Chapter 2:

Test methodology and preparation of samples
In-vitro testing methods for restoration adaptation

There are different parameters or levels to assess the quality of dental adhesives & tooth colored materials:

- Material physical properties
- Material biocompatibility
- Wear resistance
- Bond strength to dental tissues*
- Restoration marginal adaptation**
- Restoration internal adaptation**
- Restoration resistance to fracture or deformation**

* might be tested in a clinical configuration  ** usually tested in a clinically relevant configuration

Due to the aforementioned refinements in composite technology and adhesive systems, most of the limitations and drawbacks of former materials have been overcome. Actually, since modern adhesives provide a real cohesion between the restoration and dental tissues but considering the remaining resin composite polymerisation shrinkage, marginal and internal adaptation remain the chief quality parameter to be evaluated. For instance, the presence of marginal or internal gaps, favors either leakage or immediate and delayed post-operative sensitivity. It can also, on the long term, lead to a fragilization and mechanical breakdown of the restoration or dental tissues, such as the well-known cracked-tooth-syndrome (Thomas, 1989; Ehrmann and Tyas, 1990; Geursten, 1992; Kahler et al, 2000), a common problem associated to non adhesive restorations like amalgam. In other words, the restoration adaptation is perhaps the most critical parameter to determine its long term biomechanical behaviour.

The restoration marginal and internal adaptation can be evaluated after the samples were submitted to different tests or conditions (in nearly chronological order of their use in laboratory trials):

- Dye penetration
- Dye penetration and thermal cycling
- Mechanical loading
- Mechanical loading and thermal cycling
- Mechanical loading, thermal cycling & abrasion test (i.e.: with a brush)
Evaluation methods evolved in conjunction with the progresses of material properties and technology. While rather simple tests were initially applied to composite restorations, such as dye, isotope or bacteria infiltration tests (Fischer, 1949; Roulet and Reng, 1975), much more sophisticated ones are nowadays applied, including eventually thermal, chemical and mechanical simulation of the oral environment (Delong and Douglas, 1983; Krejci 1990a and b; Roulet, 1990). The scanning electron microscope has replaced visual observation or macrophotography and with the proper simulation of occlusal forces, in-vitro testing can yet almost replace in-vivo studies. Actually, clinical trials are not only time-consuming, but they are increasingly difficult to organize and, due to the observation time period required (usually more than 3 to 5 years) provide valuable information only on materials which are outdated or even already withdrawn from the market. We should therefore consider in-vitro testing as the basic evaluation tools, using in-vivo studies only for materials or techniques which proved highly successful in the laboratory and to get confirmation of their long term clinical behaviour.

The in-vitro trials presented in the next chapters are based on a basic test and an evaluation method. All materials and techniques were evaluated in a clinically relevant configuration for class II restorations, with a simulation of occlusal forces following physiological mastication patterns (Bates et al, 1975a and b; Gibbs et al, 1981a and b; Neill and Powell, 1988; Neill et al, 1989; Douglas, 1996; ).

Preparation of Specimens

Freshly extracted human third molars were used for all experiments. The inclusion criteria were that the teeth be free of decay and presented a complete apexification. The extracted teeth were kept in an isotonic solution, containing sodium azide (0.2%), at 4°C until the experiment onset, to prevent bacteria or fungus growth in the storage medium.

For each specimen, the root length was adjusted to fit in the test chamber of the mechanical loading device (Department of Cariology, & Endodontics and Laboratory of Electronics; Faculty of Medicine, University of Geneva) (Fig.2.1 to 2.3). After the specimen was properly positioned, it was fixed with light-curing composite on a metallic holder (Baltec; Balzer, Liechtenstein) and the root base was embedded with self-curing acrylic resin to complete the tooth stabilisation (Fig. 2.4). Standardised box-shaped Class II cavities (MOD) with parallel walls and rounded internal lines were prepared for all tests (Fig.2.5). The proximal margins were located 1.0 mm below (mesially) and above (distally) the cementum-enamel junction. The dimensions of the preparation were 4.0 mm in width and 2 mm in depth, at the bottom of the proximal box, and 2 x 4mm (depth x width) in the occlusal area. A parallel cavity design with bevelled enamel margins was applied to all direct techniques (Fig2.5A), while tapered cavities, with a butt margin design, was considered for all indirect restorations (Fig 2.5B).
The cavities were prepared using coarse diamond burs under profuse water-spray (Geneva Prep Set; Intensiv; Viganello, Switzerland) and finished with fine grained burs of the same shape (Geneva Prep Set). Prepared teeth were randomly assigned to one of the experimental groups, related to different adhesive systems, restorative materials or restorative techniques.

After completion of the restorative work, the pulpal chamber was perforated buccally or palatinally with a fine cylindrical bur for placing a fine metal tube (1 mm diameter), sealed with a dental adhesive and, which was connected to a reservoir of saline water (Fig. 2.3 and 2.4). This simulated pulpal circuit creates a pressure of 14 cm H2O, such as measured in vivo (Andrews et al, 1972; Ciucchi et al, 1995).

**Mechanical loading**

The loading test was carried out after a 24h delay. All specimens were successively submitted to 250'000 cycles and 50N of loading force, then 250'000 with 75N and 500'000 cycles with 100N, representing a total of 1'000'000 loading cycles. The axial loading force was exerted at a 1.5 Hz frequency, following a one-half sine wave curve. These conditions are believed to simulate about 4 yr. of clinical service (Krejci et al, 1990a and b). Restored teeth were contacted by antagonist artificial cusps, made of stainless steel, the hardness of which is similar to natural enamel (Vickers hardnesses: enamel = 320-325; steel = 315). The diameter of the metal cusps was 4 mm and they were placed 1 mm above the restoration occlusal surface, about 1.5 mm out of the central fossa. The specimen being mounted on a rubber disc, a sliding movement of the restored tooth was made possible between the first contact on the inclined plane and the central fossa. The functions of this experimental device are similar to the machine developed by Krejci et al. (1990a).

**Specimen evaluation**

*Marginal adaptation*

The restoration marginal adaptation was assessed before and after each loading phase, as well as at completion of the test. Before making each impression of the restoration proximal margins with a polyvinylsiloxane material (President light and heavy body, Coltene AG), the surfaces were cleaned with a brush and fine pumice and acid-etched with 37 % H3PO4 gel (30s on enamel and 10s on dentin). This short acid etching of restoration margins is a modification of the basic method of surface replication (Kusy and Leinfelder, 1977; Herr et al, 1981) which dramatically improves the accuracy of the SEM observation. Then, gold sputtered epoxy resin replicas were fabricated (Epofix, Struers; Copenhagen, Denmark) (Fig.2.6). The proximal tooth-restoration interface was analysed quantitatively with the scanning electron microscopy (SEM) (Digital SEM XL20, Philips, Eindhoven, Netherlands) by applying a
recognized evaluation method (Luescher et al., 1977; Roulet, 1990). The following evaluation criteria were considered for both direct and indirect restorations: “continuity”, “overfilling”, “underfilling”, “marginal opening”, “marginal restoration” or “tooth fracture” (Fig. 2.7A and 2.7B). For indirect restorations, the interfaces between the restoration and the luting cement and between the luting cement and tooth were analyzed (Fig. 2.8). The proximal limits of the restorations were observed at a standard x150 magnification. When necessary for the assessment accuracy, higher magnifications were used. Results of the restoration marginal adaptation, before and after the different loading phases, are expressed as the percentages of margins in “continuity” (or “marginal opening”) for the 3 segments under evaluation - enamel margins on the distal tooth side (E), and enamel (ED) and dentin (D) margins on the mesial tooth side (Fig. 2.5). Whenever considered appropriate, the percentages of “marginal tooth fracture” were given for the restoration adaptation to enamel. The restoration occlusal adaptation was never assessed.

**Internal adaptation**

An SEM observation of internal adhesive interfaces was judged necessary to supplement the information resulting from the standard margin quality evaluation (Luescher et al., 1977; Roulet, 1990; Krejci, 1992). This additional evaluation, performed on replicas of sample sections, proved useful to study the micromorphology and failure modes of class II adhesive restorations (Dietschi et al., 1998 and 1999).

At completion of the mechanical loading, the teeth were embedded in a slow self-curing epoxy resin (Epofix) and sectioned mesio-distally into three parts, with a central slice of 1 mm, using a slow rotating saw (Isomet 11-1180; Buehlers, Evanston IL, USA). The sections were successively polished with 200, 400 and 600 grit SiC paper and etched for 1 min with a 37 % H$_3$PO$_4$ gel. Impressions were then taken from the four available surfaces for fabricating gold sputtered resin replicas (Fig. 2.9). In order to avoid observation artefacts, special care was taken not to dehydrate the samples prior taking the impression with a “moisture tolerant” material (President light body, Coltene).

The restoration internal adaptation (or adaptation of the base-liner or luting cement to the tooth) was assessed quantitatively on the gold-sputtered replicas under the SEM, at a 150x magnification, and was judged according to two criteria: “continuity” and “interfacial opening”. Results are expressed as the percentage of interface in “continuity” (or “interfacial opening”), relative to the whole dentin interface (total) and in addition, to the following dentin segments: gingival enamel (GE), gingival dentin (GD), axial dentin (AD) and occlusal dentin (OD) (Fig. 2.10). For each sample, results are expressed as a mean value, resulting from the evaluation of the 4 sections. The micromorphology as well as the localization of bonding failures
within the adhesive interface was identified by using higher magnifications (up to 1000x). Only trained evaluators (one per study) perform SEM observations.
Figure 2.1: View of apparatus used to simulate masticatory forces.

Figure 2.2: Diagrammatic representation of the fatigue device and its function. Vertical and sliding movements of the sample are generated.
Figure 2.3: Detailed view of one of the 8 chambers of the fatigue device.

Figure 2.4: The tooth is first stabilized with composite (A), before embedding the root with resin (B). The pulpal chamber of samples is perforated (on the buccal surface) and connected to an external simulated pulpal circulation.
Figure 2.5A: Representation of cavities prepared for direct restorations. Cavities have standardized dimensions and present cervical limits in enamel (distal) and in dentin (mesial); margins are bevelled.

Figure 2.5B: Cavities for indirect restorations feature the same characteristics as for direct restorations. They are however slightly tapered and have a butt margin design.
Figure 2.6: Diagrammatic representation of the fabrication of resin replicas. The impression (IM) is poured with slow-curing resin (R) and then fixed on a special holder for SEM observation. After fixation, replicas have to be gold coated.
Figures 2.7B: Evaluation criteria for the assessment of marginal adaptation of direct restorations (excellent adaptation – underfilling – overfilling).
Figures 2.7B: Evaluation criteria for the assessment of marginal adaptation of direct restorations (marginal opening – marginal tooth fracture – marginal restoration fracture).
Figures 2.8: Evaluation criteria for the assessment of marginal adaptation of indirect restorations (tooth-cement interface and cement-restoration interface).
Figure 2.9: The internal adaptation is evaluated on 4 surfaces (1 to 4). The medium section has a 1mm thickness.

Figure 2.10: Evaluation areas considered for the internal adaptation of restorations.
References


DIETSCHI D, HERZFELD D. In vitro evaluation of marginal and internal adaptation of class II resin composite restorations after thermal and occlusal stressing. Eur J oral Sci 1988; 106: 1033-1042.


