Universidad de Granada
Departamento de Estomatología

Estudio *in vitro* de factores que afectan la durabilidad de la adhesión a dentina

Factors influencing resin-dentin bond durability: An *in vitro* study

Tesis Doctoral

Presentada por:

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Que el trabajo de investigación titulado "Estudio *in vitro* de factores que afectan la durabilidad de la adhesión a dentina" del que es autor D. Alberto Albaladejo Martínez, ha sido realizado bajo mi dirección y supervisión, y reúne en su introducción, objetivos y justificación, artículos, discusión, conclusión y resumen los requisitos para su defensa.

Y para que conste y surta efectos en el expediente correspondiente, expido la presente en Granada a cinco de diciembre de dos mil cinco.

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ÍNDICE

I. INTRODUCCIÓN

I.1. Concepto de adhesión y su proyección en Odontología.

La palabra adhesión proviene del latín *ad* y *haerere* y significa unir a (Sainz, 1967).

Adhesión se define como el estado por el que dos superficies se mantienen juntas mediante fuerzas o energías interfaciales basadas en mecanismos químicos, mecánicos o ambos con la mediación de un adhesivo (ISO/TR 11405: 1993). El material que une dos superficies se denomina adhesivo y la superficie a adherir se denomina adherente o sustrato. El espacio virtual que hay entre las superficies unidas se denomina interfase. Para que se produzca una buena adhesión tiene que existir una buena humectabilidad y un íntimo contacto entre las superficies a unir (Burke y cols., 1995; Toledano y cols., 2001). La adhesión puede estar basada en dos procedimientos:

1. Mecanismo mecánico. Consiste en el entremezclado del adhesivo solidificado en las irregularidades de la superficie del adherente. Da lugar a la adhesión mecánica, que puede ser macromecánica y micromecánica. Se denomina adhesión macromecánica si las irregularidades son apreciables a simple vista; se denomina micromecánica si las irregularidades son microscópicas.

2. Mecanismo químico. Explica la adhesión mediante la generación de enlaces químicos entre el adhesivo y el adherente. Produce adhesión química. Los enlaces implicados pueden ser primarios o fuertes (iónicos y covalentes) y secundarios o débiles (uniones por puentes de hidrógeno, interacciones por dipolos, fuerzas de van der Waals).

Los diferentes mecanismos de unión no están del todo aclarados y en esa cuestión existe controversia entre los dos tipos básicos de adhesión. De todas formas los dos mecanismos son perfectamente compatibles y, sin duda alguna, pueden darse de forma simultánea.

Existen diversos factores físicos que influyen en la adhesión. Estos factores son los fenómenos de superficie entre los que se encuentran la tensión superficial y la humectabilidad.

La adhesión no se entiende como la simple aplicación de un pegamento para unir dos superficies. En numerosas ocasiones hay que realizar pretratamientos antes de la aplicación del adhesivo. Básicamente, el proceso de unión sigue tres pasos fundamentales:

1. Acondicionamiento del adherente. Consiste en alterar su morfología y/o su estructura química.

2. Imprimación del adherente. Consiste en la aplicación de una sustancia química previa con la finalidad de hacer el sustrato más receptivo al adhesivo.

3. Aplicación del adhesivo. Consiste en aplicar el adhesivo sobre la superficie adherente.

Estos tres pasos no tienen porqué darse siempre de forma claramente diferenciada. Se pueden encontrar de forma simultánea o bien faltar alguno de ellos (Van Meerbeek y cols., 1992; Toledano y cols., 2003a).

La dentina puede ser descrita como un composite biológico, con un relleno mineral de cristales de hidroxiapatita y una matriz formada por una red de fibras de colágeno (Marshall *y cols*., 2001), así, el mecanismo básico de adhesión a dentina es esencialmente un proceso de intercambio

que envuelve el reemplazamiento de minerales removidos de los tejidos dentales duros por monómeros de resina que producen un cierre micro-mecánico en las porosidades creadas (Toledano y cols., 2001; Osorio y cols., 2003; De Munck, 2004). Este cierre fue descrito por primera vez por Nakabayashi y cols., (1982) y es referido comúnmente como "hibridación" o la formación de la capa híbrida.

I.2. Adhesión a dentina.

I.2.1. Histología de la dentina.

I.2.1. Histología de la dentina coronal.

La dentina es un sustrato biológico que actúa como adherente. Es un tejido vivo muy complejo y variable que, debido a sus peculiaridades histológicas y morfológicas, condiciona la aplicación de los sistemas adhesivos.

Desde un punto de vista histológico, la dentina es un tejido conjuntivo mineralizado y avascular. Está compuesto, en peso, por un 70% de materia inorgánica, un 18% de materia orgánica y un 12 % de agua (Mjör y cols., 1989). En cuanto al volumen, el 50% lo constituye material inorgánico, el 25% orgánico y el otro 25% agua (Mjör y cols., 1989).

La porción inorgánica de la dentina consiste básicamente en cristales de hidroxiapatita. La parte más pequeña de esta estructura se denomina unidad celular de hidroxiapatita y responde a la fórmula $3Ca_3(PO)_4$-$CA(OH)_2$. Junto a estos cristales se pueden encontrar fosfatos cálcicos amorfos, probablemente en mayor cantidad en los tejidos formados más tardíamente que en los maduros o viejos (Mjör y cols., 1989; Melfi, 1994). Los cristales están formados por varios miles de unidades

celulares y tienen forma laminar que de perfil adaptan el aspecto de agujas. Su longitud es de hasta 20 nm y el grosor puede llegar hasta los 3.5 nm. Son similares a los cristales del cemento y del hueso, pero más pequeños que los cristales del esmalte. Existen también otras sales inorgánicas como carbonatos, fosfatos cálcicos diferentes de la hidroxiapatita, sulfatos, y ciertos oligoelementos (F, Cu, An, Fe) (Davis, 1986; Melfi, 1994; Mjör y cols., 1989).

La porción orgánica (Mjör y cols., 1989; Davis, 1988; Melfi, 1994) está compuesta principalmente por fibras de colágena tipo I, en una cuantía aproximada del 17% del total del tejido, es decir, alrededor del 93% de todo el material orgánico. Además, se pueden encontrar fracciones de lípidos, glucosaminoglicanos, compuestos proteicos no identificados, constituyendo cada uno de ellos un 0.2% aproximadamente. También se puede encontrar ácido cítrico en una cantidad algo inferior al 1% (Schroeder, 1991).

En el tejido dentinario se pueden distinguir cinco unidades estructurales: odontoblastos, túbulos dentinarios, espacio periodontoblástico, dentina peritubular y dentina intertubular (Mjör y cols., 1989).

Los odontoblastos son células especializadas que tapizan la pared de la cámara pulpar y que poseen largas prolongaciones citoplasmáticas (proceso odontoblástico) que se localizan en el interior de los túbulos dentinarios.

Los túbulos dentinarios alojan la prolongación odontoblástica y se forman durante la dentinogénesis, conservando su estructura tubular en la dentina madura. El diámetro de la luz tubular cambia según la zona de la dentina, en la proximidad a la pulpa es de 3 a 4 μm y en la zona externa es de 1μm aproximadamente (Bhaskar, 1993), debido a que la superficie pulpar de la dentina es considerablemente menor que el área de las uniones amelodentinaria y cementodentinaria

(Pashley, 1991a; Schroeder, 1991; Mjör y cols., 1989). Cerca del 80% del volumen total de la dentina próxima a la pulpa está compuesta por las luces de los túbulos, mientras que éstas constituyen tan sólo cerca de un 4% del volumen de la dentina periférica. En cuanto a la superficie, la luz tubular ocupa el 1% en la dentina de la unión amelo-dentinaria y el 22% en la zona próxima a la pulpa (Pashley, 1985).

Los procesos odontoblásticos y túbulos acompañantes pueden ramificarse, especialmente, cerca de las uniones amelodentinarias y cementodentinarias. En general, las ramificaciones de los procesos de los odontoblastos son de menor tamaño y más numeroso en la dentina de la raíz que en la coronal.

El líquido en el interior de los túbulos tiene una determinada presión. La presión hidrostática es un factor a tener en cuenta en el estudio de la adhesión, debido a que proporciona un flujo permanente y constante de líquido hacia el exterior (Ciucchi, 1995).

La dentina peritubular forma la pared de los túbulos dentinarios. Por otro lado, la dentina intertubular se localiza entre los túbulos dentinarios. Es muy importante destacar las diferencias existentes entre ambas. La dentina peritubular tiene una estructura tubular. Su grosor es variable, siendo aproximadamente de 0.75 μm en la dentina externa y de 0.4 μm en la dentina interna (Bhaskar, 1993). Los cristales de hidroxiapatita son más pequeños y están agrupados muy juntos (Davis, 1986). La dentina intertubular está menos calcificada y con mayor contenido orgánico. Se halla uniformemente mineralizada, a excepción de la situada en la zona con bajo contenido mineral cercana a la pulpa, donde el grado de mineralización es inferior al común (Mjör y cols., 1989; Bhaskar, 1993). La mitad del volumen de la dentina intertubular está formada por matriz orgánica. Las fibras de colágena son su principal componente y se encuentran orientadas al azar alrededor de

los túbulos dentinarios. Las fibras tienen un diámetro variable de 0.05µm a 0.2µm (Mjör y cols., 1989).

I.2.1.2. Histología de la dentina radicular:

Los odontoblastos que forman la dentina radicular se diferencian a partir de las células epiteliales de Hertwing, lo que hace que esta dentina sea distinta en términos estructurales y de composición a la dentina coronal, en la cual, los odontoblastos se diferencian a partir de las células ectomesenquimáticas de la papila (Gómez y cols., 2002).

Las diferencias entre la dentina coronal y radicular pueden resumirse en los siguientes puntos:

- La orientación de las fibras de colágeno de la dentina del manto son diferentes. En la dentina coronal del manto, las fibras son perpendiculares a la interfase dentina-esmalte, por el contrario, las fibras de la raíz son paralelas a la interfase dentina-cemento.

- Los odontoblastos radiculares difieren un poco de los de la corona dando lugar a ramos en forma de paraguas.

- En la dentina radicular la tasa de deposición es más lenta.

- Su contenido de fósforo es menor que en la dentina coronal; además, su grado de mineralización es menor.

- En la dentina coronaria los túbulos siguen un trayecto doblemente curvo en forma de "S" itálica, sin embargo, en las cúspides y bordes incisales es prácticamente rectilíneo, mientras

que en la dentina radicular los túbulos tienen una curvatura poco pronunciada (Bhaskar, 1993).

Otra particularidad de la dentina radicular es la existencia de la "Capa Granulosa de Tomes". Esta región de la dentina es muy peculiar y sólo se encuentra en la parte más periférica de la dentina radicular, en la unión dentina-cemento. Aparecen como una serie de gránulos oscuros, sin matriz de colágeno y por lo tanto sin calcificación, que se extienden a lo largo de toda la raíz, siendo más numerosos en el vértice que en la unión cemento-esmalte. Aunque no está todavía claro su origen, se piensa que estos gránulos se forman a partir de una serie de pequeñas cámaras de aire, producidas probablemente por incurvación de lo túbulos dentinarios para formar asas en esta área (Bhaskar 1993).

Para obtener una adecuada adhesión a la dentina radicular, al igual que en la dentina coronal, es de crucial importancia la permeabilidad que presenta ésta a los agentes adhesivos, así como que la fase mineral de la dentina sea removida para producir la infiltración de los adhesivos dentro de la dentina intertubular (Gaston y cols., 1999).

I.2.1.3. Barrillo dentinario.

El barrillo dentinario o *smear layer* es un conglomerado de tejido inorgánico y orgánico, subunidades globulares originadas por fibras mineralizadas con un diámetro de 0'05-0'1µm aproximadamente, proteínas coaguladas, células sanguíneas y en algunas ocasiones microorganismos (Czonstkowsky y cols., 1990; Sen y cols., 1995; Abbott y cols., 1991). El barrillo dentinario resulta de las maniobras terapéuticas practicadas sobre el diente al cortar las superficies de los tejidos mineralizados; además, tiene gran facilidad para adherirse a las paredes de la preparación cavitaria sin poderse remover con una simple aplicación de agua o spray, representando

la interfase entre el diente y el material restaurador (Toledano y cols., 2003a). La apariencia microscópica de esta capa vista bajo el microscopio electrónico de barrido fue descrita por Brannström y cols (1980) quienes encontraron que es irregular, granular y amorfa. Boyle y cols., (1963) fueron los primeros en describir la presencia del barrillo dentinario, posterior al corte con fresas a nivel coronal. La formación de barrillo dentinario en los conductos de dientes preparados endodónticamente fue descrita por Mc Comb y Smith, (1975) quienes reportaron que la apariencia fue similar al barrillo dentinario coronal.

El barrillo dentinario posee un grosor aproximadamente de 1-5µm, aunque dicho grosor depende del tipo y filo del instrumento usado y de si en la preparación de la cavidad la dentina está seca o húmeda (Van Meerbeek y cols., 1993). Se ha identificado la capa de barrillo dentinario en dos partes, una es el barrillo superficial y la otra el barrillo compactado dentro de los túbulos dentinarios (Toledano y cols., 2003a). La penetración del material residual dentro de los túbulos es aproximadamente de 40µm de profundidad, la cual, tiene lugar por un fenómeno de capilaridad como consecuencia de las fuerzas adhesivas producidas entre los túbulos dentinarios y el material residual (Sen y cols., 1995; Cohen, 2002).

Hubo una gran controversia (Toledano y cols., 2003a) con respecto al barrillo dentinario nacida de las siguientes realidades: 1) Se ha demostrado que las bacterias pueden vivir y multiplicarse dentro de él, alcanzando la pulpa a través de los túbulos dentinarios. 2) Al cubrir la dentina, el barrillo dentinario puede interferir en los procesos de adhesión con los cementos adhesivos o con las nuevas generaciones de los adhesivos dentinarios. Los autores partidarios de su conservación se basan en que si se elimina, desaparece una barrera física que impide la entrada de bacterias a través de los túbulos; (Tay y cols., 2000a; Sano y cols., 1999; Toledano y cols., 2003b) Por lo contrario, otros autores son partidarios de eliminar el barrillo dentinario, pues por la acción

del ácido al desmineralizarse la dentina peritubular se hace mayor su diámetro, asegurándose una mayor entrada ulterior de resina (Sen *y cols*., 1995; Calt *y cols*., 2000).

Hoy en día, la conservación o eliminación del barrillo dentinario depende del tipo de adhesivo. En el caso de los sistemas autograbadores, el barrillo dentinario se mantiene incorporándose a la composición de la capa híbrida formada (Tay *y cols*., 2001; Toledano *y cols*., 2001; Osorio *y cols*., 2003). Por lo contrario, en los sistemas de grabado total, el barrillo dentinario se elimina por medio de un acondicionamiento de la dentina realizado con ácido (Van Meerbeek *y cols.,* 1998).

I.2.2. Mecanismos de adhesión a dentina

El mecanismo de adhesión a la dentina ha sido ampliamente estudiado y debatido. Al comienzo del desarrollo de la Odontología Adhesiva se buscó una adhesión química al calcio o a las fibras de colágena de la estructura dentaria. Hoy en día se habla sobre todo de adhesión mecánica o, más concretamente, adhesión micromecánica (Nakabayashi y cols., 1991; Van Meerbeek y cols., 1992).

La adhesión micromecánica a la dentina está basada en tres mecanismos (Gwinnett, 1993):

1º Adhesión mediante la infiltración de la dentina intertubular y la formación de la capa híbrida o zona de interdifusión.

2º Adhesión mediante la infiltración de los túbulos dentinarios y sus ramas laterales.

3º Adhesión superficial, por el contacto entre el adhesivo y el sustrato dentinario.

Hoy en día, la adhesión a dentina se basa en la retención micromecánica proporcionada por la capa híbrida (Nakabayashi y cols., 1991) o zona de interdifusión (Van Meerbeek y cols., 1992). El mecanismo por el que se forma la citada capa, consiste en la infiltración de un monómero adhesivo en la dentina descalcificada con las fibras de colágena expuestas que, tras polimerizar, queda entremezclado con la estructura dental desmineralizada (Nakabayashi y cols., 1991; Toledano y cols., 2003b). Es una unión micromecánica al tejido proteico (Gwinnett, 1993). La formación de una capa híbrida adecuada requiere que los péptidos dentinarios (incluidas las fibras de colágena) estén sin desnaturalizar (pues de lo contrario crearían una capa híbrida débil con gran susceptibilidad a su degradación) (Nakabayashi y cols., 1991), que el sistema adhesivo contenga resinas hidrofílicas e hidrofóbicas (para que las primeras produzcan una imprimación de la dentina haciéndola más receptiva a las segundas) (Toledano y cols., 2003a) y un catalizador que permita la polimerización en presencia de agua y oxígeno (Nakabayashi, 1991; Osorio y cols., 2005a; Nunes y cols., 2005).

Una de las principales características de la capa híbrida es la resistencia al ataque ácido (Nakabayashi, 1991), lo que la convierte en una unión resistente a una hipotética microfiltración bacteriana y le confiere estabilidad a lo largo del tiempo (Toledano y cols., 2001; Osorio y cols., 2003)

En algunos casos se puede observar una zona de vacío en la profundidad de la dentina infiltrada, que corresponde a una región en la que el adhesivo no ha llegado a penetrar (Sano y cols., 1995; Toledano y cols., 2004b). Este hecho facilita el paso de líquido y enzimas bacterianas a esta zona, produciéndose una hidrólisis de los péptidos no protegidos con la hidroxiapatita o con la resina (Nakabayashi y cols., 1992) apareciendo la nanofiltración (infiltración de bacterias debido a un inadecuado sellado de la capa híbrida con las fibras de colágeno) (Sano y cols., 1995; Osorio y cols., 2003).

La formación de la capa híbrida no es el único mecanismo de adhesión a dentina que puede ofrecer un sistema adhesivo. También puede ser proporcionado por túbulos dentinarios abiertos tras el grabado ácido, en los cuales, se produce una infiltración de resina debido al fenómeno de capilaridad, formando los *tags* de resina. Estas prolongaciones de resina son siempre mucho más largas que el grosor de infiltración en la dentina intertubular. Debido a la anchura y forma de lo túbulos y a sus ramas laterales, ofrecen una retención mecánica (Chappell y cols., 1994; Gwinnett, 1993), aunque su contribución más importante a la adhesión consiste en propiciar un correcto sellado marginal (Gwinnett, 1993). Los *tags* son una combinación de la resina y de lámina limitante que cubre la pared tubular (Titley y cols., 1995).

El tercer mecanismo de unión mecánica al tejido consiste en el contacto que se produce entre la resina y la capa de dentina parcialmente desmineralizada con el límite del frente de desmineralización. Este mecanismo constituye la adhesión superficial (Gwinnett, 1993).

Hay dos modelos fundamentales de adhesión a dentina. Uno de ellos fue propuesto por Gwinnett (Gwinnett, 1993) y el otro por Pashley (Pashley, 1990):

a) El modelo de adhesión que propone el primero considera que la fuerza de unión de la resina a la dentina depende de la superficie dentinaria de adhesión, de la capa híbrida y de los *tags* de resina. La infiltración de la dentina por el adhesivo, ya sea en la dentina intertubular como en el interior de la luz tubular, es responsable de un tercio del total de la adhesión (Gwinnet, 1993). De este tercio, la mitad se basa en la infiltración de la dentina intertubular y la formación de la capa híbrida y la otra mitad en la penetración de la resina en los túbulos y en la formación de los *tags* (Gwinnet, 1993). Los dos tercios restantes se deben a la denominada adhesión superficial que está proporcionada por las interacciones físico-químicas del adhesivo con las irregularidades de la topografía dentinaria (Gwinnet, 1993; Yoshiyama y cols., 1995).

b) El modelo de Pashley o modelo lineal simple asume que la fuerza de unión de la resina a la dentina va a depender de la profundidad de la dentina y de la resistencia de la resina asumiendo que la diferente densidad de túbulos dentinarios y presencia de dentina sólida, como consecuencia de la proximidad a la pulpa, determinan la fuerza de unión tanto a dentina superficial como profunda. Así, en la dentina superficial la fuerza de unión estará más influida por la capa híbrida que por la densidad de los túbulos, mientras que en la dentina profunda ocurriría justo al contrario De esta forma, el *tag* de resina asume un papel destacado en la fuerza de unión, sobre todo, por el trayecto convergente de los túbulos dentinarios hacia la cámara pulpar, otorgando mayor retención micromecánica al sistema. Además se va a conseguir que la resina una a los túbulos, por lo que se obtiene el sellado de la luz del mismo.

1.2.3. Sistemas adhesivos. Clasificación y características.

Un sistema adhesivo es el conjunto de materiales que sirven para realizar todos los pasos de la adhesión del material restaurador al diente, como son la preparación de la superficie del esmalte y dentina, adhesión química y/o micromecánica a esmalte y dentina y adhesión química al material restaurador (Toledano y cols 2003a). La incapacidad de las resinas compuestas para adherir directamente a los sustratos dentales, hizo que la aplicación de un sistema adhesivo fuera un paso intermedio indispensable en los procedimientos clínicos donde se utilizasen dichos materiales. El procedimiento adhesivo consta de tres componentes básicos:

1) Un acondicionador ácido, que tiene la finalidad de modificar química y morfológicamente la estructura del esmalte y la dentina para permitir a los siguientes materiales adherirse mecánica y químicamente a ella.

2) Un imprimador o *primer,* que penetra y moja toda la zona descalcificada para facilitar el contacto de la resina adhesiva con el colágeno desmineralizado. Sus funciones son mejorar la humectabilidad de la dentina acondicionada, mantener las fibras de colágeno sin colapsar y separadas entre sí y facilitar o vehiculizar la resina adhesiva hacia el interior de la dentina descalcificada (Titley y cols, 1995; Tani y cols; 1996; Perdigão y cols., 1997; Toledano y cols., 2001).

3) Una resina, la cual se disuelve con el imprimador y penetra en la dentina, sirviendo de puente entre las dos superficies a adherir, la dentina y el material restaurador. Además, la resina adhesiva confiere una flexibilidad y resistencia adecuadas a la zona de dentina infiltrada.

Los adhesivos dentinarios se pueden clasificar atendiendo a numerosos criterios. Una de las más utilizadas se basa en la cronología de la aparición de estos materiales en el mercado separando los adhesivos en generaciones. Sin embargo, esta clasificación no aclara de forma objetiva el número de pasos clínicos realizados durante la aplicación de éstos, ni tampoco como interactúan con el sustrato (Van Meerbeek y cols., 1992). Hoy en día, la manera más clara de clasificar los adhesivos, es la establecida por Van Meerbeek y cols., (1992), los cuales establecieron una clasificación según el mecanismo de acción y el número de pasos empleados.

I.2.3.1. Adhesivos que eliminan el barrillo dentinario (grabado total).

Estos adhesivos, también conocidos como sistemas de grabado total, acondicionan la dentina con un ácido que remueve totalmente el barrillo dentinario (Meerbeek y cols., 1992).

a) Adhesivos de tres pasos.

Son sistemas que constan de tres componentes que se dispensan por separado. Responden al modelo tradicional del procedimiento adhesivo a dentina y en su mecanismo de acción están basados la mayoría de los adhesivos utilizados actualmente (Van Meerbeek y cols., 1998). Requieren tres pasos clínicos. En un primer paso, se aplica un ácido que elimina todo el barrillo dentinario. Tras ello, se enjuaga la dentina grabada y se aplica un imprimador. Como tercer paso se aplica una resina adhesiva.

Estos sistemas plantean tres grandes problemas: 1) El paso separado de grabar, lavar y secar incrementa la sensibilidad de la técnica, especialmente, si se trata de una técnica húmeda (Tay y cols., 2000a; Toledano y cols., 2001); 2) La necesidad que presentan estos sistemas de secar tras enjuagar con agua, provoca un colapso de las fibras de colágeno (Gordan y cols., 1998; Toledano y cols., 2004a); 3) La profundidad de desmineralización creada por el ácido es mayor que la infiltración producida por los monómeros hidrofílicos del primer (Nakabayashi y cols., 1996; Tay y cols., 2001; Osorio y cols., 2003), dejando expuestas las fibras de colágeno desprotegidas de cristales de hidroxiapatita, las cuales, son susceptibles de hidrólisis (Watanabe y cols., 1994; Burrow y cols., 1996; Sano y cols., 1999; Toledano y cols., 2000).

En este grupo se encuentran adhesivos como Scotch Bond Multipurpose® (3M, St. Paul, MN, USA) o Aelitebond® y All Bond 2® (Bisco, Istasca, USA).

b) Adhesivos de dos pasos:

Son sistemas adhesivos que constan de dos componentes. En el primer paso se aplica un acondicionador ácido que tras su aplicación es enjuagado con la consiguiente eliminación total del barrillo dentinario. En el segundo paso, se aplica un bote donde van incluidos el imprimador y la resina adhesiva. Estos sistemas adhesivos también se conocen como adhesivos autoimprimadores, pues combinan en un solo bote, las funciones del imprimador y la resina adhesiva (Toledano y cols., 2001; Osorio y cols., 2003).

Forman parte de este grupo el Single Bond® (3M, St.Paul, MN, USA.), Prime & Bond NT® (Dentsply / De Trey GmbH, Konstanz, Alemania), o el Prime & Bond XP® (Dentsply / De Trey GmbH, Konstanz, Alemania).

I.2.3.2. Adhesivos que disuelven el barrillo dentinario.

Son sistemas adhesivos que disuelven el barrillo dentinario (mezclan de forma homogénea el barrillo con el *primer*) y simultáneamente desmineralizan la superficie del sustrato (Meerbeek y cols., 1992). Como no son lavados, el barrillo se incorpora al proceso de adhesión reduciéndose los problemas asociados a la sensibilidad de la técnica (Fritz y cols., 1999; Toledano y cols., 2001). Los sistemas adhesivos que constan de uno o dos botes y disuelven el barrillo dentinario son conocidos actualmente como autograbadores (Van Meerbeek y cols 1993; Toledano y cols., 2001).

a) Adhesivos de dos pasos.

Son sistemas formados por dos botes. El primer paso consta de un imprimador y un ácido juntos, que disuelven el barrillo dentinario tras ser aplicados en esmalte y dentina conjuntamente (Van Meerbeek y cols 1993; Toledano y cols., 2001). El imprimador incorpora el barrillo dentinario a su composición. Tras este paso se aplica una resina adhesiva.

Los adhesivos autograbadores producen un complejo híbrido que incluye una capa superior de *smear layer* infiltrado y una capa inferior de fibras de colágeno desmineralizadas e infiltradas, mezclada con grupos calcio y fosfato producto de la desmineralización de la hidroxiapatita (Tay y cols., 2000a). El uso de esos sistemas adhesivos representa un método bastante eficaz para prevenir el colapso de la trama de colágeno desmineralizado (Tay y cols., 2001; Osorio y cols., 2003). Cuando estos sistemas adhesivos se emplean, no hay necesidad de grabar, enjuagar y secar el sustrato, por lo que desaparece el riesgo de sobregrabar y sobresecar la dentina (Tay y cols., 2001; Toledano y cols., 2003). Además, el problema presentado por los sistemas adhesivos de grabado total al producir una desmineralización, por parte de los monómeros ácidos, mayor que la infiltración realizada por los monómeros hidrofílicos, se ha solventado en gran medida (Toledano y cols., 2001; Osorio y cols., 2003).

Son representantes de este grupo el Clearfil SE Bond® (Kuraray Co, Osaka, Japón), Protect Bond® (Kuraray Co, Osaka, Japón), Syntac® (Vivadent, Schaan, Liechtenstein) o el Coltène ART Bond® (Coltène, Altstätten, Suiza).

b) Adhesivos de un paso (*all-in-one*).

Son adhesivos que constan de un solo paso. Incorporan en el mismo bote los monómeros ácidos, hidrofílicos e hidrofóbicos. Estos materiales disuelven el barrillo y simultáneamente desmineralizan la superficie del sustrato (Van Meerbeek y cols., 1992). Como no deben ser lavados, el barrillo se incorpora a la capa híbrida, formando parte de ésta (Fritz y cols., 1999; Santini y cols., 2001; Toledano y cols., 2001).

Existe unanimidad en asumir las bajas fuerzas de adhesión proporcionadas por los *all-in-one* (Fritz y cols., 1999; Inoue y cols., 2000; Toledano y cols., 2001; Toledano y cols., 2003b; Osorio y cols., 2003; Osorio y cols., 2005b). La combinación de monómeros ácidos, hidrofílicos e hidrofóbicos en una solución única, puede comprometer la función de cada uno de los componentes (Toledano y cols., 2003b). Sin embargo, son capaces de disolver completamente el barrillo dentinario y formar un complejo híbrido relativamente grueso (Haller y cols., 2000; Toledano y cols., 2003; Osorio y cols., 2003).

Forman parte de este grupo los adhesivos Prompt L-Pop® (3M ESPE, Seefeld, Alemania), Etch and Prime 3.0® (Degussa AG, Hanau, Alemania), AQ Bond® (Sun Medical, Kyoto, Japón), One-Up Bond F® (Tokuyama, Tokyo, Japón), Reactmer Bond® (Shofu, Kyoto, Japan) o Xeno CF Bond® (Sankin, Tokyo, Japón).

I.2.3.3. Adhesivos que modifican el barrillo dentinario.

Son sistemas adhesivos que modifican el barrillo dentinario haciéndolo más poroso para que la resina acceda a la dentina subyacente (Toledano y cols., 2003a).

a) Adhesivos de dos pasos:

Estos adhesivos constan de dos botes. El primero contiene un *primer* con radicales acidófilos que modifican el barrillo dentinario; en el segundo paso se aplica la resina adhesiva (Toledano y cols., 2003a). Una vez que los monómeros polimerizan en el espesor del barrillo dentinario se establecen uniones químicas y micromecánicas leves, que refuerzan la nueva estructura, así como su unión a la estructura subyacente (Van Meerbeek y cols., 1992).

Al igual que aquellos sistemas adhesivos que disuelven el barrillo dentinario, consideran el *smear layer* como una barrera natural contra la penetración de las bacterias, que se desplaza por los túbulos dentinarios al interior de la cámara pulpar, al mismo tiempo que dificulta la salida del líquido tubular a la superficie de la dentina, lo que podría alterar las técnicas adhesivas (Toledano y cols., 2003a).

Son representantes de este grupo el Pentra Bond II® (Jeneric/Pentron, Wallingford, CT, USA) o el ProBond® (Detrey-Dentsply, Konstanz, Alemania).

b) Adhesivos de un paso:

Son adhesivos que constan de un solo bote compuesto de una resina adhesiva mezclada con ácidos débiles, la cual, se aplica sobre el barrillo dentinario y la dentina. La resina modifica el barrillo dentinario para poder infiltrarlo y acceder a la dentina subyacente, por lo tanto, mediante este procedimiento no tiene lugar la exposición tradicional de las fibras de colágeno como consecuencia del grabado ácido (Toledano y cols., 2003a).

Son representantes de este grupo el Ariston Liner® (Vivadent, Schaan, Liechtenstein), Hytac® (ESPE, Schaan, Liechtenstein), Compoglas® (Vivadent, Schaan, Liechtenstein) o el Solist® (DMG, Hamburg, Alemania).

1.3. Métodos de medida de la eficacia adhesiva

I.3.1. Método de medida de la eficacia adhesiva en reconstrucciones intracoronales.

Las pruebas de fuerzas de adhesión son las más usadas para cuantificar la eficacia adhesiva de diferentes sistemas. La base de este método es que la adhesión más fuerte entre el diente y el biomaterial, resistirá mejor el estrés impuesto por el sistema y la función oral (Pashley y cols., 1995).

A lo largo del tiempo, se han desarrollado diversos tests de fuerzas de adhesión (Pashley y cols., 1995). La fuerza producida en los sistemas adhesivos dentinarios se ha evaluado tradicionalmente usando el test de resistencia al cizallamiento o *shear bond strength*, el cual, resulta útil para probar materiales que fallan ante valores comprendidos entre 18-20 MPa, o menos (Chappell y cols., 1997). Sin embargo, en valores que exceden la citada cifra, a menudo no permiten diferenciar entre la fuerza del adhesivo y la fuerza cohesiva del composite o la dentina (Chappell y cols., 1997). Además, debido a que la evaluación exacta de un material adhesivo se determina mejor cuando el fallo ocurre en el propio material y no implica la dentina o el composite y, a que la mejora de los adhesivos dentinarios aumenta con el paso del tiempo, apareció la necesidad de obtener un método mejor y más eficaz (Schreiner y cols., 1998). De esta manera, se creó el test de microtensión, que hoy en día es el más usado. La técnica de microtensión para evaluar la resistencia adhesiva introducida por Sano y cols., (1994), se trata de una técnica muy laboriosa, pero presenta múltiples ventajas: (1) Con ella se pueden medir grandes fuerzas de

adhesión. (2) Permite testar la adhesión en áreas muy pequeñas y en diferentes regiones. (3) Es capaz de obtener de una sola pieza múltiples especimenes.

I.2.5. Uso de pernos de fibra en la evaluación de la eficacia adhesiva a dentina radicular.

Para la evaluación de las fuerzas de adhesión de pernos a dentina radicular, y de éstos al cemento, se han utilizado tradicionalmente las pruebas de *push-out y pull-out* (Mitchell y cols., 1994; Drummond y cols., 2000). Este tipo de pruebas presentan dos grandes problemas: 1) El área de la superficie de los pernos debe ser cuidadosamente evaluada para permitir calcular la fuerza de adhesión. 2) No se produce una distribución uniforme de las cargas a través de las muestras (Sano y cols., 1994). Los tests de microtension introducidos por Sano y cols., (1994) mejoran la distribución de las fuerzas en superficies pequeñas (0.5 x 0.5 mm), a la vez que permiten medir la fuerza de adhesión de resinas aplicadas dentro del conducto radicular. Bouillaguet y cols., (2003) realizaron un estudio de microtensión para obtener la fuerza de adhesión de pernos a dentina radicular solventando los inconvenientes que presentaban los tests de p*ush-out y pull-out.*

En la adhesión entre el perno y la dentina radicular, se encuentran dos interfases: dentina-cemento y cemento-perno. Para evaluar exclusivamente las fuerzas de adhesión de ésta última, basta con unir el perno al cemento con interposición de un adhesivo y someterlo al test de Microtensión (Goracci y cols., 2005; Monticelli y cols., 2004).

El uso de pernos adheridos al conducto radicular se considera el mejor método para medir la eficacia adhesiva en dentina radicular (Ferrari y cols., 2001a; Pegoretti y cols., 2002a; Boschian y cols., 2002; Aksornmuang y cols., 2004; Goracci y cols., 2005). Las investigaciones en el campo de la adhesión en dicho sustrato iniciadas por Mc Comb y cols., (1973), propusieron la utilización de la dentina endodonciada acondicionada para el cementado adhesivo de los sistemas de reconstrucción

de dientes no vitales. Nathanson y cols., (1980) desarrollaron una técnica de cementado pasivo de pernos, en la que propusieron para el acondicionamiento de la dentina radicular, un tratamiento con hipoclorito sódico y la utilización de pernos metálicos o sistemas de retención prefabricados. Esta técnica, debido a la presencia de un diafragma elástico, representado por el cemento entre los postes metálicos y la dentina, permite utilizar una retención de acción pasiva (Morgano y cols., 1996).

Gracias al cementado adhesivo, las técnicas de reconstrucción pueden ser menos invasivas; de hecho, la longitud del poste puede ser igual o ligeramente mayor que la altura del muñón clínico, y el diámetro se limita a reproducir la morfología del conducto preparado sin la eliminación posterior de dentina radicular (Ferrari y cols., 2004). Los beneficios de las técnicas adhesivas utilizadas para restauraciones dentales han sido bien documentados. Uno de los factores más importantes que aportan es el reforzamiento de la estructura dentaria y el aspecto estético de la restauración final (Pest y cols., 2000), por estas razones, el uso de cementos adhesivos ha sido propuesto para la cementación de pernos en dientes no vitales.

Para mejorar la estética y eliminar los problemas relacionados con las propiedades físicas de los pernos metálicos, se propuso la utilización de pernos estéticos, entre los que se encuentran los pernos de resina compuesta reforzados con fibra. Duret y cols., (1990), codificaron la utilización de pernos de resina reforzados con fibra de carbono y realizaron una técnica que evitaba la unión de materiales con características biomecánicas diferentes, así, los diferentes componentes de la restauración (poste, cemento, material de reconstrucción y dentina), constituyen un complejo estructural mecánicamente homogéneo. En esta línea, los pernos de fibra adheridos al conducto radicular son la última solución presentada para la evaluación de las fuerzas de adhesión a dentina radicular (Mannocci y cols., 2001; Bouillaguet y cols., 2003; Foxton y cols., 2003; Goracci y cols., 2005).

La eficacia de la adhesión entre perno y dentina radicular está influida por diversos factores relacionados con el poste, el cemento, la adhesión del poste al cemento y a la dentina del canal radicular. Se han evaluado los factores retentivos de los pernos, encontrando que entre las variables que afectan dichos factores, están la longitud, diámetro, diseño, material y estructura del poste (Nergiz y cols., 2002; Ferrari y cols., 2000). En lo que concierne al cemento, la retención del perno está afectada por la resistencia del cemento, la adhesión de éste sobre el poste, la dentina y otros. Utter y cols (1997), encontraron que la retención de los pernos es superior cuando se utilizan cementos resinosos en comparación, con pernos cementados con cemento de fosfato de zinc.

La selección del adhesivo y el procedimiento de cementación apropiada para la colocación de pernos en el conducto radicular están cambiando. Diferentes tipos de sistemas pueden ser utilizados en combinación con diversos cementos resinosos. Estos materiales pueden polimerizar a través de una reacción química, por proceso de fotopolimerización o combinación de ambos mecanismos, también llamado polimerización dual (Nergiz y cols., 2002; Ferrari y cols., 2000).

1.3.3. Degradación de la interfase adhesiva in vitro.

Es bastante difícil desarrollar condiciones de laboratorio que puedan testar la longevidad de la adhesión debida a los numerosos factores envueltos en la degradación de la adhesión y a que el desarrollo oral es dinámico y biológicamente complejo (Osorio y cols., 2005b). Diversos métodos han sido propuestos para reproducir una situación clínica en el medio oral, particularmente bajo condiciones en las cuales la adhesión fallaría como consecuencia de una degradación, como serían por ejemplo el ciclado mecánico, ciclado térmico, almacenamiento en agua y otras soluciones (Burrow y cols., 1993; Abdalla y cols., 1996; Kato y cols., 1998; Chan y cols., 1997; Nikaido y cols, 2002a; Yamauti y cols., 2003; Osorio y cols., 2005a)**.**

1.3.3.1. Degradación mecánica de la interfase adhesiva.

Los dientes están sujetos continuamente a un estrés durante la masticación, la deglución y los hábitos parafuncionales. El ciclado mecánico, que simula la carga oclusal ejercida por los dientes, podría acelerar el deterioro de la interfase entre la dentina y la restauración (Nikaido y cols, 2002a; Abdalla y cols., 1996; Osorio y cols., 2005b). Las cargas verticales producidas por una bola de comida entre dientes opuestos, se distribuyen sobre toda la cara oclusal, y el estrés es propagado a través de la superficie. La máxima fuerza registrada al morder en los primeros molares es aproximadamente 40-90 Kg (Bates y cols., 1975; Anderson, 1956), lo cual puede representar un desafío a la durabilidad a largo plazo de los adhesivos en dentina.

En los últimos años se han desarrollado unos sistemas simuladores de la cavidad oral para ocasionar estrés mecánico *in vitro* y evaluar el efecto del ciclado mecánico en las fuerzas de adhesión de adhesivos dentinarios autograbadores y convencionales.

La combinación del simulador de la masticación (cicladora mecánica) en combinación con el test de microtensión han mostrado que pueden proveer magníficos resultados in vitro para la evaluación de la durabilidad de la adhesión dentinaria (Nikaido y cols., 2002b). Sin embargo, existe muy poca información sobre el comportamiento in vitro de los sistemas autograbadores y convencionales tras ser sometidos a una carga cíclica.

1.3.3.2. Degradación química de la interfase adhesiva.

Estudios de envejecimiento a largo plazo in vitro (Burrow y cols., 1996; Kato y cols., 1998) usando agua como medio de almacenamiento han demostrado que el decrecimiento en las fuerzas de adhesión a lo largo del tiempo no fue uniforme para todos los materiales testados. La proporción

de disminución de las fuerzas de adhesión depende de los sistemas adhesivos aplicados. Igualmente, las fuerzas de adhesión en algunos estudios *in vivo* tienden a decrecer a lo largo del tiempo y se pueden observar alteraciones en la capa híbrida (Hashimoto y cols., 2000; Hashimoto y cols., 2001).

La dentina es un substrato formado por compuestos orgánicos e inorgánicos visto anteriormente. La fase orgánica está representada principalmente por una estructura fibrosa de colágeno que puede ser degradada por enzimas proteolíticas (Hashimoto y cols., 2000; Hashimoto y cols., 2001). Es posible que tales enzimas afecten la durabilidad de la adhesión a dentina si los componentes orgánicos, tales como el colágeno, se dejan expuesto después de la infiltración de resina. El hipoclorito de sodio (NaOCl) se ha utilizado como un sustituto de enzimas proteolíticas (Spencer y cols., 1999; Nakabayashi y cols., 1996. Yamauti y cols., 2003). La solución de NaOCl tiene un efecto proteolítico no-específico que elimina efectivamente los componentes orgánicos de dientes que han sido desmineralizados pero han quedado sin ser infiltrados por el primer.

Diversas pruebas *in vitro* se han propuesto con el objetivo de producir un envejecimiento acelerado de la interfase formada por los adhesivos dentinarios, reduciendo el tamaño de los especimenes a barritas y sumergiendo éstas en hipoclorito de sodio al 10% acuoso (NaOClaq) por un periodo de tiempo experimental corto, con la intención de determinar la habilidad de los monómeros de resina de proteger la matriz de colágeno de la actividad del NaOClaq (Yoshida y cols., 2004; Yamauti y cols., 2003). De esta manera, la evidencia proporcionada por previos estudios realizados *in vivo* e *in vitro* de la hidrólisis en los adhesivos haría del NaOCl un buen medio de prueba para analizar la durabilidad de los adhesivos (Yamauti y cols., 2003). El efecto de NaOCl podría realizar el efecto de meses de almacenamiento en agua (Yoshida y cols., 2004; Yamauti y cols., 2003).

II. OBJETIVOS Y JUSTIFICACIÓN

1. Evaluar las fuerzas adhesivas inmediatas de diferentes sistemas adhesivos a dentina coronal humana a través de un test de microtensión.

2. Evaluar el efecto del ciclado mecánico de la interfase en las fuerzas adhesivas de diferentes sistemas adhesivos a dentina coronal humana, midiendo la fuerza de unión con un test de microtensión.

3. Evaluar el efecto del test de degradación *in vitro* (inmersión en $NaOCl_{aq}$) en las fuerzas adhesivas de microtensión de adhesivos autograbadores y de grabado total en dentina coronal humana.

4. Evaluar mediante microscopía electrónica de barrido las características histomorfológicas de la capa híbrida y de los *tags* de resina principales y secundarios formados entre diversos sistemas adhesivos y dentina coronal.

5) Evaluar las fuerzas de adhesión de diferentes agentes adhesivos a dentina del canal radicular teniendo en cuenta el efecto del silano aplicado sobre la superficie del perno y el material usado como agente cementante.

La durabilidad de la adhesión resina-dentina es de crucial importancia tanto para el clínico como para el investigador, sin embargo, se sabe muy poco acerca de la estabilidad de la capa híbrida. El test de fuerzas de adhesión inmediata no puede demostrar adecuadamente los efectos que pueden tener en la durabilidad de la adhesión los poros y otros defectos internos producidos en la capa híbrida.

Después del ciclado mecánico, el efecto de estas irregularidades interfaciales que alteran la durabilidad de la adhesión puede resultar más aparente. El uso combinado del ciclado mecánico con el test de fuerzas adhesivas de microtensión (MTBS) permite la evaluación *in vitro* de la durabilidad de la adhesión resina-dentina bajo unas condiciones clínicamente más relevantes de las que son usadas normalmente en la técnica de fuerzas de adhesión estáticas. El método de degradación basado en la inmersión de los especímenes en $NaOCl_{aq}$ durante un corto periodo de tiempo resulta bastante más real que los estudios *in vitro* realizados con almacenamiento en agua durante un largo periodo de tiempo. Esta disminución de los valores de las fuerzas de adhesión obtenidas después de la inmersión es similar a los obtenidos con estudios de degradación *in vivo*.

La infiltración con resina de las fibras de colágeno desmineralizadas permiten la formación de una capa híbrida con *tags* de resina y ramas laterales, creando de esta manera, retenciones micromecánicas de la resina al substrato desmineralizado. Una de las técnicas más usadas para el estudio del mecanismo de adhesión es el microscopio electrónico de barrido (SEM).

La retención del perno en la dentina del canal radicular está afectada por diversos factores como el tipo de perno, el cemento usado y el tratamiento aplicado sobre su superficie. Los pernos de fibra han aumentado su popularidad para restaurar los dientes tratados endodónticamente; sin embargo, se han obtenido unos resultados controvertidos cuando se aplicaron diferentes adhesivos

dentinarios, y cuando se usaron materiales fluidos y composites híbridos para cementar los pernos de fibra.

II. OBJECTIVES AND JUSTIFICATION

1. To evaluate the immediate microtensile bond strength of several adhesive systems to coronal human dentin.

2. To evaluate the effect of mechanical loading on the microtensile bond strength of several adhesive systems to coronal human dentin.

3. To evaluate the effect of an *in vitro* degradation test ($NaOCl_{aq}$ immersion) on the microtensile bond strength of total etch and self etch adhesives to coronal human dentin.

4. To evaluate by scanning electron microscopy the histomorphology characteristics of the formed resin tags, adhesive lateral branches and hybrid layers of several adhesive systems to coronal human dentin.

5. To evaluate the microtensile bond strength of several bonding agents to root canal dentin taking into account the effect of post silanization and the used core material.

The durability of resin-dentin bonds between adhesive resins and coronal dentin is of critical importance and little is known regarding the stability of hybridized layers. Static bond strength tests may not adequately demonstrate the potential detrimental effects that porosities and other internal defects within the adhesive layer may have on bonding durability.

After cyclic loading, the effect of these interfacial defects on long-term bonding may be more readily apparent. The combined use of mechanical loading with microtensile bond strength (MTBS) testing permits the evaluation of the *in vitro* durability of resin-dentin bonds under more clinically-relevant conditions than are usually employed in static bond strength testing techniques. The challenging method based upon 10% $NaOCl_{aq}$ immersion of specimens during a short period of time is much more reliable than *in vitro* studies based on long-term water storage of specimens and those previously reported after $NaOCl_{aq}$ immersion are similar to the decline in bond strength obtained when *in vivo* degradation studies are performed.

The infiltration of demineralized collagen fibers with resin permits formation of a hybrid layer with resin tags and adhesive lateral branches, thus creating micromechanical retention of the resin to the demineralized substrate. One of the first and most widely used tools to study the mechanisms involved in the process of bonding has been the scanning electron microscope (SEM).

The retention of posts within root canals is affected by several factors involving the type of the post, the luting agent and the post surface treatment. Fiber posts are becoming increasingly popular for the restoration of endodontically treated teeth, however controversial results were reported when different commercially available dentin adhesive and luting cement combinations were employed for cementing fiber posts and when flowable materials and hybrid composites for building up the core onto a fiber post were used.

III.1. Effect of cyclic loading on microtensile bond strengths of total-etch and self-etch adhesives

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ABSTRACT

Objective: To evaluate the effect of mechanical loading on the microtensile bond strength (MTBS) of five adhesive systems to dentin. *Methods:* Flat dentin surfaces from 100 molars were divided into five groups, and bonded with total-etch self-priming adhesives (Single Bond -SB-, Prime&Bond NT –PNT- and Prime&Bond XP –PXP-), two-step self-etching primer (Clearfil SE Bond –SEB-), and an all-in-one adhesive (Etch & Prime 3.0 –E&P-), according to the manufacturers´ instructions. Composite build-ups were constructed incrementally with Tetric Ceram (TC). After 24 h of water storage, half of the specimens were load cycled (5000 cycles, 90 N). The teeth were then sectioned into beams of 1.0 mm^2 cross-sectional area. Each beam was tested in tension in an Instron machine at 0.5 mm/min. Data were analyzed by two way ANOVA and Student Newman Keuls multiple comparisons tests ($P<0.05$). *Results:* SEB and SB attained higher MTBS than the other three adhesives. PNT and PXP performed equally, and E&P resulted in the lowest MTBS. After mechanical loading, MTBS decreased in all groups, except for PXP. SEB, SB and PXP obtained higher MTBS than PNT. Specimens bonded with E&P resulted in premature failures and MTBS could not be measured. The type of failure was predominantly mixed, except for E&P and PNT after loading that exhibited predominantly adhesive failures. *Clinical Relevance:* All-in-one adhesives do not provide a durable bond to dentin. If dentin is acid-etched, alcohol-based adhesive systems showed the higher bond strength after mechanical loading.

INTRODUCTION

Dentin bonding systems have been simplified and improved in order to provide increased long-term strength and promote the durability and reliability of adhesive restorations (Nikaido & others, 2002a). Two main strategies are used to create durable dentin bonding: 1) the total-etch self-priming (i.e. single-bottle) bonding systems work by removing the smear layer with phosphoric acid, followed by the application of a primer and an adhesive in the same step. With these systems, incomplete expansion of the demineralized collagen matrix may impair resin infiltration and compromise bonding (Van Meerbeek & others, 1994; Pashley & others, 2003); and 2) The self-etching approach (SEB, E&P), in which increased concentrations of acid monomers enable the primer or adhesive to etch and prime the dentin simultaneously. No discrepancy between the depth of demineralisation and depth of resin infiltration is expected, since both processes occur simultaneously (Tay & others, 2000).

In the clinical situation, dentin-resin bonds are not only subjected to immediate stresses, that may disrupt the developing bonds, but also to cyclic loading during mastication that will induce generation of cracks and subsequent crack growth that challenge the long-term survival of these bonds. It has been shown that changes in the bonded interfaces *in vivo* may occur under occlusal stresses, resulting in mechanical degradation of the bonds between the restoration and dentin (Sano & others, 1999). Teeth are continuously subjected to stresses during mastication, swallowing and parafunctional habits. Maximum biting force recorded on the first molar teeth is approximately 40-90 Kg. Although masticatory loads recorded on a single molar are smaller (ca. 11-27 Kg) (Bates & others, 1975; Anderson, 1956), they may still represent a challenge to the long-term durability of resin-dentin bonds.

Static bond strength tests may not adequately demonstrate the potential detrimental effects that porosities and other internal defects within the adhesive layer may have on bonding durability (Givan & others, 1995). After cyclic loading, the effect of these interfacial defects on long-term bonding may be more readily apparent. It is anticipated that the combined use of mechanical loading with microtensile bond strength (MTBS) testing permits the evaluation of the *in vitro* durability of resin-dentin bonds under more clinically-relevant conditions than are usually employed in static bond strength testing techniques.

Thus, the objective of this study was to compare the results of mechanical loading *vs* static bond strength evaluation on the MTBSs of five total-etch and self-etch adhesives to human dentin. The null hypothesis tested was that the incorporation of mechanical loading prior to bond strength evaluation has no effect on the MTBSs of the adhesives to dentin.

MATERIAL AND METHODS

One hundred caries-free extracted human third molars that were stored in 0.5% chloramine T at 4 ºC and were used within one month after extraction. The specimens were sectioned below the dentinoenamel junction and ground flat with 180-grit silicon carbide abrasive papers under running water to provide uniform and clinically-relevant bonding surfaces. Three total-etch self-priming adhesives (Single Bond -SB-, 3M ESPE, St. Paul, MN, USA; Prime&Bond NT –PNT-, Dentsply DeTrey, Konstanz, Germany; and the experimental adhesive Prime&Bond XP –PXP-, Denstply DeTrey), a two-step self-etching primer (Clearfil SE Bond –SEB-, Kuraray Medical Inc., Tokyo, Japan), and an all-in-one self-etch adhesive (Etch&Prime 3.0 –E&P-, Dentsply Degussa AG, Hanau, Germany) were examined (i.e. five experimental groups; N=20). The mode of application, components and manufacturers of these adhesives are shown in Table 1. They were bonded to the dentin surfaces according to the manufacturers´ instructions.

After bonding, composite build-ups, each 6 mm in height, were constructed incrementally (1.5 mm) with a light-cured microhybrid resin composite (Tetric Ceram, Ivoclar-Vivadent, Schaan, Liechtenstein). Each layer of the composite was light-activated for 40 s with a Translux EC halogen light-curing unit (Heraeus-Kulzer GmbH, Hanau, Germany). Light intensity output was monitored with a Demetron Curing Radiometer (Model 100 Demetron Research Corporation, Danbury, CT, USA) to be at least 600 mW/sec.

The bonded specimens were stored in distilled water for 24 h at 37 ºC. For each experimental group, half of the specimens (N=10) were mounted in plastic rings with dental stone for load cycling under 90 N (5000 cycles, 3 cycles/sec) with the force applied longitudinally along the center of the tooth. This compressive load was applied to the flat resin composite build-ups using a spherical stainless steel plunger, 5mm in diameter, attached to a cyclic loading machine (S-

MMT-250NB; Shimadzu, Tokyo, Japan). The rest of the specimens from each group (N=10) were not subjected to cyclic loading and were stored in water until load-cycling for the other teeth was completed. Each tooth was then sectioned vertically into serial slabs. The widest center slab from each tooth was selected and further sectioned into beams with an approximate cross-sectional area of 1 mm^2, following the method described by Shono & others (1999). This resulted in the generation of 32-39 beams for each experimental sub-group.

Each beam was tested for MTBS by attaching to a modified Bencor Multi-T testing apparatus (Danville Engineering Co., Danville, CA) with a cyanoacrylate adhesive (Zapit, Dental Venture of America Inc., Corona, CA, USA). The beams were stressed to failure in tension using a universal testing machine (Instron 4411, Instron Corporation, Canton, MA, USA) at a crosshead speed of 0.5 mm/min. The fractured beams were carefully removed from the apparatus and the cross-sectional area at the site of failure was measured to the nearest 0.01 mm with a pair of digital calipers (Sylvae Ultra-Call, Li, USA). The bond strength values were calculated in MPa and analysed by two way ANOVA and Student Newman Keuls multiple comparison tests at α=0.05, to examine the contribution of the two factors, adhesive type and cyclic loading, and their interactions to the bond strength results. Fractured specimens were examined with a stereomicroscope (Olympus SZ-CTV, Olympus, Tokyo, Japan) at 40X magnification to determine the mode of failure. Failure modes were classified as adhesive, mixed, or cohesive in dentin or composite.

Representative fractured specimens from each of the ten subgroups were dehydrated for 48 h in a desiccator (Sample Dry Keeper Simulate Corp., Japan) and then mounted on aluminum stubs with carbon cement. They were then coated with gold by means of a sputter-coating unit (E500; Polaron Equipment Ltd., Watford, England) and observed with a scanning electron microscope (SEM) (Zeiss DSM-950, Karl-Zeiss, Germany) at an accelerating voltage of 20 kV, to examine the morphology of the debonded interfaces.

RESULTS

The mean MTBS values and failure modes obtained for the different groups are shown in Table 2. Both the type of adhesive ($F=25.02$; $P<0.0001$) and the use of mechanical loading ($F=41.91$; $P<0.0001$) influenced MTBS to dentin. No interaction existed between these two factors ($F=2.07$; $P=0.11$). The power of the statistical analysis for MTBS was 0.78.

Multiple comparisons tests further revealed that SEB and SB exhibited greater MTBS to dentin than the other three adhesives. PNT and PXP performed similarly, and E&P resulted in the lowest MTBS. When specimens were subjected to mechanical loading, decreases in MTBS were observed for all groups except for PXP. SEB, SB and PXP attained higher MTBS than PNT. All the specimens bonded with E&P failed prematurely during laboratory beam preparation and MTBS could not be obtained.

Most of the observed modes of failure were mixed except for specimens bonded with E&P, and for those bonded with PNT after mechanical loading, in which the failure modes were predominantly adhesive. Adhesive failures were associated with lower bond strengths. No cohesive failure of dentin or resin composite was observed in any specimen.

Fractured dentin surfaces after MTBS testing are shown in Figures 1 to 4. Mixed fracture modes showed partially cohesive failures within the adhesive resin in all groups (Figures 1A and 2A). For the simplified total-etch adhesives (SB, PNT, PXP) failures were frequently observed at either the top or the base of the hybrid layer, (Figures 1B and 2B). Partial cohesive fractures of demineralized dentin just below the hybrid layer were sometimes observed. Specimens bonded with EP failed adhesively between both tooth substrate and bonding layer (Fig. 3A and 3B). Images from

SEB specimens showed cohesive failures in both resin composite and adhesive with some fractures seen at the base of the hybrid layer (Fig. 4) or even within the underlying dentin.

DISCUSSION

Optimal dentin bonding is not always obtained in clinical practice, as normal daily functioning, malocclusion and parafunctional habits such as bruxism impose additional stresses upon the tooth and restorative system that may adversely affect the adhesive bond (Nikaido & others, 2002a). A load of 90 N was used in this study, as it was considered to be within the normal functional range (Anderson, 1956). In most of the studies 1,000 to 8,000 cycles are used; with 5,000 cycles being the median value (Abdalla & Davidson, 1996).

The one-step self-etching (all-in-one) adhesive E&P exhibited the lowest MTBS results and frequent adhesive failures (Fig. 3A). The less than optimal result achieved with this adhesive was further delineated after cyclic loading. Consensus exists in the literature that supports the poor performance of such all-in-one adhesives in bond strength measurements (Fritz & Finger, 1999; Inoue & others, 2000; Toledano & others, 2001; Toledano & others, 2003; Osorio & others 2003; De Munck & others, 2003a), although they were able to completely dissolve the smear layer, and formed a relatively thick hybridized complex (Haller & others, 2000; Cardoso, Placido & Moura, 2002; Toledano & others, 2003; Osorio & others 2003; Fritz & Finger, 1999) that incorporated the smear layer (Santini, Plasschaert & Mitchell, 2001). Several reasons have been advocated to account for the suboptimal performance of these all-in-one adhesive systems: (1) the combination of acidic hydrophilic and hydrophobic monomers into a single step may compromise the polymerization of the adhesive (De Munck & others, 2003a), (2) the stronger etching process may destabilize the collagen, leading to a decrease in bond strength (Yoshiyama & others, 1995), (3) the inherent weak strength of the adhesive polymer (Fritz & Finger, 1999; Haller & others, 2000; Inoue & others, 2000), and (4) the lower degree of polymerization of the resin monomer, due to a major solvent/oxygen inhibition effect in the photo-polymerization of these adhesives (Nunes & others

2004). The lack of adequate polymerization may also account for the inability of the specimens to withstand the occlusal loading forces, so that all specimens failed prematurely before testing.

PNT and the new experimental version of this simplified total-etch adhesive, PXP, showed similar initial MTBS values. Both adhesive systems have similar composition, containing PENTA, an acidic phosphonated monomer, which could have some kind of interaction with the calcium ions left on dentin surface, or even with the underlying dentin (Inai & others, 1998). After load cycling, MTBS values for PNT decreased but not those from PXP. Three main differences between these adhesives may account for these results: 1) PXP contains TEGDMA, which lowers the initial viscosity of the monomer mixture, enhancing its diffusion into the demineralised collagen matrix, increasing the flexibility of the hybridized dentin, and improving the rate of polymerization of the adhesive (Morgan & others, 2000; Nunes, Swift & Perdigão 2001; Nunes & others, 2004). 2) Camphorquinone is included as a photosensitizer, increasing the polymerization of monomers and bond strength to dentine (Miyazaki & others, 1995). 3) PXP contains t-butanol as solvent, (instead of acetone, in PNT). After demineralization, the collagen fibrils adhere to one another via intrafibrillar hydrogen bonding. A solvent with a solubility parameter for hydrogen bonding that approximates that of the amino acid moieties of the collagen fibrils has a better capacity in breaking up these intrafibrillar hydrogen bonds, and expanding the interfibrillar spaces to promote wetting and infiltration of the adhesive monomers (Pashley & others, 2003). It has been demonstrated that higher bond strengths were correlated with wider interfibrilar spaces and that such spaces should be properly infiltrated with resin (Eddleston & others, 2003). Application of acetone produces little solvation force affecting the further infiltration of resin monomers, while alcohols produces progressively higher solvation pressures that develop at increasing rates (Pashley & others, 2002; Reis & others, 2003). The total-etch alcohol-based adhesive systems used in the present investigation (SB and PXP) are thought to be able to maintain the collagen fibrils in an expanded condition after the evaporation of solvents, improving the monomers infiltration (Tay, Gwinnett &

Pang, 1996; Perdigão, Van Meerbeek & Lopes, 1999). This may contribute to explain the lower bond strengths of PNT after mechanical loading, because the decalcified non-infiltrated zone at the base of the hybrid layer is susceptible to degradation during aging (Hashimoto & others, 2002a; 2002b; Pashley & others, 2002). Moreover, a low rate of polymerization of the bonding resin within the hybrid layer has been shown for PNT (Hashimoto & others, 2002a), which also may lead to rapid degradation of the resin-dentin bonds.

SB and SEB obtained the highest MTBS to dentin. SB is an adhesive based on a HEMA/alcohol mixture and has been shown to obtain high bond strength values to dentin, when compared to other total-etch adhesives (De Munck & others, 2003b). The results of SB were also comparable to those of SEB (Toledano & others, 2003). A MTBS decrease is observed after mechanical loading as well as previously reported, after water degradation (De Munck & others, 2003b).

SEB is a two-step self-etching primer containing a highly hydrophilic 10-MDP monomer, which is believed to improve the wetting of the tooth surface (Van Meerbeeck & others, 1994). SEB causes minimal dissolution of smear plugs and limited opening of tubules, which reduces dentin permeability (Jackson & Söderholm, 2001) and facilitates penetration, impregnation, polymerization and entanglement of monomers with the underlying dentin to form an hybrid layer (Inoue & others, 2000; Toledano & others, 2002; Osorio & others, 2003). Moreover, 10-MDP has two hydroxyl groups that may chelate with calcium ions of dentin (Kubo & others, 2001; Nunes & others, 2003).

Within the limits of the study, we have to reject the null hypothesis as cyclic loading lowered resin-dentin bond strengths of all the total-etch or self-etching adhesive systems examined. Fatigue stress can expedite the degradation of bonds peripheral to the hybrid layer (Nikaido &

others, 2002b; Sano & others, 1999; Qvist & others, 1983). When using SEB, the loading stress seemed to have been concentrated mostly at the interface between the adhesive and the hybrid layer and within the hybrid layer, whereas specimens bonded using a total-etch approach (SB, PNT and PXP) mostly failed at the top of, or beneath the hybrid layer where demineralised collagen fibrils were exposed and the adhesive failed to envelop the collagen network (Figures 1 and 2). Such factors have been perceived to be the weakest link in achieving durable long-term bonding (Nikaido & others, 2002b; Osorio & others, 2003; Pashley & others, 2002). Although the results obtained from this study may not be directly extrapolated to the clinical situation, they provide some information regarding the susceptibility of resin-dentin bonds to deteriorate after cyclic loading. Long-term clinical data are still required to further evaluate the efficacy of these adhesives on dentin.

CLINICAL SIGNIFICANCE

The resin-dentin bond is prone to deterioration after cyclic loading, and the all-in-one adhesive examined is the least reliable system. After acid etching of dentin, alcohol/based adhesives performed better than those containing acetone as solvent.

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Table 1: Mode of Application, compositions, and manufacturers of tested adhesives.

Materials	Components	Mode/steps of application	Manufacturer
Single Bond	2-Hydroxyethylmethacrylate; water; ethanol; Bis-GMA; dimethacrylates; amines; methacrylate-functional; copolymer of polyacrylic and polyitaconic acids.	Etch for 15 seconds. Rinse with water spray for 10 seconds, leaving tooth moist. Apply two consecutive coats of the adhesive with a fully saturated brush tip. Dry gently for 2-5 seconds. Light cure for 10 seconds.	3M, St.Paul, MN, USA. Lot. 4242.
Prime & Bond NT	PENTA; UDMA resin; Resin R5-62-1; T-resin; D-resin; nanofiller; initiators; stabilizer; cetylamine hydrofluoride; acetone.	Etch for 15 seconds. Rinse with water spray for 15 seconds and remove water with a soft blow of air. Leave a moist surface. Apply ample amounts of the adhesive to saturate the surface, reapply if it is necessary. Leave the surface undisturbed for 20 seconds. Remove solvent by blowing gently with air for at least 5 seconds. Light cure for 10 seconds.	Dentsply / De Trey GmbH, Konstanz, Germany. Lot. 0209000918.
Clearfil SE Bond	Primer: 10-methacryloyloxydecyl dihydrogen phosphate; 2-hydroxyethyl methacrylate; Hydrophilic dimethacrylate; di-camphorquinone; N,N-diethanol-p-toudine, water. Bond: 10-methacryloyloxydecyldihydrogen phosphate; N,N-diethanol-p-toludine; 2-hydroxyethylmethacrylate; Bis-phenol A diglycidylmethacrylate; silanated colloidal silica; hydrophobic dimethacrylate; di-camphorquinone.	Apply Primer for 20 seconds. Mild air stream. Apply Bond. Gentle air stream. Light cure for 10 seconds.	Kuraray Co, Osaka, Japan. Lot. 390.
Etch & Prime 3.0	Universal: 2hydroxyethylmethacrylate;Water; ethanol Catalyst: Tetramethacrylocyethylpyrophophate.	Mix Etch & Prime 3.0 Universal and Catalyst. Apply for 30 seconds. Air blow gently. Light cure for 10 seconds. Repeat the above mentioned steps.	Degussa AG, Hanau, Germany. Lot.019920.
Prime & Bond XP	TCBresin; PENTA; UDMA; TEGDMA; BHT; camphorquinone; functionalised amorphous silica ethyl-4-dimethylaminobenzoate; t-butanol.	Etch for 15 seconds. Rinse with water spray for 15 seconds and remove water with a soft blow of air. Leave a moist surface. Dispense directly into a disposable brush. Apply ample amounts of the adhesive to saturate the surface, reapply if it is necessary. Leave the surface undisturbed for 20 seconds. Remove solvent by blowing gently with air for at least 5 seconds. Light cure for 10 seconds.	Dentsply / De Trey GmbH, Konstanz, Germany. Lot. 0304000987.

PENTA= penta-acrylate ester; TEGDMA= triethylene glycol-dimethacrylate; Bis-GMA= bysphenyl glycidyl methacrylate, UDMA= urethane dimethacrylate; BHT= butylated hydroxyl toluene; TCB resin = carboxylic acid modified dimethacrylate.

Table 2: MTBS values and distribution of failure modes (A: Adhesive; M: Mixed) obtained with the different adhesive systems with and without cyclic loading.

	Without load cycling			Load cycling		
	Mean (SD)	A	M	Mean (SD)	A	M
SEB	46.07 (12.1) **A**	34.5 %	65.5 %	30.61 (5.3) **B**	44.5 %	55.5 %
SB	43.34 (11.1) **A**	27.9 %	72.1 %	28.29 (8.4) **B**	40 %	60 %
PXP	29.79 (4.5) **B**	20 %	80 %	25.15 (7.9) **B**	27.9 %	72.1 %
PNT	29.08 (4.7) **B**	34 %	66 %	11.73 (3.2) **D**	77.8 %	22.2 %
E&P	16.99 (7.7) **C**	72.7 %	27.3 %	XX	XX	XX

Values are means (standard deviation) in MPa, with the number of beams chosen as the statistical unit (n=32-39). Groups with the same letter are not statistically significant (P>0.05).
XX: No MTBS data could be obtained due to premature failure of all the specimens during beam preparation.

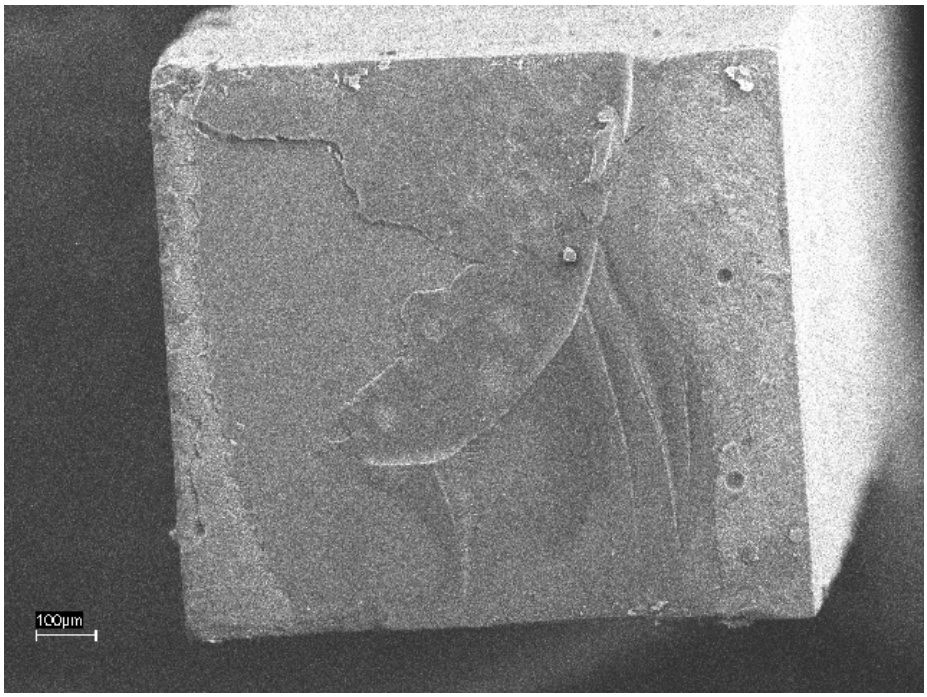

Figure 1a: SEM images of the fractured dentin surface of a specimen bonded with Single Bond after cyclic loading. A mixed failure could be observed, with resin composite present at the right and left margins, and adhesive in the central area.

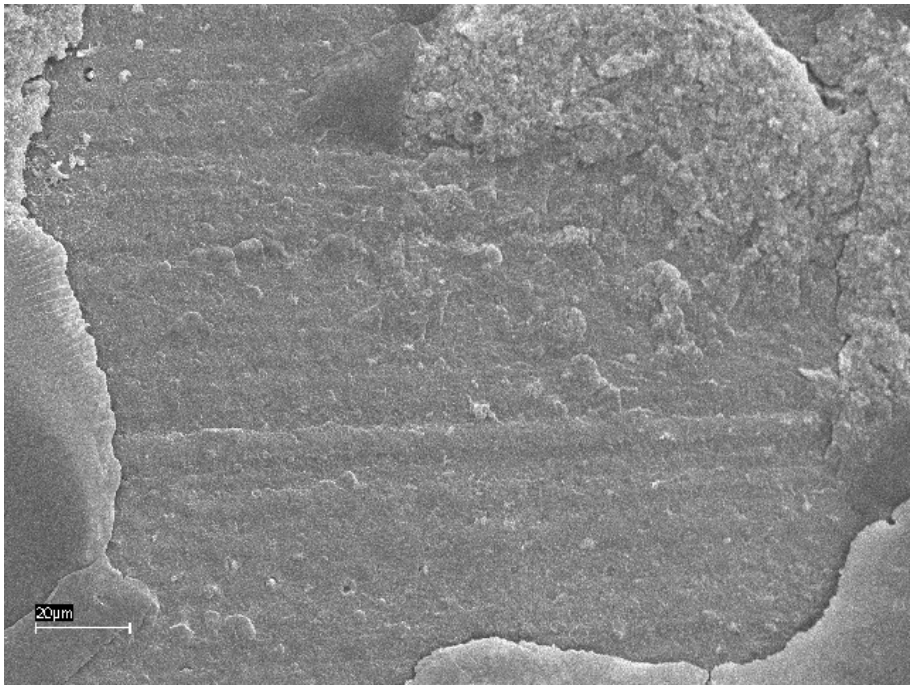

Figure 1b: SEM images of the fractured dentin surface of a specimen bonded with Single Bond after cyclic loading. A higher magnification view of the failure that occurred at the top of the hybrid layer.

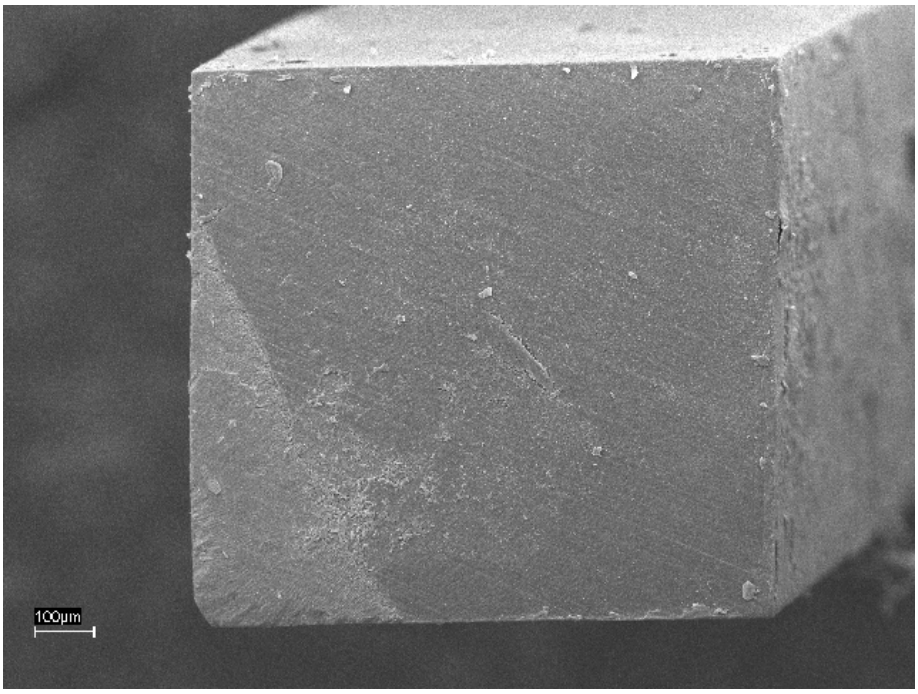

Figure 2a: SEM observations of the fractured surface along the dentin side of a specimen bonded with Prime&Bond NT, after cyclic loading. An adhesive failure, mainly at the top of the hybrid layer is observed, but a small area (left and inferior corner) failed at the bottom of the hybrid layer.

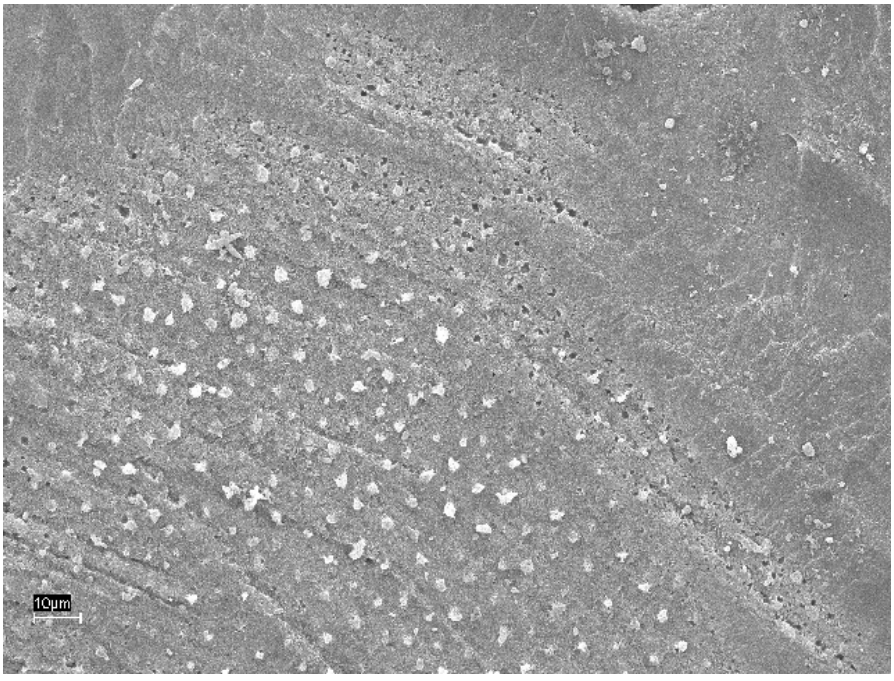

Figure 2b: SEM observations of the fractured surface along the dentin side of a specimen bonded with Prime&Bond NT, after cyclic loading. At a higher magnification, resin filled dentinal tubules are shown, but non-infiltrated dentin and porosity within the hybrid layer are also shown.

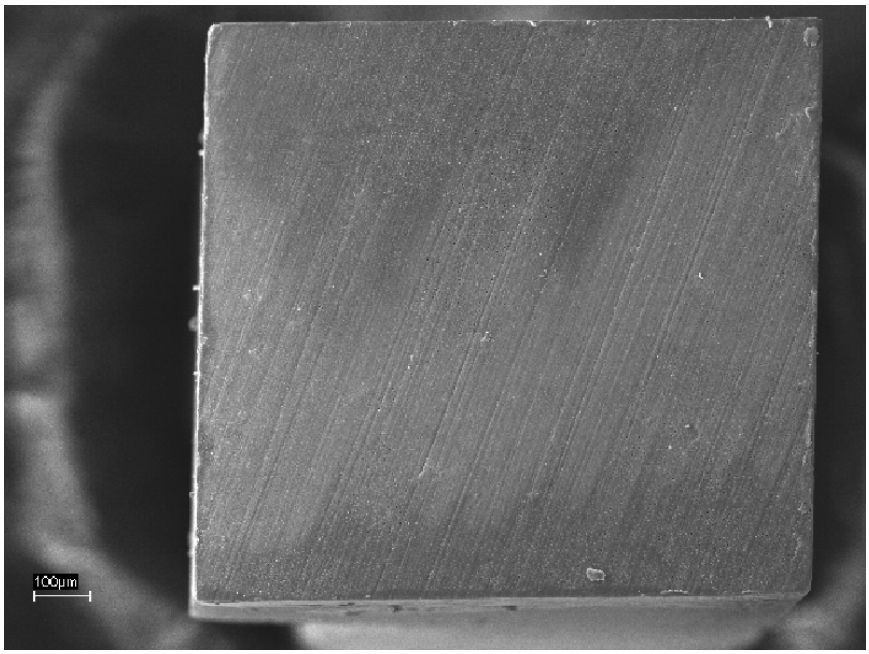

Figure 3a: SEM observations of the fractured surface along the dentin side of a specimen bonded with Etch&Prime 3.0, before cyclic loading. A general image of a typical adhesive failure. Scratches that remained from preparation of the bonding dentin surface with silicon carbide papers confirmed that the interface failed adhesively at the level between dentin and the adhesive.

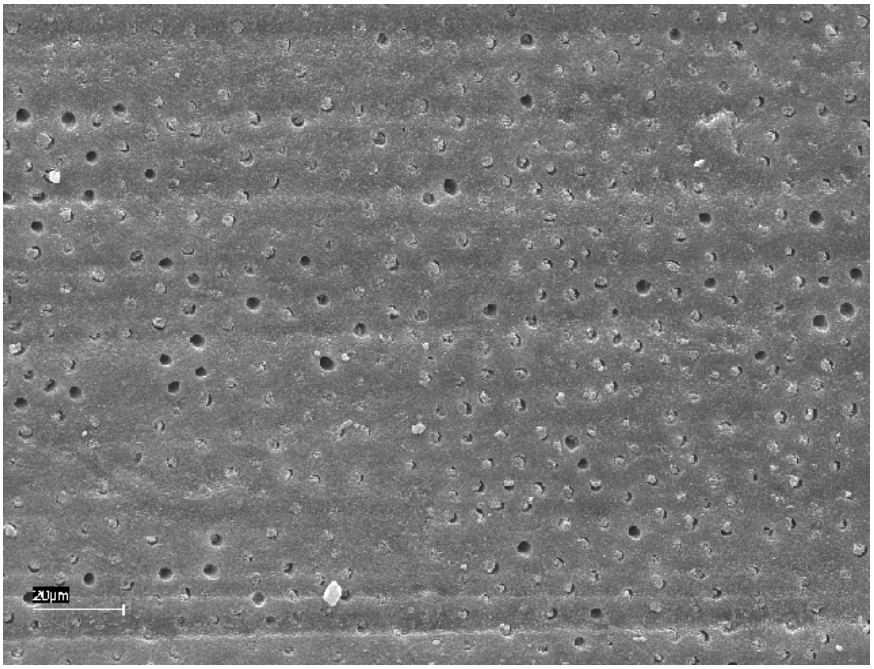

Figure 3b: SEM observations of the fractured surface along the dentin side of a specimen bonded with Etch&Prime 3.0, before cyclic loading. The enlarged entrances of the dentinal tubules could be observed, and only some of them were occluded by resin tags.

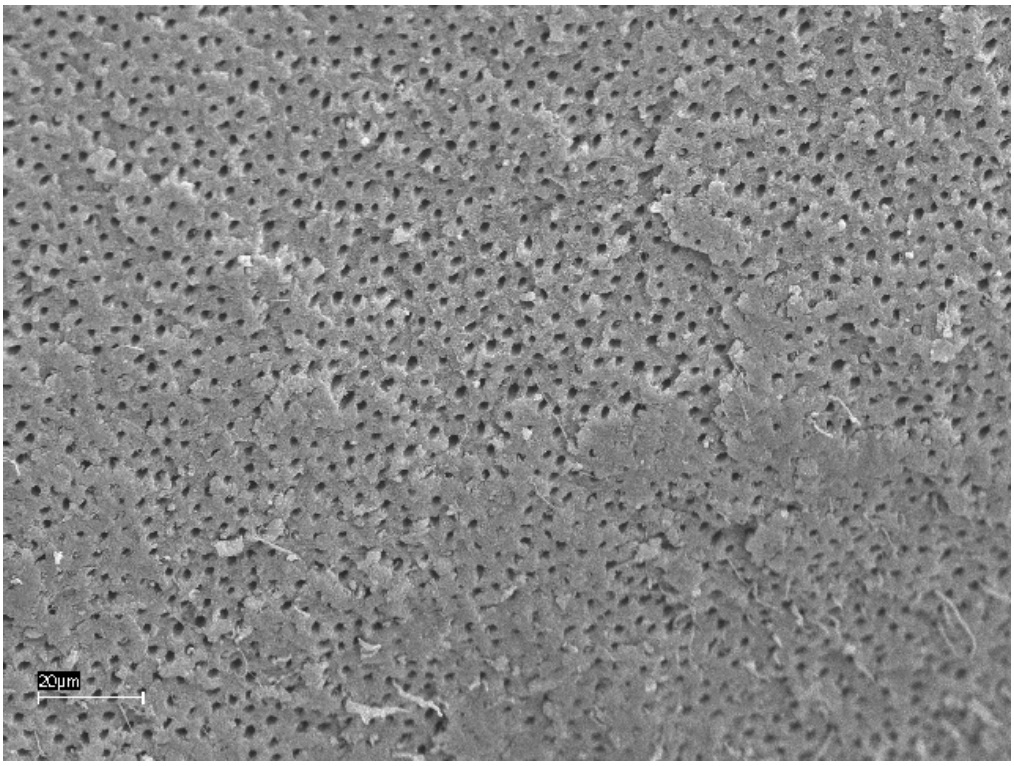

Figure 4: SEM image of a specimen bonded with CSEB, showing cohesive fractures of the dentin just below the hybrid layer.

III.2. Differential effect of *in vitro* degradation on resin-dentin bonds produced by self-etch vs. total-etch adhesives

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ABSTRACT

Objective: To evaluate the effect of an in vitro challenge (NaOCl immersion) on microtensile bond strength (MTBS) of five adhesive systems to dentin. *Methods:* flat dentin surfaces from forty molars were bonded with three total-etch adhesives (Single Bond, Prime&Bond NT and the experimental Prime&Bond XP), and two self-etching agents (Clearfil SE Bond and Etch&Prime 3.0). Composite build-ups were constructed with Tetric Ceram. Teeth were then sectioned into beams of 1.0 mm^2 cross-sectional area. Half of the beams were immersed in 10 % NaOCl aqueous solution for 5 h. Each beam was tested in tension in an Instron machine at 0.5 mm/min. Data were analyzed by 2-way ANOVA and multiple comparisons tests ($P<0.05$). *Results:* Clearfil SE Bond and Single Bond attained higher MTBS than the other three adhesives. Prime&Bond NT and Prime&Bond XP performed equally, and Etch&Prime resulted in the lowest MTBS. After NaOCl immersion, MTBS decreased in all groups. The highest MTBS values were obtained for Clearfil SE Bond and Prime&Bond XP. *Conclusions:* The extent of the resin-dentin bond degradation is material dependent. A high polymerization degree of the bonding resins, within the hybrid layer, may be a very important factor to improve the long-term durability of resin-dentin bonds.

Keywords: dentin, resin, degradation, bonding, sodium hypochlorite

INTRODUCTION

The durability of bonds between adhesive resins and dentin is of critical importance [1,2] and little is known regarding the stability of hybridized layers [3]. In general, reports show that dentin bond strength decreased during water storage over time, due to degradation of the resin and the collagen fibrils within the hybrid layer [2,4,5,6].

Dentin bonding systems have been simplified and improved in order to provide increased long-term durability of adhesive restorations [7]. Two main strategies are used to create dentin bonding: 1) the total-etch bonding systems (TE) work by removing the smear layer with phosphoric acid, followed by the application of a primer and an adhesive in two different steps or in the same one (Single Bond, Prime&Bond NT and Prime&Bond XP), but further incomplete expansion of collagen may impair resin infiltration and compromise bonding [8,9]; and 2) the self-etching approach (SE), in which the acid and the primer are combined in one solution to form an acidic monomer [10] and a final bonding step is later applied (Clearfil SE Bond). Self-etch all-in-one adhesives have also been introduced and contain all components in just one solution (Etch&Prime). When using all these self-etching systems less discrepancy is expected between the depth of demineralisation and depth of resin infiltration [10,11].

In vitro accelerated aging tests for resin-dentin bonds have been proposed, reducing specimen size and immersing the bonded sticks in 10% sodium hypochlorite aqueous solution ($NaOCl_{aq}$) for a short experimental time period, to determine the ability of resin monomers to protect the collagen matrix of dentin from proteolytic activity [12,13].

The null hypotheses to be tested are that there are not differences in dentin bond strength when using different adhesive systems, and that NaOCl immersion of specimens does not affect obtained bond strength to dentin.

MATERIAL AND METHODS

Forty caries-free extracted human third molars that were stored (4 ºC) in 0.5% chloramine T for up to one month were used. The specimens were sectioned below the dentin-enamel junction and ground flat with 180-grit silicon carbide abrasive papers under running water to provide uniform and smear-layer covered dentin surfaces. Single Bond, Prime&Bond NT, Prime&Bond XP, Clearfil SE Bond and Etch&Prime adhesives were applied following manufacturers´ instructions. Table 1 displays mode of application, components and manufacturers of the tested adhesives. Resin build-ups, each 6 mm in height, were constructed incrementally (1.5 mm) with Tetric Ceram resin composite (Vivadent, Schänn, Liechtenstein). Each layer of the composite was light-activated for 40 s with a Translux EC halogen light-curing unit (Kulzer GmbH, Bereich Dental, Wehrheim, Germany). Light intensity output was monitored with a Demetron Curing Radiometer (Model 100 Demetron Research Corporation, Danbury, CT, USA) to be, at least, 600 mW/cm^2.

After storage in distilled water for 24 h at 37 ºC, specimens were vertically sectioned into serial slabs, and further into beams giving a cross-sectional area of 1 mm^2. Half of the beams in each group were immersed in 10% NaOCl solution (Panreac Química SA, Barcelona, Spain) for 5 h, following by rinsing in water for 1 hour. Approximately 39-42 beams resulted from each subgroup, they were attached to a modified Bencor Multi-T testing apparatus (Danville Engineering Co., Danville, CA) with a cyanoacrylate adhesive (Zapit, Dental Venture of America Inc., Corona, CA, USA) and stressed to failure in tension using in a universal testing machine (Instron 4411, Instron Corporation, Canton, MA, USA) at a crosshead speed of 0.5 mm/min. The fractured beams were carefully removed from the apparatus and the cross-sectional area at the site of failure was measured to the nearest 0.01 mm with a pair of digital calipers (Sylvae Ultra-Call, Li, USA). The bond strength values were calculated in MPa and analysed by ANOVA and Student Newman Keuls multiple comparisons ($P<0.05$). Fractured specimens were examined with a stereomicroscope

(Olympus SZ-CTV, Olympus, Tokyo, Japan) at 40X magnification to determine the mode of failure. Failure modes were classified as adhesive or mixed. Representative specimens of each group were maintained for 48 h in a dessicator (Sample Dry Keeper Simulate Corp., Japan) and then mounted on aluminum stubs with carbon cement. They were then sputter-coated with pure gold by means of a sputter-coating Unit E500 (Polaron Equipment Ltd., Watford, England) and observed with a scanning electron microscopy (SEM) (Zeiss DSM-950, Karl-Zeiss, Germany) at an accelerating voltage of 20 kV, so that microscopic fracture patterns and the morphology of the debonded interface could be studied.

RESULTS

Mean MTBS values and modes of failures obtained for the different groups are shown in Table 2. The adhesive system ($F=25.06$; $P<0.001$) and $NaOCl_{aq}$ immersion ($F=150,79$; $P<0.001$) influenced MTBS to dentin. Interactions existed between both variables ($F=6.68$; $P<0.001$). The power of the multiple ANOVA analysis for MTBS was about 0.82.

Clearfil SE Bond (SE) and Single Bond (TE) give greater MTBS to dentin than the other three adhesives. Prime&Bond NT (TE) and Prime&Bond XP (TE) performed similarly, and Etch&Prime (SE) resulted in the lowest MTBS. When specimens were subjected to $NaOCl_{aq}$ immersion, decreases in MTBS were observed for all groups. The SE system -Clearfil SE Bond- and the TE adhesive –Prime&Bond XP- attained higher MTBS than the rest of the adhesives. Specimens bonded with Etch&Prime (SE) produced pretesting failures and MTBS values could not be obtained.

Most of the observed modes of failure were mixed except for specimens bonded with the one-step self-etch adhesive (Etch&Prime) that failed predominantly adhesively. Major rates of adhesive failures were associated with lower bond strengths. Specimens that undergo $NaOCl_{aq}$ immersion failed predominantly mixed, except for Prime&Bond NT (TE) that presented mostly adhesive failures. No completely cohesive failure of dentin or resin composite was observed in any specimen.

SEM of debonded dentin surfaces after MTBS testing are shown in Fig. 1 to 5. Mixed fracture modes were frequent in all groups except for Etch&Prime (SE) that failed predominantely adhesively at the top of the hybridized smear layer (Fig. 1a), and resin tags are observed occluding

the enlarged tubule entrances (Fig. 1b). In the case of the total-etch adhesives failures were frequently mixed found at the base of the hybrid layer, partial cohesive fractures of demineralized dentin just below the hybrid layer were sometimes observed (not shown), usually associated with high bond strength data. Images from Clearfil SE Bond (SE) specimens showed mixed failures found either at the top, at the base of the hybrid layer or within the hibridized smear layer, and often both within the same section. After $NaOCl_{aq}$ immersion, specimens showed partially cohesive failures within the adhesive resin (Fig. 2a to 5a). A gradual loss of adhesive from the periphery to the center portion of the bonding area is observed in all groups, and resin remaining area is smaller for Prime&Bond NT than for the rest of the adhesives (Fig. 2a to 5a). For Single Bond and Prime&Bond NT (TE) groups, fractures used to be at the top of the hybrid layer (as scratching from dentin grinding are clearly observed) (Fig. 2a and 3a), resin tags are maintained at the tubule entrances (Fig. 2b and 3b). When Prime&Bond XP (TE) specimens are examined, most of the fractures are located at the base of the hybrid layer and at the non-infiltrated underlying dentin (Fig. 4a), showing opened and enlarged dentin tubule entrances, without resin tags (Fig. 4b). Specimens bonded with Clearfil SE Bond (SE) showed most of the failures at the top of the hybrid layer (Fig. 5a), and small areas of fractures located at the base of the hybrid layer, exposing the underlying dentin, may also be observed (Fig. 5b). Tubule entrances may be observed, they are not enlarged but opened and filled with resin tags (Fig. 5b).

DISCUSSION

The total-etch self-priming adhesives Prime&Bond NT and Prime&Bond XP showed similar initial MTBS values. Both adhesive systems contain PENTA, an acidic phosphonated monomer, which could have some kind of interaction with the calcium ions left on dentin surface, or even with the underlying dentin [14]. The TE system Single Bond attained higher values, it is based on a HEMA/alcohol mixture that is able to better wet the etched dentin surface and maintain the collagen fibers in an expanded condition after the evaporation of solvents, improving the monomers infiltration, and has been shown to obtain high bond strength values to dentin, when compared to other total-etch adhesives [5,15,16].

Bond strength of the TE Single Bond to dentin was similar to that of the SE Clearfil SE Bond [17]. Clearfil SE Bond contains a highly hydrophilic 10-MDP monomer, which is believed to improve the wetting of the tooth surface and chelate to calcium ions of dentin [18]. This SE adhesive causes minimal dissolution of smear plugs and facilitates penetration, impregnation, polymerization and entanglement of monomers with the underlying dentin to form a hybrid layer [17,19,20].

Etch&Prime is a 1-step SE adhesive system (all-in-one), obtaining the lowest MTBS values and frequent adhesive failures (Fig. 1). Consensus exists about the low bond strength of most of these all-in-one adhesive systems [5,17,19,20,21]. Even though, studies have shown that the bonding agent completely dissolved the smear layer, formed a relatively thick hybridized complex [22,23,24,20,17], incorporating the smear layer and tubule entrances were not only opened but also enlarged creating thick resin tags (Fig. 2) [17,20]. Some reasons have been advocated to explain the worse bonding performance of these all-in-one adhesive systems as the inherent weak strength of the adhesive polymer [19,22,24] and the low degree of polymerization of the resin monomer,

probably due to a major solvent/oxygen inhibition effect in the photopolymerization of these adhesives [20,25].

After storage in $NaOCl_{aq}$, the MTBS fell in all specimens. $NaOCl_{aq}$ is a nonspecific deproteinizing agent, in aqueous solution superoxide radicals O_2^- are formed and induce oxidations that fragment long peptide chains of proteins [26]. Chlorination of protein terminal groups is also produced and hypochlorous acid formation evidenced [27]. Some of these amino acid-derived chloramines have also shown to increase the proteolytic susceptibility of this modified collagen [28]. The decline in bond strength is the result of both an hydrolytic process on the resin and the solubilization of unprotected collagen fibrils within the decalcified dentin [5,12,13,29,30,31].

Some studies have been conducted in order to evaluate the long-term durability of resin-dentin bonds. *In vivo* studies showed that the bond strength of a three-step total-etch adhesive may be reduced by 50-65% after 2-3 years [32], or even by 60 and 77% within 1 year when using a self-priming total-etch adhesive and a two-step self-etching adhesive respectively [33]. However, *in vitro* studies based on water storage of the specimens, attained smaller reductions in bond strength that are between 23 and 55%, [4,5,6,34], even when the dentin-resin interfaces were directly exposed to water up to six years [34]. The presented challenging method based upon 10% $NaOCl_{aq}$ immersion of specimens during a short period of time is much more reliable than *in vitro* studies based on long-term water storage of specimens. Obtained reductions in bond strength in the present study, and those previously reported after $NaOCl_{aq}$ immersion (65% to 77%) [12,13] are similar to the decline in bond strength obtained when *in vivo* degradation studies are performed [32,33]. *In vivo* studies also reported that the microestructural aspect of the debonded surfaces suggested that the exposed collagen was digested by proteolytic enzymes, which may be released from leukocytes, salivary glands, and bacteria in plaque, explaining the attained very low bond strengths [32], and

resinous materials seems to be degraded and extracted from the hybrid layer, increasing the porosities at the interface [3,33].

A larger fall in bond strength was expected for the TE adhesives, when compared to the SE systems, due to the existence of a discrepancy between the etching and the infiltrating processes [8,9,12,13]. However, it is not observed at the present results. This may account for the reported evidences supporting that for some self-etching systems an etched non-resin-infiltrated layer remains after bonding [11,35]. It may also be that when using so highly hydrophilic resins, the hydrolytic degradation of the resin is the most detrimental factor affecting long-term bonding effectiveness.

The high hydrophilicity of the adhesive resin which is an advantage during dentin bonding, may be a disadvantage in the long-term durability. As the higher water solubility of the resin and the existence of residual water within the hybrid layer may lead to: voids formation, a lower degree of cure of the adhesive resin [36,37,38], and poorly infiltrated collagen fibrils within the hybrid layer that might accelerate the degradation effect [38].

Fractographic analysis of debonded sticks showed a gradual loss of adhesive at the top of the hybrid layer or at the top of the hybridized smear layer (as grinding marks of polish may be observed on the exposed dentin surface) (Fig. 2,3,5) with this loss of adhesive being from the periphery to the center of the debonded area. The TE Prime&Bond XP and the SE Clearfil SE Bond, that attained the better resistance to the challenging of $NaOCl_{aq}$ immersion, showed the smallest resin dissolution areas (Fig. 2 to 5). The resistance of these resins to the hydrolytic degradation may be the chief reason of the lower reduction in bond strength attained by these adhesives. The least susceptibility of these resins to hydrolysis is probably due to a higher degree of

cure of the bonding resins. Prime&Bond XP contains TEGDMA, which brings down the initial viscosity of the monomer mixture, enhancing diffusion of reactive groups, increasing the flexibility, and the rates of polymerisation of the resin [25,39]. Camphorquinone is included in both adhesive systems (Prime&Bond XP and Clearfil SE Bond) as sensitier. This activator is in charge to trigger the cascade reaction of the curing, generating free radicals and increasing the polymerization of monomers [37]. Moreover, a low rate of polymerization of the bonding resin has been previously shown for Prime&Bond NT (Fig. 3) [40], and for Etch&Prime [25] leading to rapid degradation of the dentin bonds.

According to previous observations the loss of resin was responsible for the loss of bond strength [12,13]. The extent of resin dissolution is proportional to the bond strength decrease [12,13] and is material dependent. However, the deterioration patterns depend on the used adhesive approach (e.g. SE or TE). The test medium acted more aggressively with the intertubular dentin of specimens bonded by a TE procedure compared to those bonded with the SE system. After phosphoric acid etching collagen is highly susceptible to deproteinization processes [41]. When bonding with a TE adhesive, the $NaOCl_{aq}$ affected the resin-dentin bond structures following two pathways 1) the etched and non-infiltrated layer (Fig. 4b) and 2) the collagen that was resin-infiltrated but later exposed because of the bonding resin dissolution by the $NaOCl_{aq}$ (Fig. 2b,3b).

Although the results obtained from this study may not be directly extrapolated to the clinical situation, they provide some information with regard to the performance of dentin treatment procedures. This *in vitro* testing data can elucidate some specific factors, that may be more detrimental to long-term bonding effectiveness, as the hydrolytic degradation susceptibility of the used adhesive resin, and future research can focus on improving it. Even so, long-term clinical data are still required to further evaluate the efficacy of these adhesives on dentin.

The null hypothesis has to be rejected as bond strength to dentin is different for the tested adhesives and formed resin-dentin bonds are prone to *in vitro* degradation after $NaOCl_{aq}$ immersion.

CLINICAL SIGNIFICANCE

The resin-dentin bond is prone to hydrolitic degradation, and the tested all-in-one adhesive, E&P, provided the least durable bond strength. After the TE procedure, hydrolytic degradation not only of the non-resin infiltrated but also of the resin-infiltrated collagen fibers does exist, and may decline long-term bond strength of these systems to dentin. Resin dissolution rate is material dependent and a high polymerization degree of the bonding resins, within the hybrid layer, may be a very important factor to improve the long-term durability of resin-dentin bonds. The search for 2-steps self-etch systems with a high polymerization degree of the adhesive resin is encouraged.

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Table 1: Mode of Application, compositions, and manufacturers of tested adhesives.

Materials	Components	Mode/steps of application	Manufacturer
Single Bond **Total-etch** **Self-priming**	2-Hydroxyethylmethacrylate; water; ethanol; Bis-GMA; dimethacrylates; amines; methacrylate-functional; copolymer of polyacrylic and polyitaconic acids.	Etch for 15 seconds. Rinse with water spray for 10 seconds, leaving tooth moist. Apply two consecutive coats of the adhesive with a fully saturated brush tip. Dry gently for 2-5 seconds. Light cure for 10 seconds.	3M, St.Paul, MN, USA. Lot. 4242.
Prime & Bond NT **Total-etch** **Self-priming**	PENTA; UDMA resin; Resin R5-62-1; T-resin; D-resin; nanofiller; initiators; stabilizer; cetylamine hydrofluoride; acetone.	Etch for 15 seconds. Rinse with water spray for 15 seconds and remove water with a soft blow of air. Leave a moist surface. Apply ample amounts of the adhesive to saturate the surface, reapply if it is necessary. Leave the surface undisturbed for 20 seconds. Remove solvent by blowing gently with air for at least 5 seconds. Light cure for 10 seconds.	Dentsply / De Trey GmbH, Konstanz, Germany. Lot. 0209000918.
Clearfil SE Bond **Self-etch** **2-steps**	Primer: 10-methacryloyloxydecyl dihydrogen phosphate; 2-hydroxyethyl methacrylate; Hydrophilic dimethacrylate; di-camphorquinone; N,N-diethanol-p-toudine, water. Bond: 10-methacryloyloxydecyldihydrogen phosphate; N,N-diethanol-p-toludine; 2-hydroxyethylmethacrylate; Bis-phenol A diglycidylmethacrylate; silanated colloidal silica; hydrophobic dimethacrylate; di-camphorquinone.	Apply Primer for 20 seconds. Mild air stream. Apply Bond. Gentle air stream. Light cure for 10 seconds.	Kuraray Co, Osaka, Japan. Lot. 390.
Etch & Prime 3.0 **Self-etch** **1-step**	Universal: 2hydroxyethylmethacrylate;Water; ethanol Catalyst: Tetramethacrylocyethylpyrophophate.	Mix Etch & Prime 3.0 Universal and Catalyst. Apply for 30 seconds. Air blow gently. Light cure for 10 seconds. Repeat the above mentioned steps.	Degussa AG, Hanau, Germany. Lot.019920.
Prime & Bond XP **Experimental** **Total-etch** **Self-priming**	TCBresin; PENTA; UDMA; TEGDMA; BHT; camphorquinone; functionalised amorphous silica ethyl-4-dimethylaminobenzoate; t-butanol.	Etch for 15 seconds. Rinse with water spray for 15 seconds and remove water with a soft blow of air. Leave a moist surface. Dispense directly into a disposable brush. Apply ample amounts of the adhesive to saturate the surface, reapply if it is necessary. Leave the surface undisturbed for 20 seconds. Remove solvent by blowing gently with air for at least 5 seconds. Light cure for 10 seconds.	Dentsply / De Trey GmbH, Konstanz, Germany. Lot. 0304000987.

PENTA= penta-acrylate ester; TEGDMA= triethylene glycol-dimethacrylate; Bis-GMA= bysphenyl glycidyl methacrylate, UDMA= urethane dimethacrylate; BHT= butylated hydroxyl toluene; TCB resin = carboxylic acid modified dimethacrylate.

Table 2: MTBS values and distribution of failure modes (A: Adhesive; M: Mixed) obtained with the different adhesive systems with and without NaOCl challenge. Values are means (standard deviation) in MPa, with the number of beams chosen as the statistical unit (n=39-42).

		24 h evaluation			NaOCl challenge		
		Mean (SD)	A	M	Mean (SD)	A	M
Self-etch	SEB	44.79 (10.1) **A**	33 %	67 %	23.53 (4.0) a *	0 %	100 %
Total-etch	SB	42.84 (9.1) **A**	25 %	75 %	16.22 (4.9) b *	0 %	100 %
Total-etch	PXP	30.01 (5.5) **B**	24 %	76 %	20.62 (5.8) a *	0 %	100 %
Total-etch	PNT	30.08 (5.7) **B**	30 %	70 %	13.26 (2.0) b *	90 %	10 %
Self-etch	E&P	15.01 (6.5) **C**	82 %	18 %	XX	XX	XX

Within the same column, groups with the same letter are not statistically significant.
* indicates differences between subgroups at 24 h evaluation and after NaOCl immersion. (α= 0.05).
XX: No MTBS data could be obtained due to premature failure of all the specimens during beams preparation.

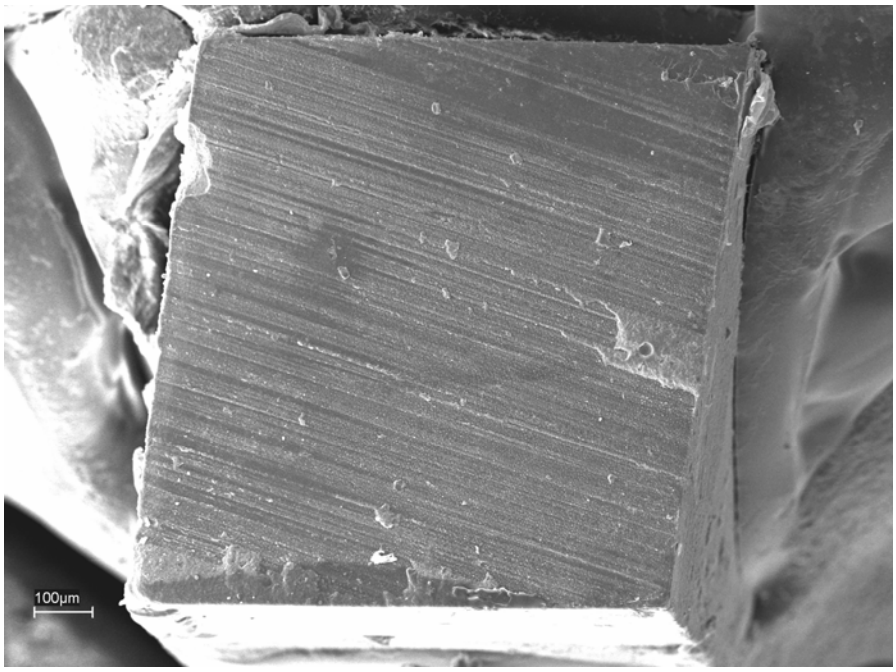


Figure 1a: SEM observations of the fractured surface along the dentin side of a specimen bonded with Etch&Prime 3.0. A general image of a typical adhesive failure. Existence of the scratches, produced by the preparation of the bonding dentin surface with carbide papers, confirmed that the interface failed adhesively at the top of the hybridized smear layer, and dentin remains covered by some adhesive resin.

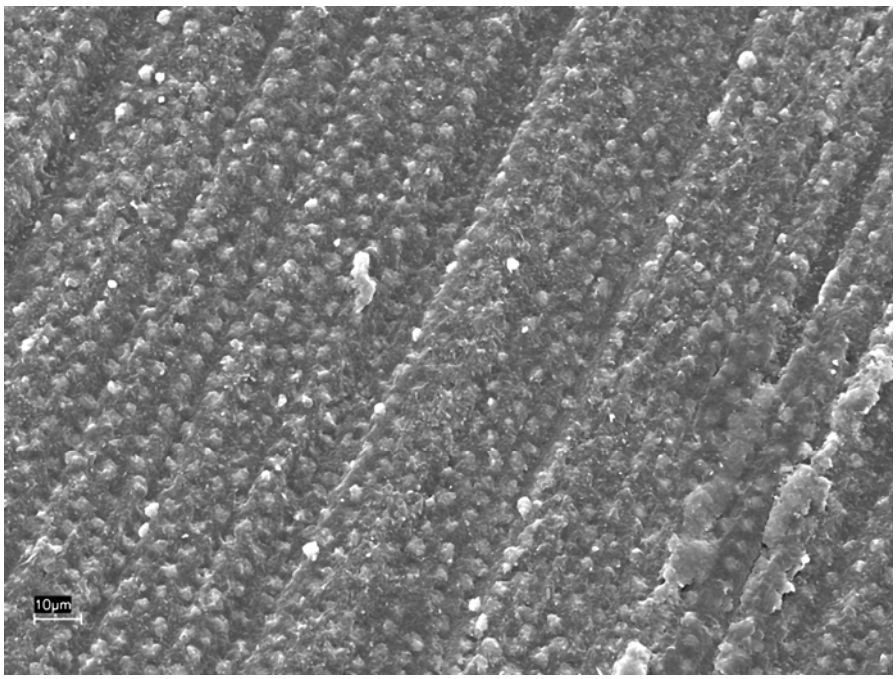


Figure 1b: SEM observations of the fractured surface along the dentin side of a specimen bonded with Etch&Prime 3.0. Enlarged entrances of the dentinal tubules occluded by resin tags are observed.

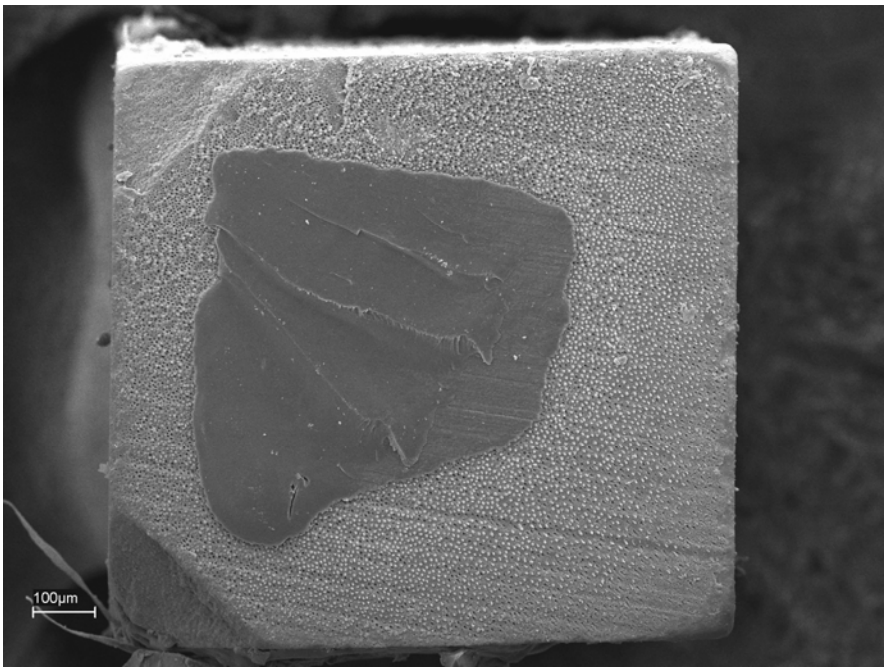


Figure 2a: SEM images of the fractured dentin surface of a specimen bonded with Single Bond and debonded after NaOClaq immersion. A mixed failure may be observed, the main fracture occurred at the top of the hybrid layer (because of the loss of the adhesive resin). Some adhesive resin is remaining at the central area.

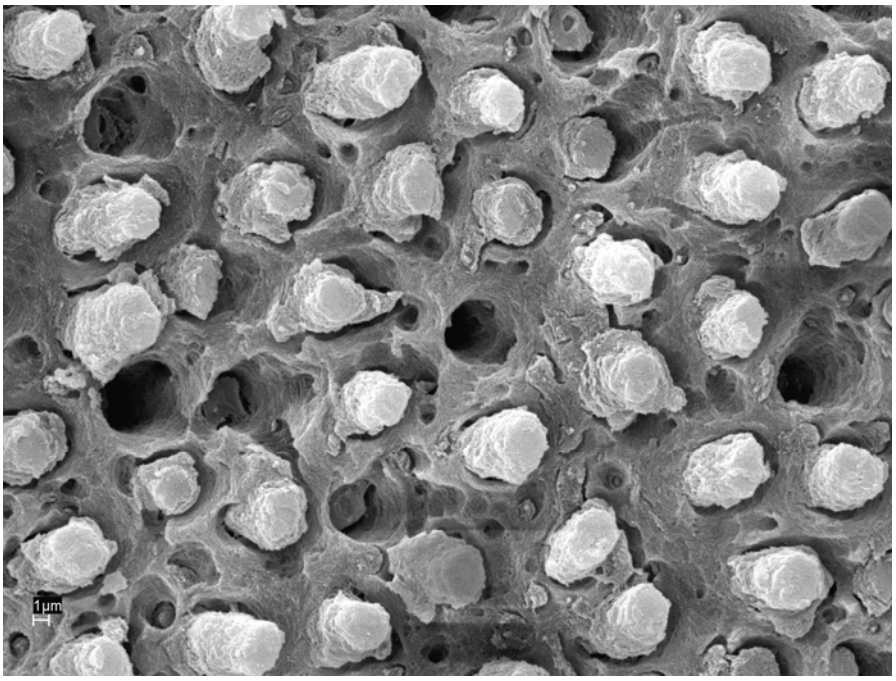


Figure 2b: SEM images of the fractured dentin surface of a specimen bonded with Single Bond and debonded after NaOClaq immersion. A higher magnification view of the failure that occurred at the top of the hybrid layer is observed, showing resin tags at the tubule entrances. Intertubular dentin appearance is that of etched and NaOClaq-treated dentin, showing no collagen and enlarged tubules with many interconnecting canals.

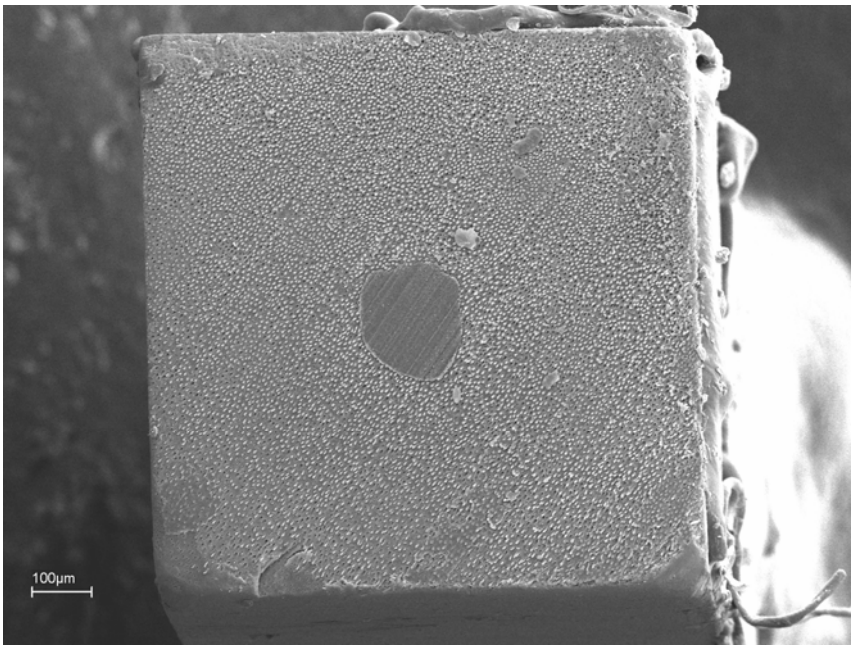


Figure 3a: SEM observations of the fractured surface on the dentin side of a specimen bonded with Prime&Bond NT and debonded after NaOClaq immersion. A mixed failure is shown to occur mainly at the top of the hybrid layer, and a very small area of adhesive resin is remaining at the central part of the debonded area may be observed.

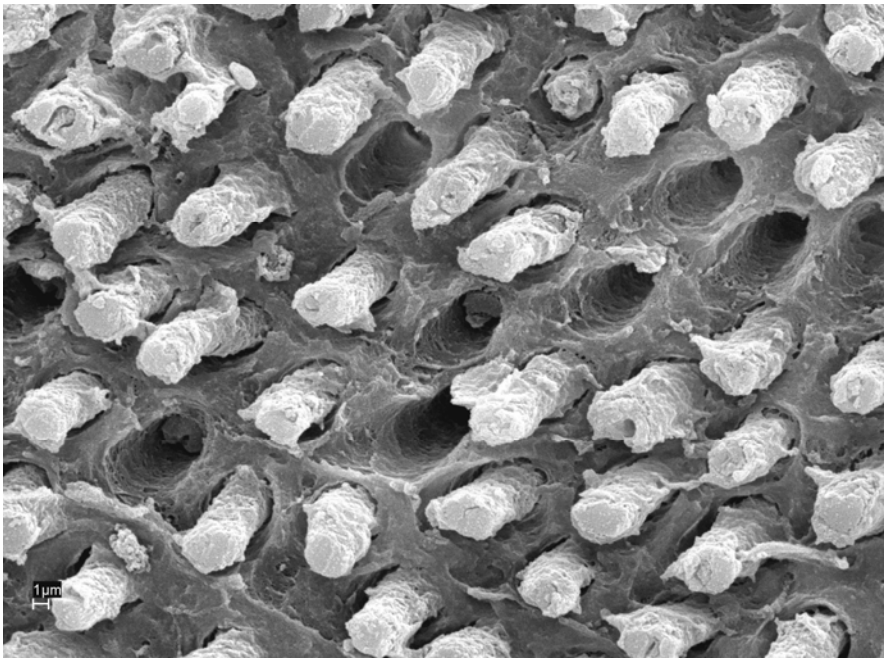


Figure 3b: SEM observations of the fractured surface on the dentin side of a specimen bonded with Prime&Bond NT and debonded after NaOClaq immersion. At a higher magnification, resin filled dentinal tubules are shown, but there is not resin remaining on the intertubular dentin. Texture and porosity of dentin are similar to those of etched and NaOClaq-treated dentin.

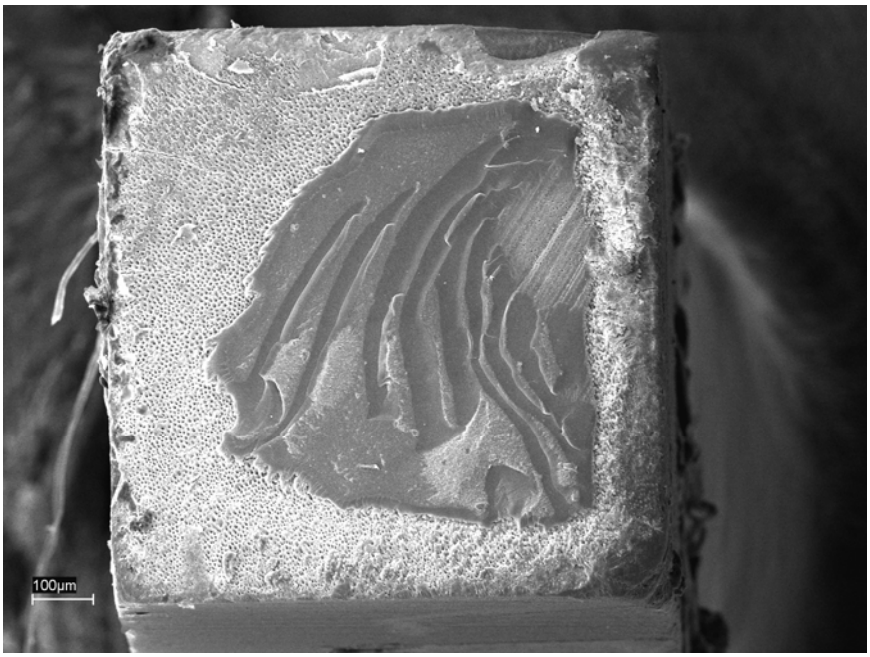


Figure 4a: SEM images of the fractured dentin surface of a specimen bonded with the experimental adhesive Prime & Bond XP (after NaOClaq immersion). A mixed failure may be observed, the main fracture occurred below the hybrid layer and a large adhesive remaining area is observed at the central part of the specimen.

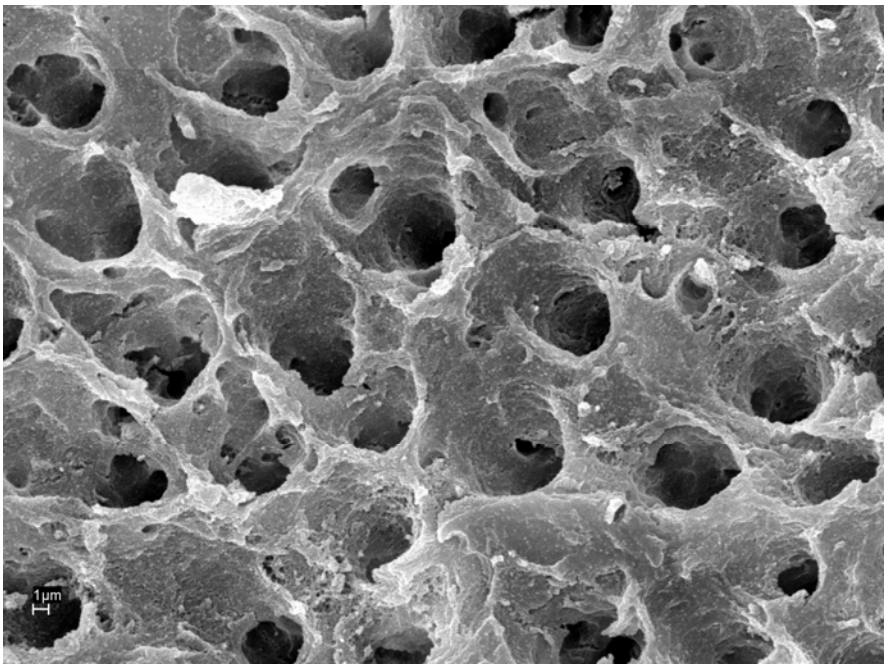


Figure 4b: SEM images of the fractured dentin surface of a specimen bonded with the experimental adhesive Prime & Bond XP (after NaOClaq immersion). A higher magnification view of the failure that occurred below the hybrid layer is showing cohesive fractures of the etched and non-infiltrated dentin. A great microestructural alteration of dentin by the test solution is evident.

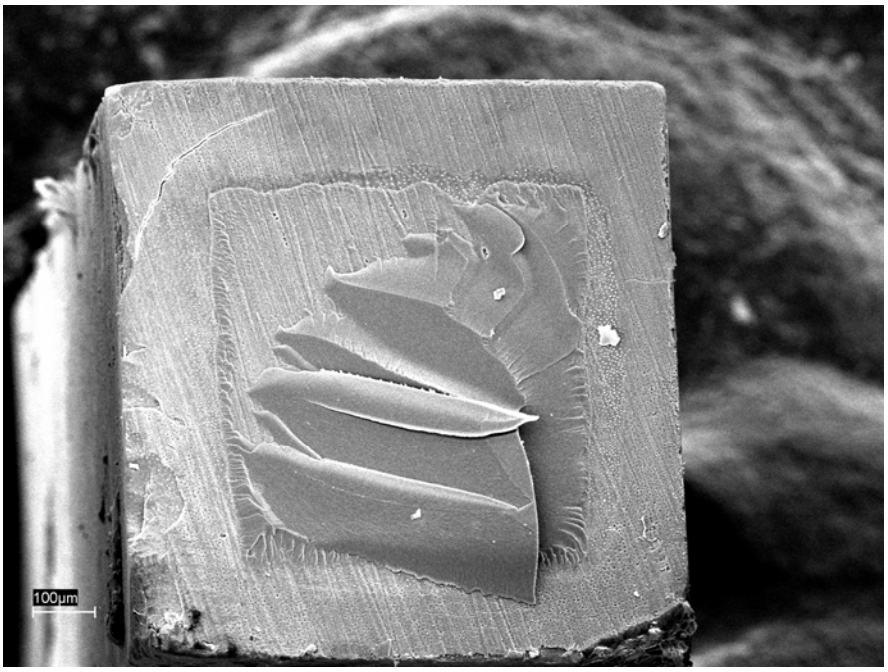
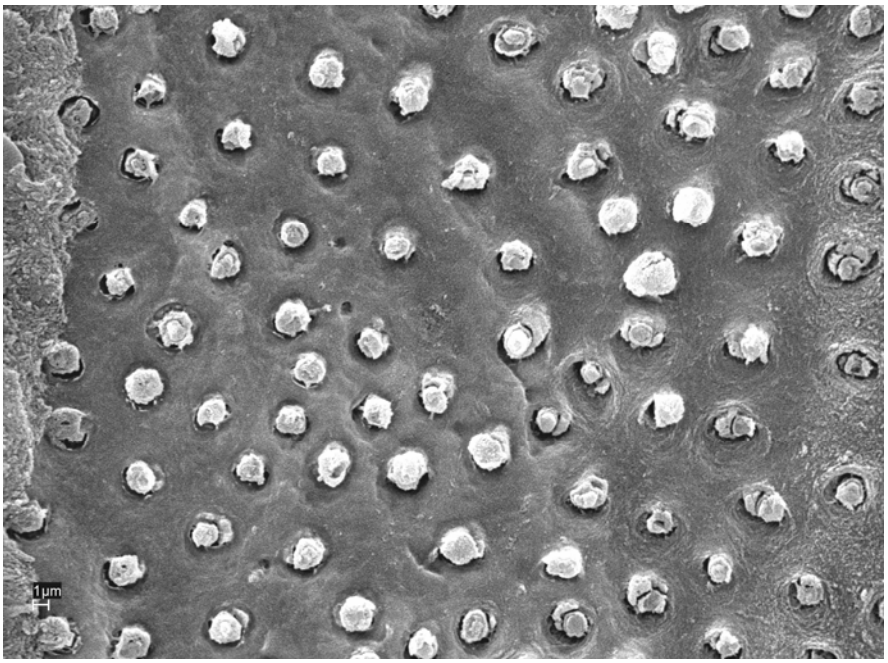


Figure 5a: SEM image of a debonded specimen after NaOClaq immersion, when using Clearfil SE Bond as adhesive agent. A mixed failure pattern, in which a large central area of remaining adhesive resin may be observed. At the periphery of the beam, alternating zones of failure at the top of the hybridized smear layer (scratches from grinding are observed) and at the base of the hybrid layer (no scratches are easily distinguished) may be observed.



III.3. Micromorphology of total etching versus self-etching adhesive systems: a SEM approach.

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ABSTRACT

Objective: To evaluate the effectiveness in the formation of resin tags, adhesive lateral branches and the hybrid layer of five adhesive systems to dentin using scanning electron microscope (SEM). **Methods:** Flat dentin surfaces from 25 molars were bonded with several adhesive systems according to the manufacturers´ instructions. Composite build-ups were constructed incrementally with Tetric Ceram. The Specimens were sectioned parallel to the long axis. One section was gently decalcified and deproteinized in order to evaluate hybrid layer formation. The other section was stored in 30% clorhidric acid to detect resin tags and adhesive lateral branches formation. **Results:** The two total-etch self-priming adhesives exhibited thicker hybrid layer than those found in self-etching adhesive systems. The resin tags formed with self-priming adhesives were much longer than those found in samples whit self-etching adhesives. Lateral branch formation was observed in total etch adhesives and Clearfil SE Bond (SEB). **Conclusion:** The formed hybrid layer obtained with the two-step self-etching adhesive systems and total etching adhesive systems were continuous and uniform in thickness. Resin tags obtained with SEB and the total etch adhesive systems had marked conical swelling at their bases and showed small lateral branches, a sign of proper resin infiltration and swelling.

Key words**:** Dentin, bonding, scanning electron microscope.

INTRODUCTION

The infiltration of demineralized collagen fibers with resin permits formation of a hybrid layer with resin tags and adhesive lateral branches, thus creating micromechanical retention of the resin to the demineralized substrate.[1,2,3] Van Meerbek *et al*[4] indicates that micromechanical retention is the chief mechanism for resin bonding to dentin with resin penetration of the intertubular dentin being of major importance in bond strength.

Dentin bonding systems have been improved in order to promote the durability and reliability of adhesive restorations.[5] Two main strategies are used to create durable dentin bonding: 1) the total-etch self-priming bonding systems (Single Bond -SB-, Prime and Bond NT -PBNT-) work by removing the smear layer with phosphoric acid, followed by the application of a primer and an adhesive in the same step leading to the formation of a thick hybrid layer;[6] and 2) the self-etching approach, in which the acid and the primer are combined in one solution to form an acidic monomer[6] and a final bonding step is later applied (Clearfil SE Bond -SEB-, ABF). The etching potential of self-etch bonding systems induces the formation of a hybridized complex comprising a surface zone made up of the hybridized smear layer with a true hybrid layer underneath.[4,7,8,9] Self-etch all-in-one adhesives have also been introduced and contain all components in just one solution (Etch & Prime -EP-).

One of the first and most widely used tools to study the mechanisms involved in the process of bonding has been the scanning electron microscope (SEM).[10] The principle of SEM is based upon a pseudo three-dimensional image that is built up point-by-point and line-by-line from secondary electrons.[10]

Thus, the objective of this study was to evaluate the hybrid layer, resin tags and adhesive lateral branches formations of five adhesive systems into dentin, describing morphological characteristics and ascertaining probable relationships with the bonding performance of these adhesive systems.

MATERIALS AND METHODS

25 extracted human third molars that were stored in 0.5% chloramine T at 4 ºC and were used within one month after extraction. The specimens were sectioned below the dentinoenamel junction and ground flat with 180-grit silicon carbide abrasive papers under running water to provide uniform and clinically relevant bonding surfaces. Two total-etch self-priming adhesives (Single Bond -SB-, 3M ESPE, St. Paul, MN, USA; Prime & Bond NT -PNT-, Dentsply DeTrey, Konstanz, Germany), two two-step self-etching adhesives (Clearfil SE Bond -SEB-, Kuraray Medical Inc., Tokyo, Japan; ABF Kuraray Medical Inc., Tokyo, Japan), and an all-in-one self-etch adhesive (Etch & Prime 3.0 -EP-, Dentsply Degussa AG, Hanau, Germany) were used. They were bonded to the dentin surfaces according to the manufacturers´ instructions (Table 1).

After bonding, composite build-ups, each 3 mm in height, were constructed incrementally (1.5 mm) with a light-cured microhybrid resin composite (Tetric Ceram, Ivoclar-Vivadent, Schäan, Liechtenstein). Each layer of the composite was light-activated for 40 s with a Translux EC halogen light-curing unit (Heraeus-Kulzer GmbH, Hanau, Germany). Light intensity output was monitored with a Demetron Curing Radiometer (Model 100 Demetron Research Corporation, Danbury, CT, USA) to be at least 600 mW/sec.

The specimens were sectioned parallel to the long axis using a diamond saw wheel (Isomet, Buehler, Lake Bluff, NY, USA) at slow speed under water coolant.

One section of each specimen was stored in water solution at room temperature. After 24 hours later the section was gently decalcified (32% phosphoric acid was applied for 30 seconds and the specimen was then washed and gently air-dried) and 2% sodium hypochlorite was applied onto the surface solution for 120 seconds in order to evaluate the hybrid layer and resin tags formation.

After being extensively rinsed with water, the specimens were exposed to increasing concentration of alcohol. Impressions were taken and prepared replica models from epoxy resin, sputter-coated with gold (Edwards Ltd., London, UK) and observed with SEM (Philips 505, Eindoven, The Nertherlands) at different standardized magnifications (X2000, X 2500, X 3000).

The other section of each sample was stored in 30% clorhidric acid for 48 hours and was washed for 20 minutes in 2% sodium hypochlorite in order to completely dissolve the dental substrate and to detect resin tag and adhesive lateral branch formation. The specimens were then processed for SEM observation. SEM photomicrographs at X500, X800, X2000 and X3000 original magnification were taken. The following aspects were evaluated by SEM: 1) the formation and uniformity of the hybrid layer along the entire length of the adhesive interface; 2) resin tags formation; 3) existence of lateral branches from the resin tags.

RESULTS

Hybrid layer observation:

All the adhesive systems showed hybrid layer formation. Self-etching adhesive systems SEB and ABF (experimental system) produced a hybridized complex of similar thickness (Fig. 1 and Fig. 2). EP resulted in a thicker hybridized complex (Fig. 3). At the interface between the hybridized complex and the restoration composite it was possible to see frequent blisters (Fig. 3). PBNT and SB exhibited similar morphology under the SEM, showing thicker hybrid layers (Fig. 4) than those found in self-etching adhesive systems.

Evaluation of resin tag formation:

SEB showed resin tags that were narrow and regularly shaped with marked conical swelling at their bases (Fig. 5). Adhesive small lateral branches sporadically were observed on the sides of the resin tags (Fig. 6). When ABF and EP were employed the tags were wide, short and funnel shaped, with no lateral braches (Fig. 7-8). The resin tags formed with self-priming adhesives PBNT and SB had marked conical swelling at their bases (Fig. 4). Both total-etching adhesives were much frequent and longer than those found in samples bonded with self-etching adhesives (Fig. 9-10). These tags showed numerous small lateral extensions of microtags branching off at right angles from the main resin tags (Fig. 11).

DISCUSSION

There are some morphological differences between the created bond structures when using a total-etch bonding system (Fig. 4) and those formed by a self-etch adhesive system. The most remarkable difference is the hybrid layer thickness.[11] Hybrid layers created by the two total-etch self-priming adhesives were thicker than those observed in the specimens bonded with self-etching adhesive systems (Fig. 1-4). Despite the physical appearance of thin hybridized complex, high immediate bond strength has been reported for these adhesive systems.[2,12,13] This suggests the absence of correlation between hybrid layer thickness and bonding efficacy as long as an uniform demineralization front is created at the intertubular dentin[14] and it is fully impregnated by resin.[16]

Most early self-etch bonding systems were hydrophobic, which did not allow them to adapt to dentin properly.[17] The adhesive must be able to diffuse and penetrate in an aqueous environment and, therefore, be hydrophilic.[18] The self-etching adhesives contain acid monomers, often mixed with water, to make the adhesive systems sufficiently acid to cross the smear layer and form a bond with the underlying dentin and to incorporate the smear layer into the hybrid layer.[17,19] The latest commercially available self-etching adhesives further incorporates all the resin monomers and photoinitiator into a single bottle and eliminates an additional mixing step.

SEB and ABF (experimental system) produced hybridized complexes of similar thickness and seems to interact with the underlying intertubular dentin (Fig. 1 and Fig. 2). The acidity of the primer was sufficient to dissolve the smear layer and smear plugs from the dentin and to demineralise the intact matrix to a depth of about 0.5 µm.[20] ABF bonding formulation is similar to that of SEB. Both self-etching adhesive systems contain 10-MDP monomer (highly hydrophilic), which interact chemically with hydroxyapatite that remains available at the partially demineralized dentin surface.[21] Moreover, MDP improves the wetting of the tooth surface,[22] causes minimal

dissolution of smear plugs and limited opening of tubules, which reduces dentin permeability[23] and facilitates penetration, impregnation, polymerization and entanglement of monomers with the underlying dentin to form hybridized complexes.[2,13,24]

The self-etching adhesives vary in their acidity by virtue of the composition and concentration of polymerizable acids and acidic resin monomers in these systems.[25] However, it seems that the pH value of self-etching adhesives does not influence the morphology of the dentin-resin interfaces.[18] The pH is not the determining factor conditioning the action of self-etching adhesives,[18] and the MTBS are neither affected by the adhesive's acidity[26] when a minimal pH of approximately 1.8 is reached by the adhesive.[27]

EP showed a thick hybridized complex (Fig. 2). Previous studies have also shown that this bonding agent is able to completely dissolve the smear layer, (pH 0.6) and to form a relatively thick hybridized complex.[28,29] When EP dentin interfaces were evaluated, many separation zones between the adhesive and the resin composite were observed (Fig. 2). These separation zones, resembling blisters, were found systematically on all the EP specimens but did not exist for the rest of the tested adhesive systems. The same technique was used for all specimens, so that the hipothesis that ligth-cured composite may not have been activated immediately, thus leading to formation of blisters, can be set aside.[18,30]

The thickness of the hybrid layer produced by SB and PBNT was approximately 3 to 4 μm according to previous studies.[31,32] The hybrid layer formed by the two total etch adhesive systems (Fig. 4), was compact and homogenous in thickness.[3] Bis-GMA, HEMA and polyalkenoic acid are the main chemical components of the SB. An aqueous HEMA solution promotes the impregnation of resin into the exposed collagen.[33,34] On the other hand, PBNT contain PENTA, an acidic

phosphonated monomer, which could have some kind of interaction within the calcium ions left on dentin surface, or even with the underlying dentin.[15]

When SEB was applied, formed resin tags were much longer than those formed by EP or by ABF (Fig. 5-7). In dentin-bonded interfaces, the contribution of the resin tags to the bond strength, relative to the role of the intertubular dentin may vary depending on the used dentin bonding agent, the orientation of the dentinal tubules and the tested dentin depth.[35] The penetration of resin tags into the dentinal tubules is believed to contribute little to the final bond strength.[1,4,36] The adaptation to the inner tubule walls probably contributes significantly much more dentin bonding.[35] The resin tags of the SEB (Fig. 5) were numerous and had marked conical swelling at their bases, which may be considered as sign of a good seal.[18] SEB showed adhesive small lateral branches (Fig. 6) sporadically observed on the sides of the resin tags, indicating that resin attempted to fill lateral canals. Mjör[37] showed that supplementary retention and sealing were offered by the formation of micro-tags in the lateral canal branch from the main tubules. EP showed short and funnel shaped tags with no lateral braches (Fig. 8), even when it has been stated that produced a deeper etching compared with SEB (pH 1.9), and tubule entrances were not only opened but also enlarged.[2]

The resin tags formed with the tested total-etch self-priming adhesives, PBNT and SB (Fig. 9 and Fig. 10) were much longer than those found in samples bonded with self-etching adhesives and both had marked conical swelling at their bases, as a result of the removal of the peribubular dentin by the previous acid etching of dentin (Fig. 4). PBNT showed resin infiltration of the funnelled dentin tubules and the collagen fibrils from the overlying.[3] PBNT and SB formed resin tags revealed numerous small lateral extensions of microtags branching off at right angles from the main resin tags (Fig. 11), which is probably a sign of proper adaptation and sealing.

CONCLUSIONS

The formed hybrid layer obtained with the two-step self-etching adhesive systems (SEB and ABF) and both total etching adhesive systems (SB and PBNT) were continuous and uniform in thickness. All adhesive systems showed resin tags formation. Resin tags obtained with the two-step self-etching adhesive system (SEB) and the total etch adhesive systems (SB and PBNT) had marked conical swelling at their bases and showed small lateral branches on the sides of the main resin tags, a sign of proper resin infiltration and swelling. EP showed blisters between the adhesive and the resin composite which could affect negatively the bond durability.

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Table 1: Mode of Application, compositions, and manufacturers of tested adhesives.

Materials	Components	Mode/steps of application	Manufacturer
Single Bond **Total-etch** **Self-priming**	2-hydroxyethylmethacrylate; water; ethanol; Bis-GMA; dimethacrylates; amines; methacrylate-functional; copolymer of polyacrylic and polyitaconic acids.	Etch for 15 seconds. Rinse with water spray for 10 seconds, leaving tooth moist. Apply two consecutive coats of the adhesive with a fully saturated brush tip. Dry gently for 2-5 seconds. Light cure for 10 seconds.	3M, St.Paul, MN, USA. Lot. 4242.
Prime & Bond NT **Total-etch** **Self-priming**	PENTA; UDMA resin; resin R5-62-1; T-resin; D-resin; nanofiller; initiators; stabilizer; cetylamine hydrofluoride; acetone; hydroxyethylmethacrylate.	Etch for 15 seconds. Rinse with water spray for 15 seconds and remove water with a soft blow of air. Leave a moist surface. Apply ample amounts of the adhesive to saturate the surface, reapply if it is necessary. Leave the surface undisturbed for 20 seconds. Remove solvent by blowing gently with air for at least 5 seconds. Light cure for 10 seconds.	Dentsply / De Trey GmbH, Konstanz, Germany. Lot. 0209000918.
Clearfil SE Bond **Self-etch** **2-steps**	Primer: 10-methacryloyloxydecyl dihydrogen phosphate; 2-hydroxyethyl methacrylate; hydrophilic dimethacrylate; camphorquinone; N,N-diethanol-p-toluidine, water. Bond: 10-methacryloyloxydecyldihydrogen phosphate; N,N-diethanol-p-toluidine; 2-hydroxyethylmethacrylate; Bis-phenol A diglycidylmethacrylate; silanated colloidal silica; hydrophobic dimethacrylate; camphorquinone.	Apply Primer for 20 seconds. Mild air stream. Apply Bond. Gentle air stream. Light cure for 10 seconds.	Kuraray Co, Osaka, Japan. Lot. 390.
Etch & Prime 3.0 **Self-etch** **1-step**	Universal: 2-hydroxyethylmethacrylate; water; ethanol Catalyst: Tetramethacrylocyethylpyrophosphate.	Mix Etch & Prime 3.0 Universal and Catalyst. Apply for 30 seconds. Air blow gently. Light cure for 10 seconds. Repeat the above mentioned steps.	Degussa AG, Hanau, Germany. Lot.019920.
ABF Experimental	Primer: hydroxyethylmethacrylate; hydrophilic dimethacrylate; 10-methacryloyloxydecyldihydrogen phosphate; N,N-diethanol p-toluidine; camphorquinone; water. Bond: Silanated silica; BisGMA; hydroxyethylmethacrylate; hydrophilic dimethacrylate; 10-methacryloyloxydecyldihydrogen phosphate; toluidine; camphorquinone; MDPB; sodium fluoride.	Apply Primer for 20 seconds. Mild air stream. Apply Bond. Gentle air stream. Light cure for 10 seconds.	Kuraray Co, Osaka, Japan. Primer. Lot. 000001 Bond. Lot 000002

PENTA= penta-acrylate ester; TEGDMA= triethylene glycol-dimethacrylate; Bis-GMA= bysphenyl glycidyl methacrylate, UDMA= urethane dimethacrylate; BHT= butylated hydroxyl toluene; TCB resin = carboxylic acid modified dimethacrylate. MDPB: 12-methacryloyloxydodecylpyridium bromide.

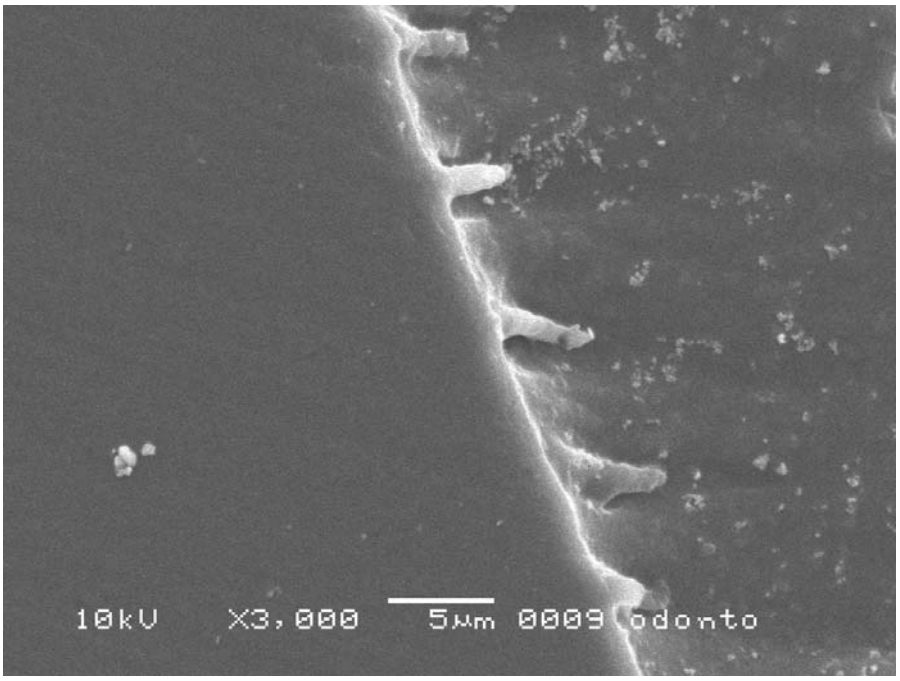

Fig. 1. Thin hybridized complex formed whit SEB (original magnification: SEM ×3000).

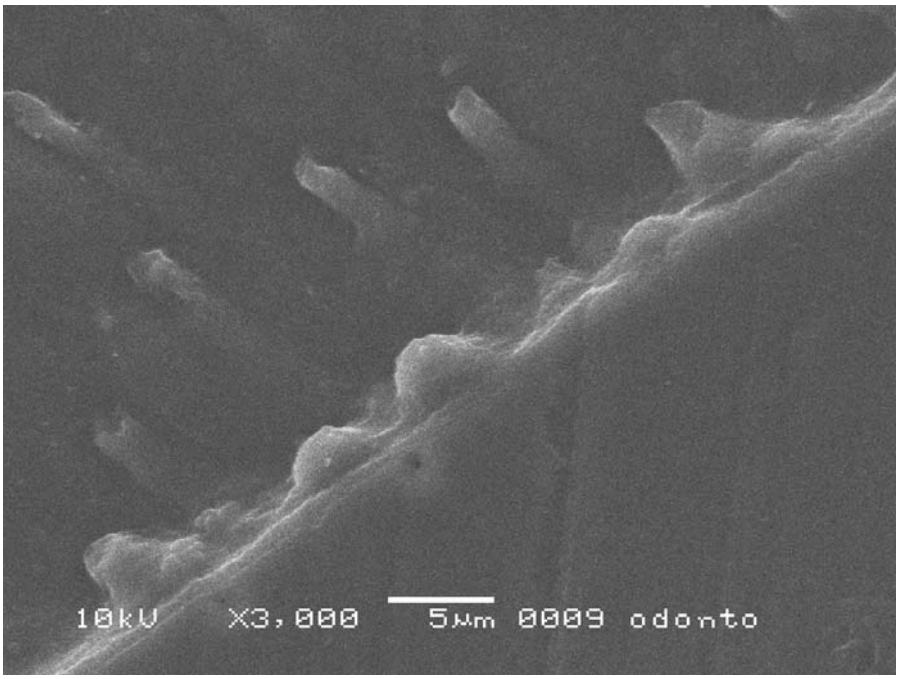

Fig. 2. Thin hybridized complex produced whit ABF (original magnification: SEM ×3000).

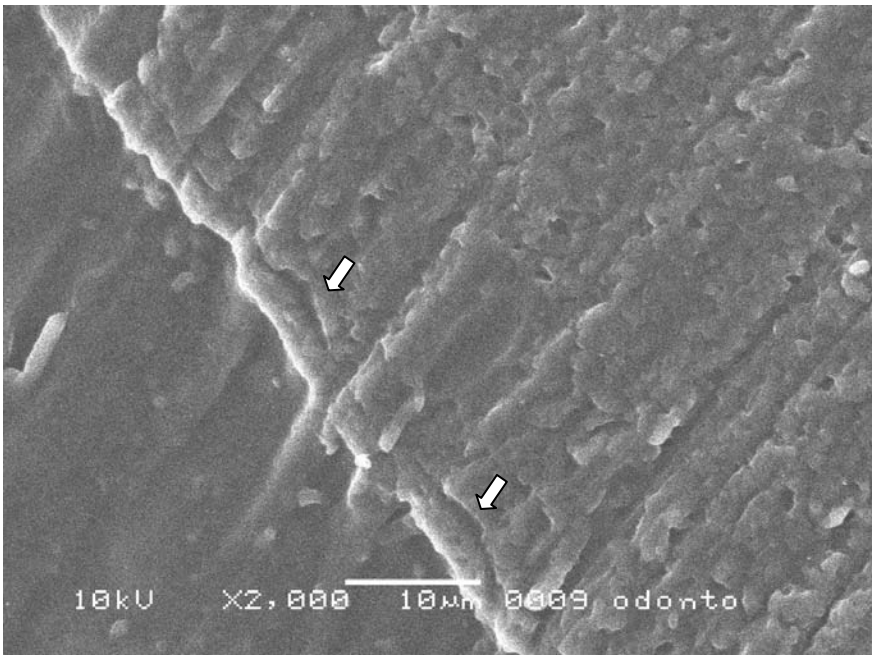

Fig. 3. Hybridized complex formed with EP. A blister (arrows) can be observed between the adhesive and the composite (original magnification: SEM ×2000).

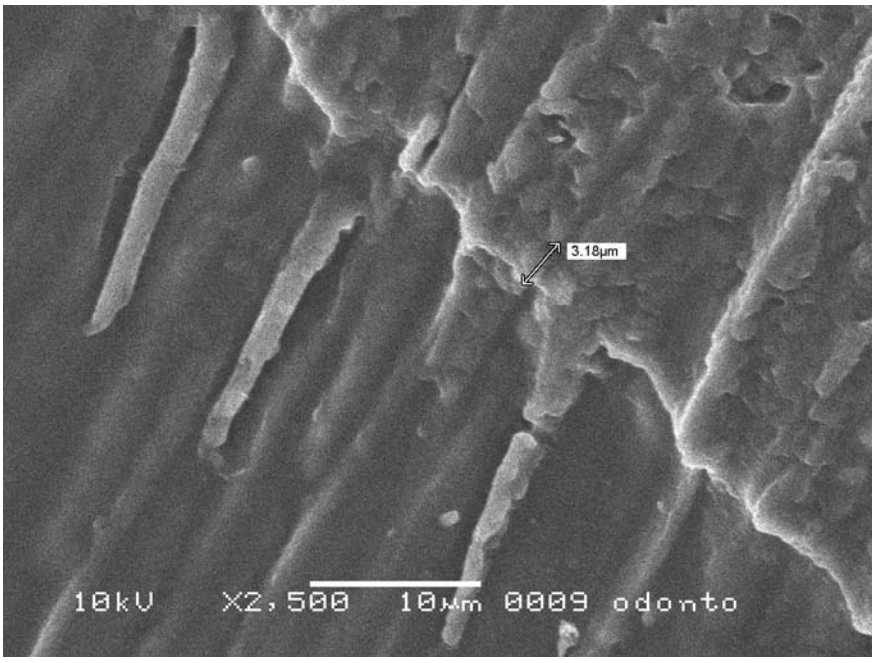

Fig. 4. Thick hybrid layer produced with PNT. Resin tags with marked conical swelling at their bases are observed (original magnification: SEM ×2500).

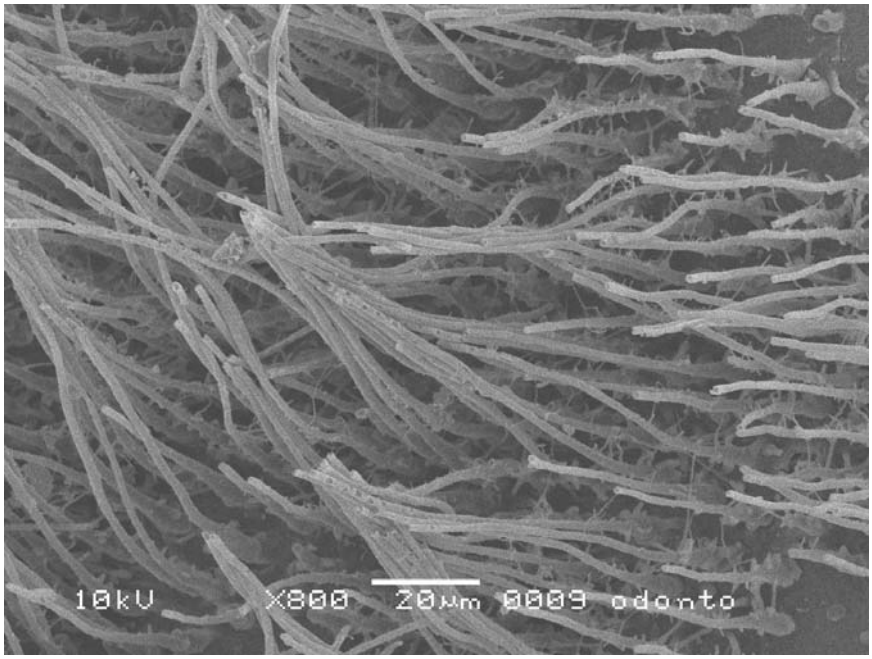

Fig. 5. Narrow and regularly shaped resin tags with marked conical swelling at their bases can be observed when bonding a dentin specimen with SEB (original magnification: SEM ×800).

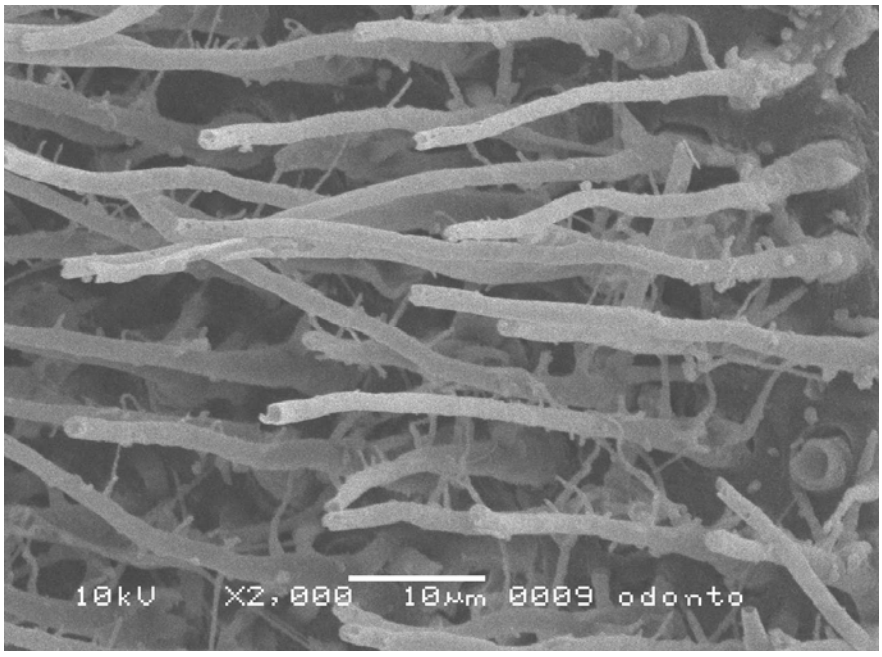

Fig. 6. Microphotograph shows adhesive small lateral branches sporadically observed on the sides of the resin tags (SEB sample) (original magnification: SEM ×2000).

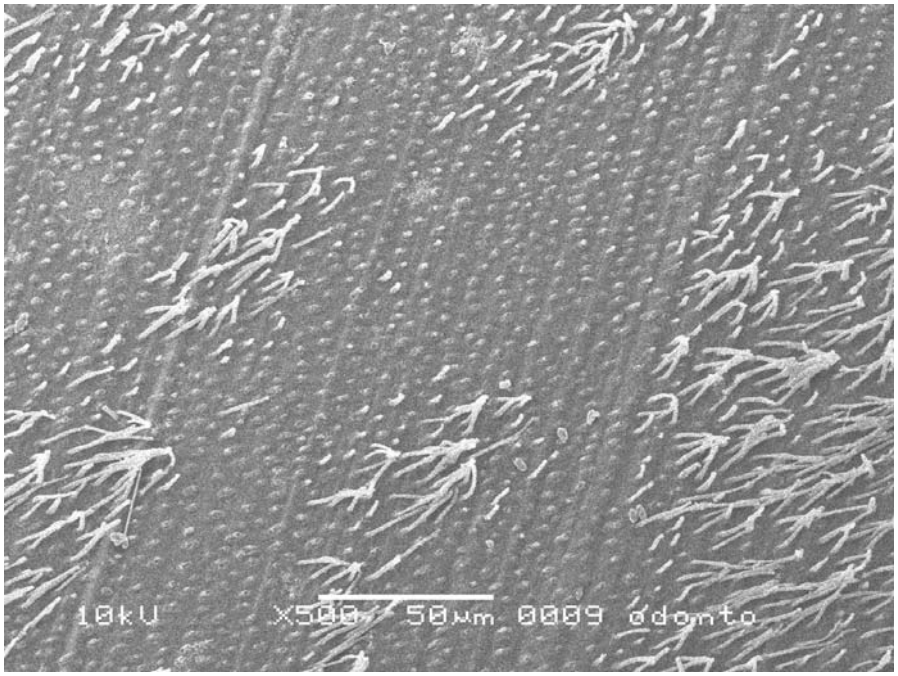

Fig. 7. Wide, short, funnel shaped and no uniform resin tags are noted whit ABF (original magnification: SEM ×500).

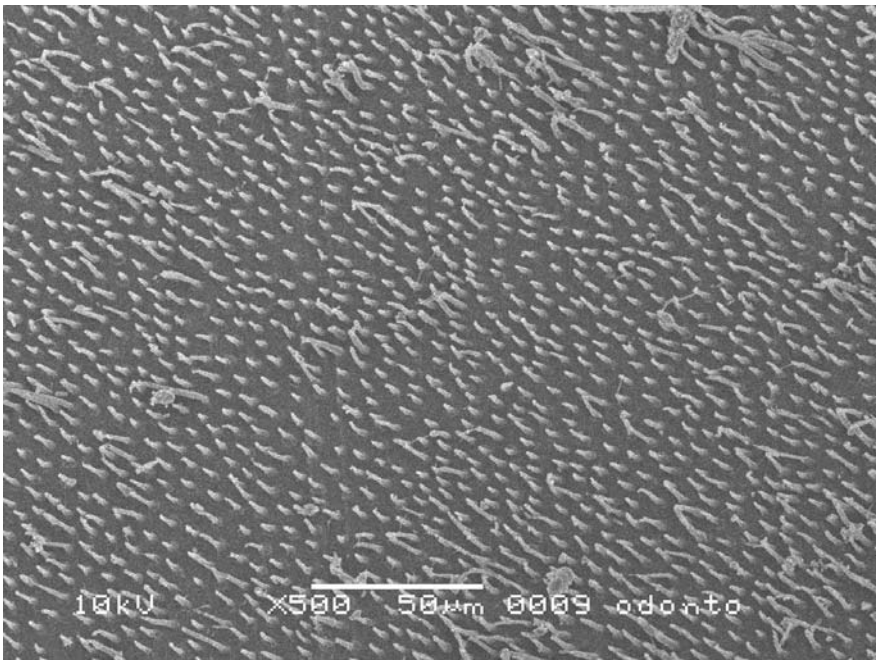

Fig. 8: Short and funnel shaped resin tags are noted with EP (original magnification: SEM ×500).

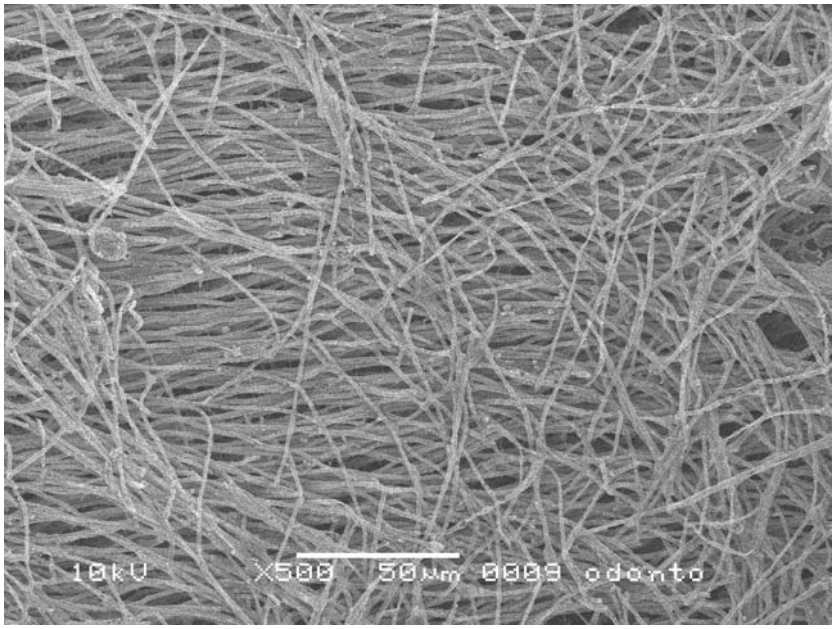

Fig. 9. Long and uniformly formed resin tags are visible with PNT (original magnification: SEM ×500).

Fig. 10. Regular and very long formed resin tags are visible with SB; also adhesive lateral branches are detectable (original magnification: SEM ×500).

Fig. 11. Resin tag and numerous small lateral extensions of microtags branching off at angles from the main resin tags are visible with PBNT (original magnification: SEM ×3000).

IV.1. Effects of adhesive systems and luting agents on bonding of fiber posts to root canal dentin.

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ABSTRACT

The study evaluated the influence of different luting materials on the microtensile bond strength of glass fiber posts to root canal dentin. Thirty maxillary premolars were endodontically treated and the roots were prepared for post cementation using the FRC Postec system (Vivadent). Two luting materials (Multilink, Vivadent and Clearfil Photo Core, Kuraray) were used in combination with three adhesive: Multilink Primer (Vivadent), Clearfil Photo Bond and Clearfil New Bond (Kuraray). A composite build-up was performed around the root to provide adequate gripping during testing. Specimens were cut to obtain beams with the post in the center and with the radicular dentin overlaid by the composite build-up on each side. Microtensile testing was performed with a universal testing machine at a cross-head speed of 0.5 mm/min. The failure mode was classified under a stereomicroscope and four representative beams of each group were selected for SEM analysis. Bond strength data that were analyzed with two-way ANOVA and Student-Newman-Keuls multiple comparisons tests revealed that adhesive systems, luting materials and the interaction between these two factors significantly influenced the bond strength results ($p<0.01$). Multilink applied with its own adhesive system obtained the best results, while the lowest bond strength was achieved with Clearfil Photo Core in combination with Multilink Primer.

Key Words: fiber post, microtensile test, self-etching adhesive, radicular dentin, curing mode.

INTRODUCTION

Fiber posts are becoming increasingly popular for the restoration of endodontically treated teeth. They provide retention for core restorations when the coronal portions of the teeth exhibited a severe loss of tooth substances (1-4).

The retention of fiber posts within root canals is affected by several factors involving the type of the post, its adaptation to the post space and the luting agent (5,6). Resin-based luting agents in combination with dentin adhesives are commonly used for the cementation of fiber posts (7,8). The rationale for using fiber posts for the rehabilitation of endodontically treated teeth is to create a restoration with an elastic modulus that is close to dentin, and to produce stress fields that are similar to those experienced by natural teeth during occlusal loading (3,9,10). The clinical results are predictable when fiber posts are closely adapted to root canal spaces, with the canal walls being surrounded by a thin and uniform film of cement (9, 11,12).

Controversial results were reported when different commercially available dentin adhesive and luting cement combinations were employed for cementing fiber posts (8,13,14). These materials may polymerize through a light-activated reaction, a chemical reaction or a combination of both mechanisms (15). Recent investigations suggested the possibility of adverse interactions between the polymerization modes of simplified dentin adhesives and chemical-cured resin composites that may affect the bond integrity (16,17).

The observation of imperfectly round root canal cross sections after endodontic treatment is not uncommon (18,19). The presence of a discrepancy between the post and the shaped canal is considered an indication for using resin composites to lute or reline the post to improve its fit and retention (9,11).

The objectives of this investigation were: 1. to compare the performance of conventional resin cement and a resin composite, and 2. to evaluate the influence of different combinations of adhesive systems and luting agents when fiber posts were luted to prepared root canals. Evaluation was performed using the microtensile bond strength test. The null hypothesis tested was that the bond strength of fiber posts to intraradicular dentin is not affected by the different combinations of dentin adhesives and luting cements.

MATERIALS AND METHODS

30 human maxillary premolars, extracted for orthodontic or periodontal reasons, were selected for the study. The teeth were stored1% Chloramine T solution at 37°C, to prevent bacterial growth, until their use. The crown of each tooth was removed 1 mm coronal to the cementoenamel junction with a water cooled diamond blade (Accutom-50, Struers, Copenaghen, Denmark). The root canals were instrumented according to a step-back technique. Manual instrumentation was performed to ISO size 35, using a series of stainless steel K-files to the working length of each tooth. The rest of the canal was prepared mechanically with Gates-Glidden drills that were used in sequence from # 2 to # 4 (Union Broach, New York, USA). The root canals were irrigated with 5.25% NaOCl at 37°C and 10% EDTA solution alternatively and dried with multiple paper points. All the teeth were obturated with a cold lateral compaction technique, using gutta-percha cones and a zinc oxide eugenol-based root canal sealer (Pulp Canal Sealer, Kerr, Romulus, MI, USA). The access to the root canal was filled with a provisional filling material (Cavit, 3M Espe, St.Paul, MN, USA).

The endodontically treated teeth were stored in deionized water at 37°C for 24 h prior to the preparation of post spaces. The coronal gutta-percha was removed from the root canal with a Largo Drill and a post space was prepared (FRC Reamer size #1, Ivoclar-Vivadent, Schaan, Liechtenstein), leaving 5 mm of gutta-percha to preserve the apical seal. Translucent glass fiber posts (FRC Postec size 1 batch no. 94017, Ivoclar-Vivadent) were used. They are made of glass fibers that are embedded in a dimethacrylate matrix. Each post was fitted to the post space and cut at adequate length.

The teeth were randomly divided into six groups of five teeth each, according to the different material selected for the luting procedure. In Group A, Multilink (ML) (batch no.G00772,

Ivoclar-Vivadent), a self-curing resin luting agent was used. For Group B, Clearfil Photo Core (CPC) (batch no. 510A Kuraray Medical Inc., Tokyo, Japan), a light-curing composite material was selected. Three adhesive systems were applied in combination with the two luting resin cements. Six experimental subgroups were thus formed: 1A. Multilink + Multilink Primer (batch no.G00772, Ivoclar-Vivadent); 2A. Multilink + Clearfil Photo Bond (batch no. 41152, Kuraray); 3A. Multilink + Clearfil New Bond (batch no. 0029A, Kuraray); 1B.Clearfil Photo Core + Multilink Primer; 2B. Clearfil Photo Core + Clearfil Photo Bond; 3B. Clearfil Photo Core + Clearfil New Bond. The composition of the tested materials are described in Table 1.

A pH meter was used for pH measurement of the adhesives employed (Micro pH 2000, Crison Instruments, Alella, Spain). Before performing the luting procedure, the surface of each post was silanized with Monobond-S (Batch no. F50602, Ivoclar-Vivadent) and gently air-dried after 60s. Monobond-S is a pre-hydrolyzed 3-methacryloxypropyl-trimethoxysilane (3-MPS) in a water/ethanol solvent. The luting procedures were performed as described in Table 1. The resinous material was inserted into the root canal with a lentulo drill.

After post insertion, composite core build-up was performed on each tooth at the coronal level to avoid the risk of coronal leakage and to provide a sufficient bulk for handling. A light-curing composite material was used for subgroups A (Tetric Ceram, Ivoclar-Vivadent), while the same Clearfil Photocore was applied following an incremental technique on specimens of subgroup B. Each increment was polymerized for 20 s (output 600 mW/cm^2, Optilux, Demetron, Sybron-Kerr, Orange, CA, USA).

Microtensile bond strength test

Specimens were stored in deionized water at 37°C for 24 h before testing. In order to provide an adequate gripping on the loading machine, additional composite build-up was made on the external root surface, following a technique previously described by Boulliaguet et al. (15). Briefly, the outer surface of each root was etched with 37% phosphoric acid for 15 s, rinsed with water and gently air-dried. An adhesive system (Single Bond, 3M Espe) was applied, air-thinned and light-cured for 20 s (Optilux, Demetron). The bulk of composite was performed with a light curing composite following an incremental technique (Tetric Ceram). Each increment was polymerized for 20 s.

Each specimen was sectioned perpendicularly to its longitudinal axis into 0.8 mm thick slabs with a diamond blade under continuous water cooling (Accutom-50, Struers, Copenhagen, Denmark). Each slab was then transversally sectioned at the outermost periphery of the post so as to obtain beams of approximately 1 mm^2 of area, the diameter of which was measured with a digital caliper (Mituyoto, Tokyo, Japan).

Each stick was attached with Zapit (Dental Ventures of America, Corona USA) to the flat grip of a Bencor Multi-T testing assembly (Danville Engineering, San Ramon, CA) and loaded in tension at a cross-head speed of 0.5 mm/min until failure, using an universal testing machine (Instron Model 4411, Instron, Canton MA, USA).

Fractured specimens were examined at 40x magnification under a stereomicroscope (Olympus SZ-CTV, Olympus, Tokyo, Japan) to determine the failure mode. Failures were classified as adhesive (at the post/cement or cement/dentin interface), cohesive (in the cement or dentin) or mixed.

The bond strength data were statistically analyzed with two-way ANOVA to evaluate the performances of the different luting materials and the interaction between adhesive systems and the luting agent. Multiple comparisons were performed with Student-Newman-Keuls test. Statistical significance was set at $\alpha = 0.05$.

Scanning Electron Microscopy analysis (SEM)

Four fractured beams of each group that were classified as mixed failures were prepared for SEM examination. Specimens were rinsed with 96% ethanol (Sigma, Aldrich Chemic, GmbH, Steinheim, Germany), air-dried, sputter-coated with gold (Polaron Equipment Ltd., Newhaven, England) and observed under an SEM at different magnifications (DSM-950, Zeiss, Germany).

RESULTS

Microtensile bond strength test

Mean microtensile bond strength values are shown in Table 2. Two-way ANOVA showed a statistically significant influence of the adhesive system ($p<0.0001$), and the luting agent ($p<0.01$) on the bond strength results. The interactions between these two variables was also significant ($p<0.01$). Student-Newman-Keuls multiple comparisons test revealed that when using Clearfil Photo Core, all adhesives performed similarly. Clearfil Photo Bond and Clearfil New Bond exhibited lower bond strengths when applied in combination with Multilink cement. Multilink Primer performed better when applied in combination with its own luting cement. Acidic pH values were obtained from all the adhesive systems. Clearfil New Bond and Clearfil Photo Core had similar pH values (2.55 and 2.52 respectively), while Multilink Primer recorded a lower value (1.95).

Microscopic evaluation

The distribution and percentages of failures are described in Table 3. Most of the recorded failures were adhesive in nature and occurred predominantly along the adhesive/dentin interface. In some groups, a higher incidence of mixed failure was registered. SEM examination revealed different fracture patterns in the tested groups. Some specimens showed a complete detachment of the luting cement from the intraradicular dentin; residuals of adhesives were only present within the tubules (Fig.1a). Others were characterized by the presence of a partial detachment of the adhesive and overlying cement from dentin (Fig.1b). Remnant cement was evident on the post surface, especially when Multilink was applied as the luting cement (Fig. 2).

DISCUSSION

In this study, the microtensile bond strength test was used for evaluating the adhesive strengths of glass fiber posts in the root canals (15), as more information could be obtained compared with “push-out” or “pull-out” test, that were employed traditionally for assessing post retention (20). As the microtensile bond strengths of the dentin adhesives were significantly different when they were used in combination with the resin-based luting cements, we have to reject the null hypothesis that the bond strength of fiber posts to intraradicular dentin is not affected by the different combinations of dentin adhesives and luting cements.

The adhesive systems selected in this study were based on two different bonding strategies: 1. Clearfil Photo Bond and New Bond are simplified etch-and-rinse adhesives that require etching and rinsing with phosphoric acid prior to bonding. Being an ionic resin monomer with acidic functional groups, 10-MDP readily diffuses into the exposed collagen fibrils of the demineralized intraradicular dentin in the absence of smear layer. Poor control of moisture and incomplete resin infiltration may affect their efficacy (21); 2. Multilink primer is a simplified self-etch adhesive, it is directly applied on the smear layer and, due to its acidic pH (1.93) it etched thorough the smear layer, and partially demineralized the underlying intact dentin (22, 23).

When Clearfil Photo Bond was applied to the post space, tensile bond strength was not affected by the choice of luting cements. Being a dual-curing adhesive system, the initiators that catalyze its setting reaction may not have unfavourable interaction with both luting cements (24-26). Conversely, the bond strengths of Clearfil New Bond were reduced when it was applied in conjunction with the self-curing luting cement (ML). The adhesive polymerized through a characteristic slow-setting chemical reaction; this aspect could be considered a favourable condition for reducing stress at the bonding interface (15,27). Some chemical incompatibility probably exists

and/or impurities (commonly water) may have penetrated the interface between the adhesive and the resin cement, affecting polymerization (26,28). Recent studies showed that the bonding efficacy of simplified etch-and-rinse adhesives to auto-cured composites/cements is hampered by the intrinsic permeability of these adhesives to water as a result of their increased hydrophilicity (29-31).

This phenomenon has been shown to occur *in vivo* in bonded vital crown dentin and recently in endodontically treated teeth (32-34). The absence of differences in moisture content between a vital and a non-vital tooth (35) and the reduction of dentin thickness due to the preparation of the dowel space, may account for this intrinsic permeability (34,36). Rinsing with water during the etching procedure, especially in narrow elliptic root canals, combined with the presence of hydrophilic monomers in the adhesives, probably resulted in the retention of remnant water within the dentinal tubules, which, in turn, may affect the bond quality (15,34). Chemically-cured composites polymerize more slowly than light-cured composites, allowing sufficient time for water to diffuse through the polymerized, simplified adhesives (28). This poor control of moisture may have contributed to the occurrence of adhesive failure along the cement-dentin interface (17). When Clearfil New Bond was applied in conjunction with Clearfil Photo Core, higher tensile bond strengths were achieved. The command cure of the composite that was induced by light activation may have prevented the diffusion of water through the adhesive layer, interfering with adhesion. In general, the amount of water movement across resin-bonded dentin when etch-and-rinse adhesives are used is greater than that with self-etching adhesives (37). The adhesive layer may have partially reduced the shrinkage stress at dentin interface (15). As a result, a higher percentage of mixed failures were recorded in this group.

Multilink Primer when applied in combination with Clearfil Photo Core achieved low bond strength values. This simplified self-etching adhesive contains a high concentration of hydrophilic

monomers. The presence of water in the solvent may affect the coupling between the adhesive and the light-cured composite. Moreover, a recent investigation revealed that the high concentration of acidic monomers in the adhesive systems negatively affects the polymerization rate of light curing composites (38).

Multilink Primer achieved the best results when it was used with Multilink cement. The technique of adhesive application on dry dentin, the maintenance of the smear plug within the tubules (22,34) and the chemical compatibility of the products produced the same manufacturer (39), could have contributed to this favourable result. Moreover, polymerization shrinkage stresses that were generated because of the highly unfavourable cavity configuration factor of the post space may be partially compensated by the use of slow-setting self-curing resin cements (15,40). This probably accounted for the relatively higher percentage of mixed failure that was seen in this group (25).

The chemical compatibility between the resinous matrix of the fiber posts and the cement (both containing methacrylate resin), may be an additional factor for the low incidence of adhesive failure along the post/cement interface (11.76 %). Moreover, the application of a silane coupling agent on the post surface also contributed to the strengthening of this interface (41). The majority of the failures showed cement remnants on the post surface (Fig. 2).

On the other hand, when the dual-cured adhesive system (Clearfil Photo Bond) was light-activated and used in combination with the light-curing composite (Clearfil Photo Core), most of the specimens failed at the cement-dentin interface (Fig.1a,1b). This could be the result of shrinkage stresses that developed from the rapid curing of the composite (42). The use of a translucent fiber post and the good polymerization rate of the selected light-cured composites probably accounted for the good results achieved in the study.

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Table 1. Mode of application and composition of tested materials.

Materials	Components	Mode/steps of application
Multilink **(Ivoclar Vivadent)**	Base and Catalyst: DMA HEMA Inorganic fillers Ytterbium trifluoride Initiators, stabilizers and pigments Primer A: Aqueous solution of initiators (sulfonate, amines) Primer B: Phosphonic acid acrylate HEMA TEDGMA Methacrylate modified polyacrylic acid	Mix Primer A and Primer B and apply for 15 s. Gently air dry. Mix the cement and apply on the teeth. Remove cement excess immediately. Wait for 120 s~~ee~~.
Clearfil Photo Core **(Kuraray)**	Silanated Silica Silanated Barium Glass Bisphenol A Diglycidylmethacrylate CQ	Apply on the restoration. Light cure for 40 s.
Clearfil Photo Bond **(Kuraray)**	K-etchant gel Catalyst liquid: Bisphenol A diglycidyl. methacrylate 10- MDP HEMA Hydrophobic dimethacrylate Benzoyl Peroxide CQ Universal Liquid: N,N`Diethanol p-toluidine Sodium benzene sulfinate Ethyl alcohol	Etch for 15 s. Rinse with water spray and gently dry with air and paper points. Mix Catalyst and Universal liquid. Apply with a brush. Gently air dry for 2-3 s. Light cure for 10 s.
Clearfil New Bond **(Kuraray)**	K-etchant gel Catalyst liquid: Bisphenol A diglycidyl methacrylate 10- MDP HEMA Hydrophobic dimethacrylate Benzoyl Peroxide Universal Liquid: N,N`Diethanol p-toluidine Sodium benzene sulfinate Ethyl alcohol	Etch for 15 s. Rinse with water spray for 10 s. Mixing Catalyst and Universal liquid. Apply with disposable brush. Dry gently for 2 or 3 s.

DMA: Dimethacrylate; HEMA: Hydroxyethyl methacrylate; TEGDMA: Triethylene glycol-dimethacrylate; 10-MDP: 10- Methacryloyloxydecyl dihydrogen phosphate; CQ: Camphorquinone.

Table 2. Mean (standard deviation) of microtensile bond strength values (MPa) obtained for each tested group. Letters show differences within the same column and numbers within the same row ($p < 0.05$).

	Clearfil Photo Core	Multilink cement
Clearfil Photo Bond	11.89 (4.34) 1a	11.37 (5.20) 1b
Clearfil New Bond	13.02 (3.39) 1a	9.57 (1.34) 2b
Multilink Primer	10.75 (2.95) 1a	15.33 (1.95) 2a

Table 3. Distribution of failure modes as observed with optical microscopy.

	Clearfil PhotoCore			Multilink		
	Mixed	Post/Cement	Dentin/Cement	Mixed	Post/Cement	Dentin/Cement
Clearfil Photo Bond	33.3%	25%	41.7%	25%	25%	50%
Clearfil New Bond	50%	20%	30%	23.08%	30.77%	46.15%
Multilink Primer	19.5%	32%	48.5%	47.06%	11.76%	41.18%

.

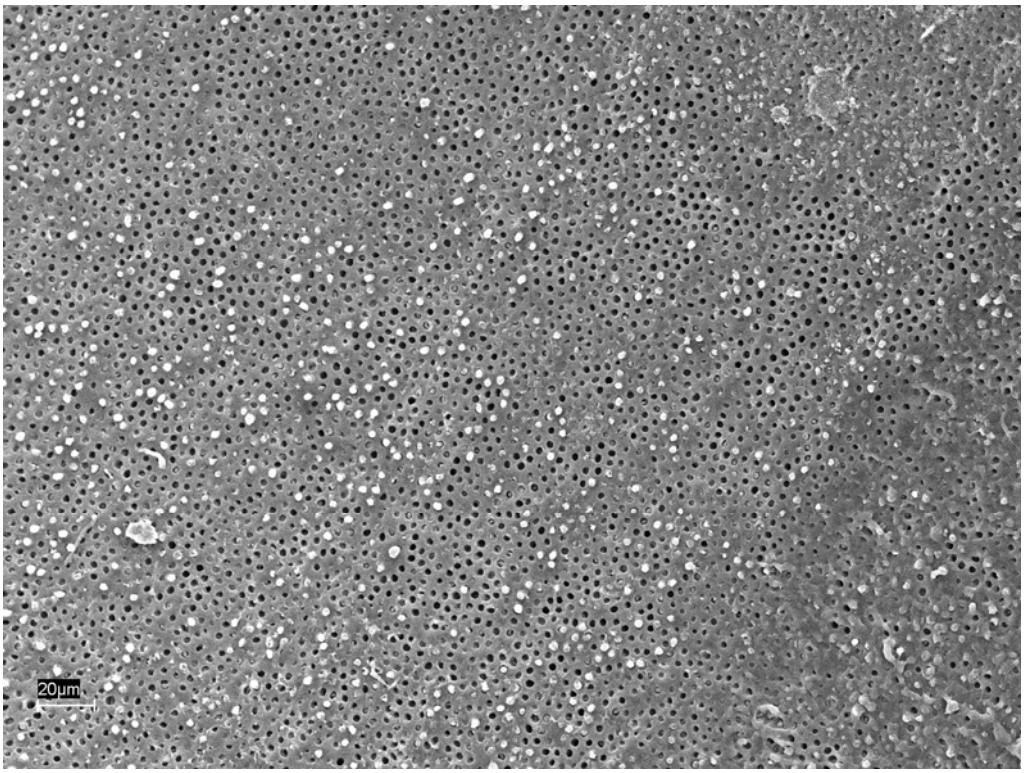

Fig. 1A. Representative SEM images of the fracture pattern on dentin. A complete exposition of the dentinal substrate with adhesive remnants at the tubular level (1000x, bar = 20 µm)

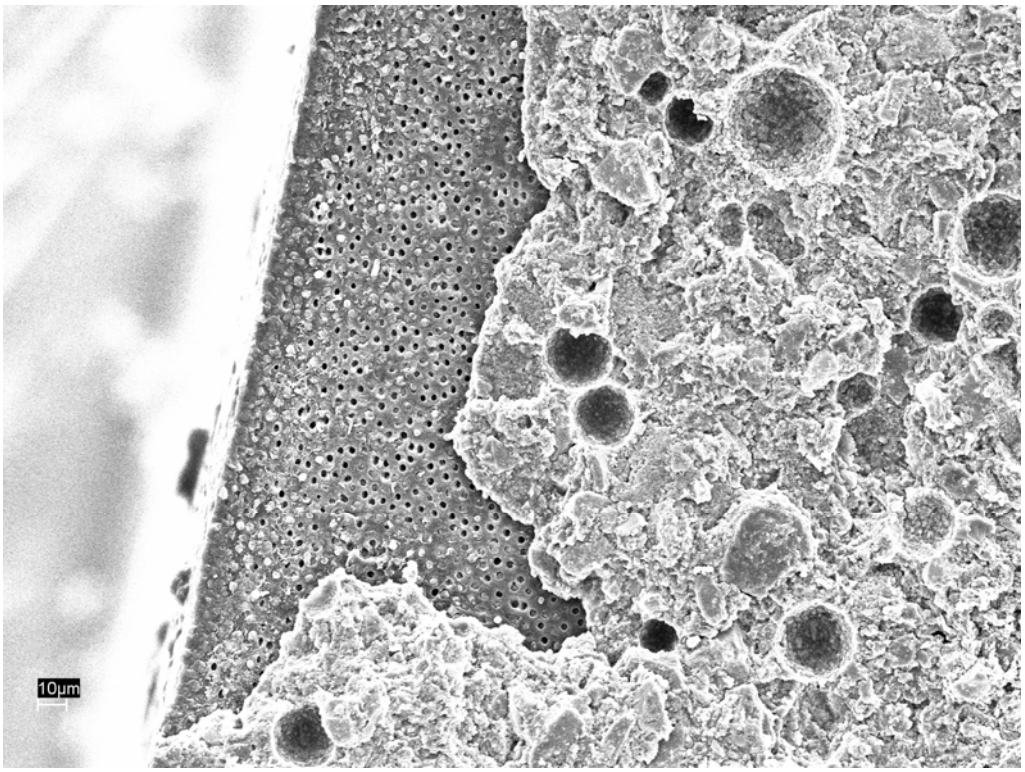

Fig. 1B. Representative SEM images of the fracture pattern on dentin A partial detachment of the luting cement from dentin were registered in the tested groups (3000x, bar = 10 µm)

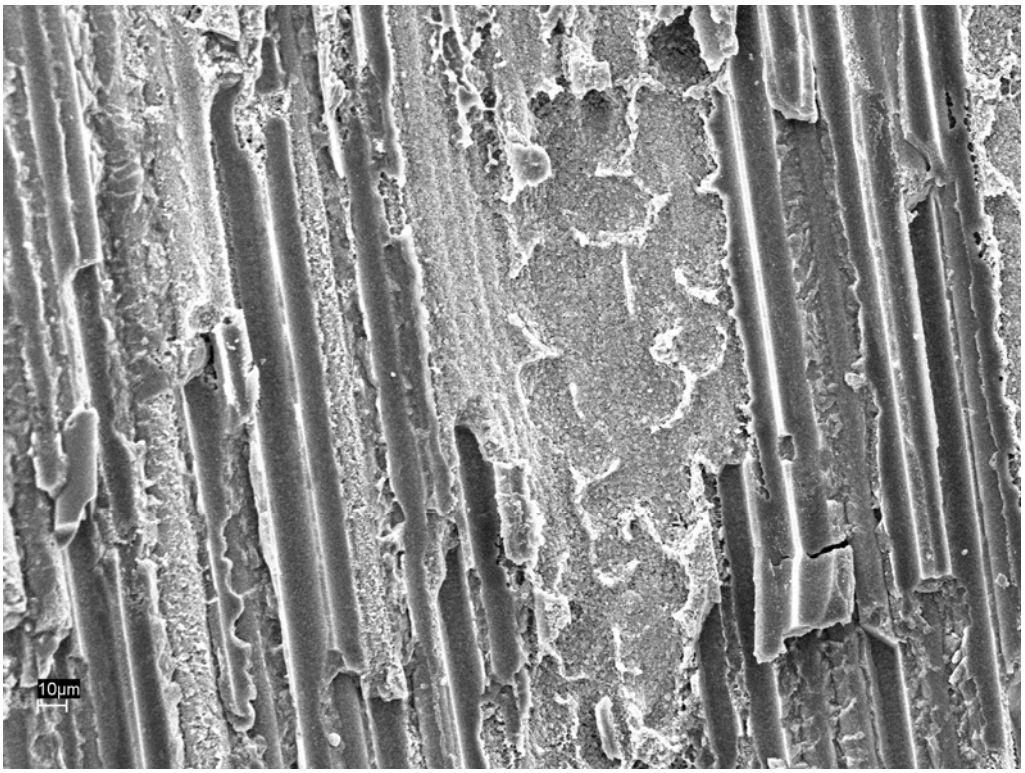

Fig. 2. Microphotograph of the post surface after testing. A thin layer of residual resin cement remained on the post surface (3000x, bar = 10 µm).

IV. 2. Post silanization improves bond strength of translucent posts to the flowable composite resins.

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ABSTRACT

Purpose: To evaluate the effect of post silanization on the microtensile bond strength (MTBS) of translucent fiber posts to seven flowable composite resins used as core material. **Materials and Methods:** A cylindrical plastic matrix was placed around the post and it was filled with different resins. In half of the specimens silanization of the post surface was performed. Two longitudinal cuts were made on two opposite sides of the post. From the slab, 1-mm thick beams were serially sectioned. Each beam was tested in tension in an Instron machine at 0.5. mm/minute. **Results:** The statistical analysis revealed that different resin composite materials used as core materials ($p<0.05$) and the post silanization procedure ($p<0.05$) had a significant effect on MTBS. X-Flow and Point 4 attained the highest MTBS regardless of the silane treatment. **Conclusion:** High filler content and low viscosity of the flowable composite resin are the main characteristics required to get high bond strength between flowables resins and the post surfaces. The application of a silane-coupling agent considerably enhances the bonding of flowable composite resins to translucent post.

Keywords: Silane, Core material, Microtensile bond strength, Flowable composite resin.

INTRODUCTION

The use of fiber posts on restoring endodontically treated teeth has increased because of the risk reduction of fracture since their modulus of elasticity can be compared to that dentin.[1, 2, 3, 4, 5]

When a significative loss of coronal structure is present, a fiber post and a build up of the abutment around it are needed. For a successful build-up of a subsequent resin core, it is necessary to establish a strong bond between resin and post as well as resin and dentin, although it has been found that bond failures at the fiber post core material interface may occur.[6, 7]

The use of flowable materials and hybrid composites for building up the core onto a fiber post has been proposed.[8, 9] The flowable materials were purposed to offer high flow, better adaptation, easier insertion and great elasticity than previously available products.[10] Also, in order to improve the interface resin-post, it has been speculate that the application of a silane coupling agent to glass fiber could enhance the interfacial shear strength and flexural properties of fiber reinforced composite.[7, 11]

Push-out test, has been used to remove posts from composite resin core materials.[12, 13] The development of the microtensile bond strength test has enabled the transverse force on small bonded areas such as the post and resin interface.[14] Some researches have reported microtensile bond strength (MTBS) data of resin cement and core materials to post and root canal dentin.[11, 15, 16, 17]

Translucent fiber post has high fatigue and tensile strength.[1] They should allow light to be transmitted into the root canal. This would increase the conversion degree of composite resins with

a consequent improvement of their mechanical properties such as modulus of elasticity and hardness.[18]

The aim of this study was to evaluate the effect of post silanization on MTBS using different flowable composites as core material bonded to translucent fiber posts.

The null hypothesis to be tested is that MTBS is not affected by post silanization or by the different flowable composites used as core materials.

MATERIALS AND METHODS

70 DT Light-Post size 2 posts with a 1.76 mm diameter in the coronal part (RTD, St Egève, France) were used for testing. On half of the posts from each group the surface was treated with a silane coupling agent (Monobond-S, Ivoclar-Vivadent, Schaan, Liechtenstein). Monobond-S contains 3-methacryl-oxypropyltrimethoxysilane (MPS) as the effective silane (1% in weight), in a solution of ethanol (52% in weight) and distilled water (47% in weight), and has a pH of 4 (15). Following manufacturer's instructions, Monobond-S was applied on the post surface with a brush. After being coated with the silane solution for 60 s at room temperature, the post surface was dried with air, and the core portion was built up around the post with the flowable composite resins reported in Table 1. On the other half of the posts, the same composite resins were used for core build-ups but post silanization procedure was not performed.

For the core build-up procedure, each post was positioned upright on a glass slab, and secured with a drop of sticky wax. Then, a cylindrical plastic matrix was placed around the post and adjusted so that the post would be exactly in the middle. The matrix was 10 mm in diameter. The flowable composite was applied in 1–2 mm thick increments, which were cured for 20 s with a halogen curing light (Optilux 401, Kerr/Demetron, Orange, CA, USA) light-output 750 m W/cm^2. The composite resin was always irradiated directly from the open upper side of the matrix and through the post. When the matrix was completely filled, the cylinder was taken off from the glass slab, and a further 20 s irradiation was done on the side of the cylinder that had faced the glass slab, in order to ensure complete polymerization of the core material. At this point, the plastic matrix was cut-off.

Each cylinder of material was secured in the holding device of an Isomet machine (Buehler, Lake Bluff, IL, USA). By means of a water-cooled diamond blade, two longitudinal cuts were then

performed on two opposite sides of the post at its outermost periphery. As a result, a slab of uniform thickness was created, that presented with the post in the center and the core build-up on each side. From the slab, 1-mm thick beams were then serially sectioned. Thirty to 35 sticks were obtained in each group. Each stick was glued with cyanoacrylate (Zapit, Dental Ventures of America, CA, USA) to the two free sliding components of a jig, which was mounted on a universal testing machine (Controls, Milano, Italy). This set-up was conceived to apply purely tensile forces to the two opposite post–core interfaces. Each specimen was loaded at a cross-head speed of 0.5 mm/min until failure occurred at either one of the two stressed interfaces. Bond strength was expressed in MPa. As the bonded interface was curved, the bonded area was calculated using a mathematical formula previously applied by Bouillaguet *et al*.[15]

Kruskall-Wallis Non-Parametric Analysis of Variance was used, followed by the Mann-Whitney U test for multiple comparisons. In all the tests the level of significance was set at $p=0.05$. Kolmogorov-Smirnov test was used to prove normal data distribution. Statistical analysis was processed by the SPSS 11.0 software (SPSS, Inc., Chicago, IL, USA).

The failure modes were determined by observation under a stereomicroscopy (Nikon type 102, Tokyo, Japan) with 30X magnification, and were classified as adhesive, mixed or cohesive within the resin composite material. Selected debonded sticks of each group were evaluated by scanning electron microscopy (SEM) analysis. Each stick was mounted on aluminum stubs, sputter-coated with gold by means of the Polaron Range SC7620 device (Polaron Equipment Ltd., Newhaven, England), and observed under SEM (DSM-950, Zeiss, Germany).

RESULTS

Microtensile bond strength mean values and standard deviation for the experimental groups are reported in the Table 2.

When silane was applied on the post surface increases in MTBS were observed for all groups. X-Flow and Point 4 attained greater MTBS than the rest of the flowable composite resins regarding of the post silane treatment.

Premature failures of experimental groups are reported in Table 2. No premature failures were found when silane was applied on the post surfaces. A highest percentage of premature failure was obtained when using Venus Flow as core material without post silanization.

By SEM analysis was evidence that most of the failures were adhesives. X-flow (Fig. 1) showed less microbubbles/voids than the other materials did (Fig. 2).

DISCUSSION

The retention of the core portion around the post depends on the chemical and micromechanical interaction between post and resin composite. According with Purton *et al*[19] one of the failure reason when fiber posts are used, is the lack of adhesion between the core material and post.

No available published data exist about MTBS of fiber post and flowable composite resins after post silanization. The results of the present study suggested that the additional application of a silane-coupling agent considerably enhanced the bonding of flowable composite to the post. Some recent data[7, 11] did also demonstrate that a preliminary silanization of the post surface may improve the bond strength between posts and other resin core materials. However, some uncertainly doubts remains around the responsible mechanism for this enhancing effect.[11] It has been suggested that the coupling agent modifies the matrix polymer morphology, either weakening (deformable layer theory) or strengthening the matrix (restrained layer theory).[20] It seems that the silane would assist substrate wetting due to its low coupling action, providing a physical adhesion by the intimate contact between the interfacing materials and van der Waals's forces which would become effective.[21]

In this study seven flowable composite materials were compared. Wave mv composite resin showed the lowest MTBS and revealed voids/bubbles wider than those noted with the other composite resins (Fig. 2). In a recent study, Bonilla *et al*[22] found that Wave mv exhibited the lowest fracture toughness. The lower required energy to initiate and propagate a crack in this material leading a catastrophic failure can justify the lowest value attained in the present study. X-Flow and Point 4 attained greater MTBS than the rest of the composite resins. X-Flow is a composite resin

combining high filler content and low viscosity that is able to achieve strong adhesion and good adaptation to the post, and exhibit a satisfactory structural homogeneity within the abutment. Point 4 has an ultra-small particle filler that is 70% loaded and it's highly filled with glass filler (approximately 70% by weight), it could account for better mechanical properties. SEM analysis revealed less presence of bubbles at the interface between flowable material and the post in X-Flow compared with the other tested materials (Fig. 1). Some *in vitro* studies have shown that the use of flowable composites reduces the occurrence of voids.[23, 24] The presence of voids/bubbles within the resin cores and the development of gaps along the interface with the post, negatively affects the strength of the abutment.[25]

When silane was applied onto the post surfaces, UniFil Low Flow Plus showed greater MTBS than UniFil Flow. UniFil Flow contains Fluoro-alumino silicate glass (50- 60%) and UniFil Low Flow Plus Fluoro-alumino silicate glass (30-40%). Its lower filler content could increase the wetting capacity of post surface. Previous studies[9, 26] have demonstrated that resin cores with high wettability show a good structural integrity and continuous adaptation around the fiber post.

Most of the failures were adhesives between the fiber post and the composite resin. DT Light Posts produced by RTD contains silanized quartz fibers and an epoxy resin. During theirs manufacturing process the fibbers are pre-stressed in tension and the soaked in resin, which is finally polymerized. For this reason, when the post is subjected to forces, the tensile stresses which are introduced can easily be absorbed, justifying the absence of cohesive failure within the fiber post.[2, 7, 8, 25]

The different flowable composite resins used in this study and the surface treatments significantly influenced the MTBS achieved, so the null hypothesis is rejected. In order to achieve

high bond strength, a difficult combination of good post adaptation and high mechanical properties is required. High filler content and low viscosity of the flowable composite resin are the main characteristics required to get high bond strength between flowables resins and the post surfaces. The application of a silane-coupling agent considerably enhances the bonding of flowable composite resins to translucent post.

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Table 1: Compositions and manufacturers of tested flowable composites.

Materials	Components	Manufacturer
UniFil Flow	2-Methacryloxyethyl, 2,2,4-trimethylhexamethylene dicarbamate, Triethylene glycol dimethacrylate, Fluoro-alumino silicate glass (50- 60%), Silica powder (10-15%).	GC, Tokyo, Japan. Lot. 0401061.
UniFil Low Flow Plus	Urethane dimethacrylate, Triethylene glycol dimethacrylate, Fluoro-alumino silicate glass (30-40%), Silica powder (5-10%), Camphorquinone.	GC, Tokyo, Japan. Lot. 0405131.
Venus- Flow	BIS-GMA, TEGDMA, low filler (particle size of Ba glass is 0.7 microns).	Heraeus Kulzer, Hanau, Germany. Lot. 010100.
Revolution 2	Uncured methacrylate ester monomers (38-53%), Inert mineral fillers, activators and stabilizers.	Kerr, Orange, Ca, USA. Lot. 3-1323.
Point 4	Uncured methacrylate ester monomers (20-35%), glass filler	Kerr, Orange, Ca, USA. Lot. 202983.

	(approximately 70% by weight), inert mineral fillers, activators and stabilizers.	
X-Flow	Strontium alumino sodium fluoro phosphate silicate glass, Di- and multifunctional acrylate and methacrylate resins , DGDMA, Highly dispersed silicon dioxide, UV stabilizer, Etyhyl –4-dimethylaminobenzoate, Camphorquinone, BHT, Iron pigments, Titanium dioxide.	Dentsply, Konstanz, Germany. Lot.0204001829.
Wave mv	Dimethacrylate resin, Camphorquinone, Tetramethyl anilina, BHT, Inorganic filler (Silica/glass powder).	Southern Dental Industries, Cologne, Germany. Lot. 030233.

Bis-GMA= bysphenyl glycidyl methacrylate; TEGDMA= triethylene glycol-dimethacrylate; DGDMA=diethylene glycoldimethacrylate; BHT= butylated hydroxyl toluene.

Table 2.

Mean (standard deviation) of microtensile bond strength values (MPa) and premature failures obtained for each tested group. Similar groups ($p>0.05$) are labelled with the same letter.

	No Silane		Silane	
	MTBS Mean (SD)	**Premature failures (%)**	**MTBS Mean (SD)**	**Premature failures (%)**
UniFil Flow	4.83 (2.01) f	47.06	6.16 (1.71) d	0
UniFil Low Flow Plus	5.04 (1.73) f	29.17	8.54 (3.04) bc	0
Venus Flow	4.41 (1.31) g	61.29	7.57 (3.00) cd	0
Revolution Formula 2	4.67 (1.74) ef	11.53	7.50 (1.97) d	0
Point 4 Flowable	6.14 (2.19) de	0	9.48 (2.70) ab	0
X-Flow	5.22 (1.69) ef	5.56	12.24 (8.01) a	0
Wave mv	3.21 (2.29) g	44.4	5.96 (3.84) de	0

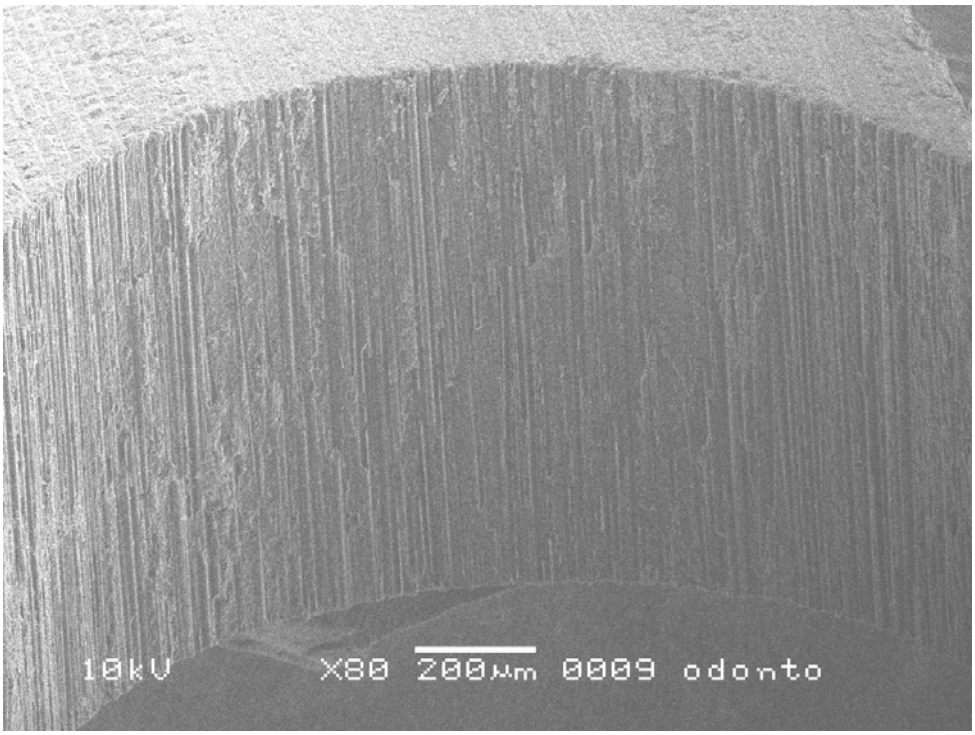

Fig 1. Flowable composite material X-Flow after being tested. An adhesive failure along the surface is detected. No voids/bubbles are noted (x80).

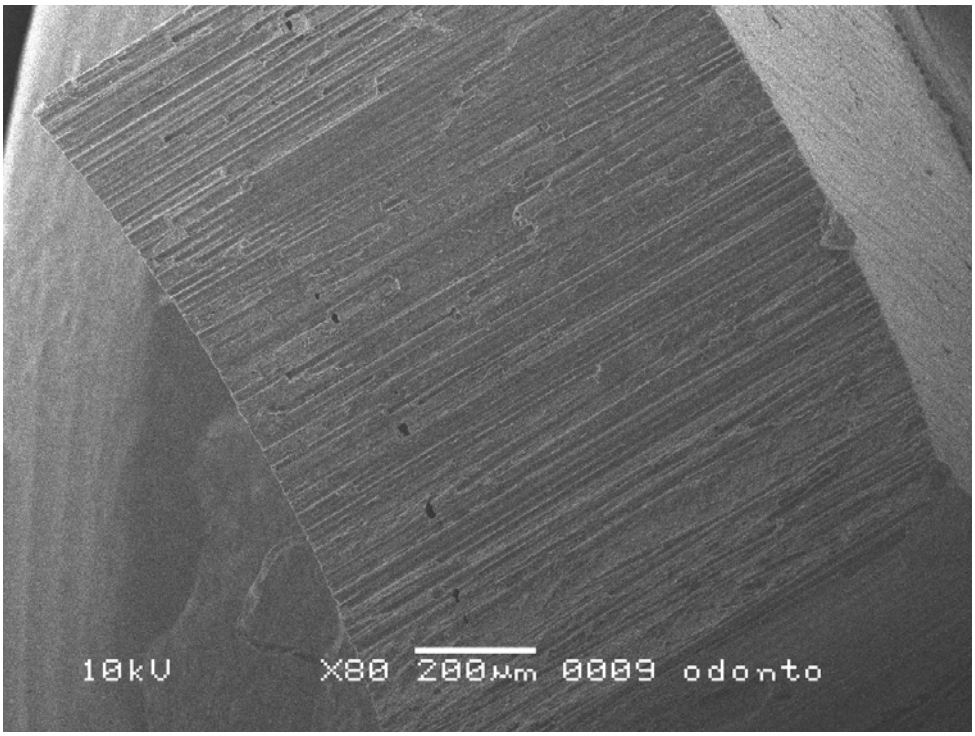

Fig 2. Flowable composite material Wave mv after being tested. An adhesive failure along the surface is detected. Voids/bubbles are noted (x80).

V. DISCUSSION

Different bond strength tests have been developed (Pashley *et al*., 1995). Currently the most commonly used are the shear and micro-tensile bond strength (MTBS) test methods. It is important to note that a bond strength value can not be considered as a material property (Van Noort *et al*., 1989), as recorded data largely depend upon experimental factors: test method, stress rate, sample size and geometry (Phurkkanon *et al*., 1998, Sudsangiam *et al*., 1999).

Microtensile bond strength test was used since MTBS testing seems most appropriate to apply *in vitro* degradation (De Munck *et al*., 2004). There are a number of advantages to the use of the microtensile testing method (Pashley *et al*., 1995): 1) More adhesive failures, fewer cohesive failures. 2) Higher interfacial bond strengths can be measured. 3) Permits measurements of regional bond strengths. 4) Means and variances can be calculated for single teeth. 5) Permits testing of bonds made to irregular surfaces. 6) Permits testing of very small areas. 7) Facilitates SEM examination of the failed bonds since the surface area is approximately $1mm^2$.

Microtensile bond strength test was used for evaluating the adhesive strengths of glass fiber posts in the root canals (Bouillaguet *et al*., 2003), as more information could be obtained compared with "push-out" or "pull-out" test, that were employed traditionally for assessing post retention (Mitchell *et al*., 1994). The microtensile test may give a better evaluation of the local bonding pattern inside the root canal when using adhesive cements (Pashley *et al*., 1999). Further, the microtensile test allowed the use of relatively flat surfaces, which served as a control not subjected to shrinkage stresses and accessibility problems, which dominate the intact canal. This type of control may not be possible in a push-out test (Bouillaguet *et al*., 2003).

To evaluate the MTBS to coronal dentin, five adhesive systems were selected: three total-etch self-priming adhesives (Single Bond -SB-, Prime & Bond XP -PXP-, Prime & Bond NT -PNT-), one self-etching adhesive (Clearfil SE Bond -SEB-), and an all-in-one adhesive (Etch & Prime -EP-). Self-etching adhesive try to solve difficulties commonly associated with the clinical application of total-etch adhesives (Toledano *et al*., 2001; De Munck *et al*., 2003a). Their application procedure is considered less time-consuming, simplifying the bonding restauration to tooth structures (Imazato *et al*., 2000) and less technique-sensitive, in particular with regard of keeping the dentin surface in an adequate state of hydration (De Munck *et al*., 2003a). Self-etching approach increase the concentrations of acid monomers enable the primer or adhesive to etch and prime the dentin simultaneously. No discrepancy between the depth of demineralisation and depth of resin infiltration is expected, since both processes occur simultaneously (Tay *et al*., 2000).

In chapter III.1 and III.2, SB and SEB obtained the highest MTBS to coronal dentin. SEB is a two-step self-etching primer containing: 1) A highly hydrophilic 10-MDP monomer, which is believed to improve the wetting of the tooth surface (Van Meerbeeck *et al.,* 1994). SEB causes minimal dissolution of smear plugs and limited opening of tubules, which reduces dentin permeability (Jackson *et al.,* 2001) and facilitates penetration, impregnation, polymerization and entanglement of monomers with the underlying dentin to form an hybrid layer (Inoue *et al*., 2000; Toledano *et al*., 2002; Osorio *et al.,* 2003). Moreover, 10-MDP has two hydroxyl groups that may chelate with calcium ions of dentin (Kubo *et al.,* 2001; Nunes *et al.,* 2003); 2) Camphorquinone as sensitizer, which increases the polymerization of monomers and bond strength to dentine (Miyazaki *et al*., 1995b); 3) Nanofiller that improve the viscosity of the resin, stabilizing and thickening the adhesive layer and improving the elastic buffering capacity when shrinking during polymerisation (Miyazaki *et al.,* 1995a; Inoue *et al.,* 2000; Gallo *et al*., 2001; Toledano *et al*., 2003); 4) Alcohol as solvent which produces progressively higher solvation pressures that develop an increaser of the infiltration of resin monomers rates (Pashley *et al*., 2002; Reis *et al*., 2003).

The one-step self-etching (all-in-one) adhesive EP exhibited the lowest MTBS. Consensus exists in the literature that supports the poor performance of such all-in-one adhesives in bond strength measurements (Fritz *et al.,* 1999; Inoue *et al.,* 2000; Toledano *et al.,* 2001; Toledano *et al*., 2003b; Osorio *et al.,* 2003; De Munck *et al*., 2003a), although they were able to completely dissolve the smear layer, and formed a relatively thick hybridized complex (Haller *et al*., 2000; Cardoso *et al*., 2002; Toledano *et al.,* 2003; Osorio *et al*., 2003; Fritz *et al*., 1999) that incorporated the smear layer (Santini *et al.,* 2001).

Several reasons have been advocated to account for the suboptimal performance of these all-in-one adhesive systems: (1) the combination of acidic hydrophilic and hydrophobic monomers into a single step may compromise the polymerization of the adhesive (De Munck *et al*., 2003a), (2) the stronger etching process may destabilize the collagen, leading to a decrease in bond strength (Yoshiyama *et al.,* 1995), (3) the inherent weak strength of the adhesive polymer (Fritz *et al*., 1999; Haller *et al*., 2000; Inoue *et al*., 2000), and (4) the lower degree of polymerization of the resin monomer, due to a major solvent/oxygen inhibition effect in the photo-polymerization of these adhesives (Nunes *et al*., 2004).

There are some morphological differences between the created bond structures when using a total-etch bonding system (Fig. 4. Chapter. III.3) and those formed by a self-etch adhesive system (Fig. 1-3 Chapter III.3). The most remarkable difference is the hybrid layer thickness (Van Meerbeek *et al*., 2001). The hybrid layers created by the two total-etch self-priming adhesives were thicker than that observed in self-etching adhesive systems (Fig. 1-4. Chapter III.3). Despite the physical appearance of thin hybridized complex, high immediate bond strength has been reported for these adhesive systems (Osorio *et al*., 2003; Toledano *et al*., 2005a, Toledano *et al*, 2005b). This suggests the absence of correlation between hybrid layer thickness and bonding efficacy as long as an uniform demineralization front is created at the intertubular dentin (Gwinnet *et al*., 1996; Vargas

et al., 1997; Inai *et al*., 1998) and it is fully impregnated by resin (Kenshima *et al*., 2005). Most early self-etch bonding systems were hydrophobic, which did not allow them to adapt to dentin properly (Van Meerbeek *et al*., 2003). The adhesive must be able to diffuse and penetrate in an aqueous environment and, therefore, be hydrophilic (Grégoire *et al*., 2005). The non-rinsing self-etching adhesives, designed to bond with dentin covered with a smear layer, contain acid monomers, often mixed with water, to make the adhesive systems sufficiently acid to cross the smear layer and form a bond with the underlying dentin and to incorporate the smear layer into the hybrid layer (Tay *et al*., 2001; Van Meerbeek *et al*., 2003). The latest commercially available self-etching adhesives further incorporates all the resin monomers and photoinitiator into a single bottle and eliminates an additional mixing step.

SEB and ABF (experimental system) produced hybridized complexes of similar thickness and seems to interact with the underlying intertubular dentin (Fig. 1 and Fig. 2. Chapter III.3). The acidity of the primer was sufficient to dissolve the smear layer and smear plugs from the dentin and to demineralise the intact matrix to a depth of about 0.5 µm (Lee *et al*., 2003). ABF bonding formulation is similar to that of SEB. Both self-etching adhesive systems contain 10-MDP monomer, a highly hydrophilic, which interact chemically with hydroxyapatite that remains available at the partially demineralized dentin surface (Nunes *et al*., 2003). Moreover, MDP improve the wetting of the tooth surface (Van Meerbeek *et al*., 1994), causes minimal dissolution of smear plugs and limited opening of tubules, which reduces dentin permeability (Jackson *et al*., 2001) and facilitates penetration, impregnation, polymerization and entanglement of monomers with the underlying dentin to form hybridized complex (Inoue *et al*., 2000; Toledano *et al*., 2003b; Osorio *et al*., 2003).

The self-etching adhesives vary in their acidity by virtue of the composition and concentration of polymerizable acids and acidic resin monomers in these systems (Pashley et al.,

2001). However, it seems that the pH value of self-etching adhesives does not influence the morphology of the dentin-resin interfaces (Grégoire *et al*., 2005). The pH is not the determining factor conditioning the action of self-etching adhesives (Grégoire *et al*., 2005), and the MTBS are neither affected by the adhesive's acidity (Kenshima *et al*., 2005).

EP showed a thick hybridized complex (Fig. 2). Previous studies have also shown that this bonding agent is able to completely dissolved the smear layer, (pH 0.6) and formed a relatively thick hybridized complex (Toledano *et al.,* 2001; Fritz *et al*., 1999). When EP hybridized complex were evaluated, many avoid zones of separation between the adhesive and the resin composite were observed (Fig. 2). These separation zones, resembling blisters, were found systematically on all the EP specimens but did not exist for the rest of the tested adhesive systems. The same technique was used for all specimens, so that the hipothesis that ligth-cured composite may not have been activated immediately, thus leading to formation of blisters, can be set aside (Tay *et al*., 2003; Grégoire et al., 2005).

Formed resin tags when SEB was applied were much longer than those formed by EP or by ABF (Fig. 5-7. Chapter III.3). The contribution to the bond strength of the resin tags, relative to the role of the intertubular dentin, in a dentin-bonded interface could vary tremendously, depending on the dentin bonding agent used, the orientation of the dentinal tubules and the tested dentin depth (Tam *et al*., 1994). The penetration of resin tags into the dentinal tubules is believed to contribute little to the final bond strength (Tagami *et al*., 1990; Van Meerbeek *et a*l., 1992; Van Meerbeek *et a*l., 1993). The adaptation to the inner tubule walls probably contribute significantly to dentin bonding (Tam *et al*., 1994). The resin tags of the SEB (Fig. 5. Chapter III.3) were numerous and had marked conical swelling at their bases, which may be considered as sign of a good seal (Grégoier et al., 2005). SEB showed adhesive small lateral branches (Fig. 6. Chapter III.3) sporadically observed on the sides of the resin tags, indicating that resin attempted to fill lateral

canals. Mjör *et al*., (1996) showed that supplementary retention and sealing were offered by the formation of micro-tags in the lateral canal branch from the main tubules

EP showed short and funnel shaped tags with no lateral braches (Fig. 8. Chapter III.3). EP produced a deeper etching compared with SEB (pH 1.9), and tubule entrances were not only opened but also enlarged (Osorio *et al.,* 2003).

To evaluate the adhesive efficacy to root canal dentin, the adhesive systems used in chapter IV.1 were based on two different bonding strategies: 1. Adhesives that require etching and rinsing with phosphoric acid prior to bonding (Clearfil Photo Bond and Clearfil New Bond). 2. Adhesives that are directly applied on the smear layer (Multilink). Multilink primer is a simplified self-etch adhesive, it is directly applied on the smear layer and, due to its acidic pH (1.93) it etched thorough the smear layer, and partially demineralized the underlying intact dentin (Toledano *et al*., 2003; Tay *et al*., 2001).

Multilink Primer when applied in combination with Clearfil Photo Core achieved low bond strength values. This simplified self-etching adhesive contains a high concentration of hydrophilic monomers. The presence of water in the solvent may affect the coupling between the adhesive and the light-cured composite. Moreover, a recent investigation revealed that the high concentration of acidic monomers in the adhesive systems negatively affects the polymerization rate of light curing composites (Suh *et al*., 2003).

Multilink Primer achieved the best results when it was used with Multilink cement. The technique of adhesive application on dry dentin, the maintenance of the smear plug within the tubules (Toledano *et al*., 2003; Chersoni *et al*., 2004) and the chemical compatibility of the products

produced the same manufacturer (Yamada *et al*., 2004), could have contributed to this favourable result. Moreover, polymerization shrinkage stresses that were generated because of the highly unfavourable cavity configuration factor of the post space may be partially compensated by the use of slow-setting self-curing resin cements (Bouillaguet *et al*., 2003; Feilzer *et al*., 1987). This probably accounted for the relatively higher percentage of mixed failure that was seen in this group (Foxton *et al*., 2003).

The chemical compatibility between the resinous matrix of the fiber posts and the cement (both containing methacrylate resin), may be an additional factor for the low incidence of adhesive failure along the post/cement interface (11.76 %). Moreover, the application of a silane coupling agent on the post surface also contributed to the strengthening of this interface (Goracci *et al*., 2004). The majority of the failures showed cement remnants on the post surface (Fig. 2. Chapter IV.1).

Derived from "traditional" three-step total etch adhesives, simplified two-step total etch adhesives have been developed that combine the primer and adhesive resin into one application step. SB is a total etch adhesive based on a HEMA/alcohol mixture and has been shown to obtain high bond strength values to dentin, when compared to other total-etch adhesives (De Munck *et al.,* 2003b). The results of SB were also comparable to those of SEB (Toledano *et al.,* 2003). On the other hand PNT and the new experimental version of this simplified total-etch adhesive, PXP, showed similar initial MTBS values. Both adhesive systems have similar composition, containing PENTA, an acidic phosphonated monomer, which could have some kind of interaction with the calcium ions left on dentin surface, or even with the underlying dentin (Inai *et al*., 1998).

The resin tags formed with total-etch self-priming adhesives PBNT and SB (Fig. 9 and Fig. 10. Chapter III.3) were much longer than those found in samples whit self-etching adhesives and

both had marked conical swelling at their bases as a result of the removal of peribubular dentin using the previous acid etching of dentin (Fig. 4. Chapter III.3). PBNT showed resin infiltration of the funnelled dentin tubule and the collagen fibrils from the overlying (Breschi et al., 2004). PBNT and SB formed resin tags revealed numerous small lateral extensions of microtags branching off at right angles from the main resin tags (Fig. 11. Chapter III.3). On the other hand, the thicknesses of the hybrid layer produced by SB and PBNT was approximately 3 to 4 μm thick according to previous studies (Frankenberger et al., 2001; Ogata et al., 2001).

The hybrid layer formed by the two total etch adhesive systems (Fig. 4. Chapter III.3), was compact and homogenous in thickness (Breschi et al., 2004). Bis-GMA, HEMA and polyalkenoic acid are the main chemical components of the SB. An aqueous HEMA solution promotes the impregnation of resin into the exposed collagen (Nakabayashi et al., 1996; Sano et al., 1994).Total etch adhesives Clearfil Photo Bond and New Bond were used to evaluate the adhesive efficacy to root canal dentin. Both are simplified etch-and-rinse adhesives that require etching and rinsing with phosphoric acid prior to bonding. Being an ionic resin monomer with acidic functional groups, 10-MDP readily diffuses into the exposed collagen fibrils of the demineralized intraradicular dentin in the absence of smear layer. Poor control of moisture and incomplete resin infiltration may affect their efficacy (Tay et al., 1995).

When Clearfil Photo Bond was applied to the post space, tensile bond strength was not affected by the choice of luting cements. Being a dual-curing adhesive system, the initiators that catalyze its setting reaction may not have unfavourable interaction with both luting cements (Oook *et al*., 2004; Foxton *et al*., 2003; Swift *et al*., 2001). Conversely, the bond strengths of Clearfil New Bond were reduced when it was applied in conjunction with the self-curing luting cement (ML).

The adhesive polymerized through a characteristic slow-setting chemical reaction; this aspect could be considered a favourable condition for reducing stress at the bonding interface (Boullaguet *et al*., 2003; Goracci *et al*., 2004). Some chemical incompatibility probably exists and/or impurities (commonly water) may have penetrated the interface between the adhesive and the resin cement, affecting polymerization (Swift *et al*., 2001; Tay *et al*., 2003). Recent studies showed that the bonding efficacy of simplified etch-and-rinse adhesives to auto-cured composites/cements is hampered by the intrinsic permeability of these adhesives to water as a result of their increased hydrophilicity (Tay *et al*., 2003; Carvalho *et al*., 2004; Chersoni *et al*., 2004a).

This phenomenon has been shown to occur *in vivo* in bonded vital crown dentin and recently in endodontically treated teeth (Chersoni *et al*., 2004b; Tay *et al*., 2004; Chersoni *et al*., 2005). The absence of differences in moisture content between a vital and a non-vital tooth (Papa *et al*., 1994) and the reduction of dentin thickness due to the preparation of the dowel space, may account for this intrinsic permeability (Chersoni *et al*., 2005; Guignes *et al*., 1996). Rinsing with water during the etching procedure, especially in narrow eliptic root canals, combined with the presence of hydrophilic monomers in the adhesives, probably resulted in the retention of remnant water within the dentinal tubules, which, in turn, may affect the bond quality (Bouillaguet *et al*., 2003; Chersoni *et al*., 2005). Chemically-cured composites polymerize more slowly than light-cured composites, allowing sufficient time for water to diffuse through the polymerized, simplified adhesives (Tay *et al*., 2003). This poor control of moisture may have contributed to the occurrence of adhesive failure along the cement-dentin interface (Sanares *et al*., 2001). When Clearfil New Bond was applied in conjunction with Clearfil Photo Core, higher tensile bond strengths were achieved. The command cure of the composite that was induced by light activation may have prevented the diffusion of water through the adhesive layer, interfering with adhesion. In general, the amount of water movement across resin-bonded dentin when etch-and-rinse adhesives are used is greater than that with self-etching adhesives (Hashimoto *et al*., 2004). The adhesive layer may have partially reduced

the shrinkage stress at dentin interface (Bouillaguet *et al*., 2003). As a result, a higher percentage of mixed failures were recorded in this group.

When the dual-cured adhesive system (Clearfil Photo Bond) was light-activated and used in combination with the light-curing composite (Clearfil Photo Core), most of the specimens failed at the cement-dentin interface (Fig.1a, 1b. Chapter IV.1). This could be the result of shrinkage stresses that developed from the rapid curing of the composite (Manocci *et al*., 2003). The use of a translucent fiber post and the good polymerization rate of the selected light-cured composites probably accounted for the good results achieved in the study.

The retention of the core portion around the post depends on the chemical and micromechanical interaction between post and resin composite. According with Purton *et al*., (1996) one of the failure reason when fiber posts are used, is the lack of adhesion between the core material and post.

No available published data exist about MTBS of fiber post and flowable composite resins after post silanization. The results of the chapter IV.2 suggested that the additional application of a silane-coupling agent considerably enhanced the bonding of flowable composite to the post according with some recent data (Goracci *et al*., 2005; Aksornmuang *et al*., 2004) that demonstrated that a preliminary silanization of the post surface can improve this interaction. However, some uncertainly remains around the mechanism actually responsible for the enhancing effect (Goracci *et al*., 2005). It has been suggested that the coupling agent modifies the matrix polymer morphology, either weakening (deformable layer theory) or strengthening the matrix (restrained layer theory) (Debnath *et al*., 2003). The surface wetting theory is the most widely accepted. The silane would assist substrate wetting due to its low coupling action, providing a physical adhesion by the intimate

contact between the interfacing materials and van der Waals's forces which would become effective (Plueddemann, 1991).

In chapter IV.2 seven flowable composite materials were compared. Wave mv performance showed the lowest MTBS and revealed voids/bubbles wider than those noted with the other composite resins (Fig. 3, Chapter IV.2). In a recent study, Bonilla *et al.*, (2003) found that Wave mv exhibited the significantly lowest fracture toughness, a measure of the energy required to initiate and propagate a crack in a material leading a catastrophic failure, which can justify its lowest value in our study. X-Flow and Point 4 attained greater MTBS than the rest of the composite resins. X-Flow is a composite resin combining high filler content and low viscosity that is able to achieve strong adhesion and good adaptation to the post, and exhibit a satisfactory structural homogeneity within the abutment (Ferrari et al., 2005). Point 4 has an ultra-small particle filler that is 70% loaded and it's highly filled with glass filler (approximately 70% by weight), it could account for better mechanical properties. SEM analysis revealed less presence of bubbles at the interface between flowable material and the post in X-Flow compared with the other tested materials (Fig. 2, Chapter IV.2). Some in vitro studies have shown that use of flowable composites reduces the occurrence of voids (Malmstrom H *et al*., 2002; Payne, 1999; Ferdianakis *et al*., 1998). The presence of voids/bubbles within the resin cores and the development of gaps along the interface with the post, negatively affects the strength of the abutment (Ferrari *et al*., 2001). UniFil Low Flow Plus showed greater MTBS than UniFil Flow when silane was applied on the post surface. UniFil Flow contains Fluoro-alumino silicate glass (50- 60%) and UniFil Low Flow Plus Fluoro-alumino silicate glass (30-40%). Its low filler content could increase the wetting capacity of post surface. Previous studies (Monticelli *et al*., 2003; Monticelli *et al*., in press) have demonstrated that resin cores with high wettability show a good structural integrity and continuous adaptation around the fiber post.

One of the most commonly used artificial aging technique is long-term water storage. The durability of bonds between adhesive resins and dentin is of critical importance (Okuda *et al*., 2002; Nakabayashi, 2004) and little is known regarding the stability of hybridized layers (Sano et al., 1999). In general, reports show that dentin bond strength decreased during water storage over time, due to degradation of the resin and the collagen fibrils within the hybrid layer (Nakabayashi, 2004; Okuda *et al*., 2001; De Munck *et al*., 2003; Reis *et al*., 2004).

Long-term water storage study attained smaller reductions in bond strength that are between 23 and 55% (Okuda et *al*., 2001; De Munck *et al*., 2003; Reis *et al*., 2004; Frankenberger *et al*., 2004), even when the dentin-resin interfaces were directly exposed to water up to six years (Frankenberger *et al*., 2004). The presented challenging method based upon 10% $NaOCl_{aq}$ immersion of specimens during a short period of time is much more reliable than *in vitro* studies based on long-term water storage of specimens and those previously reported after $NaOCl_{aq}$ immersion (65% to 77%) (Yamauti *et al*., 2003; Yoshida *et al*., 2004) are similar to the decline in bond strength obtained when *in vivo* degradation studies are performed (Hashimoto *et al*., 2000; Koshiro *et al*., 2004).

In chapter III.2 after storage in $NaOCl_{aq}$, the MTBS fell in all specimens. $NaOCl_{aq}$ is a nonspecific deproteinizing agent, in aqueous solution superoxide radicals O_2^- are formed and induce oxidations that fragment long peptide chains of proteins (Habelitz *et al*., 2002). Chlorination of protein terminal groups is also produced and hypochlorous acid formation evidenced (Weiss *et al*., 1982). Some of these amino acid-derived chloramines have also shown to increase the proteolytic susceptibility of this modified collagen (Olszowski *et al*. 2003). The decline in bond strength is the result of both an hydrolytic process on the resin and the solubilization of unprotected collagen fibrils within the decalcified dentin (De Munck *et al.,* 2003; Yamauti *et al.,* 2003; Yoshida *et al*.,2004; Takahashi *et al*., 2002; Hashimoto *et al*., 2003; Osorio *et al*., 2005).

The total-etch PXP and the SEB, that attained the better resistance to the challenging of $NaOCl_{aq}$ immersion, showed the smallest resin dissolution areas (Fig. 2 to 5, Chapter III.2). The resistance of these resins to the hydrolytic degradation may be the chief reason of the lower reduction in bond strength attained by these adhesives. The least susceptibility of these resins to hydrolysis is probably due to a higher degree of cure of the bonding resins. PXP contains TEGDMA, which brings down the initial viscosity of the monomer mixture, enhancing diffusion of reactive groups, increasing the flexibility, and the rates of polymerisation of the resin (Nunes et al., 2005; Morgan et al., 2000). Camphorquinone is included in both adhesive systems (PXP and CSEB) as sensitier. This activator is in charge to trigger the cascade reaction of the curing, generating free radicals and increasing the polymerization of monomers (Miyazaki *et al*., 2003). Moreover, a low rate of polymerization of the bonding resin has been previously shown for PNT (Fig. 3. Chapter III.2) (Hashimoto *et al*., 2002), and for EP (Nunes *et al*., 2005) leading to rapid degradation of the dentin bonds.

After phosphoric acid etching collagen is highly susceptible to deproteinization processes (Marshall *et al*., 2001). When bonding with a total-etch adhesive, the $NaOCl_{aq}$ affected the resin-dentin bond structures following two pathways 1) the etched and non-infiltrated layer (Fig. 4b. Chapter III.2) and 2) the collagen that was resin-infiltrated but later exposed because of the bonding resin dissolution by the $NaOCl_{aq}$ (Fig. 2b,3b. Chapter III.2).

Resin dissolution rate is material dependent and a high polymerization degree of the bonding resins, within the hybrid layer, may be a very important factor to improve the long-term durability of resin-dentin bonds. The search for 2-steps self-etch systems with a high polymerization degree of the adhesive resin is encouraged.

In the clinical situation, dentin-resin bonds are not only subjected to immediate stresses, that may disrupt the developing bonds, but also to cyclic loading during mastication that will induce generation of cracks and subsequent crack growth that challenge the long-term survival of these bonds. It has been shown that changes in the bonded interfaces *in vivo* may occur under occlusal stresses, resulting in mechanical degradation of the bonds between the restoration and dentin (Sano *et al*., 1999). Teeth are continuously subjected to stresses during mastication, swallowing and parafunctional habits. Maximum biting force recorded on the first molar teeth is approximately 40-90 Kg. Although masticatory loads recorded on a single molar are smaller (ca. 11-27 Kg) (Bates *et al*., 1975; Anderson, 1956), they may still represent a challenge to the long-term durability of resin-dentin bonds.

Static bond strength tests may not adequately demonstrate the potential detrimental effects that porosities and other internal defects within the adhesive layer may have on bonding durability (Givan *et al*., 1995). After cyclic loading, the effect of these interfacial defects on long-term bonding may be more readily apparent. It is anticipated that the combined use of mechanical loading with microtensile bond strength (MTBS) testing permits the evaluation of the *in vitro* durability of resin-dentin bonds under more clinically-relevant conditions than are usually employed in static bond strength testing techniques.

A load of 90 N was used in this study (Chapter III.1), as it was considered to be within the normal functional range (Anderson, 1956). In most of the studies 1,000 to 8,000 cycles are used; with 5,000 cycles being the median value (Abdalla *et al.,* 1996).

In chapter III.1 cyclic loading lowered resin-dentin bond strengths of all the total-etch or self-etching adhesive systems examined The resin-dentin bond is prone to deterioration after cyclic loading because fatigue stress can expedite the degradation of bonds peripheral to the hybrid layer

(Nikaido *et al.,* 2002b; Sano *et al.,* 1999; Qvist *et al.,* 1983). When using SEB, the loading stress seemed to have been concentrated mostly at the interface between the adhesive and the hybrid layer and within the hybrid layer, whereas specimens bonded using a total-etch approach (SB, PNT and PXP) mostly failed at the top of, or beneath the hybrid layer where demineralised collagen fibrils were exposed and the adhesive failed to envelop the collagen network. Such factors have been perceived to be the weakest link in achieving durable long-term bonding (Nikaido *et al.,* 2002b; Osorio *et al.,* 2003; Pashley *et al.,* 2002).

The one-step self-etching (all-in-one) adhesive EP exhibited the lowest MTBS results and frequent adhesive failures (Fig. 3A, Chapter III.1). The less than optimal result achieved with this adhesive was further delineated after cyclic loading.

After load cycling, MTBS values for PNT decreased but not those from PXP. Three main differences between these adhesives may account for these results: 1) PXP contains TEGDMA, which lowers the initial viscosity of the monomer mixture, enhancing its diffusion into the demineralised collagen matrix, increasing the flexibility of the hybridized dentin, and improving the rate of polymerization of the adhesive (Morgan *et al.,* 2000; Nunes, Swift *et al*., 2001; Nunes *et al*., 2004). 2) Camphorquinone is included as a photosensitizer, increasing the polymerization of monomers and bond strength to dentine (Miyazaki *et al.,* 1995). 3) PXP contains t-butanol as solvent, (instead of acetone, in PNT). After demineralization, the collagen fibrils adhere to one another via intrafibrillar hydrogen bonding. A solvent with a solubility parameter for hydrogen bonding that approximates that of the amino acid moieties of the collagen fibrils has a better capacity in breaking up these intrafibrillar hydrogen bonds, and expanding the interfibrillar spaces to promote wetting and infiltration of the adhesive monomers (Pashley *et al*., 2003). It has been demonstrated that higher bond strengths were correlated with wider interfibrilar spaces and that such spaces should be properly infiltrated with resin (Eddleston *et al*., 2003). Application of acetone

produces little solvation force affecting the further infiltration of resin monomers; while alcohols produces progressively higher solvation pressures that develop at increasing rates (Pashley *et al*., 2002; Reis *et al., 2003*). The total-etch alcohol-based adhesive systems used in the chapter 1.1 (SB and PXP) are thought to be able to maintain the collagen fibrils in an expanded condition after the evaporation of solvents, improving the monomers infiltration (Tay *et al*., 1996; Perdigão, Van Meerbeek *et al.,* 1999). This may contribute to explain the lower bond strengths of PNT after mechanical loading, because the decalcified non-infiltrated zone at the base of the hybrid layer is susceptible to degradation during aging (Hashimoto *et al.,* 2002a; 2002b; Pashley *et al.,* 2002). Moreover, a low rate of polymerization of the bonding resin within the hybrid layer has been shown for PNT (Hashimoto *et al*., 2002a), which also may lead to rapid degradation of the resin-dentin bonds.

VI. CONCLUSIONES.

1. Las fuerzas adhesivas inmediatas obtenidas con el adhesivo autograbador de dos pasos, y el sistema adhesivo de grabado total de dos pasos basado en ácido polialquenoico, fueron más altas que las obtenidas con los otros sistemas adhesivos. El sistema adhesivo de un solo paso mostró la menor fuerza de adhesión inmediata a dentina coronal.

2. Los valores de adhesión resina-dentina disminuyen tras el ciclado mecánico. Si la dentina se graba con ácido ortofosfórico, los adhesivos que tienen alcohol como solvente se comportan mejor que aquellos que contienen acetona. El adhesivo de un solo paso evaluado, mostró las fuerzas de adhesión más bajas y menos duraderas, mientras que el autograbador de dos pasos obtuvo los valores de adhesión más altos.

3. La interfaz resina-dentina puede degradarse químicamente. La disminución en las fuerzas de adhesión de todos los sistemas adhesivos es el resultado de un proceso de solubilización de la resina y de las fibras de colágeno desprotegidas en la dentina descalcificada. La extensión y velocidad de la degradación de la interfaz resina-dentina depende del adhesivo usado. El adhesivo de un solo paso evaluado, mostró las fuerzas de adhesión más bajas y menos duraderas.

4. Las capas híbridas obtenidas con los sistemas adhesivos autograbadores de dos pasos y con los de grabado total fueron continuas y con el grosor más uniforme. Los *tags* de resina obtenidos con los adhesivos autograbadores de dos pasos y los de grabado total mostraron unas bases amplias, debidas probablemente al resultado de un buen sellado de la interfaz resina-dentina, y mostraron pequeñas ramas laterales, un signo de una adecuada infiltración y humectabilidad de la dentina descalcificada.

5. Para dentina radicular, el adhesivo autograbador de dos pasos obtuvo los valores de adhesión más altos debidos probablemente a la técnica de aplicación del adhesivo, que se realiza sobre la dentina seca, al mantenimiento de los tapones de barrillo dentinario y a la compatibilidad química de los productos utilizados para el cementado del perno.

6. La aplicación de un agente silano incrementa los valores de adhesión entre los composites fluidos usados como agentes cementantes y la superficie de los pernos.

VI. CONCLUSIONS.

1. The immediate resin-dentin bond values obtained by “mild” two-step self-etch and polyalkenoic acid based two-step total etch adhesive systems are higher than those attained by the other tested adhesive systems. The tested all-in-one adhesive system provided the lower immediate bond strength.

2. The resin-dentin bonds are prone to deterioration after cyclic loading. If dentin is acid-etched, alcohol/based adhesives performed better than those containing acetone as solvent. The tested all-in-one adhesive, provided the least durable bond strength and the two-step self-etch obtained the highest MTBS to dentin.

3. The resin-dentin bonds are prone to chemical degradation. The decline in bond strength in all adhesive systems is the result of both solubilization of the resin and unprotected collagen fibrils within the decalcified dentin. The extent of the resin-dentin bond degradation is material dependent. The tested all-in-one adhesive provided the least durable bond strength.

4. The formed hybrid layer obtained with the two-step self-etching and the total etching adhesive systems were continuous and the most uniform in thickness. Resin tags obtained with the two-step self-etching and the two-step total etch adhesive systems had marked conical swelling at their bases probably resulting in good sealing of resin-dentin interfaces and showed small lateral branches on the sides of the main resin tags, a sign of proper resin infiltration and wetting of decalcified dentin.

5. When testing bonding to radicular dentin, the two-step self-etching adhesive system obtained the highest MTBS probably due to the use of a dried technique and the maintenance of the smear plugs

within the tubules. The chemical compatibility of the tested products may also be a reason to explain the better performance of this adhesive system.

6. The application of a silane-coupling agent enhances the MTBS of flowable composite resin core material to the post surface.

VII. SUMMARY

The durability of resin-dentin bonds is of critical importance and little is known regarding the stability of hybridized dentin layers. Static bond strength tests may not adequately demonstrate the potential detrimental effects that porosities and other internal defects within the adhesive layer may have on bond durability. The objectives of this thesis are: 1) to evaluate the immediate microtensile bond strength (MTBS) of several adhesive systems to coronal human dentin; 2) To evaluate by scanning electron microscopy (SEM) the histomorphology characteristics of the formed resin tags, adhesive lateral branches and hybrid layer of several adhesive systems to coronal human dentin; 3) to evaluate the effect of two *in vitro* challenges (mechanical loading and $NaOCl_{aq}$ immersion) on the MTBS of several adhesive systems to coronal human dentin; 4) to evaluate the MTBS of several bonding agents to root canal dentin taking into account the effect of post silanization and the used core material.

Resin-dentin bond strength was measured by means of a MTBS test. When bonding to coronal human dentin, flat dentin surfaces from 140 molars were obtained and bonded with several adhesive systems according to the manufacturers´ instructions. Composite build-ups were constructed incrementally. After storage for 24 h in water at 37ºC, specimens were randomly divided into three groups: 1) sectioned into beams with 1.0 mm^2 cross-sectional area, 2) load cycled for 5000 cycles (3 Hz, 90 N) and sectioned; 3) sectioned and beams stored in 10% NaOCl solution during 5h. When bonding to root canal dentin, 100 maxillary premolars were endodontically treated and the roots were prepared for post cementation using a fiber post. Different luting materials were used in combination with several adhesives. Specimens were cut to obtain beams with the post in the center. To translucent fiber posts, 70 posts were used for testing. A cylindrical plastic matrix was placed around the post and it was filled with the different resins. In half of the specimens

silanization of the post surface was performed. Two longitudinal cuts were made on two opposite sides of the post at its outermost periphery. From the slab, 1 mm^2 thick beams were then serially sectioned. Microtensile testing for all the beams (posts, coronal and root canal dentin) was performed in an Instron machine at 0.5 mm/min. The most representative beams of each group were selected for SEM analysis. All results were analyzed by Multiple ANOVA and multiple comparisons ($P<0.05$). To evaluate the histomorphology characteristics, flat dentin surfaces from 25 molars were bonded with several adhesive systems according to the manufacturers´ instructions. Composite build-ups were constructed incrementally with composite. The specimens were sectioned parallel to the long axis. One section of each specimen was gently decalcified and deproteninzed in order to evaluate hybrid layer formation and the other section of each sample was stored in 30% clorhidric acid and washed with 2% sodium hypochlorite to detect resin tag and adhesive lateral branch formation. SEM photomicrographs at differents original magnification were taken.

When bonding to coronal dentin, two-step self-etch and polyalkenoic acid based two-step total etch adhesive systems attained higher MTBS than the other adhesives. The two-step total-etch acetone and alcohol based adhesives performed equally, and the tested all-in-one obtained in the lowest MTBS. After NaOCl immersion, MTBS decreased in all groups. The highest MTBS values were obtained for two-step self-etch and the total-etch alcohol based two-step adhesive system. After mechanical loading, MTBS decreased in all adhesives, except for the total-etch alcohol based two-step adhesive system. Two-step self-etch, polyalkenoic acid based two-step total etch and total-etch alcohol based two-step obtained higher MTBS than the total-etch acetone based two-step adhesive system. Specimens bonded with the tested all-in-one resulted in premature failures and MTBS could not be measured. All the adhesive systems showed hybrid layer formation. Total etch adhesives showed thicker hybrid layers than those found in self-etching adhesive systems. The resin tags formed with total etch adhesives were much longer than those found in samples bonded whit

self-etching adhesives. Lateral branch formation was observed in total etch adhesives and in the two-step self-etch. When bonding to root canal dentin luting cement applied with its own self etch adhesive system obtained the highest MTBS while the lowest bond strength was achieved with the light-cured composite in combination with self etch adhesive. When silane was applied on the post surface increase in MTBS was observed for all groups.

In conclusion: 1) the immediate resin-dentin bond values obtained by "mild" two-step self-etch and polyalkenoic acid based two-step total etch adhesive systems are higher than those attained by the other tested adhesive systems. The tested all-in-one adhesive system provided the lower immediate bond strength; 2) the resin-dentin bonds are prone to deterioration after cyclic loading. If dentin is acid-etched, alcohol/based adhesives performed better than those containing acetone as solvent. The tested all-in-one adhesive, provided the least durable bond strength and the two-step self-etch obtained the highest MTBS to dentin; 3) the resin-dentin bonds are prone to chemical degradation. The decline in bond strength in all adhesive systems is the result of both solubilization of the resin and unprotected collagen fibrils within the decalcified dentin. The extent of the resin-dentin bond degradation is material dependent. The tested all-in-one adhesive provided the least durable bond strength; 4) the formed hybrid layer obtained with the two-step self-etching and the total etching adhesive systems were continuous and the most uniform in thickness. Resin tags obtained with the two-step self-etching and the two-step total etch adhesive systems had marked conical swelling at their bases probably resulting in good sealing of resin-dentin interfaces and showed small lateral branches on the sides of the main resin tags, a sign of proper resin infiltration and wetting of decalcified dentin; 5) when testing bonding to radicular dentin, the two-step self-etching adhesive system obtained the highest MTBS probably due to the use of a dried technique and the maintenance of the smear plugs within the tubules. The chemical compatibility of the tested products may also be a reason to explain the better performance of this adhesive system. The

application of a silane-coupling agent enhance the MTBS of flowable composite resin core material to the post surface.

RESUMEN

La durabilidad de la adhesión resina-dentina es de crucial importancia y se sabe muy poco acerca de la estabilidad de la capa híbrida. El test de fuerzas de adhesión inmediata no puede demostrar adecuadamente los efectos que pueden tener en la durabilidad de la adhesión los poros y otros defectos internos producidos en la capa híbrida. De esta manera, los objetivos de esta tesis son: 1) evaluar las fuerzas adhesivas inmediatas de diferentes sistemas adhesivos a dentina coronal humana a través de un test de microtensión; 2) evaluar el efecto de dos tests de degradación *in vitro* (ciclado mecánico e inmersión en $NaOCl_{aq}$) en las fuerzas adhesivas de microtensión de diferentes sistemas adhesivos; 3) Evaluar mediante microscopía electrónica de barrido (SEM) las características histomorfológicas de la capa híbrida formada entre diversos sistemas adhesivos y dentina coronal, y de los *tags* de resina principales y secundarios; 4) Evaluar las fuerzas de adhesión de diferentes agentes adhesivos a dentina del canal radicular teniendo en cuenta el efecto del silano aplicado sobre la superficie del perno y el material usado como agente cementante.

Las diferentes pruebas se realizaron usando el test de microtensión. Para determinar la eficacia adhesiva a la dentina coronal humana, se adhirieron a diferentes sistemas adhesivos las superficies dentinarias de 140 molares siguiendo las normas del fabricante. Se construyó incrementalmente una corona de composite. Después de almacenar los especímenes en agua a 37ºC durante 24 horas, se dividieron en tres grupos: 1) cortados en barritas con 1 mm^2 de área; 2) ciclados mecánicamente con 5000 ciclos (3 Hz, 90 N) y cortados; 3) cortados y almacenados en una solución de $NaOCl_{aq}$ al 10% durante 5h. Para determinar la eficacia adhesiva a dentina radicular, se endodonciaron 30 premolares maxilares y se prepararon las raíces para usar pernos de fibra. Se utilizaron diversos materiales de cementado en combinación con diferentes adhesivos. Se cortaron los especímenes para obtener barritas con el perno en el centro. Para los pernos de fibra

translúcidos, se testaron 70 postes. Se colocó una matriz cilíndrica de plástico alrededor del perno y se rellenó con las diferentes resinas. En la mitad de los especímenes, se silanizó la superficie de los pernos. Se realizaron dos cortes longitudinales en los dos lados opuestos. El trozo obtenido se cortó seriadamente en barritas de 1 mm^2. Una vez obtenidas las barritas de los diversos especímenes realizados (pernos de fibra translúcidos, dentina coronal y radicular), se realizó el test de microtensión para todas ellas con una máquina Instron a 0.5 mm/min. Se seleccionaron las barritas más representativas de cada grupo para un análisis con SEM. Todos los resultados se analizaron con ANOVA y múltiples comparaciones ($P<0.05$). Para evaluar las características histomorfológicas, se adhirieron las superficies dentinarias de 25 molares con diferentes sistemas adhesivos siguiendo las normas del fabricante. Se construyó incrementalmente una corona con composite. Los especímenes se seccionaron paralelos a su eje longitudinal. Una parte de cada especimen fue descalcificada y desproteinizada para evaluar la formación de la capa híbrida, y la otra parte fue almacenada en ácido clorhídrico y lavada con hipoclorito de sodio al 2% para observar los *tags* de resina principales y secundarios. Se tomaron microfotografías con SEM a diferentes aumentos.

En la dentina coronal, las uniones obtenidas por el sistema autograbador de dos pasos y el de grabado total basado en ácido polialquenoico de dos pasos, mostraron unas fuerzas de adhesión más altas que el resto de los adhesivos. Los sistemas de grabado total de dos pasos basados en acetona y alcohol actuaron de manera similar, mientras que el adhesivo de un solo paso testado obtuvo las fuerzas de adhesión más bajas. Después de la inmersión en $NaOCl_{aq}$ disminuyeron las fuerzas de adhesión en todos los grupos. El sistema autograbador de dos pasos y el de grabado total basado en alcohol de dos pasos obtuvieron las fuerzas de adhesión más altas. Después del ciclado mecánico, se produjo una disminución en las fuerzas de adhesión de todos los adhesivos, excepto en el de grabado total basado en alcohol de dos pasos. El adhesivo autograbador de dos pasos, el de grabado total de dos pasos basado en ácido polialquenoico y el de grabado total basado en alcohol de dos pasos obtuvieron unas fuerzas de adhesión más altas que el adhesivo de grabado total de dos pasos

basado en acetona. Los especímenes adheridos con el adhesivo de un solo paso se fracturaron prematuramente sin poder ser testados. Todos los adhesivos mostraron la formación de una capa híbrida. Los sistemas de grabado total formaron una capa híbrida más gruesa que aquella obtenida en los autograbadores. Los *tags* de resina creados con los adhesivos de grabado total fueron más largos que los obtenidos con los autograbadores. Se observaron t*ags* de resina secundarios en los sistemas de grabado total y en el autograbador de dos pasos. En dentina radicular, el agente cementante aplicado con su propio adhesivo autograbador obtuvo las fuerzas de adhesión más altas, mientras que las más bajas se alcanzaron con el composite fotopolimerizable en combinación con el adhesivo autograbador. Cuando el silano fue aplicado en la superficie del perno translúcido, se observó un incremento de las fuerzas de adhesión en todos los grupos.

En conclusión: 1) las fuerzas adhesivas inmediatas obtenidas con el adhesivo autograbador de dos pasos, y el sistema adhesivo de grabado total de dos pasos basado en ácido polialquenoico, fueron más altas que las obtenidas con los otros sistemas adhesivos. El sistema adhesivo de un solo paso mostró la menor fuerza de adhesión inmediata a dentina coronal; 2) los valores de adhesión resina-dentina disminuyen tras el ciclado mecánico. Si la dentina se graba con ácido ortofosfórico, los adhesivos que tienen alcohol como solvente se comportan mejor que aquellos que contienen acetona. El adhesivo de un solo paso evaluado, mostró las fuerzas de adhesión más bajas y menos duraderas, mientras que el autograbador de dos pasos obtuvo los valores de adhesión más altos; 3) la interfaz resina-dentina puede degradarse químicamente. La disminución en las fuerzas de adhesión en todos los sistemas adhesivos es el resultado de un proceso de solubilización de la resina y de las fibras de colágeno desprotegidas en la dentina descalcificada. La extensión y velocidad de la degradación de la interfaz resina-dentina depende del adhesivo usado. El adhesivo de un solo paso evaluado, mostró las fuerzas de adhesión más bajas y menos duraderas; 4) las capas híbridas obtenidas con los sistemas adhesivos autograbadores de dos pasos y con los de grabado total fueron

continuas y con el grosor más uniforme. Los *tags* de resina obtenidos con los adhesivos autograbadores de dos pasos y los de grabado total mostraron unas bases amplias, debidas probablemente al resultado de un buen sellado de la interfaz resina-dentina, y mostraron pequeñas ramas laterales, un signo de una adecuada infiltración y humectabilidad de la dentina descalcificada; 5) en dentina radicular, el adhesivo autograbador de dos pasos obtuvo los valores de adhesión más altos debido, probablemente, a la técnica de aplicación del adhesivo, que se realiza sobre la dentina seca, al mantenimiento de los tapones de barrillo dentinario y a la compatibilidad química de los productos utilizados para el cementado del perno. La aplicación de un agente silano incrementa los valores de adhesión entre los composites fluidos usados como cementos y la superficie de los pernos.

SOMMARIO

La longevità dei legami resina-dentina tra resina adesiva e dentina coronale è di fondamentale importanza e poco si conosce riguardo la stabilità degli strati ibridi.I test statici di forza di adesione possono non rappresentare adeguatamente i potenziali effetti negativi delle porosità e degli altri difetti interni dello strato adesivo sulla durata dell'adesione. Di conseguenza, gli obiettivi di questa tesi sono: 1) valutare la forza di adesione microtensile immediata di diversi sistemi adesivi alla dentina coronale umana; 2) valutare gli effetti di due condizioni *in vitro* (carico meccanico e immersione in NaOCl) sulla forza di adesione microtensile di diversi sistemi adesivi alla dentina coronale umana; 3) valutare al microscopio elettronico a scansione (SEM) le caratteristiche istomorfologiche degli zaffi resinosi presenti, degli zaffi adesivi laterali e dello strato ibrido di diversi sistemi adesivi alla dentina coronale umana; 4) valutare la forza di adesione microtensile di diversi materiali da cementazione alla dentina radicolare et valutare l'effetto del silano sulla forza di adesione microtensile di perni in fibra translucenti a diverse resine composite fluide usate come materiali da core.

Le valutazioni sono state eseguite con test di forza di adesione microtensile. Nello studio sulla dentina coronale umana, diversi sistemi adesivi sono stati applicati su superfici piane di dentina di 140 molari seguendo le istruzioni del produttore. Build-up di composito sono stati costruiti con tecnica di stratificazione incrementale. Dopo conservazione in acqua per 24h a 37°C, i campioni sono stati casualmente divisi in tre gruppi: 1) tagliati in campioni con sezione di area 1.0 mm^2; 2) meccanociclati per 5000 cicli (3 Hz, 90 N) e sezionati; 3) sezionati e i campioni ottenuti conservati in NaOCl al 10% per 5h. Nello studio su dentina radicolare, 30 premolari superiori sono stati trattati endodonticamente e le radici sono state preparate per la cementazione di un perno in

fibra. Differenti materiali da cementazione sono stati usati in combinazione con differenti sistemi adesivi. I denti sono stati tagliati allo scopo di ottenere campioni con il perno al centro. Nello studio su perni in fibra translucenti, 70 perni sono stati impiegati per il test. Una matrice cilindrica di plastica è stata posta intorno al perno e riempita con diverse resine. Il silano è stato applicato sulla superficie del perno in metà dei campioni. Due tagli longitudinali sono stati eseguiti in corrispondenza dei due lati opposti del perno, alla sua periferia più esterna. Dalla fetta così ottenuta, sono stati successivamente sezionati campioni spessi 1 mm. Per tutti i campioni (perni, dentina coronale e radicolare) il test di adesione microtensile è stato eseguito con una macchina da carico Instron alla velocità di 0.5 mm/min. I campioni più rappresentativi di ogni gruppo sono stati selezionati per l'analisi SEM. Tutti i risultati sono stati analizzati con ANOVA a due vie e confronti multipli ($P<0.05$). Per le valutazioni delle caratteristiche istomorfologiche, su superfici piatte di dentina di 25 molari sono stati applicati diversi sistemi adesivi seguendo le istruzioni del produttore. Build-up di composito sono stati ottenuti con tecnica di stratificazione incrementale. I campioni sono stati sezionati parallelamente all'asse lungo. Una sezione di ogni campione è stata leggermente decalcificata e deproteinizzata allo scopo di valutare la formazione dello strato ibrido, mentre l'altra sezione di ogni campione è stata conservata in ClH al 30% e lavata con ipoclorito di sodio al 2% al fine di valutare la formazione degli zaffi di resina principali e laterali. Quindi sono state eseguite immagini SEM a diversi ingrandimenti.

Nello studio su dentina coronale, i sistemi adesivi self-etch a due passaggi e quelli total-etch a due passaggi a base di acido polialchenoico, hanno ottenuto valori di forza di adesione microtensile più alti di quelli degli altri sistemi adesivi. Gli adesivi total-etch a due passaggi a base di acetone e quelli total-etch a due passaggi a base di alcool si sono comportati in modo simile, mentre i sistemi all-in-one testati hanno dato i più bassi valori di forza di adesione microtensile. Dopo immersione in NaOCl, la forza di adesione microtensile è risultata inferiore in tutti i gruppi. I valori più alti sono stati ottenuti per i sistemi adesivi self-etch a due passaggi e per quelli total-etch

a due passaggi a base di alcool. Dopo carico meccanico, la forza di adesione è risultata più bassa in tutti i sistemi adesivi, fatta eccezione per quelli a due passaggi a base di alcool. I sistemi adesivi self-etch a due passaggi, quelli total-etch a due passaggi a base di acido polialchenoico e quelli total-etch a due passaggi a base di alcool hanno ottenuto valori di forza di adesione più alti rispetto ai sistemi adesivi total-etch a due passaggi a base di acetone. I campioni in cui l'adesione era stata eseguita con sistemi all-in-one hanno subito fratture premature e la forza di adesione microtensile non è stata misurata. Tutti i sistemi adesivi hanno presentato la formazione di strato ibrido. I sistemi adesivi total-etch hanno presentato strati ibridi più spessi di quelli riscontrati per i sistemi adesivi self-etch. Gli zaffi resinosi formati con gli adesivi total-etch erano molto più lunghi di quelli osservati nei campioni con adesivi self-etch. La formazione di zaffi laterali è stata osservata negli adesivi total-etch e in quelli self-etch a due passaggi. Nello studio sulla dentina radicolare il cemento applicato con il proprio sistema adesivo self-etch ha ottenuto il più alto valore di forza di adesione microtensile mentre il valore più basso è stato ottenuto con il composito foto-polimerizzato in combinazione con l'adesivo self-etch. Quando il silano è stato applicato sulla superficie del perno, un aumento della forza di adesione è stata osservata in tutti i gruppi.

In conclusione 1)le forze di adesione resina-dentina ottenute con sistemi adesivi self-etch "mild"a due passaggi e con sistemi adesivi total-etch a due passaggi a base di acido polialchenoico sono più alte di quelle degli altri sistemi adesivi testati; 2) i legami resina-dentina tendono a deteriorarsi dopo carico ciclico. Sulla dentina mordenzata, i sistemi adesivi a base di alcool presentano una forza di adesione più alta dopo carico meccanico. Adesivi all-in-one non instaurano legami duraturi con la dentina; 3) i legami resina-dentina tendono a deteriorarsi dopo immersione in $NaOCl_{aq,}$ L'entità della degradazione dei legami resina-dentina è materiale dipendente; 4) lo strato ibrido e gli zaffi resinosi ottenuti con i sistemi adesivi self-etch a due passaggi erano più uniformi rispetto agli altri adesivi. Adesivi all-in-one non instaurano legami duraturi con la dentina; 5) il cemento applicato alla dentina radicolare con il proprio sistema adesivo self-etch ha ottenuto i

risultati migliori. L'applicazione del silano è risultata migliorare in modo considerevole l'adesione di resine composite fluide al perno.

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