 1636 cm^{-1} , corresponding to COO^- , followed by a peak at 1419 cm^{-1} , corresponding to the presence of CO_3 . Lastly, a peak at 1005 cm^{-1} , corresponding to the presence of PO_4 groups, was observed, which indicates the presence of carbonated hydroxyapatite in the material.

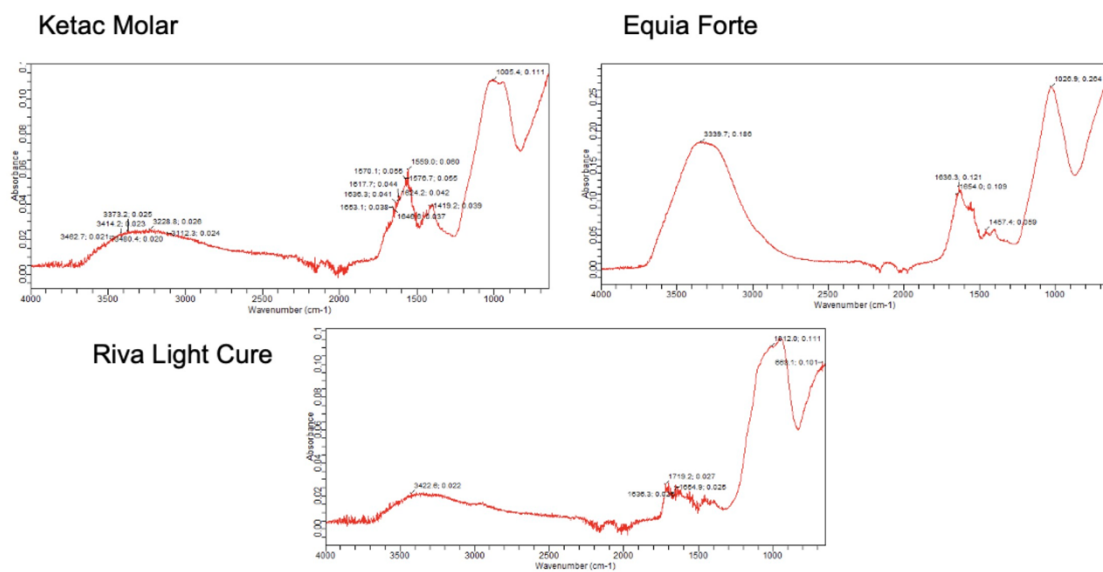


Figure 3. FTIR spectrum of the different pure materials 24 h after setting.

For Equia Forte, a marked peak was observed at 3339 cm^{-1} , corresponding to the presence of OH groups, and a peak at 1636 cm^{-1} , corresponding to COO^- , followed by a peak at 1457 cm^{-1} and a peak at 1026 cm^{-1} . Together, they indicate the presence of carbonate hydroxyapatite.

In the case of Riva Light Cure, an initial band was found at 3422 cm^{-1} , indicative of the presence of OH groups. A peak was also found at 1636 cm^{-1} (corresponding to the presence of COO^-), followed by another peak at 1020 cm^{-1} , which represents the presence of PO_4 groups. Finally, a peak was observed at 667 cm^{-1} , indicating the presence of P_2O_7 .

Figure 4 shows the graphs corresponding to Ketac Molar for the different thicknesses and times. It can be seen how at 24 h (Figure 4A), the absorbance values maintained a similar pattern in the three dentin thicknesses, highlighting only that the peak corresponding to carbonated hydroxyapatite (1399 cm^{-1}) is more pronounced in the 1 mm thick sample compared to the other two. At 7 days, the patterns were similar in the three thicknesses (Figure 4B). At 14 days (Figure 4C), it is noteworthy that at depths of 2 and 3 mm, the level of water absorption was lower (1636 cm^{-1}), and the presence of the other elements decreased compared to the 1 mm thick sample. At 28 days (Figure 4D), the graphs show a similar arrangement.

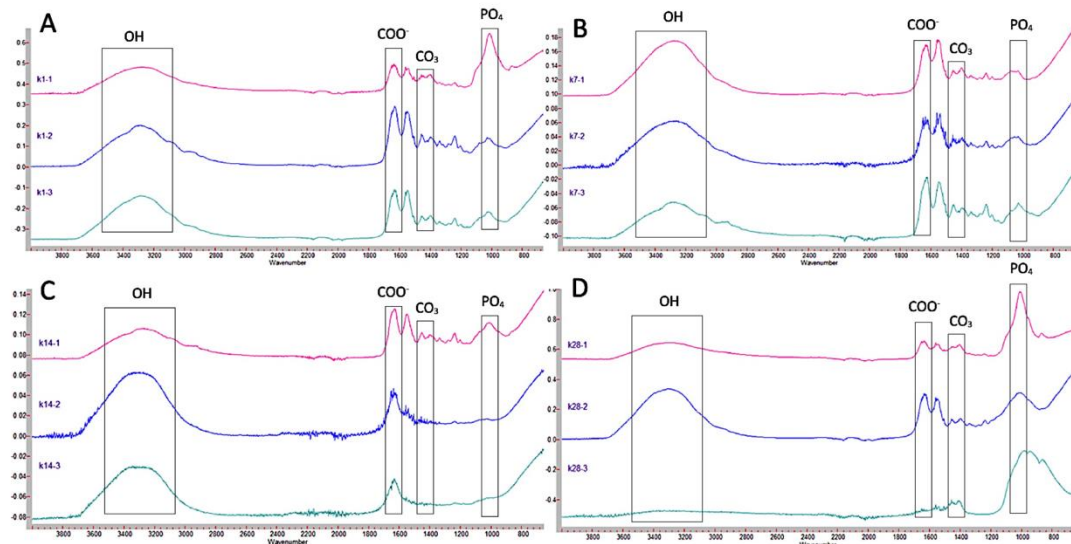


Figure 4. Comparison of Ketac Molar FTIR spectra at different thicknesses (1, 2, and 3 mm) at 24 h (A), 7 days (B), 14 days (C), and 28 days (D). The peaks of the OH, COO[−], CO₃, and PO₄ groups of interest in this study are indicated.

Figure 5 shows the graphs regarding Equia Forte. At 24 h (Figure 5A), in all thicknesses, the behavior is similar: a peak corresponding to the presence of OH bonds, followed by a peak corresponding to water absorption, then several peaks close to 1420 cm^{−1}, corresponding to CO₃, as well as a peak at 1031 cm^{−1}, close to 1021 cm^{−1}, corresponding to the presence of PO₄ groups. At 7 and 14 days (Figure 5B,C), the graphs are also similar, with peaks similar to those found at 24 h. At 28 days (Figure 5D), at 2 mm, the peak indicative of the presence of PO₄ is more pronounced compared to the other two graphs, although at 1 mm thickness, it is still a notable peak. However, at 3 mm, it is practically not marked.

Figure 6 shows the graphs regarding Riva Light Cure. At 24 h (Figure 6A), there is a peak at 3287 cm^{−1}, corresponding to the presence of OH groups, followed by a peak at 1628 cm^{−1}, corresponding to COO[−] groups. Several peaks with values at 1457 cm^{−1}, 1399 cm^{−1}, or 1340 cm^{−1} can also be seen, associated with the presence of CO₃. Finally, a peak can be seen at 1031 cm^{−1}, corresponding to the presence of PO₄ groups. At 7 days (Figure 6B), an initial section corresponding to COO[−] (1636 and 1599 cm^{−1}) is observed, followed by a peak at 1340 cm^{−1} and 1457 cm^{−1}, indicative of the presence of CO₃, and a peak at 1033 cm^{−1}, corresponding to the presence of PO₄ groups. At 14 and 28 days, the results are similar (Figure 6C,D).

The spectra of the positive (C+) and negative (C−) controls are shown in Figure 7. When analyzing the negative control (Figure 7, C− panel), an important initial peak is found, corresponding to the presence of OH groups, followed by a peak at 1636 cm^{−1}, which indicates the presence of COO[−] groups, and a peak at 1457 cm^{−1}, corresponding to the presence of CO₃. In the graph corresponding to the positive control (Figure 7, C+ panel), the disposition is similar. The initial peak, which corresponds to the presence of OH, is less marked, but the peak at 1636 cm^{−1} appears again, corresponding to the presence of COO[−]. The peaks at 1457 and 1239 cm^{−1} also appear, indicative of the presence of CO₃. Finally, a peak at 1031 cm^{−1} can also be seen, which corresponds to the presence of PO₄ groups.

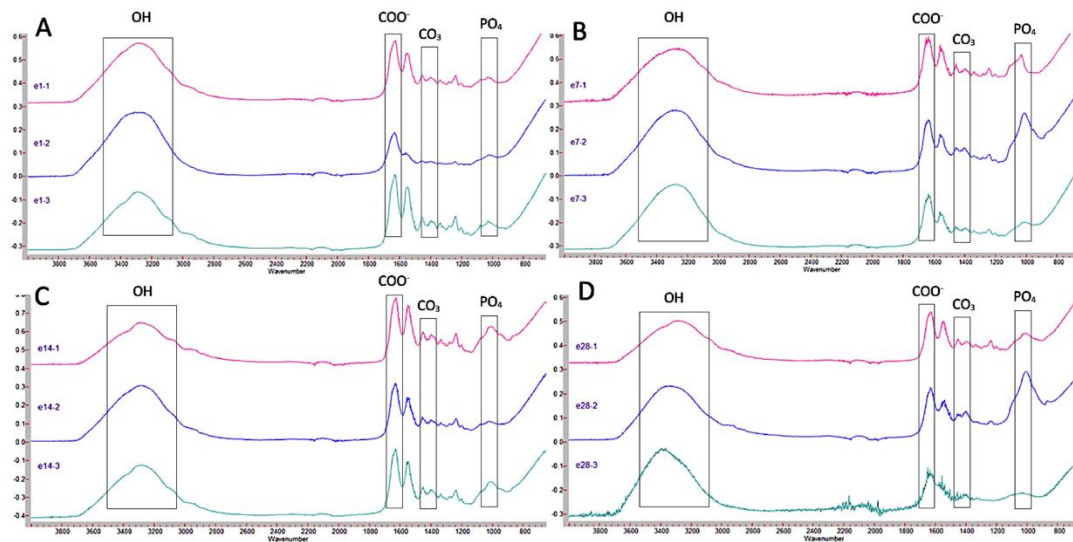


Figure 5. Comparison of Equia Forte FTIR spectra at different thicknesses (1, 2, and 3 mm) at 24 h (A), 7 days (B), 14 days (C), and 28 days (D). The peaks of the OH, COO[−], CO₃, and PO₄ groups of interest in this study are indicated.

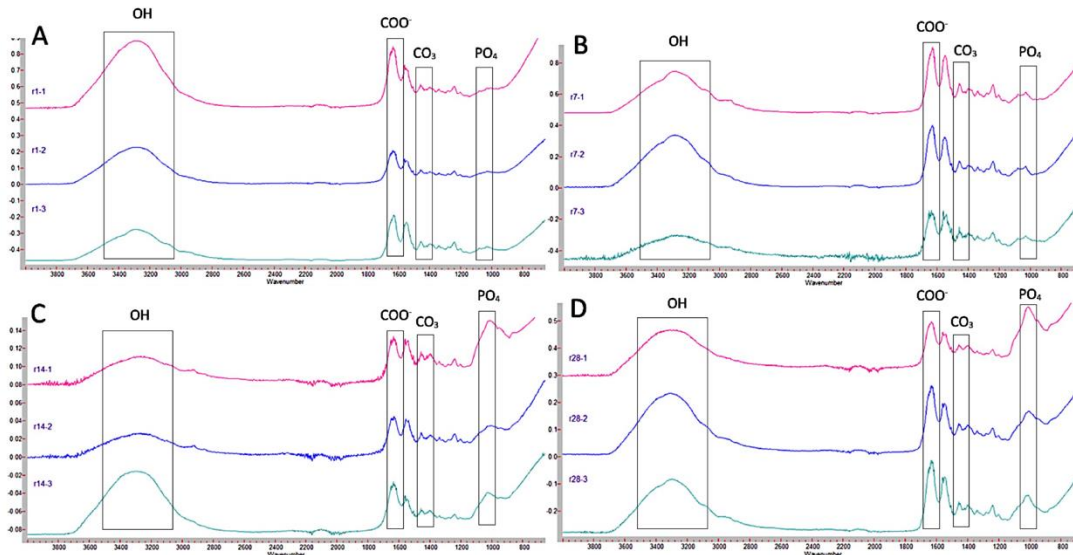


Figure 6. Comparison of Riva Light Cure FTIR spectra at different thicknesses (1, 2, and 3 mm) at 24 h (A), 7 days (B), 14 days (C), and 28 days (D). The peaks of the OH, COO[−], CO₃, and PO₄ groups of interest in this study are indicated.

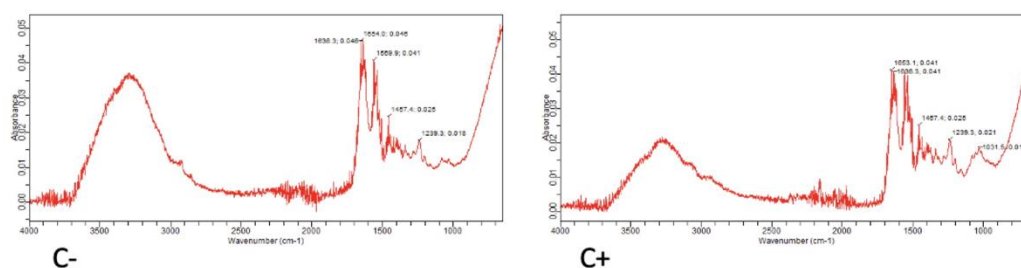


Figure 7. FTIR spectrum of the negative control (C−), corresponding to demineralized dentin, and positive control (C+), corresponding to healthy dentin.

3.2. Energy Dispersive Spectroscopy (EDX)

The Ca/P ratios (mol) were established for the control samples (1.47 for healthy dentin (positive control) and 1.40 for demineralized dentin (negative control)) and for the test groups (Table 2).

Table 2. Ca/P ratio (mol) calculated from EDX results by material, thickness, and time.

	1 Day	7 Days	14 Days	28 Days
KM 1 mm	2.23 ± 0.6	2.46 ± 0.7	2.84 ± 0.9	1.85 ± 0.8
KM 2 mm	1.52 ± 0.3	1.24 ± 0.2	1.93 ± 0.4	1.76 ± 0.5
KM 3 mm	1.17 ± 0.1	1.94 ± 0.4	2.12 ± 0.5	1.65 ± 0.6
EF 1 mm	1.09 ± 0.2	2.01 ± 0.7	1.01 ± 0.1	1.74 ± 0.5
EF 2 mm	2.21 ± 0.9	2.37 ± 0.8	2.12 ± 0.3	1.94 ± 0.2
EF 3 mm	1.91 ± 0.7	2.16 ± 0.6	1.72 ± 0.2	1.11 ± 0.4
RL 1 mm	1.98 ± 0.6	1.34 ± 0.5	1.83 ± 0.4	2.01 ± 0.5
RL 2 mm	1.65 ± 0.5	2.44 ± 0.7	1.69 ± 0.5	1.94 ± 0.6
RL 3 mm	2.09 ± 0.6	1.94 ± 0.3	1.72 ± 0.3	1.11 ± 0.2

Figure 8 shows the graphs obtained from the EDX analysis of the pure materials at 28 days.

The percentage change in the Ca/P ratio (mol) was calculated for each of the materials by thickness and time compared to the positive and negative controls, respectively (healthy dentin, C+; and demineralized dentin, C−).

Table 3 shows the percentage change in the Ca/P ratio (mol) of dentin with the different materials, times, and dentin thicknesses compared to the non-demineralized dentin. In the dentin treated with Riva Light Cure and Equia Forte, the highest percentage of remineralization was found at 2 mm and 7 days after application, with no significant differences between them, nor with Ketac Molar at 1 mm at 7 days ($p > 0.05$). However, in Ketac Molar, the highest remineralization was found at 1 mm thickness at 14 days after application, significantly higher than that found in the other two materials ($p < 0.05$). At 3 mm, Riva Light Cure and Equia Forte did not show significant differences in Ca/P gain at 1, 7, and 14 days ($p > 0.05$). At 28 days, at 3mm depth, no gain was found with these materials. In Ketac Molar, Ca/P gain was always found at 1 mm, whereas at the remaining depths, the behavior was more erratic.

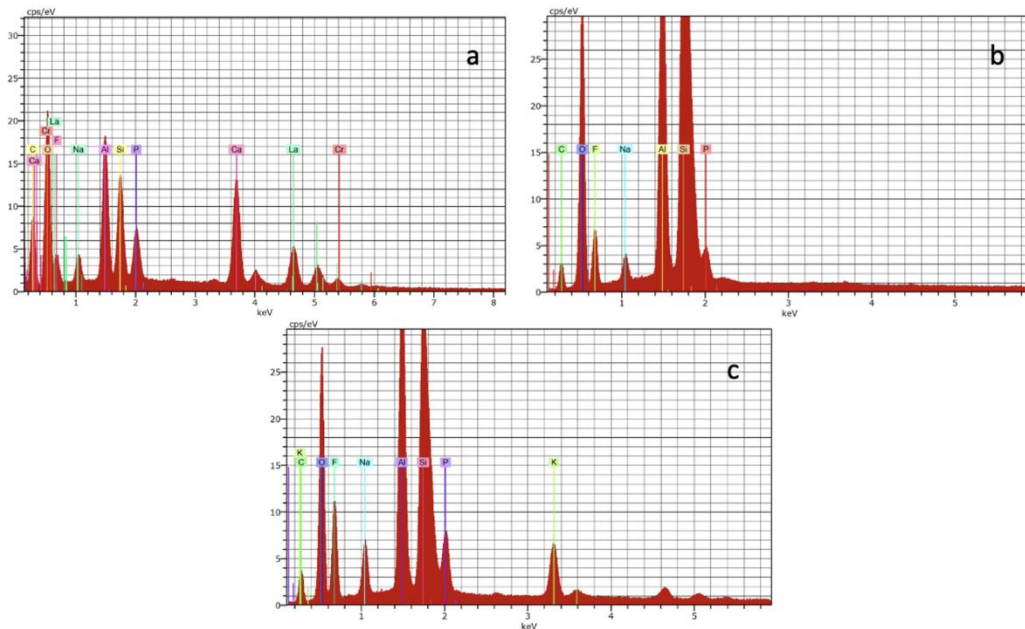


Figure 8. EDX analysis graphs of Ketac Molar (a), Riva Light Cure (b), and Equia Forte (c) pure at 28 days.

Table 3. Percentage gain in Ca/P ratio (mol), calculated from EDX results, in dentin as a function of different materials, times, and thickness compared to non-demineralized dentin.

	1 Day	7 Days	14 Days	28 Days
KM 1 mm	34.10	40.15	48.18	20.54 ^a
KM 2 mm	3.50	−18.33	23.88	16.70 ^a
KM 3 mm	−25.16	24.09	30.65	10.69
EF 1 mm	−35.05	26.89	−45.48	15.58
EF 2 mm	33.52	38.00	30.65	24.41
EF 3 mm	22.89	31.90	14.59	−32.68
RL 1 mm	25.63 ^a	−9.59	19.49	26.91 ^a
RL 2 mm	10.94	39.82	12.90 ^a	24.41 ^a
RL 3 mm	29.80 ^a	24.09	14.59 ^a	−32.68

The same letter in the superscript indicates no significant differences between thicknesses for each material and time.

Table 4 shows the percentage of gain in the Ca/P ratio of dentin by the materials, times, and dentin thicknesses compared to demineralized dentin. It was also observed that in the dentin treated with Riva Light Cure and Equia Forte, the highest percentage of remineralization was found at 2 mm and 7 days after application, without significant differences ($p > 0.05$). However, in Ketac Molar, the highest remineralization was found at 1 mm 14 days after application, with significant differences compared to the other two materials ($p < 0.05$). Ketac Molar and Equia Forte did not show significant differences between the percentage increase in the Ca/P ratio at 1 mm between 1 day and 14 days after application ($p > 0.05$). The same occurred with Equia Forte at 2 mm and Riva Light Cure at 3 mm.

Table 4. Percentage gain in Ca/P ratio (mol), calculated from EDX results, in dentin by materials, times, and thickness compared to demineralized dentin.

	1 Day	7 Days	14 Days	28 Days
KM 1 mm	37.24	43.00	50.64	24.33 ^a
KM 2 mm	8.10	−12.69	27.51	20.66 ^a
KM 3 mm	−19.20	27.70	33.96	14.94
EF 1 mm	−28.62	30.37 ^a	−38.55	19.60
EF 2 mm	36.68	40.96	33.95	28.01
EF 3 mm	26.56	35.14 ^a	18.66	−26.36
RL 1 mm	29.18	−4.37	23.32	30.40 ^a
RL 2 mm	15.18	42.68	17.05 ^a	28.01 ^a
RL 3 mm	33.14	27.70	18.66 ^a	−26.36

The same letter in the superscript indicates no significant differences between thicknesses for each material and time.

4. Discussion

Following the criteria of “minimal intervention” are the techniques of “selective caries removal” [20]. The application of these techniques requires the use of bioactive materials for the restoration. A bioactive material must induce a favorable response, which requires an interaction between the material and the dental tissues, leading to the formation of mineralized tissue [21]. Thus, the treatment of deep caries lesions involves the use of ion-releasing agents to induce the remineralization of demineralized dentin through the formation of apatite [22].

FTIR is a spectroscopic assay by which the remineralizing potential of a material can be examined, as it allows the identification of different functional groups. The presence of peaks in the zone around 1020 cm^{-1} indicates the presence of phosphate groups, which, in this case, are usually PO_4 groups. The presence of other peaks in the 1420 cm^{-1} region indicates that carbonate groups such as CO_3 are present. The association of these two peaks implies the presence of carbonated hydroxyapatite, which is expected in the dentin remineralization process [23–27].

After the analysis of the results, the null hypothesis was rejected, since materials, time, and dentin thickness influenced the remineralizing ability of the different GICs included in the present study.

After 1 day, only Ketac Molar was able to form apatite and only to a depth of 1 mm. After 7 days, Ketac Molar achieved a homogeneous effect in all thicknesses, whereas Equia Forte only achieved remineralization up to 2 mm, with a slightly greater effect at 2 mm compared to 1 mm. Finally, Riva Light Cure showed slight peaks, but did not clarify the presence of apatite at any thickness. These results were expected, since Ketac Molar had already performed better in shorter periods.

After 14 days, all the materials showed a notable presence of carbonate apatite at 1 mm higher than the greater thicknesses, except in the case of Equia Forte, where a peak of greater intensity appears at 2 mm. It should be noted that in Ketac Molar, at 2 and 3 mm thicknesses, the presence of apatite is lower than at 7 days.

Finally, at 28 days, apatite was present at all depths with all materials. Equia Forte achieved a greater presence at 2 mm, whereas Ketac Molar did so at 1 mm. Riva Light Cure showed similar results at all depths.

The non-linear remineralization behavior of Ketac Molar and Equia Forte should be highlighted. In Ketac Molar, the only constant is that its main effect is seen at 1 mm depth, whereas Equia Forte, at 7 and 28 days, shows more apatite formation at 2 mm. By contrast, Riva Light Cure shows an increase in apatite directly related to the exposure time, which was constant in the three dentin thicknesses.

On the other hand, the SEM-EDX system allows knowing the amount of calcium and phosphate present in the sample, either in simple (the pure element) or complex form (as

part of a more complex compound). With this information, the Ca/P ratio of each of the analyzed samples can be established.

According to other published studies, the increase in the Ca/P ratio is an important indicator of remineralization [28], since it allows establishing whether a material can be effective when applied on demineralized dentin in accordance with current trends of minimal intervention.

Regarding the studied materials, it is noteworthy that Ketac Molar performed better at 1 mm thickness, whereas Riva Light Cure and Equia Forte showed better results at 2 mm. This indicates that the latter two materials can remineralize at a greater depth than the former. However, at 3 mm thickness, all the materials show a decrease in the Ca/P ratio, highlighting that at 28 days, both Equia Forte and Riva Light Cure show a Ca/P ratio below that of the control. As such, at this depth and time, no remineralization is showed by the samples.

The fact that one of the studied GICs incorporates resin in its composition (Riva Light Cure) did not affect dentin remineralization, neither in terms of hydroxyapatite formation nor the Ca/P ratio found at different depths. Its behavior was very similar to that obtained by Equia Forte, which does not incorporate resin. Resin incorporation did not reduce the remineralization potential and depth achieved. Thus, from a clinical point of view, the use of a RMGIC that in addition to the chemical bonding (achieved by polycarboxylates), can also achieve micromechanical interlocking in hybridized dentine through the infiltration of the collagen network that is exposed by polyacrylic acid [29], is a good alternative in minimally invasive dentistry procedures.

When focusing on other elements, a striking fact is that despite being glass ionomers, which are materials that contain fluoride [30], in our samples, it was only detected in pure material samples, as shown in Figure 8. Its presence in the samples at 1, 2, and 3 mm thickness was detected sporadically at certain times and thicknesses and did not follow any common pattern. Consequently, this could be a subject for future study.

Strontium is indicated as present in the chemical formulation of Riva Light Cure and Equia Forte by their respective manufacturers. However, it was not detected in Equia Forte in any case. In Riva Light Cure, it was only detected at 28 days in 1 mm thickness and in the pure material. Strontium has chemical and physical properties similar to those of calcium, so it is theoretically capable of replacing Ca in hydroxyapatite [31]. Studies using strontium-doped bioactive glasses show less demineralization of enamel and dentin, but no better remineralization than bioactive glasses without strontium [32].

Regarding the limitations of the present study, a series of points should be highlighted: firstly, its inherent *in vitro* nature, which limits the application of the results to the clinical setting and requires further evidence to confirm the reported results. Secondly, the absence of a standardized methodology for this type of studies results in a substantial heterogeneity among similar studies. For example, the sample size for test groups varies among studies in the field [16,18,19,33]. In the present study, a sample size of $n = 3$ was selected for each group. The low sample size, both in the present study and similar studies, may reduce the representativeness of the results. Thus, results should be interpreted as a preliminary assessment of the remineralization ability of GICs at a laboratory level and should serve as a base for future studies.

Nevertheless, the results of this study can guide clinicians in the thickness of demineralized dentin that can be left in the cavity, as well as in the choice of GIC, depending on the clinical situation. On occasions where a rapid remineralization effect is required in minimal thicknesses, Ketac Molar will be suitable, whereas if the objective is to achieve a deeper remineralization, Riva Light Cure and Equia Forte show better results.

5. Conclusions

Carbonate apatite was found by FTIR at 28 days at all depths with all tested materials. Equia Forte achieved the greatest effect at 2mm depth, whereas Ketac Molar achieved the greatest effect at 1 mm. Riva Light Cure showed similar results at all depths.

In terms of Ca/P ratio, Equia Forte and Riva Light Cure showed similar results at all depths. Equia Forte and Riva Light Cure showed a higher dentin remineralization at 2 mm thickness, whereas Ketac Molar performed better at 1 mm thickness.

In terms of time, the formation of carbonate apatite was higher at 24 h and 7 days for Ketac Molar. However, at 14 days, it was reduced in this material and reached its peak in Riva Light Cure and Equia Forte.

According to the results of this study, long-term remineralization was not constant at 3 mm, making it inadvisable to leave such a large thickness of demineralized dentin.

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Article

Does Silver Diamine Fluoride Affect the Adaptation of High-Viscosity Resin-Modified Glass Ionomer to Dentin? An In Vitro Study

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Abstract: Background: The aim of this study was to evaluate the adaptation of a resin-modified glass ionomer to the internal walls of dentin with different conditioning systems, with or without the application of silver diamine fluoride (SDF) and potassium iodide (KI). Methods: Cervical standardized cavities were prepared on the buccal and lingual sides of 15 extracted molars. Molars were then sectioned longitudinally in a buccolingual direction, obtaining two samples from each tooth with two cavities each (60 samples). Samples were randomly divided into three groups ($n = 20$). Each group was divided into two subgroups. One subgroup did not receive dentin conditioning, one was conditioned with 25% polyacrylic acid—10 s (PA), and one with 37% orthophosphoric acid—5 s (OPA). In the other subgroup, dentine was treated with SDF/KI and not conditioned or conditioned with PA or OPA. All cavities were filled with Riva Light Cure® (RLC). The adaptation of the RLC to the cavity walls was evaluated by SEM at 100× magnification. The area of maximum interfacial gap was magnified at 1000× to measure its size. Kruskal–Wallis and Mann–Whitney U test were used for comparison. A significance level of $\alpha = 0.05$ was used. Results: No significant differences in the percentage of well-adapted samples were found in subgroups when SDF/KI was used, regardless of whether conditioner was used or not and whether PA or OPA was used ($p > 0.05$). Regardless of the gap size, dentin treatment with SDF/KI did not negatively influence ionomer adaptation to dentin walls significantly ($p > 0.05$), except for the subgroup conditioned with OPA ($p < 0.05$). Furthermore, it improved the adaptation in the axial wall of the subgroup without dentin conditioning ($p < 0.05$). Conclusion: According to the results of the present in vitro study, the use of SDF/KI did not affect RLC adaptation to the cavity walls. Subsequent use of a conditioner worsens the adaptation of the material to the cavity walls.

Keywords: dentin conditioning; silver diamine fluoride; Riva Light Cure; orthophosphoric acid; polyacrylic acid; resin-modified glass ionomer cement



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1. Introduction

Physiologically and anatomically, dentin is a complex structure, consisting of approximately 70% hydroxyapatite crystals, 18% organic matter, and 12% water and plasma-like fluids. The dentin–pulp complex is derived from the ectomesenchyme, structurally forming a true biological and functional unit [1].

Adhesion to dentin is considered more complex than adhesion to enamel due to its organic component and the presence of a smear layer that obstructs the entrance of the dentinal tubules, decreasing their permeability to adhesive systems and liners. The smear layer should be treated or eliminated, depending on the different bonding strategies and characteristics of the materials to be used [2]. Glass ionomers can chemically bond to dentin due the ion exchange layer at the interface formed by continuous diffuse of aluminum, silicon, fluorine, and strontium when present in the parent glass ionomer, and calcium and phosphorus from dentin [3].

Since Powis et al. [4] introduced polyacrylic acid, it has been used as a conditioner to improve the adhesion of glass ionomers. It contains numerous functional carboxyl ions, which form hydrogen bonds that promote substrate wettability. Its use is recommended at a concentration of 10%, 20%, or 25% [5]. Another alternative is the use of orthophosphoric acid (OPA) at concentrations around 37.5%, between 5 and 10 s [6]. Acid conditioning has been shown to improve the bond strength of glass ionomers to the dentin substrate [6–10]. In addition, the type of conditioner, its application time, and concentration can also influence bond strength [11].

Glass ionomer cements (GIC) are biomaterials that belong to the family of acid-base cements. They are the product of the reaction of weak polymeric acids with phase-separated amorphous sodium–fluorine–aluminum–silicate glass powder [12]. They set in the presence of water and the final structure contains concentrations of unset glass that act as reinforcement. They contain three fundamental components: a soluble polymeric acid, a basic glass, and water. The glass is a complex material consisting of fluorine, calcium or strontium, aluminum silicate, and phosphorus [13]. The polymeric acid solution is mainly polyacrylic acid, although maleic and itaconic acid may be present in lower concentrations to improve handling and increase working time. They set by means of an acid-base reaction 2–3 min after mixing, although the reaction can last up to 48 h. The bioactivity of glass ionomers is produced by an ionic exchange between the material and the substrate that can be maintained over time, with an initial rapid early release phase, followed by a sustained phase [14].

Resin-modified glass ionomer cements (RMGICs) contain the same essential components as conventional GICs, but incorporate a monomer, usually 2-hydroxyethyl methacrylate (HEMA), and a photoinitiator. Therefore, they exhibit two setting reactions: light-curing and the characteristic acid-base reaction of glass ionomers [15].

High density GICs are materials of very high viscosity, whose glasses have been improved by the addition of strontium, bioactive glasses, or even zirconium. This results in a reduction in their working and hardening times, and a notable improvement in their physical–chemical and mechanical properties, together with a minimum solubility. They can be self- or light-cured [16].

Silver diamine fluoride (SDF) is a cost-effective and easy-to-use alternative for the remineralization of carious dentin (13). SDF is presented in an alkaline colorless liquid form (pH 10), composed of 24–27% silver (Ag), 8.5–10.5% ammonia (NH₃), and approximately 38% fluoride (F) [9]. Currently, a triple mechanism of action of SDF against caries lesions is described: Firstly, due to the high concentrations of fluoride, a greater diffusion is achieved in enamel and dentin, favoring their remineralization. Secondly, silver possesses a bactericidal action and remains in the demineralized and hypomineralized areas, hardening them and preventing their reinvasion by means of a series of metabolites. Thirdly, ammonia stabilizes the solution and maintains the pH [17]. Furthermore, in dentin, SDF favors the maintenance the collagen matrix's integrity due to its ability to inhibit bacterial metalloproteinases, which play an important role in the enzymatic degradation of collagen. Potassium iodide (KI) decreases blackening of dentin caused by SDF [18].

The combined use of SDF/KI and bioactive restorative materials such as GICs has been proposed in minimally invasive procedures for the treatment of deep carious lesions, aiming to preserve viable demineralized dentin by exploiting their remineralization ability [19].

One problem that could arise is the adaptation of GICs to cavity walls and their bond strength to SDF/KI-treated dentin. A recent systematic review concludes, not without highlighting the methodological disparity among the included studies, that the application of SDF/KI has no adverse impact on bond strength [20]. Interestingly, some studies even indicate that it increased the bond strength of GICs on SDF/KI-treated dentin [10].

To our knowledge, there are no studies that evaluate the adaptation between RMGICs and dentin when the latter is treated with SDF/KI, nor the influence of the use or absence of a dentin conditioning system before the application of the ionomer. Accordingly, the objective of the present in vitro study was to evaluate the adaptation of a high-viscosity

resin-reinforced glass ionomer (Riva Light Cure®) to dentin walls treated with different conditioning systems, with or without the application of SDF/KI, by means of scanning electron microscopy.

The null hypothesis was that the use of SDF/KI does not influence the adaptation of the glass ionomer to cavity walls regardless of dentin conditioning.

2. Materials and Methods

2.1. Sample Preparation

An experimental *in vitro* study was devised on human molars whose extraction was indicated for periodontal or surgical reasons from donors aged between 40 and 60 years old. The protocol for this study was approved by the Ethics Committee of the Universitat de València (ref. 158063760605607). Written informed consent was obtained from the donors prior to the use of the tooth samples in the present study. The materials used for the present study are described in Table 1

Table 1. Material characteristics.

Material	Composition	Manufacturer, City, Country	Batch n°
Riva Light Cure	Liquid: Polyacrylic acid, tartaric acid, HEMA Powder: Bioactive glass (Longlass™), contains fluoride and strontium ions	SDI, Bayswater, Australia	873003
Riva Conditioner	Polyacrylic acid 25–30% in weight	SDI, Bayswater, Australia	334601
Gel Etchant	Orthophosphoric acid 37.5% in weight	Kerr, Bioggio, Switzerland	200317
Riva Star	Silver diamine fluoride: 38% silver fluoride, 15–20% anhydrous ammonia, and water. Potassium iodide	SDI, Bayswater, Australia	11557551

Once extracted, the teeth were preserved at room temperature in a 0.1% thymol solution for one week. Organic debris was removed with ultrasonic tips and curettes. The teeth were then inspected under magnification and 15 molars free of caries, cracks, or any other structural defect were selected. They were preserved in physiological saline solution until their use at 4 °C, within 6 months of extraction.

In each tooth, two class V cavities were prepared, one on the buccal side and one on the lingual/palatal side, with the coronal margin in enamel and the apical margin in dentin (1 mm apically from the dentinoenamel junction). The cavity proportions were as follows: 4 mm in length by 4 in width by 2 in depth (2 × 4 × 4). An ISO 835 diamond bur was used to perform the cavities, which was replaced after preparing five cavities. A schematic representation of the cavities view is shown in Figure 1A. Once the cavities were prepared, the teeth were sectioned longitudinally in a buccal/lingual–palatal direction with a diamond disc (ISO 806 104356524 220 Komet, Lemgo, Germany) until the cavity limit was reached, at which point they were separated by fracture by using a dental elevator (Figure 1B). In this way, two samples from each molar with two cavities each were obtained ($n = 30$ molar halves; $n = 60$ cavities).

Twenty molar halves were randomly assigned to a group, accordingly with the dentin-conditioning procedure (25% polyacrylic acid (PA) for 10 s or 37% orthophosphoric acid (OPA) for 5 s). The 10 remaining molar halves were the control group, which received no dentin conditioning. Each group was divided in two subgroups. At the same time, SDF/KI were applied to one of the cavities from each half (subgroup). The distribution and process of the different conditioning procedures is shown in Table 2.

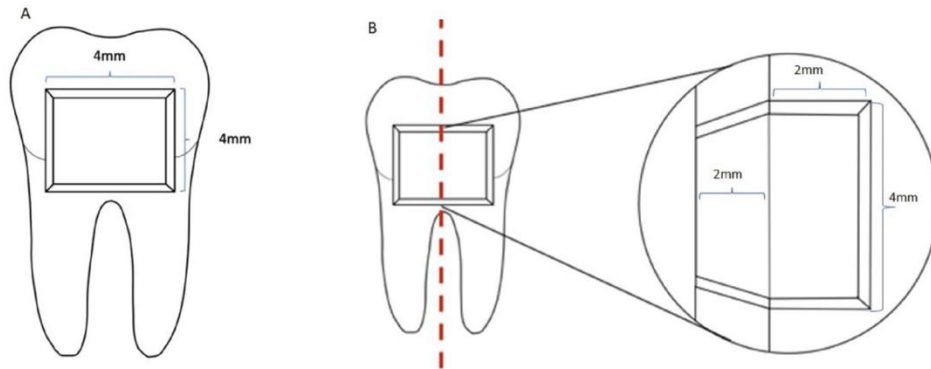


Figure 1. Cavity design (A); longitudinal view of the cavities after molar sectioning (B).

Table 2. Study subgroups accordingly dentin conditioning procedures and SDF/KI application ($n = 10$ cavities) in each subgroup.

GIC (Control)	Glass Ionomer Cement. Application in One Increment and Light Curing for 20 s
SDF/KI + GIC	SDF application for 1 min, KI application for 2 min, cavity rinsing, GIC application in one increment and light curing for 20 s.
PA + GIC	25% Polyacrylic acid. Dentin conditioning for 10 s, cavity rinsing and mild drying, GMIC application in one increment and light curing for 20 s.
PA + SDF/KI + GIC	SDF application for 1 min, KI application for 2 min, cavity rinsing, dentin conditioning with 25% PA for 10 s, cavity rinsing and mild drying, GIC application in one increment and light curing for 20 s.
OPA + GIC	37% Orthophosphoric acid. Dentin conditioning for 5 s, cavity rinsing and mild drying, GIC application in one increment and light curing for 20 s.
OPA + SDF/KI + GIC	SDF application for 1 min, KI application for 2 min, cavity rinsing, dentin conditioning with 37% OPA for 5 s, cavity rinsing and mild drying, GIC application in one increment and light curing for 20 s.

Once the cavity was conditioned according to the procedure described in Table 2, the Riva Light Cure[®] was applied. The capsule was first activated, vibrated for 10 s at 4800 rpm, placed in the applicator, and placed in the cavity. The material was adapted to the walls using a plastic instrument, an acetate strip was placed on the surface of the material and light cured with a 1500 mW/cm² LED lamp (Masters, Madrid, Spain) for 20 s.

The samples were kept at room temperature for 24 h, each specimen was stored individually in labelled Eppendorf tubes with PBS, and then thermocycled. The thermocycling process was performed manually by immersing the specimens alternately in a cold-water bath at a temperature of 5 °C and hot water at 55 °C alternately for 30 s for 1500 cycles. The bath used was Fisherbrand[™] (Fisher Scientific SL, Madrid, Spain).

2.2. Sample Visualization under Scanning Electron Microscopy (SEM)

The specimens for each group were dehydrated, mounted on aluminum stubs, and then gold-sputter coated by the Polaron Range SC7640 Sputter Coater (Quorum Technologies LTD, Ashford, Kent, UK). Scanning electron microscopy (SEM) (Hitachi S-4800, Hitachi High-Technologies Corporation, Japan) was used to assess the marginal adaptation of the RLC to the cavity walls. SEM was set on 10 kV for both magnifications (100× and 1000×). At 100×, the interface between the restorative material and dentin was observed along all cavity walls (coronal, axial, and cervical). At any point where interfacial gap was visible on each wall, the image was magnified and the maximum maladaptation at 1000× was measured in µm (Figure 2). The measurement was performed from the surface

of the material to the surface of dentin, using the SEM visualization software, as shown in Figure 2c.

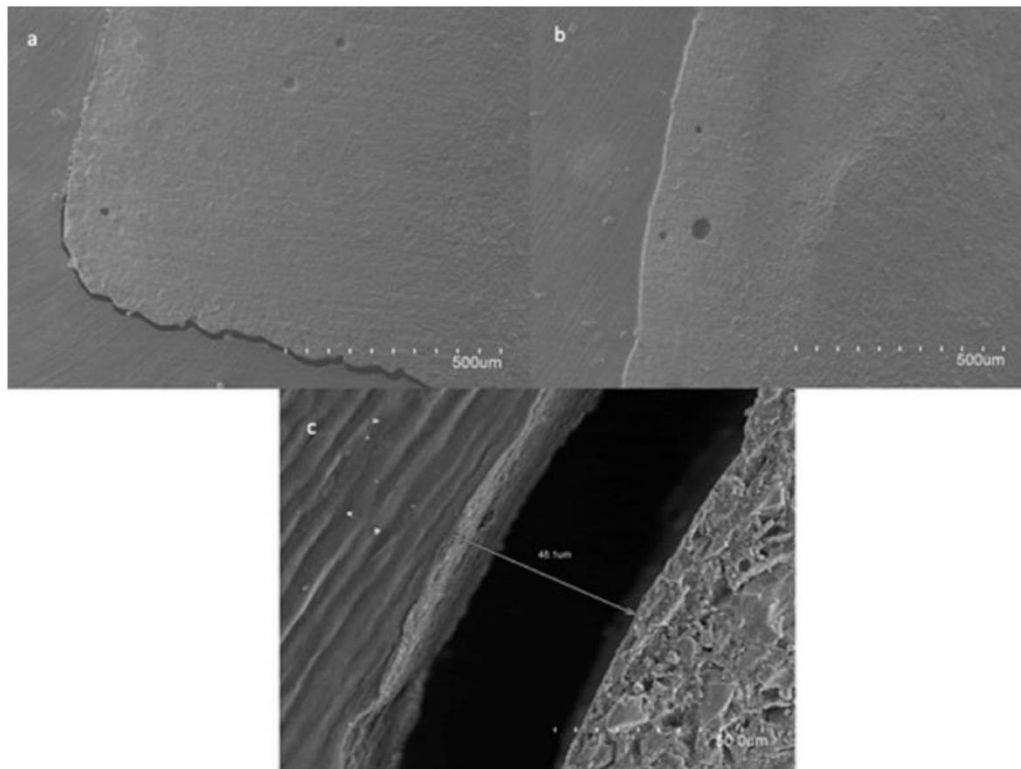


Figure 2. Representative SEM images of the samples. Images at 100× magnification with interfacial gap (a) and without interfacial gap (b), and image at 1000× magnification measuring the maximum interfacial gap (c). Scale bar: the distance between two points represents 50 μm in panels a and b (magnification ×100), and 5 μm in figure c (magnification ×1000).

2.3. Statistical Analysis

The interfacial gap values for each wall and cavity, measured in micrometers (μm), did not follow a normal distribution (Kolmogorov–Smirnov). The mismatch values, as a function of the dentin conditioning system for each wall, were compared using the Kruskal–Wallis test. Two-by-two comparisons were made with the non-parametric Dunn’s test. The Mann–Whitney U test was used to compare the interfacial gap values in the mesial and distal cavities of each molar sample, one of which had been treated with SDF/KI. The percentage of samples with interfacial gap in each subgroup was compared by means of χ^2 test. SPSS 25.0 statistical package (IBM Inc., Chicago, IL, USA) was used for the analysis. A significance level of $\alpha = 0.05$ was used in the calculations.

3. Results

3.1. Influence of SDF/KI in RLC Adaptation

In all samples in which no dentin conditioning procedure was used, regardless of whether SDF/KI was applied or not, a gap between GIC and the cavity walls was observed. In the samples in which PA was used, gaps were observed in 80% of the samples in which SDF/KI was not used, and in 90% of those in which it was used ($p = 0.237$). In the samples

in which dentin was conditioned with OPA, gaps were observed in 77.8% of the samples in which SDF/KI was used and in 62.5% of those in which it was not used ($p = 0.21$). No significant differences were found in the global percentage of samples with gaps with regards to the dentin conditioning system ($p > 0.05$). According to these results, the null hypothesis could not be rejected.

Table 3 shows the mean, standard deviation, median, and interquartile range for the interfacial gap values in each study subgroup. The gap in the cavities in which SDF/KI was used were similar to those in the cavities in which it was not used. In the coronal wall the OPA + GIC subgroup showed a significantly lower gap than the OPA + SDF/KI + GIC subgroup ($p < 0.05$). The same was observed for the total interfacial gap. In the axial wall, the SDF/KI + GIC subgroup showed a significantly lower mismatch than the GIC subgroup ($p < 0.05$). In all subgroups the lowest median interfacial gap values were found in the axial and cervical walls.

Table 3. Interfacial gap values in μm .

		Median	IQR	Mean	SD	<i>p</i>
Coronal	GIC	25.30	37.95	32.26	22.44	0.72
	SDF/KI + GIC	34.25	54.17	43.04	34.23	
	PA + GIC	52.50	68.05	58.04	44.03	0.11
	PA + SDF/KI + GIC	40.10	26.85	41.99	32.27	
	OPA + GIC	00.00	24.58	8.95	14.05	0.04
	OPA + SDF/KI + GIC	63.50	103.2	57.59	54.91	
Axial	GIC	29.10	14.70	34.17	17.09	0.04
	SDF/KI + GIC	04.93	32.37	21.70	37.19	
	PA + GIC	00.00	36.70	15.58	20.05	0.35
	PA + SDF/KI + GIC	12.50	50.30	37.54	57.77	
	OPA + GIC	00.00	00.00	03.25	09.19	0.42
	OPA + SDF/KI + GIC	00.00	110.7	42.04	59.94	
Cervical	GIC	19.30	15.71	16.19	08.56	0.63
	SDF/KI + GIC	07.38	51.87	29.05	41.15	
	PA + GIC	00.00	34.15	12.14	18.39	0.84
	PA + SDF/KI + GIC	00.00	83.20	28.76	43.25	
	OPA + GIC	00.00	19.22	8.20	11.61	0.37
	OPA + SDF/KI + GIC	00.00	99.50	40.34	58.05	
Total	GIC	21.44	20.42	27.15	12.70	0.54
	SDF/KI + GIC	18.47	43.02	31.26	30.37	
	PA + GIC	37.40	31.00	28.59	17.70	0.66
	PA + SDF/KI + GIC	29.46	33.02	36.10	36.73	
	OPA + GIC	04.70	13.82	06.80	07.61	0.04
	OPA + SDF/KI + GIC	46.00	80.18	46.66	42.15	

3.2. Comparison between Groups

In Figure 3a, it can be observed that, in the coronal wall, the OPA + GIC subgroup showed a significantly lower interfacial gap than the rest of the subgroups. In the axial wall, the interfacial gap was significantly lower in subgroups in which dentin conditioning with PA or with OPA was used, and in the SDF/KI + GIC subgroup, than in the GIC subgroup (Figure 3b). In the cervical wall from the subgroup in which the dentin was conditioned with OPA, significantly less interfacial gap was found than in GIC subgroup (Figure 3c).

Finally, when comparing the mean gap in all the walls, the OPA + GIC subgroup again showed lower gap values than the rest of the subgroups (Figure 3d).

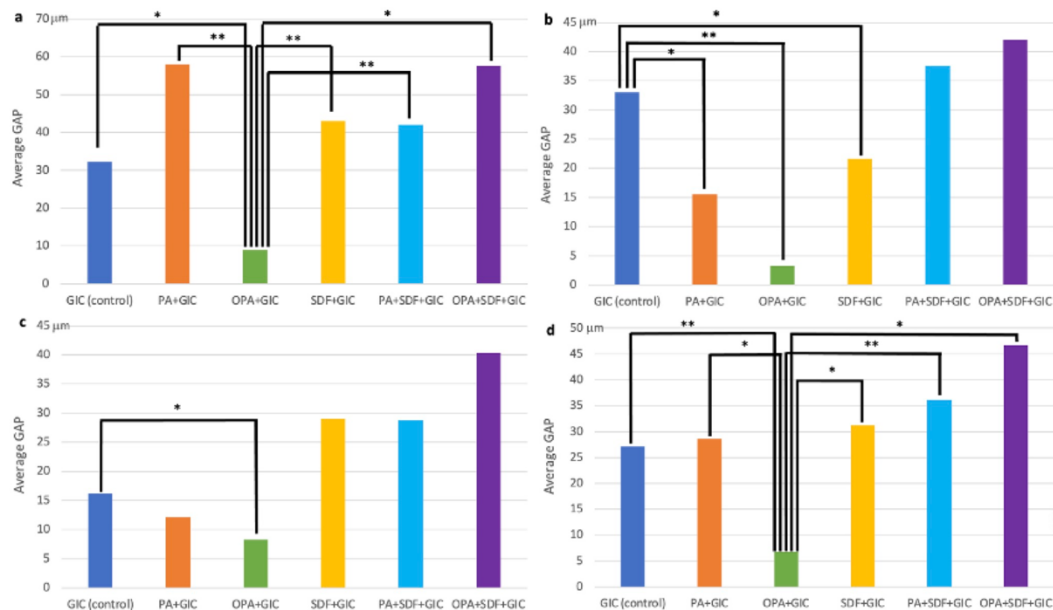


Figure 3. Gap size comparison between the study subgroups. (a) Coronal wall, (b) axial wall, (c) cervical wall, (d) mean. Statistically significant differences are indicated with an asterisk: * $p < 0.05$; ** $p < 0.01$.

4. Discussion

GICs are suitable materials for restorative procedures where there are no substantial mechanical requirements, with a highlighted application in “atraumatic restorative techniques” (ART) and in minimally invasive restorative procedures [21]. The current paradigm for the treatment of dental caries involves the approach from a biological perspective, involving the use of therapies based on the application of fluorides, calcium, phosphates, or other bioactive components. The surgical–restorative approach is reserved for deep cavitated lesions, following the criteria of the selective removal of carious dentin in combination with the use of materials with remineralizing, restorative, and/or bioinductive potential [22].

Although some studies show that RMGICs are less biocompatible than conventional ionomers [23], others claim that the pulpal inflammatory response and odontoblastic changes produced are similar to those produced by conventional GICs [7].

The adaptation of the restorative material to the cavity walls is essential for a correct sealing of the interface between the material and the substrate to avoid microleakage. Therefore, the study of the adaptation of the material to the cavity walls is an adequate criterion to evaluate interfacial sealing. The use of SEM makes it possible to evaluate the adaptation of the material around the entire perimeter and to accurately measure the possible presence of gaps [5,24]. One drawback is the possible removal of the material from the cavity walls caused by the drying of the samples and the vacuum of the SEM. However, direct scanning of the samples was selected in this study because indirect epoxy resin replicas showed some disadvantages, such as time consumption. It is also more likely that areas with excess resin or voids will remain. Furthermore, even when specimens can be replicated to preserve fidelity or to withstand the SEM vacuum conditions, it is difficult

to determine the extent to which the structures of interest have been masked or altered by the preparation or replication process [25].

In this study, we aimed to evaluate the adaptation of a high-viscosity RMGIC to cavity walls with different dentin conditioning systems followed by the use or absence of SDF/KI. For this purpose, Riva Light Cure[®] was used. This material replaces dentine and chemically bonds to the tooth. Accordingly, following the manufacturer's recommendations, no etching or bonding is required. In addition, since it is a high-density material, it can be used in restorations that need to support load [20].

A study that evaluated the interface between Riva Light Cure[®] and dentin treated with different conditioning systems found that etching with 37% OPA for 5 s or with PA for 10 s reported the presence of resin tags inside the dentin tubules, while when GIC was applied without prior conditioning, an empty interface was found between the restorative material and the dentin [6]. In the present study, it was decided to use these same dentin conditioning times and systems. It should be noted that, although interfacial gaps were found in all samples regardless of the conditioning system used, when OPA was used for 5 s, the gaps were significantly lower in all walls ($p < 0.05$). This is probably due to the infiltration of the resin component of Riva Light Cure[®] into the partially demineralized dentin. This short treatment time allows partial demineralization without a significant loss of calcium ions that could negatively affect the ionic bonding of the material to the dentin substrate [10].

Tanumiharja et al. [9] evaluated the adaptation of conventional GICs and RMGICs with and without prior conditioning with PA, finding similar adaptation with the two types of GICs with and without conditioning. However, they were able to identify hybridization areas when PA was used. In the present study, conditioning with PA alone significantly improved adaptation in the axial wall ($p < 0.05$).

The RMGIC had a thermal expansion coefficient of $25.4 \times 10^{-6} \text{ }^{\circ}\text{C}^{-1}$, which was quite high compared with the tooth structure. Therefore, after thermocycling, there were slight changes in marginal adaptation [26]. The samples in this study were subjected to thermocycling to simulate oral cavity conditions, although our samples exposed cavity walls that are not exposed under physiological conditions. This may have influenced our results, constituting a limitation of this study.

This study aimed to evaluate the effect of SDF/KI on the adaptation of Riva Light Cure[®] to dentin followed or not by conditioning with 10 s of PA or 5 s or OPA. Other authors have evaluated the influence of SDF/KI on the bond strength of restorative materials to enamel and dentin. A systematic review and meta-analysis [27] concluded that pre-application of SDF/KI does not alter the adhesion of GICs, and even improves adhesion [28]. The results obtained in this study indicate that SDF/KI did not cause significant changes in the presence of interfacial gaps ($p > 0.05$) independently of the dentin conditioning procedure. Even in the subgroup without conditioning, a significantly higher adaptation was found with SDF/KI in the axial wall ($p < 0.05$). These results agree with a recent meta-analysis performed by Fröhlich et al. [12], in which it was found that the adhesion of GICs to SDF/KI-treated dentin did not differ significantly from untreated dentin, even improving in some cases, which could be explained by the chemical adhesion that GICs exhibit with the dentin substrate. In this meta-analysis, no difference was found in GIC adhesion with or without dentin rinsing after SDF/KI placement, as is the case when dentin bonding systems are used. The study by François et al. showed that the interface between a GIC and healthy dentin was not affected by the use of SDF/KI [29].

Lastly, it should be highlighted that SDF was used with a double layer of KI to reduce the dark staining caused by silver. According to the literature reviewed, KI does not seem to influence GICs adaptation [21].

The cavities were cut in half in order to have a sample that could be visualized on SEM once prepared, so as not to disturb the interface between the GIC and the dentine wall. On the other hand, we had samples with identical characteristics in the SDF/KI and non-

SDF/KI subgroups. Although this could be a limitation, as it does not reproduce the clinical situation, we believe that it could allow a better assessment of the interface adaptation.

5. Conclusions

According to the results of the present in vitro study, the use of SDF/KI did not affect the Riva Light Cure[®] adaptation to the cavity walls. The size of gap was higher when SDF/KI was followed by dentin conditioning with OPA. The gap size in the SDF/KI was not affected by the post conditioning with PA. In the axial wall SDF/KI improves adaptation when used without any conditioner. Thus, from a practical point of view, the use of SDF/KI does not interfere with the adaptation of the glass ionomer to the cavity walls. Subsequent use of a conditioner does not improve the adaptation of the material to the cavity walls.

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