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RAMAN ANALYSIS OF DENTIN-ADHESIVE INTERFACE

(Settore Scientifico Disciplinare Med 28)

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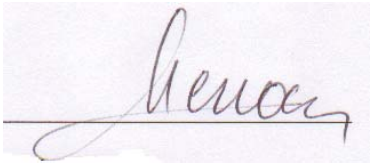

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TABLE OF CONTENTS

Chapter 1: Introduction 1

Chapter 2: Tooth anatomy 11

Chapter 3: Dental adhesion. 19

Chapter 4: Raman spectroscopy: a useful tool for polymerisation monitoring and chemical investigating. 35

Chapter 5: Degree of conversion of Filtek Silorane Adhesive System and Clearfil SE bond within the hybrid and adhesive layer: an *in-situ* Raman analysis. 51

Chapter 6: Degree of conversion influences interfacial nanoleakage expression of one-step self-etch adhesives.. 75

Chapter 7: The effect of curing mode on extent of polymerization and microhardness of dual-cured, self-adhesive resin cements.. 99

Chapter 8: Enamel and Dentin Bond Strength Following Gaseous Ozone Application**.** 115

Chapter 9: Summary, conclusions and future directions 137

Riassunto, conclusioni e direzioni future 145

Curriculum Vitae

The thesis was written based upon the publications and articles reported below:

Chapter 1: Introduction

Chapter 2: Tooth anatomy

Perdigão J, Breschi L - Chapter 11 Current Perspectives on Dental Adhesion, 1st edition, 2008, Montage Media Corporation, 1000 Wyckoff Avenue, Mahwah NJ 07430, USA; ISBN 0-9673009-4-0.

Chapter 3: Dental adhesion.

Chapter 4: Raman spectroscopy: a useful tool for polymerisation monitoring and chemical investigating.

L. A. Lyon, C. D. Keating, A. P. Fox, B. E. Baker, L. He, S. R.. Nicewarner, S. P. Mulvaney, M. J. Natan. Raman Spectroscopy. Anal. Chem. 1998, 70, 341R-361R

J. R. Ferraro K. Nakamoto. Introductory Raman Spectroscopy. 2nd edition, 1994, Academic Press, San Diego

.

Chapter 5: Degree of conversion of Filtek Silorane Adhesive System and Clearfil SE Bond within the hybrid and adhesive layer: an *in-situ* Raman analysis.

***C.O. Navarra**, M. Cadenaro, S.R.. Armstrong, J. Jessop, F. Antoniolli, V. Sergo, R.. Di Lenarda, L. Breschi. Degree of conversion of Filtek Silorane Adhesive system and Clearfil SE Bond within the hybrid and adhesive layer: an in-situ Raman analysis. (Submitted).*

Chapter 6: Degree of conversion influences interfacial nanoleakage expression one-step self-etch adhesives: a correlative micro- *Raman* and FEI-SEM analysis.

***C.O. Navarra**, M. Cadenaro, B. Codan, A. Mazzoni, V. Sergo, E. De Stefano Dorigo R.. L. Breschi. Degree of conversion influences interfacial nanoleakage expression one-step self-etch adhesives: a correlative micro-Raman and FEI-SEM analysis. (Submitted).*

Chapter 7: The effect of curing mode on extent of polymerization and microhardness of dual-cured, self-adhesive resin cements.

*M. Cadenaro, **C.O. Navarra,** F. Antoniolli, R.. Di Lenarda, F. Rueggeberg, L. Breschi. The effect of curing mode on extent of polymerization and microhardness of dual-cured, self-adhesive resin cements. (Submitted).*

Chapter 8: Enamel and dentin bond strength following gaseous ozone application.

*M. Cadenaro, C. Delise, F. Antoniolli, **C.O. Navarra,** R. Di Lenarda, L. Breschi. Enamel and dentin bond strength following gaseous ozone application. (In press)*

CHAPTER 1:

INTRODUCTION

The concept of adhesion on dental tissues was introduced in dentistry in the middle of the past century, when the swiss chemist Oskar Hagger developed the archetype of the adhesive monomers [1], a system based on glycerophosphoric acid dimethacrylate, used for the first time by McLean and Kramer for a dental filling: in 1952 they published the first paper on dentinal bonding agents (DBA) [2]. Because of poor adhesion of restorative materials to prepared tooth, early attempts to restore teeth emphasized surgical removal of sound tissue by preparing the cavity to provide mechanical retention through such features as dovetails, grooves, undercuts, sharp internal angles and so forth. But the real "adhesive revolution" began in the Fifty after Michael Buonocore introduced acid etch on dental hard tissues as preconditioning process of the enamel before restoration to increase the fillings retention on the tooth surface [3]; at that time the "Adhesive Dentistry age" started: traditional mechanical methods of preparing the teeth for filling based on the concept "extension for prevention" proposed by Black [4] were replaced by more conservative approaches. Though adhesive techniques were initially employed in operative dentistry to bond fillings to the tooth, in recent times they are used also in prosthetics, orthodontics, and pediatric dentistry.

Indication of Adhesive Dentistry

The development of materials able to create a tight bond with dental tissues and to resist to the oral environment brought a radical transformation of the dentistry techniques, especially in operative dentistry: now it is possible not only to fill the teeth without sacrificing sound tissue for the mechanical retention to the tooth, but also to correct esthetic flaws (of colour, shape, dimension, etc) without a prosthetic approach, saving sound tissue, time and money. The introduction of endodontic posts which are capable to reach a

good bond with the dentin in the canal permits to create the core build-up for crowns. In prosthetics bonding agents can be used to cement crowns or partial dentures; in orthodontics using adhesives to place brackets is easier and faster for dentist and patient than in the past. The use of adhesives material in pediatric dentistry leads to fill the baby-teeth very fast, a necessary requirement with children and the use of sealants is worldwide part of preventive programs against the childhood caries.

The concept of adhesion

The etymology of "adhesion" is derived from the latin word *adhaerere,* which is a compound verb of *ad* (to) and *haerere* (to stick, to attach).

The American Society for Testing and Materials, one of the most important society of coding and regulation of the commercial material in the world, defines adhesion between two material as "*the condition in which two surfaces are held together by interfacial forces which may consist of valence forces (physical and chemical) or interlocking action (mechanical), or both of them*" [6]. Two materials can be defined as adhesive materials if they are able to join, to resist separation and to transmit loads across the interface. The adhesive in adhesive terminology is the *adherent*, and the substrate which is locked is the *adherend*. The *bond strength* is the strength necessary to divide the two adherends. The *durability* is the time in which the bond is effective.

Five theories have been proposed to explain the mechanism of adhesion:

1. Mechanical Adhesion

Adhesive materials fill the voids or pores of the surfaces and hold surfaces together by micromechanical interlocking.

2. Chemical Adhesion

Two materials may form a compound at the interface. The strongest joins are where atoms of the two materials swap (ionic bonding) or share (covalent bonding) outer electrons. A weaker bond is formed if oxygen, nitrogen or fluorine atoms of the two materials share a hydrogen nucleus (hydrogen bonding).

3. Dispersive Adhesion or Adsorption

Two materials may be held together by Van der Waals forces. A Van der Waals force is the attraction between two molecules, each with has a region of a positive or negative charge. In the simplest case, such molecules are therefore polar with respect to average charge density, although in larger or more complex molecules, there may be multiple "poles" or regions of greater positive or negative charge. These positive and negative poles may be a permanent property of a molecule (Keesom forces) or a transient effect which can occur in any molecule, as the random movement of electrons within the molecules may result in a temporary concentration of electrons in one region (London forces).

4. Electrostatic Adhesion

Some conducting materials may pass electrons to form a difference in electrical charge at the join. This results in a structure creates an attractive electrostatic force between the materials.

5. Diffusive Adhesion

When the molecules of both materials are mobile and soluble in each other the materials may merge at the interface by diffusion. This would be

particularly effective with polymer chains where one end of the molecule diffuses into the other material. It is also the mechanism involved in sintering. When metal or ceramic powders are pressed together and heated, atoms diffuse from one particle to the next, joining the particles into one.

The strength of the adhesion between two materials depends on which of the above-said mechanisms occurs between the two materials, on the surface area over which the two materials get in contact and on the wetting of the adherends: materials that wet against each other tend to have a larger contact area than those that don't. The amount of wetting depends on the surface tensions of the materials. The theory of wetting claims that a solid with high surface tension is more wettable than one with low surface tension, and the energy of the adhesive should be smaller than the one of the solid to obtain a good adhesion; this means that the free energy of the bonding agent should be as big as possible to let it penetrate into the surface gaps. The angle between the adhesive and the surface is the contact angle (θ), and it describes the degree of wetting: if the contact angle is low, the wetting will be very favorable because the fluid cover a larger area of the surface; if the angle is high, the wetting will be unfavorable, because the fluid will remain compact on the surface (Fig. 1).

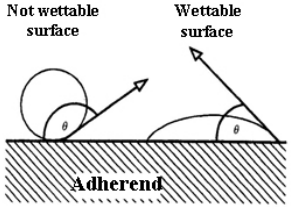

***Fig.1a**: Wetting theory in relationship with the contact angle (θ).*

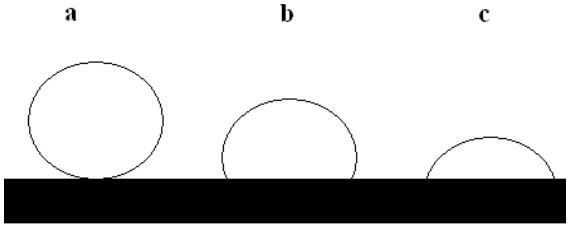

***Fig. 1b**: Fluids with crescent wetting (from a to c).*

The contact angle θ and the surface tension of the material are joined by the following equation:

$$\gamma_{SV} = \gamma_{SL} + \gamma_{LV} \cos\theta$$

where γ is the surface tension between the two materials and S, V, L are the solid, gaseous or liquid substance respectively in contact angle.

A contact angle of 90° or greater generally characterizes a surface as not-wettable, and one less than 90° as wettable. In the context of water, a wettable surface may also be defined hydrophilic and a non-wettable surface hydrophobic. So wetting is a key characteristic for the durability of the bond permitting a void-free interface: in fact if a load is applied on the adherend, the created strength lines are spreading without discontinuity through the adhesive layer; if there is a void into the adherent, the strength lines are spreading discontinuously deviating from it (Fig. 2). Therefore, the forces are increased near void area characterized by high curvature radius; in that area there is a force intensification. In these cases a concentration of stress is originated and it could decrease many of the physical properties of the adhesive bond [7].

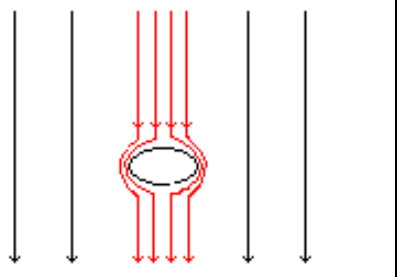

***Fig. 2**: Strengths distribution in presence of voids.*

Advantages of adhesion

The adhesive bonds respect mechanical bonds don't show high concentration of stresses, and are able to use fully the properties of the adherends; but the adhesive bond requires a much higher area than a mechanical bond to bear the same load. The bonding agents are in the majority polymeric materials that don't require application of force to be fastened: they permit also to assemble fragile materials (such as ceramic) that cannot be attached with mechanical bond. One of the biggest disadvantages of adhesives is that they rely on adhesion for the transfer of load through the assembly: as the adhesion is a surface phenomenon it is clear how the properties of the adhesive bond are directly correlated with the surface properties and the necessity of an adequate surface is a priority [7]. Compared to mechanical bond the adhesive bond has many advantages such as bigger resistance to fatigue, vibrations and corrosion and it is capable to seal and bond at the same time. Advantages and disadvantages are summarized in table 1.

Table 1. *Advantages and disadvantages of adhesive techniques.*

Advantages of adhesives	Disadvantages of adhesives
• Absence of stresses concentration due to piercing of the adherend • Resisting fatigue and vibration • Resisting corrosion • Sealing and insulating • Smooth contours	• Bond strength related on the surface condition • Lack of non destructive quality control methods

The dental bonding agent are synthetic, thermoset, with multiple components, acrylic adhesives.

Objectives

The aim of the studies was to investigate if change in composition or in application technique can improve the properties of dentinal adhesives.

The second aim was to characterize the structure created by the interaction of commercial adhesives and dentin in order to study the properties of commercial simplified adhesives.

The technique used was the Raman micro-spectroscopy, which is a useful tool investigating the properties of polymer.

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CHAPTER 2:

TOOTH ANATOMY

Teeth are composed by tissues with different chemical compositions and a different mineralization: enamel, dentin, cement and pulp. Adhesion is possible on enamel and dentin: because their composition is different, the strategies for adhesion are distinct.

The Enamel

The enamel is the shell of the tooth covering the crown; it is translucent and tends to wear over time. It is the hardest and most mineralized tissue of human body: is composed of 96% by weight of inorganic compounds (for the most part Ca, P, CO_2, Na, Mg, Cl e K and trace of F, Zn, Fe, Cu, etc) in form of hydroxyhapatite crystals (Fig. 1, Fig. 2, Fig. 3), being the remainder of the tissue formed by an organic matrix consisting of proteins (ie, amelogenins and enamelins) and trace amounts of water. The proteins are organized in branched fibrils, the complex structures of proteoglycans.

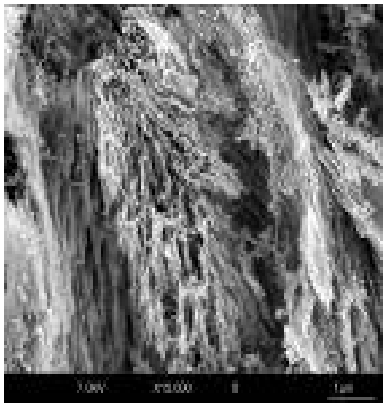

***Fig. 1**. Hydroxyapatite crystals of enamel. The picture shows human enamel after etching with 35% orthophosforic acid (FEISEM image, 15000x)*

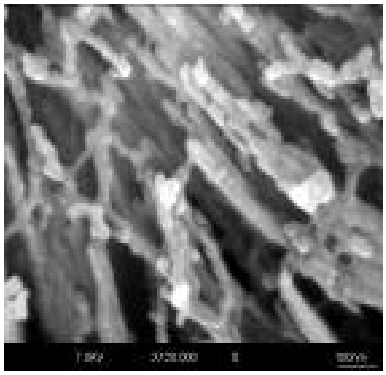

***Fig. 2**. Higher magnification (120000x) of the same region: the nanocrystals are evident.*

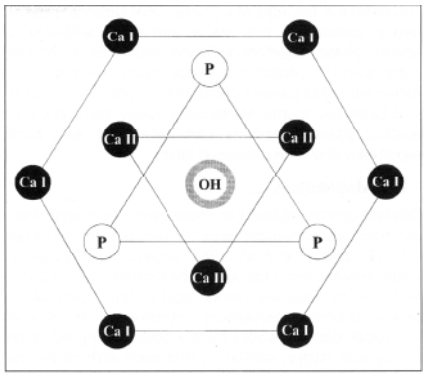

***Fig. 3**. Crystal structure of hydroxyapatite: the overall planar hexagonal nature of the arrangement of calcium and phosphate ions around the central c-axis hydroxyl column can be seen [18]*

The carbonated-apatite crystals are long (possibly up to 1 mm), 50 nm wide by 25 nm thick, extending from the dentin toward the enamel surface [1]. It is thought that they may actually extend unbroken from dentin to the enamel surface. They are arranged in bundles of approximately 1000 crystals, the so-called enamel prisms. The cross-sectional profile of the prisms varies from circular to keyhole-shaped (Fig. 4). The hydroxyapatite crystals are primarily arranged with their long (c-) axes parallel to the long axes of the prisms (Fig. 5). At the periphery of each prism, however, the crystals deviate somewhat from this orientation, producing an interface

between prisms where there tends to be more intercrystalline space, forming the interrod enamel [2].

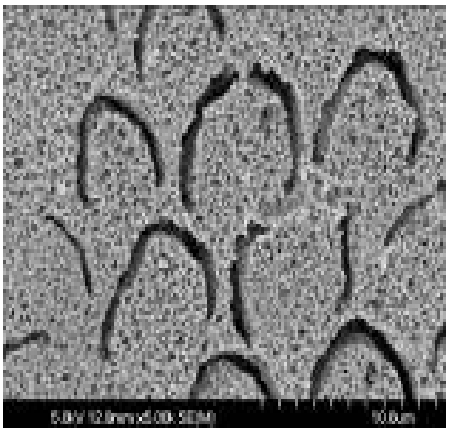

***Fig. 4**. Enamel prisms with the typical key-hole shape.*

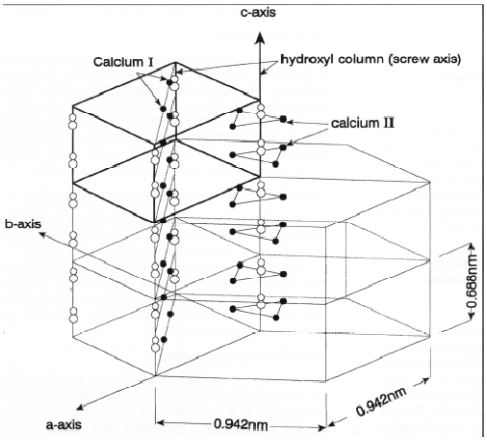

***Fig. 5**. The rhomboidal crystallographic unit cell (shown in heavier lines) is here represented [18].*

The Dentin

Ectodermic and mesenchymal components interact to form this tissue inducing the odontoblasts, the denting-forming cells, to start the dentinogenesis. During the dentin formation, the odontoblasts secret a matrix around their extensions forming the dentinal tubules that have different density, diameter and orientation depending on the site of the tooth [4] (Fig. 6, Fig. 7). The tubules diameter is 2.5 µm near the pulp chamber and 0.63 µm near the dentinal-enamel junction [5]. Similarly the number of the tubules is 45,000/mm^2 near the pulp and 20,000/mm^2 near the enamel [6]. The tubular net is liable for the intrinsic moisturizing of the tissue making the dentin a semi-permeable tissue.

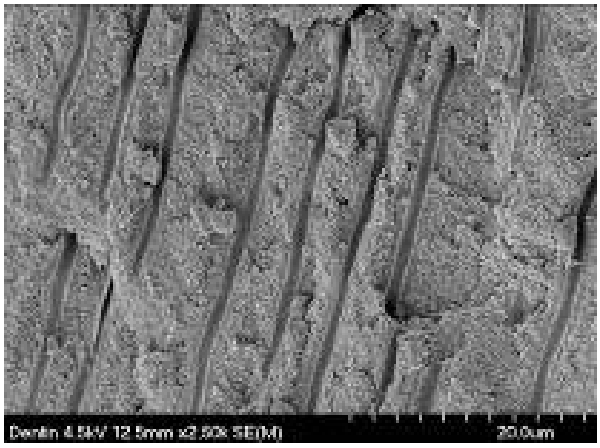

Fig. 6. *Transversal section of dentinal tubules (SEM image 2,500x)*

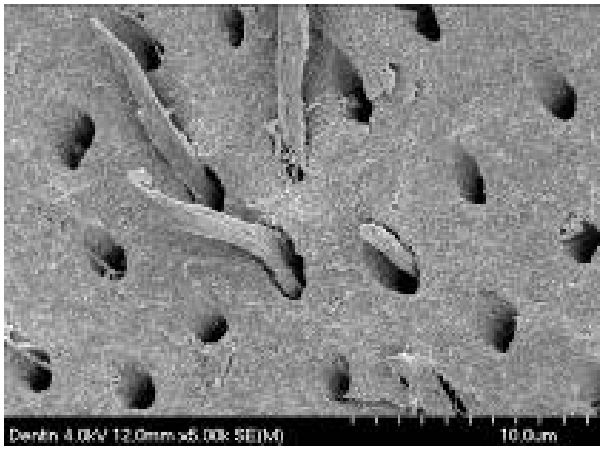

Fig. 7. *Dentinal tubules and odontoblastic extensions (SEM 5,000x)*

A high mineralized matrix produced by the odontoblasts is surrounding the tubules forming the peritubular dentin. Across the space between each tubule there is the intertubular dentin. The odontoblastic extensions are immersed in a fluid with the pressure of 25-30 mm Hg [7], and because they are in communication with the pulp they transmit the sensitive stimulus

changing the intratubular pressure [8]: the biggest difference between enamel and dentin is that dentin is a live and dynamic tissue [9,10].

The micro-structure of dentin is composed by an organic and an inorganic matrix: hydroxyhapatite crystals and salts such as carbonates, sulphates, phosphates, etc., and traces of F, Cu, Zn, Fe, etc., form the inorganic part. The organic part is made in the majority by collagen. Collagen fibers are organized in bands with a 40nm periodicity.

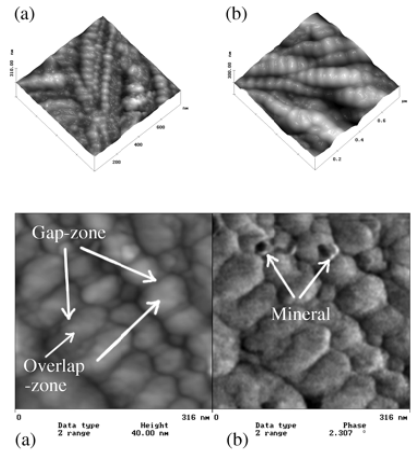

Fig. 8*. AFM images of hydrated collagen fibers. Note the regular ‘‘banded’’ structure as indicated in the higher magnification micrograph [11].*

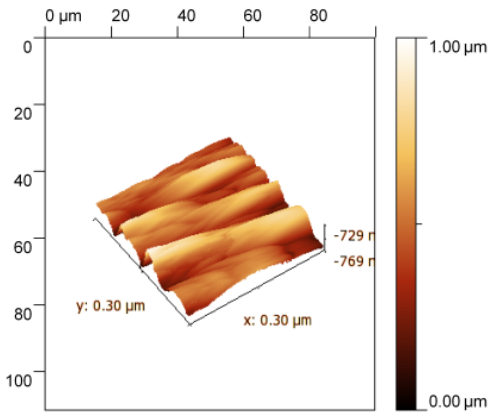

Fig. 9 *AFM image of hydrated collagen fibers obtained in our laboratories. The periodicity of 40nm of fibers is well visible.*

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CHAPTER 3:

DENTAL ADHESION

3.1 Mechanism of Adhesion

The sealing properties of dental adhesives are based on the ability to adhere to dental hard tissues, enamel and dentin, and simultaneously to bind the lining composite with a co-polymerization of residual double bonds C=C in the oxygen inhibition layer. The basic mechanism of bonding to enamel and dentin is essentially an exchange process involving replacement of minerals removed from the hard dental tissue by resin monomers, which, upon setting, become micro-mechanically interlocked in the created porosities[1,2,3]; diffusion and capillarity are primary involved to obtain micro-mechanical retention. Microscopically, this process is called 'hybridization' [4].

Bonding to dental tissues has been one of the most challenging issues since the introduction of the acid-etch technique nearly 50 years ago [5]. Acid etching transforms the smooth enamel into an irregular surface (Fig. 1, Fig. 2).

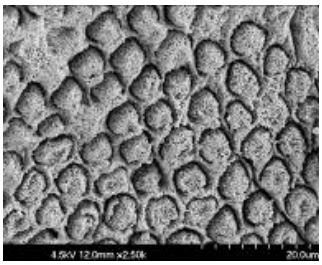

Fig. 1: Effect of the acidic etch with 35% phosphoric acid on the surface of enamel tissue (FEISEM image at 15,000): the enamel crystal distribution reveals the typical prism structure.[6]

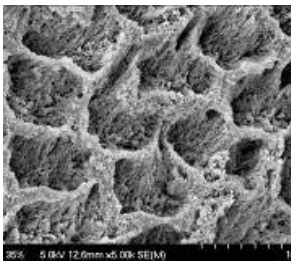

Fig. 2: Higher magnification of the same specimen showing empty areas between the nano-crystals available for bonding infiltration (120,000X). [6]

A mixture of resin monomers or adhesive is applied on the enamel surface. The resin is drawn into the micro porosities of the enamel surface by capillary action. The monomers in the fluid resin polymerize and become

mechanically interlocked with the enamel structure. The formation of resin microtags (Fig.Fig. **3** and 4) within the enamel structure has been considered the essential mechanism of adhesion of resin to enamel [7, 8]. When tooth structure is prepared with a bur or other instrument, residual organic and inorganic components form a “smear layer” of debris on the surface [9]. The smear layer obstructs the entrance of dentin tubules (Fig. 5), decreasing dentin permeability by up to 86% [10]. Sub-micron porosity of the smear layer still allows flow of dentinal fluid [11]. In dentin this process involves the exposed collagen fibres with the creation of a chemical bond between dental tissue and adhesive.Although the layer acts as a physical obstacle that decreases the permeability of dentin, it can also be considered a barrier that must be removed or made permeable, so that monomers can contact and interact with the dentin surface.

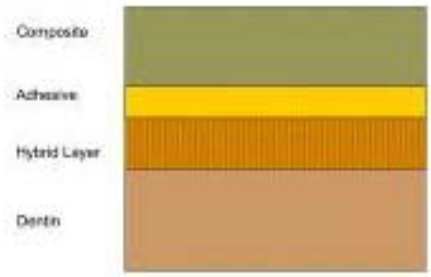

Fig. 3: Most adhesive joints involve only two interfaces, a bonded composite restoration is an example of a more complex adhesive joint.[6]

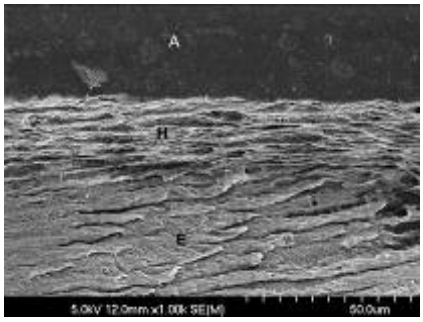

Fig. 4: SEM micrograph of enamel-resin interface, 1,000X. *(E = Enamel; H = Resin microtags and hybrid layer; A = Adhesive).*[6]

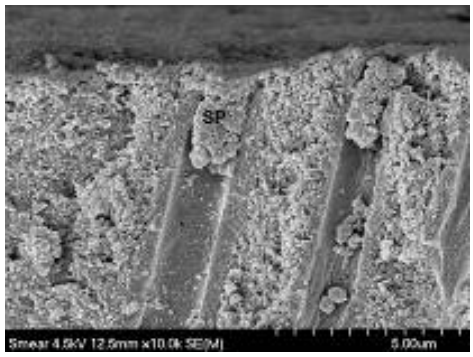

Fig. 5: SEM micrograph of smear layer and smear plug (SP) at 10,000X. [6]

Manufacturers of dentin/enamel adhesives use one of two major strategies for overcoming the barrier (Fig. 6, Fig. 7):

- **Self-etch**: Adhesives containing monomers that simultaneously condition and prime dentin and enamel surface with a non-rinsing solution of acidic monomers in water. These bonding systems do not remove the smear layer, but make it permeable to the monomers subsequently applied. Regarding user-friendliness and technique-sensitivity, this approach seems clinically most promising. This approach eliminates the rinsing phase, which not only lessens the clinical application time, but also significantly reduces the technique-sensitivity or the risk of mistakes during application. There are basically two types of 'self-etch' adhesives: 'mild' and 'strong' [1] 'Strong' self-etch adhesives have a very low pH (< 1) and exhibit a bonding mechanism and interfacial ultra-morphology in dentin resembling that produced by etch-and-rinse adhesives. 'Mild' self-etch adhesives (pH of around 2) dissolve the dentin surface only partially, so that a substantial number of hydroxyapatite crystals remain within the hybrid layer. Specific carboxyl or phosphate groups of functional monomers can then chemically interact with this residual hydroxyapatite [12]. This two-fold bonding mechanism (*i.e.*, micro-mechanical and chemical bonding) is believed to be

advantageous in terms of restoration durability. It has a micro-mechanical bonding component that may in particular provide resistance to abrupt debonding stress. The chemical interaction may result in bonds that better resist hydrolytic break-down and thus keep the restoration margins sealed for a longer period.

- Total-etch: 'Etch-and-rinse' adhesives involve a separate etch-and-rinse phase. An acid gel (mostly 30-40% phosphoric acid) is applied to treat dentin and enamel for 15 to 30 seconds and rinsed off. The acid dissolves the smear layer and the top 1 µm to 6 µm of hydroxyapatite (Fig. 1, Fig. 2). This conditioning step is followed by a priming step and application of the adhesive resin, resulting in a three-step application procedure. Simplified two-step etch-and-rinse adhesives combine the primer and adhesive resin into one application. Total-etch has led to significant improvements in the *in vitro* bond strengths of resins to dentin.

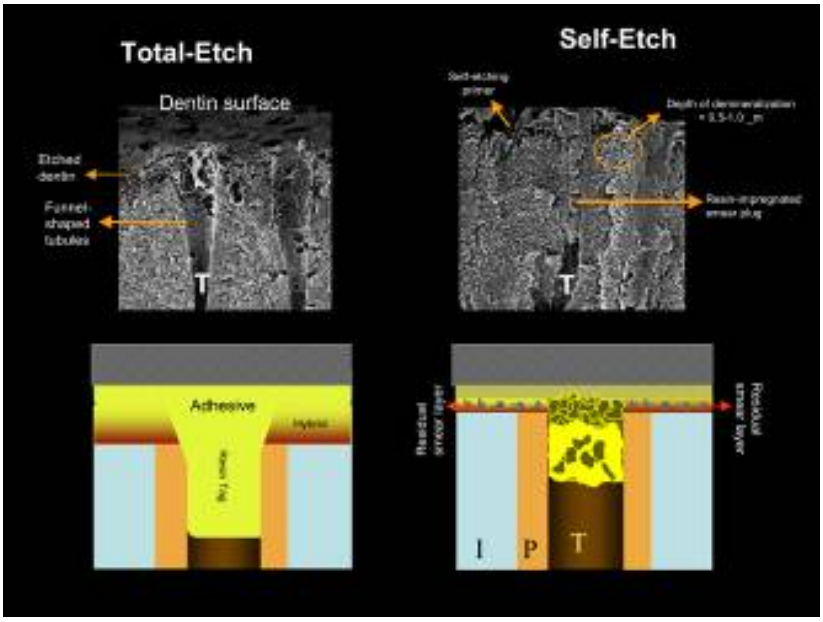

Fig. 6: Diagram showing the current adhesion strategies—self-etch and total-etch. [6]

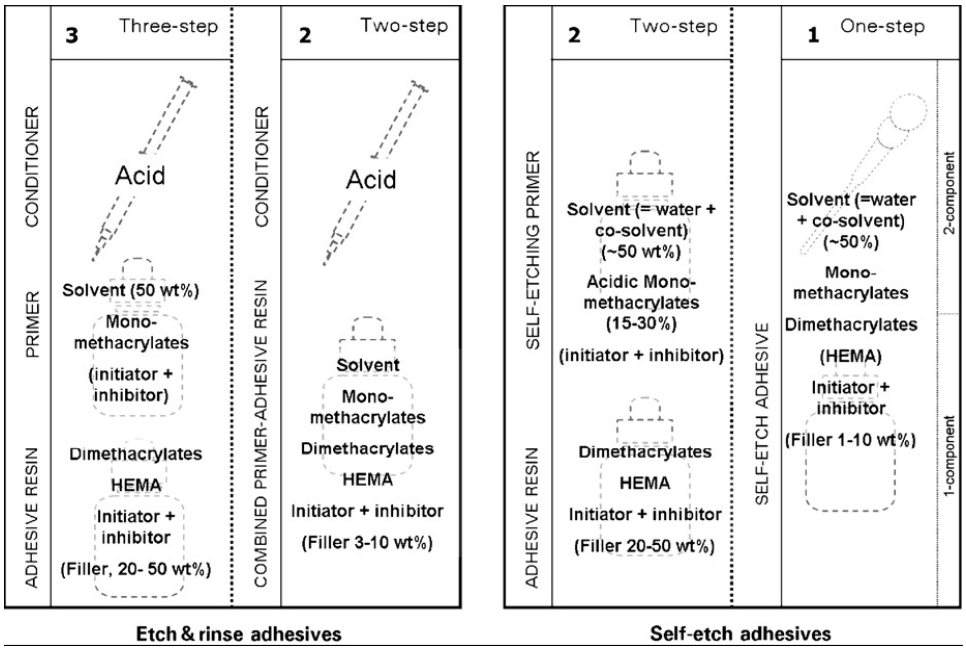

Fig. 7: Classification of contemporary adhesives indicating the main components [3]. Most adhesives contain methacrylate-based monomers. Two-step etch&rinse adhesives are often referred to as 'one-bottle'systems. As such, one-step self-etch adhesives are often subdivided in one- and two-component systems.

3.2 Chemical composition

The chemical composition of adhesives is aimed at interacting with both dentin and enamel. Traditionally, adhesives contain acrylic resin monomers, initiators, inhibitors, solvents and filler particles: every component has a specific function.

3.2.1 Resin components

Dental adhesives contain resin monomers that are similar to those in composite restorative materials to promote a good covalent bond between the adhesive and the restorative material. The matrix provides structural continuity and thus physical-mechanical properties such as strength. Monomers are the key constituents of adhesives. Basically, two kinds of

monomers can be distinguished: cross-linkers and functional monomers: the firsts have only one polymerizable group, cross-linkers have two polymerizable groups (vinyl-groups or –C=C–) or more. Most functional monomers exhibit a chemical group, which will impart monomer-specific functions, the so-called functional group. Functional monomers will form linear polymers upon curing, in contrast to cross-linkers that form cross linked polymers. Compared to linear polymers, the latter have proven to exhibit better mechanical strength, and cross-linking monomers are therefore important to reinforce the adhesive resin [13,14].

The structure of monomers can be divided in three distinct parts: one or more polymerizable groups grafted onto a spacer (Fig. 8) and a functional group (Fig. 9).

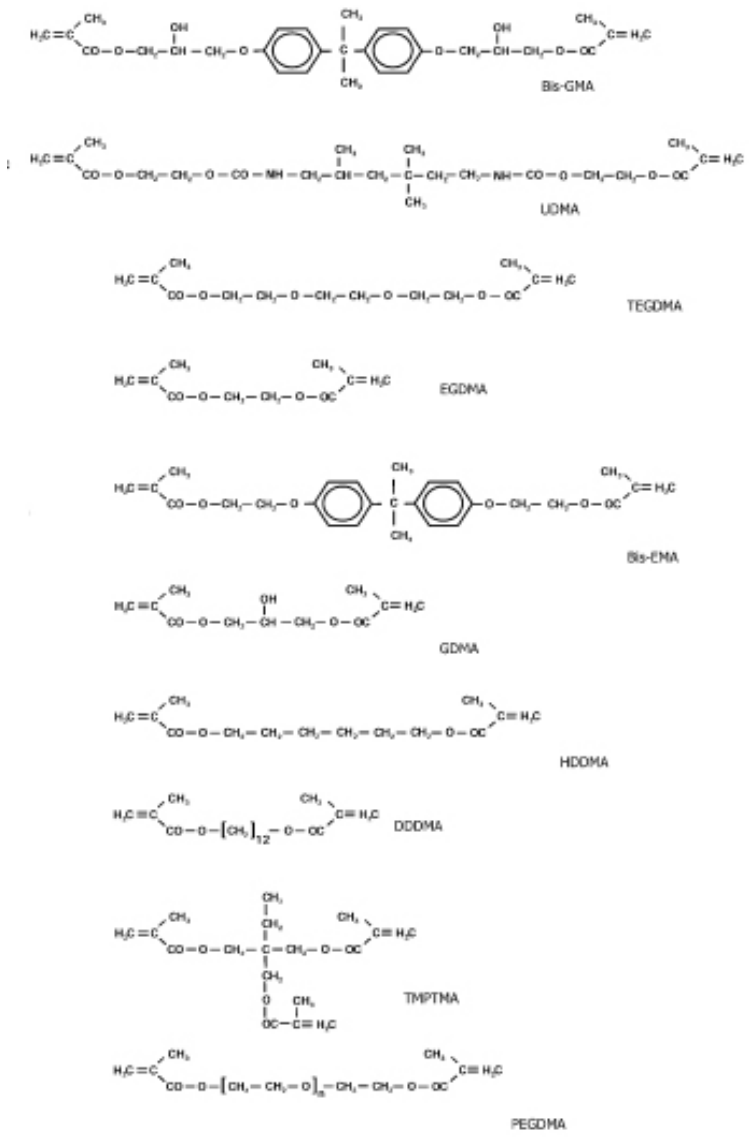

Fig. 8: Principals cross-linking monomers used in dental bonding agents. [15]

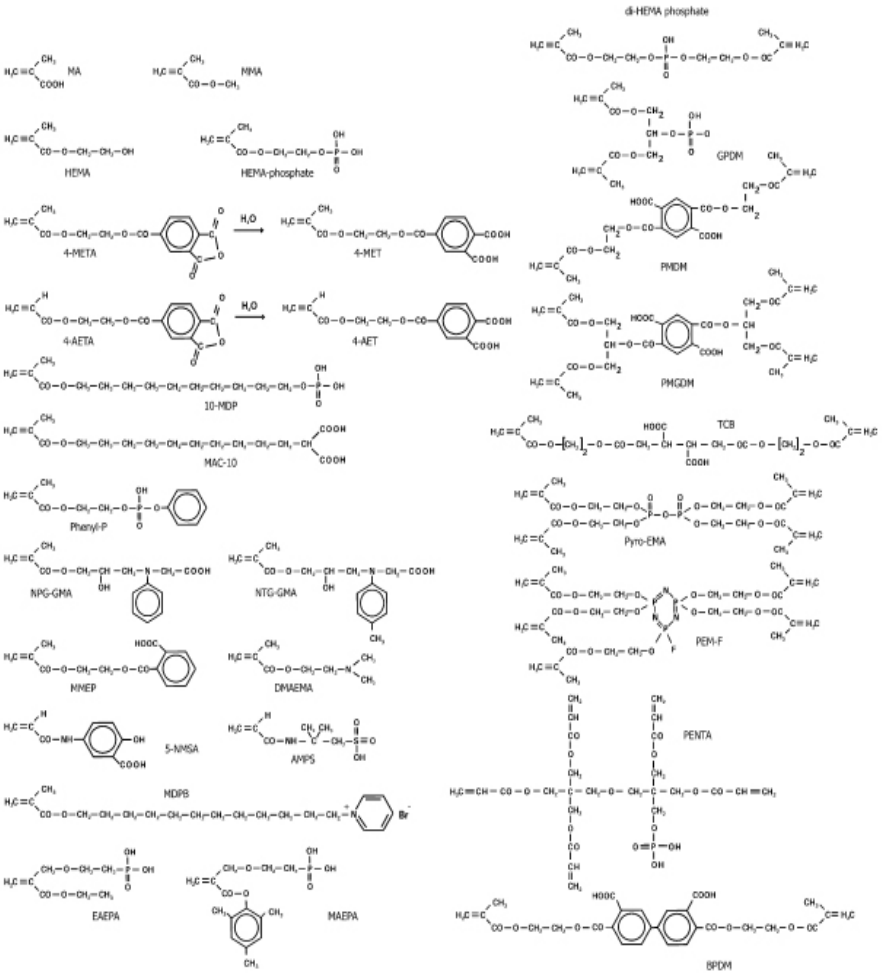

Fig. 9: Principals functional monomers used in dental bonding agents.[15]

3.2.2 Initiator system

The monomers in dental resins polymerize thanks to a radical polymerization reaction. In order to set off this reaction, small amounts of initiator are required, which will be consumed during the polymerization reaction [16]. Initiators are generally molecules that possess atomic bonds with low dissociation energy that will form radicals under certain conditions

[17]. Those radicals will set off the radical polymerization reaction. Radicals can be produced by a variety of thermal, photochemical and red ox methods [16].

- ***Photoinitiators***

Photo initiating systems based on camphorquinone (CQ) are the widely and most successfully used visible-light photoinitiators in dental restorative resins [18, 19]. Although CQ is able to start the photo polymerization process by itself, the process will occur at a low rate. To speed up this reaction, co-initiators or accelerators are needed. In general, tertiary amines are highly effective hydrogen donors. Despite their good clinical acceptance, photo initiating systems based on camphorquinone and amines present some drawbacks. Although used in very small amounts (ppm), the yellow-coloured camphorquinone significantly influences the composite colour. Moreover the low pH of self-etching enamel-dentin adhesives reduce the concentration of amine able to initiate the polymerization, resulting in insufficient monomer conversion [20]. To overcome these problems macromolecular photoinitiators like aromatic tert-amine with a bisphenol-A skeleton have been recommended [21]. CQ can be replaced by polymeric radical photoinitiators bearing the photosensitive CQ groups in the side chain [22]. Another promising way to overcome the use of amines as accelerators is found in Norrish Type I photoinitiator systems. They are based on photochemical cleavage of aldehydes and ketones into two free radical intermediates, which initiate polymerization without the need for an additional amine (i.e. acyl phosphonates, acylphosphine oxides, bisacylphosphine oxides, and titanocenes) [23]. However, only acylphosphine oxides, such as Lucirin TPO (2,4,6-trimethylbenzoyldiphenylphosphine oxide), are used in commercially available products. The effective wavelength range for activating camphorquinone has been reported to be between 400 and 500 nm, with a

maximum excitation wavelength at *ca*. 470 nm. Consequently, this initiator is perfectly adapted to the emission spectra of all curing unit types.

- ***Chemical initiators***

The use of chemical initiators is usually restricted to cements and resin that cannot rely (solely) on light curing for polymerization. Adhesives that are chemically curing standardly need the admixture of the initiator with the co-initiator, after which the setting reaction will start. Inherently, they consist of two separate bottles, the content of which need to be mixed before application onto the tooth surface. The most common initiator in self-curing resins would be benzoylperoxide (BPO) in conjunction with a tertiary amine [24]. BPO will react with the tertiary amine as a co-initiator, yielding radicals. It is a colourless, crystalline solid, that is very limitedly soluble in water, but soluble in ethanol and acetone.

The same neutralization in acidic solutions that arise from the use of amines with photoinitiators, occurs also in self curing systems with amines.

3.2.3 Inhibitors

Inhibitors added to dental resins are actually antioxidants that are able to scavenge free radicals originating from prematurely reacted initiators. The effect of inhibitors on the actual polymerization is negligible since only minute amounts are used. The most frequently used inhibitors in adhesives are butylated hydroxytoluene, also butylhydroxytoluene (BHT) and monomethyl ether hydroquinone (MEHQ) (Fig. 10). Whereas BHT is most often used in composites and hydrophobic adhesive resins, MEHQ is preferred for more hydrophilic resins.

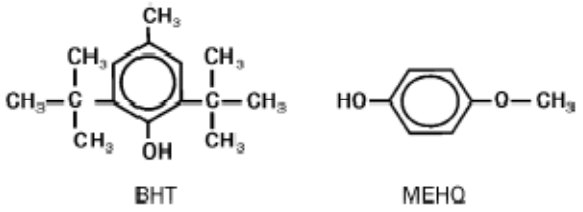

Fig. 10: Chemical structure of the two most frequently used inhibitors in adhesive systems (BHT: butylated hydroxytoluene; MEHQ: monomethyl ether hydroquinone). [15]

3.2.4 Solvents

Hydrophilic resin monomers are often dissolved in volatile solvents, such as acetone and ethanol. The inclusion of these volatile solvents aids in the displacement of water from the dentine surface, facilitating penetration of the resin monomers into the micro porosities of the exposed, acid-demineralised collagen network [25]. The inclusion of solvent in dentine bonding has led to an increase in bond strength [26]. The presence of water is also essential for adhesives. Water has a plasticizing effect on the collagen fibrils and decreases the stiffness of the collapsed fibrils, which is important for the expansion of the dried dentine collagen. In self-etch adhesives, it provides an ionisation medium to facilitate self-etching activity to occur.

3.2.5 Filler

Dental adhesives can be ‘filled’or‘unfilled’ adhesives. The commonly used systems for bonding direct restorations to tooth tissue do not contain filler particles. Fillers can be added to adhesives to fortify the adhesive layer or to modify their viscosity. Fillers can also provide in fluoride release and radio-opacity.

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CHAPTER 4:

RAMAN SPECTROSCOPY:

A USEFUL TOOL FOR POLYMERIZATION MONITORING AND CHEMICAL INVESTIGATING.

Raman spectroscopy is a spectroscopic technique used to study vibrational, rotational and other low frequencies modes in a chemical system. The ever-increasing range of science brances to which Raman spectroscopy is applied has generated a broad field of applications also in biological research.

Historical Background of Raman Spectroscopy

In 1928, when Sir Chandrasekhra Venkata Raman discovered the phenomenon that bears his name, only crude instrumentation was available. Sir Raman used sunlight as the source and a telescope as the collector; the detector was his eyes. That such a feeble phenomenon as the Raman scattering was detected was indeed remarkable. Gradually, improvements in the various components of Raman instrumentation took place. Early research was concentrated on the development of better excitation sources. Various lamps of elements were developed (e.g.,helium, bismuth, lead, zinc) [1,2,3]. These proved to be unsatisfactory because of low light intensities. Mercury sources were also developed. Currently monochromatic lasers are used as light sources. Developments in the optical train of Raman instrumentation took place in the early 1960s. Holographic gratings appeared in 1968, which added to the efficiency of the collection of Raman scattering in commercial Raman instruments. These developments in Raman instrumentation brought commercial Raman instruments to the present state of art. Moreover, Raman spectra can also be obtained by Fourier transform (FT) spectroscopy. FT-Raman instruments are being sold by all Fourier transform infrared (FT-IR) instrument makers, either as interfaced units to the FT-IR spectrometer or as dedicated FT-Raman instruments.

Lasers are ideal excitation sources for Raman spectroscopy due to the following characteristics of the laser beam:

(1) Single lines from large continuous wave lasers can easily provide 1-2 W of power, and pulsed lasers produce huge peak powers on the order of 10-100 MW.

(2) Laser beams are highly monochromatic (band width ~ 0.1 cm^{-1} for Ar^{+} laser), and extraneous lines are much weaker. The extraneous lines can be eliminated easily by using notch filters or pre-monochromators.

(3) Most laser beams have small diameters (1-2 mm), which can be reduced to 0.1mm by using simple lens systems. Thus all the radiant flux can be focused on a small sample, enabling fruitful studies of microliquids (~ 1 μL) and crystals (~ 1 mm^3). In the case of Raman microscopy, sample areas as small as ~ 2 μm in diameter can be studied.

(4) Laser beams are almost completely linearly polarized and are ideal for measurements of depolarization ratios.

(5) It is possible to produce laser beams in a wide wavelength range by using dye lasers and other devices.

Energy Units and Basic Theory

To understand how the Raman effect is generated, a brief illustration of basic theory and energy units is needed. Fig. 1 illustrates a wave of polarized electromagnetic radiation travelling in the z-direction. It consists of the electric component (x-direction) and magnetic component (y-direction), which are perpendicular to each other.

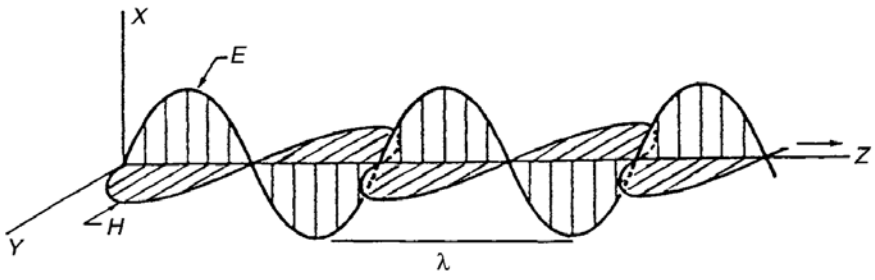


***Fig. 1**. Plane-polarized electromagnetic radiation.*

The distance between two points of the same phase in subsequent waves is called the "wavelength", λ, which can be measured in units such as Å (angstrom), nm (nanometer), mμ (millimicron), or cm (centimeter). The relationships between these units are:

$$1\ Å=10^{-8}cm=10^{-1}nm=10^{-1}m\mu$$

The frequency, ν, is inversely proportional to wavelength λ. It is equal to the light velocity dived by λ. Thus,

$$\nu = c/\lambda$$

where *c* is the velocity of light (3×10^{10}cm/s). If λ is in the unit of centimetres, frequency's dimension is (cm/s)/(cm) = 1/s. This "reciprocal second" unit is also called the "hertz" (Hz).

The third parameter, which is most common to vibrational spectroscopy, is the "wavenumber," ν, defined by

$$\nu =\nu/c$$

The difference between ν and ν is obvious. It has the dimension of (l/s)/(cm/s) = 1/cm. By combining the two formulas we have:

$$\nu =\nu/c=1/\lambda$$

and

$$\nu = c/\lambda = c\,\nu$$

As shown earlier, the wavenumber (ν) and frequency (ν) are different parameters, yet these two terms are often used interchangeably.

Raman spectra are intimately related to electronic transitions [Fig. 2]: in Raman spectroscopy, the sample is irradiated by a laser beams in the UV-visible region to induce the Raman effect, and the scattered light is usually observed in the direction perpendicular to the incident beam. The scattered light consists of two types: one, called *Rayleigh scattering* [Fig. 3] is strong and has the same frequency as the incident beam (ν), and the other, called *Raman scattering,* is very weak (~ $10^{-5}$of the incident beam). The light hits the molecule. The incident photon excites one of the electrons into a virtual state, and depending on the state of the electron Stokes Raman scattering [Fig. 3] or anti- Stokes Raman scattering can be generated. In the spontaneous Raman effect, the molecule will be excited from the ground state to a virtual energy state, and relax into a vibrational excited state, which generates Stokes Raman scattering. If the molecule was already in an elevated vibrational energy state, the Raman scattering is then called anti-Stokes Raman scattering.

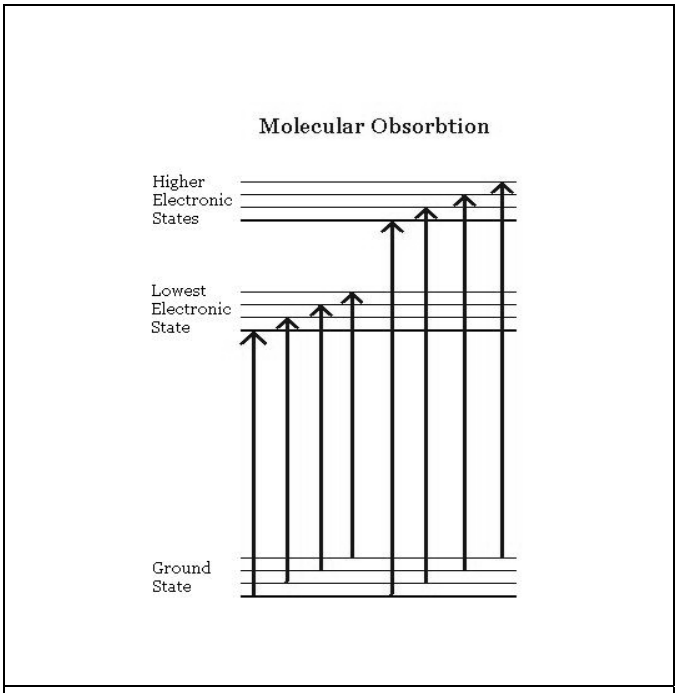


***Fig. 2**.Electronic transition after excitation.*

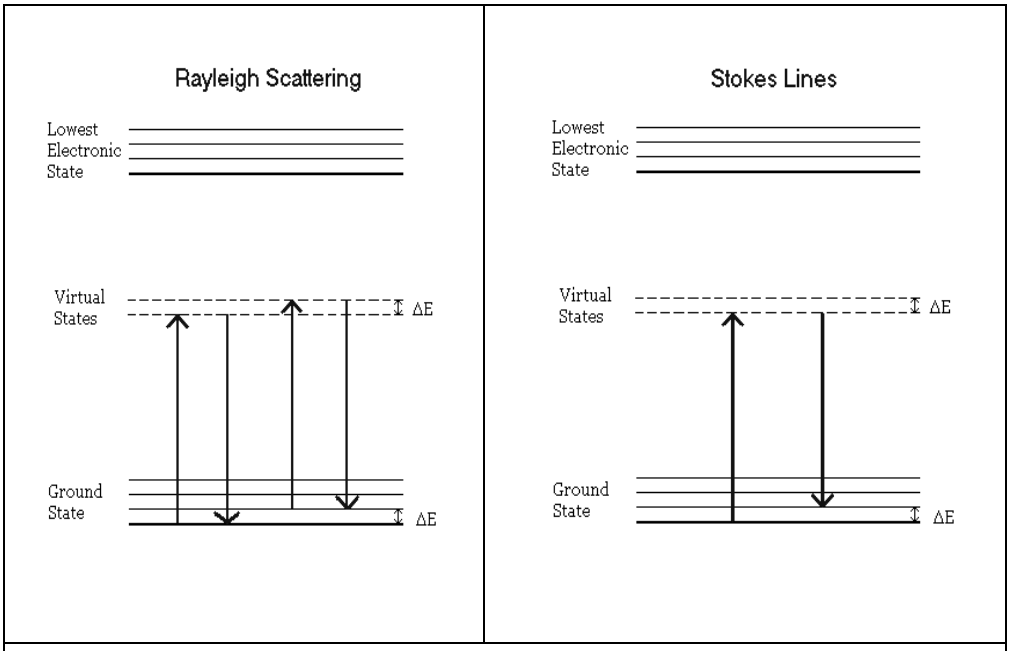


***Fig. 3.** Rayleigh scattering and Stoke effect.*

In contrast to IR spectra, Raman spectra can be measured also in the UV-visible region where the excitation as well as Raman lines appear.

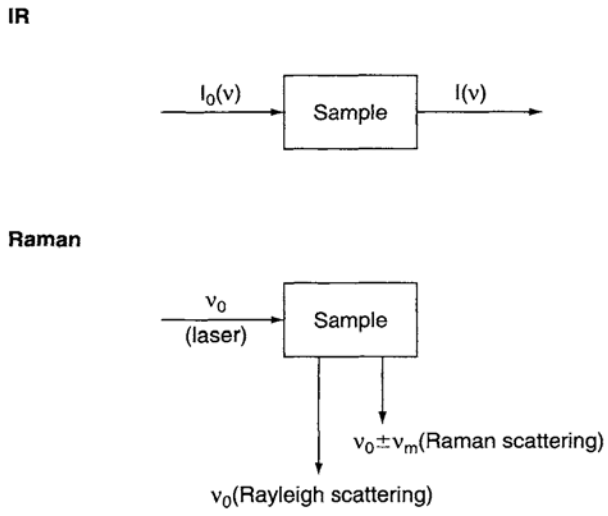


Fig. 4: *Differences in mechanism of Raman vs. IR.. I_0 and I denotes the intensity of the incident and transmitted beams, respectively; ν_0 is the frequency of the laser source and ; ν_m is the vibration frequency of the molecule.*

General Instrumentation

Four major components make up the commercially available Raman spectrometer. These consist in the following:

(1) Excitation source, which is generally a continuous-wave (CW) laser

(2) Sample illumination and collection system

(3) Wavelength selector

(4) Detection and computer control/processing systems

A schematic view of a typical arrangement of these components is shown in Fig. 5. Nowadays, a wide variety of systems with this general format has been marketed. The major categories for different instruments are defined by the wavelength region for the excitation sources, the type of wavelength,

selector, and the detection system. Most of the instrumentation will have similar sample illumination and collection systems.

The techniques used for these studies will be here presented.

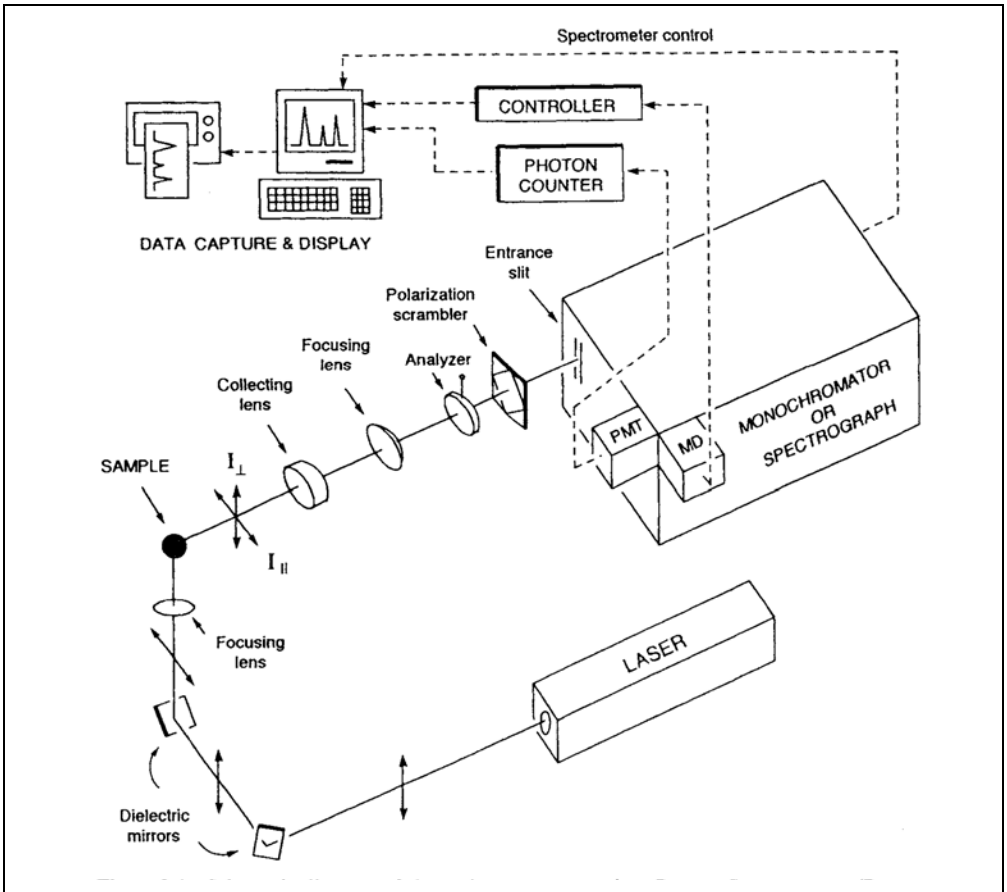


***Fig. 5**. Schematic diagram of the major components in a Raman Spectrometer.*

Raman Microscopy

Raman microscopy was developed in the 1970s. The technique provides the capability of obtaining analytical-quality Raman spectra with 1μm spatial resolution using samples in the picogram range. The major limitations in the design of a Raman microprobe are related to the feeble Raman effect and the minute sample size. It is necessary to optimize the Raman signal, and a high

numerical aperture (NA) objective is necessary to collect the light scattered over a large solid angle to assure that more light from the sample is detected. A large-aperture collector is used, which minimizes elastic and inelastic scattering from the substrate. The substrate must give a weak fluorescence signal in the region of interest. The Raman microprobe has provided applications in a widerange of scientific fields.

Raman mapping

Two modes of illumination are available for Raman illumination. One is "point illumination", and the other is "area (or global) illumination". In the first case, the laser beam is focused on one chosen point under the microscope image, and the Raman spectrum of this particular point is measured. For the area illumination, a wide area is measured with a two-dimensional CCD camera. By deconvoluting one particular vibration from the whole spectrum, it is possible to obtain a microscopic image of the distribution of the component associated with this vibration. This Raman mapping technique is utilized extensively to study the distribution of various components in biological samples and on the surfaces of solids such as the tooth surfaces.

Confocal microspectroscopy

The use of a confocal microscope greatly improves Raman microspectroscopy. Confocal microscope has a pinhole (25 to 100 um in diameter) which rejects out-of-focus signals. The smaller the pinhole size, the better the rejection. As a result, the background (substrate) signals can be reduced significantly. Furthermore, it is possible to measure Raman spectra of the sample at different depth by moving the focal plane in the axial direction (Fig. 6).

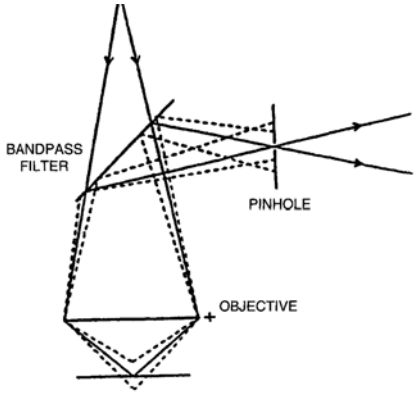


***Fig. 6**. Suppression of out-of-focus signal contributions by means of confocal signal detection.*

Biochemical and Medical Applications

Raman spectroscopy continues to be widely used as a structural and functional probe in biochemical systems and also as a quantitative technique for biomarkers. Motivation for its use is driven by difficulties associated with acquisition of infrared vibrational data for water-soluble species, by enhanced availability of ultraviolet and near-infrared excitation sources (where fluorescence interference is minimized), and by the increased realization that unique, fingerprint-like spectra can be obtained for species as small as low-molecular-weight metabolites and as large as living organisms. Raman spectroscopy is ideal for studies of biochemical and medical systems mainly for two reasons:

(1) Since water is a weak Raman scatterer, it does not interfere with Raman spectra of solutes in aqueous solution.

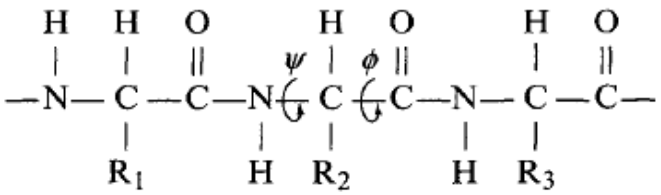
(2) By taking advantage of resonance Raman scattering, it is possible to selectively enhance particular chromophoric vibrations using a small quantity of biological samples. As a result, biochemical and medical applications of Raman spectroscopy have increased explosively in recent years.

Now it will concern with applications of Raman spectroscopy to study proteins such as collagen and polymers, such as the dental resins. In contrast to biochemical samples, medical samples contain a number of components such as proteins, nucleic acids, carbohydrates and lipids, etc. Thus, Raman spectra of medical samples are much more complex and must be interpreted with caution.

Peptides and Proteins

Several different approaches have been used to probe protein secondary, tertiary, and quaternary structure. A common feature in these experiments is the presence of conformationally and/or environmentally sensitive Raman bands. For example, amides exhibit five conformationally sensitive vibrational bands.

The peptide (—CO—NH—) groups in proteins are nearly planar because of resonance stabilization involving the C=O and C—N bonds.



However, the torsional angles (ψ and φ) between two —CO—NH— groups can vary depending upon the amino acid residues involved. This aspect, together with hydrogen bonding between the CO of one peptide and the NH of the other, produces several secondary structures of proteins such as α-helix, β-sheet and random coil.

Raman spectroscopy provides structure-sensitive bands for differentiating these secondary structures. The table below lists observed frequencies and band assignments of structure-sensitive amide vibrations. The general trends

shown in the table were found by correlating x-ray structural data with Raman frequencies.

	Amide I	Amide III
α-Helix	1,645–1,600	1,300–1,260
β-Sheet	1,680–1,658	1,243–1,230
Random coil	1,665–1,660	1,243

Tab.1. *Bands assigned with amides in relationship with the steric shape.*

Polymers

Raman spectroscopy is an essential tool in characterization of the structure, environment, and dynamics of polymeric materials. The method's high sensitivity to polymer primary and secondary structure, as well as to solvation state, has allowed detailed information to be obtained on a wide variety of "soft" materials. Furthermore, the portability of the technique allows for its use in on-line process monitoring. Raman spectroscopy has great utility in the characterization of polymeric fibers because of low fluorescence background and the ability to obtain both second- and fourth-order orientation functions from polarized data [5]. Thus, Raman spectroscopy was used to monitor the effects of firing temperature and polymer drawing during manufacturing [6]. Cis/trans ratios in polymeric composites have been studied as a function of temperature [7]. The stress in a composite multifiber has been observed and related to manufacturing flaws, ultimately leading to design improvements [8]. Use of Raman spectra to determine polymer conformation has been the subject of several publications. Raman has been used to observe conformational disorder in waxes [9], solvent reorientation in polyacrylamide gels [10], and strain

inhomogeneities in highly oriented gel-spun polyethylene [11] and for monitoring curing in divinyl ethers and epoxies [12-15].
Several papers have explored polymer-based electrolytes [16-19].

Prospectus

Several factors are combining to make the future of Raman extraordinarily promising:

1) Recent advances in instrumentation (e.g., compact diode lasers) have been coupled with previous innovations (e.g., CCD detectors) to make low-cost and portable instruments a reality.
2) Recent publication of extensive Raman spectral databases will raise Raman closer to the status of IR for characterization of organic compounds.
3) As real-world applications of new materials appear, the use of Raman as a characterization tool will become pervasive.
4) New data demonstrating single-molecule detection by SERS have offered the first real challenge to fluorescence in bioanalytical chemistry.
5) The use of Raman in medical diagnostics, while still in its infancy, has the potential to move this technique out of laboratory and into the clinic and the hospital.

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CHAPTER 5:

DEGREE OF CONVERSION OF FILTEK SILORANE ADHESIVE SYSTEM AND CLEARFIL SE BOND WITHIN THE HYBRID AND ADHESIVE LAYER: AN *IN-SITU* RAMAN ANALYSIS

Introduction

Composite formulations have continued to evolve since BisGMA was first introduced in 1962 to dentistry by Bowen [1,2]. Recent developments in material science technology have considerably improved physical properties of resin-based composites and expanded their clinical applications [3]. Nevertheless, despite the differences among their chemical formulation, all composite resins are essentially characterized by a polymerization reaction occurring between methacrylate groups of monomers, resulting in cross-linking of mobile molecules to form rigid polymers.

The main adverse effect of this methacrylate polymerization reaction is volumetric shrinkage, which contributes to stress formation along the bonded interfaces of restorations [4,5]. For this reason, research mainly focused on dental adhesion improvements to achieve higher bond strength and to improve the stability of the adhesive interface over time [6,7]. Despite significant increases in immediate bond strength, the occurrence of microleakage and gap formation mainly at the adhesive-dentin interface has not been completely resolved [8-10].

Based on the intensive research on shrinkage and stress development, different approaches have been proposed to reduce polymerization shrinkage and the effects of contraction stress in dental composites. These methods include incremental placement techniques [11], development of soft-start polymerization [12], use of low-modulus intermediate layers [13], and modifications of the current resin-based composites [14,15]. Additionally, several low-shrinkage materials have been recently developed as an alternative to dimethacrylate resins, but none of them has been able to equal BisGMA-based composite properties [16].

Silorane [3], a low-shrinkage, tooth-colored restorative material (as claimed by the manufacturer, 3M ESPE, St. Paul, MN, USA), has been recently

introduced in the dental market. Silorane was so named by the manufacturer to indicate a hybrid compound of siloxane and oxirane functional moieties [3]. While the siloxane determines the highly hydrophobic nature of the siloranes, the cycloaliphatic oxirane functional groups are responsible for lower shrinkage when compared to methacrylate-based composites. Oxiranes, which are cyclic ethers, polymerize by a cationic ring-opening mechanism, while methacrylates polymerize via a free-radical mechanism [17].

The adhesive system of the Filtek Silorane (3M ESPE) is a methacrylate-based, two-step self-etch system (Silorane Adhesive System, 3M ESPE). The polymerizable self-etching primer is applied, followed by the photopolymerization of the adhesive resin containing a hydrophobic dimethacrylate monomer. This adhesive resin promotes association with and covalent bonding to the hydrophobic oxirane-based composite. Previous studies revealed higher marginal adaptation and reduced microleakage formation and lower cuspal deflection when silorane-based materials were used compared to methacrylate composites [18-21]. However, little is known about the effective formation of a stable hybrid layer (HL) or about the polymerization reaction occurring at the adhesive interface.

The degree of conversion (DC) of dental adhesive films has been previously evaluated using direct methods such as differential scanning calorimetry (DSC) [22,23], Fourier-transform infrared (FTIR) spectroscopy, and Fourier-transform Raman spectroscopy and micro-Raman analyses [24-31]. DSC measures the heat generated during resin polymerization, which is proportional to the percentage or concentration of reacted monomers (i.e., extent of polymerization or DC) [22,23]. However, micro-Raman and FTIR spectroscopies offer the advantage to obtain precise information about chemical composition, as well as the ability to determine the DC by

measuring the amount of converted double bonds [25-31]. Raman spectroscopy, in particular, enables spatially resolved chemical analysis of the HL through direct examination of specimens without compromising their integrity [25,26,28-31], thus allowing *in-situ* mapping of the adhesive interface.

The objectives of the study were to assay the DC of the Silorane Adhesive System within the HL and to compare it with a well-known reference material for two-step, self-etch adhesive systems, namely Clearfil SE-Bond (Kuraray Dental, Tokyo, Japan), using micro-Raman spectroscopy. The hypothesis tested was that no difference occurs between the DC of the two-step, self-etch adhesives studied.

Table 1: *: Composition and technique guide of the solutions contained within the Silorane Adhesive System and Clearfil SE Bond as reported by the manufacturers.*

Adhesive system		Composition (Wt%)	Application mode
Group #1: **Filtek Silorane Adhesive**	**Self-Etching/Primer**	• 2-Hydroxyethyl Methacrylate (HEMA) (15-25) • Bisphenol A Diglycidyl Ether Dimethacrylate (BisGMA) (15-25) • Water (10-15) • Ethanol (10-15) • Phosphoric Acid-Methacryloxy-Hexylesters Mixture (5-15) • Silane Treated Silica (8-12) • 1,6-Hexanediol Dimethacrylate (5-10) • Copolymer of Acrylic and Itaconic Acid (<5) • (Dimethylamino)Ethyl Methacrylate (<5) • dl-Camphorquinone (<3) • Phosphine Oxide (<3)	• Apply on tooth surface and brush for 15 sec • Expose to a gentle air stream • Cure10 sec
	Bond	• Substituted Dimethacrylate (70-80) • Silane Treated Silica (5-10) • Triethylene Glycol Dimethacrylate (TEGDMA) (5-10) • Phosphoric Acid Methacryloxy-Hexylesters(<5) • dl-Camphorquinone (<3) • 1,6-Hexanediol Dimethacrylate (<3)	• Agitate the bottle • Apply on tooth surface • Expose to a gentle air stream • Cure 10 sec
Group #2: **Clearfil SE Bond**	**Self-etching/Primer**	• 2-Hydroxyethyl Methacrylate (HEMA) (20-30) • 10-Methacryloyloxydecyl Dihydrogen Phosphate (MDP) • Hydrophilic Aliphatic Dimethacrylate • dl-Camphorquinone • Water • Accelerators • Dyes and others	• Apply on tooth surface and leave it for at least 20 sec Evaporate the volatile ingredients with a mild air stream.
	Bond	• 2-Hydroxyethyl Methacrylate (HEMA) (25-35) • 10-Methacryloyloxydecyl Dihydrogen Phosphate (MDP) • Bisphenol A Diglycidylmethacrylate (Bis-GMA) • 2-Hydroxyethyl Methacrylate (HEMA) • Hydrophobic Dimethacrylate • dl-Camphorquinone • N,N-Diethanol-p-toluidine • Silanated Colloidal Silica	• Apply on tooth surface • Expose to a gentle air stream • Cure 10 sec

Materials & Methods

Teeth preparation and bonding procedures

Ten non-carious human third molars were collected after patient's informed consent was obtained under a protocol approved by the University of Trieste (Italy). Occlusal enamel was removed using a low-speed diamond saw under water-cooling (Micromet, Remet, Bologna, Italy) and middle/deep dentin was exposed, and an uniform smear layer was produced with 180 grit silicon carbide paper. The ten dentin disks were then randomly and equally assigned to the two tested groups (N=5): Group 1 was bonded with Silorane Adhesive (LOT N°7AY, 3M ESPE), and Group 2 was bonded with Clearfil SE-Bond (primer: LOT, 00026A; bond: LOT 00040A, Kuraray Dental) in accordance with manufacturer's instructions. Adhesive composition and application modes are listed in *Table 1*.

Specimens were photopolymerized at room temperature using a halogen curing light (Elipar 2500, 3M ESPE, St Paul, MN, USA) at a distance of 1 mm from the specimens. The irradiance at the tip of the curing light was 600 mW/cm^2 as measured by a radiometer having a wavelength range of 400-500 nm (manufacturer's information). A build-up material (Group 1: Silorane Filtek Restorative, 3M ESPE, LOT N°7AM; Group 2: Clearfil APX, Kuraray Dental, LOT 00945A) was applied in 2 mm thick increments and cured for 40 s. Specimens were stored in distilled water for 24 h before being sectioned perpendicular to the bonded surface to expose the adhesive interface and to obtain 2 mm thick slabs, which were placed directly on glass microscope slides during micro-Raman spectroscopic analysis. Two slabs were obtained from each tooth, for a total of ten slabs for each group. The adhesive interfaces were polished for 30 s with wet SiC papers up to 800 grit, followed by 6, 1, and 0.05 μm diamond pastes, with distilled water rinsing between each step. Specimens were cleaned with soap and sonicated

for 30 minutes, then bonded interfaces were rubbed with a cotton swab saturated with 5% sodium hypochlorite to rid the surface of smeared proteins produced by polishing.

Micro-Raman spectroscopy

Raman spectra of the specimens were collected using a computer-controlled laser Raman microprobe equipped with a Leica DM/LM optical microscope with an 100x objective (NA 0.9) and CCD detector attached to a modular research spectrograph (Renishaw InVia, Renishaw plc, Gloucheshire, UK). The 100x objective was used to increase the precision of the acquired chemical data, resulting in a laser spot size of ≤1μm. A monochromatic, near-infrared diode laser operating at 785 nm was used to induce the Raman scattering effect. The spectral coverage of this model ranges from 100 to 3450 cm^{-1} with an average spectral resolution of 5 cm^{-1}. A comparison with a silicon standard was performed to calibrate wavelength and intensity using the calibration system integrated with the software (WiRE 2.0, Renishaw) before each experiment according to manufacturer's specifications. The exposure time for each scan was 40 s, and the laser power on the specimen was ~8 mW.

The 20 dentin slabs were analyzed by acquiring spectra in three line-scans across each specimen in intertubular regions of the bonded interface, starting in the sound mineralized dentin and ending in the adhesive resin layer. Six line-scans were done for each tooth, for a total of 30 line-scans for each group. The adhesive/dentin interface was analyzed acquiring spectra in steps of 1 μm intervals using a computer motorized x-y-z stage. A digital image of each analyzed site was acquired to identify the exact position of each obtained spectrum (Fig 1a and b). The spectra were analyzed over the spectral region of 400 to 1900 cm^{-1}. The acquired data were analyzed with

software developed for spectrographic analysis (Grams/AI 7.02, Thermo Galactic Inc., USA).

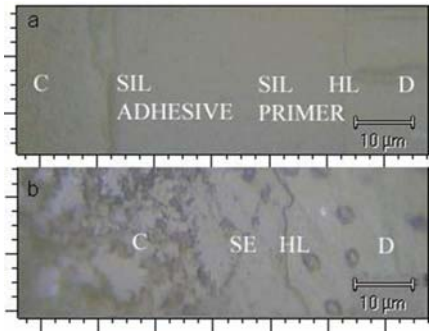


Fig. 1a and 1b: *Digital light microscopy photograph of the bonded tooth specimens through the 100x objective: Silorane Adhesive System (SIL) interface (1a) compared with Clearfil SE adhesive (SE) interface (1b). (D: dentin; HL: hybrid layer; C: composite layer).*

Conversion calculation

Representative Raman spectra acquired in human dentin are shown in Fig. 2.

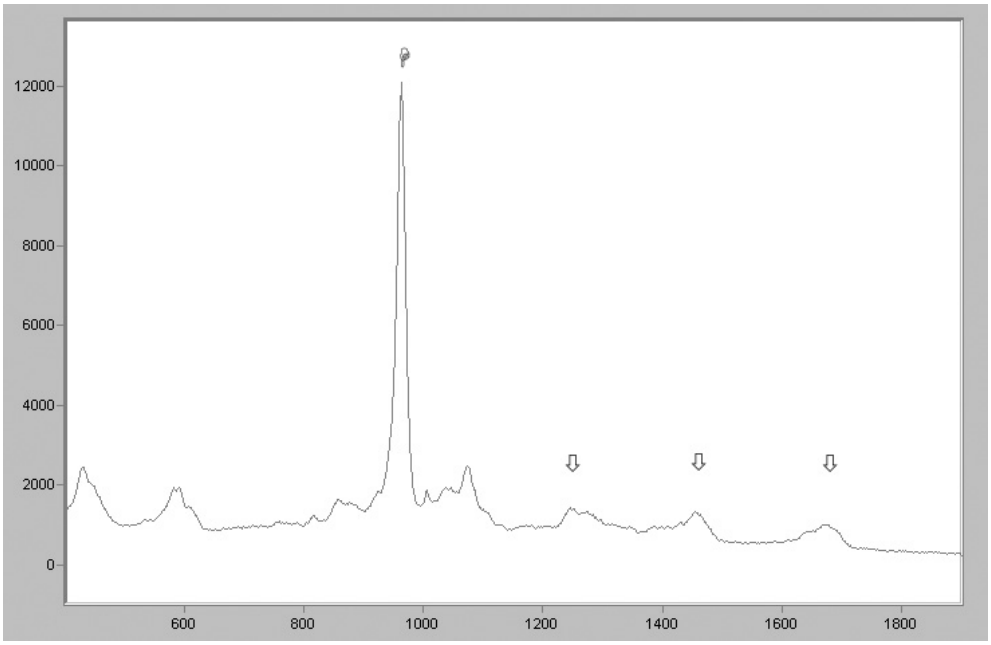


Fig. 2*. Raman spectrum of mineralized dentin. The P-O band at 960 cm^{-1} of the mineral component is well represented (pointer). The peaks at 1245, 1450, and 1667 cm^{-1} are associated with organic components (arrows).*

The strong peak at 960 cm^{-1} is associated with the P-O group of the dentin mineral component, while peaks at 1245 (C-N), 1450 (CH_2), and 1667 cm^{-1} (C=O) represent the dentin organic components [25].

Raman spectra of the primer and the adhesive, both uncured and cured, were collected to identify the reference and reaction peaks for each adhesive system.

As shown in Fig. 3a-d, the spectra of both primers and adhesives were characterized by common intense bands typical of these methacrylate-based adhesive systems: 605 cm^{-1} (carbonyl, C-C-O), and 1640 cm^{-1} (C=C group) [25, 31]. The 605 cm^{-1} peak was selected as a reference peak, which remains stable and unchanging during polymerization, and the 1640 cm^{-1} was selected as a reaction peak.

The 960 cm^{-1} peak was used as a reference to identify the position of each analyzed spectrum in the HL as exemplified in Fig. 4. The degree of conversion was calculated using the ratio between the reaction (I_{rxn}) and internal reference (I_{ref}) peak intensities as follows:

$$\%conversion = 1 - \frac{[I_{rxn}(p)/I_{ref}(p)]}{[I_{rxn}(u)/I_{ref}(u)]} x100\%$$

where “u” refers to unpolymerized and “p” to polymerized adhesive system.

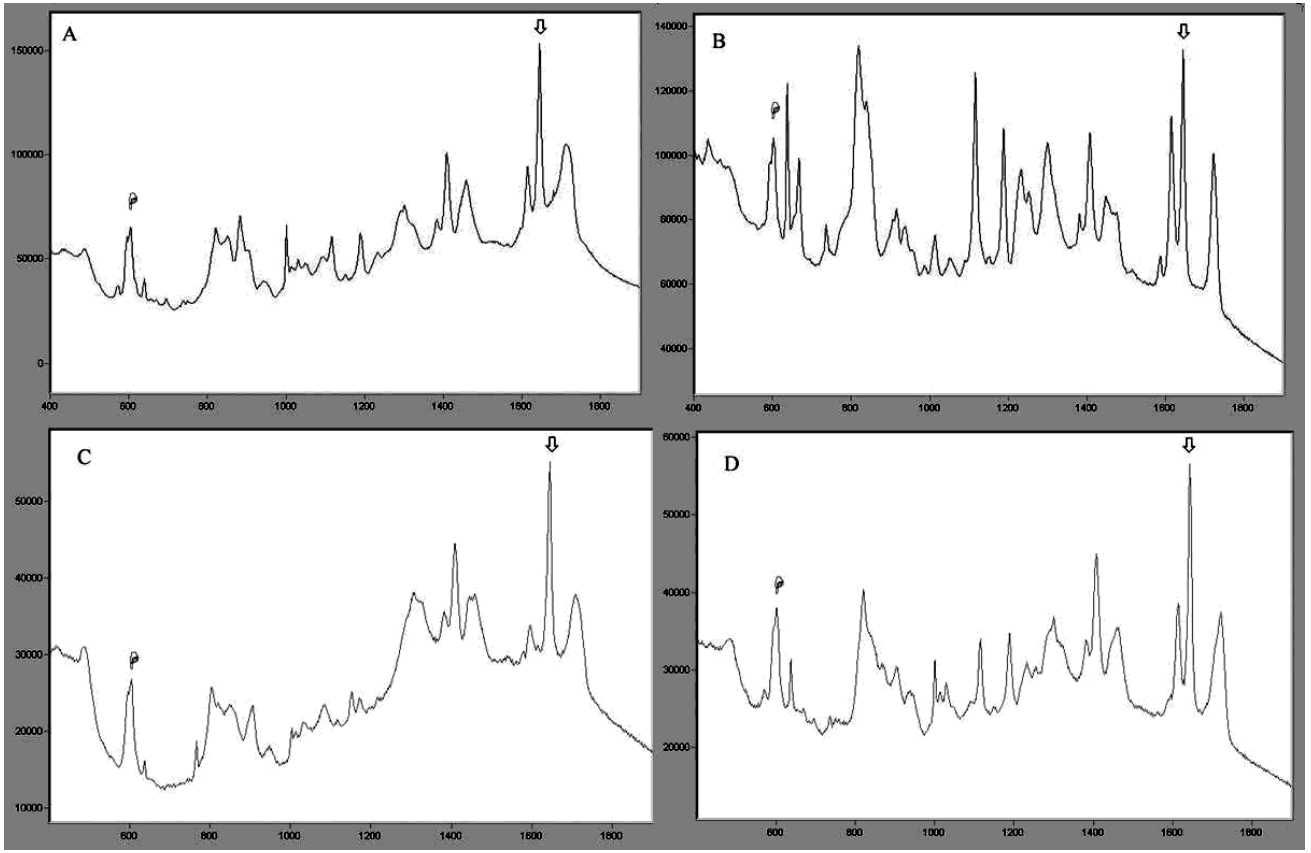
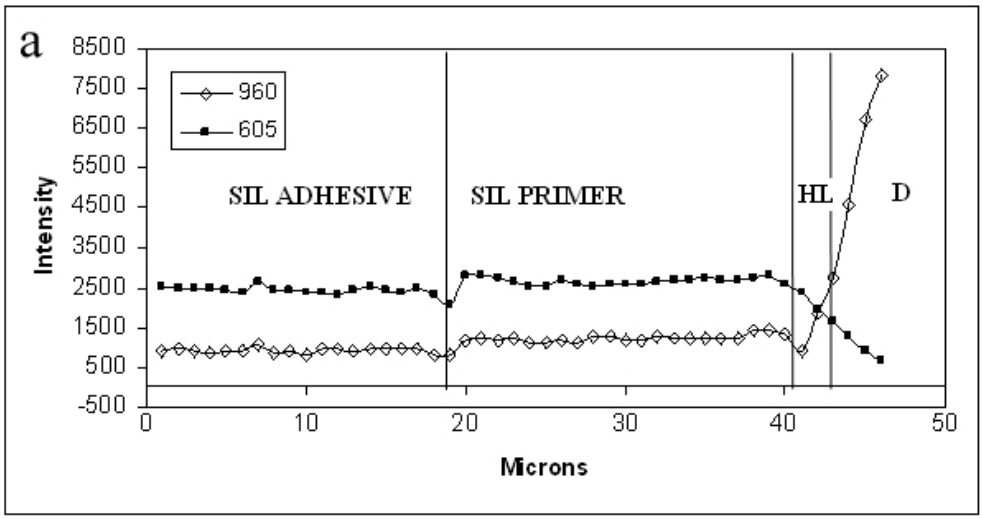


Fig. 3a-d: *Spectra of uncured* primer (a) and adhesive (b) of the Silorane Adhesive System and uncured primer (c) and adhesive (d) of the Clearfil SE Bond. Peaks at 605 *cm^{-1}* (carbonyl, C-C-O; pointer) *and 1640 cm^{-1} (C=C group; arrow) were considered as reference and reaction* peaks, respectively, for the calculation of the degree of conversion of both the examined systems**.**



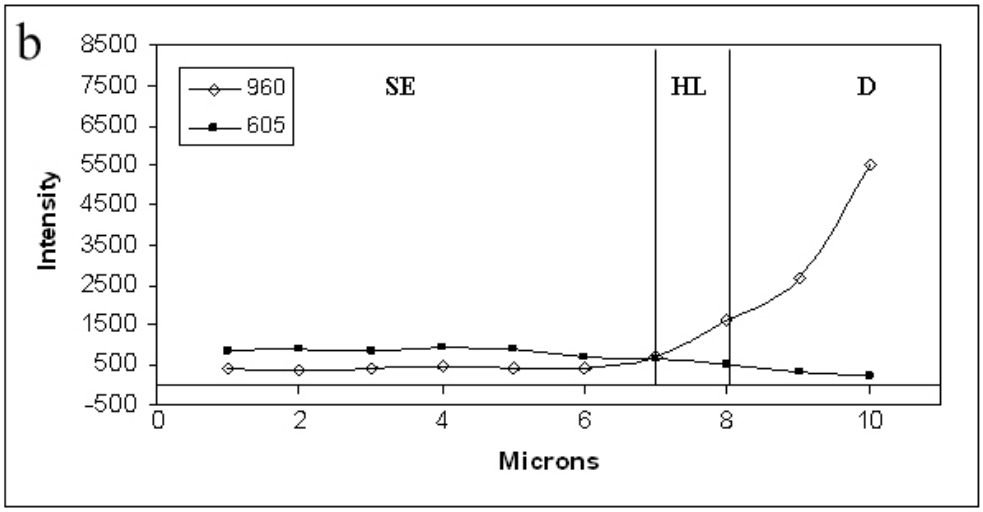


***Fig. 4a and 4b**: The figures are showing the trend of the intensities of the representative peaks of the mineralized tissue (P-O; 960 cm^{-1}) and of the bonding agents' layers (C-C-O; 605 cm^{-1}) in one Raman line-scan acquired into the interface of Silorane Adhesive System (4a) and Clearfil SE Bond (4b). It is clearly shown the transit from one layer to the other in relationship with the thickness of the interface. (D: dentin; HL: hybrid layer; Silorane Adhesive System: SIL; Clearfil SE adhesive: SE).*

Statistical analysis

Since values were normally distributed (Kolmogorov-Smirnof test), DC values were analyzed with a two-way ANOVA (tested variables were: adhesive system and adhesive-dentin layer) and Tukey's post hoc test. Statistical significance was set at $p<0.05$.

Results

Conversion calculation

Means and standard deviations (SD) of DC and statistical differences between layers of interest (Fig. 1a and b) and adhesives are reported in table 2.

Table 2 *Degree of conversion (DC) of the Silorane Adhesive System and Clearfil SE-Bond expressed as percentages (%). The table reports DC of the hybrid layer (HL), primer layer, and adhesive layer obtained from six line-scans for each tooth (30 line-scans for each group) and averaged per total number of scans. Means of DC followed by the same superscript letter indicate no statistical difference at the 95% confidence level (p<0.05).*

Materials	HL	Primer	Adhesive
Group #1: Filtek Silorane Adhesive	91.6±8.8%[a]	93.0±4.8%[a]	68.7±6.8%[b]
Group #2: Clearfil SE/APX	83.4±2.6%[c]		85.4±2.6%[c]

The DC of the Silorane Adhesive system, obtained from Raman spectra (Fig. 5a), was 68±6% in the adhesive layer, increasing ($p<0.05$) within the primer

layer and HL (93±4% and 91±8%, respectively). The DC of Clearfil SE Bond, obtained from Raman spectra (Fig. 5b), was lower than the DC of the Silorane Adhesive in the HL (83±2%, $p<0.05$), but higher within the adhesive layer (85±2%, $p<0.05$). Overlap of the spectra from the same region proved the reproducibility of the technique

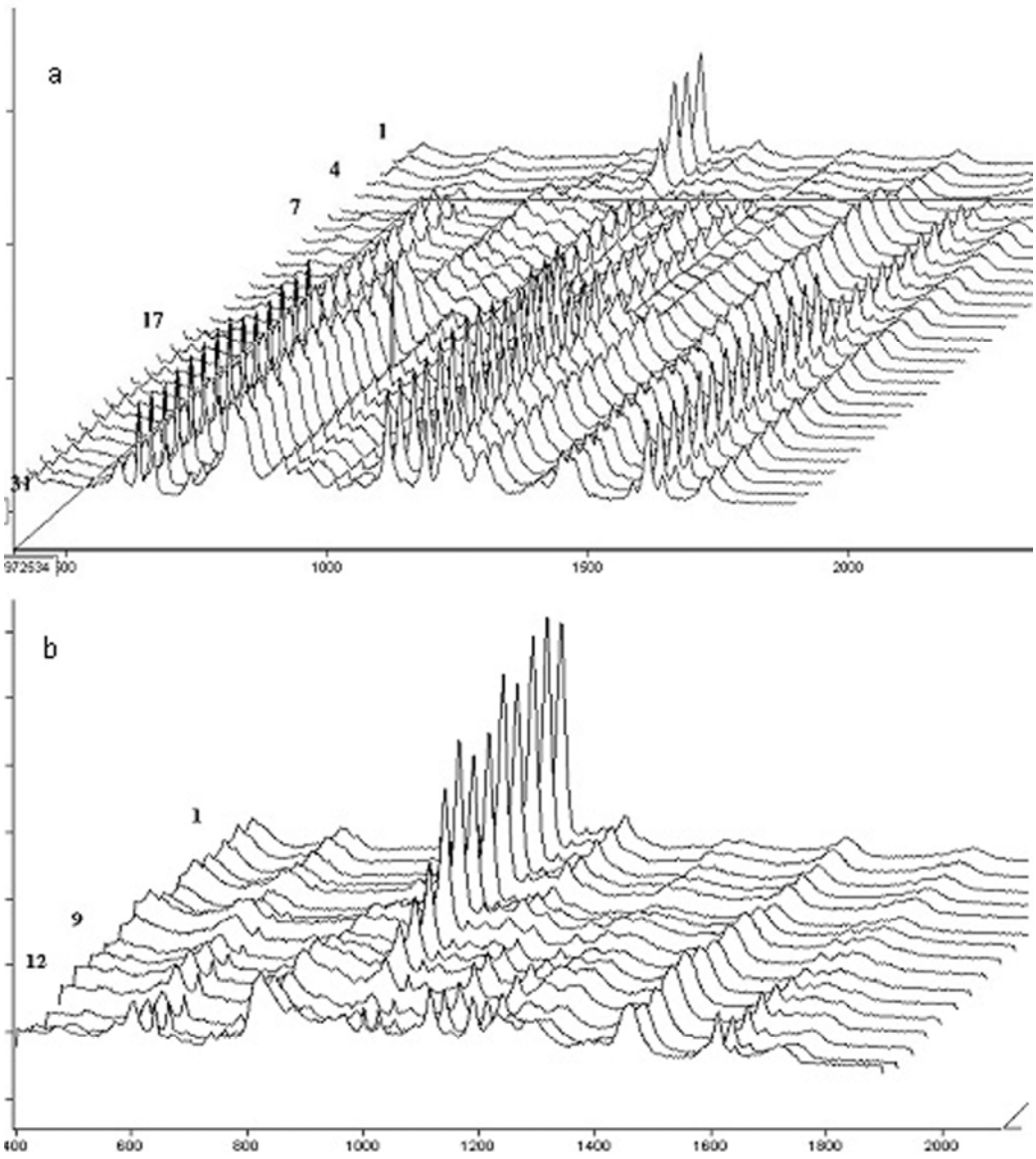


Fig. 5a and 5b: *Raman line-scan spectra acquired at 1 µm intervals across the adhesive-dentin interface created by Silorane Adhesive System (a) and Clearfil SE Bond (b). In Figure 5a, Spectra 1-3 were in mineralized sound dentin, Spectra 4-6 were taken along the hybrid layer created by Silorane Adhesive System, Spectra 7-17 were collected on the primer layer, and Spectra 17-31 are representative of the adhesive layer. In Figure 5b, Spectra 1-9 were in mineralized sound dentin, Spectra 10-12 were taken along the hybrid layer created by Clearfil SE Bond , and from Spectra 13-16 are representative of the adhesive layer.*

Discussion

The tested hypothesis was rejected since differences were found in the DC calculated from Raman spectra of bonded interfaces created by the Silorane Adhesive vs. Clearfil SE Bond. Silorane Adhesive showed a high DC within the HL and the primer (>90%, Table 2), while DC decreased within the hydrophobic adhesive bonding layer (<70%). Clearfil SE Bond showed lower DC within the HL and higher DC within the adhesive layer when compared to the Silorane Adhesive System ($p<0.05$). The high degree of conversion showed by the Silorane Adhesive System confirms the occurrence of a proper polymerization reaction of the methacrylate-based polymeric structure within the hybrid and primer layer.

Since high DC corresponds to optimal mechanical properties of light-polymerized dental materials [32], the DC of the resin film created by the adhesive is one of the parameters that determine the inherent strength and stability of the bond over time [33]. Previous DSC reports [22,23] revealed that the DC of the adhesive systems is dependent on their respective formulations. Whether a halogen [22] or LED [23] light activating source was used, polymerization of the adhesive film was severely compromised (even under a nitrogen atmosphere) for simplified adhesives in which the bonding agent was combined with either the primer (i.e., two-step, etch-and-rinse adhesive) or the etching/primer solution (i.e., one-step, self-etch adhesive). However, higher conversions were measured for unsimplified adhesive systems, characterized by a hydrophobic and relatively unsolvated layer of bonding across the conditioned dentin surface (i.e., three-step, etch-and-rinse and two-step, self-etch adhesives) [22,23]. It was hypothesized that the higher percentage of hydrophilic monomers and the presence of water in simplified adhesives compromised their polymerization reaction, leading to suboptimal curing of the associated resin films.

The same studies demonstrated that adhesive conversion was inversely correlated with the intrinsic permeability of the adhesive films [22,23]. The correlation between conversion and permeability confirms that partially-cured adhesives are more permeable to fluid movement [34], and the invasive water could interfere with polymerization of the dentin adhesives and resin composites [28,29,33,35]. In addition, since the presence of greater amounts of uncured monomers would expedite water sorption and compromise the integrity of the hybrid and adhesive layer, bonds created in simplified etch-and-rinse and self-etch adhesives would degrade faster [36-40].

In contrast with all other two-step, self-etch systems, the self-etching/primer agent of the Silorane Adhesive must be polymerized. The hydrophilic compounds contained within this first step contribute only to the HL formation, and no mixing occurs between these hydrophilic monomers and the hydrophobic bonding layer. In this way, the coupling between the primed hydrophilic dentin tissue and the extremely hydrophobic silorane-based restorative material can be achieved.

Previous studies have shown that the hydrophobicity of the adhesive layer is also correlated with bond durability [40]. For this reason, adding a hydrophobic resin layer onto a polymerized one-step adhesive agent has been previously proposed to create a two-step, self-etch adhesive [41,42] and to promote bond durability over time [7]. Likewise, the hydrophobic coating of the Silorane Adhesive may provide additional stability to the bonded interface by reducing the amount of water uptake over time.

The DC of the Silorane hybrid and primer layer was higher than that in the bonding layer. This difference in DC among the layers may be attributed to the difference in viscosity between the primer and adhesive. In a previous study, increased DC of the adhesive films was correlated with lower

viscosity formulations of the comonomer blends due to increased polymer chain mobility and increased the rate of diffusion of radicals [43]. In addition, the difference in DC among the layers may be an artifact of the monomer functionality in the primer and adhesive. The primer contains both monofunctional and difunctional methacrylates, while the adhesive contains only difunctional methacrylates. The difunctional methacrylate system most likely will vitrify sooner, trapping unreacted pendant methacrylate groups. In comparison, the Clearfil SE Bond primer and adhesive contain both monofunctional and difunctional methacrylates, and the DC of the HL and bonding layers are similar.

The polymerizable self-etching primer of the Silorane Adhesive may mimic a one-step, self-etch adhesive, since it contains the three cardinal steps for bonding (i.e., etching, priming, and bonding), leading to proper impregnation of the dentin substrate and optimal polymerization. The DC of a one-step, self-etch adhesive system (Adper Prompt L-Pop, 3M ESPE) was investigated at different depths *in situ* [29]. The DC of resin tags at 50 μm deep with respect to the surface was only ~ 79% due to the presence of water within the dentin tubules. It was hypothesized that water interferes with the polymerization reaction leading to the presence of unpolymerized acidic monomers and oligomers that would continuously etch the surrounding dentin, finally jeopardizing the bond and contributing to nanoleakage formation [44]. However, the DC of the HL of the Adper Prompt L-Pop was comparable to the value measured for the Silorane Adhesive System.

The DC measured in the Silorane Adhesive primer layer was considerably higher than other comparable all-in-one systems tested in previous studies [24,29,45-48]. These DC may be "apparent" conversion caused by the elution of unreacted HEMA during the storage of the specimens in distilled water and specimen preparation, increasing the amount of water penetration

into the HL [46]. The DC of the Silorane Adhesive bonding layer would be unaffected by water storage since the adhesive contains only dimethacrylates and, in general, at least one of the methacrylate functional groups would be linked to the polymer network after photopolymerization.

In conclusion, the Silorane Adhesive showed a very high DC within the HL that should significantly contribute to the formation of a stable adhesive interface. Further *in-vitro* and *in-vivo* investigations are needed to determine how much this greater extent of reaction in the Silorane Adhesive contributes to the stability of the bond created between this new low-shrinkage material and dentin.

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CHAPTER 6:

DEGREE OF CONVERSION INFLUENCES INTERFACIAL NANOLEAKAGE EXPRESSION OF ONE-STEP SELF-ETCH ADHESIVES.

Introduction

Dentin bonding agents are blends of hydrophobic and hydrophilic monomers in different percentages to obtain amphiphilic compounds that allow the adhesion between the hydrophobic restorative material and the intrinsically wet dentin substrate. One-step self-etch adhesives contain the highest percentages of hydrophilic monomers among the currently available adhesive systems [1, 2]. Despite their simplified clinical application, they exhibit lower immediate bond strength values compared to unsimplified counterparts. The stability of the adhesive interface created by one-step self-etch adhesives is also a matter of concern due to expedited aging phenomena affecting the hybrid layer [3, 4]. Different studies revealed significant bond strength reduction and extensive interfacial nanoleakage expression within the adhesive interface created by simplified adhesive after only 6 to 12-month aging under either under *in vitro* or *in vivo* conditions [1, 5, 6, 7].

The lack of a relatively hydrophobic and unsolvated bonding agent as separated final step of the adhesive procedure has been accounted as one of the main reason for the instability of simplified adhesive. Due to the relatively high percentage of hydrophilic monomers blended within their formulation, the adhesive interface created by one-step self-etch adhesives behaves as semi-permeable membrane after polymerization, as fluids are allowed to cross the interface in relation to osmotic pressure gradients [4, 8]. For this reason simplified adhesives are characterized by increased water sorption that expedites polymer swelling and other water degrading phenomena [4, 9, 10, 11].

Recent studies revealed that adhesive permeability well correlates also with the presence of un-reacted monomers within the hybrid layer. In particular one-step self-etch adhesives revealed the highest permeability associated with the lowest degree of cure (DC) when compared to the unsimplified

multi-step systems [3, 12, 13]. Indeed the presence of water and high concentration of hydrophilic domains affects the polymerization reaction leading to suboptimal DC, reducing bond longevity due to elution of un-reacted monomers. The final consequence of this process is the formation of a porous hybridoid structure along the adhesive interface with reduced sealing ability.

Since it is well known that high DC of resin polymers corresponds to improved mechanical properties of light cured resin materials [14], reduced DC of the simplified adhesive films also reduces their bonding effectiveness [3, 12, 15].

Among the different method used to investigate DC of resin polymers (differential scanning calorimetry, micro-hardness, Fourier Transform Infrared Spectroscopy), micro-Raman analysis represents a useful tool to obtain an *in situ* evaluation by a direct measure of the converted double bond percentage [16, 17, 18, 19, 20, 21, 22]. Indeed micro-Raman spectroscopy is a non-destructive technique that has the advantage to allow a spatially resolved chemical analysis of the hybrid layer after minor specimen preparation steps. Spectra of the uncured material are acquired to determine the double-bond peak ratio (which is considered as DC of 0%) and then the fully cured material is investigated to assess the 100% possible conversion rate.

A (meth)acrylamide-based one-step self-etch adhesive has been formulated (iBond, Heraeus Kulzer, Hanau, Germany). Higher stability in aqueous environment has been supported for (meth)acrylamide-based resin blends compared to methacrylate-based systems [23]. While the use of (meth)acrylamide may reduce the well-know problems related to the hydrolytic degradation of one step self-etch adhesives, other properties such as DC have not been extensively investigated.

Increased nanoleakage expression has also been described in simplified adhesive interfaces and silver nitrate deposits within the hybrid layers were frequently associated with hydrophilic domains and residual water-filled areas. However, the correlation between nanoleakage and unreacted hydrophilic monomers has not been clarified.

Thus, the aims of this study were to characterize the hybrid layers created by a (meth)acrylamide-based and two methacrylate-based one-step self-etch adhesives on dentin using micro-Raman spectroscopy and to correlate the DC with the interfacial nanoleakage expression. The null hypotheses tested were that (1) DC has no influence on interfacial nanoleakage expression of simplified adhesives and (2) no differences can be detected among the tested adhesives.

Materials and methods

Teeth preparation and bonding procedures

Nine non-carious human third molars were collected after patient's informed consent was obtained under a protocol approved by the University of Trieste (Italy). After removal of occlusal enamel using a low-speed diamond saw under water-cooling (Micromet, Remet, Bologna, Italy), middle/deep dentin was exposed and a uniform smear layer was produced with 180 grit silicon carbide paper. Specimens were equally (N=3) and randomly assigned to one of the tested adhesives: Group 1: AdheSE One (Vivadent, Schaan Liechtenstein; lot # K10655); Group 2: Adper Prompt L-Pop (L-Pop; 3M ESPE, St Paul, MN, USA; lot # A 50801239125); Group 3: iBond (Heraeus Kulzer, Hanau, Germany; lot # 010081). Adhesive composition and application mode (in accordance with manufactures' instructions) are listed in Table 1. A halogen-curing unit with a previously tested output of 600 mW/cm^2 was used to polymerize the adhesive

interfaces (Elipar 2500, 3M ESPE) with the distance of the curing tip was set at 1mm from the adhesive surface. A 2mm-thick increment of composite (Filtek Z250, 3M ESPE, lot 6NM) was applied on the bonded surfaces and cured for 40 s. Specimens were stored in deionized water for 24h at 37°C and then perpendicularly sectioned in 2mm-thick slabs to expose the bonded interface. Three slabs were obtained from each bonded specimen. The interfaces were polished to obtain a smooth surface suitable for micro-Raman analysis in accordance with Zou et al. [17].

Table 1: Composition and application modes of the tested adhesives as reported by manufacturers.

Adhesive system	Composition (Wt%)	Application mode
Group 1: AdheSE One	• Bis-acrylamide derivative (40-50) • Water (20-30) • Bis-methacrylamide dihydrogenphosphate, Amino acid acrylate, Hydroxyalkyl methacrylamide (20-40) • Silicon dioxide (<5) • Initiators and stabilizers (<1)	1. Apply on tooth surface and brush for 30 s; 2. Expose to a strong air stream; 3. Light cure for 10 s.
Group 2: Adper Prompt L-Pop	**Liquid 1:** • Di-HEMA phosphate (75-90) Bisphenol A Diglycidyl Ether Dimethacrylate (Bis-GMA) (10-15) • Ethyl 4-Dimethyl Aminobenzoate (<2) • DL-Camphorquinone (1-1.5) • Water (70-80) 2-Hydroxyethyl Methacrylate (HEMA) (17-28)	1. Apply on tooth surface with moderate finger pressure for 15 s; 2. Expose to a gentle air stream; 3. Light cure for 10 s.

Raman microspectroscopy

A modular research spectrograph (Renishaw InVia, Renishaw plc, Gloucheshire, UK) connected to an optical microscope (Leica DM/LM

optical microscope, Wetzlar, Germany) was used to investigate in situ the DC of the adhesive interfaces. A near-infrared diode laser operating at 785nm was used to induce the Raman scattering effect. The spectral coverage ranged from 100 to 3450cm^{-1} with an average spectral resolution of 5cm^{-1}. Instrument calibration was determined before each data acquisition by comparison of spectra from a standard (silicon, 520cm^{-1}).

Specimens were placed under an optical microscope on a computer-controlled X-Y-Z stage focusing the laser beam with 60x lens (Olympus, NA 1.4, Hamburg, Germany) to provide a spot size ≤1μm and to properly identify the position in which spectra were then collected. To preserve the proper specimen hydration, micro-Raman analysis was performed maintaining the specimens immersed in purified water. A digital image of the site was recorded before each acquisition and spectra were acquired in line-scans across the adhesive\dentin interface at steps of 1μm intervals in intertubular regions of the bonded interface to avoid resin tags. The exposure time for each scan was 40s, and the laser power on the specimen surface was ~8mW with a spectral region of 400 to 1900cm^{-1}, including the fingerprint region of the methacrylate-based bonding agents. Three line scans were acquired for each specimen for a total of 27 measurements for each adhesive group (3 teeth x 3 slabs x 3 line scans). Acquired data were analyzed with spectrographic analysis software (Grams/AI 7.02, Thermo Galactic, USA).

Conversion calculation

A preliminary spectrum of mineralized dentin was acquired and characterized by a P-O peak at 960 cm^{-1} [24] and by the collagen organic components that were represented by peaks at 1245 (C-N of amide III), 1450 (CH_2) and 1667cm^{-1}(C=O of the amide I; Fig. 1) [16].

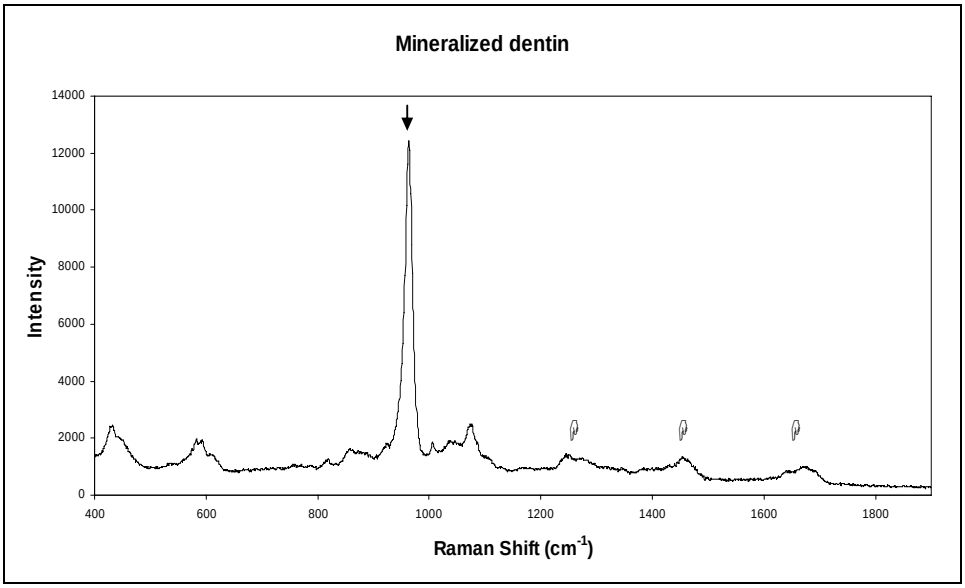

Fig. 1: *Micro-Raman spectrum of mineralized dentin. The P-O band at 960 cm^{-1} of the mineral component is well represented (arrow). The peaks at 1245, 1450 and 1667 cm^{-1} are associated with organic components (pointers).*

Micro-Raman spectra of uncured and cured adhesive solutions were collected to identify a reference peak (the most stable and best-represented peak for each adhesive system that was unaffected by the polymerization reaction) and a reaction peak (that identify the double reactive bond at 1640 cm^{-1} [17] as to calculate the degree of conversion for each adhesive system (Fig.2). The reference peak for iBond and Adper Prompt L-Pop was set at 605cm^{-1} (carbonyl, C-C-O) [17]. The reference peak for AdheSE One was set on the phenyl C=C at 1610cm^{-1} (clearly distinguishable from the 1640cm^{-1} peak), [17, 25] since the 605cm^{-1} peak was frequently undefined (Fig.2). The degree of conversion was calculated using the ratio between a reactive (I_{rnx}) and reference (I_{ref}) peak intensities as follows:

$$DC\%conversion = \left\{1 - \frac{I_{rnx}(p)/I_{ref}(p)}{I_{rnx}(u)/I_{ref}(u)}\right\} x100\%$$

where "u" refers to uncured and "p" to polymerized adhesive system.

Statistical analysis

As values were normally distributed (Kolmogorov-Smirnof test), DC data were analyzed with a one-way ANOVA and Tukey's post hoc test. Statistical significance was set at $p<0.05$.

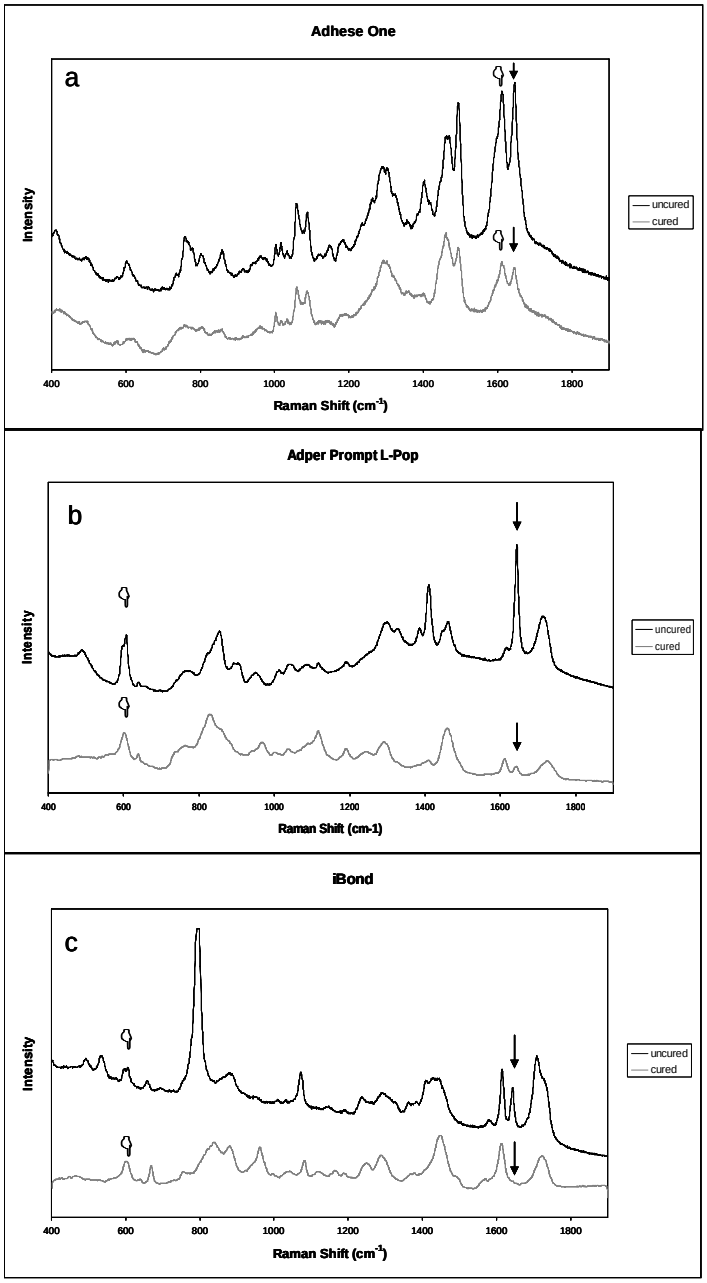

***Fig. 2**: Micro-Raman spectra of uncured and polymerized 40s adhesive for each tested system. The reference peak at 1640 cm^{-1} (arrows) is well recognizable in all of the spectra and it decreases proportionally in function of the number of C=C double bonds that has been converted in C-C during the polymerization. The reference peak (pointer) was for iBOND and Adper Prompt L-Pop the carbonyl peak at 605 cm^{-1} (C-C-O), and for AdheSE One the peak of the phenyl C=C at 1610 cm^{-1}.*

Nanoleakage analysis

After micro-RAMAN analysis, specimens (N=9 bonded slabs per group) were covered with nail varnish, leaving 1 mm exposed at the interface and processed for interfacial nanoleakage evaluation. Bonded interfaces were immersed to 50 wt% ammoniacal $AgNO_3$ solution according to the protocol described by Tay et al. [6]. After immersion in the tracer solution, thoroughly rinsed in distilled water and immersed in photodeveloping solution, the silver-impregnated specimens were fixed, dehydrated, embedded in epoxy resin (Epon 812, Fluka, Switzerland) and processed for light microscopy (LM).

In brief specimens were fixed on glass slides using a cyanoacrilate glue and flattened with 600, 800, 1200 and 2400-grit SiC paper under running water (LS2, Remet, Bologna, Italy). Specimens were then stained with 0,5% acid fuchsin for 15 min and analyzed with a LM at 100x (Nikon E 800, Nikon, Japan). Images of all interfaces were obtained at a magnification of x100 and the amount of silver tracer along the interface was evaluated by scoring nanoleakage interfacial expression by two observers as reported in Table 2. Images assigned to the same score were statistically evaluated and differences between the groups were analyzed with Chi-square test ($p<0.05$).

Additional specimens were prepared for high resolution-SEM analysis. Silver-impregnated bonded interfaces were exposed and polished with SiC papers of increasing fineness (up to 4000 grit). The specimens were carefully rinsed in 0.02M Tris-buffered saline (TBS) at pH 8.2 and in deionised water. Specimens were then subsequently fixed in 2.5% glutaraldehyde in 0.1M cacodylate buffer at pH 7.2 for 4 hr at room temperature, rinsed in 0.15M cacodylate buffer, dehydrated in an ascending ethanol series (25%, 50%, 75%, 90%, 95%, 100%), and dried with

hexamethyldisilazane (Sigma Chemical Co., St. Louis, USA) according to the method of desiccation reported by Perdigão et al. [26]. Specimens were then mounted on microscope stubs and coated with a 1 nm thick layer of evaporated carbon using a Balzers Med 010 Multicoating System (Bal-Tec AG, Liechtenstein). Observations were performed using a Field Emission In-lens SEM (JSM 890, JEOL, Tokyo, Japan) at 7 kV accelerating voltage and $1x10^{-12}$ Amp probe current. Images were obtained with both secondary electron (SEI) and back-scattered (BSI) signals.

Results

Means and standard deviations (SD) of DC% and statistical differences between the adhesives are reported in Table 2. Micro-RAMAN spectra of the hybrid layer of AdheSE One showed a DC% of 48.1±16.1% (Fig. 3a) while significantly higher DC% ($p<0.05$) was found for Adper Prompt L-Pop and iBond (85.5±2.6% and 89.96±6.1% respectively; Fig. 3b, c). As shown in figure 3 the transition from dentin to HL is suggested by the appearance of the reference peak (in Fig. 3a, 3b and 3c in spectrum #4) and the presence of the spectrum of neat adhesive signed the top of the HL (Spectrum #7 in Fig. 3a and 3b, and spectrum #6 in Fig. 3c).

The highest nanoleakage expression was found for AdheSE One, while Adper Prompt L-Pop and iBond showed lower nanoleakage (Table 2; $p<0.05$).

Table 2: Mean and standard deviations of DC% as revealed by the micro-Raman spectroscopic analysis. For each group of specimens interfacial nanoleakage expression was evaluated under light microscopy on all images taken along the entire interface and number of sections for each assigned nanoleakage scores are reported.

Adhesive system	**DC%**				**Nanoleakage**		
	1µm	**2µm**	**3µm**	**Total**	**Score**	**% of adhesive interface showing nanoleakage**	**%**
Group 1: AdheSE One	35.2±19.5	55.5±26.2	48.0±15.5	48.1±16.1[a]	**0**	no nanoleakage	0
					1	< 25 % with nanoleakage	11
					2	25 ≤ 50 % with nanoleakage	36
					3	50 ≤ 75 % with nanoleakage	20
					4	>75 % with nanoleakage	31
Group 2: Adper Prompt L-Pop	85.4±3.0	81.6±1.8	83.7±1.6	85.5±2.6[b]	**0**	no nanoleakage	3
					1	< 25 % with nanoleakage	23
					2	25 ≤ 50 % with nanoleakage	31
					3	50 ≤ 75 % with nanoleakage	38
					4	>75 % with nanoleakage	4
Group 3: iBond	89.0±6.2	87.4±6.2	90.6±4.0	89.9±6.1[b]	**0**	no nanoleakage	3
					1	< 25 % with nanoleakage	24
					2	25 ≤ 50 % with nanoleakage	26
					3	50 ≤ 75 % with nanoleakage	37
					4	>75 % with nanoleakage	9

Means of DC% followed by the same superscript letter indicate no statistical difference at the 95% confidence level ($p<0.05$).

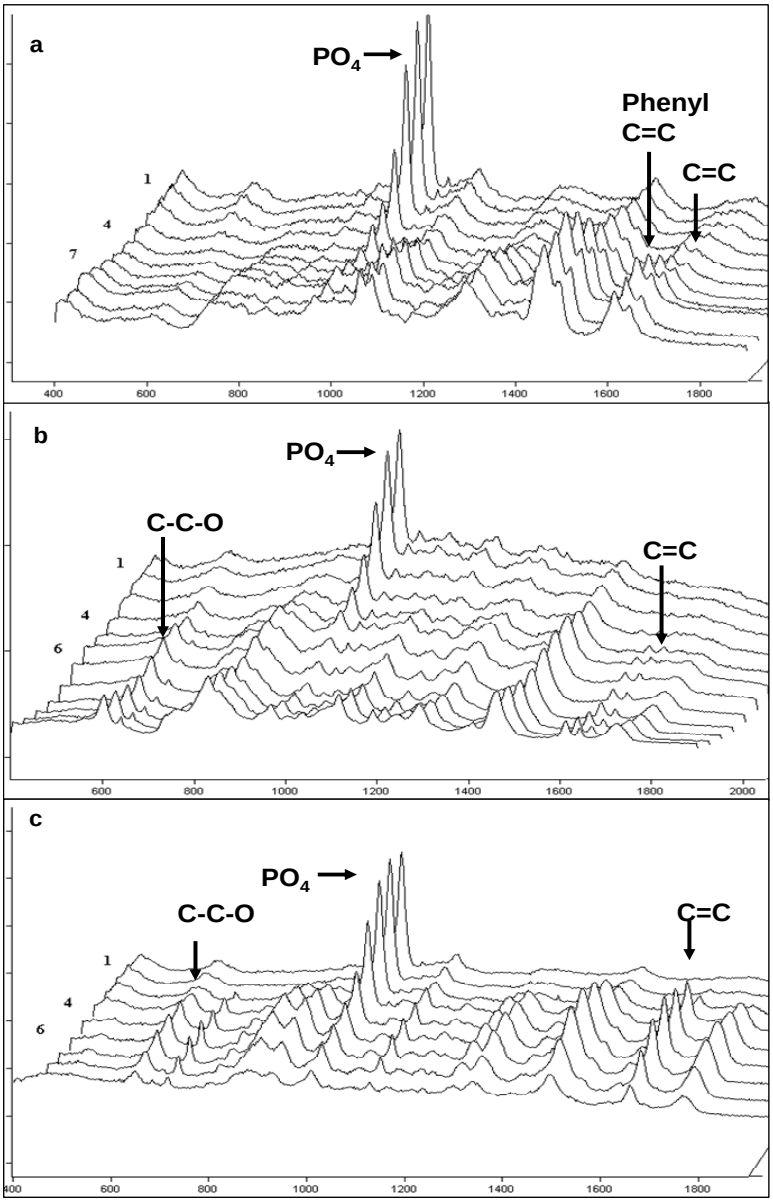

***Fig 3a-c**: Micro-Raman spectra acquired across the adhesive-dentin interface created by AdheSE One (3a), Adper Prompt L-Pop (3b) and iBond (3c). In **Fig. 3a** spectra #1-3 were collected in mineralized dentin. The appearance of the 1640 cm^{-1}peak in spectrum #4 suggested the beginning of the HL. Spectra #7-11 evidenced the presence of neat adhesive. In **Fig. 3b** spectra #1-3 represent mineralized dentin. The bottom of the HL was recognized in the spectrum #4. Spectrum #7 represents the deepest part of the adhesive layer. In **Fig. 3c** the spectrum #4 evidences the bottom of the HL. The contemporary decrease of the phosphate peak and the increase of the adhesive peaks are clearly shown in the spectra #4-5. Spectrum #6 is acquired in adhesive layer.*

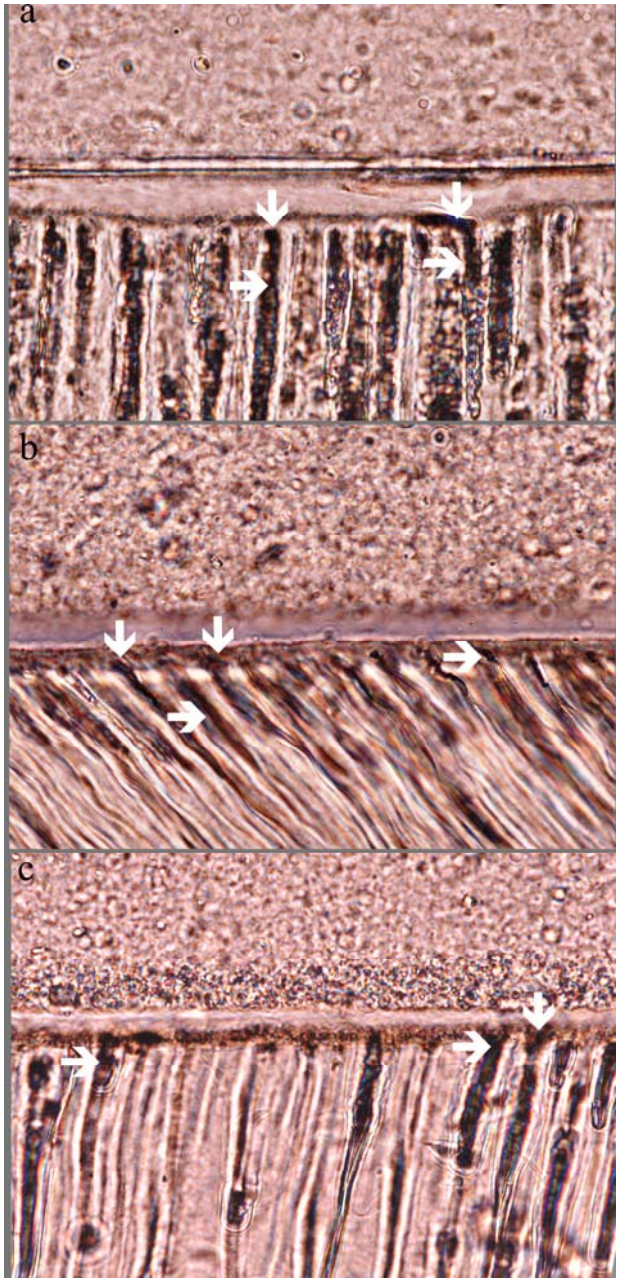

***Figure 4a-c**: Light microscope images revealing interfacial nanoleakage expression along the hybrid layers created by the tested one-step self-etch adhesives. As shown by arrows, the highest nanoleakage expression where found in AdheSE One interface (4a).*

Discussion

The results of the study support the rejection of both null hypotheses since (1) the lowest DC was associated with the highest interfacial nanoleakage expression (AdheSE One) and (2) differences were found among the tested adhesives (L Pop=iBond>AdheSE One; $p<0.05$).

The DC results of this study are comparable with previous investigations on dentin adhesive interfaces created by AdheSE One[27], Adper Prompt L-Pop [20] and iBond [28]. Conversely, when the polymerization kinetic of the pure formulation of Adper Prompt L-Pop was analyzed (not applied on dental tissues), DC was reported to be near the 40% [29]. Similar observations were found for XENO III [12, 30]. This could be explained by the lack of interaction between the acidic adhesive and the substrate. Since Adper Prompt L-Pop contains methacrylated phosphoric acid-HEMA esters [31], it needs water from the dentin to dissociate into HEMA and phosphoric acid and to reach a higher DC [32]. However this dissociation continues over time in wet conditions after curing, expediting hydrolysis [20] and increasing interfacial nanoleakage expression over time.

AdheSE One showed the lowest DC among the three adhesives. This could be related to its different chemical composition compared to the other tested adhesives. While Adper Prompt L-Pop and iBond are methacrylate-based adhesives, AdheSE One contains (meth)acrylamides characterized by an amide group (-CO-NH- or –CO-N) instead of an ester group (-CO-O-R-) to promote H-H bond formation between the carboxyl and amide groups of the monomers with the carboxyl groups of the collagen [32, 33]. Nie et al. [34] showed that the presence of copolymerized Bis-GMA within (meth)acrylamide monomer blends positively affect the polymerization. As Bis-GMA is not present within AdheSE One formulation, its conversion may be significantly reduced. Additionally, AdheSE One exhibited a

significantly prolonged reaction time, probably related to the presence of filler that can soar the stiffness of the adhesive and oppose the monomers mobility slowing the polymerization and decreasing the DC as previously reported for composite resins [35].

Furthermore, solvents influence the polymerization of dental adhesives [36, 37]. As AdheSE One is a water-based adhesive (while iBond is mainly solved in acetone), insufficient solvent evaporation may jeopardize the curing reaction of the hydrophilic methacrylamides monomers [38].

Several previous studies investigated interfacial nanoleakage expression of one-step self-etch adhesives. According to its original definition, nanoleakage in self-etch adhesives identifies regions of increased permeability within the resin matrices [13] in which presence of high concentration of hydrophilic monomers has been hypothesized [13, 7]. The results of this study support the hypothesis that a correlation between nanoleakage and suboptimal polymerization of these hydrophilic domains exists. AdheSE One showed the highest nanoleakage expression at the bottom of the hybrid layer (Fig. 4a, Tab. 2) and at the peritubular level. These areas represent the most hydrophilic regions of the hybrid layer due to their intimate contact with dentinal fluids. These findings well correlate with the micro-Raman analysis showing that the lowest DC for AdheSE One was localized within the deepest part of the hybrid layer (Tab. 2, 1 μm). The lack of polymerization of the hydrophilic portion of the simplified adhesives occurred also for Adper Prompt L-Pop and iBond since silver nitrate precipitation within the deepest part of the hybrid layer and along the resin tags was found (Fig. 4b, c).

Since all bonded interfaces created by simplified adhesives exhibit presence of bound and unbound water [38, 39], phase separation phenomena affect the polymer structure forming separated hydrophobic (Bis-GMA and

UDMA rich areas) and hydrophilic domains (poly(HEMA, [40]. Ye et al. [41, 42] using tapping mode atomic force microscopy imaging, confirmed a nano-scale phase separation for all cross-linked polymethacrylates related to BisGMA/HEMA and water concentration [42]. While HEMA is a good solvent for BisGMA in the absence of water forming homogenous resin solutions, indeed the presence of water (either blended within adhesive formulation or derived from the dentin substrate) changes the solubility within the polymer network creating poly(HEMA)-rich segments linked to water, and separating them from poly(BisGMA)-rich hydrophobic areas. Hydrophobic groups can properly cure due to the initiation of the polymerization obtained by camphorquinone (that is hydrophobic itself) together with ethyl-4-(dimethylamino)benzoate (hydrophobic co-initiator), while the separated hydrophilic domains (mainly impregnating the peritubular dentin and the water-filled dentinal tubules) may suboptimally cure due to lack of polymerization initiation. To improve the DC of simplified adhesives, the use of additional hydrophilic photoinitiators in addition to standard camphorquinone activation could be beneficial (can adhesive be cured CQ). As acid-base reaction between amines and acidic monomers has been described and phosphine oxides are not hydrolytically stable [23], the use of [3-(3,4-dimethyl-9-oxo-9H-thioxanthen-2-yloxy)-2-hydroxypropyl] trimethyl ammonium chloride has been suggested. Indeed the addition of a hydrophilic component to initiators systems reduced the detrimental effect of phase separation and improved the performances of the adhesives promoting polymerization of both hydrophilic and hydrophobic domains [42].

In conclusion, this study revealed that insufficient polymerization, probably related to inappropriate initiation of the hydrophilic domains, can be found within simplified adhesive interfaces, especially for those based on

(metha)acrylamides. Further studies should investigate the role of different compounds in order to optimize the curing process, and focus on the addition of hydrophilic initiators or extended polymerization intervals to improve the quality of the adhesive interface.

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CHAPTER 7:

THE EFFECT OF CURING MODE ON EXTENT OF POLYMERIZATION AND MICROHARDNESS OF DUAL-CURED, SELF-ADHESIVE RESIN CEMENTS.

Introduction

Because of their enhanced mechanical properties, good aesthetic qualities, and ease of handling, resin cements are widely used to lute fibre posts and composite or ceramic indirect restorations [1]. Because of the tremendous reduction in polymerization light reaching the resin cement, the so called "dual-cure" cements were developed [2]. Besides being able to photo-polymerize, these materials are characterized by their ability to undergo a totally dark, self-curing reaction in the absence of light [3]. Recently, self-adhesive dual-cure cements were introduced in order to simplify the luting procedure. Besides the photo- and self-activating components, these materials contain acidic monomers and priming agents that cause tooth demineralization and hybridize the dentin surface simultaneously, without additional surface pre-treatment. Thus, etching, priming, bonding, and cementing are combined in one single product and into one clinical step. The acidic monomers in self-adhesive cements inhibit the amine co-initiator [4], which in-turn, adversely affects polymerization of self-cured and dual-cured materials. Aromatic sulfinic acid sodium salts are sometimes added to avoid this inhibitory effect [4, 5]. On a different topic, adverse effect of hydrophilic monomers on polymerization kinetics been recently advocated as one of the main reason for the intrinsic instability of these simplified self-adhesive systems, in which highly acidic and hydrophilic monomers are usually blended in large quantities. Using differential scanning calorimetry [6, 7], microhardness testing [6] and micro-Raman spectroscopy [8], it was found that these simplified adhesives (one-step self-etch systems) exhibit lower degrees of conversion than do conventional adhesives (three and two-step etch-and-rinse and two-step self-etch systems). It was speculated that the quality of the bonds created by these self-etch adhesives would degrade faster, since the presence of greater amounts of uncured monomer expedites

water sorption and compromises hybrid and adhesive layer longevity [9]. Moreover, the low degree of conversion compromises mechanical properties [10]. With respect to this knowledge base, it may be speculated that self-adhesive cements would also demonstrate lower degrees of conversion than conventional resin-based cements [11, 12]. A variety of methods have been employed to determine the extent of resin monomer polymerization. Differential scanning calorimetry (DSC) is a direct method that analyses the extent of polymerisation based on the assumption that heat generated during resin polymerization (i.e. the exothermic heat of polymerization) is proportional to the amount of reacted monomer [13, 14, 15]. Microhardness has been shown to be a simple [16] and reliable indicator of double bond conversion, and has been used as an indirect measurement of the extent of polymerization [17, 18]. The purpose of this study was to compare effect of utilizing either the light- or self-curing modes of dual-cure adhesive cements on the extent of polymerization and the microhardness, compared to a conventional, non-adhesive dual-cure cement (control). The research hypotheses tested for each cementing system were that (1) extent of polymerization and microhardness when light-activating the dual-cured, self adhesive cements will be greater than when letting the cements self-cure only, and that (2) the extent of polymerization and microhardness of the self-adhesive cements are lower than those of the conventional dual-cure material (control), regardless of mode of polymerization activation.

Materials & Methods

Two commercial self-adhesive cements were tested: Maxcem (Batch No.5-1173,Kerr, Orange, CA, USA) and RelyX Unicem (Batch No. 224842, 3M ESPE, St. Paul,MN, USA). Additionally a conventional, dual-cured, non-

adhesive resin cement, Panavia F2.0 (Batch no. 41153, Kuraray Medical, Tokyo, Japan) was used as a control. Cement compositions are reported in Table 1.

Table 1. *Composition of the tested cements. (Supplied by manufacturer)*

Maxcem	Glycerol phosphate dimethacrylate (GPDM) Co-monomers (mono-, di- and tri-functional methacrylate monomers Water, acetone and ethanol Camphorquinone-based photo-initiator system Proprietary Redox initiators Fillers: Barium glass fillers, fumed silica, sodium hexafluorosilicate (66 wt%)	Self-adhesive one-step 2 paste/"auto-mix" dual barrel syringe
RelyX Unicem	Methacrylated phosphoric ester, dimethacrylate Pigment Substituted pyrimidine Peroxy compound Sodium persulfate Acetate Initiator Stabilizer Fillers: Glass powder, fumed silica, calcium hydroxide, (72 wt%)	Self-adhesive one-step Capsule/mechanically mixed
Panavia F2.0	**ED Primer II A&B** HEMA 10-MDP 5-NMSA Water Accelerator Sodium benzene sulphinate **Cement Only** 10-MDP, 5-NMSA, BPEDMA, BPO, dimethacrylates Dibenzoyl peroxide Sodium aromatic sulfinate N,N-diethanol p-toluidine Photoinitiators Fillers: Barium glass, silanized silica, sodium fluoride (70.8 wt%)	Dual-cure 2 paste/hand-mixed

Measuring the extent of polymerization

A "heat flux" differential scanning calorimeter (DSC) (Q10 TA Instruments, New Castle, DE, USA) was used at a constant temperature (35°C), and in a

nitrogen atmosphere to avoid oxygen inhibition. Two aluminium pans (diameter = 6.5, 1.2 mm thick) were placed on the sample platforms of the instrument. The first pan was filled with 10 mg of cement material, previously mixed and prepared following the manufacturer instructions. The second pan was left empty, acting as a thermal reference. An aluminium cover with a 8 mm diameter quartz window was employed to allow light to pass through and to permit specimen photo-curing inside the DSC chamber. During photo-polymerization, the halogen curing-unit tip (Elipar 2500, 3M ESPE, St. Paul, MN, USA) was positioned 5 mm from the target pan using a custom made support, which allowed full irradiation of the adhesive-containing pan. Polymerization of the dual-curable cements was performed under two different conditions:

1. The specimen was immediately irradiated for 20s, after which the DSC was maintained isothermally for 2 h;
2. The specimen was not exposed to the curing light, and the DSC was maintained isothermally for 2 h to detect heat derived only from the self-curing chemical reaction.

A second DSC analysis was performed in order to evaluate the amount of monomer that did not react, but that still potentially could. For this analysis, the same specimens in groups 1 and 2 were irradiated with an additional 2 min of light exposure. A third irradiation (2 min) showed that no supplementary reaction occurred either in light-cured and self-cured specimens, thus the heat obtained from this last irradiation represented the heat generated only by the light unit and was subtracted from the previous DSC curves [6], while the heat of reaction obtained from the first 2 min exposure could be considered as the maximum reaction attainable for each

cement. The exothermic heat of reaction due to cement polymerization was evaluated as the integral of heat flux detected from the DSC over the time of observation, as described from the following equation:

$$H_p = \int_0^t W(t)dt \qquad (1)$$

where

Hp = exothermic heat from the polymerization reaction

W(t) = heat flux detected from the DSC

t = time

dt = time differential

In such a way, the extent of polymerization (E_p) after light-cure or self-cure was evaluated as the relative percentage of the maximum reaction attainable for each cement according to the following equation:

$$E_p = \frac{H_{1p}}{H_{1p} + H_{2p}} \cdot 100 \qquad (2)$$

where H_{1p} represents the exothermic heat detected in the first calorimetric analysis and H_{2p} represents the value obtained in the second DSC analysis.

Microhardness testing

Vickers microhardness measurements (VMHT, Leica Microsystems, Milano, Italy) were obtained using a 50g load applied for and 15s. New cement specimens (N=10 for each luting agent for each group) were prepared and polymerized as described in the above DSC analysis section. Following that treatment, the microhardness of the exposed surface was taken at three randomized locations. These values were averaged, reporting a single Vickers hardness for each specimen.

Statistical analyses

A two-way ANOVA was used to evaluate the DSC and microhardness data. The two variables were resin cement and mode of cure. Differences between groups were identified using Tukey's multiple comparison test at $\alpha = 0.05$.

Results

Differential scanning calorimetry

Figures 1a-b represent DSC curves of the two modes of cure (photo- and selfcure) of the three tested cements. For all materials light-cure produced an initial high and narrow peak rapidly decreasing after light exposure, while in the self-cured specimens the peak was lower and broader. Extent of polymerization of all cements was higher when both the photoactivated and self-activated modes were used ($p<0.05$; Table 2) in comparison to the self-cure polymerization alone. No difference was found among the three cements polymerized when using the light-activation mode. RelyX Unicem and Panavia F2.0 polymerized using only the self-cure mode showed lower values than Maxcem ($p<0.05$;Table 2).

Table 2. *Extent of polymerization (mean ± SD) for the tested materials in the two different curing modes.*

Cement	Initial 20s light 2h self-cure	2h self-cure
Maxcem	54.6 ± 1.9^{a}	40.5 ± 3.8^{b}
RelyX Unicem	54.4 ± 1.4^{a}	31.7 ± 1.4^{c}
Panavia F2.0	48.6 ± 1.1^{a}	31.4 ± 1.8^{c}

Values having similar superscript letter are not significantly different
N = 10/group

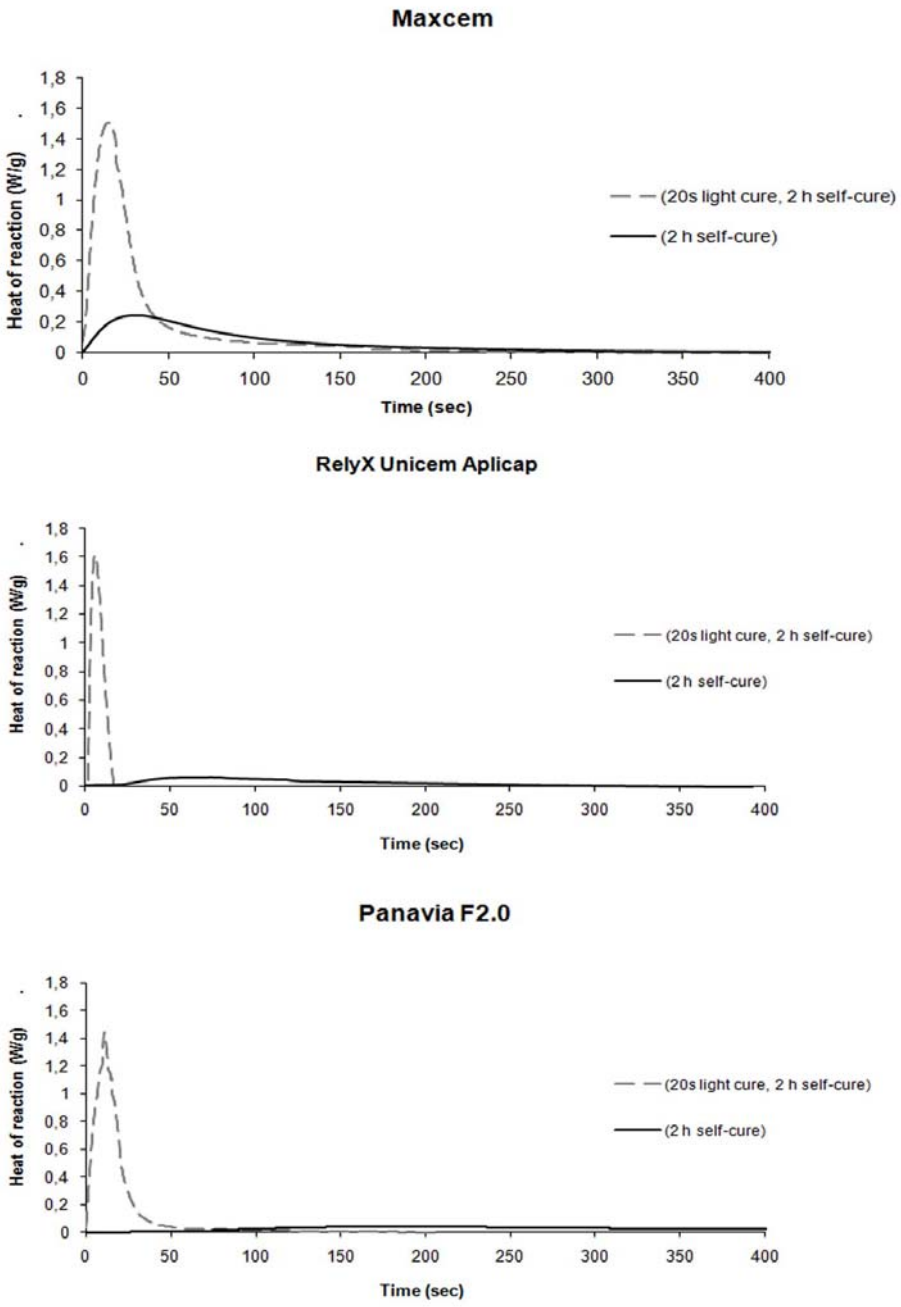

Fig. 1*: Representative DSC curves of the cements tested in different curing modes. A narrow and high peak was obtained with light-cure mode, while self-cured specimens showed a smaller and broader peak related to the slower speed of reaction. 99x164mm (300 x 300 DPI)*

Microhardness evaluation

Microhardness values of all cements was higher when the light activation of the dual cured material was utilized ($p<0.05$; Table 3). Panavia F2.0 showed the lowest values in the light-activation mode, while RelyX Unicem showed the lowest microhardness in the self-cure mode ($p<0.05$; Table 3).

Discussion

The first hypothesis stating that extent of polymerization of all tested cements would be higher when the dual-cure systems were initially light-activated compared to self-cure mode was accepted. These observations were in accordance with previous data [11, 12, 19, 20] showing that self-cure polymerization leads to lower degrees of conversion values than does utilizing the light-curing mode. As the tested cements are marketed for cementation of fixed prostheses, inlays, or endodontic posts (i.e. clinical conditions in which light activation is dramatically attenuated or even absent), the results of this study suggest that all these cements may be inadequately polymerized if no light activation can be performed.

The current study is the first to report on the extent of polymerization of Maxcem. On the other hand, two previous studies tested the degree of polymerization of RelyX Unicem and Panavia F2.0 [1211, 12]. When testing these products in self-cure mode, lower degrees of polymerization were found than those than those obtained in the current. However, the duration of time the materials were allowed to self-cure in the present study was longer (2 h) than that used in the other studies cited above (15 min). Conversely, those same studies described higher polymerization values of RelyX Unicem and Panavia F2.0 when polymerized in the light-cure mode than those obtained in the present investigation. This finding may be explained by the reduced irradiation time (20s) used in the current study (a value that was in

accordance with manufacturers' instructions) compared to the 40s duration used by the previous investigations above. The extent to which monomer interacts to form polymers is expected to affect the properties of dental resins. Several studies report a direct correlation between the degree of monomer conversion and the resulting mechanical properties of dental restorative resins [17, 18, 21]. In the current research, materials showed lower microhardness values when left to self-cure without light activation. This finding has also been reported by others [22] Microhardness data are comparable only within the same resin system [23], as they are not linearly correlated to the degree of cure if compared across different materials. Thus, the data derived from the present study were useful in comparing the polymerization achieved with different curing modes in a particular cement [24]. The results supported the hypothesis that the light-activation mode of all tested dual-cured resin cements improves their microhardness by increasing the extent of polymerization compared to using only the self-cure polymerization mode. In addition, extent of polymerization of the self-adhesive cements was comparable to that of the conventional dual-cure material, both when using light activation and when relying totally on the self-cure mode. Thus, the second tested hypothesis was rejected. Deactivation of the tertiary amine used in the chemically-cured resins by acidic resin monomers accounts for the reported incompatibility between acidic monomers and chemically-cured materials. Addition of aromatic sulfinic acid sodium salts, however, may counteract this negative effect and allow an adequate degree of conversion of self-cured materials, even in presence of acidic monomers [5]. Among the tested materials, RelyX Unicem contains sodium persulfate, which may explain the comparable results with the non-acidic, dual-curing resin cement control (Panavia F2.0), whereas no information is provided by the manufacturer for Maxcem related

to presence of these salts in its formulation. Data from the current work were not in accordance with previous studies [11, 12] that revealed lower degrees of conversion for RelyX Unicem than for Panavia F2.0 using Fourier transformed infrared spectroscopy. It should be stressed that the current work utilized DSC instead. The previous studies cited looked only at reaction of the monomeric C=C units, whereas DSC is insensitive to the type of functional group undergoing the reaction process. Thus, any other types of simultaneous, secondary reactions, such as a glass ionomer one, would be detected by the DSC, but unless investigators specifically monitored that reaction during IR analysis, it would be missed. Indeed, an additional acid-base setting reaction has been reported for RelyX Unicem, since its acidic monomers are claimed to chemically interact with the basic inorganic fillers of the cement [25]. Similar information is not provided for MaxCem. Previous observations reported that Maxcem is characterized by a poor bonding ability to both enamel and dentin, independently [26, 27] and that the product has a limited potential for removal or modification of the smear layer and for resin infiltration into the underlying dentin [26]. On a similar note, RelyX Unicem has been shown to produce an inadequate hybrid layer [28, 29, 30] and a low bond strength to enamel [28, 31, 32], while its bond strength to dentin seems to be comparable to other resin cements [31, 32]. These findings suggest that the etching potential of self-adhesive cements may be insufficient [26]. Low bond strength may also depend on the incorporation of acidic monomers in these self-adhesive cements, which may have interfered with formation of an optimal cross-linked polymer network. Thus, even though the overall extent of polymerization might have been high, the fragility of the polymer network formed would have conferred low mechanical properties and inadequate bond strength results. Under this perspective, use of extent of polymerization as a predictor of the

properties of resin cements, and thus of its potential for clinical success, may be not totally unequivocal. In conclusion, further studies are needed to evaluate if extent of polymerization, as measured using DSC, of the recently introduced self-adhesive cements may affect their bonding ability and influence their long-term stability in the oral environment.

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CHAPTER 8:

ENAMEL AND DENTIN BOND STRENGTH FOLLOWING GASEOUS OZONE APPLICATION

Introduction

Conventional removal of carious tissue and cavity preparation procedures do not guarantee the complete elimination of oral cariogenic bacteria eventually entrapped within the dentin tubules or the smear layer that may induce secondary caries or pulpal inflammation.[35,39] For this reason the use of ozone gas has recently been supported to improve cavity disinfection prior to restorative procedures.[39]

The antibacterial property of ozone is due to its strong oxidizing activity[10,11] that involves rupture of the cell membranes and destruction of intracellular components through an oxidation reaction process.[30,40] It was hypothesized that the elimination of cariogenic bacteria[31] and the demineralization process promoted by ozone could arrest or even reverse tooth caries development. In addition, the application of gaseous ozone was proven to be painless and atraumatic.[17,40] For all the above-mentioned reasons gaseous ozone has been proposed as an alternative to the traditional "drill & fill" approach in the treatment of small cavities or as a disinfectant of the cavity surface in conventional caries removal techniques. [3,4, 5,6,7,,24,25,26, 34,37]

Although the use of gaseous ozone could be advantageous to remove residual bacteria, the effect of ozone gas on dental bonding procedures is still unclear as previous studies demonstrated that oxygen and other oxidant agents (such as whitening agents) have a negative influence on bond strength of dental adhesives. Decreased shear and microtensile bond strength values have been reported by testing adhesion after the application of whitening peroxides at different concentrations and formulations.[1,33] Reduction in adhesion strength has been attributed to residual active oxidant chemicals within the dental tissues which may interfere with monomers polymerization.[8,13] Conversely, other studies supported the hypothesis that

oxygen induces alterations of the mechanical properties of enamel and dentin that may be responsible of bond strength reduction.[42] In particular, denaturation and destabilization of the quaternary structure of proteins constituting the fibrillar network of the dentin organic matrix has been suggested as responsible of decreased bond values.[1,2,28,33]

While a previous study revealed no effect of ozone gas on bonding of a three-step etch-and-rinse system, [39] up to our knowledge no data are available for self-etch systems and in particular for the most simplified one-step self-etch systems whose degree of conversion has been shown to be particularly susceptible to residual oxygen radicals present either on dentin[13] and enamel[8] after whitening procedures.

The purpose of this study was to challenge the influence of ozone gas on bond strength of a two-step and a one step self-etch adhesive system. The hypothesis tested was that gaseous ozone negatively interferes with enamel and dentin bond strength.

Materials & Methods

Shear bond strength test on bovine enamel

Ninety-six recently extracted bovine incisors were selected, cleaned, stored in 0.5% chloramine T solution at 4°C and used within one month after extraction. The vestibular surface of each tooth was ground under water cooling with a 180 grit diamond paper to produce a flat surface covered by a clinically relevant smear layer. The root of each tooth was then embedded in a cylindrical mold (diameter 1 cm) filled with self-polymerizing poly-methyl-methacrylate resin (Rèsine Mécaprex KM-U, Presi, Grenoble, France) with the vestibular surface perpendicular to the horizontal plane. Specimens were randomly and equally divided into 4 treatment groups

(N=24, Table 1). Specimens of Group 1E (Table 1) were treated with gaseous ozone application for 80 s. Ozone gas was delivered on the vestibular surface using the HealOzone system (KaVo, Biberach/Riss, Germany) keeping an hermetic seal with the silicone cup. Disinfected enamel surfaces were then bonded with Clearfil Protect Bond (Kuraray Medical, Tokyo, Japan), a two-step self-etch adhesive system, in accordance with manufactures' instructions. In brief the self-etching/primer of Clearfil Protect Bond was applied on enamel surface for 20 s and uniformly spread by a gentle air-stream. The bonding agent was then applied with a continuous brushing technique for 20 s, blown to a thin layer and polymerized for 10 s with Optilux 501 (SDS, Kerr, USA) that delivered an irradiation of 600 mW/cm^2. Specimens of Group 2E (Table 1) were similarly bonded with Clearfil Protect Bond without preliminary ozone treatment (control).

Specimens of Group 3E (Table 1) were submitted to the ozone treatment (similarly to Group 1) and then bonded with XENO III (Dentsply DeTrey, Konstanz, Germany), a one-step self-etch adhesive system, in accordance with manufacturers' instructions. In brief XENO III was applied on the enamel surface after mixing its two components for approximately 5 s, then after 20 s the adhesive was gently air-dried and light cured for 10 s with an halogen curing light (Optilux 501). Specimens of Group 4E (Table 4) were bonded with XENO III without preliminary exposure to the ozone treatment (control).

A composite resin cylinder (Filtek™ Z-250, 3M ESPE, Dental Products, USA) was built on the top of the bonded surface using a 3 mm-thick cylindrical silicone mold with a diameter of 4 mm in two consecutive increments that were individually polymerized for 40 s (Optilux 501). All specimens were then subjected to the shear bond test with a universal testing

machine (Galdabini Sun 500, Varese, Italia), using a tangential blade applied on the adhesive interface at a crosshead speed of 1 mm/min. Specimens were stressed until failure and load per area unit was calculated. Failure mode of each specimen was analyzed under a stereomicroscope (Zeiss Stemi 2000-C, Carl Zeiss Jena GmbH, Zeiss Group, Jena, Germany) and classified as cohesive or adhesive.

Microtensile bond strength test on human dentin

Forty non carious extracted human molars were selected after informed consent form for research purpose had been signed by patients under a protocol approved by the University of Trieste (Italy). The teeth were stored in 0.5% chloramine T solution at 4°C and used within one month after extraction. Occlusal enamel and root dentin were removed perpendicular to the long axis of each tooth by a low speed diamond saw under water irrigation (Micromet, Remet, Bologna, Italy) and a standardized smear layer was created on the exposed coronal dentin with 180-grit silicon carbide paper. Specimens were then randomly and equally divided in 4 groups (N=10). Specimens of Group 1D (Table 1) were treated with gaseous ozone application for 80 s using the HealOzone system and then bonded with Clearfil Protect Bond following the manufactures' instructions. Specimens of Group 2D (Table 1) were similarly bonded with Clearfil Protect Bond without the preliminary ozone treatment (control). Specimens of Group 3D and 4D (control) were bonded with XENO III with or without exposure to ozone treatment respectively.

A 4 mm thick build-up of composite resin Filtek Z250 was prepared and incrementally (2 mm thick layers) polymerized on the dentin surface of all specimens. Each composite layer was photo-polymerized for 20 s with Optilux 501. Sticks with a surface area of approximately 0.9 x 0.9 mm and a

standardized height of 8 mm were created from each specimen, by means of a low-speed saw under water irrigation using a microtensile non–trimming technique. The dimension of each stick was individually measured with a digital caliper, to the nearest 0.01 mm, and the bonding area was calculated for subsequent bond strength evaluation. The specimens were observed under a stereomicroscope (Zeiss Stemi 2000-C, Carl Zeiss Jena GmbH, Zeiss Group, Jena, Germany) to avoid the inclusion of sticks containing residual enamel. Premature failures of each specimens were recorded. Sticks were then attached to a modified jig for microtensile testing and stressed under tension until failure with a simplified universal testing machine (Bisco Inc., Schaumburg, IL, USA) at a crosshead speed of 1 mm/min. Failure modes were evaluated under stereomicroscope (Zeiss Stemi 2000-C) and classified as cohesive or adhesive. The number of prematurely debonded sticks per group during specimen preparation was also recorded.

Statistical analysis

Since data were normally distributed (Kolmogorov–Smirnof test), a two-way ANOVA was applied to investigate statistical differences (variables: use of ozone and type of adhesive). Microtensile bond strength data were analyzed either considering or not premature failures as 0 bonds.

***Table1**: Composition of dental bonding systems tested in the study.*

Adhesive	Composition	Mode of application
Clearfil Protect Bond	*Etching/Primer:* - 2-hydroxyethylmethacrylate (HEMA) - Hydrophilic dimethacrylate - 10-methacryloyloxydecyl - Dihydrogen phosphate - 12-methacryloyloxydodecylpyridinium - Bromide - Water *Bond*: - 10-methacryloyloxydecyl dihydrogen phosphate (MDP) - Bis-phenol A diglycidylmethacrylate (Bis-GMA) - 2-hydroxyethyl methacrylate (HEMA) - Hydrophobic dimethacrylate - Camphorquinone - N,N-diethanol-p-toluidine - Silanated colloidal silica - Surface treated sodium fluoride	*Group 1 Ozone + adhesive* *Group 2 Adhesive only*
XENO III	*Liquid A*: - 2-hydroxyethyl methacrylate (HEMA) - Butylated hydroxy toluene (BHT) - Highly dispersed silicon dioxide - Purified Water - Ethanol *Liquid B*: - Phosphoric acid modified methacrylate resin - Mono fluoro phosphazene modified methacrylate resin - Urethane dimethacrylate resin - Butylated hydroxy toluene (BHT) - Camphorquinone - Ethyl-4-dimethylaminobenzoate	*Group 3 Ozone + adhesive* *Group 4 Adhesive only*

Results

Shear bond strength on bovine enamel

Means and standard deviations of shear bond strength expressed in MPa are shown in Table 2. Ozone treated specimens revealed no statistical difference with controls for both the tested adhesive systems ($p>.05$). Clearfil Protect Bond showed significantly higher bond strength values than Xeno III ($p<.05$). The predominant failure mode in both groups was adhesive, with fractures occurred between resin and enamel (Table 2).

Table 2: *Mean and standard deviation of shear bond strength values and failure modes obtained on bovine enamel. No statistical difference was found between 1E vs 2E ($p>.05$) and between 3E vs 4E ($p>.05$), thus no effect was found by ozone application on bond strength on enamel. Statistical difference was found between the adhesives as Clearfil Protect Bond > Xeno III ($p<.05$)*

Group	Bond strength	Failure modes	
		Adhesive	Cohesive
1E: Clearfil Protect Bond + ozone	17.26±4.76[a] MPa [24/0]	14	10
2E: Clearfil Protect Bond control	18.61±3.35[a] MPa [24/0]	24	0
3E: Xeno III + ozone	11.86 ±3.62[b] MPa [24/0]	20	4
4E: Xeno III control	12.25±3.03[b] MPa [24/0]	22	2

† Values of shear bond strength are mean±standard deviation [number of intact specimens tested/premature failures]. Difference between groups with the same superscripts are not statistically significant ($p>.05$).

Microtensile bond strength test on human dentin

Means and standard deviations of microtensile bond strength expressed in MPa are shown in Table 3. No statistical significant difference was found in

bond strength values of ozone-treated specimens vs controls for both adhesive systems (p>.05) either considering or not 0-bond values. No significant differences were observed between Clearfil Protect Bond and Xeno III (p>.05).

In both groups fractures were observed mostly between resin and tooth (adhesive failure) (Table 3).

Table 3*: Mean and standard deviation of microtensile bond strength (with or without including pre-testing failures as 0 bond values) and failure modes obtained on human dentin. No statistical difference was found between 1D vs 2D (p>.05) and between 3D vs 4D (p>.05), thus no effect was found by ozone application on bond strength on dentin. Statistical difference* was not found between the adhesives, i.e. Cleafil Protect Bond vs Xeno III, p>.05.

Group	Bond Strength		Failure modes	
	With 0-bonds	Without 0-bonds	Adhesive	Cohesive
1D: Clearfil Protect Bond + ozone	27.7±10.6 MPa	28.9±9.2 MPa	252	14
	[278/12]			
2D: Clearfil Protect Bond control	29.3±11.0 MPa	30.5±9.4 MPa	262	18
	[287/7]			
3D: Xeno III + ozone	26.3±11.7 MPa	27.6±10.4 MPa	248	14
	[275/13]			
4D: Xeno III control	29.1±8.6 MPa	29.0±8.7 MPa	268	4
	[288/16]			

† Values of microtensile bond strength are mean±standard deviation [number of intact specimens tested/premature failures].

Discussion

This study was conducted by means of different adhesion test methods on bovine or human substrates due to the known difficulties related to the use of microtensile bond strength on human enamel. Numerous investigation pointed out that the microtensile bond strength test is superior to shear bond test in assessing the adhesive force at the adhesive interface.[14,15] However, while microtensile bond strength test is greatly effective in investigating the dentin/adhesive interface, the use of microtensile test to investigate the enamel/adhesive interface is not widely accepted due to the inherent fragility of the enamel substrate in the small cross-sections of microtensile specimens, in particular in the non-trimming technique.[22] For this reason shear bond strength test is still considered one of the most used technique in adhesion testing on enamel.

In addition, in shear bond strength testing a quite wide and flat tooth surface to build the composite specimen is needed for the shear force application.[12,36] However, human teeth are becoming difficult to obtain due to preventive dentistry advances and reduced need for extraction for periodontal reasons.[29] For this reason, due to the size and availability, bovine incisors are often preferred for shear bond strength tests, even though some concern about whether data obtained from bovine teeth can be applied to human teeth has been raised.[12] Nevertheless, several studies demonstrated that there is no significant difference between bovine and human enamel in bond testing.[20,27,29]

The results of this study showed that the tested hypothesis was rejected as no difference was found between enamel shear bond and dentin microtensile bond strength of both the two-step and the one-step self-etch adhesive if

gaseous ozone was used prior to application of the bonding agent (Tables 2,3).

The presence of oxygen can determine a delay or even an inhibition of the polymerization process that may adversely affect the bond strength of dental adhesives.[8,13,18,21,32,38] [32] Previous investigations revealed that sub-optimal polymerization of the polymeric matrices occurs by residual free radicals if oxidant agents such as hydrogen peroxide and sodium hypochlorite are used on dental substrate.[8,13,19,28,41,43-46] Additionally, hydrogen peroxide-based bleaching agents were shown to jeopardize bond strength determining structural alterations of the enamel and dentin substrates.[1,2,28,33]

Due to its oxidative potential and reactivity, it can be hypothesized that gaseous ozone affects bond strength of adhesive systems in the same way of hydrogen peroxide, thus both by modifying the polymerization kinetic (thus reducing the final degree of conversion of the polymer) and affecting the structural integrity of the dental substrate. As etch-and-rinse adhesives are applied on a demineralized substrate due to preliminary application of phosphoric acid followed by extensive rinsing with water, we may speculate that the etching removes both superficial mineralized components and residual oxidants. Conversely, as the bonding agent of self-etch systems is applied directly on the oxidized substrate since no rinsing occurs after the application of the self etching/primer agent (i.e. etch-and-dry approach),[9] we may speculate that self-etch systems are more susceptible to residual oxygen eventually incorporated within the smear layer and smear plugs. Conversely, the results of this study showed that no significant bond reduction occurs if self-etch systems are applied either on enamel or dentin treated with ozone gas (Tables 2,3). These data are in agreement with those obtained by Schmidlin et *al.*[39] using a three-step etch-and-rinse adhesive system, thus no different susceptibility to the ozone pre-treatment can be postulated between

the different classes of dental adhesives. As bond strength seems to be unrelated to the preliminary ozone treatment, different hypothesis can be formulated.

Since ozone gas is characterized by high instability and reactivity due to the presence of a third oxygen atom weakly bonded to the other two atoms, gaseous ozone is unable to maintain its chemical stability for a long time and as it meets unsatured organic molecules it releases the unstable oxygen atom.[40] Proteins, carbohydrates and phospholipidic compounds of dental plaque are ideal substrates for gaseous ozone's oxidizing action due to their unsaturated molecular structure.[4,24] Once delivered to the carious cavity, gas completely decomposes reacting instantaneously with organic compounds,[4, 23,24] thus we may speculate that only a few or even no gaseous ozone residuals are present on the dental substrate during bond application. For this reason we may hypothesize that no inhibition of the photo-polymerization process occurs.

Despite its instability, a second hypothesis that may explain the relative unsusceptibility of bonding to ozone can be related to the very short ozone gas application time if compared to bleaching agents, in which reverse of oxidant effect is achieved only after 2 weeks from the application.[8,13,19,41,43-45] While bleaching requires multiple applications, gaseous ozone is applied once for 80 s (maximum contact time recommended by manufacturer is 120 s) thus minimal residual oxygen can be retained by the cavity surface.

A third hypothesis can accept presence of minor ozone gas residuals eventually entrapped within the smear layer, but functionally not influencing bonding. In fact these residual active chemicals could interfere with the adhesive polymerization process through two different and opposite reactions. Likewise other oxidants, gaseous ozone can combine with free radicals forming less reactive species or it can also react with the

propagating methacrylate monomers.[21,32] This could cause a premature chain termination with formation of shorter polymeric chains, but also involve a greater number of free radicals available for the reaction with still vacant monomers. This may result in a greater degree of conversion without any adverse effects on polymerization and bond strength.

In relation to possible substrate modifications induced by gaseous ozone, it was shown that, differently from bleaching agents, gaseous ozone does not modify enamel and dentin structure.[16] A 40 s ozone gas application showed no influence on enamel physical properties, such as microhardness, contact angle and etching resistance or free surface energy that would in turn influence bonding.[16] This implies that ozone dehydration on enamel does not impair free surface energy nor affect bond strength. On the contrary, the dehydration of dentin caused by ozone could produce the collapse of collagen fibrils decreasing dentin wettability by adhesive systems, but at the same time ozone may also oxidize and degrade organic compounds of the dentinal matrix, opening dentinal tubules and promoting the diffusion of resin monomers into the dentin structure.[3,26]

Additionally it should be considered that the operative protocol recommended by the HealOzone manufacturer suggests the application of a reductant solution on the treated surface. This solution is formulated to neutralize bacterial acids and provide minerals and fluoride to promote remineralization. The rationale of the application of this solution on the oxidized substrate is related to previous observations[43] that the application of 10% sodium ascorbate following bleaching treatments allows to reverse the negative effect of oxidizing agents. To avoid the influence of this factor on bonding and to analyze the effect of the gaseous ozone application only, in the present study the reductant liquid was purposely not used. However,

Schmidlin *et al.*[39] demonstrated that the application of this reductant solution reduced bond strength if compared to specimens treated with ozone only.

Further research is essential to confirm that gaseous ozone has no effect on immediate bond strength on enamel and dentin and that the ozone gas does not interfere with or improve the stability of the adhesive interface over time. In addition further *in vivo* studies should be carried out to better investigate the real disinfecting potential and oxidant ability of gaseous ozone in dentistry.

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CHAPTER 9:

SUMMARY, CONCLUSIONS AND FUTURE DIRECTIONS

Summary

Contemporary restorative techniques are based on the adhesive properties of tooth colored resin-based materials. Despite both sealing and bonding abilities of dental adhesives have been improved over years the adhesive/dentin interface remains the weakest area of tooth-colored restorations and is the key factor to guarantee longevity for clinical durability of composite dental fillings [1]. The bonded interface is exposed to the oral environment and phenomena such as nanoleakage, water tree formation, degradation of the organic matrix by host enzymes, and consequently marginal discoloration, secondary caries and loss of integrity of the restorations, can occur [2,3]. The hybrid layer is formed when an adhesive resin penetrates a demineralized or acid-etched dentin surface and infiltrates the exposed collagen fibrils. If this ideal was achieved, the collagen fibers of the demineralized dentin would be completely embedded in the adhesive resin and thus protected from the hydrolytic action of oral or dentin fluids. Thus this hybrid zone consists of a complex composite structure, highly sensitive both to the demineralization process and to specific characteristics of the bonding adhesive system.

Manufacturers have attempted to address the limitations associated with dentin bonding by eliminating as many steps as possible in the bonding protocol. Theoretically, this approach increases the efficiency of the procedure and reduces technique sensitivity. These trends are reflected in the introduction of all-in one, single-step adhesive and cement systems; the increased concentration of acidic resin monomers in these systems allows for simultaneous etching and priming (and sealing in case of cements) of the prepared dentin surface.

The study described in this thesis aimed to provide an overview of the new trends in dental adhesion, as well as to elucidate how factors related to chemical composition and to polymerization may impair or improve the bond strength.

It is well known that high DC of resin polymers corresponds to improved mechanical properties of light cured resin materials [4], thus the extent and rate of the polymerization of functional vinyl monomers were analyzed in this study. Raman microspectroscopy is an effective tool for investigating the chemistry of these interfaces because it does not rely on homogenization, extraction, or dilution, but rather each structure is analyzed *in situ* with a high spatial resolution.

In chapter 5 the degree of conversion (DC) of the adhesive interfaces created by Filtek Silorane Adhesive were analyzed compared to a competitor (Clearfil SE Bond) using micro-Raman spectroscopy. Silorane [5], a low-shrinkage, tooth-colored restorative material based on siloxane and oxirane functional moieties has been recently introduced in the dental market with a specific bonding system. While the siloxane determines the highly hydrophobic nature of the siloranes, the cycloaliphatic oxirane functional groups are responsible for lower shrinkage when compared to methacrylate-based composites. Oxiranes, which are cyclic ethers, polymerize by a cationic ring-opening mechanism, while methacrylates polymerize via a free-radical mechanism [6]. The adhesive system of the Filtek Silorane (3M ESPE) is a methacrylate-based, two-step self-etch system (Silorane Adhesive System, 3M ESPE), consisting of a polymerizing self-etching primer and an adhesive resin containing a hydrophobic dimethacrylate monomer. This adhesive resin promotes association with the hydrophobic oxirane-based composite. In this study the adhesives were applied on human dentin in accordance with manufacturer's

instructions. Specimens were cut to expose the bonded interfaces to the micro-Raman beam. Raman spectra were collected along the dentin/self-etching primer/adhesive interface at 1µm intervals. The relative intensities of bands associated with mineral and adhesive components within the bonded interface were used to detect monomer penetration into the dentin matrix and to calculate the degree of conversion. Data were statistically analyzed with two-way ANOVA. Filtek Silorane Adhesive showed a higher DC than Clearfil SE Bond in the hybrid layer, but a lower DC in the adhesive.

In chapter 6 the hybrid layer created by three one-step self-etch adhesives was characterized *in situ* using Raman micro-spectroscopy and the DC was correlated with nanoleakage expression. Dentin disks were bonded with AdheSE One, Adper Prompt L-Pop or iBond. 2 mm-thick composite build-ups were layered on the polymerized adhesive surfaces and the adhesive/dentin interfaces were exposed to a micro-Raman beam. The adhesive penetration was calculated using the relative intensities of bands associated with mineral and adhesive and the degree of conversion (DC) was evaluated. Data were statistically analyzed with two-way ANOVA. Interfacial nanoleakage expression was evaluated on the same specimens. AdheSE One showed higher nanoleakage expression and lower DC than iBond and Adper Prompt L-Pop.

Because of their enhanced mechanical properties, good aesthetic qualities, and ease of handling, resin cements are widely used to lute fibre posts and composite or ceramic indirect restorations [7]. Recently, self-adhesive dual-cure cements were introduced in order to simplify the luting procedure: no additional surface pre-treatment is needed because etching, priming, bonding, and cementing are combined in one single product and into one clinical step. Differential scanning calorimetry (DSC) is a

convenient tool for the analysis of the extent of polymerization (EP) of dental resin monomers by measuring the calorimetric value of the exothermal peak generated during polymerization, that is proportional to the amount of reacted monomer [8]. Microhardness has been shown to be a simple [9] and reliable indicator of double bond conversion, and has been used as an indirect measurement of the extent of polymerization. Small amounts of two dual-cured, self-adhesive resin cements (Maxcem and RelyX Unicem) and a conventional, dual-cured resin cement as control (Panavia F2.0) were polymerized within the DSC chamber. Ten specimens were light-cured immediately (20s) and left undisturbed for 2h and ten additional specimens were left to self-cure in the dark for 2h. Following DSC analysis, Vickers microhardness of the specimens was measured. For each test parameter, data were analyzed with a two-way ANOVA and the Tukey post-hoc test. %EP and microhardness of all cements were higher when the light-cure mode of dual-activation was used ($p<0.05$) instead of only self-curing. No significant difference in %EP was found between either self-adhesive cement and the control using either the light- or self-curing modes.

Our research focused also on a technique that can indirectly improve the longevity of dental adhesion: the use of ozone as a disinfectant pretreatment. Conventional removal of carious tissue and cavity preparation procedures do not guarantee the complete elimination of oral cariogenic bacteria eventually entrapped within the dentin tubules or the smear layer that may induce secondary caries or pulpal inflammation [10]. For this reason the use of ozone gas has recently been supported to improve cavity disinfection prior to restorative procedures [11]. The antibacterial property of ozone is due to its strong oxidizing activity that involves rupture of the cell membranes and destruction of intracellular components through an

oxidation reaction process. The purpose of this study was to evaluate the effects of gaseous ozone application on enamel and dentin bond strength produced by two self-etch adhesive systems (Clearfil Protect Bond and Xeno III). Shear bond strength test was conducted to assess adhesion on bovine enamel (Protocol 1), while microtensile bond strength test was performed on human dentin (Protocol 2). Specimens were stressed until failure occurred and failure modes were analyzed. Shear bond and microtensile bond strength data were analyzed using two-way ANOVA and Tukey's post hoc. No statistical differences were found between ozone treated specimens and controls, both on enamel and dentin irrespective of the tested adhesive. Clearfil Protect Bond showed higher bond strength to enamel than Xeno III, irrespective of the ozone treatment.

Conclusions

Within the limitations of this study the following conclusions may be drawn:

1. Since the polymerizable self-etch primer of the Silorane Adhesive may mimic a one-step, self-etch adhesive, the application of a hydrophobic coating may provide additional stability to the bonded interface reducing the amount of water uptake over time.
2. Increased nanoleakage expression was associated with the adhesive (AdheSE One) showing the lowest DC. A correlation between high nanoleakage and degree of conversion was confirmed. Low DC may affect the quality and the long-term stability of the adhesive interface due to the elution of unreacted monomers forming a porous and highly permeable hybrid layer.
3. No differences were found between the conventional dual-cure cement and the self-adhesive cements.

4. The use of ozone gas had no influence on immediate enamel and dentin bond strength.

Future directions

Manufacturers have attempted to address the limitations associated with dentin bonding by eliminating as many steps as possible in the bonding protocol. Theoretically, this approach increases the efficiency of the procedure and reduces technique sensitivity. However, presently, the clinical and in vitro behavior of self-etch adhesives is not comparable to that of etch-and-rinse agents. The future research directions should investigate how improve their performances.

The Raman microspectroscopic technique offers distinct advantages, including minimal sample preparation and both qualitative and quantitative analysis at ~1-μm spatial resolution. The nondestructive nature of this analysis also allows investigation of the same specimen using complementary techniques. This technique can be considered as a valuable mathod for future *in vitro* and *in vivo* investigations of the dentin/adhesive interface.

In this *in vitro* study, results allowed a preliminary evaluation of the interface created by self-etch systems. More investigations are needed to assess their mechanical properties and clinical studies are necessary to support laboratory data, in order to point out if simplified systems may be really provide restoration longevity.

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RIASSUNTO, CONCLUSIONI E DIREZIONI FUTURE

Riassunto

La moderna odontoiatria conservativa si fonda su tecniche adesive rese possibili dalle proprietà di materiali da restauro polimerici a base resinosa. Sebbene le capacità di legame ai tessuti dentali abbiano dimostrato notevoli progressi qualitativi nel corso degli anni, l'interfaccia tra dentina ed adesivo rimane il punto debole dei restauri in composito e rimane tuttora il fattore chiave per garantire stabilità e longevità ai restauri conservativi [1].

Poichè l'interfaccia adesiva è esposta all'ambiente orale, si possono facilmente verificare fenomeni quali nanoleakage, formazione di water tree e degradazione della componente organic da parte di enzimi endogeni, portando a discolorazione dei margini del restauro, carie secondaria e perdita di integrità del restauro stesso [2,3].

Lo strato ibrido si forma nel momento in cui la resina adesiva penetra nella dentina demineralizzata o mordenzata, infiltrando le fibrille collagene esposte. Nella condizione ottimale, tali fibrille sono completamente permeate dall'adesivo e quindi protette dall'azione idrolitica dei fluidi orali o dentinali. Data la natura stessa dello strato ibrido, che consiste in una struttura complessa in cui materiale a base metacrilica e tessuto dentale si compenetrano, esso è notevolmente influenzato sia dalle modalità di demineralizzazione del substrato, sia dalle caratteristiche specifiche del sistema adesivo utilizzato.

Le case produttrici di tali materiali hanno cercato di superare i limiti intrinseci al legame adesivo dentinale limitando il più possibile il numero di passaggi nel protocollo di applicazione, con il fine di ridurre la sensibilità legata alla tecnica. Questa tendenza si riflette nell'introduzione sul mercato dei sistemi adesivi all-in one, adesivi e cementi ad un passaggio: in questi sistemi un'aumentata concentrazione di monomeri acidi permette la

contemporanea applicazione sulla dentina preparata di mordenzante, primer e adesivo (e cemento nel caso dei prodotti per la cementazione) in un unico passaggio.

Il lavoro descritto in questa tesi ha lo scopo di offrire una visione sulle nuove tendenze in odontoiatria adesiva e di elucidare come fattori correlati alla composizione chimica ed al processo di polimerizzazione degli adesivi dentinali possano influire sulla qualità del legame adesivo.

È noto come ad un alto grado di conversione delle resine polimeriche corrispondano elevate proprietà meccaniche [4], motivo per cui la valutazione del grado di conversione dei materiali fotopolimerizzabili è stato l'oggetto dei nostri studi attraverso l'uso della microspettroscopia Raman. La microspettroscopia Raman è un efficace strumento per lo strudio chimico dell'interfaccia adesiva in quanto non necessita di processi manipolativi del substrato come omogeneizzazione, estrazione o diluizione, bensì l'analisi viene condotta *in situ* con un alta risoluzione spaziale.

Nel capitolo 5 il grado di conversione (DC) dell'adesivo nello strato ibrido creato dal Filtek Silorane Adhesive è stato analizzato con la microspettroscopia Raman comparandolo a quello di un composito convenzionale (Clearfil SE Bond). Il silorano [5], un nuovo materiale da restauro conservativo a bassa contrazione basato sulla combinazione di molecole silossaniche ed ossiraniche, è stato recentemente introdotto sul mercato con un suo specifico sistema adesivo. Il silossano è il componente che determina la grande idrofobicità del materiale, mentre il gruppo funzionale cicloalifatico ossiranico è responsabile della contrazione da polimerizzazione più bassa se comparata ad un composito a base di metacrilati. Gli ossirani, che sono degli eteri ciclici, presentano una polimerizzazione cationica con apertura dell'anello, mentre i metacrilati polimerizzano con un meccanismo radicalico [6]. Il sistema adesivo del

composito Filtek Silorane (3M ESPE) è un sistema adesivo self-etch su base metacrilica (Silorane Adhesive System, 3M ESPE) a due passaggi e prevede una prima applicazione di un primer, seguito da uno strato di resina idrofobica contente monomeri dimetacrilici che ha lo scopo di promuovere l'associazione con il composito ossiranico altamente idrofobico. Dopo aver applicato tale sistema adesivo seguendo le indicazioni del produttore, i campioni adeguatamente preparati sono stati sottoposti ad analisi Raman. Spettri Raman sono stati acquisiti lungo l'interfaccia adesiva ad intervalli di un micron e le intensità relative delle bande associate a componenti minerali e adesivi sono state utilizzate per individuare la penetrazione monomerica nella matrica dentinale e per calcolare il grado di conversione. I dati sono stati analizzati statisticamente con metodo two-way ANOVA. Il Filtek Silorane Adhesive ha dimostrato un DC maggiore del Clearfil SE Bond nello strato ibrido, minore in quello adesivo.

Nel capitolo 6 lo strato ibrido creato da tre sistemi adesivi self-etch ad un passaggio è stato caratterizzato *in situ* mediante microspettroscopia Raman ed il loro grado di conversione è stato correlato al grado di nanoleakage dell'interfaccia. Sono stati preparati dischi di dentina con AdheSE One, Adper Prompt L-Pop ed iBond. È stato poi preparato un build-up di composito di 2 mm e le interfacce adesive sono state esposte al laser per effettuare l'analisi spettroscopica. La penetrazione dell'adesivo è stata calcolata utilizzando le intensità relative associate al minerale ed all'adesivo ed è stato valutato il grado di conversione per ogni micron dello strato ibrido. L'analisi statistica è stata effettuata mediante two-way ANOVA. L'espressione del nanoleakage all'interfaccia è stata valutata sugli stessi campioni. AdheSE One ha dimostrato un'espressione del nanoleakage e un DC più elevati rispetto iBond ed Adper Prompt L-Pop.

Grazie alle loro buone proprietà meccaniche, ottime qualità estetiche e facilità d'uso, i cementi resinosi sono ampiamente utilizzati per la cementazione di perni e compositi o di restauri indiretti in ceramica [7]. Recentemente per semplificare la procedura di cementazione sono stati introdotti sul mercato dei cementi duali autopolimerizzanti, che permettono di eseguire mordenzatura, priming, bonding e cementazione in un'unico passaggio clinico. Il calorimetro differenziale a scansione (DSC) è un efficace strumento in grado di fornire il grado di conversione delle resine odontoiatriche dall'analisi del picco esotermico originatosi durante la polimerizzazione, in quanto tale picco è proporzionale alla quantità di monomeri che hanno reagito [8]. La microdurezza è un indicatore della conversione dei monomer vinilici semplice ed affidabile [9] ed ampiamente utilizzato. Piccole quantità di due cementi duali self-adhesive (Maxcem and RelyX Unicem) e di un cemento duale convenzionale (Panavia F2.0) utilizzato come controllo sono stati polimerizzati nella camera della DSC. Dieci campioni sono stati polimerizzati immediatamente (20s) e lasciati indisturbati per due ore, mentre altri dieci campioni sono stati lasciati autopolimerizzare al buio per due ore. Dopo l'analisi DSC, è stata misurata la loro microdurezza (Vickers). Per ogni parametro testato i dati sono stati analizzati statisticamente con metodi two-way ANOVA e Tukey post-hoc test. Il grado di conversione e la microdurezza di tutti i cementi sono stati maggiori se fotopolimerizzati piuttosto che autopolimerizzati. Non è emersa una differenza significativa nel grado di conversione tra i vari cementi.

La nostra ricerca si è successivamente indirizzata su un metodo che ha le potenzialità di migliorare la durata del legame adesivo, ossia l'uso dell'ozono come pretrattamento disinfettante del substrato dentario. La rimozione convenzionale del tessuto cariato e la procedura di preparazione della cavità non garantiscono la completa eliminazione di eventuali batteri

cariogeni rimasti intrappolati nei tubuli o nel fango dentinali, che possano indurre carie secondaria o infiammazione della polpa [10]. Per questo motivo recentemente è stato promosso l'uso del gas ozono come disinfettante della cavità prima della procedura di restauro [11]. La proprietà antibatterica dell'ozono è dovuta alla sua forte attività riducente che determina attraverso un processo ossidativo la rottura delle membrane cellulari e la distruzione di componenti intracellulari. L'obiettivo di questo studio è stato quello di valutare gli effetti dell'applicazione di ozono gassoso sulla forza di legame tra due adesivi self-etch (Clearfil Protect Bond e Xeno III) ed i tessuti dentari. In un primo protocollo lo shear bond strength test è stato condotto per valutare l'adesione su smalto bovino, mentre in un secondo protocollo è stato effettuato il microtensile bond strength test su dentina umana. I dati sono stati analizzati statisticamente e non sono emerse differenze significative tra i campioni pretrattati con ozono e non pretrattati, sia su smalto che su dentina. Clearfil Protect Bond ha dimostrato una forza di legame sullo smalto maggiore che Xeno III, indipendentemente dall'uso di ozono.

Conclusioni

Nell'ambito di questa ricerca, possono esser tratte le seguenti conclusioni:

1. Pochè il self-etch primer del Silorane Adhesive mima in effetti un adesivo one-step self-etch in quanto racchiude tutti i tre passaggi cardinali dell'adesione (etching, priming e bonding), l'applicazione di uno strato idrofobico può garantire una maggiore stabilità dell'interfaccia adesiva riducendo l'assorbimento d'acqua nel tempo.
2. Un grado di nanoleakage maggiore è stato osservato con l'adesivo che ha dimostrato il grado di conversione più basso (AdheSE One). Un basso grado di conversione può minare la qualità e la stabilità a

lungo termine dell'interfaccia adesiva a causa dell'eluizione di monomeri non polimerizzati, portando alla formazione di uno strato ibrido poroso e altamente permeabile.

3. I cementi duali self-adhesive non hanno mostrato differenze di micro durezza e DC rispetto a un cemento duale convenzionale.
4. L'uso di ozono non ha influenzato la forza di legame immediata su smalto e dentina.

Prospettive future

Le case produttrici hanno cercato di superare i limiti intrinseci associati all'adesione dentale eliminando il più possibile il numero di passaggi: in teoria questa semplificazione porterebbe ad una maggior efficacia ed ad una riduzione dell'influenza dell'operatore nella tecnica. Attualmente il comportamento degli agenti resinosi self-etch non è paragonabile a quello degli adesivi etch&rinse: le future direzioni di ricerca dovranno individuare i metodi per migliorare le loro caratteristiche adesive.

La tecnica di microspettroscopia Raman offre numerosi vantaggi, tra i quali quello di necessitare di una minima preparazione del campione e di offrire un'alta risoluzione spaziale (~1-μm) con grande precisione delle informazioni chimiche raccolte; la natura non-distruttiva della tecnica permette inoltre di analizzare gli stessi campioni con tecniche complementari. La tecnica Raman racchiude considerevoli promesse per future ricerche sia *in vitro* che *in vivo* dell'interfaccia dentina/adesive.

In questo studio *in vitro*, i risultati hanno permesso una prima valutazione dell'interfaccia creata da sistemi adesivi self-etch. Ulteriori sperimentazioni sono necessarie per studiare le loro proprietà meccaniche e studi clinici sono fondamentali per supportare i dati di

laboratorio, con lo scopo di chiarire se sistemi adesivi semplificati possano garantire la longevità dei restauri.

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