A proposal of microtomography evaluation for restoration interface gaps

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Summary. Nowadays, several adhesive systems are used in dental restoration and they’re evaluated by clinical research. In vitro evaluations are often made by means of traditional observation techniques (for example scanning electron microscope (SEM), while 3D cone-beam microtomography technique (3D micro-CT), that can be able to generate 3D sample images without any sample treatment during acquisition data, is going to be used a lot in the next few years. In dental cavity restored with composite, it is possible to predict the presence of gaps due to polymerization shrinkage; that is the reason this work purpose is to reveal by 3D images and measure by micro-CT analysis the voids generated applying the most used adhesive systems at the moment. By means of microtomographic analysis is proposed an aid to overcome bidimensional SEM investigation limits like random observation of sample surface, sample sectioning (to see inside it with the relative possible structural alterations induced on the same sample) and the gold sputtering treatment. For this experimental work, human crown teeth have been selected, all restored with the same composite material, using five adhesive systems. After about 48 hours each tooth has been acquired by means of Skyscan 1072 micro-CT instrument and then processed by 3D reconstruction and micro-CT analyser software. Three adhesive systems have showed 3D micro-CT images with not as much voids as expected, with a very little extent. This kind of micro-CT in vitro evaluation proposal suggests a method to observe and quantify the voids generated after polymerization shrinkage during tooth restoration.

Key words: adhesive systems, dental restoration, interface gaps, microtomographic analysis.

INTRODUCTION

One of the most important discoveries in dentistry was the acid-etching technique introduced in 1955 [1, 2] that allowed to bond hard dental tissue to resin material. Many studies were produced by industry in the past years to promote a strong adhesion capable to counteract polymerization composite shrinkage, to last more and to shorten chair time. The efficacy of bond-
ing to adhesive interface, briefly, guarantees stable adhesion and clinical success of dental reconstruction. Nowadays, adhesive dentistry consists of total etching and self etching techniques.

The total etching technique (three step system) is composed by many phases: etching-rinsing-drying, priming, bonding that allows the formation of the resin tags and an hybrid layer that can be able to create a bond between dentin and filling material, that is proper of micromechanical nature [2, 3]. To simplify and shorten this technique, it is possible to use the single bottle primer and bonding (two step system). In the three step system, the removal of smear layer and smear plug, the deep demineralization of dentin, the possibility that resin may not penetrate so deeply, may cause nanoleakage [4, 5] with consequent decreasing of dentin-resin bonding values [6-8] and long term degradation of bonding caused by hydrolytic alteration of unprotected collagen network and resinous material [4, 9]. Another consequence is the increase of dentin permeability, due to outward dentinal fluid movement from the pulp to the dentin that causes degeneration of collagen fibres not covered by resin and early degeneration of resin (aging) [10].

During chew process, the movement of flowed out fluid (increased by some iper-osmotic resin components) may cause displacement of odontoblasts and stretching of nervous fibres with consequent pain, postoperative sensibility, flexural restoration movements, pulp pathology and carries recidivism [11-14]. The two step system uses one-bottle for primer and bonding. It shorten procedures but create the same problems as total etching, due to strong acid action. Moreover, needs more layer because of its monomer dilution and needs of longer polymerization time [15-17]. The self etching technique is easier and shorter. It consists of two steps: self etching-priming and bonding. This products have lower acidity than phosphoric acid and don’t need rinsing. There are also products one-bottle self etching-primer bonding (one step) available on the market to simplify procedure.

These products don’t require the removal of smear layer and smear plug that remain in situ.

Basing on their own pH, self etching-priming products may causes a removal or only a partial dissolution of smear layer, going deep into interacting with the under-laying dentin. On the base of their acid behaviour, they are classified into mild, moderate and aggressive [11]. The simultaneous resin infiltration and polymerization in loco, prevents collapse of demineralised collagen [5], create an hybridized complex (consisting of hybridized smear layer and an underlying thin real hybrid layer) and resin tags [12] that, despite the shorter appearance than the one in total etching, seem to assure a satisfactory seal if the smear plugs are left intact [6]. As smear layer thickness on resin monomer diffusion seems to have no influence into (on) the underlying intact dentin creating a true hybrid layer, on the other hand, hybridized layer thickness doesn’t appears as it’s influencing bond strength [13]. Taking intact smear layer and smear plugs simultaneously infiltrated by resin, self etching primer systems, seems to reduce nano-leakage [6], dentin permeability [14], outward dentinal fluid movement, postoperative sensitivity, flexural restoration movements. Self etching priming products seems also to guarantee a higher bond strength than all the etching products [7, 8]. They are more simple to use and can reduce the chair-side time, which is very important and helpful in pedodontics with old and handicapped patients.

Basing on literature review [1] the simplified systems (one-bottle primer/bonding in total etching technique and one step in self-etching technique) seems to show the worst results because of insufficient dentin resin impregnation and the necessity of a longer polymerization time. The presence of hydrophilic monomers determines an hybrid layer that behave as permeable membrane to pulpal fluids creating micro-channel (“water trees”) even after polymerization with fluid movements into adhesive interface [15] and consequent degradation of hybrid layer collagen and resin [16].

All these factors reduce longevity of the bonding interface. On the other hand, separate use of bonding made of hydrophobic monomers, show higher extent of polymerization and less permeability to water of adhesive interface [17] with a longer consequent durability in time.

In conclusion, the most important factor to assure a clinical success is the achievement of a stable and lasting adhesion that is guaranteed by a satisfactory hybridization of demineralised dentin. It is extremely important, then that collagen fibrils result completely encapsulate by adhesive to avoid degradation [9]. Under shrinkage effect, gaps occur when strength is higher than cohesive bond on adhesive interface [18]. Gaps represent the local bonding failure and more gaps are present at the interface, less effective are the products in creating a real bond with dental surface.

Until now, adhesive interfaces were examined mainly using SEM. This technique, even if is a powerful investigation method, needs samples to be subjected to severe stress for sectioning them in more portions with deep/hard sample-portion alteration and possible crack formations.

In this study we proposed to analyze gap formation to adhesive interface using a new technique like three-dimensional X-ray micro-CT that doesn’t need any treatment or stress or sectioning of samples.

**MATERIALS AND METHODS**

In this experimental work 25 coronal portions of human third molars extracted from patients (20-35 age) suffering for dysodontiasis and stored in physiological solution have been used.

Only one composite material (Filtek™ Silorane 3M ESPE) has been choose to restore the cavity in all teeth.
The selected adhesive systems used to prepare the samples were:
1. TECO (DMG, Germany), total etch bonding system for all light-curing restorative materials, with unique single dose application;
2. Adper™ Scotchbond™ SE Self-Etch Adhesive (3M ESPE, USA), two-bottle self-etch adhesive system;
3. CONTAX (DMG, Germany), self-etching and self-conditioning adhesive system;
4. Adper™ Scotchbond™ 1 XT (3M ESPE, USA), total etching simplified technique with monobottle
5. Bond - 1 SF PENTRON CLINICAL All-in-one (Pentron Clinical Technologies LLC, USA), light-cure, one-coat, self-etch Solvent-Free adhesive.

The 25 coronal portions of human teeth were randomly collected/divided in 5 groups, i.e. 5 samples for each adhesive systems. Each tooth has been analyzed five times by means of a microtomography instrument mod. SkyScan 1072 (SkyScan, Kartuizersweg 3B, 2550 Kontich, Belgium).

Sample preparation
In all teeth a cavity has been created in the central portion of occlusal surface, then measured and evaluated by means of microtomographic technique. All tooth cavity dimensions were computed on 4 orthogonal sections for each samples and in 3 point along each dimension. The dimensional mean values of the created cavities were 2.8 height and 5.1 diameter. To avoid angle formation, in each tooth the cavity was prepared using a round diamond bur with 2.5 mm diameter for about 3.0 mm mean depth. All cavities were cleaned with air-water spray and softly dried to ensure wet surface. The five selected adhesive systems were applied according to manufacturer’s instructions and all cavities were filled using the same Filtek™ Silorane (3M ESPE, USA). The composite was photopolymerised in layer of 2 mm and then all teeth were served at composite-dentine interface.

All the procedures have been carried out each time from the same dentist. Increasing layer’s polymerization (2 mm maximum thickness) of the same composite has been applied for a period of 45 seconds for each layer, using a LED rechargeable blue lamp (mod.655/00, De Giorgi, Italy) with 420-480 nm wavelength.

Sample micro-CT acquisition
Each tooth has been extracted from physiological solution, placed on a stub and glued on plasticine to avoid any movement during micro-CT acquisition. The acquisition parameters were the following:
- voltage: 100 kV;
- current: 98 μA;
- filter material: 1 mm di alluminio;
- spatial resolution: 14-15 micron voxel/pixel size;
- rotation step, rotation angle: 0.45°;
- frame averaging: 2 frames;
- amount of axial micro-CT slices: about 800;
- slice thickness: 10 micron.

Sample reconstruction
At the end of micro-CT acquisition, each sample has been subjected to reconstruction process that reproduces a sample by means of about 6 or 8 hundred slices that can be displayed by means of a dynamic software, named Tview (SkyScan, Kartuizersweg 3B, 2550 Kontich, Belgium). With these slice set of data, each sample has been 3D reconstructed by means of CT-Rec (SkyScan, Kartuizersweg 3B, 2550 Kontich, Belgium) and displayed by 3D image. By means of another software, named CT-Analyser (SkyScan, Kartuizersweg 3B, 2550 Kontich, Belgium), volumetric and dimensional parameters have been obtained for each sample. This software showed the same parameters also for the restorative material and the gap at dentine-material interface, selecting a specific Region Of Interest or Volume Of Interest (ROI, VOI).

Reconstructed sample images have been processed displaying the tooth crown portion with transparency effect and colouring with blue the composite material volumes and with red the void volumes observed at composite-dentine interface.

The reconstruction parameters were the following:
- number of layers, this value depends to chosen sample section to reconstruct;
- lower and upper vertical position, these values depend on sample dimensions and/or on selected section to reconstruct;
- pixel size, 15 micron for a magnification of 20x;
- lower and upper grey threshold, these values depend on sample radiopacity that shows the two limit levels between for reconstruct processing.

At the end of reconstruction process it is possible to obtain some dimensional parameters for each sample, like bone histomorphometry nomenclature (19): total volume (TV), expressed in mm³, object volume (Obj.V), expressed in mm³, percent object volume (Obj.V/TV), expressed in %, total surface (TS), expressed in mm², object surface (Obj.S), expressed in mm², object surface / volume ratio (Obj. S/Obj.V), expressed in 1/mm.

RESULTS
Table 1 shows the mean volumetric dimension values of the acquired tooth-crown sample (Vtot), the restorative composite material (Vm) and the gap at composite-dentine interface (Vg), obtained by micro-CT analysis and processed according to about 15 μm pixel size corresponding to 20x magnification. In Table 1, a parameter (Vs) obtained by material volume (Vm) plus gap volume (Vg) that represents the volume of cavity created in the middle of occlusal surface is shown. Observing this table, it’s possible to evaluate that sample 5 showed a Vg lower value while the sample 3 showed a Vg greater value.

To better understand the previous data, in Table 2 are showed other parameters, P1, P2, P3, calculated to evaluate, in percentage, the proportion between the created cavity and the total sample volume (P1...
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Between the gap and the restorative material volume (P2 = 100 × Vg/Vm) and between the gap and the cavity volume (P3 = 100 × Vg/Vs), P2 and P3 have been then related to P1 in order to decrease the coronal and cavity volume value influence and to obtain a better understanding of adhesive performance for each tested material. In Table 3 the percentage numerical values when dividing P2 and P3 by P1 are showed.

Table 3 obtained values show better adhesive properties for adhesive system no. 5 and greater gap volume value for adhesive system no. 3, according to Tables 1 and 2 results. These parameters, in a good proportional equilibrium, can be an indication of a long life restoration.

DISCUSSION

The adhesive evaluation of restorative materials and dentine structure is one of the most difficult goals for any kind of analytic investigation technology. It has been used in the research field from many years in order to display, understand and improve dentine-restoration interface to obtain a long life restoration.

The failure of adhesion interface shows the presence of gaps (gap making) that are generated when the polymerization shrinkage of composite material is stronger than composite-dentine interface. The presence of gaps has been shown by means of SEM observations on a sample section obtained after restored tooth cutting.

Another problem that is yet to be solved and evaluated, is due to the aging alterations of restoration-dentine interface that, with passing of time, shows gaps and detachment making.

From several years the micro-CT analysis is working in different sections of dentistry, providing an aid to well understand and observe innovative dental materials and devices by means of 3D information (data), in spite of bi-dimensional ones obtained with SEM and histological analysis [20-23]. This new kind of analysis (3D analysis) shows more positive characteristics than traditional investigations, as the sample subjected to micro-CT analysis does not need any treatment or cutting and it is possible in this way to analyse the same sample many times.

In this work, micro-CT analysis has been carried on with observations of dental crowns restored with composite materials using five different adhesive dental systems to evaluate their performances and to support the wide adhesion study in literature. Micro-CT analysis provided two kinds of results; the first qualitative data/results generate the 2D and 3D images obtained on all 360° sample acquisition, the second quantitative data/results generate numerical values like hystomorphometric parameters. By 2D images it is possible to observe, analyse and measure the different components of an analysed sample, slice by slice for all the sample size.

In this proposal of micro-CT study, the restoration structure and gaps of the adhesive interface after the composite polymerization shrinkage have been analysed with virtual horizontal and vertical

Table 1 | Mean volumetric values obtained by microtomographic analysis

<table>
<thead>
<tr>
<th>Samples</th>
<th>Vtot (mm³)</th>
<th>Vm (mm³)</th>
<th>Vg (mm³)</th>
<th>Vs (mm³) = (Vm + Vg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>406.94 + 0.01</td>
<td>42.43 + 0.01</td>
<td>0.80 + 0.01</td>
<td>43.23 + 0.54</td>
</tr>
<tr>
<td>2</td>
<td>641.44 + 0.01</td>
<td>88.24 + 0.01</td>
<td>0.70 + 0.01</td>
<td>88.51 + 1.26</td>
</tr>
<tr>
<td>3</td>
<td>414.34 + 0.01</td>
<td>55.32 + 0.01</td>
<td>2.67 + 0.01</td>
<td>57.99 + 0.22</td>
</tr>
<tr>
<td>4</td>
<td>499.55 + 0.01</td>
<td>96.59 + 0.01</td>
<td>1.00 + 0.01</td>
<td>97.59 + 0.98</td>
</tr>
<tr>
<td>5</td>
<td>490.06 + 0.01</td>
<td>67.91 + 0.01</td>
<td>0.06 + 0.01</td>
<td>67.93 + 11.32</td>
</tr>
</tbody>
</table>

Table 2 | Calculated parameters obtained by microtomographic analysis

<table>
<thead>
<tr>
<th>Samples</th>
<th>P1 (%)</th>
<th>P2 (%)</th>
<th>P3 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.62</td>
<td>1.88</td>
<td>1.85</td>
</tr>
<tr>
<td>2</td>
<td>13.80</td>
<td>0.31</td>
<td>0.30</td>
</tr>
<tr>
<td>3</td>
<td>14.00</td>
<td>4.83</td>
<td>4.60</td>
</tr>
<tr>
<td>4</td>
<td>19.54</td>
<td>1.03</td>
<td>1.02</td>
</tr>
<tr>
<td>5</td>
<td>13.86</td>
<td>0.03</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Table 3 | Percentage of P2 and P3 divided by P1

<table>
<thead>
<tr>
<th>Samples</th>
<th>P2/P1 (%)</th>
<th>P3/P1 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.18</td>
<td>0.17</td>
</tr>
<tr>
<td>2</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>3</td>
<td>0.34</td>
<td>0.39</td>
</tr>
<tr>
<td>4</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>5</td>
<td>0.002</td>
<td>0.002</td>
</tr>
</tbody>
</table>
cut or slice images. By means of reconstruction and processing software of the used micro-CT system, it has been possible to show dental crown volumetric structure as transparent while restored material with gaps along all sample structure have been highlighted with different colours. Other images have been processed and obtained without restoration material but with the same transparent effect for the dental crown volumetric structure and with gaps highlighted in red. In this way, air bubbles due to reconstruction process glued in composite material and gaps due to polymerization shrinkage, have been well observed then highlighted with different colours. The best adhesive materials and methods generate very small and little gaps. When the gaps are observed by means of micro-CT images, it is possible to measure them and calculate their volumetric values. For any kind of selected horizontal and vertical slice, it is possible to measure dentine-restoration gaps in any wished point or carry out any other dimensional measure. After performing the measures previously mentioned, it has been proposed the computing of some parameters that can contribute to an evaluation of used restoration-structure proportion to obtain a weighted evaluation of measured gap values.

These parameters were calculated basing on volumetric results obtained by means of micro-CT analysis.

In this experimental work proposal of micro-CT analysis for an evaluating study of the adhesive system, now widely used in conservative restoration, have been analysed five dental crown restored with the same material and method to obtain the same polymerization shrinkage, but actually five different kind of adhesive system technologies have been used.

These adhesive system technologies are all that now can be used to do conservative restorations and they consist in single or multiple procedures that use more or few aggressive substances respect to the dental tissue. From performed micro-CT analysis it has been underlined that the “all in one” adhesive material shows less quantity and less large gaps than other adhesive system and there seems to be no correlation between the restoration dimension and gap making. Micro-CT analysis results obtained in this study may be due to the use of a particular polymerization technique defined layer by layer deposition, but will be verified with statistical evaluation on a larger number of samples.

Fig. 1 | 3D reconstruction images of the tooth samples.
CONCLUSIONS

This proposal of microtomography evaluation for restoration interface gaps seem to be an innovative and very useful technique that can substitute the traditional SEM investigations used until now, even if the tooth sample cutting and treatment for SEM observations surely causes alterations like gaps or creeps on the same sample. With micro-CT analysis it is possible:
- to analyse a sample without any treatment or cutting, as requested for SEM investigations, that can provide alterations on the sample structure;
- to study every point of adhesive interface by means of a lot of horizontal or vertical slices;
- to display very well the presence of adhesive interface gaps due to polymerization shrinkage and calculate the gap volumetric values;
- to analyse the same sample several times with micro-CT instrument, i.e. a no treated tooth sample can be subjected to a first micro-CT analysis then may be subjected to different aging process or other treatments and again analysed by micro-CT. In this way it is possible to observe the dimensional differences, or gaps making, or other kind of alterations induced on the same sample after application of various aging treatments in time and evaluate the possible adhesive interface degradation of aged composite-restoration interface.

Conflict of interest statement

There are no potential conflicts of interest or any financial or personal relationships with other people or organizations that could inappropriately bias conduct and findings of this study.

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References


