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Dental resin materials *in vivo* — TEM results after one year: A pilot study

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Abstract Dental resins deteriorate clinically due to chewing forces, temperature changes, chemical agents or biological attack. Findings concerning these influences on the different components of a resin are limited. The aim of this study was to evaluate an alternative method for assessing the influence of the oral cavity on dental materials and their individual components as well as analyzing degradation effects over time. Seven dental composite and resin materials were inserted into the upper complete dentures of two subjects and evaluated after one year with a transmission electron microscope. The various resin components showed different degrees or deterioration. Composites with an urethandimethacrylate matrix were less vulnerable. A layer of salivary proteins (pellicle) was found on all materials but the polymethylmethacrylate reference. An accumulation of pellicle on filler particles and the crevice between filler and matrix was noted. We conclude that the tested method is effective for evaluating the interaction between the material's components and the biological environment. Further studies are needed to confirm these observations.

1. Introduction

Dental resins show traces of wear attack or superficial cracking over time when orally applied [1]. Chewing forces, temperature changes, chemical agents or biological attack, among other variables, can adversely affect the different material components. Scanning electron microscopy (SEM) showed a superficial deterioration of the resins and a drop out of fillers [2], although the filler components are silanized and more or less chemically bonded to the methacrylate resin matrix [3]. Alcohol, acids, and water attack the silane coupling or fillers, such as barium glass [4]. Enzymes or oral bacteria interact with the resin [5], and low viscous monomers like triethylenglycoldimethacrylate (TEGDMA) are even metabolized [6, 7]. Accumulated bacteria produce further acids, alcohol or degrading enzymes which continue the destructive attack.

Much information has been presented concerning the deterioration of dental materials under clinical conditions [8, 9] and single components have been investigated in detail in the laboratory [10, 11]. Such tests were performed with isolated components, but the combination of matrix, silane coupling and filler has been neglected. Furthermore the microscopic size of certain components, such as SiO_2, prevents clinical examination since only specialized laboratory equipment can provide detailed insight into this microscopic world. SEM enables a high resolution, but only superficial information is provided. Transmission electron microscopy (TEM) allows a higher resolution and cross-sections of the surface can be examined. However, the hardness of the highly filled resins, which is caused by up to 80 weight% filler content, combined with a soft layer of proteins and bacteria, restricts the preparation of ultra-thin specimens. Therefore, images to date have only been able to show bonding surfaces [12] or protein and bacterial layers [13] where the supporting material was removed, for example by etching.

The aim of this study was to evaluate an alternative assessment method using TEM and to illustrate the influence

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of the oral cavity on dental materials and their individual components. The interaction between the layer of salivary proteins (pellicle) or bacterial layers and the materials was investigated after one year of oral application for assorted composite and resin tooth materials.

2. Materials and methods

Seven materials representing variations of clinically used veneering composites (effect, incisal, dentine masses) and denture tooth material were provided for examination. Similar to commercially available composites, all materials were based on Urethandimethacrylate (UDMA) and differed only in the type of additional matrix components (Bisphenolglycidyl-methacrylate (bis-GMA), Decandioldimethacrylate (DDMA)) and filler particles (Tab. 1). As a reference, a Polymethylmethacrylate-based (PMMA) resin without filler was used. The tooth composite was selected for a high affinity for oral adhesion, whereas the PMMA reference showed a low bacterial film *in vivo* [14].

Specimens (4.5 mm × 3.0 mm × 1.0 mm) of each material were arranged into one sample, which was inserted in the posterior buccal area of a maxillary complete denture and subsequently polished (universal polishing paste, Ivoclar-Vivadent AG, Schaan, Liechtenstein). The complexity of sectioning, sample preparation and TEM analysis forced us to restrict our analyses to commonly used materials and only two patients (male/female), but may nonetheless provide adequate information for estimating the usability of the presented method.

After 12 months of oral service, the samples were removed and stored in water with 0.2% sodium azide. Specimens were cut into cubes (1 mm × 1 mm × 2 mm), embedded in agarose and fixated in a 0.1 M cacodylate buffer with 2.5% glutaraldehyde and 2% formaldehyde. Specimens were dehydrated in graded ethanol and embedded in epoxy resin (Embed 812, Science Services, Munich, Germany). 80 nm thin sections (Reichert Ultracut S, Leica, Bensheim, Germany) were contrasted with uranyl acetate and lead citrate. The slices were collected on copper grids and examined at magnifications between 1,600 × and 40,000 × (EM912 AB, LEO, Oberkochen, Germany). As a control, specimens without intra-oral service were investigated.

3. Results

With regard to the patients, the TEM images of the materials showed comparable characteristic properties and only minor differences in the amount of pellicle and bacterial film. In addition to the translucent matrix components, three types of inorganic fillers could be detected: nanofiller ($<0.001\,\mu$m), microfiller ($\sim 0.02\,\mu$m) and macrofiller ($\sim 2\,\mu$m) [15]. The materials A, B and G were not affected by the intra-oral service. A translucent layer below the material surface indicates alterations in the subsurface of the veneering materials (C, D, E; thickness: 650 nm–1700 nm) and the denture tooth composite (F; thickness: 120 nm–400 nm). The non-worn control materials had no superficial alterations. All materials, with the exception of the non-filled denture tooth PMMA reference (G), showed a fibrillar and globular pellicle between 30 nm and 1000 nm. The pellicle cumulated in cavities (C) and was denser when filler particles of the veneering composites (A; B; C; D; E) were exposed. Pellicle was even found at the interfaces between matrix and macrofillers (C and E). The denture tooth composite (F) showed the highest amount of pellicle (up to 1000 nm). Bacterial films between 0.8 μm and 10 μm were found on all materials but the PMMA reference (G). Representative images of specimens and controls are shown in Fig. 1.

Table 1 Materials and composition

	Material			
	Veneering composites	Matrix	Filler type	Filler content
A	High filler content composite (experimental)	UDMA	nano, micro, macro	80% (w/v)
B	Reduced filler content composite (experimental)	UDMA	nano, micro, macro	50% (w/v)
C	Effect mass composite	UDMA, Bis-GMA, DDMA	nano, macro	77% (w/v)
D	Incisal composite	UDMA, Bis-GMA, DDMA	nano, macro	77% (w/v)
E	Dentin composite	UDMA, Bis-GMA, DDMA	nano, micro, macro	76% (w/v)
	Denture tooth materials			
F	Denture tooth composite	UDMA	nano	
G	Denture tooth resin	PMMA	—	—

All materials provided by Ivoclar-Vivadent AG, Schaan, Liechtenstein
Abbreviations: Bis-GMA = Bisphenolglycidylmethacrylate; DDMA = Decandioldimethacrylate; PMMA = Polymethylmethacrylate; UDMA = Urethandimethacrylate; Nano = Nanofiller (SiO_2); Micro = Microfiller (mixed oxides);. Macro = Macrofiller (bariumglass); (w/v) = weight per volume

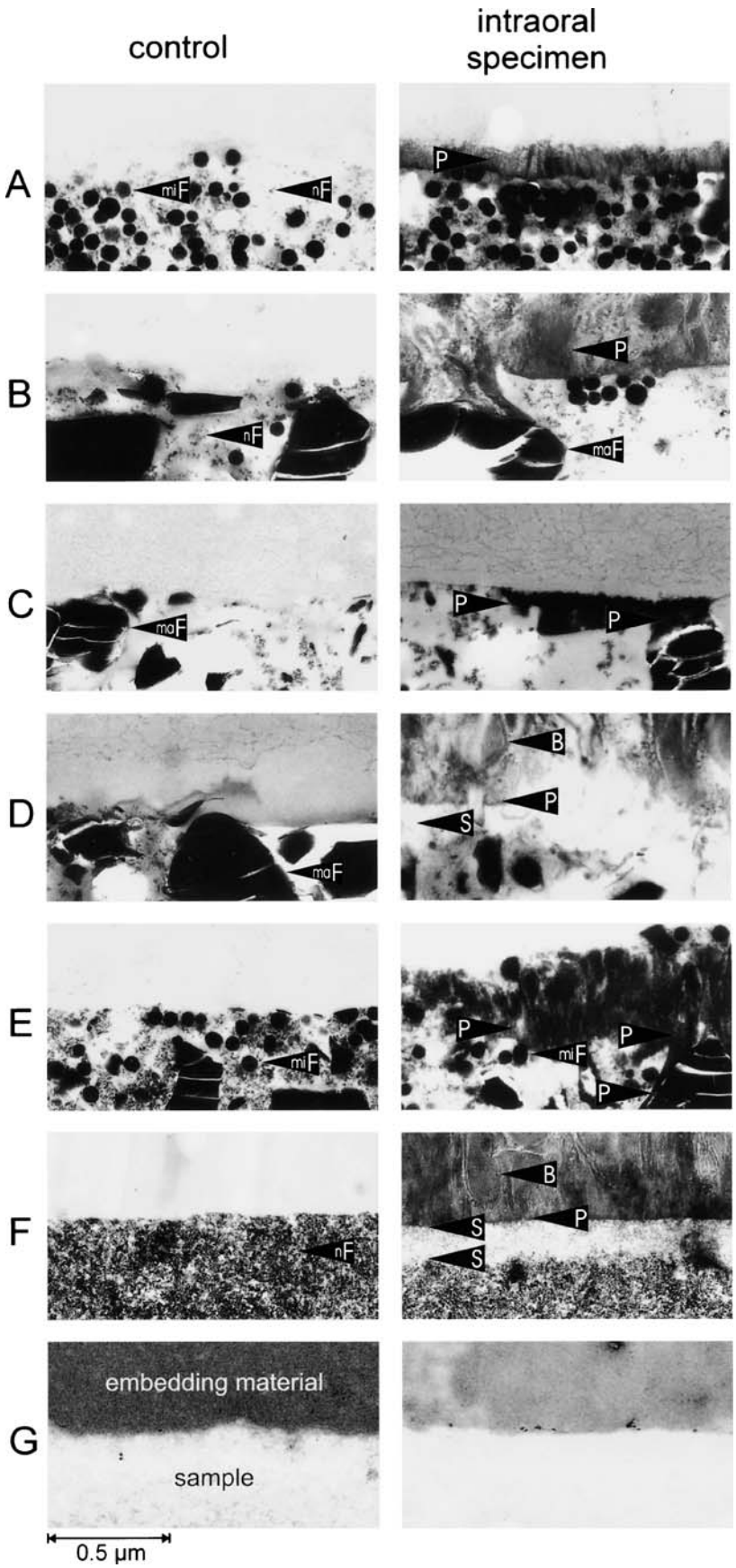

Fig. 1 TEM images of the specimen surfaces, magnification 10,000, scale = 0.5 μm; Controls are shown on the left, intraorally worn specimens on the right. **(A)** high filler content composite: accumulation of high fibrillar pellicle on microfillers **(B)** reduced filler content composite; pellicle reaches macrofillers **(C)** effect mass composite: dense pellicle in cavities **(D)** incisal composite: translucent surface layer, pellicle and bacterial layer **(E)** dentin composite: pellicle in subsurface areas in contact to macrofiller **(F)** denture tooth composite, reference: translucent surface layer, pellicle and bacterial layer **(G)** denture tooth resin, reference: no discernable surface layer, material is less dense than embedding material. Symbols used: nF = nanofiller; miF = microfiller; maF = macrofiller; P = salivary protein layer (pellicle); B = bacteria; S = less dense surface layer.

4. Discussion

Thin-film cutting of highly filled materials results in artefacts and fusion of individual components or even damage to the cutting knife. To circumvent this problem, previous studies removed hard materials by etching, only evaluating the pellicle or bacteria [16] or cut materials with a more evenly distributed hardness, such as dentin and resin [12].

The techniques described above using TEM analysis allowed for the combined illustration of the hard dental composite including the sensitive biological layer without the need for a potentially harmful etching process. This may provide a more accurate impression of the interaction between dental material and environment compared to previous studies. The presented TEM images of dental resins and composites showed differences in type and amount of the pellicle and bacterial film, as well as changes in the materials. After oral service, the composite materials and the denture tooth reference showed a superficially brightened and less dense layer. Whether this effect indicates degradation or increased water uptake with a subsequent swelling cannot be determined by TEM analysis. Bis-GMA, DDMA (or some of their derivates) or SiO_2 components seem to be responsible for this, whereas materials with only UDMA matrix seem insensitive. Ethanol, acids or enzymes are known to degrade resins [4] and might have promoted the damage to the surfaces of the sensitive materials. A correlation between pellicle thickness and the alterations may only be surmised.

The structure of the dense basal or globular and fibrillar pellicle and the overlying bacterial film are in accordance with previous studies [17, 18]. Because this layer is easily removed by conventional cleaning methods, we presume that the TEM images show a common layer younger than 12 hrs [13]. The appearance of greater amounts of pellicle in protected cavities has been described previously [13]. The accumulation of pellicle on filler particles or even in the material substance between filler and matrix is most interesting. A high affinity of the pellicle to the different fillers or silanized surfaces may cause deterioration, loss of the fillers or degradation of the filler-matrix bonding. Whether hydrolytic attacks [19] or a high protein affinity, particularly to the Si-O bonding, contribute to this result has to be clarified in further detailed studies. The images provide a preliminary indication that not only matrix, but also inorganic fillers and the silane-coupler may contribute to the degradation process of a composite material, thereby promoting wear and superficial degradation.

A bacterial layer could be obeserved on the pellicle layer, but no evidence was found to conclude that the amount or location of bacteria contributes to the degradation of the materials. The growth of the bacterial layer generally depends on cleaning and dietary habits [20].

Although extremely sensitive and effective, TEM images can only provide a snapshot which is limited to a small section of the investigated material. Furthermore, the significance of this study is limited due to the low number of patients and investigated materials. However, the alternative method presented may serve to encourage further detailed investigations on dental materials and their interaction with the oral environment in the future and to ultimately improve the clinical value of such products.

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