Clinical performance of resin composite restorations: the value of accelerated in-vitro testing
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Clinical performance of resin composite restorations: The predictive value of accelerated in vitro testing

Franklin García-Godoy
Clinical performance of resin composite restorations:
The value of accelerated in-vitro testing

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CHAPTER 1

Introduction
Tooth-colored materials such as resin-based composites are today the treatment option of choice for the majority of patients. This is mainly attributed to esthetic reasons and not primarily related to lifetime-expectancy of individual restorative approaches. Amalgam was a successful dental restorative material for over 200 years. However, on one hand it was repeatedly alleged to be toxic, and on the other hand it is dark or at best argentic and therefore simply not invisible nor tooth-colored. Today we also know that amalgam, among all toxicologic issues, also has clinical disadvantages: missing adhesive stabilization of remaining tooth hard tissues, cracked teeth after long service times, and mandatory cement linings are not desirable in today's restorative therapy of carious lesions. Resin composites are invisible, adhesively stabilizing to both enamel and dentin, and appropriately sealing. Nevertheless, is the distinct change towards resin composites really better in terms of overall clinical performance? What are resin composite restorations able to perform, especially compared to amalgam in larger stress-bearing posterior cavities? Today it is well-proven that adhesive restorations are successful, having been repeatedly reported for pit and fissure sealings, direct and indirect resin composites, and bonded indirect ceramic restorations. In the focus of the present thesis only directly applied resin composite restorations were investigated, especially as posterior restorations for amalgam replacement.

It is well-known from several studies in the field of adhesive dentistry that durable adhesion to enamel and dentin is a fundamental prerequisite for successful adhesive dentistry because polymerization shrinkage of resin-based composites is still a major issue. Bonding to phosphoric acid etched enamel is generally accepted as clinically successful. The same is true for dentin, however, it is not completely understood whether the etch-and-rinse or the self-etch approach may represent the ultimate way of durable bonding to dentin. And technique sensitivity with bonded restorations still is problematic facing a 1:12 failure rate ratio in a clinical trial with identical materials but different clinical operators. In the literature, many short term evaluations are published, but medium to long-term investigations are still needed. Therefore, bonding degradation over time is an important aspect. In dentin, the last decade revealed an array of innovative findings with regards to bond
degradation, especially in dentin.\textsuperscript{14,35-38} Both water treeing and enzymatic degradation investigations clearly showed that there is a long way to go for durable dentin bonding also in the nanoscale aspect.\textsuperscript{14,30,31,36-38} Results up to three years are presented here, giving some more aspects of longevity in vitro (Chapter 2).

Moreover, adhesion to enamel is even more important because the main retention is provided by appropriate bonding to enamel margins, and the absence of marginal staining is primarily provided by a tight enamel seal.\textsuperscript{4,17-19,28,32,39} When cavities get larger and larger, dentin support of the biomechanical tooth-restoration complex gets lost and restoration margins in enamel receive even more stress besides residual shrinkage stress and intraoral chewing forces.\textsuperscript{19,20,23-27} Therefore, long-term evaluations over six years also were carried out (Chapter 3). In this chapter, in vitro results were not thought to stand alone. When the setups for a clinical study were designed, it was an aim of the author to simultaneously start both in vivo and in vitro studies; i.e. in vitro evaluations regarding marginal quality were started with the same materials at the same time like the prospective randomized clinical trial dealing with posterior resin composite restorations. So after several years of clinical service, one should be able to directly compare in vitro results with in vivo outcomes.

Resin-dentin and resin-enamel bonding behavior is important for clinical success, but resin composite research is not adhesion alone. Adhesion is certainly an important issue as described above, but restorative material properties such as wear, flexural strength, and flexural fatigue behavior are important as well.\textsuperscript{2,3,32,40-45} In former times (being represented by older studies in the literature of the field), the predominant failure mechanism of resin composite restorations was gap formation and subsequent recurrent caries.\textsuperscript{34} Residual stresses led to gap formation over time, supported by different coefficients of thermal expansion.\textsuperscript{1-3,32,34,40} Gaps are colonized by biofilms consequently resulting in secondary caries. This scenario was believed to be the major failure reason for decades and is still in the back of dentists’ minds all over the world. However, facing more recent prospective clinical trials dealing with modern resin composite materials for posterior use, it becomes evident that more material-dependent issues like bulk fractures and chipping have displaced secondary caries as the No. 1 failure scenario with this class of materials.\textsuperscript{2,3,13,16,19,20,26,27,40,42,46-48} This may be due to the better understanding of long-term bonding to both enamel and dentin,
but it may also be attributed to enhanced materials properties in terms of shrinkage and shrinkage stress.\textsuperscript{2;5;23;25;32}

Randomized prospective clinical trials are still the ultimate instrument for evaluating dental restoratives such as resin composites, ceramics, cements, and prosthodontic restorations.\textsuperscript{4;15;16;46-49} However, the predominant problem with these clinical trials is that once valuable results are available after several years of clinical service, the material under investigation may not be in the market anymore. This is a frustrating situation which often occurred with many research groups. Another problem is when publishable results are obtained, the peer review periods and publication backlog of top dental journals add considerable time and actual publication is postponed significantly. Legislation in the field of medical products also contributes negatively here; it becomes harder and harder to get ethical permission to carry out clinical trials, costs for clinical centers have to be readjusted, and finally the manufacturer may not have enough budget for the study anymore. Due to this considerable number of reasons, preclinical \textit{in vitro} investigations are more important than ever, but it is still not fully understood whether these tests are able to reliably predict clinical behavior.

As already mentioned, the performance of bulky resin composites is of importance to counteract bulk fractures over time.\textsuperscript{17;32;40-42;50;51} Therefore, a thorough in vitro vs. in vivo comparison of flexural fatigue characteristics of resin composites must be addressed here (\textit{Chapter 4}). In this case, the strategy was the same as in bonding investigations of Chapter 3. Again, we simultaneously started both in vitro and in vivo branches of the overall investigation plan. However, compared to the informative value of thermomechanical loading for marginal quality estimation, loading of resin composite beams is controversially discussed. On one hand, any fatigue loading design is superior to pure initial loading alone.\textsuperscript{17;32;40-42;50;51} Initially, high loading forces seldomly lead to clinical failures in real life.\textsuperscript{13;20;43} It is more the subcritical, repeated load that degrades dental restorations over several years of clinical service. Loading of beams - with fatigue or not - may not be very close to the clinical situation, because in the oral cavity no resin composite beam may be found to be loaded. Intraorally, resin composites are always bonded more or less successfully to dental hard tissues, i.e. bending forces such as those observed in a classical three- or four-point flexural strength evaluation may not occur similarly.\textsuperscript{13;20;43} Nevertheless, the practical advantage of the presented four-point flexural fatigue evaluation design
is of a very thoroughly standardized quality and moreover, a sound database of many restoratives exists for comparison.\textsuperscript{32;41;42} So we decided to include this particular way of stressing resin composite specimens in order to simulate bulk fatigue over time. And finally, again we were able to correlate the results to clinical outcome directly.

The ultimate instrument is still the randomized clinical trial. Therefore, a prospective clinical long-term trial was set up and reported (Chapter 5). As mentioned above, both materials under investigation are no longer on the market. On the other hand, a certain amount of valuable information results from the present long-term evaluation of Grandio and Tetric Ceram. The longitudinal approach allows for the correlation of initial cavity size with marginal degradation over time. It is possible to measure wear in both areas, occlusal contact area and contact-free area, and distinct differences among materials can be worked out. Chapter 5 is the heart and center of the present thesis, because it allows a clear view on what is happening with adhesively bonded resin composites in stress-bearing areas of posterior teeth.\textsuperscript{4;16;18;46\textasciitilde48} It is possible to distinguish between smaller, minimally invasive resin composite restorations, and larger restorations with considerable occlusal contacts in resin composite instead of being underpinned by more wear resistant enamel cusps. Last but not least, a prospective clinical long-term trial offers the possibility of getting hints about the ultimate question of how resin composite restorations behave over time and what failure scenarios are really in the center of interest. This is the clear advantage compared to cross-sectional studies offering important observation, but unfortunately in a clearly retrospective nature.\textsuperscript{25;34}

The final question remains and should be addressed with the last issue of the present thesis, i.e. what is this all about or in different words: Is clinical performance of bonded restoratives predictable in the lab (Chapter 6)? It was extensively discussed previously, and it has to be the final statement of the present thesis. The key idea for valuably estimating this particular and ultimate question was to start with in vitro and in vivo research simultaneously.\textsuperscript{17;29} Only in this way was it possible to truly compare preclinical outcome and clinical observations. And only when evaluated parameters in both branches approximately match, the predictive quality of the chosen in vitro setup is appropriate. This is the simple truth regarding the major question of the present thesis, before smart materials avoiding classical material disadvantages are considered as alternative.\textsuperscript{52}
Chapter 1

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CHAPTER 2

Degradation of resin-bonded human dentin after 3 years of storage
Introduction

Considerable evidence in adhesive dentistry has accumulated over the past decade, based on *in vitro* and *in vivo* work. Dentin bonds created by resin-based adhesives may not be as durable as previously conjectured.\(^1\) Although the current strategies of incorporating ionic and hydrophilic resinous components in etch-and-rinse and self-etch adhesives arise from the need to bond to an intrinsically wet substrate,\(^2\) they create potentially unstable resin matrices that slowly degrade via water sorption.\(^3\) This is particularly so when resin-dentin bonds are not protected by enamel, and when the durability of these bonds was challenged by reducing adhesive interfaces into smaller portions, to expedite aging effects and increasing their interactions with water.\(^4-6\)

Another mechanism of bond degradation is the potential instability of the demineralized dentin collagen matrix, that was manifested as the thinning or disappearance of collagen fibrils from aged, bonded dentin,\(^7,8\) or the failure of aged hybrid layers to take up heavy metal stains.\(^4\) This issue of collagen instability has caused concern, with the demonstration of the potential involvement of host-derived matrix metalloproteinases (MMPs), a class of independent endopeptidases, in the breakdown of the collagen matrices in dentin caries\(^9-11\) and the periodontium.\(^12-14\) In the context of dentin bonding, residual collagenolytic activity was observed in mineralized dentin of extracted teeth that accounted for the disintegration of collagen fibrils from unbonded, aged acid-etched dentin, in the absence of the contribution from bacterial or salivary MMPs. This low but persistent endogenous collagenolytic activity was strongly inhibited by the use of protease inhibitors, the incorporation of which preserved the structural integrity of the collagen fibrils.\(^1\) In that study, the demineralized collagen matrices were not protected by adhesives. Thus, it remains to be resolved whether these endogenous enzymatic activities can result in the proteolysis of the resin-infiltrated collagen network in aged, adhesive-bonded dentin.

Using a model that consisted of soluble fluorescein-labeled Type I collagen and gelatin in a previous study, both collagenolytic and gelatinolytic activities were identified in powdered mineralized human dentin. Retention of these activities was evident even upon autoclaving of the mineralized dentin power in water. Conversely, these activities were sufficiently suppressed after treatment of the powder with phosphoric acid. The results derived from this model suggested that if resin-bonded dentin is permeable to endogenous enzymes released from the underlying mineralized dentin, complete cleavage of the collagen fibrils within the hybrid layer is possible,
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first into ¾- and ¼-length fragments, and subsequently into smaller peptides. Thus, the objective of the present study was to investigate the morphologic correlations of these endogenous enzymatic activities in aged, adhesive-bonded dentin. As water molecules are putatively required for the activity of zinc-dependent MMPs, the null hypothesis tested was that there was no difference in the ultrastructure of resin-dentin bonds that were aged in mineral oil or artificial saliva.

Materials and Methods

Twenty-one non-curious human third molars were collected after the subject’s informed consent had been obtained under a protocol reviewed and approved by the Human Assurance Committee of the Medical College of Georgia, USA. Within 1 month of extraction, the occlusal enamel and roots of these teeth were removed using a slow-speed saw (Isomet) under water-cooling. The exposed dentin surfaces were polished with wet 180-grit silicon carbide papers.

Experimental design: Three total-etch adhesives were examined. They included two two-step systems (Prime&Bond NT and Excite), and a multi-step system (All-Bond 2). Six teeth were used for each adhesive. Each tooth was etched with 32-37% phosphoric acid gel for 15 seconds, and rinsed with water for 20 seconds. The teeth were then bonded with the respective adhesives according to the manufacturers’ instructions, and restored with a microfilled resin composite (EPIC-TMPT). After allowing the bonds to mature for 24 hours, each tooth was sectioned longitudinally into 0.9 mm-thick serial slabs. Two slabs from the center part of each tooth were further sectioned into 0.9 x 0.9 mm beams. The composite-dentin beams from each adhesive group were randomly divided into two equal portions and stored respectively in 5 ml aliquots of artificial saliva or mineral oil at 55°C for 3 years, according to the accelerated aging protocol described by Tay et al. The artificial saliva contained (mmoles/L): CaCl₂ (0.7), MgCl₂•6H₂O (0.2), KH₂PO₄ (4.0), KCl (30), NaN₃ (0.3) and HEPES buffer (20). The rationale for using artificial saliva was to prevent demineralization of the mineralized dentin during aging. The artificial saliva was replaced every month during the 3-year period. Sodium azide was added to prevent bacterial growth, and to ensure that the only available source of MMPs was derived from the dentin substrate. The resin-bonded dentin beams that were to be aged in mineral oil (Dow-Coming 200 Fluid), were wiped with lint-free gauze and briefly air-dried to remove excess water prior to immersion in oil. They served as controls in that collagenolytic and gelatinolytic activity cannot occur in a non-aqueous medium.
To further ensure that degenerative changes observed after accelerated aging were not caused by the increased aging temperature (55°C), an "extreme" control was performed for each adhesive, using an additional tooth that was bonded in the same manner. Composite-dentin beams prepared from these teeth were subjected to an autoclave cycle at 121°C and 103 kPa for 30 minutes in water before further laboratory processing.

Transmission electron microscopy (TEM) - For each adhesive, 10 specimens were randomly retrieved from those aged in mineral oil, and another 10 from the artificial saliva at time zero and after 3 years of incubation. Half of the specimens were immersed in a 50 wt% ammoniacal silver nitrate solution for 24 hours, according to the tracer protocol for nanoleakage examination. These specimens were processed for TEM examination without further laboratory demineralization. The remaining specimens were completely demineralized in ethylene diamine tetra-acetic acid. Both undemineralized and demineralized, epoxy resin-embedded, 90 µm-thick sections were prepared according a TEM protocol. Undemineralized sections were examined without further staining. Demineralized sections were stained with 2% uranyl acetate and Reynold's lead citrate for examining the characteristics of the resin-dentin interfaces, and with a specific collagen staining technique (1% phosphotungstic acid and 2% uranyl acetate) for examination of the status of the collagen fibrils. The sections were examined using a TEM (Philips EM208S®) operating at 80 kV.

Results
The "extreme" control specimens that were autoclaved at 121°C showed that collagen fibrils were not denatured at this temperature when they were protected by adhesive resin or apatite minerals (not shown). Their ultrastructural features were similar to those observed in control specimens that were aged in mineral oil at 55°C for 3 years. For example, in undemineralized sections of Prime&Bond NT that were aged in mineral oil, a 5-7 µm thick zone of demineralized dentin with sparse silver deposits was observed (Fig. 2.1A), that corresponded with the electron-dense hybrid layer in stained, demineralized sections (Fig. 2.1B). Collagen fibrils with normal dimensions and organization could be identified both within the hybrid layer (Fig. 2.1C) and the underlying dentin (Fig. 2.1D).
By contrast, extensive nanoleakage was observed in Prime&Bond NT specimens that were aged in artificial saliva (Fig. 2.2A). The corresponding hybrid layer was
abnormal and only discontinuous patches of stained fibrillar remnants were observed (Fig. 2.2B). They consisted of grossly disintegrated, short microfibrillar fragments (Fig. 2.2C). The collagen matrix from the underlying mineralized dentin was also denatured, but to a lesser extent, and appeared as swollen, partially unraveled fibrils that lacked cross banding (Fig. 2.2D).

Whereas the Excite specimens that were aged in mineral oil exhibited minimal nanoleakage (Fig. 2.3A) and a highly electron-dense hybrid layer (Fig. 2.3B), those that were aged in artificial saliva demonstrated substantial degenerative changes. Extensive patches of nanoleakage could be seen in the zone of demineralized dentin that extended into the underlying mineralized dentin (Fig. 2.3C). In such regions, stained fibrillar components were absent from the hybrid layer. Collagen fibrils from the underlying dentin were sparsely distributed among abnormally wide interfibrillar spaces (Fig. 2.3D).

Stained fibrillar components were evident throughout the entire hybrid layer in All-Bond 2 specimens that were aged in mineral oil (Fig. 2.4A). These banded collagen fibrils exhibited the characteristic unraveling of their severed ends along the dentin surface (Fig. 2.4B). In specimens that were aged in artificial saliva, crystalline deposits (Fig. 2.4C) derived from the supersaturated artificial saliva (Pashley et al.¹) were seen along the adhesive-hybrid layer interface. Although nanoleakage was only identified in discrete parts of the demineralized collagen matrix (Fig. 2.4C), the entire hybrid layer was completely devoid of stained fibrillar components (Fig. 2.4D).
Figure 2.1: Control resin-bonded dentin beams of Prime&Bond NT that were aged in mineral oil for 3 years. C: resin composite; A: adhesive; D: intertubular dentin. A. Unstained, undemineralized section of a specimen that has been immersed in ammoniacal silver nitrate. The electron-lucent zone of demineralized dentin (between open arrows) corresponded with the stained hybrid layer in Fig. 2lB. Areas of incomplete resin infiltration within this zone are represented by reticular patterns of silver deposits (nanoleakage; pointer). B. The corresponding image of the electron-dense hybrid layer (H) from a demineralized section that was stained with uranyl acetate and lead citrate. C. A demineralized section that was stained with phosphotungstic acid and uranyl acetate. Although the hybrid layer was electron-dense due to the intense mordanting effect of the adhesive solution, cross banding could be identified in longitudinally-oriented collagen fibrils (arrow). D. Normal collagen dimensions and organization could be identified from the laboratory demineralized, epoxy resin-infiltrated dentin beneath the hybrid layer.

Figure 2.2: Experimental resin-bonded beams of Prime&Bond NT that were aged in artificial saliva for 3 years. C: resin composite; A: adhesive; D: intertubular dentin. A. Unstained, undemineralized section after immersion in ammoniacal silver nitrate. Extensive silver impregnation (arrow) was present in the hybrid layer (between open arrows). The intertubular dentin appeared normal in the undemineralized section. B. Phosphotungstic acid and uranyl acetate stained, demineralized section showing the breaking down of collagen fibrils (pointer) within the hybrid layer (H). A substantial part of the hybrid layer was devoid of stainable fibrillar components. Oblique sections of resin tags (open arrowheads) could also be seen in the disintegrated hybrid layer. No bacteria was observed in the section. C. A high magnification view of the adhesive-hybrid layer interface, showing the stainable fibrillar remnants that were present within the hybrid layer (H). Collagenolysis resulted in the appearance of loose strands of gelatin microfibrils (arrow). D. A high magnification view of the hybrid layer dentin junction where grossly disintegrated fibrillar remnants (pointer) were observed at the base of the hybrid layer (H). Further gelatinolysis by matrix metalloproteinases that diffused from the underlying dentin probably resulted in the breakdown of the gelatin strands into smaller peptides. This may account for the absence of stainable fibrillar components from the rest of the hybrid layer. Collagen fibrils in the laboratory demineralized dentin were denatured to a lesser extent, probably due to the protection rendered by the mineral phases. There was a loss of cross banding from these partially denatured and swollen fibrils, in which only the microfibrillar architecture could be identified (arrow).
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**Figure 2.3:** Control (Figs. A, B) and experimental (Figs. C, D) resin-bonded dentin beams of Excite after 3 years of accelerated aging. C: resin composite; A: adhesive; D: intertubular dentin. A. Unstained, undemineralized section following immersion in ammoniacal silver nitrate. The control specimen that was aged in mineral oil exhibited relatively little silver uptake (pointer) within the demineralized dentin zone (between open arrows). B. The corresponding uranyl acetate and lead citrate-stained, demineralized section showing the presence of a highly electron dense hybrid layer (H) in which collagen fibrils could be clearly distinguished (arrow). C. Unstained, undemineralized section following immersion in ammoniacal silver nitrate. The experimental specimen that was aged in artificial saliva exhibited extensive areas of heavy silver deposits within the demineralized dentin zone (between open arrows) that extended into the underlying mineralized dentin (asterisk). The rest of the mineralized dentin appeared normal when undemineralized sections were examined. D. The corresponding uranyl acetate and lead citrate-stained, demineralized section demineralized section showing a palely-stained hybrid layer (H) in which fibrillar components could not be identified. Stained collagen fibrils in the underlying intertubular dentin were sparse and separated by abnormally wide interfibrillar spaces (open arrow).

**Figure 2.4:** Control (Figs. A, B) and experimental (Figs. C, D) resin-bonded dentin beams of All-Bond 2 after 3 years of accelerated aging. C: resin composite; A: adhesive; D: intertubular dentin. A. Phosphotungstic acid and uranyl acetate stained, demineralized section of a control specimen that was aged in mineral oil, showing the presence of stained fibrillar components within the 5-6 µm thick hybrid layer (H and between open arrows). B. A high magnification view of the area depicted by the box in Fig. 2.4A. Collagen fibrils within the hybrid layer (H) were partially obscured by the infiltrated adhesive resins. Nevertheless, banded collagen fibrils could be identified (pointer), with unraveling of microfibrillar strands along the surface of the cut dentin (arrow). C. Unstained, undemineralized section of an experimental specimen that was aged in artificial saliva and examined after immersion in ammoniacal silver nitrate. Crystalline deposits (open arrowhead) derived from the supersaturated artificial saliva were present between the adhesive and the surface of the cut dentin. The presence of segregated silver deposits within demineralized dentin zone (between open arrows) indicated that the latter was an intact layer, despite the absence of stainable fibrillar components (Fig 2..4D). D. The corresponding phosphotungstic acid and uranyl acetate-stained, demineralized section of a specimen that was aged in artificial saliva. The hybrid layer (H) was completely devoid of stainable fibrillar components, and exhibited a similar staining characteristic as the underlying resin tag (pointer) that is supposed to consist predominantly of adhesive resin. The activity of the phosphotungstic acid dissolved the crystalline deposits along the cut dentin surface (open arrowhead). At higher magnification (not shown), collagen fibrils within the underlying dentin were denatured and appeared as microfibrillar strands that were devoid of cross banding (Fig. 2.2D).
Discussion
The null hypothesis had to be rejected as pronounced differences were present between the mineral oil and artificial saliva specimens. The extent of nanoleakage, interfacial staining characteristics, and the conditions of the collagen fibrils in both the hybrid layers and the underlying dentin from all the adhesives were examined. As the glass transition temperature of mineralized dentin is 166.7°C,\(^{21}\) it is not surprising that the temperature of superheated steam (121°C) was insufficient to denature collagen in mineralized or resin-infiltrated dentin. As the structural integrity of the collagen fibrils were also preserved in all control specimens that were aged at 55°C in mineral oil, one may confidently assert that the degenerative changes that occurred after accelerated aging in artificial saliva was not caused by the increase in aging temperature. In the absence of salivary and bacterial MMP activities, these changes must have been initiated by the endogenous collagenolytic and gelatinolytic activities of the mineralized dentin, confirming the results of our previous long-term study.\(^{1}\)

The inability to take up heavy metal stains from water-aged hybrid layers has previously been reported by DeMunck et al.\(^{4}\) The authors surmised that such a phenomenon was due to the decline in polar groups along the surfaces of degrading collagen fibrils. Using a specific collagen stain, we demonstrated the existence of discontinuous patches of grossly disintegrated microfibrillar fragments in some of the artificial saliva-aged interfaces (Fig. 2.2C). These fragments probably represented remnant \(\frac{3}{4}\)- and \(\frac{1}{4}\)-length fragments that resulted from collagenolysis,\(^{22}\) but were retained by the adhesive resins within the hybrid layer. The existence of mild to moderate nanoleakage (\textit{i.e.} silver uptake) in the hybrid layers that were aged in oil suggested that these hybrid layers were initially permeable to water that was utilized by residual MMP hydrolases to degrade the collagen matrix of the hybrid layer and the underlying mineralized dentin. In resin-bonded specimens incubated in artificial saliva for 3 years, the coexistence of areas that did and did not take up specific collagen stains and areas exhibiting a complete lack of stainable fibrillar components from other specimens (Figs. 2.3D, 2.4D) indicates that the degenerated microfibrillar fragments have further been degraded beyond detection. Such a process may occur via gelatinolytic MMPs released from the mineralized dentin, with the gelatin breaking down into peptides of lower molecular weight (kDa). Such a phenomenon is analogous to the appearance of clear bands in Coomassie bluestained gels, when the cleavage products of gelatin were subjected to Western blotting after treatment with MMP-2 (Gelatinase A) or MMP-9 (Gelatinase B).\(^{23,24}\)
Although the collagenase MMP-8 has been shown to exist in carious human dentin, its endogenous origin has not been established. Conversely, the gelatinase MMP-2 has been shown to be present in human dentin. Apart from its gelatinolytic activity via fibronectin-like domains, MMP-2 is also capable of collagenolysis, albeit at a slower rate, via its hemopexin domain. Thus, even in absence of an endogenous collagenase source, endogenous MMP-2 from the dentin matrix can apparently result in the slow but complete cleavage of the entire resin-infiltrated collagen network over 3 years.

Further immunolabeling of MMP-2 within freshly-prepared and aged dentin hybrid layers should be performed to establish the role played by this proteolytic enzyme in the degradation of the demineralized collagen matrix.

It is disturbing to observe consistent partial degradation of the underlying mineralized dentin. Degradation was less severe than the hybrid layers, probably because the collagen fibrils were better protected by apatites than by adhesive resins. Nevertheless, this provides evidence that under specific circumstances, mineralized dentin is capable of self-destruction by its own matrix-bound enzymes. Such a process may require the activation of host-derived MMPs with acids, as purported in the pathogenesis of dentin caries. A provocative clinical concern is why self-degradation and accompanying weakening of mineralized dentin has not been reported in teeth bonded in vivo with dentin adhesives. A possible explanation is the disturbance of the balance between MMPs and their natural inhibitors, TIMPs (tissue inhibitors of metalloproteinases) when extracted teeth are used for aging experiments. It is known that both TIMP-1 and TIMP-2 can complex with active MMP-2 and inhibit proteolytic activity. As these natural MMP inhibitors have shorter half-lives than MMPs, prolonged interruption of MMP-TIMP interaction such as the cessation of dentin fluid flow (i.e. in vitro conditions) may prevent the replenishment of pulpal TIMPs out into peripheral dentin. While such ideas remain highly speculative, a similar degradation of collagen fibrils has recently been shown in endodontically-treated teeth that have undergone long-term clinical function. Clearly, more work is required to establish the relationship between MMPs and their natural inhibitors, or the use of synthetic inhibitors such as chlorhexidine or doxycycline, in prolonging the longevity of resin-dentin bonds and or non-vital dentin in endodontically-treated teeth.

a. Buehler Ltd., Lake Bluff, IL, USA.
b. Dentsply DeTrey, Konstanz, Germany.
c. Ivoclar-Vivadent, Schaan, Liechtenstein.
d. Bisco Inc., Schaumburg, IL, USA.
e. Parkell Inc., Edgewood, NY, USA.
f. Dow-Corning Corp., Midland, MI, USA.
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28. Ward RV, Hembry RM, Reynolds JJ, Murphy G. The purification of tissue inhibitor of metalloproteinases-2 from its 72 kDa progelatinase complex. Demonstration of the biochemical similarities of tissue inhibitor of


CHAPTER 3

Long-term degradation of enamel and dentin bonds:
6-year results in vitro vs. in vivo.
Introduction
Tooth-colored materials such as resin-based composites are today the treatment option of choice for the majority of patients, primarily due to esthetic demands. It is well-proven that adhesive restorations are successful e.g. pit and fissure sealings, direct and indirect resin composites, and bonded indirect ceramic restorations. Durable adhesion to enamel and dentin still represents the fundamental prerequisite due to polymerization shrinkage of resin-based composites. Bonding to phosphoric acid etched enamel is widely accepted as clinically viable, however, in dentin it is not completely clear whether the etch-and-rinse or the self-etch approach may be more successful in getting durable bonds. Irrespectively, technique sensitivity with bonded restorations remains problematic facing a 1:12 failure rate ratio in clinical trials with identical materials but different clinical operators.

The primary goal of preclinical screening of dental materials should be to mimic the clinical situation in order to predict clinical behavior. Therefore, researchers try to predict clinical behavior of restoratives with laboratory in vitro investigations. Of course, randomized clinical trials remain the ultimate instrument for evaluating dental restoratives, however, a major problem with clinical trials is that when they give valuable results after several years of clinical service, the material under investigation may no longer be available in the market. Thus, preclinical in vitro investigations are more important than ever, however, it is still not fully understood whether these tests are able to reliably predict clinical behavior. So the aim of this investigation was to compare preclinical results of a large marginal quality in vitro database with clinically recorded marginal qualities of the same materials.

Materials and Methods
In vitro study: Thirty-two intact, non-carious, unrestored human third molars, extracted for therapeutic reasons with patients’ approval, were stored in an aqueous solution of 0.5% chloramine T at 4°C for up to 30 days. The teeth were debrided of residual plaque and calculus, and examined to ensure that they were free of defects under a light microscope at x20 magnification. Standardized class II cavity preparation (MO, 4mm in width bucco-lingually, 2mm in depth at the bottom of the proximal box) with proximal margins located 1-2 mm below the cementoenamel
junction were performed. The cavities were cut using coarse diamond burs under profuse water cooling (80 µm diamond, Komet, Lemgo, Germany), and finished with a 25 µm finishing diamond (one pair of diamonds per four cavities). Inner angles of the cavities were rounded and the margins were not bevelled to deliver comparable results to previous experiments.\textsuperscript{11,15}

The prepared cavities (n=8) were treated with two different adhesives according to the manufacturers’ instructions (n=16 with Syntac, Ivoclar Vivadent, Schaan, Principality of Liechtenstein, and n=16 with Solobond M, Voco, Cuxhaven, Germany; Table 3.1). The dentin adhesives and resin composite were polymerized with a Translux CL light-curing unit (Elipar Trilight, 3M Espe, Seefeld, Germany). The intensity of the light was checked periodically with a radiometer (Demetron Research Corp, Danbury, CT, USA) to ensure that 600 mW/cm\textsuperscript{2} was always delivered during the experiments. The adhesive was polymerized for 40 s prior to application of the resin composite in all cases. The resin composites Tetric Ceram (Ivoclar Vivadent; shade A2) and Grandio (Voco; shade A2) were used for all experimental restorations. Each cavity preparation was bonded with the respective adhesive and restored incrementally with the resin composite in layers up to 2 mm thickness. The increments were separately light-cured for 40 s each with the light source in contact with the edge of the cavity. Prior to the finishing process, visible overhangs were removed using a posterior scaler (A8 S204S, Hu-Friedy, Leimen, Germany). Margins were finished with flexible disks (SofLex Pop-on, 3M ESPE, St. Paul, USA).

After storage in distilled water at 37°C for 21 days, impressions (Provil Novo, Heraeus Kulzer, Hanau, Germany) of the teeth were taken and a first set of epoxy resin replicas (Alpha Die, Schuetz Dental, Rosbach, Germany) was made for SEM evaluation. One pair of groups was subjected to storage in distilled water at 37°C for 2190 days.\textsuperscript{14} After storage, impressions were taken and thermo-mechanical loading of specimens was performed in an artificial oral environment (“Quasimodo” chewing simulator, University of Erlangen, Germany).
Table 3.1: Experimental setup in laboratory groups (TML: thermomechanical loading / WS: water storage).

Two specimens were arranged in one simulator chamber in proximal contact, similar to the oral situation with the two restored marginal ridges in a normal intercuspation. The two adjacent lateral ridges were occluded against a steatite (a multi-component semi-porous crystalline ceramic material) antagonist (6 mm in diameter) for 100,000 cycles at 50 N at a frequency of 0.5 Hz. The specimens were subjected to 2,500 thermal cycles between +5°C and +55°C by restoration the chambers with water in each temperature for 30 s. The mechanical action and the water temperature within the chewing chambers were checked periodically to ensure a reliable thermo-mechanical loading (TML) effect. After completion of TML, a second set of replicas was manufactured for later SEM analysis. The other pair of groups was subjected to TML only.

In vivo study: In the course of a randomized clinical study with approval of a local ethics committee, 30 subjects (23 female, 7 male, mean age 32.9 (24-59) years) with a minimum of two restorations to be replaced in different quadrants received at least two different Class II restorations (52 MO/OD, 16 MOD or more surfaces, no cusp replacements) in a random decision. Thirty six Grandio restorations were bonded with Solobond M (Voco) and 32 Tetric Ceram restorations were bonded with Syntac/Tetric Ceram (n=16) or Solobond M/Grandio (n=16) replicas after 21 days water storage (baseline / n=16).

<table>
<thead>
<tr>
<th>In vitro specimens (n=32)</th>
<th>Syntac/Tetric Ceram (n=16)</th>
<th>Solobond M/Grandio (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>replicas after 21 days water storage (baseline / n=16)</td>
<td>replicas after 21 days water storage (baseline / n=16)</td>
<td></td>
</tr>
<tr>
<td>TML</td>
<td>TML</td>
<td></td>
</tr>
<tr>
<td>100,000 x 50 N</td>
<td>100,000 x 50 N</td>
<td></td>
</tr>
<tr>
<td>2,500 x 5°C/55°C</td>
<td>2,500 x 5°C/55°C</td>
<td></td>
</tr>
<tr>
<td>replicas (n=8)</td>
<td>replicas (n=8)</td>
<td></td>
</tr>
<tr>
<td>TML</td>
<td>TML</td>
<td></td>
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<tr>
<td>100,000 x 50 N</td>
<td>100,000 x 50 N</td>
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<tr>
<td>2,500 x 5°C/55°C</td>
<td>2,500 x 5°C/55°C</td>
<td></td>
</tr>
<tr>
<td>replicas (n=8)</td>
<td>replicas (n=8)</td>
<td></td>
</tr>
</tbody>
</table>

Two specimens were arranged in one simulator chamber in proximal contact, similar to the oral situation with the two restored marginal ridges in a normal intercuspation. The two adjacent lateral ridges were occluded against a steatite (a multi-component semi-porous crystalline ceramic material) antagonist (6 mm in diameter) for 100,000 cycles at 50 N at a frequency of 0.5 Hz. The specimens were subjected to 2,500 thermal cycles between +5°C and +55°C by restoration the chambers with water in each temperature for 30 s. The mechanical action and the water temperature within the chewing chambers were checked periodically to ensure a reliable thermo-mechanical loading (TML) effect. After completion of TML, a second set of replicas was manufactured for later SEM analysis. The other pair of groups was subjected to TML only.

In vivo study: In the course of a randomized clinical study with approval of a local ethics committee, 30 subjects (23 female, 7 male, mean age 32.9 (24-59) years) with a minimum of two restorations to be replaced in different quadrants received at least two different Class II restorations (52 MO/OD, 16 MOD or more surfaces, no cusp replacements) in a random decision. Thirty six Grandio restorations were bonded with Solobond M (Voco) and 32 Tetric Ceram restorations were bonded with Syntac/Tetric Ceram (n=16) or Solobond M/Grandio (n=16) replicas after 21 days water storage (baseline / n=16).
Long-term degradation of enamel and dentin bonds: 6-year results in vitro vs. in vivo

(Ivoclar Vivadent). The cavities were cut using coarse diamond burs under profuse water cooling (80 µm diamond, Komet, Lemgo, Germany), and finished with a 25 µm finishing diamond. Inner angles of the cavities were rounded and the margins were not bevelled. After cleaning and drying under rubber dam isolation (Coltene/Whaledent Inc., Altstätten, Switzerland), adhesive procedures were performed with Solobond M (2-step etch-and-rinse adhesive) and Syntac (4-step etch-and-rinse adhesive). The resin composite materials were applied into the cavity in layers of approximately 2 mm thickness and adapted to the cavity walls with a plugger. Each layer was light cured for 40 s (Elipar Trilight, 3M Espe, Seefeld, Germany). The occlusal region was modeled as exactly as possible under intraoral conditions, avoiding visible overhangs. The light-emission window was placed as close as possible to the cavity margins. The intensity of the light was checked periodically with a radiometer (Demetron Research Corp., Danburg, CT, USA) and was found to be consistently above 650 mW/cm².

As soon as polymerization was completed, the surface of the restoration was checked for defects and corrected when necessary. Visible overhangs were removed with a scaler and the rubber dam was removed. Contacts in centric and eccentric occlusion were checked with foils (Roeko, Langenau, Germany) and adjusted with finishing diamonds (Komet Dental, Lemgo, Germany), shaped with flexible discs (3M Dental, St. Paul, USA), super-fine discs (3M Dental, St. Paul, USA) and polishing brushes (Hawe-Neos Dental, Bioggio, Switzerland). A fluoride varnish (Elmex Fluid, GABA, Lörrach, Germany) was used to complete the treatment. Impressions were used to make epoxy replicas (Alpha Die, Schütz Dental, Germany); 22 of them (n=11 per group) were subjected to scanning electron microscopic (SEM) analysis (Table 3.2). The replicas with the longest evaluable margins were selected randomly.

<table>
<thead>
<tr>
<th>In vivo specimens (n=22)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Syntac/Tetric Ceram (n=11)</td>
<td>Solobond M/Grandio (n=11)</td>
</tr>
<tr>
<td>replicas at initial recall (baseline / n=11)</td>
<td>replicas at initial recall (baseline / n=11)</td>
</tr>
<tr>
<td>6 years of clinical function</td>
<td></td>
</tr>
<tr>
<td>replicas (n=11)</td>
<td>replicas (n=11)</td>
</tr>
</tbody>
</table>

Table 3.2: Procedure for in vivo groups.
All in vitro and in vivo replicas were mounted on aluminum stubs, sputter-coated with gold and examined under a SEM (Leitz ISI 50, Akashi, Tokyo, Japan) at x200 magnification. SEM examination was performed by one operator having experience with quantitative margin analysis who was blinded to the restorative procedures. The marginal integrity between resin composite and enamel was expressed as a percentage of the entire judgeable margin length. Marginal qualities were classified according to the criteria “continuous/gap-free margin” and “gap/irregularity”, non-judgeable parts and artifacts were excluded. Afterwards the percentage “gap-free margins” in relation to the individual judgeable margins was calculated as marginal integrity. For in vivo specimens, all non-judgeable parts such as overhangs were excluded with the remaining criteria having been either “continuous margin” or “gap/irregularity” (crevice, negative step formation, or marginal fracture in enamel or resin composite). For better comparison with in vitro results, gap-free margins were added whether there was a crevice or not. Marginal quality in dentin was not recordable with the impression/replica technique. However, clinical criteria such as secondary caries were evaluated.

Statistical analysis was performed using SPSS 14.0 (SPSS Inc., Chicago, IL, USA). As the majority of groups in each of the two investigations did not exhibit normal data distribution (Kolmogorov-Smirnov test), non-parametric tests were used (Wilcoxon matched-pairs signed-ranks test, Mann-Whitney U-test) for pairwise comparisons at the 95% significance level.

**Results**

An overview of the results is shown in Table 3.3. In both the in vitro and in vivo scenario, marginal quality of resin composite restorations was significantly deteriorated over time (p<0.05; Wilcoxon test).

In the laboratory processed specimens, all restorations initially revealed nearly 100% gap-free margins (p>0.05; Mann-Whitney U-test). After TML alone, continuous margins dropped to 87-90% in enamel and 55-66% in dentin (p<0.05; Wilcoxon test; in dentin with a significantly higher portion of gap-free margins for Syntac/Tetric Ceram; p<0.05; Mann-Whitney U-test). After water storage for 6 years alone, gap-free margins dropped to 97-99% in enamel and 67-75% in dentin (p<0.05; Wilcoxon test; in dentin with a significantly higher portion of gap-free margins for Syntac/Tetric Ceram; p<0.05; Mann-Whitney U-test). After water storage and TML,
marginal quality in enamel ranged from 85-87%, and in dentin 42-52% (p<0.05; Wilcoxon test; in dentin with a significantly higher portion of gap-free margins for Syntac/Tetric Ceram; p<0.05; Mann-Whitney U-test). Thermomechanical loading had a more detrimental effect on marginal quality than 6 years water storage alone (p<0.05).

<table>
<thead>
<tr>
<th>Materials</th>
<th>Substrate</th>
<th>Initial</th>
<th>TML</th>
<th>WS</th>
<th>WS + TML</th>
<th>Initial</th>
<th>6 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syntac +</td>
<td>Enamel</td>
<td>100</td>
<td>90(3)</td>
<td>99 (2)</td>
<td>87 (8)</td>
<td>90 (9)</td>
<td>80 (18)</td>
</tr>
<tr>
<td>Tetric Ceram</td>
<td>Dentine</td>
<td>100</td>
<td>66 (11)</td>
<td>75 (10)</td>
<td>52 (16)</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>Solobond M +</td>
<td>Enamel</td>
<td>100</td>
<td>87 (4)</td>
<td>97 (2)</td>
<td>85 (9)</td>
<td>86 (10)</td>
<td>74 (19)</td>
</tr>
<tr>
<td>Grandio</td>
<td>Dentine</td>
<td>98 (2)</td>
<td>55 (14)</td>
<td>67 (11)</td>
<td>42 (14)</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

Table 3.3 Overview of results with percentages of gap-free margins in % (SD). TML: Thermomechanical loading; WS: Water storage for 2190 days.

For the in vivo replicas, only marginal quality in enamel was recordable by the impression/replica technique. In enamel, continuous margins were initially at 86-90% and after 6 years of clinical service at 74-80%, however, whether initially (1-3%) nor after 6 years (4-5%) severe gap formation was found. Non-continuous margins were attributed to clinical wear and consecutive negative step/crevice formation/marginal fractures having been slightly more pronounced for Grandio (p<0.05; Mann-Whitney U-test). For proximal margins being located in dentin, clinical observations neither revealed severe staining nor secondary caries formation over 6 years, so the absence of enamel at the bottom of the proximal box did not affect results.
Discussion

The idea to compare in vitro and in vivo outcome of resin-based composite restorations is not new. As first approach in the history of the field, Abdalla and Davidson published their “comparison of the marginal integrity of in vitro and in vivo Class II composite restorations” in 1993, dealing with microleakage scores in both laboratory and ex vivo specimens. In this paper, microleakage was substantially more severe in the in vivo group, so the unanimous conclusion was that it may not be possible to reliably predict clinical behavior of bonded restorations in the lab. In this context, Peumans et al. reported the clinical outcome of different adhesives in non-carious cervical lesions, because Class V setups do not provide macromechanical retention but considerable amounts of dentin margins. However, Class V trials completely fail to prove whether bonded resin-based composites may compete with amalgam in posterior cavities under occlusal load. Although in Class II setups macro-retention is normal, at least in replacement cavities and considerably less dentin margins are routinely found, there are also advantages. Recording of postoperative hypersensitivities is a valid instrument to estimate dentin seal, and secondary caries is a well-suited indicator of loss of bonding performance, especially in proximal areas of the restorations.

Former publications of our workgroup already dealt with resin composites and their corresponding adhesives in both aspects, in vitro and in vivo. The first one aimed to elucidate in vitro performance of resin composites focusing on microtensile bond strengths to enamel and dentin, flexural fatigue behavior, and wear behavior. Ariston pHc and Solitaire showed some differences compared to other contemporary resin composites, Ariston provided significantly less adhesion to enamel and dentin, Solitaire provided inferior flexural fatigue behavior. The corresponding part showed clinical findings of the previously described resin composites being completely unacceptable. So in both cases, clinical performance of resin composites in Class II cavities was predictable from laboratory results.

However, the literature in the field of adhesive dentistry still requires some evidence given from in vitro – in vivo comparisons. Based on the previously described prospective clinical trial, also in vitro experiments with the identical materials were performed. As artificial aging protocol six years water storage was chosen with and without thermomechanical loading. TML setups are frequently used to mimic the clinical situation, however, it is unclear how many cycles mean how much lifetime in
the mouth of the patient. Therefore, setups and cycle numbers are subjected to a wide variation from 4,000 to 1.2 million cycles combined with separate or simultaneous thermocycling. Derived from Swiss data from the 1980’s, it was widely accepted that in vitro fatiguing of resin-tooth interfaces for 1.2 million cycles may be somewhat equivalent to 5 years of clinical service.\textsuperscript{3,7,20,21} Even for fatigue simulations on core build-up restorations as postendodontic restorations, this benchmark was used from time to time.\textsuperscript{25,26} However, the basis of this approach was just counting chewing cycles and not a true in vitro – in vivo comparisons of dental restoratives under simulated and clinical load.

A previous study of our workgroup demonstrated that 100,000 load cycles combined with 2,500 thermocycles was able to simulate clinical behavior of a 2-year period for Class I cavities restored with bonded resin composites.\textsuperscript{11} A distinct advantage for etch-and-rinse adhesives compared to self-etch adhesives was also shown.\textsuperscript{11}

Also the results of the present \textit{in vitro} – \textit{in vivo} comparison revealed a certain relationship between preclinical and clinical observations. Percentage of gap-free margins was chosen as a main criterion, because this way it was possible also to include clinical crevices without detectable gap formations. The present results indicate that TML alone led to a higher loss of marginal integrity than 6 years’ water storage (Table 3.3). On the other hand, a combined WS/TML scenario exhibited the most pronounced effect on marginal quality over time. Nevertheless, in vitro and in vivo results show a certain similarity. However, this is also attributed to the fact that gap-free irregularities were also included in “gap-free” margins. Otherwise, completely perfect margins in vivo (i.e. without any crevice or negative step formation were \textasciitilde55\% initially and 20-30\% after 6 years of clinical service.

Thus, marginal quality prediction is possible from laboratory findings, however, marginal integrity is only one among several crucial factors responsible for clinical success or clinical failure over time, meaning that more interfacial gaps after thermal and mechanical stressing in vitro raise the probability of the same scenario to occur under clinical circumstances in the oral cavity. However, some important publications about secondary caries clearly demonstrated that the presence of marginal gaps \textit{in vivo} do not necessarily have to be accompanied with secondary caries.\textsuperscript{16,17}
Altogether, a particular problem with adhesive restorations remains in general: A given combination of adhesive and resin composite may obtain high percentages of gap-free margins \textit{in vitro}, so it may consequently have also a promising clinical behavior related to marginal quality. The residual question is: What percentage of marginal quality may be promising or below what borderline is there no guarantee for clinical success? In the present case, in both groups (Syntac/Tetric Ceram vs. Solobond M/Grandio) the in vitro results were promising, so also clinical outcome was sufficient. For inferior materials, not enough data are collected and furthermore ethically doubtful.

Facing the ultimate question, it has to be taken into account that several studies dealing with evaluated marginal quality of restorations \textit{in vitro} were designed in order to elucidate primarily experimental questions where clinical trials would never pass an ethics committee. In these cases only \textit{in vitro} studies are possible, showing appropriate ways for clinical application of dental materials. Facing different laboratory approaches to predict clinical behavior such as bond strength testing or microleakage assessment, thermomechanical loading with a SEM marginal analysis still is the setup being closest to the clinical situation, however, it is still the most extensive and time-consuming way of evaluating restorative materials.
References


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CHAPTER 4

Fatigue behavior of dental resin composites: Flexural fatigue

*in vitro* vs. six years *in vivo*
Introduction
During the last decades, resin-based composite materials were optimized with a special focus on amalgam replacement.\textsuperscript{1-4} Thus, resin composites were more and more improved for application even in occlusally stressed areas.\textsuperscript{5-10} In order to simulate clinical conditions in the laboratory, it is important to observe fatigue phenomena as an important factor during biodegradation processes of dental biomaterials.\textsuperscript{11-15}

In former times that have been characterized by less effective adhesive technology and higher polymerization stresses generated by older resin composite materials, gap formation and recurrent decay were the predominant reason for failures of bonded resin composite restorations.\textsuperscript{5,16} Today, this is still true, but fatigue fractures after several years under clinical load gain importance as reason for failure.\textsuperscript{2-4, 9,10,22}

Fatigue in dental restoratives is influenced by corrosive water attack at 37°C and by subcritical cyclic masticatory forces having been estimated to be 5-20 MPa.\textsuperscript{6,17,18} Contemporary approaches to fatigue principles describe fracture processes in three stages: crack initiation, slow crack growth, fast fracture.\textsuperscript{6,17,18} Especially crack initiation time and slow crack growth time are determining fatigue resistance of an individual material. Crack initiation originates from surface and subsurface microcracks, porosities, or filler particles.\textsuperscript{11,14,15,19} Cyclic loading is able to drive a crack, and additional water exposure causes a variety of further weakening effects on resin composite materials, i.e. degradation of the filler–matrix interface, swelling, or visco-elastic effects on the matrix which all are able to accelerate slow crack growth.\textsuperscript{11,14,15,19}

Today it is still not fully understood whether and to what extent we are able to predict clinical behavior of dental biomaterials such as resin composites based on in vitro research. Therefore, the aim of this study was to compare fatigue behavior of two different resin composites using an array of laboratory parameters (Young's modulus, flexural strength, flexural fatigue limit) and analyze resin composite restorations in the course of a randomized prospective clinical long-term trial over 6 years. This is the maximum approach to thoroughly evaluate dental materials simultaneously in vitro and in vivo.
Materials and Methods

Grandio

Grandio (shade A3, Voco, Germany), a nanofilled hybrid resin composite, was used in this study. The resin matrix consisted of bisphenol glycidyl methacrylate (BisGMA) and triethylene glycol dimethacrylate (TEG DMA), mixed in a ration of 3:1. Further 71.4 vol% (87 wt%) inorganic fillers were compounded, being 30% nanosized fillers. Camphoroquinone served as photoinitiator. Grandio is clinically indicated as a universal composite for anterior and posterior restorations.

Tetric Ceram

The light-curing dental restorative Tetric Ceram (shade A3, Ivoclar Vivadent, Liechtenstein) was used in this study. The material was based on a bisphenol glycidyl methacrylate (BisGMA)/urethane dimethacrylate (UDMA)/triethylene glycol dimethacrylate (TEGDMA) resin matrix, with camphoroquinone as photoinitiator and 60 vol % (78 wt%) inorganic filler content. Tetric Ceram is clinically indicated as a universal fine particle hybrid resin composite for anterior and posterior restorations.

Specimen preparation

Rectangular specimens with the dimension 2 x 2 x 25 mm were produced using a metal/glass mold and light-curing on five overlapping spots of 8 mm diameter. The upper and lower side of the bar were cured with a commercial halogen light curing unit (Elipar Trilight (750 mW/cm²), 3M Espe, Germany). The illumination time on a single spot was 40s. The procedure followed the manufacturers’ recommendation and ISO 4049 standard. The specimen flanges were ground under an angle of 45° using SiC paper (1200 grit). All specimens were stored for 14 days in distilled water at 37°C, deviating from the ISO 4049 standard.

Elastic Modulus (EM)

To determine the elastic response of the composites, the Young’s moduli were calculated from load-displacement curves in a four-point bending test setup. The specimens (n=10) were positioned in the test rig (distance between the lower supports: 20mm, upper fins: 10mm) of a universal testing machine (Zwick Z2.5, Zwick, Ulm, Germany) and loaded up to 20 MPa with a crosshead speed of 0.75 mm/minute. By the linear-elastic relationship between stress (in the range between 10 to 20 MPa) and
strain the EM was calculated. An extensometer served to measure the true deflection (system compliance) directly at the lower side of the specimen.

**Fracture strength (FS)**

To evaluate the initial flexural strength (FS), the four-point bending test was used (n = 15). Fifteen bars of each material were brought into the four-point test rig and loaded until fracture with a crosshead speed of 0.75 mm/minute in a universal testing machine (Z 2.5, Zwick, Germany). The measurements were carried out in distilled water at 37°C.

**Flexural Fatigue Limit (FFL)**

The flexural fatigue limits (FFL) of the composite materials were determined for $10^5$ cycles under equivalent test conditions at a frequency of 0.5 Hz (n=25). The “staircase” approach was used for fatigue evaluation. For every cycle the stress alternated between 1 MPa and the maximum stress. Tests were conducted sequentially, with the maximum applied stress in each succeeding test being increased or decreased by a fixed increment (5 MPa) of stress, according to whether the previous test resulted in failure or not. The first specimen was tested at approximately 50% of the initial flexural strength value. As the data are cumulated around the mean stress, the number of specimens required is less than with other methods (n=25). The test stopped at a certain level below which no further failure occurred.

The mean flexural fatigue limit (FFL) was determined using Eq. 1 and standard deviation, using Eq. 2, respectively:

$$FFL = X_0 + d \left( \frac{\sum n_i}{\sum n_i} \pm 0.5 \right)$$  \hspace{1cm} (1)

$$SD = 1.62d \left( \frac{\sum n_i \sum i^2 n_i - \left( \sum i n_i \right)^2}{\left( \sum n_i \right)^2} + 0.029 \right)$$  \hspace{1cm} (2)

where $X_0$ is the lowest stress level considered in the analysis and $d$ is the fixed stress increment. To determine the FFL, the analysis of the data was based on the least frequent event (failures versus non failures). In Eq. 1 a negative sign was used when...
the analysis was based on failures. The lowest stress level considered was designated as \( i = 0 \), the next as \( i = 1 \), and so on, and \( n_i \) was the number of failures or non-failures at the given stress level.\(^6\),\(^13\),\(^17\)

**Statistical treatment**

The strength data (FS and FFL) were statistically treated using a one-way ANOVA test followed by a modified LSD post-hoc routine (\( p < 0.05 \)).

**Clinical study**

In the course of a randomized clinical study with approval of a local ethics committee (University of Erlangen, Germany), 30 subjects (23 female, 7 male, mean age 32.9 (24-59) years) with a minimum of two restorations to be placed in different quadrants received at least two different Class II restorations (52 MO/OD, 16 MOD or more surfaces, no cusp replacements) in a random decision.\(^26\) Thirty six Grandio restorations were bonded with Solobond M (Voco) and 32 Tetric Ceram restorations were bonded with Syntac (Ivoclar Vivadent). The cavities were cut using coarse diamond burs under profuse water cooling (80 µm diamond, Komet, Lemgo, Germany), and finished with a 25 µm finishing diamond. Inner angles of the cavities were rounded and the margins were not bevelled. After cleaning and drying under rubber dam isolation (Coltene/Whaledent Inc., Altstätten, Switzerland), adhesive procedures were performed with Solobond M (2-step etch-and-rinse adhesive) and Syntac (4-step etch-and-rinse adhesive). The resin composite materials were applied into the cavity in layers of approximately 2 mm thickness and adapted to the cavity walls with a plugger. Each layer was light cured for 40 s (Elipar Trilight, 3M Espe, Seefeld, Germany). The occlusal region was sculpted as exactly as possible under intraoral conditions, avoiding visible overhangs. The light-emission window was placed as close as possible to the cavity margins. The intensity of the light was checked periodically with a radiometer (Demetron Research Corp., Danburg, CT, USA) and was found to be consistently above 650 mW/cm\(^2\). Visible overhangs were removed with a scaler and the rubber dam was removed. Contacts in centric and eccentric occlusion were checked with foils (Roeko, Langenau, Germany) and adjusted with finishing diamonds (Komet Dental, Lemgo, Germany), shaped with flexible discs (3M Dental, St. Paul, USA), super-fine discs (3M Dental, St. Paul, USA) and polishing brushes (Hawe-Neos Dental, Bioggio, Switzerland). A fluoride varnish (Elmex Fluid, GABA, Lörrach, Germany) was applied with a foam pellet (Pele-Tim, Voco, Cuxhaven, Germany) to complete the treatment.
Impressions (Dimension Penta and Garant, 3M ESPE, Seefeld, Germany) were taken at baseline, after 4 and 6 years and were used to make epoxy replicas (Alpha Die, Schütz Dental, Germany) for x30 magnification cast analysis under a scanning electron microscope (SEM) evaluating fatigue characteristics such as voids, chippings or cracks within the resin composite, and 22 of them (n=11 per group) were subjected to SEM margin analysis at x200. The replicas with the longest evaluable margins were selected randomly. All in vitro and in vivo replicas were mounted on aluminum stubs, sputter-coated with gold and examined under a SEM (Leitz ISI 50, Akashi, Tokyo, Japan) at x200 magnification. SEM examination was performed by one operator having experience with quantitative margin analysis who was blinded to the restorative procedures. The marginal quality between resin composite and enamel was expressed as percentage of the entire judgeable margin length, i.e. margins having been accessible by the impression material in vivo. Marginal qualities were classified according to the criteria “continuous/gap-free margin” and “gap/irregularity” for another study, here the focus was set on marginal breakdown and cracks. Non-judgeable parts and artifacts were excluded. Afterwards the percentage of “marginal fracture areas” in relation to the individual judgeable margin was calculated as marginal fracture score. For better comparison with in vitro results, gap-free margins were scored whether there was a negative step or not.

Statistical analysis was performed using SPSS 14.0 (SPSS Inc., Chicago, IL, USA). As the majority of groups in each of the two investigations did not exhibit normal data distribution (Kolmogorov-Smirnov test), non-parametric tests were used (Wilcoxon matched-pairs signed-ranks test, Mann-Whitney-U test) for pairwise comparisons at the 95% significance level.

Results
Results of the laboratory part are displayed in Table 1. It was shown that Grandio exhibited a significantly higher elastic modulus (p<0.05) and flexural fatigue limit (p<0.05). Initial fracture strengths were not significantly different (p>0.05). Results of the clinical investigation are shown in Tables 3 and 4. Irrespective of the resin composite used, significant changes over time were found for all criteria applied in clinical examinations (Friedman test; p<0.05). The main reason for the degradation of the occlusal surface of the restorations was an increased surface roughness (41%
after 4 and 27% after 6 years) and more chipping especially in the proximal ridge area (36% after 4 and 35% after 6 years; Fig. 2). Voids were obvious mainly after 6 years (clinically 44% and SEM 73%; Table 3 and 4). Except for the criterion “wear” after 6 years (Pearson correlation coefficient 0.671) no correlation was calculated between the clinical and cast evaluation (p>0.05; two-tailed correlation). Neither restorative materials nor localization of the restorations (upper or lower jaw) had a significant influence on the surface degradation at baseline and after 4/6 years (p>0.05; Mann-Whitney U-test). However, molar restorations performed worse than premolar restorations regarding the clinical criterion “wear” after both 4 and 6 years (premolars: 22% bravo after 4 and 44% after 6 years vs. molars: 61% bravo after 4 and 69% after 6 years).

Criteria and results of the x30 magnification cast analysis under the SEM are displayed in Table 4. Here also, no significant differences were computed between the materials Grandio and Tetric Ceram. Only in the x200 SEM analysis of restoration margins, marginal breakdown areas were more often recorded for Tetric Ceram (7.9%) vs. Grandio (4.8%). Examples for clinical images and corresponding SEM pictures are explained in Figures 1-5. Predominant findings in both species of investigations were slightly exposed restoration margins after 6 years of clinical service due to mainly wear in the contact free areas (CFA; Figures 1b, 2b, 3b, 4b) and more roughened surfaces in the occlusal contact areas (OCA).

Discussion

Biomaterials labs daily try to simulate clinical conditions in order to predict clinical behavior of dental biomaterials.5,20-23 This is the only and most important justification of the existence of this kind of laboratory. Without clinical relevance, what should be the reason for in vitro research?

Beside other co-factors for clinical success with resin-based composites such as marginal integrity, wear, biocompatibility and absence of postoperative hypersensitivities2,3,9,10,24,25, fatigue is a major factor during biodegradation of restorative materials. Fatigue loading has gained importance in materials science not only regarding adhesion,5,22-24,26 since even for restorative materials the initially high flexural strength values suffered a reduction of 50% after fatigue loading.
The chosen in vitro setup of the present study was shown to give reliable and reasonable results in several studies with an array of different classes of materials.\textsuperscript{14,15,30-32} On first sight it may be awkward to evaluate a restorative material as simple beam in a 4-point bending device, since the same material is always adhesively bonded under clinical conditions. However, results from previous studies as well as the present results demonstrate that FFL analysis of dental biomaterials gives important results for their intraoral use in stress-bearing restorations.\textsuperscript{14,15,30-32} Facing the present results in both aspects in vitro and in vivo, it is crucial to achieve equal or even similar test conditions in both setups.

One fundamental point here is light polymerization. On one hand, we had to stick to previously published curing protocols to get comparable results to former investigations;\textsuperscript{6,13,17} on the other hand, light-curing under clinical conditions should at least be achievable. So it might be a consequence that in vitro specimens are still more intensively light-cured than the same materials having been applied in vivo. The same is true for storage times in vitro, so we used the well-established protocol of 2 weeks water storage\textsuperscript{6,13,17} whereas loading began immediately intraorally.

Another critical point in the present simulation of clinical circumstances is the in vitro setup in general. In vitro we used simple beams having been mechanically loaded as bars and not as bonded resin composite restorations being stabilized inside the tooth. Here, thermomechanical loading may be much closer to the real clinical situation, however, also in a chewing simulation chamber with more complex and individually styled restorations in natural teeth forces are more complex to handle.\textsuperscript{23}

The normal way of testing dental biomaterials is to conduct preclinical issues first and then carry out clinical trials. However, when in vitro and in vivo research is carried out simultaneously as in the present paper, an appropriate comparison of both ways of investigations is possible.\textsuperscript{4,23,26} So since 1998, we decided to perform in vitro and in vivo research with the same materials simultaneously with both resin composites and ceramic materials. This may be the most appropriate way to explain what happened clinically and why it happened as well. Furthermore it is easier to determine predictable parameters in the laboratory.

Compared to other classes of restorative materials such as glass ionomer cements, both resin composites under investigation provided sufficient initial flexural strengths
of >100 MPa. However, both Young’s modulus and flexural fatigue limit were significantly higher for Grandio. In the present study, FFL of investigated materials was 63 MPa for Grandio and 44 MPa for Tetric Ceram. These findings were not statistically different at the initial stage of flexural strength. After mechanical fatigue, the FFL of Grandio was 55% of the initial flexural strength, whereas Tetric Ceram dropped to 45% of its initial flexural strength values. Regarding both aspects, physicochemical characteristics were slightly better for Grandio which also exhibited a somewhat increased filler load of 87%wt vs. 78%wt in Tetric Ceram. Facing the overall clinical performance of both materials, slightly superior materials characteristics for Grandio did not result in a significantly better clinical outcome, at least after 6 years of clinical service. Only when dental restorative materials have flexural fatigue limits of less than 20 MPa, it seems to be critical for clinical survival with more frequent catastrophic bulk fractures even after considerably shorter clinical observation periods. In a previous study under identical test conditions in vitro as well as in vivo, Solitaire provided a FFL of 18 MPa which lead to unacceptably high marginal and bulk fracture rates in vivo in the course of a prospective clinical study.

Finally, the significantly higher FFL for Grandio when compared to Tetric Ceram led to clinical consequences having been detected under the SEM during the marginal quality evaluation: Grandio restorations exhibited significantly less marginal breakdown (4.8%) than Tetric Ceram restorations (7.9%). Although Grandio restorations showed inferior marginal quality in a previous clinical trial, they performed slightly better according to marginal fatigue characteristics in the present investigation. However, these are microscopic findings suffering limited clinical impact for the present observation period of 6 years when it comes to clinical survival as the major criterion.

Up to now, wear was the predominantly detected degradation factor over time. In contrast, the influence of the clinical operator is still a major factor for clinical success, maybe more than the difference between an FFL of 40 MPa vs. 50 MPa.
References


CHAPTER 5

Microhybrid vs. nanohybrid resin composites in extended cavities after 6 years.


**Introduction**

Although a prospective clinical trial is not an in vitro study as reflected by the topic of the present thesis, clinical data are the one and only desirable comparison tool for dental restorative materials. Moreover, they can be linked to preclinically evaluated in vitro data. Therefore, it is important to compare the presented in vitro data with clinical outcome of directly bonded dental biomaterials such as resin-based composites.

Cavitated carious lesions are today predominantly restored by use of resin composites. Durable adhesion to tooth hard tissues still is the fundamental prerequisite for pit and fissure sealings, direct and indirect resin composites, and bonded all-ceramic restorations. When adhesion mechanisms fail, gap formation and secondary caries corroborate clinical success of restorations.

Bonding to enamel is clinically durable at least when the etch-and-rinse approach is applied, but also clinically acceptable sealing is obtainable to limit the occurrence of postoperative hypersensitivity. Although bonded resin composites have proven to durably seal dentin especially with multi-step adhesives, it is still unclear whether it is possible to maintain a stable proximal seal in Class II cavities with proximal margins extending beyond the amelocemental junction. In vitro studies give varying results after thermomechanical loading and/or long-term storage, mostly with distinct advantages for two- or three-step adhesives compared to simplified adhesive systems. Prospective clinical trials remain ultimate instruments, but preclinical in vitro investigations are still needed for experimental questions and preclinical screening.

One of the most severe problems of clinical trials of dental materials is apparent: while affording acceptable results after some years of clinical service, there is a certain risk that the adhesive and/or resin composite under investigation is not in the market anymore. However, it is not a matter of course that this kind of clinical trial reveals favorable results, so also catastrophic outcomes were observed when fundamental prerequisites are neglected such as hygroscopic expansion or flexural fatigue behavior. And it may be still true that, e.g. amalgam, may be superior to resin composites for restoration of very extended defects.
Research and development of resin-based composites during the last decade generated different subspecies of restorative materials, such as hybrid resin composites, fine hybrid resin composites, nanohybrid resin composites, or even nano resin composites (Filtek Supreme XTE, 3M ESPE, Seefeld, Germany). Especially the latter ones entered the market with claims of less polymerization shrinkage, lowered shrinkage stress and even higher wear resistance.\textsuperscript{32-37} In most of the cases, however, a truly better clinical outcome is not proven. On the other hand, it is stated from recent in vivo results that modern nano hybrid resin composite may provide an enamel-like wear behavior.\textsuperscript{38,39}

Therefore, the aim of this clinical trial was to investigate two different restorative material systems (i.e. adhesive and resin composite) in extended Class II cavities over 6 years in order to observe differences between conventional (Tetric Ceram) and partially nanofilled (Grandio) resin composites. The null-hypothesis tested was that there would be no difference between the different resin composites with their respective adhesives under investigation.

**Methods and Materials**

Patients selected for this study met the following criteria: 1) Absence of pain from the tooth to be restored; 2) possible application of rubber dam during luting of restoration; 3) no further restorations planned in other posterior teeth; 4) high level of oral hygiene; 5) absence of any active periodontal and pulpal disease; 6) restorations required in two different quadrants (split mouth design).

Thirty patients (23 female, 7 male, mean age 32.9 (24-59) years) with a minimum of two restorations to be replaced in different quadrants received at least two different restorations in a random decision according to recommendations of the CONSORT statement.\textsuperscript{40} Thirty-six Grandio restorations were bonded with Solobond M (Voco, Cuxhaven, Germany) and 32 Tetric Ceram restorations were bonded with Syntac (Ivoclar Vivadent, Schaan, Liechtenstein). All restorations (only Class II, 52 MO/OD, 16 MOD or more surfaces, no cusp replacements) were re-restorations made by one dentist in a private practice (31 upper bicuspids, 12 upper molars, 14 lower bicuspids, 11 lower molars). Reasons for replacement were caries (n=19), insufficient esthetics (n=2), and secondary caries (n=47). For all teeth receiving restorations, current X-rays (within 6 months of the procedure) were present. After evaluating the radiographs, 53
cavities (78%) were treated as caries profunda. Twenty-four cavities (35%) revealed no enamel at the floor of the proximal box, while 33 cavities (49%) exhibited a proximal enamel width of <0.5 mm.

All restorations were inserted in permanent vital teeth without pain symptoms. An extension for prevention was disregarded for maximal substance protection; however, the majority of restorations were previously prepared with undercuts for amalgam retention. The cavities were cut using coarse diamond burs under profuse water cooling (80 µm diamond, Komet, Lemgo, Germany), and finished with a 25 µm finishing diamond. Inner angles of the cavities were rounded and the margins were not bevelled. After cleaning and drying under rubber dam isolation (Coltene/Whaledent Inc., Altstätten, Switzerland), adhesive procedures were performed with Solobond M (2-step etch-and-rinse adhesive) and Syntac (4-step etch-and-rinse adhesive). The resin composite materials were applied into the cavity in layers of approximately 2 mm thickness and adapted to the cavity walls with a plugger. Each layer was light cured for 40 seconds (Elipar Trilight, 3M ESPE, Seefeld, Germany). The occlusal region was modeled as exactly as possible under intraoral conditions, avoiding visible overhangs. The light-emission window was placed as close as possible to the cavity margins. The intensity of the light was checked periodically with a radiometer (Demetron Research Corp., Danburg, CT, USA) and was found to be constantly above 650 mW/cm².

As soon as polymerization was completed, the surface of the restoration was checked for defects and corrected when necessary. Visible overhangs were removed with a scaler and the rubber dam was removed. Contacts in centric and eccentric occlusion were checked with foils (Roeko, Langenau, Germany) and adjusted with finishing diamonds (Komet Dental, Lemgo, Germany), shaped with flexible discs (3M Dental, St. Paul, USA), super-fine discs (3M Dental, St. Paul, USA) and polishing brushes (Hawe-Neos Dental, Bioggio, Switzerland). A fluoride varnish (Elmex Fluid, GABA, Lörrach, Germany) was used to complete the treatment.

At the initial recall (baseline, i.e. within 2 weeks), and after 6 months, 1, 2, 4, and 6 years, all restorations were assessed according to the modified United States Public Health Service (USPHS) criteria (Tables 5.1 and 5.2) by two independent investigators using loupes with x3.5 magnification, mirrors, probes, bitewing radiographs, impressions (Dimension Penta and Garant, 3M-ESPE, Seefeld, Germany), and intraoral photographs. Replicas were collected for later marginal and
wear analysis (studies in preparation). Recall assessments were not performed by the clinician who initially placed the restorations.

Statistical appraisal was computed with SPSS for Windows XP 14.0 (SPSS Inc., Chicago, IL, USA). Statistical unit was one tooth, differences between groups were evaluated using Mann-Whitney U-test, changes over time were calculated with the Friedman test (p=0.05).

<table>
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<tr>
<th>Modified criteria</th>
<th>Description</th>
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<tr>
<td>“Excellent”</td>
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<tr>
<td>“Good”</td>
<td>Slight deviations from ideal performance, correction possible without damage to tooth or restoration</td>
<td>“alpha”</td>
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<tr>
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<td>Few defects, correction impossible without damage to tooth or restoration.</td>
<td>“bravo”</td>
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<td>Severe defects, prophylactic removal for prevention of severe failures</td>
<td>“charlie”</td>
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<tr>
<td>“Poor”</td>
<td>Immediate replacement necessary</td>
<td>“delta”</td>
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Table 5.1: Evaluated clinical codes and criteria.

Results
The overall success rate was 100% after 6 years of clinical service, while drop out of patients was 0%. Results of the clinical investigation are displayed in Tables 5.2-5.7. Neither restorative material nor localization of the restoration (upper or lower jaw) had a significant influence on any criterion after 6 years (p>0.05; Mann-Whitney U test). However, molar restorations performed worse than premolar restorations regarding marginal integrity (4 years), restoration integrity (6, 12, 24, 48 months), and tooth integrity (4 and 6 years; Table 5.8). Irrespective of the resin composite used, significant changes over time were found for all criteria applied in clinical
examinations (Friedman test; p<0.05). Marginal integrity started with a major portion of overhangs in all marginal areas having been detected until the 1-year recall and distinctly dropping afterwards (overhangs at baseline 44%; 6 months: 65%; 1 year: 47%; 2 years: 6%; 4 years: 4%; and 6 years: 3%). Beyond the 1-year recall, more and more negative step formations due to wear were detected (Table 5.5). This phenomenon was earlier seen in molars (87 % bravo after 4 years) than in premolars (51 % bravo after 4 years; Table 5.8a).

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<td>44% 54% 2%</td>
<td>60% 40%</td>
<td>34% 66%</td>
<td>41% 59%</td>
</tr>
<tr>
<td>73.3 months</td>
<td>91% 9%</td>
<td>40% 47% 13%</td>
<td>29% 56% 15%</td>
<td>31% 48% 21%</td>
</tr>
<tr>
<td>Hyper-sensitivity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpha1 Alpha2 Bravo</td>
<td>[%]</td>
<td>[%]</td>
<td>[%]</td>
<td>[%]</td>
</tr>
<tr>
<td>1.2 months</td>
<td>100%</td>
<td>99% 1%</td>
<td>93% 7%</td>
<td>82% 18%</td>
</tr>
<tr>
<td>24.4 months</td>
<td>94% 6%</td>
<td>93% 7%</td>
<td>84% 13% 3%</td>
<td>84% 16%</td>
</tr>
<tr>
<td>49.2 months</td>
<td>44% 54% 2%</td>
<td>60% 40%</td>
<td>34% 66%</td>
<td>41% 59%</td>
</tr>
<tr>
<td>73.3 months</td>
<td>91% 9%</td>
<td>40% 47% 13%</td>
<td>29% 56% 15%</td>
<td>31% 48% 21%</td>
</tr>
<tr>
<td>Radiographic assessment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpha1 Alpha2 Bravo</td>
<td>[%]</td>
<td>[%]</td>
<td>[%]</td>
<td>[%]</td>
</tr>
<tr>
<td>1.2 months</td>
<td>100%</td>
<td>99% 1%</td>
<td>93% 7%</td>
<td>82% 18%</td>
</tr>
<tr>
<td>24.4 months</td>
<td>94% 6%</td>
<td>93% 7%</td>
<td>84% 13% 3%</td>
<td>84% 16%</td>
</tr>
<tr>
<td>49.2 months</td>
<td>44% 54% 2%</td>
<td>60% 40%</td>
<td>34% 66%</td>
<td>41% 59%</td>
</tr>
<tr>
<td>73.3 months</td>
<td>91% 9%</td>
<td>40% 47% 13%</td>
<td>29% 56% 15%</td>
<td>31% 48% 21%</td>
</tr>
</tbody>
</table>

Table 5.2: Descriptive statistics for all assessed restorations.
<table>
<thead>
<tr>
<th>Criterion</th>
<th>Baseline (n=36)</th>
<th>2 Years (n=36)</th>
<th>4 Years (n=36)</th>
<th>6 Years (n=36)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alpha1</td>
<td>Alpha2</td>
<td>Bravo</td>
<td>Alpha1</td>
</tr>
<tr>
<td>Surface roughness</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>97</td>
<td>3</td>
<td>92</td>
</tr>
<tr>
<td>Color match</td>
<td>92</td>
<td>8</td>
<td>92</td>
<td>8</td>
</tr>
<tr>
<td>Marginal integrity</td>
<td>50</td>
<td>47</td>
<td>3</td>
<td>53</td>
</tr>
<tr>
<td>Tooth integrity</td>
<td>86</td>
<td>14</td>
<td>47</td>
<td>42</td>
</tr>
<tr>
<td>Restoration integrity</td>
<td>100</td>
<td></td>
<td>11</td>
<td>45</td>
</tr>
<tr>
<td>Proximal contact</td>
<td>94</td>
<td>3</td>
<td>3</td>
<td>89</td>
</tr>
<tr>
<td>Change of sensitivity</td>
<td>100</td>
<td>100</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Hyper-sensitivity</td>
<td>97</td>
<td>3</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Radiographic assessment</td>
<td>89</td>
<td>3</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

*Table 5.3: Descriptive statistics for all Grandio restorations.*
## Table 5.4: Descriptive statistics for all Tetric Ceram restorations.

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Baseline (n=32)</th>
<th>2 Years (n=32)</th>
<th>4 Years (n=32)</th>
<th>6 Years (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alpha1</td>
<td>Alpha2</td>
<td>Bravo</td>
<td>Alpha1</td>
</tr>
<tr>
<td>Surface roughness</td>
<td>100</td>
<td>100</td>
<td></td>
<td>94</td>
</tr>
<tr>
<td>Color match</td>
<td>97</td>
<td>3</td>
<td></td>
<td>94</td>
</tr>
<tr>
<td>Marginal integrity</td>
<td>37</td>
<td>63</td>
<td></td>
<td>69</td>
</tr>
<tr>
<td>Tooth integrity</td>
<td>97</td>
<td>3</td>
<td></td>
<td>31</td>
</tr>
<tr>
<td>Restoration integrity</td>
<td>85</td>
<td>9</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Proximal contact</td>
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<td>3</td>
<td>3</td>
<td>75</td>
</tr>
<tr>
<td>Change of sensitivity</td>
<td>94</td>
<td>6</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Hyper-sensitivity</td>
<td>84</td>
<td>13</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>Radiographic assessment</td>
<td>94</td>
<td>6</td>
<td></td>
<td>94</td>
</tr>
</tbody>
</table>

## Table 5.5a: Descriptive statistics regarding “marginal integrity” (all restorations).

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Baseline (n=68)</th>
<th>24 months (n=68)</th>
<th>48 months (n=68)</th>
<th>72 months (n=68)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha I Excellent</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slight defects, easily correctable</td>
<td>Negative step Overhang</td>
<td>8.8 % 44.1 % 1.5 %</td>
<td>44.1 % 5.9 % 10.3 %</td>
<td>29.4 % 4.4 % 0.0 %</td>
</tr>
<tr>
<td>Stained overhang</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bravo Slight defects, not correctable without damage</td>
<td>Gap / negative step Staining</td>
<td>1.5 % 16.2 % 0.0 %</td>
<td>16.2 % 23.5 % 23.5 %</td>
<td>23.5 % 42.6 % 47.2 %</td>
</tr>
<tr>
<td>Criterion</td>
<td>Baseline (n=36)</td>
<td>24 months (n=36)</td>
<td>48 months (n=36)</td>
<td>72 months (n=36)</td>
</tr>
<tr>
<td>-----------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>-----------------</td>
</tr>
<tr>
<td><strong>Alpha I</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excellent</td>
<td>50.0 %</td>
<td>0.0 %</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td><strong>Alpha II</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slight defects, easily correctable</td>
<td>Negative step</td>
<td>5.6 %</td>
<td>38.9 %</td>
<td>27.8%</td>
</tr>
<tr>
<td>Overhang</td>
<td>38.9 %</td>
<td>2.8 %</td>
<td>8.3%</td>
<td>2.8%</td>
</tr>
<tr>
<td>Stained overhang</td>
<td>2.8 %</td>
<td>11.1 %</td>
<td>0.0 %</td>
<td>2.8%</td>
</tr>
<tr>
<td><strong>Bravo</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slight defects, not correctable without damage</td>
<td>Gap / negative step</td>
<td>2.8 %</td>
<td>19.4 %</td>
<td>25.0%</td>
</tr>
<tr>
<td>Staining</td>
<td>0.0 %</td>
<td>27.8 %</td>
<td>38.9%</td>
<td>52.8%</td>
</tr>
</tbody>
</table>

**Table 5.5b:** Descriptive statistics regarding “marginal integrity” (Grandio restorations).

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Baseline (n=32)</th>
<th>24 months (n=32)</th>
<th>48 months (n=32)</th>
<th>72 months (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alpha I</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excellent</td>
<td>37.5 %</td>
<td>0.0 %</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td><strong>Alpha II</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slight defects, easily correctable</td>
<td>Negative step</td>
<td>12.5 %</td>
<td>50.0 %</td>
<td>31.3%</td>
</tr>
<tr>
<td>Overhang</td>
<td>50.0 %</td>
<td>9.4 %</td>
<td>0.0 %</td>
<td>0.0 %</td>
</tr>
<tr>
<td>Stained overhang</td>
<td>9.4 %</td>
<td>9.4 %</td>
<td>0.0 %</td>
<td>0.0%</td>
</tr>
<tr>
<td><strong>Bravo</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slight defects, not correctable without damage</td>
<td>Gap / negative step</td>
<td>0.0 %</td>
<td>12.5 %</td>
<td>21.9%</td>
</tr>
<tr>
<td>Staining</td>
<td>0.0 %</td>
<td>18.8 %</td>
<td>46.9%</td>
<td>40.6%</td>
</tr>
</tbody>
</table>

**Table 5.5c:** Descriptive statistics regarding “marginal integrity” (Tetric Ceram restorations).
<table>
<thead>
<tr>
<th>Criterion</th>
<th>Baseline (n=68)</th>
<th>24 months (n=68)</th>
<th>48 months (n=68)</th>
<th>72 months (n=68)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alpha I</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excellent</td>
<td>91.2 %</td>
<td>39.7 %</td>
<td>29.4 %</td>
<td>30.9 %</td>
</tr>
<tr>
<td><strong>Alpha II</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slight defects, easily correctable</td>
<td>Enamel chipping</td>
<td>1.5 %</td>
<td>4.4 %</td>
<td>0.0 %</td>
</tr>
<tr>
<td></td>
<td>Enamel crack</td>
<td>7.4 %</td>
<td>42.6 %</td>
<td>55.9 %</td>
</tr>
<tr>
<td></td>
<td>Wear</td>
<td>0.0 %</td>
<td>0.0 %</td>
<td>0.0 %</td>
</tr>
<tr>
<td><strong>Bravo</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slight defects, not correctable without damage</td>
<td>Enamel chipping</td>
<td>0.0 %</td>
<td>10.3 %</td>
<td>14.7 %</td>
</tr>
<tr>
<td></td>
<td>Enamel crack</td>
<td>0.0 %</td>
<td>2.9 %</td>
<td>0.0 %</td>
</tr>
<tr>
<td></td>
<td>Wear</td>
<td>0.0 %</td>
<td>0.0 %</td>
<td>0.0 %</td>
</tr>
</tbody>
</table>

**Table 5.6a:** Descriptive statistics regarding “tooth integrity” (all restorations).

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Baseline (n=36)</th>
<th>24 months (n=36)</th>
<th>48 months (n=36)</th>
<th>72 months (n=36)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alpha I</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excellent</td>
<td>86.1 %</td>
<td>47.2 %</td>
<td>30.6 %</td>
<td>33.3 %</td>
</tr>
<tr>
<td><strong>Alpha II</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slight defects, easily correctable</td>
<td>Enamel chipping</td>
<td>2.8 %</td>
<td>2.8 %</td>
<td>0.0 %</td>
</tr>
<tr>
<td></td>
<td>Enamel crack</td>
<td>11.1 %</td>
<td>38.9 %</td>
<td>58.3 %</td>
</tr>
<tr>
<td></td>
<td>Wear</td>
<td>0.0 %</td>
<td>0.0 %</td>
<td>0.0 %</td>
</tr>
<tr>
<td><strong>Bravo</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slight defects, not correctable without damage</td>
<td>Enamel chipping</td>
<td>0.0 %</td>
<td>8.3 %</td>
<td>11.1 %</td>
</tr>
<tr>
<td></td>
<td>Enamel crack</td>
<td>0.0 %</td>
<td>2.8 %</td>
<td>0.0 %</td>
</tr>
<tr>
<td></td>
<td>Wear</td>
<td>0.0 %</td>
<td>0.0 %</td>
<td>0.0 %</td>
</tr>
</tbody>
</table>

**Table 5.6b:** Descriptive statistics regarding “tooth integrity” (Grandio restorations).
### Microhybrid vs. nanohybrid resin composites in extended cavities after 6 years

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Baseline (n=32)</th>
<th>24 months (n=32)</th>
<th>48 months (n=32)</th>
<th>72 months (n=32)</th>
</tr>
</thead>
</table>
| **Alpha I**
Excellent | 96.9 % | 31.3 % | 28.1 % | 28.1 % |
| **Alpha II**
Slight defects, easily correctable
Enamel chipping
Enamel crack | 0.0 % | 6.3 % | 0.0 % | 6.3 % |
| | 3.1 % | 46.9 % | 53.1 % | 40.6 % |
| **Bravo**
Slight defects, not correctable without damage
Enamel chipping
Enamel crack | 0.0 % | 12.5 % | 18.8 % | 18.8 % |
| | 0.0 % | 3.1 % | 0.0 % | 6.3 % |

**Table 5.6c:** Descriptive statistics regarding “tooth integrity” (Tetric Ceram restorations).

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Baseline (n=68)</th>
<th>24 months (n=68)</th>
<th>48 months (n=68)</th>
<th>72 months (n=68)</th>
</tr>
</thead>
</table>
| **Alpha I**
Excellent | 92.6 % | 8.8 % | 1.5 % | 2.9 % |
| **Alpha II**
Slight defects, easily correctable
Chipping
Crack
Roughness / Abrasion | 2.9 % | 0.0 % | 0.0 % | 1.5 % |
| | 0.0 % | 1.5 % | 0.0 % | 0.0 % |
| | 1.5 % | 39.7 % | 25.0 % | 32.4 % |
| **Bravo**
Slight defects, not correctable without damage
Chipping
Crack probing
Abrasion
Roughness
Void | 0.0 % | 2.9 % | 7.4 % | 2.9 % |
| | 2.9 % | 0.0 % | 4.4 % | 1.5 % |
| | 0.0 % | 30.9 % | 51.5 % | 58.8 % |
| | 0.0 % | 4.4 % | 7.4 % | 0.0 % |
| | 0.0 % | 11.8 % | 2.9 % | 0.0 % |

**Table 5.7a:** Descriptive statistics regarding “restoration integrity” (all restorations).
### Table 5.7b: Descriptive statistics regarding “restoration integrity” (Grandio restorations).

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Baseline (n=36)</th>
<th>24 months (n=36)</th>
<th>48 months (n=36)</th>
<th>72 months (n=36)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alpha I</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excellent</td>
<td>100.0 %</td>
<td>11.1 %</td>
<td>2.8%</td>
<td>2.8%</td>
</tr>
<tr>
<td><strong>Alpha II</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slight defects, easily correctable</td>
<td>Chipping 0.0 %</td>
<td>0.0 %</td>
<td>0.0 %</td>
<td>0.0 %</td>
</tr>
<tr>
<td></td>
<td>Crack 0.0 %</td>
<td>0.0 %</td>
<td>0.0 %</td>
<td>0.0 %</td>
</tr>
<tr>
<td></td>
<td>Roughness / Abrasion 0.0 %</td>
<td>44.4 %</td>
<td>27.8%</td>
<td>38.9%</td>
</tr>
<tr>
<td><strong>Bravo</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slight defects, not correctable without damage</td>
<td>Chipping 0.0 %</td>
<td>5.6 %</td>
<td>8.3%</td>
<td>2.8%</td>
</tr>
<tr>
<td></td>
<td>Crack probing</td>
<td>0.0 %</td>
<td>2.8%</td>
<td>0.0 %</td>
</tr>
<tr>
<td></td>
<td>Abrasion 0.0 %</td>
<td>16.7%</td>
<td>50.0%</td>
<td>55.6%</td>
</tr>
<tr>
<td></td>
<td>Roughness</td>
<td>0.0 %</td>
<td>5.6%</td>
<td>8.3%</td>
</tr>
<tr>
<td></td>
<td>Void 0.0 %</td>
<td>16.7%</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
</tbody>
</table>

### Table 5.7c: Descriptive statistics regarding “restoration integrity” (Tetric Ceram restorations).

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Baseline (n=32)</th>
<th>24 months (n=32)</th>
<th>48 months (n=32)</th>
<th>72 months (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alpha I</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excellent</td>
<td>84.4 %</td>
<td>6.3 %</td>
<td>0.0 %</td>
<td>3.1 %</td>
</tr>
<tr>
<td><strong>Alpha II</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slight defects, easily correctable</td>
<td>Chipping 6.3 %</td>
<td>0.0 %</td>
<td>0.0 %</td>
<td>3.1 %</td>
</tr>
<tr>
<td></td>
<td>Crack 0.0 %</td>
<td>3.1 %</td>
<td>0.0 %</td>
<td>0.0 %</td>
</tr>
<tr>
<td></td>
<td>Roughness / Abrasion 3.1 %</td>
<td>34.4 %</td>
<td>21.9%</td>
<td>25.0%</td>
</tr>
<tr>
<td><strong>Bravo</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slight defects, not correctable without damage</td>
<td>Chipping 0.0 %</td>
<td>0.0 %</td>
<td>6.3%</td>
<td>3.1%</td>
</tr>
<tr>
<td></td>
<td>Crack probing</td>
<td>6.3 %</td>
<td>6.3%</td>
<td>3.1%</td>
</tr>
<tr>
<td></td>
<td>Abrasion 0.0 %</td>
<td>46.9 %</td>
<td>53.1%</td>
<td>62.5%</td>
</tr>
<tr>
<td></td>
<td>Roughness</td>
<td>0.0 %</td>
<td>3.1%</td>
<td>6.3%</td>
</tr>
<tr>
<td></td>
<td>Void 0.0 %</td>
<td>6.3 %</td>
<td>6.3%</td>
<td>0.0 %</td>
</tr>
</tbody>
</table>
### Table 5.8a: Descriptive statistics of premolars vs. molars regarding “marginal integrity”. No significant difference could be calculated after 72 months (p=0.073) in contrast to the result after 48 months (p=0.007; Mann-Whitney U-test).

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Premolars 48 months</th>
<th>Premolars 72 months</th>
<th>Molars 48 months</th>
<th>Molars 72 months</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alpha I</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excellent</td>
<td>2.2%</td>
<td>0.0%</td>
<td>4.3%</td>
<td>0.0%</td>
</tr>
<tr>
<td><strong>Alpha II</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slight defects, easily correctable</td>
<td>40.0%</td>
<td>44.4%</td>
<td>8.7%</td>
<td>26.1%</td>
</tr>
<tr>
<td>Overhang</td>
<td>6.7%</td>
<td>2.2%</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Stained overhang</td>
<td>0.0%</td>
<td>2.2%</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td><strong>Bravo</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slight defects, not correctable without damage</td>
<td>20.0%</td>
<td>8.9%</td>
<td>26.1%</td>
<td>17.4%</td>
</tr>
<tr>
<td>Gap / negative step</td>
<td>31.1%</td>
<td>42.2%</td>
<td>60.9%</td>
<td>56.5%</td>
</tr>
</tbody>
</table>

### Table 5.8b: Descriptive statistics of premolars vs. molars regarding “tooth integrity”. Significant differences could be calculated after 48 (p=0.013) and 72 months (p=0.003; Mann-Whitney U-test).

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Premolars 48 months</th>
<th>Premolars 72 months</th>
<th>Molars 48 months</th>
<th>Molars 72 months</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alpha I</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excellent</td>
<td>37.8%</td>
<td>42.2%</td>
<td>13.0%</td>
<td>8.7%</td>
</tr>
<tr>
<td><strong>Alpha II</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slight defects, easily correctable</td>
<td>0.0%</td>
<td>6.7%</td>
<td>0.0%</td>
<td>13.0%</td>
</tr>
<tr>
<td>Enamel chipping</td>
<td>53.3%</td>
<td>35.6%</td>
<td>60.9%</td>
<td>52.2%</td>
</tr>
<tr>
<td>Enamel crack</td>
<td>0.0%</td>
<td>2.2%</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Wear</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td><strong>Bravo</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slight defects, not correctable without damage</td>
<td>8.9%</td>
<td>4.4%</td>
<td>26.1%</td>
<td>8.7%</td>
</tr>
<tr>
<td>Enamel chipping</td>
<td>0.0%</td>
<td>4.4%</td>
<td>0.0%</td>
<td>8.7%</td>
</tr>
<tr>
<td>Enamel crack</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Wear</td>
<td>0.0%</td>
<td>2.2%</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Criterion</td>
<td>48 months</td>
<td></td>
<td>72 months</td>
<td></td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td>-----------</td>
<td>------------------</td>
<td>-----------</td>
<td>------------------</td>
</tr>
<tr>
<td></td>
<td>premolars</td>
<td>molars (n=23)</td>
<td>premolars</td>
<td>molars (n=23)</td>
</tr>
</tbody>
</table>
| **Alpha I**
Excellent                        | 2.2 %     | 4.3 %            | 0.0 %     | 8.7 %            |
| **Alpha II**
Slight defects, easily correctable
Chipping                                     | 2.2 %     | 0.0 %            | 2.2 %     | 0.0 %            |
| Crack                                         | 0.0 %     | 0.0 %            | 0.0 %     | 0.0 %            |
| Roughness / Abrasion                          | 33.3 %    | 4.3 %            | 40.0%     | 17.3%            |
| **Bravo**
Slight defects, not correctable without damage
Chipping                                       | 4.4 %     | 8.7 %            | 4.4 %     | 0.0 %            |
| Crack probing                                  | 4.4 %     | 4.3 %            | 2.2 %     | 0.0 %            |
| Abrasion                                       | 40.0 %    | 73.9 %           | 51.1 %    | 73.9%            |
| Roughness                                      | 8.9 %     | 4.3 %            | 0.0 %     | 0.0 %            |
| Void                                           | 4.4 %     | 0.0 %            | 0.0 %     | 0.0 %            |

Table 5.8c: Descriptive statistics of premolars vs. molars regarding “restoration integrity”. No significant difference was calculated after 72 months (p=0.321) in contrast to the 48 month findings (p=0.018; Mann-Whitney U-test).

Tooth integrity significantly deteriorated due to increasing enamel cracks over time (p<0.05; Table 5.6). Enamel chippings or cracks were significantly more frequently observed in molars (26% bravo after 4 years / 35 % bravo after 6 years) than in premolars (9% bravo after 4 years/11% brav o after 6 years). The main reasons for decreasing “restoration integrity” were visible signs of surface roughness and distinct wear traces (28% after 1 year, 75% after 2 years, 84% after 4 years, 91% after 6 years; Table 5.7). Visible wear of both materials under investigation was detectable earlier in molars (74% bravo after 4 years) than in premolars (40% bravo after 4 years; Table 5.8c, Figures 5.1 and 5.2).
Figure 5.1
Clinical view of a Grandio restoration after 6 years (upper left first premolar). A discoloration at the palatal proximal margin was detectable, probably due to a small overhang. Wear and negative step formation in the occlusal portion of the restoration was obvious. The surface appears rough.

Figure 5.2
Clinical view of a Tetric Ceram restoration after 6 years (upper right first premolar, same patient as in Figure 5.1). No discoloration was detectable clinically. Wear and negative step formation in the occlusal portion of the restoration was detectable. In the occlusal contact area of the lateral ridge, the surface show signs of surface fatigue (small cracks) and the surface appears rough.

Discussion
Fundamental prerequisites for clinical success with resin-based composites as posterior restorative material still must be met in order to achieve clinical success with this particular group of dental materials.\textsuperscript{10-12,18,30,41} There is no doubt that resin composites are almost perfect for minimally invasive posterior cavities, however, it is to the date not fully understood how far we can go in terms of cavity extension. So it is still frequently argued that resin composites suffer some disadvantages in very
extended cavities and should therefore be replaced by other materials and techniques such as indirectly bonded restorations.\textsuperscript{21} When large cavities need to be restored, the major objections against resin composites are the dangers of developing recurrent caries and the non-predictable wear rates over time.\textsuperscript{42} The problem with secondary caries is even more common when proximal Class II margins are located in dentin. At least from in vitro and in vivo investigations dealing with indirect ceramic inlays and onlays, it is proven that even margins extending beyond the amelocemental junction can be safely restored.\textsuperscript{6,16,25,42} For Class V restorations it is similar, although 50\% of margin length is located in dentin.\textsuperscript{4,19,20,22} On the other hand, proximal marginal seal in dentin-bordered cavities restored with direct resin composite restorations, is underrepresented in the literature of the field.\textsuperscript{21} Thus, the setup of this clinical trial excluded minimally invasive cavities and was mainly restricted to amalgam replacement restorations resulting in 35\% of cavities with no proximal-cervical enamel and 49\% with <0.5 mm proximal enamel width. Finally, after 6 years of clinical service, these restorations did not reveal significantly worse clinical outcomes, and moreover, neither recurrent caries nor severe marginal staining was detected.

The most recent recommendations for clinical trials with restorative materials\textsuperscript{31} could not be addressed, because these recommendations were published considerably after the beginning of the present study. Therefore, it was not possible to include more evaluation aspects among well-suited protocols such as the CONSORT statement.\textsuperscript{15,16,40,42}

Direct and indirect resin composites as well as all-ceramic inlays have to be bonded for acceptable clinical outcome.\textsuperscript{5,10,11,14,30} The selection of materials for this study was carried out after thorough in vitro testing with promising results for both materials used, in terms of good marginal adaptation and long-term stability,\textsuperscript{24,27,28,43} because previous studies clearly indicated that it may be dangerous to make clinical trials with materials having failed some preclinical screenings.\textsuperscript{15,43}

Although the different adhesives used in the present investigation required special bonding protocols, i.e. wet bonding with the acetone-based Solobond M, this obviously did not negatively influence clinical results in terms of postoperative hypersensitivities. Initially, restorations bonded with Syntac exhibited slightly more hypersensitivities (baseline to 6 months) (3\% vs. 0\% bravo scores), but this played
no role past the 1-year recall. Therefore, both the internal sealing of dentin and tight
dentin margins were possible with both adhesives under investigation. Even so,
dealing with the clinical outcome of complete restorative systems (i.e. adhesive plus
resin composite) always is more complicated than evaluating two adhesives with one
resin composite in order to minimize variables. This paper is not able to completely
elucidate this particular problem, but the promising results over the 6-year period
support the assumption that both systems are clinically acceptable.

Including nanofillers in recent resin composites is widespread in adhesive dentistry
today. Major advantages are primarily enhanced translucency effects and increased
polishability.\textsuperscript{12,44,45} Irrespective of these aspects, clinical reports dealing with this
class of materials exhibited no significant advantages \textit{in vivo}.\textsuperscript{12} In the present
investigation, Tetric Ceram was used as fine hybrid resin composite (without
nanofillers), and Grandio was used as one of the first resin composites with
incorporated nanofillers among conventional hybrid type fillers as so-called
nanohybrid resin composite.\textsuperscript{12,32,37}

Altogether, the null hypothesis of the present investigation was confirmed because
there was no difference in the clinical behavior between Grandio and Tetric Ceram
used for extended Class II posterior restorations.
References


31. Hickel R, Roulet JF, Bayne S et al. Recommendations for conducting
controlled clinical studies of dental restorative materials. Science Committee Project 2/98--FDI World Dental Federation study design (Part I) and criteria for evaluation (Part II) of direct and indirect restorations including onlays and partial crowns. *J Adhes Dent* 2007; 9 Suppl 1:121-147.


CHAPTER 6

General discussion:
Is the clinical performance of bonded restoratives predictable in the laboratory?
Introduction

Dental biomaterials are subjected to considerable degradation processes during clinical service over time.\(^1\)\(^-\)\(^5\) After amalgam, having been the standard restorative for posterior restorations for more almost two centuries, today tooth-colored materials such as resin-based composites are the treatment option of choice for the majority of patients.\(^6\)\(^-\)\(^13\) Adhesive dentistry's long-term success is proven for pit and fissure sealing, direct and indirect resin composites, and ceramic inlays.\(^11\)\(^,\)\(^13\)\(^-\)\(^25\) Nevertheless, even in the era of nano-optimized resin-based composites, polymerization shrinkage still relies on durable adhesion to enamel and dentin as a fundamental prerequisite, and vice versa, without successful adhesion, gap formation potentially jeopardizes clinical long-term success.\(^4\)\(^,\)\(^26\)\(^-\)\(^36\)

Adhesion to phosphoric acid etched enamel is no longer a concern for dentists due to its clinically proven durability,\(^4\)\(^-\)\(^6\)\(^,\)\(^31\)\(^,\)\(^35\)\(^-\)\(^40\) however, durability of self-etch adhesives in heavily loaded Classes I and II is still not clinically proven, whereas in cervical lesions, medium-term results are promising.\(^12\)\(^,\)\(^21\)\(^,\)\(^22\)\(^,\)\(^41\)\(^,\)\(^42\) Knowledge about adhesion to dentin is different; the self-etch approach - at least with two steps - seems to be the most promising way to get durable bonds beside multi-step etch-and-rinse systems.\(^30\)\(^,\)\(^31\)\(^,\)\(^35\)\(^,\)\(^40\)\(^,\)\(^43\)\(^,\)\(^44\) Comparing enamel and dentin as adhesive substrates still reveals dentin to be the weaker substrate due its tubular structure and intrinsic wetness which leads to permeability problems with all-in-one self-etch adhesives.\(^27\)\(^,\)\(^31\)\(^,\)\(^33\)\(^-\)\(^35\)\(^,\)\(^44\)\(^-\)\(^47\)

More or less all degradation processes found for dental biomaterials are related to fatigue.\(^10\)\(^,\)\(^37\)\(^-\)\(^55\) Especially with resin-based composites, fatigue is not only a matter of loss of adhesive performance over time (adhesive fatigue) but also of bulk fatigue (fracture) and surface fatigue (wear). For resin composites, two fatigue phenomena (adhesive fatigue leading to recurrent caries and bulk fatigue leading to fractures) are responsible for the vast majority of clinical failures observed during clinical long-term trials.\(^6\)\(^,\)\(^7\)\(^,\)\(^11\)\(^,\)\(^16\)\(^,\)\(^56\) Wear is in most of the cases less clinically relevant because worn resin composites may still be clinically serviceable, however, loss of anatomic form over time may lead to occlusal interferences.\(^10\)\(^,\)\(^57\)\(^-\)\(^59\) Research in restorative dentistry is, since decades, focused on predicting clinical issues in the lab.\(^60\) Of course, clinical long-term trials remain the ultimate instrument for thoroughly evaluating dental biomaterials. However, the main problem with clinical trials is that when they give
valuable results after several years of observation time, the adhesive and/or resin composite may no longer be on the market.

Therefore, preclinical in vitro investigations are very important, however, it is contradictorily reported in the literature whether these tests are able to reliably predict clinical behavior. So the aim of this paper was to investigate this particular question with a special focus on marginal integrity, bulk fatigue (fracture), and surface fatigue (wear).

Materials and Methods
Publications in dental and biomaterial journals with dental materials since 1990 were retrieved in PubMed, MedLine, Dimdi, and Embase. Search key words were: margin, gap, marginal integrity, marginal adaptation, enamel margin, dentin margin, marginal quality, fracture, chipping, bulk fracture, abrasion, and wear. We only chose papers dealing with resin composites. Congress abstracts were completely ignored. Top cited papers were retrieved from www.scopus.com in order to prioritize publications that were frequently referred to. As indicator for top cited papers, the frequency of citations per year (CPA) was set >5.

Results
An overview of top cited papers dealing with marginal integrity, fatigue, and wear of bonded restorations is displayed in Tables 6.1-6.3.

Marginal quality
Papers reporting direct comparisons between in vitro and in vivo results are scarce in the literature in the field of adhesive dentistry. However, several evaluations of marginal integrity from the preclinical point of view exist as do a few papers focusing on marginal adaptation in vivo. Marginal integrity papers in vitro repeatedly report a superior performance of etch-and-rinse adhesives in enamel bonding, however, again there are contradictory reports of Hannig et al. claiming self-etch adhesives being an alternative to phosphoric acid even in stress-bearing cavities. For dentin bonding, Frankenberger and Tay
reported equal results for etch-and-rinse adhesives and two-step self-etch adhesives in dentin margins of Class II cavities after thermomechanical loading, and significantly worse results when all-in-one adhesives were used for bonding of resin composites in vitro.\textsuperscript{35}

Abdalla and Davidson directly compared \textit{in vitro} and \textit{in vivo} applied resin composite restorations in Class II cavities with less microleakage in the laboratory.\textsuperscript{65} Two papers of our group investigated the resin composites Ariston pHc and Solitaire both in vitro and in vivo. In the in vitro part, both restoratives exhibited some shortcomings,\textsuperscript{36} however, these previously reported materials properties led to catastrophic outcomes in vitro with mean survival times for both materials of 2.4 years.\textsuperscript{66} Frankenberger et al. also compared the same materials in vitro and in vivo with respect to marginal adaptation of Class I resin composite restorations in molars.\textsuperscript{39} Here, some minor differences were noticed between the in vitro and the in vivo situation, however, the rankings regarding the adhesive’s performances were the same, revealing superior results for etch-and-rinse adhesives when compared to self-etch adhesives.\textsuperscript{39} And among the self-etch adhesives, two-step self-etch adhesives were more effective than one-step self-etch adhesives.\textsuperscript{39} Another publication observed marginal quality for Grandio and Tetric Ceram restorations both in vitro and in vivo over 6 years.\textsuperscript{4} Also here, in vitro and in vivo results for marginal quality were similar until the 6-year recall with a combined 6-year water storage/thermomechanical loading scenario.\textsuperscript{4} Heintze et al. compared results of clinical studies with bonded Class V resin composite restorations with two different in vitro stressing regimens for a variety of 37 adhesives.\textsuperscript{44} They concluded that the systematic analysis of the correlation between laboratory data of marginal adaptation and the outcome of clinical trials of Class V restorations revealed that the correlation was weak and only present if studies were compared which used the same composite for the \textit{in vitro} and \textit{in vivo} evaluation.\textsuperscript{44}

\textit{Bulk fatigue / fracture behavior}

Regarding the fatigue behavior related to flexural strength, some studies evaluated the flexural fatigue behavior in terms of a so-called flexural fatigue limit (FFL).\textsuperscript{48,49,67-79} The flexural fatigue limits (FFL) of the composite materials were determined for $10^5$
cycles under equivalent test conditions at a frequency of 0.5 Hz (n = 25). The “staircase” approach was used for fatigue evaluation. For every cycle the stress alternated between 1 MPa and the maximum stress. Tests were conducted sequentially, with the maximum applied stress in each succeeding test being increased or decreased by a fixed increment of stress, according to whether the previous test resulted in failure or not. The first specimen was tested at approximately 50% of the initial flexural strength value. As the data are cumulated around the mean stress, the number of specimens required is less than with other methods. The mean flexural fatigue limit (FFL) is determined using Eq. 1 and standard deviation, using Eq. 2, respectively:

$$\text{FFL} = X_0 + d \left( \frac{\sum i n_i}{\sum n_i} \pm 0.5 \right)$$  \hspace{1cm} (1)

$$\text{SD} = 1.62d \left( \frac{\sum n_i \sum i^2 n_i - (\sum in_i)^2}{(\sum n_i)^2} + 0.029 \right)$$  \hspace{1cm} (2)

$X_0$ is the lowest stress level considered in the analysis and $d$ is the fixed stress increment. To determine the FFL, the analysis of the data was based on the least frequent event (failures versus nonfailures). In Eq. 1 a negative sign was used when the analysis was based on failures. The lowest stress level considered was designated as $i = 0$, the next as $i = 1$, and so on, and $n_i$ was the number of failures or non-failures at the given stress level. Turssi et al. evaluated FFLs of microfill versus nanfilled resin composite with equal to worse outcome for the nanomaterials. Abe et al. compared an array of resin composites with results having been inferior for most of the packable resin composites under investigation. Lohbauer et al. evaluated the FFL behavior of different resin composites for posterior use and concluded that high initial flexural strengths do not automatically mean high FFLs. In a direct in vitro - in vivo comparison of resin-based materials regarding FFL and clinical outcome, the low FFL for the resin composite Solitaire led to unacceptably high fracture rates in vivo, so the authors concluded that FFL >30 MPa is the critical threshold value for bulk fatigue in order to withstand masticatory forces and clinical fatigue life over time.
Surface contact fatigue/clinical wear

Early abrasion studies during the first stages of resin composite testing were cast analyses according to some scales such as the Leinfelder scale allowing estimates of clinical wear in terms of calibrated casts in 50 µm steps.\(^1\),\(^{52,53,82-88}\) Compared to preclinical screenings where normally only the test material is abraded,\(^{54,55,89,90}\) clinical wear measurements in restorative dentistry always deal with enamel and restorative materials and an exact determination of reference points is only possible with demanding 3D laser devices.\(^{57-59,91-93}\) Due to the expensive tool and sophisticated software issues, very few 3D laser scan studies dealing with clinical wear phenomena are available in the literature, reporting wear rates after 3 years of clinical service of ~80 µm.\(^{91,92}\)

Discussion

In vitro research on dental adhesive biomaterials is necessary, because a) special experimental research questions would never pass an ethics committee and b) not every adhesive and/or resin-based composite can be the subject of randomized prospective clinical trials because these are time-consuming and expensive. Nevertheless, it is still not fully understood whether and what we can really simulate in the lab and where major shortcomings are. So, the objective of this paper was to clarify the question “Is clinical performance of dental biomaterials predictable in the lab?”. Roulet thought about this topic years ago indicating that in vitro research suffers from interpretation problems and even in vivo studies always reveal significant limitations.\(^{94}\)

Today, there are still only a handful of studies directly comparing in vitro with in vivo results from the same workgroup. Abdalla and Davidson published the first “Comparison of the marginal integrity of in vitro and in vivo Class II composite restorations” being unique so far. This investigation dealt with microleakage in laboratory and ex vivo specimens.\(^{65}\) Whereas only 40% of in vitro specimens revealed microleakage after mechanical loading, 100% of in vivo restorations exhibited microleakage.\(^{65}\) Therefore, the authors concluded that laboratory studies may not be able to completely predict clinical behavior of adhesive junctions in the oral cavity.\(^{65}\)
Clinical studies are mainly performed in Classes V or II cavities being the latter the most difficult to obtain. Major advantages of clinical trials in Class V cavities were referred to as non-existing macromechanical retention, considerable amounts of dentin margins, probably less influence of the particular resin composite, and easy judgement of retention vs. retention loss. However, the main problem in adhesive dentistry is not retention of Class V restorations, it is still to prove whether bonded resin composites are able to fully replace amalgam in stress-bearing posterior cavities. However, disadvantages of Classes I and II are that retention is often provided by undermining dentin decay and subsequent undercuts, and less presence of clinically judgeable dentin margins. So marginal quality assessments in posterior stress-bearing resin composite restorations may be less suitable to investigate adhesives alone compared to non-carious Class V restorations, however, clinical importance facing millions of stress-bearing posterior resin composite restorations is great.

Opdam et al. reported marginal integrity and postoperative sensitivity in Class II restorations in vivo finding that etch-and-rinse adhesives showed good results in enamel bonding and self-etch adhesives produced less postoperative hypersensitivity. When clinical staining is related to inferior or loss of enamel bonding durability, and postoperative hypersensitivities are linked to inferior or loss of dentin bonding quality, this was predictable from laboratory investigations.

Four recent publications of our workgroup aimed to evaluate resin composites and their corresponding adhesives in both aspects, in vitro and in vivo. Frankenberger et al. reported in vitro performance of resin composites by means of microtensile bond strengths to enamel and dentin, flexural fatigue behavior, and wear behavior. The resin composites Ariston pHc and Solitaire were different from contemporary resin composites, i.e. Ariston exhibited significantly less adhesion, Solitaire revealed an inferior flexural fatigue limit. Krämer et al. reported clinical findings of identical materials demonstrating catastrophic clinical outcome with several bulk fractures of Solitaire, and even more failures of Ariston restorations caused by postoperative hypersensitivities and enamel fractures. Frankenberger et al. compared different classes of adhesives in vitro and in vivo with identical enamel bonding rankings for the different bonding approaches. The same was true for a recent publication showing 6-year results in vitro and in vivo with again similar
outcomes over time.\textsuperscript{4} So in all cases, clinical performance of resin composites in Classes I and II cavities was predictable from laboratory results, especially significant differences between etch-and-rinse adhesives and self-etch adhesives in enamel bonding durability. So even the results of Heintze et al.\textsuperscript{43,44} actually match the outcome of our workgroup where always the same resin composites were used \textit{in vitro} and \textit{in vivo} which may have contributed to the more consistent values.\textsuperscript{4,39}

All these findings clearly reflect that marginal quality prediction is possible from laboratory studies, however, marginal integrity is only one among several crucial factors for clinical outcome with bonded tooth-colored materials. A high amount of gaps after thermomechanical challenge in vitro increases the probability of the same scenario \textit{in vivo}. However, this does not necessarily lead to recurrent decay because the presence of marginal gaps \textit{in vivo} does not necessarily lead to secondary caries. One ultimate question is still unclear: when e.g. resin composite restoration achieves good results in an in vitro marginal quality assessment, it is rather predicable that its clinical marginal quality will not cause significant problems. On the other hand, can we conclude this also from the other side of the scale? Probably not. We still do not know below which percentage of gap-free margin it is not safe to use the material combination also clinically.

The final reason in favor of in vitro research regarding marginal quality is that many studies focus on experimental questions that would never pass an ethics committee for a clinical trial. In these cases \textit{in vitro} studies are the only way, giving important tendencies for clinical application of dental biomaterials. Among all in vitro approaches to predict clinical outcome, thermomechanical loading and subsequent marginal analysis is the closest scenario to the clinical situation, however, it is almost as intricate as a clinical trial.

As the frequency of citations as well as the presence of top cited papers clearly reflects, flexural fatigue behavior of dental biomaterials receives far less attention. However, a few top papers indicate that flexural fatigue behavior of dental biomaterials is closely related to clinical outcome in terms of fracture behavior.\textsuperscript{36,48,49,66,67,70,71,95} Compared to the multiple questions about marginal quality, FFL measurements in vitro are able to exactly define a kind of lower borderline at ~30 MPa flexural fatigue limit, because below that level clinically much more fractures were observed.\textsuperscript{66} On the other hand, there are still not enough clinical data
proving that higher and higher initial and fatigue values for flexural strength automatically lead to less fractures observed in clinical recalls after several years of clinical service.  

Wear is an important consequence of occlusal interactions. If not controlled, wear could lead to poor masticatory function with a concomitant reduction in quality of life. However, for most of the investigated resin-based composites this is simply not the case. Compared with other modes of in vitro testing of dental biomaterials, preclinical wear simulation is the most sophisticated branch. Clinical wear is a very complex scenario being influenced by several factors such as pH, contact-free abrasion, occlusal contact fatigue, and antagonist structure and material. Most of the in vitro regimens are only able to mimic one of these several co-factors. Analyzed as an array, many different wear simulation scenarios could finally result in an appropriate estimation of clinical wear. Also here it is clearly visible in Table 6.3 that the scientific importance of wear investigations decreased during the last decade and top cited papers are scarce. 3D lasercans are the ultimate instrument to evaluate clinical wear, however, there is an urgent need for more clinical data with different restoratives.

In times of ranking publications according to their journal impact factor (JIF), citations are an important tool in order to judge the importance of individual papers in the literature. High JIF regularly result from frequently cited papers. As performed before with a substantial amount of citations, the authors again decided to include this aspect by focussing on frequently cited papers being indicated by CPA (citations per anno; Tables 6.1-6.3). For the top cited paper published by Van Meerbeek et al. this means that this single publication receives an individual or "true" impact factor of 50 compared to the journal impact factor of ~3 meaning that the importance of the paper exceeds the importance of the journal by means of 16. Although citation rankings and measurement are always criticized to be somewhat subjective, it is the only way to judge or rank scientific outcome. The same is true for the journal impact factor, i.e. it may not be an optimum tool for author evaluations, however, is there a better alternative? So finally, the inclusion of top cited papers is of at least some relevance and should not be underestimated.
Conclusions
Clinical marginal quality is predictable from in vitro adhesive fatigue investigations with thermomechanical loading, but it is not possible to determine a cut off for clinically successful marginal quality. Flexural fatigue can be appropriately determined in the lab as well, having been successful in defining lower borderlines for additional clinical safety. To compare in vitro and in vivo results according to wear phenomena, valid in vivo results are too seldom. Altogether, it has to be taken into account that the described co-factors are only a few among several important aspects in restorative dentistry, i.e. overall clinical performance is not predictable from fatigue aspects alone.

<table>
<thead>
<tr>
<th>Year</th>
<th>Author</th>
<th>Title</th>
<th>Citations per year (CPA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>Van Meerbeek et al.</td>
<td>Adhesion to enamel and dentin: Current status and future challenges</td>
<td>50.9</td>
</tr>
<tr>
<td>1997</td>
<td>Mehl et al.</td>
<td>Physical properties and gap formation of light-cured composites with and without soft start polymerization</td>
<td>14.5</td>
</tr>
<tr>
<td>1995</td>
<td>Feilzer et al.</td>
<td>Influence of light intensity on polymerization shrinkage and integrity of restoration-cavity interface</td>
<td>12.1</td>
</tr>
<tr>
<td>2005</td>
<td>Frankenberger &amp; Tay</td>
<td>Self-etch vs etch-and-rinse adhesives: effect of thermo-mechanical fatigue loading on marginal quality of bonded resin composite restorations</td>
<td>10.8</td>
</tr>
<tr>
<td>1990</td>
<td>Kemp-Scholte et al.</td>
<td>Complete marginal seal of Class V resin composite restorations effected by increased flexibility</td>
<td>10.3</td>
</tr>
<tr>
<td>1999</td>
<td>Hannig et al.</td>
<td>Self-etching primer vs. phosphoric acid: an alternative concept for composite-to-enamel bonding</td>
<td>10.0</td>
</tr>
<tr>
<td>2000</td>
<td>Peumans et al.</td>
<td>Porcelain veneers: A review of the literature</td>
<td>9.0</td>
</tr>
<tr>
<td>2000</td>
<td>Frankenberger et al.</td>
<td>Technique sensitivity of dentin bonding: Effect of application mistakes on bond strength and marginal adaptation</td>
<td>8.6</td>
</tr>
<tr>
<td>1998</td>
<td>Opdam et al.</td>
<td>Marginal integrity and postoperative hypersensitivity in Class II resin composite restorations in vivo</td>
<td>6.8</td>
</tr>
<tr>
<td>2007</td>
<td>Heintze</td>
<td>Systematic reviews: I. The correlation between laboratory tests on marginal quality and bond strength. II. The correlation between marginal quality and clinical outcome</td>
<td>6.0</td>
</tr>
<tr>
<td>2000</td>
<td>Frankenberger et al.</td>
<td>Leucite-reinforced glass ceramic inlays and onlays after six years: clinical behavior</td>
<td>6.0</td>
</tr>
<tr>
<td>1990</td>
<td>Kemp-Scholte &amp; Davidson</td>
<td>Marginal integrity related to bond strength and strain capacity of composite resin restorative systems</td>
<td>5.8</td>
</tr>
<tr>
<td>2007</td>
<td>Frankenberger et al.</td>
<td>Marginal integrity: Is the clinical performance of bonded restorations predictable in vitro?</td>
<td>5.7</td>
</tr>
</tbody>
</table>

Table 6.1: Top cited papers (CPA >5) regarding marginal adaptation of resin composites.
### Table 6.2: Top cited papers (CPA >5) regarding bulk fatigue behavior of resin composites.

<table>
<thead>
<tr>
<th>Year</th>
<th>Author</th>
<th>Title</th>
<th>Citations per year (CPA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1997</td>
<td>Gladys et al.</td>
<td>Comparative physico-mechanical characterization of new hybrid restorative materials with conventional glass-ionomer and resin composite restorative materials</td>
<td>11.4</td>
</tr>
<tr>
<td>2003</td>
<td>Drummond et al.</td>
<td>Static and cyclic loading of fiber-reinforced dental resin</td>
<td>8.9</td>
</tr>
<tr>
<td>2005</td>
<td>Lohbauer et al.</td>
<td>The effect of different light-curing units on fatigue behavior and degree of conversion of a resin composite</td>
<td>5.6</td>
</tr>
</tbody>
</table>

### Table 6.3: Top cited papers (CPA >5) regarding surface fatigue / wear of resin composites.

<table>
<thead>
<tr>
<th>Year</th>
<th>Author</th>
<th>Title</th>
<th>Citations per year (CPA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1998</td>
<td>Bayne et al.</td>
<td>A characterization of first-generation flowable composites</td>
<td>11.3</td>
</tr>
<tr>
<td>2005</td>
<td>Sarrett et al.</td>
<td>Clinical challenges and the relevance of materials testing for posterior composite restorations</td>
<td>9.6</td>
</tr>
<tr>
<td>1996</td>
<td>Mair et al.</td>
<td>Wear: Mechanisms, manifestations and measurement. Report of a workshop</td>
<td>7.4</td>
</tr>
<tr>
<td>2005</td>
<td>Turssi et al.</td>
<td>Filler features and their effects on wear and degree of conversion of particulate dental resin composites</td>
<td>7.4</td>
</tr>
</tbody>
</table>
Figure 6.1: Biodegradation of a resin composite restoration in a lower first molar after 6 years of clinical service. R: Resin composite. E: Enamel. Clinical wear is clearly visible around the occlusal margins. Gap formation does not play a major role in this case.\textsuperscript{4}
References


Summary
Tooth-colored materials such as resin composites should be safely used for patients in terms of clinical behavior. Esthetics are more or less a side aspect in the posterior region. However, it is probably the most important issue for patients all over the world to receive invisible restorations. This summary section picks up relevant issues having been addressed in the introduction section (Chapter 1) to build a thematic bridge in this thesis and to provide some framework for future thoughts.

Resin composite materials are well-suited materials to bond to tooth hard tissues and furthermore to support enamel and dentin in terms of cuspal adhesive stabilization. It is well-proven that adhesion in dentistry can be safely applied in many ways, such as pit and fissure sealings, direct and indirect resin composites, and bonded ceramic restorations. Bonding to phosphoric acid etched enamel is accepted to be clinically successful, however, marginal staining or gap formation have been also reported as major incidents already after medium-term observations. Also dentin can be bonded and sealed successfully, being rather quickly represented by the absence of postoperative hypersensitivities. When no hypersensitivities occur, the dentin seal should be estimated to be sufficient, at least to avoid fluid movement inside the dentin tubules. Long-term bonding degradation over time is interesting to observe, therefore it was the topic of the second part of the thesis to do so (Chapter 2). Also, the central idea was to accelerate degradation in vitro, in this case degeneration of resin-dentin bonds by artificial saliva compared to mineral oil (control). Resin-dentin beams bonded with three different etch-and-rinse adhesives (two-step: Prime&Bond NT, Excite/three-step: All-Bond 2) were prepared after 3 years of storage in the described media and subjected to transmission electron microscopy (TEM). An "extreme control" was introduced by autoclaving at 121°C and 103kPa. The TEM images clearly showed that the extreme control scenario did not result in denatured collagen when having been protected by adhesive resin. The control group with mineral oil exhibited almost intact hybrid layers over 3 years with minimal silver deposits, whereas in the test group with artificial saliva, extensive nanoleakage was observed. All ultrastructural parameters, i.e. extent of nanoleakage, interfacial staining characteristics, and the structure of the collagen fibrils in hybrid layers and underlying dentin were completely different and revealed considerable aging processes when storage was accelerated using artificial saliva.
Dentin bonding is more responsible for proper dentin seal and consequent reduction of postoperative hypersensitivities. In contrast, enamel bonding is important for restoration retention and marginal integrity, i.e. unstained margins, especially in the visible area. Therefore, in the next stage marginal quality in enamel and dentin was evaluated simultaneously in vitro and in vivo. This was achieved by starting both investigations at the same time to get comparable results between preclinical and clinical investigations (Chapter 3). In this section, 32 in vitro specimens were compared to 22 in vivo specimens regarding marginal adaptation in enamel and dentin. Clinically, replicas of baseline investigations and 6-year recalls were compared, and in vitro we investigated replicas initially and after 2190 days of water storage with and without thermomechanical loading for accelerated aging processes under a SEM. Due to inferior proximal access to dentin margins, marginal adaptation to dentin was only assessed in vitro. Here, percentages of gap-free margins dropped from 98-100% at the beginning to 55-66% after thermomechanical loading alone and 67-75% after water storage alone and to 42-52% after both accelerated aging mechanisms together. Enamel margins remained 100% gap-free in vitro and 86-90% in vivo at the beginning, dropping to 85-87% after water storage and thermomechanical loading in vitro and 74-80% in vivo. So besides some more artifacts and overhangs due to the more challenging clinical situation, results in marginal quality were almost identical between in vitro and in vivo specimens.

After having worked out the value of accelerated aging on resin-dentin and resin-enamel bonding behavior, the following step was to focus on material properties of resin composites for posterior use. Especially flexural strength and flexural fatigue behavior have been of special interest for us due to the fact that it received increased attention facing increasing marginal and bulk fracture rates in clinical trials dealing with direct resin composites. A thorough evaluation of flexural fatigue characteristics was therefore the aim of the next chapter of the present thesis (Chapter 4). Following the same strategy as in Chapter 3, here we also simultaneously started both in vitro and in vivo evaluations. For the in vitro part, elastic modulus, flexural strength, and flexural fatigue limit according to the staircase method were assessed. In vivo, clinical fracture behavior in terms of marginal breakdown and bulk fractures/chippings were observed and correlated to the outcome detected in vitro. In vitro results showed similar values initially, however, flexural fatigue limits were higher for Grandio compared to Tetric Ceram. In contrast, no such differences occured in vivo up to the
6-year recall of the same materials having been under investigation. Only at a closer view, facing x200 magnification of marginal breakdown sites, revealed that Tetric Ceram showed more areas of marginal defects (7.9%) compared to Grandio (4.8%).

The ultimate instrument for definitive estimation and judgement of dental biomaterials is still the randomized clinical long-term trial. A prospective clinical long-term trial was carried out over a 6-year observation time, using the same restorative materials (Solobond M/Grandio, Syntac/Tetric Ceram) previously described (Chapter 5). Thirty patients received at least two different restorations in a random decision according to recommendations of the CONSORT statement. Thirty-six Grandio restorations were bonded with Solobond M, 32 Tetric Ceram restorations were bonded with Syntac (only Class II, 52 MO/OD, 16 MOD or more surfaces). Twenty-four cavities (35%) revealed no enamel at cervical margin, 33 cavities (49%) exhibited less than 0.5 mm cervical enamel width. At the baseline initial recall, after 6 months, 1, 2, 4, and 6 years, all restorations were assessed according to modified United States Public Health Service (USPHS) criteria by two independent investigators using loups with x3.5 magnification, mirrors, probes, bitewing radiographs, impressions, and intraoral photographs. The overall success rate was 100% after 6 years of clinical service, while drop out of patients was 0%. Neither restorative materials nor localization of the restorations had a significant influence on any criterion after 6 years. However, molar restorations performed worse than premolar restorations regarding marginal integrity (4 years), restoration integrity (6, 12, 24, 48 months), and tooth integrity (4 and 6 years). Irrespective of the resin composite used, significant changes over time were found for all criteria applied in clinical examinations. Marginal integrity started with a major portion of overhangs in all marginal areas having been detected until the 1-year recall and distinctly dropping afterwards (overhangs at baseline 44%; 6 months: 65%; 1 year: 47%; 2 years: 6%; 4 years: 4%; and 6 years: 3%). Beyond the 1-year recall, more and more negative step formations due to wear were found. Tooth integrity significantly deteriorated due to increasing enamel cracks over time. Enamel chippings or cracks were significantly more frequently observed in molars than in premolars. Main reasons for decreasing “restoration integrity” were visible signs of surface roughness and distinct wear traces. Visible wear of both materials under investigation was earlier detectable in molars (74% bravo after 4 years) than in premolars (40% bravo after 4 years).
The final question remains and is addressed with the last issue of the present thesis, i.e. what is this all about or in different words: Is clinical performance of bonded restoratives predictable in the lab (Chapter 6)? It could be repeatedly shown that the easiest way of predicting clinical behavior is assessment of marginal integrity. Here the best correlation between in vitro and in vivo results can be found. The explanation is clear: restoring real teeth with real restorations and loading them with real forces being similar to subcritical loads in vivo finally results in realistic estimations of later observable clinical behavior. It was clearly shown that by use of etch-and-rinse adhesives a tight enamel seal is provided both in vitro and in vivo being well-suited to counteract polymerization forces and to withstand occlusal stresses in the oral cavity. However, this estimation is not correlated to the risk for secondary caries, because gap formation not always, nor quickly results in the formation of secondary caries. Here a complex biofilm acts on different tooth hard tissues, which is extremely hard to simulate under in vitro conditions using an artificial mouth. So the final correlation gap-caries is clearly not yet achieved with present investigations having been carried out for the present thesis. Regarding fatigue behavior related to flexural strength, the literature reveals that promising initial flexural strengths do not logically mean high FFLs and an FFL of >30 MPa may be the critical threshold value for bulk fatigue in order to withstand masticatory forces and clinical fatigue over its lifetime. This furthermore supports the aspect that thorough in vitro screening of dental biomaterials is only sufficient when long-term fatigue phenomena are considered in order to get accelerated aging. Regarding clinical wear, few 3D laser scan studies are available in the literature, reporting wear rates after 3 years of clinical service of ~80 µm. Altogether, there is a certain value for accelerated in vitro testing of dental biomaterials which is very highly esteemed because financial resources get smaller, and ethics committees’ approval are harder to obtain.

In conclusion, the present thesis clearly shows that in vitro research will be even more important during the next decade of research in restorative dentistry.
Samenvatting
Voor een veilige toepassing van tandkleurige vulmaterialen, zoals kunststof composieten (hierna zonder meer aangeduid als composiet), is het klinisch gedrag van groot belang. Esthetiek is in het posterieure gebied minder van belang. Desondanks hechten onze patiënten wereldwijd veel belang aan ‘onzichtbare’ restauraties. Deze samenvatting richt zich op de relevante zaken die aan de orde komen in de inleiding (hoofdstuk 1) van dit proefschrift om een thematische brug op te bouwen en om een zeker kader te bieden voor toekomstige gedachtenvorming.

Composiet restauratie-materialen zijn zeer geschikt om te hechten aan de harde tandweefsels (glazuur en dentine) wat van belang is om het tandweefsel te ondersteunen waardoor het herstel van het gebitselement wordt ondersteund. Het is aangetoond dat hechting in de tandheelkunde in vele klinische situaties veilig kan worden toegepast. Bijvoorbeeld in fissuur-afdichtingen, directe en indirecte composietrestauraties, en in adhesief bevestigde keramische restauraties. Hechting aan met fosforzuur geëtst glazuur is klinisch een betrouwbare gebleken methode. Desondanks zijn randverkleuring en rand-spleetvorming gerapporteerd als optredende verschijnselen bij middellange-terminonderzoek. Ook kan aan tandbeen (dentine) worden gehecht waarbij dit levende weefsel met succes wordt afgedicht en afgeschermd van omgevings-invloeden wat de afwezigheid van postoperatieve overgevoeligheden verklaart. Als er geen hypergevoeligheid optreedt, wordt de dentine afdichting als voldoende ingeschat om de vloeistofbeweging in de dentinetubuli te voorkomen. Degradatie van de hechting aan dentine met de tijd, op de lange termijn, is een interessant fenomeen dat het onderwerp van onderzoek is van het tweede deel van het proefschrift (Hoofdstuk 2). Het centrale uitgangspunt van dit onderzoek was om de degradatie te versnellen in vitro door de kunststof-dentine-verbinding aan kunstspeeksel of minerale olie (controle) bloot te stellen. Kunststof-dentine-staafjes, aan elkaar gehecht door middel van drie verschillende ets-en-spoel adhesiefsystemen (twee-staps systemen: Prime & Bond NT, Excite; drie-staps systeem: All-Bond 2) werden toegepast. Na 3 jaar opslag in de beschreven media werd het hechtvlak met transmissie elektronenmicroscopie (TEM) beoordeeld. Een "extreme controlegroep" werd in de autoclaaf aan 121°C en 103kPa druk blootgesteld. De TEM-beelden laten duidelijk zien dat dit extreme controle scenario niet leidt tot het ontstaan van gedenatureerd collageen wanneer het dentine is afgedekt met een kunststof van een dentine hechtsysteem. De hybride laag van de controlegroep die werd blootgesteld aan minerale olie bleef vrijwel intact over de onderzoeksperiode.
van 3 jaar met een minimum aan zilverneerslag, terwijl in de testgroep blootgesteld aan kunstmatig speeksel, uitgebreide nanolekkage werd waargenomen. Alle ultrastructurele parameters, dat wil zeggen: de omvang van nanolekkage, interface verkleuringen en de structuur van de collageenfibrillen in de hybride lagen en in het onderliggende dentine waren totaal verschillend en illustreren intense verouderingsprocessen die in dit onderzoek werden versneld met behulp van kunstmatig speeksel.

Hechting aan dentine is verantwoordelijk voor een goede afdichting van het tandbeen en de daaruit voortvloeiende vermindering van postoperatieve overgevoeligheden. Daarentegen is hechting aan glazuur van belang om de restauratie houvast te geven en voor een goede ‘onzichtbare’ overgang tussen tandweefsel en restauratie, de randaansluiting. Daarom werd in de volgende fase van het onderzoek van dit proefschrift de kwaliteit van de randaansluiting van een vulling met glazuur en met dentine gelijktijdig in vitro en in vivo geëvalueerd. Dit werd gerealiseerd door het gelijktijdig starten van beide onderzoeken om zo vergelijkbare resultaten tussen de preklinische en klinische onderzoeken (hoofdstuk 3) te verkrijgen. In dit deelonderzoek is de randaansluiting van composiet restauraties aan glazuur en aan dentine met elkaar vergeleken, in vitro met 32 monsters en in vivo met 22 monsters. Klinisch werden replica's van de baseline-resultaten met die van 6-jaar recalls vergeleken, en in vitro werden baseline replica’s vergeleken met replica’s na 2.190 dagen blootstelling aan water met of zonder thermomechanische belasting. De (versnelde) verouderingsprocessen werden met Scanning Electron Microscopy (SEM) geëvalueerd. Vanwege de met direct zicht slecht toegankelijke approximale dentine-randaansluitingen werd de randaansluiting aan dentine alleen in vitro onderzocht. In vitro daalde het percentage van de lekvrije randaansluitingen van 98-100% bij aanvang tot 55-66% na uitsluitend thermomechanische belasting en tot 67-75% na het uitsluitend blootstellen aan water en tot 42-52% na blootstelling aan beide versnelde verouderingsmechanismen. De glazuurrandaansluiting was voor de in vitro monsters aan het begin 100% lekvrij terwijl in vitro dit variëerde van 86 - 90%. Blootstelling aan water en thermomechanische belasting leidde tot een daling naar 85 - 87% in vitro en naar 74 - 80% in vivo. Ondanks het feit dat de klinische situatie gepaard gaat met wat meer artefacten en overhangende restauraties, waren de gevonden in vitro en in vivo onderzoeksresultaten nagenoeg identiek.
Na meer zicht te hebben gekregen op de waarde van versnelde veroudering van de kunststof hechting aan dentine en aan glazuur was de volgende stap in dit onderzoek om zich te concentreren op de materiaaleigenschappen van composieten voor toepassing in de posterieure delen van het gebit. Vooral inzicht in buigsterkte en (buig-)vermoeingsgedrag zijn van bijzonder belang voor het verkennen van de randlekkage en bulkfracturen die in klinische studies met directe composieten vaak worden gevonden. Een grondige evaluatie van de buig-vermoeidheid karakteristieken was dan ook het doel van het volgende hoofdstuk van dit proefschrift (hoofdstuk 4). Volgens dezelfde strategie als in hoofdstuk 3, werd hier ook tegelijkertijd aangevangen met zowel in vitro als in vivo onderzoek. In het in vitro deel werd de elasticiteitsmodulus, buigsterkte en buigingsvermoeingsgrens volgens de “staircase” methode bepaald. In vivo, werd het klinisch gedrag in termen van randbreuk, bulkfracturen en ‘chipping’ beoordeeld en gecorreleerd met de uitkomsten van het in vitro onderzoek. In vitro resultaten tonen bij aanvang dezelfde waarden voor de verschillende vulmaterialen, echter de buigings/vermoeingsgrenzen waren hoger voor Grandio in vergelijking met Tetric Ceram. Daarentegen werden in vivo, waarbij dezelfde materialen zijn onderzocht, geen verschillen gevonden, zelfs niet na 6 jaar. Alleen bij een beter zicht, met x200 vergroting van de plaatsen waar randbreuk werd gevonden, bleek dat Tetric Ceram meer randafwijkingen (7,9%) liet zien ten opzichte van Grandio (4,8%).

Het ultieme instrument voor de definitieve bepaling van de klinische geschiktheid van tandheelkundige biomaterialen is nog steeds gerandomiseerd klinisch onderzoek. Met gebruikmaking van dezelfde vulmaterialen (Solobond M / Grandio, Syntac / Tetric Ceram), eerder beschreven in de vorige hoofdstukken, werd een prospectieve klinische langetermijnstudie uitgevoerd met een observatiertijd van 6-jaar (hoofdstuk 5). Dertig patiënten ontvingen ten minste twee verschillende restauraties waarbij de materiaalkeuze op basis van aanbevelingen van de CONSORT verklaring willekeurig werd bepaald. Zesendertig Grandio restauraties werden met Solobond M als dentine hechtsysteem geplaatst, 32 Tetric Ceram restauraties werden met Syntac als dentine hechtsysteem geplaatst (alleen Klasse II, 52 MO / OD, 16 MOD of meer oppervlakken). In vierentwintig caviteiten (35%) was geen glazuur op de cervicale rand aanwezig, 33 caviteiten (49%) vertoonden minder dan 0,5 mm cervicale glazuurbreedte. De restauraties werden bij aanvang, na 6 maanden, 1, 2, 4 en 6 jaar beoordeeld, op basis van de gemaaliseerde United States Public Health Service
Samenvatting

(USPHS) criteria door twee onafhankelijke onderzoekers met behulp van loepen met x3.5 vergroting, spiegels, sondes, bitewing röntgenfoto's, afdrukken, en intra-orale foto's. Het totale succespercentage van klinisch functioneren was 100% na 6 jaar, alle patiënten zijn gedurende deze periode in onderzoeksgroep gebleven. Noch vulmateriaal, noch lokalisatie van de restauraties hebben een significante invloed gehad op het bepalen van de kwaliteit van functioneren na 6 jaar. Echter, molaarrestauraties presteerden slechter dan premolaarrestauraties met betrekking tot de integriteit van de randaansluiting (4 jaar), restauratie integriteit (6, 12, 24, 48 maanden), en tand integriteit (4 en 6 jaar). Ongeacht het gebruikte composiet, werden significante veranderingen in de tijd gevonden voor alle criteria die in het klinisch onderzoek werden geëvalueerd. De beoordeling van de randaansluiting bij aanvang leidde tot de constatering van relatief veel overhangende randen (1 jaar) en namen daarna af (overhang baseline 44%, 6 maanden 65%; 1 jaar: 47%; 2 jaar: 6%; 4 jaar: 4% en 6 jaar: 3%). Naast de 1-jaar recall, werden meer en meer randhoogteverschillen als gevolg van slijtage gevonden. Tandintegriteit verslechterde als gevolg van het toenemen van glazuurbarsten in de tijd. Glazuur-'chipping’ of -barsten werden significant vaker waargenomen in molaren dan in de premolaren. De belangrijkste redenen voor het verminderen van "restauratie integriteit" waren zichtbare tekenen van ruwheid van het oppervlak en duidelijke slijtagesporen. Voor beide materialen was de zichtbare slijtage eerder detecteerbaar in molaren (74% bravo na 4 jaar) dan bij premolaren (40% bravo na 4 jaar).

In hoofdstuk 6 wordt de vraag waar het in dit proefschrift om draait behandeld: zijn klinische prestaties van adhesief geplaatste restauraties te voorspellen met laboratoriumonderzoek? Meerdere malen is aangetoond dat de makkelijkste manier van het voorspellen van het klinische gedrag de evaluatie van de marginale integriteit is. Op dit gebied kan de optimale correlatie tussen in vitro en in vivo resultaten worden gevonden. De verklaring hiervoor is duidelijk: testresultaten met natuurlijke tanden en kiezen die zijn voorzien van echte restauraties die worden belast met de krachten die vergelijkbaar zijn met de subkritische belastingen in vivo resulteren uiteindelijk in een realistische inschatting van het latere klinisch waarneembare gedrag. Het is duidelijk aangetoond dat door toepassing van ets-en spoel adhesief systemen een stevige afdichting van het glazuur, zowel in vitro als in vivo, wordt verkregen en dat deze hechting zeer geschikt is om de polymerisatiekrachten op te vangen en om de functionele occlusale spanningen in de mondholte te weerstaan.
Echter deze schatting is niet gecorreleerd met het risico op secundaire cariës. Randspleetvorming leidt niet altijd tot de vorming van secundaire cariës. Een complexe biofilm ontstaat in de randspleet welke verschillend werkt op de verschillende harde tandweefsels. De biofilm is uiterst moeilijk te simuleren onder in vitro omstandigheden. Dus inzicht in de correlatie tussen randspleetvorming en hetontstaan van cariës is duidelijk nog niet verkregen met de onderzoeken uit dit proefschrift. Met betrekking tot vermoeingskarakteristieken in relatie tot buigsterkte, blijkt in de wetenschappelijke literatuur dat een veelbelovende buigsterkte waarde bij aanvang niet per definitie betekent dat dit resulteert in een hoge vermoeingslimiet (FFL). Een FFL-waarde van > 30 MPa lijkt een kritische drempelwaarde voor bulkvermoeidheid te zijn om de kauwkrachten langdurig te weerstaan. Dit ondersteunt het feit dat grondige in-vitro screening van tandheelkundige biomaterialen slechts toereikend is wanneer vermoeidheidskarakteristieken worden meegewogen. Met betrekking tot klinische slijtage zijn er enkele studies beschikbaar in de literatuur waarbij gebruik wordt gemaakt van 3D laser scantechnologie, waarbij de gerapporteerde slijtage na 3 jaar klinische dienst is in de orde van grootte van ~ 80 μm. De waarde voor versnelde in-vitro testen van tandheelkundige biomaterialen is van groot belang, de financiële middelen voor klinisch onderzoek nemen af en de goedkeuring van ethische commissies is steeds moeilijker te verkrijgen.

Tot slot, dit proefschrift laat duidelijk zien dat het belang van in-vitro-onderzoek in de restauratieve tandheelkunde tijdens het komende decennium zal toenemen.
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