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Minimizing tooth-restoration interface degradation.

The use of micro-CT in quantitative 3D analysis of interfaces and the mechanical behavior of direct and indirect restorations

By

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Declaration of interests

I hereby declare that, the contents and organization of this dissertation constitute my own original work and does not compromise in any way the rights of third parties, including those relating to the security of personal data. Financial disclosures and conflicts of interest, if present, are reported at the end of each published research paper.

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I would like to dedicate this thesis to the woman that sustained me with her love and caring for all these years. I love you, Alice.

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Abstract

Restorative dentistry has been rapidly evolving in the past 20 years, with the aim of matching aesthetic requests of patients and preservation of dental tissues. However, even if adhesive-composite combinations are nowadays used for most direct and indirect restorative procedures, their durability at the interface level is still questioned by multiple systematic reviews. The tooth-restoration interface is namely composed by three main entities: the biological substrate, the adhesive/cement layer and the restorative material (direct or indirect). Moreover, it is constantly subjected to biological, chemical, physical and mechanical stresses, that lead to its degradation over time. As a matter of facts, the biological substrate topic is strictly related to clinical procedures and patient-specific factors that cannot be changed through bioengineering. Adhesive systems, both for direct and indirect purposes, could be improved, but they have already been widely studied and literature is clear about their pros, cons, recommendations and protocols. On the other hand, recently introduced restorative materials, such as milled ceramics and newer resin bond composites still severely lack literature data about their interfacial performance, particularly when simulating their degradation in clinical-like scenarios. Thus, the present PhD thesis aimed to expand knowledge about restorative materials interfacial behavior, in particular when subjected to chewing simulation, through micro-CT interfacial analysis

Contents

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1. Introduction

1.1 Tooth-restoration interface (TRI)

Restorative dentistry has been rapidly evolving in the past 20 years, with the aim of matching aesthetic requests of patients and preservation of dental tissues [1],[2].

This approach has been made possible thanks to the introduction in dentistry of adhesive systems and resin-based composite (RBC) materials, that do not need a retentive cavity design compared to older metal-based materials, such as amalgam [3]. These adhesives and composite materials have been around even before 1955, when Buonocore, alongside other pioneers, started to study and publish papers regarding adhesion of RBC in dentistry [4]. Today, thanks to technological advancement, fast and predictable procedures are available. However, even if adhesive-composite combinations are used for most direct and indirect restorative procedures, their durability at the interface level is still questioned [5]. A Cochrane systematic review in 2021 reported that resin composite restorations may have almost double the failure rate of amalgam restorations and a much higher risk of developing secondary caries [6]. Secondary caries has been defined as "lesions at the margins of existing restorations" and is still controversial whether these lesions are a result of the presence of the dental restoration or simply a new primary lesion that forms in the same region [7]. In any case, the presence or recurrence of these lesions is typically associated with the external margins of the restoration, and it has been stated that 80% to 90% of secondary caries will be found at the gingival margin (for class II to V restorations), irrespective of the type of restorative material [8]. This recurrence is also clinically confirmed by narrative and systematic reviews, such as the one performed by Jokstad in 2016, reporting that secondary caries as the most common reason for re-restoration of teeth, regardless of restorative material [9],[7]. Figure 1A-C represents some typical clinical cases, in which restoration underwent failure related to their TRI: marginal gap and discoloration, marginal fracture, secondary caries, aesthetic issues.

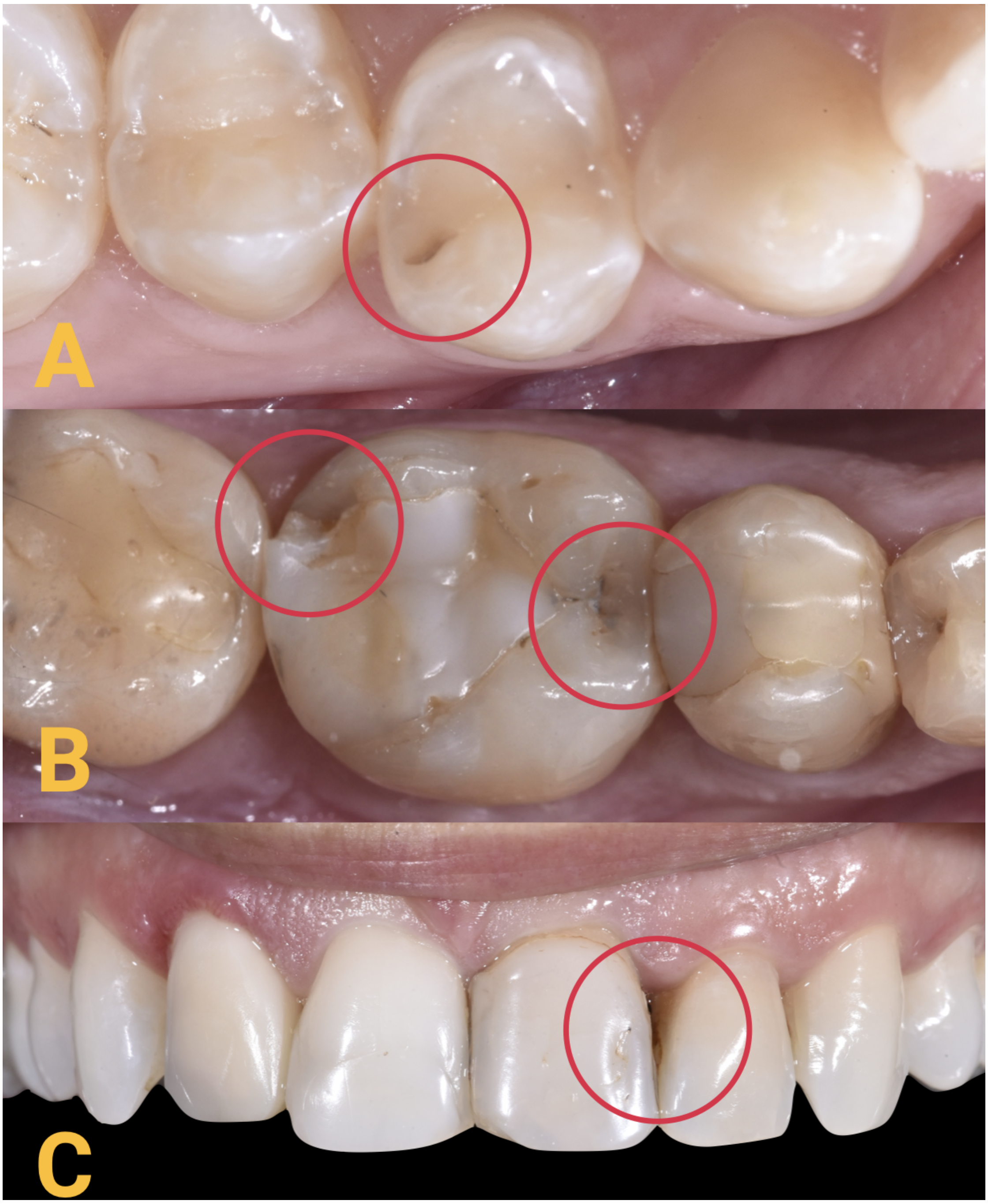


Figure 1: Common TRI failures that can be clinically observed. 1A marginal discoloration associated with interfacial gap. 1B marginal fracture and secondary caries along the margins of a resin composite restorations on 4.6. 1C aesthetic failure due to margin discoloration and pigmentation.

Given these premises, it is clear that the integrity of the TRI, where secondary caries occur, plays a fundamental role in the restoration quality and longevity. The TRI is namely composed by three main entities: the biological substrate, the adhesive/cement layer and the restorative material (direct or indirect). Moreover, it is key to remember that this interface is subject to mechanical loads, thermal variations and biochemical aggression by acids and bacteria. All these aspects have an influence on the progressive onset of an interfacial gap, that always progressively lead to TRI failure. Figure 2 reports a summary of the topic regarding the adhesive interface and its degradation. All these topics will be assessed and widely discussed in the following sections of the introduction, focusing on the aspects that can be optimized through bioengineering.

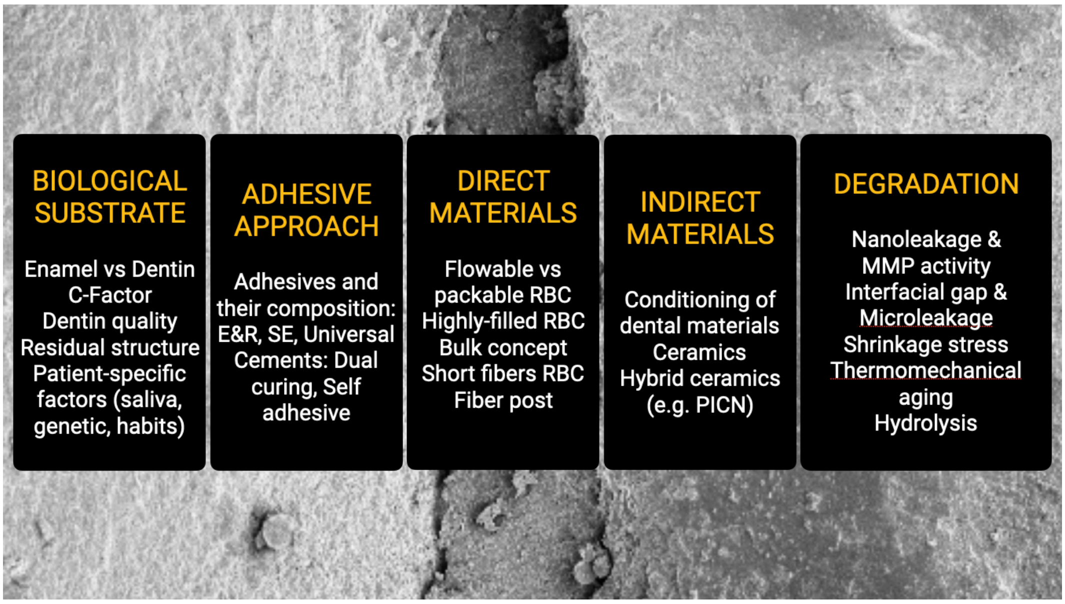


Figure 2. Factors that have an influence on adhesive interface and its performance over time.

1.2 Biological Substrate

The biological substrate on which restorative materials are placed is strictly related to the patient and the damage that the tooth suffered. The initial “conditio sine qua non” for all restorative adhesive procedure consists in proper diagnosis, cleaning and finishing of the cavity. This is made to obtain a proper substrate for adhesion and optimal marginal seal. Clinical parameters and procedures will not be discussed, since they would result off-topic from the aim of the thesis. However, a quick lecture about the most important factors related to the biological substrate will be now presented in the following paragraphs.

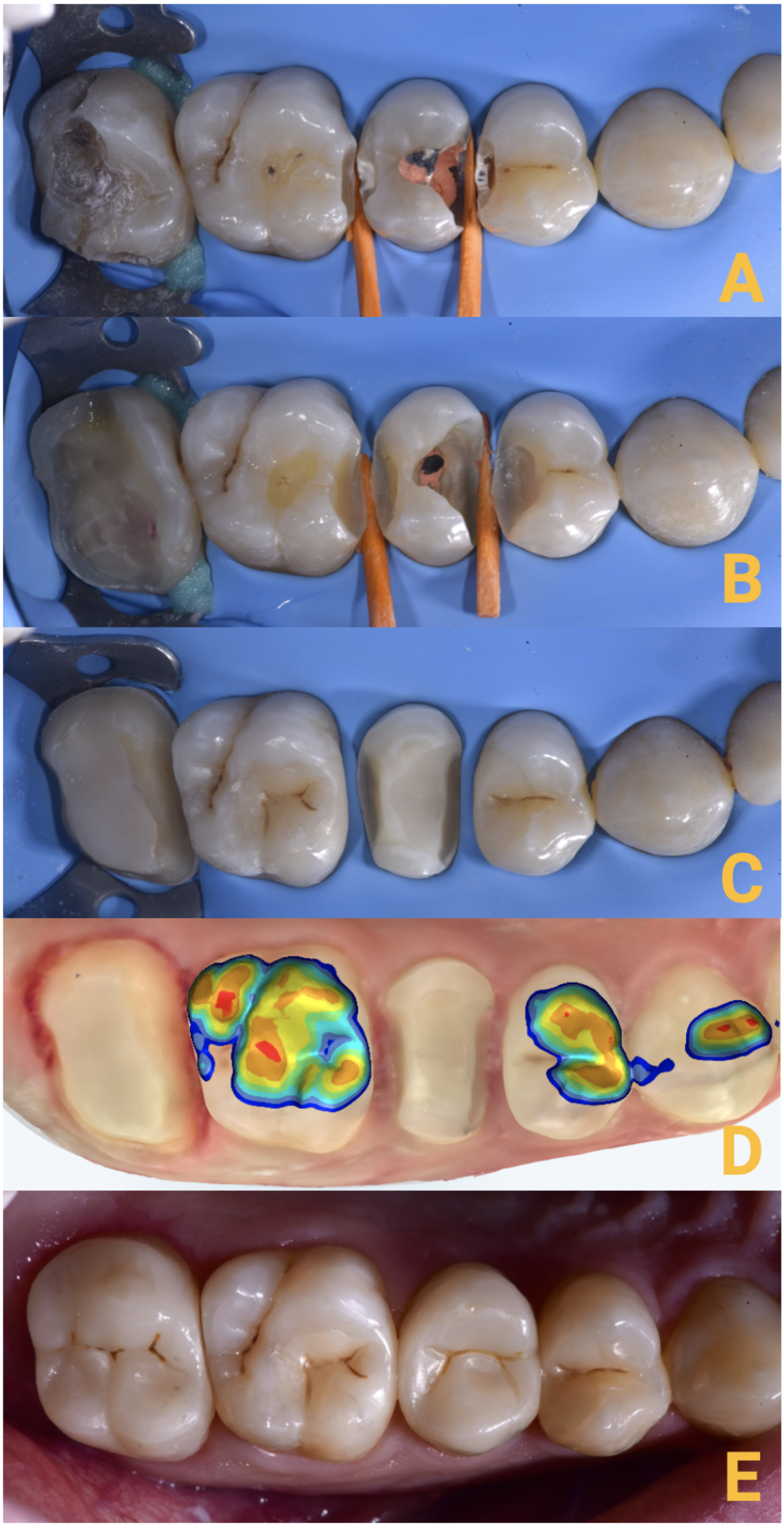
Two different biological substrates could be available at the same time during an adhesive procedure: enamel and dentin. The prognosis for adhesive restorations with margins made entirely of enamel is excellent, thanks to optimal and stable adhesion [10],[11]. This is related to the histological aspects of enamel, which is easy to dehydrate, demineralize and infiltrate, when properly pre-treated with etching [12]. On the other hand, it is difficult to achieve optimal adhesion on dentin due to its permeability related to dentinal tubule and the presence of a higher water and organic quotes [13]. Studies have also shown that what have been called wall lesions may form independent of surface lesions, though not likely due to microleakage through very small gap spaces in the clinical situation [14]. Therefore, preserving enamel whenever possible is considered a key factor in adhesive dentistry, even if this is not always clinically possible. Peculiar clinical situations that require adhesion, such as the presence of sclerotic dentin or intact enamel, must be managed accordingly to the substrate characteristics with minor adjustments on adhesive protocols [15],[16]. Bleaching procedures, on the other hand, due to the oxygen release, have been demonstrated to impair adhesion for two weeks [17]. In fact, oxygen inhibits the formation of covalent dual bonds, reducing the degree of conversion and ultimately bond strength [17].

Another important aspect to consider after cavity finishing is the so-called "Cavity factor", also known as C-factor. The concept was introduced by Davidson and Feilzer et al. and is defined as the ratio of the bonded surface area to the unbonded surface area [18],[19]. The higher the C-factor, the higher the amount of restrained areas that do not allow a free volumetric contraction of the composite, the higher the shrinkage stress at the adhesive interface [19]. Precisely surfaces extension, rather than the number of surfaces, is key for distributing shrinkage forces: deep class I cavities would have a higher C-factor than superficial cavities [20]. This is an important clinical aspect to consider, but again cavity design is correlated to the tooth damage and cannot be modified without furtherly remove sound tooth structure, which is ethically unacceptable.

Another aspect of great importance in the biomechanics of the substrate is the vitality of the tooth. In fact, endodontically treated teeth (ETT) are well-known to be much more subjected to mechanical failure by fracture compared to vital ones [1],[21]. This is related to the pathology itself and the consequent tooth substance loss, but also to clinical procedures performed during the root canal therapy, even if effects of irrigants and medicaments seem to play a secondary role on fracture resistance [22]. ETT can be therefore considered a challenge for restorative materials and, consequently, several strategies have been proponed to avoid catastrophic failures. Many authors since the past century demonstrated that these teeth need an indirect approach in order to reinforce the residual tooth structure. In 2002, Aquilino and Caplan showed that cuspal coverage could increase up to six times the survival rate of ETT posterior teeth [23], making the full crown to be considered the gold standard therapeutic approach for years [24]. In the past, the only available technique for these indirect restorations consisted in combining a metal post with a full coverage metal-ceramic or metal-resin crown [25]. However full crown preparations tend to remove a large amount of healthy dental structure both in anterior and posterior teeth [26],[27]. With the possibility of an adhesive approach, rather than a retentive approach, a large number of recent studies focused on partial preparation designs, which ensure higher sound tissue preservation [28]. Surprisingly, it has been recently demonstrated that onlays, overlays and endowcrowns can equally be effective compared to traditional crowns, in terms of mechanical, functional and aesthetic properties [29], [30]. As a consequence, a big number of materials have been proposed for these restorations, that will be discussed in the dedicated section of this introduction.

Last but not the least, the “patient” factor has a huge importance on TRI durability. This topic includes genetic factors [31], individual biofilm and saliva properties [32], oral and feeding habits [33]. All these factors are hardly controllable by clinicians, and they are rather of competence of epidemiologists and governmental politics, that should invest on prevention and to improve patient’s knowledge of the problem starting from early childhood [34].

Figure 3 represent a clinical case of a single quadrant, in which different substrates, C-factors and pulp conditions are present at the same moment. It is therefore not surprising that different techniques and materials must be used, in a tooth-by-tooth biomechanically oriented approach.



A
B
C
D
E

Figure 3. Quadrant I rehabilitation in a young patient. 3A-B: initial situation and cavity cleaning, Tooth 1.7 is vital, with pulp exposure and severe structural loss due to secondary caries. Tooth 1.6 and 1.4 are both vital, with class II cavities. Tooth 1.5 has been recently endodontically treated and in the mesial aspect no enamel is available, while a good amount of structure is available both buccally and on the palatal side. 3C: direct restorations are performed on 1.6 and 1.4, while partial adhesive preparations are performed on 1.7 and 1.5 after buildup and deep margin elevation on 1.5 mesial side. 3D: impression through intraoral scanning and assessment on restorative thickness. 3E: 1-year follow up of the finished case. This case has been awarded as the best under-31 restorative case by the AIC academy, and is available, with full steps and decision making, on its website.

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1.3 Adhesion in direct restorations

Creating a proper adhesive layer is a crucial aspect to achieve a stable TRI in direct restorations. In order to do that, different strategies can be used nowadays almost similar bond strength results.

The most important evolutionary steps of these materials have been well summarized by Sofan et al., in their 2017 review [35]. An effective usage of adhesives was firstly proposed in the mid-late 60's, even if first studies on dental adhesion lead back to the late '40s. The first generation of dental adhesive however, had an extremely low adhesion to dentin (1-2 Mpa), for the biological concepts previously described. Adhesives evolved with the concepts of "total etch" and "smear layer" in the 1970s, leading to the second and third generations, up to the acceptance of the "hybrid layer" concept in 1990s. Fourth and fifth generation took place in this period, while in the late 90s, self-etch adhesives were developed, leading to the sixth, seventh and eight generation. More recently, with the expiration of 10-Methacryloyloxydecyl dihydrogen phosphate (10-MDP) patent in 2010, new monomers have been introduced, leading to the development of different universal adhesives that are evolving up to now.

Generally speaking, three adhesive systems can be used today when a proper surface conditioning is performed on the substrate: 3 step etch-and-rinse (3SER) from 4th generation, 2 step self-etch (2SSE) from 6th generation and universal systems from 8th generation. On the other hand, simplified 2 step ER and 1 step SE systems that tried to combine primer and bonding in one solution, present a reduced ability to infiltrate the dentin substrate, producing a suboptimal hybridization, inferior bond strength and are being therefore abandoned [36].

3SER systems have been considered the state of art for dental adhesion for many years. These adhesives involve three steps: acid-etching with 32-37% phosphoric acid (pH 0.1-0.4) of both enamel (20-60 s) and dentin (15-20 s), priming with an amphiphilic agent (e.g hydroxyethyl methacrylate, HEMA) and adhesive in separate steps [37]. The etching treatment on enamel allow for a higher adhesion due to the increased roughness and the penetration into acid-etched prisms of the bonding agent [38]. The same treatment, for a lower amount of time, must be performed on dentin as well to create the so-called hybrid layer firstly described by Nakabayashi et al. in 1982 [39]. In fact, the bonding process on dentin is not only related to resin tags inside the dentinal tubules, but it is mostly mediated by the infiltration of the resin agent into a collagen fibrils network created by the acid [37].

This network must be maintained sufficiently hydrated to permit the infiltration and avoid fiber collapse, according to the wet-bonding concept proposed by Kanca et al. [40]. Even if 3SER systems are the oldest of the marketed adhesives, their separation of key ingredients offers more therapeutic and research flexibility than combined adhesives. However, multiple steps and specific timing of each one might lead to including mistakes in the procedure, making them harder to use compared to other simplified systems.

The second category of modern adhesives is constituted by 6th generation 2SSE systems. Differently from 3SER adhesives, 2SSE do not require a separate etching step of dentin, as they contain acidic resin monomers with this function, even if they still need etching of enamel for optimal sealing [41]. For these reasons they have been claimed to be more user-friendly, less technique sensitive and less aggressive on dentin. Indeed, clinical studies found less postoperative sensitivity due to the impossibility of dentinal over-etching [42]. Generally speaking, the mechanism of dentin adhesion of 2SSE systems rely on the smear layer [43], which is created during cavity cleaning, rather than researching an optimal collagen network. In fact, with 2SSE dentin is demineralized and infiltrated simultaneously and monomers are gradually buffered with the increasing depth, creating a chemical interlock [44],[45]. This ability of penetrating and creating a stable chemical bond depends on the pKa and the nature of the monomers and has been explained with the so-called "Adhesion-Decalcification concept", which is strictly related to chemical bonds and reaction equilibrium [41]. This concept also provides an explanation to why strong self-etch adhesives do not work so well, and therefore a "mild" approach is now applied. As a consequence, the etching effect on enamel will be insufficient and ultimately enamel will need a selective etching procedure [41]. Selective enamel etching has been proved to guarantee a more durable bond and seal thus protecting dentin bond as well [46].

The last category, according to Sofan et al. [35], is the 8th generation of universal adhesive systems, which has been developed and optimized in recent years. The idea behind these adhesives was to give the dentist the opportunity to decide which adhesive strategy to use (ER or SE) with a single product and under the "all-in-one" concept. This was possible thanks to the introduction of new acidic functional monomer molecules, such as the well-known 10-MDP, that show higher bonding performance. A 2021 review by Fehrenbach et al. well summarized the chemical composition of these adhesives [47]. Indeed, several monomers have been tested, with inferior performances compared to 10-MDP, such as PENTA, 6-MHP, 4-META, pyrophosphate esters, monomers derived from sulfonic acid. On the other hand, bond strength values similar to 10-MDP were verified in materials containing PEM-F, acrylamide phosphates, 4-AET, MAC-10 and monomers derived from polyacrylic and phosphonic acids. Adhesives based on GPDM were the only ones that resulted in greater bonding potential than the 10-MDP-based group. Moving to the clinical protocols, several reviews looked up to the bond strength of these systems and how to pre-treat the substrate to achieve the best outcome. In 2015 Rosa et al. reported that enamel bond strength of universal adhesives is improved with prior phosphoric acid etching, while the etching effect was not evident for dentin when using mild universal adhesives, just like 2SSE [48]. This was confirmed in 2019, by another review by Cuevas-Suárez et al., that stated again the necessity of selective enamel-etching and the usage of a mild pH strategy. As a matter of durability of the bond, in 2019 a review by Carrilho et al. confirmed the stability of universal adhesives bond and suggested a "scrubbing technique" to improve the penetration of the adhesive system, which needs time to infiltrate, hybridize and form MDP-Ca bonds [49]. If all above mentioned protocols are strictly followed, universal adhesives can be considered a good choice even when compared to conventional 3SER and 2SSE systems [50].

1.4 Adhesion in indirect restorations

Creating a proper adhesion is a crucial step when dealing with indirect restorations, since it allows to perform minimally invasive partial preparations that do not rely on retentive parameters. In order to do that, resin-based, dual-curing cements have been developed. Compared to adhesives, cements are loaded with filler particles similarly to RBC, therefore showing much superior mechanical properties, at the expense of a higher viscosity. This allows to compensate the interfacial misfits that are always present between the prepared surface of the tooth and the restorative material. To ensure a sufficient degree of conversion (DC) of the monomers even under opaque restorative materials, auto-polymerization through chemical activators was implemented in light-curable materials. Indeed, it is obvious that material thickness and translucency may not allow a sufficient light transmission to the cement layer, ultimately resulting in a low DC [51],[52]. However, translucency of the tooth-restoration complex is critical for the aesthetic outcome of the restoration, meaning that we cannot use highly-translucent materials and transparent cement shades, especially with highly-chromatic substrates [53]. Relying only on chemical curing does not appear to be an effective strategy: light curing seems able to improve DC and consequently materials mechanical properties, making also clinical manipulation easier [54]. Several light curing strategies have been proposed, but will not be discussed in the present section, since they are related to specifical materials and their polymerization kinetic. Generally speaking, in order to optimize shrinkage stress and avoid gap formation, it is known that light curing must be performed after an initial "resting" phase in which the chemical self-curing process occurs [55]. This phase timing is related to the cement composition and polymerization kinetic. If light curing is performed too soon, the material may undergo the so-called "early vitrification" in which cross-linked polymer network traps the free radicals of the reaction, decreasing the efficacy of chemical curing and causing the development of stresses [55].

Up to date, two different kinds of luting cements are available: conventional dual-curing cements (CDCC) and self-adhesive cements (SACE). Both these types of cements are used for indirect restorations and fiber post cementation, but they offer different protocols and advantages.

CDCC represent the older generation, with a large number of papers that studied their performance over time, both in vitro and in vivo. When properly applied and cured they achieve good performances both on dental tissues and under most modern restorative materials [56]. In order to properly bond tooth structure, they need several clinical steps as they rely on the same adhesive systems previously described for direct restorations, often mixed with chemical activators. This raised scientific doubts, since adhesives might have influence on the polymerization kinetic of CDCC and may therefore result operator-dependent compared to conventional cements. However, based on literature, clinical long-terms studies indicate that conventional cements have long-term high survival rates, but resin-based cements have even greater success [57].

SACE were developed in order to reduce the number of clinical steps, with a similar concept to 2SSE systems. It has been reported that these materials are able to adhere to tooth structure without the requirement of a separate etching step and application of an adhesive/bonding agent [58]. This is possible thanks to the presence of functional acidic monomers and fillers capable of neutralizing the initial low pH of the cement [59]. Regarding the physical/mechanical/wear properties of SACE, few studies examined and compared them to other types of cements. A study by Piwowarczyk et al. compared the flexure and compressive strength of a self-adhesive resin cement, to two zinc phosphates, two glass–ionomers, three resin-modified glass–ionomers and four dual-cure resin cements, concluding that resin cements in general had better performance compared to conventional ones [60]. As a matter of adhesion to dental substrate, SACE generally perform worse than CDCC applied with adhesive systems when micro-tensile or shear tests are performed [61]. This is confirmed by the fact that they do not form a classical hybrid layer with dentin when analyzed through microscope, despite they have a fairly strong bond to it in push-out tests [62]. It is therefore assumable that, compared to CDCC that rely only on "classic" adhesion concept, SACE rely both on adhesion and retention thanks to their low volumetric contraction.

Bonding performances of resin cements towards restorative materials, such as fiber posts, zirconia, glass ceramics and milled RBC, is mainly related to the physicochemical conditioning of the substrates, even if the cement composition might have an influence [63]. These aspects will be further discussed in indirect restorative materials section.

1.5 Modern direct restorative materials

Direct restorative materials are RBCs and derivates that can be applied directly in the patient's mouth to restore missing tooth structure. These materials, for commercial reasons, are evolving so fast that it is objectively difficult to properly test them and research their long-term behavior. However, some categories can be recognized in literature that are widely applied in contemporary dentistry. Packable, flowable, bulk-fill and fiber-reinforced are the most common, but RBC can also be classified based on the filler content and dimension (microfilled, nanofilled, nanohybrid, highly-filled) [64]. Other materials, such as glass-ionomers and sealants find usage in pediatric and geriatric dentistry due to their easier manipulation, but cannot be considered long-lasting materials when compared to properly-managed RBC [65],[66]. Worth to mention, dental amalgam is still used in many countries of the world, showing great and well-documented long-term performances [67]. As reported by Frankenberger et al., this material benefits of a high longevity and low technique sensitivity [68], with even better performances compared to RBC [67]. However, due to aesthetic reasons, the toxicity during manipulation phases and the necessity of reducing anthropogenic mercury release into the environment (Minamata Convention 2013) this material is being progressively abandoned.

All RBC have a similar core composition, constituted by an organic matrix (monomers), inorganic phase (filler), coupling agents (silane) and modulators of the reaction (initiators, co-initiators or activators and inhibitors).

The organic matrix can be constituted by a variety of monomers, that have been developed since the '60s. Dr Bowen was the first to replace epoxy resin with methacrylate to produce the dimethacrylate known as Bis-GMA (Bisphenol A-glycidyl methacrylate) [69]. Since then, several monomers have been proposed in order to improve the resin mechanical performances, DC, polymerization kinetic and shrinkage. The most commonly used have been summarized in Figure 4 (chemical structure) and Figure 5 (basic properties) for quicker consultation.

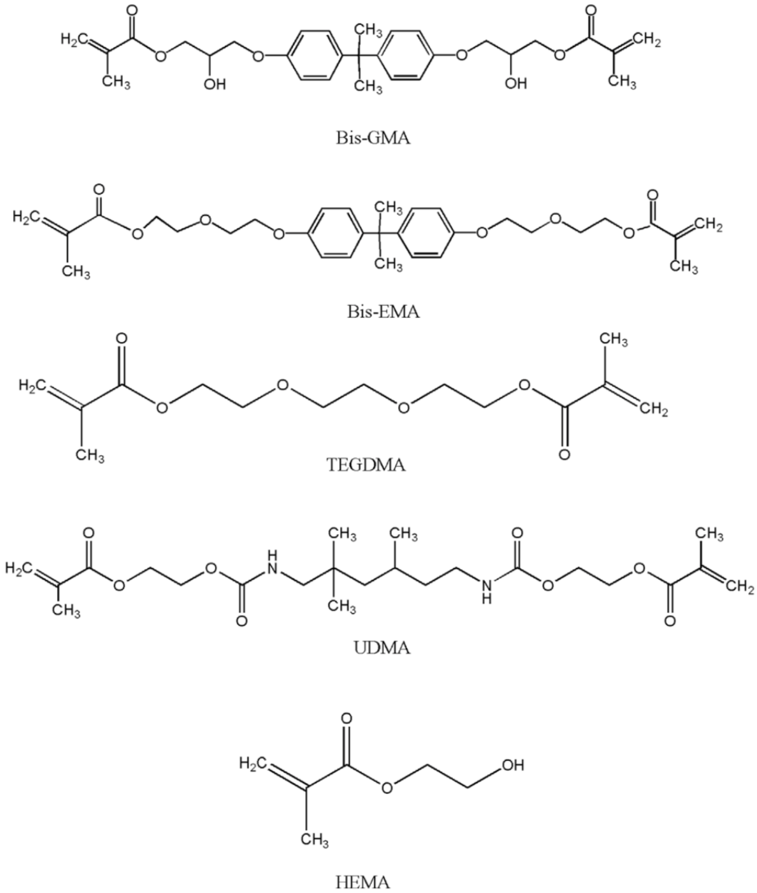

Figure 4. Molecular structure of different monomers used in RBC (Bis-GMA: Bisphenol A-glycidyl methacrylate; Bis-EMA: Ethoxylatedbisphenol-A-dimethacrylate; TEGDMA: Triethylene glycol dimethacrylate; UDMA: Urethane dimethacrylate) [69]

Monomer	Molecular Weight	Concentration of double bonds (mol/kg)	Viscosity (Pa.s)	Refractive Index	Density
Bis-GMA	512.59	3.9	700	1.5497	1.16
Bis-EMA	540	3.7	3	1.532	1.12
TEGDMA	286.3	6.99	0.05	1.46	1.09
UDMA	470	4.25	8.5	1.485	1.12
HEMA	130.14	*	*	1.452	1.07
PPGDMA	600	*	0.09	1.45	1.01

* Not Known.

Figure 5. Basic properties of the most common dental resin monomers according to a recent review (2019) by Pratap et al. [69].

Bis-GMA consists of Bisphenol A (BPA) and glycidyl methacrylate [70]. It is highly hydrophobic, so it must be managed with organic solvents. It possesses a stiff central core of phenyl ring and two pendant hydroxyl groups, which are responsible for its high viscosity, lesser degree of conversion (due to viscosity itself that impairs monomer movements) and high water-sorption capacity. However, compared to small-sized dental monomers such as methyl-methacrylate (MMA), it has lower shrinkage (5.2%), higher Young modulus, fair impact strength, high refractive index and reduced toxicity due to lower volatility and diffusivity into tissues [69]. Generally speaking, Bis-GMA is a good choice for dental RBC, but must be combined with more flexible monomers, such as TEGDMA, to deal with its viscosity. A hydrophobic analog of Bis-GMA is the Bis-EMA, in which a hydroxyl group has been replaced with an epoxy specie. It possesses lower viscosity, water sorption and polymerization shrinkage, making it suitable as diluent. However, the DC it can achieve is low, due to steric hindrances (congestion caused by the physical presence of the surrounding ligands that slow or prevent the reaction). Another interesting monomer is TEGDMA, which is formed by triethylene glycol (TEG) reacting with methacrylic acid. The fact that TEGDMA possesses a long, linear structure makes him flexible and explain its low viscosity. It is often used as diluent, but has high water sorption, reduced mechanical properties and low color stability. One more monomer, mainly used in dental adhesive systems rather than composites, is HEMA, which is formed by methacrylic acid reaction with ethylene glycol or ethylene oxide. The peculiarity of HEMA is in its hydrophilic properties, making it ideal to perform a bridge between dental tissues and composite systems. Lastly UDMA is synthesized from hydroxyalkyl methacrylates and disocyanates. Again, due to the absence of phenol rings it is flexible, mobile and tough, with 100 times lesser viscosity compared to Bis-GMA. It is more viscous than TEGDMA and Bis-EMA due to NH groups, but it shows greater DC and polymerization rate, making it a good choice to replace Bis-GMA either partially or totally.

All these monomers undergo polymerization through a chain reaction consisting of initiation, propagation and termination, which is started by initiators. Initiator molecules have either bonds that are cleaved due to light curing or excitable chemical groups. The result of both the processes is the creation of a reactive molecule that start the chain reaction of monomers. The most common photo-initiator in dental materials is camphoroquinone (CQ), which absorbs light between 360-510 nm. The major drawback of CQ is the yellow color and its toxicity, that led to develop several other molecules that work similarly, such as Diphenyl-phosphine oxide (TPO) and 1-phenyl-1,2propanedione (PPD) [71]. Other agents, such as amines, are used as accelerators that donate electrons to the reaction in order to improve initiators efficiency and increase DC. Initiators, co-initiators and inhibitors concentration, light permeability and monomer composition will significantly influence DC of the system, its volumetric shrinkage and shrinkage stress development [72].

Last but not the least, fillers and their coupling agents are extremely important in the overall performance of RBC. The first-ever developed RBC had “macro-fillers”, with a particle size of 10-50 µm, with low possibility to polish and high wear rate (due to large porosities formed after filler enucleation due to wear). Micro -filled RBC were later introduced to improve aesthetic, with particle size of about 40 nm, but those particles were too small to improve the material strength due to low filler loading. In the last 15 years, nanohybrid packable RBC (5-100 nm) became an important advancement in dental materials, balancing mechanical properties, aesthetic and wear behavior, making them the universally accepted gold-standard of RBC. Filler surface treatment with coupling agents is also a key topic in RBC performance. Coupling agents create charged surfaces, consequently causing a proper filler dispersion in the material. Moreover, they improve the cohesion between the organic matrix and the inorganic part, consequently improving DC, mechanical properties and filler loading amount [73]. Among coupling agents, zirconate, titanate and silanes are the most commonly-used [74],[75]. Silanes are mainly constituted by organic silicides and represent the most commonly employed coupling agents, due to their ability of creating a bond both with the inorganic filler (through covalent bonding with alkoxy group) and the organic resin (through the reactive organic group). Thanks to its properties, silanes also improve hydrolytic stability preventing water to enter the silica-matrix interface [76].

The general composition of composites can be modified in different ways, which led to the creation of the above-mentioned categories of RBC, that will now be discussed in terms of mechanical and clinical performances.

RBC can come in different handling characteristics and viscosity ranges, to improve their versatility for different clinical procedures. An example is the so-called flowable RBC, which is extremely common in clinical practice due to the high fluidity of the material that facilitate adaptation. In order to reduce the viscosity, these flowable RBC generally have a reduced filler loading (37-53% volume) compared to packable conventional materials (50-70% volume) [77]. As reported by Baroud et al. in their 2015 systematic review, flowable materials have several advantages such as high fluidity, possibility to form layers of minimum thickness, high flexibility and low elastic modulus [77]. Due to the reduced filler content however, the volumetric shrinkage is higher and the physical properties are generally lower. Wear in particular seems to be higher, making them indicated in low-stress internal areas [78]. On the other hand, these materials have been proposed as liners under nanohybrid packable composites due to their easy manipulation and favorable elastic modulus. Results of this technique are contradictory, with some studies supporting their usage, while other showing a higher microleakage in flowable groups especially after thermos-mechanical aging treatments [77],[79]. On the opposite, it has been shown that flowable RBC might reduce shrinkage stress at the bonded interface and create a stress-absorbing layer due to the stress-relieving effect of their elastic modulus [80]. Further information is needed in order to assess the interfacial gap behavior around these RBC, especially after aging treatments.

Another category is represented by the so-called "bulk-fill" RBC. These composites were developed with the concept of simplifying clinical procedures, allowing to significantly reduce the number of composite layers needed to perform a direct restoration. Several papers, both in vivo and in vitro, claim that these materials can be polymerized in up-to 4 mm layers, for both their low shrinkage and optical properties, showing no differences compared to conventional packable RBC [81],[82]. Low shrinkage is necessary in order to avoid severe interfacial gap formation, while optical properties are essential to allow light-penetration in depth, therefore reaching a proper DC at the cavity base. The first goal was reached by modifying the composition of a RBC, by lowering filler content, increasing the dimensions of filler particles (>20 microns) and using high-molecular-weight monomers to improve marginal adaptation [83]. Simultaneously, to improve light penetration, refractive indexes of resin monomers and fillers in the un-polymerized material were equalized, highly reactive photoinitiators were incorporated and changes were made in the translucency, reducing the pigment content [83]. Due to the different polymerization kinetic and the presence of strong initiators, concerns were raised about the performance of these materials in terms of interfacial gap presence. However, a recent study clearly showed that volumetric shrinkage and interfacial adaptation are not directly correlated, but rather are material-dependent: bulk fill materials showed a lower volumetric shrinkage compared to conventional RBC, but the interfacial adaptation of tested materials was the same [84]. Another study with micro-CT volumetrically showed that most of the interfacial gap occurred at the adhesive-tooth interface rather than adhesive-material interface when low-shrinkage bulk materials were applied [85]. Despite the amount of studies about bulk-fill RBC, a 2021 Meta-analysis by Zotti et al. concluded that bulk-fill materials are clinically interesting, but there are still insufficient data to explore the relationship between bulk-fill RBC and microleakage performance. Moreover, the reduction in filler content might have an influence on their mechanical outcome, making them more prone to degradation over time. An in-vitro study published in 2013 about this topic revealed similar flexural strength values as the class of packable nanohybrid RBCs and significantly higher values when compared to flowable RBCs [86]. The modulus of elasticity, the indentation and the Vickers hardness classified bulk-fill RBCs between packable hybrid RBCs and flowable RBCs; in terms of creep, flowable bulk-fill and flowable RBCs perform similarly, both showing a significantly lower creep resistance when compared to the nanohybrid RBCs.

In order to improve RBC mechanical properties, possibly of bulk RBC as well, ultimately optimizing both clinical procedure and mechanical outcome, short-fibers RBC were developed. The concept derives from engineering and in architectural applications and has been proved to improve material strength and fracture toughness. In 2013, the first dental composite containing short fibers was introduced in commerce (EverX Posterior, GC, Tokyo, Japan), with the goal of mimicking dentin stress-absorbing capability, reinforcing tooth structure and preventing catastrophic vertical fractures. A 2018 review by Garoushi et al. well summarized the key points associated to short-fiber reinforced RBC, that will now be presented [87]. In terms of mechanical properties, they possess superior fracture toughness (2.4-2.9 MPa m1/2), flexural strength (124-201 Mpa), flexural modulus (9.5 GPa) and fatigue limit (0.9-1.1 MPa m1/2) compared to bulk-fill and packable nanohybrid RBC. These parameters are in function of fiber diameter, length, orientation and loading. In particular, millimeter-scale short fibers seem to be able to stop crack propagation and increase fracture resistance. Moreover, their volumetric shrinkage seems lower (0.17%) and vary according to fibers orientation, even if this is not confirmed by all studies. Shrinkage stress has been reported around 5 Mpa, with packable nanohybrid RBC and bulk fill RBC ranging 3.94-10.45 Mpa. Shouha et al. reported that the addition of 5 wt% of short fiber fillers to resin matrix did not affect shrinkage stress values, whereas 10 and 20 wt% loading resulted in higher stress values [88]. According to them, an increase in shrinkage stress from the fiber load of >5 wt% could arise from increases in modulus and stiffness of the material. In terms of interfacial adaptation and microleakage, due to their low shrinkage stress, short-fibers RBC showed promising results, even if it is still controversial if their performance is better than nanohybrid packable RBC. The presence of fibers also increases overall translucency of the material and its internal scattering, giving them advantages in terms of polymerization depth. Little evidence is available regarding their bonding performance, which seems good, both to universal adhesives and, thanks to a thicker oxygen inhibition layer, to other RBC layers. In general, literature is in agreement to the fact that short-fibers RBC are suitable as core materials, but they must be covered by other RBC, in order to achieve a proper aesthetic and polishing [89].

Whenever a tooth is endodontically treated, clinicians can also use the root canal system to insert a post that serve as pre-prosthetic base, with the aim of reinforcing the surrounding RBC. In the past, this post was made of metal, and it was always combined with a full crown restoration. More recently, fiber posts have been introduced in combination with cements and RBC, showing excellent clinical results [90]. The main advantages, compared to metal posts, are the favorable elastic modulus, the aesthetic outcome, the less invasive treatment and the reduced amount of vertical root fractures. A 2021 meta-analysis analyzed the effects of fiber post insertion on the mechanical properties of the tooth-restoration complex, demonstrating that the use of glass fiber posts increases the fracture resistance of endodontically treated teeth [91]. Moreover, fiber posts have been shown to be fatigue-resistant, have high tensile strength and similar elastic modulus to that of dentin, which might also avoid stress concentration during function [92]. However, few information is available on how fiber post systems influence the interfacial behavior of the restoration [93]. This is particularly important if we consider that both clinically and in-vitro, the most frequent failure of fiber post combined with RBC is represented by debonding [94]. This is surely connected to the difficulties, previously reported in the substrate paragraph, connected to radicular dentin adhesion, however the mechanical properties changes in the restoration complex caused by a fiber post insertion might be important as well. Indeed, Ausiello et al. showed, through a finite element analysis (FEA), that a hybrid composite post could be able to optimize stress distribution, dissipating forces from the coronal to the apical end [95]. This was confirmed by Kemaloglu et al., that showed how fiber network might change stress dynamics at the interfaces suggesting that it might influence marginal gap progression as well [96].

Less popular in the daily clinical practice, but worth to mention for their research development and future perspectives, self-adhesive RBC, compomers, siloranes and ormocers represent a possible alternative to conventional RBC.

Self-adhesive RBC have been developed in the early 2010s to simplify clinical procedures and could prove particularly useful for clinical situations with precarious isolation. The concept, similarly to self-adhesive cements, is to take advantage of functional acidic monomers that interact with dentin and chemically bond exposed minerals. These materials can be applied with or without dedicated adhesive systems and are still ongoing research. Up to date, the application of additional primer/bonding agents still seems to improve marginal sealing and microtensile bond strength [97,98]. In order to overcome this issue, different biological substrate pretreatments are being tested. For example, Er:YAG and Er,Cr:YSGG lasers have been reported to enhance shear bond strength values by eliminating the smear layer, opening dentinal tubules and increasing resin infiltration [99]. The same can be said for their specific composition, that is still ongoing optimization to achieve high flexural strength, conversion degree, while simultaneously reducing shrinkage [100].

Compomers are polyacid-modified composites that found applications in children's dentistry due to their bioactivity. As reported by Nicholson et al. in their review, their core composition is similar to RBC, but they contain additional acidic monomers and reactive glass powder that give them unique properties that will now be discussed [101]. Compared to nanohybrid RBC, they are designed to uptake small amounts of water, triggering an acid-base reaction between the reactive glass filler and the acidic monomers: this causes fluoride to be released, therefore obtaining an anticariogenic effect. However, concerns were raised in the literature studies about the interfacial stresses and polymerization values obtained by these materials. Moreover, compomers also have significantly inferior fracture toughness compared to nanohybrid RBC due to the water uptake phenomenon, making them suitable for children's prevention and dentistry rather than adult restorative dentistry.

Siloranes have been developed as composite materials with low volumetric shrinkage, with a reported contraction inferior than 1% [102]. However, as previously mentioned, shrinkage stress, which is what really matters for the stability of the adhesive interface, is not determined by volumetric shrinkage alone. A number of other factors have a major impact on the final stress, including the elasticity modulus, degree of cure, coefficient of thermal expansion, the silanization characteristics at the resin-filler interface, as well as the rate of cure and polymerization kinetics, the C-factor and the compliance of the remaining tooth structure. When siloranes were analyzed for their stress development, high polymerization stress values were reported by several authors, as stated in a recent review, raising concerns about the marginal integrity that they might achieve over time [103]. Additionally, compared to nanohybrid methacrylate-based composites, they showed similar or inferior bond strength to tooth structure, an inferior cusp deflection, more adhesive failures after aging and similar mechanical properties [103]. These data are supported by clinical trials, that often found an inferior marginal adaptation of these materials, even if long-term studies are still needed to better assess the performance of these materials. For these reasons, siloranes have been discontinued in clinical practices.

Ormocers are RBC in which the methacrylate has been partially replaced by an inorganic network [104]. More specifically, they possess long inorganic silica chain with organic lateral chains, able to react due to photoinitiators during light-curing. The larger size of the monomer molecules may reduce polymerization shrinkage, wear, and leaching of monomers [104], and the materials are expected to combine the advantages of both organic polymers (e.g. flexibility and impact resistance) and inorganic materials (e.g. thermal stability, mechanical strength and chemical resistance). In 2011 it was reported that ormocers have a good marginal adaptation compared to methacrylate-based RBC in-vitro [105]. However, despite these good premises, a 2022 meta-analysis reported that nano-filled and nanohybrid RBC possess superior clinical longevity compared to ormocers in posterior restorations, meaning that the interfacial behavior of these materials is yet to be understood [106].

1.6 Modern indirect restorative materials

Indirect restorations are meant to reinforce the residual tooth structure whenever a substantial loss happens due to caries or fractures. The concept is that, compared to direct restorative materials, indirect restorative materials possess far superior mechanical and physical properties, making them more suitable to restore severely compromised teeth, ensuring a proper fracture resistance of the restoration. In recent years, due to aesthetic reasons, metal-ceramics have been progressively abandoned in favor of the so-called “metal-free” materials. These materials include polycrystalline ceramics such as zirconia, glass-matrix ceramics such as lithium disilicate and a variety of hybrid materials [107].

Zirconium dioxide (ZrO2), also known as zirconia, is probably the most used among these materials, due to his high mechanical performances and good aesthetic outcome, that can be modulated changing its chemical composition. In general, as reported in a 2022 meta-analysis, this material presents the following properties: fair translucency related to its cubic phase (that can be modulated), high flexural resistance (781.92-936.04 MPa), high fracture toughness (4.46-5.34 MPa) and high hardness (11.49-12.03 GPa) [108]. The amount of cubic phase, which is optically isotropic, improves aesthetic and translucency at the expense of strength and toughness, due to the lack of transformation toughening and a coarser microstructure [109]. A recent study pointed out that cubic grains are wider than tetragonal ones and generate more stabilizing oxides, making the tetragonal phase more prone to aging [110], raising concerns regarding its interfacial behavior after fatigue. Moreover, the absence of a glassy phase makes the bonding mechanisms of zirconia to dental tissues more difficult, because it is not possible to etch the surface, meaning that the interfacial seal is again a crucial aspect that bioengineering should improve. A systematic review and meta-analysis in 2015 reported that MDP-based resin cements tend to present higher bond strength results towards zirconia than those of other cements types, but there is still lack of data and standardization of tests [111]. This was confirmed in 2021 in another systematic review, in which again a standardized adhesive protocol to zirconia wasn’t established due to lack of evidence [63]. As a matter of fact, several pre-treatments have been proposed to optimize adhesion, with airborne-particle abrasion and tribo-chemical silica coating having the more evidence in literature, but no evidence support a universal protocol [112].

Among glass-matrix ceramics lithium disilicate (LiSi2) and its derivates are the most commonly used since the ‘90s. This material presents excellent aesthetic, fair fatigue resistance and comparable survival rates after adhesive cementation compared to zirconia, even if its mechanical properties are inferior [113],[114]. As most indirect materials, lithium disilicate can be treated and chemically-modified in several ways in order to improve its mechanical performances, for example adding zirconia grains into its matrix or thermo-pressing it rather than milling it [115]. However, even zirconia-reinforced lithium disilicate achieve flexural strength of 400-500 MPa and an elastic modulus of 60-67 GPa, that are not comparable with zirconia itself [116]. On the other hand, a key aspect that differentiates this material from zirconia is that this material can be efficiently etched with hydrofluoric acid. This creates a micro-rough surface and consequently promote a good adhesion, especially if a dedicated ceramic-primer is used in combination with the acidic treatment [117]. Apart of high bond strength, lots of papers described the interfacial adaptation of lithium disilicate restorations, showing that it is clinically acceptable especially if the material treated with press technique [118]. However, few study analyzed lithium disilicate interface degradation over time, especially in clinical-like scenario and on partial preparation designs.

Last but not the least, resin-based materials milled through CAD-CAM procedures are becoming increasingly popular, due to their good aesthetic, wear-behavior, repairability, reduced cost and the possibility of being used in simplified chairside procedures (without firing or sintering processes) [119]. We can distinguish resin-based ceramics (e.g. Lava Ultimate, 3M-ESPE) that contain a polymer matrix with at least 80% nano-ceramic filler particles and hybrid ceramics (e.g. VITA Enamic, VITA-Zahnfabrik and Cerasmart, GC) made of a ceramic network infiltrated with a polymer using polymer-infiltrated ceramic network (PICN) technology [120]. In 2018 a literature review by Facenda et al. reported that the mechanical properties of PICN are equivalent or superior to nano-filled RBC, even if inferior to lithium disilicate [121]. Moreover, these materials can be adhesively treated with the same procedures reported for RBC, simplifying clinical protocols and ensuring a good bond strength. Short-term clinical performances are promising, but these materials are too young to have a proper follow-up yet [122]. Regarding their mechanical performances, resin-based ceramics show flexural strengths up to 230 MPa and relatively low Young’s modulus, a factor that might have an important influence on the interfacial behavior, crack propagation, forces distribution and resistance to loads [120]. Among CAD-CAM resin-based materials are worth to mention polymethyl methacrylate (PMMA), polyether-ether-ketone (PEEK) that are both usable for provisional and long-term provisional restorations, giving also the opportunity of being loaded with ceramic particles becoming hybrid materials. All these materials can achieve high bond strength through chemical and mechanical surface treatments, as stated by Mine et al. in their review [123]. Based on this paper, the initial process of bonding should aim to create micro-retentive surfaces either by sandblasting or hydrofluoric acid etching. A second phase should be the silanization to improve chemical adhesion, and then a conventional adhesive system can be used. Properly following manufacturer's specific instructions, can allow to obtain compressive bond strength above 20 MPa.

However, despite the good bond strength, few studies investigated the effect of the mechanical properties of these materials on the interfacial behavior of different preparation designs. The elastic mismatch between dental tissues and the RBC restoration creates complex stresses and strains at the interface, possibly altering the TRI and pumping cariogenic fluids in micro-gaps [124]. Figure 6 summarizes the mechanical properties and chemical composition of the most common CAD/CAM materials in dentistry [120].

Material Type	Product examples	Flexural Strength (MPa)	Elastic Modulus (GPa)	Hardness (HV)
Composites	Vita CAD-Temp (VITA)	80	2.8	N/A
Aluminum oxide ceramics	Vita In-Ceram ALUMINA (VITA)	419	410	2035
Leucite-Reinforced Glass Ceramics	IPS Empress CAD (Ivoclar)	160	62	632.2
Zirconium oxide ceramics	IPS e.max zirCAD (Ivoclar)	1200	206.3	N/A
Lithium silicate ceramics	IPS e.max CAD (Ivoclar)	353.1	102.7	617
Resin-based ceramics	Lava Ultimate (3M)	200	12	96
PMMA	Polident PMMA (Polident)	114	2.77	26
PEEK	PEEK-OPTIMA (Invibio)	165	3.70	N/A
Hybrid Ceramics	VITA ENAMIC (Vita)	150-160	30	200

Figure 6. Exemplificative products alongside their mechanical properties and composition of the most common CAD/CAM materials applied in dentistry [120].

1.7 Degradation of the TRI

As explained in the sections above, ideally bonding agents should penetrate the dentinal collagen network and completely replace the water of the area. However, due to the presence of residual solvent and fluid movements out of dentinal tubules into the hypertonic comonomer mixtures [125],[126], water replacement is never ideal [127]. The local areas that are water-rich and resin-poor have been named "nanoleakage" and might occur both in hybrid layer and adhesive layer, increasing with time and function and eventually leading to a true interfacial gap [128]. Nanoleakage can also occur as a consequence of adhesive solvent evaporation on the kinetics of water diffusion [129]. The complex chemistry of adhesive materials, such as hydrophilic/hydrophobic ingredients, water and solvents can potentially influence this water kinetic and affect nanoleakage, as happened for 2SER systems [130]. Moreover, in accordance with a 2018 meta-analysis, substrate pretreatment, such as etching, significantly influence nanoleakage [131]. It is nearly impossible to completely avoid nanoleakage, but it is reasonable to assume that with proper adhesive selection and following the dedicated protocols, this phenomenon can already be clinically managed with efficiency [132].

Another aspect correlated with nanoleakage and therefore TRI performance over time, is the degradation of the hybrid layer by collagenase/gelatinase endogenous enzymes. Hybrid layer degradation can indeed create empty spaces in the TRI, with subsequent water uptake and nanoleakage occurrence [133]. These endogenous enzymes are released with the acid etching and significantly decrease bond strength over time. This happens through hydrolytic degradation and enzymatic action of different metalloproteinases (MMPs) on collagen fibrils, but also salivary esterases and other enzymes such as cysteine proteases [134],[135],[136]. Several experimental strategies aimed to prevent or at least reduce degradation: increasing the degree of conversion and esterase resistance of hydrophilic adhesive, use of inhibitors of collagenolytic enzymes, collagen cross-linking agents/promoters, ethanol wet-bonding and biomimetic remineralization of resin-dentin bonds [137]. Most of these strategies did not find a clinical application, due to the instability of the used components or the impossibility to apply them in clinical routines. For example, removing interfibrillar highly hydrated negatively-charged proteoglycans hydrogel is a very effective strategy to improve bond strength up to 49-63%, but the process needs 24 hours [138]. Up to date, the most commonly applied agent is chlorhexidine (CHX), that seems to improve significantly the bond strength after aging accordingly to a 2021 Meta-Analysis by Kiuru et al. [139], while also acting as antimicrobial agent [140]. Another strategy that has been incorporated in 3SER adhesive systems is the previously cited ethanol wet-bonding through primer application step. This technique chemically dehydrates acid-etched dentin and reduce the hydrophilicity of collagen matrix [141]. Ethanol wet-bonding has also been proved to reduce nanoleakage and micro-permeability, preventing long-term degradation of resin-dentin bonds through a reduction of MMPs activity [37]. Other agents, such as benzalkonium chloride, can be incorporated with etching agents in order to reduce MMP activity and serve as an antimicrobial agent [142]. Figure 7 shows an example of MMP activity, detected with in situ-zymography and the effects of both prolongated etching and ethanol wet bonding (courtesy of Dr. Allegra Comba and Prof. Lorenzo Breschi, UNIBO).

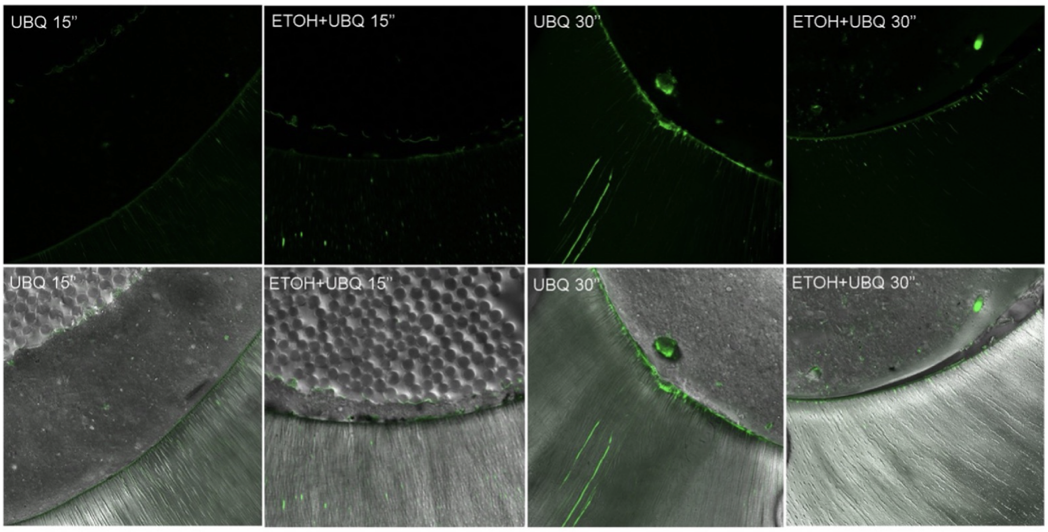

Figure 7. Representative confocal laser scanning microscopy images. Top images were acquired in the green channel. Bottom images were produced by merging the differential interference contrast image and the image acquired in green channel. Green represent MMP activity, both inside the dentinal tubules and the TRI (hybrid layer).

On a different scale compared to nanoleakage, microleakage and interfacial gap are the most important manifestation of the TRI degradation that are observable both in-vitro research and clinically. Interfacial gap can be thought as a discontinuity in the tooth-restoration complex, which ideally should be perfectly in contact, with a uniform layer of adhesive and/or cement interposed. Interfacial gap mechanically weakens the adhesive interface and might cause infiltration of molecules, fluids and bacteria, a phenomenon also known as microleakage [143],[9]. This can lead to secondary caries formation or micro-fracture of the TRI, with subsequent clinical failure of the restoration itself. This is demonstrated by several studies that focused their attention in reproducing secondary caries formation around dental composites in-vitro, even with severe limitations due to the complexity of the TRI and the oral bacterial biofilm [9]. These studies concluded that many factors may affect caries formation, including marginal gap formation, gap size, the local chemical environment, the durability of the bonded interface, the extent of bacterial penetration, and the presence of mechanical loading [14]. In particular, interfacial gap size and mechanical loading have been shown to be related to lesion severity within in vitro models, even if these results do not correspond exactly with those obtained from in situ studies [14].

Even if still debated, external gaps that exceed a width of 60-120 μm have been defined as “clinically critical”, meaning that they could likely lead to bacterial products accumulation, ultimately leading to sensitivity and increased chance of secondary caries or periodontal problems [144],[145],[146]. With the introduction of modern full-ceramic indirect materials and adhesive cements, this range has been reviewed in several studies, and there is still no consensus about the proper range. However, it has been demonstrated that larger gaps harbor a greater number of bacteria, even around dental amalgam [147], creating what has been defined as “macroleakage” [8]. As a logical deduction to this preface, it can be concluded that the key to eliminating or minimizing lesion recurrence is maintaining a biological and chemical seal of the interface. This seal should prevent the formation of a lesion along the restoration [148], even if it cannot probably prevent another primary lesion to occur in the same area [149]. Figure 8 reports representative interfacial gap images, that can be considered clinically relevant.

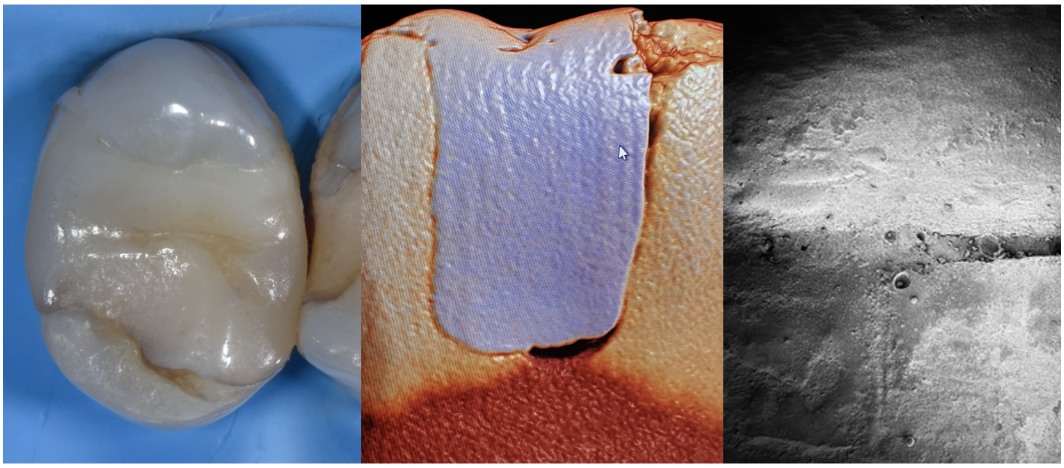

Figure 8. Clinically relevant TRI gap images. Left picture: in-vivo TRI gap in a MOD restoration. Central picture: micro-CT 3D reconstruction of a restoration (blue volume) with an interfacial gap (black area). Right-picture: 66x magnification SEM image of a TRI gap about 300 μm width.

Moving to the causes of interfacial gap, two main reasons have been highlighted in the literature: shrinkage stress development due to material polymerization (baseline stress-relieving gap) and thermomechanical degradation due to function (progressive gap onset).

Volumetric shrinkage is an intrinsic, material-dependent phenomenon that occurs when monomers are converted into polymer chains creating C=C covalent bonds [150]. During the shrinkage process and depending on the material mass and polymerization kinetic, bonded surfaces are subjected to increasing forces related to the shrinkage, that translate into stresses [151]. These stresses can remain unrelieved in the TRI, weakening the tooth-restoration bond, or give origin to stress-relieving gaps, namely baseline interfacial gaps [152]. This problem has been largely investigated through Finite Element Analysis and reduced with new generation materials and novel techniques [153]. However, it is impossible to completely avoid stresses on the bonded surfaces, considering that a high conversion of the monomer bonds is desirable to achieve sufficient DC that translate into good mechanical properties [154]. Worth to mention, a recent study by Sampaio et al. demonstrated that volumetric shrinkage is not linearly correlated to gap development [84]. A good balance between material properties, such as elasticity modulus, DC and polymerization kinetic is therefore crucial in order to achieve both good baseline (less shrinkage stress) and over-time (better mechanical properties) performances.

The second cause of interfacial gap formation or progression, is well-known since 1983, when Qvist reported that "functional mastication has a major influence on the marginal adaptation of composite restorations in the oral environment" [155]. As a matter of facts, the immediate bonding effectiveness of modern systems is excellent, but it severely decreases over time due to function [156]. Even if a lot of studies focus on static shear and tensile tests to determine the effectiveness of bond strength, a recent review (2018) clearly stated that cyclic loading experiments are more reliable predictors of the mechanical performance of contemporary adhesive restorative materials [157]. Indeed, it is rare during function to develop forces that exceed the bond strength of adhesive systems, but more likely continuous loading might have an effect on interfacial gap through fatigue weakening and peeling effect on the adhesive layer [158]. The "fatigue weakening" does not occur only due to mechanical forces, but also through thermal fluctuations caused by patient diet. In fact, all restorative materials have a different expansion coefficient compared to tooth substrates, meaning that a variation in temperature will cause a stress in the TRI, as reported by several studies on different materials [159],[160]. Again, the mechanical properties of restorative materials are crucial, and could be optimized through bioengineering. Lastly, it should be considered that gap can occur inside the adhesive layer or the restorative material (cohesive failure) or between the adhesive layer and the substrate (adhesive failure). As specified in a comparative methodology study by Campos et al. in 2018 [161], cohesive failures are not significant of the bond performance, since they are connected to the mechanical properties of the adhesive and the restorative material themselves.

Since the degradation of the TRI is the core topic of the present thesis, further paragraphs will describe the available methods to assess interfacial gap and how to simulate thermomechanical cyclic fatigue. Moreover, stress development topic will be expanded in the specific material section.

1.8 Available methods for gap analysis

At this point, it is clear that dental materials, both direct and indirect, possess intrinsic excellent mechanical and physical properties, but there is still much to understand about their interfacial behavior and whether there are or not strategies to avoid gap formation and propagation. In order to do that, several methods have been proposed for marginal integrity and gaps analysis in literature since the '90s, with results varying considerably [162].

The traditional laboratory methods require organic tracers, such as basic fuchsin, methylene blue and rhodamine, combined with microscopy techniques or scanning electron microscopy (SEM) [163],[164]. The advantages of these techniques are the possibility to detect extremely small gaps and the high-quality micro-images obtainable. However, several disadvantages limit the usage of these protocols, such as the invasiveness (the sample must be sectioned), semiquantitative results, limitations in representing a tridimensional geometry. Moreover, measurements are taken by an operator on an arbitrary scale (usually graded from 0-25% to 75-100%), with possible operator and sample section biases. Worth to mention, the penetration of the tracer is also not directly correlated to clinically relevant gaps and it is impossible to evaluate the effects of artificial aging on samples after a baseline analysis, due to the sectioning procedure. To overcome this last issue, replica method was proposed. This method consists in the impression of the samples with high-quality polyvinyl siloxane and the subsequent creation of epoxy resin models that can therefore be "sacrificed" for SEM analysis [165]. The procedure has been proved effective, but it only allows the analysis of the external margin and such results can be affected by the accuracy of the impression/pouring procedure [166]. Figure 9 shows pictures obtained with SEM combined with replica technique on a pilot study of Spreafico et al. [165].

Figure 9. Left picture: SEM image of TRI after thermomechanical loading at 67x magnification. The restoration is marked with "R", while the tooth with "T". Specifically, the picture is showing an interproximal area of an indirect adhesive restoration. Right picture: detail (150x magnification) of the green-cornered area highlighted in the left image. It is worth to notice the high quality of the images, that can easily detect defects of the interface, such as cracks and undercuts (red arrows).

In order to bypass these issues, recently developed techniques focus on 3D tomography non-invasive imaging, through optical coherence tomography (OCT) and x-rays micro-computed tomography (micro-CT).

OCT has been introduced in dental research in 2000 and it is today applied to evaluate interfacial adaptation and microleakage of RBC restorations in several study designs. OCT is a time-domain low-coherence interferometric technique that provides high-resolution subsequential cross-sectional (two-dimensional) images by quantifying the reflection of light from dental structures [167]. The resolution of OCT is approximately 5-15 µm, which is higher compared to clinical CT images, but far lower compared to modern micro-CT machines used for TRI analysis [168]. On the other hand, OCT can be applied in vivo thanks to dedicated points and its quicker acquisition time (in the order of minutes compared to the hours of micro-CT), even if it is not possible to work at extremely high resolutions due to patient movements. OCT initially had limitations in detecting gaps in deep cavities, due to low light transmission through dental tissues and materials, but new techniques and equipment are progressively overcoming this problem [169]. Unfortunately, the biggest limitation of OCT, well highlighted in a review by Sahyoun et al., is that image scaling, registration and fusion methods still must be implemented to superimpose OCT data onto each other or with external 3D volumes, making difficult to analyze baseline and after aging images alongside [170]. This is also related to the fact that obtained images are hard to interpret since grayscale changes with refraction indexes rather than radiodensity, making this method suitable for expert operators and hardly consistent in between acquisitions. Figure 10 shows an OCT image of a class V cavity, with highlights on an internal TRI gap (bright area indicated by the red arrow).

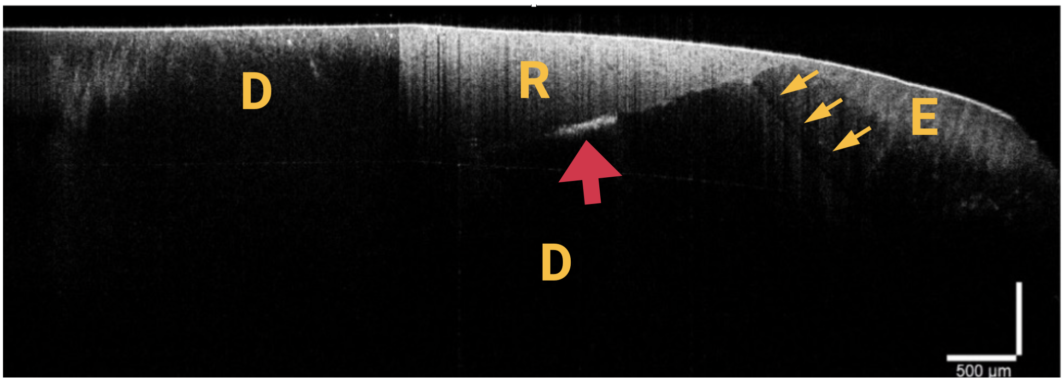

Figure 10. Axial image of a class V restoration (R) obtained through OCT technique. Enamel (E) limit with dentin (D) is represented by a black line (highlighted with the yellow arrows). An interfacial gap (highlighted with the red arrow) appears as a bright area between the restoration and the dentin.

X-rays micro-CT, on the other hand, uses multiple x-rays exposures combined with sample rotation to directly acquire the full sample volume, regardless sample size [85]. Images are then reconstructed through dedicated algorithms and sliced by software for qualitative analyses, measurements, segmentation and 3D rendering. The main drawback, compared to OCT, is that micro-CT can be used only for in-vitro research and is way more time-consuming. However, achievable resolution is extremely high (under 1 µm or even less with modern nano-CT) and it can detect gap at any depth or location. It is therefore not surprising that micro-CT is often considered the gold standard method for internal adaptation analysis, especially when compared to conventional techniques [171]. Some authors also combined the two techniques, using a radiopaque tracer such as silver nitrate, to better detect interfacial gaps with micro-CT [85],[172]. Therefore, thanks to the high resolution of micro-CT, most studies are today avoiding these complex procedures, demonstrating that micro-CT scanning without tracers is able to detect gaps and properly analyze the adhesive layer [173]. The usage of tracers could be interesting in discriminating the location of the gap with respect of the adhesive layer, but the procedure can also cause scattering, artifacts and possibly change the TRI biomechanics. Moreover, if the samples must be subjected to further tests and then micro-CT scans, they have to be treated a second time with the tracer, a procedure that might cause an overestimation of gap progression. Even without tracers, a recent review by Contrepois et al., defined micro-CT as the only method that allows both a precise identification of critical gaps and sufficient measurements to define margin conditions [174]. Moreover, thanks to dedicated algorithms, micro-CT led to important advances in polymerization shrinkage assessment and 3D mapping of this important phenomenon [151],[175],[176]. However, even if micro-CT showed amazing results and several advantages, it must be considered that the majority of papers evaluate dataset only through axial slicing and manual measurements [177] This can lead to over or under-estimations, since the interfacial surface is only analyzed in a few points, and to operator bias since the measurements are manually taken from pixel to pixel. If we consider that it is defined "clinically acceptable" a gap range between 60 μm and 150 μm, and the voxel size of micro-CT is usually set between 8 μm − 15 μm, it is easy to assume that a slight mis-click during linear measurement might cross the line of what is considered acceptable and vice-versa. Moreover, if aging protocols are used to simulate clinical conditions, it is difficult to compare linear data before and after aging, since small misalignments might lead to evaluate different points of the interface, with obvious consequences on the results. Given these considerations, micro-CT seems to be the best method for TRI analysis, but still need improvements in terms of analysis workflow. Even the usage of 3D workflows is still under development in terms of data interpretation, since there are few studies in literature to compare with. Moreover, the 3D analysis is able to detect all defects, but specific information about the defect geometry (e.g. width, depth..) must be manually assessed. Figure 11 shows conventional micro-CT images of an overlay restoration at baseline, after a first chewing simulation that caused heavy occlusal wear and after fracture due to second chewing simulation. It is noticeable how images are fairly aligned. Images can then be precisely segmented for further 3D rendering or analysis (e.g. Finite Element Analysis).

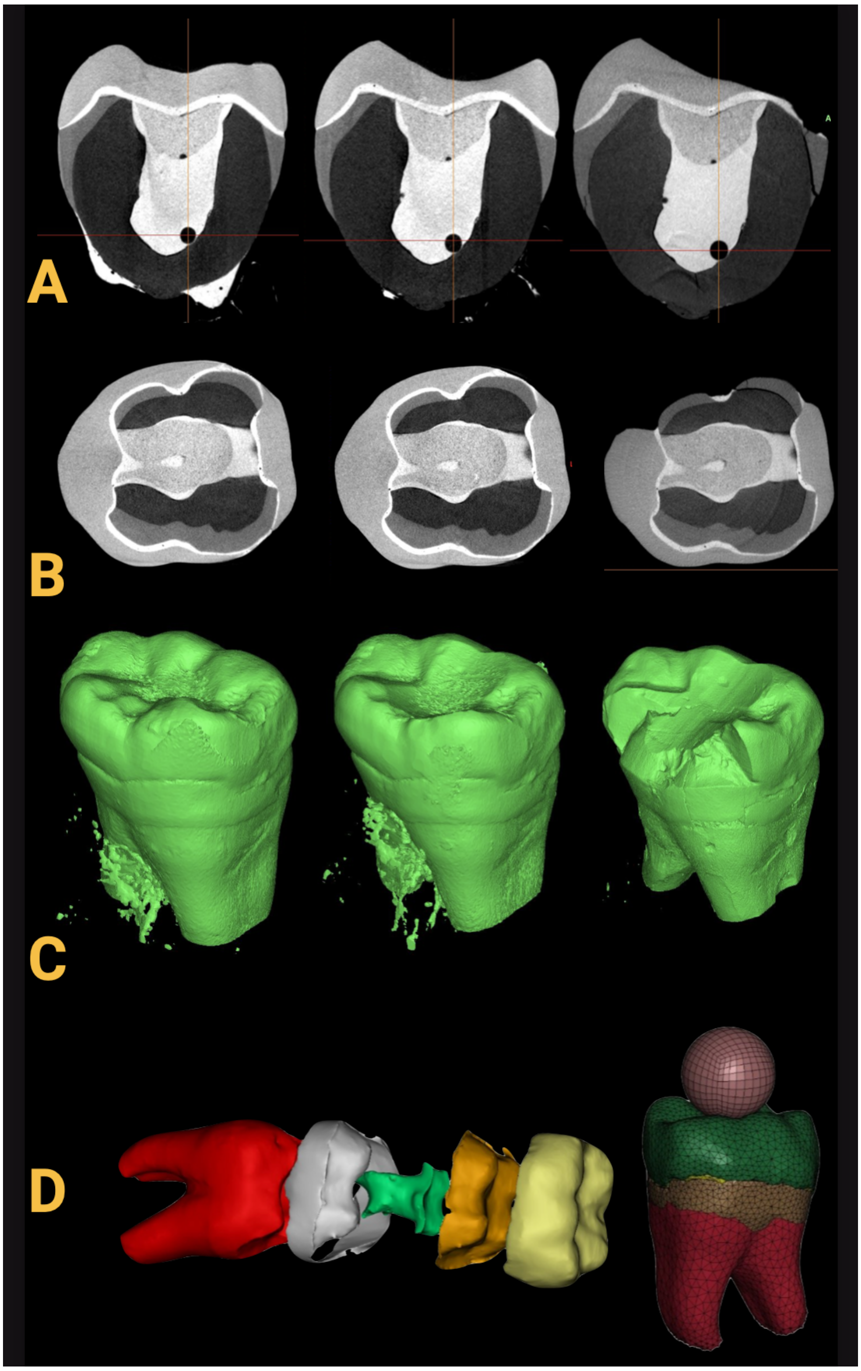

Figure 11. Micro-CT images of an overlay restoration, axial view (11A), coronal view (11B) and 3D rendering (11C). Left images represent baseline, central images the same sample after chewing simulation (see occlusal wear) and right images sample after fatigue failure. Images are well aligned and can be furtherly processed and segmented for other analyses (11D).

Another limitation of micro-CT imaging is related to the severe scattering caused by high-radiodensity structures, such as gutta-percha, zirconia and metals. These artifacts might be not relevant for a general analysis of the sample, but crucial if next to the interfacial area of interest. Most of these artifacts can be minimized optimizing both acquisition (with higher x-rays intensity) and reconstruction (using dedicated algorithms), as shown in Figure 12. Automatic workflows, such as 3D segmentation of the TRI, highly suffer from this kind of artifacts, since the software is not able to discern them from real gaps. Operator experience, in such cases, plays an important role in evaluating the outcome of the procedure.

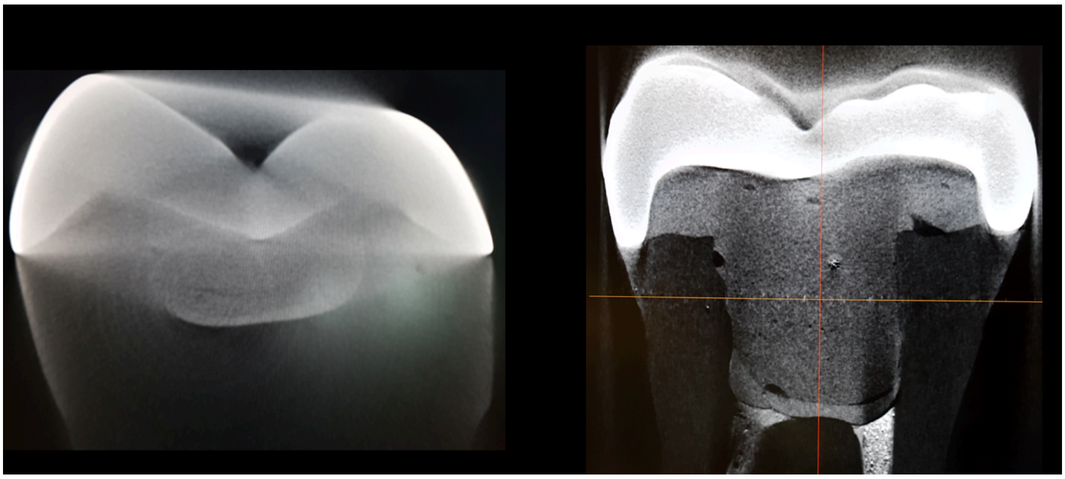

Figure 12. Micro-CT images of the same zirconia restoration, before (left image) and after (right image) optimization. In this case, the point of interest was the cervical and internal area of the restorative material. Thus, sample was positioned properly in order to have artifacts in the occlusal area rather than cervical area. Additionally, 0.5 mm Alluminum and 1 mm Copper filters were applied in the acquisition and a beam-hardening correction was applied in reconstruction (Nrecon, Bruker).

1.9 Available methods for artificial aging

According to what discussed up to this point, material sealing and bonding properties are surely important at baseline, but the majority of interfacial failures occur under sub-critical loadings due to progressive TRI and restorative material weakening for both resin-based materials and ceramic materials [156],[178]. Indeed, it was highlighted in a recent review that fatigue parameters obtained from cyclic loading experiments are more reliable predictors of the performance of contemporary restorative materials rather than quasi-static tests, such as fracture resistance test [157]. This was confirmed in a review conducted by the Academy of Dental Materials that aimed to establish guidance about mechanical properties of composite resins [179]. This review ranked the available mechanical tests, from the most significant to the least significant, in terms of being the most useful, applicable, supported by the literature and with a correlation with clinical findings. Results showed that fatigue was ranked in the highest priority group, and it was stated that "it may be the most important property for dental materials that are exposed to periods of cyclic loading while chewing food". Indeed, damage accumulation over time produced by subcritical forces, can propagate cracks or initiate new defects in areas of high stress concentration, such as sharp edges or imperfections. These defects and cracks can then propagate towards the TRI, ultimately leading to restoration fracture or adhesive TRI gap and failure. Another review specifically focused on adhesive interface degradation confirmed that the main mechanisms of TRI degradation are chemical, mechanical and thermal [180]. Chemically, the exposure to water, saliva and enzymes progressively causes hydrolysis and plasticizing of the resin components, with subsequent microleakage and gap formation. This happens both at the external interface and the internal one through nanoleakage and MMP activity that have been previously described. Mechanically, chewing cycles can cause stress concentration, in particular spots, higher than the interfacial fracture strength, leading to crack initiation and propagation. Finally, thermal variations due to diet can cause water sorption and expansion/contraction stresses. A 2022 review also highlighted that, while in sound teeth loads are transmitted mainly as compressive forces inside the tooth structure, restored teeth also develop tensile and shear stresses along the TRI [181]. This is related to the different elastic properties between restorative materials and tooth structure, coupled with the complex geometry of the restorations.

Given these assumptions it is clear that the artificial aging protocol to which the samples will be subjected to, is crucial to test TRI durability over time and obtain clinically-relevant results. Among artificial aging methods, different storage, mechanical and thermal protocols are provided by literature.

Aging by storage has been used for several years, with the only side effect of being severely time-consuming. The principles are again water sorption, nanoleakage and hydrolyzation. The different available protocols have been summarized in a 2007 review and will now be illustrated [180]. Available solutions for these protocols include water combined with an antimicrobial agent (e.g., 0.5% chloramine T) and 10% NaOCl solutions. Time of storage can vary from months to years, and is accelerated by solution routine refresh and sample sectioning. NaOCl solutions are described as a quicker method to simulate the degradation, by the removal of organic components through oxidation and fragmentation of peptide chains. When using NaOCl solutions, 1 hour seems to be not enough to affect bond strength values and hybrid layer, while 5 hours are effective on micro-tensile tests. However, NaOCl is responsible only of the chemical degradation of the organic content, and has little effect on resin degradation, which is essentially related to water uptake, plasticization and chemical decomposition. It is worth to mention that all these systems are efficient in degrading bonded TRI, even if they do not simulate enzymatic activity of saliva. For this reason, processed human saliva has been applied too, but its enzymatic activity is short lived, making the replenishment frequent and hard to handle [182].

Storage is crucial also for mechanical tests, that will now be extensively discussed. In fact, static liquid storage could influence, through polymer degradation, the response of the TRI to cyclic loading. Storage time prior to mechanical tests has been reported ranging from 24 hours to 7days, with distilled water as common medium [181]. The usage of saliva might elute more filler particle, leading to an inferior TRI performance to the test.

Aging through mechanical tests can be divided in non-anatomical sample tests, that have the advantage of being standardized, and anatomical sample tests, with the advantage of being more realistic in stress distribution.

Among non-anatomical sample mechanical fatigue tests, the Academy of Dental Materials illustrated the fatigue resistance (fatigue limit) and the fatigue strength (staircase method) tests [179]. These tests are meaningful to determine the intrinsic properties of resin-based direct and indirect restorative materials, however no information can be drowned about how their interfacial behavior. The fatigue resistance test consists in applying a force in the middle of a dumbbell-shaped sample, with a frequency representing its usage condition (1-2 Hz). The initially applied stresses are near the tensile stress of the material, and the test proceeds until failure. The test is then repeated with lower loads, progressively plotting stresses and cycles to failure, until the specimen is cycled for 100.000 cycles. The fatigue strength test consists in applying a force in 3-4 points of a beam-shaped sample.

The frequency is again 1-2 Hz, while the initial load is approximately ½ of the fracture strength of the material, and the number of cycles is 5.000-10.000. If the sample survive, a second new specimen is tested in the same conditions with a 5% higher force and vice versa. After 20-30 samples, the fatigue strength is plotted as the average of the applied stresses that did not cause a failure. This method determinates the number of cycles representative of the sample life, but the test is not conducted in a wide force range or extreme stress values, which could mask real material performance [183].

Among anatomical sample mechanical aging protocols, chewing simulation is the most common. Anatomical chewing simulation has the advantage of having a real TRI, constituted by an adhesive or a cement system, connecting the restoration to the substrate. This fact makes this type of test suitable for both material and TRI analyses, while also better reproducing occlusal cyclic forces of function and parafunctional activities. When load is applied, compressive stress is created in correspondence of the loading points, while, due to flexion, tensile and shear stresses are generated on opposite ends of the tooth, along the TRI [180]. According to Frankenberger and Tay, compressive forces might squeeze preexisting water channels out of the interface decreasing nanoleakage and vice-versa, concluding that the complexity of this mechanism are still to be investigated [184]. These forces, due to elastic mismatch between dental tissues and restorative materials, create stresses at the TRI, disturbing the demineralization-remineralization equilibrium and indirectly promoting the dissolution of enamel and dentin through a pumping action of cariogenic fluids in and out of gaps and increased water sorption in preexisting water channels within the adhesive system [124],[180]. Furthermore, loads directly influence the physicochemical behavior of dental hard tissues by inducing complex strain and stress fields on the crystal scale, promoting the dissolution of the apatite [124] and significantly influencing bond-strength [37]. However, different mechanical aging protocols are described in literature and, as a consequence, there is severe heterogeneity in terms of results [113]. A 2020 paper by Valero et al. tried, with a systematic review on chewing simulators, to assess the reality and reproducibility of in vitro studies using this technique. The concept behind chewing simulator is the attempt to recreate mastication patterns as much as possible, both in terms of direction and forces. These machines can analyze implants, restorations, fixed prostheses and jaw models, using as antagonist natural teeth or spheres of different materials and diameters. Unfortunately, high variability was reported in this review as well, with several different setup in terms of sample shape, sample materials, antagonist and periodontal ligament simulation. Moreover, chewing simulators can be programmed in the three axes depending on their constructive characteristics, meaning that they can simulate a 3D chewing cycle, a vertical compression only, an eccentric or lateral force only or a combination of vertical-lateral forces without the 3D motion. In addition, load sensors should be equipped to better control forces transmitted through the axes and a medium can be applied to simulate the wet oral environment (water, saliva, abrasive/erosive medium). Talking about load entities, maximum voluntary bite forces have been reported to be around 150-650 N, depending on measurement location, age, gender and type of measurement used [185]. However, teeth are rarely subjected to such forces: during chewing function forces are high, but the interposed food acts like a cushion, during swelling (about 2000 times per day) the contact is much lighter, during bruxism forces can be heavy and prolonged over time, but follow a grinding pattern. A review by Naumann et al. tried in 2009 to summarize the test parameters appliable for post-endodontic restorations [186]. Even if this review focused on static tests, it also reported some dynamic protocols, stating that "at least 100.000-1.000.000 cycles are necessary" to create fatigue weakening. A simulation of 240.000 cycles at 50 N was assumed to correspond with 1 year of clinical service, while 1.200.000 cycles correspond to 5-years, even if this correlation seems dependent from the tested type of restoration. Other studies report similar data, with around 300.00 cycles corresponding to a year on average [182]. A 2022 review by Lima et al., reported that 1.2 million cycles is the number of mechanical cycles most often used [181]. Studies used forces ranging 30–250 N (average 50-90 N [185]), with a frequency of 0.5-2 Hz (0.5 Hz closer to in-vivo [180]) and general poor standardization. Coherently, a 2022 narrative review reported that loads of 30-250 N, cycles from 50.000 to 5 million, frequencies from 0.5 to 3 cycles per second can be generally considered clinically representative [187]. It is important to underline, as reported in a 2022 review, that adopting higher frequencies to speed up the process can influence crack propagation and interfacial gap exposure to environmental attack [183]. The effect of different frequencies depends on tested material, so the authors suggest to "carefully consider" that changing them can lower test predictability of in-vivo behavior. The same review also explores the topic of the load applicators, suggesting to properly consider material, shape and diameter of the applicator according to the aim of the tested scenario. Depending on the sample-antagonist configuration, according to Lima et al., different types of stresses can be generated [181]. The starting point is commonly a tripod contact (mesiobuccal, distobuccal and lingual cusps), with load applied from the central fossa eccentrically on lingual cusps. Another setup consists in applying the load from the lingual surface, at variable angulation (30-105°) to the antagonist.

Another possible anatomical mechanical approach derived from staircase method, is the one proposed by Strand et al., in which a gradual cycling force of 50 N is used with a frequency of 2 Hz for 500 cycles, increasing of 50N and 500 cycles until failure [187]. This accelerated fatigue method is overall less time consuming, but it is possible to test only one specimen at a time and it is focused on material failure rather than TRI degradation, unless multiple analyses are carried out during the procedure with non-destructive methods.

A third option to reproduce the cyclic stresses the interface is subjected to, consists in thermocycling. In fact, the linear coefficient of thermal expansion is severely different between the restorative material and the tooth [188]. Thermocycles can possibly cause crack initiation or propagation in the TRI, with subsequent gap formation and water absorption [180]. Clinically speaking, this also reproduces thermal shocks that might occur due to cold/hot foods and beverages associated with modern diets. It was also reported that hot temperatures accelerate interfacial hydrolysis of the TRI, facilitating water uptake and increasing the aging of the sample [156]. According to a review [180], cycling number ranges from 100 to 50.000, making results hard to compare. It is estimated that 10.000 cycles correspond to 1 year of clinical function (20-50 times a day), making the ISO standard 500 cycle insufficient to simulate long-term TRI durability [180],[182]. It was also observed, in a 2022 review, that at least 30.000 cycles are needed to affect the flexural properties of RBC and therefore [176]. Regarding bath temperatures, a range between 0°C and 67° C can be acceptable to simulate diet intake [182]. Most studies are nowadays using 5°C-55° C, a range that well reproduces the temperatures actually occurring in the oral cavity and reflect ISO recommendations [189]. Concerning bath time, ISO standard reports that it should be at least 20 s, with most studies ranging from 15 s to 60 s (even from 10 s to 240 s according to a 2011 meta-analysis [189]). However, it has been pointed out that patients cannot tolerate long direct contact with extremely cold/hot foods, making a dwell time of 15 s more recommendable to simulate clinical conditions, and longer times better to create higher stresses. In between baths, it has been suggested an intermediate bath at 37° C or an interval ranging 3-15 s, with shorter times better reproducing the abrupt changes that occur in-vivo.

Last but not the least, it must be highlighted that most of these tests can be performed in different medium and, consequently, different pH. It has already been demonstrated that acidic environments cause greater damage to RBC compared to distilled water or artificial saliva [190]. However little information concerns the influence of pH cycling on the TRI degradation [180]. It seems that water droplets increase after acidic treatment, augmenting hydrolysis and consequently matrix decomposition. Moreover, at the TRI, a low pH also damages the mineral part at the restoration margin, enhancing gap formation and flow of fluids. Further studies should provide additional information on cyclic-pH aging methods before creating standardized in-vitro protocols.

1.10 Aim of the PhD thesis

According to the present narrative literature review, degradation of the TRI has been widely demonstrated to be a key point in restorative dentistry future improvements. As a matter of facts, the biological substrate topic is strictly related to clinical procedures and patient-specific factors that cannot be changed through bioengineering. Adhesive systems, both for direct and indirect purposes, could be improved, but they have already been widely studied and literature is clear about their pros, cons, recommendations and protocols. On the other hand, recently introduced restorative materials, such as milled ceramics and newer RBC still severely lack literature data about their interfacial performance. This is particularly true if we consider that most standard tests are performed without simulating clinical-like scenarios.

It is therefore crucial to combine proper in-vitro aging tests, aiming to thermo-mechanically simulate oral environment, with TRI progressive analysis using non-destructive methods with low operator bias, such as micro-CT. This could consent to appreciate a 360° biomechanical evaluation of the tooth-restoration complex, based on which material optimization can be developed.

Thus, the present PhD thesis aimed to expand knowledge about restorative materials interfacial behavior, in particular when subjected to chewing simulation, through micro-CT 3D interfacial analysis.

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2. Research studies

2.1 Decision making process

The first step of my PhD course consisted in the optimization of the conventional (linear) micro-CT workflow (see paragraph 2.2). The aim was making the process faster, reducing operator bias and improving the effectiveness of the analysis. In order to do that, a segmentation software was applied to micro-CT dataset, with the aim of obtaining 3D data regarding the TRI integrity. 3D analysis allowed to highlight clinically relevant situations that were underestimated or undetected by the conventional analysis. Moreover, the workflow was faster, standardized and allowed to export the volumes for further evaluations and comparison with after-aging results. On the other hand, conventional linear analysis was able to better describe the morphology of the gap, with some limitations correlated to the number of points selected for the analysis.

Given these premises, the second step consisted in validation, applying the developed 3D workflow to analyze a simple clinical scenario in an exploratory study (see paragraph 2.3). Class I cavities and well-known commercial bulk, ormocer and nanohybrid materials were selected, in order to understand if obtained 3D results were consistent, in terms of statistical trend, with other literature papers that used similar procedures. Results confirmed that the protocol was effective. Thus, it was decided to test different direct scenarios before and after cyclic fatigue, to quantify TRI progressive degradation.

Three studies were structured to analyze different modern direct materials. The concept was to understand if some chemical composition or mechanical reinforcement could lead to superior TRI behavior compared to others. All materials were tested in clinical-simulated scenarios, according to their indications, and analyzed before and after aging. A first study focused on material viscosity and filler loading, applied in a deep margin elevation scenario (see paragraph 2.4). Tested materials included nanohybrid flowable and packable RBC, nanohybrid flowable and packable ormocers, nano-filled RBC and nano-filled bulk RBC. The second study concentrated on the possible effects of including glass fibers inside the material itself (see paragraph 2.5). Similarly, the third study analyzed the effects of introducing vertical bundle fibers or fiber-posts inside the restorative material as a core to the restoration (see paragraph 2.6).

With the same conceptualization, two additional studies were structured to test different materials for indirect restorations. Again, the aim was to investigate TRI behavior before and after fatigue, to assess if any material could be superior both at baseline or after aging. Indeed, Young modulus and adhesive ability could influence the interface severely. Materials tested covered the most important categories available in commerce: milled RBC, lithium silicate, reinforced lithium silicate and high translucency zirconia (see paragraphs 2.7 and 2.8).

Lastly, using the same workflow of micro-CT data segmentation, a finite element model of a tooth-restoration complex was created and validated. In this way, different material combinations were assessed in order to improve knowledge about the interfacial stress distribution (see paragraph 2.9). The idea was to optimize the stress at the TRI when different materials are combined, for example utilizing or not an elastic material as a liner.

2.2 Application of a 3D segmentation software to micro-CT imaging of dental materials interfaces

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Abstract

Objectives: In order to analyze the interfacial behavior of dental materials, micro-CT imaging has been widely applied in research, since it allows to obtain non-destructive images with high resolution. However, most of the papers analyze the obtained volumes through 2D-sectioning and linear manual measurements. This paper aimed to be an exploratory study that applies a 3D segmentation software to micro-CT dataset in order to achieve a comprehensive analysis of the interface and compare it with conventional measurements.

Materials and Methods: Twenty teeth were prepared, crowned and scanned with micro-CT before and after artificial aging in order to evaluate the marginal gap presence and progression. Conventional linear analysis consisted in four manual linear measurements in sixteen marginal points. On the other hand, 3D segmentation was performed to automatically detect the volume representing discontinuity along the tooth- restoration complex.

Results: Data showed no correspondence between obtained results when comparing volumetric and linear analyses. Linear measurements allowed a better characterization of the discrepancy in terms of vertical and horizontal extension. On contrary, 3D segmentation workflow was standardized and less time-consuming.

Conclusions: Severe yet localized interfacial failures were detected only by 3D evaluation. Further studies are necessary in order to better evaluate the accuracy of the procedure, which showed promising results.

Introduction

One of the most common procedures in restorative dentistry is the manufacturing of indirect restorations that are used to strengthen the residual tooth structure and avoid catastrophic fractures [1]. In order to match the clinical need of better mechanical properties and aesthetic, dental materials have been evolving in the last decade towards an adhesive, minimally invasive, yet operator-dependent approach [2]–[5].

However, even if modern materials are extremely performing compared to outdated materials, both in terms of aesthetic and mechanical properties, the interface between the tooth and the restoration is still considered a critical point, subjected to failures correlated to the precision in their margins [6]. Nevertheless, restorative interfaces are subjected to heavy loads, temperature fluctuations and biochemical damage. Over time, these stimuli lead to interfacial gap formation, which is clinically recorded as a marginal discontinuity between the tooth and the restoration that ultimately leads to the clinical failure of the restoration itself [7]. It is therefore not surprising that dental research focused its attention on dental material interfaces with many different techniques [8].

Traditional in vitro methods to detect microleakage and gap in the tooth-restoration interface involve the use of organic dyes in conjunction with microscopy techniques or scanning electron microscopy (SEM) [9], [10]. After the sample has been infiltrated, linear measurements are taken by an operator, in order to determine, which percentage of the interface was dyed (usually graded from (0 − 25)% to (75 − 100)%). The disadvantages of these analyses include semi-quantitative results, operator bias and limited ability to represent the complex geometry of the marginal area [11]. Moreover, the usage of SEM to determine the presence of internal and marginal gaps requires sample sectioning, which eliminates the possibility of evaluating the effects of artificial aging on samples after a baseline analysis. To obviate this problem, epoxy replicas have been proposed, but it can only allow the analysis of the external margin. Moreover, such results can be affected by the accuracy of the impression, adding another bias to the procedure [12].

More recently, non-contact digitalization and measuring techniques, like computed tomography, 3D scanners and photogrammetry are becoming often applied in several fields, as automotive, industry, reverse engineering, architecture, cultural heritage and medicine [13]–[16]. Among these, in the dentistry field, tomography techniques, including optical coherence tomography (OCT) and micro computed tomography (micro-CT) have been employed to evaluate interfacial adaptation in composite resins and ceramics. OCT provides cross-sectional images by quantifying the reflection of light from structures [17]. On the other hand, micro-CT uses multiple x-ray exposures with sample rotation to acquire the desired volume that can be subsequently reconstructed and sliced [18]. The resolution of OCT is around 10 μm − 15 μm, which is more than current clinical CT images [19], but less than micro- CT (2 μm or less with novel nano-CT) [20]. Moreover, OCT is not able to reach high depth maintaining its high resolution, therefore Micro-CT has been applied as a gold standard for internal adaptation analyses in literature [21].

Micro-CT also has the advantages of being non-destructive, quantitative and has the opportunity to reconstruct the full 3D volume. These features made this method markedly more comprehensive and therefore frequently employed for interfacial analysis of dental materials [22]. In a recent review by Contrepois et al., micro-CT was defined the only method that allows both a precise identification of critical gaps and sufficient measurements to define margin conditions [21]. However, even if micro-CT showed interesting results and advantages, the majority of papers evaluate dataset only through axial slicing and manual measurements, as shown by Pelekanos et al. [23]. This might lead to over or underestimations, since the interfacial surface is only analyzed in a few points, and to operator bias since the measurements are manually taken from pixel to pixel. If we consider that it is defined "clinically acceptable" a gap range between 60 μm and 150 μm [24], and the voxel size of micro-CT is usually set between 8 μm − 15 μm, it is easy to assume that a slight mis-click during linear measurement might cross the line of what is considered acceptable and vice-versa. Moreover, if aging protocols are used to simulate clinical conditions, it is difficult to compare linear data before and after aging, since small mis-alignments might lead to evaluate different points of the interface, with obvious consequences on the results. As a direct consequence, some authors are trying to evaluate and export the interfacial gap volume to achieve a comprehensive evaluation of the interface, also with the advantage of being able to better align baseline and after-aging results [25]–[27].

The present study aimed to be an exploratory study that applied a standardized workflow through a 3D segmentation software to micro-CT dataset in order to achieve a comprehensive analysis of the interfacial gap volume. The first aim was comparing the results trend with the conventional linear workflow. The second aim was evaluating if the progression of gap due to aging is better detected by either of the workflows. Tested null hypotheses were that (1) there are no significant differences between workflows in terms of obtained results trend and (2) both the workflows are equally able to detect interfacial gaps progression after aging.

Materials & Methods

Twenty sound single rooted teeth (n = 20) extracted for periodontal reasons within 3 months, were selected and stored in 0.5% chloramine solution at 4° C. The sample size was calculated based on previous studies and accordingly to the fact that this was an exploratory study. After debridement, the following inclusion criteria were applied: no carious lesions, demineralization, abrasions or cracks under 6× optical magnification with transillumination, intact cement-enamel junction (CEJ).

Specimens were prepared for crown restorations by the same expert operator (>10years of experience in restorative field). A chamfer finishing line was positioned 1 mm above the CEJ, following its shape. A common clinical workflow was applied in order to obtain the restorations. Specimens were scanned with an intraoral scanning system (Cerec Omnicam, Dentsply Sirona) at maximum resolution, in order to digitalize the dental surface. The restorations were designed by a dedicated computer assisted design (CAD) software (Cerec System 5.1, Dentsply Sirona), following manufacturer instructions with 1 mm thickness to optimize cement properties [28]. Finally, crowns were milled (Cerec MC XL, Dentsply Sirona) at default settings using a glass ceramic material commonly employed (lithium disilicate, CeltraDUO, Dentsply Sirona). Crowns were then sintered, glazed and polished according to material instructions in the dedicated system (Cerec Speedfire, Dentsply Sirona). This entire procedure has been extensively described in literature and confirmed to be clinically acceptable. It is worth to mention that during the CAD phase, the software automatically arranges an 80 μm spacing in the occlusal and axial walls of the crown that will be occupied by the resin cement layer, while in the chamfer area (also known as the marginal area) no spacing is given in order to optimize the precision of the restoration.

A universal dual-curing cement (G-Cem One, GC) was applied in order to lute the restoration to the prepared tooth. The correct pretreatment to improve adhesive cement performances was performed on both restoration and tooth substrates. The crown was treated as follows: hydrofluoridric acid 9.6% for 30 s, five-minute ultrasonic bath in 98% alcohol, dry, double-layer ceramic primer application (G-Multi Primer, GC, Tokyo, Japan). The tooth was treated with sandblasting (1 cm, 1.5 bar) with 30 μm aluminum oxide powder and peripheral enamel etching with orthophosphoric acid 35% for 20 s, then rinse and dry.

After the proper crown setting, cement excesses were removed. Then, after 60 s of chemical cure stabilization, light curing was performed with a light emission diode (LED) lamp (Cefalux 2, Voco) at 1400 mW/cm2 for 2 min. The luting and curing processes were performed with the sample positioned in a gypsum model in order to simulate the clinical condition. The curing-light tip was positioned in contact with the buccal, incisal and oral surfaces. Irradiance of light-curing unite was monitored periodically with a radiometer (CM-2500, DEI Italia). All samples were confirmed to be clinically acceptable by the same expert operator.

All samples were subjected to a Micro-CT scan (SkyScan 1172 Micro-CT, Bruker), with the following parameters: voltage= 100 kV; current = 100 μA; aluminum and copper (Al+Cu) filter; pixel size = 10 μm; averaging = 5; rotation step = 0.6◦. Images were reconstructed (Nrecon, Bruker) in order to obtain DICOM files, with standardized parameters: beam hardening correction = 15%, smoothing = 2, ring artifact reduction = 9. To reveal interfacial gap progression between the tooth and the crown after artificial aging, specimens were also subjected to a second scan, with the same baseline parameters, after a standard thermal treatment.

After the first micro-CT scan, all samples were subjected to artificial aging through a thermal treatment. The treatment consisted in 10.000 cycles between 5 ◦C and 55 ◦C in distilled water, with a dwell time of 1 min each. This standardized protocol was taken from literature, to have consistency with well-known results [29].

Since the present study also evaluated the interfacial gap progression with artificial aging, in order to perform the conventional linear analysis on the same point before and after the test, baseline data and post-aging data had to be aligned. As a matter of facts, it is nearly impossible to physically position a sample in the exact same position before and after artificial aging in the micro-CT chamber. Therefore, baseline and after-ageing reconstructed dataset were also digitally aligned (DataViewer TM, Bruker). After initial manual alignment, the “3D registration” function was used. This function uses a best-fit algorithm to optimize the superimposition. The process was repeated twice and checked by an expert operator, that confirmed it was successful before proceeding to further analyses.

Reconstructed files of each scan were imported into the segmentation software, that also allows for linear measurements, as shown in Fig. 1 (Mimics Medical 24.0, Materialise). Samples were oriented according to their position during the scanning procedure, with the incisal area up and the root down. A total of eight slices, four in ZY and four in ZX planes, were created forming a uniform grill. This allowed to analyze the marginal area in a total of sixteen points.

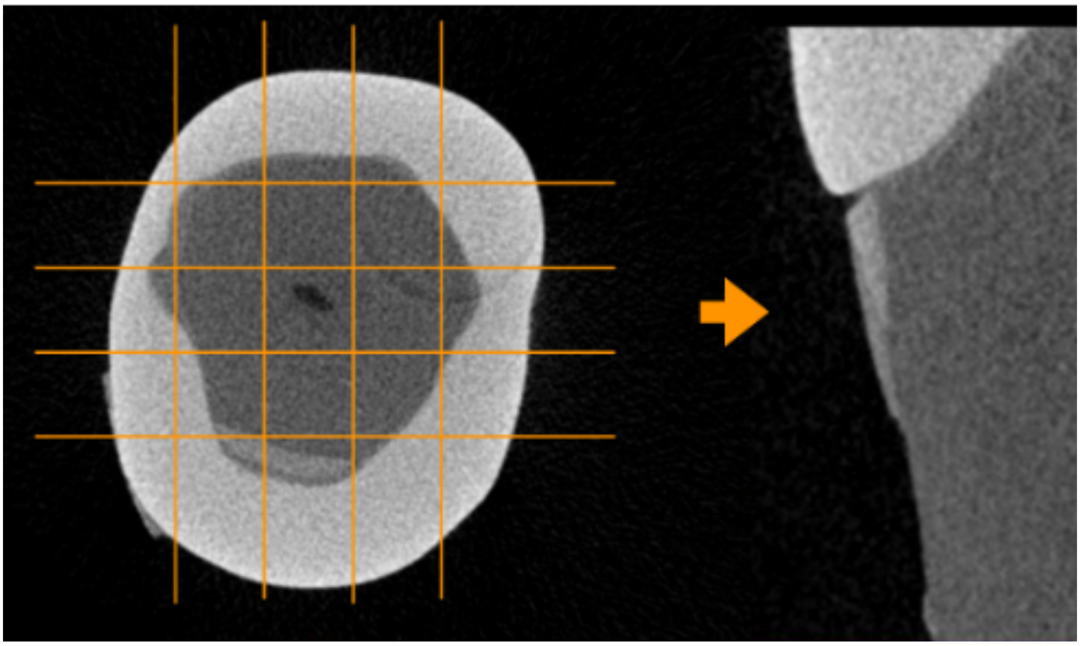

Fig. 1. The sample was divided, with a 4x4 grill properly scaled, in a total of eight slices (16 points of the margin) as shown in the left picture. An example of the obtained respective axial image in a single point is reported in the right picture.

Data collection was performed accordingly to previous papers protocol in order to achieve consistency with literature data [23], [30], as shown in Figure 2.

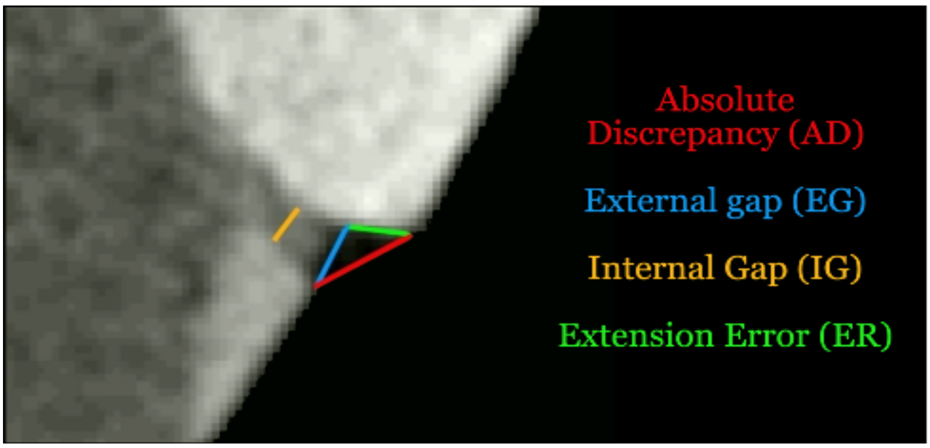

Fig. 2. Graphical summary of linear measurements that were performed (400% magnification)

The following measurements were defined and recorded for each interface point:

- External gap (EG): perpendicular distance between the external point of the tooth margin and the crown
- Internal gap (IG): perpendicular distance between the tooth surface and the crown surface measured in the internal area (0.5 mm from the external point of the tooth margin)
- Extension error (ER), also known as over/under contouring: distance between the external point of the crown margin and the perpendicular projection on the crown counter of the external point of the tooth margin
- Absolute discrepancy (AD): distance between the external point of the tooth margin and the external point of the crown margin. It is also defined as the angular combination of EG and ER

All measurements were performed by the same experienced operator at 400% magnification, with the “linear distance” software function applied manually. The function “angle” with setting “90°” was used in order to create the perpendicular projections. Data were collected, then an average value was calculated for the single parameters of each tooth.

Reconstructed files of each scan were imported a second time into the dedicated segmentation software (Mimics Medical 24.0, Materialise). The first step consisted in creating a region of interest (ROI) corresponding to the ideal scenario, in which there is a perfect continuity between the tooth and the prosthetic crown. In order to do that, thresholding was performed in order to select the four spikes of the Houndsfield scale (Hu) corresponding to dentin, enamel, cement and the lithium disilicate used for the restoration. The selected voxel volume was then modified through the following functions: “region growing”, “open = 4”, “wrap = 0.03, gap closing distance = 1 mm” and “reduce = 3, connectivity = 9”. The concept behind this process is that the gap is just a discontinuity in the theoretically perfect tooth-restoration surface. After that, a second thresholding was performed in order to select voxels corresponding to voids (Hu range: −1024 to −333). The mask was checked, in order to confirm that all marginal gaps were included. Then, boolean intersection was performed between the two masks, in order to separate the voxels representing voids inside the ROI. The final mask was then cropped with “region growing” function in order to comprehend only the tooth-restoration interface, since small discontinuities along the restoration are common and the software mis-include them into the gap volume. Fig. 3 reports the significant steps of the segmentation for clarification.

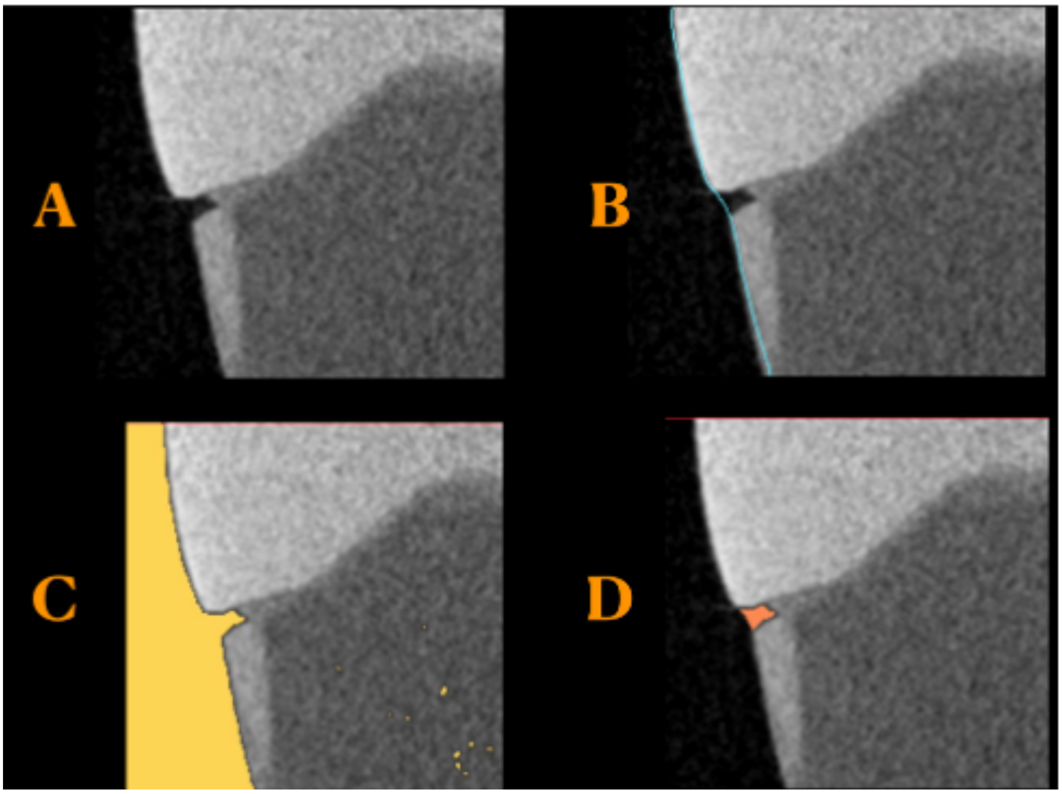

Fig. 3. Illustrate the process that was used to obtain the final gap volume. Fig. 3A represent the initial axial situation, where it is evident a marginal discontinuity. Fig. 3B shows the ROI that the software generated (light blue line). Fig. 3C represents the second thresholding that was meant to select the void voxels. Finally, Fig. 3D shows the intersection between the ROI and the void volume.

Furthermore, with the selected software, it was possible to export the final voxel volume representing the interfacial gap in standard tessellation language (.stl). The exporting parameters were optimal quality and sample rate 1:1. Then, through the "stl registration" function of the software it was possible to perform a superimposition of baseline gap volume and after-aging gap volume. This is particularly significant in order to understand, on the whole interface, where the aging had a significant effect, and to immediately link it to the respective 2D slice to eventually deeply analyze it. An example of this procedure is shown in Fig. 4.

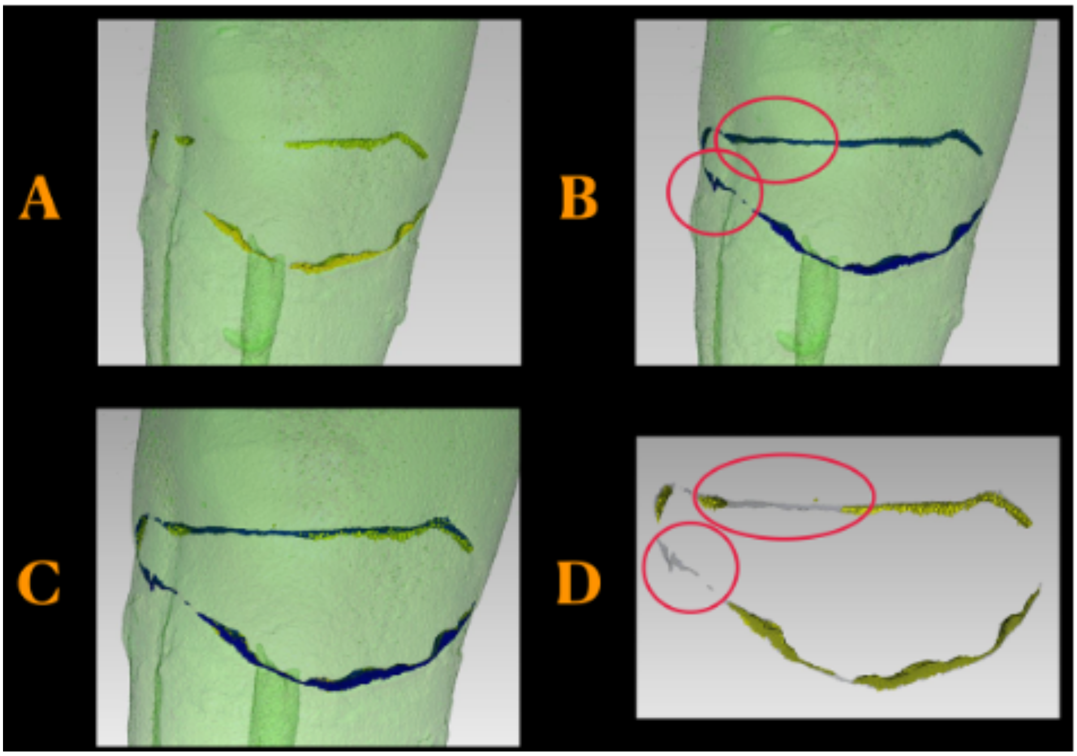

Fig. 4. Represent baseline (Fig. 4A) and after aging (Fig. 4B) gap volume. Green volume represents the tooth-restoration complex (in transparency), yellow volume represents baseline gap and blue volume after aging gap. Red circles highlight gap progression caused by the thermal process. Notice how it is possible to align these two volumes (Fig. 4C) for further analysis. A detail of Fig. 4C is reported in Fig. 4D.

It is worth to mention that this procedure sometime showed pixel noise or small artifacts. However, with the 3D preview, it was easy to detect and remove them. After this final check, through "volume information" function, the final volume of the interfacial gap was collected in mm^3.

In order to understand if the workflow is able to detect interfacial gap progression, a comparison between baseline and after-aging values was performed through a one-way analysis of variance (ANOVA). On the other hand, since data derived from the two workflows are not directly comparable, the trend was analyzed through interpolation of the obtained average values.

Results

Baseline and after-aging linear measurement results, expressed as average of each sample, are graphically reported in Fig. 5.

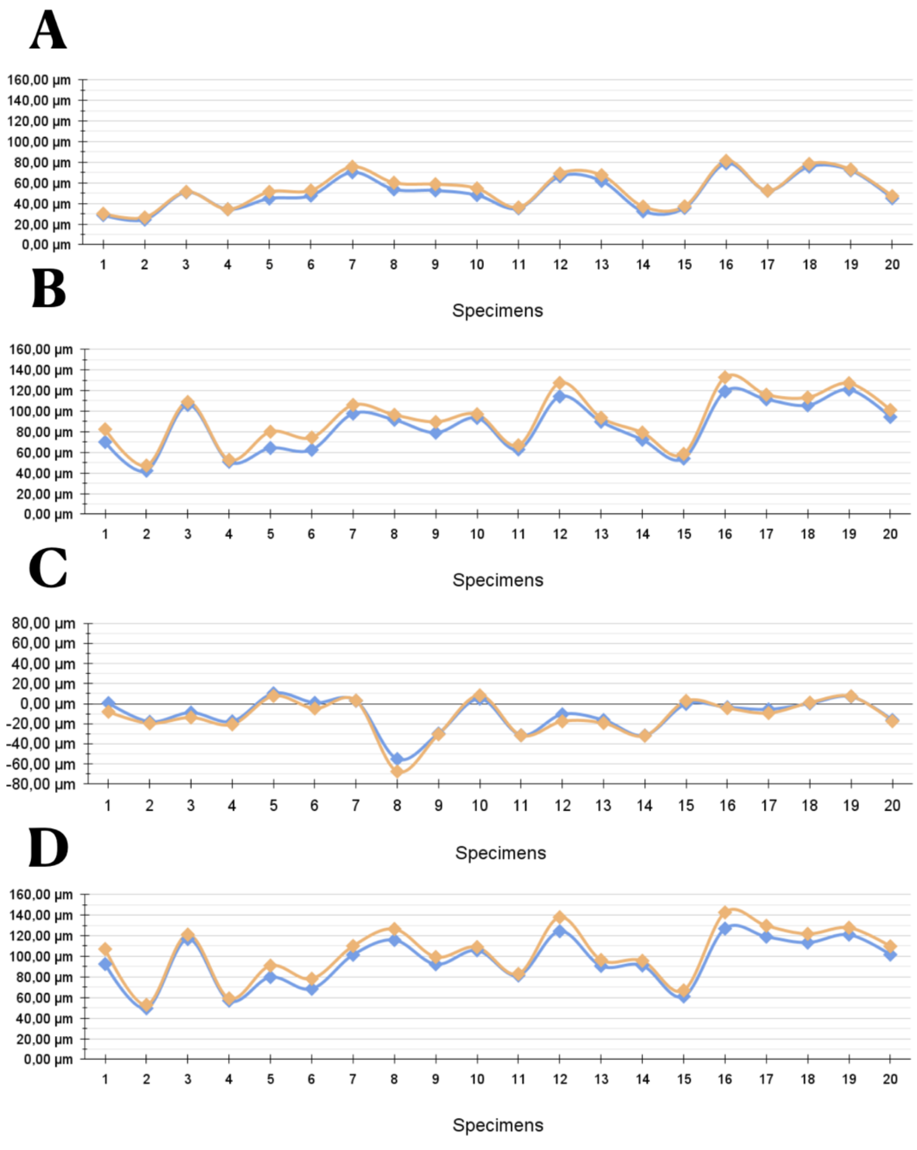

Fig. 5. Linear gap results expressed as average (µm), respectively for IG (Fig. 5A), EG (Fig. 5B), ER (Fig. 5C) and AD (Fig. 5D). The blue line represents the baseline value, while the orange line the after-aging value (post- thermocycling).

One way ANOVA test did not report any significant difference between before and after aging simulation for all the tested parameters (AD: $p = 0.61$; EG: $p = 0.54$, IG: $p = 0.74$; ER: $p = 0.89$).

Baseline and after-aging volumetric gap measurement results are graphically reported in Fig. 6. One way ANOVA test did not report any significant difference between before and after aging simulation ($p = 0.42$).

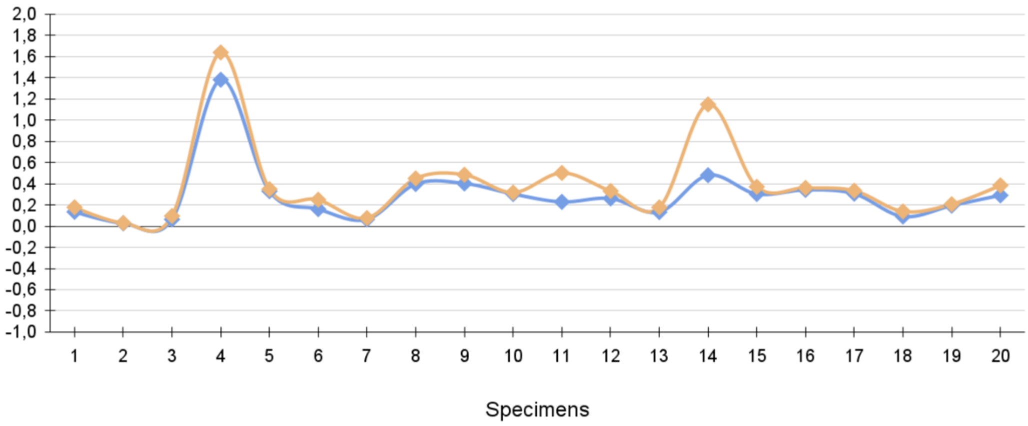

Fig. 6. Volumetric gap results (mm^3). The blue line represents the baseline value (pre-thermocycling), while the orange line the after-aging value (post- thermocycling).

Discussion

Obtained data brought to reject the first null hypothesis, since no correspondence in terms of trend was found between the tested volumetric analysis and the conventional linear measurements. This was not surprising, since a volume is hardly directly comparable with linear measurements of such a complex geometry, in according with previous studies [26], [27].

Results of linear analysis were comparable with the ones found in literature for similar procedures, with all the sample being clinically acceptable [31]. Generally speaking, volumetric data showed a more linear trend, with severe differences only for a few samples. This might be explained by the fact that volumetric analysis is more comprehensive, therefore results might reflect more the overall prosthetic procedure performance. Sample #4 volumetric results, both baseline and after-aging, were very different both from linear and other volumetric data. This was explained by the 3D preview of the void mask: even if the marginal area seemed intact externally, an interfacial cement bubble occurred from the margin through the restoration-crown interface as shown in Fig. 7.

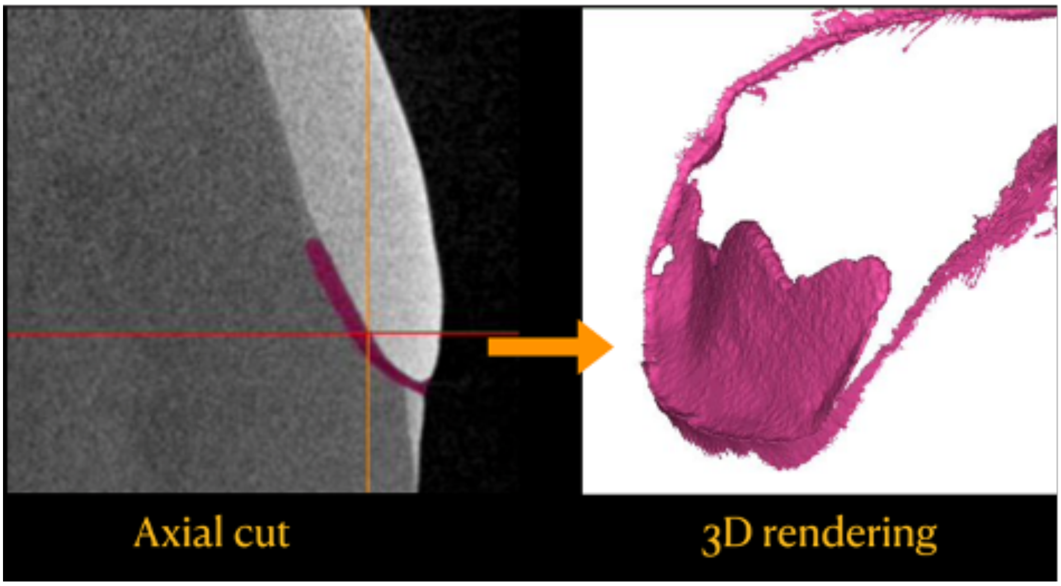

Fig. 7. Sample #4 analysis. Notice the bubble extension when 3D rendering is performed, compared to the simple measurement done in the axial cut.

During linear measurements this void was detected as well, but the IG parameter does not differentiate between cement layer thickness or void. On the other hand, during the 3D procedure, this void was included, massively changing the gap volume. From a clinical point of view, this sample should not be considered clinically acceptable, since the defect is a clear locus minoris resistenitae that will influence the performance of the restoration, thus it is important to use a measurement procedure with the ability to detect it.

Samples #11 and #14 showed a massive change in gap volume after thermocycling, that was not detected in linear measurements of the same samples, thus these specimens were investigated through 3D rendering. As shown in Fig. 8, it was possible to observe that these samples presented severe interfacial failures, with prosthetic crown marginal chipping. This aspect is clinically relevant and could be considered a crucial aspect of the analysis. However, due to the fact that this failure was localized in a limited area of the margin, the linear analysis probably did not detect, or at least under-estimated with the average calculation, the damage of the restorative interface.

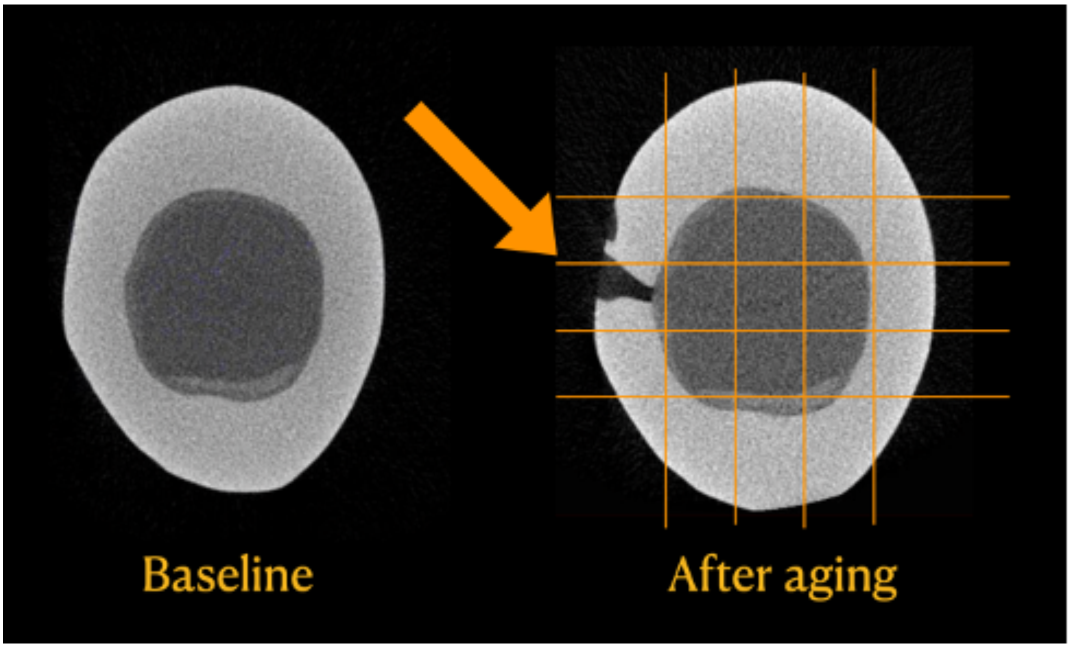

Fig. 8. Sample #14 baseline and after aging coronal view. It is possible to notice a severe fracture of the prosthetic margin that was highly underestimated by linear analysis.

Lastly, both the methods did not report significant differences between baseline and after-aging samples, thus the second null hypothesis was rejected. It is worth to mention that all samples showed a deterioration of marginal quality in terms of EG, IG, AD and gap volume, even if not statistically significant. This might be explained by the aging treatment performed, which might have been insufficient to cause a significant variation in terms of tested parameters. As suggested by Schmid-Schwap et al. In their meta-analysis, there is lack of standardization of experimental conditions to ensure comparability of various studies, thus it is hard to compare obtained results with other papers [32].

Conclusions

- 3D analysis allowed to highlight clinically relevant situations that were underestimated or undetected by the conventional analysis.

- 3D analysis workflow was faster and standardized and allowed to export the volumes for further evaluations and comparison with after-aging results.
- Conventional linear analysis was able to better describe the morphology of the gap, but with some limitations correlated to the number of points selected for the analysis.
- Thermal aging caused interfacial degradation that was highlighted by both procedures, even if not statistically significant.

Further studies are needed to evaluate the accuracy of the 3D procedure, which showed promising results and to assess the effect of thermal aging on interfacial gap behavior.

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2.3 Influence of curing mode and layering technique on the 3D interfacial gap of bulk-fill resin composites in deep class I restorations: a micro-CT volumetric study

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Abstract

Objectives: To evaluate 3D interfacial gap of bulk-fill composite resins, applied in deep class I restorations with different layering techniques and curing modes.

Methods: Ninety-six (n = 96) samples were prepared with standardized deep class I cavities and adhesive procedures. Four materials were tested: SDR (SDR), SonicFill2 (SF), Admira Fusion X-Tra (AFXT), Filtek Supreme XTE (FS). Four subgroups (n = 6) were created according to layering and curing techniques: 2+2 mm with soft start curing (SG1), 2+2 mm with conventional curing (SG2), 4 mm with soft start curing (SG3), 4 mm with conventional curing (SG4). All samples underwent micro-CT scans and afterwards, voids surrounding restorations underwent automatically thresholding procedure (Mimics, Materialise; Geomagic Studio 12, 3D Systems) in order to analyze 3D interfacial gap. Statistics were performed using three-way ANOVA with Tukey test (significance $p < 0.05$).

Results: Significant differences were reported between materials, layering techniques and their interaction. No significant differences were reported for polymerization mode. Bulk-fill materials showed average values of interfacial gap ranging from 0.031 mm^3 to 0.200 mm^3, while FS showed values ranging from 0.416 mm^3 to 1.200 mm^3.

Conclusions: All bulk-fill materials performed significantly better than FS ($p < 0.05$), without differences between each other. Curing mode did not influence volumetric interfacial gap presence in any group ($p > 0.05$), while layering

technique influenced volumetric interfacial gap presence only in FS group, performing better when incrementally applied. Regarding gap localization, the floor of the cavity resulted to be the area with the highest likelihood of gaps in all samples.

Clinical significance: Interfacial gaps in deep class I restorations, which mainly concentrate at the cavity floor, could be reduced with bulk-fill composites independently of the layering technique and the curing mode.

Introduction

Composite resins are widely employed in dentistry, especially for posterior direct restorations. However, their durability remains an issue (16). One of the main problems related to longevity is the volumetric contraction of composite resins, which is related to the conversion of monomer into polymer chains (6) and can cause clinical problems such as post-operative sensitivity, margin discoloration (33), enamel and dentin cracks (22) and interfacial gap formation (27). The data on the volumetric shrinkage of composite resins reported in the literature is highly variable, with a range of 1.35–7.1% and an average of 2–3% (21). This variability is related to the mechanical properties of the materials used, especially their viscosity, the quantity of monomer present, and the polymerization kinetics (41),(36),(43). The data on the shrinkage stress that the volumetric contraction can generate at the adhesive interface (18), which is the weakest area of the restoration (7), varies even more. Indeed, several factors can influence the quality of the tooth–restoration interface.

An important role in the tooth-restoration interface quality is played by the restorative material itself. Recently, bulk-fill composite materials have been introduced to increase the curing depth to up to 4 mm and minimize shrinkage stress. The manufacturers of these composite resins claim that the shrinkage stress of the materials is lower than that of either flowable or non-flowable traditional composites. Moorthy et al. reported that the minor shrinkage stress exhibited by bulk-fill flowable composites resulted in lower cuspal deflection compared to traditional composites fitted using an oblique layering technique (23). By contrast, an in vitro study by Furness et al. showed that flowable and non-flowable bulk-fill materials resulted in a similar proportion of gap-free external marginal interface as conventional composites (12). Yet, another paper by Oglakci et al. reported that different types of bulk-fill composite resins affected gap formation differently and that low-viscosity bulk-fill composites exhibited better adaptation to cavity walls (25).

Several layering techniques have also been proposed to optimize interfacial adaptation as much as possible. These include composite incremental layering techniques (8), (19) and the use of a liner with low elastic modulus (20). A recent study by Alqudaihi et al. reported that an incremental technique is crucial for achieving high adaptation and reducing gap formation, even when using new bulk-fill composite materials (3).

Light-curing modes can also affect polymerization kinetics (21). The process of composite resins polymerization involves a pre-gel and a post-gel phase. During the pre-gel phase, the reactive species can flow and undergo molecular rearrangement to compensate for the volumetric shrinkage without generating significant amounts of internal and interfacial stresses. When the resin reaches its post-gel phase, the formation of a semi-rigid polymer network hinders plastic deformation. The resin reaches a higher modulus of elasticity and transmits the stress generated by polymerization shrinkage to the tooth–restoration interface, potentially leading to several clinical disadvantages, such as postoperative sensitivity, microleakage, enamel cracking, cusp deflection and marginal gaps. It has been reported that soft start curing techniques lengthen the pre-gel phase, leading to a low monomer conversion rate, thus increasing material flow and improving shrinkage behavior and marginal adaptation (42), (28).

Despite the existence of many papers on bulk-fill materials, there is no consensus on how they behave compared to traditional composites with regards to volumetric interfacial gaps presence. Moreover, little is known about the influence of horizontal or bulk layering strategies and conventional vs soft start curing modes on three-dimensional (3D) interfacial gap presence in cavities restored using bulk composites. Thus, the aim of the present in vitro study is to evaluate 3D interfacial gap presence in deep class I cavities restored with different bulk materials, incremental layering strategies, and curing modes.

The null hypotheses are that 3D interfacial gaps presence in deep class I restorations are not influenced by the material used (conventional composite vs bulk fill composite) (1), the layering strategy (horizontal vs bulk) (2) or the curing mode (conventional vs soft-start) (3).

Materials & Methods

Ninety-six (n = 96) human molars extracted for periodontal reasons within the previous 3 months were selected and stored in distilled water after being disinfected with an ultrasonic device. The study was granted ethics approval by the local ethics committee of the Dental School, University of Turin, Italy (DS-2018_No.001). The selected teeth had no previous restorations, carious lesions, demineralization, or cracks under 20x optical magnification (SZX9; Olympus Optical Co., Ltd., Tokyo, Japan).

A single experienced operator (years of practice >10 y) performed a class I cavity preparation on each tooth using calibrated burs, maintaining 360° enamel margins and following these parameters: 3 mm mesiodistal (±0.5 mm), 3 mm oral-buccal (±0.5 mm), and 4 mm depth (±0.5 mm). After preparation, each linear measurement was carefully checked using a periodontal probe.

All cavities were subjected to the same adhesive procedure: selective enamel etching for 30seconds with 35% phosphoric acid (K-etchant, Kuraray Noritake Dental, Mie, Japan), rinsing with water for 30s and air-drying. A two-step self-etch adhesive system (Clearfil SE Bond 2; Kuraray Noritake Dental, Mie, Japan) was then applied following manufacturer's instructions: 20 s brushing primer application, 5 s dry with mild air, bonding application, gentle air-flow to make the layer uniform, light curing for 20 s with a multi-LED curing unit (Translux 2Wave; Kulzer, Hanau, Germany) at 1400 mW/cm^2. Irradiance was periodically checked with a radiometer (CM-2500, DEI Italia).

Specimens were then divided into four groups (n = 24 each) according to the restorative material employed, following manufacturer's instructions (except G4, SG3-4 that were used as control):

- Group 1: Surefill SDR, Dentsply, Konstanz, Germany (SDR). The cavity was restored with a flowable bulk-fill material. A setting time of 10 s was allowed before light curing to achieve optimal adaptation of the material to the cavity walls.
- Group 2: SonicFill 2 Kerr, West Collins, Orange, CA, USA. The cavity was restored with a sonically applied (SonicFill Handpiece; Kerr, West Collins, Orange, CA, USA) bulk-fill composite, selecting an extrusion speed of 2 for better control.
- Group 3 - Admira Fusion X-Tra, VOCO GmbH, Cuxhaven, Germany (AFXT). The cavity was restored with an ormocer bulk-fill material. A specific instrument (Composculp #3/4, Hufriedy Italy, Milan, Italy) was used to compact the material and achieve proper adaptation.
- Group 4- Filtek Supreme XTE, 3MEspe, St Paul, MN, USA (FS). The cavity was restored with a standard nanohybrid composite packable composite. A

specific instrument (Composculp #3/4, Hufriedy Italy, Milan, Italy) was used to compact the material and achieve proper adaptation.

Each group was further divided into four subgroups (n = 6) according to the layering technique and the polymerization mode. Polymerization was carried out with the same multi-LED curing unit (Translux 2Wave; Kultzer, Hanau, Germany) at 1400 mW/cm2:

- Subgroup 1 (SG1): The restoration was performed by applying two horizontal layers, each 2 mm thick and light cured for 20 s with a soft-start curing program (light intensity increased from 50% to 100% in 2 s).
- Subgroup 2 (SG2): The restoration was performed by applying two horizontal layers, each 2 mm thick and light cured for 20 s with a conventional program.
- Subgroup 3 (SG3): The restoration was performed by applying a single layer of composite, 4 mm thick and light cured with a soft-start curing program as described for SG1.
- Subgroup 4 (SG4): The restoration was performed by applying a single layer of composite, 4 mm thick and light cured with a conventional curing program as described for SG2.

Each layer was cured with the same multi-LED lamp (Translux2Wave, Hanau, Germany) using either a conventional or soft-start curing program. The curing tip was placed at a standardized distance of 3 mm from the occlusal surface of the specimen. A radiometer (CM-2500, DEI Italia, Varese, Italy) was used to monitor the curing lamp output at the beginning of each subgroup preparation. The surfaces were finished and polished with diamond burs and silicon points to obtain a smooth surface without over or under contouring.

A summary of the materials employed, including a general description, manufacturer details, composition and volumetric shrinkage are presented in Table 1.

	General description	Manufacturer	Composition	Volumetric shrinkage (%)
Smart dentin replacement (SDR)	Flowable bulk-fill resin composite	Dentsply, Konstanz, Germany	Resin matrix: Modified UDMA EBPADMA, TEGDMA. Inorganic filler: Barium and strontium fluoroalumino silicate glasses (4.2 μm). Camphoroquinone, BHT, UV stabilizer, titanium dioxide, iron oxide. 68 wt%, 44 vol%	3.38% (44)
SonicFill 2 (SF)	Sonically applied bulk-fill packable resin composite	Kerr, West Collins, Orange, CA, USA -	Resin matrix: Bis-GMA, TEGDMA, EBPDMA. Inorganic filler: SiO_2 glass oxide, barium glass, YbF3, mixedoxide. 81.3 wt%	2.03% (1)
Admira Fusion X-Tra (AFXT)	ORMOCER bulk-fill packable resin composite	VOCO GmbH, Cuxhaven, Germany	Resin matrix: ORMOCER (aromatic and aliphatic dimethacrilates, methacrylate-functionalized polysiloxane). Inorganic filler: Ba-Al-glass, pyrogenic SiO2. Photoinitiator: camphoroquinone. Synergist: NI. 84 wt%, 78 vol%	1.24% (29)
Filtek Supreme XTE (FS)	Nanohybrid packable resin composite	3MEspe, St Paul, MN, USA	Resin matrix: BisEMA, BisGMA UDMA, TEGDMA, PEGDMA. Inorganic filler: SiO2 (20nm), ZrO2 (4-11nm), aggregated ZrO2/SiO2 cluster filler. 78.5wt%, 63.3vol%	1.21% (2)

Table 1. General description, manufacturer details, composition and volumetric shrinkage (%) of the main materials (reference number in the brackets).

After preparation, samples were stored in distilled water for 24h before the micro-CT scanning, paying attention to avoid any light exposure during storage. After 24h, samples were scanned using micro-computed tomography (micro-CT) (SkyScan 1172; Bruker, Billerica, MA, USA). High-resolution scans were performed using the following parameters: voltage = 100 kV; current = 100 μA; aluminum and copper (Al+Cu) filter; pixel size = 10 μm; averaging = 5; rotation step = 0.5°. Images were reconstructed using NRecon software (Bruker, Billerica, MA, USA) to obtain DICOM files with standardized parameters: beam hardening correction = 25%, smoothing = 2, ring artifact reduction = 7, total scan time = 55 minutes.

A recently developed 3D method was used to analyze the internal interfacial gap presence (34), (35). Mimics software (ver. 20.0; Materialise, Ann Arbor, MI, USA) was used to automatically perform the thresholding of voids surrounding the restoration within a 300 µm range with a Hounsfield unit (HU) range of 1,024 to 970 to maximize void visualization (Figure 1).

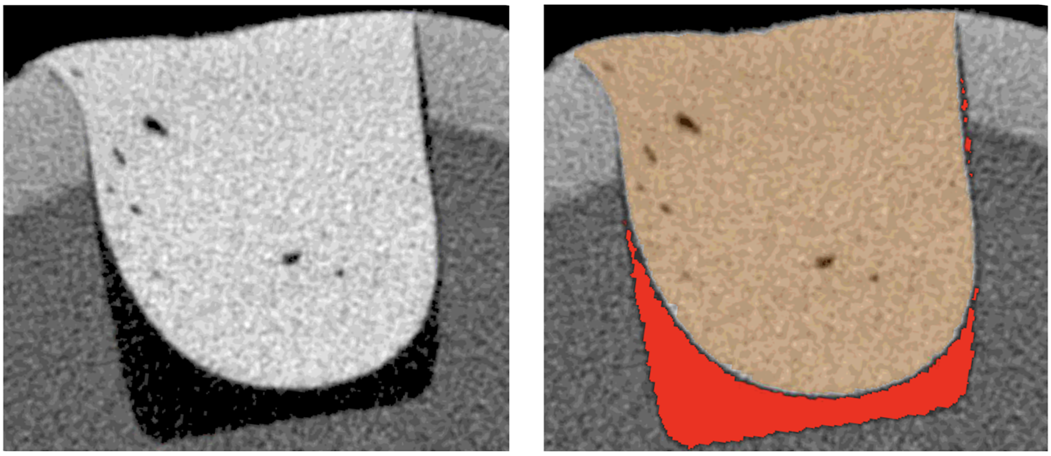

Figure 1. Technique representative image (control group G4, SG4). Random sample segmentation performed with Mimics software (ver. 20.0, Materialise). The orange area represents the restoration, while the red one represents the analyzed void volume.

To ensure consistency across the data, the same protocol with the same HU parameters was applied to all samples. Standard Triangulation Language (.stl) files were then created at an optimal quality (sampling ratio of 1:1), and volumetric calculation of the resulting mask was performed on the. Stl files using Geomagic Studio 12 software (3D Systems, Rock Hills, USA). Volume data expressed in mm^3 were collected for all samples (Figure 2).

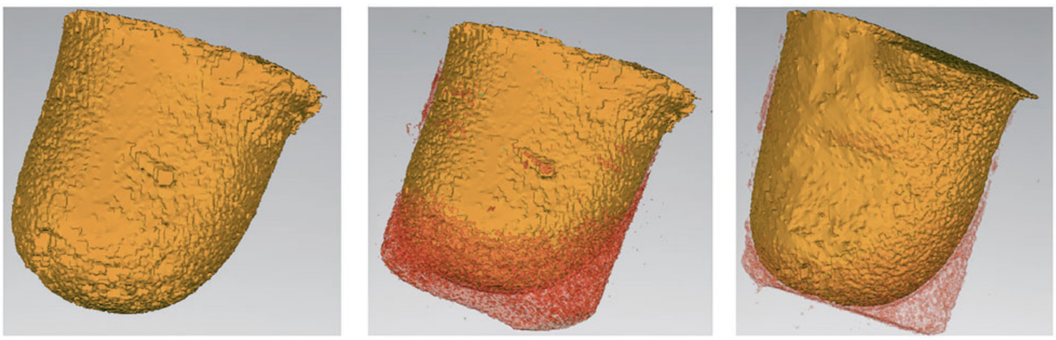

Figure 2. Technique representative image (control group G4, SG4). Three-dimensional rendering using Geomagic Studio 12 software (3D systems) of the same sample shown in Figure 1. The orange area represents the restoration, while the red area represents the analyzed void volume. The areas have different translucencies for better visualization.

To examine the effects of the variables "material," "layering strategy," curing mode," and their interactions on interfacial gap formation, a three-way analysis of variance (ANOVA) was conducted. Post-hoc pairwise comparison was performed using Tukey's test. A p-value < 0.05 was considered significant. All statistical analyses were performed using STATA software (ver. 12.0; StataCorp, College Station, TX, USA).

Results

Interfacial gap data, expressed as means and standard deviations, for the soft-start curing (SG1 and SG3) and conventional curing (SG2 and SG4) modes are summarized in Table 2.

	SG1 (2+2mm, soft start)	SG2 (2+2mm, conventional program)	SG3 (4mm, soft start)	SG4 (4mm, conventional program)
SDR	0.031 ± 0.016^{aA}	0.052 ± 0.028^{aA}	0.133 ± 0.094^{aA}	0.058 ± 0.030^{aA}
SF	$0.200 \pm 0{,}093^{aA}$	0.186 ± 0.104^{aA}	0.115 ± 0.037^{aA}	0.112 ± 0.046^{aA}
AFXT	0.152 ± 0.037^{aA}	0.125 ± 0.068^{aA}	0.062 ± 0.049^{aA}	0.079 ± 0.045^{aA}
FS	$0.530 \pm 0{,}161^{bA}$	0.416 ± 0.135^{bA}	1.200 ± 0.781^{bB}	0.740 ± 0.561^{bB}

Table 2. Summary of interfacial gap data, expressed as mean ± standard deviation (mm^3). Same superscript capital letters indicate no difference between row results. Same superscript lower-case letters indicate no difference between column results.

The results of the three-way ANOVA showed significant differences between materials ($p < 0.001$), layering technique ($p = 0.024$) and their interactions ($p < 0.001$). No significant differences were reported for the polymerization mode variable (p = 0.21). Tukey's post-hoc test showed that FS performed significantly worse in terms of interfacial volumetric gaps presence than all other tested materials. Moreover, the FS 4 mm layering subgroups performed significantly worse than the FS 2+2 mm subgroups. No significant differences were reported for bulk-fill materials in terms of layering technique.

The 3D-rendering of all restorations with associated interfacial gaps presence showed that the bottom of the cavity was, in all samples, the most subjected area to volumetric gap presence. On the other hand, cavity axial walls showed inferior volume of interfacial gaps. Moreover, subgroup 1 showed a small number of gaps and air bubbles in the interface between the two layers. Figure 3 shows the interfacial gaps in a random sample from each group and subgroup.

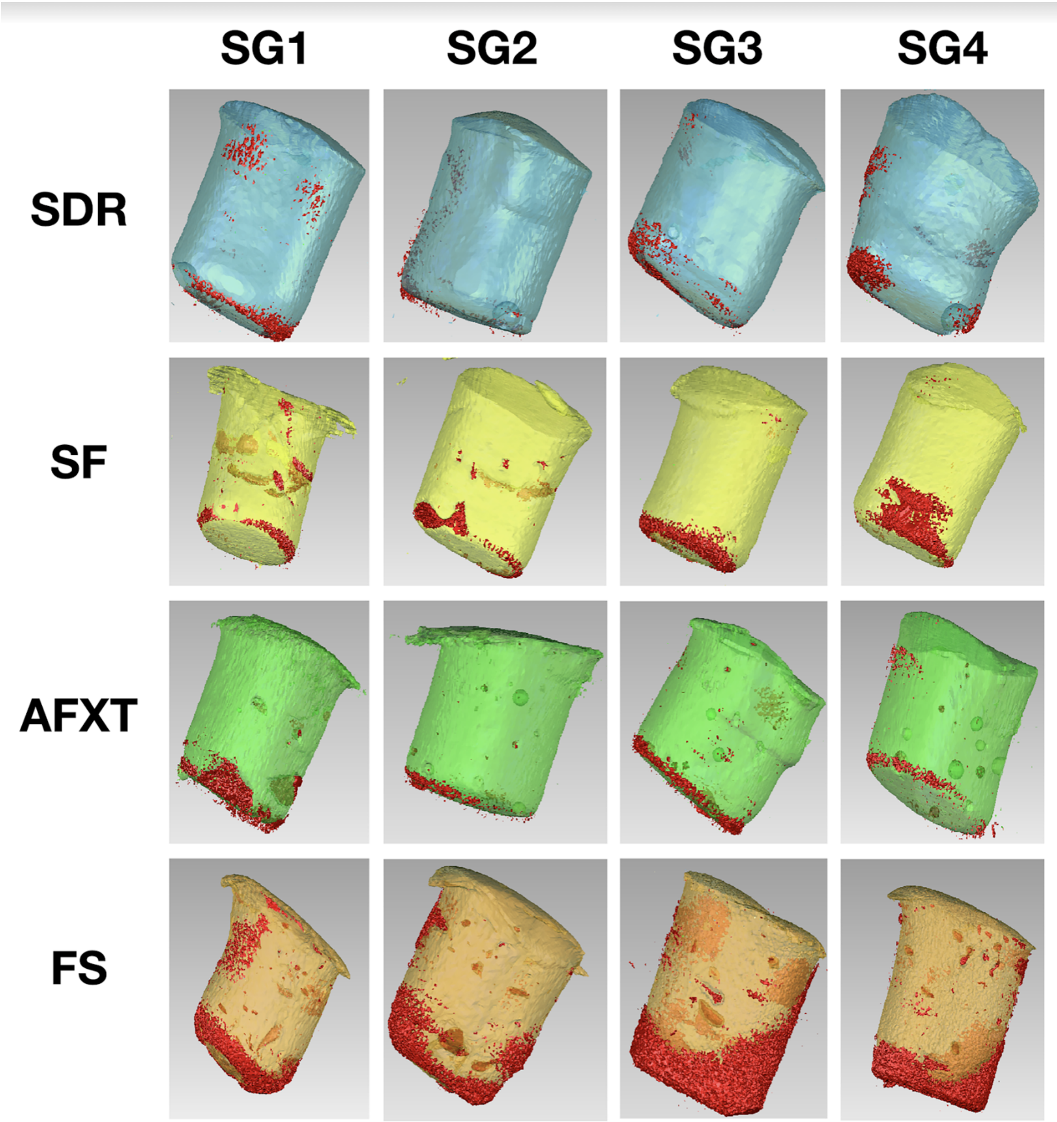

Figure 3. Technique representative image. Random samples from each group and subgroup. The red areas represent the gaps. It should be noted that internal bubbles were automatically excluded, by filling voids in the mask of the composite, to focus the analysis specifically on the interface. Red area was analyzed calculating the STL volume using Geomagic software.

Discussion

Although there are strategies to reduce its extent, volumetric interfacial gap presence is still a major issue that contributes to adhesive restoration failure. The present study investigates volumetric gap presence and location in deep class I restorations reconstructed using different bulk-fill composite resins with different layering strategies and polymerization modes.

Over the last decade, different methods have been used to analyze the interfacial gap presence (28), (38). Optical coherence tomography (OCT) has recently been used for this purpose, sometimes combined with contrasting agents (11), (15), (34). Initial limitations in detecting gaps in deep cavities, related to light transmission ability through dental tissues and materials, have been overcome in recent years with new techniques and equipment (4). However, a review by Sahyoun et al. showed that image scaling, deformable registration, and fusion methods must still be implemented to superimpose OCT data onto 3D surfaces (31). Micro-CT imaging, which enables high-quality 3D reconstructions with a non-destructive approach (24), is an alternative option for studying and evaluating interfacial gaps presence. However, micro-CT images are usually analyzed using linear measurements and two-dimensional reconstructions, which can lead to operator bias (17), (39). Recent studies have demonstrated a non-destructive, standardized 3D method for evaluating gaps, involving quantitative measurement of the gap volume without operator bias and qualitative evaluation of the gap location through 3D rendering (35), (34), (32).

In accordance with previous studies, in deep class I cavities incremental layering techniques have been recommended and are considered the gold standard (20), (40). However, restoring deep cavities with multiple increments of resin composite is time-consuming and increases the risk of incorporating air bubbles or contaminants between the increments (10).

Regarding the first null hypothesis, all bulk-fill materials tested showed less volumetric interfacial gap presence compared to conventional nanohybrid composites, regardless to their formulation (packable or flowable). Thus, the first null hypothesis is rejected. This result contrasts with the findings of a study by Furness et al. (12), which showed that bulk-fill materials, both flowable and non-flowable, resulted in a similar proportion of gap-free marginal interface compared to a conventional composite. However, the two-dimensional evaluation of the adhesive interface in association with dye leakage penetration might explain the discrepancy between their results and those of the present study, which found no gap-free surfaces in any sample. It is worth to mention that dye leakage penetration technique is able to evaluate the presence of the infiltration at the level of the hybrid layer, even if some limitation related to the two-dimensional technique itself has been reported (9), (13). Besides, 3D micro-CT analysis allows non-destructive observation at the level of the interface and a more comprehensive analysis of the samples, that could result in a higher mean presence of gaps (13). Another recent study by Sampaio et al. highlighted the fact that volumetric shrinkage and interfacial gap are related but not completely corresponding (32) since stress development depends on the molecular characteristics of the material itself (11). Similarly, the present in vitro study shows that volumetric shrinkage has no linear correlation with interfacial gaps, since mean shrinkage values reported in literature (Table 1) did not correlate with volumetric interfacial gap presence results.

As concerns layering technique, the second null hypothesis was partially rejected since stratification technique significantly influenced volumetric gap presence only in the FS group when comparing 2+2 mm incremental layering to 4 mm bulk layering. These results are supported by recent in vitro (with scanning electron microscopy) and in vivo (with clinical sensitivity tests) studies, which have found that bulk layering techniques applied on traditional composites are inferior to incremental ones (21), (20), (26). This might also be related to the degree of conversion: it has been demonstrated that conventional composites cannot guarantee proper monomer conversion into polymer chains at 4 mm depth, whereas bulk-fill composites can (44), (43). The results of the present study are, therefore, aligned with those of other papers, which have reported that the layering technique significantly influences the performances of traditional nano-filled composites (23), (30). Regarding interfacial gaps, Haak et al. found no significant differences between traditional layered and bulk-fill composites in terms of marginal or internal gaps after artificial aging (14). However, this might be explained by the different cavity design and depth tested in their study. Moreover, micro-CT may be more specific for internal gap analysis compared to slice sectioning since the cutting procedure can produce biases and artifacts.

Since the curing mode did not significantly influence volumetric interfacial gap presence, the third null hypothesis was accepted. A recent review showed that there are debates regarding whether the longer pre-gel phase, facilitated by ramp curing, and the consequent lower stress at the interface is not as important as other parameters in preventing interfacial gap formation (21). Another review confirmed that even if the rationale for ramp curing is solid, there is no consensus on the benefits of different light-application protocols. Moreover, the small amount of clinical data available does not show whether such light-curing protocol provides significant benefits at the level of the adhesive interface (37).

Finally, 3D rendering showed that interfacial gaps were mostly concentrated at the cavity floor in all groups. This may align with the findings of Ausiello et al., who reported a high concentration of stress in this area when applying shrinkage forces on a finite element analysis model (5), even if the present study did not focus on shrinkage stress itself but it concentrates on volumetric interfacial gap presence between cavity walls and restorative materials. Hayashi et al. drew similar conclusions when using real-time OCT to analyze the sealing floor area percentage (SFA%) (15). The previously cited study by Furness et al. (12) also reported a significantly lower percentage of gap-free margins at the pulpal floor interface than at the enamel interface, which confirms that this might be area mostly subjected to interfacial gap presence for bulk composites. However, one of the biases concerning gap presence at the bottom of the cavity, could be the operator experience in composite layering. On the other hand, specifically designed studies should be conducted to better analyze the influence operator experience on material adaptation to cavity floors. Further studies are needed to analyze micro-gaps three-dimensionally and determine how they might be prevented efficiently.

Conclusions

Within the limitations of the present study, it can be concluded that volumetric interfacial gaps presence:

- Is not related to either the layering technique or the curing mode when using bulk-fill materials
- Is influenced by the layering technique when using conventional composite resins, with incremental application leading to better performance
- Is significantly lower in all tested bulk-fill materials compared to a conventional nanohybrid resin composite
- Is mostly concentrated in the cavity bottom area, regardless of the material employed

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2.4 Tridimensional Evaluation of the Interfacial Gap in Deep Cervical Margin Restorations: A Micro-CT Study

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Abstract

Objectives: The purpose of this laboratory study was to perform a tridimensional interfacial gap evaluation of class II cavities with enamel and dentin cervical margins, before and after cyclic fatigue, restored with different nanohybrid composite resins.

Methods and Materials: Standardized class II cavities were performed on 48 intact maxillary premolars, placing the mesial cervical margin 1 mm above the cement–enamel junction (CEJ) and the distal cervical margin 1 mm below the CEJ. Specimens were treated with two-step self-etch adhesive (Clearfil SE Bond2) and divided into six groups according to the resto- ration technique. Microcomputed Tomography imaging was executed before and after 1,000,000 cycles of chewing simulation at 50 N. Tridimensional interfacial gaps, expressed as cubic millimeters, were analyzed through a standardized software flowchart (Mimics). Data were analyzed with a two-way analysis of variance and Tukey post hoc tests ($p = 0.05$).

Results: Restoration technique ($p = 0.001$) and chewing simulation ($p = 0.00001$) significantly influenced interfacial gap on dentin but not on enamel. The post hoc test showed that, on dentin margins, flowable resins had a lower gap at baseline but a higher gap after chewing simulation, especially when a 2 mm-thick layer was applied, compared with nanohybrid and bulk-fill composites.

Conclusions: Based on the obtained results, no differences in interfacial gap volume were found on enamel margins. On dentin margins, flowable resins showed better marginal seal at baseline, but they seem to be more prone to interfacial degradation during chewing simulation than traditional composites.

Introduction

Composite resins are the most widely used materials in direct posterior restorations, and consequently, interest in the longevity and reliability of these materials has grown over time.[1] Although widely used, composites still present various problems. During the polymerization phase, these materials are subject to shrinkage stress, which can lead to debonding, causing an interfacial gap.[2,3] The resulting poor marginal seal is associated with postoperative sensitivity, secondary caries, periodontal problems, and infiltration of bacteria, liquids, and molecules, leading to marginal discoloration and failure of the restoration itself.[4,5]

The clinical prognosis for adhesive restorations with margins made entirely of enamel is excellent,[6,7] but the same cannot be stated for deep cavities; with cervical margins extending beyond the cement–enamel junction (CEJ), it is difficult to obtain an effective and durable marginal seal.[8-10] It has been reported that restorations with cervical margins in dentin and cementum are more susceptible to marginal staining, postoperative sensitivity, and the formation of secondary caries.[4,11]

Different techniques and materials have been tested to improve the sealing of deep cervical margins, such as the open-sandwich technique,[12,13] the use of ceramic inlays,[14-16] and margin elevation with composites.[16]

Several studies have shown how the use of flowable composites, interposed between the cavity floor of the interproximal box and the restorative material, can reduce the interface stress related to volumetric contraction while curing.[17,18] These materials have a low elasticity modulus, which allows increased elastic deformation and therefore greater absorption of contraction stress caused by polymerization; this minimizes the interfacial gap, especially in the cervical area.[8,19] Another property of these materials is improved wetting, which facilitates adaptation, ensuring a more intimate contact with the cavity walls.[20] However, flowable resins have inferior mechanical properties compared with conventional composites.[21]

Some studies have reported a better marginal fit with use of flowable composite layering with reduced thickness.[22,23] However, Malmstrom and others showed that neither the thickness nor the presence of flowable composite as an initial cervical increment significantly influenced the marginal microleakage,[24] probably due to lower mechanical properties and a reduced resistance to deformation compared with conventional composite resins.[25]

Bulk-fill flowable composites have been introduced to minimize internal polymerization stresses via a longer pre-gel phase. A study by Moorty and others showed that minor contraction stress exerted by bulk-fill flowable composites translates into reduced cuspal deflection compared with traditional composites placed with an oblique layering technique.[26] However, a laboratory study by Furness and others showed that bulk-fill materials, both flowable and non-flowable, resulted in a similar gap-free marginal interface compared with conventional composites.[27]

Interfacial gap formation has been evaluated in the literature by means of different destructive tests,[28-32] hindering a more detailed analysis of the interface before and after polymerization. Micro- computed Tomography (micro-CT) enables creation of a three-dimensional (3D) map of the tooth–restoration interface and detection of the deepest marginal leakage,[33,34] allowing a detailed assessment not only of the entity but also of the topography of the interfacial gap.

To the best of our knowledge, no studies have performed a tridimensional interfacial gap analysis when the margin elevation technique with different resin composite materials and techniques for deep class II cavities is necessary. Thus, the aim of the present laboratory study was to evaluate the volumetric interfacial gap of composite restorations in class II cavities, with enamel and dentin cervical margins, before and after cyclic fatigue.

The null hypotheses were that the tridimensional interfacial gap during cervical margin elevation technique is not influenced by (1) the material used or (2) cyclic fatigue.

Materials & Methods

Forty-eight intact human maxillary premolars, extracted for periodontal reasons within the last three months, were selected and stored in distilled water after disinfection with an ultrasonic device. The selected teeth had no carious lesions, demineralization, cracks, or signs of wear. Two class II cavities, one mesial and one distal, of similar shape and size were created on each specimen by the same operator. The cavities were 4 mm in the buccal– lingual direction and 3 mm in the mesio-distal direction; the mesial cavity had an enamel cervical margin 1 mm above the cement–enamel junction (CEJ), whereas the distal cavity had a dentin cervical margin 1 mm below the CEJ. Calibrated burs were used and cavity parameters were carefully checked after preparation with a probe. A circumferential steel matrix was applied (Automatrix, Dents- ply, Sirona, Germany) and tightened until a perfect fit with the cervical margin was achieved. Then, all specimens were subjected to the same adhesive procedure: selective enamel etching for 40 seconds with 35% phosphoric acid (K-etchant, Kuraray Noritake Dental, Mie, Japan), rinsing for 30 seconds, and air-drying. A two-step self-etch adhesive system (Clearfil SE Bond2, Kuraray Noritake Dental) was then applied following the manufacturer's instructions and lightly air-dried before light-curing for 40 seconds with a light-emitting diode (LED) lamp (Cefalux2, VOCO, Cuxhaven, Germany). Specimens were then divided into six groups (n=8 each) using the following restoration techniques.

- Group 1 (G1): A 1-mm-thick horizontal layer of flowable resin (Grandioso Heavy Flow, VOCO) was applied over the cervical margin. The restoration was then finalized with 2-mm-thick oblique layers of nanofilled composite (Grandioso, VOCO).
- Group 2 (G2): A 1-mm-thick horizontal layer of ormocer flowable resin (Admira Fusion Flow, VOCO) was applied over the cervical margin. The restoration was then finalized with 2-mm-thick oblique layers of nanofilled ormocer (Admira Fusion, VOCO).
- Group 3 (G3): The same technique used in G1 was applied but with 2 mm of flowable composite (Grandioso Heavy Flow, VOCO).
- Group 4 (G4): The same technique used in G2 was applied but with 2 mm of flowable ormocer (Admira Fusion Flow, VOCO).
- Group 5 (G5): A nanohybrid composite (Filtek Supreme XTE, 3M ESPE, St Paul, MN, USA) was used, applying 2-mm-thick oblique layers.
- Group 6 (G6): A bulk restoration was performed using a bulk nanofilled composite (Filtek Bulk-Fill Posterior, 3M ESPE)

A summary concerning the used materials is given in Table 1.

Material	Classification	Main Components
Grandioso heavy flow (VOCO GmbH Cuxhaven)	Nanohybrid flowable composite resin	Bis-GMA, Bis-EMA, TEGDMA Fillers (83 wt% = 68 vol%): glass ceramic (average size: 1 μm), functionalized SiO_2 nanoparticles (20-40 nm)
Grandioso (VOCO GmbH Cuxhaven)	Nanohybrid composite resin	Bis-GMA, Bis-EMA, TEGDMA Fillers (89 wt% = 73 vol%): glass ceramic and silicone dioxide nanoparticles (20-40 nm, 0.5-3 μm)
Admira Fusion flow (VOCO GmbH Cuxhaven)	Nanohybrid flowable ormocer	Matrix: resin ormocer Fillers (74 wt%): silicon dioxide (unspecified filler size)
Admira Fusion (VOCO GmbH Cuxhaven)	Nanohybrid ormocer	Matrix: resin ormocer Fillers (84 wt%): Silicon dioxide nanofillers (20-50 nm) and silicon oxide–based hybrid fillers (1 μm)
Filtek Supreme XTE (3M Maplewood, MN, USA)	Nano-filled composite resin	Bis-GMA, Bis-EMA, PEGDMA, TEGDMA Fillers (65 wt% = 46 vol%): ytterbium trifluoride (0.1-5 μm), nonagglomerated/nonaggregated surface modified 20-nm and 75-nm silica fillers, surface modified aggregated zirconia/silica fillers (cluster average size: 0.6-10 μm)
Filtek Bulk-Fill Posterior (3M, Maplewood, MN, USA)	Bulk-fill nanofilled composite	Bis-GMA, UDMA, Bis-EMA, PEGDMA, TEGDMA Fillers (64.5 wt% = 42.5 vol%): ytterbium trifluoride (0.1-5 μm), zirconia/silica (0.01-3.5 μm)

Abbreviations: Bis-EMA, bisphenol A diglycidyl methacrylate; Bis-GMA, bisphenol A-glycidyl methacrylate; TEGDMA, triethylene glycol dimethacrylate; PEGDMA, Poly(ethylene glycol) dimethacrylate; UDMA, Urethane Dimethacrylate.

Table 1. Summary of used materials with respective classification and main components

In all specimens, each composite layer was light- cured with an LED lamp (Cefalux2, VOCO) at 1400 mW/cm2 for 20 seconds. Finishing and polishing procedures were then performed with abrasive disks (SofLex, 3M ESPE) and silicon points (Enhance, Dentsply).

The marginal adaptation of each restoration was evaluated using a micro-CT scanner (SkyScan 1172, Bruker, Billerica, MA, USA). High-resolution scans were performed on each specimen using the following parameters: voltage = 100 kV; current = 100 μA; aluminum and copper (Al+Cu) filter; pixel size = 10 μm; averaging = 5; rotation step = 0.18; and total scan duration = five hours.

A CS-4.4 chewing simulator (SD Mechatronik, Feldkirchen-Westerham, Germany) was used for fatigue-cycling mechanical aging of the specimens. The resilience of the human periodontium was simulated by coating the roots of the teeth with a 1 mm polyether layer (Impregum, 3M ESPE). A 6 mm-diameter steatite sphere was used with the following settings: occlusal load = 50 N; frequency = 1 Hz; downward speed = 16 mm/s; and sliding movement = 2 mm over the buccal triangular crest. All restored specimens had a standardized anatomy and were similarly positioned to center the sphere exactly on the central fossa of the tooth. The test was performed for 1,000,000 cycles in distilled water.

To reveal interfacial gap progression between the restorations and the tooth after cyclic fatigue, specimens were subjected to a second scan, with the same baseline parameters used to ensure consistency of the grayscale values. NRecon (Bruker, Kontich, Belgium) was used to reconstruct samples and obtain DICOM files (Digital Imaging and Communications in Medicin; dcm) with the same Hounsfield unit (Hu) parameters and the following software corrections: beam hardening = 30%, smoothing = 3, smoothing kernel = 2 gaussian, and ring artifact correction = 7. A novel tridimensional method was used to analyze the interfacial gap. Using Mimics software (ver. 20.0, Materialise, Leuven, Belgium), thresholding of voids surrounding the restoration was performed automatically to include all voids surrounding the restoration in a 200-lm range. The Hu values representative for gap voids (-1024/-990) were selected by an expert operator on the first sample and therefore applied to all samples. Using dynamic region growing and region growing functions, only internal and marginal gaps were included in the present analysis (Figures 1 and 2).

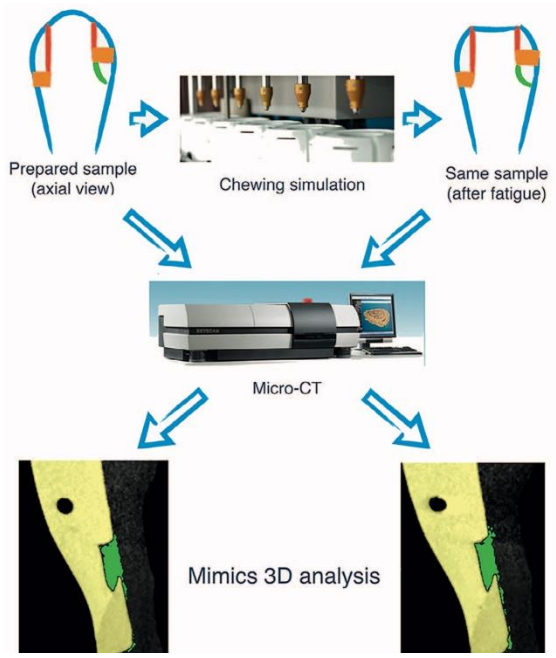


Figure 1. Representative scheme of the protocol.

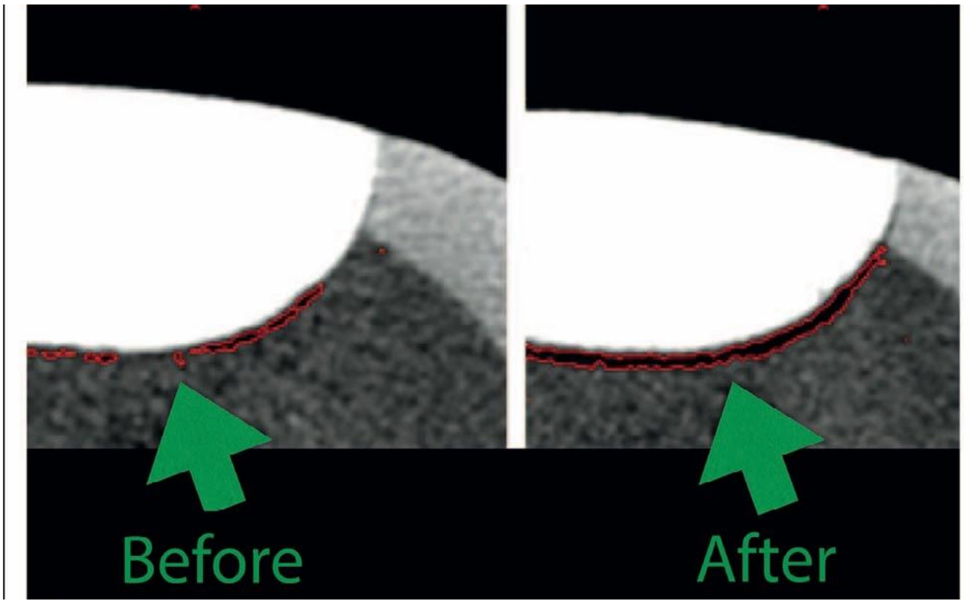
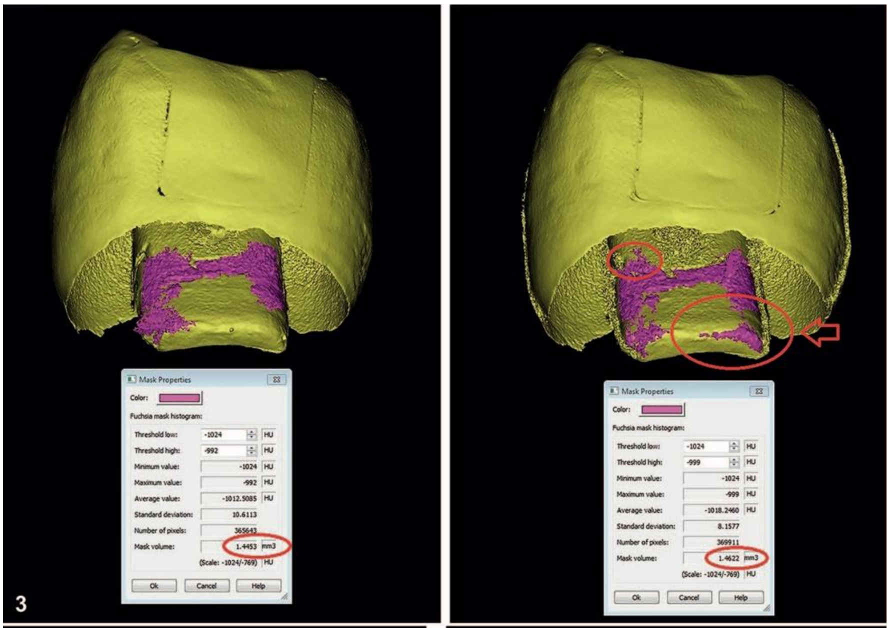


Figure 2. Same coronal cuts of a random sample, before (left side) and after cyclic fatigue (right side). Void mask contour is highlighted with a red line. It is noticeable that the restoration interface degraded after cyclic fatigue, ultimately leading to a marginal gap opening internally.

Volumetric calculation of the resulting mask was performed by the software, and volume data (expressed in cubic millimeters) were collected for both the dentin (Figure 3) and enamel interfaces (Figure 4).



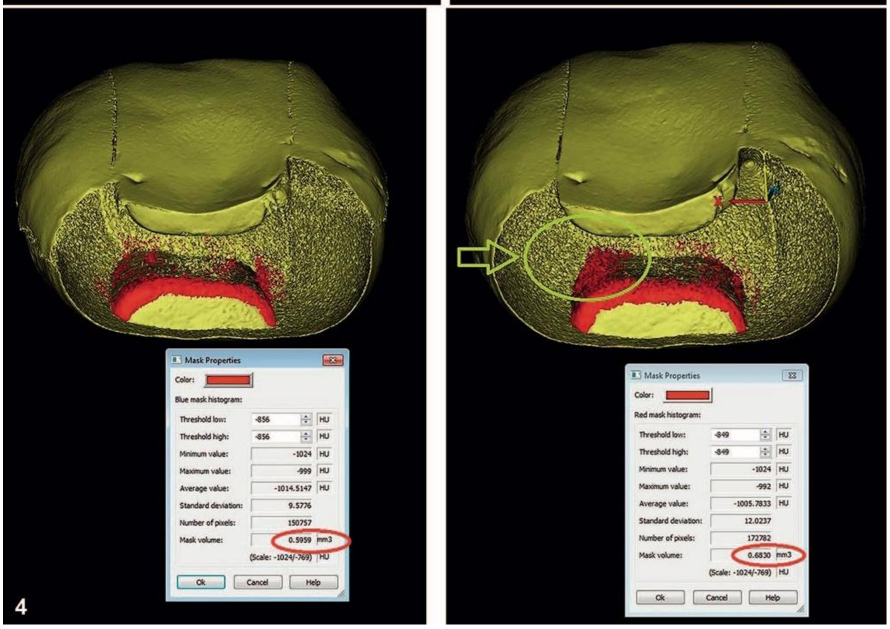


Figure 3. The same sample as Figure 2 is represented with 3D rendering before (left side) and after chewing simulation (right side). Yellow volume represents enamel and restorations. Violet volume represents interfacial voids of the deep restoration. It is noticeable that some areas showed a visible interfacial gap opening.

Figure 4. The same sample as Figure 3 before (left side) and after chewing simulation (right side). Yellow volume represents enamel and restorations. Red volume represents

interfacial voids of the superficial restoration. It is noticeable that some areas showed a visible interfacial gap opening.

To evaluate the effects of materials and techniques, a two-way analysis of variance (ANOVA) and post hoc Tukey tests were performed. The significance level was set to 95% ($p < 0.05$). All statistical analyses were performed using the Stata software package (Stata- Corp, College Station, TX, USA).

Results

Interfacial volumetric gaps (6SD; expressed in cubic millimeters) are shown in Tables 2 (enamel cervical margins) and 3 (dentin cervical margins). The results of the ANOVA showed that restoration technique ($p = 0.001$) and chewing simulation ($p = 0.00001$) significantly influenced the interfacial gap on dentin but not on enamel. The post hoc test showed that, for deep dentin margins, flowable resins, either 1 or 2 mm, were better able to seal the interface before the chewing simulation but were more prone to interfacial degradation than nano-hybrid and bulk-fill composites. After cyclic fatigue, only the dentin margins closed with 2 mm of flowable composites showed greater interfacial gap than the other groups. On enamel margins, no differences were found between the restoration techniques tested.

Material	Cyclic Fatigue	
	Before	After
Grandioso Heavy Flow, 1 mm	0.857 ± 0.254 Aa	1.162 ± 0.355 Aa
Admira Fusion Flow, 1 mm	0.727 ± 0.279) Aa	1.222 ± 0.246 Aa
Grandioso Heavy Flow, 2 mm	0.699 ± 0.388 Aa	1.457 ± 0.504 Ab
Admira Fusion Flow, 2 mm	0.879 ± 0.590) Aa	1.244 ± 0.446 Aa
Filtek Supreme XTE	1.322 ± 0.381 Aa	1.543 ± 0.159 Aa
Filtek Bulk-Fill Posterior	1.081 ± 0.485 Aa	1.665 ± 0.401 Aa

[a] *Same lowercase letters in rows indicate no difference between storage time (p>0.05); same uppercase letters in columns indicate no difference between luting procedures (p>0.05).*

Table 2. Interfacial volumetric gaps of the enamel cervical margin (mm^3), before and after cyclic fatigue. Same superscript capital letters indicate no difference between row results. Same superscript lower-case letters indicate no difference between column results.

Material	Cyclic Fatigue	
	Before	After
Grandioso Heavy Flow, 1 mm	0.931 ± 0.310 Aa	1.528 ± 0.363 Ab
Admira Fusion Flow, 1 mm	0.820 ± 0.276 Aa	1.592 ± 0.692 Ab
Grandioso Heavy Flow, 2 mm	0.719 ± 0.334 Aa	2.057 ± 0.506 Bb
Admira Fusion Flow, 2 mm	0.975 ± 0.206 Aa	2.298 ± 0.399 Bb
Filtek Supreme XTE	1.608 ± 0.209 Ba	1.922 ± 0.411 Aa
Filtek Bulk-Fill Posterior	1.422 ± 0.335 Ba	1.851 ± 0.178 Aa

[a] *Same lowercase letters in rows indicate no difference between storage time ($p>0.05$); same uppercase letters in columns indicate no difference between luting procedures ($p>0.05$).*

Table 3. Interfacial volumetric gaps of the dentin cervical margin (mm^3), before and after cyclic fatigue. Same superscript capital letters indicate no difference between row results. Same superscript lower-case letters indicate no difference between column results.

Discussion

Based on the results of the present study, the first null hypothesis was partially rejected because the use of flowable resins yielded a significantly better marginal seal on deep cervical margin elevation at baseline. The sealing ability of flowable resins also showed a significant reduction after artificial aging; therefore, the second null hypothesis was rejected.

Several laboratory studies have tested the performance of adhesive systems by evaluating marginal gap formation around restorations of extracted teeth.[35] This method assumes that if the forces generated by polymerization shrinkage or thermomechanical strain exceed the bond strength, an observable gap will form at the margin of the restoration. Although there is no clear correlation between laboratory gap formation and interfacial failures observed clinically, it is reasonable to assume that this marginal gap formation is clinically relevant.[36] Many studies have found that all current adhesives appear incapable of completely sealing the restoration margins in a sub-micron scale due to the fact that the hybrid layer is always incomplete[37]. In complex cavities, such as deep cervical margins, this can potentially lead to actual micro-leakage [38].

Many techniques have been used to assess microleakage, and the results vary considerably.[28] Traditional laboratory methods to detect microleakage between a restoration and composite use organic dyes, such as basic fuchsin, methylene blue, and rhodamine, in conjunction with microscopy techniques[29] or transmission electron microscopy.[30] The disadvantage of these analyses include invasiveness, semiquantitative results, and limited ability to represent tridimensional geometry.[31] Using scanning electron microscopy (SEM) to determine the presence of internal cracks or voids requires sample sectioning, which eliminates the possibility of evaluating the effects of artificial aging on samples after a baseline analysis. Epoxy replicas can also be used and evaluated with SEM, but this allows analysis only of the external margin. Moreover, such results can be affected by the accuracy of the impression, and a weak-to-moderate correlation with clinical findings has been reported.[32] More recently, optical coherence tomography (OCT) has been used to evaluate interfacial adaptation and microleakage in composite restorations. OCT is a time-domain low-coherence interferometric technique that provides high-resolution cross-sectional (two-dimensional) or volumetric (tridimensional) images without x-ray exposure by quantifying the reflection of infrared light from dental structures.[39] The resolution of OCT is approximately 5-15 microns, which is more than radiographic or current clinical CT images.[40] OCT initially showed some limits in detecting gaps in deep cavities due to the light transmission ability through dental tissues and materials, but new techniques and equipment are overcoming this problem.[41] However, a recently published review by Sahyoun and others stated that image scaling, deformable registration, and fusion methods still must be implemented to superimpose OCT data onto another 3D surface.[42] In the present study, micro-CT was used without any radio-opaque tracer to evaluate interfacial gaps. This approach has the advantages of being nondestructive, quantitative, and tridimensional. Specifically, it allows tridimensional visualization of the spatial distribution of the interfacial leakage along the cavity walls and floor, which cannot be obtained easily using traditional techniques that require sectioning of the specimen. These features make this method markedly more comprehensive. By contrast, traditional methods for microleakage studies can provide only limited, or even unrepresentative, information unless multiple sections of the sample are analyzed.[34] The volumetric evaluation of interfacial gaps between tooth hard tissues and restorations allows not only standardized, tridimensional measurement of the gap progression after cyclic fatigue, but also qualitative visualization of where the gap occurs. Stress propagation along adhesive restoration interfaces could be related to several factors and visualized by superimposition of baseline and after-chewing scans to determine the weakest point of the restoration–tooth interface.

When considering interface analysis between resin composite and tooth tissue, the adhesive system could represent a variable in marginal gap formation, particularly when margins are positioned under the CEJ. Bonding to dentin is different compared with enamel due to morphologic, histologic, and compositional differences; dentin contains a substantial proportion of water and organic materials, which impairs the bonding mechanism.[43] To improve the evaluation of restorative materials conducted in the present study, the same adhesive protocol was applied to all samples, as described in the previous section. Previous studies have shown an absence of resin tags in the cementum area,[44,45] which reinforces the notion that cervical margins are the hardest to infiltrate among all areas of class II restorations. A two-step self-etch adhesive procedure with pre-etching of the peripheral enamel was performed on each sample in this study. As demonstrated in the literature, a mild etching effect causes a reduction in bond strength to enamel compared with that achieved using phosphoric acid–selective enamel etching.[46] This allows a considerable increase in the depth of resin penetration (longer resin tags), resulting in a better adhesion performance along enamel margins.[47] The results of the present study showed that the restoration techniques did not significantly influence the interfacial gap values of enamel margins at baseline or after chewing simulation. This can be explained by the greater adhesive reliability achievable on enamel substrate.[48]

Consistent with previous studies, class II cervical margins in dentin, which usually yield to the deep margin elevation technique, showed a significantly greater marginal gap than those in enamel because dentin is a highly hydrophilic tissue that is only partially dehydratable and, therefore, more difficult to infiltrate by hydrophobic adhesives.[29] Water may persist within the adhesive layer on solvent evaporation, permeate the adhesive interface from the outer environment, or diffuse from the wet underlying dentin substrate. The amount of water uptake within the interface increases with time as bond strength decreases. In nonaqueous media, long-term preservation of dentin bond strength seems to be strongly linked to interface sealing.[49]

The results of the present study also showed that marginal gaps along the dentin margins, treated with a deep margin elevation technique, were smaller when a flowable composite was used as the first horizontal layer, independent of its layer thickness and type. This finding is consistent with the results of two previous studies. Fabianelli and others reported that the open-sandwich technique was associated with significantly less dye penetration than the closed-sandwich technique,[12] whereas Korkmaz and others reported that the closed-sandwich technique required greater operator skill and achieved worse marginal adaptation.[50] Sadeghi and Lynch showed how better marginal adaptation could be achieved using an intermediate layer of flowable composites or compomers, especially when the cavity extended below the CEJ.[51] Moreover, it has been widely reported that stresses generated during placement of a composite restoration can significantly influence the immediate marginal leakage, especially when dentin margins are present.[52] With a low elastic modulus and better wettability, flowable composites can create an intermediate flexible layer between the adhesive system and the composite resin, reducing contraction stress and improving the restoration seal.[53]

Several authors reported significant effects of flow on the cavity floor, reducing microleakage in class II restorations. A previous study evaluated microleakage with and without flowable liners and concluded that flowable composites reduced, but did not eliminate, microleakage at the gingival cavosurface margins apical to the CEJ.[51] The use of flow materials could reduce C-factor effects, leading to a reduction in polymerization stress and associated problems when applied in a 1 mm-thick layer. Lowering the C-factor may reduce the internal stresses within the composite restoration. However, the benefit of the gingival liner for reducing polymerization contraction stress is still somewhat controversial: some studies have reported that the use of flow did not reduce microleakage in class II restorations with margins below the CEJ,[7,54] where- as other studies exhibited discordant results regarding microleakage.[7,9,55,56] However, the methods used to evaluate marginal gaps were not precise or standardized, leading to greater variability in results due to differences in sample preparation, sectioning, and data collection procedures. Contrasting results were also presented by Kim and Park, who used micro-CT to evaluate the internal adaptation of composites.[16] They showed that bulk-fill and layered composite resins had similar marginal sealing quality over dentin. However, differences in restorative materials, flowable liners, adhesive systems, and above all, testing procedures may explain variations in results.

The present laboratory study showed how flowable composites exhibited greater interfacial deterioration than nanohybrid composites, with a significantly increased gap volume after artificial aging procedures, especially when applied in thicknesses of 2 mm. Furthermore, the 3D analysis of the interfacial gap progression after cyclic fatigue allowed visualization of the microleakage increasing more at the level of the angle between the axial pulp wall and gingival floor. This could be related to the flowable resin's mechanical properties: Bayne and others evaluated the filler percent, wear, compressive strength, diametral tensile strength, indented biaxial flexure strength, and toughness of eight flowable and two hybrid composites.[25] Mechanical properties were approximately 60%-90% of conventional composites, resulting in a conclusion that flowable materials should be used with caution in high stress-bearing areas. More recently, Baroudi and others found that the edge-fracture resistance of flowable composites was lower toward the margins than toward the center of a restoration,[57] explaining how, in the present study, the area between the axial pulpal wall and gingival floor, near the cervical margin, showed the greatest increase in the interfacial gap. Even if the flowable composite's elastic modulus permits a stress-absorbing action, its higher amount of monomer could attenuate its mechanical resistance in long-term simulations, particularly in the area were internal stress consequent to functional loads concentrates. This would explain how flowable resins, if applied in 2 mm layers, showed greater interfacial deterioration. Pongprueksa and others also reported that conventional composites released significantly fewer monomers than flowable or bulk-fill composites, and a higher total monomer elution was recorded for both flowable composites, irrespective of the application method.[58] It is therefore assumable that higher quantities of unpolymerized monomers could lead to overlapping deformations. By consequence, fatigue micro-failures would be more likely to appear in early stages of the simulation compared with a rigid material, with a more regular molecular structure that can dissipate forces.

Conclusions

- All composite materials performed significantly better on enamel than on dentin.
- All flowable materials, regardless of the first horizontal layer thickness, were able to create a significantly better marginal seal than nanohybrid composites at baseline.
- Nanohybrid and bulk-fill composites may be able to better maintain a marginal seal over time, because their use was not associated with any significant alteration of the marginal seal after mechanical treatment.

Our results suggest that longitudinal clinical trials are necessary for precise clinical indications on the ideal approach to restoring cavities with deep cervical margins.

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2.5 3D Interfacial Gap and Fracture Resistance of Endodontically Treated Premolars Restored with Fiber-reinforced Composites

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Abstract

Objectives: To evaluate interfacial gap and fracture resistance of endodontically treated maxillary premolars, restored with different glass-fiber reinforced materials.

Materials and Methods: Eighty-four extracted intact premolars were endodontically treated and MOD cavities prepared. Specimens were divided into 7 groups (n = 12 for each) as follows: sound teeth (G1); no restoration (G2); direct composite restoration with fiber-reinforced composite (Ever-X Posterior) (G3); direct composite restoration (Filtek Supreme XTE) (G4); a horizontal layer of high-viscosity flowable composite (G-ænial Flow, GC) was placed on pulp chamber floor, 10 mm x 3 mm glass fibers (everStick NET, GC) were inserted into the cavity (G5); same procedure as in Group 5 except the direct restoration was made incrementally with FSXTE (3M Oral Care) (G6); composite overlays were placed (G7). Specimens were scanned with micro-CT to evaluate 3D interfacial gaps before and after chewing simulation using Mimics software to calculate voids between restoration and tooth (dentin and enamel). These data (in mm^3) were collected for statistical analysis. Thereafter, specimens were loaded to fracture using a universal testing machine. Maximum breaking loads were recorded in Newton (N). The data obtained were analyzed using two-way ANOVA and post-hoc Tukey's test ($p < 0.05$).

Results: ANOVA showed that horizontal glass-fiber insertions statistically significantly reduced interfacial gaps after chewing simulation. No differences in fracture resistance were found between Filtek Supreme and Ever-X; moreover, glass-fiber insertion did not significantly improve fracture resistance in either case. Composite overlays achieved significantly better fracture toughness than did direct restorations.

Conclusions: For the direct restoration of endodontically treated premolars, the insertion of glass fibers into direct composite restorations was unable to guarantee a significant increase in the fracture resistance or a significant change in the fracture pattern. However, it significantly reduced interfacial gap volume after cycling fatigue.

Introduction

Restoration of endodontically treated teeth remains a challenge for clinicians, since non-vital posterior teeth generally are less stiff and more susceptible to fracture than vital teeth. This can be due to the loss of a large amount of tissue through carious lesions, making endodontic access, and root canal preparation. Fracture resistance further decreases when such endodontic treatment is associated with mesio-occlusal-distal (MOD) cavities, since the loss of marginal ridge integrity decreases the tooth's stiffness. Based on in vitro studies, maxillary premolars with deep MOD cavities are susceptible to fracture if extrinsic forces are applied.[65,67] In particular, fractures of the palatal cusps occur more frequently due to their anatomic form, an unfavorable crown/root ratio, dental arch position, and exposure to shear and compressive forces.[50,61] Thus, the remaining tooth structure and the efficacy of the restorative procedures to replace lost structural integrity are crucial for the longevity of endodontically treated teeth.[19] Different treatment strategies have been proposed, including intra-coronal post systems, modified directly placed restorations, different core materials and designs, and adhesive techniques, all of which exhibit certain advantages and disadvantages.[37,73] Regardless of the foundation core, a full-crown restoration remains the most proven solution in literature owing to its high longevity.[59,68] However, less invasive bonded clinical solutions such as indirect onlays, overlays, or endocrowns have been suggested as more conservative approaches for full-coverage restorations.[36,52]

Despite the significant development of bonded restorations, composite resins fail predominantly due to occlusal wear or secondary caries.[45,46] A common complication potentially contributing to the loss of integrity and influencing the resistance of a restored tooth is interfacial microleakage.[57,64] This can be caused by polymerization of composite resin, which is accompanied by contraction stress. The concomitant volume reduction generates a tensile force at the weakest area of the tooth-restoration interface, and stress-relieving gaps form which promote microleakage. If these gaps exceed ca 60 µm in width, postoperative sensitivity and secondary caries may form at the outer margin of the restoration.[28] Furthermore, during oral function, the tooth-restoration complex is exposed to fatigue stress resulting from cyclic loading, with the progressive onset of gap formation and interfacial microleakage.[42] A recent method to detect interfacial gaps is x-ray micro-computed tomography (μCT). Without destroying the original specimen, x-rays penetrate through it and images are collected by a detector slice-by-slice. This two-dimensional information is processed using special algorithms; a three-dimensional reconstruction is generated. Studies using μCT in restorative dentistry are increasingly being performed, since this technique has proven effective for the evaluation of the internal adaptation of composite resin restoration,[31,71] as well as the magnitude and direction of polymerization shrinkage.[14,20] Furthermore, it quantifies interfacial leakage with silver nitrate infiltration.[12,72]

Nowadays direct resin composite restorations are the most widespread, useful, and least invasive approach to restore endodontically posterior teeth.[13,48] To increase fracture resistance, glass fibers and a fiber post have been inserted into direct composite restorations.[2,33] Particularly, ultrahigh-molecular-weight polyethylene fiber (PWT) with an ultrahigh elastic modulus was tested to reinforce the polymer-based materials.[11,15] Some studies showed that their network changed the stress dynamics at the enamel-composite interface;[35] therefore, their effect on fracture resistance reported in literature is contradictory.[7,54] Moreover, knowledge is still limited about interfacial gap progression after fatigue stress and fracture resistance of glass-fiber-reinforced composite restorations in endodontically treated posterior teeth. Thus, this in vitro study aimed to evaluate the interfacial gap, fracture resistance, and failure pattern of endodontically treated maxillary premolars restored with glass-fiber–reinforced composites. The null hypothesis was that glass fibers do not increase the fracture resistance of direct composite restorations in endodontically treated teeth (1) and do not influence interfacial gap (2).

Materials & Methods

Eighty-four extracted intact maxillary premolars with mature apices, extracted for orthodontic and periodontal reasons, were selected. The inclusion criteria were: sound teeth with nearly similar crown sizes (7 mm ± 1 mesio-distally, 10 mm ± 1 bucco-orally) and no cracks under transillumination and magnification, extracted within 1 month of testing. Scaler and a hand-scaling instrument were used for surface debridement, followed by cleaning with a rubber cup and pumice slurry. The teeth were stored in distilled water at room temperature until required.

Endodontic treatment was carried out in all specimens, except in the control group (intact teeth). Samples were endodontically instrumented using Pathfiles (1-2-3) and ProTaper Next X1 and X2 (Dentsply Maillefer; Ballaigues, Switzerland) to a working length set at 1 mm short of the visible apical foramen. Irrigation was performed with 5% NaOCl (Niclor 5, Ogna; Muggiò, Italy) alternating with 10%

EDTA (Tubuliclean, Ogna) using a 2 ml syringe and 25-gauge needle. Thereafter, specimens were obturated with gutta-percha (gutta-percha points, medium, Inline B.M. Dentale; Torino, Italy) using the Down Pack heat source (Hu-Friedy; Chicago, IL, USA) and an endodontic sealer (Pulp Canal Sealer EWT, Kerr; Orange, CA, USA). Backfilling was performed with the Obtura III system (Analytic Technologies; Redmond, WA, USA).

After 48 h of storage in distilled water, a standardized MOD cavity was prepared by the same operator in all specimens, except in the positive control group. For cavity preparation, cylindrical diamond burs (#806314014; Komet; Schaumburg, IL, USA) under copious air-water cooling were used in a high-speed handpiece (Kavo; Biberach, Germany). The residual thickness of the buccal and oral cusps at the height of the contour was 1.5 ± 0.2 mm in all specimens, with the medial and distal cervical margins located 1 mm coronal to the CEJ. After preparation, all internal edges were smoothed and rounded.

Standardized adhesive procedures were performed in all specimens. The enamel margins were etched with 36% phosphoric acid (Ultraetch, Ultradent; South Jordan, UT, USA) for 40 s, while dentin was etched for 15 s. Thereafter, specimens were washed and gently air-dried with an air syringe, preventing the dentin from dehydrating. A multi-mode adhesive (G-Premio Bond, GC; Tokyo, Japan) was applied following the manufacturer's instructions and cured for 20 s with an LED curing light (Valo, Ultradent) at 1400 mW/cm^2. Later, specimens were randomly assigned to 7 groups (n = 12 each) according to the restorative material employed (Fig 1):

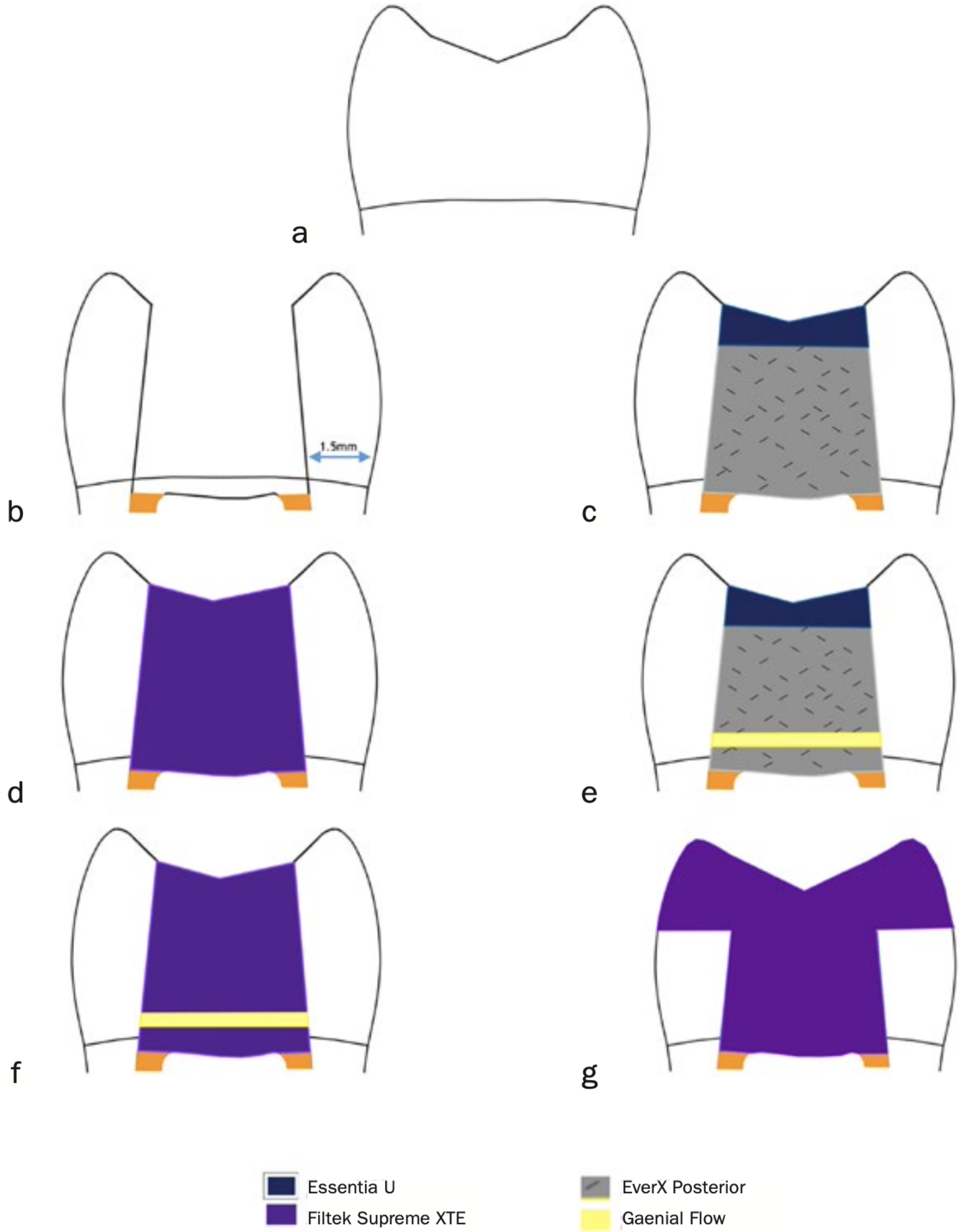

Fig 1. Sample preparation with wall thickness measurement area for all groups. a) Group 1: sound teeth; b) Group 2: MOD cavity without restoration; c) Group 3: direct restoration with Ever-X; d) Group 4: direct restoration with Filtek Supreme XTE; e) Group 5: direct restoration with everStick NET in the bottom of the cavity; f) Group 6: direct restoration with Filtek Supreme XTE and fiber (EverStick.NET) on the bottom of the cavity; g) Group 7: overlay on Filtek Supreme XTE buildup.

- Group 1 (G1, positive control): sound teeth (no cavity preparation or root canal treatment);
- Group 2 (G2, negative control): the MOD cavity was not restored;
- Group 3 (G3): the MOD cavity was incrementally restored with short-fiber–reinforced composite (Ever-X Posterior, GC; Tokyo, Japan), curing each 1.5- to 2 mm-thick layer with an LED curing light (Valo) at 1400 mW/cm2, leaving 2 mm for placement of top layer using micro-hybrid composite (Essentia U, GC);
- Group 4 (G4): the MOD cavity was restored with a nano-hybrid resin composite (Filtek Supreme XTE, 3M Oral Care; St Paul, MN, USA, FSXTE) applied in 1.5 to 2 mm layers using an oblique incremental technique. Each layer was light cured with an LED curing light (Valo) at 1400 mW/cm2;
- Group 5 (G5): a horizontal layer of high-viscosity flowable composite (G-ænial Flow, GC) was placed over the pulp chamber floor. The glass fibers (everStick NET, GC) were cut to measure 10 mm long and 3 mm wide, inserted into the cavity, and adapted onto the pulpal floor in a buccal-oral direction, remaining 1 mm from the occlusal enamel margins. After light curing for 20 s with an LED lamp (Valo), a direct composite restoration was performed as described in Group 3;
- Group 6 (G6): specimens were restored with the same procedure described for Group5 except for the material used. Direct restoration was performed with FSXTE, applied in 2 mm layers following an incremental oblique technique. Each layer was light cured with an LED curing light (Valo) at 1400 mW/cm2;
- Group 7 (G7): a buildup with nanohybrid composite (Filtek Supreme XTE, 3M) was performed with a 2 mm oblique layering technique. Thereafter, a standardized overlay preparation with 2 mm cusp reduction was per formed. Composite overlays of equal thickness were prepared on a gypsum cast obtained after taking a mono- phase bicomponent impression with a light-body putty silicone material (Flexitime; Heraeus Kulzer). Overlays were post-cured (Labolight LV-III; GC, Tokyo, Japan) for 5 min and cemented using a dual-curing luting system (G- Cem Link Force, GC) following manufacturer's instructions. The overlays were inserted into the cavities and fixed in place manually, applying pressure to the occlusal surface with a large plugger. Excess luting composite was removed with a fine spatula along all sample margins. Polymerization was performed with an LED curing unit (Valo) for 60 s/surface. The luting composite was cured for an additional 10 s/surface after applying a thin layer of glycerin gel to eliminate the oxygen-inhibition layer on the surface of the luting composite.

All restorations were made by the same experienced operator, who aimed to obtain an intercuspidal angle of 90 degrees to standardize the cusp inclination, thus allowing reproducible positioning of the steel sphere during compressive tests. All restored specimens were finished using a fine diamond bur (8379314016, Komet, Gebr. Brasseler; Lemgo, Germany) and polished with fine Sof-Lex disks (3M Oral Care) and silicone cups (Dimanto, Voco; Cuxhaven, Germany). They were then stored in distilled water at 37°C for 1 week.

The marginal integrity of each restoration was evaluated using a Micro-CT scan (SkyScan 1172 Micro-CT, Bruker Optik; Ettlingen, Germany). Specimens were scanned with parameters set for high resolution: voltage = 100 kV, current = 100 µA, aluminum and copper (Al+Cu) filter, 10 µm pixel size, averaging = 5, rotation step = 0.1 °, total scan duration = 6 h. NRecon software (Bruker Optik) and Data Viewer software (Bruker Optik) were used to reconstruct specimens and obtain 3D images.

Specimens were stored in distilled water at 37°C for 24 h and then cleaned for 10 min by sonication. A CS-4.4 chewing simulator (SD Mechatronik; Feldkirchen-Westerham, Ger- many) performed fatigue cycling to mechanically age specimens, which were embedded in light-curing acrylic resin. Resilience of the human periodontium was simulated by coating the tooth roots with a 1 mm layer of polyether (Impregum, 3M Oral Care)[55] before embedding them in light-curing acrylic resin. A 6 mm-diameter steatite sphere was ap- plied using an occlusal load of 50 N, a frequency of 1 Hz, a downward speed of 16 mm/s, and a 2 mm sliding movement over the palatal triangular crest. All restored specimens possessed a standardized anatomy and were similarly positioned for the sphere to apply pressure onto the mesio- buccal, disto-buccal, and palatal cusps (tripod contacts). The test was performed for 500,000 cycles in distilled water.

To reveal the marginal gap progression between the restoration and tooth structure after cycling fatigue, specimens were scanned a second time with the same baseline parameters to ensure consistency in the greyscale values. Initial scans were aligned with post-chewing scans using the DataViewer software (Bruker microCT) and reconstructed with Nrecon using the same protocol. Thresholding was performed automatically with the Mimics Medical 20.0 software (Materialise; Leuven, Belgium), to obtain a void mask representing the voids between the restoration and the tooth (dentin and enamel). Using the dynamic region growing function, only the external gap was considered in this analysis. Volume data, expressed in mm^3, were calculated and collected for statistical analysis (Fig 2).

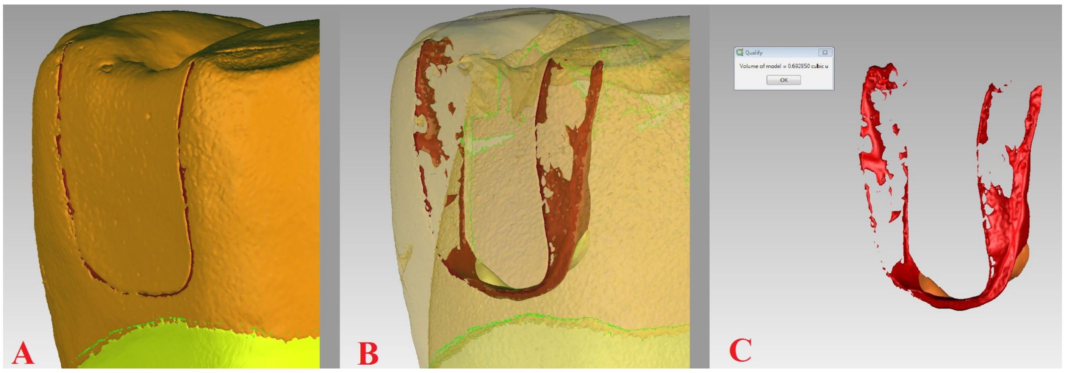

Fig 2. Micro-CT 3D images of specimens to calculate interfacial gap volume. a: every specimen was 3D reconstructed dividing enamel, dentin, restoration, and voids using Hounsfield scale's spikes on Mimics software. Optimal quality STL images were then imported into Geomagic Qualify for analysis. b: same view with enamel, dentin, and restoration set to transparency 60% to better visualize void areas in the interface. c: volume calculation was automatically performed by Geomagic Qualify on void STL images, setting units in millimeters.

Specimens were submitted to a static fracture resistance test using a universal testing machine (Instron; Canton, MA, USA) with a 6 mm-diameter steel-sphere crosshead welded to a tapered shaft and applied to the specimens at a constant speed of 0.5 mm/min and an angle of 30 degrees to the long axis of the tooth. Load was applied perpendicular to the triangular crest of the palatal cusp. Samples were loaded until fracture; the maximum breaking loads were recorded in Newton (N). Broken specimens were analyzed under a stereomicroscope (SZX9, Olympus Optical; Tokyo, Japan). The types of failure were determined and compared, particularly with a distinction made between catastrophic (irreparable, below the CEJ) and non-catastrophic fractures (reparable, above the CEJ).

Interfacial gaps and fracture resistance are expressed as means ± standard deviation (SD). The Kolmogorov-Smirnov test for normality revealed a normal data distribution. Statistical analysis was conducted with a two-way ANOVA to examine the effects of the factor "fibers" and "restoration" (Filtek vs Ever-X vs Overlay) and their interactions on fracture resistance and interfacial marginal gap progression. Post-hoc pairwise comparison was performed using Tukey's test. For all statistical analyses, statistical significance was pre-set at $p < 0.05$. All statistical analyses were performed using Stata 12.0 (StataCorp; College Station, TX, USA).

Results

Means (± SD) of interfacial gaps, expressed in mm^3, before and after fatigue load, obtained in different groups are displayed in Table 1. Two-way ANOVA showed a significant increase in marginal gaps after chewing simulation in G3 ($p = 0.0001$), G4 ($p = 0.0001$), and G7 ($p = 0.00001$). Thus, the insertion of horizontal glass fibers reduced interfacial gap propagation after chewing simulation and with composite overlays.

	Ever-X Posterior	Filtek Supreme XTE	Ever-X Posterior + Fiber	Filtek Supreme XTE + Fiber	Composite Overlay
Before	0.415^{Aa} (±0.123)	0.499^{Aa} (±0.145)	0.424^{Aa} (±0.156)	0.434^{Aa} (±0.172)	0.322^{Ba} (±0.112)
After	0.705^{Ab} (±0.189)	0.788^{Ab} (±0.175)	0.568^{Ba} (±0.145)	0.551^{Ba} (±0.199)	0.398^{Ca} (±0.982)

Table 1. Means and SD of interfacial gap, expressed as mm^3, before and after chewing simulation obtained in different groups. Same superscript capital letters indicate no difference between row results. Same superscript lower-case letters indicate no difference between column results.

Fracture resistance values (in N) obtained in different groups are listed in Table 2. Two-way ANOVA showed a significant difference for the variable restoration ($p = 0.00001$). The post-hoc Tukey test showed that sound teeth had a significantly higher fracture resistance than other groups (934.91±143.08 N), while non-restored cavities presented significantly lower values (100.80±12.28 N). No differences in fracture resistance were found between Filtek Supreme (451.92±60.39 N) and Ever-X (465.36±66.71 N); fiber insertion did not significantly improve the fracture resistance of either (respectively 499.79±66.77 and 515.96±72.54 N). Additionally, composite overlays achieved significantly better fracture toughness than the direct restoration techniques tested, regardless of materials used (705.70±123.62 N).

Group	n	Fracture Load (Mean ± SD)	Minimum	Max
Sound tooth	12	934.91 ± 143.08^{a}	569.66	1039.45
Unrestored Cavity	12	100.80 ± 12.28^{d}	86.51	120.10
Ever-X Posterior	12	465.36 ± 66.71^{b}	376.01	630.01
Filtek Supreme	12	451.92 ± 60.39^{b}	383.70	587.24
Ever-X Posterior with glass-fibers	12	515.96 ± 72.54^{b}	480.79	773.19
Filtek Supreme with glass-fibers	12	499.79 ± 66.77^{b}	307.77	699.43
Composite overlay	12	705.70 ± 123.62^{c}	519.86	939.46

Table 2. Mean fracture load, expressed in Newton, obtained in different groups. Differences were considered significant at p < 0.05

The fracture analysis revealed that in all fractured restorations, the origin of the fracture was always at the occlusal surface, mainly from the major contact loading area of the sphere in the stepwise fatigue test. The direction of fracture propagation was corono-apical. The number of fractures per group is shown in Fig 4. Reparable fractures started from the occlusal surface and ended above the CEJ, while irreparable fractures progressed in a mesio-distal vertical direction, which split the restoration and ended under the CEJ. In groups with a direct restoration (G3, G4, G5, G6), the main fractures were always adhesive. The debonding of the restoration, which started from the occlusal surface, occurred on the wall loaded. Some mixed fractures (adhesive-cohesive) occurred, predominantly in G7. Fracture analysis showed that the presence of glass fibers was unable to significantly alter fracture propagation, which mainly ended above the CEJ.

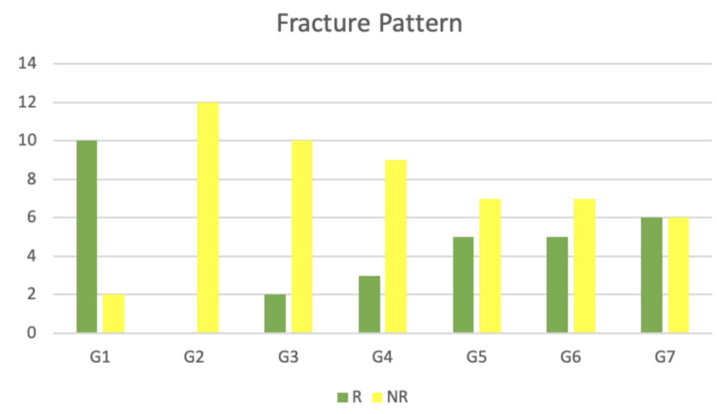

Fig 4. Number of reparable (R) or irreparable (IR) fractures identified in the different groups.

Discussion

Based on the results obtained, the null hypothesis was partially rejected, since glass fibers did not significantly increase the fracture resistance of the direct composite restorations in endodontically treated teeth. However, they did significantly reduce interfacial marginal gap progression after fatigue loading. Biomechanical analysis of endodontically treated teeth shows that coronal destruction due to caries and the loss of marginal ridge integrity decreases tooth rigidity[3,66,67] and thus the fracture resistance. In the current study, MOD cavities were prepared to decrease fracture resistance as much as possible and to better evaluate the reinforcement abilities of the tested restorative techniques. MOD cavities represent the worst clinical condition to restore in endodontically treated posterior teeth. Several studies have shown that MOD preparation and endodontic treatment accentuated the concentration of stress inside the tooth, mainly due to the loss of marginal ridges[38,50,68] and the resistance to cuspal fracture.[22,58] Thus, ideal restoration of endodontically treated teeth should improve mechanical resistance, reinforcing the weakened remaining structure, to prevent fracture and to ideally restore the fracture strength to that of an intact tooth.

The literature supports the idea that non-vital posterior teeth should be treated with a cuspal coverage restoration to increase fracture resistance.[60] However, saving sound tooth structure is crucial. Today, the good quality of adhesives and the high-performance properties of resin composite materials [40,50] have enabled minimally invasive approaches; they offer a valid option for the restoration of endodontically treated teeth which could be comparable to full-crown restorations.[16,37] It is reported that adhesive restorations better transmit and distribute functional stresses at the bonding interface to dental hard tissues, potentially reinforcing the weakened tooth structure, especially in large cavities.[44] Furthermore, they decrease cusp flexion.[43] However, there is currently no consensus on the ideal final coronal restoration of endodontically treated posterior teeth.

Previous studies have demonstrated that regardless of the posterior tooth type (premolar or molar), class II resin-based composite restorations most frequently fail due to marginal leakage when the synergism of the tooth-composite interface, mediated by the adhesive bond is compromised.[17,70] Initially, a gap may originate through polymerization shrinkage and failure to obtain a good bond. Thereafter, occlusal stresses generated during mastication and especially through parafunctional habits, such as bruxism, have been shown to have a deleterious effect on the marginal adaptation of composites,[49] especially at gingival margins where occlusal forces tend to concentrate.[23] These mechanical stresses repeated over time lead to the fatigue or weakening of the adhesive interface. Once the concentrated stresses exceed the interfacial fracture toughness, a crack can form, which in turn may lead to further gap formation and microleakage.[39] In the present study, to evaluate the interfacial marginal gap of resin-based restorations, specimens were scanned before and after chewing simulation with microCT, which has proven to be an easy and accurate method to detect and evaluate 3D volumetric gaps.[41] The literature contains little on the interfacial behavior of resin-based materials examined with non-destructive techniques. However, in the oral cavity, materials are subjected to mechanical, thermal, and chemical processes; they induce fatigue damage which progresses from substructural and microscopic changes to microscopic cracks to structural instability and complete fracture.[62] Thus, interfacial analysis is crucial to better understand the kinetics of biomechanical failure. A limitation of the present study was the absence of thermal stress; intra-oral temperature changes exert an effect on the composite-tooth interface similar to mechanical stress, since composites and adhesives have a higher thermal contraction/expansion coefficients than do hard dental tissues.[25] Moreover, only marginal gaps were evaluated in the present study, not the internal adaptation of the restoration. Marginal gap formation is the result of a localized bond failure;[10] it is a concern where micro-gaps are found in the interface between restorative material and tooth substrate, resulting in leakage. Nevertheless, the marginal seal may be different from internal adaptation, because localized debonding may produce micro-gaps that are not always associated with the outside margin and are not readily apparent.[29]

Measuring the fracture strength is a static test used to predict the failure of restored teeth under compression.[63] In accordance with previous studies, no statistically significant difference was found in fracture resistance between the different direct restorative materials.[4] Short-fiber–reinforced composites are expected to enhance the longevity of medium-to-large sized composite restorations in posterior teeth,[24] because the fracture toughness of the short-fiber composite resins is generally higher than that of conventional composite resins, as shown in several studies.[18,26] This property is ascribed to the millimeter-scale short fibers, which exceed the critical fiber length,[69] enabling stress transfer from the matrix to the fibers. Furthermore, the presence of fibers results in an anisotropic property that has been suggested to relieve stress and prevent crack propagation.[69] However, a significant improvement of fracture resistance was observed between the direct techniques tested without glass-fibers insertions, which led to a slight but significant increase in load resistance independent of the composite material used. Nevertheless, it is important to highlight that none of the restoration techniques tested could re-establish the fracture resistance equivalent to that of a sound maxillary premolar.

The results of the present in vitro study showed that the insertion of glass fibers in direct composite restorations significantly influenced marginal adaptation after fatigue; yet this did not statistically increase the fracture resistance of endodontically treated maxillary premolars. This result could be due to the effect of the insertion of horizontal fibers into the composites, which significantly improved their mechanical properties,[9,26,27] particularly their flexural strength.[32] This could lead to a lower cuspal deflection under cyclic loading, which is directly correlated to a reduction of marginal leakage that creates a gap at the tooth-restoration interface with consequent marginal infiltration.[1] The presence of glass fibers in the resin composite could even alter the elastic modulus of the material itself, thus modifying the stress distribution and transmission to residual cavity walls. As mentioned above, an anisotropic characteristic[69] may also play a role. However, any significant improvement of fracture resistance was observed between the direct techniques tested without glass-fibers insertions, which led to a slight but significantly increased load resistance regardless of the composite used. However, it is important to emphasize that none of the restoration techniques tested could re-establish fracture resistance equivalent to that of a sound maxillary premolar. The obtained results agreed with those of Rodrigues et al.,[54] who found that fibers placed into MOD cavities did not reinforce teeth. Those authors reasoned that cusp deflection resilience occurred due to adhesive and composite resin, not due to the glass fibers insertion, which instead could have a protective effect on fracture propagation towards the pulp chamber floor. Furthermore, Cobankara et al. [15] showed no difference between a resin composite restoration with or without fibers in MOD cavities in molars.

In the present study, glass fibers were inserted in a buccal-oral direction (u-shaped), similar to the method suggested by Belli et al.[6,7]. Some authors reported that the form and direction of fibers, their composition, fiber/resin volume ratio and the bond strength between fibers and resin had an influence on the reinforcing effect.[8,44] Moreover, there is evidence that the mechanical properties of the composite depend on the type, extension, and length of the fibers.[56] Belli et al[7] showed that the use of polyethylene ribbon fibers under composite restorations increased the fracture resistance thanks to their ability to connect the residual walls and modify stress transmission and distribution along the restoration-dentin interface. However, that study was conducted on molars which were not subjected to cyclic loading before fracture. A similar effect was found by Karzoun et al.[34] They placed a horizontal fiber post into the post-endodontic composite restoration, joining palatal and buccal walls of a MOD cavity. This technique showed a slight, nonsignificant increase in fracture resistance, even if the horizontal post did not prevent catastrophic fractures.

Both in terms of marginal gap formation and fracture resistance, the best result was obtained with composite overlays. This can be explained by the strengthening effect of buccal and oral cusp connection provided by this therapeutic option. Other authors demonstrated the efficacy of the luted indirect techniques, based on shrinkage limited to the very thin layer of luting material.[5] Previous studies have shown that cuspal coverage with adhesive restorations is a valid option to increase the tooth fracture resistance of endodontically treated teeth.[8] However, Rocca et al., [53] who placed bi-directional E-glass fibers over the pulpal chamber area of devitalized molars restored with CAD-CAM resin composite overlays, found that such restorations did not benefit from the simultaneous use of glass fibers. Moreover, Fennis et al. [21] obtained similar results: the incorporation of fiber-reinforced composite did not increase the load-bearing capacity of premolars with cusp-covering restorations. This could be related to the fact that the overlay thickness puts distance between the glass fiber and the loading impact area. In fact, Oskoee et al. [47] suggested that the fracture resistance increased when fibers were placed close to the point where force was exerted, as this led to a shorter working arm and a lower input force, according to the lever principle of Archimedes. Additionally, placing fibers on the occlusal surfaces keeps buccal and lingual cusps together, resulting in higher fracture resistance. Thus, placing glass fibers in the cervical to middle thirds did not significantly increase the fracture resistance.

Conclusions

- MOD cavity preparations significantly reduced the fracture resistance of endodontically treated premolars, but none of the restorations tested were able to restore the original fracture resistance.
- The insertion of glass fibers into direct composite restorations could not ensure a significant increase in fracture resistance or a significant deviation of the fracture pattern
- The insertion of glass fibers into direct composite restorations was able to significantly reduce the interfacial gap opening after cycling fatigue.

Further studies are necessary to confirm these results.

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2.6 Could different direct restoration techniques affect interfacial gap and fracture resistance of endodontically treated anterior teeth?

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Abstract

Objectives: To evaluate different direct restoration techniques on various cavity designs in anterior endodontically treated teeth (ETT).

Materials and Methods: Ninety upper central incisors (n = 90) were selected, endodontically treated and divided into three groups (n = 30) accordingly to the cavity design: minimal endodontic cavity access (Group A), endodontic access + mesial class III cavity (Group B), endodontic access + two class III cavities (Group C). Three subgroups (n = 10) were then created accordingly to the restoration technique: nano hybrid composite restoration (Subgroup a), glass fibre post + dual-cure luting cement (Sugroup b), bundled glass fibre + dual-cure luting cement (Subgroup c). Samples underwent micro-CT scan, chewing simulation and a second micro-CT scan. 3D quantification (mm^3) of interfacial gap progression was performed, then samples underwent fracture resistance test. Data were statistically analyzed setting significance at $p < 0.05$.

Results: Groups A and B showed significantly lower interfacial gap progression compared to group C ($p < 0.001$). Subgroup b performed significantly better compared to subgroup a ($p < 0.001$) and c ($p = 0.005$). Improved fracture strength was reported for group C compared to group A ($p = 0.005$), while both subgroups b and c performed better than subgroup a ($p < 0.001$).

Conclusions: Cavity design significantly influenced interfacial gap progression and fracture resistance. Fibre posts significantly lowered gap progression and improved

fracture resistance while bundled fibers only increased fracture resistance. A significant reduction of non-repairable fractures was recorded when fibers were applied.

Clinical Relevance: A minimally invasive approach, conserving marginal crests, should be applied whenever possible. Inserting a fibre post is indicated when restoring anterior ETT, in order to reduce gap progression, improve fracture resistance and avoid catastrophic failures.

Introduction

Restoration of endodontically treated teeth (ETT) remains a challenge for dental clinicians, as the endodontic treatment weakens the tooth structure in terms of biomechanical behavior compared to the vital counterpart. In fact, ETT are more brittle due to structural changes in dentin, loss of water and weakened collagen cross-linking [1]. These changes lead to increased cuspal deflection during function, with consequent higher occurrence of fractures [2,3]. For this reason, post-endodontic restoration challenge is to recover the biomechanical behavior of the tooth and prevent catastrophic fractures.

Several types of restorations have been proposed in literature to restore and reinforce ETT. In the past, traditional full coverage crowns in combination to metal post showed enhanced longevity, in the expense of an invasive procedure [4–6]. Thanks to the introduction of adhesive techniques, less invasive procedures are nowadays available to restore compromised teeth. Recent studies reported that the mechanical resistance and the longevity of ETT directly depends on the amount of residual tooth structure, meaning that a minimally invasive approach should be applied whenever possible. Direct resin composite restorations represent the least invasive approach in order to preserve the much sound structure possible. For this reason, they have been frequently studied to evaluate their efficacy when restoring an ETT, showing a significant increase in fracture resistance when the direct restoration was reinforced by fiber posts [7–10]. This trend was also confirmed by the in-vivo evidence that highlighted a positive correlation between post insertion and restoration longevity [11–13]. However, despite great evidence regarding posterior teeth, few information concerning the direct restorations efficacy in endodontically treated anterior teeth are available.

In addition to the previously introduced concepts, it has to be considered that anterior restorations are subjected to high masticatory loads and parafunctional forces. Thus, fracture is a relatively common clinical failure that occur over time [14,15]. A recent review by Heintze et al. reported that the lack of mechanical retention in class IV restoration must be considered an adhesive challenge and seems to lead to twice as high failure rate than Class III restorations. A higher prevalence of failure in Class IV restorations in bruxers was also reported by van Dijken et al. [16], showing that overloading and increased mechanical stresses in the restorations are making them more prone to fracture and secondary caries.

The evaluation of a direct restoration efficacy should not be focused on the tooth structure reinforcement effect only. Indeed, occlusal stresses generated during mastication and, especially, during parafunctional activities, such as bruxism, were shown to have a deleterious effect on the marginal adaptation of composites [17]. These mechanical stresses repeated over time lead to fatigue weakening of the adhesive interface, ultimately generating a gap that may further lead to microleakage [18]. Even if a direct correlation between microleakage and clinical parameters has not been proved [19], gaps that exceed a width of 60 μm could possibly lead to bacteria accumulation, ultimately leading to sensitivity and increased chance of secondary caries [20–22].

The aim of the present in vitro study was to evaluate the effect of different direct restoration techniques on endodontically treated anterior teeth with different cavity designs, analyzing interfacial gap and fracture resistance. The null hypothesis tested were that the cavity design (1) and the restoration technique (2) do not affect the interfacial gap and the fracture resistance of endodontically treated central incisors.

Materials & Methods

Ninety upper central incisors (n = 90) with similar crown and root size (length>14±2 mm), extracted within four months for periodontal reasons, were selected. Manual scaling was performed for surface debridement, followed by cleaning with a rubber cup and pumice. Specimens were disinfected in 0.5% chloramine for 48 h and then stored in 4% thymol solution at room temperature until use. Samples were double-checked with optical 4.5x magnification to exclude teeth with caries, previous restorations and visible cracks.

Selected teeth were endodontically treated using Pathfiles and ProTaper Next (Dentsply Maillefer, Ballaigues, Switzerland) to the working length, set at 1 mm short of the visible apical foramen. Irrigation was performed with 5% NaOCl (Niclor 5, Ogna, Muggiò, Italy) alternated with 10% EDTA (Tubuliclean, Ogna, Muggiò, Italy). The root canals were filled with gutta-percha cones trough a warm vertical condensation technique. Specimens were then divided into three groups (n = 30 each) accordingly to the cavity design, which were performed by the same experienced operator.

- Group A: specimens exclusively presented a minimal endodontic cavity access at the cingulum level. Gutta-percha was removed up to 3 mm below the Cemento-Enamel Junction (CEJ).
- Group B: additional to the cavity access, a single class III cavity was prepared on the mesial side using an egg-shaped diamond bur. To ensure reproducible cavity dimensions as much as possible, the mesio-distal, linguo-buccal and cervical-incisal extent of the tooth crown were measured with a caliper. Class III cavities included one third of the mesio-distal and linguo-buccal lengths and one quarter of the cervical-incisal extent. The cervical margin of the cavity was performed in enamel, ensuring a distance to the CEJ of 1 mm. Due to the selected mesio-distal dimension, the median part of the cavity was always connected to the endodontic cavity access.
- Group C: same as group B, but two class III cavities were prepared on mesial and distal side of each sample.

After cavity preparation, specimens were divided into three subgroups accordingly to the employed restoration technique (n = 10 each):

- Subgroup a: Cavity was etched with phosphoric acid (Conditioner 36, Dentsply, Konstanz, Germany) for 15 s, rinsed with water, and air-dried. A universal adhesive (Futurabond U, Voco, Cuxhaven, Germany) was applied uniformly at all cavity surfaces for 20 s using a micro brush, air-dried for 5 s and light-cured for 20 s with a multiLED lamp (1400 mW/cm^2; Bluephase Style, Ivoclar, Schaan, Luxembourg). A direct restoration with nano hybrid composite (Filtek Supreme XTE, 3M) was performed applying 2 mm thick layers with horizontal layering technique.
- Subgroup b: Post-space was prepared with dedicated drills for a total of 8 mm depth (Rebilda Post Drill, diameter 1.2 mm). A dedicated fiber post (Rebilda Post, Voco) was luted with a dual-cure luting cement (Rebilda GT, Voco) following manufacturer instruction. After light-curing for 40 seconds with a multiLED lamp (1400 mW/cm^2; Bluephase Style, Ivoclar), a direct composite restoration was performed as described for subgroup a.
- Subgroup c: Same as subgroup b, but using a bundled glass-fiber reinforced composite post (Rebilda Post GT, Voco).

All the restored specimens were finished and polished with fine-grit diamond burs and silicon points in order to obtain a smooth surface without over or under contouring, and then stored in distilled water. Figure 1 schematically reports the study design.

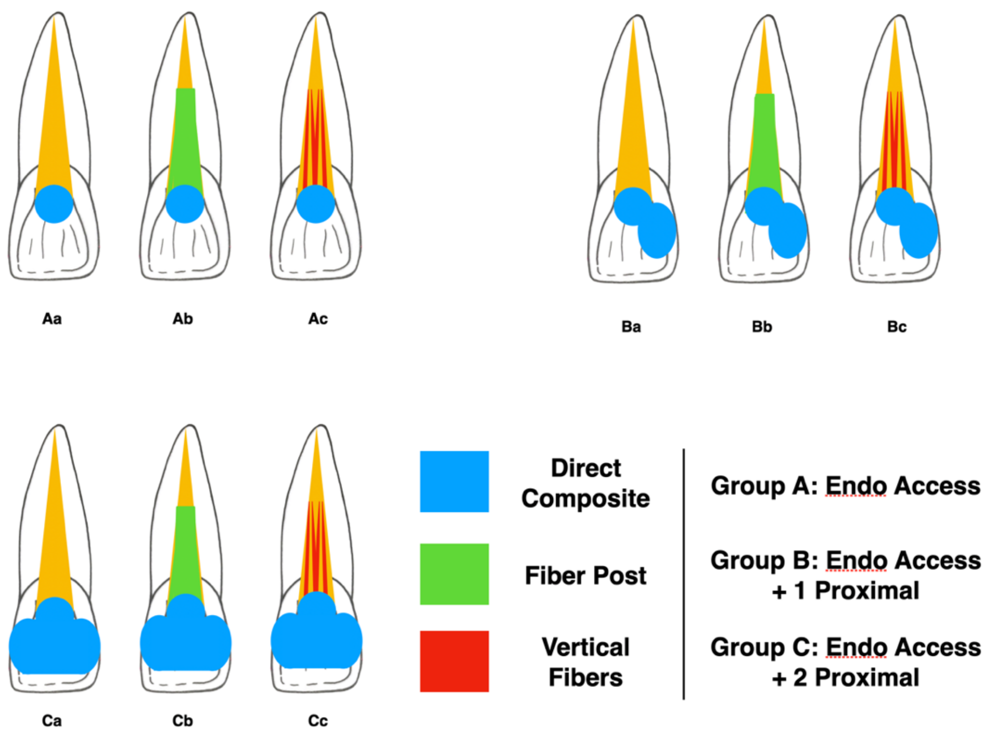

Figure 1. Schematic representation of the present study sample preparation protocol.

Each sample underwent a micro computed tomography (micro-CT) scan (SkyScan 1172; Bruker, Billerica, MA, USA) to evaluate interfacial gap. High-resolution scans were performed using the following parameters: voltage = 100kV; current = 100µA; aluminum and copper (Al+Cu) filter; pixel size = 15 µm; averaging = 5; rotation step = 0.5°. Images were reconstructed though NRecon software (Bruker, Billerica, MA, USA) in order to obtain DICOM files, with standardized parameters: beam hardening correction = 20%, smoothing = 3, ring artifact reduction = 9.

A CS-4.4 chewing simulator (SD Mechatronik; Feldkirchen- Westerham, Germany) was used for mechanical aging of the specimens. A 4 mm diameter metal cone was employed, using the following parameters: occlusal load = 50 N, frequency = 1 Hz, downward speed = 16 mm/s and 2 mm sliding movement. The movement pattern was set from the palatal cingulum towards the incisal edge. The test was performed for 500.000 cycles in water at room temperature.

To reveal interfacial gap progression between the restoration and the tooth structure after cyclic fatigue, samples were subjected to a second scan with same baseline parameters to ensure consistency in the greyscale values. Initial scans were then reconstructed with NRecon using the same protocol and aligned with post-chewing scans using DataViewer TM software (Bruker, Billerica, MA, USA). Thresholding was performed automatically with Mimics Medical 20.0 software (Materialise, Ann Arbor, MI, USA), in order to obtain a void mask representing gaps and voids inside the tooth-restoration complex, with external boundaries set at 1 mm from the direct restoration. A Hounsfield unit (HU) range of 1,024 to 950 was selected to maximize void visualization. The volume of the mask was automatically calculated by the software and recorded in mm^3. In order to specifically analyze gap progression and exclude composite internal bubbles volume, the result obtained from the baseline scan was subtracted from the volume of the second scan. Figure 2 reports the 3D rendering of a random sample (restoration and voids), seen from the inner surface (in contact with the tooth), before and after chewing simulation.

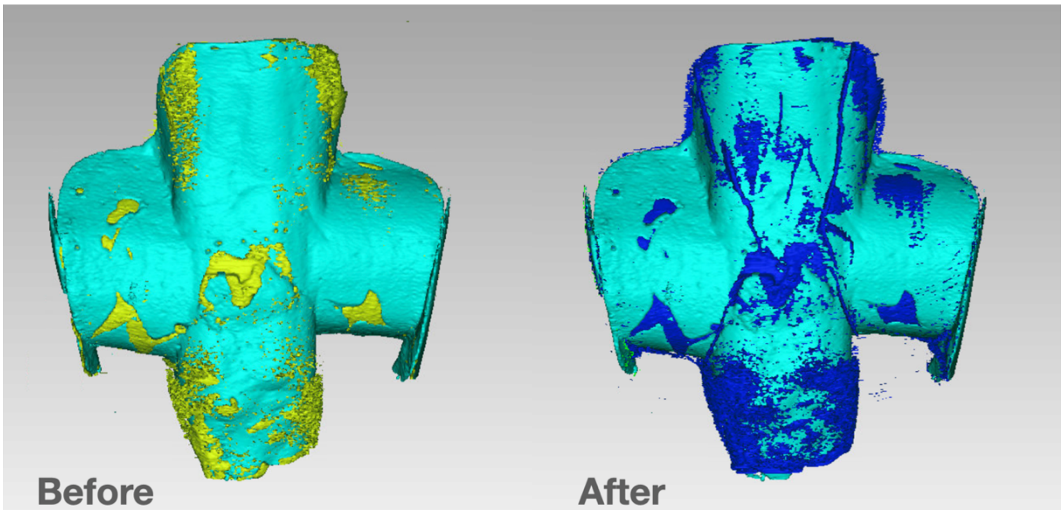

Figure 2. Random sample before (left) and after (right) chewing simulation. Light blue volume represents the restoration, seen from the inner surface. Yellow volume represents baseline void volume, while blue volume represents final void volume after cyclic fatigue. It is noticeable that many areas underwent degradation due to mechanical stresses and

crack lines appeared. To specifically analyze interfacial gap progression, final data recorded consisted in blue volume minus yellow volume.

Samples were then submitted to a static fracture resistance test using a universal testing machine (Instron 10-S; Canton, MA, USA) with a 4 mm-diameter metal cone crosshead welded to a tapered shaft and applied to the sample at a constant speed of 0.5 mm/min and an angle of 30° to the long axis of the tooth. Load was applied on the palatal cingulum until fracture and the maximum breaking loads were recorded in Newton (N).

Broken specimens were analyzed under a stereomicroscope (SZX9; Olympus Optical Co., Ltd., Tokyo, Japan). The types of failure were determined and compared, distinguishing between catastrophic fractures (non-reparable, below the CEJ) and non-catastrophic fractures (reparable, above the CEJ). Figure 3 reports two different fractures, as well as a schematic representation for clarification.

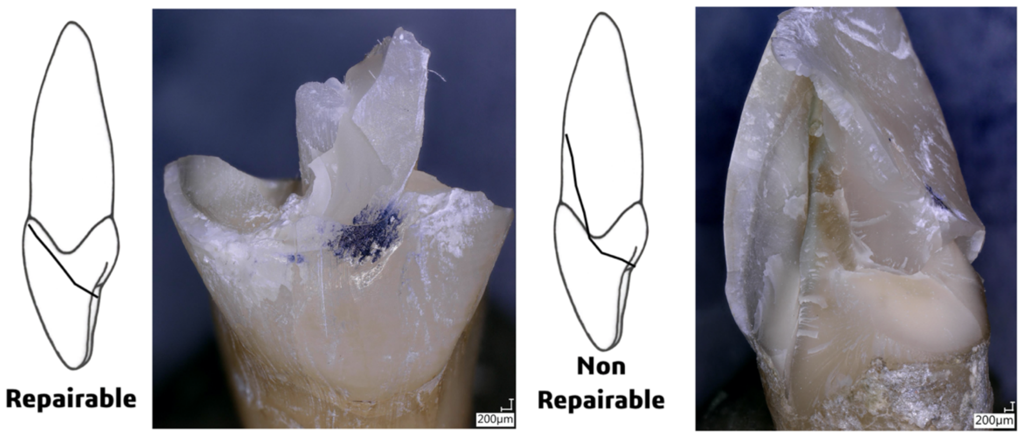

Figure 3. Random fractures recorded among samples. Notice how CEJ was taken as a reference point to distinguish reparable and non-reparable fractures.

To examine the effects of the factors “cavity design” and “restoration technique” on interfacial gap progression and the fracture resistance, a two-way analysis of variance test (ANOVA) was conducted. Post-hoc pairwise comparison was performed using Tukey test. All statistical analyses were performed using STATA software (ver. 12.0; StataCorp, College Station, TX, USA).

Results

Interfacial gap progression data, expressed as means ± standard deviation in mm^3, and fracture resistance, expressed in N, are summarized respectively in Table 1 and Table 2. Two-way ANOVA showed that interfacial gap was significantly related to the cavity design ($p < 0.001$) as well as to the restoration technique ($p < 0.001$), as well as the interaction between the two factors ($p < 0.001$). Tukey post-hoc test revealed that group A and B showed significantly lower interfacial gap increase after cyclic fatigue compared to group C and subgroup b showed significantly reduced gap formation compared to subgroup a and c.

	Subgroup a (No post)	Subgroup b (Fiber post)	Subgroup c (Bundled Fibers)
Group A (endodontical access)	0.12±0.06	0.29±0.09	0.27±0.08
Group B (mesial class III cavity)	0.27±0.09	0.19±0.08	0.22±0.07
Group C (mesial and distal class III cavities)	0.67±0.19	0.35±0.10	0.48±0.15

Table1. Mean interfacial gap variations ± standard deviation, expressed as mm^3, for each group and subgroup.

	Subgroup a (No post)	Subgroup b (Fiber post)	Subgroup c (Bundled Fibers)
Group A (endodontical access)	542.6±207.2	667.2±243.3	660.4±231.7
Group B (mesial class III cavity)	507.7±143.1	718.7±149.7	643.6±208.8
Group C (mesial and distal class III cavities)	335.8±86.5	663.1±166.3	537.8±108.2

Table2. Mean fracture resistance ± standard deviation, expressed in Newton (N), for each group and subgroup.

Concerning fracture resistance, two-way ANOVA showed a significance difference both for the factor “cavity design” ($p = 0.023$) and for the factor “restoration technique” ($p < 0.001$). The Tukey post-hoc test highlighted statistical improved fracture strength for subgroup b ($p < 0.001$) and c ($p = 0.005$) compared to the subgroup a. Concerning the cavity design factor, Tukey test showed statistical significance when group C was compared with group A ($p = 0.005$), with Group C performing significantly worse (lower fracture resistance). Recorded fracture patterns, classified between repairable and non-repairable, are reported in Table 3.

	Subgroup a (No post)		Subgroup b (Fiber post)		Subgroup c (Bundled Fibers)	
	Rep	Non-rep	Rep	Non-rep	Rep	Non-rep
Group A (endodontical access)	2	8	8	2	7	3
Group B (mesial class III cavity)	2	8	8	2	8	2
Group C (mesial and distal class III cavities)	0	10	7	3	5	5

Table 3. Fracture patterns for each group and subgroup, divided between repairable (rep) and non-repairable (non-rep).

After an accurate analysis of the reconstructed images, it was also observed, from a qualitative point of view, that some of the samples randomly presented pre-existent micro-cracks, not visible at 4.5x magnification, that propagated as a consequence of chewing simulation. Micro-cracks showed a tendency to continue inside the composite buildup when no fibers were applied (subgroup a) compared to samples reinforced with fibers (subgroups b and c). Figure 4 illustrates an example of this trend, showing the propagation of initial micro-cracks in two random samples from subgroups a and c, before and after chewing simulation.

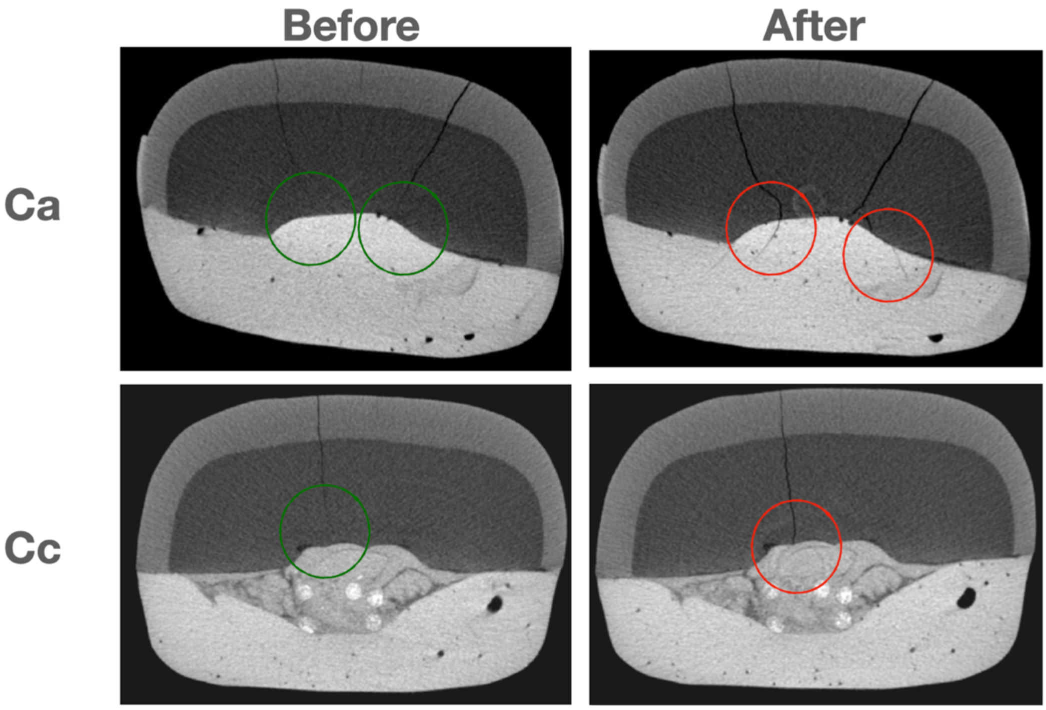

Figure 4. The first row shows a random sample (Ca) before and after chewing simulation. It is noticeable that cracks propagated from the tooth structure to the buildup itself. The second row shows another sample reinforced with fibers (Cc), where the crack propagation is clearly limited to the tooth structure.

Discussion

Clinical studies already demonstrated that incisors and canines have an overall higher failure rate compared to posterior teeth, as the occlusal forces are more transverse [23,24]. The cyclic fatigue derived from chewing, especially transversal forces, cause a progressive degradation and therefore "opening" of the adhesive interface [17,18]. The consequent marginal leakage is of critical concern when referring to composite restorations since it might lead to secondary caries and cracks, letting the tooth more prone to fracture [20,21]. Moreover, in ETT, marginal leakage led to a potential bacterial recolonization of the root canal system, ultimately causing endodontic failure [25].

Basaran et al. showed that a percentage of dye leakage at the interface between the post and the root canal was always present, regardless of the fiber post or the adhesive technique employed [26]. However, to date, two-dimensional techniques for the analysis of the interfaces are to be considered obsolete and limited compared to three-dimensional investigation methods. A recent technique to detect interfacial gaps is represented by μCT, that allows, without destroying the specimen, to generate 3D images. The number of studies using μCT in restorative dentistry is increasing, as this technique has proved effective for the evaluation of internal adaptation of composite resin restoration [27–32]. In the present study, cyclic intermittent loading induced an interfacial gap opening in all specimens, corroborating in vivo and in vitro previous findings that showed functional and parafunctional stresses, especially transversal forces, are able to cause marginal gap opening on adhesive interfaces [17,18].

Based on the present study results, the cavity extension as well as the use of fiber post were crucial in reducing the interfacial gap progression after cyclic fatigue; thus, the first null hypothesis was rejected. Interfacial gap openings occur during fatigue when cyclic forces induce a tooth flexion which is higher in non-vital teeth due to their reduced stiffness [33]. Loss of tooth structure is a key factor for stress resistance of endodontically treated teeth, in anterior as well as in posterior teeth. As demonstrated by Reeh et al. referring to premolars, the loss of marginal ridges can lead to a diminished fracture resistance going from 44 to 66% [33]. Obviously, if more tooth structure is preserved, cyclic forces find a higher resistance to flexion, thus leading to less interfacial gap formation. The present study showed that the loss of one or two marginal ridges is immediately correlated to increased interfacial gap, because they represent the anatomical portion in anterior teeth that provides resistance to traversal loads. The use of a fiber post is indeed crucial when extended cavities are present as their mechanical properties are close to the dentin [34,35]. Consequently, they can reproduce the natural load transmission mechanisms to the tooth structure reducing the risk and entity of gap formation. Moreover, an increased flexural strength when using a fiber post compared to composite-only build-up has already been demonstrated by several authors [36]. The higher flexural strength of fiber post might mediate loads between dentin and restoration materials, therefore resulting in a more homogenous stress distribution [37]. On the other hand, the placement of vertical bundled glass-fibers within the root canal did not significantly reduce the gap increase during cyclic fatigue, probably due to the lower flexural strength of this restorative solution if compared to the traditional glass fiber post.

Possible ways to restore compromised ETT were studied and analyzed in the past by many authors [4,38,39], who demonstrated an important reduction in tooth fracture when a full coverage crown was performed. However, this option is very demanding in terms of economical and biological costs for the patient. This concept is particularly true when referring to anterior teeth, whose fracture resistance is similarly correlated to the presence of residual tooth structure [40,41], but it is subjected to different biomechanical stresses during function and parafunction.

The present study results clearly showed that the cavity configuration in anterior teeth is directly correlated to the fracture resistance, which could be partially recovered by using a fiber-supported composite restoration. Thus, the second null hypothesis was rejected. It has been recently suggested, in order to improve fracture resistance in ETT, to insert fibers within direct resin composite restorations [42,43]. Thanks to their elastic modulus similar to dentin and stress bearing capabilities, fibers might reinforce the structure and lead to fewer root fractures. Literature however is not unanimous about the usage of fibers, with studies affirming that there is no significant difference in the use of a classic composite build-up and its corresponding post system [44]. On the other hand, other authors affirm that for anterior ETT fiber post placement seems advisable to improve static loads resistance, especially in cases with extensive loss of coronal tissues [45]. This is in accordance with the present study results, which reported ETT performing significantly better in fracture resistance test when a post or vertical bundled fibers were used. This reinforcement effect was mainly advisable in group C, probably because buccal enamel, incisal margin and oral cingulum are less involved in the tooth structure reinforcement compared to proximal ridges. This could also explain the different results obtained by Lausnitz et al.: a less invasive cavity design surely helps the specimen in resisting both fatigue cycles and fracture loads [46]. This is also accordance with the results of Vadini et al. that reported a significant benefit in resistance to static loads when a post was placed, particularly in cavity designs with extensive loss of coronal tissues (two Class II cavities) [45]. Anyway, further studies should focus and evaluate the contribution to the resistance to occlusal loads of the anatomical components of the anterior teeth in order to better understand the impact of cavity configuration and extension on their resistance to fatigue phenomena.

As demonstrated by Newman et al., [47] fiber-supported composite appears to dissipate forces along the root canal system, reducing peak stresses on the root and therefore moving the critical fracture point coronally, ultimately leading to repairable fractures [48,49]. On the other hand, rigid posts such as carbon fiber or cast posts and core seem to be more prone to cause non-repairable root fractures due to their elastic modulus [50]. Hayashi et al. studied the fracture mode when teeth restored with different post system were subjected to oblique and vertical load, concluding that vertical loadings caused crack propagation in the middle and apical portion of the roots, while with oblique loads most of the fractures occurred in the cervical part of the root when fiber posts were used, and in the middle part, when prefabricated metallic or cast metallic post-core were used [51]. Chieruzzi et al. showed that when a fiber-post is used, the stress generated through dentin, cement and post is well-distributed and without any relevant peak. Therefore, it can be concluded that the use of glass fiber allows to simulate the mechanical behavior of natural tooth [52]. The fracture pattern analysis performed in the present study confirmed previous findings, as all samples restored with fiber posts showed more favorable fracture patterns. In this context, vertical bundled fibers showed better performance compared to a direct composite restoration, but inferior performances compared to fiber post-supported composite restoration, especially where an extensive loss of structure was simulated.

Lastly, in some sample it has been noted that fiber seem to limit or avoid the propagation of micro-cracks, as previously shown in Figure 4, ultimately acting as force-breakers. In most of the samples of subgroup a (no post) the propagation of dentinal cracks, which were randomly present before cyclic fatigue test, continued in the composite restoration, while in subgroups b and c fibers were able to block or reduce this trend. This data could be important to understand the resistance to cyclic loads, even considering that the majority of dental restorations fail under subcritical, cyclic occlusal loads over an extended period of time, during which the interfacial bond degrades progressively.

Conclusions

Based on the obtained results, it can be concluded that:

- Cavity design significantly influence interfacial gap progression, fracture resistance and fracture pattern.
- Fiber post-supported composite significantly reduced gap progression and improved fracture resistance of ETT anterior teeth. Thus, the insertion of a fiber post is indicated, even to improve the probability of a favorable fracture pattern.
- Vertical bundled fibers were not able to reduce interfacial gap progression significantly, but they increased fracture resistance and slightly improved fracture pattern, even if not as much as conventional fiber post.

Further in vitro studies are necessary to evaluate the crack propagation during fatigue.

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2.7 External Marginal Gap Variation and Residual Fracture Resistance of Composite and Lithium-Silicate CAD/CAM Overlays after Cyclic Fatigue over Endodontically-Treated Molars.

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Abstract

Objectives: The purpose of this in vitro study was to evaluate the external marginal gap variation with a 3D quantitative method and the residual fracture resistance after cyclic fatigue in endodontically treated molars restored with overlays of different materials, with and without fiber posts-supported buildups.

Materials and Methods: Forty-eight human maxillary molars were selected, endodontically treated, prepared with standardized MOD cavities and randomly allocated into 6 study groups considering the “core strategy” (build-up with composite resin; build-up with composite resin supported by a fiber post); and the “restorative material” of the indirect adhesive overlay (GrandioBlocks, Voco; Cerasmart, GC; CeltraDuo, Dentsply). All procedures were executed according with manufacturers guidelines. Micro-CT analysis prior and after cyclic fatigue were executed, followed by scanning electron microscope analysis and fracture resistance test.

Results: The Two-Way ANOVA analysis showed that interfacial gap progression was significantly influenced by the “core strategy” ($p < 0.01$) but not of “restorative material” ($p = 0.59$). Concerning fracture resistance, “restorative material” was statistically significant ($p < 0.01$), while “core strategy” ($p = 0.63$) and the interaction ($p = 0.84$) were not.

Conclusions: The fiber post presence within the build-up promoted a lower interfacial gap opening after fatigue, evaluated through micro-CT scans. In terms of fracture resistance, teeth restored with Cerasmart and Celtra Duo were statistically similar, but superior to GrandioBlocks.

Introduction

Preservation of healthy dental tissue is a fundamental factor in the longevity of restorations, especially when dealing with endodontically treated teeth (ETT), whose mechanical failure by fracture is more common compared to vital ones [1,2]. This increased fragility is strictly related to the pathology itself, but also to the procedures performed to devitalize and restore the tooth. On the other hand, pulp vitality loss, effects of irritants, medicaments and bacteria seem to play a secondary role on the fracture resistance [3,4]. In the past, there was the opinion that ETT needed a root canal post and full coverage crown rehabilitation [5]. Aquilino and Caplan showed that cuspal coverage could increase up to six times the survival rate of non-vital posterior teeth [6]. Therefore, the full crown has been considered the gold standard therapeutic approach for large cavities in ETT for years [7]. However, full crown preparations tend to remove a large amount of healthy dental tissue from teeth that have already lost a huge quantity of sound tooth structure due to pathology and endodontic procedures [8]. Hence, the majority of recent studies have focused more on partial direct or indirect bonded restorations, which ensure higher sound tissue preservation than traditional fixed full crowns [9]. It has recently been demonstrated that onlays, overlays and endocrowns can equally be effective compared to traditional crowns, in terms of mechanical, functional and esthetics proper-ties, while simultaneously preserving tooth structure [10,11]. For these restorations, the use of different materials has been successfully proposed, such as glass-reinforced ceramics, composite resins, and hybrid ceramics [12–14]. These materials, which can be all processed through CAD/CAM workflows, showed good performance in both in vitro and in vivo studies [14–16].

To improve mechanical properties of the tooth-restoration complex, fiber posts have been indicated in association with direct restorations as well as during buildup procedures which support indirect adhesive restorations [17,18]. A recent study by Ausiello et al. showed, through a FEA analysis, how a hybrid composite post should be sufficient to optimize the stress distribution, dissipating stress from the coronal to the apical end [19]. Kemaloglu et al. (2015) showed that a fiber network might change stress dynamics at the interfaces [20] and recent studies suggested that this fact might influence marginal gap progression [21,22]. Different results concerning their effect on fracture resistance have been reported in literature [23–25], and it has been suggested that fiber reinforced materials might lower the number of clinically unrepairable fractures [26] even if ferrule effect must be considered as primary importance [19,27].

Literature clearly supports that ETT, especially posterior ones, should be treated with cuspal coverage restorations to increase fracture resistance [28]. It has also been reported that adhesive indirect restorations are able to well-transmit and distribute functional stresses to dental hard tissues, potentially reinforcing the weakened tooth structure while preserving sound tissues [25,29]. However, despite the significant development of adhesive protocols [30] and restorative materials, failures related to secondary caries, restoration fracture or debonding are still a major issue when dealing with indirect partial adhesive restorations on ETT [16,31]. It should be considered that, upstream of a catastrophic failure, the marginal gap formation could potentially lead to secondary caries formation, and also contribute for lowering tooth resistance [11,32,33]. Leakage can be caused by several factors: the volume reduction of the luting cement related to chain assembling generates tensile forces and subsequent stress-relieving gaps which could appear inside the tooth-restoration interface. If these gaps exceed a width of approximately 60 μm at the outer margin of the restoration, an increment of postoperative sensitivity and secondary caries might be reported [34]. Furthermore, during oral function, the tooth-restoration complex is exposed to fatigue stress derived from cyclical intermittent loading with the progressive onset of marginal leakage [35]. Consequently, the analysis of marginal degradation is today crucial to better understand biomechanical failures that could occur clinically.

Despite the presence in literature of a great number of in vitro studies which focuses on resistance of direct and indirect adhesive solutions on ETT [36], there are few papers regarding the tooth-restoration interface behavior of bonded cuspal coverage rehabilitations after exposition to cyclic intermittent loading. Considering the importance to study the effect of fatigue on the external margins of an adhesive restoration, the aim of the present in vitro study was to evaluate the tridimensional marginal gap and the consequent fracture resistance after cyclic fatigue in ETT restored with overlays of different CAD/CAM materials, with and without fiber posts-supported buildups (FPSbu). The initial null hypotheses are that both marginal gaps opening, and fracture resistance are not influenced (1) by the presence/absence of a FPSbu and (2) by the CAD/CAM material employed.

Materials & Methods

The general description of the main materials used in the present study, their manufacturers and composition are listed in Table 1.

Material	General Description	Manufacturer	Composition
Grandioso X-Tra	Nanohybrid bulk resin composite	Voco	86% *w/w* filler content, Bis-GMA, UDMA, TEGDMA
Cerasmart 270	Hybrid ceramic	GC	71 wt% silica and barium nano glass, Bis-MEPP, UDMA, dimethacrylate co-monomers
Celtra DUO	Zirconia reinforced lithium disilicate	Dentsply	58% silicon dioxide, 10.1% crystallized zirconium dioxide, 10% zirconium dioxide, 5% phosphorous pentoxide, 2.0% ceria, 1.9% alumina, 1% terbium oxide
Grandio Blocks	Nanohybrid reinforced composite	Voco	86% *w/w* inorganic filler in a polymeric matrix
Rebilda Post #15	Glass fiber reinforced post	Voco	Solid composite of glass fibers, inorganic fillers, PDMA

Table 1. General description of the main materials used in the present study.

This study was designed in 6 study groups (n = 8), where the specimens were randomly allocated (www.randomizer.org) considering:

(i) "Core build-up" in 2 levels, being one condition where the build-up core was done only using a bulk-fill composite resin (Grandioso X-tra, Voco, Cuxhaven, Germany); or another condition where it was done associating composite resin and a fiber post (Rebilda Post #15, Voco, Cuxhaven, Germany);

(ii) "CAD/CAM blocks" in 3 levels: after core build-up, 3 different CAD/CAM restorative materials were tested: a nanohybrid composite resin (GB, GrandioBlocks, Voco, Cuxhaven, Germany), a flexible hybrid ceramic (CS, Cerasmart 270, GC, Tokyo, Japan), or a zirconia reinforced lithium silicate (CD, Celtra Duo, Dentsply, Konstanz, Germany).

Forty-eight (n = 48) human upper maxillary molars with mature apices, extracted for periodontal reasons within the last 4 months, were selected and stored in distilled water at room temperature. The inclusion criteria were as follow: sound teeth, similar root (length > 12 mm) and crown size (10 mm ± 2 mesio-distal, 10 mm ± 2 bucco-oral) and no crack or demineralization under visual examination with light trans-illumination and magnification. Ultrasonic scaling and polishing were performed for surface debridement. All samples were collected with informed consent in the Department of Cariology and Oper Dent, University of Turin. The ethical committee of the University of Turin approved the study protocol (DS_00071_2018).

Endodontic treatment was carried out in all specimens by the same expert operator (Pathfiles 1-2-3 and ProTaper Next X1-X, Dentsply Maillefer, Ballaigues, Switzerland) to the working length, set at 1 mm short of the visible apical foramen. Irrigation was per-formed with 5% NaOCl (Niclor 5; Ogna, Muggiò, Italy) alternated with 10% EDTA (Tubuliclean, Ogna, Milan, Italy). Thereafter, specimens were obturated with gutta-percha points (GuttaPercha Points Medium, Inline; B.M. DentaleSas,Turin, Italy) using down Pack (Hu-Friedy, Chicago, IL, USA) and an endodontic sealer (Pulp Canal Sealer EWT; Kerr, Orange, CA, USA). After that, gutta-percha backfilling was performed (Obtura III system, Analytic Technologies, Redmond, WA, USA).

A single and experienced (>10years of experience in restorative field) operator prepared the standardized MOD cavities setting residual wall thickness of buccal and oral cusps at the height of the contour to 1.5 ± 0.2 mm and placing mesial and distal cervical margins 1 mm coronally to the CEJ. For cavity preparation, cylindrical diamond burs (model 835KR; Komet, Schaumburg, IL, USA) under copious air-water cooling were used in a high-speed headpiece (Kavo Dental GmbH, Biberach, Germany). All internal edges were then smoothed and rounded with an Arkansas point (FG 645, Komet, Schaumburg, IL, USA), in order to remove non-sustained enamel.

Considering the factor "core build-up", samples were divided in 2 groups (n = 24) according to the build-up technique. In the first group (G1) cavities were subjected to the following adhesive procedure: Selective enamel etching 30 s with 35% phosphoric acid (K-etchant, Kuraray Noritake Dental, Tokyo, Japan), rinsing 30 s and air-drying. A universal adhesive system (Futurabond U, Voco, Cuxhaven, Germany) was applied in self-etch mode following the manufacturer instruction and light-cured for 20 s with a LED light curing unit at 1000 mW/cm^2 (Cefalux 2, Voco, Cuxhaven, Germany). The MOD cavity was horizontally incrementally restored with a bulk fill material (Grandioso X-Tra, Voco, Cuxhaven, Germany). Each layer, maximum 3 mm thick, was light cured with the same curing LED lamp for 30 s.

In the second group (G2) a single 8 mm post-space was prepared in the palatal root employing dedicated drills (Rebilda Post #15 Voco, Cuxhaven, Germany). The correct length and adaptation of each post (Rebilda Post #15 Voco, Cuxhaven, Germany) was verified. Post spaces were then rinsed and dried with paper points, while fiber posts were cleaned with ethanol for 30 s. A universal adhesive system (Futurabond U, Voco, Cuxhaven, Germany) was applied on post spaces and over each fiber post following the manufacturer instruction and light-cured for 20 s. Dual-cure luting cement (Bifix QM, Voco, Cuxhaven, Germany) was applied according to the manufacturer's instructions and injected into the post-space with a suitable sized mixing tip. Fiber posts were slowly inserted into the post-space and the excess cement was removed. Each specimen was light cured for 2 min using the same LED lamp and a composite build-up was performed as described for G1.

In each sample, 360° enamel margins were exposed with an overlay beveled preparation, in order to obtain 2 mm space for the restoration. In order to standardize preparations, an initial anatomical occlusal reduction was performed with 1.8 mm diameter cylindric bur (model 835KR; Komet, Schaumburg, IL, USA). After that, mesial and distal boxes were prepared with dedicated sonic points (n°34 and n°35, SonicFlex, Kawo, Shangai, China), cervically exposing enamel and remaining 1 mm above CEJ level. Occlusal sharp edges were beveled with a football-shaped bur angulated at 45° (model 8379-021, Komet, Schaumburg, IL, USA). Finishing was performed with same-shape burs with fine and extra-fine grit, then Arkansas (FG 645, Komet, Schaumburg, IL, USA) and rubber points were used to smooth all the corners. Specimens were then scanned with an intraoral camera (Cerec Omnicam AC, Dentisply, Sirona, Konstanz, Germany) and each group was divided in 3 subgroups (n = 8) according to the CAD/CAM restorative material: a nanohybrid composite (Grandio Blocks, Voco, Cuxhaven, Germany; GB), a flexible hybrid ceramic (Cerasmart 270, GC, Tokyo, Japan; CS), and a zirconia reinforced lithium silicate (Celtra Duo, Dentsply, Konstanz, Germany; CD). All overlays were designed with a CAD system, that allowed to standardize a 2 mm thickness of the restorations (Cerec 4.5.2 software, Dentisply, Sirona, Konstanz, Germany) and milled with material-specific default settings in extra-fine mode (Cerec MC XL, Dentsply, Sirona, Konstanz, Germany). Once milled, zirconia reinforced lithium silicate was crystallized (Cerec Speedfire, Dentisply, Sirona, Konstanz, Germany) according to the manufacturer instructions. Each overlay was then luted with universal adhesive (Futurabond U, Voco, Cuxhaven, Germany) and a dual-curing cement (Bifix QM, Voco, Cuxhaven, Germany) following manufacturer instructions. After overlay adaptation and cement excess removal with brushes, light curing was performed for 60 s for each side with the same LED lamp. A final 20 s/side polymerization was performed after covering the specimen with transparent air barrier gel. Finishing and polishing with diamond burs and silicone cups was performed to obtain a perfectly smooth surface.

A summary of the specimen preparation protocol is presented in Figure 1.

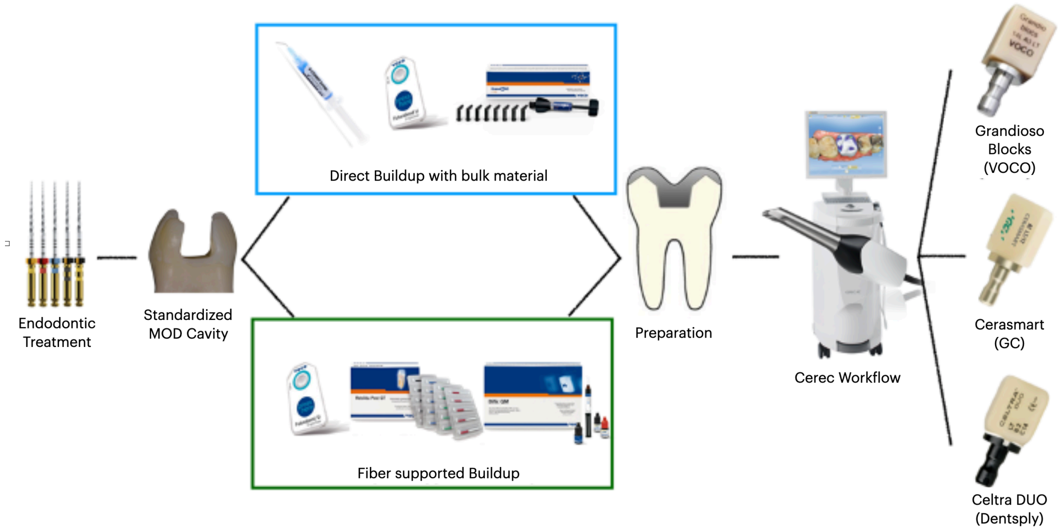

Figure 1. Specimen preparation workflow.

Specimens were scanned with X-ray micro computed tomography (micro-CT) for high-resolution scans (SkyScan 1172 Micro-CT, Bruker, Billerica, MA, USA), using following parameters: Voltage = 100 kV, current = 80 A, source-object distance = 80 mm, source-detector distance = 220 mm, pixel binning = 292, exposure time/projection = 3; aluminum and copper (Al + Cu) filter; pixel size = 10 µm; averaging = 5; rotation step = 0.4°. Images were reconstructed (NRecon, Bruker, Billerica, MA, USA) to obtain DICOM files, with standardized parameters: beam hardening correction = 25%, smoothing = 3, ring artifact reduction = 7. The same procedure, with the same parameters, was performed after cyclical intermittent loading in order to maintain consistency between data.

Specimens were subjected to cyclic intermittent loading in distilled water using a CS-4.4 chewing simulator (SD Mechatronik, Feldkirchen-Westerham, Germany). A 50 N force was applied using 6 mm diameter steatite balls as antagonist, accordingly to previous studies on fatigue testing [25,26], with the following settings: frequency = 1 Hz, speed = 16 mm/s, sliding = 2 mm over the buccal triangular crest, number of cycles = 500,000.

Twelve specimens, two for each subgroup, were randomly selected after mechanical aging and cleaned in an ultrasonic bath with alcohol (TUC-150; Telsonic AG, Bronschhofen, Switzerland) for three minutes and then air-dried. Polyvinylsiloxane impressions were taken (Flexitime Light Flow, Heraeus Kulzer) and poured with epoxy resin (EpoFix; Struers) to produce replicas, which were mounted on aluminum stubs and sputter-coated (100 s, 50 mA) with gold/palladium by use of a sputter coating device (Balzers SCD 050; Balzers, Liechtenstein). Replicas were examined under a scanning electron microscope (Emission Scanning Electron Microscopy, Zeiss Supra 40 Field). Different magnification (66×; 150×; 500×; 1000×) images were obtained with following settings: WD = 10 mm, aperture size = 30.00 μm, EHT = 5.00 kV, signal A = In Lens, stage at T = 0°.

Specimens were submitted to static fracture resistance test using a universal testing machine (Instron, Canton, MA, USA) with a 6 mm diameter steel sphere crosshead welded to a tapered shaft and applied to the specimens at a constant speed of 2 mm/min and at an angle of 30° to the long axis of the tooth. Maximum fracture loads were recorded in Newton with statistical purposes. Fractured specimens were assessed for failure modes: Catastrophic fractures (non-reparable, below the CEJ) and non-catastrophic fractures (reparable, above the CEJ). Classification was based on an agreement between three examiners.

To reveal marginal gap progression between the indirect restoration and the tooth after cyclic loading, a tridimensional method of analysis was used. Through a dedicated software (Mimics Medical, ver. 23.0; Materialise, Belgium), thresholding of voids surrounding the restoration was performed automatically to include marginal voids only. Volumetric calculation of the resulting mask was performed by the software, and overall volume data of the residual marginal gap, expressed in mm^3, were collected (Figure 2).

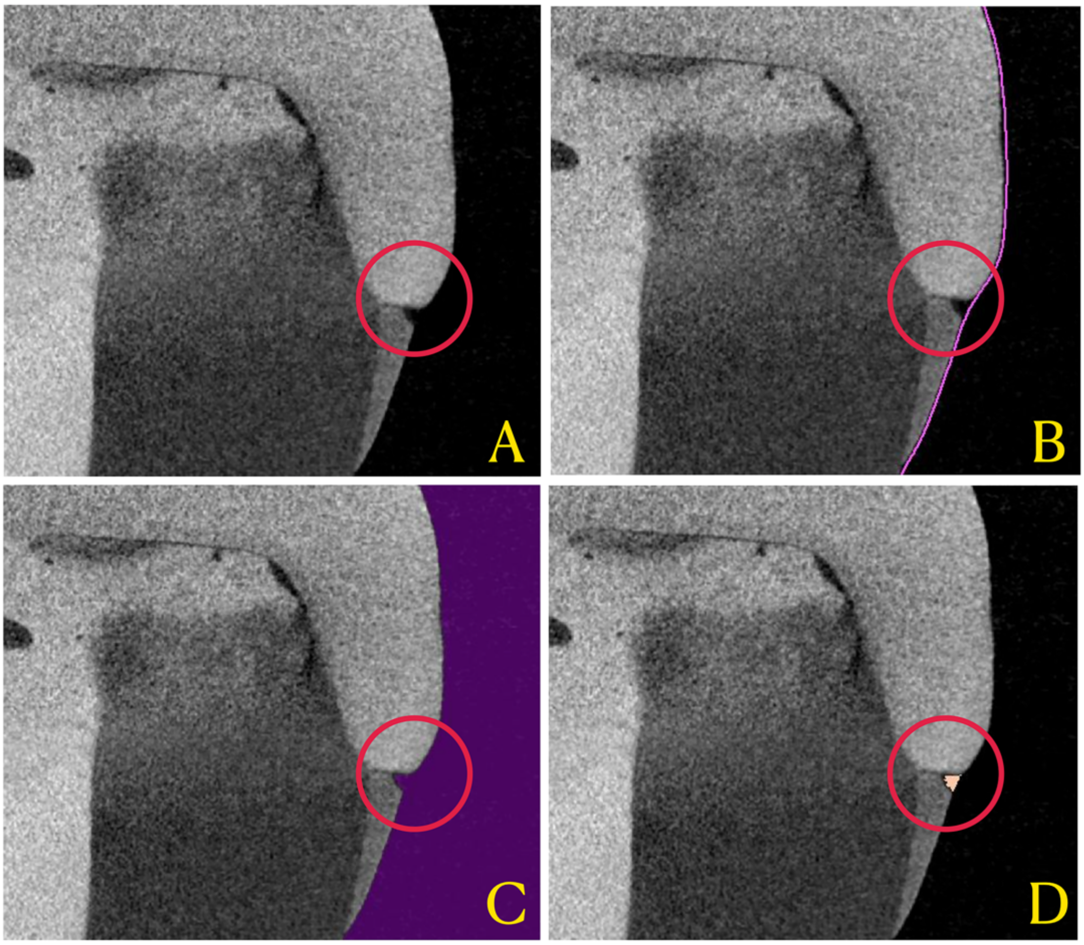

Figure 2. Applied workflow for 3D interfacial gap analysis. (A) represents the obtained micro-CT reconstructed image, imported in the segmentation software (Mimics 23, Materialise, Belgium). (B) shows the region of interest (ROI) defined by the software for gap analysis (pink line). (C) shows the void thresholding performed (violet mask) that defines "void" concept through all samples. (D) shows the intersection between the ROI and the void mask, ultimately representing interfacial gap (orange mask).

In order to examine the effects of the study factors (core build-up and CAD/CAM materials) and the interactions between them on the marginal gap progression and the fracture resistance, a two-way analysis of variance test (ANOVA) was conducted. Post-hoc pairwise comparison was performed using Tukey test. All statistical analyses were performed using a software (STATA 12, ver. 12.0; StataCorp, College Station, TX, USA) and differences were considered significant for $p < 0.05$.

Results

Results of marginal gap variation, expressed in mm^3 (T1 after cyclical intermittent loading minus baseline T0) are reported in Table 2. Results of the ANOVA test showed that marginal gap was significantly influenced by the core build-up ($p < 0.001$) but not by the restorative material employed ($p = 0.59$). The interaction between the factors showed a significant influence on the marginal gap variation ($p = 0.039$). Tukey post hoc revealed that the fiber post presence promoted better gap results (lowered gap) compared to the resin composite core alone, apart from restorative material.

	CS		**GB**		**CD**	
	Fiber Post (-)	**Fiber Post (+)**	**Fiber Post (-)**	**Fiber Post (+)**	**Fiber Post (-)**	**Fiber Post (+)**
Marginal Gap Variation (mm^3)	0.52^{a} ±0.08	0.44^{b} ±0.07	0.52^{a} ±0.06	0.45^{b} ±0.04	0.59^{a} ±0.09	0.41^{b} ±0.05

Table 2. Mean interfacial gap variations ± standard deviation, expressed as mm^3, for each subgroup. Same superscript letters indicate no significant differences.

Mean fracture resistance to static load, expressed in N, obtained in different groups was reported in Table 3. Two-way ANOVA test showed that fracture resistance was significantly related to the CAD/CAM material employed ($p < 0.001$) but not the FPSbu ($p = 0.63$). The interaction between the factors showed a not significant influence on the fracture resistance ($p = 0.84$). Tukey post hoc revealed that CS and CD groups had no statistical difference to each other and higher resistance than GB. Registered fracture patterns are reported in Table 4.

	CS		GB		CD	
	Fiber Post (-)	Fiber Post (+)	Fiber Post (-)	Fiber Post (+)	Fiber Post (-)	Fiber Post (+)
Fracture Resistance (N)	1481.21 [a] ±195.27	1576.22 [a] ±220.51	1136.43 [b] ±202.37	1203.86 [b] ±149.88	1351.52 [a] ±208.08	1484.45 [a] ±179.05

Table 3. Mean fracture resistance ± standard deviation, expressed as Newton, for each subgroup. Same superscript letters indicate no significant differences.

		CS		GB		CD	
		Fiber Post (-)	Fiber Post (+)	Fiber Post (-)	Fiber Post (+)	Fiber Post (-)	Fiber Post (+)
	Catastrophic	5	4	4	2	3	1
	Non-catastrophic	3	4	4	6	5	7

Table 4. Fracture patterns for each subgroup.

SEM micrographs of adhesive margins showed marginal gaps after mechanical loading in all groups (Figure 3). Independently of the buildup, with or without fiber post, and the indirect restorative material tested, the typical localization of gaps was mainly located in mesial and distal areas of the interproximal box. It can also be noted that there is correspondence with the 3D reconstructions obtained from the renderings of the acquisitions via micro-CT (Figure 4), which, however, allowed for a quantitative and not only qualitative analysis of the marginal gap formation.

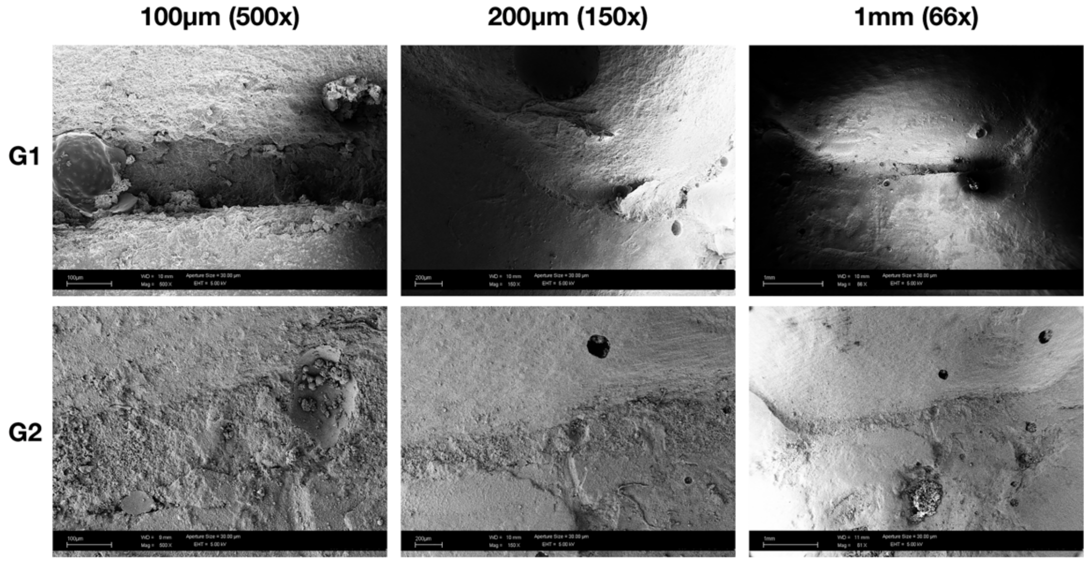

Figure 3. Representative SEM micrographs of the mesial surface from two random samples at different magnification. It is possible to notice that both present marginal degradation after cyclical intermittent loading, mainly at the corners of the box area.

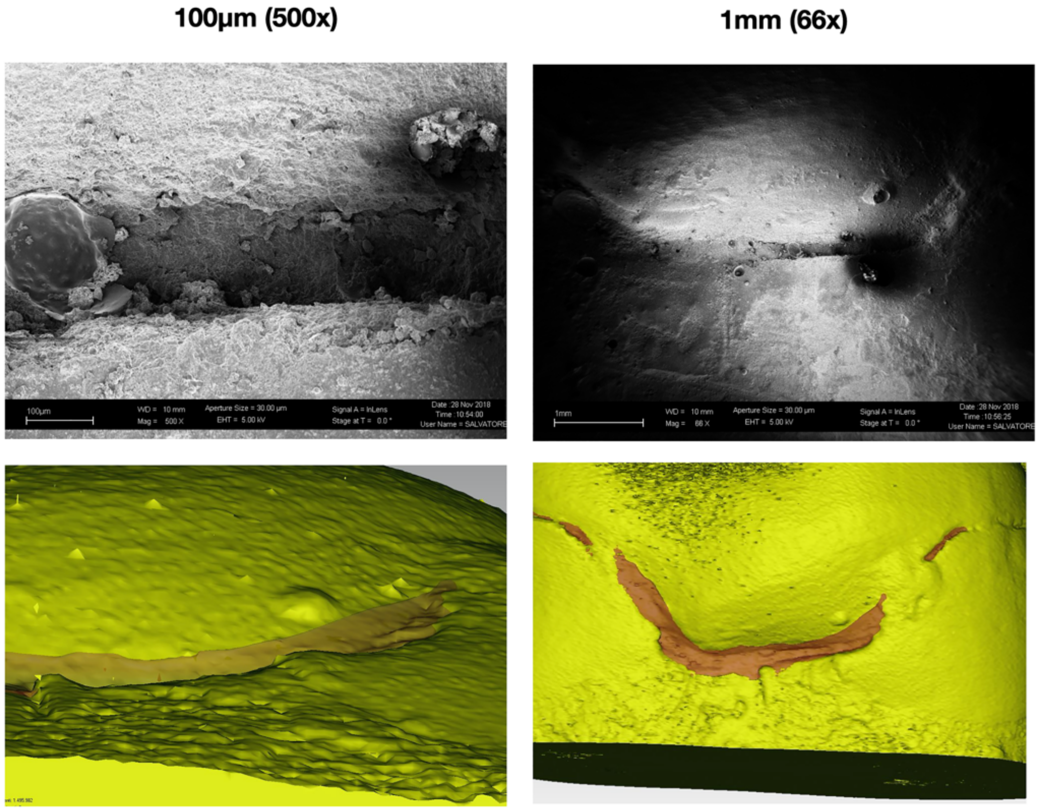

Figure 4. Previous sample from Figure 3 aside of micro-CT tridimensional gap analysis. For the present figure, images have been imported to an external software (Geomagic Qualify 12, 3D Systems, Rock Hills, SC, USA) and the analysis limited to the single box area for better visualization. Yellow volume represents the tooth-restoration complex, while the transparent red volume represents the marginal 3D gap that was calculated and analyzed.

Discussion

Modern restorative procedures on ETT aim to improve their mechanical properties, which are inferior to those of their vital counterparts, while being minimally invasive to healthy dental tissues. To accomplish these goals, ETT are more and more frequently restored with adhesive approaches and partial luted restorations which represent a valid alternative to conventional crowns [11,37].

Based on the present study results, the first null hypothesis was partially rejected since FPSbu significantly influenced external marginal gap variation but not the residual fracture resistance after cyclic fatigue. A marginal gap opening was observed after intermittent loading in all specimens, corroborating in vivo and in vitro previous findings that showed how functional and parafunctional stresses, especially transversal forces, can cause degradation of the adhesive interface and a marginal gap variation [38,39]. Present findings showed how fiber post insertion within the composite build-up significantly reduced the gap opening. The higher flexural strength of fiber posts might mediate loads between dentin and CAD/CAM luted restoration, therefore resulting in a more homogenous stress distribution compared to composite-only build-up [40,41]. Moreover, in this study the indirect restorations performed were supported in all specimens by a composite build-up which had, independently of the fiber post presence, lower flexural strength and mechanical properties compared to the CAD/CAM material employed for the overlay fabrication. Thus, the build-up could represent the weakest part of the restoration complex together with the adhesive system. It is therefore reasonably to assume that a more rigid core build-up could bring mechanical benefits to the whole indirect adhesive restoration complex, reducing marginal stresses accumulation which could cause an opening during function. It should also be considered that the present study was designed to simulate both compressive and lateral forces during chewing simulation. Horizontal chewing patterns could produce a shear effect at the adhesive interface, with a high probability of causing progressive gap opening and debonding. The presence of a fiber post within the build-up might mitigate these forces, dissipating them among a wider adhesive interface and through the root canal system [42].

A positive interaction in terms of external gap opening was highlighted when zirconia reinforced lithium silicate was used in combination with the fiber post-supported build-up. It has been shown that resin-based composites blocks have inferior flexural modulus and flexural strength values compared to glass-reinforced ceramics [43,44]. According to that, CAD/CAM materials with higher flexural strength could better benefit from the augmented strength of the core build-up, which makes mechanical properties of the system more homogeneous, also considering the increased fragility of an ETT. Moreover, rigid materials could be more prone to transmit forces directly to the under-neath structure [38], thus the ability of the fiber post to dissipate and distribute functional loads along the adhesive interfaces could be more evident.

SEM micrographs, above all at higher magnification, still offer a gold standard qualitative analysis of the external margins of adhesive restorations, as shown in Figure 4. Micro-CT, on the other hand, has the advantage of being a non-destructive method of analysis [45,46] that can offer not only a bi-dimensional, but also a tridimensional analysis of the sample before and after chewing simulation, therefore measuring gap progression in qualitative and quantitative ways. By contrast, SEM could be used to assess the presence of internal cracks even if sample sectioning is needed [47] and, when epoxy replicas are performed, only external margins can be inspected. Moreover, it must be noticed that micro-CT is also able to measure gap among the whole adhesive interface and not just external margins: Since forces also concentrate on internal edges, the study of internal gaps might be useful in the future. Analyzing gap localization through qualitative micrographs from SEM, it was also reported that margin opening seems to occur mostly in the interproximal boxes area. This can be considered in accordance with another study by Ausiello et al., which reported a high concentration of stresses in this area when applying forces on a finite element analysis (FEA) model [48].

After cyclic fatigue test, specimens were submitted to static fracture resistance test. Based on the study results, the second null hypothesis was partially rejected since the tested CAD/CAM materials significantly affected fracture resistance with GB showing a significantly lower resistance than other tested materials. This is probably related to the composition of the nano-hybrid CAD/CAM block, which has lower resistance compared to hybrid ceramics or zirconia-reinforced lithium silicate [49]. In general CAD/CAM composites, thanks to their more compact and cured tridimensional structure, show greater flexural and compressive strength values compared to traditionally layered composites. However, they are not still able to have a biomechanical behavior comparable to glass-reinforced ceramics [50]. Previous studies showed slightly different fracture resistance values [51], probably due to different build-up techniques and the different tooth preparation for the restoration. Furthermore, another reason for these inconsistencies with the present study results could be related to the 30° angle applied during the fracture resistance test with the universal machine, which could affect the fracture values as well as the fracture pattern [52].

On the other hand, this in vitro study did not highlight a significant correlation between the fiber post-supported build-up and the residual fracture resistance, though the second null hypothesis was partially rejected. This is in accordance with a similar study conducted on endodontically treated premolars restored with partial ceramic restorations, which reported that the fracture resistance was not improved by the insertion of glass or quartz fibers posts [53]. Similar results were reported by Scotti et al., assessing composite onlay [54], and by Krejci et al. on several indirect adhesive composite configurations [55]. Moreover, a recent study by Magne et al. on all-ceramic leucite-reinforced glass ceramic crowns confirmed that insertion of a fiber-reinforced post does not enhance the load-bearing capacity of the tooth [56]. Thus, in terms of fracture resistance, the fiber post use could be considered clinically irrelevant compared to other factors such as the ferrule effect and the cuspal coverage itself.

For what concerns fracture pattern, a reduction of catastrophic failures in association with fiber post occurred in all groups. This is in accordance with a literature review by Goracci et al., which reported reduced risk of vertical root fractures when glass fiber post is applied [42]. Moreover, Newman et al. suggested that fiber posts might dissipate forces along the root canal, reducing stresses on the root and therefore preventing catastrophic failures [57]. It has also been hypothesized that, when forces exceed tolerance of the system, fiber posts might be able to concentrate stresses in the coronal portion, ultimately resulting in a repairable failure pattern [22,32]. This is also in accordance with a recent in-vivo review, which concluded that failures of fiber posts were mainly due to post loss of retention, compared to metal post that presented a higher amount of root fractures [58].

A limitation of the present study was the absence of thermal stresses during the cyclic fatigue test that could mimic intra-oral temperature changes: since composites and adhesives have a higher thermal contraction/expansion coefficient than hard tooth tissues, this might influence gap formation and progression.

Conclusions

Based on the results of the present study, it can be concluded that

- The use of a fiber post within the composite build-up which support adhesive overlays in ETT had a significant positive effect for the external marginal gap opening after cyclic intermitted loading. Thus, from a clinical point of view, it could be speculated that its use could promote a marginal leakage reduction during oral function.
- Different CAD/CAM restorative materials were not able to significantly affect the interfacial gap behavior.
- Rigid restorative material, such as zirconia-reinforced lithium disilicate, seem to benefit the most from the insertion of a fiber post in terms of gap reduction.
- The tridimensional method used in this study to quantify the interfacial gap progression seems to give encouraging results.
- Considering the residual fracture resistance after cyclic fatigue, Cerasmart and Celtra Duo better performed if compared to Grandio Blocks.
- The fiber post insertion was not a parameter which influenced the tooth-restoration complex resistance.
- Encouraging results on fracture pattern were found when fiber post was applied.

Further studies are necessary to confirm the obtained results, in order to offer precise protocols to clinicians regarding indirect partial adhesive restorations on ETT.

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2.8 External gap progression after cyclic fatigue of adhesive overlays and crowns made with high translucency zirconia or lithium silicate

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Abstract

Objectives: To evaluate three-dimensional external gap progression after chewing simulation of high translucency zirconia (HTZ) and zirconia-reinforced lithium silicate (ZLS) applied on endodontically-treated teeth with different preparation designs.

Materials and Method: Endodontically treated molars were prepared with low-retentive (adhesive overlay) and high-retentive (full crown) designs above cementum-enamel junction and restored with HTZ and ZLS. Micro-computed tomography analysis was assessed before and after chewing simulation to evaluate three-dimensionally the external gap progression. Results were statistically analyzed with two-way ANOVA and post-hoc Tukey test.

Results: High-retentive preparation design had a significantly inferior gap progression compared to the overlay preparation ($p < 0.01$); ZLS exhibited a significant inferior gap progression compared to HTZ ($p < 0.01$).

Conclusions: High-retentive preparations restored with ZLS seems to better perform in maintaining the sealing of the external margin after cyclic fatigue. The clinician should pay attention to the proper combination of preparation designs and ceramic material selection for an endodontically treated molar restoration. High translucency zirconia seems to perform worse than lithium silicate in terms of marginal sealing, still showing lacks in resistance to cyclic fatigue when adhesive preparations are performed.

Introduction

Modern restorative procedures on endodontically treated teeth (ETT) aim to improve their mechanical properties, which are inferior to those of their vital counterparts [1],[2], while being minimally invasive to healthy dental tissues. To accomplish these goals, ETT are frequently restored with adhesive procedures and partial restorations which represent a valid alternative to conventional crowns [3],[4],[5].

Several materials have been successfully applied in full-coverage adhesive restorations on ETT, such as glass-reinforced ceramics, composite resins, and hybrid materials [6],[7],[8]. These materials showed good performance in both in vitro and in vivo studies [9],[10]. However, every year, new restorative materials are developed and produced with the aim of restoring the optical and mechanical properties of natural teeth, even in severely compromised teeth.

Among the recently introduced monolithic CAD/CAM materials that can be used for cuspal coverage indirect restorations on severely damaged teeth, zirconia has certainly experienced the greatest evolution. In particular, high translucency zirconia (HTZ) has been recently introduced in restorative dentistry, replacing the tetragonal version, especially for monolithic single-tooth restorations. The introduction of a variable amount of cubic phase, which is optically isotropic, was meant to improve the translucency of the material, at the expense of strength and toughness due to the lack of transformation toughening and the coarser microstructure [11]. As a recent study pointed out, cubic grains are wider than tetragonal ones and generate more stabilizing oxides, making the tetragonal phase more prone to aging [12]. As result, HTZ was initially considered less suitable for posterior restorations and indicated only for the anterior area. Today, however, industries have been able to produce various types of zirconia with varying percentages of cubic phase, ultimately creating HTZ specifically indicated for the posterior sectors and with a good balance between optical and mechanical proprieties [13]. On the other hand, an alternative ceramic material with high mechanical and esthetic performances suitable for cuspal coverage restorations is the zirconia-reinforced lithium silicate ceramics (ZLS). Its microstructure has a homogeneous glassy matrix which contains a crystalline component made of round and submicrometric elongated grains of lithium metasilicates and lithium orthophosphates; in addition to these, tetragonal zirconia fillers are added, aimed at increasing strength values, obtain favorable optical properties within increased mechanical characteristics compared to other glass-ceramics [14],[15].

A crucial consideration when dealing with adhesive preparations is the luting protocol and its efficiency, since the adhesive preparation design is, by definition, less macro-mechanically retentive than a conventional crown. Despite significant developments in adhesive protocols towards enamel and dentin, failures related to secondary caries are still the major issue when adhesive restorations are addressed [9],[10], above all with unexperienced operators [16]. It should be considered that, prior to clinical dramatic failure, such as the restoration debonding or fracture, the interfacial gap formation plays an important role. Interfacial gap creates a hard-to-clean weakened area [5],[17] and it can lead to bacterial recolonization of the tooth crown and the root canal system, with subsequent endodontic failure [18]. These interfacial gaps tend to progressively expand during oral function and parafunction due to fatigue stresses from cyclic loading [19],[20],[21]. Therefore, as highlighted in a recent review, fatigue parameters obtained from cyclic loading experiments should be considered more reliable predictors of the mechanical performance of contemporary adhesive restorative materials than quasi-static mechanical properties [22]. Moreover, the scientific community has put forth significant effort in testing and proposing adhesive treatments able to ensure effective bonding and interfacial seals using HTZ [23] to let the material be employable in low-retentive minimally invasive preparations. The absence of a glassy phase makes the bonding mechanisms of HTZ to dental tissues more difficult [24]: recent studies showed how the physico-chemical conditioning method tends to increase the bond strength of resin-based cements towards zirconia [23],[25]. However, to the best of our knowledge, no studies reported the effects of fatigue cycling on the external gap opening of ETT restored with indirect adhesive restorations made with HTZ or ZLS.

The aim of the present in vitro study was to evaluate the external gap progression after cyclic fatigue of HTZ and ZLS applied on ETT with low and high retentive preparation designs. The following null hypotheses were tested: (1) there is no difference in terms of external gap progression between low-retentive and high-retentive preparation designs, and (2) there is no difference between HTZ and ZLS.

Materials & Methods

This study was designed in 4 study groups (n =12 each), where the specimens were randomly allocated considering:

- "Preparation design" in 2 levels: extracted molars, once endodontically-treated, were prepared for a cuspal coverage restoration with two different designs: a low-retentive adhesive overlay preparation and a high-retentive full crown preparation with margin located 1 mm above cementum-enamel junction (CEJ).

- "Restorative material" in 2 levels: Cuspal coverage adhesive restorations were performed using 2 different cad-cam monolithic materials: a high translucency zirconia designed for posterior teeth (Katana STML, Kuraray Noritake) and a zirconia-reinforced lithium silicate (Celtra Duo, Dentsply).

The materials employed in the present study are detailed in Table 1.

	Description	Manufacturer	Composition
KATANA STML	High translucency zirconia	Kuraray Noritake	Zirconium oxide (wt%: 59.9% c-ZrO_2, 39.5% t-ZrO_2, 0.4% m-ZrO_2, 0.2% r-ZrO_2), 4.8% Y_2O_3, pigments
Celtra Duo	Zirconia-reinforced lithium silicate	Dentsply	58% silicon dioxide, 10.1% crystallized zirconium dioxide, 10% zirconium dioxide, 5% phosphorous pentoxide, 2.0% ceria, 1.9% alumina, 1% terbium oxide
CLEARFIL MAJESTY ES-2	Nanohybrid resin composite	Kuraray Noritake	Bisphenol A diglycidyl methacrylate, barium glass, pre-polymerized organic filler, hydrophobic aromatic dimethacrylate, hydrophobic aliphatic dimethacrylate dl-Camphorquinone, accelerators, initiators, pigments
PANAVIA V5	Dual resin cement	Kuraray Noritake	Bis-GMA, TEGDMA, aromatic and aliphatic multifunctional monomer, accelerators, dl-Camphorquinone, surface-treated barium glass, fluoroaluminosilicate glass, fine particulate
CLEARFIL SE BOND 2	Two-bottle self-etch adhesive	Kuraray Noritake	Primer: 10-MDP, HEMA, hydrophilic dimethacrylate, photoinitiator, water Bond: 10-MDP, dimethacrylate resins, HEMA, Vitrebond copolymer, ethanol, water, filler, initiators, silane

Table 1. General description of the main materials used in the present study.

A total of 48 (n = 48) human sound upper molars were selected for the present study within two months from extraction due to periodontal reason. The inclusion criteria were as follow: sound teeth, similar root (length>12 mm) and crown size (10 mm ± 2 mesio-distal, 10 mm ± 2 bucco-oral) and no crack or demineralization under visual examination with light trans-illumination and magnification. After proper disinfection (ultrasonic scaling and 0.5% chloramine for 48 hours), selected teeth were stored in distilled water at 37°C.

Each specimen was endodontically treated by the same operator using PathFiles (1-2-3) and ProTaper Next (X1/X2) (Dentsply Maillefer) to reach the working length, set at the visible apical foramen. Irrigation was performed with 5% NaOCl (Niclor 5, OGNA) alternated with 10% EDTA (Tubuliclean, OGNA). Thereafter, specimens were obturated with gutta-percha points (Gutta-Percha Points Medium, Inline, B&M Dental) using a Down Pack heat source (Hu-Friedy) and an endodontic sealer (Pulp Canal Sealer EWT, Kerr). Backfilling was performed with the Obtura III system (Analytic Technologies).

A standardized mesio-occlusal-distal cavity was prepared by an expert operator setting the residual wall thickness of the buccal and oral cusps at the height of the contour to 1.5 ± 0.2 mm, measured with a conventional caliper. Mesial and distal boxes were finished with dedicated sonic points (n°34 and 35, SONICflex, KaVo) to standardize their dimensions. A core composite build-up was performed for all specimens, following the same protocol. A 30-second selective enamel etching was performed with 35% phosphoric acid (K-ETCHANT, Kuraray Noritake Dental), then rinsed for 30 seconds and air-dried. Then, a self-etch adhesive was applied (CLEARFIL SE BOND 2, Kuraray Noritake) following the manufacturer's instructions. Build-up restoration was performed with a nanohybrid resin composite (CLEARFIL MAJESTY ES-2, Kuraray Noritake) with a 2 mm-thick oblique layering technique. Light curing of both adhesive and resin composites was accomplished with an LED curing lamp (Celalux 2, VOCO) using a conventional program for 20 seconds at 1000 mW/cm^2.

Samples were randomly allocated to one of two groups (n = 24 each) using https://www.randomizer.org/ according to the selected preparation design:

- Low-retentive (ADH). A standardized 1.5 mm occlusal reduction was performed with a cylindrical bur (6836 KR 014, Komet) following occlusal anatomy. Boxes were finished with dedicated sonic points (n°34 and 35, SONICflex, KaVo) to remove eventual built-up composite excesses. Finally, the occlusal margins were beveled with a football-shaped bur (8368 L, Komet), and all corners were rounded with an Arkansas tip (661, Komet) and a rubber point (9436 M, Komet).
- High-retentive (CRW). A standardized 1.5 mm full preparation was executed with a chamfer margin 1 ± 0.5 mm above cement-enamel junction (CEJ). Both initial preparation and finishing were performed with dedicated chamfer burs (6881 014, Komet; 8881 014, Komet). Finally, all corners were rounded with an Arkansas tip (661, Komet) and a rubber point (9436 M, Komet).

An exemplificative image reporting transversal sections of a low-retentive and high-retentive designs is reported in Fig.1.

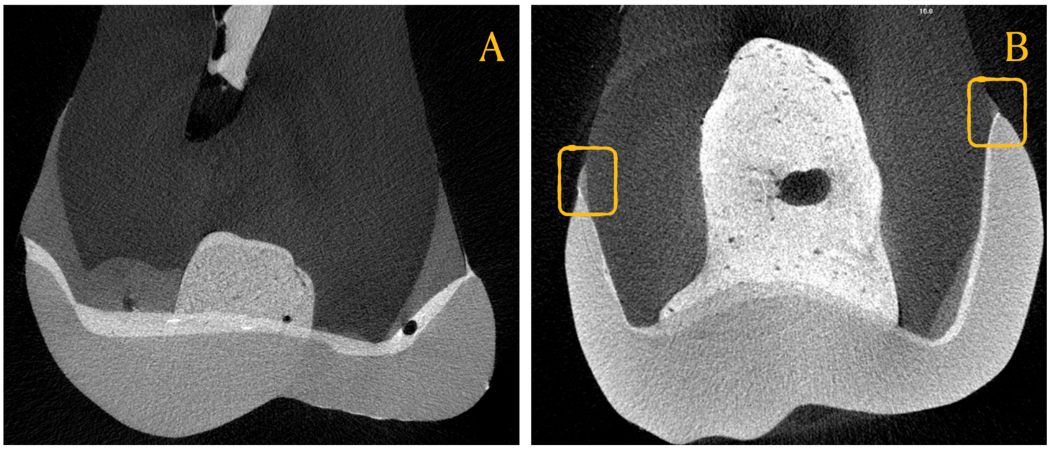

Figure 1. Random samples transversal sections of a low-retentive design (Fig. 1A) and high-retentive design (Fig. 1B). Both the restorations were performed above the CEJ level, as highlighted in Fig. 1B.

Samples were scanned with an intraoral scanner (CEREC Omnicam, Dentsply) and divided into two subgroups (n = 12 each) according to the CAD/CAM material employed: HTZ (KATANA, Kuraray Noritake) and ZLS (Celtra Duo, Dentsply). All restorations were designed with a CAD system, that allowed to standardize a minimum of 1.5 mm thickness (Cerec 4.5.2 software, Dentisply, Sirona, Konstanz, Germany) and milled with material-specific default settings in extra-fine mode (Cerec MC XL, Dentsply, Sirona, Konstanz, Germany). In all specimens the parameters for luting space and minimum occlusal ceramic thickness were set to 80 µm and 1.5 mm, respectively. Once milled, ZLS was crystallized (Cerec Speedfire, Dentisply, Sirona, Konstanz, Germany) and HTZ was sintered according to the manufacturer instructions. Each restoration was luted with a dual-cure resin cement, following the manufacturer's instructions (PANAVIA V5, Kuraray Noritake). Either ADH either CRW were cemented with digital pressure applied by the same operator, with more than 10 years of clinical experience, until fully seated onto the tooth margin. Table 2 reports the details of the adhesive procedures performed on both teeth and restorative materials. Excesses of cement were removed with a micro-brush, then, after three minutes of setting, photopolymerization was carried out for a total of three minutes (approximately 40 seconds per surface) with an LED lamp at 1000mW/cm^2 (Celalux 2, Voco). Finishing and polishing were performed with fine and extra-fine diamond burs and rubber points on a handpiece. Margins were double-checked to exclude samples with under-contours, while over-contours were corrected with a new cycle of finishing and polishing. All samples were confirmed to be clinically acceptable by an expert operator (more than 10 years of experience in prosthodontic field).

Substrate	Adhesive Procedure Performed
Tooth	Enamel etching for 15 s, rinse and dry, apply tooth primer (PANAVIA V5 kit, Kuraray Noritake) for 20 s, dry with air
HTZ	Dry sandblasting with 50 µm alumina powder (RONDOflex Plus 360, KaVo), five-minute ultrasonic bath in 98% alcohol, dry, apply CERAMIC PRIMER PLUS (PANAVIA V5 kit, Kuraray Noritake) for 20 s, dry, apply PANAVIA V5 cement through dedicated mixing tips
ZLS	9.6% hydrofluoric acid (Porcelain Etch Gel, Pulpdent) for 30 seconds, five-minute ultrasonic bath in 98% alcohol, dry, apply CERAMIC PRIMER PLUS (PANAVIA V5 kit, Kuraray Noritake) for 20 s, dry, apply PANAVIA V5 cement through dedicated mixing tips

Table 2. Detailed adhesive procedures performed on different materials.

Samples first underwent a micro-CT scan (Skyscan 1172, Bruker) with the following parameters: voltage = 100 kV, current = 100 A, aluminum and copper (Al + Cu) filter, pixel size = 10 µm, averaging = 4, and rotation step = 0.1°. Images were reconstructed (NRecon, Bruker) to obtain DICOM files with standardized parameters: beam hardening correction = 15%, smoothing = 5, and ring artifact reduction = 6. HTZ samples (SG1) showed a higher radiopacity than ZLS, which was managed by doubling the aluminum and copper filters, using averaging = 7, and properly positioning the sample.

Fatigue simulation was accomplished with a chewing simulator (CS-4.4, SD Mechatronik) using 6mm diameter steatite balls as antagonists with the following settings: load = 50 N, frequency = 1 Hz, speed = 16 mm/s, sliding = 2 mm over the buccal triangular crest, and number of cycles = 500,000. A loading force of 50 N was selected in accordance with previous studies on fatigue testing [26],[27],[28].

Specimens which survived to chewing simulation were submitted to a second micro-CT scan, with the same parameters as the baseline, to maintain data consistency and evaluate the effect of fatigue on external gap progression. The obtained DICOM data were imported into a segmentation software (Mimics Medical 20.0, Materialise). A standardized workflow consisting of thresholding, region growing, and Boolean and morphological operations was used to specifically analyze the external interfacial gap. Segmented masks were converted into optimal quality STL files and imported into the analysis software (Geomagic Studio 12, 3D Systems) for noise removal and volume calculation (mm^3). Figure 2 (A–D) presents a schematic representation of the protocol steps for clarification.

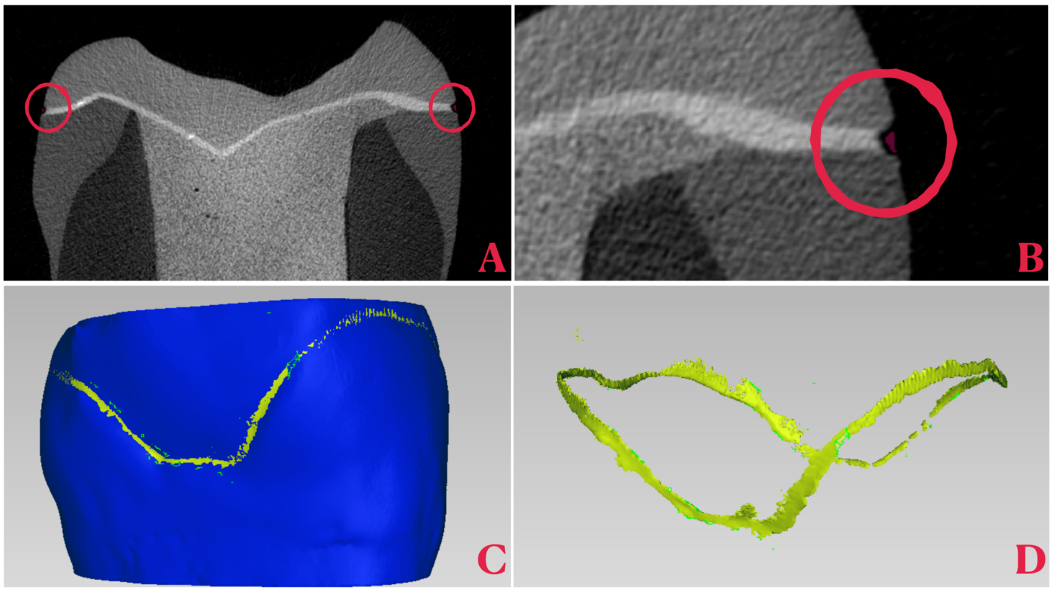

Figure 2. Random sample external gap analysis (ADH, ZLS) in stages A–D. Figure A presents a random cross-section with external gaps highlighted. Figure B is a magnification of Figure A, showing in red the pixels corresponding to the external gap used in the analysis. Figure C shows a 3D rendering (Geomagic Studio 12, 3D Systems) of the tooth-restoration complex (in blue) and the analyzed gap (in yellow). Figure D presents the analyzed gap in yellow.

To have significant data to discuss and to highlight the interfacial gap progression caused by cyclic fatigue, a subtraction was made between the final gap volume and the baseline gap volume. Figure 3 presents a random sample gap analysis, before and after the chewing simulation, with the external gap progression highlighted.

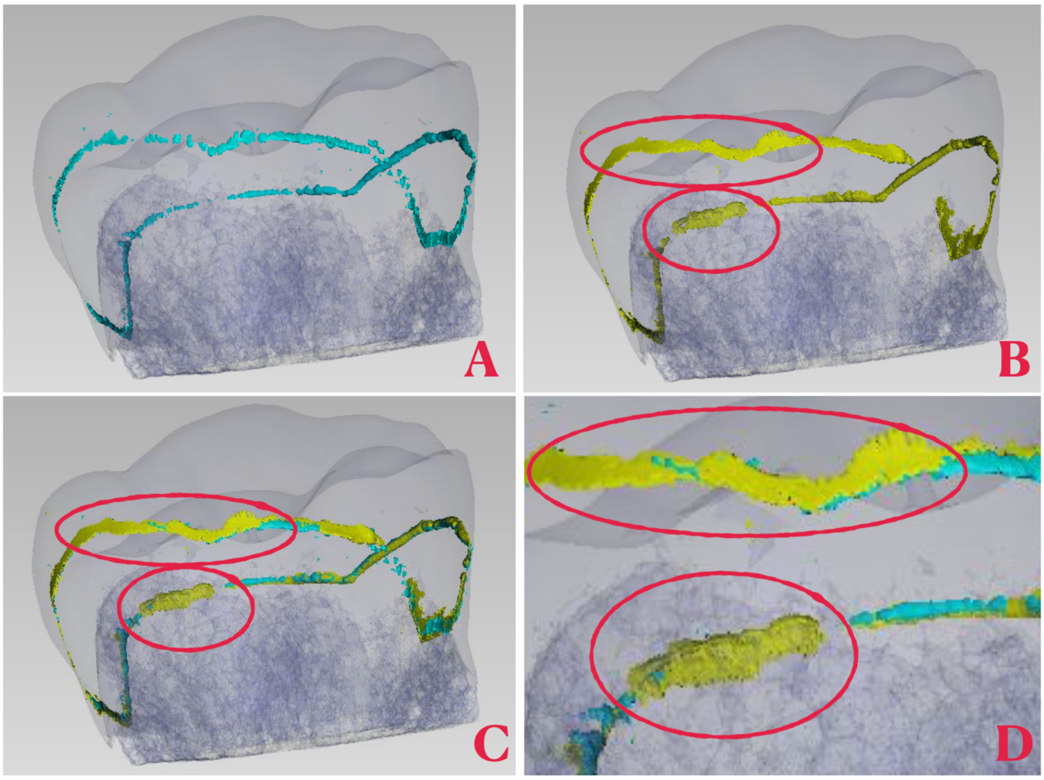

Figure 3. Random sample (ADH, HTZ) external gap progression analysis. Figure A presents the baseline gap in light blue aligned with the transparent blue tooth-restoration complex. It is worth mentioning that even if a gap is reported throughout the whole interface, it is extremely thin, making its total volume almost irrelevant. Figure (B) presents the same sample gap after fatigue simulation in yellow, with red circles indicating some of the area that showed a significant gap progression. Figure (C) presents the superimposition of the baseline (light blue) on the final gap (yellow), with the same highlights presented in Figure B. Figure (D) presents a detailed view of Figure C for better understanding.

A Shapiro-Wilk test revealed that the data were normally distributed. To evaluate the effect of materials and preparation design on the tridimensional interfacial gap progression, a two-way analysis of variance (ANOVA) and post-hoc Tukey test were performed. The significance level was set to 95% ($p < 0.05$). All statistical analyses were performed using the STATA software package (ver. 14.0, StataCorp, College Station).

Results

None of the tested specimens showed critical cracks, fractures or debonding after cyclic fatigue. The external gap progression data (± SD, expressed in cubic millimeters) of the tested specimens are shown in Table 3.

	HTZ	ZLS
ADH	0.16 ± 0.08 ^{Aa}	0.10 ± 0.06 ^{Aa}
CRW	0.11 ± 0.09 ^{Aa}	0.02 ± 0.02 ^{Bb}

Table 3. External gap progression, expressed as mean ± standard deviation (mm^3) for all tested subgroups. Same superscript capital letters indicate no difference between row results. Same superscript lower-case letters indicate no difference between column results.

Two-way ANOVA reported significant differences between the tested materials ($p = 0.0001$) and preparation designs ($p = 0.005$), while their interaction did not show a significant difference ($p = 0.75$). Pairwise comparison showed that the high-retentive preparation design had a significantly inferior gap progression compared to the overlay preparation. However, ZLS exhibited an inferior gap progression compared to HTZ.

Discussion

Degradation of restorative interfaces is a key topic in better understanding and preventing biomechanical and microbiological failures of modern restorations that use adhesion to properly reinforce tooth structures while preserving dental tissues [29],[30]. In the present study, cyclic fatigue simulation induced external gap progression in all specimens, in agreement with several papers that previously demonstrated that fatigue stresses are able to cause degradation of adhesive interfaces [31],[32]. Although there is no clear correlation between in vitro gap formation and interfacial failures observed in vivo, none of the specimens showed external gap higher than 150 µm after cyclic fatigue, which is considered clinically acceptable [33].

Based on the present study's results, the first null hypothesis was rejected, since the high-retentive preparation design showed lower external gap progression than the low-retentive ones. Several explanations might be offered for this finding. First, adhesive cementation helps to distribute forces, ultimately improving a restoration's fatigue resistance [34],[35]. The tested high-retentive design possessed a wider adhesive interface, which might have acted as a cushion, better dissipating forces and preventing gap progression. Second, the fatigue simulation included a sliding movement meant to increase the lateral forces applied to the restorative material, forcing the system to flex. Therefore, the axial walls of the crown design probably dissipated some of these lateral forces, acting like a ferrule and increasing not only the retention but also the stability of the system [36],[37]. Finally, gap progression in low-retentive restorations was probably augmented due to the direction of the chewing sliding pattern, which started from the central fossa and moved along the buccal triangular crest. In fact, in the selected adhesive overlay design, buccal and oral cusps had the lowest stability due to the lack of vertical walls. Moreover, the different margin configuration and the consequent restoration marginal profile could also justify the external gap progression showed in the present study. In fact, the beveled chamfer of the low-retentive preparation corresponds to a wider amount of enamel exposure but a slightly thinner restoration in the external part, that might be more prone to chipping [38]. Partially in disagreement with the present study's results, a recent paper by Gupta et al. reported that both zirconia crowns and overlays had similar marginal behavior after fatigue [39]. However, they performed their analysis with SEM and focused on microcracks and marginal integrity rather than volumetrically quantified gaps; thus, it is impossible to directly compare results. As underlined in a review on marginal adaptation, micro-CT is the only method that allows both a precise identification of critical gaps and sufficient measurements to define margin conditions [40].

The results of the present study also showed significant differences between ZLS and HTZ in terms of gap progression; thus, the second null hypothesis was rejected. A first possible explanation regarding the external gap progression results concerns the adhesive cementation. In fact, it has already been demonstrated that ZLS can be successfully luted, achieving high bond strength, if the surface is properly treated [41]. However, HTZ, due to the absence of any glassy matrix, cannot be conditioned with conventional acid etching techniques and, consequently, might be considered less suitable for adhesive procedures [42],[43]. Thus, the stability of the HTZ cement-restoration interface might be inferior compared to that of ZLS. A recent study on tensile bond strength in ZLS showed good performance and stability with aging, even if thermocycling significantly influenced the bond strength values [44]. Similar studies on zirconia, however, reported major loss of bond strength after thermocycling, with prevalent adhesive failures, even if data were cement-dependent [45],[46]. Another possible explanation relates to the mechanical proprieties of HTZ compared to those of ZLS. HTZ has a flexural strength of approximately 600–800 MPa and an elastic modulus of 200–210 GPa [11], while ZLS flexural strength of 400–500 MPa and an elastic modulus of 60–67 GPa [47]. Several papers support the fact that low elastic modulus restorative materials have better biomechanical performance when applied to full-coverage adhesive restorations. They demonstrate a better stress distribution due to the partial absorption of stress [48],[49] which might cause interfacial overloading in HTZ samples, ultimately bringing to premature fatigue micro-failure of the restorative interface, recorded as volumetric gap progression in the present study's methodology.

Within the limitations of the present study, it is worth mentioning the difficulty encountered in HTZ sample analysis due to the presence of x-ray artifacts. Micro-CT has been widely used to analyze the internal and marginal fit of zirconia crowns [50],[51] and therefore can be considered a reliable method of qualitative analysis. However, when it comes to quantitative evaluation through software-automated analysis, thresholding of gap was found to be harder in HTZ than in ZLS. The scattering effect of HTZ caused pixel blurring that the software sometimes incorrectly included in the region of interest. This problem was managed with a few manual adjustments and a modification of the acquisition phase, as described in the materials and methods section.

Conclusions

Based on the obtained results and within the limitations of the present study, it can be concluded that:

- External gap progression was significantly inferior for the high-retentive preparation design and significantly lower for ZLS compared to HTZ.
- Clinically speaking, it seems that materials with high adhesive properties (ZLS) and retentive preparations are able to better preserve marginal integrity when subjected to fatigue.

Further studies are necessary to confirm the given results, to provide a better understanding of the biomechanical behavior of HTZ and ZLS in minimally invasive dentistry and to find a possible correlation between the marginal gap progression and the interfacial bacterial colonization in indirect adhesive restorations.

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2.9 Highly-filled flowable composite in deep margin elevation: FEA study obtained from a microCT real model.

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Abstract

Objectives: To evaluate shear stress (SS) and normal pressure (NP) at the tooth-restoration interface of highly-filled flowable resin composite applied to deep margin elevation technique through FEM analysis generated by a microCT scan.

Materials and Methods: A reference maxillary molar with two class II cavities was prepared according to deep margin elevation protocol. A geometrical model was segmented from a micro-CT scan generating separate volumes of enamel, dentin and restorative materials. The 3D Finite Element (FE) model was subsequently built-up and an axial chewing load was simulated. Data concerning the tooth-restoration interface were analyzed in terms of SS and NP. Different materials and techniques were tested in order to evaluate the effects of the restorative material, the usage of a highly-filled flowable composite as liner and the substrate of the cervical area.

Results: Both SS and NP presented similar distribution, but showed significant differences between tested materials. Composites showed more homogeneous behavior in stress distribution compared to ceramic. The use of a highly-filled flowable composite as liner on the cervical margin significantly reduced SS and NP on the cavity floor and the cervical margin area. Lastly, stress distribution in the cavity floor area varied according to the cervical margin substrate: enamel showed a protective role in stress distribution.

Conclusions: Highly-filled flowable composite resins showed encouraging results when applied to deep margin elevation from an interfacial mechanical point of

view. Further studies are needed to validate these data and to better define the role of cervical enamel in stress distribution.

Introduction

Carious class II cavities with juxtagingival and subgingival margins in posterior teeth represent a daily challenge for clinicians. These interproximal lesions present technical difficulties mainly related to rubber dam isolation and cervical margin finishing procedures [1]. The subgingival extension also poses problems on adhesive procedures, since little or no enamel is usually available in the cervical area. It is globally accepted that adhesion is stable and efficient on enamel [2], while on dentin it is less reliable due to substrate morphology [3], being also influenced by the adhesive type [4] and the application technique [5]. Another aspect that must be considered is that thermo-mechanical load conditions could cause premature bond degradation in this critical area, ultimately leading to restoration failure [6,7].

In order to avoid periodontal surgery when the biological width is maintained [8], the cervical margin relocation procedure, subsequently renamed "Deep margin elevation", has been proposed [9,10]. Different materials have been used for this technique and two main protocols have been proposed. The open-sandwich protocol consists in placing a first horizontal layer of resin-modified glass-ionomer or, more recently, of flowable resin composite on the cervical margin, then finalize the restoration with a conventional material [11]. The closed-sandwich protocol, on the other hand, consists in placing the composite or, eventually, a ceramic inlay immediately, as unique material [11]. Flowable composite application in the open-sandwich protocol is based on the rational that a flowable material should be easier to manipulate and adapt to the deep cavity floor than the glass-ionomer [12,13]. Moreover, the lower elasticity modulus of flowable resins has been reported to potentially decrease shrinkage stress and the consequent interfacial gap formation during polymerization [14]. This might help reducing postoperative sensitivity, secondary caries and periodontal problems [15,16]. From a mechanical point of view, another aspect that has been recently highlighted is that an open-sandwich protocol might decrease ceramic fracture when preparation margins are located below CEJ [17]. However, there is no consensus about the influence of deep margin elevation technique on fracture strength of different adhesive restorations [18,19]. Flowable materials present lower mechanical properties compared to traditional composites, due to the lower percentage of filler load [12], and it has been recently suggested that this might lead to premature adhesive failure when cyclic loads are applied [20]. In order to merge the best proprieties of flowable and conventional composite resins, highly-filled flowable composites have been recently introduced [21]. These composites take advantage of new monomers, that contribute to maintain flowability even if highly-loaded with fillers, ultimately improving mechanical performance [22].

Nowadays, materials are in constant evolution, so it is difficult to test and compare their performances. In this sense, Finite Elements Method (FEM) is useful to perform fast, predictable investigations comparing different materials in the exact same conditions, without the need of prototyping [23]. FEM consists in discretizing the 3D geometry of the tooth with small elements, calculating load conditions and deformation state for each one and then assembling the results for the whole structure. FEM has been validated by several studies [24], that measured shrinkage forces and residual stresses of different restorative materials [25,26]. However, most of the FEM studies are based on CAD-generated restorations and focus on the material behavior itself rather than working on realistic restorations and analyzing their interfacial behavior.

To the best of our knowledge there is no consensus about which materials and techniques should be used in deep margin elevation to achieve the best interfacial performances. Moreover, there is a lack of data regarding highly-loaded flowable composites applied to deep margin elevation and their behavior under load. Thus, the aim of the present study was to evaluate, through FEM analysis focusing on shear stress (SS) and normal pressure (NP) consequent to axial load, the interfacial behavior of a new highly-loaded flowable composite, applied with different techniques and materials, to a deep margin elevation scenario.

The null hypotheses tested were that the interfacial load conditions are not influenced by (1) the occlusal restorative material, (2) the usage of a highly-filled flowable composite as liner and (3) the substrate of the cervical margins.

Materials & Methods

A single intact human upper molar, extracted for periodontal reasons within the last month, was selected and stored in distilled water after disinfection with an ultrasonic device. The study was granted ethics approval by the local ethics committee of the Dental School, University of Turin (DS-2018_No.001). The selected tooth was examined and confirmed to be sound, with no carious lesions, demineralization, cracks, or signs of wear under 20x magnification optical microscope.

Two standardized class II cavities, one mesial and one distal, were created on the specimen by the same expert operator with the following dimensions: 4 mm in the buccal–oral direction and 3 mm in the mesio-distal direction. The mesial cavity cervical margin was set 1 mm above the CEJ, whereas the distal cervical margin was set 1 mm below the CEJ.

A circumferential steel matrix was applied (Automatrix; Dentsply, Konstanz, Germany) and tightened until a perfect fit with the cervical margins was achieved. Then, the specimen was subjected to the adhesive procedure: selective enamel etching for 30 s with 35% phosphoric acid (K-etchant, Kuraray Noritake Dental, Mie, Japan), rinsing for 30 s, and air-drying. A two-step self-etch adhesive system (Clearfil SE Bond2; Kuraray Noritake Dental, Mie, Japan) was then applied following the manufacturer's instructions, and slightly air-dried before light-curing for 40 s with a light-emitting diode (LED) lamp at 1400 mW/cm^2 (Cefalux2; Voco, Cuxhaven, Germany). Then, 2 mm standardized composite layers were applied progressively (Filtek Supreme XTE; 3M ESPE, St Paul, MN, USA), each being light-cured with the same LED lamp for 20 s. Finishing and polishing procedures were then performed with abrasive disks (SofLex; 3M ESPE, St Paul, MN, USA) and silicon points (Enhance; Dentsply, Konstanz, Germany). The final restorations were confirmed to be clinically acceptable by an expert operator. An overview of the simulated restorative materials, their manufacturers, classification and composition are reported in Table 1.

Material	Manufacturer	Classification	Composition
e.max CAD	Ivoclar Vivadent, Schaan, Lichtenstein	Lithium disilicate	SiO_2, Li_2O, K_2O, P_2O_5, ZrO_2, ZnO, Al_2O_3, MgO
Filtek Supreme XTE	3M ESPE, St. Paul, MN, USA	Nano-filled resin composite	Organic part: Bis-GMA, Bis-EMA, PEGDMA, TEGDMA Fillers (65 wt% = 46 vol%): ytterbium trifluoride (0.1-5 lm), nonagglomerated/nonaggregated surface modified 20-nm and 75-nm silica fillers, surface modified aggregated zirconia/silica fillers (cluster average size: 0.6-10 lm)
Majesty ES Flow Super low	Kuraray Noritake Dental, Tokyo, Japan	Highly-filled flowable resin composite	Organic part: TEGDMA, hydrophobic-aromatic dimethacrylate, dl-CQ, PI Fillers (78 wt% = 64 vol%): Barium glass filler, silica filler

Table 1. Simulated materials of the present study, alongside with their respective manufacturer, classification and composition.

A Micro-CT scan (SkyScan 1172; Bruker, Billerica, MA, USA) was employed to recreate an accurate 3D model of the tooth structure and the restorations. High-resolution scan was performed using the following parameters: voltage = 100 kV; current = 100 µA; aluminum and copper (Al+Cu) filter; pixel size = 20,27 µm; averaging = 5; rotation step = 0.6°. NRecon software (Bruker, Billerica, MA, USA) was used to reconstruct the images and obtain DCM files (DICOM, Digital Imaging and Communications in Medicine) applying the following corrections: beam hardening = 15%, smoothing = 4, smoothing kernel = 2 gaussian, ring artifact correction = 7.

The obtained files were imported into a segmentation software (Mimics Medical ver. 20.0; Materialise, Ann Arbor, MI, USA). Thanks to the different radiodensity among structures, it was possible to automatically create multiple segments made up of pixel sets representative of enamel, dentin and restorative material. In order to ensure proper contacts between different masks, morphology operations, alongside with region growing algorithm and boolean operations were used. The final parts were then converted into optimal-quality STL files (STereo Lithography interface format), with a sample ratio 1:1.

A graphical workflow of the performed steps is reported in Figure 1.

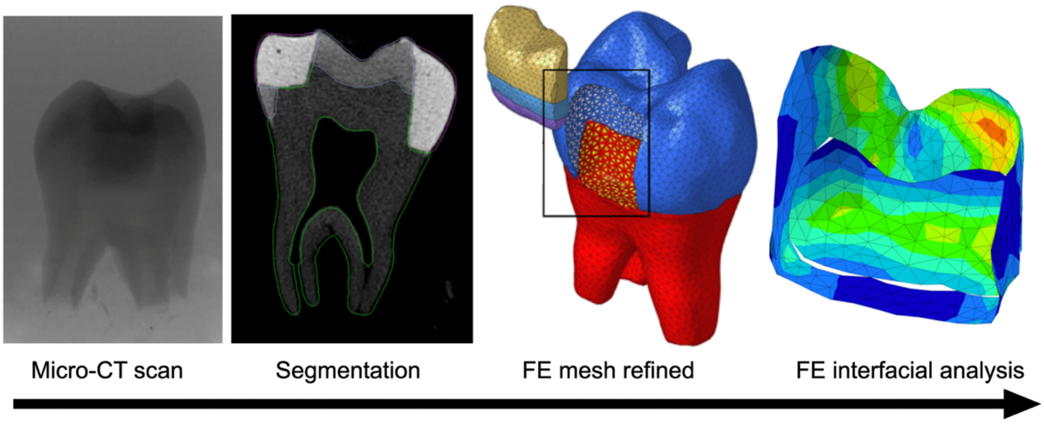

Figure 1. Summary of the performed steps from micro-CT to FE analysis. A maxillary molar was restored with two class II cavities and scanned with micro-CT. Segmentation allowed to create different meshes for dentin (red volume), enamel (blue volume) and restorations (separate volume consisting of different layers). Finite Element analysis was performed focusing on the interfacial behavior of the restorative materials tested.

The obtained STL files were imported into Meshlab (ISTI, CNR, Pisa, Italy) in order to perform a refinement process. The process consisted in the decimation of the FEs and in the elimination of the degenerated elements. Moreover, holes on the geometry were filled and the normal of the elements were reoriented in the same direction for all the elements making the mesh suitable for volume elements generation. The two-dimensional model was successively converted into a tetrahedral mesh using Altair Hyperworks (Altair Engineering, Troy, Michigan, USA). Subsequently, in order to virtually reproduce the loading cycle of a chewing simulator machine, the sphere used in the experimental test to apply the load was discretized using shell elements with rigid material and the tooth was positioned within a resin disk, as shown in Figure 2. The total number of elements of the FE model, including resin disk and sphere, was approximately 135.000.

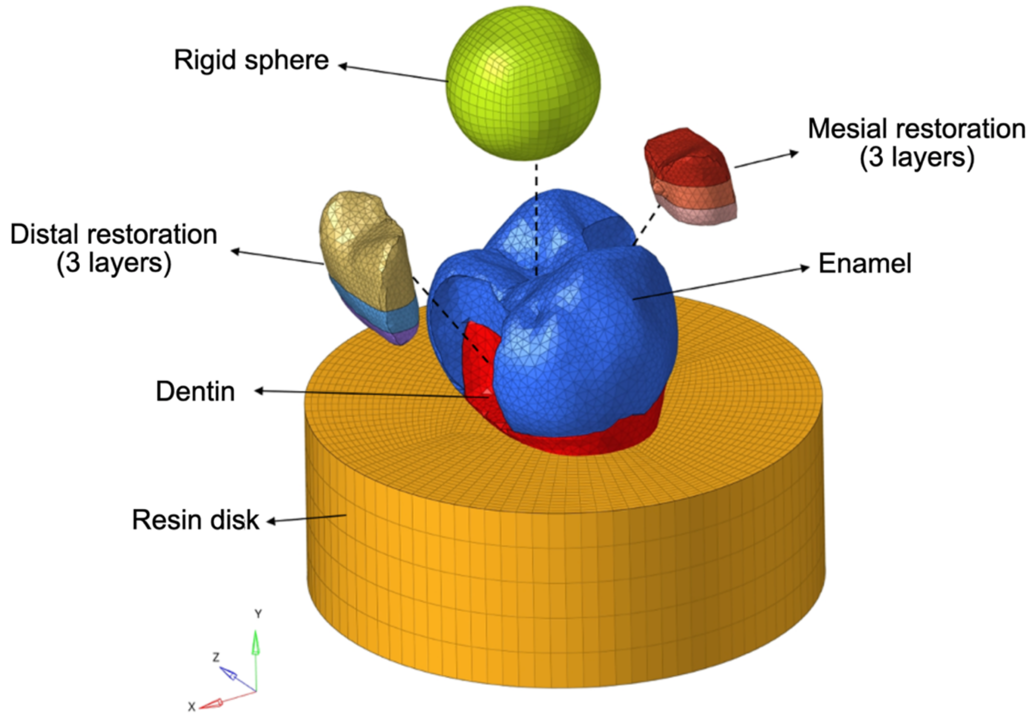

Figure 2. Representation of the simulated FE model. Each volume is reported alongside its description, as follows: green = rigid chewing sphere, blue = enamel, red = dentin, orange = resin disk. Mesial and distal restorations were divided into three segments in order to simulate different models, as explained in the text.

Restorations were segmented in three parts, in order to simulate a total of six different models (Figure 3), using different restorative materials and techniques as follows:

- Model A: the entire restoration was simulated with lithium disilicate (IPS e.max CAD MT, Ivoclar Vivadent, Schaan, Liechtenstein);
- Model B: the entire restoration was simulated with nano-filled conventional packable composite (Filtek Supreme XTE, 3M ESPE, St. Paul, MN, USA);
- Model C: a 1.5 mm thick horizontal layer of highly-filled flowable resin composite (Majesty ES Flow super low, Kuraray Noritake Dental, Tokyo, Japan) was simulated as a first layer, over the cervical margin; the remaining

restoration was simulated with nano-filled traditional composite (Filtek Supreme XTE, 3M ESPE, St. Paul, MN, USA);

- Model D: same scenario of model B, but with 3 mm of highly-filled flowable composite (Majesty ES Flow super low, Kuraray Noritake Dental, Tokyo, Japan).
- Model E: same as scenario C, using lithium disilicate (IPS e.max CAD MT, Ivoclar Vivadent, Schaan, Liechtenstein) instead of the nano-filled traditional composite
- Model F: same as scenario E, but with 3 mm of highly-filled flowable composite (Majesty ES Flow super low, Kuraray Noritake Dental, Tokyo, Japan).

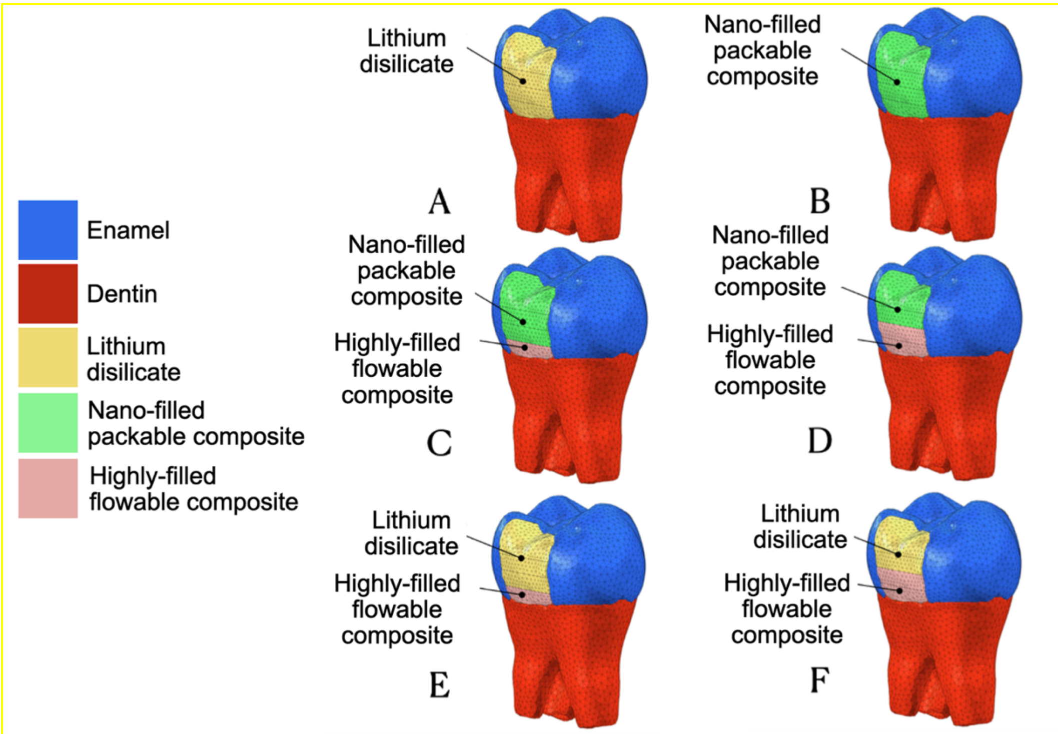

Figure 3. Schematic representation of the six simulated FE models. Materials are represented as follows: red = dentin, blue = enamel, yellow = lithium disilicate, green = nano-filled conventional packable composite and pink = highly-filled flowable resin composite.

Dentin, enamel and restorations were modelled with a linear elastic material model, whereas the resin disk and the chewing sphere were modelled as rigid materials, because the deformation of these parts is negligible compared to the other parts of the model subjected to load. Sphere-tooth contact was considered with an automatic surface-to-surface contact algorithm (Fig.4).

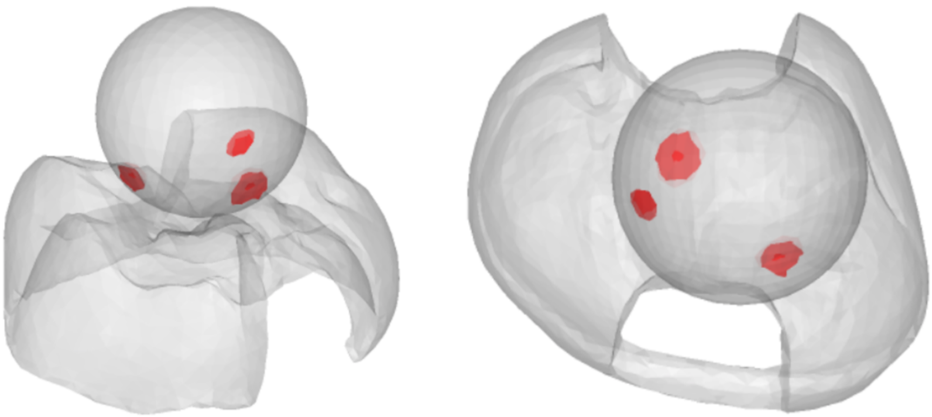

Figure 4. Contact points (red area) determined with a surface-to-surface contact algorithm.

The FE simulation was performed using LS-Dyna code in its explicit formulation. In order to reach the load applied during the experimental test, the simulation was set-up in the following way. Considering the explicit method, to increase the stability of the simulation, a motion law was applied to the sphere. In particular, a constant velocity in the vertical direction (Y axis in negative direction, referring to Figure 2), was applied to the sphere up to the applied load reached a value of 50 N.

The contact between the sphere and the tooth was set as a penalty-based formulation, whereas the joining between the other parts (enamel, dentin and restorations) were defined with a tied contact formulation with offset option, according to software house indications. The load was aimed to simulate a single load cycle. Normal pressure and shear stress at the tooth-restoration interface were recorded (Figure 5).

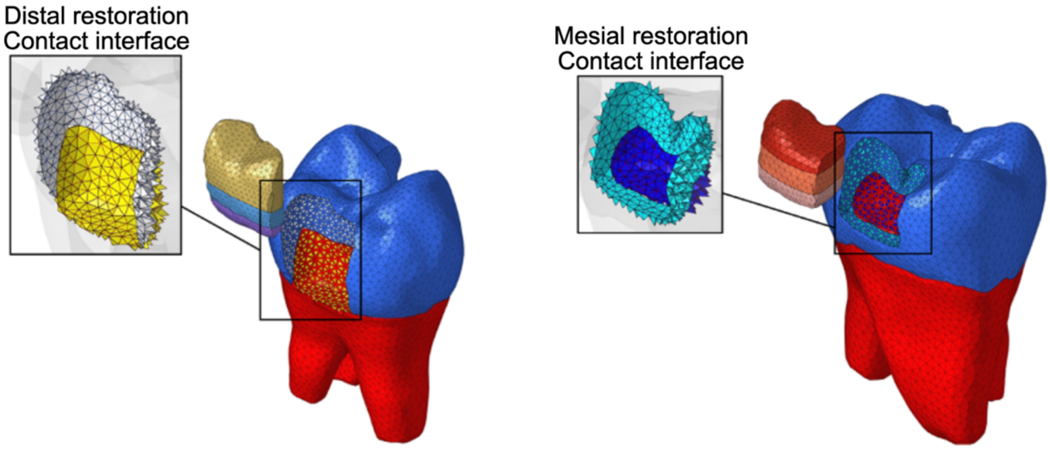

Figure 5. Highlights of the elements at the tooth-restoration interfaces of which normal pressure and shear stresses were evaluated. Left picture present a highlight of the distal restoration, while right picture of the mesial one.

Table 2 reports a summary of the mechanical characteristics given to the simulated materials, which were taken from the literature [27,28] or directly provided from the manufacturers. Table 3 reports a summary of volumes, expressed as mm^3, and elements, expressed as n°, for the different parts in different scenarios.

Material	**Young's modulus (GPa)**	**Poisson ratio**	**Density (g/cm^3)**
Enamel	78	0.33	3
Dentin	18	0.23	2.2
Allumina sphere	80	0.23	2.7
Resin Disk	3.5	0.33	1.54
Lithium disilicate (e.max CAD)	95	0.23	2.5
Nano-filled packable composite (Filtek Supreme XTE)	12.7	0.35	1.9
Highly-filled flowable composite (Majesty ES Flow Super low)	6.7	0.33	1.85

Table 2. Material properties of the FE parts, obtained from literature or provided from the manufacturers.

		SPHERE	RESIN DISC	ENAMEL	DENTIN	DISTAL	MESIAL		
Scenario A,B	volume	111.62	1685.30	158.23	238.69	32.24	25.18		
	elements	7000	3280	15638	24057	14834	13206		
						DISTAL		**MESIAL**	
		SPHERE	**RESIN DISC**	**ENAMEL**	**DENTIN**	**Lithium disilicate/Nano-filled packable composite**	**Highly-filled flowable composite**	**Lithium disilicate/Nano-filled packable composite**	**Highly-filled flowable composite**
Scenario C, E	volume	111.62	1685.30	158.23	238.69	26.99	5.25	17.26	7.92
	elements	7000	3280	15638	24057	11700	3134	9080	4126
						DISTAL		**MESIAL**	
		SPHERE	**RESIN DISC**	**ENAMEL**	**DENTIN**	**Lithium disilicate/Nano-filled packable composite**	**Highly-filled flowable composite**	**Lithium disilicate/Nano-filled packable composite**	**Highly-filled flowable composite**
Scenario D, F	volume	111.62	1685.30	158.23	238.69	18.98	13.26	7.34	17.84
	elements	7000	3280	15638	24057	7720	7114	3903	9303

Table 3. Number of elements and volume expressed in mm^3 for each single part in different tested scenario.

Results

Figures 6a and 6b illustrate the contour maps of the normal pressure (at peak load) at the interface between the tooth and the restorations for the mesial cavity (enamel cervical margin) and the distal cavity (dentin cervical margin). All the pictures are representative of the tooth-restoration interfaces observed from outside the tooth, as previously shown in Figure 5. The results are presented with relative percentage values referred to model A to increase the comparability of the results between the different cases.

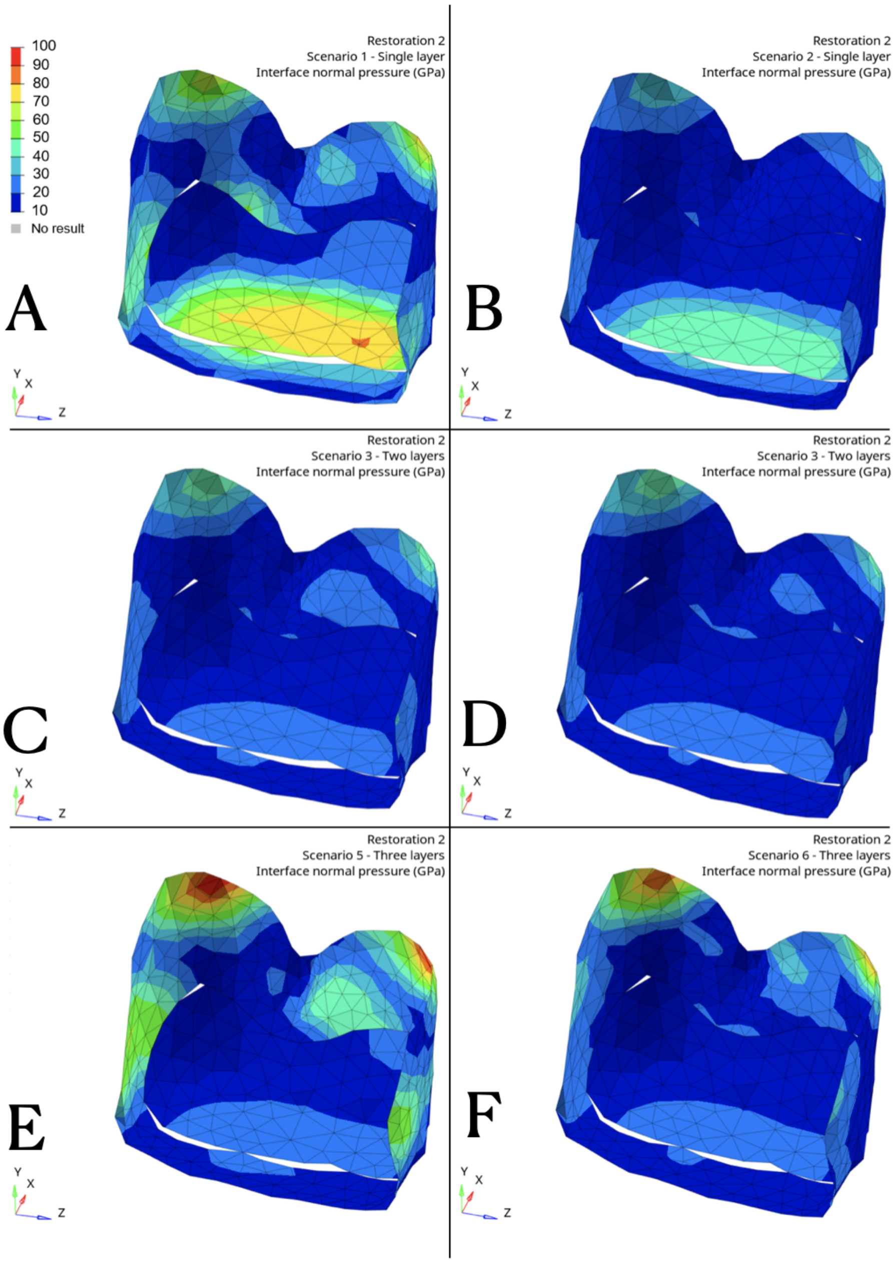

Fig.6a. Normal pressure distribution on the tooth-restoration interface, at the peak load, for the mesial cavity (enamel cervical margin). The legend reported to the left shows the maximum and the minimum values, respectively red and blue.

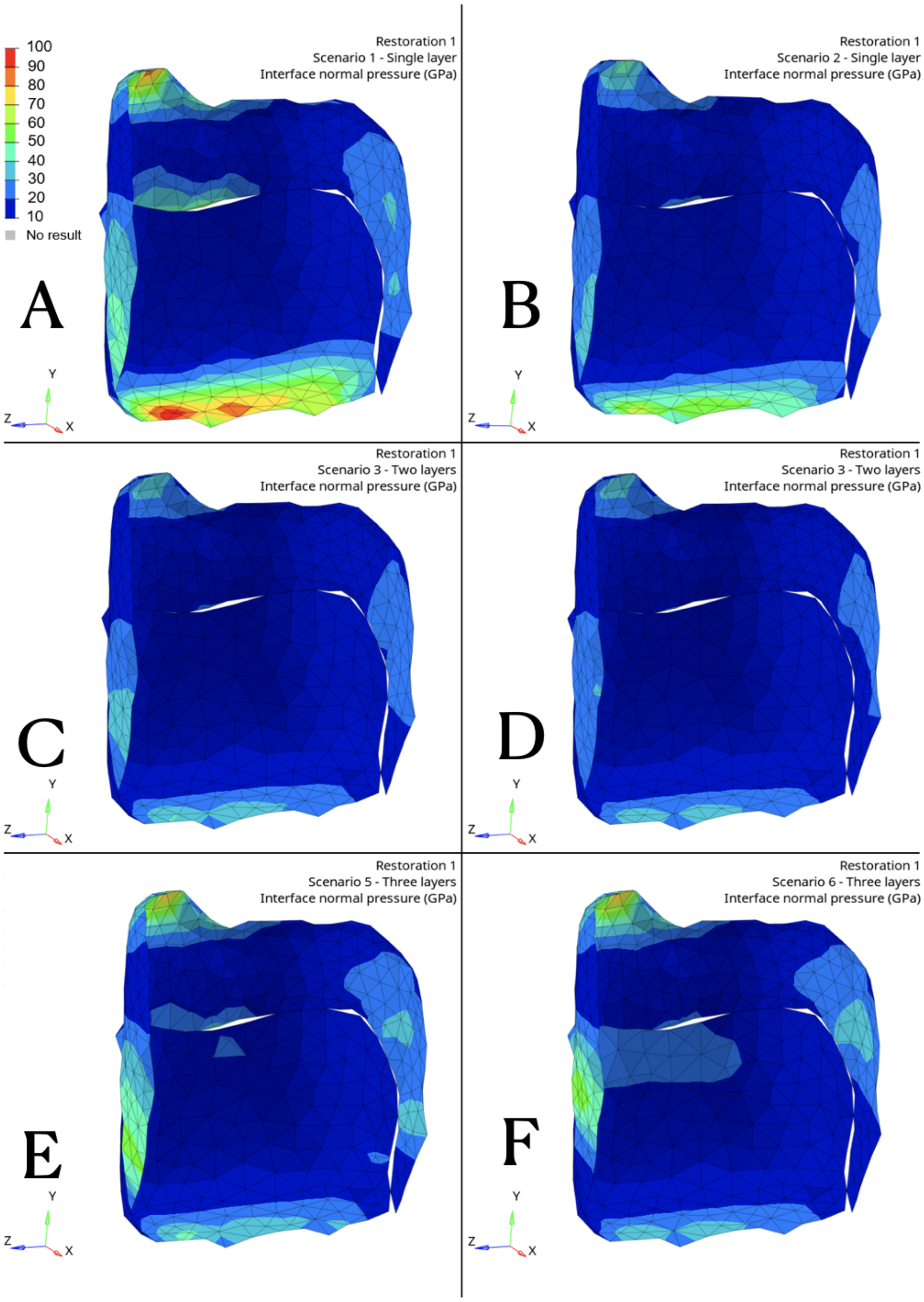

Fig.6b. Normal pressure distribution on the tooth-restoration interface, at the peak load, for the distal cavity (dentin cervical margin). The legend reported to the left shows maximum and minimum values, respectively red and blue.

For what concerns the mesial restoration (Fig. 56), the model that presented higher interfacial stresses is the model A. Most of the stresses appear to be concentrated on the inner cavity floor, while lower and not homogeneous stresses were found on the axial enamel and the cervical enamel margin. The model E presented even higher interfacial stresses on the axial enamel, especially in the coronal part, but performed significantly better regarding the axial dentin, the cervical enamel margin and the cavity floor area compared to the model A. The model F showed a similar pressure distribution but it performed even better than the model E, because the high stresses in the coronal enamel are reduced. The model B showed minor stresses on the cavity floor and the cervical enamel margin, with a homogeneous distribution among the whole surface in particular compared to the model A. The models C and D both performed significantly better than the others, appearing as equally the best solutions. It was also possible to observe that, in all the models, cervical enamel margin showed less stresses compared to the inner cavity floor, therefore probably acting as a force-breaker.

As regards to the distal restoration (Fig. 6b), the results confirmed the trend obtained on the mesial restoration. The model A showed the worst behavior, with high stresses on the cavity floor and a peak load in the cervical margin. Minor stresses were also reported among axial enamel, with a homogenous distribution. The models E and F showed again better results compared to the model A, except for the axial enamel stresses that increased and were localized more coronally. In this case, the model F is slightly worse than the model E because the pressure distributions are a little bit higher. The model B showed an overall good stress distribution, but the models C and D performed again significantly better in the cavity floor area.

Figures 7a and 7b illustrate the contour maps of shear stresses (at peak load) at the interface between the tooth and the restoration for the mesial cavity (enamel cervical margin) and the distal cavity (dentin cervical margin). The results are presented with relative percentage values referred to model A in order to increase the comparability of the results between the different cases.

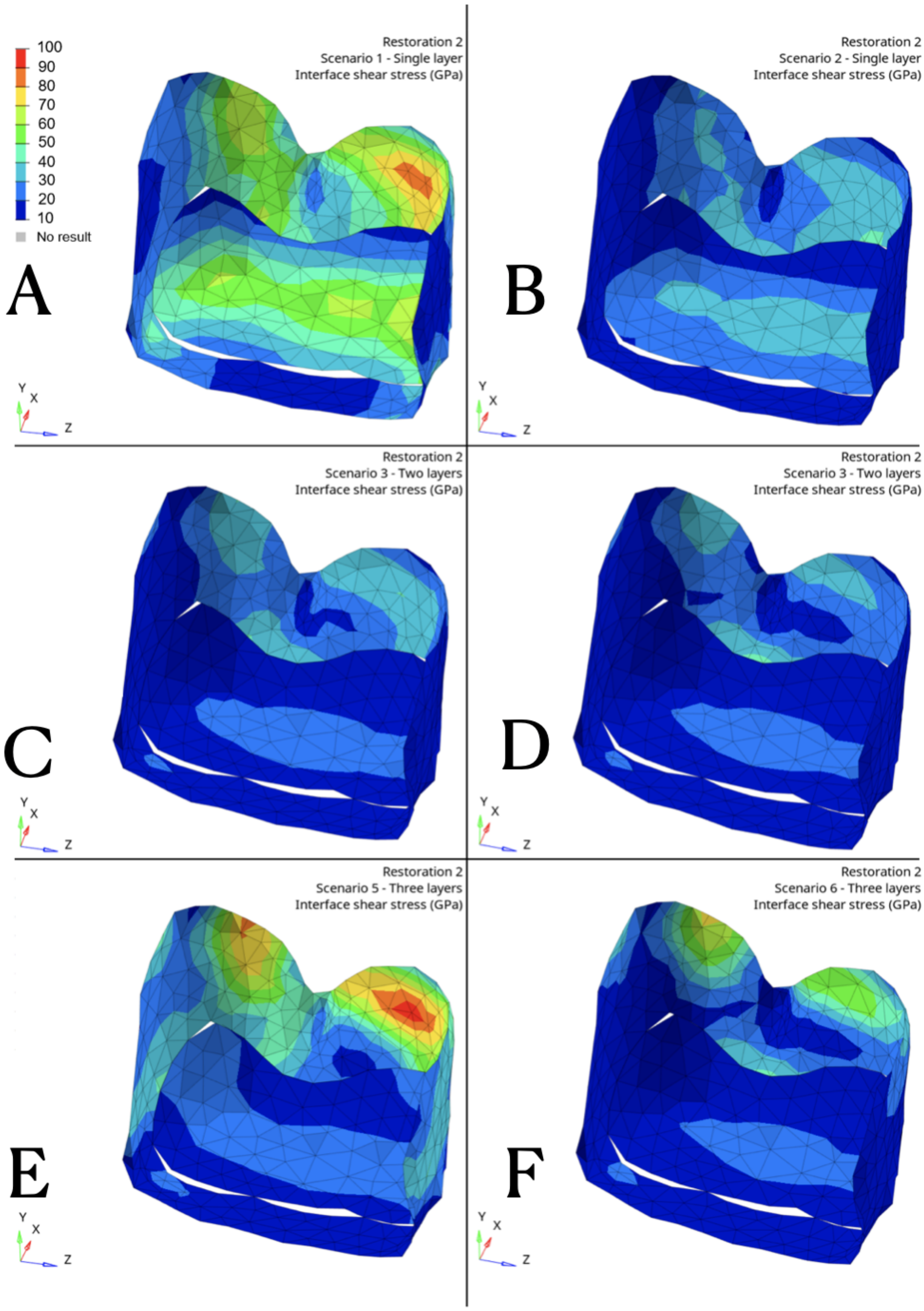

Fig.7a. Shear stress distribution on the tooth-restoration interface, at the peak load, for the mesial cavity (enamel cervical margin). The legend reported to the left shows the maximum and the minimum values, respectively red and blue.

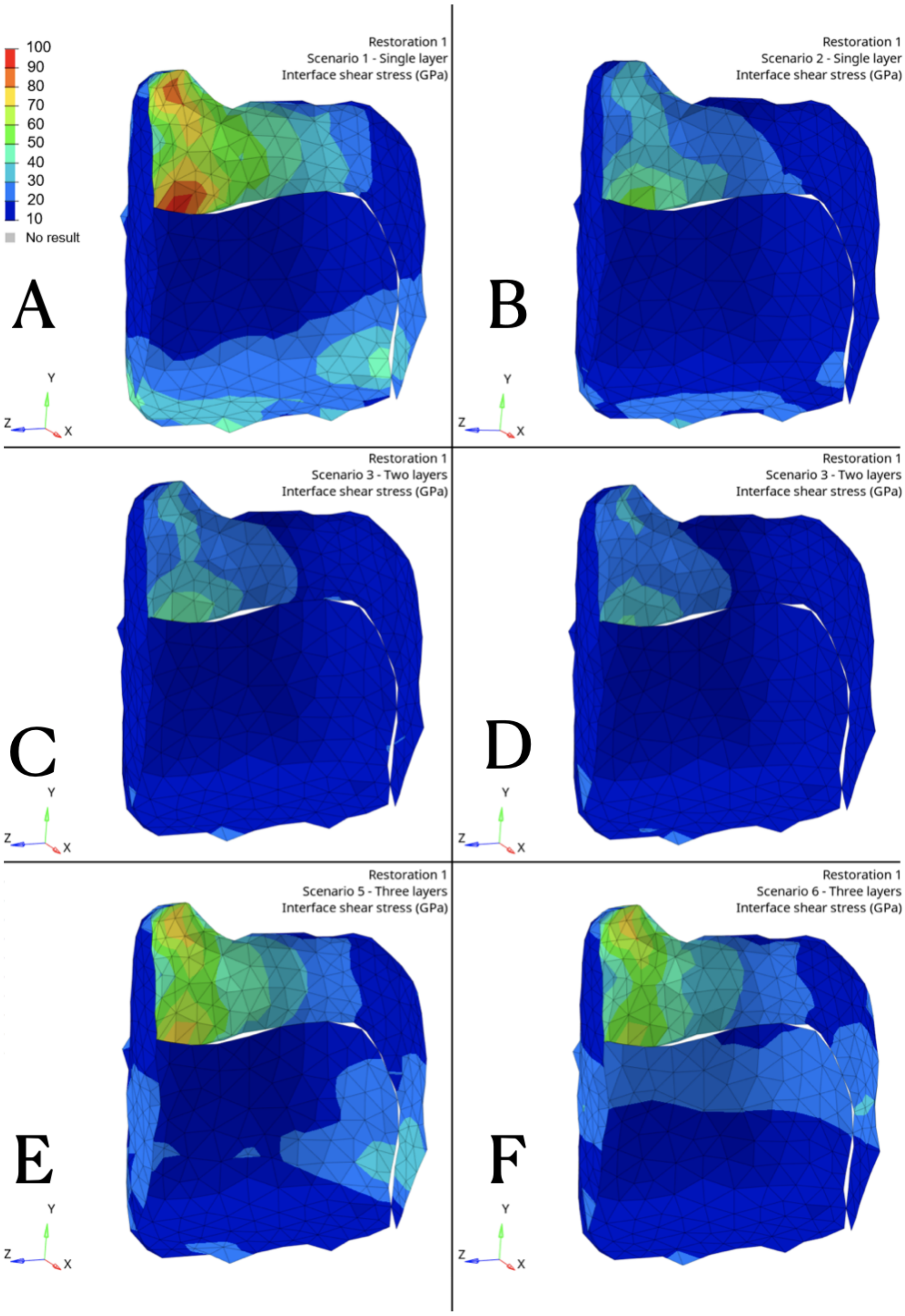

Fig.7b. Shear stress distribution on the tooth-restoration interface, at the peak load, for the distal cavity (dentin cervical margin). The legend reported to the left shows the maximum and the minimum values, respectively red and blue.

Regarding the mesial cavity (Fig. 7a), the model A showed the worst overall behavior, with high stresses on the cavity floor, the cervical and axial enamel margins. Alike to what previously reported in the normal pressure analysis, the models E and F performed significantly better than the model A, even if the model E showed higher stresses on axial enamel margins, because the stress distribution showed average lower values. The models B, C and D showed low and well distributed stresses, with the models C and D performing slightly better than B on the cavity floor area.

As for the distal cavity (Fig. 7b), the model A showed the worst interfacial response, especially on the axial enamel and the cavity floor. The stress distribution showed the highest average stress values moreover with two important peaks. The model E performed significantly better than A, but few noticeable stresses persisted on the cavity floor area and the axial enamel. In this scenario, the model F worked slightly better than E, showing only low axial stresses in a very coronal position, with complete preservation of the cavity floor. Once more, the model B only showed few stresses in the coronal enamel and in the cavity floor. The models C and D had again the optimal performance, with minimum stresses only in the coronal enamel.

Discussion

The present study analyzed the interfacial mechanical behavior of highly-filled flowable resin composite employed in deep margin elevation through FEM analysis. Based on the obtained results, all the three null hypotheses tested were rejected.

As regards the first null hypothesis, both tensile and shear stresses showed significant differences between tested occlusal materials. In particular, overall higher stresses were reported for lithium disilicate inlay compared to the nano-filled composite filling, both when they were employed alone (model A vs B) and when they were leaned on the highly-filled flowable composite (model C vs E and model D vs F). Being more specific, when the materials were employed alone (model A vs B), lithium disilicate presented higher shear stresses and normal pressure on axial walls, cavity floor and cervical margin. Contrastingly, when lithium disilicate was applied on the highly-filled flowable composite (models E and F), higher stresses were reported only on axial walls, in the closest area to the contact point of the load. This can be explained by the different mechanical properties of the materials, as ceramic has a higher Young modulus [29], and therefore a stiffer behavior that creates higher stresses on the same deformation [30]. On the other hand, composite materials have a low Young modulus because of the resin matrix [31], that might cause a more homogeneous distribution of the forces, as confirmed by the present study results [32]. When restoring multiple teeth or in case of hard-to-manage interproximal areas, even if the indirect solution with lithium disilicate could be less common than the direct composite approach, ceramic inlays clinically showed excellent mechanical proprieties and long-term performances, [33,34]. Moreover, as for direct restorations, ceramic inlays need adhesive cementation in order to increase over-time performances, therefore isolation must be considered a crucial factor [35]. As consequence and accordingly to the present study results, in order to make isolation and cementation procedures easier and to prevent stress concentration, deep margin elevation with highly-filled flowable composite resins could be successfully applied under ceramic inlays [36].

The second null hypothesis was also rejected, because the use of a highly-filled flowable composite as liner on the cervical margin significantly influenced the resulting shear and tensile stresses, with better performances (lower stresses and more uniform distribution) in the cavity floor and cervical margin areas whenever a deep margin elevation technique was applied (model A vs E-F and Model B vs C-D). This is in accordance with Ausiello et al. that already showed with FEM study how Young's modulus values of the restorative materials play an essential role in the stress distribution [37]. Other studies indirectly came to the same conclusions analyzing the interfacial behavior of flowable composite used as cavity liners, even if data cannot be compared directly to the present study due to different methodology and materials [38,39]. Data also showed a slight trend of developing inferior shear stresses and normal pressure when two layers of highly-filled flowable material were applied instead of a single layer (model C vs D and model E vs F). This trend is consistent with the previously reported discussion: the thicker the elastic layer, the higher the elastic deformation that can distribute stresses [28]. Given the present results, it is reasonable to assume that the highly-filled flowable composite could be useful to improve tensile and shear stresses distribution in the cervical area.

Lastly, the third null hypothesis was rejected, because a different stress distribution was observed in the cavity floor area according to the substrate (mesial vs distal cavities). In particular, enamel cervical margin seemed to play a protective role in the stress distribution, always showing inferior stress values compared to dentin cervical margin. This stress-breaker effect could be explained by the different mechanical proprieties of the dentin and enamel when related to the elasticity of the employed materials [40]. Dentin mechanical proprieties are more similar to that of composite compared to ceramics and this might lead to a more homogeneous distribution of forces. The exact opposite can be said for enamel and ceramic, that directly transmit forces to the adjacent structures [41]. As consequence, the absence of cervical enamel should be considered a critical factor, not only from an adhesive point of view, but also biomechanically. Without cervical enamel a major attention should be given to the management of stresses in the cavity-base, since stresses will be more prone to concentrate in the outer interfacial area, leading to possible interfacial gap formation.

Another aspect to highlight is that the position of the loading point has a correspondence with the stress peaks distribution, as it has been shown in numerous other FEM studies [28,42]. Indeed, it can be observed that the contact points of the sphere determined the localization of the maximum stress area on the tooth-restoration interface in the nearest point in projection. Even if the sphere tries to realistically replicate a conventional 3-contact point chewing, the stress peaks depend on the initial position of the sphere, and therefore the occlusal area with higher stress. As a consequence, cavity design and occlusal morphology will play a crucial role to avoid stress concentration. In accordance, it could be indirectly speculated that the occlusal contact points should not be placed on the tooth-restoration interface, but rather away from it, in order to avoid stress peaks along the adhesive interface.

For what concerns the methodology, FEM has already been proved to be an efficient and predictable method to simulate and analyze the mechanical behavior of dental materials [24]. Outdated FEM studies used 3D CAD models, with severe limitations in terms of realism [43]. More recently, 3D model reconstruction from micro-CT has been introduced, bringing to the creation of a more realistic virtual model [24,44]. However, most of the studies with micro-CT only segmented enamel and dentin, subsequently creating the restorations through CAD software, with limitations in terms of realism, especially in the corner area, since burs are not able to create perfect geometrical shapes [28,45]. In the present study, a healthy tooth was first cavitated and restored, then scanned and fully segmented aiming to better reproduce a clinical scenario [44]. Real restorations are not comparable to perfect shapes, indeed presenting voids, gaps and complex individual geometries which could have an impact on mechanical simulation and stress distribution. In previous works [28], FEM was used to study the global stress state in a restored tooth by means of linear analysis. In the present work non-linear analysis were carried out, allowing to obtain the complete stress state on the whole interface surfaces of a restored tooth. The stress state at the interface was studied considering both the shear stresses and the normal component (normal pressure). The analysis of these two entities allowed to understand how the tooth and the adhesive layer are stressed. These informations are also useful to study potential long-term effects on the restoration.

A limitation of the FE models adopted in this work concerns the absence of the adhesive layer mesh. This might affect ceramic performances since it has been demonstrated that glass ceramics mechanical behavior benefit from adhesive cementation [46]. However, at the state of the art, it is quite difficult to get a realistic and precise micro-CT volume definition for the adhesive layer that could be easily translated in a FE model, due to its thickness and material properties [47,48]. Excluding the adhesive layer allows faster computation (both considering the pre-process and the solution time) and avoid introducing potential mistakes in this critical area. Moreover, all the materials were simulated with homogeneous and isotropic law, despite the dentin and the enamel having an anisotropic behavior in the reality. However, several other studies [49,50] applied the same FEM hypothesis, due to the fact that anisotropy can be observed in a microscopic scale, whereas the tooth is modelled in a macroscopic scale. Lastly, it should be considered that FEM analysis only considers the mechanical point of view, without considering the biological performance of adhesives, but this is a limitation correlated to the methodology itself.

Conclusions

- FEM can be efficiently used to study interfacial behavior of dental materials in different clinical situations. In particular, all FE meshes, even the restorations, were created starting from micro-CT in order to create realistic dental models.
- Shear stresses and normal pressure presented similar distributions at the interfaces, and they were influenced in the same way by the different materials considered for the restoration.
- Composite resins showed a more homogeneous behavior in stress distribution compared to ceramic.
- The highly-filled flowable resin composite showed encouraging results when applied as liner, with a significant reduction of shear stresses and normal pressure on the cavity floor and the cervical margin area, especially when the load was applied on an occlusal ceramic material.
- Cervical enamel margin seemed to play a protective role in the stress distribution, acting as a stress breaker.

Further studies are needed to better assess the influence of shrinkage stress at the interface before loading on the mechanical behavior of different tested materials.

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3. Overall conclusions

The present chapter is meant to draw general conclusions from the performed researches and to emphasize the key points that have been dealt with.

First of all, a new digital workflow was developed to better quantify interfacial gaps through micro-CT dataset volume segmentation (paragraph 2.2). This new protocol allows to highlight clinically relevant situations that might be underestimated or even undetected by conventional linear analysis. Moreover, it is faster, less operator-dependent and thus easier to standardize. With the used software, it is also possible to perform a 3D rendering of the gap volume, export selected voxels and align obtained data, thus allowing a better comparison between different dataset (e.g., before and after fatigue simulation).

Through this new workflow, all performed studies reported a deterioration at the TRI, both with thermal and mechanical aging protocols, even if it was not always significant from a statistical point of view. In particular, 10.000 thermal cycles alone (5-55°C, dwell time 1 min) did not seem to cause a significant TRI deterioration However, accordingly to paragraph 1.9, this is probably related to the insufficient number of performed cycles and the absence of mechanical loading. On the other hand, all the other published researches used a standardized chewing simulation protocol (50 N, 1 Hz, 2 mm sliding, 500.000-1.000.000 cycles) that caused significant interfacial deterioration in most direct and indirect scenario. It is worth to mention that the deterioration observed is in the order of tenth of mm^3 and is yet to be confirmed if this is clinically relevant or not.

Regarding direct restorative materials, bulk RBC (packable, flowable and ormocer packable) performed better in terms of baseline gap compared to conventional nanohybrid RBC (paragraph 2.3). In particular, these bulk materials seem less technique-sensitive, meaning that can be layered more easily. Another interesting aspect is that the viscosity of the RBC seems to play an important role when dealing with a complex cavity design, with flowable performing better than packable at baseline (paragraph 2.4). However, packable RBC showed inferior gap progression after chewing simulation compared to flowable materials, especially on dentin substrate, probably due to higher physical properties. According to these two studies, an ideal direct material could be a highly-filled flowable bulk RBC, easy to manipulate, with low shrinkage, high elasticity and a sufficient amount of filler to sustain mechanical loads.

When fibers are applied, our studies concluded that short fibers inside RBC do not have an influence on gap progression (paragraph 2.5). Horizontal fibers applied in buccal-oral direction, on the other hand, improved gap performance with cyclic fatigue, even if they did not influence fracture strength or pattern. On anterior ETT vertical fibers did not influence gap progression, while fiber posts positively did that (paragraph 2.6). Thus, fiber might surely improve gap behavior, but their orientation and form are crucial. The objective when using fibers to improve TRI behavior, is opposing to cuspal flexion, while simultaneously increasing flexural strength of the tooth-restoration system. This last aspect has yet to be investigated, because it seems to play a crucial role in TRI stress development.

Similar results were also found in indirect materials (paragraph 2.7), with fiber posts reducing gap progression even when placed under indirect adhesive restorations. The material of the indirect restoration, on the other hand, did not influence gap behavior when the restoration was adhesively cemented. However, lithium disilicate performed better than zirconia (paragraph 2.8), meaning that the stability of the TRI is also related to the efficiency of the adhesion. Thus, future researches should focus on creating a material not only with sufficient mechanical properties to withstand chewing and thermal forces, but also with high elasticity and high bonding performances in order to preserve TRI. Moreover, more retentive preparation designs could improve flexural strength of the tooth-restoration complex and reduce TRI degradation and should therefore be preferred if compatible with biological preservation.

Finally, finite element study showed encouraging interfacial results when applying highly-filled flowable RBC as liners, with reduced shear stresses and normal pressure on the cavity floor and the cervical margin area (paragraph 2.9). This is again related to the high elasticity of these material, that help stress-distribution. Further studies are necessary to better understand TRI behavior under simulated fatigue. However, it is clear that creating finite element models from micro-CT could both accelerate and improve material testing process, helping to design materials with proper elasticity to ensure TRI preservation.

Unfortunately, with the present work it was not possible to find a straight-forward solution to minimize the TRI gap, as the issue is multi-factorial. However, several inputs to further studies were found, including: (1) direct RBC chemical optimization and testing, (2) optimization of fiber positioning with focus on the consequent TRI behavior, (3) indirect material testing in different combinations, (4) FEM modelling of different scenario to foresee and prevent interfacial failure and (5) clinical studies confirming obtained data.

4. Other published papers

J Adhes Dent. 2019;21(4):329-335. doi: 10.3290/j.jad.a42932. “Effect of Er:YAG and Burs on Coronal Dentin Bond Strength Stability”. Allegra Comba, **Andrea Baldi**, Riccardo Michelotto Tempesta, Aristea Cedrone, Giorgia Carpegna, Annalisa Mazzoni, Lorenzo Breschi, Mario Alovisi, Damiano Pasqualini, Nicola Scotti. PMID: 31432047 DOI: 10.3290/j.jad.a42932

Int J Prosthodont. 2021 Feb 23. doi: 10.11607/ijp.6970. “Effect of different fiber-reinforced solutions on fracture strength and pattern of endodontically treated molars”. Michelotto Tempesta Riccardo, Saratti Carlo Massimo, Rocca Tommaso, Pasqualini Damiano, Alovisi Mario, **Baldi Andrea**, Comba Allegra, Scotti Nicola

Scotti N., **Baldi A.**, Vergano E.A., Kubo C.H., Torres C.R.G. (2020) Light-Curing Units. In: Torres C. (eds) Modern Operative Dentistry. Textbooks in Contemporary Dentistry. Springer, Cham. https://doi.org/10.1007/978-3-030-31772-0_13

J Dent. 2021 Jan;104:103510. doi: 10.1016/j.jdent.2020.103510. “Journal of Dentistry”; “A 5-year retrospective evaluation of direct composite restorations in orthodontically treated patients”. Comba A, Vergano EA, **Baldi A**, Alovisi M, Pasqualini D, Castroflorio T, Stura I, Migliaretti G, Scotti N.

Applied Sciences. 2021; 11(9):3971. https://doi.org/10.3390/app11093971 “Bond Strength stability of different dual-curing adhesive cements towards CAD-CAM resin nanoceramic: an in vitro study” Edoardo Alberto Vergano, **Andrea Baldi**, Allegra Comba, Edoardo Italia, Giorgio Ferrero, Rossella Femiano, Felice Femiano, Scotti Nicola

Int J Prosthodont. 2021 Apr 16. doi: 10.11607/ijp.7486. PMID: 33871486. “Does curing delay affect the bond strength of fiber post with self-adhesive cements?”. Comba Allegra, **Baldi Andrea**, Juzikis Elvinasc, Vergano Edoardo Alberto, Pasqualini Damiano, Alovisi Mario, Berutti Elio, Scotti Nicola

Applied Sciences. 2021; 11(11):5060. https://doi.org/10.3390/app11115060; Digital procedures compared to conventional gypsum casts in the manufacturing of

CAD/CAM adhesive restorations: 3D surface trueness and interfacial adaptation analysis. **Andrea Baldi**, Allegra Comba, Edoardo Alberto Vergano, Michail Vakalis, Mario Alovisi, Pasqualini Damiano, Giorgio Ferrero, Edoardo Italia, Riccardo Michelotto Tempesta, Domenico Baldi, Nicola Scotti

Materials (Basel). 2021 Jul 14;14(14):3920. doi: 10.3390/ma14143920. Do chemical-based bonding techniques affect the bond strength stability to cubic zirconia? Allegra Comba, **Andrea Baldi**, Riccardo Michelotto Tempesta, Massimo Carossa, Letizia Perrone, Carlo Massimo Saratti, Giovanni Tommaso Rocca, Rossella Femiano, Felice Femiano, Nicola Scotti

Polymers (Basel). 2021 Aug 30;13(17):2915. doi: 10.3390/polym13172915. Influence of low-pH beverages on two-body wear of CAD/CAM monolithic materials. Nicola Scotti, Andrei Ionescu, Allegra Comba, **Andrea Baldi**, Eugenio Brambilla, Alessandro Vichi, Raffaele Ciardiello, Andrea Tridello, Davide Salvatore Paolino, Daniele Botto

J Contemp Dent Pract. 2021 Dec 1;22(12):1477-1482. Endodontic treatment of a molar with peculiar anatomy: case study with CBCT and 3D printed model. Pietro Costamagna, Giorgia Carpegna, Caterina Chiara Bianchi, **Andrea Baldi**, Damiano Pasqualini, Nicola Scotti, Mario Alovisi

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5. Acronyms and abbreviations

3SER: 3-step etch and rinse
2SSE: 2-step self-etch
10-MDP: 10-Methacryloyloxydecyl dihydrogen phosphate
AD: Absolute discrepancy
ANOVA: Analysis of variance
Bis-EMA: Ethoxylatedbisphenol-A-dimethacrylate
Bis-GMA: Bisphenol A-glycidyl methacrylate
Bis-MEPP: Bisphenole A ethoxylate dimethacrylate
BPA: Bisphenol A
CAD: Computer assisted design
CAM: Computer assisted manufacturing
CDCC: Conventional dual-curing cements
C-Factor: Cavity factor
CEJ: Cement–enamel junction
CHX: Chlorhexidine
CQ: Camphoroquinone
DC: Degree of conversion
DICOM: Digital Imaging and Communications in Medicin
EDTA: Ethylenediaminetetraacetic acid
EG: External gap
ER: Extension error
ETT: Endodontically treated teeth
FE: Finite element
FEA: Finite element analysis
FEM: Finite element model
FPSbu: fiber posts-supported buildup
HEMA: Hydroxyethyl methacrylate
HTZ: High translucency zirconia
IG: Internal gap
LED: Light emission diode
Micro-CT/ µCT: Micro-computed tomography
MMA: Methyl methacrylate
MMP: Metalloproteinases
MOD: Mesio-occlusal-distal
NP: Normal pressure
OCT: Optical coherence tomography
PEEK: Polyether-ether-ketone
PEGDMA: Polyethylene glycol dimethacrylate
PICN: Polymer-infiltrated ceramic network
PMMA: polymethyl methacrylate
PPD: 1-phenyl-1,2propanedione
PWT: Ultrahigh-molecular-weight polyethylene fiber
ROI: Region of interest
RBC: Resin based composite
SACE: self-adhesive cements
SEM: Scanning electron microscopy
SS: shear stress
TEGDMA: Triethylene glycol dimethacrylate
TPO: Diphenyl-phosphine oxide
TRI: Tooth Restoration interface
UDMA: Urethane dimethacrylate
ZLS: Zirconia-reinforced lithium silicate