

Nano-Layering adds strength to the adhesive interface

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Abstract:

X-ray diffraction (XRD) surface analysis and ultrastructural interfacial characterization using transmission electron microscopy (TEM) confirmed that the functional monomer 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) self-assembles into nano-layers at adhesive-tooth interfaces. Self-assembled nano-layering is thought to contribute to the durability of bonding to tooth dentin, although this has not been proven yet. In order to disclose this potential bond-durability contribution of nano-layering, we observed the 3D-spreading of nano-layering by a series of focused-ion-beam (FIB)-milled cross-sections by scanning electron microscopy (FIB-SEM) and examined the mechanical properties of self-assembled nano-layering using scanning probe microscopy (SPM). A commercial 10-MDP-containing two-step self-etch adhesive partially demineralized dentin up to submicron depth, forming a submicron hydroxyapatite-rich hybrid layer. TEM chemically and ultra-structurally confirmed the formation of interfacial nano-layering. FIB-SEM 3D-reconstructions disclosed a 3D-network of self-assembled nano-layering extending from the hybrid layer up to within the adjacent adhesive-resin layer. SPM revealed that nano-layering within the adhesive-resin layer possessed a higher elastic modulus than that of the surrounding adhesive resin, hereby suggesting that nano-layering contributes to the mechanical strength of adhesives like filler particles do. Nano-layering's three-dimensionally expanded structure is expected to strengthen the surrounding resin, as well to better interconnect the adhesive-resin layer to the hybrid layer. In conclusion, this exploratory study demonstrated that nano-layering constitutes a strong phase at the adhesive interface, which may contribute to the clinical longevity of the 10-MDP-based bond to dentin.

Introduction

Self-etch adhesives are user-friendly, not requiring separate etching, as they contain acidic functional monomers that simultaneously 'condition' and 'prime' tooth tissue. Key constituents of self-etch adhesives are functional monomers (Van Meerbeek *et al.*, 2020), which directly determine interfacial ultrastructure and chemical interaction with direct impact on bond durability (Yoshihara *et al.*, 2010, 2019). The adhesion-decalcification (AD) concept mechanistically explained how acidic molecules chemically interact with HAp-based tissue following either an adhesion or decalcification route (Van Meerbeek *et al.*, 2020). Acidic functional monomers produce hybrid layers with different ultrastructure depending on the dissolution rate of their Ca-salts. In brief, acidic monomers with soluble Ca-salts produce thicker HAp-poor/free hybrid layers at dentin with abundant collagen exposure, as representative for etch-and-rinse adhesives. Contrastingly, functional monomers producing stable Ca-salts partially/shallowly demineralize dentin, resulting in submicron hybrid layers with collagen remained protected by HAp, as representative for mild self-etch adhesives. Besides providing micromechanical interlocking through etching, additional primary chemical interaction of functional monomers with HAp stabilizes the adhesive interface. In particular, 10-MDP revealed intense and stable chemical bonding efficacy, which was later also documented for some fluoro-carbon analogues (Yoshihara *et al.*, 2014). In continued research, Yoshihara *et al.* (2019) discovered that 10-MDP self-assembled into about 4-nm nano-layers, consisting of two sublayers of parallel oriented 10-MDP monomers in opposite direction with one nano-layer connected to the next one via Ca that formed a stable ionic bridge connecting the phosphate groups of adjacent 10-MDP's. This theoretical molecular structural model of nano-layering was proposed based on X-ray diffraction (XRD) data (Yoshida *et al.*, 2012). Nano-layering at adhesive interfaces was next visualized using transmission electron microscopy (TEM) by Yoshihara *et al.* (2010). The mechanisms of interfacial nano-layering were characterized ultra-morphologically and chemically, collecting data from specimens prepared in a similar manner as done clinically, this correlatively using scanning TEM with energy-dispersive X-ray spectrometry (STEM-EDS), XRD and solid-state nuclear magnetic resonance (NMR). Most recently, water-stable 10-MDP_Ca salts were found to consist of the very stable CaRPO_4 , meaning that the two OH-groups of 10-MDP's phosphate group ionically reacted with Ca (Yoshihara *et al.*, 2019).

Hybrid layers have abundantly been documented to degrade with time (Breschi *et al.*, 2018). Immediate and aged micro-tensile bond-strength tests revealed the hybrid layer to fracture as being the most degradation-sensitive part of the adhesive assembly (Frassetto *et al.*, 2016). Many researchers attempted to strengthen the hybrid layer, for example by optimizing the adhesive's composition, improving its degree of conversion, or by adding novel agents such as collagen crosslinkers, all of which having been investigated amply (Spencer *et al.*, 2014; Frassetto

et al., 2016).

Until the date of this paper, no mechanical property data of 10-MDP_Ca-salt nano-layering are available in light of its potential contribution to adhesive interfacial stability. In this study, we therefore mapped elastic modulus across cross-sectioned interfaces to assess the mechanical properties of self-assembled nano-layering using scanning probe microscopy (SPM). We also observed the 3D-spreading of nano-layering by reconstructing a series of focused-ion-beam (FIB) milled adhesive-dentin cross-sections imaged by scanning electron microscopy (FIB-SEM). The null hypothesis tested was that no difference in EM was measured between 10-MDP_Ca-salt nano-layering and the surrounding adhesive resin.

Materials and Methods

Specimen preparation for FIB-SEM, TEM and SPM

The occlusal crown of four extracted non-carious human third molars (approved by the Commission for Medical Ethics of Okayama University Hospital under file number 1606-020) was removed using a diamond saw (Accutom 50, Struers, Ballerup, Denmark), upon which the exposed dentin was wet-ground using 600-grit SiC paper (WTCC-S, Nihonkenshi, Fukuyama, Japan) to prepare a flat and uniform smear layer, enabling good visualization of interfacial ultrastructural detail. The 10-MDP-containing two-step self-etch adhesive Clearfil SE Bond 2 ('C-SE2'; Kuraray Noritake) was applied on dentin according to the manufacturer's instructions, involving application of C-SE2 primer using a microbrush, followed by air-blowing at 20 sec after application. Next, an experimental adhesive resin with the same composition as that of C-SE2 but without filler (Kuraray Noritake) was applied, so to avoid filler interference with the SPM mapping of EM across the adhesive interface. The adhesive resin was cured for 10s using the LED light-curing unit G-light Prima II (GC, Tokyo, Japan). Then, the low-viscosity composite resin Clearfil Protect Liner F (Kuraray Noritake) was applied and light-cured. Upon 24-h water storage, the adhesive-treated dentin specimens were mid-sectioned in two halves, upon which per specimen half up to five about 1x1-mm rectangular slabs were cut. All slabs were successively fixed in glutaraldehyde (Nacalai Tesque, Kyoto, Japan), gradually dehydrated in ethanol and embedded in epoxy resin (TAAB Laboratories Equipment, Aldermaston, UK). Further specimen preparation/processing for FIB-SEM, TEM and SPM is detailed below.

FIB-SEM

Eight slabs embedded in epoxy resin with the cross-sectioned adhesive-dentin interface exposed at the surface were mechanically polished using diamond lapping films (3M, St.Paul, MN, USA) and argon-ion polishing (SM-090101 Cross-Section Polisher, JEOL, Tokyo, Japan). Orthogonal FIB-

SEM (SMF-1000, HITACHI, Tokyo, Japan) imaging was conducted for 3D-ultrastructural characterization. The FIB and SEM ion/electron beams were orthogonally aligned, instead of being set at the standard angle of 60°, to obtain high spatial resolution and high-contrast SEM photomicrographs (Wirth, 2009). Lucis Pro (Microtechnics, Granite Bay, CA, USA) and Stacker and Visualizer-Kai (System In Frontier, Tokyo, Japan) software packages were utilized for 3D reconstruction. SEM was performed at 0.5 kV. Images were obtained using an annular in-lens secondary electron detector and an annular in-lens energy-selected backscattered electron detector. The observed area was 10x10 μm with 10 nm/pixels each. Serial sectioning observations were carried out with a slice pitch of 10 nm for 990 sheets. The obtained images were rendered by removing resin and pseudo-colored according contrast.

TEM

From eight slabs, originating from four teeth, numerous ultra-thin adhesive-dentin cross-sections were cut using routine ultra-microtomy (LEICA EM UC6, Leica, Vienna, Austria), prior to being observed by TEM at 200 kV, utilizing a JEM-2100 microscope (JEOL).

Quantitative mechanical property mapping by SPM

Similar to the FIB-SEM specimen-preparation procedure, eight embedded specimens were mechanically polished using diamond lapping films (3M) and argon-ion polished (SM-090101 Cross-Section Polisher, JEOL). The SPM system employed was a Bruker MultiMode 8 SPM equipped with a Nanoscope V controller and Nanoscope analysis software (Bruker Nano Surface Business, Santa Barbara, CA, USA). Cross-sectioned surfaces were quantitatively characterized using the PeakForce QNM (Quantitative NanoMechanics) SPM module under ambient conditions. Elastic modulus (EM) was calculated by NanoScope analysis software using the Derjaguin-Muller-Toropov (DMT) model (Derjaguin *et al.*, 1975; Wang *et al.*, 2013). Samples were scanned using a probe (RTESPA-525, Bruker) with a nominal radius of 8 nm and a nominal spring constant of 200 Nm^{-1} with 0.501 Hz scan rates, a 250-mV amplitude set point and a 150-nm peak force amplitude. After SPM, the samples were observed by SEM (JSM-6701F, JEOL).

S/TEM observation of a clinically C-SE treated tooth

A tooth extracted because of periodontal pathology (approved by the Commission for Medical Ethics of Okayama University Hospital under file number 1901-036) was collected. The tooth was adhesively restored 9 years ago using Clearfil SE Bond ('C-SE' as pre-cursor of C-SE2; Kuraray Noritake) and the resin-based composite Clearfil AP-X (Kuraray Noritake). The tooth was fixed in glutaraldehyde, gradually dehydrated in ethanol and embedded in epoxy resin. After the

adhesive-dentin interface was exposed, the interface was mechanically polished using diamond lapping film (3M, St. Louis, MO, USA), followed by broad argon-ion-beam polishing using a cross-section polisher (SM-09020 CP, JEOL). Subsequently, a thin layer of carbon was deposited on the surface (JEE-420T, JEOL), upon which the specimens were examined with Feg-SEM (JSM-6701F, JEOL) operated at 5 kV and employing an annular semiconductor detector. For TEM, ultrathin sections were cut (Leica EM UC7, Leica, Vienna, Austria), prior to being examined with high-resolution 200-kV TEM utilizing a JEM-2100 (JEOL) microscope.

Results

FIB-SEM

FIB-SEM 3D-reconstruction resulted in a 10- μm^3 block of the mild self-etch adhesive-dentin interface, revealing a 500-nm to 1- μm thick HAp-rich hybrid layer with abundant nano-layering produced on top (Fig. 1a). 10-MDP_Ca salts were detected within the hybrid layer and widely spread along the interface within the adhesive-resin area. By adding pseudocolor in Fig. 1b and digitally removing resin in Fig. 1c, 10-MDP_Ca salt nano-layering was observed more clearly. Within the composite resin, filler particles were visible.

TEM

TEM of the interface of C-SE2 bonded onto dentin revealed a 0.5-0.7 μm thick hybrid layer, in which HAp was found to have remained without hardly any collagen exposure (Fig. 2b). At the hybrid-layer top, regular longitudinally layered and often curved structures were observed. At higher magnification, these layers represented nano-layers with a periodicity of approximately 3.5 nm (Fig. 2c).

SPM

DMT-EM mapping clearly indicated differences in surface brightness with the brighter regions representing areas exhibiting higher EM, corresponding to stiffer phases, versus lower EM at the darker regions, corresponding to softer phases (Fig. 3). Across the adhesive-dentin interface, EM varied from 1.1 to 10.2 GPa. As mineralized tissue, unaffected dentin underneath the interface appeared brightest, with the highly mineralized peritubular dentin exhibiting clearly higher EM than intertubular dentin. The upper area showed the low-viscosity composite resin, clearly indicating the higher brightness and thus higher EM of the contained silica filler. The surrounding resin matrix was darker, representing a softer (less stiff) surface, as was also detected for the unfilled adhesive layer between low-viscosity composite resin and hybrid layer, for which similar EMs were recorded. High magnification showed a darker (lower EM) hybrid layer than dentin, but was brighter (higher EM) than the adhesive resin. Characteristic brighter extensions within

the adhesive resin, originating at the hybrid layer, represent the formed nano-layers that were identified as regions with higher EM than that of the surrounding resin within the adhesive-resin layer.

SEM

Upon SPM, the same FIB-sectioned specimens were observed by SEM (Fig. 4). Low magnification revealed a 10- μm thick adhesive-resin layer between the low-viscosity composite resin and dentin. At high magnification, abundant nano-layering extending from the hybrid layer was detected and fully corresponded to that disclosed by SPM.

S/TEM observation of a clinically C-SE treated tooth

The clinical case of an adhesively restored tooth extracted after 9 years of clinical service revealed light-microscopy (LM) photographs of the exposed composite-tooth interface (Fig. 5). SEM disclosed a tight composite-adhesive-dentin interface, while the adhesive and composite resin closest to dentin contained several voids, which should be attributed to de-bonded silica filler in the adhesive layer. TEM revealed a 500-nm thick hybrid layer. High-magnification TEM clearly disclosed nano-layering close to the hybrid layer, which was detected after 9-year *in-vivo* service.

Discussion

The study's aim was to measure the mechanical strength of nano-layering in potential contribution to adhesive interfacial stability. Considering its nanometer size and regionally varying occurrence, measurement of mechanical properties of nanometer-sized interfacial structures is challenging. SPM methods have been developed to detect interactions of the cantilever tip with the sample surface; among diverse applications, they have enabled measuring mechanical properties such as EM on the micrometer to subnanometer scale. When adhesive-dentin interfaces were mapped using the DMT-EM SPM module, the self-assembled nano-layering of 10-MDP_Ca salts presented with higher EM than the surrounding adhesive resin. Additionally, this study imaged nano-layering distribution along the adhesive-dentin interface in 3D using 3D-reconstruction of consecutive FIB cross-sections. Full nano-layering identification was obtained by complementing XRD chemical surface analysis with TEM/SEM ultra-structural interfacial analysis.

Having been recorded as most effective functional monomer, 10-MDP is often the main functional monomer contained in the newest generation of universal adhesives (Van Meerbeek *et al.*, 2020). 10-MDP has a characteristic molecular structure with a long spacer group ending in a polymerizable methacrylate group, well separated from the phosphate group at the other

monomer's end with chemical binding potential to Ca of HAp (Yoshihara *et al.*, 2018). 10-MDP interacts with dentin in three ways (Yoshihara *et al.*, 2019): (1) achieving shallow surface micro-retention by etching without fully uncovering collagen; (2) binding electrostatically to calcium of HAp remaining around collagen; and (3) self-assembling into nanometer-sized structures consisting of 10-MDP_Ca salts, known as nano-layering (Yoshihara *et al.*, 2010). Nano-layering is confirmed by XRD, revealing three characteristic peaks representing the regularly ordered 10-MDP_Ca salts. Based on the molecular structure of 10-MDP, 10-MDP analogues with nano-layering potential were synthesized and appeared as effective as 10-MDP with some even possessing better hydrolytic resistance (Yoshihara *et al.*, 2014).

Much adhesive dentistry-related research involves morphologic examination of hybrid-layer integrity, often to study bond-degradation mechanisms (Frassetto *et al.*, 2016). Mechanical properties of adhesive interfaces are most often quantitatively assessed in terms of bond strength measured in different modes (De Munck *et al.*, 2012). Basically, the overall strength of the complete adhesive-dentin assembly is measured, commonly also influenced by the involved tooth substrate and restorative material. In order to evaluate interface quality, nano-indentation has been employed to measure nano-hardness and nano-EM across adhesive interfaces (Van Meerbeek *et al.*, 1993). However, this technique is limited regarding indent size/depth, being often too large/deep, by which mixed substrate/material property data are collectively recorded. New opportunities arose with the introduction of atomic force microscopy (AFM) equipped with nano-indentation. AFM-cantilever tips have a radius of approximately 20 nm, although reaching nano-level resolution using AFM nano-indentation remains difficult, mainly in case of highly varying surface topography (Schulze *et al.*, 2005). Pioneering AFM research was conducted to measure *in-situ* mechanical properties of fully hydrated calcified tissues and across the dentinoenamel junction using AFM-based nano-indentation (Kinney *et al.*, 1996; Marshall *et al.*, 2001). Likewise, AFM nano-indentation was used to determine hardness and EM across adhesive-dentin interfaces and of hybrid layers in particular (Oliveira *et al.*, 2004, Schulze *et al.*, 2005). As appeared for this AFM nano-indentation (Ho *et al.*, 2004), most challenging for SPM is the need to prepare artifact- and smear-free, and nearly completely flat cross-sections. Metallographical grinding and polishing of interface specimens cannot prevent polishing debris be smeared across the interface and damage the softer collagen fibrils within the hybrid layer (Yoshida *et al.*, 1999; Ho *et al.*, 2004). Ultra-microtomy sectioning with a diamond knife was therefore used to prepare clean adhesive interfaces, enabling AFM-imaging of hybrid-layer collagen (Van Meerbeek *et al.*, 1999; Yoshida *et al.*, 1999). However, due to differences in surface properties, the interface specimens revealed a clear dip at the adhesive-resin layer in between the harder dentin substrate and particle-filled composite resin. In a prior pilot study, metallographical polishing and ultra-microtomy were found inapplicable to assess nano-layering

with SPM, as both methods induced surface distortions and scratches. We next prepared interfacial cross-sections using broad argon-ion-beam cross-section polishing, which resulted in much flatter surfaces with minimum damage and distortion (Erdman *et al.*, 2006). Such gentle argon-ion-beam cross-sectioning appeared effective to assess mechanical properties across adhesive-tooth assemblies that consist of different materials/substrates with varying properties, as there are unaffected dentin, the hybrid and adhesive-resin layers, and the composite resin.

10-MDP_Ca-salts are stable and insoluble in water (Yoshida *et al.*, 2012), with 10-MDP_Ca-salt nano-layering outgrowing from the hybrid layer in a hairy fashion into the adhesive-resin layer (Yoshihara *et al.*, 2019). In this way, the adhesive interface can be regarded as a functionally graded material (Marshall *et al.*, 2001), often being referred to as FGM, with properties such as stiffness (EM) to gradually change from the mineralized unaffected dentin across the partially demineralized hybrid layer towards the adhesive resin, this to the direct benefit of producing a stress-withstanding/absorbing adhesive interface. The formed nano-layering, consisting of two rows of regularly aligned 10-MDP molecules, with one nano-layer strongly connected by stable Ca-salt formation to the next nano-layer in a 3D perspective, further optimizes this functional gradient at the adhesive-dentin interface, since the nano-layered structure appeared in this study to possess mechanical properties in between that of the hybrid and adhesive-resin layer.

The actual contribution of nano-layering to adhesive interfacial stability however remained unknown up to the publication date of this work. This knowledge is nevertheless of high interest, for instance to know if interfacial nano-layering intentionally should be intensified to make adhesive interfaces more durable. Anecdotal clinical evidence of nano-layering stability is provided by a clinical case with nano-layering still detected after 9-year clinical service at the adhesive-dentin interface of a composite restoration bonded to dentin using Clearfil SE Bond (Kuraray Noritake), being the commercial precursor of the self-etch adhesive used in this study (Fig. 5).

SPM, or atomic force microscopy (AFM), enables analysis at atomic level. Among several SPM measurement methods, PeakForce Quantitative Nanomechanical Property Mapping (QNM) probes surface topography and mechanical properties at the same time by making a force-distance curve at each spot and fitting this force-distance curve with the DMT model of elastic contact (Drijkonigen *et al.*, 2014). SPM in DMT mode revealed higher EM for dentin, attributable to HAp, and for the composite-resin filler consisting of silica glass. The intermediary EM mapped at the submicron hybrid layer must be ascribed to the solely partial dentin demineralization with HAp still surrounding collagen. EM of the adhesive resin was lower as compared to that of dentin and the low-viscosity composite resin. However, EM of nano-layers surpassed that of the adhesive resin, by which the null hypothesis that no difference in EM was measured between 10-MDP_Ca-salt nano-layering and the surrounding adhesive, failed to be

accepted. This higher EM of nano-layers should be attributed to the compactly ordered structure of interconnected 10-MDP_Ca-salts. Having better mechanical properties than those of the surrounding adhesive resin, these nano-layers are expected to contribute to the stability of the adhesive-dentin interface. EM mapping of adhesive-dentin interfaces by SPM may be useful to study bond-degradation processes when adhesive interfaces are exposed to accelerated aging regimes.

Dental adhesives often contain filler particles in order to increase viscosity, to provide radiopacity, to improve the adhesive's mechanical properties and to act as film-thickness regulator with shock-absorbing potential (Lohbauer *et al.*, 2010; Van Meerbeek *et al.*, 2020). Adding filler to adhesives resulted in different effects on their mechanical properties, while these depends also on the type of filler and their composition, but also on the test method applied. Optimized micro/nano-silica filler content in adhesives was shown to improve bond strength (Miyazaki *et al.*, 1995; Kim *et al.*, 2005; Lohbauer *et al.*, 2010). TEM confirmed that filler particles hardly infiltrate hybrid layers (Tay *et al.*, 1999), while they can penetrate with resin within opened dentin tubules, strengthening resin tags (Frankenberger *et al.*, 2001; Lohbauer *et al.*, 2010). Otherwise, Miyazaki *et al.* (1995) revealed that higher amounts of silica filler reduced the degree of conversion and resulted in lower bond strength. Others found that 1-10% filler increased the adhesive's flexural (Kim *et al.*, 2005) and cohesive strength (Conde *et al.*, 2009). The formed nano-layers could be considered as self-formed filler, promoting the adhesive resin's strength. Moreover, while micro/nano-filler particles possess a spheric structure, 10-MDP_Ca salts self-assembled into a three-dimensional structure that grew and expanded on the hybrid-layer top into the adhesive-resin layer, hereby strengthening both the hybrid and adhesive-resin layer, as well as their interconnection.

In conclusion, this study found that 10-MDP_Ca-salt nano-layers possess higher EM than the surrounding adhesive resin. Their presence within the hybrid layer extending into the overlying adhesive resin strengthens both the hybrid and adhesive-resin layer. Their 3D-continuous structure results in enhanced strengthening as compared to filler, while it may also more tightly interconnect the hybrid layer with the adhesive-resin layer. While *in-vivo* case evidence of nano-layer stability exists, further research should test the nano-layering's resistance against hydrolytic degradation.

Author Contributions

Yoshihara K, contributed to conception, design, data acquisition, analysis and interpretation, drafted and critically revised the manuscript; Nagaoka N, contributed to conception, design, data acquisition, analysis and interpretation, and critically revised the manuscript; Nakamura A, contributed to conception, design, data acquisition, analysis and critically revised the

manuscript; Hara T, contributed to conception, design, data acquisition, analysis and critically revised the manuscript; Yoshida Y, contribute to conception, interpretation, and critically revised the manuscript; Van Meerbeek B, contribute conception, interpretation, drafted and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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Figure captions:

Figure 1 3D-reconstructed FIB-SEM cross-sections of a mild self-etch adhesive-dentin interface in **(a)**, with pseudocolors added in **(b)** and additional digital removal of resin in **(c)**.

Figure 2. Collage of TEM photomicrographs of the interface of Clearfil SE Bond 2 (Kuraray Noritake) bonded to dentin, imaged at different magnifications and revealing a submicron hybrid layer in and nano-layering with a periodicity of approximately 3.5 nm.

Figure 3. Derjaguin-Muller-Toporov (DMT) elastic modulus (EM) mapping by SPM across the adhesive-dentin interface produced by Clearfil SE Bond 2 (Kuraray Noritake) bonded to dentin at different magnifications.

Figure 4. SEMs of the FIB cross-sectioned interface of Clearfil SE Bond 2 (Kuraray Noritake) bonded to dentin and imaged at the same area as scanned by SPM in Figure 3. (a) Low-magnification photomicrograph revealed a tight adhesive-dentin interface with disclosure of the grey-colored submicron hybrid layer and nano-layering extending from the hybrid layer within the overlying adhesive resin in (b) and (c). The SEM photomicrograph in (c) was taken at about the same position and magnification as the SPM-scanned image in (d).

Figure 5. Clinical case of an extracted adhesively restored tooth, of which ultra-structural SEM and TEM interfacial characterization revealed nano-layering after 9-year clinical service at the adhesive-dentin interface of a composite restoration bonded to dentin using Clearfil SE Bond (Kuraray Noritake), being the commercial precursor of the 10-MDP-based self-etch adhesive applied in this study.

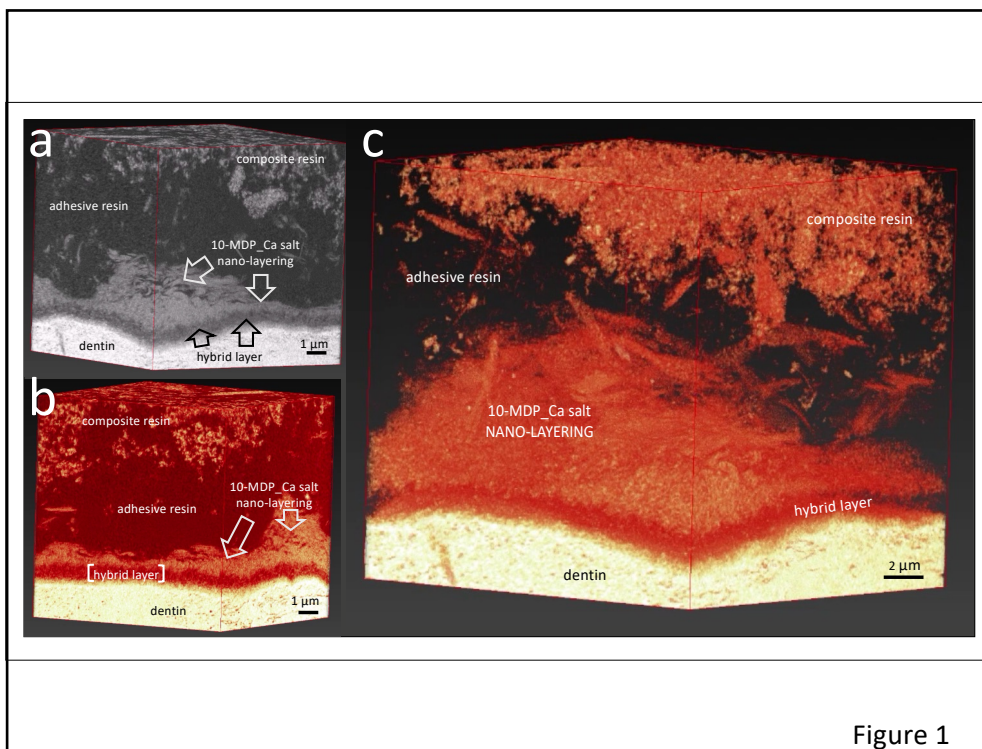


Figure 1

1

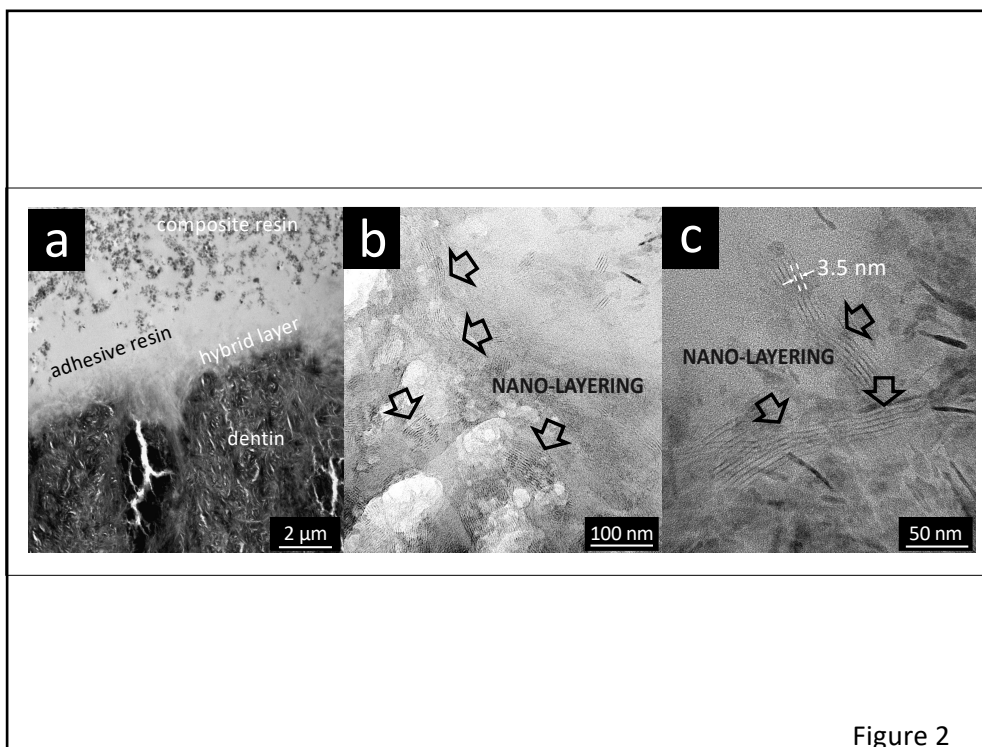


Figure 2

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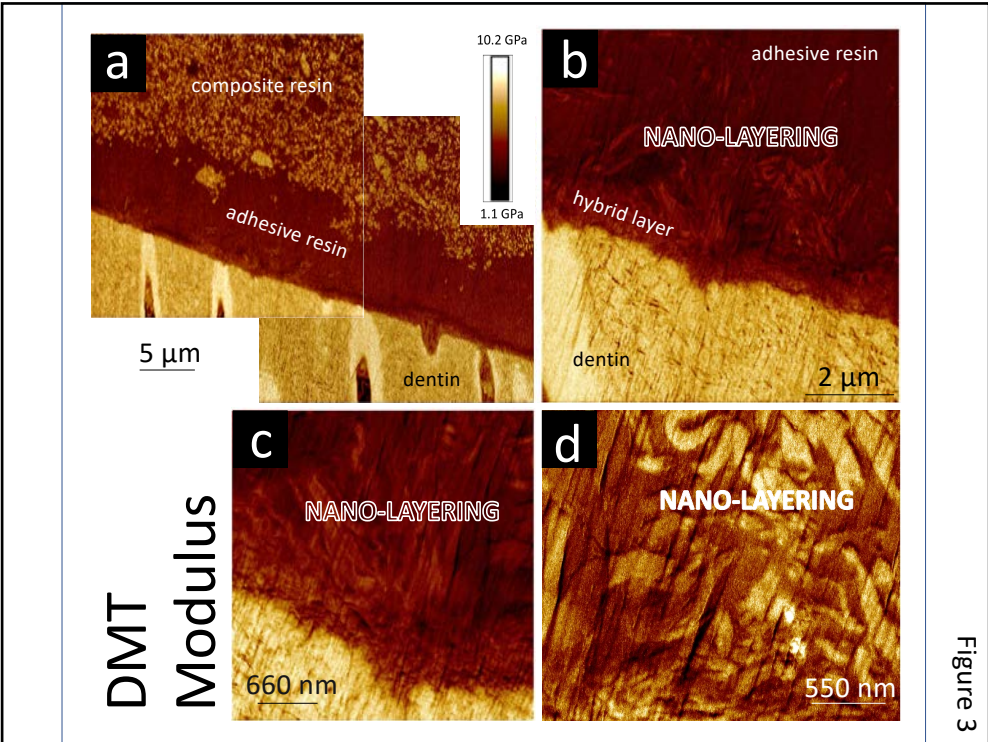


Figure 3

3

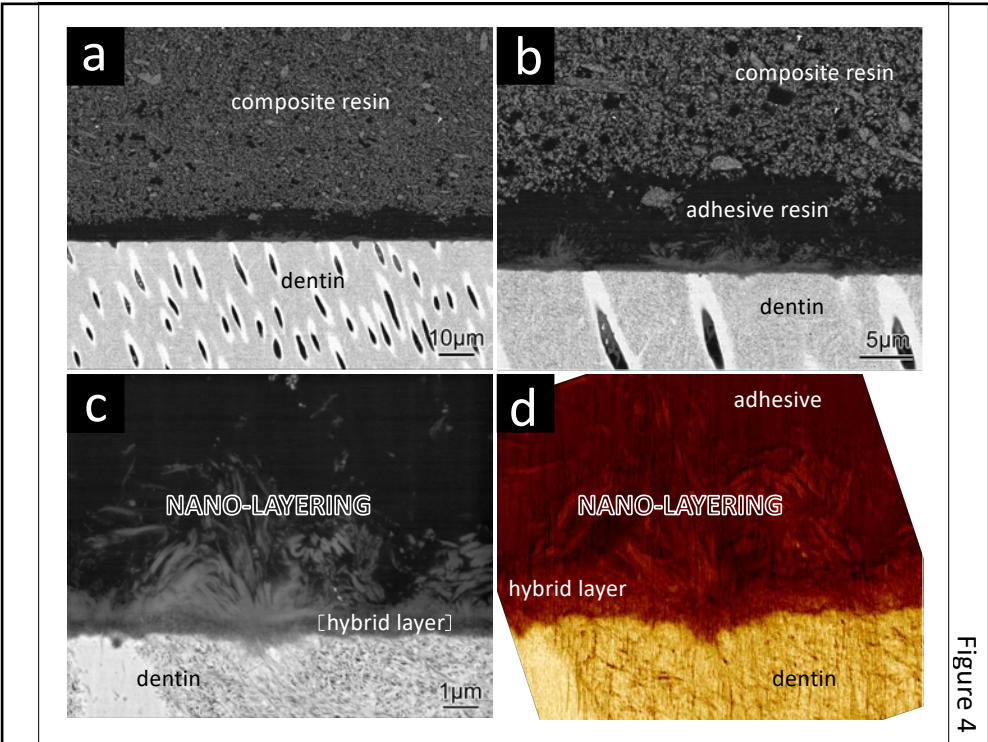


Figure 4

4

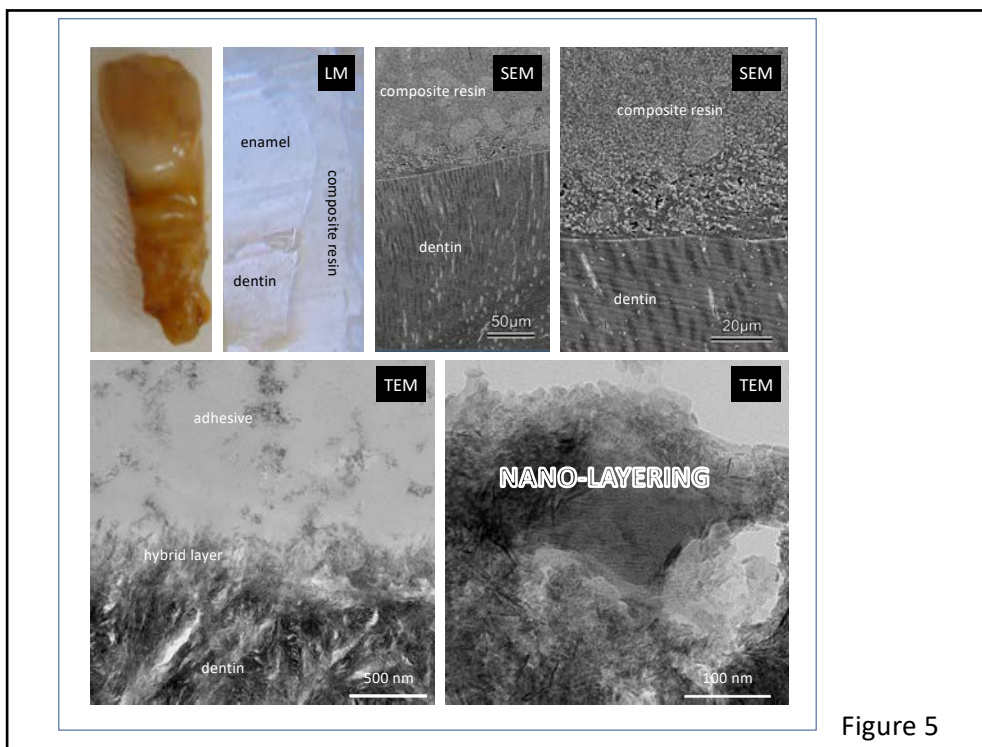


Figure 5

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