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DISSERTATION

Effects of Different Finishing Procedures and Materials on Surface Roughness of Infiltrated Subsurface Bovine Enamel Lesions

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Yang Fan
aus Hangzhou, Zhejiang, China

Gutachter: 1. Prof. Dr. Andrej M. Kielbassa
2. Priv.-Doz. Dr. C. Gernhardt
3. Priv.-Doz. Dr. T. Wrbas

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Contents

1 Introduction

In modern dentistry, it has been widely accepted that no cavity design or restorative material will cure caries. Once the operative approach has been realised, the original anatomy, strength, and aesthetics of the tooth are lost forever, at least in terms of the mean lifetime of any tooth (or restoration type). Furthermore, traditional operative treatment will lead to the continuum of replacement dentistry, with repeatedly enlarged restorations and increased damage of hard tissues [KIELBASSA *et al.* 2009]. Therefore, initial enamel caries is usually not treated operatively to avoid any inevitably sacrifice of adjacent sound tissues. At this stage, the non-operative preventive treatment includes stimulation of the natural repair process of remineralisation by application of fluorides, education by means of improving oral hygiene and implementation of a proper diet. However, this regimen usually takes a considerably long time, and would have to highly rely on a perfect oral hygiene of patients. Unfortunately, an adequate oral hygiene of enamel lesions, particularly in the case of proximal lesions, seems hardly to be achievable, and cariogenic biofilms (plaque) can only be completely eliminated by patients themselves. Thus, any remineralisation of initially carious lesions would not be realized, finally leading to a need for operative treatment [KIDD 1984]. Therefore, there is a strong need for an alternative non-operative treatment of initial (proximal) caries lesions that arrests the progression of enamel lesions.

In recent decades, instead of removing demineralised and porous dental hard tissues at later stages of disease progression, occluding or filling the microspaces and microporosities of the lesions which act as diffusion pathways for acids and dissolved minerals at a much earlier stage of development with light-curing resins, such as dental adhesives or sealants, has been studied extensively to increase tissue preservation. This promising approach has been called infiltration technique, and has been considered as a treatment regimen relating to minimal intervention dentistry [KIELBASSA *et al.* 2009]. Achievable penetration depths and capacity of arresting the progression of caries using different low-viscous materials, various pretreatments and suitable application times have been determined by several studies [GRAY and SHELLIS 2002, MEYER-LUECKEL *et al.* 2006, MUELLER *et al.* 2006, MEYER-LUECKEL and PARIS 2008a]. A dedicated resin (Icon caries infiltrant; DMG, Hamburg, Germany) with low viscosity and high penetration coefficient (PCs) has been developed by the Berlin research group, and is commercially available on the market since March 2009 [KIELBASSA *et al.* 2009].

Since the surface layer of enamel caries lesions has a lower pore volume compared with that of the lesion body underneath [SILVERSTONE 1973], it forms a barrier which might hamper the infiltration of the lesion body. Therefore, an acid-etching procedure before infiltration is necessary to remove the surface layer [GRAY and SHELLIS 2002, MEYER-LUECKEL *et al.* 2007]. With the infiltration technique, excessive resin is removed from the tooth surface before light-curing without the need for any resin coat on lesion surfaces [MUELLER *et al.* 2006]. Therefore, no sealant margins are produced on the tooth surface that could enhance plaque accumulation, increase the risk of secondary caries, or cause periodontal inflammation [KIELBASSA *et al.* 2009]. It seems reasonable to consider the infiltrated lesion as a composite of etched enamel prisms and resin that has penetrated into the pores.

A thorough finishing of the infiltrated surfaces is vital for a good long-term prognosis of the treated teeth because the remaining roughness would facilitate plaque accumulation and thereby promote demineralisation or the development of secondary carious lesions. Therefore, the surface morphology (roughness and profile) resulting from infiltration treatment is important for evaluation, and in particular for further improvement of the infiltration technique. However, it remains unclear so far how to finish or polish the infiltrated lesion surface. Furthermore, no study has hitherto focused on the changes of surface morphology during the procedure of infiltration of the initial enamel caries lesion, and, up to now, no recommendations on how to finish an infiltrated lesion have been published by the manufacturer.

In order to provide the basic information for the further improvement of the infiltrated lesion's surface quality, the present study is primarily focused on qualitative and quantitative evaluation of the surface morphology (roughness and profile) of the treated lesions for assessing of roughness effects of two different finishing ways (polishing and non-polishing) of infiltration treatment; moreover, evaluation of other materials (differing from Icon) on the surface quality of infiltrated lesion to elucidate potential effects on surface quality.

2 Literature Review

2.1 Enamel

2.1.1 Enamel Structure

It is now well accepted that dental enamel is the hardest tissue of the mineralized tissues in the body; enamel, in principle, should be fully mineralized when a tooth erupts into the oral cavity [EGGERT *et al.* 1973, GUENTHER *et al.* 1977]. This acellular tissue comprises 95% mineral and 5% water and organic matrix by weight. The corresponding figures on volume basis are 86% mineral, 2% organic material and 12% water. The mineral component of enamel is basically a substituted calcium hydroxyapatite, with the stoichiometric formula for hydroxyapatite being $Ca_{10}(PO_4)_6(OH)_2$ [ROBINSON *et al.* 1971, ROBINSON *et al.* 1983, ELLIOTT 1997]. The high inorganic content confers a glass-like appearance and translucent quality to the enamel. The yellow-white colour of teeth is therefore imparted by the dentin shining through the translucent enamel.

The crystals are up to 1 mm long, 50 nm wide and 25 nm thick, and the distribution of these compositions is not homogenous; instead, the crystals usually are arranged in bundles of approximately 1,000 crystals, the so-called enamel prisms or rods, which are the basic microscopic component of enamel originating in the region close to the amelodentinal junction and extending towards the enamel surface. However, not all rods reach the surface, and a homogenous zone of enamel devoid of rod markings can be found throughout the crowns of primary teeth; this is also common in the pit, fissure, and cervical regions of permanent dentition [STACK and FEARNHEAD 1965, RIPA *et al.* 1966, GWINNETT 1967]. In specific locations, like occlusal fissures, the porosity, protein, and crystal distribution may be quite complex and the prismatic structure may be very complicated. The rather low mineral and high protein content, indicative of a higher porosity, is probably due to a poorer prismatic packing [ROBINSON *et al.* 1971, ROBINSON *et al.* 1983]. The packing of crystals is slightly looser along the rod periphery than in the rod and interrod enamel. Furthermore, at the periphery of each prism, the crystals deviate somewhat from this orientation. Therefore, each crystal is separated from its neighbours by tiny intercrystalline spaces. These spaces are filled with water and organic material, and are supposed to form a fine network and offer diffusion pathways within the tissue, which are often referred to as micropores (or simply pores), and are regarded as an important feature with respect to caries onset [CARLSTRÖM 1963].

It has been demonstrated that the very outermost enamel is rather porous; the perikymata grooves act as larger diffusion pathways. Moreover, a varying number of developmental defects, designated focal holes, small irregular fissures and microholes less than 1 µm in diameter, are observed in the enamel [JOHNSON 1967, FEJERSKOV *et al.* 1984]. These diffusion pathways, irrespective of whether they are large or small, are all occupied by water (12% by volume) and organic material (2% by volume) under in vivo conditions. It is therefore reasonable to consider dental enamel as a microporous solid composed of tightly packed crystals.

2.1.2 Enamel Reactions During Eruption

With the eruption of a tooth into the oral cavity, the enamel surface is immediately coated by a salivary protein pellicle, and plaque starts to accumulate on the surfaces; this biofilm constantly undergoes a dynamic modification at all times [POT *et al.* 1977]. Although in theory enamel is composed of numerous crystals of hydroxyapatite (HAP), the actual circumstance is that most mineral crystals in enamel have a certain proportion of substitutions for calcium, phosphate and hydroxyl groups. Calcium may be replaced with magnesium, sodium, zinc, selenium and/or strontium. Phosphates may be substituted by carbonates and hydrogen phosphates. Fluoride incorporation is thought to occur by fluoride ions filling hydroxyl vacancies or replacing hydroxyl ions [YOUNG 1975, MARGOLIS *et al.* 1999, ZERO 1999, ROBINSON *et al.* 2000]. The partially erupted teeth offer more favourable conditions for bacterial accumulation than fully erupted teeth, because it does not participate in mastication until functional occlusion is obtained. During this period, innumerable minute processes of mineral dissolution and re-deposition occur at the enamel-plaque interface. When the tooth approaches complete occlusion, shear forces from functional chewing will modify microbial accumulation, and hence cusps are often devoid of dental plaques [CARVALHO *et al.* 1989, 1991, CARVALHO *et al.* 1992].

The central feature of the roles of plaque and saliva in the dynamic transformation of enamel in the oral cavity is the generation of organic acids by acidogenic plaque bacteria following the dietary intake of fermentable carbohydrates (such as sucrose). Saliva performs two direct functions to combat enamel dissolution by these acids: (a) the continuous flow of saliva acts to clear the acids from the mouth and (b) the supply of a number of diverse salivary constituents that have caries-protective activity. The latter constituents can act on the acids themselves (via buffering or neutralisation), on

the bacteria (via inhibition of the metabolic processes involved in acid production), and on the enamel (by maintaining chemical super saturation in the adjacent plaque fluid). A key indirect function of saliva is as a medium for the transfer of potentially active therapeutic agents, such as fluorides, to the site of action. It should be remembered that the entire enamel surface should be regarded as being in a dynamic equilibrium with its surrounding oral fluid at all time.

2.2 Caries

2.2.1 Defining the Disease

Dental caries is perhaps the most ubiquitous disease that has afflicted mankind. People are susceptible to the disease throughout their lifetime [FEATHERSTONE 2000, PITTS and STAMM 2004]. While it is normally not a fatal condition, it can cause a great deal of pain and distress, and loss of teeth has profound consequences in terms of eating, speaking, and social behaviour in general [KIDD *et al.* 2000].

The term dental caries is used to describe the results – the signs and symptoms – of a localized chemical dissolution of the tooth surface caused by acidic by-products from bacterial fermentation of dietary carbohydrates taking place in the biofilm (dental plaque) covering the affected area [KIDD *et al.* 2000]. This acid causes the local pH value to fall below a critical value resulting in demineralization of tooth tissues [CAUFIELD and GRIFFEN 2000, FEATHERSTONE 2000, 2004]. This process usually begins with demineralization of enamel and proceeds to the underlying dentin and finally will reach the dental pulp.

There are several features of dental caries:

First, dental caries is a chronic disease that progresses slowly in most people. In principle, the dental caries lesion may develop at any tooth site in the oral cavity where a biofilm develops and remains for a period of time, in particular at more or less protected sites where biofilms are allowed to accumulate and mature over time. Such sites include pits, grooves and fissures in occlusal surfaces, approximal surfaces cervical to the contact area and along the gingival margin.

Second, dental caries is a result of an imbalance in the physiologic equilibrium between tooth mineral and biofilm fluid. Therefore, dental caries starts with microbiological shifts within the complex biofilm and can be affected by any factor that influ-

ences the metabolic process, such as salivary flow and composition, exposure to fluoride, consumption of dietary sugars, and by preventive behaviours (cleaning teeth).

Third, at any given point in time the net mineral loss or gain is part of a continuous spectrum of events. The very early changes in the enamel can not be detected with traditional clinical and radiographic methods. In other words, no clinically detectable caries lesion actually does not implicate that mineral loss has not occurred.

Last, the disease is initially reversible and can be halted at any stage, even if parts of dentine or enamel already have been destroyed (cavitated), as long as the biofilm can be removed.

2.2.2 Enamel Caries

Enamel caries can be regarded almost exclusively as a chemical process, since enamel is an entirely acellular substrate [ROBINSON *et al.* 2000]. The caries lesion develops where microbial deposits are allowed to form biofilms that are not frequently removed or disturbed by mechanical wear (mastication, attrition, abrasion from brushing, flossing or toothpaste). De- and remineralisation are two dynamic processes of dental caries, in which chemical composition plays a key role. These processes take place frequently during the day in most people. Over time this process will lead to either cavitation within the tooth or repair and reversal of the lesion, or maintenance of the status quo [FEATHERSTONE 2004]. The driving force for de- and remineralisation of tooth mineral is the degree of saturation with respect to dental minerals in the adjacent liquid. The main inorganic anions are chlorides and inorganic phosphates, whilst short-chain organic acids include lactic, acetic, propionic, succinic, formic, pyruvic and butyric acids [DUCKWORTH and GAO 2006]. The main cationic components are ammonium, potassium, magnesium, and, in particular, calcium.

After repeated episodes of prolonged exposure to acidic conditions consistently below the critical pH (5.5) for enamel dissolution, demineralization starts as a distinct dissolution of apatites from the enamel prisms. Demineralization begins at the crystal level after the bacteria metabolize fermentable carbohydrates, producing organic acids that diffuse into the tooth through the water amongst the crystals. The acid dissolves calcium and phosphate into the surrounding aqueous phase between the crystals when it reaches a crystal surface. Previous experiments demonstrated that

the surface partly dissolves from the very beginning of lesion formation with enlargement of intercrystalline diffusion pathways [THYLSTRUP and FREDEBO 1982, THYLSTRUP *et al.* 1983, HOLMEN *et al.* 1985, THYLSTRUP *et al.* 1994]. Histological examination of caries lesions of enamel has consistently suggested that the most accessible and/or most soluble materials, are removed from the periphery of the prisms [DARLING 1961].

Remineralization is the body's natural repair process for subsurface non-cavitated carious lesions [TEN CATE and DUIJSTERS 1982]. Calcium and phosphates, originating from saliva or other sources, will diffuse into the tooth and build on existing crystals [TEN CATE and FEATHERSTONE 1991]. In this process, fluorides have a considerable function of speeding up the process. If fluoride ions adsorb to the crystal surface, these ions can attract calcium ions, which then attract phosphate ions, and finally build a fluorapatite-like remineralised veneering material in the crystal surface. This kind of surface is less soluble and more difficult for the acid to dissolve than the original carbonated hydroxyapatite mineral. This also means that the demineralisation by acid can be markedly inhibited by a sufficient concentration of fluoride ions on the crystal surface [TEN CATE and FEATHERSTONE 1991].

In the case of failure to remove plaque from retentive tooth areas, a diet high in frequently consumed refined carbohydrates, the dynamic equilibrium between demineralisation and re-mineralisation will be tipped towards demineralisation, and this will result in clinically detectable white spot lesions [HICKS *et al.* 2004]. The characteristic chalky surface of the white spot lesion is because there is an increase in the internal enamel porosity due to demineralisation, which causes a loss of translucency and also because direct surface erosion. The white spot lesion is the earliest clinical sign that can be seen by the human eye, and yet, by this time the process has been going on for months. It has been demonstrated that when such lesions were re-exposed to the oral environment experimentally, none of them continued to progress. Furthermore, the whitish appearance diminished after 1 week, and the surfaces that had been brushed resumed the hardness as well as the shiny appearance of normal enamel after 2 or 3 weeks [HOLMEN *et al.* 1987, NYVAD and FEJERSKOV 1987]. This means that at this stage, prior to cavitation, therapeutic intervention can arrest or reverse the process [FEATHERSTONE 2000].

When an air-dried ground section of the enamel lesion is examined in polarized light microscope the porous lesion appears as a wedge-shaped defect with the base at

the enamel surface. When the same section is examined in transmitted light after imbibition with quinoline, four porosity-related zones can be described [DARLING 1956, DARLING 1961].

(1) Translucent zone

An apparently structureless translucent zone may be seen at the advancing front of the lesion. It may vary from 5 to 100 µm in width with a pore volume of slightly more than 1% when examined in dry air. Much of this first loss also appeared to derive from interprismatic areas and from the prism peripheries, in part due to an easier flux of ions through these regions [DARLING 1961, ARENDS and TEN CATE 1981].

(2) Dark zone

The dark zone is a more constant feature of the advancing front of carious lesions than is the translucent zone. Thus, the dark zone occurs in 90-95% of lesions, and if the translucent zone is present, the dark zone is located between the latter and the body of the lesion. Polarized light studies of the dark zone indicate a pore volume between 2 and 4%. The dark appearance indicates that this zone contains very small pores which are impermeable to the large quinoline molecule. It is suggested to be a result of parts of the large pores that may be reduced by remineralisation and the natural repair process [DARLING 1961, SILVERSTONE 1967]. Supporting this concept is the observation that if lesion development occurs over a relatively long period of time, the dark zone will be wide, otherwise, the dark zone will not form and there will be rapid advancement of the front with a large, heavily demineralised body of lesion and a surface zone of minimal thickness.

(3) Body of the lesion

Further demineralisation produces the body of the lesion with a 25-50% porosity, and with pores constantly increasing until mechanical destruction of the tissues (cavitation) [DARLING 1961].

(4) Surface zone

The surface layer has a varying thickness of some 40 µm. The porosity of this surface zone amounts to 1-2%, which is fairly close to that of sound tissue. This zone often persists until cavitation occurs [DARLING 1961]. The maintenance of an intact surface during caries formation is quite remarkable. At first, this was considered to be unique to surface enamel, for example, high concentrations of fluoride, which stabilizes apatite, low carbonate and low magnesium, which have a reverse, destabilizing

effect. This would favour a lower acid solubility for mineral in this tissue region, effectively protecting the enamel from dissolution [WEATHERELL *et al.* 1968, WEATHERELL *et al.* 1972, ROBINSON *et al.* 1981, ROBINSON *et al.* 1983]. Currently, this is considered to occur, for the most part, by redeposition of material dissolved from deeper layers and potentially with some contribution from plaque fluid. For example, at the same time, penetration of acid into the deeper, more soluble layers would remove interior mineral in preference to the outer enamel surface (such as fluorides). The outer tissue could then continue to accumulate fluorides and become even more acid-resistant [WEATHERELL *et al.* 1968, ROBINSON *et al.* 1981, THEUNS *et al.* 1986].

2.3 Therapy of Initial Enamel Caries (Subsurface White Spot Lesions)

2.3.1 Traditional Treatment Options

In clinical practice, caries management by operative treatment, despite its constraints and its inherent tendency to promote repeated restorations [ELDERTON 1990] is still the favoured method in many countries. However, it should be borne in mind that once a tooth has been treated by an operative procedure, the likelihood of losing the tooth with age is higher than for a sound tooth and may be as high as having a non-treated caries lesion because of the comparably short durability of restorations, the propensity of new caries lesions to form at the margins of restorations if the causes of the disease are not removed [MJOR and TOFFENETTI 2000] and the harm caused by excessive sacrifice of tooth substance [CAUFIELD and GRIFFEN 2000, TYAS *et al.* 2000, PITTS and STAMM 2004].

Biofilms constantly form and grow on any tooth surface; meanwhile, de- and remineralisation as two dynamic processes can occur at random [MANJI *et al.* 1991]. Without regular mechanical disturbance of dental plaque, and with continuing demineralization (and without the benefit of remineralisation) an initial subsurface lesion will appear [HOLMEN *et al.* 1988]. Thus, caries lesion development and progression can be controlled by controlling the metabolism in the microbial biomass. It can be considered that disease control concerns influencing biofilm formation and growth, or modifying the dissolution kinetics of the apatites, or both. Therefore, mechanical removal of plaque [HICKS *et al.* 2003], chemical (antimicrobial) modification of plaque [SCHIOTT 1973], using fluorides and proper diet [BURT and PAI 2001], which can modify the metabolic process, should make it possible to control or even arrest the initial subsur-

face caries lesion. Thus, initial enamel caries should generally not be treated operatively to avoid removal of adjacent sound tissues. At this stage, the common treatment includes education in oral hygiene, application of antimicrobials (i.e. chlorhexidine), stimulation of the natural repair process of remineralisation by application of fluorides, and proper diet.

Tooth brushing and flossing are considered the most effective mechanical means used by patients themselves to remove the dental plaque for modifying the metabolic process of initial non-cavitated caries lesion [HICKS *et al.* 2003]. However, while flossing in particular seems to be a reasonable recommendation for proximal surfaces, its preventive effect has not been supported by evidence up to now, neither with regard to gingival health [BERCHIER *et al.* 2008] nor with respect to proximal caries [HUJOEL *et al.* 2006], and only the professional use on a supervised basis has been identified to reduce caries risk (in children) [WRIGHT *et al.* 1977, HUJOEL *et al.* 2006]. Nonetheless, it is widely accepted that this way, arrest of the lesion may be achieved, and remineralisation becomes possible. However, optimal conditions are mandatory to ensure repair or healing by deposition of mineral on existing damaged crystals or nucleation and *de novo* crystal formation [HICKS *et al.* 2004].

Dental caries is caused by acidic by-products from bacterial fermentation of dietary carbohydrates taking place in the biofilm (dental plaque) covering the affected area. Therefore, it seems reasonable to use the antimicrobials to control for bacteria if control of caries is the goal. However, the expected benefit should always be weighed against the potential adverse effect for antimicrobials use. Actually, some studies could not demonstrate any protective effect on enamel and dentine against demineralisation by using chlorhexidine as the active agent [VAN STRIJP *et al.* 1997, TIMMONS *et al.* 2007, VAN STRIJP *et al.* 2008]. Moreover, chlorhexidine obviously hampers fluoride accumulation on a tooth's hard substance [RIEBEN *et al.* 2008]. Due to the current lack of evidence on long-term clinical outcomes and reported side effects, chlorhexidine rinse, which is currently the only treatment mode available in the US, should not be recommended for caries prevention [AUTIO-GOLD 2008].

There is evidence that the application of topical fluorides (toothpaste, gels, varnishes, paint-on application and mouthrinses) can inhibit caries progression by promoting remineralisation of early caries lesions and reducing sound tooth enamel demineralisation. In particular during orthodontic treatment, the use of topical fluoride in its various forms has been the most commonly used caries preventive protocol for at-risk

patients [VAN DER VEEN and DE JOSSELIN DE JONG 2000]. Unfortunately, it remains unclear which method or combination of methods to deliver the fluoride is the most effective. Based on current best practice, for which there is evidence, it has been recommended that patients with fixed braces rinse daily with a 0.05% sodium fluoride mouth rinse [BENSON *et al.* 2004]. Therefore, it should be reasonable to consider the use of fluoride as a primary prevention component.

With proximal caries, a school-based fluoride mouth rinsing (FMR), as a supplement to the daily use of fluoride toothpastes, being able to reduce the caries incidence on proximal surfaces in adolescents with low to moderate caries risk has been reported [MOBERG SKOLD *et al.* 2005a]. In the same year, another study showed that school-based F varnish treatment every 6 months in 13- to 16-year-olds was excellent to prevent proximal caries in medium and high caries risk areas [MOBERG SKOLD *et al.* 2005b]. Furthermore, the professional application of a 10% SnF_2 solution combined with the home use of a SnF_2 dentifrice being the most effective treatment in retarding the development of initial proximal lesions for high school students was advocated more than 25 years ago [POWELL *et al.* 1981]. All in all, fluorides have been considered an efficient therapeutic and prevention strategy for proximal caries. However, it should be kept in mind that a complete and long-term remineralisation of white spot enamel lesion by application of fluoride can not be reached in most cases [TEN CATE *et al.* 1981, AL-KHATEEB *et al.* 2000, GUSTAFSSON *et al.* 2000]. Regular exposure of dental enamel to the various forms of topical fluorides has been found to have a greater effect in the prevention of enamel demineralisation rather than in the remineralisation of existing lesions [JEANSONNE and FEAGIN 1979, O'REILLY and FEATHERSTONE 1987].

It has been documented that one of the fluoride effects on white spot lesions is a preferential deposition of minerals in the surface layer of the enamel, resulting in arrestment of these lesions [TEN CATE *et al.* 1981]. However, this relatively thick and highly mineralised surface layer might act as a barrier, and, thus, has also been suspected to inhibit re-mineralisation. Thus, etching of enamel lesions has been investigated and suggested to increase the surface porosity and enhance remineralisation of the incipient lesions [HICKS and SILVERSTONE 1984a, b, AL-KHATEEB *et al.* 2000]. However, a remineralisation study showed that the enhancing effect of remineralisation by fluoride was only temporary and the process reached a plateau for all groups after a few weeks. It was concluded that full remineralisation was achieved neither by

etching, nor by the addition of fluorides, nor by the combination of both treatment regimens [AL-KHATEEB *et al.* 2000].

Indeed, a proximal caries will progress very slowly, and average survival times of proximal lesions confined to enamel of up to 8 years have been reported [LITH *et al.* 2002]. On the other hand, it has been argued that once a proximal caries lesion is cavitated, it can no longer be cleaned by means of flossing by the patient, and it would be difficult (or even impossible) to arrest further progression; hence, these lesions tend to progress slowly [ESPELID and TVEIT 1986, WAGGONER and ASHTON 1989, KIELBASSA *et al.* 2006]. Therefore, from this threshold an operative intervention is generally recommended to prevent further lesion progression. Unfortunately, the bitewing radiograph does not give any direct information on the surface integrity of proximal lesions [KIELBASSA *et al.* 2006]. This could explain clinical findings that fluoridation and improved oral hygiene can only slow down the progression of proximal caries but are not suitable to reverse progression by full re-mineralization [MEJARE *et al.* 1998]. Actually, there are only a few studies that presented true remineralisation of proximal lesions [PITTS 1986, PITTS and LONGBOTTOM 1987, ALTENBURGER *et al.* 2007]. Therefore, a considerable number of professionals still tends to favour the invasive approach; with lesions confined to enamel, the decision to prepare a cavity (invasive treatment strategy) ranged from 19% in Norway [TVEIT *et al.* 1999] to nearly 50% in Mexico [MAUPOME and SHEIHAM 1997] and Brazil [TRAEBERT *et al.* 2007], but with even higher proportions in other countries [DOMEJEAN-ORLIAGUET *et al.* 2004, GHASEMI *et al.* 2008]. Obviously, these situations could result in over-treatment, considering the slow progression of enamel lesion development.

2.3.2 The Resin Infiltration Concept

It is well accepted that surface features of active initial carious lesions exhibit widened intercrystalline spaces and frequently minor fractures of the perikymata edges which act as diffusion pathways for acids and dissolved minerals. On the other hand, the restoration of initial enamel lesions results in an unfavourable damage of the teeth and the traditional treatment regimens for initial enamel caries lesions based on fluoride application are not as efficient as expected in many cases, especially in the case of proximal surfaces. Therefore, instead of removing porous dental hard tissues at later stages of disease progression, filling microspaces and microporosities of the

lesion at a much earlier stage of development has been considered. Thus, the sealing of initial enamel lesions by resins might be a promising approach, as it can be illuminated from the fissure sealing technique [SIMONSEN 1991].

The first study describing the infiltration of carious lesions with organic resins was done in 1976 by Robinson and colleagues. Those authors demonstrated a reduction in pore volume (up to 60% of the lesion pore volume had been occluded) following the application of resorcinol-formaldehyde resin (which was unsuitable for clinical uses due to its toxic nature). The first criteria for an ideal infiltration material (hydrophilic, highly surface-active and with low viscosity, bacteriostatic, non-toxic to oral tissue, polymerisable to a solid state, resistant against chemical and mechanical challenges of the cavity, and cosmetically acceptable) has been established from that study [ROBINSON *et al.* 1976]. Since then, a number of dental adhesives (sealants, bonding agents) have appeared on the market which exhibit some of the properties originally postulated for such infiltrative treatments. A main advantage of these materials is that they are already in use within the oral environment. Many studies reported a significant reduction of pore volume after sealing the caries-like lesions with those materials [DAVILA *et al.* 1975, RODDA 1983, TANTBIROJN *et al.* 2000, ROBINSON *et al.* 2001, GRAY and SHELLIS 2002, MEYER-LUECKEL *et al.* 2006]. Some of these *in vitro* studies showed that 60% or more of the pore volume of initial artificial lesions were occluded following infiltration with unfilled resins and the infiltration depth of 60 µm was sufficient to prevent further demineralization [DAVILA *et al.* 1975, ROBINSON *et al.* 2001, GRAY and SHELLIS 2002].

Some studies have demonstrated that the natural white spot lesions should be acid-etched prior to infiltration, due to the high mineral content of the surface layer and organic substances to be found in natural caries [DAVILA *et al.* 1975, ROBINSON *et al.* 1976, MEYER-LUECKEL *et al.* 2007]. In a recent study, it was found that treatment with 15% hydrochloric acid gel for 90-120 s led to a virtually complete removal of the surface layer and therefore seemed to be more suitable for the pretreatment of natural enamel lesions prior to resin infiltration than 37% phosphoric acid which was normally used for artificial caries-like lesions [MEYER-LUECKEL *et al.* 2007]. This discrepancy might be explained by differences in lesion structures, in particular with regard to the surface layer. The surface layers of natural lesions obviously are inhomogeneous and may show higher mineral contents compared to artificial lesions, which is due to the dynamic de- and remineralisation process in the oral cavity [MEYER-LUECKEL *et al.*

2007]. Moreover, 30 s might be the suitable penetration time for optimising the seal of the lesion resulting in higher penetration depths and a more compact resin layer has been proved by another recent vitro study [MEYER-LUECKEL *et al.* 2006].

Furthermore, the viscosity, surface tension and contact angle on the capillary wall have been proved to be important properties that influence the ability of the resins to penetrate into porous enamel [FAN *et al.* 1975]. A high penetration coefficient (PC) can be achieved from high surface tension, low viscosities and low contact angles. Sealants with a low viscosity have a greater potential to penetrate into the fissures and the microporosities in the etched human enamel, which has be mentioned previously [PERCINOTO *et al.* 1995]. For artificial lesions, significant differences in the depths of penetration were revealed when various resin infiltrants with different penetration coefficients were used [PARIS *et al.* 2007a]. This has been corroborated with natural lesions recently, thus indicating that resin infiltrants with high penetration coefficients are able to penetrate more deeply into subsurface lesions [PARIS *et al.* 2007b]. The highest PCs have been found for mixtures containing tetraethyleneglycol dimethacrylate (TEGDMA), 2-hydroxyethyl methacrylate (HEMA) and 20% Ethanol [MEYER-LUECKEL and PARIS 2008b].

Under cariogenic conditions, the sealed lesions are significantly more resistant to demineralisation if compared to untreated lesions [GOEPFERD and OLBERDING 1989, ROBINSON *et al.* 2001, MUELLER *et al.* 2006, PARIS *et al.* 2006]. Many commercially available adhesives and sealants, such as Resulcin Monobond (Merz, Luetjenburg, Germany), Helioseal (Ivoclar Vivadent, Schaan, Liechtenstein), Excite (Ivoclar Vivadent), and Heliobond (Ivoclar Vivadent) have proved good capability of penetration into the micropores and reduction of lesion progression, especially after double application [MUELLER *et al.* 2006] or longer penetration times (30 s) [PARIS *et al.* 2006].

Adolescents treated with fixed orthodontic appliances may be considered as a risk group due to the accumulation of dental plaque, and the incidence of early enamel demineralization (white spot lesions, WSL) adjacent to the brackets has been estimated to be 15–85% [MITCHELL 1992]. It seems to be reasonable that fluoride may reduce the number of white spots developing during the treatment with brackets [O'REILLY and FEATHERSTONE 1987, GEIGER *et al.* 1992, BOYD 1993, SCHMIT *et al.* 2002]. However, the effectiveness of these fluorides is highly dependent on the patient's full compliance, so that partial or sporadic compliance might result only in lim-

ited benefits [STRATEMANN and SHANNON 1974, GEIGER *et al.* 1988, GEIGER *et al.* 1992].

Therefore, application of resin sealant on the enamel surface around and beneath the orthodontic bracket or on the white-spot surface after orthodontic treatment should be an option to reduce the white-spot formation or inhibit progression of the initial lesion, especially for patients without good compliance. Sealing the entire labial surface with the unfilled resin has been advocated by some researchers [HUGHES *et al.* 1979]. However, in a recent *in vitro* abrasion and demineralisation study, a toothbrush wear simulator was used to simulate the extensive toothbrush abrasion and a microhardness tester to measure the mineral change after demineralisation. The unfilled sealant (Light Bond Sealant; Reliance Orthodontic Products, Itasca, Illinois, USA) showed nearly no protective effect to the underlying enamel, due to the low abrasion and wear resistance to tooth brushing. On the other hand, the highly filled sealant (Pro Seal; Reliance Orthodontic Products) offered adequate resistance against wear during the tooth brushing and essentially complete protection against decalcification. The authors suggested that once the covered sealant wore off, the enamel was exposed to acid attack directly, and demineralisation could develop further [HU and FEATHERSTONE 2005]. This result is consistent with some reported clinical studies [ZACHRISSON *et al.* 1979, ARTUN and THYLSTRUP 1989, BANKS and RICHMOND 1994] and some *in vitro* studies [SILVERSTONE 1977, ROBINSON *et al.* 2001, SCHMIDLIN *et al.* 2005].

These studies all implied that the protection afforded to the enamel surface relies on the retention of the superficial resin coverage rather than the resin which penetrates into the pores. However, a similar study (toothbrush machine and microhardness tester were used) demonstrated that a well sealed (Estilux glaze; Kulzer, Hanau, Germany) enamel layer would function cariostatically for at least another two years after the bulk of the sealant was worn due to the resin tags [DAVIDSON and BEKKE-HOEKSTRA 1980]. Interestingly, another quite similar investigation, in which a similar abrasive procedure (toothbrush wear simulator) and materials (unfilled resin, Delton; Dentsply Professional, York, Pennsylvania, USA, and high filled resin, Pro Seal; Reliance Orthodontic Products) were used, showed that both the two materials provided significant reductions in enamel demineralization, although Pro Seal showed more protection than Delton. A directly visualized measurement with a polarized light mi-

croscope instead of the microhardness tester was used to test the extent of lesions [BUREN *et al.* 2008].

It seems that some discrepancies exist between these studies. Furthermore, some other recent studies have demonstrated that even after the resin coats on top of the lesions were removed mechanically after light curing or the overlying resin was wiped away before light curing, the remaining enamel surfaces without resin coat are also resistant to carious attacks, which should be due to the infiltrated material or the resin tags. The visualising measurement methods such as confocal laser microscope (CLSM), transversal microradiography (TMR), or polarized light microscope were used in these studies [MUELLER *et al.* 2006, MEYER-LUECKEL and PARIS 2008b]. Thus, the discrepancies between these studies might be due to the different measurement means, as well as different pre-treatment regimens, different times of application, and different characteristics of the materials which could influence the quality of infiltration. Compared to microhardness measurements to determine the extent of carious lesions which have been proved to be not completely correct [DAVIDSON and BEKKE-HOEKSTRA 1980], the visualized means should be more reliable due to directly visual and without requirement of removing the surface layer of enamel. Further investigations are mandatory on this field.

On the other hand, the resin coats on top of the lesion surfaces would be difficult to clean and increase the risks of secondary caries and periodontitis, particularly on proximal sites. Nevertheless, polishing stripes could be used to remove the overlying resins after light curing of proximal surfaces, but it seems difficult to avoid damage to the lesion surface and adjacent sound enamel. Therefore, the protective effect provided by infiltrated materials without coats on enamel surface should be exactly required for proximal initial caries lesion. It could be postulated that the highly filled sealant is only acceptable for labial or buccal surfaces, but not for the proximal ones. Therefore, infiltrants able to either offer complete infiltration, or to result in an infiltrated surface with high resistance of abrasion and wear, should be the goal.

Only few long-term clinical evaluations of methods for managing proximal tooth surfaces with initial caries lesions have been reported. A 2-years clinical study was performed on a sealant (Concise Sealant; 3M ESPE) to arrest the progression of non-cavitated proximal posterior carious lesions in 50 patients. This study revealed that about 93% and 88% of the surfaces with enamel caries showed no progression after sealant or fluoride varnish treatment. Posterior bitewing radiographs from baseline

and the 2-year study were evaluated under optimal conditions [GOMEZ *et al.* 2005]. Another recent 18-months clinical study on sealed (Gluma One Bond; Heraeus Kulzer; or Concise Sealant; 3M ESPE) proximal early active lesions in 72 patients showed by subtraction radiography that 43.5% of the sealed and 84.1% of the untreated control surfaces had progressed over the study period, thus revealing a considerably reduced progression rate. The difference in the progression rates between the two clinical studies could be due to the different evaluation methods. A SEM study on the microstructure of sealant (Clinpro Sealant; 3M ESPE; with and without a preceding bonding) penetration in the enamel of *in vivo* sealed proximal (non-cavitated) incipient caries lesion showed that the resin tags in the lesion area were twist, curved and irregular, while in the sound enamel, a more regular pattern and normal shape was observed. The length of the resin tags ranged between 4.2 and 5.5 µm [GOMEZ *et al.* 2008]. The relatively short resin tags might be due to the surface zone were revealed after the application of phosphoric acid gel instead of the hydrochloric acid gel which was recommended in another *in vitro* study [MEYER-LUECKEL *et al.* 2007]. Furthermore, bonding agent (Single Bond; 3M ESPE) showed no increased penetration depths [GOMEZ *et al.* 2008]. However, more clinical studies are needed.

In conclusion, from the foregoing review it seems obvious that the resin infiltration technique might be a promising micro-invasive approach to arrest initial caries lesions and preserve (demineralised) enamel. This therapeutic approach is in accordance with the concept of minimally invasive dentistry which has focused on maximum conservation of sound tissues. Compared to traditional operative approaches, the infiltration concept has many advantages. These include: reducing the porosity and assess of acid and egress of dissolved material, affording mechanical support to the tissue, inhibiting or delaying lesion progression, avoiding inevitably removing adjacent sound tissue, resisting future acid attack, delaying of restorative intervention for longer periods, no risk of gingivitis and periodontitis, improving aesthetic outcome, minimal relying on patients' compliance and high patient acceptance [KIELBASSA *et al.* 2009]. Therefore, this micro-invasive approach can provide a real wait-and-see position to both the clinician and the patient.

Up to now, only few clinical trials are available from the literature. Therefore, more high-quality clinical investigations are required. More clinical studies on the difference in the effectiveness of infiltrated lesions and sites to be preserved by oral hy-

giene/fluoride programs, on a clinically efficient way of finishing the infiltrated surface, on the retention of sealants in oral environment and the aesthetic outcome of this regiment, and on variations of application procedures are required to further improve this micro-invasive therapeutic approach.

Many laboratory studies on the extent of occlusion of lesion porosity and on the capability of prevention of lesion progression have been performed in artificial caries lesions (either human teeth or bovine teeth) and natural caries lesions (human teeth). Various pre-treatments (phosphoric acid or hydrochloride acid; with or without bonding), different application times (15 s or 30 s; single or double application) have been well tested to improve arresting the progression of caries lesions and resisting future acid attack. Even so, studies on variations of application procedures (repeated application after different time intervals, possible need for re-infiltration regimens within preventive orientated recalls) will still be fields of major interest.

From another perspective, even if it has been well accepted to ensure a good long-term prognosis of the treated teeth, a high-quality treated surface should be mandatory, because the remaining roughness or the thick resin coat on the lesion surface facilitates plaque accumulation and thereby promotes demineralization or the development of the secondary carious lesions, no study has hitherto focused on the morphology (roughness and profile) of the infiltrated lesion surface or the suitable finishing way to enhance the quality of infiltrated lesion surface. Moreover, as has been mentioned above, the resin materials with low viscosity and high penetration coefficients (PCs) have been proved to have a greater potential to penetrate into the fissures and the microporosities [PARIS *et al.* 2007b]. Manifold materials (some commercially available adhesives, fissure sealants, and experimental infiltrants) exhibit some of the properties originally postulated for such infiltrative treatments [MUELLER *et al.* 2006].

A dedicated resin (Icon caries infiltrant, DMG, Hamburg, Germany) with low viscosity and high penetration coefficient (PCs) has been developed and is commercially available on the market since March 2009 [KIELBASSA *et al.* 2009]. However, up to now, no study has focused on the potential effects of the various materials on the surface quality of infiltrated lesions. It has been considered that the resin materials with different surface properties, such as bonding agents (i.e. Excite; Ivoclar Vivadent), composite sealant (i.e. Fortify; Bisco, Schaumberg, USA) or high-gloss varnish (i.e. Glaze & Bond; DMG, Hamburg, Germany), and caries infiltrant (i.e. Icon;

DMG), etc, may have different effects on the roughness of the infiltrated lesion surface. Therefore, further studies on the potential effects of different finishing ways and the different properties of resin materials on the surface morphology of the infiltrated lesion should be mandatory for the improvement of the infiltrated lesion surface quality.

2.4 Different Evaluation Methods of Surface Characteristics in Dentistry

Various techniques can be used for assessing surface morphology for surfaces of teeth and dental materials, such as scanning electron microscopy (SEM), contact and non-contact profilometry, confocal laser scanning microscopy (CLSM), or atomic force microscopy (AFM). SEM is a common and powerful method for studying details in the surface structure of enamel and dentine, provided that the specimen preparation is done properly [TEN BOSCH and ANGMAR-MANSSON 1991, JOHANSSON *et al.* 2001]. The principle of SEM is based upon a pseudo three-dimensional image that is built up point-by-point and line-by-line from secondary electrons [VAN MEERBEEK *et al.* 2000]. It is probably the most widely used microscopy technique in dental materials science, because of its relatively high performance (with especially a large depth of field and relatively high resolution), and perhaps also because of its ease of use. However, SEM has some limitations in defining surface topography. The electron beam technique does not allow visualization of three-dimensional surface texture. Also, because with beam techniques the contrast relies on the different emission of electrons, these cannot give contrast on flat homogeneous surface materials [KAKABOURA *et al.* 2007]. On the other hand, the sample preparation which includes covering non-conducting surfaces with gold or carbon rules out further processing of the surface. Moreover, although SEM can give spectacular images of the surface details due to the long depth of focus, the topography can not be quantified from such an image, unless one uses cross-sections – in which case only a line profile is obtained.

Contact diamond profilometry probably is the most commonly and conventionally applied technique for surface profile and roughness measurements [BOLLEN *et al.* 1997, REISNER *et al.* 1997, WHITEHEAD *et al.* 1999, JONIOT *et al.* 2000, KAKABOURA *et al.* 2007]. However, this kind of profilometry cannot penetrate to certain micro-irregularities, because of its stylus size, and the stylus tip may damage the enamel

surface by scratching the soft, eroded enamel [BARBOUR and REES 2004] or distort flexible materials such as impression materials and abrade hard surfaces such as dental stones [RODRIGUEZ *et al.* 2009]. Moreover, this technique cannot be used to depict the 3-D characteristics of a dental surface, and can only determine the 2-D surface roughness parameter (R_a) which is considered to be less realistic than the 3-D surface roughness parameter (S_a) [KAKABOURA *et al.* 2007].

The use of non-contact optical profilometer-based laser scanning techniques overcomes some of these disadvantages as they do not touch the surface of the sample and the diameter of the light spot is much smaller than that of a usual stylus tip. No specimen pretreatment is required, and thus non-contact optical profilometers may be used to evaluate the same sample longitudinally (i.e. before and after treatment). The 3D topographic map of teeth or materials surfaces can be recorded using the confocal principle [RODRIGUEZ *et al.* 2009], but it cannot display the surface in true colours, which is a clear limitation when trying to display the surface features before and after treatment [REN *et al.* 2009].

CLSM is also widely used in dental materials science. The major advantage of CLSM is that it does not require special specimen processing, as observations can be carried out under normal environmental conditions. High-resolution confocal microscopic images may be made from either the surface of a sample or from just beneath the surface. These images can be likened to optical tomograms, giving thin (> 0.35 µm) slices up to 200 µm below the surface of a transparent tissue. With microscopes running under normal conditions, the optical section thickness will be >1 µm, and the effective penetration into enamel and dentin will achieve a maximum of 100 µm. These features provide a great potential to examine resin-dentin interfaces [WATSON 1997]. Moreover, surface profiles and 3D images can be reconstructed from extended-focus computer images derived from multiple image planes; therefore, many useful measurements, such as average roughness (R_a) can be carried out over the complete area in view [RADFORD *et al.* 1997]. However, the resolution of CLSM does not allow submicron characterization of tooth structures [VAN MEERBEEK *et al.* 2000], which limits the applications of this technique when measuring surface submicron morphologies. In a previous study, the CLSM was used to obtain 3D images and roughness values of dental surfaces, but the standard deviations were several times larger than the measured values [AZZOPARDI *et al.* 2004].

Recently, atomic force microscopy (AFM) has been used for dental researches. AFM is capable of providing three-dimensional detailed topographical images of surface roughness at a nanometer resolution, thereby providing 3-D surface parameters (such as S_a). The most unique feature of AFM is the ability to carry out observations in liquid [WATARI 1999], when compared to the conventional microscopes. It does not require extensive and special specimen processing, such as chemical fixation, dehydration and drying, or conductive metal coating. Such characteristics render it suitable for the in situ observation of the etching process [WATARI 2005]. Moreover, compared to the 5 µm (most cases) diamond stylus of the 2-D profilometer, the AFM usually is equipped with a 0.01 µm SiN3 tip, thus permitting more precise tracings. However, even though AFM is very useful for probing nanometer features, it is difficult to measure precisely the topography and profile of thick and rough layers in the micrometer range due to its limited vertical range [PECHEVA *et al.* 2007]. Additionally, the dimensional scale for AFM is much smaller than for profilometry, and thus AFM can only provide complementary topographical information in some cases [ZHANG *et al.* 2000].

In the present investigation, a novel Focus Variation 3D scanning microscopy (InfiniteFocus G4 Microscopy; IFM, Alicona Imaging, Grambach/Graz, Austria) was first used for the longitudinal observation of the morphology (roughness and profile) changes on lesion surface before and after each implementation step of the infiltration technique. Since the small depth of focus of an optical system with vertical scanning and a high vertical resolution are combined, this technique is able to provide both true-colour information of 3D topography and the quantitative information for dimensional measurement, surface analysis and characterisation. Moreover, it does not require sample preparation and does not involve the stylus tip touching the sample, thus allowing longitudinal observations of enamel surface changes in real-time. Therefore, this technology has been considered to have great potential in dental studies involving qualitative and quantitative evaluation of surface topography.

3 Purposes

Remineralisation of initial (proximal) carious lesions seems hardly to be achievable in most cases. Invasive treatment (surgery) of the proximal enamel caries is often associated with a large loss of healthy enamel. The resin infiltration technique should be a promising micro-invasive approach to arrest initial caries lesions, thus at least postponing (if not avoiding) sacrifice of sound structures. The achievable penetration depths and capacity of arresting the progression of caries using different low-viscous materials, various pretreatments and suitable application times have been determined by many *in vitro* studies. A dedicated resin (Icon caries infiltrant, DMG) with low viscosity and high penetration coefficient (PCs) has been developed and is commercially available on the market since March 2009. However, no study has hitherto focused on the morphology (roughness and profile) of the infiltrated lesion surface or on enhancing the quality of infiltrated lesion surface, which should be vital for a good long-term prognosis of the treated teeth, since the remaining roughness facilitates plaque accumulation and thereby promotes demineralization or the development of the secondary carious lesions. Therefore, the present study was designed to observe the changes in lesion surface morphology (roughness and profile) before and after each implementation step of the infiltration technique both in qualitative and quantitative terms. For this study, the novel Focus Variation 3D scanning microscope (InfiniteFocus G4 Microscopy; IFM, Alicona Imaging, Grambach/Graz, Austria) for surface analysis was used. The major points of interest of this study were:

1. To evaluate the effects of two different finishing ways (polishing with abrasive strips after light curing, and non-polishing but removing excess materials by using a rubber cup before light curing) on the surface morphology (roughness and profile) of infiltrated lesion.
2. To investigate the potential effects of different materials on the surface quality of infiltrated lesion.

The following null hypotheses were set up:

1. The two different finishing ways have similar effects on the roughness of infiltrated lesion surface.
2. The four different materials have similar effects on the surface quality of infiltrated lesion.

These null hypotheses were tested against the alternative hypothesis of a difference.

4 Materials and Methods

4.1 Specimen Preparation

A total of 40 bovine teeth were routinely cleaned under running tap water using a scalpel (Aesculap, Tuttlingen, Germany) to remove plaque, blood and soft tissues. The cleaned teeth were then either used immediately or stored in 0.9% sodium chloride solution at room temperature until required.

From the 40 bovine incisors, 80 enamel specimens (approximately 5×4×4 mm) were prepared (Band Saw Exakt 300cl; Exakt Apparatebau, Norderstedt, Germany). After embedding into epoxy resin (Technovit 4004; Heraeus Kulzer, Hanau, Germany), enamel surfaces were ground flat and polished (Polishing Machine Exakt 400cs; Abrasive Paper 1200, 2500, 4000; Exakt Apparatebau), thereby removing some 150 µm of the outer enamel. An acid resistant varnish (Dupli-Color; Kurt Vogelsang, Hassmersheim, Germany) was applied on the left part (A, control; Fig.1) of the enamel surface of every specimen to protect sound enamel. The other part of the surface was not covered by the varnish, and was left unprotected (B; Fig.1).

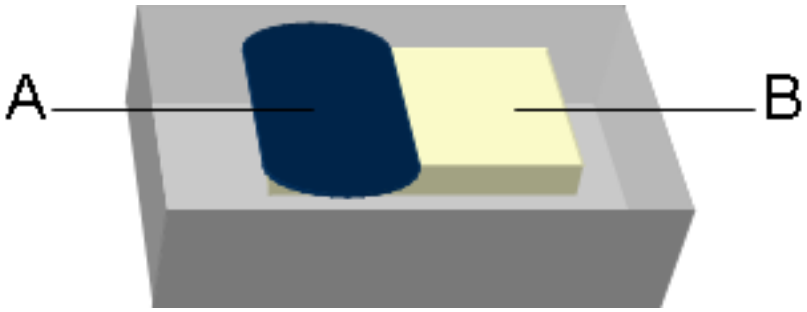

Fig. 1: Experimental set-up: sound enamel area A (control; covered by varnish), and unprotected part B (effect) of specimen surface

To create artificial caries-like (subsurface) lesions in the unprotected areas, specimens were stored in a demineralising solution (Table 1) [BUSKES *et al.* 1985] for 28 days (pH 4.95; 37 °C). The pH value was checked daily, and, if necessary, corrected with small portions of acetic acid or potassium hydroxide solution.

4.2 Experimental Setup

4.2.1 Etching

After the specimens were demineralised, the varnish on area A was removed carefully (scalpel no.15; Aesculap, Tuttlingen, Germany). The artificial lesion (part B) on each specimen was etched with 20% phosphoric acid gel (5 s; Gluma Etch 20 Gel; Heraeus Kulzer, Hanau, Germany). Then, etching gel was thoroughly washed by

means of an air/water spray (30 s), and the surface was dried with oil-free compressed air (30 s).

Table 1: Contents of the demineralising solution [BUSKES *et al.* 1985]

Concentration	Composition	Quantity
3 mM	$CaCl_2 \cdot \times 2H_2O$	2205.00 mg
3 mM	KH_2PO_4	2040.00 mg
50 mM	CH_3COOH	15.16 g
10 M	(KOH added to pH=5)	~47 ml
6 µM	$(MHDP)CH_2$	5.28 mg
	thymol	traces
	H_2O	5 litre

4.2.2 Resin Materials Application

The specimens were randomly divided into eight groups (n = 10 specimens/group). Four different resinous materials were applied onto the unprotected enamel part (B) of the specimens (Fig. 2, Table 2). Two groups were created with each material

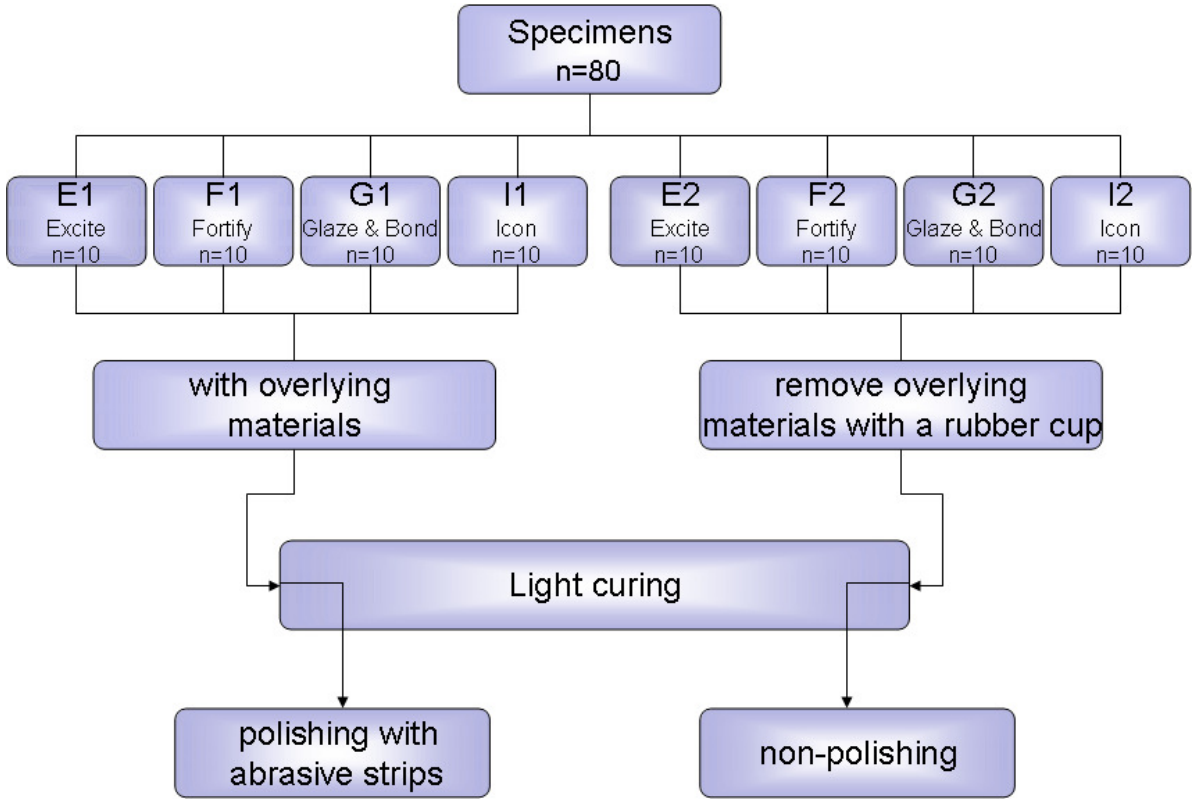

Fig. 2: Group assignment and overview of the experimental procedures

(Excite - E1/E2; Fortify - F1/F2; Glaze & Bond - G1/G2; Icon - I1/I2). All products were carefully applied (in excess) using a micro-brush (3M ESPE, Seefeld, Germany) for an application time of 30 s without rubbing.

Subsequently, the resin materials were light-cured (3 mm distance from the light-source; 60 s, 800 mW/cm^2) directly with overlying material on the surfaces (groups E1, F1, G1, I1). In the other groups (E2, F2, G2, I2), excess material was removed by hand with a rubber cup (Brasseler, Lemgo, Germany) before light curing (3 mm, 60 s, 800 mW/cm^2).

Tab. 2: Tested materials (information as given by the manufacturers)

Material	Manufacturer	Composition	Batch No.
Excite (light-cured bonding agent)	Ivoclar Vivadent, Schaan, Liechtenstein	Phosphoric acid acrylate, HEMA, BisGMA, Alcohol, Di-methacrylates, Silicon dioxide, Initiators, Stabilizers	L53322
Fortify (light-cured composite surface sealant)	Bisco, Schaumberg, USA.	UDMA, ethoxylated bisphenol A, Di-methacrylate	0900001125
Glaze & Bond (light-cured varnish)	DMG, Hamburg, Germany	Multifunctional acrylates, methyl methacrylates, catalysts, stabilizers, additives	627235
Icon (light-cured resin for caries lesion infiltration)	DMG, Hamburg, Germany	Methacrylate-based resin matrix, initiators, additives	621424

4.2.3 Polishing Procedure

After light curing, the treated surfaces of groups E1, F1, G1, I1 were polished (Sof-Lex Finishing and Polishing Strips system; coarse/medium, fine/superfine, 3M ESPE, Seefeld, Germany) using a modified polishing device (Fig. 3). To simulate the clinical situation and to standardise the polishing procedure, a force of 50 N (derived by some pre-tests) was provided by a metal weight on the top of the device. The polishing strip with the functional side at the top was fixed on a metal platform which could be moved in the direction of the y-axis by a push-pull manoeuvre performed manually. The resin block containing the specimen was fixed under the metal cylinder which connected the metal weight on the top and the specimen at the bottom. The surface of the specimen was in touch with the polishing strip at the corresponding site. The push-pull manoeuvre was performed 15 times in 15 s for coarse, medium, fine, and superfine procedures, respectively, thus amounting to a total of 60 strokes and 60 s

for each specimens of group E1, F1, G1, and I1. All samples were polished by one operator, and the polishing strips were renewed for every specimen. The polished surfaces were washed and dried by air/water spray (30 s).

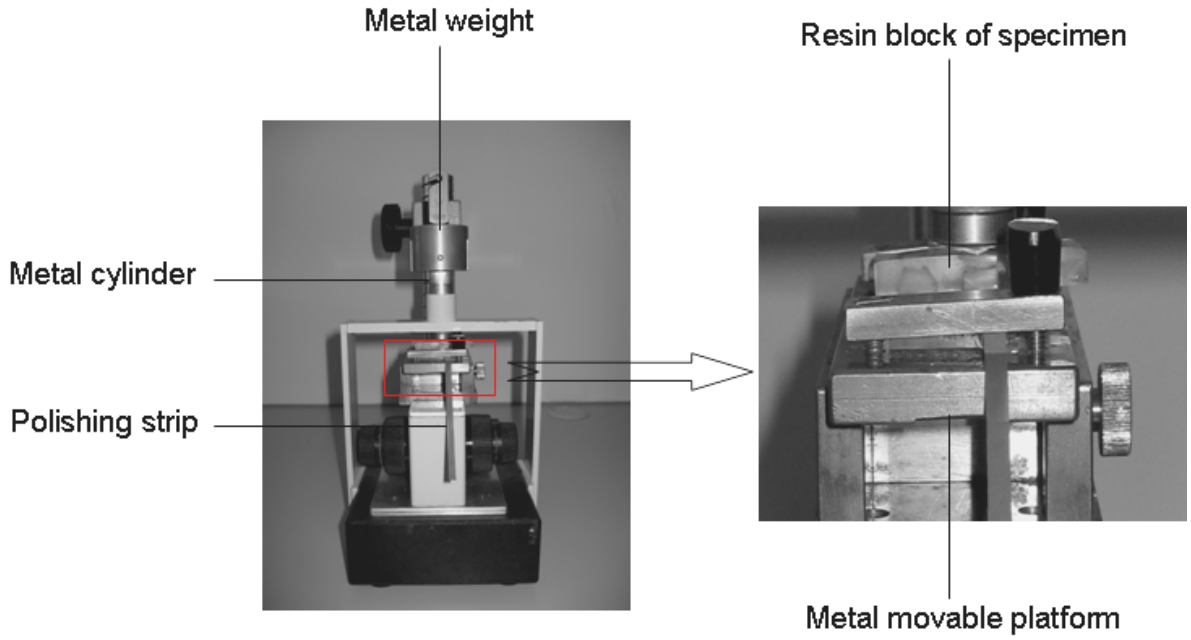

Fig. 3: Standardised polishing device

4.2.4 Measurements

The 3D topography images of the specimens' surface were captured by a novel Focus Variation 3D scanning microscope (IFM; Alicona Imaging, Grambach/Graz, Austria), based on a variation of focus procedure, which combines the functions of metrology and microscopy in a single optical instrument; IFM G4 software (Alicona Imaging) was used for analysis. The specimen to be measured was placed onto the motorized stage (with part A on left and part B on right) and was illuminated with modulated white light. The coaxial white light was provided by a light source delivered through a beam splitter. The specimens' reflected light was projected through the beam splitter onto a colour digital sensor. As the distance between the object and objective was varied, images were continuously captured. Each position in depth was differently imaged depending on the 3D structure of the specimen. Both the topographic and colour information were registered to the 3D data file, and then reconstructed by the software. Consequently, the 3D measurements, such as the average

roughness (S_a), the differences of height (DH) between surface B and reference surface A, and the marginal angles of overlying materials, could be performed directly.

A magnification of 50 and a vertical resolution of 20 nm were used to capture the 3D images for S_a measurements. Three different 283 µm × 200 µm-size rectangle areas with no visual defects were chosen and measured for each sound enamel surface A. The mean S_a value of the three measurements was calculated and recorded for each specimen. A small round cavity which had been prepared by using a diamond bur with a 0.5 mm diameter (Brasseler, Lemgo, Germany) in the central region of the surface B for each specimen served as a reference point. One size of 283 µm × 200 µm rectangular area (located just below the reference mark and kept directly in touch with the lower edge of the cavity at the midpoint of its upper borderline) was selected and measured for each area B (Figs. 4a, 4b). The area roughness analysis mode was used for S_a measurement, and a filter (λ_c = 50 µm) was set to separate waviness and roughness from the overall form.

A magnification of 10 and a vertical resolution of 200 nm were used to capture the 3D images for profile measurements. One size of 1429 µm × 1088 µm rectangular area, which was located at the central region of the specimens (with the borderline in the middle of the visual field) was captured for each specimen.

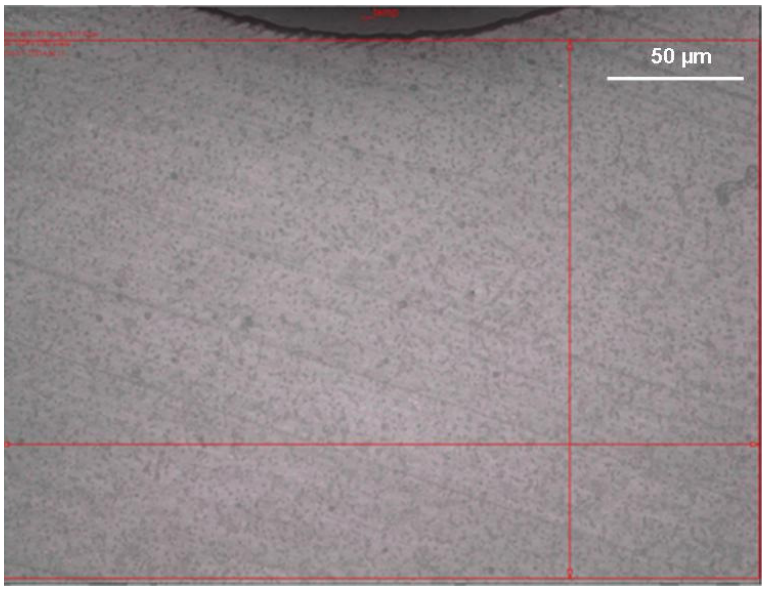

Fig. 4a: One size of 283 µm × 200 µm rectangular area of surface B (located just below the reference mark and kept directly in touch with the lower edge of the cavity at the midpoint of its upper borderline) was selected.

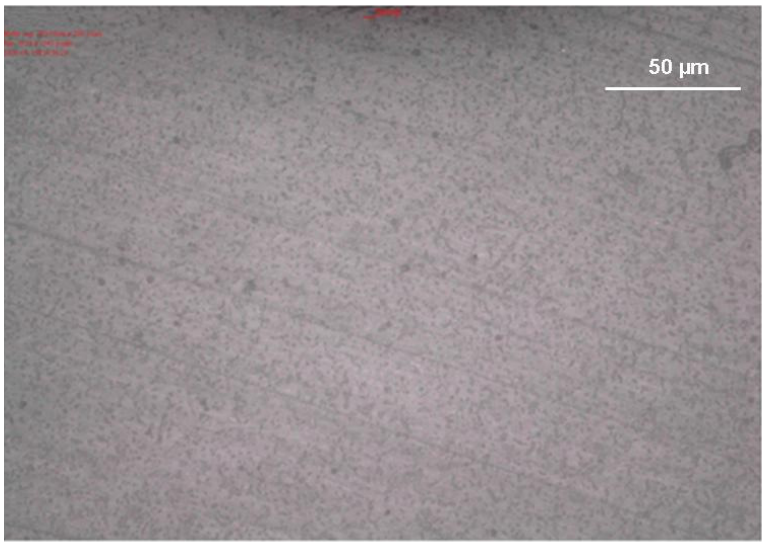

Fig. 4b: The selected region was captured.

A transverse line (100 points width, minimal 300 µm length) crossing the reference surface A and ending at the borderline was manually drawn for defining the profile path. Thus, the corresponding profile line of reference surface was obtained. The average height (*z*) of the selected region on reference surface A was adjusted at the zero level using the function of Adjust Reference Plane program in the software (error less than 0.05 µm; Fig. 5a).

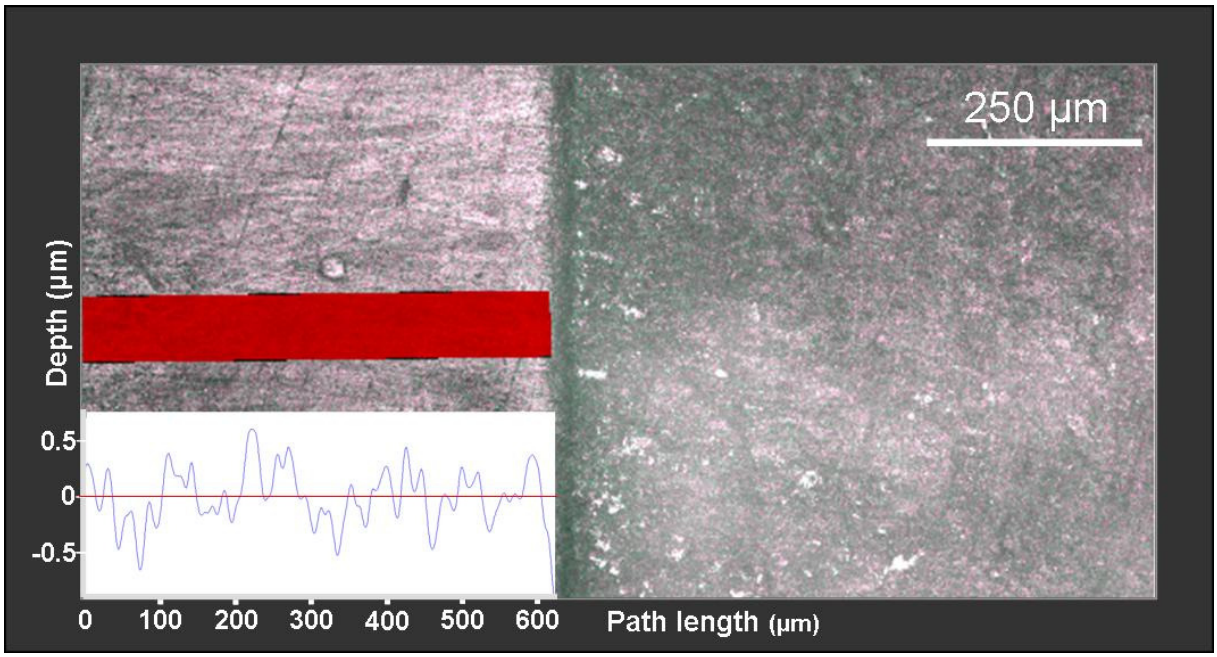

Fig. 5a: A dimension line located on the reference surface was drawn and the corresponding profile line was obtained (see window). The average height (*z*) of the selected region on reference surface A was adjusted close to the zero level.

Then, the profile path was extended to the end of surface B (minimal 1 mm length), and the corresponding profile line covering surface A and B was obtained (Fig. 5b). The starting point of the relatively flat part of surface B could then be defined from the profile line in order to exclude the area near the borderline where the profile was abnormally deep from the analysis (Fig. 5b).

Thus, the profile path only traversing the relatively flat part of surface B was determined and drawn from the starting point which had been decided previously to the end of the surface B (300 µm length), and the average height (z) of the selected region on surface B, which was provided quantitatively by the software in the parameters segment, was recorded as difference of height (DH; Fig. 5c).

The same means were used for all situations (polished surfaces) without abnormally deep profile areas near the borderline. Three measurements were performed successively at different locations with no visual defects, and the mean value of DH was calculated.

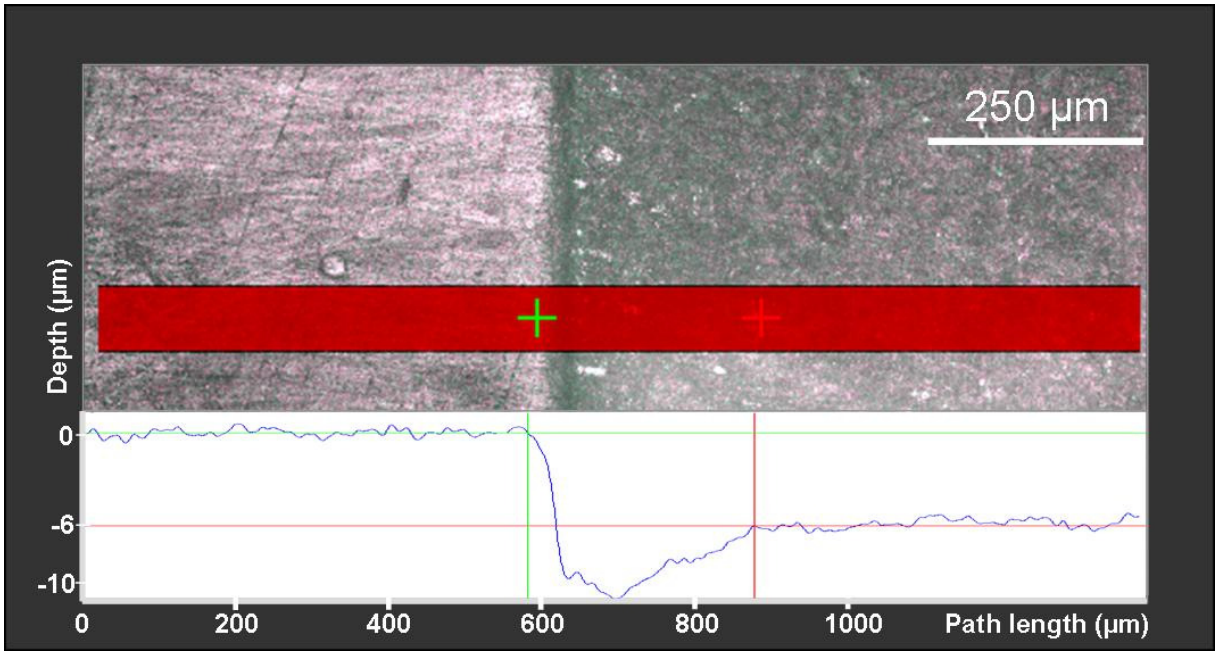

Fig. 5b: The profile path was extended to the end of surface B and the corresponding profile line was obtained. The starting point of the evenly flat part of surface B was decided from the profile line to exclude the abnormal deep region near the borderline.

For evaluation of thickness of overlying materials on the specimen surfaces of groups E1, F1, G1, and I1 after light curing and before the polishing procedures, one transverse profile path (1000 points width, 1 mm length) crossing the borderline was drawn for each specimen, and the corresponding profile line of the boundary region between A and B was obtained.

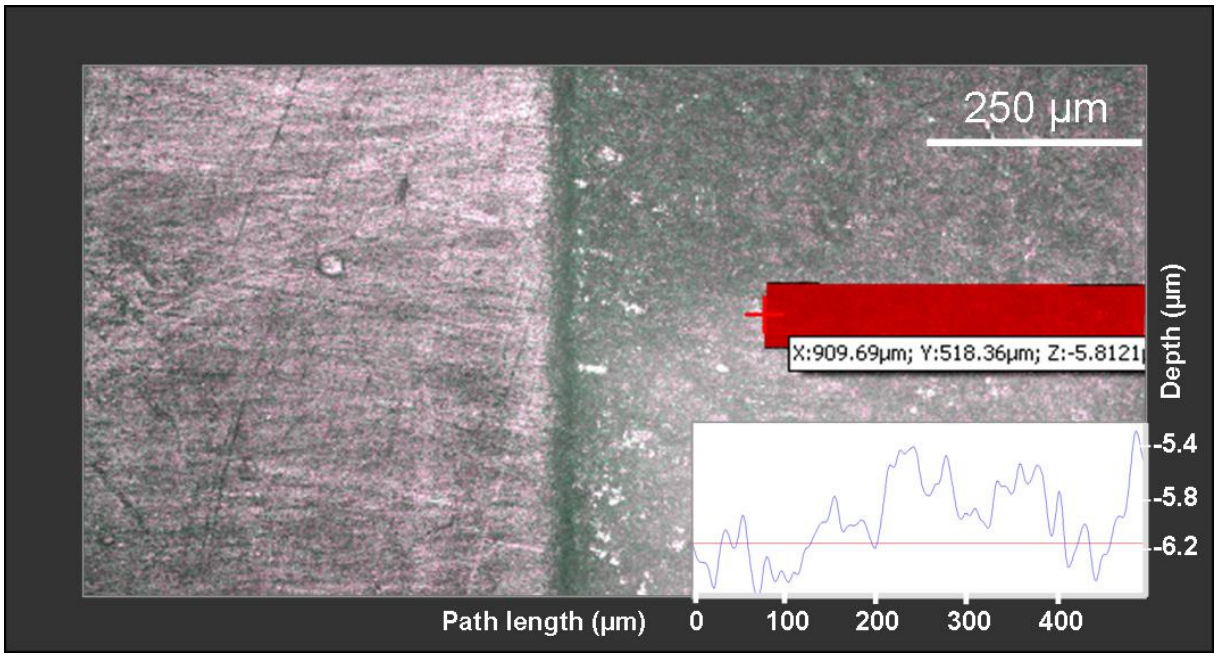

Fig. 5c: The dimension line only covering the evenly flat part of surface B was defined from the starting point to the end of the surface B and the corresponding profile line was obtained.

The differences of heights DH 1 (200 µm away from the borderline) and DH 2 (500 µm away from the borderline) were recorded. The average marginal angle of overlying materials was also measured and recorded at the same two points (Figs. 6a, 6b).

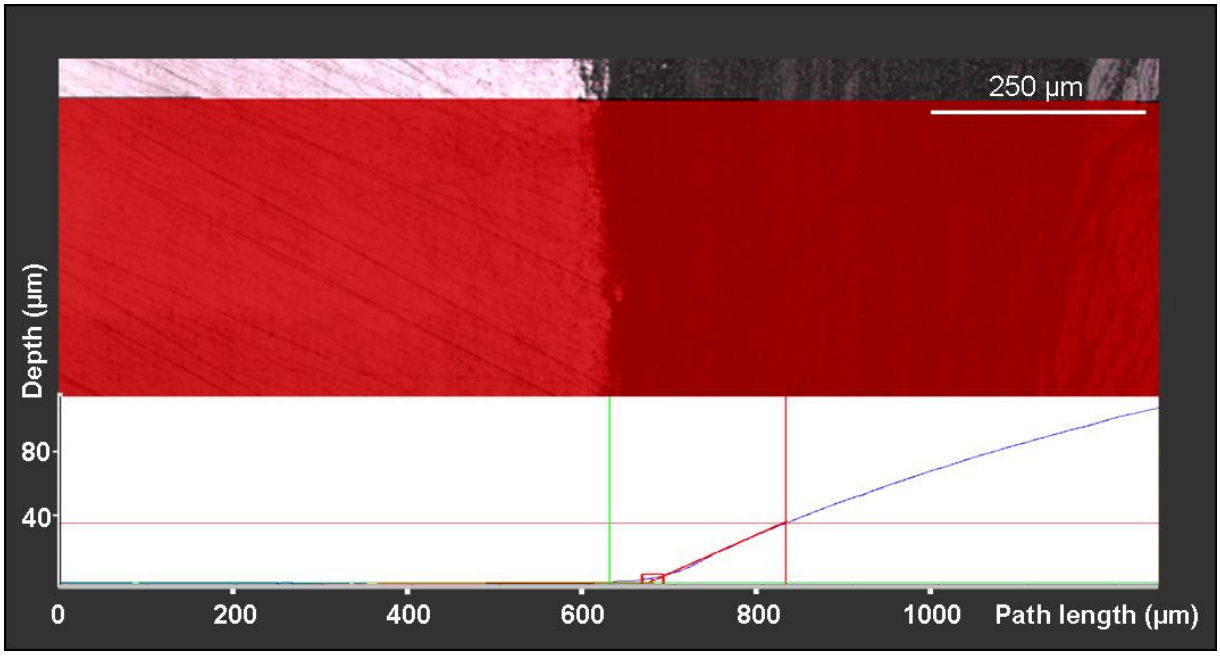

Fig. 6a: The dimension line (1000 points width) crossing the borderline was drawn and the corresponding profile line was obtained. The first difference of height (DH 1, 200 µm away from the borderline) and the marginal angle were measured and recorded.

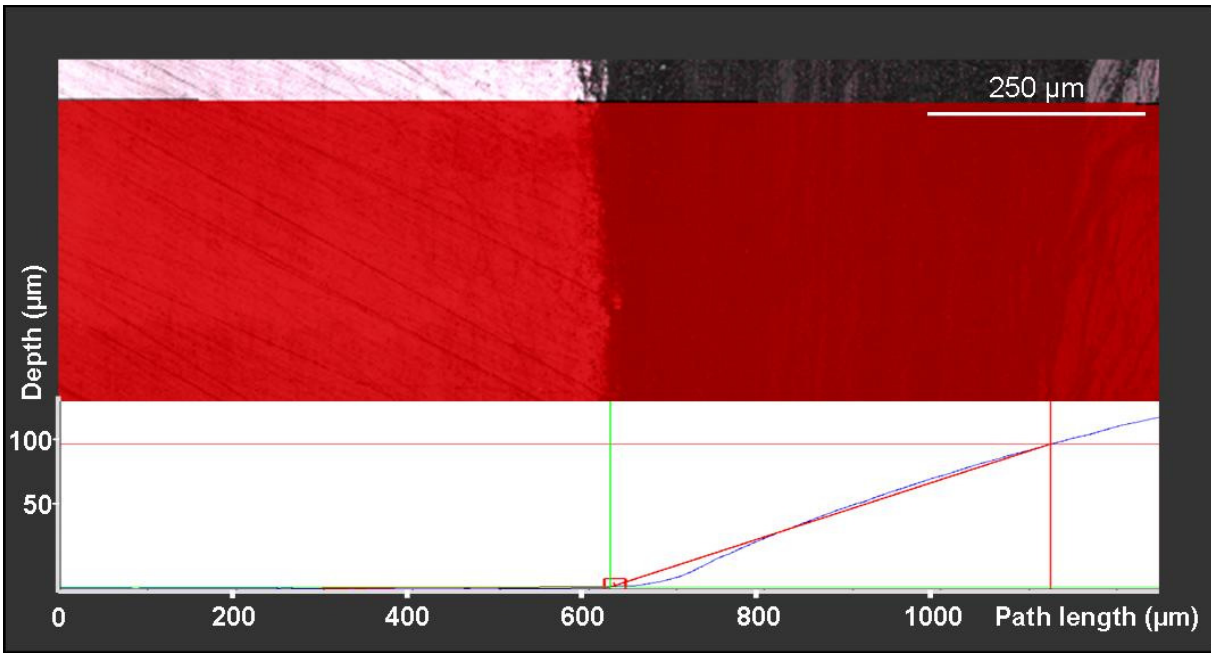

Fig. 6b: The dimension line (1000 points width) crossing the borderline was drawn and the corresponding profile line was obtained. The second difference of height (DH 2, 500 µm away from the borderline) and the marginal angle were measured and recorded.

In total, the S_a values were obtained from the surfaces of sound enamel (control), those of the artificial caries like lesions (subsurface lesions), the etched lesions, the infiltrated and polished lesions (excess material was removed after light curing with abrasive strips), and the infiltrated (but non-polished) lesions (excess material was removed with a rubber cup before curing). DH was measured for surfaces of artificial caries-like lesions, etched lesions, polished and non-polished infiltrated lesions. The marginal angle as well as DH 1 and DH 2 were measured for the surfaces of infiltrated lesions with overlying materials after light curing and before polishing (Fig. 7).

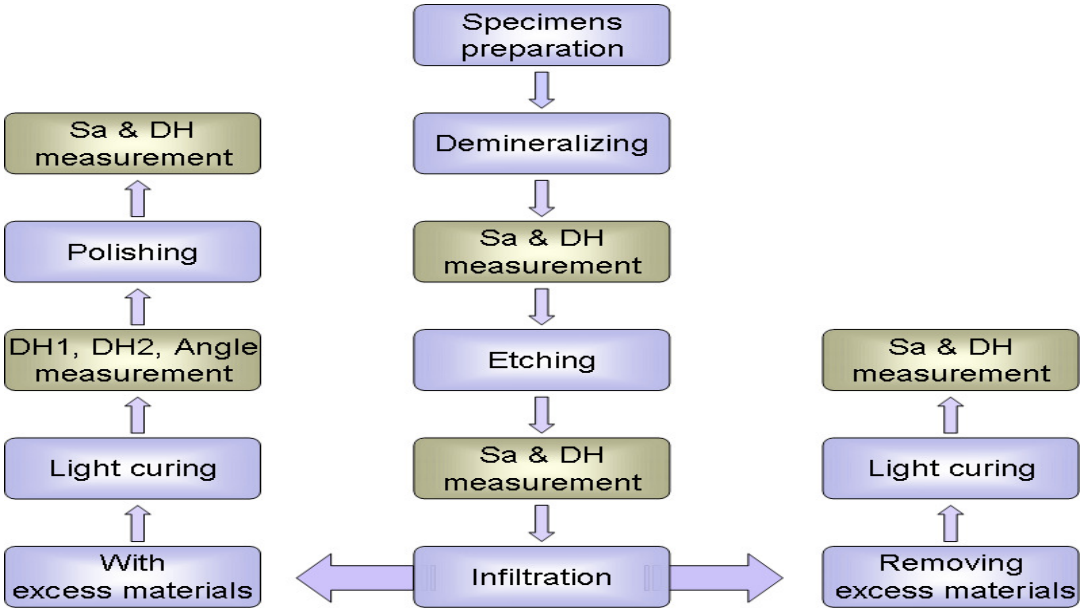

Fig. 7: Experimental setup

4.3 Statistical Analysis

Statistical analysis was performed with the Statistical Package for Social Science (SPSS 13.0; SPSS, Chicago, USA). The mean values of S_a, DH, and marginal angle of the different subgroups were statistically analyzed using one-way analysis of variance (ANOVA) and *post hoc* testing according to Tukey. In each study group, different surface conditions were compared by two-sided *t*-tests for paired samples. In dependence of the number of the groups, p values were adjusted according to Bonferroni. All tests were performed at a 5% level of significance.

5 Results

5.1 Average Roughness (S_a) Changes

After demineralisation, surface B showed a chalky white colour in all cases. The 3D topography images of surfaces at three different stages are shown in Figures 8a-c.

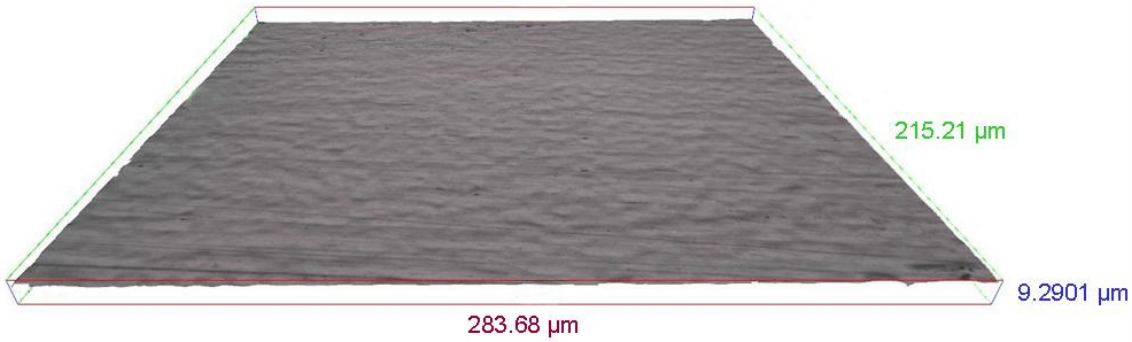

Fig. 8a: Representative micrograph of a polished sound enamel surface (area size 283µm × 215µm, 50× magnification)

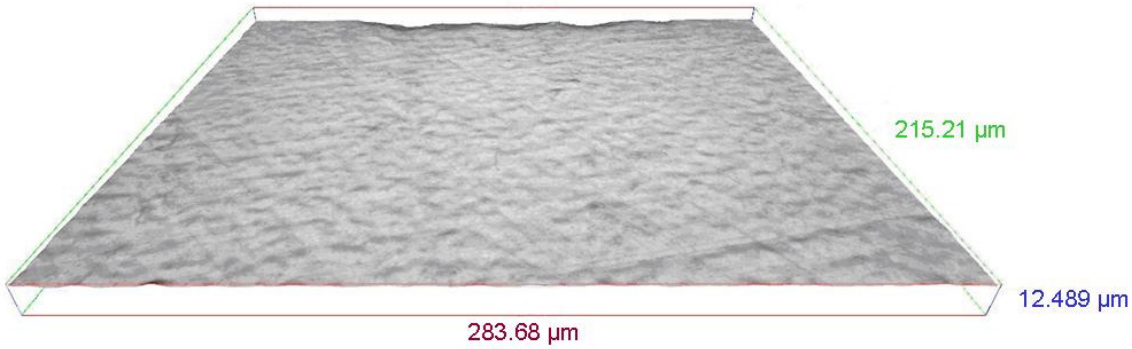

Fig. 8b: Representative micrograph of an artificial caries like subsurface lesion surface (area size 283µm × 215µm, 50× magnification)

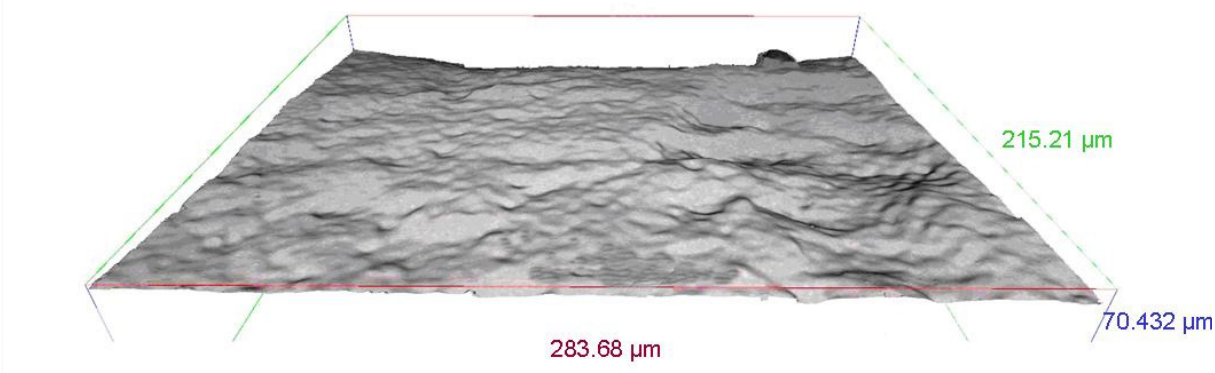

Fig. 8c: Representative micrograph of an etched artificial caries like (subsurface) lesion surface (area size 283µm × 215µm, 50× magnification)

The mean S_a of artificial caries-like (subsurface) lesion surfaces (65.8 ± 12.3 nm) was significantly higher in comparison with the sound enamel surfaces (59.3 ± 12.7 nm; mean difference 6.5 nm; $p<0.01$, *t*-test). Not surprisingly, the mean S_a of lesion surfaces increased significantly after etching (315.0 ± 144.3 nm; mean difference 249.2 nm; $p<0.001$, *t*-test). In groups E1, F1, G1, and I1, the mean S_a of the infiltrated and polished lesion surfaces (257.2 ± 126.0 nm) was significantly higher than that of sound enamel surfaces (55.7 ± 12.7 nm; mean difference 201.5 nm; $p<0.001$, *t*-test with Bonferroni correction). In groups E2, F2, G2, and I2, the S_a-values of infiltrated (but non-polished; the excess material was removed by a rubber cup before light curing) lesion surfaces (230.9 ± 138.3 nm) was also significantly higher than that of sound enamel surfaces (62.9 ± 11.7 nm; mean difference 168.0 nm; $p<0.001$, *t*-test with Bonferroni correction). The S_a-values of infiltrated lesion surfaces were in all cases (polished and non-polished specimens) significantly higher in comparison to demineralized enamel ($p<0.001$, *t*-test). Furthermore, no significant differences in S_a were found between the polished and the non-polished infiltrated lesion surfaces ($p>0.05$, *t*-test).

Figure 9 shows the mean S_a values of the reference areas and the differently treated enamel surfaces. Baseline S_a values between subgroups G1 (50.7 ± 13.6 nm) and I2 (71.1 ± 10.9 nm) as well as between I1 (52.2 ± 11.3 nm) and I2 (71.1 ± 11.2 nm) differed significantly ($p<0.01$ and $p<0.05$, resp.; ANOVA, Tukey's *post-hoc* test); no further differences could be found at baseline. For artificial caries-like lesions ($p>0.05$; ANOVA) or etched lesion surfaces ($p>0.05$, ANOVA), no significant differences in S_a were observed amongst the eight subgroups. S_a values for infiltrated and polished lesion surfaces were similar amongst the subgroups E1, F1, G1, and I1 ($p>0.05$, ANOVA). No significant differences in S_a were found for the infiltrated (but non-polished) lesion surfaces amongst the subgroups E2, F2, G2, and I2 ($p>0.05$, ANOVA).

S_a values between the polished infiltrated lesion surfaces and the etched lesion surfaces before resin infiltration were similar within the subgroups E1, F1, G1, and I1 ($p>0.05$, *t*-test with Bonferroni correction). S_a values of infiltrated (but non-polished) lesion surfaces also corresponded to the means of the etched lesion surfaces (before resin application) in subgroups E2, F2, and I2 ($p>0.05$, *t*-test with Bonferroni correction), while a significant reduction of S_a was observed in subgroup G2 after infiltration

(mean difference 166.9 ± 73.2 nm; p<0.001, *t*-test with Bonferroni correction). For review of all statistical comparisons, see Fig. 9.

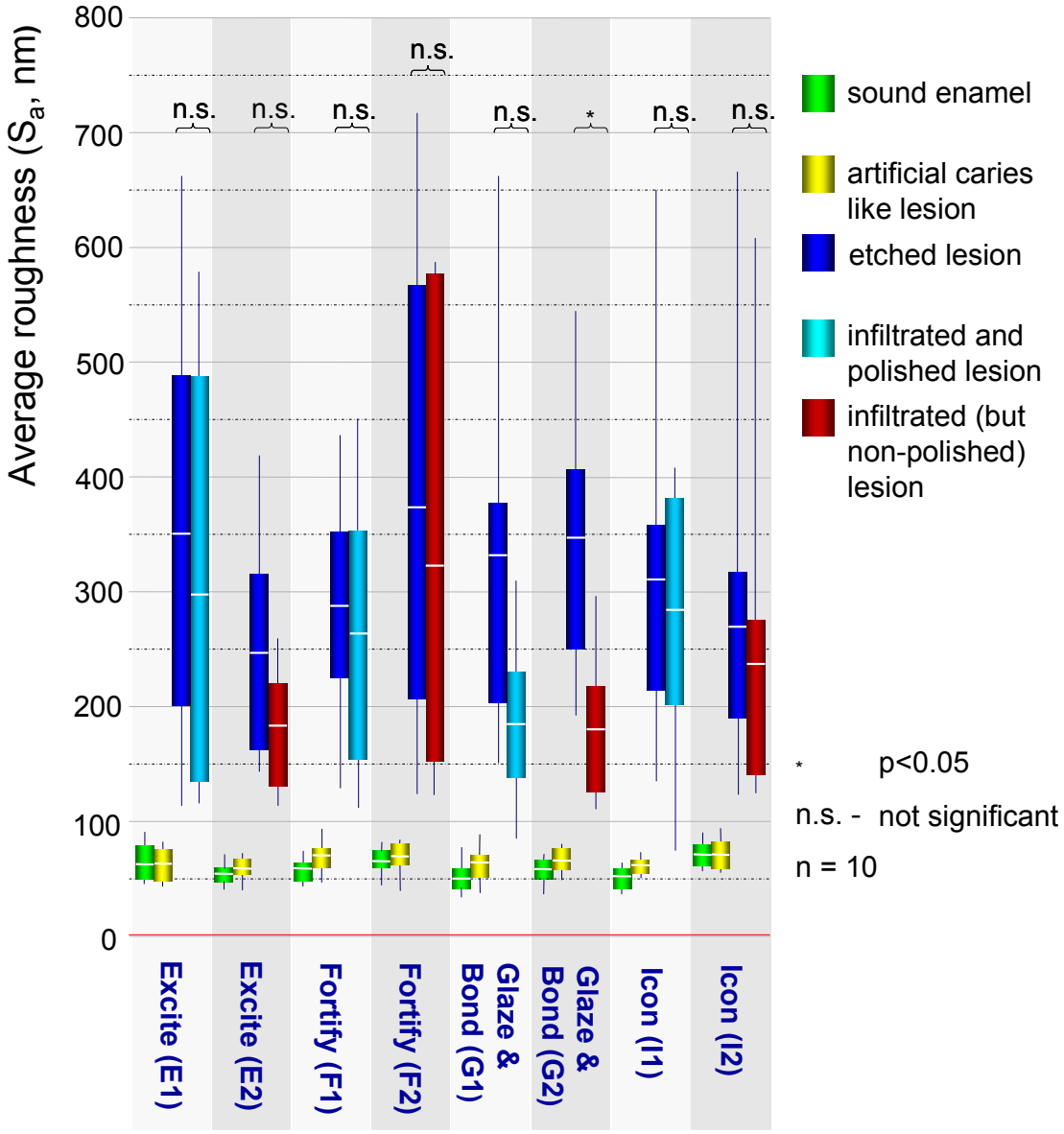

Fig. 9: Box-and-whisker-plot of average surface roughness (Sa) for sound enamel, artificial caries like (subsurface) lesion, etched lesion, infiltrated and polished lesion, and infiltrated (but non-polished) lesion for each group

Figures 10a-d show representative 3D topography images of infiltrated and polished lesion surfaces with the different resin materials.

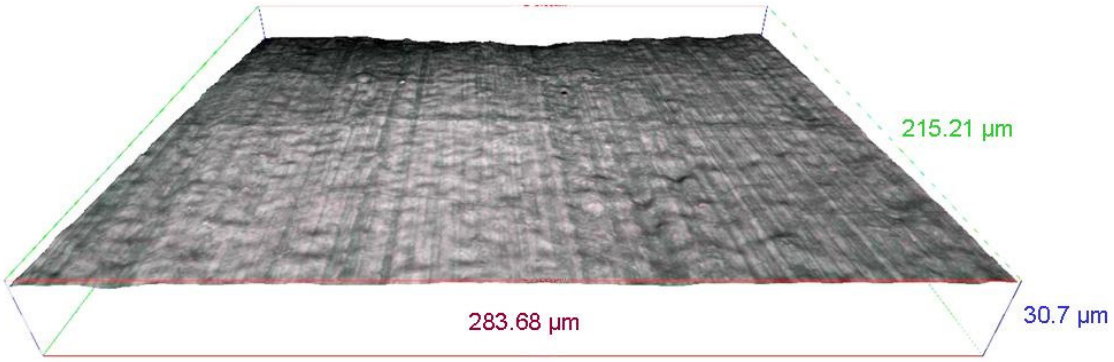

Fig. 10a: Representative micrograph of an infiltrated and polished lesion surface in group E1 (treated with Excite; size 283 µm × 215 µm, 50× magnification)

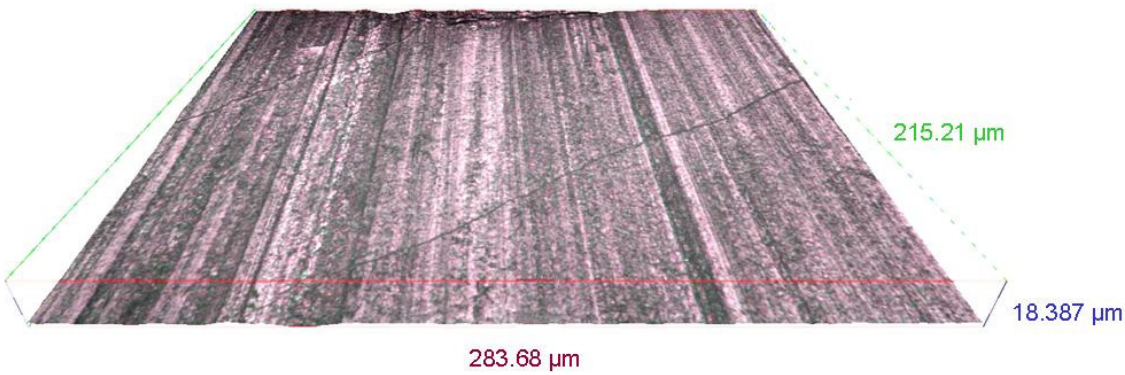

Fig. 10b: Representative micrograph of an infiltrated and polished lesion surface in group F1 (treated with Fortify; size 283 µm × 215 µm, 50× magnification)

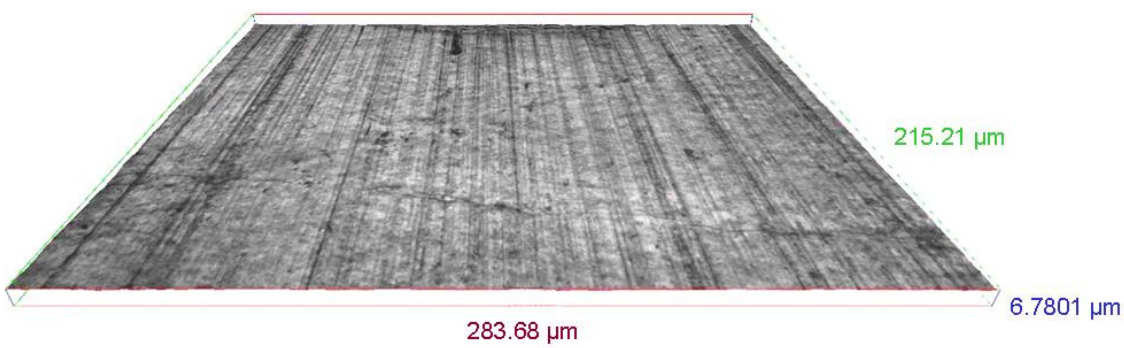

Fig. 10c: Representative micrograph of an infiltrated and polished lesion surface in group G1 (treated with Glaze & Bond; size 283 µm × 215 µm, 50× magnification)

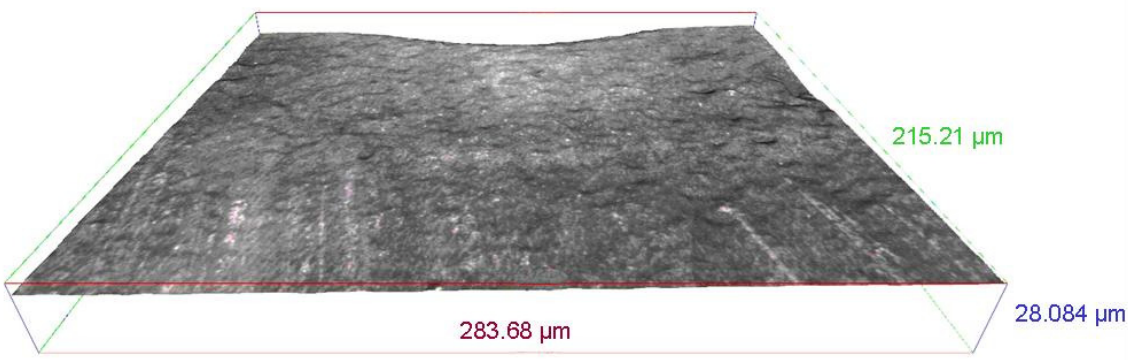

Fig. 10d: Representative micrograph of an infiltrated and polished lesion surface in group I1 (treated with Icon; size 283 µm × 215 µm, 50× magnification)

Figures 11a-d show representative 3D topography images of infiltrated (but non-polished) lesion surfaces with the different resin materials (the excess materials were

removed with a rubber cup before light curing). It was noted that small amounts of resin were left on the etched lesion surface in some cases.

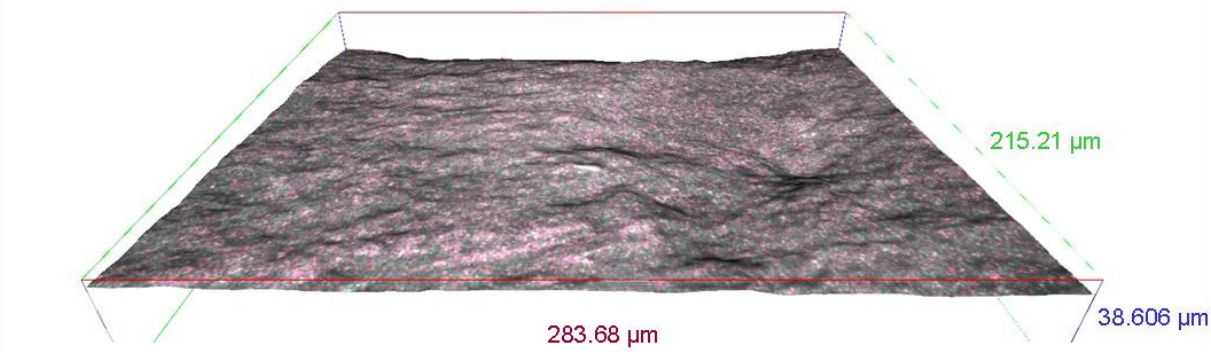

Fig. 11a: Representative micrograph of an infiltrated (but non-polished) lesion surface in group E2 (treated with Excite; size 283 µm × 215 µm, 50× magnification)

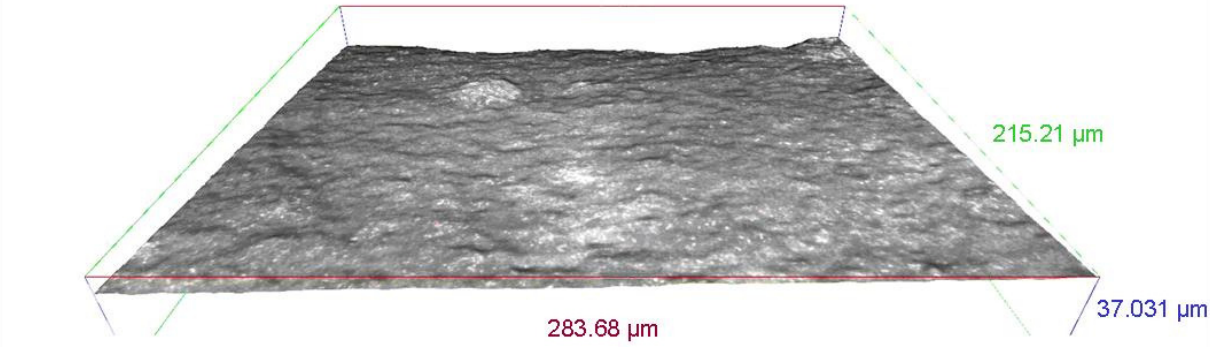

Fig. 11b: Representative micrograph of an infiltrated (but non-polished) lesion surface in group F2 (treated with Fortify; size 283 µm × 215 µm, 50× magnification)

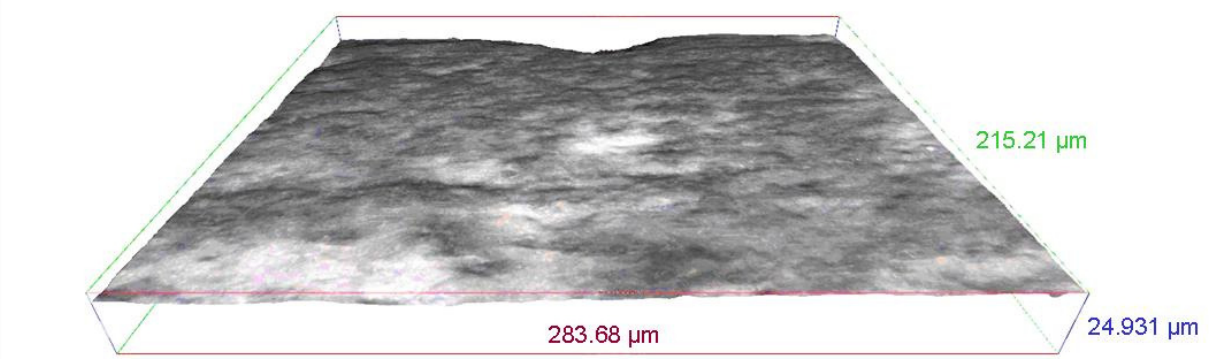

Fig. 11c: Representative micrograph of an infiltrated (but non-polished) lesion surface in group G2 (treated with Glaze & Bond; size 283 µm × 215 µm, 50× magnification)

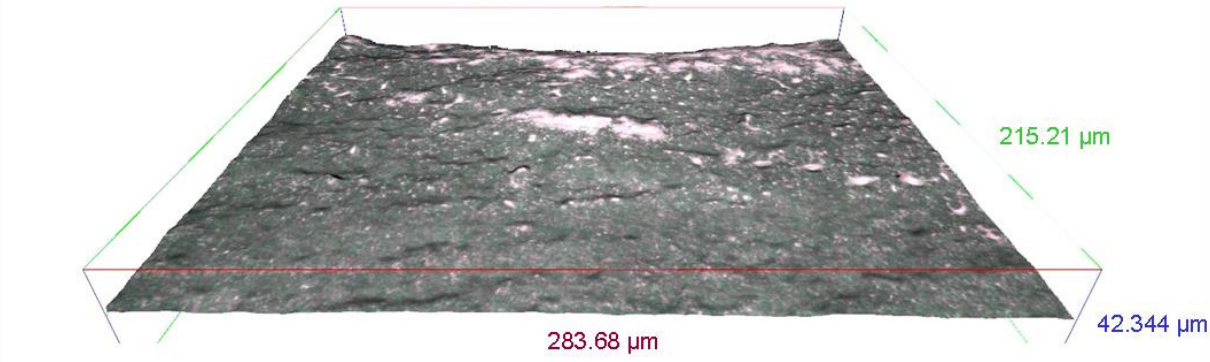

Fig. 11d: Representative micrograph of an infiltrated (but non-polished) lesion surface in group I2 (treated with Icon; size 283 µm × 215 µm, 50× magnification)

5.2 Surface Height Changes

Representative 3D topography images and corresponding profile lines of the boundary regions between reference surfaces A and treated surfaces B before and after each treatment steps are given in Figures 12a-e. The different colours on the 3D topography images represent the different depth levels. After demineralisation, an abnormally deep trench (width <300 µm) appeared on the surface B near the borderline, whereas the consecutively flat part of surface B showed almost a comparable height, similar like the reference surface A (here, the differences were negligible, see Fig. 12a).

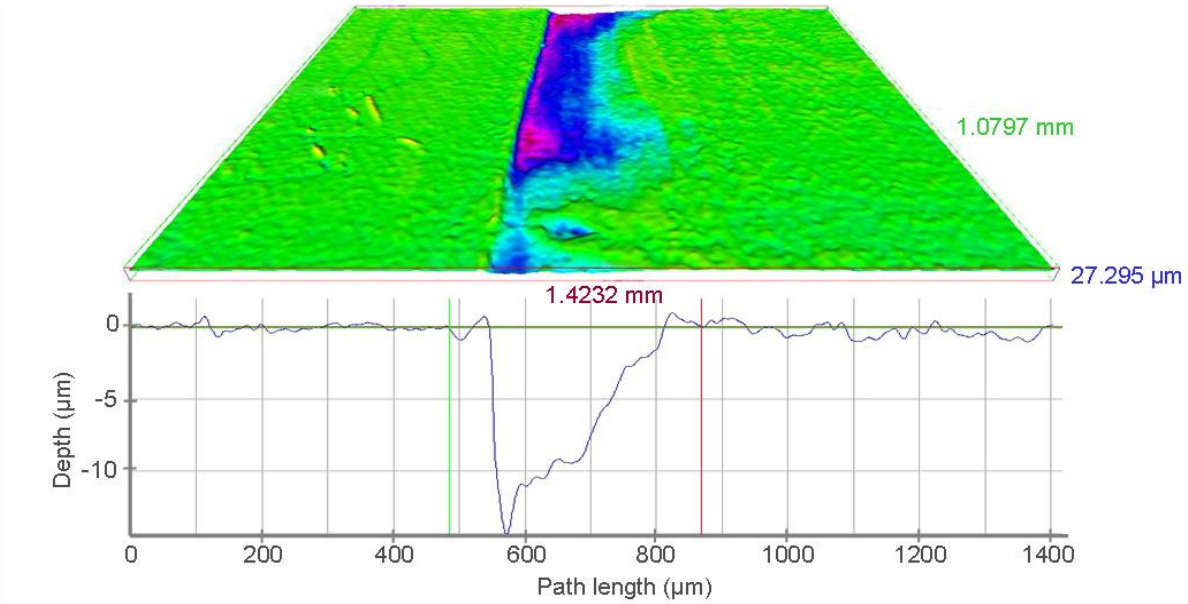

Fig. 12a: Representative 3D topography image (size 1429 µm × 1088 µm, magnification 10×) with the corresponding profile line of the boundary region between A and B after demineralization (an abnormally deep trench near the borderline is observably)

A clear surface height reduction resulted from etching on surface B, while the depth of trench also increased (see Fig. 12b).

Resin application on surface B resulted in some overlying material; however, an intact resin coat was left on lesion surfaces in most cases. Resin coats of Icon were observed to be extremely thin, and did not seem to be intact in some cases, but with a poor/non-polymerised layer on the surfaces. The profile lines of overlying materials showed more or less gently inclined curves (see Fig. 12c). Therefore, DH was measured at two different locations (DH 1, 200 µm away from the borderline and DH 2, 500 µm away from the borderline). The marginal angles were also measured at the same two locations.

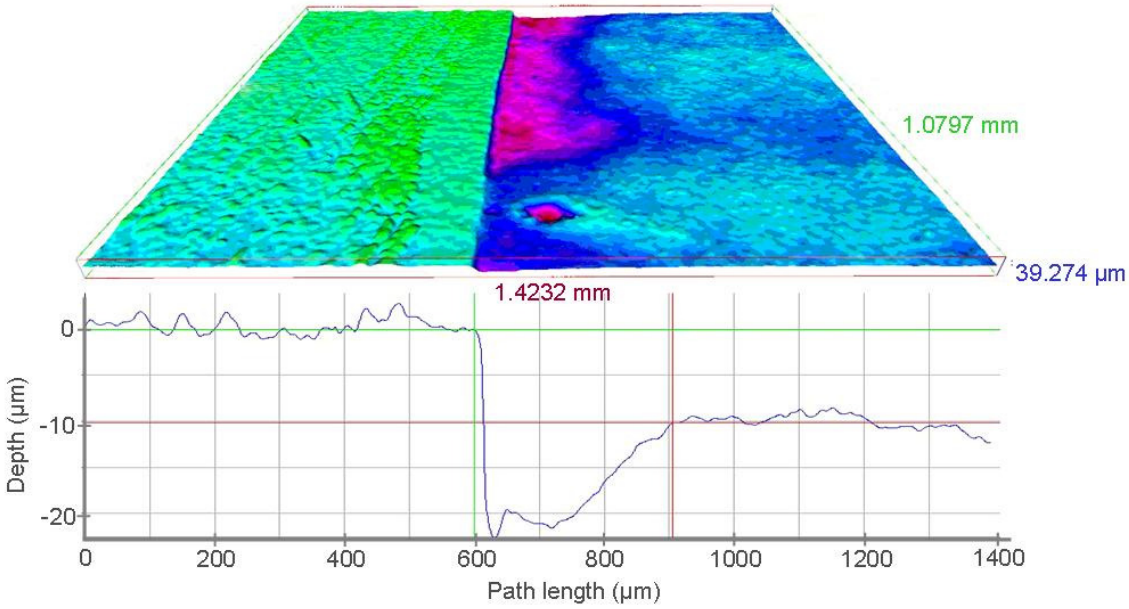

Fig. 12b: Representative 3D topography image (size 1429 µm × 1088 µm, magnification 10×) with the corresponding profile line of the boundary region between A and B after etching (a clear difference of height step with an abnormally deep trench close the borderline is observably)

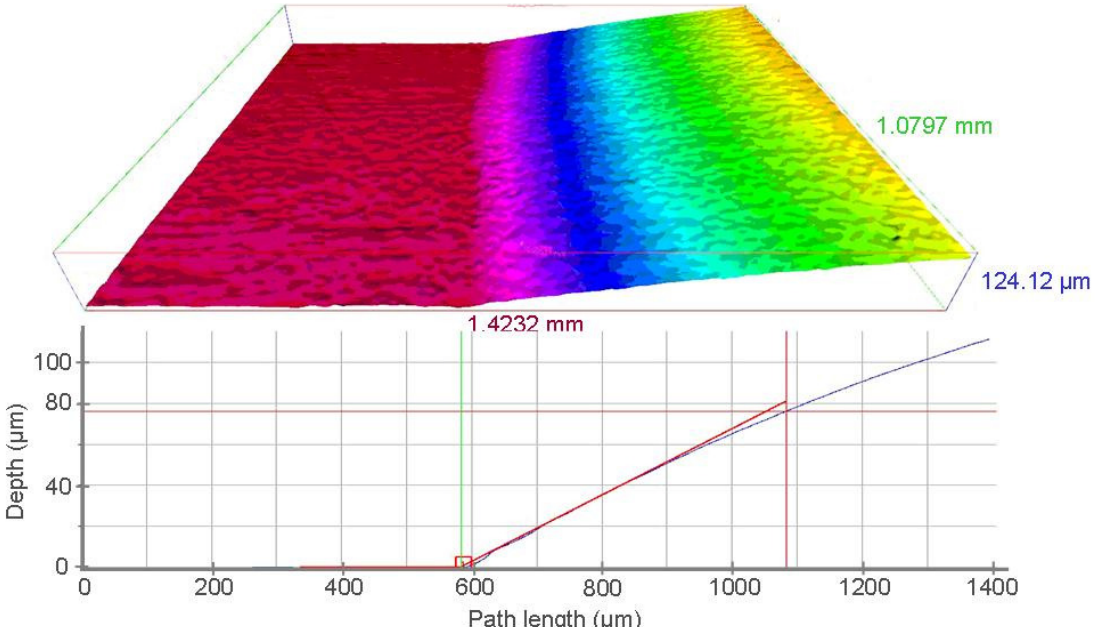

Fig. 12c: Representative 3D topography image (size 1429 µm × 1088 µm, magnification 10×) with the corresponding profile line of the boundary region between A and B after infiltration and light curing for groups E1, F1, G1 and I1 (a gradual upward curve within the range of the observation)

After polishing (groups E1, F1, G1, and I1), the overlying material was reduced by means of abrasive strips. The DH values between reference surfaces and polished surfaces presented either negative (in most cases, over-abrasion) or positive (some cases, some overlying material left), without the trench close to borderline (Fig. 12d).

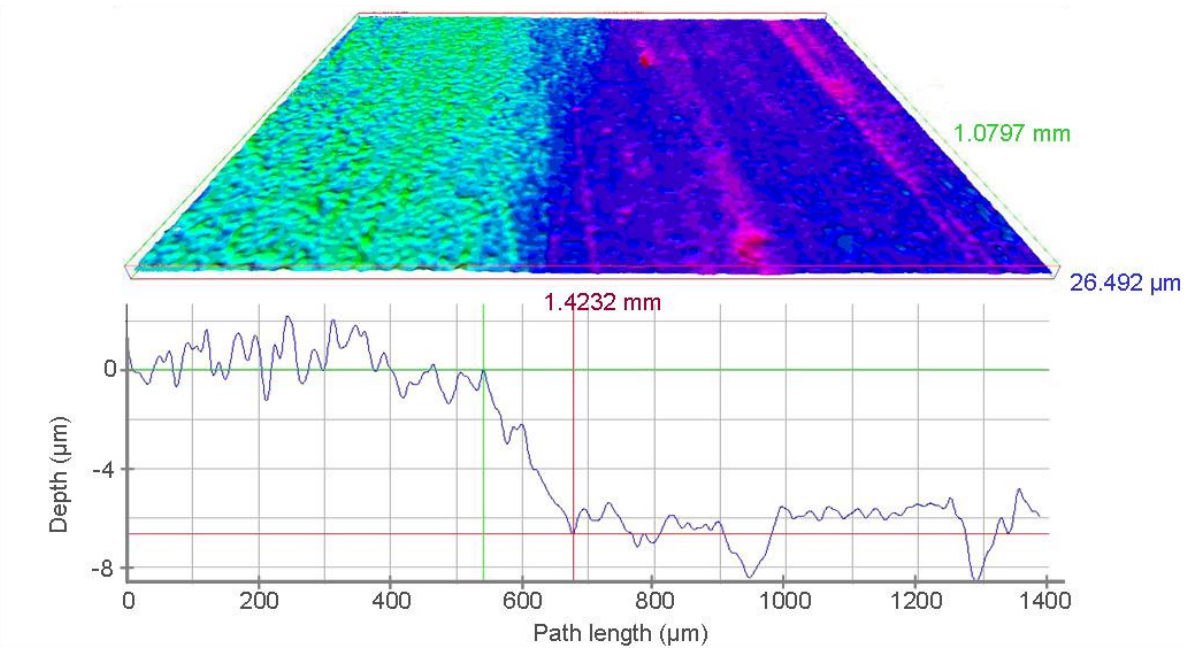

Fig. 12d: Representative 3D topography image (size 1429 µm × 1088 µm, magnification 10×) with the corresponding profile line of the boundary region between A and B after polishing for groups E1, F1, G1 and I1 (difference of height without the trench close the borderline)

In the associated group, the overlying material was removed with a rubber cup before light curing. The profile features/measurements were similar to the etched lesion surfaces before resin application, which could be characterized as a clear height reduction with a deep trench close to the borderline (see Fig. 12e).

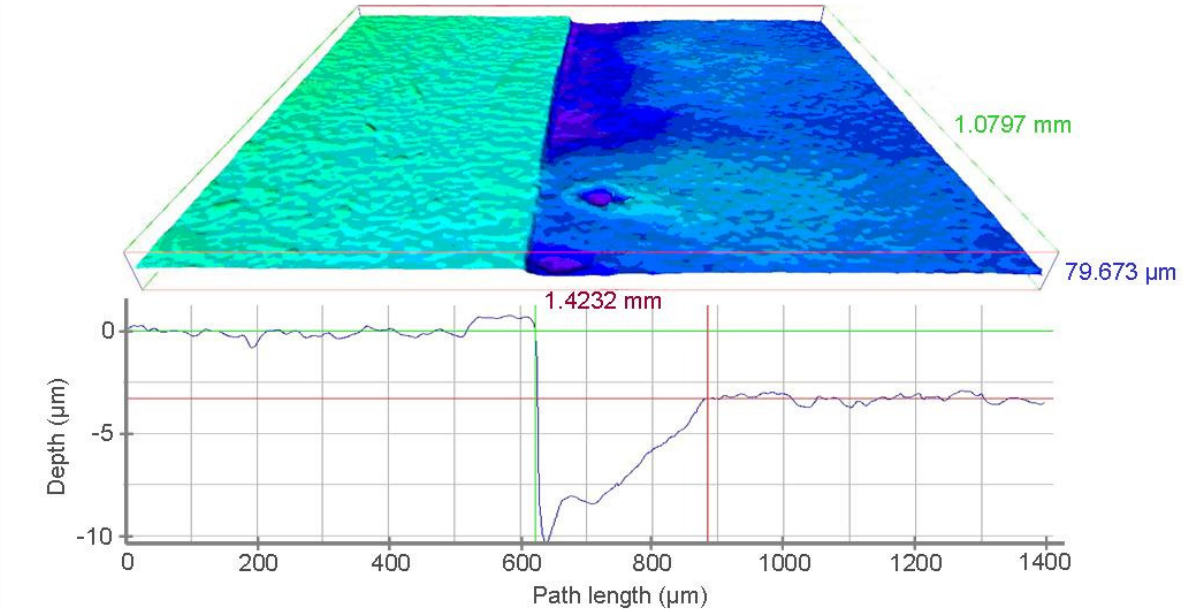

Fig. 12e: Representative 3D topography image (size 1429 µm × 1088 µm, magnification 10×) with the corresponding profile line of the boundary region between A and B after removing the excess materials and light curing for groups E2, F2, G2 and I2 (abnormally deep trench near the borderline still left after removing the excess materials)

Figure 13 presents the differences of heights in each group. The reduction in height of surface area B after etching was uniform (total mean 6.6 ± 2.0 µm), and no significant differences in DH could be found amongst the eight different groups at this stage ($p>0.05$, ANOVA). The mean DH value of infiltrated and polished lesion surface (40 specimens in sum of groups E1, F1, G1, and I1) did not significantly differ from the etched lesion surface (mean difference of DH 0.2 µm, $p>0.05$, *t*-test with Bonferroni correction). However, a (small but) significant increase in DH was noted on infiltrated (but non-polished) lesion surfaces (40 specimens in sum of groups E2, F2, G2 and I2), even though the excess material was removed with a rubber cup before light curing, compared to the etched lesion surface (mean difference of DH -1.0 µm, $p<0.001$, *t*-test with Bonferroni correction).

Furthermore, the thickest resin coats (highest DH 1 and DH 2 value) were found in the Excite group (E1; $p<0.001$, ANOVA, Tukey's *post-hoc* test) in comparison to the other groups. This was followed by the Fortify group (F1) and Glaze & Bond group (G1), with no significant differences between F1 and G1 ($p>0.05$, ANOVA, Tukey's *post-hoc* test). The lowest DH 1 means were obtained in the Icon group in comparison to the other groups (I1; $p<0.01$, ANOVA, Tukey's *post-hoc* test). The lowest DH 2 means were also found in the Icon-group (I1; here the values did not significantly differ from group G1; $p>0.05$, ANOVA, Tukey's *post-hoc* test).

The results of marginal angle measurements were consistent with the results of DH 1 and DH 2 measurements. The means of marginal angles (measuring positions at DH 1 and DH 2) of the Excite group (E1) were significantly higher in comparison with the other materials ($p<0.05$, ANOVA, Tukey's *post-hoc* test). This was again followed by the Fortify group (F1) and the Glaze & Bond group (G1) (no significant differences were found between F1 and G1; $p>0.05$, ANOVA, Tukey's *post-hoc* test). The means of marginal angle (in measuring positions DH 1) of Icon was significantly smaller in comparison with the other materials ($p<0.05$, ANOVA, Tukey's *post-hoc* test). In addition, no significant difference in the marginal angle (in measuring positions DH 2) between groups I1 and G1 was found ($p>0.05$, ANOVA, Tukey's *post-hoc* test). For more detailed information on results, please see Appendix (Chapter 11).

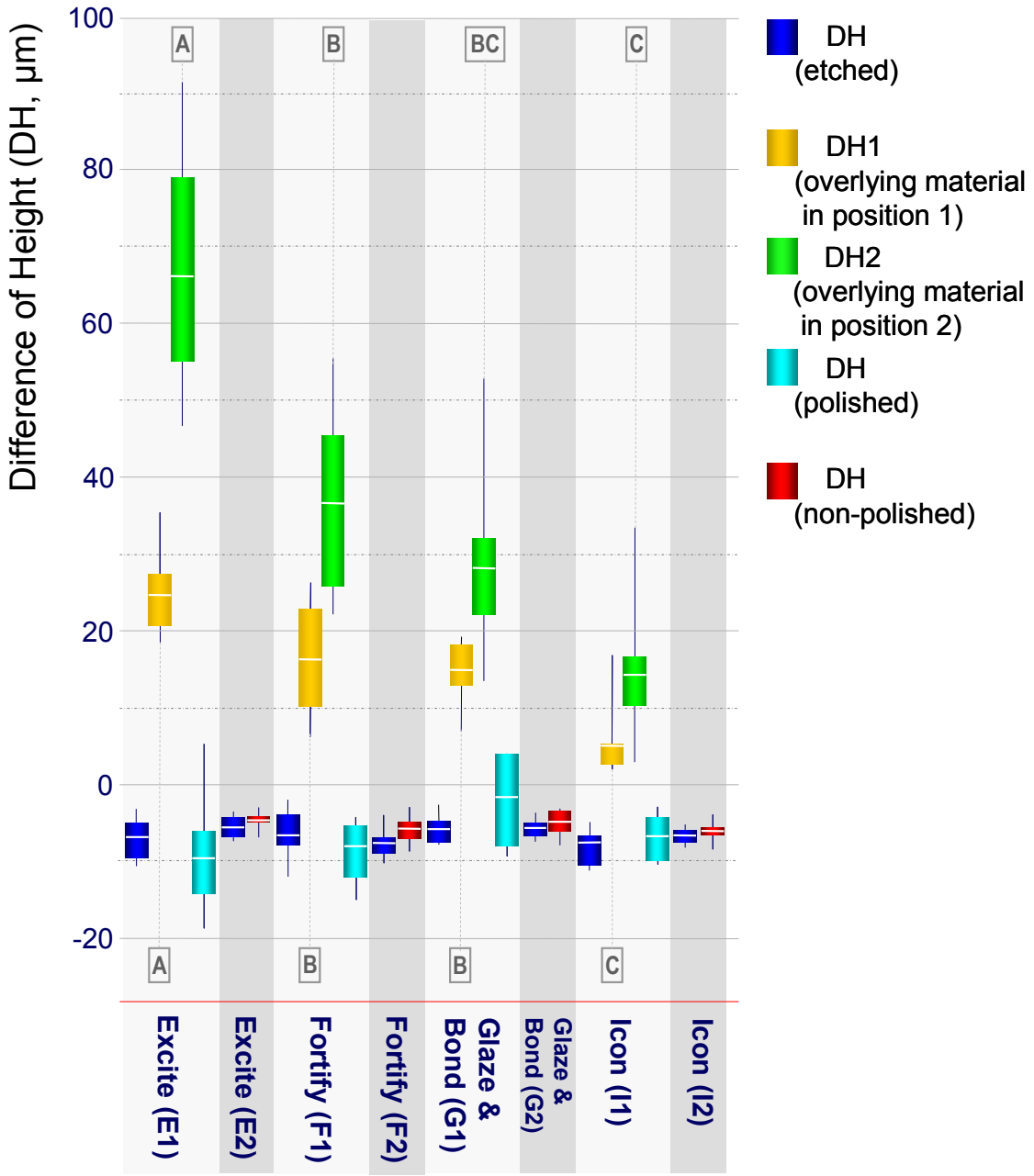

Fig. 13: Box-and-whisker-plot of average differences of height (DH) between surface B and reference surface A for artificial caries like lesions, etched lesions, infiltrated and polished lesions, and infiltrated (but non-polished) lesions; DH 1 (position 1, 200 µm away from the borderline) and DH 2 (position 2, 500 µm away from the borderline) for the surfaces of infiltrated lesions with overlying materials after light curing and before polishing. Groups with different letters are significantly different from each other ($p<0.05$, ANOVA, Tukey's *post-hoc* test).

6 Discussion

The infiltration technique of incipient (proximal) enamel lesions with resin materials of low-viscosity could be an alternative approach to the widely used treatment concepts of remineralisation or restorative treatment [KIELBASSA *et al.* 2009]. The penetration ability of different materials has been demonstrated and improved by several studies [ROBINSON *et al.* 2001, MEYER-LUECKEL *et al.* 2006, PARIS *et al.* 2007b, MEYER-LUECKEL and PARIS 2008a], along with an inhibitory effect of the infiltration technique on further lesion progress under demineralising conditions, which has been proved by several investigations [ROBINSON *et al.* 1976, ROBINSON *et al.* 2001, MUELLER *et al.* 2006]. Thus, this new treatment regimen should gain wide popularity within the concept of minimally invasive dentistry.

However, it has to be kept in mind that to ensure a good long-term prognosis of the treated teeth a high-quality treated surface should be an essential requirement. The term high-quality surface refers to a restoration surface with low roughness, anatomical contour and restoration without excess of material, which should prevent the formation of discolouring films, avoid plaque accumulation, reduce wear rate, and enhance fracture resistance [DE JAGER *et al.* 2000]. Previous publications have indicated that an intra-oral hard surface with an average roughness (R_a) of more than 0.2 µm is positively correlated with increased accumulation of supra- and subgingival plaque [QUIRYNEN *et al.* 1990, QUIRYNEN *et al.* 1993]. Therefore, the so called “threshold R_a” was suggested to be located around 0.2 µm [BOLLEN *et al.* 1996]. It would seem reasonable that these requirements should also hold true for the infiltrated lesion surface to prevent the occurrence of secondary caries, and to lower the caries risk for any adjacent surfaces of neighbouring teeth. However, no study has hitherto focused on the changes of lesion surface morphology resulting from the implementation of the infiltration technique.

6.1 Discussion of Materials and Methods

For the present study, freshly extracted bovine teeth were used. Many studies on demineralization of enamel have been conducted on bovine teeth, because the number of extracted caries-free human teeth is insufficient to meet the research needs [MELLBERG 1992] and the chemical structure of bovine enamel is quite similar to that of human enamel [DAVIDSON *et al.* 1973]. The teeth were either used immediately or

stored in 0.9% sodium chloride solution (saline) at room temperature until required. All cutting, grinding, and polishing operations on the enamel samples were run under constant water cooling. Between the various processing steps, the samples were kept in water to avoid errors caused by dehydration [HERKSTROTER *et al.* 1989].

The demineralising solution used in the present study has been shown to produce subsurface caries like lesions on bovine teeth in several previous studies [BUSKES *et al.* 1985, KIELBASSA *et al.* 2005, MUELLER *et al.* 2006]. Initial enamel caries (white spot lesion) is characterized by a subsurface demineralisation (with a pore volume of 25 percent or more) resulting from the action of acidic metabolites of bacteria in plaque with a highly mineralized surface (representing a pore volume of less than 5 percent) [SILVERSTONE 1968]. The so-called caries-like lesions hitherto created *in vitro* by many working groups have been defined as lesions with a subsurface demineralisation covered by a surface layer which appears relatively unaffected by the acid attack [SILVERSTONE 1968]. Diphosphonate (MHDP) was added as a surface dissolution inhibitor, which could result in the slow precipitation of a calcium MHDP phase or simply adsorption of MHDP to the crystals [WHITE and NANCOLLAS 1980]. Therefore, the surface changes caused by the MHDP-containing model system were confined to a general enlargement of the intercrystalline spaces over the entire exposed enamel surface. Thus, a relatively intact surface overlying a subsurface demineralization was created in the present study.

Moreover, four resin materials with different properties were applied on the etched lesions' surfaces in different groups. Excite (Ivoclar/Vivadent, Schaan, Liechtenstein) is a filled (nano-fillers), light-curing, single-component bonding agent for enamel and dentin bonding in conjunction with the total-etch technique, and it has developed an excellent clinical reputation in recent years (according to the manufacturer). Its penetration behaviour in enamel caries lesions has been proven to be the best among those of some common commercially available bonding agents [MUELLER *et al.* 2006]. Therefore, Excite was used in the present study to investigate the lesion surface roughness after infiltration by bonding agents.

Fortify (Bisco, Schaumberg, USA) is a light-cured, low viscosity, unfilled resin formulation which is intended for use as a surface sealant. This material is considered to be able to penetrate into the micro defects on the surface of composite restorations by capillary action and repair the damaged surface after polishing and finally enhance the smoothness and wear resistance of composite restorations (according to the

manufacturer). Thus, it was speculated to have good penetration abilities into the microporosities in caries lesions and considerably positive effects on the surface roughness of the infiltrated lesions. Therefore, this material was tested in the present study.

With Glaze & Bond (DMG, Hamburg, Germany), a light-cured one-component material was used pursuing a similar objective; this material provides a highly glossy surface, and has been shown to clinically reduce plaque accumulation (according to the manufacturer).

Icon (DMG, Hamburg, Germany) is a light-cured unfilled resin with low viscosity and a high penetration coefficient (PC) for micro-invasive treatment (infiltration) of incipient caries lesions. Up to now, Icon is the only professional material for infiltration treatment; thus, this commercially available infiltrant was included in the present study.

In the present investigation, a relatively novel technique, Focus Variation 3D scanning microscopy (InfiniteFocus microscopy; IFM), was first used for longitudinal observation of the changes of lesion surface morphology (roughness and profile) before and after each implementation step of the infiltration technique. This novel technique is referred to as “topomicroscopy”, and is considered to combine the functions of metrology and microscopy in a single optical instrument. Its operating principle combines the small depth of focus of an optical system with vertical scanning to provide topographical and colour information from the variation of focus. IFM can simultaneously capture the entire surface topographic information in combination with its true colour information. Both the topographic and colour information is registered to the 3D data file. Compared with other surface measurement techniques, there are several advantages to the IFM method used in present study. First, the use of IFM enabled longitudinal observations of the same sample surface at both baseline and post-treatment stages, because no specimen preparation is required for this technique implementation, which would not be possible by using SEM. Second, IFM has no stylus tip, which is always equipped with contact profilometers and involves touching the sample, thus avoiding any damage to the sample surface. Third, IFM can measure areas instead of single profile lines. The area analysis mode which was used in the present study for the roughness measurement could give more repeatable results than the linear sweep analysis mode of profilometry; thus, considerably more information (with more than 1,800,000 data points on an image with 283 μm × 200 μm size) could be obtained from the whole measurement area (if compared to a single measurement line). Therefore, like AFM, IFM could provide the 3-D surface roughness S_a from 3-D

measurement areas instead of 2-D surface roughness R_a from 2-D profiles. On the other hand, since the dimensional scale for IFM was larger than that for AFM, it seemed to be more suitable for the present study, in which the specimens were relatively large. Forth, IFM has a high vertical resolution (< 10 nm), which is much higher than that of CLSM, for quality assurance in the micro- and nano-range. Moreover, 3D measurements could be performed directly on the optical colour images. This visual link between the surface and its 3D information (including colour information) provided the possibility to make a precise determination of the area that was intended to be measured. Therefore, abnormal areas such as the deep trench close to the borderline of surface B, which might be due to a lower local acidity of the demineralization solution around the varnish surface or the stress that was generated at the junction of the two different surfaces, could be detected and then easily excluded from the analysis area in the present study. Even though the trench has a gradual transition into the flat parts of the enamel, it will cause a systemic error in the height difference (if it would have been included with the areas to be analyzed). Therefore, in the present study only the relatively flat parts of the profiles were chosen and analysed.

The surface roughness parameter most often used is R_a (average roughness) and is defined as the mean deviation of the profile from the centre line, where the centre line (sometimes called mean reference line) is derived from the profile by filtering out its short wavelength components. R parameters (according to EN ISO 4287) were originally developed for two-dimensional, stylus type profiling applications. Many of these statistics were later adapted for three-dimensional use as well for systems such as optical profilers which are capable of true 3D measurement. S parameters were defined in 1991 by the attendees of the first EC Workshop on 3D Surface Measurement and Characterization. These statistics are well defined for measuring 3D data arrays, such as those generated by IMF or AFM. The S parameters (according to EN ISO 25178) provide 3D equivalents to the standard 2D R parameters (S_a for R_a, Ssk for Rsk), as well as additional information relevant to 3D surfaces only. Other surface roughness parameters, such as R_q (S_q; root-mean-square of roughness), R_p (S_p; maximum peak height), R_v (S_v; maximum valley height), and R_z (S_z, maximum height) are also in common use, but the present study was limited to the most commonly used roughness parameter S_a (R_a).

6.2 Discussion of Results

During the development of artificial caries, an opening of intercrystalline spaces at the initial step to the subsequent progression of subsurface demineralisation was observed in some SEM or polarized light microscopy studies [FEATHERSTONE *et al.* 1985, HOLMEN *et al.* 1985]. These spaces remain at the outermost surface as the lesion will progress below the surface. Therefore, an increase in the surface roughness after a caries-like lesion created was expected by some researchers [FEATHERSTONE *et al.* 1985, HOLMEN *et al.* 1985]. Rather few quantitative surface roughness studies of enamel in relation to demineralization have been reported. In an optical profilometric study, an increase in the surface roughness after demineralisation was studied quantitatively. However, that experiment only covered a demineralisation time of 0-70 h [ZHANG *et al.* 2000], which could not include the substantial subsurface demineralisation stage used in the present study; thus, the speculations of some previous qualitative studies (relating that artificial caries like lesion surface was rougher than sound enamel surface) have been confirmed [FEATHERSTONE *et al.* 1985, HOLMEN *et al.* 1985].

Furthermore, no detectable surface height reduction could be revealed after demineralisation in the present study. This result is in accordance with previous findings showing that the surface changes caused by the MHDP-containing model system were confined to a general enlargement of the intercrystalline spaces resulting from partial dissolution of the peripheries of the individual crystals without a direct dissolution of the outer surface [HOLMEN *et al.* 1985].

The infiltration of enamel caries lesion with resin materials is mainly driven by capillary forces. The highly mineralized surface layer of the lesions might hamper resin penetration. Therefore, an acid-etching procedure before infiltration is required to remove or alter the surface layer [GRAY and SHELLIS 2002, MEYER-LUECKEL *et al.* 2007]. The thickness of the surface layer varies from some 10 µm [ARENDS *et al.* 1979] up to 50 µm [FEJERSKOV O *et al.* 2003] in natural lesions. In the present study, a lower concentration of phosphoric acid gel (20%) was applied on the lesion surface for 5 s, since the width of artificial lesion surface layers has been found to be considerably smaller than that of natural lesions [SILVERSTONE 1968, KIELBASSA *et al.* 2005]. With this etching procedure, the roughness of surface significantly increased to more than 450% (if compared with the S_a values after demineralization), while the height of the lesion surface was reduced to 6.6 ± 2.0 µm in the present study. However, it should

be emphasized that optical measurement of etched enamel surfaces is considered difficult, due to very frail surface which tends to scatter and absorb light [HOLME *et al.* 2005]. This phenomenon was confirmed in the present study, revealing artefacts in some specimens after etching of the demineralised surfaces. Therefore, the present outcome of etched surfaces should be regarded as a strong tendency, but absolute values should not be overestimated.

After etching, resin materials were carefully applied on surface B. In the clinical situation, a smooth surface can be obtained after simply polymerizing the material against a matrix. However, even if care is taken with the placement of the matrix, removal of excess material and re-contouring of restored surfaces will frequently be necessary. Unfortunately, these procedures significantly increase surface roughness [SCHEIBE *et al.* 2009]. On the other hand, a thick resin layer on top of the lesion, which might enhance plaque accumulation and cause periodontal inflammation, is not required for this technique, because only the infiltrated material is assumed to influence the progression of demineralisation [MUELLER *et al.* 2006, PARIS *et al.* 2007c].

In the present study, the excess material was removed either after light curing (with abrasive strips, in groups E1, F1, G1, and I1) or before light curing (using a rubber cup, in group E2, F2, G2, and I2), and the corresponding surface roughness resulting from these two different finishing ways (polished or non-polished surfaces) were measured and compared with each other. However, the use of abrasive strips after light curing could not show any improved smoothness (roughness) if compared with the use of a rubber cup before light curing. After use of the abrasive strips, a lot of scratches could be evaluated. Although a threshold for unacceptable surface roughness has not yet been agreed, a 2-D surface roughness (R_a) above 0.2 µm obviously results in an increase of plaque accumulation, thus resulting in higher risks for caries and periodontal inflammation [BOLLEN *et al.* 1996]. It should be emphasized that the mean S_a values of infiltrated lesions (polished or non-polished) obtained in the current study were both above the threshold roughness, while those of polished enamel surfaces and initial enamel (subsurface) caries like lesion surfaces were significantly lower than the reported thresholds.

However, this result should be interpreted with caution, since arithmetic R_a values from a 2-D profilometer cannot to be simply compared with the S_a values from a 3-D measurement. Some significantly lower arithmetic values of S_a recorded by AFM compared with 2-D profilometer R_a have been reported in a previous study

[KAKABOURA *et al.* 2007]. Therefore, such comparisons are not possible for S_a values facilitated by IFM; so far, such data are not available in the literature for infiltrated lesion surfaces.

Furthermore, the mean height value of infiltrated and polished lesion surface (-6.6 µm) was similar to that of etched lesion surface, while the standard deviation ($\pm$6.1 µm) was quite high. The height of infiltrated and polished lesion surfaces B of the different specimens was either negative (in most cases due to over abrasion) or positive (in some cases, with some overlying material left) compared to the height of the reference surface A. This would imply that the amount of abrasion was difficult to control, even though the times and forces of polishing procedure had been standardised. The infiltrated (but non-polished) lesion surfaces (here, the excess material had been removed with a rubber cup before light curing) were found to be slightly higher than the etched lesion surfaces, and this outcome might be interpreted as resulting from a small amount of resin left on the surface or in some irregular regions of etched lesion surface. Moreover, since the standard deviation of the evaluated surplus was relatively small (±1.5 µm), the surface height of various specimens remained fairly constant at this stage.

On the other hand, in the clinical situation, it would be even more difficult to control the amount of abrasion with abrasive strips, in particular in the proximal areas without any direct visible access. Thus, excessive abrasion of adjacent sound enamel or insufficient removal of excess materials might occur in many cases. Considering the various factors mentioned above, removal of excess material before light curing should be recommended to simplify the treatment procedure and to avoid any unexpected abrasion resulting from the use of abrasive strips. Furthermore, in the clinical situation, the teeth to be sealed could be separated with orthodontic elastics to gain access to the proximal space and then seal the proximal lesion [KIELBASSA *et al.* 2009]. However, the use of a rubber cup on proximal tooth surfaces is not always applicable, even though a small proximal space can be obtained in most cases. Therefore, the use of dental floss is more practical in some cases, but difficult to control as well. Therefore, a more suitable instrument should be developed in order to meet these demands.

In total, mean percentage reduction of S_a values in the infiltrated groups (if compared to the etched surfaces) ranged from 9% (I2, F2) to 25% (E1), and increased to some

45% in G1 and G2 subgroups. Regarding the non-polished groups of the present study, infiltrated lesion surfaces were still significantly rougher than demineralized enamel in all cases, and no significant difference in S_a values for the infiltrated lesions could be evaluated with the different materials. Only Glaze & Bond appeared to reduce the surface roughness of the etched lesion surfaces after infiltration. The favourable performance of Glaze & Bond in reducing the surface roughness may partially be attributed to the absence of an oxygen inhibited layer usually forming after light curing (information according to the manufacturer). However, the observed improvement of S_a values in the G2 group should be interpreted with caution (and not be overrated), since some area with unsatisfactory surface quality outside the measurement regions could be observed in many cases. In contrast, even if a positive trend of mean values could be evaluated in all subgroups, Excite, Fortify and Icon did not show any significant effects on the roughness outcome. All in all, the null hypothesis that the four different materials have equal effects on the surface quality of infiltrated lesion had to be partially rejected. However, it is difficult to fully explain these results from comparing commercial materials since their full constituents are rarely divulged by the manufacturers; nevertheless, the physical and chemical properties of the materials used in the present study should be the major factors leading to differences in surface roughness. As no comparable studies are available from the literature, this is the first 3D metrology report on infiltrated subsurface lesions, and further investigations in this field should be mandatory.

The values of height and marginal angle of overlying materials may to some extent reflect the thickness of different resin coats on lesion surfaces. In the present study, the use of Excite resulted in a considerable thicker resin coat on lesion surfaces than the other three materials did, and this may be partially explained by the low solvent content and a certain content of extremely fine fillers (nano-fillers) (according to the manufacturer). At the same time, the resin coats of Icon were observed to be obviously thinner than those of the other materials, which might be attributed to its prominent feature of low viscosity and small contact angel. Nevertheless, it could be assumed that excessively thin adhesive layers may suffer from incomplete resin polymerization, which might be due to the fact that the entire fluid might be oxygen inhibited and will not set [RUEGGEBERG and MARGESON 1990, VAN LANDUYT *et al.* 2007].

On the other hand, the higher the proportion of a functional diluent (being responsible for the low viscosity) the resin contains, the greater the potential of polymerization shrinkage during curing [RUEGGEBERG and MARGESON 1990]. Moreover, too much evaporation of solvent (ethanol or acetone) also might lead to inhomogeneous resin layers. These factors may be related to the observed phenomena that the resin coat of Icon was relatively inhomogeneous and even incomplete in many cases, while this was not the case with the other three materials. However, resin coat thickness does not depend on the properties of materials only, but is clearly associated with the amount of material. In the present study, the resinous materials had been continuously coated on the lesion surface until the material spilled over the borderline (overlying material), and this was considered clinically relevant. Notwithstanding, a more precise control of the amount of material application is required for further studies.

Furthermore, Icon is a caries infiltrant that in particular has been designed for the micro-invasive infiltration technique. Therefore, a low-viscosity resin seems essential to obtain a sufficiently deep penetration into the lesion body [PARIS *et al.* 2007b]. However, it has been proved that materials having lower viscosities as a result of greater amounts of diluenting agent content have greater thickness of air-inhibited layers, since the rate of oxygen diffusion in resin increases with decreasing viscosity of the liquid [RUYTER 1981, BAN and HASEGAWA 1984]. Thus, the thin layer of Icon on the lesion surface (especially after removing the excess materials by a rubber cup) could be inhibited by oxygen and then washed away (e.g. by salivary flow or mechanical action), which may lead to exposure of enamel substance and subsequent acid attack. Therefore, the need for a re-covering regimen using a resin that would have better surface properties should be re-considered.

7 Conclusions

With the help of the 3-D measurement method (IFM) presented here, the changes in lesion surface morphology (roughness and profile) before and after each implementation step of the infiltration technique were observed by means of qualitative and quantitative methods. The present study's null hypotheses were partially rejected.

Within the limitations of the present study, it can be concluded that:

1. An increase in surface roughness was detected after creating artificial caries like (subsurface) lesions.
2. After etching with 20% phosphoric acid gel (5 s), the average roughness of lesion surface increased significantly, while the surface height decreased (6.6 ± 2.0 µm).
3. The use of abrasive strips after light curing did not show any advantage in reducing the roughness of infiltrated lesion surfaces. Since the amount of abrasion was not easy to control, excess material should be removed before light curing.
4. With the benefits of the infiltration concept (and the hampered lesion progress) in mind, the surface quality of incipient lesions infiltrated with resinous infiltrants seems to be perfectible.
5. The resin coat of Excite showed the largest thickness, while that of Icon was the thinnest one. The resin coats of Excite, Fortify, and Glaze & Bond were found to be homogeneous and intact on the lesion surfaces, while Icon performed worse in this respect.

Future research should both focus on both penetration behaviour and the surface quality. Thus, a two-step treatment regimen should be taken into account, which can be defined as to (1) obtain a good penetration depth into the porous bed of initial lesions with the use of low-viscosity resin materials (such as Icon), and to (2) additionally cover the infiltrated lesion surface with a resinous material that should enhance the surface property. More investigations are needed to evaluate the potential effects of the materials with different properties on the morphology of infiltrated lesion surface. The improvement of two-step regimens is considered one of the fields of major interest.

8 Abstract

Statement of problem: Surface morphology of infiltrated subsurface enamel lesions requires documentation. **Objectives:** To evaluate the effects of two different finishing procedures and four different materials on the surface roughness of infiltrated subsurface bovine enamel lesions. **Materials and methods:** Eighty enamel specimens were prepared from bovine incisors, and surfaces were partially varnished (control). The non-varnished areas were demineralised (pH 4.95, 28 d), and etched with phosphoric acid gel (20 %, 5 s). Specimens were randomly divided into eight groups (n=10; E1/E2-Excite, Vivadent; F1/F2-Fortify, Bisco; G1/G2-Glaze & Bond, DMG; I1/I2-Icon, DMG). In subgroup 1, resin materials were polymerised and polished using abrasive strips by means of a polishing device. In subgroup 2, excess material was removed with a rubber cup before polymerisation (without polishing). Mean surface roughness (S_a) and difference of height (DH) between control and treated surfaces were determined using a dedicated focus-variation system (Alicona). **Results:** Demineralised enamel was significantly rougher than sound enamel ($p<0.01$; *t*-test), while the DH was negligible. After etching, S_a-values increased significantly ($p<0.001$; *t*-test), while DH decreased (-6.6 ± 2.0 µm). Infiltrated lesion surfaces were significantly rougher than demineralized enamel in all cases ($p<0.001$; *t*-test), while no significant difference in S_a was found between polished and non-polished groups ($p>0.05$; *t*-test). Regarding the non-polished groups, no significant difference in S_a could be evaluated with the different materials ($p>0.05$; ANOVA). Application of Glaze & Bond appeared to reduce S_a values of etched lesions ($p<0.001$, G2; *t*-test-Bonferroni), while with all other subgroups S_a values improved only marginally. For infiltrated lesions, mean DH of subgroup 1 was similar to that of the etched lesions ($p>0.05$; *t*-test-Bonferroni), while a significant increase was noted in subgroup 2 ($p<0.001$; *t*-test-Bonferroni). **Conclusions:** Regarding surface roughness, the use of abrasive strips does not seem to be advantageous; excess material should be removed before light curing. The surface quality of incipient lesions infiltrated with resinous infiltrants seems to be perfectible.

Keywords: infiltration; initial caries; surface roughness; polishing procedures

Clinical Significance: With the infiltration technique of incipient carious lesions, removal of the excess material before light curing should be recommended, rather than the use of abrasive strips after polymerisation. The need for a re-covering regimen using a resin that would enhance surface properties should be re-considered.

9 Zusammenfassung

Problemstellung: Die Oberflächenmorphologie von infiltrierten initialen Schmelzläsionen ist in der verfügbaren Literatur nicht beschrieben. **Zielsetzung:** Ziel dieser Studie war, zwei unterschiedliche Bearbeitungsmethoden auf die Oberflächenqualität von vier unterschiedlichen, zur Infiltration von initialen Schmelzläsionen geeigneten Materialien zu untersuchen. **Material und Methoden:** Aus den Labialflächen von Rinderschneidezähnen wurden 80 Schmelzproben präpariert, die teilweise mit Nagellack abgedeckt wurden (Kontrolle). Die nicht abgedeckten Bereiche wurden demineralisiert (pH 4,95; 28 d), und die Läsionsbereiche wurden anschließend mit Phosphorsäuregel konditioniert (20 %; 5 s). Nach randomisierter Verteilung der Proben auf acht Gruppen (n=10) erfolgte die Infiltration der Materialien (Excite-E, Vivadent; Fortify-F, Bisco; Glaze & Bond-G, DMG; Icon-I, DMG). In Untergruppe 1 wurden die Kunststoffe polymerisiert; anschließend erfolgte die standardisierte Ausarbeitung mit Polierstreifen in einer Polierapparatur (polierte Gruppen E1, F1, G1, I1). Bei den Proben der Untergruppe 2 wurde das überschüssige Material vor der Polymerisation mit Gummikelchen abgezogen (nicht polierte Gruppen E2, F2, G2, I2). Die mittlere Oberflächenrauheit (S_a) und die Höhendifferenz (DH) zwischen den Kontrollbereichen und den infiltrierten Arealen wurde in beiden Untergruppen mit der Fokusvariation (Alicona) bestimmt. **Ergebnisse:** Die Oberflächenrauheit der demineralisierten Proben war im Vergleich zu den polierten Ausgangssituationen signifikant erhöht ($p<0,01$; *t*-test). Nach der Konditionierung mit Phosphorsäure kam es zu einem weiteren, signifikanten Anstieg der mittleren Rauheit ($p<0,001$; *t*-test), während die Höhendifferenz zunahm (-6,6 ± 2,0 µm). Die S_a-Werte der infiltrierten Läsionen waren in allen Gruppen (polierte versus nicht polierte Proben) signifikant höher als bei den gesunden, unbehandelten Proben ($p<0,001$; *t*-test). Zwischen den polierten und den nicht polierten Proben waren keine Unterschiede erkennbar ($p>0,05$; *t*-test). Lediglich nach Applikation von Glaze & Bond wurde im Vergleich zu den konditionierten Proben in der ein signifikant reduzierter S_a-Wert beobachtet ($p< 0,001$, G2; *t*-test-Bonferroni); die Infiltration der anderen Materialien zeigte nur eine gering ausgeprägte Auswirkung auf die Rauheit der Oberflächen ($p>0,05$, I1, I2; *t*-test-Bonferroni). **Schlussfolgerungen:** Die Verwendung von Poliersteifen nach der Polymerisation ist hinsichtlich der Oberflächenrauheit nicht vorteilhaft; überschüssiges Material sollte vor der Aushärtung entfernt werden. Die Oberflächenqualität von Infiltranten (Icon) scheint verbesserungswürdig.

Schlagwörter: Infiltration; initiale Schmelzkaries; Oberflächenrauheit; Politur

Klinische Bedeutung: Bei der Infiltrationstherapie der initialen Schmelzkaries ist die Entfernung von Überschüssen vor der Polymerisation einer nachträglichen Bearbeitung mit Polierstreifen vorzuziehen. Die Abdeckung der infiltrierten Areale mit einer Kompositschicht mit verbesserten Oberflächeneigenschaften scheint ratsam.

10 References

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11 Appendix

List of materials

Mat. 1 Abrasive Paper 1200, 2500, 4000; Exakt Apparatebau, Norderstedt, German

Mat. 2 Acetic acid >99%; Merck, Darmstadt, Germany

Mat. 3 Analytical Balance Typ AG204; Mettler, Giesen, Germany

Mat. 4 Band Saw Exakt 300cl; Exakt Apparatebau, Norderstedt, Germany

Mat. 5 Calcium chloride dihydrate > 99,5%; Merck, Darmstadt, Germany

Mat. 6 Diamond bur; Gebr, Brasseler, Lemgo, Germany

Mat. 7 Disposable scalpel; Aesculap AG, Tuttingen, Germany

Mat. 8 Dupli-Color; Kurt Vogelsang, Hassmersheim, Germany

Mat. 9 Excel 2000; Microsoft GmbH, Unterschleissheim, Germany

Mat. 10 Excite; Ivoclar/Vivadent, Schaan, Liechtenstein

Mat. 11 FORTIFY; Bisco, Schaumberg, USA

Mat. 12 Glaze & Bond; DMG, Hamburg, Germany

Mat. 13 Gluma Etch 20 Gel; Heraeus Kulzer, Hanau, Germany

Mat. 14 Icon; DMG, Hamburg, Germany

Mat. 15 IFM G4 software; Alicona Imaging, Grambach/Graz, Austria

Mat. 16 InfiniteFocus microscopy; Alicona Imaging, Grambach/Graz, Austria

Mat. 17 Methylenehydroxydiphosphonic acid >98%; Merck, Darmstadt, Gernamy

Mat. 18 Micro-brush; 3M ESPE, Seefeld, Germany

Mat. 19 Oven BR 6000; Heraeus, Hanau, Germany

Mat. 20 PH-/Redox-Temperatur Meter GMH 3510; Greisinger, Regenstauf, Germany

Mat. 21 Polishing Machine Exakt 400cs; Exakt Apparatebau, Norderstedt, Germany

Mat. 22 Potassium chloride 99,5-100,5%; Merck, Darmstadt, Germany

Mat. 23 Potassium dihydrogen phosphate min 98%; Merck, Darmstadt, Germany

Mat. 24 Potassium hydrogen -Plätzchen min. 85,0%; Merck, Darmstadt, Germany

Mat. 25 Rubber cup; Brasseler, Lemgo, Germany

Mat. 26 Sodium chloride 0.9% solution; DeltaSelect GmbH, Pfullingen, Germany

Mat. 27 Sof-Lex Finishing and Polishing Strips system; 3M ESPE, Seefeld, Germany

Mat. 28 SPSS 13.0 for windows; SPSS GmbH, Munich, Germany

Mat. 29 Spurr Resin Kit; Plano GmbH, Wetzlar, Germany

Mat. 30 Technovit 4004; Heraeus Kulzer, Hanau, Germany

Mat. 31 Thymol > 99%; Merck, Darmstadt, Germany

List of results

Table 1: Means of average roughness (S_a) and standard deviations (nm) of all sound enamel, artificial caries like (subsurface) lesions, etched lesions, infiltrated (and polished) lesions, and infiltrated (but non-polished) lesions surfaces

Surface	N	Mean	Std. Deviation
sound enamel	80.00	59.3	12.7
artificial caries like (subsurface) lesion	80.00	65.8	12.3
etched lesion	80.00	315.0	144.3
Infiltrated and polished lesion	40	257.2	126.0
Infiltrated (but non-polished) lesion	40	230.9	138.3

Table 2: Means of average roughness (S_a) and standard deviations (nm) of sound enamel, artificial caries like (subsurface) lesions, etched lesions, and infiltrated (and polished) lesions in groups E1, F1, G1, and I1

Surface	E1 (n =10)		F1(n =10)		G1(n =10)		I1(n =10)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
sound enamel	61.7	15.7	58.3	9.8	50.7	13.6	52.2	8.9
artificial caries like lesion	63.2	14.1	69.5	13.8	64.3	15.3	61.9	6.9
etched lesion	350.7	184.4	287.6	91.5	330.8	148.3	310.7	140.6
infiltrated and polished lesion	297.4	170.6	262.6	114.5	184.8	73.4	283.9	113.1

Table 3: Means of average roughness (S_a) and standard deviations (nm) of sound enamel, artificial caries like (subsurface) lesions, etched lesions, and infiltrated (but non-polished) lesions in groups E2, F2, G2, and I2

Surface	E2 (n =10)		F2 (n =10)		G2 (n =10)		I2 (n =10)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
sound enamel	55.2	9.3	65.4	11.2	59.6	10.9	71.1	10.9
artificial caries like lesion	60.7	9.6	69.7	13.4	65.7	10.1	71.1	12.9
etched lesion	247.1	98.1	373.7	196.0	347.2	104.4	271.9	157.8
infiltrated (but non-polished) lesion	183.3	53.1	322.7	196.3	180.3	60.4	237.3	152.2

Table 4: Means and standard deviations (µm) of differences of heights (DH) of etched lesions, infiltrated and polished lesions, and infiltrated (but non-polished) lesions

Surface	N	Mean	Std. Deviation
etched lesion	80	-6.6	2.0
infiltrated and polished lesion	40	-6.6	6.0
infiltrated (but non-polished) lesion	40	-5.4	1.5

Table 5: Means and standard deviations of the differences of heights (µm) (DH1, DH2; 200 µm, 500 µm away from the borderline), and the marginal angles (°) in measuring positions DH 1 and DH 2 of the overlying materials for groups E1, F1, G1 and I1

	E1 (n =10)		F1 (n =10)		G1 (n =10)		I1 (n =10)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DH1	25.1	5.0	16.6	7.3	15.3	3.9	5.2	4.4
DH2	67.4	14.8	37.3	12.1	28.7	11.7	14.5	8.2
angle in DH 1	7.1	1.4	4.7	2.1	4.4	1.1	1.5	1.3
angle in DH 2	7.7	1.7	4.3	1.4	3.3	1.3	1.7	0.9

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Curriculum Vitae

For reasons of data protection, the Curriculum vitae is not published in the online version.

Declaration in lieu of an oath

„Ich, Yang Fan, erkläre, dass ich die vorgelegte Dissertationsschrift mit dem Thema: *Effects of Different Finishing Procedures and Materials on Surface Roughness of Infiltrated Subsuface Bovine Enamel Lesions* selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt, ohne die (unzulässige) Hilfe Dritter verfasst und auch in Teilen keine Kopien anderer Arbeiten dargestellt habe.“

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