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Instructions for use

Interfacial interactions of bioadhesive materials with human hard tissues

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ABSTRACT : Recently, in dental practice, tooth reconstruction can be performed using dental adhesive technology following either a "glass-ionomer", an "etch-and-rinse" or a "self-etch" approach. Glass-ionomer cements are well-known to possess an auto-adhesive capability without requiring any surface pre-treatment. The fundamental bonding mechanism of resin-based materials to enamel and dentin is essentially based on an exchange process, in which minerals removed from the dental hard tissues are replaced by resin monomers. These resin monomers, upon polymerization become micro-mechanically interlocked in the created porosities. Besides micro-mechanical interlocking through hybridization, an additional chemical interaction between functional monomers/polymers and tooth substrate components has been found to be important. In this review, we focus on how the chemical interaction of the biomaterial-hard tissue interface can improve bond durability, especially using chemical analytical techniques.

Key Words : glass-ionomer, etch-and-rinse, self-etch, polyalkenoic acid, functional monomer, XPS, XRD

Introduction

Adhesive technology has evolved rapidly since it was introduced more than sixty years ago. Today, decayed or fractured teeth can be reconstructed minimal-invasively and nearly invisibly using adhesive technology. Dental adhesives can be classified into three categories based on their bonding strategy : glass-ionomer adhesives, etch-and-rinse adhesives and self-etch adhesives[1]. Although the three adhesive approaches are totally different and are achieved through different bonding mechanisms, the success of each approach depends to a large extent on the properties of the resultant adhesive material-tooth tissue interface[2-4]. In other words, the longevity of the adhesive tooth restorations depends on the quality of the formed hybrid layer very much. Theoretically, a better hybrid-layer quality can be achieved through more intense and chemical interaction of the adhesive materials with the different tooth-tissue components available at the interface. In this paper, we aimed to provide some more insight in the hybridization mechanisms at adhesive material-tooth material interfaces.

Morphological investigations

The fundamental bonding mechanism of resin-based adhesive systems following an "etch-and-rinse" or a "self-etch" approach is essentially based on an exchange process, in which minerals removed from the dental hard tissues are replaced by resin monomers that upon polymerization become micro-mechanically interlocked in the created porosities[1, 5, 6]. This process, which is called "hybridization", involves infiltration and subsequent *in situ* polymerization of resin within porosities created on the surface of tooth substrates, and thus is a process primarily based upon diffusion. Therefore, adhesive–hard tissue

interfaces (hybrid layers) have been thoroughly studied ultra-morphologically using diverse techniques such as scanning electron microscopy (SEM)[7-10], transmission electron microscopy (TEM)[11-18], confocal laser microscopy[19, 20] and even atomic force microscopy[2, 21]. However, the complex molecular interactions at the interface have hardly been investigated and are far from understood. This causes also delaying in the development of theoretically designed materials with long-lasting adhesive potential.

Glass-ionomer cements

Although glass-ionomer cements are well-known to possess an auto-adhesive capability without requiring any kind of surface pre-treatment, the inherent mechanism of the postulated chemical bonding was not fully demonstrated for many years. Amongst several chemical analytical tools, infra-red (IR) spectroscopy has most frequently been used in an attempt to demonstrate the chemical bonding process of glass-ionomer cements[22-24]. However, IR could never reveal indisputable evidence of chemical bonding. Whilst the reaction of carboxyl groups with calcium can be detected using IR, it is not possible to distinguish between carboxyl groups of the polyalkenoic acid that have chemically interacted with calcium at the hydroxyapatite (HAp) interface and those that merely participated in gelation through reaction with calcium extracted from apatite. To detect true chemical bonding at the interface, chemical information must be gathered exclusively from the bonded layer within a few nm at the interface. Indeed, one of the most difficult problems in material science is to study the chemistry at interfaces. X-ray Photoelectron Spectroscopy (XPS) is a highly selective and specific method of surface analysis[25]. The method allows the upper 1 to 10 atomic layers (0.5 to 5 nm) to be investigated with a detection limit of 0.1-1 atom%. Therefore, to acquire detailed chemical information of the interface between the two materials using XPS, an ultrathin film of the molecule with chemical bonding potential is present on top of the substrate (Fig. 1).

XPS wide-scan spectra of untreated enamel and enamel treated with polyalkenoic acid is shown in Fig. 2. Although a C 1s peak was already present at untreated enamel, application of the polyalkenoic acid significantly increased its intensity. The detailed spectrum of the C 1s region of untreated enamel shows an asymmetric peak with a main C-C, C-H peak at 284.6 eV and weaker C-O,
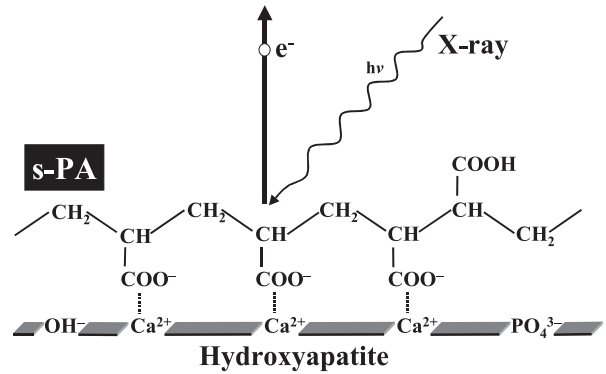

Fig. 1 Schematic presentation showing the principle of XPS that involves the detection of ejected electrons (e⁻) as a result of the impact of an X-ray with a certain kinetic energy (hν) on a monolayer-coated substrate[25].
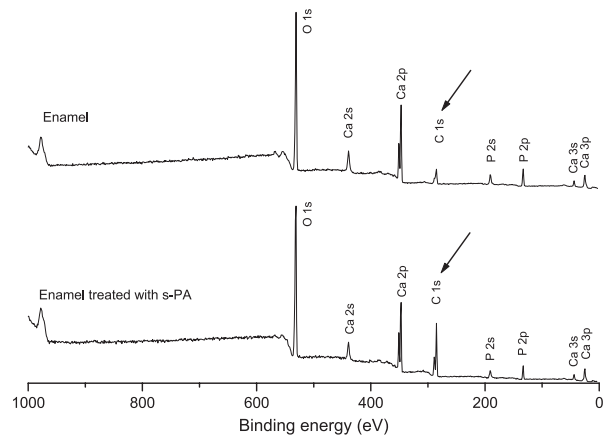

Fig. 2 XPS wide-scan spectra of untreated enamel and enamel treated with s-PA. Note the significant increase in intensity of the C 1s peak and its twofold composition as result of the application of s-PA on enamel (arrows)[25].

$C=O$, $-COO-$ and CO_3^{2-} peaks (Fig. 3). The complete C 1s peak should be attributed to the specific composition of enamel and any common carbon contamination in surplus. Comparing the narrow-scan spectra of the C 1s region of enamel treated with the polyalkenoic acid with that of the polyalkenoic acid alone revealed a significant shift of the carboxyl peak to a lower binding energy (Fig. 4).

However, this shift could not indisputably be attributed to the formation of ionic bonds with Ca of HAp in enamel. It is impossible to exclude the possibility that this apparent shift could also be the result of the simple combination of the narrow-scan spectrum of the C 1s region of polyalkenoic acid (Fig. 4 : dotted spectrum) with the asymmetric C 1s peak of untreated enamel (Fig. 3). As mentioned before, the latter asymmetric C 1s peak should be attributed to the specific composition of enamel
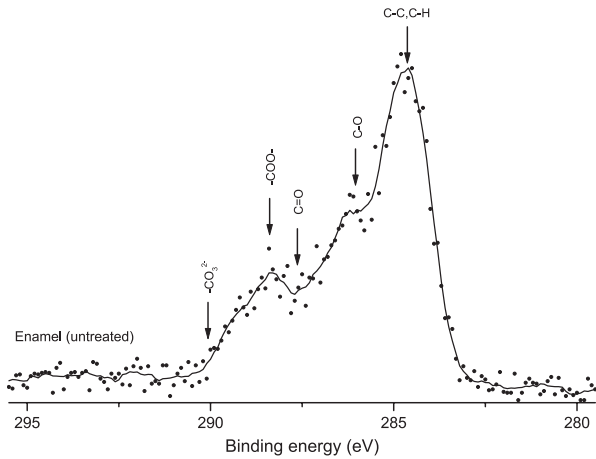
Fig. 3 XPS narrow-scan spectrum of the C 1s region of untreated enamel. Note the presence of an asymmetric peak with a main C-C,C-H peak at 284.6 eV and weaker C-O, C=O, -COO- and CO_3^{2-} peaks, which should be attributed to the specific composition of enamel and any common carbon contamination in surplus[25].
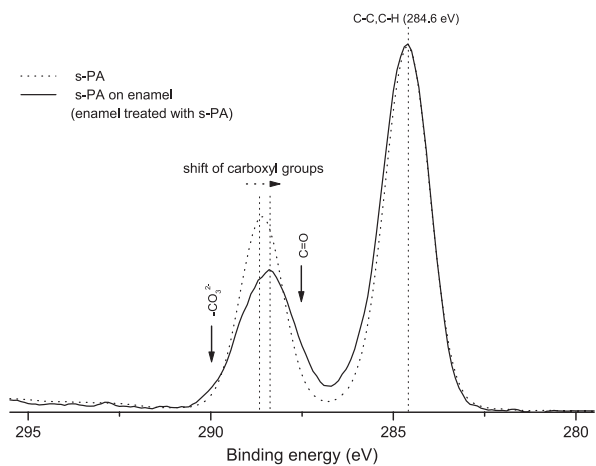

Fig. 4 XPS narrow-scan spectra of the C 1s region of s-PA and of s-PA applied on enamel. Note the shift of the carboxyl peak to a lower binding energy[25].

and any common carbon contamination in surplus. Therefore, in order to characterize the complete process of ionic bonding of polyalkenoic acids to HAp, the effect of polyalkenoic acid on pure, synthetic HAp should have been investigated as well.

The wide-scan spectra of untreated HAp and of HAp treated with polyalkenoic acid are similar, except for a twofold peak at a binding energy of approximately 285 eV that appeared when HAp was exposed to polyalkenoic acid (Fig. 5). The single C 1s peak of untreated HAp can be attributed to C-C and C-H bindings expected at 284.6 eV and to C-O bindings expected at 286.0 eV (Fig. 6). However, it does not show the peak representing

carboxylic groups, which should have appeared at a binding energy of around 288.6 eV. The narrow-scan spectrum of HAp treated with polyalkenoic acid shows the twofold peak that should be attributed to carbon (C 1s) from polyalkenoic acid (Fig. 7). It has a peak at a binding energy of 284.6 eV, mainly representing the backbone of polyalkenoic acid (C-C, C-H bindings), and a peak at 288.6 eV, representing the carboxyl groups of pure polyalkenoic acid. When polyalkenoic acid was applied to HAp, the latter peak significantly shifted to a lower binding energy. Furthermore, its FWHM (full width at half maximum) became larger, while the FWHM of the 284.6 eV peak remained almost constant. Deconvolution of this shifted peak disclosed two components (Fig. 8).
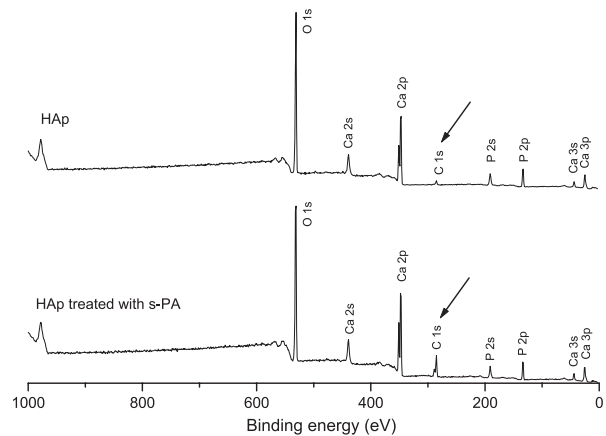

Fig. 5 XPS wide-scan spectra of untreated HAp and HAp treated with s-PA. Note that the spectrum of pure HAp at the top shows only a small and single-fold peak at C1s versus a significantly more intense and two-fold peak after treatment with s-PA (arrows)[25].
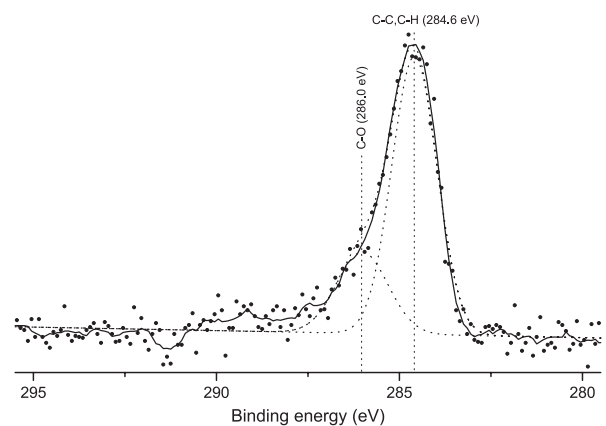

Fig. 6 Detailed spectrum of the C1s region spectrum of untreated HAp. It shows a common carbon contamination asymmetric line with a main C-C, C-H peak at 284.6 eV and a weak C-O peak at about 286.0 eV[25].
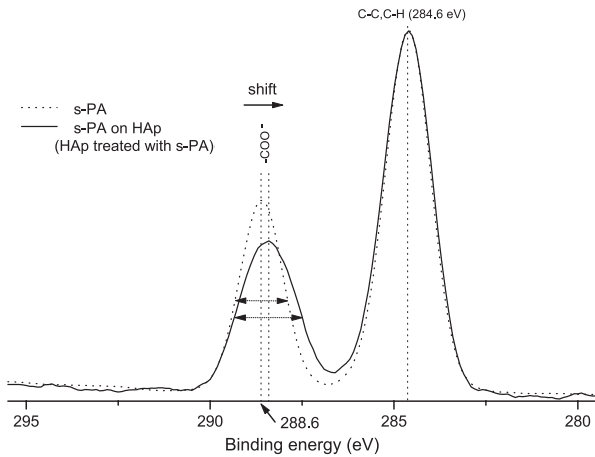
Fig. 7   XPS narrow-scan spectra of the C 1s region of s-PA and s-PA applied on HAp. When HAp was treated with s-PA, a peak representing carboxyl groups from s-PA appeared and shifted to a lower binding energy[25].
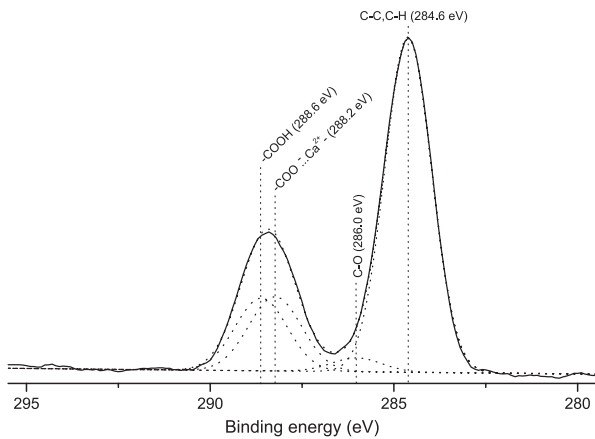

Fig. 8   Deconvolution of the shifted peak in Fig. 6 disclosed a peak at 288.6 eV representing unreacted -COOH and a peak at 288.2 eV resulting from carboxyl groups bonded to calcium of HAp[25].

The peak at 288.6 eV represents unreacted -COOH, which corresponds to that of pure polyalkenoic acid (see Fig. 6). The use of pure HAp allowed us to determine the chemical bonding potential of the polyalkenoic acid to HAp. In other words, this control measurement enabled to quantify the percentage of bonded carboxyl groups versus the non-bonded ones. The percentage of bonded carboxyl groups (-COO- ... Ca^{2+}) versus unreacted, free -COOH was calculated by dividing the deconvoluted -COO- ... Ca^{2+} peak area by the original non-deconvoluted peak area.

Based on the chemical interaction of polyalkenoic acid with HAp, the obtained XPS data clearly indicate that the carboxyl groups of the polyalkenoic acid bonded chemically to calcium of HAp based on the following:

(i)   Calcium salts, such as $CaHPO_4$ and $Ca(H_2PO_4)_2$, were not detected at the treated surface, because the binding energies of Ca 2p and P 2p of treated HAp and the difference between the binding energies of Ca 2p and P 2p, Δ(Ca 2p, P 2p), are significantly different from those of $CaHPO_4$ and $Ca(H_2PO_4)_2$ (Table). Consequently, any phosphate detected in the spectra of HAp treated with polyalkenoic acid must be attributed to HAp, and cannot originate from PO_4^{3-} extracted by the polyalkenoic acid (Fig. 9).

(ii)   The binding energies of Ca 2p for respectively HAp and enamel treated with polyalkenoic acid (Table : 346.91 and 346.86 eV) are significantly different from the binding energy of Ca 2p in the calcium polyalkenoate control (Table : 347.23 eV). Therefore, the XPS-spectra of HAp treated with polyalkenoic acid could only have been recorded from an ultra-thin layer bonded to the substrate. Carboxyl groups that merely participated in gelation of the polyalkenoic acid through reaction with calcium extracted from HAp did not remain on the surface (Fig. 9) (see also (iii)).

(iii)   The FWHM of Ca 2p of HAp treated with polyalkenoic acid (1.39 ± 0.03 eV) did not significantly differ ($P>0.1$) from that of untreated HAp (1.38 ± 0.06 eV). In addition, deconvolution of the Ca 2p peak of HAp treated with polyalkenoic acid did not show a peak at 347.2eV, which would have represented calcium polyalkenoate (Table). This excludes the final possibility that the Ca 2p peak of HAp treated with polyalkenoic acid could have represented partial bonding of a multi-layer calcium polyalkenoate gel to HAp with areas of attachment interspersed with areas of non-attachment (Fig. 10). In the latter case, the FWHM of the Ca 2p peak would have been expected to be larger, because it would then originate from both calcium polyalkenoate and pure HAp.
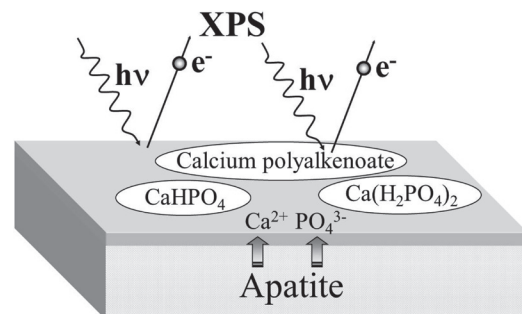

Fig. 9   Schematic illustration of a possible apatite surface treated with polyalkenoic acids. Calcium salts, such as calcium polyalkenoate, $CaHPO_4$ and $Ca(H_2PO_4)_2$, should be detected at such treated surface, using XPS.

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Table   Average binding energy in eV per functional group or atom.

	COO⁻	C-C,C-H	Ca 2p	P 2p	Δ(Ca 2p,P 2p)
Synthesized polyalkenoic acid (s-PA)	288.60 (0.03)	284.60*	----	----	----
Enamel	----	284.60*	346.79 (0.10)	132.81 (0.12)	213.98 (0.15)
Hydroxyapatite (HAp)	----	284.60*	346.67 (0.19)	132.70 (0.14)	213.97 (0.16)
s-PA on enamel	288.30 (0.10)	284.60*	346.86 (0.08)	132.79 (0.10)	214.06 (0.07)
s-PA on HAp	288.27 (0.08)	284.60*	346.91 (0.08)	132.88 (0.08)	214.03 (0.06)
CaHPO₄	----	284.60*	347.24 (0.16)	133.44 (0.17)	213.80 (0.05)
Ca(H₂PO₄)₂.H₂O	----	284.60*	347.74 (0.23)	134.38 (0.36)	213.28 (0.03)
Calcium polyalkenoate	288.31 (0.06)	284.60*	347.23 (0.12)	----	----

*Binding energy taken from literature and used as calibration reference;
Values connected by line are not statistically different (Student t Test: P>0.01);
n > 5 for CaHPO₄ and Ca(H₂PO₄)₂.H₂O; n > 10 for the other measurements (n = number of measurements).
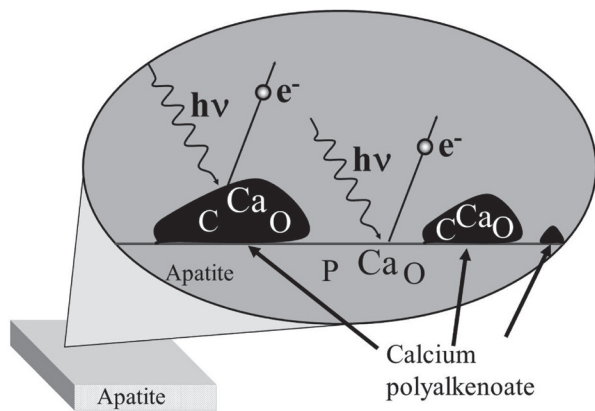

Fig. 10   Schematic illustration of a possible apatite surface treated with polyalkenoic acids. At such treated surface, the Ca 2p peak of apatite treated with polyalkenoic acids was represented partial bonding of a multi-layer calcium polyalkenoate gel to HAp with areas of attachment interspersed with areas of non-attachment.

Extrapolation of above-mentioned findings on HAp (Table and Figs. 9 and 10) to the XPS-data obtained by application of polyalkenoic acid on enamel (Table and Figs. 2-4) allows us to state that the observed shift of the carboxyl peak in Fig. 4 represents the formation of ionic bonds of the carboxyl groups of polyalkenoic acid to Ca of enamel HAp. In addition, XPS of HAp and enamel treated with polyalkenoic acid disclosed surfaces enriched in Ca and reduced in P, indicating that P was extracted at a relatively higher rate than Ca. The Ca/P ratio of HAp and enamel significantly increased from 1.30 (+/−0.02) to 1.59 (+/−0.04), respectively from 1.40 (+/−0.04) to 1.67 (+/−0.13) when treated with polyalkenoic acid. All these XPS results support the proposed mechanism in which carboxylic groups replace PO_4^{3-} ions of the substrate and make ionic bonds with Ca ions of HAp. The fact that these effects were detected after thorough ultrasonic cleaning suggests that polyalkenoic acid has a strong chemical bonding potential to calcium-containing substrates. It was also demonstrated that the actual molecular formula of the polialkenoic acid significantly influences the chemical bonding potential[26]. XPS clearly showed that a polyalkenoic acid based upon 10 : 1 acrylic/maleic acid units has about two third of its carboxyl groups bonded to HAp vs only half of the carboxyl groups of pure polyacrylic acid. The difference in bonding potential was confirmed by the considerably lower adhesiveness of pure polyacrylic acid-based glass-ionomer cement to enamel and dentin as compared to that of the polyalkenoate cement containing the polyalkenoic acid based upon 10 : 1 acrylic/maleic acid units. It indicates that the molecular structure of the polyalkenoic acid significantly influences the chemical bonding efficacy to HAp-based substrates[26].

Resin-based adhesives

Today's resin-based adhesives either follow an 'etch-and-rinse' or a 'self-etch' approach, which differ significantly in the manner they deal with tooth tissue. For etch-and-rinse adhesives, bonding to tooth tissue is essentially based on micro-mechanical interlocking, through the formation of a hybrid layer or hybridization. Nevertheless, it should be stated that both approaches have performed successfully in laboratory as well as clinical research, while obviously there also exists a high product-dependency.

Etch-and-rinse adhesives

Adhesive bonding begins by acid-etching to increase the permeability of resins to enamel and dentin. Nakabayashi *et al*. were the first to demonstrate true hybrid layer

formation in acid-etched dentin[27]. Acid-etching with 30-40wt% phosphoric acid completely demineralizes the surface of the intertubular dentin matrix to create nanometer-sized porosities within the underlying collagen fibrillar matrix. After the dentin surface is conditioned, it has been recommended to maintain in a moist state prior to bonding; this clinical technique is commonly reffered to as "wet-bonding"[28-31]. Water left on the dentin surface is thought to keep the exposed collagen web flexible and permeable to subsequent primer infiltration. Air-drying of the conditioned dentin surfaces has been shown to cause the unsupported collagen web to shrink and collapse, preventing monomers of the primer and adhesive resin from efficiently wetting and infiltrating the conditioned surface. However, the wet-bonding technique can guarantee efficient resin interdiffusion only if all of the remaining water on the dentin surface is completely eliminated and replaced by resin monomers during the subsequent priming step. When the water inside the collagen network is not completely displaced, the polymerization of resin inside the hybrid layer can be affected, or at least, the remaining water will compete for space with resin inside the demineralized dentin. The risk that all moisture on the dentin surface is not completely replaced by primer monomers was ultramorphologically documented as overwetting phenomena[32]. In such overwet conditions, excess water that was incompletely removed during priming appeared to cause phase separation of the monomer components, resulting in weaken the bond and incompletely sealed tubules. This is the limitation of etch-and-rinse adhesives.

Self-etch adhesives

Different from etch-and-rinse adhesives, self-etch adhesives do not require a separate etching step, as they contain acidic monomers that simultaneously condition and prime the dental substrate. Consequently, this approach has been claimed to be user-friendlier (shorter application time, less steps) and less technique-sensitive (no wet-bonding), thereby resulting in a reliable clinical performance[33-36], although this appeared very product-dependent. Another important clinical benefit of self-etch adhesives is the absence of, or at least lower incidence of post-operative sensitivity experienced by patients (as compared to that associated with etch-and-rinse adhesives)[37-39]. All these favourable key-features have lead to the steadily growing popularity of self-etch

adhesives in today's dental practices.

In general, self-etch adhesives have the advantage to demineralize and infiltrate the tooth surface simultaneously to the same depth, theoretically ensuring complete penetration of the adhesive[40]. On the other hand, the quality of hybrid layer strongly depends on its nano-structure and reactants formed by monomers-tooth reaction. With increasing depth, the acidic monomers are gradually buffered by the mineral content of the substrate, loosing their ability to further etch dentin[41, 42]. The morphological features of the adhesive-tooth interface produced by self-etch adhesives depend to a great extent on the manner in which their functional monomers interact with the dental substrate[43]. The actual bonding performance attained by self-etch adhesives varies a great deal, depending on the actual composition and more specific on the actual functional monomer included in the adhesive formulation.

Chemical interaction between tooth tissues and self-etch adhesives

In case of self-etch adhesives, chemical interaction is achieved through specific functional monomers, such as 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP), 4-methacryloxyethyl trimellitic acid (4-MET) and 2-methacryloxyethyl phenyl hydrogen phosphate (Phenyl-P). XPS revealed that the chemical bonding promoted by 10-MDP is not only more effective, but also more stable in water than that provided by 4-MET and phenyl-P, in this order (Fig. 11)[44]. The dissolution rate of the respective calcium salts of these three monomers, as measured by atomic absorption spectroscopy (AAS), was inversely related to their chemical bonding potential, as revealed by XPS : the more intense the chemical bonding potential, the less the resultant calcium salt could be dissolved[44].

Confirming these experimental chemical data, the bond strength to dentin of the 10-MDP-based two-step self-etch adhesive Clearfil MegaBond (marketed as Clearfil SE Bond outside Japan ; Kuraray Medical, Tokyo, Japan) remained high after long-term thermo-cycling, while that of Unifil Bond (GC, Tokyo, Japan) that contains 4-MET, significantly dropped (but only after 100,000 cycles) and that of Clearfil Liner Bond II (Kuraray Medical, Tokyo, Japan) that contains Phenyl-P, gradually decreased when the bond was exposed to thermo-cycling for the longer periods (Fig. 12)[45]. Clearfil SE Bond has been proven to
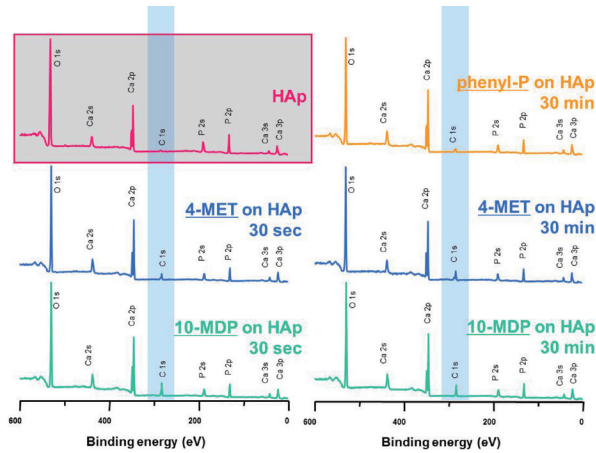
Fig. 11   XPS wide-scan spectra of untreated HAp, of HAp treated with 15% (w/w) 4-MET for 30 sec, of HAp treated with 15% (w/w) 10-MDP for 30 sec, of HAp treated with 15% (w/w) 4-MET for 30 min, of HAp treated with phenyl-P for 30 min, and of HAp treated with 10-MDP for 30 min[44].
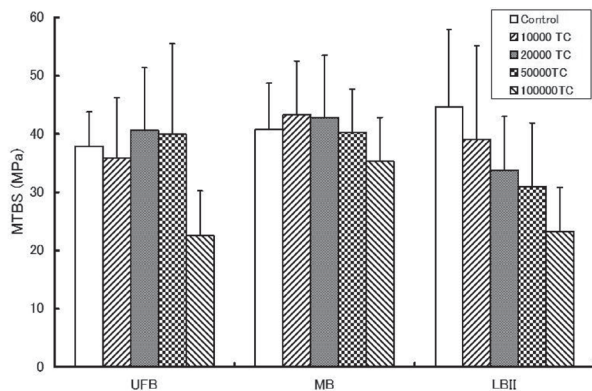

Fig. 12   Micro tensile bond strength of three commercially available self-etch systems, Unifil Bond (UFB), Clearfil MegaBond (MB) and Clearfil Liner Bond II (LBII), before and after thermo-cycling (TC)[45].

yield reliable results in terms of bonding effectiveness and durability when compared to other commercially available self-etch adhesives, this in laboratory as well as clinical research[46-50].

For the functional monomer Phenyl-P, its functional groups (hydrogen phosphates) ionically bond to Ca at the HAp surface (Fig. 13a). This first phase is largely determined by the number of ionized acidic monomers. In the second phase, the formed Phenyl-P_Ca bindings easily dissociate in the solution. Since at the same time abundant phosphate (PO_4^{3-}) and hydroxide (OH^-) ions are extracted from the apatite surface by hydronium ions (H_3O^+), saturation of these ions in the acidic solution is readily achieved, and leads to the very fast deposition of dicalcium phosphate dihydrate (DCPD : $CaHPO_4 \cdot 2H_2O$)

in the third phase (Fig. 13a). As a result, Phenyl-P severely decalcifies apatite around collagen fibrils. TEM of adhesive-dentin interfaces produced by the Phenyl-P-based adhesive indeed disclosed that almost all apatite was dissolved and collagen exposed up to a depth of about 1 μm. DCPD can not protect collagen as well as the original apatite crystals, and thus makes the intermediate monomer-infiltrated collagen layer less stable[51].

On the contrary, 4-MET has a weak chemical bonding potential in comparison with 10-MDP[37]. This weak reactivity to apatite for 4-MET leads to the formation of a submicron hybrid layer with the apatite crystals that remain around collagen fibrils (Fig. 13b). TEM of adhesive-dentin interfaces produced by the 4-MET-based adhesive did reveal relatively shallow interaction with dentin, consisting of both shallow demineralization and collagen exposure[45, 51]. It may be the reason why the hybrid layer of the 4-MET-based adhesive enhanced the degradation resistance of the adhesive-dentin bond and thus extends the bond longevity as compared to that formed with the Phenyl-P based adhesive, Clearfil LinerBond II[45].

In contrast to Phenyl-P and 4-MET, hydrogen phosphate groups of 10-MDP form ionic bonds to Ca at the apatite surface in the first phase. These bonds hardly dissociate in the second phase, indicating that this reaction hardly proceeds (Fig. 13c). X-ray diffraction (XRD) revealed that 10-MDP continuously forms a regularly layered structure at the apatite surface. Each layer of this self-assembled nano-layered structure consists of two 10-MDP molecules with their methacrylate groups directed towards each other and their functional hydrogen phosphate groups directed away from each other (Fig. 14)[52]. High-resolution TEM of 10-MDP-treated HAp powder confirmed this ca. 4-nm layered structure (Fig. 15)[51, 53]. Although phosphate and hydroxide ions are also extracted by hydronium ions (H_3O^+) from the apatite surface and then brought into solution, the concentration of these ions is not high (Fig. 13c). This explains why both XRD and nuclear magnetic resonance (NMR) revealed that upon interaction of 10-MDP with HAp, DCPD was only very slowly deposited in the solution. This high chemical affinity of 10-MDP to HAp along with nano-layering was first demonstrated on pure synthetic HAp using XRD and later confirmed by NMR[52]. Apatite in natural dentin is carbonated and also contains trace amounts of Na, Mg, Sr, and Al among others[54]. Direct evidence of the formation of a nano-layered structure on natural dentin was later provided
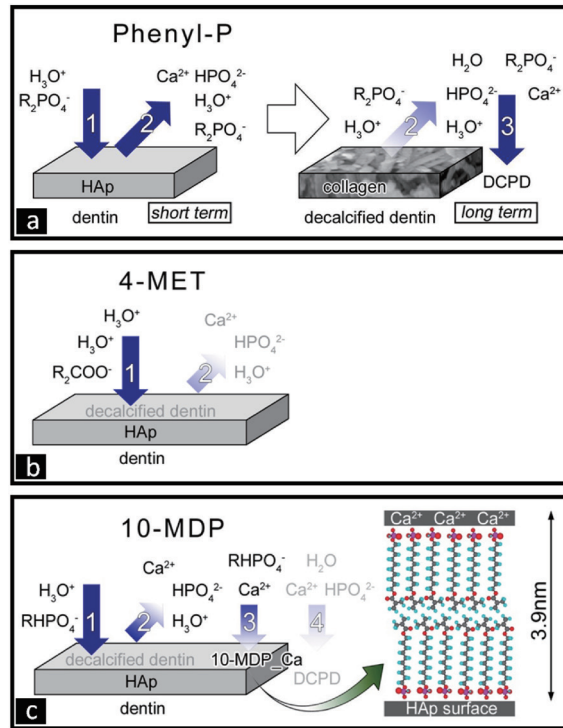
Fig. 13　Nano-controlled molecular interaction at the biomaterial-hard tissue interface.

(a) Schematic diagram of decalcification of dentin induced by Phenyl-P adsorption in the aqueous solution. The initial chemical reaction involves the formation of Phenyl-P_Ca bindings and its dissociation, accompanied by superficial dissolution of HAp through the attack of hydronium ions (H_3O^+). When abundant calcium, phosphate ions (HPO_4^{2-}) and hydroxide ions (OH-) are extracted from the apatite surface, saturation of the acidic solution by these ions leads to the deposition of DCPD ($CaHPO_4 \cdot 2H_2O$).

(b) Schematic diagram of decalcification of dentin induced by 4-MET adsorption in the aqueous solution. The initial chemical reaction involves the formation of 4-MET_Ca bindings that are chemically stable in contrast to the phenyl-P_Ca bindings in (a). This process is accompanied by superficial dissolution of HAp through the attack of hydronium ions (H_3O^+). When calcium, phosphate ions (HPO_4^{2-}) and hydroxide ions (OH^-) are extracted from the apatite surface in a sufficient amount, saturation of the acidic solution by these ions leads to the deposition of DCPD ($CaHPO_4 \cdot 2H_2O$) ; such preciperation did not occur soon.

(c) Schematic diagram of decalcification of dentin induced by 10-MDP adsorption in the aqueous solution and subsequent co-precipitation of calcium salts with low solubility. The initial chemical reaction involves the formation of 10-MDP_Ca bindings that are chemically stable in contrast to the phenyl-P_Ca and 4-MET_Ca bindings in (a) and (b). This process is accompanied by superficial dissolution of HAp through the attack of hydronium ions (H_3O^+). When abundant calcium ions are extracted from the apatite surface, saturation of the acidic solution by calcium ions leads towards calcium salt formation of 10-MDP. The nucleation and growth of 10-MDP_Ca crystals occurs at the HAp surface, where the self-assembled nano-layered structure consists of two MDP molecules with their methacrylate groups directed towards each other and their functional hydrogen phosphate groups directed away from each other. Only at a very late stage when abundant calcium, phosphate (HPO_4^{2-}) and hydroxide ions (OH^-) are extracted from the apatite surface, saturation of the acidic solution by these ions leads to DCPD formation[10].
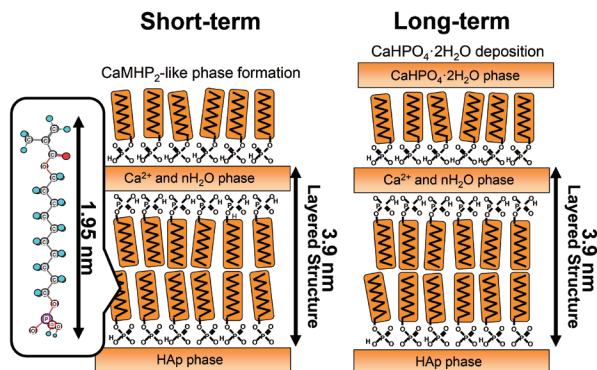

Fig. 14　Proposed mechanism of MDP adsorption onto HAp and precipitation of $CaMHP_2$ and $CaHPO_4 \cdot 2H_2O$[52].
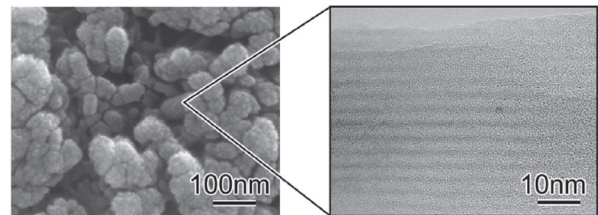

Fig. 15　SEM (left) and TEM (right) (including the electron diffraction pattern) images illustrating 10-MDP-treated HAp particles. Note that a nano-layered structure was observed by TEM[10].

by TEM (Fig. 16), and structurally by XRD of 10-MDP-treated dentin samples (Fig. 16)[53]. Furthermore, rubbing the primer solution on the dentin surface intensified the nano-layering, which may explain why this 'active' application technique increases the bond strength as observed in previous studies[53].
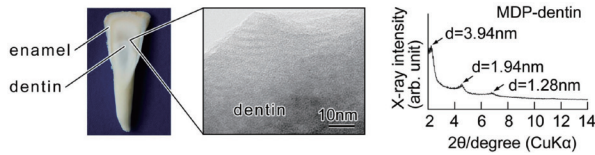

Fig. 16 Direct evidence of the formation of nano-layered structure on dentin (left) was provided by TEM (middle), and also by thin film X-ray diffraction of 10-MDP–dentin samples (right)[10].

Adhesion-decalcification concept (ad-concept)

The way molecules interact with apatite-based tissues can be described in the so-called 'AD-concept' or 'Adhesion-Decalcification concept' (Fig. 17)[55, 56]. This model shows that initially all acids chemically (ionically) bond to calcium of HAp. This first bonding phase goes together with release of PO_4^{3-} and hydroxide (OH^-) ions from HAp into the own solution, such that the surface remains electro-neutral. Whether the molecule will remain bonded or will de-bond, depends on the stability of the formed bond to Ca, or in other words on the stability of the respective calcium salt.

Molecules like 10-MDP, as mentioned above, the functional monomer in self-etch adhesives, and polyalkenoic acids which are main components of glass-ionomer cements, chemically bond to Ca of HAp, forming stable calcium-phosphate and calcium-carboxylate salts, respectively, along with only a limited surface-decalcification effect.

The bonding mechanism of 10-MDP-based self-etch adhesives closely resembles that of resin-modified glass-ionomer cements[5, 57]. The self-etch adhesives including 10-MDP and indeed only superficially interact with enamel and dentin, and hardly dissolve apatite crystals, but rather keep them in place within a thin submicron hybrid layer. Resin-modified glass-ionomer cements also typically present with a submicron hybrid layer that still contains substantial apatite. In this respect, glass-ionomer cements could even be regarded as a kind of mild self-etch adhesives. Polyalkenoic acid is a polymer with a multitude of carboxyl functional groups that
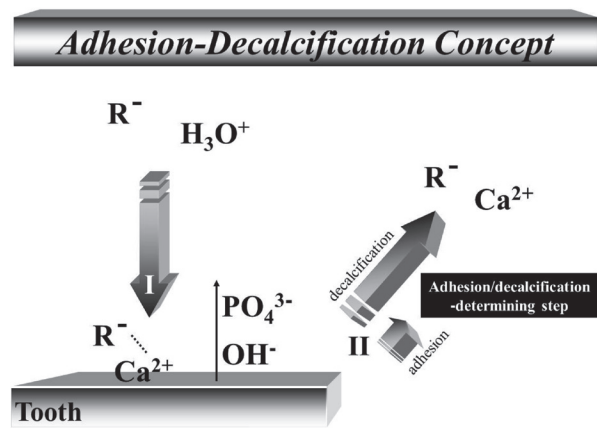

Fig. 17 Schematic illustration of the Adhesion-Decalcification concept or 'AD-concept'. After ionic interaction of the functional monomer (usually a carboxyl or phosphate group), there are two options : either the ionic bond is hydrolytically stable, resulting in a Ca-monomer salt that can co-polymerize with the monomers of the adhesive resin, or the ionic bond is not stable, resulting in decalcification and release of calcium and phosphate from the tooth surface[55].

grab individual Ca-ions along the mineral substrate as chemical 'hands'. This chemical bonding, combined with micro-mechanical interlocking through shallow hybridization, establishes the unique self-adhesiveness of glass-ionomer cements (even without any form of beforehand treatment). Glass-ionomer cements have indeed been recorded with the lowest annual failure rate with regard to Class-V adhesive restorations[33, 58]. The basic difference with 'true' resin-based self-etch adhesives is that the latter possess functional monomers with usually only one or two functional chemical groups with affinity to HAp. They provide individual monomers that become upon polymerization a polymer that is linked to HAp, versus glass-ionomer cements that make use of an already existing (polyalkenoic-acid) polymer with multiple functional groups that are attached to the polymer backbone and can 'grab' Ca at different and remote sites. The additional chemical bonding provided by glass-ionomer cements and self-etch adhesives is believed to be advantageous in terms of bond durability[45, 59].

On the contrary, molecules like phosphoric and maleic acid, but also functional monomers of self-etch adhesives like Phenyl-P, will initially bond to Ca of HAp, but will readily de-bond. The negatively loaded phosphate ions (or carboxyl groups for carboxyl-based monomers/acids) will remove the positively loaded (and thus electro-statically attracted) Ca ions from the surface, up to a certain depth depending on the application time. This results in a severe decalcification

or 'etching' effect, as it is best known for phosphoric acid that is used as 'etchant' as part of the 'etch-and-rinse' approach. Because the calcium-phosphate/carboxylate bond originally formed at the enamel/dentin surface is not stable, the bond will dissociate, leading to a typical etch pattern at enamel and a relatively deep (3-5 μm) hybrid layer at dentin that does no longer contain any apatite crystals.

Keeping apatite at the interface is important to protect collagen and generate chemical interaction receptiveness. Dentinal collagen exposed by an etch-and-rinse procedure has been documented to be highly vulnerable to hydrolytic and enzymatic degradation processes[60-62]. Actually, the fact that an etch-and-rinse hybrid layer can be demineralized, confirms the relatively permeable nature of the resin-impregnated collagen layer and perhaps its consequent instability on the long term. On the other hand, it also underlines the great advantage of mild self-etch adhesives as they keep collagen not only encapsulated and thus protected by HAp, but also provide the potential to chemically interact with HAp[5, 44].

Importance of polymerization for bonding

Despite the high chemical interaction potential of 10-MDP and the related nano-layering, it was shown that the application of an experimental 10-MDP : EtOH : H_2O self-etching primer followed by the bonding agent of the commercially available Clearfil SE Bond did not suffice to reach a bond strength comparable to that of the complete Clearfil SE Bond system (using also the commercially available 10-MDP-based self-etching primer)[44]. However, when camphorquinone (CQ) was added as photo-initiator to the experimental 10-MDP : EtOH : H_2O self-etching primer, an equally high bond strength to dentin was measured like that of Clearfil SE Bond (of which the self-etching primer also contains CQ). This finding highlights the need for adequate polymerization.

Conclusion

XPS provided evidence of chemical bonding of glass-ionomer cements to apatitic substrates. For self-etching adhesives, essential are the functional monomers with a strong chemical affinity to calcium of HAp. Correlative XRD and solid-state NMR disclosed that Phenyl-P and 10-MDP should be regarded as the two extremes : Phenyl-P rather 'etches', while 10-MDP rather 'bonds' to HAp, with 4-MET behaving somewhat in between.

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