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The effect of aging on composite-to-composite repair strength

Margareta Rinastiti

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Chapter 1

General Introduction

1 The history of dental composites

Aesthetic concepts in restorative dentistry started around 30 years ago and were the driving force in the development of some dental restorative materials.[1] The development of aesthetic material started with the invention of silicate cement in 1908. In 1947, direct-filling with methyl methacrylate resin was introduced in order to replace silicate cement. Restoration with methyl methacrylate was associated with many problems, such as a polymerization shrinkage up to 20-25%, poor color stability, low stiffness and lack of adhesion to the tooth structure.[2] To solve the shrinkage problem, a new type of restorative material was developed by adding inorganic filler particles into the resin.[2] Due to the absence of a coupling agent between the filler particles and the matrix, this material showed high wear and discoloration.[3] In the early 1950s, a silane coupling compound was introduced to enhance bonding between the resin and ceramic surfaces.[4] In 1955, Michael Buonocore discovered the concept of acid-etching to improve the adhesion of acrylic resin to the enamel surface.[2] A notable development of composite materials is the invention of bisphenol glycidyl dimethacrylate (Bis-GMA) by Bowen in 1962.[5] The advantages of Bis-GMA over traditional polymethyl methacrylate are lower volatility and diffusion into tissues, higher cross linking ability, less polymerization shrinkage and rapid hardening under oral conditions.[6] From 1970 on, composite materials were polymerized using ultraviolet light-curing resulting in command-setting composite.[2]

The introduction of new bonding systems and composites has had a major impact on restorative dentistry. It not only brought a change in materials and techniques, but also a change in treatment philosophy,[7] called minimal invasive dentistry.[8,9] Composites allow the possibility of preserving sound tooth structure during cavity preparation[10] and represent a significant aesthetic treatment option, enabling the fabrication of restorations with a natural appearance.[11]

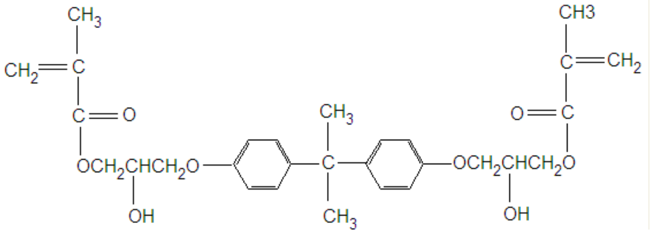
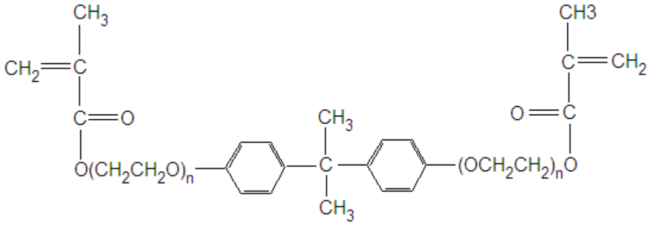
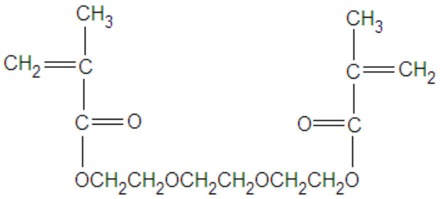
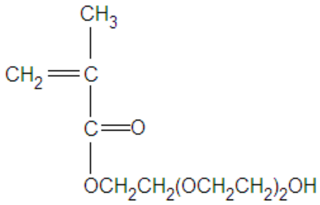
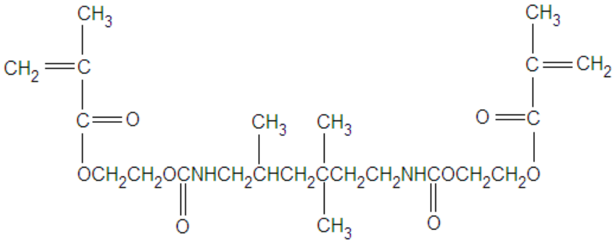
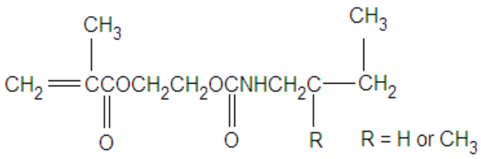
2 Composition of dental composites

Composites are engineered materials consisting of at least two different classes of materials i.e. metals, ceramics, and polymers[12] with significantly different physical and chemical properties. Composites need components that will stabilize the material. Composite resins consist of an organic resin matrix, inorganic fillers, a coupling agent and additional component, like an initiator, stabilizer and pigments to produce the different shades.[13]

2.1 Resin monomer

The resin component of a cured composite is a polymeric matrix that is built from repetitive units called monomers, which are bound together. Polymerization occurs through the carbon-carbon double bonds of two methacrylate groups.[14] The polymeric matrix usually is based on a mixture of dimethacrylates like Bis-GMA, UDMA, Bis-EMA, TEGMA, TEGDMA, and DUDMA (see Table 1).[12,14-17] The type of monomer influences the reactivity, viscosity, polymerization shrinkage, mechanical properties and water uptake of the composite.[18]

Table 1 Nomenclature of monomer matrix of dental composites used in this thesis

Abbreviations	Monomer matrix	Molecular structure
Bis-GMA	bisphenol glycidil methacrylate	
Bis-EMA	ethoxylated bisphenolglycidil methacrylate	
TEGDMA	triethylene glycol dimethacrylate	
TEGMA	triethylene glycol methacrylate	
UDMA	urethane dimethacrylate	
DUDMA	diurethane dimethacrylate	

2.2 *Filler particles*

The concentration of filler in a composite resin is generally 70 to 80% by weight.[19] Filler particles have a considerable influence on the physical and mechanical properties of the composite. They reduce the coefficient of thermal expansion, polymerization shrinkage, provide radio-opacity, improve handling and increase wear and aesthetic results.[20] Most current composites are filled with radiopaque silicon dioxide, boron silicate and lithium aluminum silicates.[21] In some composites, heavy metal particles such as barium, strontium, zinc, aluminum or zirconium are added.[22] Composite resins can be categorized according to particle size:

- traditional composite (10-20 μm),
- microfilled (0.01 – 0.05 μm),
- hybrid (large particle: 10-20 μm; colloidal silica: 0.01 – 0.05 μm),
- microhybrid (0.1 – 6.0 μm),
- nanohybrid (microfiller: 0.1 – 2.5 μm; nanofiller: 20-50 nm) and
- nanofilled (5 – 100 nm) [23]

New developments in composites are to minimize the filler size and maximize the filler load in order to fulfill all requirements set to a composite resin.[12]

2.3 *Coupling agent*

Good adhesion between matrix and filler prevents erosion of the filler surface and facilitates stress transfer between those two components, thus increasing the mechanical properties of composite resins.[24] To improve the bonding between the filler-matrix interface, inorganic fillers are coated with silanes.[25] An additional benefit is the improved dispersion of these silane-coated filler particles in the matrix monomer.[26] Basically the synergy between polymer matrix and inorganic filler particle is mediated by organofunctional silane as the interfacial phase. Organofunctional silane ($YRSiX_3$) is a bifunctional molecule capable of reacting with the silanol groups of glass or ceramic fillers via its silane functional group (-SiX$_3$) to form Si-O-Si- bonds with filler surfaces, and with the resin phase by graft copolymerization via its Y functional group. R is a hydrocarbon or fluorocarbon chain bound to at least one reactive functional group Y. Usually, Y is a methacrylic vinyl group.[27] X can be an alkoxy substituent, e.g., -OCH$_3$, or -OCH$_2$CH$_3$. The most commonly used organofunctional silanes in dental-composites is γ-methacryloxypropyl-trimethoxysilane (γ-MPS).[12]

2.4 *Other components*

Dental composites also contain chemical substances to initiate and promote the polymerization reaction such as benzoyl or lauryl peroxide, various tertiary amines and camphorquinone.[28] Butylated hydroxytoluene and hydroquinone are typically used in composites to prevent polymerization during storage and avoid uncontrolled photopolymerization by normal room light during the restoration of a cavity.[29] To produce composite resins in the right color, perfectly matching the natural tooth tissue, iron oxides, aluminum oxide or titanium dioxide are added as pigments.[30,31]

3 The stability of dental composites *in vivo*

3.1 *Aging in the oral cavity*

Aging of composite resins in the oral cavity is very complex. Thermal changes, food, beverages, saliva and biofilm result in degradation of the composite resin.[32-34] Intra-oral temperature changes, induced by eating, drinking and breathing,[35] result in crack formation and propagation through debonding of the filler and matrix interface.[36] These gaps between restoration and tooth structure allow oral fluids going in and out of the gap.[35] Various food and beverage constituents are extrinsic factors that can degrade and age composite restorations. Yap *et al.*[37] and Wu *et al.*[38] reported that food simulating liquids, like e.g. citric acid, lactic acid, heptane and ethanol, softened the composite surface. Staining of the superficial layer of composites also relates to dietary habits.[39] Consumption of coffee, soft drinks, tea, alcoholic beverages and even water or fluoride may affect the aesthetic and physical properties of composite resins.[40]

Biological elements in the oral cavity such as saliva, containing enzymes and bacteria, as well as biofilm (dental plaque) have an important role in the aging process of composite resins. It may lead to composite degradation,[41] leaching of monomer components, filler dissolution, reduction of the hardness[42] and increased surface roughness.[43] Composite surfaces also become more porous as a result of the acids produced by bacteria in the biofilm.[41,44]

3.2 Modes of degradation of dental composite resins

Composite material can be degraded via three principal modes,[17] i.e.: mechanical degradation,[45] physical degradation and chemical degradation.[34,46] Mechanical degradation is mainly described by different types of wear, a natural process which occurs when two bodies move in contact. Wear in the oral cavity, such as adhesive wear, abrasive wear, corrosive wear and fatigue can result in loss of the anatomical structure of the tooth or composite material.[45]

Physical degradation is comprised by sorption, dissolution and elution of composite resins exposed to the aqueous oral environment.[41] Water molecules diffuse into the composite and are largely absorbed by the matrix in a diffusion-controlled process resulting in chemical degradation which leads to release of the degradation product.[47,48] The physical changes of the matrix due to the water exposure are swelling and softening[49] which can cause microcrack formation, debonding of the filler-matrix interfaces or even hydrolytic degradation of the filler.[50,51] In addition, water can act as a plasticizer which weakens the polymer structure.[11,28]

Chemical degradation has been associated with hydrolysis stimulated by saliva, bacteria and pH change[42,52] or oxidation.[53] Salivary esterase and other enzymes are able to degrade the dimethacrylate resin matrix, resulting in production and release of methacrylic acid.[42] Bacteria can generate hydrolytic and proteolytic enzymes and acids such as acetic, propionic and lactic acids that are produced by bacterial metabolims leading to a pH decrease.[52] These factors potentially affect structural integrity of restorative material and discoloration associated with microleakage.[41]

3.3 *Survival rate of dental composite restorations*

Degradation of composite restorations challenges their integrity and longevity over time. Issues concerning the longevity of restorations are complex, and render interpretation and comparison of the results of different experimental studies difficult. The expected life-span of a single-surface composite restoration is 6-7 years while that of a multi-surface restoration is about 4 years.[54] Few studies report a life-time of 7-10 years,[55-58] but composite restoration in posterior teeth can have a life-time of more than 10 years.[59] For anterior composite restorations, 60–80% remain acceptable till about five years.[60-62] Annual failure rates of composite restorations range between 0–14.4%.[63]

4 The concept of minimally invasive dentistry

4.1 *Traditional repair of failed composite restorations*

Aging of composite resin restorations in the oral environment results in discoloration, degradation, microleakage, wear, ditching at the margins, delamination or fracture and/or chipping. All are often experienced in clinical situations. These failures may require repair or replacement of the restoration.[64-66]

Total replacement of a restoration is the most common procedure in daily clinical practice to repair a damaged composite restoration.[67] However, this approach may be regarded as overtreatment, since in most cases a large portion of the restoration is clinically and radiographically free of failures. Moreover, accelerated cyclic re-restoration of teeth is inevitably followed by weakening of the tooth which predisposes to fracture, unnecessary grinding of intact dental tissues and repeated injuries to the pulp.[68] In addition, replacement of restorations occupies half the general practitioners time and represents substantial expenses.[69] Therefore repairing the restoration would have a significant effect on the conservation of the tooth integrity and is economically more attractive.

4.2 Repair of failed composite restorations using minimal invasive dental concepts

One of the latest evolutions in the dental practice, is a change in treatment philosophy from "extension for prevention" to "prevention for extension" (minimal invasive dentistry). One of the minimal invasive dentistry concepts is a new technical procedure for the repair of defects in restorations based on layering new composite resin onto the existing, aged one. A major problem of this procedure is the establishment of a long lasting bonding between aged and fresh composite resin.[70] It has been demonstrated that the adhesion of composite repair reaches only 20% to 80% of the initial bond strength.[71]

The longevity of composite-to-composite repair depends on the surface conditioning method and the age of the restoration.[72,73] Clinically, bonding between two composite layers is achieved in the presence of an oxygen-inhibited layer of unreacted monomers.[72] A great variety of surface conditioning methods and adhesive agents has been proposed to improve the repair strength of composite resins, such as roughening,[74] acid etching with hydrofluoric acid[75] or phosphoric acid, application of intermediate adhesive resin[76,77] or air-borne particle abrasion using (functionalized) aluminum oxide or silica-coated particles with and without silanes.[78] Even though the literature presents several comparative studies,[79-81] there is no consensus for the best surface treatment to achieve optimal composite-to-composite repair strength. The best surface treatment for optimal repair is depending on the surface characteristics of the composite resins after aging, which is not easily assessed on an experimental basis.

Aims of this thesis

Aging of composite restorations in the oral cavity involves complex factors and results in composite resin degradation and damage of the restorations. In the literature, there is no consensus about the best model to mimic composite aging and degradation. Therefore, the first aim of this study is to find the best model for simulating intra-oral aging of composite resins. The second aim is to determine the best possible conditioning method for composite-to-composite repair based on the bond strength analyses.

References

1. Christensen GJ (1997) The state of the art in esthetic restorative dentistry. *J Am Dent Assoc* 128:1315-1317
2. Schulein T (2005) Significant events in the history of operative dentistry. *J Hist Dent* 53:63-72
3. Knock FE, Glenn JF (1951) Dental material and method. *US Patent* 2,558,139:
4. Bjorksten J, Yaeger L (1952) Vinyl silane size for glass fabric. *Modern Plastic* 29:124-188
5. Bowen R (1963) Properties of a silica-reinforced polymer for dental restorations. *J Am Dent Assoc* 66:57-64
6. Khatri CA, Stansbury JW, Schultheisz CR, Antonucci JM (2003) Synthesis, characterization and evaluation of urethane derivatives of Bis-GMA. *Dent Mater* 19:584-588
7. Roeters FJM, Opdam NJM, Loomans BAC (2004) The amalgam-free dental school. *J Dent* 32:371-377
8. Murdoch-Kinch MA, McLean ME (2003) Minimally invasive dentistry. *J Am Dent Assoc* 134:87-95
9. Tyas MJ, Anusavice KJ, Frencken JE, Mount GJ (2000) Minimal intervention dentistry - a review - FDI Commission Project 1-97. *Int Dent J* 50:1-12
10. Leinfelder K (1997) A conservative approach to placing posterior composite resin restorations. *J Am Dent Assoc* 127:743-748
11. Gordan V, Mjør I, Blum I, Wilson N (2003) Teaching students the repair of resin-based composite restorations: a survey of North American dental schools. *J Am Dent Assoc* 134:317-323
12. Ferracane JL (1995) Current trends in dental composites. *Crit Rev Oral Biol Med* 6:302-318
13. Ferracane JL (2001) Direct esthetic anterior restoratives. Materials in dentistry : principles and applications. 2nd ed. Lippincott Williams & Wilkins, Philadelphia, USA, pp 88-105
14. Peutzfeldt A (1997) Resin composites in dentistry: the monomer systems. *Eur J Oral Sci* 105:97-116
15. Bowen RL, Marjenhoff WA (1992) Dental composites/glass ionomers: the materials. *Adv Dent Res* 6:44-49
16. Eckerman M, Suuronen K, Jolanki R, Alanko K (2004) Methacrylates in dental restorative materials. *Contact Dermatitis* 50:233-237
17. Ferracane JL (1994) Elution of leachable components from composite. *J Oral Rehabil* 21:441-452

18. Moszner N, Salz U (2001) New development of polymeric dental composites. *Prog Polym Sci* 26:535-576
19. Tanimoto Y, Nemoto K (2004) Influence of particle size of fillers on frictional wear of dental composite. *Compos Interfaces* 11:15-24
20. Labella R, Lambrechts P, Van Meerbeek B, Vanherle G (1999) Polymerization shrinkage and elasticity of flowable composites and filled adhesives. *Dent Mater* 15:128-137
21. Xu H (1999) Dental composite resins containing silica-fused ceramic single crystalline whiskers with various filler levels. *J Dent Res* 78:1304-1311
22. Hosoda H, Yamada T, Inokoshi S (1990) SEM and elemental analysis of composite resins. *J Prosthet Dent* 64:669-676
23. Van Noort R (2002) Clinical dental materials. Introduction to dental materials. 2nd ed. Mosby, London, UK. pp 96-123
24. Lin C, Lee S, Keh E, Dong D, Huang H, Shih Y (2000) Influence of silanization and filler fraction on aged dental composites. *J Oral Rehabil* 27:919-926
25. Chen TM, Brauer GM (1982) Solvent effets on bonding organo-silane to silica surface. *J Dent Res* 61:1439-1443
26. Mohsen N, Craig R (1995) Effect of silanation of fillers on their dispersability by monomer systems. *J Oral Rehabil* 22:183-189
27. Antonucci J, Dickens S, Fowler BO, Xu H, McDonough W (2005) Chemistry of silanes: interfaces in dental polymers and composites. *J Res Natl Inst Stand Technol* 110:541-558
28. Ruyter IE (1988) Composites - characterization of composite filling materials: reactor response. *Adv Dent Res* 2:122-133
29. Klapdohr S, Mozner N (2005) New inorganic components for dental filling composites. *Monatsh Chem* 136:21-45
30. Kim O, Shim W (2001) Studies on the preparation and mechanical properties of esthetic polymeric dental restorative using silane treated silica microfiller via freeze-drying. *Polym Composite* 22:650-659
31. Yoshida K, Taira Y, Atsuta M (2001) Properties of opaque resin composite containing coated and silanized titanium dioxide. *J Dent Res* 80:864-868
32. Lee Y, Lim B, Powers J (2004) Color changes of dental resin composites by salivary enzyme. *J Biomed Mater Res B Appl Biomater* 70B:66-72
33. Fúcio Z, Carvalho FG, Sobrinho LC, Sinhoreti M, Puppin-Rontani RM (2008) The influence of 30-day-old *Streptococcus mutans* biofilm on the surface of esthetic restorative materials--An *in vitro* study. *J Dent* 36:833-839

34. Oilo G (1992) Biodegradation of dental composites/glass-ionomer cements. *Adv Dent Res* 6:50-54

35. Gale M, Darvell B (1999) Thermal cycling procedures for laboratory testing of dental restorations. *J Dent* 27:89-99

36. Kawano F, Ohguri T, Ichikawa T, Matsumoto N (2001) Influence of thermal cycles in water on flexural strength of laboratory-processed composite resin. *J Oral Rehabil* 28:703-707

37. Yap AUJ, Low J, Ong LKFL (2000) Effect of food-simulating liquids on surfac characteristics of composite and poly-acid. *Oper Dent* 25:170-176

38. Wu W, Toth E, Moffa J, Ellison J (1984) Subsurface damage layer of *in vivo* worn dental composite restorations. *J Dent Res* 63:675-680

39. Schulze K, Marshall S, Gansky S, Marshall G (2003) Color stability and hardness in dental composites after accelerated aging. *Dent Mater* 19:612-619

40. Badra V, Faraoni J, Ramos R, Palma-Dibb R (2005) Influence of different beverages on the microhardness and surface roughness of resin composite. *Oper Dent* 30:213-219

41. Santerre JP, Shajii L, Leung B (2001) Relation of dental composite formulations to their degradation and the release of hydrolyzed polymeric-resin-derived products. *Crit Rev Oral Biol Med* 12:136-151

42. Munksgaard EC, Freund M (1990) Enzymatic hydrolysis of (di)methacrylates and their polymers. *Scand J Dent Res* 98:261-267

43. Beyth N, Bahir R, Matalon S, Domb A, Weiss E (2008) *Streptococcus mutans* biofilm changes surface-topography of resin composites. *Dent Mater* 24:732-736

44. Asmussen E (1984) Softening of Bis-GMA-based polymers by ethanol and by organic acids of plaque. *Scand J Dent Res* 92:257-261

45. Mair L, Stolarski T, Vowlest R, Lloyd C (1996) Wear: mechanisms, manifestations and measurement. Report of a workshop. *J Dent* 24:141-148

46. Winkler M, Greener E, Lautenschlager E (1991) No-linear *in vitro* wear of posterior composites with time. *Dent Mater* 7:258-262

47. Geurtsen W (1998) Substances released from dental resin composites and glass ionomer cements. *Eur J Oral Sci* 106:687-695

48. Ruyter IE (1995) Physical and chemical aspects related to substances released from polymer materials in an aqueous environment. *Adv Dent Res* 9:344-347

49. Ferracane JL (2006) Hygroscopic and hydrolytic effects in dental polymer networks. *Dent Mater* 22:211-222

50. Ferracane JL, Marker VA (1992) Solvent degradation and reduced fracture toughness in aged composites. *J Dent Res* 71:13-19

51. Sarkar NK (2000) Internal corrosion in dental composite wear: its significance and simulation. *J Biomed Mater Res B Appl Biomater* 53B:371-380

52. Prakki A, Cilli R, Mondelli FL, Kalachandra S, Pereira JC (2005) Influence of pH environment on polymer based dental material properties. *J Dent* 33:91-98

53. Øysaed H, Ruyter IE, Kleven U (1988) Release of formaldehyde from dental composites. *J Dent Res* 67:1289-1294

54. Mjor I, Jokstad A, Qvist V (1990) Longevity of posterior restorations. *Int Dent J* 40:11-7

55. Collins CJ, Bryant RW, Hodge K-LV (1998) A clinical evaluation of posterior composite resin restorations: 8-year findings. *J Dent* 26:311-317

56. Mair L (1998) Saucer-shaped cavity preparations for posterior approximal resin composite restorations: observatons up to 10 years. *Quintessence Int* 29:5-11

57. Pallesen U, Qvist V (2003) Composite resin filling and inlays. An 11 year evaluation. *Clin Oral Invest* 7:71-79

58. Raskin A, Michotte-Theall B, Vreven J, Wilson NHF (1999) Clinical evaluation of a posterior composite 10-year report. *J Dent* 27:13-19

59. da Rosa Rodolpho PA, Cenci MS, Donassollo TA, Loguqrcio AD, Demarco FF (2006) A clinical evaluation of posterior composite restorations: 17-year findings. *J Dent* 34:427-435

60. Millar B, Robinson P, Inglis A (1997) Clinical evaluation of an anterior hybrid composite resin over 8 years. *Brit Dent J* 182:26-30

61. Van Meerbeek B, Perdigpo J, Lambrechts P, Vanherle G (1998) The clinical performance of adhesives. *J Dent* 26:1-20

62. Van Noort R, Davis LG (1993) A prospective study of the survival of chemically activated anterior resin composite restorations in general dental practice: 5-year results. *J Dent* 21:209-215

63. Manhart J, Chen H, Hamm G, Hickel R (2004) Buonocore memorial lecture. Review of the clinical survival of direct and indirect restorations in posterior teeth of the permanent dentition. *Oper Dent* 29:481-508

64. Mjor IA (1981) Placement and replacement of restorations. *Oper Dent* 6:49-54

65. Roulet JF (1988) The problems associated with substituing composite resins for amalgam: a status report on posterior composites. *J Dent* 16:101-113

66. Swift E.J. (1987) Wear of composite resins in permanent posterior teeth. *J Am Dent Assoc* 115:584-588
67. Swift EJ, Le Valley BD, Boyer DB (1992) Evaluation of new methods for composite repair. *Dent Mater* 8:362-365
68. Mjor IA, Moorhead JE, Dahl JE (1999) Selection of restorative materials in permanent teeth in general dental practice. *Acta Odontol Scand* 57:257-262
69. Jokstad A, Bayne S, Blunck U, Tyas M, Wilson N (2001) Quality of dental restorations. FDI commission project 2-95. *Int Dent J* 51:117-158
70. Boyer DB, Chan K, Reindhart J (1984) Build-up and repair of light-cured composites: bond strength. *J Dent Res* 63:1241-1244
71. Roulet JF (1987) Degradation of Dental Polymers. Karger, Basel, Germany
72. Soderholm KJ, Zigan M, Ragan M, Fischlschweiger W, Bergman M (1984) Hydrolytic degradation of dental composites. *J Dent Res* 63:1248-1254
73. Yap AUJ, Sau CW, Lye KW (1999) Effects of aging on repair bond strengths of a polyacid-modified composite resin. *Oper Dent* 24:371-376
74. Kupiec K, Barkmeier W (1996) Laboratory evaluation of surface treatments for composite repair. *Oper Dent* 24:358-360
75. Passos S, Özcan M, Vanderlei A, Leite F, Kimpara E, Bottino M (2007) Bond strength durability of direct and indirect composite systems following surface conditioning for repair. *J Adhes Dent* 9:443-447
76. Chiba K, Hosoda H, Fusayama T (1989) The addition of an adhesive composite resin to the same material : bond strength and clinical techniques. *J Prosthet Dent* 61:669-675
77. Shahdad SA, Kennedy JG (1998) Bond strength of repaired composite resins: an *in vitro* study. *J Dent* 26:685-694
78. Özcan M (2002) The use of chairside silica coating for different dental applications. *J Prosthet Dent* 87:469-472
79. Brendeke J, Özcan M (2007) Effect of physicochemical aging conditions on the composite-composite repair bond strength. *J Adhes Dent* 9:399-406
80. Özcan M, Barbosa S, Melo R, Galhano G, Bottino M (2007) Effect of surface conditioning methods on the microtensile bond strength of resin composite to composite after aging conditions. *Dent Mater* 23:1276-1282
81. Özcan M, Valandro L, Amaral R, Leite F, Bottino M (2009) Bond strength durability of a resin composite on a reinforced ceramic using various repair systems. *Dent Mater* 25:1477-1483

Chapter 2

Biofilm Formation on Dental Restorative and Implant Materials

H.J. Busscher, M. Rinastiti, W. Siswomihardjo, H.C. van der Mei
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1 Introduction

Biofilms form on nearly all surfaces exposed to the natural environment.[1] Biofilm formation in the oral cavity is undoubtedly the most well-known example of biofilm formation and combating oral biofilm formation is an everlasting daily struggle for all of us. Biofilms not only form on dental hard and soft tissues as the major cause of caries and periodontal diseases,[2] but also on the multitude of biomaterial surfaces used for the restoration of function in the oral cavity. Although at a first glance, biofilm formation on biomaterial surfaces in the oral cavity may appear relatively harmless dependent on their location, its consequences may be severe. Similar to the development of periodontitis, biofilms on dental implants may lead to peri-implantitis.[3] An overhanging Class II restoration located in gingival margin is prone to bacterial colonization with an impact on gingival health.[4,5,6] Biofilm formation on resin composites not only degrades the material and roughens its surface,[7] but also causes colonizing bacteria to invade the interface[8] between the restoration and the tooth, leading to secondary caries[9] and pulp pathology[10] Biofilms around brackets in orthodontic treatment may cause demineralization of the surrounding enamel as a negative side-effect of the treatment[11,12] Consequently, the interest in new dental materials attracting less biofilm or releasing antimicrobial compounds is increasing.

The scope of this review is confined to the biomaterials properties affecting biofilm formation in the oral cavity, and the performance of different materials, including possible preventive, biomaterial-associated measures. At this point it is important to emphasize that biofilm formation in the oral cavity always occurs to a conditioning film existing of adsorbed salivary proteins preceeding adhesion of the first colonizing microorganisms, unless in cases of severe xerostomia. This review is not intended to deal with salivary protein adsorption as a separate step in biofilm formation and it suffices to say that salivary protein adsorption levels out differences in surface properties of different materials to an extent determined by the thickness,[13] composition [14] and conformation of proteins in the adsorbed layer.[15]

2 Mechanisms of biofilm

Despite many decades of research, a generally valid mechanism for bacterial adhesion and biofilm formation to surfaces does not yet exist.[16,17] The forces that mediate bacterial adhesion to surfaces, and for that matter also the adsorption of selective salivary proteins,[13] have been reasonably well identified in the past, and include ubiquitously present attractive Lifshitz-Van der Waals forces, electrostatic interactions and acid-base bonding. Using the extended DLVO theory,[18] named after Derjaguin-Landau-Verweij and Overbeek, these forces can be combined and a distance dependent interaction energy can be calculated, as shown in Fig. 1. Most naturally occurring surfaces are negatively-charged and electron-donating, while possessing a small hydrogen-donating component, which makes that the interaction energy at close approach is dominated by acid-base attraction, unless strong mono-polar repulsion occurs.

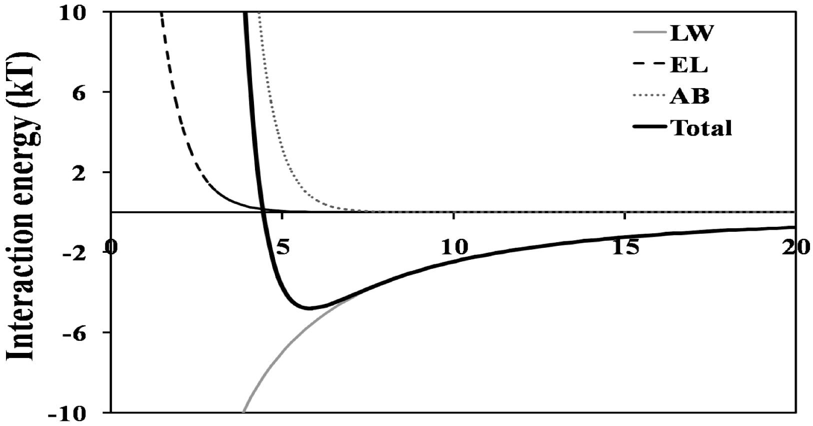

Fig. 1 Example of the DLVO-interaction energy as a function of distance for a situation of electrostatic repulsion between bacteria and substratum surfaces and acid-base attraction. Lifshitz-Van der Waals component (LW); Acid-base component (AB); Electrostatic component (EL); Total interaction energy (Total).
Note that a negative interaction energy indicates that adhesion is favourable.

In vitro, relationships have been described between substratum hydrophobicity, surface free energy and charge, depth of DLVO-interaction energy minima, or surface roughness and numbers of adhering bacteria. However, the number of studies describing exceptions is equally high as the number of studies establishing these relationships.[16,19] Often these relationship cover a range of a factor of two to utmost three in bacterial adhesion numbers and the differences between substrata with different properties rapidly disappear once the adhering bacteria are allowed to start growing into a more mature biofilm. *In vitro* relationships also depend heavily on experimental conditions, like the presence of shear, conditioning of substrata by an adsorbed salivary protein film, the bacterial strain or combination of strains used.[19] Moreover, although having been a focus point in the past, it is not a priori obvious that the depth of any interaction energy minimum should relate with numbers of adhering bacteria. Possibly, bacterial binding forces or the ease with which adhering bacteria or biofilms can be detached from substrata are more likely to relate with the different features from DLVO-interaction energy curves.

In vivo, the situation may appear more complex than *in vitro*, but surprisingly *in vivo* research has turned out much more decisive in indicating the parameters that influence oral biofilm formation.[20] Whereas *in vitro*, bacteria adhere to virtually every surface, regardless of its properties, supra-gingivally hydrophobic surfaces in the oral cavity attract far less plaque than more hydrophilic ones, as has been established over a nine days time period (see Fig. 2).[21]

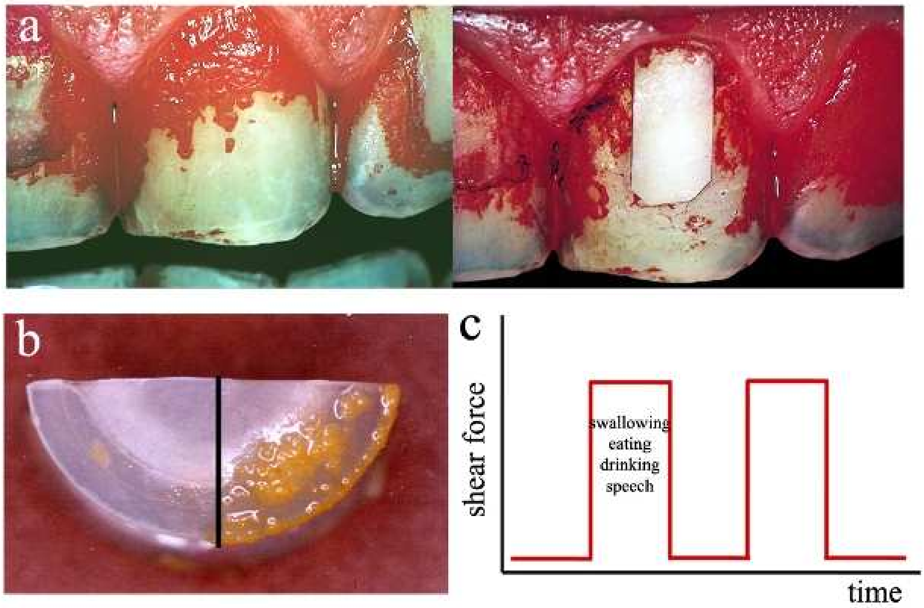

Fig. 2 The role of substratum hydrophobicity and fluctuating shear in biofilm formation in the oral and oropharyngeal cavity

- **a)** Nine days undisturbed plaque formation on a human front incisor (intermediate hydrophobicity) and a Teflon strip (hydrophobic) glued on a front incisor (from Quirynen *et al*., 1989).[21]
- **b)** Six weeks biofilm formation on a dual-sided hydrophobic (left-hand side)-hydrophilic (right-hand side) voice prosthesis (from Everaert *et al*., 1997).[22]
- **c)** Shear forces in the oral cavity can vary during the day between wide ranges, creating periods of bacterial adhesion and detachment (from Busscher *et al*., 1992).[23]

This observation has been confirmed in a study on oropharyngeal biofilm formation on voice prostheses,[22] encompassing a timescale of even six weeks (see also Fig. 2). Interestingly, these timescales allow extensive adsorption of salivary conditioning films, interactions with dietary components and adhesion of multiple bacterial strains and species. Yet, a hydrophobic surface harvests far less biofilm than a more hydrophilic one. Most likely, this is due to the fluctuating shear conditions in the oral cavity, as *in vitro* bacteria adhere also to hydrophobic surfaces, at least under constant shear conditions.[24] Hydrophobic surfaces placed sub-gingivally, for instance, do not harvest significantly less biofilm than hydrophilic surfaces,[25] which suggests that under fluctuating shear, as existing supra-gingivally, biofilm is occasionally sloughed off during periods of high oral shear. This is not withstanding the fact that bacteria do adhere to hydrophobic surfaces.

Nearly all studies indicate, that, under *in vivo* conditions, smooth surfaces attract less biofilm than rough ones.[26,27] From a series of split-mouth studies, it could be concluded that both an increase in surface roughness above a threshold of 0.2 µm and/or of the surface free energy facilitates biofilm formation on restorative materials. When both surface characteristics interact with each other, surface roughness was found to be predominant.[26]

3 Biofilms on acrylic resin denture base

Acrylic resin or polymethyl methacrylate has a wide variety of applications, such as impression trays, artificial teeth and denture bases. The material was developed in 1928 in various laboratories and was brought to market in 1933 by the Rohm and Haas Company. Soon after its introduction to the market, the material was widely used by the dental profession and by 1946, 98% of all dentures were constructed from methyl methacrylate polymers.[28]

One of the main clinical problems associated with the use of acrylic dentures is the adhesion of *Candida* which can lead to stomatitis,[29] although bacteria too adhere to acrylic surfaces.[30] *Candida albicans* is a dimorphic fungus that is commensal in the gastro- intestinal and reproductive tracts of healthy individuals[31] and is capable of initiating a variety of recurring superficial diseases, especially in the oral mucosa.[32] *In vitro C. albicans* has been described to be able to form a biofilm on biomaterial surfaces.[33] *C. albicans* in the oral cavity is mostly detected in a mixed biofilm with bacteria,[34,35] and it has been suggested that bacterial adhesion enhances subsequent adhesion of *Candida*.[30] Mechanical or chemical removal of fungal biofilms represents a significant problem,[32] as yeasts are known to adhere quite strongly to denture base materials,[36] possibly as a result of the presence of microporosity on the denture surface. Indeed, *C. albicans* adhesion is enhanced if the roughness of denture base materials is increased.[37] Recently it has been demonstrated that *C. albicans* biofilm formation on polyethylene oxide-modified denture base materials is discouraged *in vitro*.[33]

4 Biofilms on metallic biomaterials

Five days old oral biofilms on gold and amalgam surfaces *in vivo* are known to be thick and fully covering the substratum surfaces, but on the other hand were found to be little viable.[38] Earlier work showed that pieces of amalgam placed intra-orally for 24 and 72 h attracted about half the number of viable bacteria than titanium oxide.[39] There is limited knowledge about possible differences in mechanisms of bacterial adhesion to metal surfaces as compared with non-conducting polymer surfaces, but it has been suggested that electron-transfer plays a role in bacterial adhesion to conducting materials, like gold and amalgam.[40] In addition, upon approach of a negatively charged bacterium to a conducting material, an oppositely charged image may develop in the conducting material, creating a strong electrostatic attractive force.[41]

The low viability of oral biofilms on amalgam surfaces is probably due to the release of toxic compounds from the alloy. Amalgam consists for approximately 50% mercury, that may slowly diffuse from the amalgam into the biofilm. Therewith, it also becomes possible that bacteria develop resistance against mercury. *In vitro*, a higher number of bacteria resistant to mercury was found in biofilms grown on amalgam than on enamel,[42] but mercury resistance levels returned to baseline after 72 h. Interestingly, resistance to mercury was concurrent with resistance to a number of antibiotics, most notably tetracycline. The genes encoding for resistance to metals such as mercury, were found on the same mobile genetic elements as resistance to antibiotics. Note, that although the release of mercury from amalgam restorations may stimulate antibiotic resistance in oral bacteria, mercury-resistant bacteria were found in 71% of children without amalgam fillings and previous exposure to amalgam.[43]

The low viability (less than 2%) of oral biofilms on gold can not be due to the release of toxic compounds, since gold is completely inert.[38] Possibly full coverage by a relatively thick biofilm hampers the supply of nutrients into the biofilm leading to a low viability.

5 Biofilms on ceramics

Little is known about biofilms on ceramic surfaces. Inlays of two types of ceramic surfaces collected less plaque with reduced viability over a three days period of no oral hygiene than the natural tooth surface. Compared to gold and amalgam however, biofilms on ceramic biomaterials formed *in vivo* during five days[38] were relatively thin, but highly viable (34% to 86%). Note that this supports the suggestion above that thick biofilms are less viable than thin ones due to a hampered supply of nutrients into a thick biofilm.

6 Biofilms on resin composites and glass ionomer cements

Biofilm formation on resin composites and glass ionomer cements leads to a negative spiral of events,[7] in which the colonizing organisms cause severe deterioration of the surface (see Fig. 3), which on its turn promotes biofilm formation and therewith more extensive deterioration of the surface. The clinical manifestation of this downward spiral is the development of caries around or below a restoration.[44]

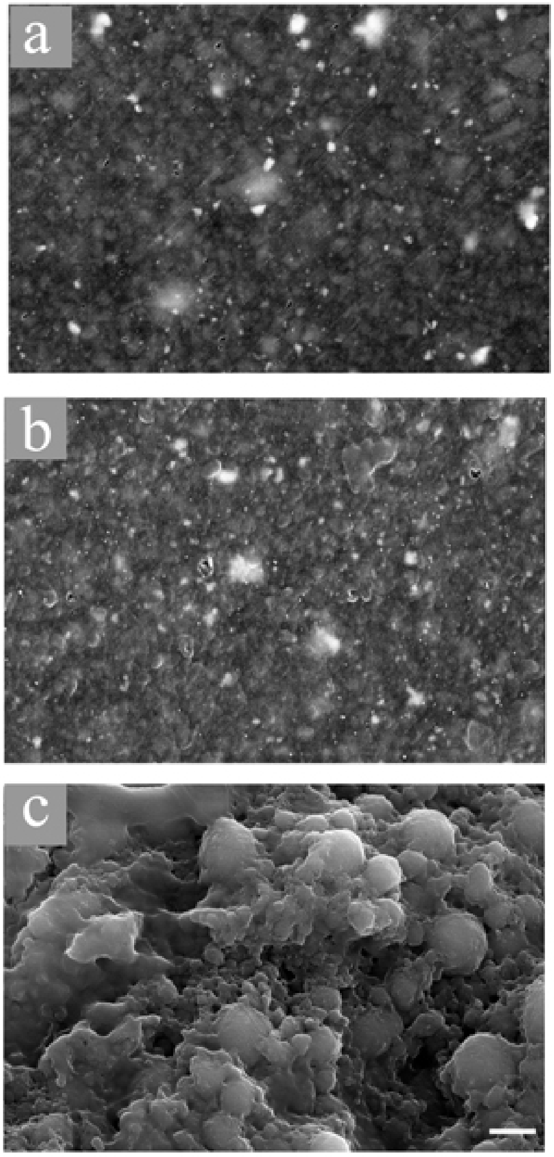

Fig. 3 Scanning electron micrographs of nanohybrid-resin composite surfaces
- **a)** prior to biofilm formation for 14 days *in vitro*
- **b)** after biofilm formation for 14 days *in vitro*
- **c)** after having been 180 days *in vivo*

Bar marker represent 2 µm.

Surface deterioration of resin composites and glass ionomer cements has been demonstrated by increased roughness and sometimes a decreased microhardness of the materials upon exposure to biofilms *in vitro*. After one month exposure to a *Streptococcus mutans* ATCC27351 biofilm, a BIS-GMA, UDMA resin composite with filler particle sizes between 0.04 and 0.2 µm showed an increase in surface roughness from less than 10 to above 40 nm without affecting the microhardness, suggesting the removal of filler particles based on the roughness dimensions created. Resin composites with larger sized (0.01 to 3.5 µm) filler particles became significantly less rough (around 15 nm) after biofilm growth.[7] In a similar study, one month exposure to *S. mutans* biofilms showed an increase in surface roughness from around 0.1 µm to well over 1 µm of resin-modified and conventional glass ionomer cements, while only the microhardness of the resin-modified cement was negatively affected.[45] Clearly, *in vivo* the presence of biofilm is just one of the factors that may stimulate surface degradation, other factors being acidic fluid intake, temperature fluctuations or simply the presence of an aqueous environment as such.

The degree of conversion of resin composites is never complete and approximately 5% to 10% of unpolymerized monomer can be extracted in water. It has been suggested that especially the release of EGDMA (ethyleneglycol dimethylacrylate) and TEGDMA (triethyleneglycol dimethacrylate) from resin composites may enhance the growth of cariogenic bacteria, like mutans streptococci and lactobacilli, organisms found mostly along the margins of composite fillings,[46] and also enhance the glucosyltransferase activity in *Streptococcus sobrinus*.[47] More recently, Khalichi *et al.*[48] found that triethyleneglycop, as the ether portion of TEGDMA, modulates the expression levels of glucosyltransferase B (gtf B) involved in biofilm formation and yfuV as a putative transcription regulator in *S. mutans*. Interestingly, Takahashi *et al.*[49] found that growth stimulation of *S. sobrinus* and *Streptococcus sanguis* by EGDMA monomers as measured by optical density was not accompanied by an increase in the numbers of colony forming units harvested. Consequently, the increase in optical density was ascribed to an increased size of the bacteria rather than to increased bacterial concentrations, which could be microscopically confirmed by the presence of a vesicular material surrounding the bacteria. Extraction and chemical analysis of this vesicular material showed a composition comparable with EGDMA-polymer. Effects of monomer release became smaller upon increasing the light curing time of the composites.[48,50] Also components of dentine bonding agents, such as HEMA or TEGDMA, have been shown to stimulate the growth of cariogenic organisms like *S. sobrinus* and *Lactobacillus acidophilus*.[51] Direct extrapolation of these *in vitro* studies to the clinical situation is difficult, as composite surfaces are usually polished, affecting the surface properties,[52] while most importantly the volume in which monomers are released is large and continuously refreshed by salivary excretion and fluid intake.

The setting of glass ionomer cements is via an acid-base reaction between fluoroaminosilicate glass particles and a polyacrylic acid solution, yielding a structure that is dimensionally more stable than composites. Hence, the use of glass ionomer cements potentially reduces microleakage by adhering to tooth structure and enhances fluoride release with a potential impact on oral biofilm formation. Fluoride can act as a buffer to neutralize acids produced by bacteria [53] and suppresses the growth of caries-related oral bacteria.[54] Glass ionomer cement indeed collects a thin biofilm with a low viability (2% to 3%), possibly as a result of fluoride release.[38] Especially levels of Streptococci, including *S. mutans*[55] and *S. sanguis*[56] appeared reduced. However, an *in vitro* study[57] also showed that glass ionomer cements containing fluoride did not reduce the amount of bacterial growth and biofilm formation on the surfaces bathed in saliva. This suggests that either fluoride is not a dominant factor in controlling biofilm formation or that its concentration is too low to be effective.[58]

7 Material-associated preventive measures

Fluoride-releasing composites have been developed in order to reduce biofilm formation and its effects on the surrounding enamel. However, the amount of fluoride released from composites is lower than from glass ionomer cement and often not effective in inhibition of bacterial growth[59] and fluoride release decays over time. This is a general drawback of antimicrobial releasing materials, and therefore antibacterial components should be immobilized on biomaterials surfaces in a way that antibacterial efficacy is maintained. For that reason, resin composites have been modified by adding antibacterial components, such as 12-methacryloyloxydodecylpyridinium bromide combined with quaternary ammonium and a methacryl group in the resin matrix. This material inactivated bacteria upon coming into contact with its surface, hence inhibiting biofilm formation on the composite surface,[60,61,62] even after contact with saliva.[63] Whether or not these modification yield clinically significant results under the dynamic conditions of the oral cavity remains to be seen however.[64] For titanium implants, anodization by discharge in a NaCl solution has been suggested to reduce adhesion of viable bacteria, as peroxidation products of Ti-Cl were able to destroy surface structures of adhering bacteria,[65] but here too clinical efficacy remains to be demonstrated.

References

1. Moons P, Michiels CW, Aertsen A (2009) Bacterial interactions in biofilms. *Crit Rev Microbiol* 35:157-168.
2. Sbordone L, Bortolaia C (2003) Oral microbial biofilms and plaque-related diseases: microbial communities and their role in the shift from oral health to disease. *Clin Oral Invest* 7:181-188.
3. Grossner-Schreiber B, Teichmann J, Hannig M, Dorfer C, Wenderoth DF, Ott SJ (2009) Modified implant surfaces show different biofilm compositions under *in vivo* conditions. *Clin Oral Imp Res* 20:817-826.
4. Jansson L, Ehnevid H, Lindskog S, Blomlöf L (1994) Proximal restoration and periodontal status. *J Clin Periodontol* 21:557-582.
5. Joseph I, Blomster S, Forsgardh A, Bergman E, Berglund E. Foss L, Reinhardt EL, Sjoberg B (1997) Interactory effect between marginal plaque and subgingival restorations on periodontal pocket depth. *Swed Dent J* 21:77-83.
6. Cenci MS, Lund RG, Pereira CL, de Carvalho RM, Demarco FF (2006) *In vivo* and *in vitro* evaluation of Class II composite resin restorations with different matrix systems. *J Adhes Dent* 26:311-317.
7. Beyth N, Bahir R, Matalon S, Domb AJ, Weiss EI (2008). *Streptococcus mutans* biofilm changes surface-topography of resin composites. *Dent Mater* 24:732-736.
8. Carvalho RM, Pereira JC, Yoshiyama M, Pashley DH (1996). A review of polymerisation contraction: the influence of stress development versus stress relief. *Op Dent* 21:17-24.
9. Collins CJ, Bryant RW, Hodgel KLV (1998). A clinical evaluation of posterior composite resin restorations: 8-year findings. *J Dent* 26:311-317.
10. Pashley DH (1990). Clinical considerations of microleakage. *J Endod* 16:70–7.
11. Papaioannou W, Gizani S, Nassika M, Kontou E , Nakou M (2007). Adhesion of *Streptococcus mutans* to different types of brackets. *Angle Orthod* 77:1090-1095.
12. Mitchell L (1992). Decalcification during orthodontic treatment with fixed appliances--an overview. *Br J Orthod* 19:199-205.
13. Hannig C, Hannig M (2009). The oral cavity—a key system to understand substratum-dependent bioadhesion on solid surfaces in man. *Clin Oral Investigations* 13:123-139.
14. Vroman L (2008). Finding seconds count after contact with blood. *Colloids Surf B Biointerfaces* 62:1–4.

15. Norde W (2008). My voyage of discovery to proteins in flatlan and beyond. *Colloids Surf B Biointerfaces* 61:1–9.
16. Bos R, Van der Mei HC, Busscher HJ (1999). Physico-chemistry of initial microbial adhesive interactions-its mechanisms and methods for study. *FEMS Microbiol Rev* 23:179-230.
17. Hermansson M (1999). The DLVO theory in microbial adhesion. *Coll Surf B: Biointerfaces* 14:105-119.
18. Van Oss CJ (1995). Hydrophobicity of biosurfaces-Origin, quantitative determination and interaction energies. *Coll Surf B: Biointerfaces* 5:91-110.
19. Bakker DP, Postmus BR, Busscher HJ, Van der Mei HC (2004). Bacterial strains isolated from different niches can exhibit different patterns of adhesion to substrata. *Appl Environ Microbiol* 70:3758-3760.
20. Glantz PO (1969). On wettabilty and adhesiveness. *Odontol Rev* 20:1–132.
21. Quirynen M, Marechal M, Busscher HJ, Weerkamp AH, Arends J, Darius PL, Van Steenberghe D (1989). The influence of surface free-energy on planimetric plaque growth in man. *J Dent Res* 68:796-799.
22. Everaert EPJM, Mahieu HF, Wong Chung RP, Verkerke GJ, Van der Mei HC, Busscher HJ (1997). A new method for *in vivo* evaluation of biofilms on surface-modified silicone rubber voice prostheses. *Eur Arch Oto-Rhino-Laryngol* 254:261-263.
23. Busscher HJ, Cowan MM, Van der Mei HC (1992). On the relative importance of specific and non-specific approaches to oral microbial adhesion. *FEMS Microbiol Rev* 88:199-210.
24. Boks NP, Kaper HJ, Norde W, Van der Mei HC, Busscher HJ (2009). Mobile and immobile adhesion of staphylococcal strains to hydrophilic and hydrophobic surfaces. *J Coll Interf Sci* 331:60-64.
25. Quirynen M, Marechal M, Busscher HJ, Weerkamp AH, Darius PL, Van Steenberghe D (1990). The influence of surface free-energy and surface roughness on early plaque formation. An *in vivo* study in man. *J Clin Periodontol* 17:138-144.
26. Teughels W, Van Assche N, Sliepen I, Quirynen M (2006). Effect of material characteristics and/or surface topography on biofilm development. *Clin Oral Imp Res* 17(Suppl. 2):68-81.
27. Verran J, Maryan CJ (1997). Retention of *Candida albicans* on acrylic resin and silicone of different surface topography. *J Prosthet Dent* 77:535-539.
28. Powers JM, Sakaguchi RL (2006). Craig's Restorative Dental Materials. 12th ed. Mosby Elsevier, pp. 514.

29. Ramage G, Tomsett K, Wickes BL, Lopez Ribot JL, Reddingf SW (2004). Denture stomatitis a role for Candida biofilm. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 98:53 – 59.
30. Verran J, Motteram KL (1987). The effect of adherent oral streptococci on the subsequent adherence of Candida albicans to acrylic *in vitro*. *J Dent* 15:73-76.
31. Elguezabal N, Maza JL, Dorronsoro S, Ponton J (2008). Whole saliva has a dual role on the adherence of *Candida albicans* to polymetylmethacrylate. *Open Dent* J 2:1-4.
32. Samaranayake YH, Samaranayake LP (2001). Experimental oral candiasis in animal models. *Clin Microbiol Rev* 14:398-429.
33. Chandra J, Patel JD, Li J, Zhou G, Mukherjee PK, McCormick TS, Anderson JM, Ghannoum MA (2005). Modification of surface properties of biomaterials influences the ability of *Candida albicans* to form biofilms. *Appl Environ Microbiol* 71:8795-8801.
34. Bamford CV, d'Mello A, Nobbs AH, Dutton LC, Vickerman MM, Jenkinson HF (2009). *Streptococcus gordonii* modulates *Candida albicans* biofilm formation through intergeneric communication. *Infect Immun* 77:3696-3704.
35. Holmes AR, Cannon RD, Jenkinson HF (1995). Interactions of *Candida albicans* with bacteria and salivary molecules in oral biofilms. *J Ind Microbiol* 15:208-213.
36. Nalbant AD, Kalkanci A, Filiz B, Kustimar S (2008). Effectiveness of different cleaning agents against the colonization of *Candida* spp and the *in vitro* detection of the adherence of these yeast cells to denture acrylic surfaces. *Yonsei Med J* 4:647-654.
37. Radford DR, Sweet SP, Challacombe SJ, Walter JD (1998). Adherence of *Candida albicans* to denture-base materials with different surface finishes. *J Dent* 26:577-583.
38. Auschill TM, Arweiler NB, Brecx M, Reich E, Sculean A, Netuschil L (2002). The effect of dental restorative materials on dental biofilm. *Eur J Oral Sci* 110:48-53.
39. Leonhardt A, Olsson J, Dahlen G (1995). Bacterial Colonization on Titanium, Hydroxyapatite, and Amalgam Surfaces *in vivo*. *J Dent Res* 74:1607–1612.
40. Poortinga AT, Bos R, Busscher HJ (1999). Measurement of charge transfer during bacterial adhesion to an indium tin oxide surface in a parallel plate flow chamber. *J Microbiol Methods* 38:183-189.
41. Mei L, Van der Mei HC, Ren Y, Norde W, Busscher HJ (2009). Poisson analysis of streptococcal bond strengthening on stainless steel with and without a salivary conditioning film. *Langmuir* 25:6227-6231.

42. Ready D, Pratten J, Mordan N, Watts E, Wilson M (2007). The effect of amalgam exposure on mercury-and antibiotic-resistance bacteria. *Int J Antimicrob Agents* 30:34-39.

43. Ready D, Qureshi F, Bedi R, Mullany P, Wilson M (2003). Oral bacteria resistant to mercury and to antibiotics are present in children with no previous exposure to mercury amalgam restorative materials. *FEMS Microbiol Lett* 223:107-111.

44. Sousa RP, Zanin ICJ, Lima JPM, Vasconcelos SMLC, Melo MAS, Beltrao HCP, Rodrigues LKA (2009). In situ effects of restorative materials on dental biofilm and enamel demineralisation. *J Dent* 37:44-51.

45. Fucio SBP, Carvalho FG, Sobrinho LC, Sinhoreti MAC, Puppin-Rontani RM (2008). The influence of 30-day-old *Streptococcus mutans* biofilm on the surface of esthetic restorative materials-An *in vitro* study. *J Dent* 36:833-839.

46. Hansel C, Leyhausen G, Mai UEH, Geurtsen W (1998). Effects of various resin composite (co)monomers and extracts on two caries-associated microorganisms *in vitro*. *J Dent Res* 77:60-76.

47. Kawai K, Tsuchitani Y (2000). Effects of resin composite components on glucosyltransferase of cariogenic bacterium. *J Biomed Mater Res* 51:123-127.

48. Khalichi P, Singh J, Cvitkovitch DG, Santerre JP (2009). The influence of triethylene glycol derived from dental composite resins on the regulation of *Streptococcus mutans* gene expression. *Biomaterials* 30:452-459.

49. Takahashi Y, Imazato S, Russell RRB, Noiri Y, Ebisu S (2004). Influence of resin monomers on growth of oral streptococci. *J Dent Res* 83:302-306.

50. Brambilla E, Gagliani M, Ionescu A, Fadini L, García-Godoy F (2009). The influence of light-curing time on the bacterial colonization of resin composite surfaces. *Dent Mater* 25:1067-1072.

51. Schmalz G, Ergucu Z, Hiller KA (2004). Effect of dentin an the antibacterial activity of dentin bonding agents. *J Endod* 30:352-358.

52. Carlen A, Nikdel K, Wennerberg A, Holmberg K, Olsson J (2001). Surface characteristics and *in vitro* biofilm formation on glass ionomer and composite resin. *Biomaterials* 22:481-487.

53. Nicholson JW, Aggarwal A, Czarnecka B, Limanowska-Shaw H (2000). The rate of change of pH of lactic acid exposed to glass-ionomer dental cements. *Biomaterials* 21:1989-1993.

54. Nakajo K, Imazato S, Takahashi Y, Kiba W, Ebisu S, Takahashi N (2009). Fluoride released from glass-ionomer cement is responsible to inhibit the acid production of caries-related oral streptococci. *Dent Mater* 25:703-708.

55. Seppa L, Korhonen A, Nuutinen A (1995). Inhibitory effect on *S. mutans* by fluoride-treated conventional and resin-reinforced glass ionomer cements. *Eur J Oral Sci* 103:182-185.

56. Hengtrakool C, Pearson GJ, Wilson M (2006). Interaction between GIC and *S. sanguis* biofilms: Antibacterial properties and changes of surface hardness. *J Dent* 34:588-597.

57. Al-Naimi OT, Itota T, Hobson RS, McCabe JF (2008). Fluoride release for restorative materials and its effect on biofilm formation in natural saliva. *J Mater Sci: Mater Med* 19:1243-1248.

58. Wiegand A, Buchalla W, Attin T (2007).Review on fluoride-releasing restorative materials – Fluoride release and uptake characteristics, antibacterial activiity and influence on caries formation. *Dent Mater* 23:343-362.

59. Yap AUJ, Khor E, Foo SH (1999). Fluoride release and antibacterial properties of new-generation tooth colored restoratives. *Oper Dent* 24:297-305.

60. Imazato S, Torii M, Tsuchitani Y, McCabe JF, Russell RRB (1994a). Incorporation of bacterial inhibitor into resin composite. *J Dent Res* 73:1437–1443.

61. Imazato S, McCabe JF (1994b). Influence of incorporation of antibacterial monomer on curing behavior of a dental composite. *J Dent Res* 73:1641–1645.

62. Imazato S, Tarumi H, Kato S, Ebisu S (1999). Water sorption and colour stability of composites containing the antibacterial monomer MDPB. *J Dent* 27:279–283.

63. Ebi N, Imazato S, Noiri Y, Ebisu S (2001) Inhibitory effect of resin composite containing bactericide-immobilized filler on plaque accumulation. *Dent Mater* 17:485-491.

64. Imazato S (2003) Antibacterial properties of resin composites and dentin bonding systems. *Dent Mater* 19:449-457.

65. Omari S, Shibata Y, Arimoto T, Igarashi T, Baba K, Miyazaki T (2009) Microorganism and cell viability on antimicrobially modified titanium. *J Dent Res* 88:957-962.

Chapter 3

Comparison of Shear and Microtensile Failure Stresses in the Repair of Dental Composite Restorations

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Submitted to Dental Materials

1 Introduction

Composite resins are widely used in dentistry for the restoration of anterior and posterior tooth surfaces. Restoration not only involves bonding of a composite resin to dental hard tissues, but frequently failure of existing restorations can be repaired by bonding of an additional layer of composite resin to an existing composite restoration.[1,2] Composite restorations may need immediate repair, when the anatomical shape and aesthetics are not satisfactory. Alternatively, due to composite degradation under the influence of the dynamics in the oral environment, discoloration, microleakage, wear, ditching at the margins, delamination or simply fracture may occur, necessitating repair.[3-5] However, repair of aged composite restorations does not yield the same bond strengths as found in immediate repair.[6] In order to enhance composite-to-composite bonding in both immediate repair and repair after aging, various surface conditioning methods have been developed and compared, including intermediate adhesive resin application (IAR-application) or chair side silica coating and silanization followed by application of its specific IAR (SC-application).[1,7,8]

In vitro, analysis of composite-to-composite bonding can be done either by applying a shear or microtensile forces.[9-12] Shear bond strength evaluation requires easier specimen preparation and alignment during measurement than microtensile bond strength measurement.[13-15] However, measurement of shear bond strength has been suggested to be less reliable than of microtensile bond strength,[16] because the adhesive interface in microtensile bond strength analysis is relatively small, invoking a more uniform stress distribution and therewith allowing better access to the true interfacial bond strength.[17] To date, there is no consensus on whether the strength of composite-to-composite bonding should be evaluated in a shear or microtensile mode. Clinically it can be argued that, in the repair of composite restorations, applied forces are predominantly in the shear mode. Standard deviations in mean values of bond strengths are always high and sometimes even 100%. McCabe and Carrick[18] have taken advantage of these large standard deviations to calculate a so-called dependability of the bond or Weibull modulus from measured bond strength data over a large number of specimens. The Weibull distribution expresses the probability of failure as a function of the applied force or stress,[18,19] and can be used to calculate the Weibull modulus. Low dependabilities or Weibull moduli indicate a failure distribution over a wide range of applied force values, whereas high Weibull moduli refer to a more narrow range of applied forces or stresses causing failure. Weibull moduli should thus be taken into account next to a simple comparison of failure stress values, especially in case of brittle fracture modes, as for composite-to-composite bonding, that have high standard deviations. Weibull analysis has been used in dentistry to evaluate strength of ceramics and adhesives,[20-22] but a direct comparison of Weibull analyses for shear and microtensile data has never been performed.

Therefore, the objectives of this study were firstly to compare shear and microtensile repair bond strengths for nanohybrid and nanofilled composite resins by comparison of failure shear and microtensile stresses, failure type and Weibull analyses. Secondly, it is investigated whether aging by thermocycling and surface conditioning by IAR- or SC-application affects the comparison of both modes of bond strength evaluation.

2 Materials and methods

2.1 Composite resins and specimens preparation

The product names, manufacturers, chemical compositions and batch numbers of the materials used in this study are listed in Table 1.

Table 1 Product and company names, chemical compositions, abbreviations and batch numbers of the materials used in this study.

Product name	Company name	Chemical composition	Abbre-viations	Batch Number
COMPOSITE RESIN				
Tetric Evo Ceram (nanohybrid)	Ivoclar Vivadent AG, Schaan, Liechtenstein	Bis-GMA, TEGDMA, UDMA , Ba-Al-F-B-Silicate, SiO_2, mixed oxide, YbF3 containing fillers (48.5 v%)	TE	J04088
Filtek Supreme XT (nanofilled)	3M ESPE, St. Paul, MN, USA	TEGDMA, UDMA, Bis-EMA, Zirconia-Silica, Silica containing fillers (57.7 v%)	FS	6 BG
TRIBOCHEMICAL SILICA COATING KIT (for SC-application)				
CoJet®-Sand	3M ESPE AG, Seefeld, Germany	Aluminum trioxide particles coated with silica, particle size: 30 µm	SC	165092
ESPE®-Sil	3M ESPE AG, Seefeld, Germany	3-methacryloxypropyltrimethoxy-silane, ethanol	Silane	152745
INTERMEDIATE ADHESIVE RESIN (for IAR-application)				
Visio™-Bond (for tribochemical silica coating treatment)	3M ESPE AG, Seefeld, Germany	Dicyclopentyldimethylene diacrylate 2-propenoic acid, 2-methyl, 2-[(2-hydroxyethyl)(3-methoxypropyl)amino]ethyl ester	VB	161808
Multilink (for TE)	Ivoclar Vivadent AG, Schaan, Liechtenstein	Primer A: Water, initiators (sulfonate, amines) Primer B: Phosphonic acid acrylate, HEMA, TEGDMA, methacrylate modified polyacrylic acid	M	Primer A: H10145 Primer B: 09713
Adper Scotchbond 1XT (for FS)	3M ESPE, St. Paul, MN, USA	Dimethacrylate, HEMA, polyalcenoic acid copolymer, silane treated colloidal silica, ethanol, water, photoinitiator	A	4BM

Two commercially available composites were used. For shear bond strength evaluation, cylindrical undercut cavities (diameter: 5.5 mm, depth: 3.5 mm) were prepared in auto-polymerized polymethylmethacrylate surrounded by a polyvinyl carbon ring (3 specimens per ring) and filled with composites, as described below. The composites for microtensile bond strength measurements were inserted into a rectangular silicone mold (5 mm x 6 mm x 6 mm). Cavities and molds were filled with unpolymerized composite resins using a hand instrument and photo-polymerized incrementally in layers with a thickness less than 2 mm. Each increment was photo-polymerized with a halogen photo-polymerization unit (Optilux 501, Kerr, USA) for 20 s at a distance of 2 mm from the surface. Light intensity was higher than 400 mW/cm^2, as verified by a radiometer after every 6 specimens (Demetron LC, Kerr). During final photo-polymerization, the top layer was covered with a 100 µm thick translucent Mylar strip (KerrHawe SA, Bioggio, Switzerland) in order to create a smooth surface and to prevent the formation of an oxygen inhibition layer. Composite blocks for microtensile bond strength measurements were removed from their molds and all specimens were kept in the dark after polymerization, and randomly assigned to one of the surface conditioning methods.

2.2 *Surface conditioning and aging methods*

Forty specimens for shear bond strength- and 32 specimens for microtensile bond strength evaluation were randomly divided into four groups, comprising non-aged specimens and specimens aged by thermocycling, both for two different types of surface conditioning. Thermocycling was done by 5000x cycles in water from 5 to 55°C with a dwell time of 30 s at each temperature and a transfer time from one water bath to the other of 5 s (Willytec, Gräfelfing, Germany[6]). Surface conditioning of the non-aged and thermocycled composites was done by applying two surface conditioning methods :

Intermediate adhesive resin (IAR)-application: IARs of the corresponding composite (Table 1) were applied in a thin layer on the substrates using a micro-brush. Subsequently, the solvent was gently air-thinned under compressed air and finally photo-polymerized for 20 s, according to manufacturer's instructions.

Tribochemical silica coating (SC)-application: Tribochemical treatment was done using an intra-oral air-abrasion device (Dento-Prep™, RØNVIG A/S, Daugaard, Denmark) filled with 30 µm alumina particles coated with silica from a distance of approximately 10 mm at a pressure of 2.5 bars for 4 s. Following surface conditioning, loose particles were gently air blown. The conditioned substrates were then coated with a 3-methacryloxypropyl-trimethoxysilane coupling agent, γ-MPS allowing 5 min for reaction. Finally, an IAR specific to the SC procedure was applied with a microbrush, air thinned and light-polymerized for 20 s.

2.3 *Bond strength evaluation*

For shear bond strength evaluation, corresponding composite resins were bonded onto their substrates using translucent polyethylene molds (inner diameter: 3.5 mm, height: 5 mm). Bonding procedures were carried out in accordance with the manufacturers` instructions (see also above). Polyethylene molds were then gently removed from the test specimens. Shear bond strength was determined using a Universal Testing Machine (Zwick ROELL X2.5MA 18-1-3/7, Zwick, Ulm, Germany). Specimens were mounted to the jig of the testing machine, and a shear load was applied vertically at a crosshead speed of 1 mm/min to the composite-composite interface and the maximum force to produce debonding was recorded.

For microtensile bond strength evaluation, unpolymerized composite resin was incrementally bonded onto rectangular composites blocks, prepared, aged and conditioned as described above, until a height of 5 mm using a hand instrument and rectangular shaped, and photo-polymerized. Composite–composite blocks were sectioned using a diamond saw (Microdont, São Paulo, Brazil, n. 34570) at low-speed under water cooling. The blocks were positioned perpendicularly with respect to the diamond blade. The first 1 mm sections taken from the sides were discarded and only central specimens were used for experiments yielding sticks with a length of 6 mm and adhesive area of 1 ± 0.1 mm^2. The sticks were positioned parallel to the loading axis of a Universal Testing Machine (EMIC DL-1000, EMIC, São José dos Pinhais, Brazil), and microtensile test measurement was performed at a cross speed 1 mm/min.

After debonding, fracture surfaces were examined with a light microscope (MP 320, Carl Zeiss, Jena, Germany; x40) to distinguish adhesive and cohesive failure in the composite surfaces. Failures types were categorized as cohesive failure in the substrate, or adhesive failure at the interface.

2.4 Weibull Analysis

For most cases, the Weibull distribution is given by

$$P_\sigma = 1 - \exp\left\{ -\left(\frac{\sigma}{\sigma_0} \right)^m \right\} \qquad (1)$$

in which P_σ denotes the failure probability, σ the applied stress (shear or microtensile) and m the dependability of the bond or Weibull modulus. σ_0 is a normalization parameter, without physical significance.[18] The failure probability for the different experimental failure stresses P_σ is obtained from a ranking of the failure bond stresses measured in ascending order according to[18]

$$P_\sigma = \frac{n}{N+1} \qquad (2)$$

where n is the rank number of the data point and N is the total number of data. Subsequently, the double logarithm of Eq. 1 was plotted, i.e. $\ln(\ln(1 - P_\sigma))$ against $\ln(\sigma)$, yielding a straight line from which the Weibull modulus m and the normalization parameter σ_0 can be calculated by linear regression, together with their respective confidence intervals and a linear correlation coefficient to indicate the goodness of the fit to the Weibull equation.

2.5 Statistical analysis

The means of each group were analyzed by three-way analysis of variance (ANOVA), with bond strength as the dependent variable and aging, conditioning and composite types as the independent factors (SPSS 15.0

software for Windows, SPSS Inc., Chicago, IL, USA). *P* values less than 0.05 were considered to be statistically significant in all tests.

3 Results

3.1 Failure analysis

The mean failure shear and microtensile stresses with their percentages of cohesive failure are shown in Table 2 Composite type, aging and surface conditioning method affected both shear and microtensile failure stresses significantly ($p < 0.05$).

Mean failure stresses in shear are approximately three-fold lower than in a microtensile mode. Standard deviations are high up to 50%, regardless of the mode of evaluation, as common in bond strength measurements.[23] Failure modes after shear application were more frequently cohesive than after application of a microtensile force, with the exception of thermocycled composites after IAR-application.

Table 2 Mean failure stresses and percentages of cohesive failure in the substrate for the two different composite resins prior to (non-aged) and after thermocycling. Composite surfaces were either conditioned by IAR- or SC-application. ± denotes standard deviations over 10 specimens for shear bond strength and 32 specimens for microtensile bond strength measurements. For abbreviations, see Table 1

Composite	**Surface Conditioning**	**Shear Mode**		**Microtensile Mode**	
		Failure Stress (MPa)	**Cohesive Failure (%)**	**Failure Stress (MPa)**	**Cohesive Failure (%)**
		Non-aged			
TE	IAR-application	21.7 ± 7.9	20	53.7 ± 19.3	10
	SC-application	18.0 ± 4.9	100	47.7 ± 13.1	15
FS	IAR-application	22.8 ± 8.7	100	46.9 ± 20.9	43
	SC-application	28.0 ± 5.8	100	51.1± 13.6	23
		After thermocycling			
TE	IAR-application	12.9 ± 3.1	10	32.3 ± 8.7	10
	SC-application	15.9 ± 7.0	60	43.9 ± 15.8	8
FS	IAR-application	7.0 ± 3.1	0	38.1 ± 9.6	8
	SC-application	17.7 ± 9.7	60	45.2 ± 10.5	14

3.2 *Weibull analysis*

The Weibull moduli and the stresses predicted to yield 5% failure ($\sigma_{0.05}$) are presented in Table 3.

Table 3 Weibull moduli (m), and their 95% confidence intervals (CI) and the stress ($\sigma_{0.05}$, MPa) expected to yield a 5% chance of failure for two different composite resins prior to (non-aged) and after thermocycling. Composite surfaces were either conditioned by IAR- or SC-application.

Composite	Surface Conditioning	Shear Mode			Microtensile Mode		
		m	CI	$\sigma_{0.05}$	m	CI	$\sigma_{0.05}$
Non-aged							
TE	IAR-application	2.6	1.9 – 3.6	8.0	2.8	2.3 – 3.4	21.3
	SC-application	3.5	2.5 – 4.8	8.6	4.1	3.5 – 4.8	25.3
FS	IAR-application	1.9	1.4 – 2.6	5.7	2.1	1.6 – 2.7	13.4
	SC-application	4.3	3.1 – 5.9	15.4	4.0	3.3 – 4.8	26.9
After thermocycling							
TE	IAR-application	3.9	2.8 – 5.4	6.4	4.0	3.4 – 4.7	16.9
	SC-application	1.7	1.2 – 2.3	3.3	4.0	3.4 – 4.8	19.9
FS	IAR-application	2.2	1.6 – 3.0	1.9	2.8	2.3 – 3.3	17.3
	SC-application	1.4	1.0 – 1.9	2.6	4.7	4.0 – 5.5	26.2

Correlation coefficients of the fits to the Weibull equation Eq. (1) varied between 0.93 and 0.99, indicating that the data fit the Weibull distribution well.[22] Weibull moduli for shear data are similarly low than obtained for microtensile data. The cumulative failures distribution according the Weibull equation for shear and microtensile stresses are presented in Fig. 1.

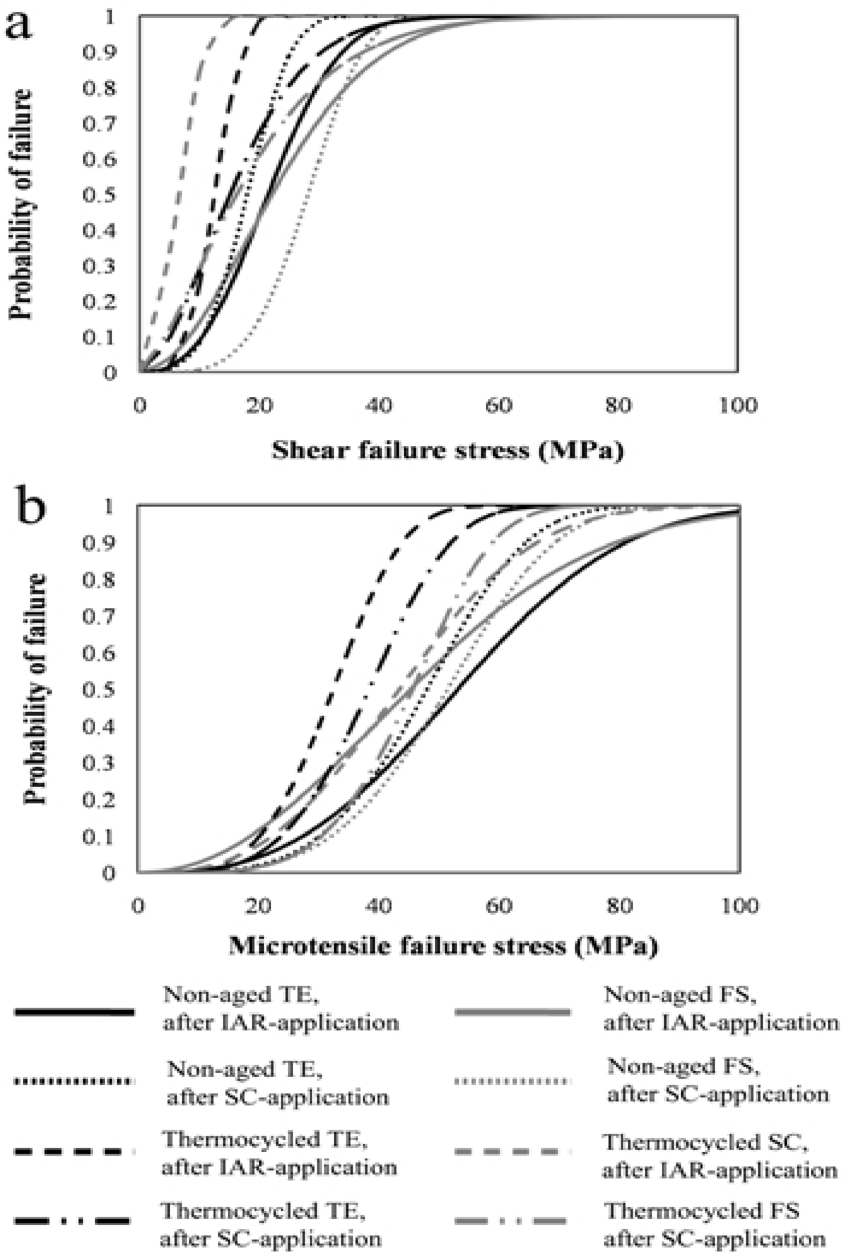

Fig. 1 Probability of failure versus

- **a)** the shear stress applied
- **b)** the microtensile stress applied

for two different composite resins prior to (non-aged) and after thermocycling. Composite surfaces were either conditioned by IAR- or SC-application.

From Figs. 1a and 1b it can be seen, that 5% failure in a microtensile mode requires higher stresses than required to cause 5% failure by shear. Interestingly, there is a good correlation between $\sigma_{0.05}$ and the mean failure shear stresses (see Fig. 2a), with a correlation coefficient of 0.8. In a microtensile mode, no correlation (R = 0.5) exists between the mean failure microtensile stress and $\sigma_{0.05}$, obtained from the Weibull distribution (see Fig. 2b).

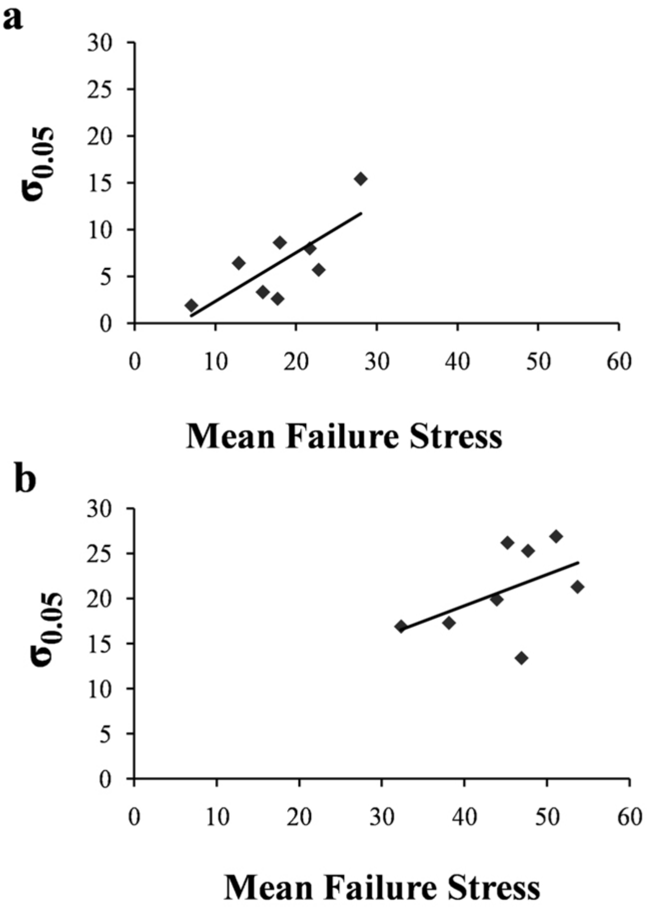

Fig. 2 Shear **(a)** and microtensile **(b)** stresses causing 5% failure as a function of the mean failure shear and microtensile stresses, respectively.

4 Discussion

In this paper, we compared shear and microtensile repair bond strengths for a nanohybrid and nanofilled composite resin by failure shear and microtensile stresses, failure type and Weibull analyses, prior to and after aging by thermocycling and after IAR- or SC-application. This is the first time Weibull analyses for shear and microtensile evaluations of composite-to-composite bonding has been compared in one and the same study. Weibull moduli were similar for shear and microtensile evaluations and generally low, indicating that bonds were not very reliable. 5% failure levels indicated by the Weibull distributions corresponded with mean failure shear stresses, but not with mean microtensile stresses. The Weibull moduli may be influenced by the methodology of failure stress measurements and the dependability of bond strength itself, but both factors can not be separated. Weibull moduli found here however, are comparable with values for failure shear stress distributions of composite-to-dentin bonding (2.8 - 4.1)[24] and failure microtensile stress distributions of composite-to-composite bonding after aging (3.3 – 6.4).[25] Exact values for the Weibull modulus depend on experimental conditions, but from a clinical point of view, the values obtained here must all be regarded as low. Failure modes after application of shear forces were predominantly cohesive in the substrate, especially after SC-application, whereas in a microtensile mode adhesive failure at the interface was mostly seen. In a tensile mode, the force is equal on all layers of material causing failure at the weakest layer, which for composite-to-composite bonding appears to be the adhesive interface (see Fig. 3). After SC-application the adhesive interface is very rough due to the capture of silica-coated particles, which strongly confines the shear stress to the interface causing sliding of weakest layers over each other. According to the data in Table 3, the weakest layers exist in the old composite resin, as schematically outlines in Fig. 3 as well.

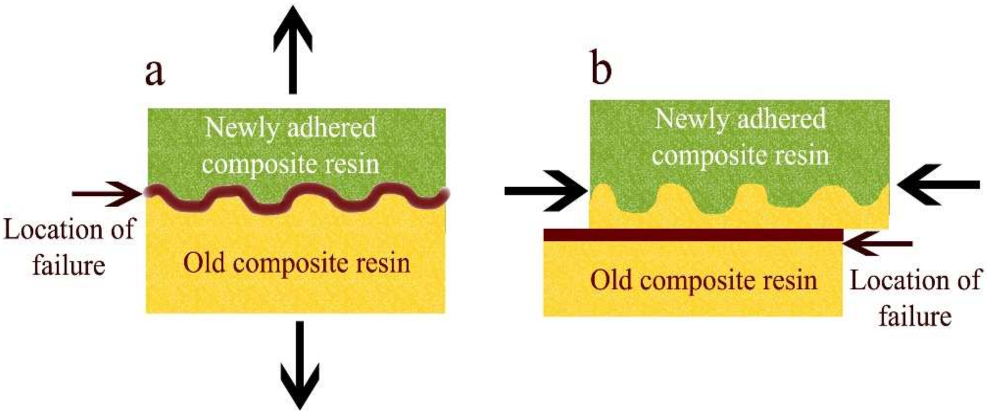

Fig. 3 Schematics, explaining the predominance of adhesive failure at the interface after tensile application **(a)** and of cohesive failure in the substrate after shear application **(b)**.

In two previous studies,[6,26] we extensively compared different aging methods and surface conditionings based on mean failure shear stresses and concluded that SC-application gave similar bond strengths but more cohesive failure in immediate repair compared with IAR-application. After thermocycling, however, SC-application gave higher bond strengths than IAR-application. In engineering, including dentistry, a slightly lower mean failure stress is often considered more acceptable with a high Weibull modulus (more dependable) than a bonding with higher mean failure strength but with a low Weibull modulus.[18] Moreover, also the mode of failure should be taken into account, which makes a comprehensive evaluation of all available data difficult.

Since Weibull moduli are similar for shear and microtensile evaluations, comparisons on the basis of mean failure stresses can be made without accounting for the minor differences in Weibull moduli. The mean failure shear stresses in the current study confirm previous comparisons of IAR- and SC-applications[26] and effects of thermocycling.[6,10] Although in general 5% failure levels obtained from Weibull distributions correlate with failure shear stresses, detailed analysis of Tables 2 and 3 show that 5% failure levels in a shear mode are higher after SC-application than after IAR-application, especially for nanofilled FS. This may indicate that SC-application yields higher bond strengths than IAR-application. In a microtensile mode, data for non-aged composite repairs yield the same conclusions as follow from shear bond evaluation. After thermocycling in the current study, SC-application yields consistently higher shear and microtensile failure stresses than IAR-application, as found before in a shear mode.[6] However since in a microtensile mode, no correlation existed between the mean failure microtensile stresses and $\sigma_{0.05}$, obtained from the Weibull distribution, analysis of the data based on the Weibull distribution gives a different conclusion. 5% failure level analysis for thermocycled composite resins, indicates that in shear IAR-application yields higher failure stresses than SC-application for nanohybrid TE, with an opposite conclusion for nanofilled FS. On the other hand, from microtensile analysis of 5% failure levels, SC-application consistently yields higher failure levels (see Fig. 2b). Most likely, analyses of the Weibull distribution are to be preferred, because demonstration of statistical significance based on mean values is greatly hampered by large standard deviations, while the Weibull analysis makes use of the spread in the data. In fact, this is directly related with the Weibull modulus, as can be seen in Fig. 1a, where clearly the distributions for aged composites intersect each other. Thus, a less strict comparison of, for instance 50% failure levels, would have brought the conclusions from mean failure stress analyses better in line with the ones from a Weibull analysis.

Both an analysis on the basis of mean failure stresses as well as the Weibull analysis, neglect whether failure is cohesive in the substrate or adhesive at the interface. Clinically, cohesive failure indicates that the bond is stronger than the composite resin itself and hence attempts to enhance the bond strength must be considered futile.[27] Clinically, restorations are mainly exposed to shear rather than to microtensile forces and shear evaluations are to be preferred over microtensile ones, also since Weibull moduli are similarly low for both modes. Hence, considering the high percentages of cohesive failure (as also found in other studies[10,28]) in shear combined with the low Weibull moduli, this study indicates that the weakest and least dependable link in composite-to-composite bonding is the composite resin itself and not the adhesive interface, with the exception of bonding created after thermocycling by IAR-application.

5 Conclusion

Failure modes after shear application are more frequently cohesive than after application of a microtensile force, Weibull moduli are similarly low for shear and microtensile evaluations. In general 5% failure levels obtained from Weibull distributions correlate with failure shear stresses, but not with failure microtensile stresses.

References

1. Bouschlicher MR, Reindhardt JW, Vargas MA (1997) Surface treatment techniques for resin composite repair. *Am J Dent* 10:279-283
2. Shahdad SA, Kennedy JG (1998) Bond strength of repaired anterior composite resins: an *in vitro* study. *J Dent* 26:685-694
3. Mjör I, Moorhead J, Dahl J (2000) Reasons for replacement of restorations in permanent teeth in general dental practice. *Int Dent J* 50:361-366
4. Roulet JF (1988) The problems associated with substituting composite resins for amalgam: a status report on posterior composites. *J Dent* 16:101-113.
5. Swift E.J. (1987) Wear of composite resins in permanent posterior teeth. *J Am Dent Assoc* 115:584-588.
6. Rinastiti M, Özcan M, Siswomihardjo W, Busscher H (2009) Effects of surface conditioning on repair bond strengths of non-aged and aged microhybrid, nanohybrid and nanofilled composites. *Clin Oral Investig* Submitted.
7. Chiba K, Hosoda H, Fusayama T (1989) The addition of an adhesive composite resin to the same material : bond strength and clinical techniques. *J Prosthet Dent* 61:669-675.
8. Özcan M, Barbosa SH, Melo RM, Galhano GP, Bottino MA (2007) Effect of surface conditioning methods on the microtensile bond strength of resin composite to composite after aging conditions. *Dent Mater* 23:1276-1282.
9. Amaral A, Özcan M, Bottino MA, Valandro LF (2006) Microtensile bond strength of a resin cement to glass infiltrated zirconia-reinforced ceramic: The effect of surface conditioning. *Dent Mater* 22:283-290.
10. Brendeke J, Özcan M (2007) Effect of physicochemical aging conditions on the composite-composite repair bond strength. *J Adhes Dent* 9:399-406
11. Cesar P, Meyer Faara P, Miwa Caldart R, Gastaldoni Jaeger R, da Cunha Ribeiro F (2001) Tensile bond strength of composite repairs on artglass using different surface treatments. *Am J Dent* 14:373-377.
12. Oilo G (1993) Bond strength testing--what does it mean? *Int Dent J* 43:492-498.
13. Cardoso PEC, Sadek FT, Goracci C, Ferrari M (2002) Adhesion testing with the microtensile method: effects of dental substrate and adhesive system on bond strength measurements. *J Adhes Dent* 4:291-297.

14. Inoue S, Vargass M, Abe Y, Lambrechts P, Vanherie G, Sano H, Van Merbeek B (2001) Microtensile bond strength of eleven contemporary adhesives to dentin. *J Adhes Dent* 3:237-245.
15. Rasmussen S (1996) Analysis of dental shear bond strength tests, shear or tensile. *Int J Adhesion and Adhesives* 16:147-154.
16. Pashley DH, Carvalho RM, Sano H, Nakajima M, Yoshiyama M, Shono Y, Fernandes CA, Tay F (1999) The microtensile bond test: a review. *J Adhes Dent* 1:299-309.
17. Della BA, Anusavice K, Shen C (2000) Microtensile strength of composite bonded to hot-pressed ceramics. *J Adhes Dent* 2:305-313.
18. McCabe JF, Carrick TE (1986) A statistical approach to the mechanical testing of dental materials. *Dent Mater* 2:139-142.
19. Weibull W (1951) A statistical distribution function of wide applicability. *J Appl Mech* 18:293-297.
20. Armstrong S, Vargas M, Fang Q, Laffon J (2003) Microtensile bond strength of a total-etch 3-step, total-etch 2-step, self-etch 2-step, and a self-etch 1-step dentin bonding system through 15-month water storage. *J Adhes Dent* 5:45-56.
21. Burrow MF, Thomas D, Mike V, Tyas M (2004) Analysis of tensile bond strength using Weibull statistics. *Biomaterials* 25:5031-5035.
22. Robin C, Scherrer SS, Wiskott HWA, de Rijk WG, Belser UC (2002) Weibull parameters of composite resin bond strengths to porcelain and noble alloy using the Rocatec system. *Dent Mater* 18:389-395.
23. Palin WM, Fleming GJP, Trevor Burke FJ, Marquis PM, Randall RC (2003) The reliability in flexural strength testing of a novel dental composite. *J Dent* 31:549-557.
24. Braga RR, Cesar PF, Gonzaga CC (2000) Tensile bond strength of filled and unfilled adhesives to dentin. *Am J Dent* 13:73-76.
25. Rodrigues Junior S, Ferracane J, Della Bona A (2009) Influence of surface treatments on the bond strength of repaired resin composite restorative materials. *Dent Mater* 25:442-451.
26. Rinastiti M, Özcan M, Siswomihardjo W, Busscher HJ (2009) Immediate repair bond strengths of microhybrid, nanohybrid and nanofilled composites after different surface treatments. *J Dent* 38:29-38.
27. Lucena-Martin C, Gonzlez-Lopez S, de Mondelo JMN-R (2001) The effect of various surface treatments and bonding agents on the repaired strength of heat-treated composites. *J Prosthet Dent* 86:481-488.
28. Tezvergil A, Lassila LVJ, Vallittu PK (2003) Composite-composite repair bond strength: effect of different adhesion primers. *J Dent* 31:521-525

Chapter 4

Immediate Repair Bond Strength of Microhybrid, Nanohybrid and Nanofilled Composites after Different Surface Treatments

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1 Introduction

Resin-based composites (hereon: composite resin) are increasingly used in dentistry in order to restore damaged parts of hard dental tissues or to reconstruct missing teeth. Composite resins have good aesthetic properties and by employing adhesive technologies, they allow for minimally invasive interventions which may not always require tooth preparation.[1] After the removal of rubber dam, due to mishandling of the material, dentists not fully mastering the matrix technique or finishing procedures or due to an inadequate colour shade selection or form mismatch, composite restorations may require immediate correction.[2] In these cases, total replacement may neither be necessary nor desirable. Total replacement is costly and time-consuming.[3] Removing the existing restoration leads to more tissue loss and in some situation pulp injury.[4,5] Hence, addition of a new layer of composite resin onto an existing, already polymerized composite resin is often preferable.

The success of composite-to-composite adhesion depends on the chemical composition of the surface, its roughness,[1,6], wettability,[7] and the surface conditioning procedure applied.[8] In clinical practice, composite resin is exposed to atmospheric oxygen creating an oxygen-enriched surface layer that remains unpolymerized.[9] The oxygen-inhibited layer is viscous and contains unreacted C=C bonds.[10] The unreacted C=C bonds of the functional groups on the surface of a polymerized matrix will allow the monomer of new composite resin to bond, thereby improving adhesion.[8] A variety of surface conditioning methods and intermediate adhesive resins (IARs) have been developed to improve composite-to-composite adhesion. While some studies have reported enhanced repair bond strength with the use of an IAR,[11,12] others claim better results with physical conditioning of the composite surface.[1,13] One such example to the latter is chair-side tribochemical silica-coating that creates both micromechanical and chemical reaction sites on substrate surfaces. In this technique, the surface is air-abraded with silica-coated particles, followed by application of a silane coupling agent and an IAR.[14,15] The application of silane on the composite resin was suggested to improve the wettability of the fillers on the composite surface and consequently adhesion of the composite resin.[16]

Understanding composite-to-composite adhesion, including the type of failure[17] necessitates physico-chemical characterization and modeling of the composite surface and relating this to the actual bond strengths and its failure type. The wettability of composite surfaces can be examined by contact angle measurements with liquids.[18,19] Surface roughness can be calculated from three-dimensional topographic images obtained using Atomic Force Microscopy (AFM)[20] or Scanning Electron Microscopy (SEM).[3,21] Elemental surface composition of the outermost composite surface can be analyzed quantitatively using X-ray Photoelectron Spectroscopy (XPS).[22] Various *in vitro* testing methods have been developed in order to evaluate the bond strength of adhesive systems, including conventional tensile, microtensile[11,23] and shear bond tests.[14,15] Shear bond tests have been more widely used than tensile bond strength tests, because of their relative simplicity.[24]

In order to achieve long-lasting composite restorations,[3,25] recent developments in polymer science focus on reducing filler particle sizes and at the same time maximizing particle loading in composite resins. This has led to the development of new filler particles, although there is no consensus on the final target value to be reached, sometimes suggested to be around 15-30 MPa.[26] The size of new filler particles ranges between 5 - 100 nm and particles are used in clusters or dispersed forms,[27] while the size of conventional filler particles in microhybrid composite resins lies around 0.7 - 3.6 µm.[28] Composite-to-composite adhesion of nanofiller composite resins has not been compared with those of microhybrid composite resins, nor have the merits of silanization of nanofiller composite resins been determined. However, it can be hypothesized that different polymer matrices and filler fractions will influence the repair bond strength of composite resins during relayering. Therefore, the objectives of this study were to compare the immediate repair bond strengths and failure types of microhybrid, nanohybrid and nanofilled composite resins with and without surface conditioning methods, including the merits of silanization in these composite resin systems. Secondly, the interacting composite surfaces will be physico-chemicaly characterized by their surface composition and roughness.

2 Materials and methods

2.1 Specimens preparation

The product name, manufacturer, chemical composition, abbreviations and batch numbers of the materials used in this study are listed in Table 1.

Disc shaped specimens (N = 160) were fabricated by placing unpolymerized composite resins incrementally into cylindrical undercut cavities (diameter: 5.5 mm, thickness: 3.5 mm) that were prepared in auto-polymerized polymethylmethacrylate (PMMA) (Autoplast, Candulor, Wangen, Switzerland) surrounded by a PVC cylinder (3 specimens per cylinder, see Fig. 1).

The unpolymerized composite resins were packed into the cavities with a hand instrument and photo-polymerized incrementally in layers with a thickness less than 2 mm. Each increment was photo-polymerized with a halogen photo-polymerization unit (Optilux 501, Kerr, USA) for 40 s at a distance of 2 mm from the surface. Light intensity was higher than 400 mW/cm^2, as verified by a radiometer after every 6 specimens (Demetron LC, Kerr). During final photo-polymerization, the top surface layer was covered with a 100 μm thick translucent Mylar strip (KerrHawe SA, Bioggio, Switzerland) in order to create a smooth surface and to prevent the formation of an oxygen inhibition layer. Specimens of each composite resin group were randomly assigned to one of the surface conditioning methods and immediately further processed.

Table 1 Product and company names, chemical compositions, abbreviations and batch numbers of the materials used in this study.

Product name	Company name	Chemical composition	Abbreviations	Batch Number
COMPOSITE RESIN				
Quadrant Anterior Shine (microhybrid)	Cavex BV, Haarlem, The Netherlands	Bis-GMA, DUDMA, silica, silicate glass, Ba-glass, fluoride containing fillers (63 v%)	AS	010100
Grandio (nanohybrid)	Voco GmbH, Cuxhaven, Germany	Bis-GMA, UDMA, TEGMA, Glass-ceramic, SiO_2 containing fillers (71.4 v%)	G	621332
Tetric Evo Ceram (nanohybrid)	Ivoclar Vivadent AG, Schaan, Liechtenstein	Bis-GMA, TEGDMA, UDMA , Ba-Al-F-B-Silicate, SiO_2, mixed oxide, YbF3 containing fillers (48.5 v%)	TE	J04088
Filtek Supreme XT (nanofilled)	3M ESPE, St. Paul, MN, USA	TEGDMA, UDMA, Bis-EMA, Zirconia-Silica, Silica containing fillers (57.7 v%)	FS	6 BG
TRIBOCHEMICAL SILICA COATING KIT (for SC-application)				
CoJet®-Sand	3M ESPE AG, Seefeld, Germany	Aluminum trioxide particles coated with silica, particle size: 30 µm	SC	165092
ESPE®-Sil	3M ESPE AG, Seefeld, Germany	3-methacryloxypropyl-trimethoxysilane, ethanol	Silane	152745
INTERMEDIATE ADHESIVE RESIN (for IAR-application)				
Visio™-Bond (for tribochemical silica coating treatment)	3M ESPE AG, Seefeld, Germany	Dicyclopentyldimethylene diacrylate 2-propenoic acid, 2-methyl, 2-[(2-hydroxyethyl)(3-methoxypropyl)amino]ethyl ester	VB	161808
Quadrant Unibond (for AS)	Cavex BV, Haarlem, The Netherlands	Bis-GMA, TEGDMA, silicate glass fillers, silica, polycarboxylic acid, champorquinone	Q	010049
Solobond Plus (for G)	Voco GmbH, Cuxhaven, Germany	Bis-GMA, TEGDMA, HEMA, champorquinone	S	591583
Multilink (for TE)	Ivoclar Vivadent AG, Schaan, Liechtenstein	Primer A: Water, initiators (sulfonate, amines) Primer B: Phosphonic acid acrylate, HEMA, TEGDMA, methacrylate modified polyacrylic acid	M	Primer A: H10145 Primer B: 09713
Adper Scotchbond 1XT (for FS)	3M ESPE, St. Paul, MN, USA	Dimethacrylate, HEMA, polyalcenoic acid copolymer, silane treated colloidal silica, ethanol, water, photoinitiator	A	4BM

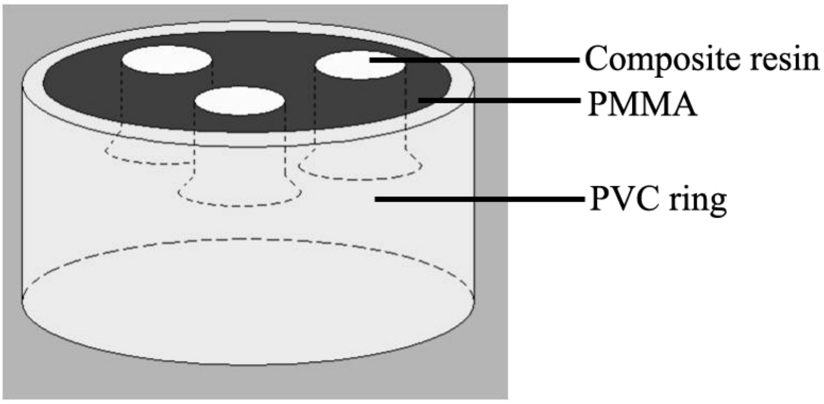

Fig. 1 Schematic drawing of the cylindrical undercut cavities that were prepared in auto-polymerized polymethylmethacrylate surrounded by a PVC cylinder and the assembly used to measure the shear bond strength.

2.2 *Surface conditioning methods*

Control: The top layer of composite resin was polymerized. No surface conditioning was applied.

Control, with strip: The top layer of composite resin was polymerized against a Mylar strip. No surface conditioning was applied after removal of the Mylar strip.

Intermediate adhesive resin (IAR): The top layer of composite resin was polymerized against a Mylar strip. IARs of the corresponding composite resin (Table 1) were applied in a thin layer on the substrates using a micro-brush. Subsequently, the solvent was gently air-thinned under compressed air and finally photo-polymerized for 20 s, according to manufacturer's instructions.

Tribochemical silica coating (SC): The top layer of composite resin was polymerized against a Mylar strip. Tribochemical treatment was done using an intraoral air-abrasion device (Dento-Prep™, RØNVIG A/S, Daugaard, Denmark) filled with 30 µm alumina particles coated with silica (CoJet®-Sand, 3M ESPE AG, Seefeld, Germany) from a distance of approximately 10 mm at a pressure of 2.5 bars for 4 s. Following surface conditioning, loose particles were gently air blown. The conditioned substrates were then coated with a 3-

methacryloxypropyltrimethoxysilane coupling agent, γ-MPS (ESPE®-Sil, 3M ESPE AG) allowing 5 min for reaction. Finally, an IAR specific to the SC procedure was applied (Visio™-Bond, 3M ESPE AG) with a microbrush, air thinned and light-polymerized for 20 s.

2.3 *Repair composite resin application*

Following surface conditioning, composite resins of the same kind as their substrates were immediately adhered onto the conditioned substrates using translucent polyethylene molds (inner diameter: 3.6 mm; height: 5 mm) and photo-polymerized. Bonding procedures were carried out by the same operator throughout the experiments. The composite resin was packed against the substrate incrementally with a hand instrument and light polymerized in two layers with a thickness of less than 2 mm. For easy retrieval of the composite resin after polymerization, the mold was filled 1 mm below its height. Each layer was polymerized for 40 s from a distance of 2 mm. After polymerization, specimens were gently removed from the polyethylene molds and kept in the dark, never longer than 24 h in dry condition.

2.4 *Shear bond strengths and failure analysis*

Specimens were mounted in the assembly of a universal testing machine (Zwick ROELL Z2.5 MA 18-1-3/7, Ulm, Germany) and a shear force was applied using a shearing blade parallel to the adhesive interface (Fig. 2) until failure occurred. The load was applied to the adhesive interface, as close as possible to the surface of the substrate at a cross-head speed of 1.0 mm/min and the stress-strain curve was analyzed with the machine's software program (TestXpert®, Zwick ROELL, Ulm, Germany).

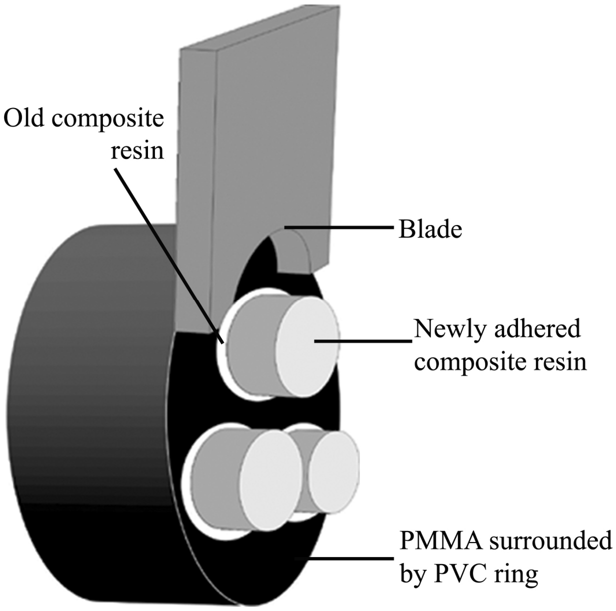

Fig. 2 Schematic drawing of the assembly used to measure the sear bond strength

Experiments were carried out at room temperature (23°C) and a relative humidity of 53%. Subsequently, digital photos (Nikon D1, Micro Nikon 60 lens, Tokyo, Japan) were taken from the substrate surfaces and specimens were evaluated under light microscopy (MP 320, Carl Zeiss, Jena, Germany) (x40). Failures types were categorized as cohesive failure in the substrate appearing as small indents, or adhesive failure at the interface showing as a completely smooth surface.

2.5 Additional specimens for surface characterization

For contact angle measurements (3 droplets per group), composite resins were packed into a silicone mold (25 x 2 x 2 mm) covered with a Mylar strip and glass plate that were pressed together in order to create a flat surface. For XPS, SEM (1 specimen per group) and AFM (3 specimens per group) examinations, composite resin blocks (5 x 6 x 6 mm) were produced, as described above. All physico-chemical surface characterizations were carried out on composite resins prior to and after silica coating.

2.5.1 Contact angle measurements

Contact angles are indicative of the surface composition of a material. Here, contact angles were measured with water, γ-MPS silane (ESPE®-Sil) and IARs, employing the sessile drop technique. Three microlitre liquid droplets were placed on the composite surface and equilibrium contact angles were measured with a home-made contour monitor. Slightly larger droplets of 6 μ were employed to determine the advancing and receding water contact angles on a composite surface. Advancing and receding contact angles were achieved by keeping the needle in the droplet and increasing or decreasing the droplet volume until the contact angles appeared maximal or minimal, respectively.

2.5.2 XPS analysis

XPS can measure the elemental surface composition of a material. Here, XPS was performed using an S-Probe spectrometer (Surface Science Instruments, Mountain View, CA, USA), equipped with an aluminum anode (10 kV, 22mA) and a quartz monochromator. The direction of the photoelectron collection angle to the specimens was 55 degrees and the electron flood gun was set at 10 eV. A survey scan was made with a 1000 x 250 μm spot and a pass energy of 150 eV. Binding energies were determined by setting the binding energy of the C_{1s} component due to carbon-carbon bonds at 284.8 eV.

2.5.3 SEM analysis

In order to visualize the morphology of composite surfaces, cold field emission SEM (JSM-6301F, Jeol Instruments, Tokyo, Japan) images of fresh composite resin prior to and after SC application were taken at 25 kV at a magnification of x5000. Surfaces were first sputter-coated with a 13 nm thick layer of gold/paladium (80/20) prior to examination.

2.5.4 AFM analysis

The average surface roughnesses (R_a) of composite resins were assessed using AFM (Nanoscope IIIa Dimension™ 3100, Digital Instruments, Santa Barbara, CA, USA). The microscope was operated in the contact mode, using a Si_3N_4 cantilever tip (DNP from Veeco, Woodbury, NY) with a spring constant of 0.06 N/m. The composite resin was placed on a glass slide using double sided sticky tape. The specimens were placed below the cantilever of the AFM to obtain three dimensional images (70 x 70 µm) of the surface at three randomly selected places per specimen.

2.6 Modeling of the composite surfaces

The advancing and receding water contact angles as well as the XPS data allow to model the surfaces in terms of matrix composition and amount of filler particles. The equilibrium contact angle, determined in part by the matrix and the filler particles exposed, can be expressed according to Cassie and Baxter[29] as follows :

$$\cos\theta_E = f_{1,CA} \times \cos\theta_A + \left(1 - f_{1,CA}\right) \times \cos\theta_R$$

where "θ_E" is the equilibrium water contact angle, "θ_A" the advancing water contact angle reflecting the more hydrophobic matrix, "θ_R" the receding water contact angle reflecting hydrophilic silica particles and "$f_{1,CA}$" the matrix fraction exposed at the surface as inferred from contact angle data.

XPS data can also be used to model the composite surface. For a fully resin matrix covered surface, the molecular structure of PMMA yields a theoretical value $(O/C)_{theoretical}$ that can be compared with the experimental value for $(O/C)_{experimental}$ due to the resin matrix and Si-containing filler particles. The experimental values for $(O/C)_{experimental}$ can be expressed in matrix values $(O/C)_{matrix}$ by subtracting the oxygen arising from Si (since silica is SiO_2, which involves subtracting twice the amount of measured Si from the measured O percentage). Subsequently, the following formula can be applied to calculate the matrix fraction exposed at the surface as derived from XPS data, $f_{1,\ XPS}$

$$f_{1,\ XPS} = (O/C)_{matrix} / (O/C)_{theoretical}$$

2.7 Statistical analysis

Statistical analysis was performed using SPSS 15.0 software for Windows (SPSS Inc., Chicago, IL, USA). Effects of surface conditioning and composite type on bond strength and surface roughness were compared using two way-ANOVA and LSD post-hoc tests. Failure types were analyzed using Kruskall-Wallis. *P* values less than 0.05 were considered to be statistically significant.

3 Results

3.1 Bond strength and failure types

Type of composite resins and surface conditioning methods both showed significant effect on the results ($p < 0.05$, 2-way ANOVA, see Table 2). Regardless of composite type, there was no bond strength difference between both control groups ($p > 0.05$, 2-way ANOVA, LSD post-hoc test), and in both contol groups bond strengths were highest for the nanohybrid composite resin G.

Table 2 Results of 2-way analysis of variance for average of surface roughness (*$p<0.05$)
* Statistically significant difference at the level of 5%.

Source	DF	SS	MS	F	P
Composite resin	3	610.196	203.399	6.235	0.001*
Surface treatment	1	1705.943	568.648	7.432	0.000*
Interaction (Composite resin -Surface treatment)	3	835.515	92.946	2.849	0.004*
Error	132	4305.925	32.621		
Total	147	55637.176			

Table 3 The mean immediate repair bond strengths of four different composite resins after application of intermediate adhesive resins (IARs) and tribochemical silica coating (SC). For abbreviations see Table 1. Data are averages ± standard deviations over 10 specimens.

Composite resin	Control	Control, with strip	IAR-application	SC-application
AS	$15.1 \pm 4.1^{a,A}$	$14.5 \pm 5.7^{a,A}$	$15.0 \pm 6.6^{a,A}$	$25.0 \pm 8.5^{a,B}$
G	$17.7 \pm 6.5^{a,b,A}$	$20.0 \pm 5.2^{b,A}$	$15.8 \pm 5.9^{a,b,A}$	$26.3 \pm 7.9^{a,B}$
TE	$10.5 \pm 4.6^{a,c,A}$	$14.8 \pm 4.6^{a,A,C}$	$19.9 \pm 4.1^{a,c,B,D}$	$15.9 \pm 4.6^{b,C,D}$
FS	$14.6 \pm 4.6^{a,A}$	$17.4 \pm 4.1^{a,A,B}$	$21.3 \pm 6.7^{b,c,B}$	$27.4 \pm 5.6^{a,C}$

* Same small letters indicate an insignificant difference between the row, same capital letters denote an insignificant difference between the column

Failure types of all composite resin were predominantly (> 90%) cohesive in the control group not polymerized against a strip, whereas controls, polymerized against strips showed between 40% - 70% cohesive failures. IAR application increased the bond strength significantly only in TE compared to both control groups (Table 3). Upon application of their corresponding IARs, FS showed mainly cohesive failure in the substrate (90%), while TE yielded more adhesive failures (100%).

After application of SC, significantly higher ($p < 0.05$) bond strengths were observed for AS and FS than for their two corresponding controls and IAR groups (Table 3). However, no higher bond strengths were found for TE than obtained in the control, with strip or IAR groups (p > 0.05, Table 3). Application of SC, yielded exclusively (100%) cohesive failures in the substrate for all composite resins.

3.2 Contact angle measurements

Equilibrium and advancing water contact angles were similar across all composite resins and typically ranged between 70 - 80 degrees, while receding angles amounted around 45 degrees (Fig. 3). Silane spread completely over the composite surfaces (0 degrees), while the VB contact angle was also 0 degrees. Equilibrium contact angles with IARs varied across the different composite resins and ranged from 11 degrees for S to 39 degrees for Q.

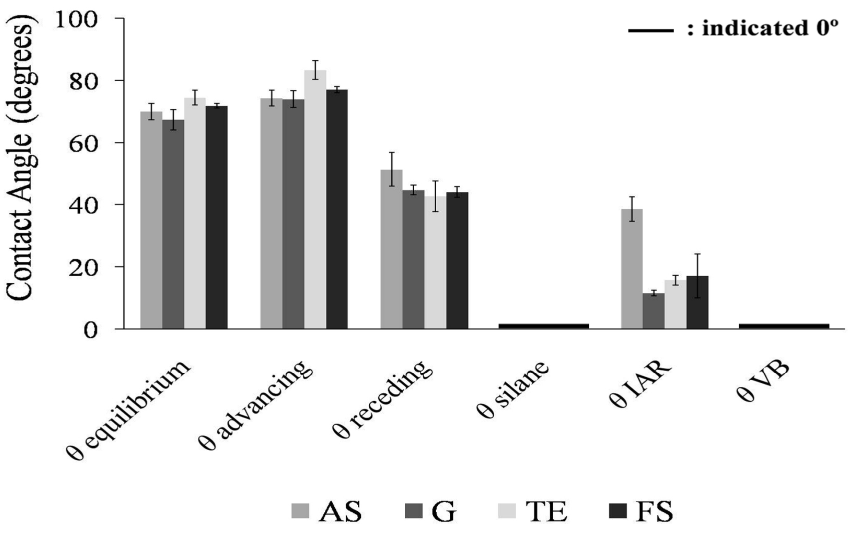

Fig. 3 Equilibrium, advancing and receding contact angles for water, silane, IARs and VB on the various resin composite resins. Error bars indicate the standard deviations over 3 droplets.

3.3 XPS analysis

There were only small differences in surface composition across the different composite resins. Analyses typically indicated the presence of oxygen and carbon as the major constituents on the surface and a small amount of silica, reflecting the filler particles (Table 4). After application of SC, the percentage of Si increased about 1.5 times for AS and up to 4 times for G, TE and FS.

Table 4 Elemental surface composition of the four composite resins prior to and after SC.

Composite Resin	**Elemental Surface Composition (%)**							
	Si		**O**		**C**		**Other**	
	Prior to SC	**After SC**	**Prior to SC**	**After SC**	**Prior to SC**	**After SC**	**Prior to SC**	**After SC**
AS	3.2	5.4	23.4	7.5	68.7	87.0	N 4.7	
G	4.7	15.5	25.8	35.5	70.2	47.2	-	
TE	4.0	15.5	20.9	32.9	70.7	46.6	N 4.5	N 2.3 Al 2.7
FS	3.5	14.5	26.6	34.3	68.9	48.2	Zr 1.0	

Si : Silicon
O : Oxygen
C : Carbon
N : Nitrogen
Al : Aluminum
Zr : Zirconium

3.4 AFM analysis

The mean roughness of all composite resins in the control group, polymerized against strips was always below 10 nm. This is likely because the roughness prior to conditioning is more the result of the final surface treatment applied, in this case the application of a Mylar strip, rather than an intrinsic property of the composite resin. However, after SC (Fig. 4), the surface roughness of all composite resins increased significantly ($p < 0.05$, 2-way ANOVA), with higher surface roughnesses for AS and FS than for G and TE.

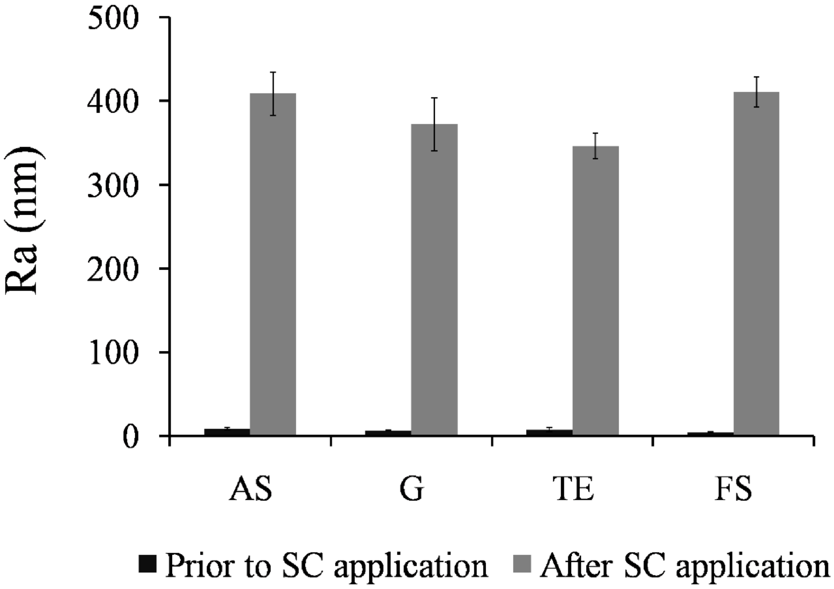

Fig. 4 Mean surface roughness of the composite surfaces prior to and after SC conditioning. Error bars denote the standard deviations over 3 different specimens.

3.5 SEM analysis

Fig. 5 shows SEM images of the composite resins prior to (left panel) and after SC (right panel). The microfilled composite resin, AS, clearly showed larger filler particles than nanofilled G, TE and FS, as evidenced by the white regions. It should be noted that, particularly since they appear somewhat blurred, these regions need not necessarily be at the surface but may also be slightly underneath the surface, covered by a thin resin matrix layer. After SC, similarly rough surfaces were evident in all composite resins (Fig. 5, right panel).

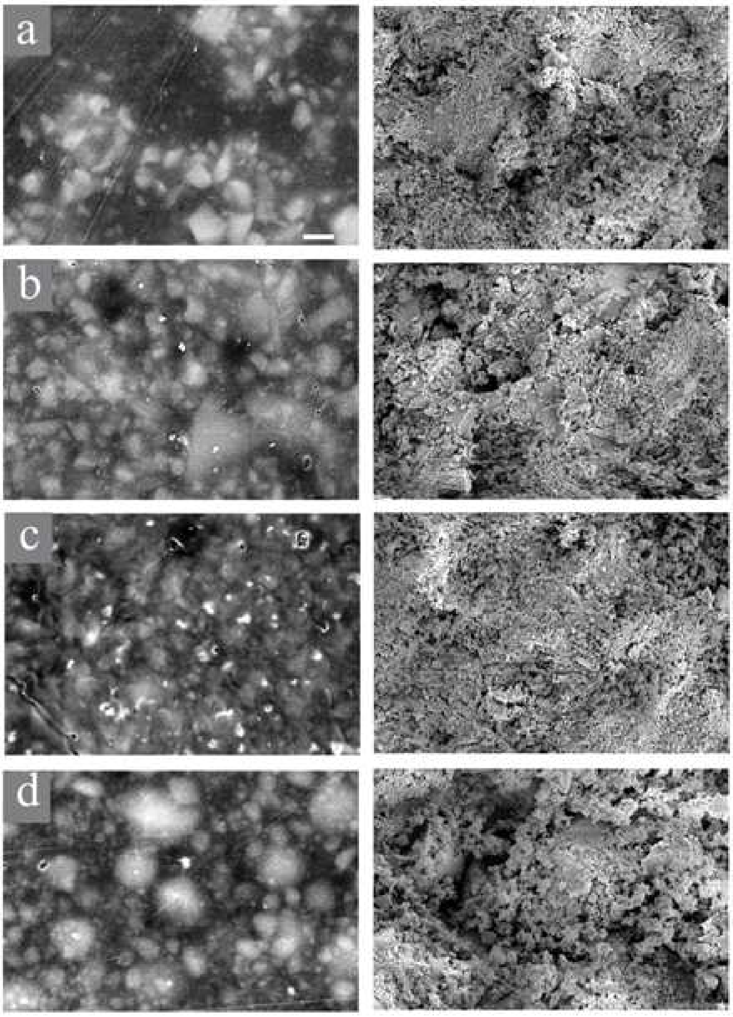

Fig. 5 SEM micrographs of the different composite resins prior to (left panel) and after silica coating (right panel).

- **a)** Anterior Shine (AS)
- **b)** Grandio (G)
- **c)** Tetric Evo Ceram (TE)
- **d)** Filtek Supreme XT (FS)

Bar marker indicates 1 µm.

3.6 Modeling of the composite surfaces

The surfaces of all composite resins appeared dominated by the resin matrix (Table 5). Although there was no exact numerical correspondence between surface modeling based on contact angle measurements and XPS, modeling based on XPS data confirmed the modeling based on contact angle data, as both indicated that the composite surfaces are dominated by the matrix. It should also be noted that the percentage surface coverage by fillers was far below their presence in the bulk in both modelings.

Table 5 Filler exposure at the composite surface (%) calculated from contact angle and XPS data, as compared with the bulk filler composition provided by the manufacturers. *For abbreviations see Table 1

Composite Resin	Filler exposure at the surface (%) From Contact Angles	From XPS	Bulk filler composition (v%) according to the manufacturer
AS	21	20	63.0
G	26	27	71.4
TE	32	41	48.5
FS	18	14	57.7

* note that filler exposure at the surface is expressed in area percentages while bulk filler compositions are given in volume percentages.

4 Discussion

In this manuscript we compared immediate repair bond strength of composite resins. Composite surfaces were made against Mylar strips as used for proximally for shaping proximal surface, pulling and distributing the composite resin evenly to the lingual, labial, palatal, buccal to reduce voids,[30] and avoid oxygen inhibition.[31] This yielded smooth and reproducible surfaces, without affecting the clinical relevance of the study because bond strengths of composite resins polymerized and not polymerized against Mylar strips were not significantly different. However, failure type analysis showed more cohesive failure in the control group not polymerized against strips than in the control group, with strip. The effect of the oxygen inhibited layer on resin composite resin repair is still controversial[9] and negated by the lack of a significant difference in bond strength between both controls polymerized in the absence and presence of a Mylar strip, in accordance with previous studies.[10,32] This leads to the conclusion that an oxygen-inhibited layer for bonding with composite resins is not necessary. Application of an intermediate adhesive resin resulted in non-significant increases in bond strengths when compared to the non-conditioned control group, while application of silica coating caused a major increase. The increase was especially noted in AS and G. Interestingly, silica coating yielded exclusively cohesive failures in the substrate in all composite resins. This indicates that the adhesive strength at the joint interface exceeded the cohesive strength of the composite resin substrate. This type of failure usually indicates the clinical reliability of the adhesion. In this study, a shear bond test was employed, in which tensile forces occur close to the load application area, which may affect the substrate more than the adhesive interface itself.[33] The microtensile test, suggested by Sano *et al.*[34] asseses the bond strength of specimens with reduced areas of adhesive joints where fractures occur basically at the adhesive interface. At the same time, Sau *et al.*[35] reported that loading the specimens under shear could be considered to be clinically more relevant than flexural or tensile loading, since it produces elements of shear, tensile and compressive stresses that often occur during chewing. For this reason, we used a shear bond test. It was interesting to note that the incidence of the adhesive failures in the non-conditioned control group, was high, except for FS. This indicates that a shear test does not exclusively result in cohesive failure of the substrate.

The non-significant differences between bond strengths of the different composite resins in the control group may be related to the dominance of the resin matrix at the surface, as inferred from contact angle and XPS data. Since surface energy dictates good spreading of the resin matrix over the high energy silica particles, the composite surface may easily become dominated by resin matrix, as indicated clearly by the surface modeling performed in this study. These findings in turn, essentially falsify the assumption that the silanized filler particles on the composite surface contribute to the bond strength when relayering or repair is performed.[11,36]

The application of an intermediate adhesive resin on composite resins and tooth surfaces has been described to have an important role in adhesion, as combined with light irradiation it may lead to radical reactivation. When applied on the tooth surface, a layer of intermediate adhesive resin is generally considered as a weak link.[37] *In vitro* studies on aged composite resins have indicated that the repair bond strength increases after the application of an intermediate adhesive resin.[11,12] Our study shows that this is not the case when intermediate adhesive resins are applied on fresh composite surfaces, since no significant improvement was found. Possibly the degree of conversion in fresh composite resins is still high and beneficial effects of an intermediate adhesive resin do not become evident.

Silica coating resulted in the high shear bond strengths in AS, G and FS, while also the roughnesses of the composite surfaces after silica coating were much higher than in the control group. The abrasion process removes loose contaminated layers and the roughened surfaces provide a means for mechanical interlocking or "keying" with the adhesive, therewith creating a larger surface area for the bond.[38] In addition, the changes brought about by tribochemical treatment, affect the surface energy and wettability of the composite surface.[35] After silica coating, water contact angles decreased due to the increased surface roughness, along with an increase in the percentage Si exposed at the surface. Silane adsorbs strongly to the silica-coated particles and promotes the wetting of the rough surface, facilitating the diffusion of the composite resin into micromechanical porosities in the substrate.[39] Finally, monomeric ends of VB react with methacrylate group enhance the affinity of the two composite resin layers.

Interestingly, silica coating of TE resulted in lower bond strengths as compared to other composite resins. This possibly relates to the percentage of filler exposure in TE obtained by modeling (see Table 5) that was highest prior to treatment. Possibly, due to the high filler content in TE, silica-coated particles bounce from the surface, as suggested by the relatively small increase in the roughness of the TE surface after silica coating. TE also presented exclusively adhesive failures after intermediate adhesive resin application that could be a consequence of its resin matrix composition. Monomer of TE is composed of Bis-GMA and UDMA and FS is composed of TEGDMA, UDMA and Bis-EMA. Sideridou *et al.*[40] found that the maximum polymerization degree of UDMA and Bis-GMA was higher than of TEGDMA and Bis-EMA. Thus TE might not have sufficient unreacted monomers to create a good adhesion between the substrate and new composite resin.

The results in this study showed that groups with equal bond strengths do not always have the same failure types. Furthermore, when a composite resin repaired tends to fracture cohesively, a more durable adhesion can be expected under occlusal load.[41] Consequently, it is suggested that failure analysis should be one of the considerations to assess the adhesion between substrate and new composite resin in immediate repair. Moreover, from the clinical standpoint, since repairs may be performed also after exposure to the oral environment, future studies are warranted to study the aging effects on the physical and chemical properties of composite surfaces and their consequences on adhesion.

5 Conclusions

With the limitation of the current study, the following conclusions can be drawn:

1. The benefit of surface conditioning in composite-to-composite immediate repair varied depending on the composite type. Application of an intermediate adhesive resin did not yield improved shear bond strength, but in general, silica coating created bond strengths close to the upper target value published in the literature.[26]
2. A high bond strength after immediate repair not always concurred with high incidence of cohesive failure in the composite resin substrate.

3. The composite surface is dominated by the matrix and tribochemical silica coating followed by silanization increased the percentage of filler particles exposed at the surface and therewith the immediate relayering bond strengths.
4. Surface modeling showed minor differences among microhybrid, nanohybrid and nanofiller composite resins.

References

1. Bouschlicher MR, Reindhardt JW, Vargas MA (1997) Surface treatment techniques for resin composite repair. *Am J Dent* 10:279-283.
2. Lewis G (1998) Shear bond strength immediately repaired light-cured composite resin restorations. *Oper Dent* 23:121-127.
3. Ferracane JL (1995) Current trends in dental composites. *Crit Rev Oral Biol Med* 6:302-318.
4. Murray P, Windsor L, Smyth T, Hafez A, Cox CF (2002) Analysis of pulpal reactions to restorative procedures, materials, pulp capping, and future therapies. *Crit Rev Oral Biol Med* 13:509-520.
5. Ohshima H (1990) Ultrastructural changes in odontoblasts and pulpe capillaries cavity preparation in rat molars. *Arch Histol Cytol* 53:423-438.
6. Rosales-Leal JI, Osorio R, Holgadi-Terriza JA, Cabrrerizo-Vilches MA, Toledano M (2001) Dentin wetting by four adhesive systems. *Dent Mater* 17:526-532.
7. Rosales-Leal J, Osorio R, Holgadi-Terriza J, Cabrrerizo-Vilches M, Toledano M (2001) Dentin wetting by four adhesive systems. *Dent Mater* 17:526-532.
8. Özcan M, Matinlinna J, Valittu PK, Huysmans M-C (2004) Effect of drying time of 3-methacryloxypropyltrimethoxilane on the shear bond stregth of a composite resin to silica-coated base/noble alloys. *Dent Mater* 20:586-590.
9. Dall'Oca S, Papacchini F, Goracci C, Cury AH, Suh BI, Tay FR, Polimeni A, Ferrari M (2007) Effect of oxygen inhibition on composite repair strength over time. *J Biomed Mater Res B Appl Biomater* 81:493-498.
10. Suh BI (2004) Oxygen-inhibited layer in adhesion dentistry. *J Esthet Restor Dent* 16:316-323.
11. Shahdad SA, Kennedy JG (1998) Bond strength of repaired composite resins: an *in vitro* study. *J Dent* 26:685-694.
12. Tezvegril A, Lassila LVJ, Vallittu PK (2003) Composite-composite repair bond strength: effect of different adhesion primers. *J Dent* 31:521-525.
13. Swift EJ, Le Valley BD, Boyer DB (1992) Evaluation of new methods for composite repair. *Dent Mater* 8:362-365.
14. Brendeke J, Özcan M (2007) Effect of physicochemical aging conditions on the composite-composite repair bond strength. *J Adhes Dent* 9:399-406.
15. Chiba K, Hosoda H, Fusayama T (1989) The addition of an adhesive composite resin to the same material : bond strength and clinical techniques. *J Prosthet Dent* 61:669-675.

16. Özcan M, Alander P, Vallittu PK, Huysmans MC, Kalk W (2005) Effect of three surface conditioning methods to improve bond strength of particulate filler resin composites. *J Mat Sci Mater Med* 16:21-27.
17. Gregory WA, Pounder B, Bakus E (1990) Bond strengths of chemically dissimilar repaired composite resins. *J Prosthet Dent* 64:664-668.
18. Attal J, Asmussen E, Degrange M (1994) Effects of surface treatment on the free surface energy of dentin. *Dent Mater* 10:258-264.
19. Erickson R (1992) Surface interactions of dentin adhesive materials. *Oper Dent* Suppl 5:81-94.
20. Fano L, Fano V, Ma WY, Wang XG, Zhu F (2005) Adhesiveness of dental resin-based restorative materials investigated with atomic force microscopy. *J Biomed Mater Res B Appl Biomater* 73:35-42.
21. Asaka Y, Miyazaki M, Aboshi H, Yoshida T, Takamizawa T, Kurokawa H, Rikuta A (2004) EDX fluorescence analysis and SEM observations of resin composites. *J Oral Sci* 46:143-148.
22. Gerth HU, Dammaschke T, Zuchner H, Schafer E (2006) Chemical analysis and bonding reaction of RelyX Unicem and Bifix composites--a comparative study. *Dent Mater* 22:934-941.
23. Özcan M, Barbosa S, Melo R, Galhano G, Bottino M (2007) Effect of surface conditioning methods on the microtensile bond strength of resin composite to composite after aging conditions. *Dent Mater* 23:1276-1282.
24. Oilo G (1993) Bond strength testing-what does it mean? *Int Dent J* 17:492-498.
25. Beun S, Glorieux T, Devaux J, Vreven J, Leloup G (2007) Characterization of nanofilled compared to universal and microfilled composites. *Dent Mater* 23:51-59.
26. Senawongse P, Sattabanasuk V, Shimada Y, Otsuki M, Tagami J (2004) Bond strengths of current adhesive systems on intact and ground enamel. *J Esthet Restor Dent* 16:107-115.
27. Schmalz G (2009) *Resin-based composites*. In: Schmalz G, Arenholt-Bindslev D (eds) Biocompatibility of dental materials. Springer, Berlin, pp 99-138.
28. Venhoven BAM, de Gee AJ, Werner A (1996) Influence of filler parameters on the mechaneical coherence of dental restorative resin composites. *Biomaterials* 17:735-740.
29. Cassie A, Baxter S (1944) Wettability of porous surfaces. *T Faraday Soc* 40:546-551.
30. Lombardi V (2002) *Placement and finsihing*. In: Albers H (ed) Tooth-colored restoratives: Principles and techniques. 9th edn. BC Decker Inc, Hamilton, pp 159-181.

31. Truffier-Boutry D, Place E, Devaux J, Leloup G (2003) Interfacial layer characterization in dental composite. *J Oral Rehabil* 30:74-77.

32. Eliades G, Caputo A (1989) The strength of layering technique in invisible light-cured composites. *J Prosthet Dent* 61:31-38.

33. da Silva EM, Poskus LT, Guimaraes JGA (2008) Influence of light-polymerization modes on the degree of conversion and mechanical properties of resin composites: A comparative analysis between a hybrid and a nanofilled composite. *Oper Dent* 33:287-293

34. Sano H, Shono T, Sonoda H, Takatsu T, Ciucchi B, Carvalho R, Pashley DH (1994) Relationship between surface area for adhesion and tensile bond strength -- Evaluation of a micro-tensile bond test. *Dent Mater* 10:236-240.

35. Sau CW, Oh GSY, Koh H, Chee CS, Lim CC (1999) Shear bond strength of repaired composite resins using a hybrid composite resin. *Oper Dent* 24:156-161.

36. Oztas N, Alacam A, Bardakci Y (2003) The effect of air abrasion with two new bonding agents on composite repair. *Oper Dent* 28:149-154.

37. Li Y, Swartz ML, Phillips RW, Moore BK, Roberts TA (1985) Effect of filler content and size on properties of composites. *J Dent Res* 64:1396-1401.

38. Valandro L, Özcan M, Bottine M, Bottino M, Scotti R, Bona A (2006) Bond strength of a resin cement to high-alumina and zirconia-reinforced ceramics: The effects of surface conditioning. *J Adhes Dent* 3:175-181.

39. Hisamatsu N, Atsuta M, Matsumura H (2002) Effect of silane primers and unfilled resin bonding agents on repair bond strength of a prosthodontic microfilled composite. *J Oral Rehabil* 29:644-648.

40. Sideridou I, Tserki V, Papanastaiou G (2002) Effect of chemical structure on degree of conversion in light-cured dimethacrylate-based dental resins. *Biomaterials* 23:1819-1829.

41. Lucena-Martin C, Lopez S, de Mondelo J (2001) The effect of various surface treatments and bonding agents on the repaired strength of heat-treated composites. *J Prosthet Dent* 86:481-488.

Chapter 5

Effects of Surface Conditioning on Repair Bond Strengths of Non-aged and Aged Microhybrid, Nanohybrid and Nanofilled Composite Resins

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1 Introduction

Advances in adhesive technologies strongly influenced current concepts in restorative dentistry with the goal of conserving healthy dental tissues and reducing the number of interventions needed.[1,2] Application of resin-based composites does not require mechanical retention enabling minimal invasive treatment.[3] However, dynamic conditions in the oral environment, such as pH changes in saliva, diet, and rapid temperature alterations, may degrade the composite resin.[4,5] *In vivo* aging involves various phenomena including discoloration, microleakage, wear, ditching at the margins, delamination or simply fracture and may ultimately require replacement.[6-8] Total replacement of a restoration is the most common procedure in daily clinical practice.[6] However, this approach may be regarded as overtreatment, since in most cases, a large portion of the restoration is clinically and radiographically intact. Complete removal of a restoration inevitably leads to weakening of the tooth structure, unnecessary grinding of sound dental tissues and sometimes repeated injuries to the pulp.[9,10] For this reason, repair of existing restorations through relayering is considered as an alternative for total replacement.[6] The success of composite-to-composite bonding in repair through relayering depends on the condition of the composite resin surface, including its composition,[11] roughness,[12] wettability[13] and the surface conditioning methods applied.[11,14-16]

Due to aging of the composite resin surface in the dynamic oral environment, the adhesive strength of composite-to-composite restorations decreases by 25 to 80% compared to their original strength.[16,17] Therefore, various surface conditioning methods have been developed to improve adhesion between aged and non-aged composite resins. The use of an intermediate adhesive resin is known to enhance the repair bond strength significantly,[14,16] while also chairside air-borne particle abrasion with small silica-coated particles, followed by silanization has been reported to yield a significant increase in composite-to-composite bonding,[12,15,18] but there is no consensus about possible benefits of the use of silica-coating over application of intermediate adhesive resins for aged composite resins.

In laboratory studies, aging of composite resins has been simulated by storage in water,[19,20] citric acid immersion[15,21] or subjecting them to thermocycling.[22,23] Among all these methods, water storage is considered to have detrimental effects on the composite resin surface due to hydrolysis and release of filler particles as well as water uptake in the resin matrix.[24,25] Thermocycling generates stresses due to differences between thermal expansion of various materials involved in a restoration that could result in bond failure at the tooth-restoration or filler-matrix interface.[26] Aging of composite resins through citric acid challenges has not been frequently applied, but may be advocated to mimic effects of acidic food and beverages. Citric acid immersion is known to cause release of filler particles, similar as observed after water storage.[15] Composite-to-composite bonding after aging has been investigated in several studies[27,28] without a common consensus on which aging method represents the worst case or clinically most relevant scenario.

The objectives of this study were to compare the strengths of composite-to-composite bonding for microhybrid, nanohybrid and nanofilled composite resins after different aging methods and to analyse the effect of different aging conditions on the composite surface.

2 Materials and methods

2.1 Composite resins, specimen preparation and aging conditions

Product names, manufacturers, chemical compositions, abbreviations and batch numbers of the materials used in this study are listed in Table 1.

Table 1 Product and company names, chemical compositions, abbreviations and batch numbers of the materials used in this study.

Product name	Company name	Chemical composition	Abbre-viations	Batch Number
COMPOSITE RESIN				
Quadrant Anterior Shine (microhybrid)	Cavex BV, Haarlem, The Netherlands	Bis-GMA, DUDMA, silica, silicate glass, Ba-glass, fluoride containing fillers (63 v%)	AS	010100
Grandio (nanohybrid)	Voco GmbH, Cuxhaven, Germany	Bis-GMA, UDMA, TEGMA, Glass-ceramic, SiO_2 containing fillers (71.4 v%)	G	621332
Tetric Evo Ceram (nanohybrid)	Ivoclar Vivadent AG, Schaan, Liechtenstein	Bis-GMA, TEGDMA, UDMA , Ba-Al-F-B-Silicate, SiO_2, mixed oxide, YbF3 containing fillers (48.5 v%)	TE	J04088
Filtek Supreme XT (nanofilled)	3M ESPE, St. Paul, MN, USA	TEGDMA, UDMA, Bis-EMA, Zirconia-Silica, Silica containing fillers (57.7 v%)	FS	6 BG
TRIBOCHEMICAL SILICA COATING KIT (for SC-application)				
CoJet®-Sand	3M ESPE AG, Seefeld, Germany	Aluminum trioxide particles coated with silica, particle size: 30 µm	SC	165092
ESPE®-Sil	3M ESPE AG, Seefeld, Germany	3-methacryloxypropyl-trimethoxysilane, ethanol	Silane	152745
INTERMEDIATE ADHESIVE RESIN (for IAR-application)				
Visio™-Bond (for tribochemical silica coating treatment)	3M ESPE AG, Seefeld, Germany	Dicyclopentyldimethylene diacrylate 2-propenoic acid, 2-methyl, 2-[(2-hydroxyethyl)(3-methoxypropyl)amino]ethyl ester	VB	161808
Quadrant Unibond (for AS)	Cavex BV, Haarlem, The Netherlands	Bis-GMA, TEGDMA, silicate glass fillers, silica, polycarboxylic acid, champorquinone	Q	010049
Solobond Plus (for G)	Voco GmbH, Cuxhaven, Germany	Bis-GMA, TEGDMA, HEMA, champorquinone	S	591583
Multilink (for TE)	Ivoclar Vivadent AG, Schaan, Liechtenstein	Primer A: Water, initiators (sulfonate, amines) Primer B: Phosphonic acid acrylate, HEMA, TEGDMA, methacrylate modified polyacrylic acid	M	Primer A: H10145 Primer B: 09713
Adper Scotchbond 1XT (for FS)	3M ESPE, St. Paul, MN, USA	Dimethacrylate, HEMA, polyalcenoic acid copolymer, silane treated colloidal silica, ethanol, water, photoinitiator	A	4BM

A flow-diagram of the experimental design and techniques applied is presented in Fig. 1.

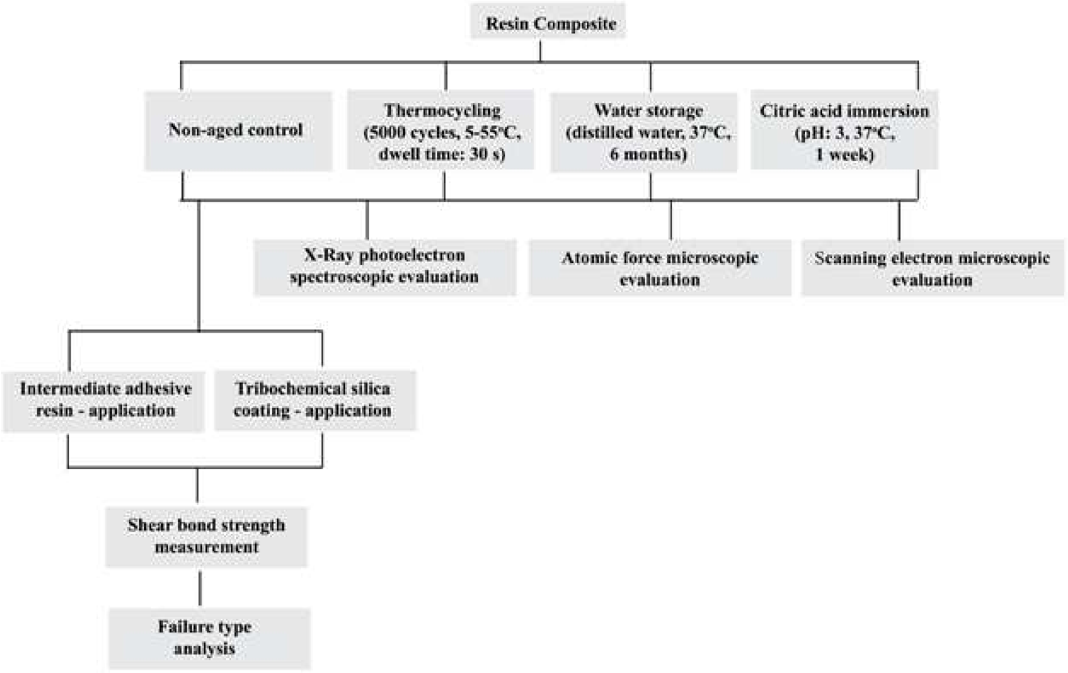


Fig. 1 Flow-diagram of the experimental design and techniques applied.

Disc shaped specimens were made by placing unpolymerized composite resins into cylindrical undercut cavities (diameter: 5.5 mm, thickness: 3.5 mm) prepared of polymethylmethacrylate surrounded by a polyvinylchloride cylinder (3 specimens per cylinder). The unpolymerized composite resins were packed in increments of 2 mm into the cavities with a hand instrument and photo-polymerized. Each increment was photo-polymerized with a halogen photo-polymerization unit (Optilux 501, Kerr Co., Orange, CA, USA) for 20 s (G, TE and FS) or 40 s (AS) at a distance of 2 mm from the surface. Light intensity was higher than 400 mW/cm^2, as verified by a radiometer (Demetron LC, Kerr Co.), after every six specimens. During final photo-polymerization, the top surface layer was covered with a 100 µm thick translucent Mylar strip (KerrHawe SA, Bioggio, Switzerland) in order to create a smooth surface and to prevent the formation of an oxygen-inhibited layer. Specimens of each composite resin group were subjected to one of the three aging conditions: 1) 5000 Thermocycles in water from 5 to 55°C with a dwell time of 30 s at each temperature and a transfer time from one water bath to the other of 5 s (Willytec GmbH, Gräfelfing, Germany); 2) Immersion in deionized water at 37°C during six months; 3) Immersion in citric acid (pH 3.0) at 37°C during one week. A non-aged group acted as the control.

After aging, the composite resin surfaces were conditioned with one of the following two procedures:

Intermediate adhesive resin (IAR-application): IARs of the corresponding composite resins (Table 1) were applied in a thin layer on the substrates using a micro-brush. Subsequently, the solvent was gently air-thinned under oil-free compressed air and finally photo-polymerized according to each manufacturer's instructions.

Tribochemical silica coating (SC-application): A silica coating (see also Table 1) was applied using an intraoral air-abrasion device (Dento-Prep™, RØNVIG A/S, Daugaard, Denmark) filled with 30 µm alumina particles coated with silica from a distance of approximately 10 mm at a pressure of 2.5 bars for 4 s. Following surface conditioning, loose particles were gently air blown. The conditioned substrates were then coated with a 3-methacryloxypropyl-trimethoxysilane coupling agent, γ-MPS allowing 5 min for its reaction. Finally an intermediate adhesive resin, specific to the silica coating procedure was applied with a microbrush, air thinned and photo-polymerized for 20 s. The entire procedure described above will be referred to in this manuscript as SC-application.

2.2 *Repair bond strengths and failure analysis*

Following surface conditioning, composite resins of the same kind as their substrates were adhered onto the conditioned substrates using translucent polyethylene molds (inner diameter: 3.6 mm; height: 5 mm). The composite resin was placed against the substrate incrementally with a hand instrument and photo-polymerized in two layers with a thickness of less than 2 mm. Each layer was polymerized according manufacturer instruction, from a distance of 2 mm from the mold. After polymerization, specimens were gently removed from the polyethylene molds.

Specimens were secured in a mounting jig of a universal testing machine (ROELL Z2.5 MA 18-1-3/7, Zwick GmbH, Ulm, Germany). The force was applied at the adhesive interface using a semicircular blade with 45 degrees inclination at the tip, fitting around the cylindrical composite specimen, at a cross-head speed of 1 mm/min until failure. The mode of failure was determined under optical light microscopy (MP 320, Carl Zeiss GmbH, Jena, Germany) at a x40 magnification and recorded as cohesive in the substrate, appearing as small indents or as adhesive failure at the interface showing a completely smooth surface.

2.3 Surface characterization

For surface characterization, composite blocks (5 x 6 x 6 mm) were produced, as described above.

Elemental compositions of the outermost composite surfaces can be determined quantitatively by X-ray photoelectron spectroscopy. In this study, X-ray photoelectron spectroscopy was performed using an S-Probe spectrometer (Surface Science Instruments, Mountain View, CA, USA) equipped with an aluminum anode (10 kV, 22 mA) and a quartz monochromator. The direction of the photoelectron collection angle to the specimens was 55 degrees and the electron flood gun was set at 10 eV. A survey scan was made with a 1000 x 250 µm spot and pass energy of 150 eV. Binding energies were determined by setting the binding energy of the C_{1s} component due to carbon-carbon bonds at 284.8 eV.

X-ray photoelectron spectroscopy data were used to model the composite surface. For a fully resin matrix covered surface, the molecular structure of the resin yields a theoretical value $(O/C)_{theoretical}$ that can be compared with the experimental value for $(O/C)_{experimental}$. Since the experimental value for (O/C) is due to the resin matrix and silica-containing filler particles, the experimental values for $(O/C)_{experimental}$ can be expressed in a matrix part $(O/C)_{matrix}$ by subtracting the oxygen arising from silica (for SiO_2, this involves subtracting twice the amount of measured Si from the amount of measured O). Subsequently, the fraction of the surface covered by filler particles can be derived from

$$f_{filler} = 1 - (O/C)_{matrix} / (O/C)_{theoretical}$$

Surface roughness can be calculated from three-dimensional topographic images produced by atomic force microscopy. The atomic force microscope (Nanoscope IIIa Dimension™ 3100, Digital Instruments, Santa Barbara, CA, USA) was operated in the contact mode, using a Si_3N_4 cantilever tip (DNP from Veeco, Woodbury, NY) with a spring constant of 0.06 N/m. The composite resin was placed on a glass slide using double-sided sticky tape. Specimens were placed below the cantilever of the atomic force microscope to obtain three dimensional images (70 x 70 μm) of the surface at three randomly selected positions on each specimen from which the average surface roughness was calculated.

A cold field emission scanning electron microscope (JSM-6301F, Jeol Instruments, Tokyo, Japan) was used to image the morphology of the composite surfaces prior to and after aging. Images were taken at 25 kV at a magnification of x10000. Surfaces were first sputter-coated with gold/palladium (80/20) prior to examination.

2.4 *Statistical analysis*

Statistical analysis was performed with the SPSS software package (version 14.0; SPSS, Chicago, IL, USA). The results of the normality and homogeneity test (Kolmogorov-Smirnov) indicated that the residual values were normally distributed when plotted against predicted values. Effects of composite types, surface conditioning procedures and aging methods on bond strengths were compared using three-way ANOVA and LSD post-hoc tests. The differences in surface roughnesses were analyzed using Mann-Whitney U. In all comparisons, statistical significance was accepted if the p value was less than 0.05.

3 Result

3.1 *Bond strength and failure types*

The shear bond strengths of the composite resins are shown in Table 2. Three-way ANOVA indicated significant effects ($p < 0.05$) of composite types, surface conditioning procedures and aging methods on repair bond strengths. Significant interactions were identified between composite types with surface conditioning procedures, composite types with aging methods, and surface conditioning procedures with aging method. LSD post-hoc tests showed that bond strengths after IAR-application were significantly lower in aged composite resins than in non-aged control ones ($p < 0.05$), except for TE and FS after aging in citric acid. SC-application, however, led to significantly higher bond strengths than IAR-application, especially after water storage. Regardless of the composite type, SC-application yielded more cohesive failures in the substrate (70, 88, and 70% for thermocycling, water storage and citric acid immersion, respectively) than after application of the corresponding IARs (5, 15, and 48% for thermocycling, water storage and citric acid immersion, respectively).

Table 2 Mean repair bond strengths (MPa) for the four different composite resins after different aging conditions. Composite surfaces were either conditioned application of an intermediate adhesive resin (IAR) or silica-coating (SC).
± denotes standard deviations over 10 specimens. For abbreviations see Table 1.

Composite resin	**After IAR-application**				**After SC-application**			
	Non aged control	**Thermo-cycling**	**Water storage**	**Citric acid immersion**	**Non-aged control**	**Thermo-cycling**	**Water storage**	**Citric acid immersion**
AS	19.8 ± 4.6	2.8 ± 0.8	3.8 ± 2.6	13.8 ± 6.4	23.2 ± 1.3	18.7 ± 6.7	28.3 ± 7.4	11.4 ± 4.0
G	31.8 ± 5.7	10.4 ± 3.8	12.7 ± 3.7	13.7 ± 3.3	32.2 ± 4.2	23.0 ± 8.0	45.2 ± 5.0	13.8 ± 3.1
TE	22.2 ± 6.5	11.9 ± 2.4	8.4 ± 4.6	18.0 ± 7.1	20.0 ± 5.2	16.9 ± 6.5	32.2 ± 8.3	21.7 ± 8.6
FS	27.6 ± 6.6	7.1 ± 3.1	13.0 ± 8.1	23.0 ± 9.8	29.2 ± 6.4	20.9 ± 7.1	11.2 ± 3.9	17.5 ± 6.9

The changes in bond strengths after aging with respect to those of the non-aged controls are presented in Fig. 2. IAR-application on aged composite resins did not improve the repair bond strengths to the level obtained in the non-aged controls, regardless of the aging method and type of composite resin involved. Also SC-application did not restore bond strengths of composite surfaces aged by thermocycling and citric acid immersion to the level of non-aged controls, but it did improve the bond strength after water storage (except for FS).

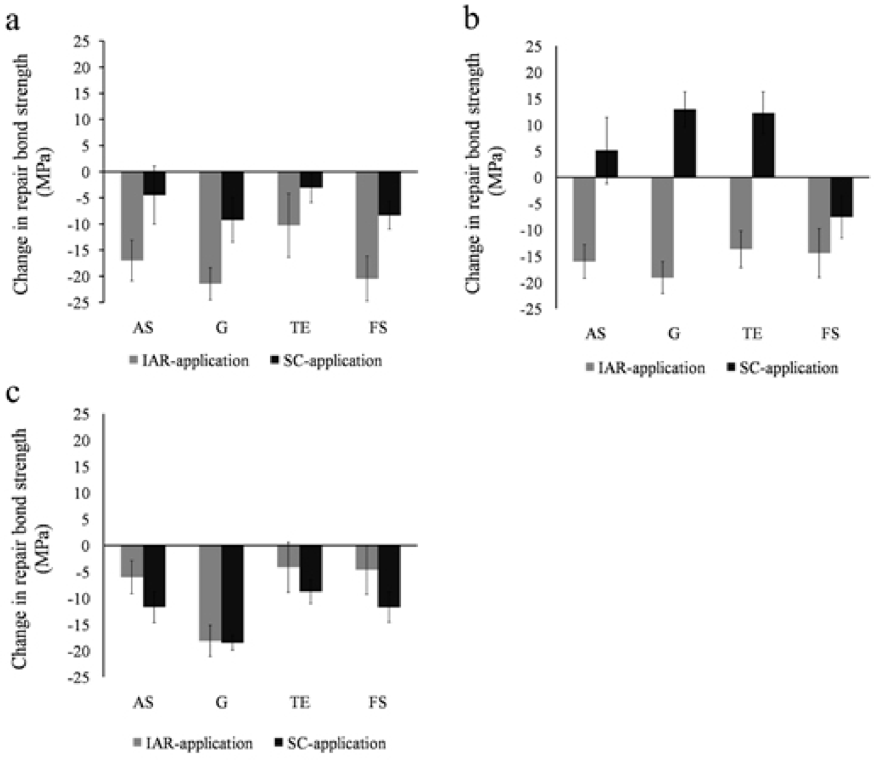


Fig. 2 Changes in repair bond strengths with respect to non-aged controls for the four composite resins involved in this study, following conditioning using either IAR- or SC-application and after

- **a)** thermocycling,
- **b)** water storage and
- **c)** citric acid immersion.

Error bars denote the SD over ten different specimens per group.

3.2 *Surface analysis*

The percentage filler exposure at the composite surfaces prior to and after aging is presented in Table 3 for non-aged and aged composite resins. Thermocycling and citric acid immersion increased filler particle exposure at the surface in all composite types with respect to the non-aged control. Water storage showed only minor effects on filler exposure, except for AS, which was the only composite type showing a lower filler exposure after water storage.

Table 3 Filler exposure at the composite surface prior to and after different aging conditions, calculated from X-ray photoelectron spectroscopic (XPS) analyses and comparison to the bulk filler composition provided by the manufacturers. For abbreviations see Table 1.

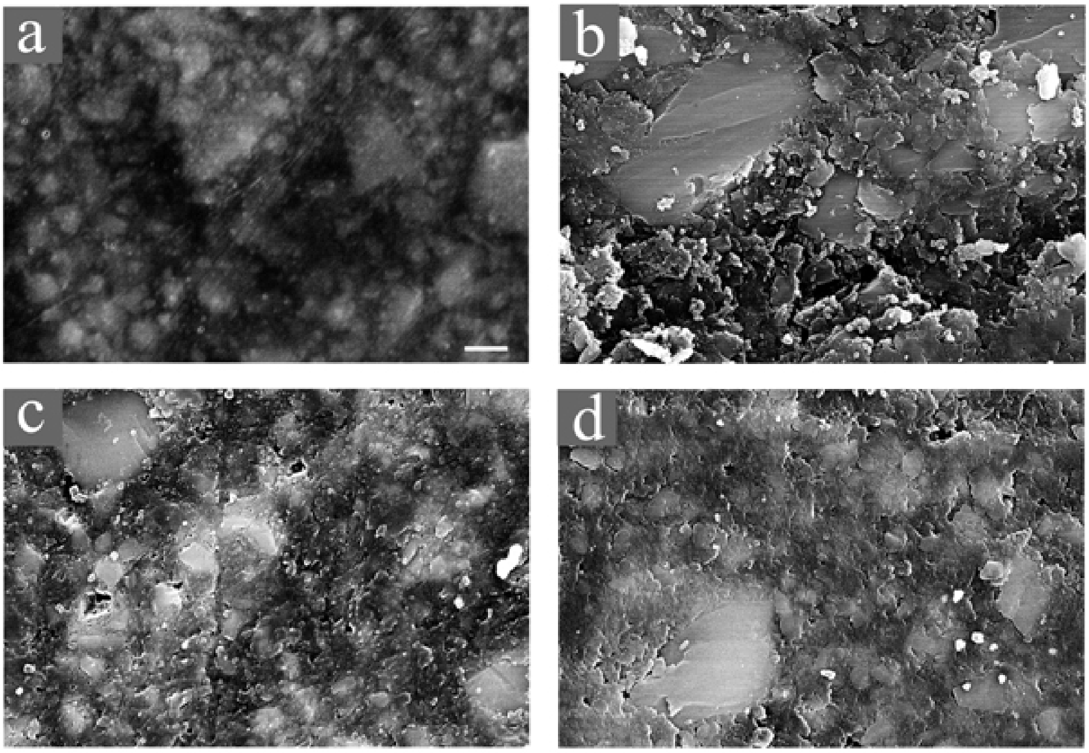
Composite resin	Filler exposure at the surface from XPS (%)				Bulk filler composition (v%) according to the manufacturer
	Non-aged control	Thermo-cycling	Water storage	Citric acid immersion	
AS	20	22	7	25	63
G	27	56	21	51	71
TE	41	60	42	64	48
FS	14	49	24	68	58

The roughness of the non-aged controls ranged between 4 and 9 nm, and increased significantly ($p < 0.05$) after aging by a factor of five to ten (19 to 79 nm), as can be seen in Table 4. Subsequent SC-application yields another significant increase by a factor of ten ($p < 0.05$). However, the effects of silica-coating are variable, depending on the aging condition. Thermocycling generally yields significantly higher surface roughnesses than the non-aged control, whereas after water storage and citric acid immersion similar roughnesses are observed as for the non-aged control.

Table 4 Mean surface roughness (nm) of the four composite resins after different aging conditions. Surface roughness was measured prior to and after silica-coating. ± denotes standard deviations over 3 specimens. For abbreviations, see Table 1

Composite	**Prior to silica-coating**				**After silica-coating**			
resin	**Non-aged control**	**Thermo-cycling**	**Water storage**	**Citric acid immersion**	**Non-aged control**	**Thermo-cycling**	**Water storage**	**Citric acid immersion**
AS	9 ± 1	37 ± 6	54 ± 21	39 ± 18	409 ± 26	510 ± 69	360 ± 14	335 ± 12
G	7 ± 1	43 ± 17	53 ± 7	20 ± 12	351 ± 24	511 ± 29	392 ± 13	342 ± 41
TE	8 ± 3	79 ± 13	30 ± 5	25 ± 2	346 ± 15	494 ± 124	378 ± 13	364 ± 8
FS	4 ± 1	65 ± 24	41 ± 7	22 ± 5	411 ± 18	463 ± 74	418 ± 20	329 ± 111

Scanning electron micrographs of composite surfaces prior to aging, show scattered white regions, probably indicative of filler particles covered by a thin layer of matrix. All aging conditions clearly roughen the surface, as illustrated in Fig. 3 for the nanohybrid composite resin G**.**



Figs. 3 Representative scanning electron micrographs of the nanohybrid composite resin, Grandio (G).

- **a)** non-aged control
- **b)** after thermocycling,
- **c)** after water storage and
- **d)** after citric acid immersion.

Bar marker indicates 1 µm.

4 Discussion

The durability of composite-to-composite repair bonding depends on the adhesion between the polymerized substrate and the adhering composite resin. The aging condition of the substrate may affect the strength of the adhesive joint. In this manuscript, we evaluated the effects of composite type, surface conditioning procedure and aging method on the repair bond strengths of microhybrid, nanohybrid and nanofilled composite resins and analyzed surface properties of the composite resins after aging. The results indicated that composite type, surface conditioning procedure and aging method all influenced the repair bond strengths and the prevalence of cohesive failure.

In vitro, analysis of composite-to-composite bonding can be done either by applying a shear or microtensile forces. Shear bond strength evaluation requires easier specimen preparation and alignment during measurement than microtensile bond strength measurement.[29] However, shear bond strength evaluation has been suggested to be less reliable than microtensile bond strength evaluation,[30] because the adhesive interface in microtensile bond strength analysis is relatively small, invoking a more uniform stress distribution and therewith allowing better access to the true interfacial bond strength. To date, there is no consensus on whether the strength of composite-to-composite bonding should be evaluated in a shear or microtensile mode, although it can be argued that clinically, in the repair of composite restorations, applied forces are predominantly in the shear mode, as applied here.

Previously,[31] we have extensively discussed the effects of IAR- and SC-application in immediate repair, i.e. in non-aged composite resins. SC-application was found to create significantly higher bond strengths than IAR-application in all four composite resins. Also in the current study, repair bond strengths of non-aged composite resins after SC-application were slightly higher or similar than after IAR-application, but not to the extend as seen before. In repair after aging of a composite resin, simulated here by three different aging methods, neither IAR- nor SC-application were generally able to create bond strengths comparable to the ones observed in immediate repair, i.e. non-aged controls in the current study, although it is known that different aging methods degrade the composite surface in different ways. In this respect, it is interesting that water storage followed by SC-application was the only combination studied that yielded an increase in bond strength with respect to non-aged controls (see Fig. 2). Hypothetically, we attribute this to increased capture of silica-coated particles by the softened resin matrix.

Thermocycled specimens have been subjected to temperature fluctuations, generating thermal stresses and leading to microcracks in the matrix or failure at the filler/matrix interface.[22,23] Moreover, exposure to water may cause hydrolytic degradation of filler's silane coating[23,32] or swelling of the matrix.[33] Filler exposure after thermocycling never decreased in the current study, regardless of the composite type, indicating that particle detachment is highly unlikely. Differences in filler exposure after thermocycling are thus most likely due to matrix degradation, leading to exposure of underlying filler particles and an increased surface roughness. Composite resins containing hydrophilic components, like TEGDMA or TEGMA as a matrix (G, TE and FS), may be more susceptible to matrix degradation[32] than AS, lacking TEGDMA or TEGMA that enable easier water penetration due to their hydrophilicity.

There is limited information about the effect of citric acid immersion on composite degradation. Aging in citric acid takes place at a low pH and in an aqueous environment, but at the same time it lacks temperature fluctuations as in thermocycling. Yet, citric acid immersion had similar effects on filler particle exposure than thermocycling, but caused a smaller increase in surface roughness. Thus, whereas matrix degradation due to fluctuating temperatures is the likely cause for increased filler particles exposure after thermocycling, citric acid may cause matrix degradation by the same effects. Water exposure causes an increase in surface roughness, which is probably more due to swelling rather than to matrix degradation, because filler particle exposure after water storage hardly increases.

The severe aging effects caused by aggressive thermocycling and citric acid immersion negatively impacted the repair bond strengths compared to non-aged composite resins, regardless of the composite type and conditioning applied. Similarly, IAR-application after water storage also had a negative impact on repair bond strengths, but surprisingly SC-application after water storage yielded higher bond strengths for AS, G and TE. Possibly, increased filler particle exposure as after thermocycling and citric acid immersion causes silica-coated particles to bounce off the filler dominated surfaces, whereas after water storage, causing predominantly swelling, the swelled matrix at the composite surface will yield increased capture of silica-coated particles and a more positive effect of SC-application. Interestingly, SC-application did not give any benefit for FS aged by water storage.

Regardless of the aging method and composite type, cohesive failure in the substrate appeared much more frequent than adhesive failure after SC-application. IAR-application on aged composite resins resulted in more adhesive failures. The cohesive failures observed after SC-application indicate that the adhesive strength at the interface exceeded the cohesive strength of the underlying composite resin, and thus the repair as such can not be considered the weakest link. Moreover, if a composite repair fractures cohesively in the substrate, one can assume that the approach selected for repair was appropriate to bear the occlusal loads.[34] Thus based on failure type analysis, SC-application should generally be preferred for aged composite resins, although it has been argued that also a non-functional abrasive powder, such as Al_2O_3 may produce the same results.[35] However, it remains to be investigated whether the durability of the bond that can be obtained with a non-functional powder is equally high as observed for functionalized one. A silica-functionalized surface is chemically more reactive to the resin. Silane molecules react with water to form three silanol groups (–Si–OH) from the corresponding methoxy groups

(–Si–O–CH_3). The silanol groups then react further to form a siloxane (–Si–O–Si–O–) network with the silica surface. Monomeric ends of the silane molecules react with the methacrylate groups of the adhesive resins by a free radical polymerization process. Clearly, no siloxane network will form in case of a non-functional powder.[36]

5 Conclusions

Thermocycling, water storage and citric acid immersion affect the surface of composite resins with an impact on the repair bond strengths. Bond strength analysis is indecisive on whether aged composite resins should be repaired using IAR- or SC-application. Failure type analysis however, strongly indicates that in general SC-application should be favoured for the repair of aged composite resins.

References

1. Tyas MJ, Anusavice KJ, Frencken JE, Mount GJ (2000) Minimal intervention dentistry - a review. FDI Commission Project 1-97. *Int Dent J* 50:1-12.
2. White JM, Eakle WS (2000) Rationale and treatment approach in minimally invasive dentistry. *J Am Dent Assoc* 131 Suppl:13S-19S.
3. Murdoch-Kinch MA, McLean ME (2003) Minimally invasive dentistry. *J Am Dent Assoc* 134:87-95.
4. Akova T, Ozkomur A, Uysal H (2006) Effect of food-simulating liquids on the mechanical properties of provisional restorative materials. *J Dent* 22:1130-1134.
5. Sarkar NK (2000) Internal corrosion in dental composite wear: its significance and simulation. *J Biomed Mater Res B Appl Biomater* 53:371-380.
6. Mjor IA (1981) Placement and replacement of restorations. *Oper Dent* 6:49-54.
7. Roulet JF (1988) The problems associated with substituting composite resins for amalgam: a status report on posterior composites. *J Dent* 16:101-113.
8. Swift EJ (1987) Wear of composite resins in permanent posterior teeth. *J Am Dent Assoc* 115:584-588.
9. Lewis G, Johnson W, Martin W, Canerdy A, Claburn C, Collier M (1998) Shear bond strength of immediately repaired light-cured composite resin restorations. *Oper Dent* 23:121-127.
10. Mjor IA, Moorhead JE, Dahl JE (1999) Selection of restorative materials in permanent teeth in general dental practice. *Acta Odontol Scand* 57:257-262.
11. Papacchini F, DallÓca S, Chieffi N, Goracci C, Sadek FT, Suh BI, Tay FR, Ferrari M (2007) Composite-to-composite microtensile bond strength in the repair of a microfilled hybrid resin : Effect of surface treatment and oxygen inhibition. *J Adhes Dent* 9:25-31.
12. Bouschlicher MR, Reindhardt JW, Vargas MA (1997) Surface treatment techniques for resin composite repair. *Am J Dent* 10:279-283.
13. Rosales-Leal JI, Osorio R, Holgadi-Terriza JA, Cabrrerizo-Vilches MA, Toledano M (2001) Dentin wetting by four adhesive systems. *Dent Mater* 17:526-532.
14. Chiba K, Hosoda H, Fusayama T (1989) The addition of an adhesive composite resin to the same material : bond strength and clinical techniques. *J Prosthet Dent* 61:669-675.
15. Özcan M, Barbosa S, Melo R, Galhano G, Bottino M (2007) Effect of surface conditioning methods on the microtensile bond strength of resin composite to composite after aging conditions. *Dent Mater* 23:1276-1282.

16. Shahdad SA, Kennedy JG (1998) Bond strength of repaired anterior composite resins: an *in vitro* study. *J Dent* 26:685-694.
17. Turner CW, Meiers JC (1993) Repair of an aged, contaminated indirect composite resin with a direct, visible-light-cured composite resin. *Oper Dent* 18:187-194.
18. Swift EJ, Le Valley BD, Boyer DB (1992) Evaluation of new methods for composite repair. *Dent Mater* 8:362-365.
19. Cesar P, Meyer Faara P, Miwa Caldart R, Gastaldoni Jaeger R, da Cunha Ribeiro F (2001) Tensile bond strength of composite repairs on Artglass using different surface treatments. *Am J Dent* 14:373-377.
20. Frankenberger R, Kramer N, Sindel J (2000) Repair strength of etched vs silica-coated metal-ceramic and all-ceramic restorations. *Oper Dent* 25:209-215.
21. Yap AUJ, Sau CW, Lye KW (1999) Effects of aging on repair bond strengths of a polyacid-modified composite resin. *Oper Dent* 24:371-376.
22. Hakimeh S, Vaidyanathan J, Houpt ML, Vaidyanathan TK, Von Hagen S (2000) Microleakage of compomer Class V restorations: Effect of load cycling, thermal cycling, and cavity shape differences. *J Prosthet Dent* 83:194-203.
23. Kawano F, Ohguri T, Ichikawa T, Matsumoto N (2001) Influence of thermal cycles in water on flexural strength of laboratory-processed composite resin. *J Oral Rehabil* 28:703-707.
24. Cattani-Lorente M, Dupuis V, Moya F, Payan J, Meyer JM (1999) Comparative study of the physical properties of a polyacid-modified composite resin and a resin-modified glass ionomer cement. *Dent Mater* 15:21-32.
25. Soderholm KJ, Zigan M, Ragan M, Fischlschweiger W, Bergman M (1984) Hydrolytic degradation of dental composites. *J Dent Res* 63:1248-1254.
26. Crim GA, Garcia-Godoy F (1987) Microleakage: The effect of storage and cycling duration. *J Prosthet Dent* 57:574-576.
27. Brentel AS, Özcan M, Valandro LF, Alarca LG, Amaral R, Bottino MA (2007) Microtensile bond strength of a resin cement to feldpathic ceramic after different etching and silanization regimens in dry and aged conditions. *Dent Mater* 23:1323-1331.
28. Padipatvuthikul P, Mair LH (2007) Bonding of composite to water aged composite with surface treatments. *Dent Mater* 23:519-525.
29. Rasmussen S (1996) Analysis of dental shear bond strength tests, shear or tensile. *Int J Adhesion and Adhesives* 16:147-154.

30. Della BA, Anusavice K, Shen C (2000) Microtensile strength of composite bonded to hot-pressed ceramics. *J Adhes Dent* 2:305-313.

31. Rinastiti M, Özcan M, Siswomihardjo W, Busscher HJ (2009) Immediate repair bond strengths of microhybrid, nanohybrid and nanofilled composites after different surface treatments. *J Dent* 38:29-38.

32. Örtengren U, Andersson F, Elgh U, Terselius B, Karlsson S (2001) Influence of pH and storage time on the sorption and solubility behaviour of three composite resin materials. *J Dent* 29:35-41.

33. McCabe JF, Rusby S (2004) Water absorption, dimensional change and radial pressure in resin matrix dental restorative materials. *Biomaterials* 25:4001-4007.

34. Lucena-Martin C, Lopez S, de Mondelo J (2001) The effect of various surface treatments and bonding agents on the repaired strength of heat-treated composites. *J Prosthet Dent* 86:481-488.

35. Rathke A, Tymina Y, Haller B (2009) Effect of differnet surface treatments on the composite-composite repair bond strength. *Clin Oral Invest* 13:317-323.

36. Özcan M, Vallittu PK (2003) Effect of surface conditioning methods on the bond strength of luting cement to ceramics. *Dent Mater* 19:725-731.

Chapter 6

Effect of Biofilm on the Repair Bond Strengths of Composite Resins

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1 Introduction

Microorganisms in the oral environment not only form a biofilm on all available surfaces, including hard and soft tissue surfaces, but also on biomaterials used for restoration of function or aesthetics.[1,2] Composite materials in restorative dentistry are used more and more, although composites are not inert to the conditions prevailing in the oral cavity. Exposure to food components, acidic beverages, temperature changes,[3,4] chewing, saliva and biofilm[5] lead to degradation of composite surfaces.[3,6] Degraded composite surfaces may have increased roughness, sometimes accompanied by decreased microhardness and increased exposure of filler particles or matrix swelling.[7,8] Repair of composite restorations not necessarily involves removal of an entire restoration with its associated damage to surrounding dental hard tissues. Often, repair can be done by relayering, i.e. bonding of a composite layer onto an existing one.[9] Previous studies have shown that exposure of composite surfaces to biofilm may degrade the composite surface, but the extent to which this affects composite-to-composite bonding has never been determined.

In order to enhance composite-to-composite bonding, different surface conditioning methods can be applied, like intermediate adhesive resin (IAR) application,[10,11] or silica-coating followed by silanization and IAR-application (SC-application).[12-14] The efficacy of different surface conditioning methods depends on the aging conditions and the type of composite,[10,15] but have not yet been evaluated after aging of composites by exposure to oral biofilm.

Therefore, the present study aims to determine the strength of composite-to-composite bonding for microhybrid, nanohybrid and nanofilled composites after exposure to a mixed oral-species biofilm *in vitro* and the effects of IAR- and SC-application on the resulting bond strengths. In addition, surface degradation by exposure to biofilm will be evaluated.

2 Material and methods

2.1 Composites, specimen preparation and biofilm growth

The composites used in this study are microhybrid Anterior Shine (abbreviated AS), nanohybrid Grandio (G), nanohybrid Tetric Evo-Ceram (TE), nanofilled Filtek Supreme^{XT} (FS), as detailed in Table 1. Unpolymerized composites were placed into cylindrical undercut cavities prepared in auto-polymerized polymethylmethacrylate surrounded by a PVC cylinder. Composites were polymerized incrementally with a halogen lamp according to manufacturer's instruction. During final photo-polymerization, the top surface layer was covered with a Mylar strip in order to create a smooth surface and to prevent the formation of an oxygen inhibiting layer. Details have been previously described.[16] Specimens of each composite group were sterilized with plasma-treatment and subsequently exposed to an oral biofilm for aging. A non-aged group acted as a control.

Mixed species oral biofilms were made according to the Zürich biofilm model.[17] The supra-gingival strains included were *Streptococcus oralis* J22, *Streptococcus sobrinus* HG1025, *Actinomyces naeslundii* T14V-J1, *Fusobacterium nucleatum*, *Veillonella parvula* and *Candida albicans* GB1/2. Bacterial strains were maintained on blood agar (OXOID, Basingstoke, UK), while *C. albicans* GB1/2 was grown on trypticase soy agar (TSA, OXOID). All strains were incubated anaerobically at 37°C. One colony of each strain was inoculated into 10 mL universal medium supplemented with 5% fetal bovine serum (mFUM)[18] and incubated as pre-cultures for 24 h. Mixed cultures were prepared by inoculation 50 mL mFUM with 50 µL *S. oralis*, 100 µL *S. sobrinus,* 750 µL *A. naeslundii*, 500 µL *F. nucleatum,* 100 µL *V. parvula* and 1000 µL *C. albicans* pre-culture and incubated for 16 h. Different amounts of each strain were used in order to obtain a biofilm in which all strains were detectable for at least eight days.

Composite specimens for bond strengths measurement and surface characterization were placed in 24 or 12 wells plates, respectively and exposed for 4 h to 3 mL suspension of the six strains in mFUM under anaerobic conditions at 37°C, after which the suspension was replaced by medium. mFUM was refreshed every 48 h. At day fourteen, the biofilm was removed from the composite surfaces using a cotton swab and transferred into 4.5 mL RTF, vortexed vigorously for 2 min and serially diluted. The dilutions were plated on blood and TSA plates and cultured anaerobically for 72 h, after which colonies were counted and expressed as CFU's per cm^2 composite surface.

2.2 *Repair bond strengths and failure analysis*

Prior to composite-to-composite bonding, composite surfaces after biofilm removal were additionally cleaned five times by sonication for 1 min in demineralized water. Then, composite specimens were conditioned with one of the following two procedures:

Intermediate adhesive resin application: IARs of the corresponding composites (Table 1) were applied in a thin layer on non-aged composites and composites aged by exposure to biofilms, air-thinned under oil-free compressed air and finally photo-polymerized according to manufacturer's instructions.

Tribochemical silica-coating: A silica-coating was applied using an intraoral air-abrasion device (Dento-Prep™, RØNVIG A/S, Daugaard, Denmark) filled with 30 µm alumina particles coated with silica (Table 1). The conditioned substrates were then coated with a silane coupling agent followed by intermediate adhesive resin application, specific to the silica-coating procedure (Table 1).

Following surface conditioning, composites of the same kind were adhered onto the conditioned composites using translucent polyethylene molds. Composite was incrementally packed against the treated composite and polymerized according the manufacturer instructions. After polymerization, specimens were gently removed from the polyethylene molds.

Table 1 Product and company names, chemical compositions, abbreviations and batch numbers of the materials used in this study.

Product name	Company name	Chemical composition	Abbre-viations	Batch Number
COMPOSITE RESIN				
Quadrant Anterior Shine (microhybrid)	Cavex BV, Haarlem, The Netherlands	Bis-GMA, DUDMA, silica, silicate glass, Ba-glass, fluoride containing fillers (63 v%)	AS	010100
Grandio (nanohybrid)	Voco GmbH, Cuxhaven, Germany	Bis-GMA, UDMA, TEGMA, Glass-ceramic, SiO_2 containing fillers (71.4 v%)	G	621332
Tetric Evo Ceram (nanohybrid)	Ivoclar Vivadent AG, Schaan, Liechtenstein	Bis-GMA, TEGDMA, UDMA , Ba-Al-F-B-Silicate, SiO_2, mixed oxide, YbF3 containing fillers (48.5 v%)	TE	J04088
Filtek Supreme XT (nanofilled)	3M ESPE, St. Paul, MN, USA	TEGDMA, UDMA, Bis-EMA, Zirconia-Silica, Silica containing fillers (57.7 v%)	FS	6 BG
TRIBOCHEMICAL SILICA COATING KIT (for SC-application)				
CoJet®-Sand	3M ESPE AG, Seefeld, Germany	Aluminum trioxide particles coated with silica, particle size: 30 µm	SC	165092
ESPE®-Sil	3M ESPE AG, Seefeld, Germany	3-methacryloxypropyl-trimethoxysilane, ethanol	Silane	152745
INTERMEDIATE ADHESIVE RESIN (for IAR-application)				
Visio™-Bond (for tribochemical silica coating treatment)	3M ESPE AG, Seefeld, Germany	Dicyclopentyldimethylene diacrylate 2-propenoic acid, 2-methyl, 2-[(2-hydroxyethyl)(3-methoxypropyl)amino]ethyl ester	VB	161808
Quadrant Unibond (for AS)	Cavex BV, Haarlem, The Netherlands	Bis-GMA, TEGDMA, silicate glass fillers, silica, polycarboxylic acid, champorquinone	Q	010049
Solobond Plus (for G)	Voco GmbH, Cuxhaven, Germany	Bis-GMA, TEGDMA, HEMA, champorquinone	S	591583
Multilink (for TE)	Ivoclar Vivadent AG, Schaan, Liechtenstein	Primer A: Water, initiators (sulfonate, amines) Primer B: Phosphonic acid acrylate, HEMA, TEGDMA, methacrylate modified polyacrylic acid	M	Primer A: H10145 Primer B: 09713
Adper Scotchbond 1XT (for FS)	3M ESPE, St. Paul, MN, USA	Dimethacrylate, HEMA, polyalcenoic acid copolymer, silane treated colloidal silica, ethanol, water, photoinitiator	A	4BM

Specimens were positioned on a shear-testing machine (Zwick ROELL Z2.5 MA 18-1-3/7, Ulm, Germany). The shear strength of composite-to-composite bonding was measured at a cross-head speed of 1.0 mm/min. The mode of failure was determined under optical light microscopy (MP 320, Carl Zeiss, Jena, Germany) at x40 magnification and recorded as cohesive in the substrate, appearing as small indents or as adhesive failure at the interface, showing a completely smooth surface.

2.3 *Physico-chemical surface characterization*

Surface characterization was done on non-aged composites and composites after exposure to biofilm. After biofilm removal, composites were cleaned five times by sonication for 1 min in demineralized water. Scanning electron micrographs were taken with a cold field emission SEM (JSM-6301F, Jeol Instruments, Tokyo, Japan) at 25 kV and x10000 magnification. Roughnesses of the composite surfaces were examined by optical profilometry (Proscan 2000, Scantron, Taunton, England). Specimens were scanned in two different directions, performing three measurements per specimen, and mean R_a surface roughnesses calculated. Filler particle exposure at the composite surfaces was determined from X-ray photoelectron microscopic (XPS) analyses of the outermost composite surface, using a previously published model, based on the prevalence of silicon and oxygen.[16]

2.4 *Statistical analysis*

Statistical analysis of mean failure stresses was performed with the SPSS software package (version 14.0; SPSS, Chicago, IL, USA). Normality and homogeneity tests (Kolmogorov-Smirnov) indicated that residual values were normally distributed when plotted against predicted values. Effects of composite types, surface conditioning procedures and biofilm exposure on failure stresses were compared using three-way ANOVA and LSD post-hoc tests. Surface roughnesses were analyzed using Mann-Whitney test. In all comparisons, statistical significance was declared if the *p*-value was less than 0.05.

Since mean failure stresses contain high standard deviations, statistical analysis according to Weibull was done as well.[19] In the Weibull analysis, a failure probability is calculated as a function of the applied stress, from which a Weibull modulus is calculated. Low Weibull moduli or dependabilities indicate a failure distribution over a wide range of applied stresses, whereas high Weibull moduli refer to a more narrow range of applied stresses causing failure. For most cases, the Weibull distribution is given by

$$P_\sigma = 1 - \exp\left\{ - \left(\frac{\sigma}{\sigma_0} \right)^m \right\} \qquad (1)$$

in which P_σ denotes the failure probability at an applied shear stress σ and m the Weibull modulus. σ_0 is a normalization parameter, without physical significance.[19] The failure probability for the different experimental failure stresses P_σ is obtained from a ranking of the failure bond stresses measured in ascending order according[19]

$$P_\sigma = \frac{n}{N+1} \qquad (2)$$

where n is the rank number of the data point and N is the total number of data. Subsequently, $\ln(\ln(1 - P_\sigma))$ was plotted against $\ln(\sigma)$, yielding a straight line according to Eq. (1) from which the Weibull modulus m can be calculated by linear regression, together with a linear correlation coefficient to indicate the goodness of the fit to the Weibull equation.

3 Result

3.1 *Biofilms on composites*

Although all strains appeared in biofilms on the composite surfaces up to day 8th, only *S. oralis, S. sobrinus* and *V. parvula* were detected in the biofilms at day 14th. The total number of CFU's per cm^2 amounted $1.8 \pm 0.9 \times 10^8$ on AS and $2.3 \pm 0.6 \times 10^8$ on G, which is significantly higher than on the surfaces of TE ($1.0 \pm 0.8 \times 10^8$) and FS ($0.8 \pm 0.3 \times 10^8$) composites.

3.2 *Surface characteristics*

Examples of SEM micrographs for TE (Fig. 1) clearly indicate a rougher surface after aging by exposure to biofilm, in contrast to the non-aged control where the filler particles are covered by a thin layer of matrix.

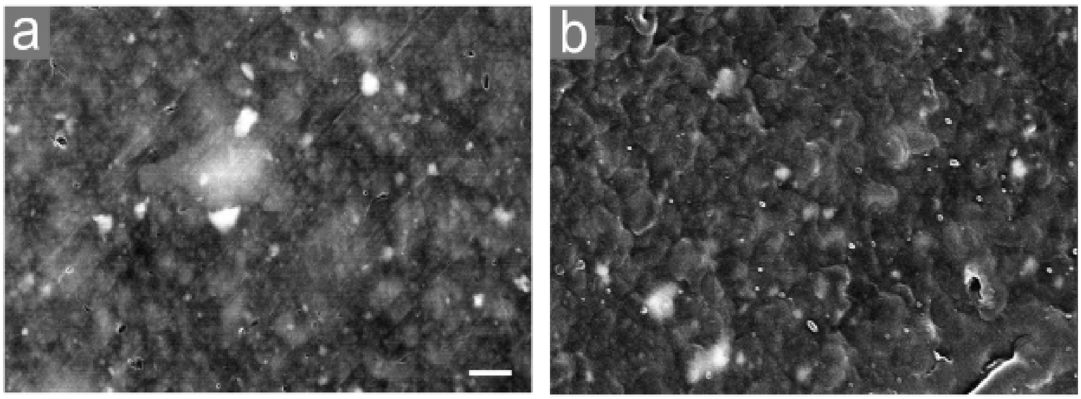

Fig. 1 Representative SEM micrographs of the
a) non-aged nanohybrid, Tetric Evo Ceram (TE)
b) Tetric Evo Ceram, after aging by exposure to biofilm
Bar marker indicates 1 µm.

The roughnesses of nanohybrid and nanofiller composites after exposure to biofilm were significantly higher than of non-aged composites. No significant effect of exposure to biofilm on surface roughness was observed for the microhybrid composite AS (Table 2). Application of the silica-coating significantly increased the roughnesses of non-aged composites, with little or no systematic effects on roughnesses of composite surface aged by exposure to biofilms. Filler exposure at the composite surfaces after aging by exposure to biofilm was lower than prior to aging. (Table 2).

Table 2 Mean surface roughness and filler particle exposure at the surface of the four resin composites prior to and after aging by exposure to biofilm. Surface roughness was also measured after silica-coating, while filler particle exposure was only measured prior to silica-coating. ± denotes standard deviations over 3 specimens.

Composite Resin	**Non-aged control**		**After biofilm exposure**		**After silica-coating**	
					Non-aged control	**After biofilm exposure**
	Surface roughness (µm)	**Filler particle exposure (%)**	**Surface roughness (µm)**	**Filler particle exposure (%)**	**Surface roughness (µm)**	**Surface roughness (µm)**
AS	8 ± 2	20	15 ± 7	4	43 ± 8	19 ± 10
G	19 ± 2	38	44 ± 4	4	56 ± 7	54 ± 10
TE	13 ± 4	30	48 ± 6	7	36 ± 7	37 ± 12
FS	13 ± 3	13	33 ± 7	8	18 ± 3	23 ± 17

3.3 Failure analysis

IAR- and SC-application yielded similar failure stresses in all composites prior to aging, while exposure to biofilms affected the composite-to-composite repair in all composite types (Table 3).

Table 3 Failure stress analysis including mean failure stresses and percentages of cohesive failure in the substrate, Weibull moduli (m) and their 95% confidence intervals (CI_m) and the stress ($\sigma_{0.05}$, MPa) expected to yield a 5% chance of failure for four different resin composites prior to (non-aged) and after aging by exposure to biofilm. Composite surfaces were either conditioned by IAR- or SC-application. ± denotes standard deviations over 10 specimens*.

Composite resin	**Non-aged**					**After exposure to biofilm**				
	Mean failure stress (MPa)	**Cohesive failure (%)**	m	**CI_m**	**$\sigma_{0.05}$**	**Mean failure stress (MPa)**	**Cohesive failure (%)**	m	**CI_m**	**$\sigma_{0.05}$**
After IAR-application										
AS	17.5 ± 6.5 ^{a,A}	90	2.1	1.6-2.5	4.8	4.6 ± 1.5 ^{a,B}	30	2.8	2.4-3.2	1.8
G	24.1 ± 10.0 ^{b,c,A}	60	2.2	1.7-2.6	7.1	12.9 ± 3.1 ^{b,B}	30	3.6	2.7-4.4	6.2
TE	20.3 ± 3.8 ^{a,c,e,A}	40	5.1	4.5-5.7	12.3	11.6 ± 4.9 ^{b,B}	30	2.7	1.4-3.9	4.8
FS	24.5 ± 7.4 ^{b,d,e,A}	100	2.8	2.3-3.3	9.7	11.0 ± 3.1 ^{b,B}	20	3.3	2.9-3.6	5.0
After SC-application										
AS	21.3 ± 6.1 ^{a,c,e,A}	100	2.7	1.9-3.5	8.1	18.0 ± 5.9 ^{c,A}	100	4.4	3.7-5.0	12.0
G	29.4 ± 6.6 ^{d,A}	90	3.6	2.6-4.6	14.3	25.0 ± 7.4 ^{d,A}	100	2.3	1.5-3.1	8.1
TE	17.9 ± 5.7 ^{a,A}	100	3.2	2.4-4.0	7.9	23.2 ± 5.7 ^{d,B}	90	2.6	1.4-3.8	8.6
FS	29.0 ± 4.5 ^{b,d,A}	100	6.3	5.4-7.3	19.4	27.5 ± 4.0 ^{d,A}	90	6.4	5.7-7.3	18.5

* same small letters indicate an insignificant difference between the rows of different composites, same capital letters denote an insignificant difference between the columns of non-aged composites and composites after exposure to biofilms.

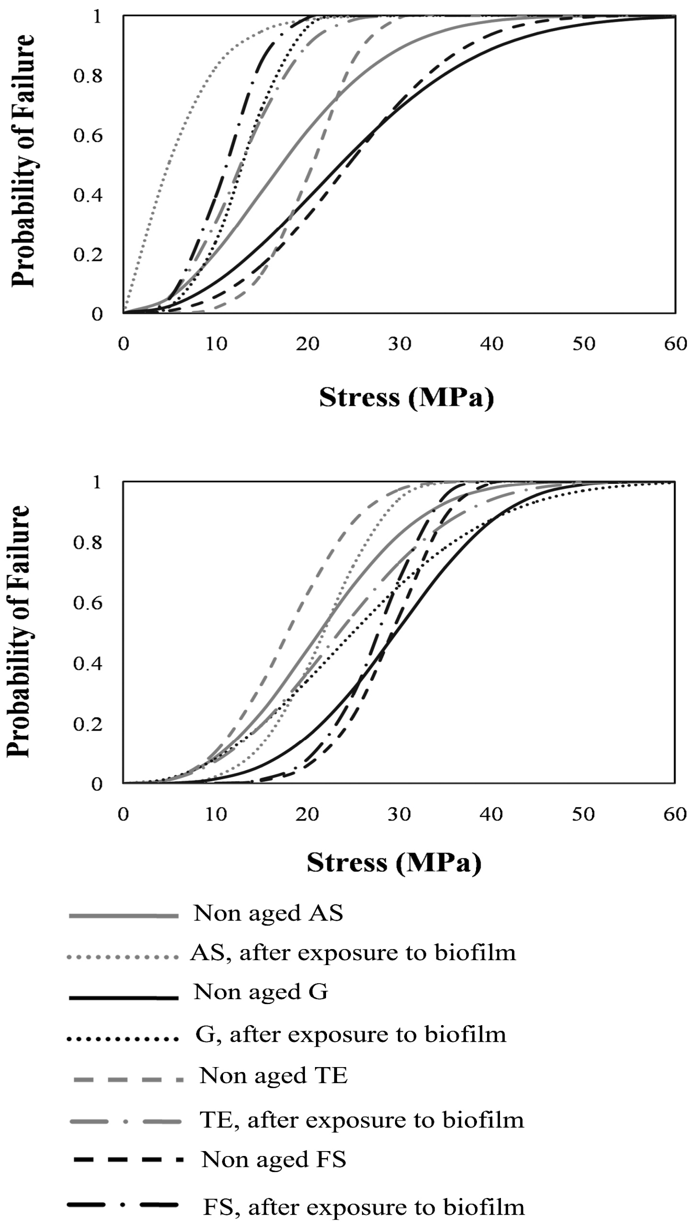

Fig. 2. Probability of failure as a function of the shear stress applied for four different resin composites prior to (non-aged) and after aging by exposure to biofilms. Composite surfaces were either conditioned by IAR- (top graph) or SC-application (bottom graph).

Mean failure stresses of composites conditioned using their corresponding IARs were significantly lower after aging by exposure to biofilms than prior to aging. SC-application could maintain the repair bond strengths after aging at the same level as obtained prior to aging. For TE an even higher mean failure stress was observed after aging than prior to aging. Failure modes of both non-aged and biofilm aged composites conditioned by SC-application were more frequently cohesive in the substrate than when conditioning was done by IAR-application.

Correlation coefficients of the fits to the Weibull equation (Eq. 1), varied between 0.86 and 0.99, indicating that the data fitted the Weibull distributions (Fig. 3) well.[20] Weibull moduli were generally low, indicating a low dependability of the bond. Only for FS after SC-application a meaningful increase in Weibull modulus was seen with respect to the other composite types. Stresses expected to yield 5% failure revealed similar effects of aging by exposure to biofilms and surface conditioning as did the mean failure stresses.

4 Discussion

In this study surface degradation of composite materials is indicated by decreased filler particle exposure for nanohybrid and nanofilled composites and increased surface roughness in all composites types after exposure to mixed species oral biofilms *in vitro*. Whereas IAR- and SC-application yielded similar bond strengths in non-aged samples, bond strengths after IAR-application on samples aged by exposure to biofilms were significantly lower than of non-aged controls, while SC-application maintained the same failure stress level in aged and non-aged samples. Moreover, SC-application always yielded the clinically desirable cohesive failure in the substrate,[21] indicating that the bond itself is not the weakest link.

We have studied filler exposure for the same four composites also after aging by thermocycling, water immersion and citric acid exposure and invariably found increased filler particle exposure after aging.[4] Thus aging by biofilm exposure must proceed according to a very different mechanism. Certain organic acids of oral biofilms are known to induce softening of composites by permeation and extraction of substances in the polymer.[22] These acids cause a surface swelling of composites[23] with an impact on the surface roughness and evidently causes coverage of filler particles at the composite surface. Clinically, the roughening of composite surfaces exposed to oral biofilm leads to a vicious circle of events, in which the roughened surface is more prone to renewed biofilm formation after cleansing, leading to more severe roughening.[24]

Actual aging of composite surfaces in the oral cavity involves more than sole exposure to biofilms, and also temperature variations, immersion in water or acidic fluids from food components may contribute to the clinical process of aging. Thermocycling, immersion in water and citric acid exposure on composite surfaces have recently been shown to degrade composite surfaces with various effects on the repair bond strengths[4] and without a clear conclusion on whether to apply intermediate adhesive resins or silica-coating for conditioning. The current study unequivocally indicates that composite restorations aged by exposure to biofilm can be conditioned best by SC-application and not by application of an intermediate adhesion resin. Probably the superiority of SC-application in all composite types here is due to matrix swelling and roughening of the composite surface, providing enhanced capture of silica-coated particles and mechanical interlocking with the adhesive to create a larger surface area for the bond.[25]

5 Conclusion

Bond strength and failure type analyses indicate that SC-application yields superior composite-to-composite bonding compared with IAR-application for composite surfaces aged by exposure to biofilms.

References

1. Carlen A, Nikdel K, Wennerberg A, Holmberg K, Olsson J (2001) Surface characteristics and *in vitro* biofilm formation on glass ionomer and composite resin. *Biomaterials* 22:481-487.
2. Ikeda M, Matin K, Nikaido T, Foxton R, Tagami J (2007) Effect of surface characteristics on adherence of S. mutans biofilms to indirect resin composites. *Dent Mater J* 26:915-923.
3. Akova T, Ozkomur A, Uysal H (2006) Effect of food-simulating liquids on the mechanical properties of provisional restorative materials. *J Dent* 22:1130-1134.
4. Rinastiti M, Özcan M, Siswomihardjo W, Busscher HJ (2009) Effects of surface conditioning on repair bond strengths of non-aged and aged microhybrid, nanohybrid and nanofilled composites. *Clin Oral Investig* Submitted.
5. Fúcio Z, Carvalho FG, Sobrinho LC, Sinhoreti M, Puppin-Rontani RM (2008) The influence of 30-day-old *Streptococcus mutans* biofilm on the surface of esthetic restorative materials--An *in vitro* study. *J Dent* 36:833-839.
6. Øilo G (1992) Biodegradation of dental composites/glass-ionomer cements. *Adv Dent Res* 6:50-54.
7. Göpferich A (1996) Mechanisms of polymer degradation and erosion. *Biomaterials* 17:103-114.
8. Soderholm KJ (1981) Degradation of glass filler in experimental composites. *J Dent Res* 60:1867-1875.
9. Rodrigues Junior S, Ferracane J, Della Bona A (2009) Influence of surface treatments on the bond strength of repaired resin composite restorative materials. *Dent Mater* 25:442-451.
10. Chiba K, Hosoda H, Fusayama T (1989) The addition of an adhesive composite resin to the same material : bond strength and clinical techniques. *J Prosthet Dent* 61:669-675.
11. Shahdad SA, Kennedy JG (1998) Bond strength of repaired anterior composite resins: an *in vitro* study. *J Dent* 26:685-694.
12. Bouschlicher MR, Reindhardt JW, Vargas MA (1997) Surface treatment techniques for resin composite repair. *Am J Dent* 10:279-283.
13. Swift EJ, Le Valley BD, Boyer DB (1992) Evaluation of new methods for composite repair. *Dent Mater* 8:362-365.
14. Yap AUJ, Sau CW, Lye KW (1999) Effects of aging on repair bond strengths of a polyacid-modified composite resin. *Oper Dent* 24:371-376.

15. Brendeke J, Özcan M (2007) Effect of physicochemical aging conditions on the composite-composite repair bond strength. *J Adhes Dent* 9:399-406.
16. Rinastiti M, Özcan M, Siswomihardjo W, Busscher HJ (2010) Immediate repair bond strengths of microhybrid, nanohybrid and nanofilled composites after different surface treatments. *J Dent* 38:29-38.
17. Guggenheim B, Guggenheim M, Gmür R, Giertsen E, Thurnheer T (2004) Application of the Zürich biofilm model to problems of cariology. *Caries Res* 38:212-222.
18. Gmür R, Guggenheim B (1983) Antigenic heterogeneity of *Bacteroides intermedius* as recognized by monclonal antibodies. *Infect Immun* 42:459-470.
19. McCabe JF, Carrick TE (1986) A statistical approach to the mechanical testing of dental materials. *Dent Mater* 2:139-142.
20. Robin C, Scherrer SS, Wiskott HWA, de Rijk WG, Belser UC (2002) Weibull parameters of composite resin bond strengths to porcelain and noble alloy using the Rocatec system. *Dent Mater* 18:389-395.
21. Lucena-Martin C, Gonzlez-Lopez S, de Mondelo J (2001) The effect of various surface treatments and bonding agents on the repaired strength of heat-treated composites. *J Prosthet Dent* 86:481-488.
22. Lee S, Huang H, Lin C, Shih Y (1998) Leached components from dental composites in oral simulating fluids and the resultant composite strengths. *J Oral Rehabil* 25:575-588.
23. Asmussen E (1984) Softening of BISGMA-based polymers by ethanol and by organic acids of plaque. *Scand J Dent Res* 92:257-261.
24. Beyth N, Bahir R, Matalon S, Domb A, Weiss E (2008) *Streptococcus mutans* biofilm changes surface-topography of resin composites. *Dent Mater* 24:732-736.
25. Özcan M, Barbosa S, Melo R, Galhano G, Bottino M (2007) Effect of surface conditioning methods on the microtensile bond strength of resin composite to composite after aging conditions. *Dent Mater* 23:1276-1282.

Chapter 7

Degradation of Composite Resins by Intra-Oral Aging

-A Pilot Study-

1 Introduction

Composites resins have developed remarkably fast over the past decade for application as a restorative material in dentistry. However, after composite resins are placed in the oral cavity, composite restorations must withstand continuous mechanical and environmental loads. Food components, acidic beverages, temperature changes,[1] humidity, mastication forces, saliva and biofilm[2] all contribute to progressive physical and chemical degradation of the material.[3] These processes influence the longevity of composite restorations and cause discoloration, microleakage, wear, ditching at the margins, loss of anatomical shape, delamination or simply fracture that ultimately require replacement or repair.[4-6]

Numerous different *in vitro* models were developed to evaluate the effect of the oral environment on composite resins and each model proposed to simulate different factors. Storage in water simulates the effect of humidity in the oral environment.[7,8] Water has detrimental effects on the composite surface due to hydrolysis and release of filler particles as well as water uptake in the resin matrix.[9,10] Absorbed water causes softening of the matrix, microcrack formation, resin degradation and debonding of the filler-matrix interfaces.[11] The effect of organic and inorganic components in saliva is imitated by using artificial saliva that provoked filler leaching[12] and decreased microhardness.[13] Food simulating liquids, as e.g. ethanol and heptane induce a softening of composite restorations[1] and citric acid caused release of filler particles.[14] Thermocycling is the most common method to simulate thermal changes in the oral cavity due to eating, drinking and breathing.[15] Thermocycling resulted in bond failure at the tooth-restoration or filler-matrix interface.[16] A biochemical model where the composite is exposed to enzymes,[17] bacteria or biofilms[2,18] showed hydrolysis, increased surface roughness and exposure of inorganic filler.

In vitro aging models for composites study only single factors thus lacking the synergy of factors operative in the oral cavity. In many cases, the correlation between *in vitro* data and clinical performance is not clear.[19] To reflect what actually occurs in the oral environment, *in situ* studies should be carried out to forward a "gold standard"[20] for comparison of results from *in vitro* aging methods.

Therefore, the aim of this study was to investigate the *in situ* degradation of four different composites by measuring the filler exposure, surface roughness and morphology of mycrohybrid, nanohybrid and nanofilled composites in an intra-oral aging model.

2 Materials and methods

2.1 In situ model

Seven healthy volunteers, aged 20-30 years, not using any medication within one month prior to and during the study and free of active caries lesions participated in this study. They received verbal and written information concerning the study. Consent forms were signed prior to enrollment and the research protocol was approved by the Ethics Committee of the Faculty of Dentistry, Gadjah Mada University, Yogyakarta, Indonesia.

2.1.1 Preparation of the palatal device

Participants wore custom-made acrylic palatal appliances (Fig. 1) containing four different composite resins (Table 1).

Table 1 Product and company names, chemical compositions, abbreviations and batch numbers of the materials used in this study.

Product name	Company name	Chemical composition	Abbreviations	Batch Number
COMPOSITE RESIN				
Quadrant Anterior Shine (microhybrid)	Cavex BV, Haarlem, The Netherlands	Bis-GMA, DUDMA, silica, silicate glass, Ba-glass, fluoride containing fillers (63 v%)	AS	010100
Grandio (nanohybrid)	Voco GmbH, Cuxhaven, Germany	Bis-GMA, UDMA, TEGMA, Glass-ceramic, SiO$_2$ containing fillers (71.4 v%)	G	621332
Tetric Evo Ceram (nanohybrid)	Ivoclar Vivadent AG,Schaan, Liechtenstein	Bis-GMA, TEGDMA, UDMA , Ba-Al-F-B-Silicate, SiO$_2$, mixed oxide, YbF3 containing fillers (48.5 v%)	TE	J04088
Filtek Supreme XT (nanofilled)	3M ESPE, St. Paul, MN, USA	TEGDMA, UDMA, Bis-EMA, Zirconia-Silica, Silica containing fillers (57.7 v%)	FS	6 BG
TRIBOCHEMICAL SILICA COATING KIT (for SC-application)				
CoJet®-Sand	3M ESPE AG, Seefeld, Germany	Aluminium trioxide particles coated with silica, particle size: 30 µm	SC	165092
ESPE®-Sil	3M ESPE AG, Seefeld, Germany	3-methacryloxypropyltri-methoxysilane, ethanol	Silane	152745
INTERMEDIATE ADHESIVE RESIN (for IAR-application)				
Visio™-Bond (for tribochemical silica coating treatment)	3M ESPE AG, Seefeld, Germany	Dicyclopentyldimethylene diacrylate, 2-propenoic acid,2-methyl,2-(2-hydroxylethyl) (3-methoxypropyl)(amino]ethyl ester)	VB	161808
Quadrant Unibond (for AS)	Cavex BV, Haarlem, The Netherlands	Bis-GMA, TEGDMA, silicate glass fillers, silica, polycarboxylic acid, champorquinone	Q	010049
Solobond Plus (for G)	Voco GmbH, Cuxhaven, Germany	Bis-GMA, TEGDMA, HEMA, champorquinone	S	591583
Multilink (for TE)	Ivoclar Vivadent AG, Schaan, Liechtenstein	Primer A: Water, initiators (sulfonate, amines) Primer B: Phosphonic acid acrylate, HEMA, TEGDMA, methacrylate modified polyacrylic acid	M	Primer A: H10145 Primer B: 09713
Adper Scotchbond 1XT (for FS)	3M ESPE, St. Paul, MN, USA	Dimethacrylate, HEMA, polyalcenoic acid copolymer, silane treated colloidal silica, ethanol, water, photoinitiator	A	4BM

For retention, the appliances had metal clasps on the molars or pre-molars. Four cavities (diameter: 5.5 mm, thickness: 2 mm) were prepared on the left and right side in the bottom of the appliance. Each cavity was filled with unpolymerized composites using a hand instrument and photo-polymerized with a halogen photo-polymerization unit (Optilux 501, Kerr, Orange, CA, USA) for 20 s (G, TE and FS) or 40 s (AS) at a distance of 2 mm from the surface. Light intensity was higher than 400 Mw/cm^2, as verified by a radiometer (Demetron LC, Kerr). The surface layer was covered with a 100 µm thick translucent Mylar strip (KerrHawe SA, Bioggio, Switzerland) in order to create a smooth surface and to prevent the formation of an oxygen inhibiting layer.

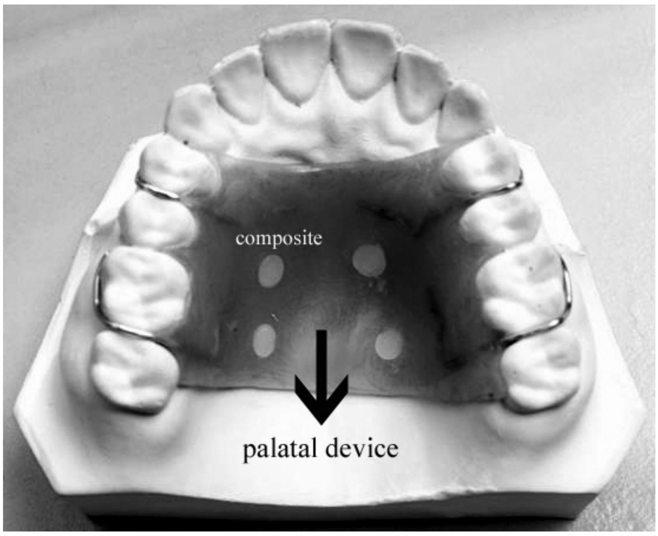

Fig. 1 Dental cast with palatal appliance containing four resin composites.

2.1.2 *Clinical phase*

The participants were instructed to wear the appliances during the entire day for a time period of 180 days and the appliance was only removed during sleeping for safety reasons. There was no restriction on the consumption of food and beverages. The participants brushed the palatal appliance with water, two times per day. After the experimental clinical phase, the composites were removed from the palatal appliances and the composite surfaces were evaluated. Newly prepared composite resins were employed as a non-aged control.

2.2 Surface characterization

Degradation of the composites was evaluated after intra-oral aging and compared with non-aged controls. Surface characterization was carried out using X-ray photoelectron spectroscopy (XPS) in order to determine the elemental composition, optical profilometry for surface roughness and scanning electron microscopy (SEM)[21,22] to study the surface morphology. Prior to surface evaluation, composite surfaces were cleaned five times by sonication for 1 min in demineralized water.

2.2.1 Elemental composition

Elemental composition of the outermost composite surface was determined quantitatively with XPS using an S-Probe spectrometer (Surface Science Instruments, Mountain View, CA, USA) equipped with an aluminum anode (10 Kv, 22Ma) and a quartz monochromator. The direction of the photoelectron collection angle to the specimens was 55 degrees and the electron flood gun was set at 10 Ev. A survey scan was made with a 1000 x 250 µm spot and pass energy of 150 Ev. Binding energies were determined by setting the binding energy of the C_{1s} component due to carbon-carbon bonds at 284.8 Ev. Filler particle exposure at the composite surfaces prior to and after intra-oral aging was determined from XPS analyses of the outermost composite surface.[22] For a fully resin matrix covered surface, the molecular structures of the organic matrix yields a theoretical value $(O/C)_{theoretical}$ that can be compared with the experimental value for $(O/C)_{experimental}$ due to the resin matrix and Si-containing filler particles. The experimental values for $(O/C)_{experimental}$ can be expressed in matrix values $(O/C)_{matrix}$ by subtracting the oxygen arising from Si (since silica is SiO_2, twice the amount of measured Si needs to be subtracted from the measured percentage of oxygen). Subsequently, the following formula can be applied to calculate the percentage filler particle exposed at the surface

$$\% filler = 1 - \left((O/C)_{matrix} / (O/C)_{theoretical} \right)$$

2.2.2 *Surface roughness*

Roughnesses of the composite surfaces after intra-oral aging acid were assessed using optical profilometry (Proscan 2000, Scantron, Taunton, England). Specimens were scanned in two different directions (5 x 5 mm), performing three measurements per specimen, and mean R_a surface roughnesses were calculated.

2.2.3 *Morphology of composite surface*

A cold field emission SEM (JSM-6301F, Jeol Instruments, Tokyo, Japan) was used to image the morphology of the composite surfaces. Prior to examination, surfaces were first sputter-coated with gold/palladium (80/20) and images were taken at 25 Kv at a magnification of x10000.

2.3 *Statistical analysis*

Filler exposures and surface roughnesses were analyzed using Mann-Whitney U and statistical significance was declared if the *p*-value was less than 0.05.

3 Results

The roughnesses of all composite resins prior to and after intra-oral aging are presented in Table 2. The surface roughness increased significantly by a factor of two to five when compared to the non-aged controls (Table 2). The percentage filler exposure at the composite surfaces after intra-oral aging decreased zero and was significantly lower than prior to aging except for TE

(Table 2). Note that our XPS model for calculating the percentage filler exposure has been validated by contact angle measurements[22] for composite resin surfaces that have not been exposed to the oral environment. Composite resins after intra-oral aging show negative filler exposure percentages, probably due to an overestimation of the amount of oxygen arising from the polymer matrix. Additional oxygen might arise from remnants of salivary conditioning films or oral biofilm, although bacteria were never seen in scanning electron micrographs.

Table 2 Mean surface roughness and filler particle exposure at the surface of the four composite resins prior to and after intra-oral aging. ± denotes standard deviations over three specimens, worn by three different volunteers.

Com-posite	Non-aged control		After intra-oral aging	
	Surface roughness (µm)	Filler particle exposure (%)	Surface roughness (µm)	Filler particle exposure (%)
AS	9 ± 4	20 ± 14	39 ± 5	0*
G	17 ± 6	24 ± 9	40 ± 10	0*
TE	17± 6	35 ± 12	63 ± 10	28 ± 19
FS	16 ± 3	18 ± 12	46 ± 9	0*

* filler exposure was calculated to be slightly negative, i.e. -23 ± 20% for AS, -3 ± 17% for G and -9 ± 10% for FS.

Micrographs of composite surfaces prior to aging (Fig. 2) show scattered white regions, probably indicative of filler particles covered by a thin layer of matrix. Intra-oral aging clearly roughens the surface and TE has the roughest surface and shows clear matrix degradation.

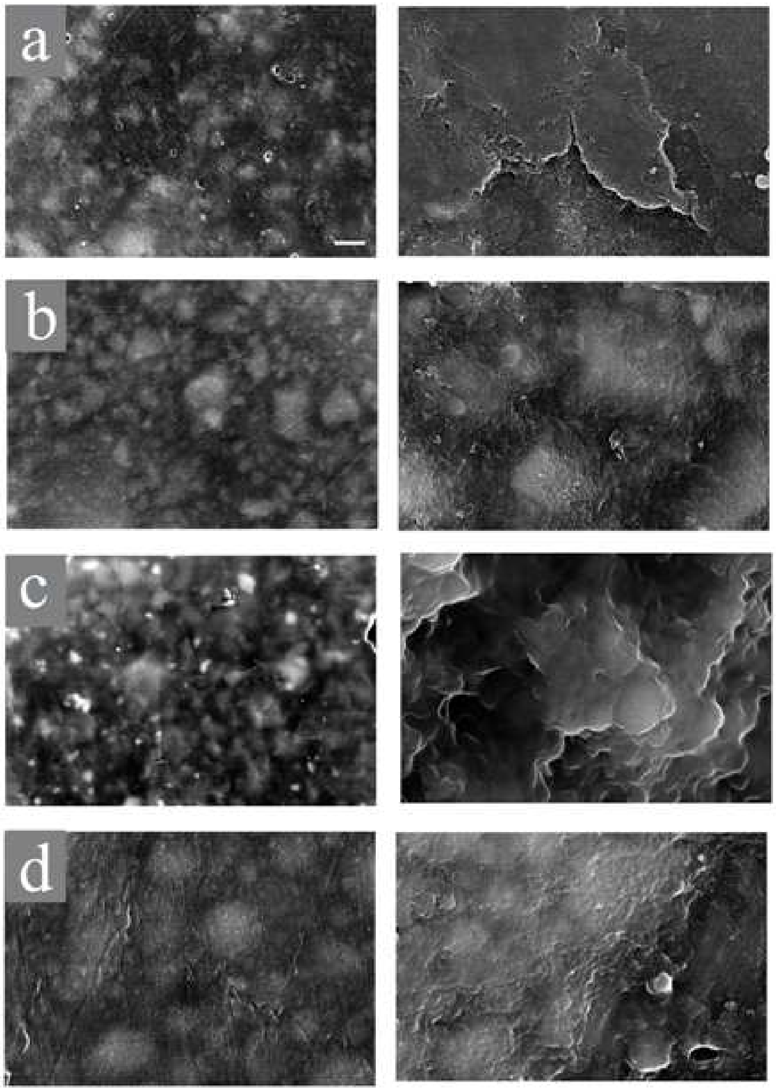

Fig. 2 SEM micrographs of the different composite resins prior to (left panel) and after intra-oral aging (right panel) .

a) Anterior Shine (AS);
b) Grandio (G)
c) Tetric Evo Ceram (TE)
d) Filtek Supreme XT (FS)
Bar marker indicates 1 µm.

4 Discussion

The dynamics in the human oral cavity influences the longevity of composite restorations. Since it is hard to mimic the interplay of all factors operative in the oral cavity under laboratory conditions, in situ studies are more relevant and may eventually be applied to determine the most relevant *in vitro* conditions.

In this study, *in situ* surface degradation of composite materials is indicated by decreased filler particle exposure and increased surface roughness of all composites after intra-oral aging. Once composite restorations have been polymerized, their surfaces are constantly interacting with the oral environment. Saliva for example, consists for 99.4% of water and 0.6% of hormones, proteins, enzymes and microorganisms.[23] Absorption of water and enzymes from human saliva[24] as well as of organic acids from oral biofilms [25,26] will cause swelling and softening of the resin matrix. Moreover, the intra-oral fluids are good solvents for composite resins, and solvent effects are largest when there is minimal mismatch in solubility between the solvent and the matrix itself.[27,28] A polymer matrix will swell when placed in a good solvent, because the attractive forces between the polymer chains are exceeded by the attraction between the solvent molecules and the polymer chains.[29]

The swelled polymer matrix covers filler particles on the composite surface, which is confirmed by the decrease in filler particles exposure observed. Interestingly, only nanohybrid TE remained to show a relatively high filler particle exposure after intra-oral aging despite the fact that among all four composite resin types, the volume % filler of TE is the lowest (48.5 v%, see Table 1). Lower filler loading is known to cause a greater disparity between diffusion coefficients in absorption and desorption of the water and hence water clusters can be expected to accumulate not only in the matrix but also in the filler-matrix interface of TE.[30] The formation of water cluster and the hydrolytic degradation of the silane coupling agent may cause filler-matrix debonding and a loss of matrix layer. The net effects of filler-matrix debonding and loss of matrix likely cancel each other, leaving the filler particle exposure at the surface of TE unaffected.

Surface roughness is principally determined by the presence of protruding filler particles above the resin matrix and intruding porosities.[31] However, the present study shows that matrix swelling and mechanical trauma probably also influence the surface roughness. Composite resins that were embedded in the palatal device receive mechanical trauma from brushing and also food also may increase the surface roughness. The exposure to saliva and mechanical trauma promote a cyclic effect: saliva softens the composite superficial layer and this layer is subsequently more easily damaged by brushing or food components. Matrix swelling evidently causes coverage of filler particles at the composite surface. Therefore, filler particle exposure on the composite surface reduced significantly except for TE.

5 Conclusions

Intra-oral aging of composite resins cause increased surface roughness and decreased filler particle exposure due to swelling of the matrix. For a composite resin with a relatively low volume loading of filler particles, loss of matrix and filler-matrix debonding cancel each others effects on filler particle exposure, which remains largely unaffected by intra-oral aging.

The above conclusions are drawn however, on the basis of a pilot study involving only a limited number of participants. Extension of the group size is necessary in order to put these conclusions on a firmer basis, while also more extensive cleansing of composite resin specimens must be carried out in order to remove salivary conditioning film. In this way, we anticipate to be able to avoid the calculation of negative filler exposures.

References

1. Akova T, Ozkomur A, Uysal H (2006) Effect of food-simulating liquids on the mechanical properties of provisional restorative materials. *J Dent* 22:1130-1134.
2. Fúcio Z, Carvalho FG, Sobrinho LC, Sinhoreti M, Puppin-Rontani RM (2008) The influence of 30-day-old *Streptococcus mutans* biofilm on the surface of esthetic restorative materials - An *in vitro* study. *J Dent* 36:833-839.
3. Drummond J (2008) Degradation, fatigue, and failure of resin dental composite materials. *J Dent Res* 87:710-719.
4. Mjor IA (1981) Placement and replacement of restorations. *Oper Dent* 6:49-54.
5. Roulet JF (1987) Degradation of dental polymers. Karger, Basel, Germany.
6. Swift EJ (1987) Wear of composite resins in permanent posterior teeth. *J Am Dent Assoc* 115:584-588.
7. Cesar P, Meyer Faara P, Miwa Caldart R, Gastaldoni Jaeger R, da Cunha Ribeiro F (2001) Tensile bond strength of composite repairs on Artglass using different surface treatments. *Am J Dent* 14:373-377.
8. Frankenberger R, Kramer N, Sindel J (2000) Repair strength of etched vs silica-coated metal-ceramic and all-ceramic restorations. *Oper Dent* 25:209-215.
9. Cattani-Lorente M, Dupuis V, Moya F, Payan J, Meyer JM (1999) Comparative study of the physical properties of a polyacid-modified composite resin and a resin-modified glass ionomer cement. *Dent Mater* 15:21-32.
10. Soderholm KJ, Zigan M, Ragan M, Fischlschweiger W, Bergman M (1984) Hydrolytic degradation of dental composites. *J Dent Res* 63:1248-1254.
11. Ferracane JL, Marker VA (1992) Solvent degradation and reduced fracture toughness in aged composites. *J Dent Res* 71:13-19.
12. Soderholm KJ, Mukherjee R, Longmate J (1996) Filler leachability of composites stored in distilled water or artificial saliva. *J Dent Res* 75:1692-1699.
13. Mayworm C, Camargo Jr S, Bastian F (2008) Influence of artificial saliva on abrasive wear and microhardness of dental composites filled with nanoparticles. *J Dent* 36:703-710.
14. Özcan M, Barbosa S, Melo R, Galhano G, Bottino M (2007) Effect of surface conditioning methods on the microtensile bond strength of resin composite to composite after aging conditions. *Dent Mater* 23:1276-1282.

15. Gale M, Darvell B (1999) Thermal cycling procedures for laboratory testing of dental restorations. *J Dent* 27:89-99.
16. Crim GA, Garcia-Godoy F (1987) Microleakage: The effect of storage and cycling duration. *J Prosthet Dent* 57:574-576.
17. Santerre J, Shajii L, Tsang H (1999) Biodegradation of commercial dental composites by cholesterol esterase. *J Dent Res* 78:1459-1468.
18. Carlen A, Nikdel K, Wennerberg A, Holmberg K, Olsson J (2001) Surface characteristics and *in vitro* biofilm formation on glass ionomer and composite resin. *Biomaterials* 22:481-487.
19. Øilo G (2009) Biodegradation of dental composites/glass-ionomer cements. *Adv Dent Res* 6:50-54.
20. Ten Cate J (1992) Patient selection and appliance design in intra-oral models. *J Dent Res* 71 Spec No:908-910.
21. Kakaboura A, Fragouli M, Rahiotis C, Silikas N (2007) Evaluation of surface characteristics of dental composites using profilometry, scanning electron, atomic force microscopy and gloss-meter. *J Mat Sci Mater Med* 18:155-163.
22. Rinastiti M, Özcan M, Siswomihardjo W, Busscher HJ (2010) Immediate repair bond strengths of microhybrid, nanohybrid and nanofilled composites after different surface treatments. *J Dent* 38:29-38.
23. Eiriksson SO, Pereira PNR, Swift EJ, Heymann HO, Sigurdsson HO (2004) Effects of saliva contamination on resin-resin bond strength. *Dent Mater* 20:37-44.
24. Larsen I, Munksgaard E (1991) Effect of human saliva on surface degradation of composite resin. *Scand J Dent Res* 99:254-261.
25. Asmussen E (1984) Softening of BisGMA-based polymers by ethanol and by organic acids of plaque. *Scand J Dent Res* 92:257-261.
26. Lee SY, Greener EH, Menis DL (2009) Detection of leached moieties from dental composites in fluid simulating food and saliva. *Dent Mater* 11:348-353.
27. McKinney JE, Wu W (1985) Chemical softening and wear of dental composites. *J Dent Res* 64:1326-1331.
28. Wu W, McKinney JE (1982) Influence of chemicals on wear of dental composites. *J Dent Res* 61:1180-1183.
29. Ferracane JL (2006) Hygroscopic and hydrolytic effects in dental polymer networks. *Dent Mater* 22:211-222.
30. Santos C, Clarke RL, Braden M, Guitian F, Davy KWM (2002) Water absorption characteristics of dental composites incorporating hydroxyapatite filler. *Biomaterials* 23:1897-1904.

31. Gladys S, Van Meerbeek B, Braem M, Lambrechts P, Vanherle G (1997) Comparative physico-mechanical characterization of new hybrid restorative materials with conventional glass-ionomer and resin composite restorative materials. *J Dent Res* 76:883-894.

Chapter 8

General Discussion

Improvements in composite materials have enabled dentists to use composite resins for a large variety of restorations. Due to the mishandling of the composite materials or interaction with the oral environment, composite resin restorations need to be repaired, either immediately or after aging. In this thesis, different methods to simulate aging of composite resins are compared and their effects on composite-to-composite bonding determined. This thesis aimed to answer two relevant clinical questions:

1. What is best *in vitro* aging method for simulating intra-oral aging of composite resins?
2. What is the best conditioning method for composite-to-composite repair?

1 Comparison of *in vitro* aging with aging during intra-oral exposure

Answering the question "What is best *in vitro* aging method for simulating intra-oral aging of composite resins?" requires a "gold standard". In this thesis, we assume that the results of our *in situ* pilot study will constitute this "gold standard". Apart from the gold standard to be derived in a pilot study, the gold standard proposed suffers from some other possible weaknesses, like the lack of use of toothpastes during wear of the intra-oral appliance and the absence of mechanical loading due to mastication. Yet, we believe our gold standard to be acceptable.

In our studies, degradation of composite resins was assessed primarily on the basis of surface roughness and filler particle exposure. In Tables 1, 2 and 3 we summarize the results from the different studies with respect to surface rouhghness and filler particle exposure. Note, that surface roughness was measured by two different methods: profilometry and atomic force microscopy (AFM). AFM was used from the onset of the study, but surface degradation by exposure to biofilm *in vitro* and intra-oral aging yielded surfaces that were too rough for AFM analysis.

Table 1 Mean surface roughness (nm) measured by atomic force microscopy of four composite resins prior to and after thermocycling, water storage and citric acid immersion. Values within brackets denote the relative surface roughness of aged- to non-aged composite resins.

Composite resin	Non-aged	Thermocycling	Water storage	Citric acid immersion
AS	9	37 (4.1)	54 (6.0)	39 (4.3)
G	7	43 (6.1)	53 (7.6)	20 (2.9)
TE	8	79 (9.9)	30 (3.7)	25 (3.1)
FS	4	65 (16.2)	41 (10.2)	22 (5.5)

Table 2 Mean surface roughness (µm) measured by profilometry of four composite resins prior to and after *in vitro* exposure to biofilm and after *in situ* aging. Values within bracket denote the relative surface roughness of aged- to non-aged composite resins.

Composite resin	Non-aged	Biofilm exposure *in vitro*	*In situ*
AS	8	15 (1.9)	39 (4.9)
G	18	44 (2.4)	40 (2.2)
TE	15	48 (3.2)	63 (4.2)
FS	15	33 (2.2)	46 (3.1)

Table 3 Mean filler particle exposure (%) at the surface of the four composite resins prior to and after *in vitro* and *in situ* aging.

Composite resin	Non-aged	Thermo-cycling	Water storage	Citric acid immersion	Biofilm exposure *in vitro*	*In situ*
AS	20	22	7	25	4	0
G	24	56	21	51	4	0
TE	35	60	42	65	7	28
FS	18	49	24	68	8	0

The surface roughnesses of non-aged composite resin measured by AFM were lower than those measured by profilometry. This may be attributed to the smaller sample area measured by AFM (70 x 70 µm) as compared to profilometry (5 x 5 mm). The minimum height that can be measured by profilometry is 0.01 µm, while AFM measures differences in height up to 0.1 nm. This yields a principal difference between both methods, since AFM measures the high-frequency height variations over the low-frequency height variations measured by profilometry, i.e. both adapt a different base line. Therefore roughness values from profilometry may not be directly compared with AFM,[1] although both profilometry and AFM point to severe roughening of composite resin surfaces during aging.

Based on the data summarized in Tables 1, 2 and 3, we forward a model for the composite resin surface after different aging conditions (see Fig. 1).

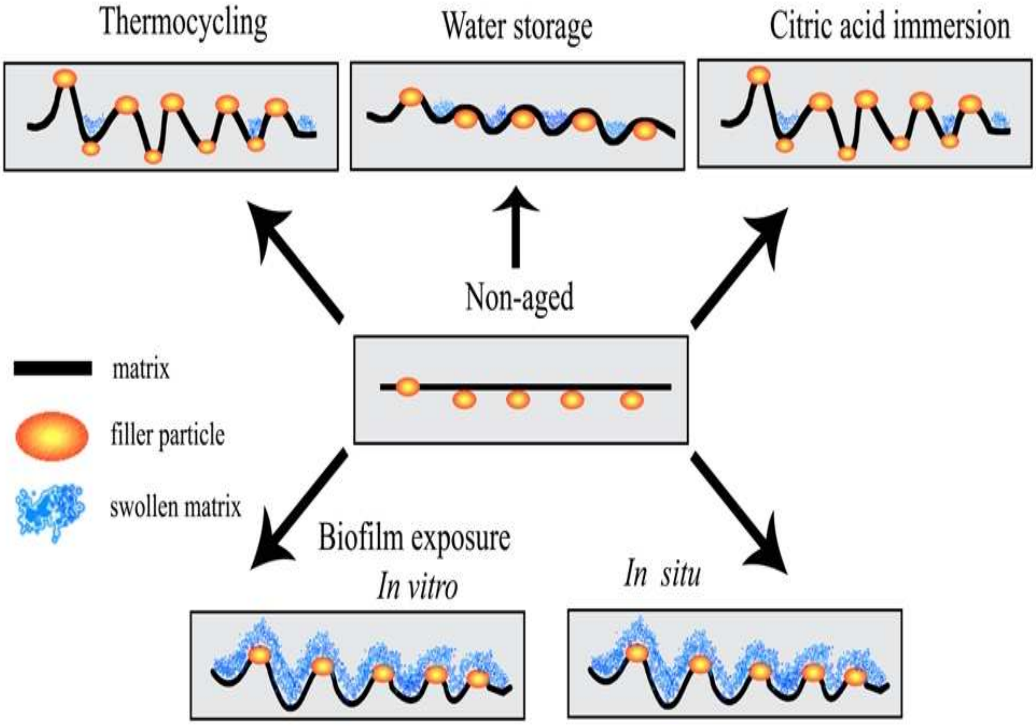

Fig. 1 Schematic presentation of composite resin surfaces after thermocycling, water storage, citric acid immersion, exposure to biofilm *in vitro* and *in situ* aging.

All aging methods evaluated in this study involve an aqueous environment. The aqueous environment may cause water uptake, swelling and matrix dissolution.[2] However, water is considered as an inactive fluid without chemical interaction with the composite resins, having only a mildly degrading effect. Thermocycling and citric acid immersion involve thermal and acid attack that may affect the composite resin integrity through the matrix or failure at the filler/matrix interface, yielding more severe degradation than water. This is in line with the current observations on changes in surface roughness and filler particle exposure in this thesis.

Restorations in the oral cavity are never fully immersed in water or citric acid for a prolonged period of time, neither do they undergo 5000 thermocycles per week. Rather, clinically 5000 thermocycles between 50°C to 55°C for hot food consumption and 5°C to 10°C for cold beverages intake[3] would be received by a composite resin restoration in a period of six months. Depending on the site of the restoration, the composite restoration will be subject to brushing and is unlikely to collect a thick layer of biofilm as applied here. Clearly, neither of these aging conditions are responsible as a single factor for *in situ* composite resin degradation. Moreover, all aging conditions represent an exaggeration of the true clinical situation, and we do not know the effect of this exaggeration. Based on the comparison of our *in vitro* and *in situ* aging models, it can be concluded that exposure to biofilm is the best choice for *in vitro* aging of composite resin surfaces.

2 Comparison of IAR- and SC-application on the composite-to-composite repair

Answering the question "What is the best conditioning method for composite-to-composite repair?" requires thorough comparison of different conditioning methods, including after aging. In Table 4 we summarize the mean repair shear bond strengths and failure types as observed in the different chapters of this thesis.

Table 4 Mean repair shear bond strengths (Mpa) for the four different composite resins after different aging conditions. Composite surfaces were either conditioned by application of an intermediate adhesive resin (IAR) or silica-coating (SC). The values within brackets denotes the %cohesive failure in the substrate.

Composite resin	Non-aged	Thermocycling	Water storage	Citric acid immersion	Biofilm *in vitro*
After IAR – application					
AS	17.4 (80)	2.8 (0)	3.8 (0)	13.8 (40)	4.6 (30)
G	23.9 (60)	10.4 (10)	12.7 (10)	13.7 (10)	12.9 (30)
TE	20.8 (40)	11.9 (10)	8.4 (20)	18.0 (80)	11.6 (30)
FS	24.5 (90)	7.1 (0)	13.0 (30)	23.0 (80)	11.0 (20)
After SC – application					
AS	23.2 (100)	18.7 (100)	28.3 (90)	11.4 (80)	18.0 (100)
G	29.3 (100)	23.0 (60)	45.2 (100)	13.8 (40)	25.0 (100)
TE	17.9 (100)	16.9 (60)	32.2 (100)	11.2 (70)	23.2 (90)
FS	28.5 (100)	20.9 (40)	21.7 (70)	17.5 (100)	27.5 (90)

Based on the data from Table 4, the interactions of IAR- and SC-application with non-aged and aged composite surfaces can be described as schematically presented in Fig. 2.

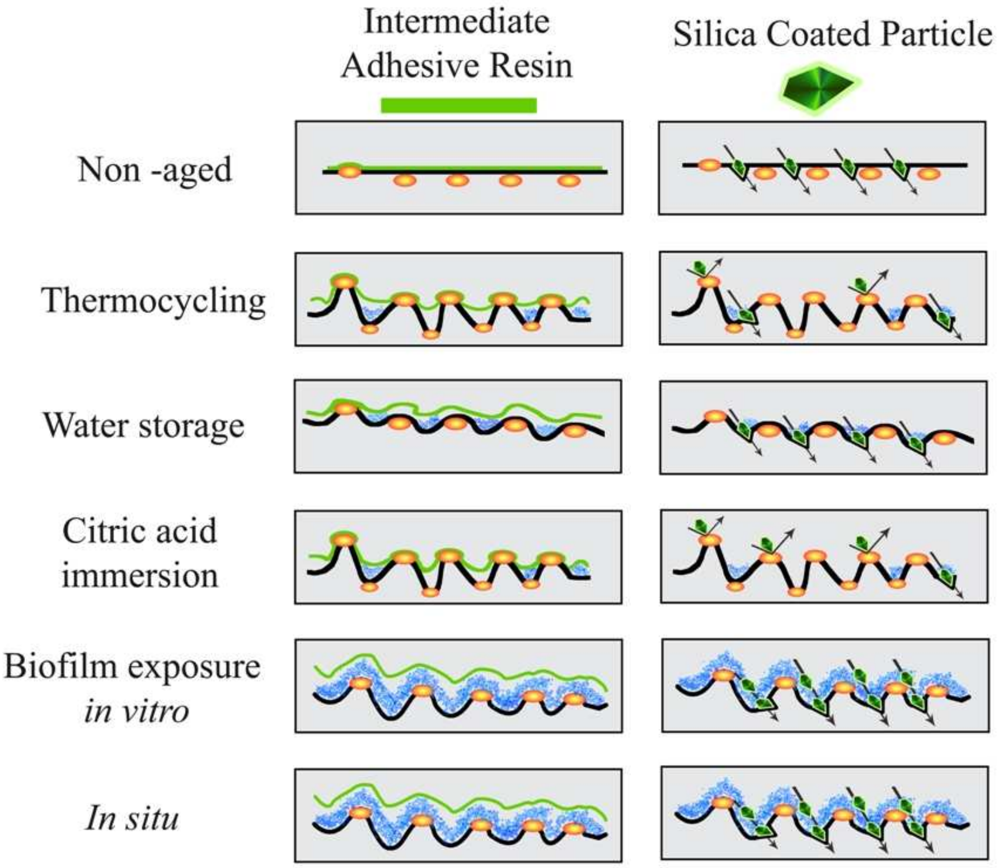

Fig. 2 Schematic presentation of the interaction of the composite surface after different aging conditions with intermediate adhesive resin and silica-coated particles.

Intermediate adhesive resin can enhance the strength of composite-to-composite bonding either by chemical bonds to the exposed filler particles and/or micromechanical retention caused by penetration of the monomer of the intermediate adhesive resin into crevices in the matrix surface.[4] Table 4 shows that IAR-application is never able to restore the composite-to-composite bond strength to the values observed for non-aged composite. By comparison of Tables 3 and 4, it can be seen that filler exposure is not determinant for the effects of IAR-application on the final bond strength. Although some degree of surface roughness is required for proper action of an IAR, frequently after aging the roughness of composite surfaces is too rough, impeding penetration.

SC-application is in essence a physico-chemical reaction to repair the composite resins, and it yields considerably more benefit than IAR-application. According to our data, and as schematically presented in Fig. 2, SC-application is more effective for composite resins with a swollen matrix due to increased capture probabilities of the silica-coated particles. On the other hand, exposure of filler particle on the surface inhibits capture of particles, as the incoming particles are more likely to bounce off the surface. Bond strength is only one aspect in the repair of composite restorations, and failure mode is equally important. SC-application yields a higher occurrence of cohesive failure in the substrate than IAR-application according to the mechanism outline in Fig. 3.

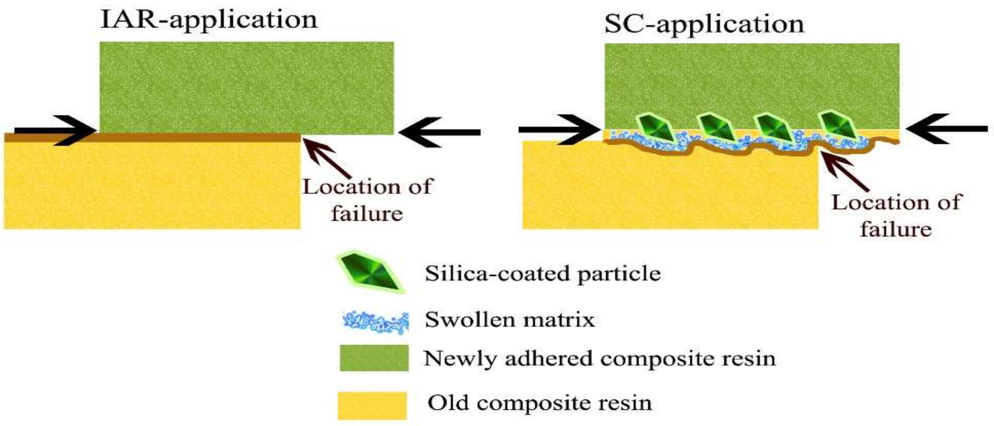

Fig. 3 Schematic presentation of adhesive failure at interface after IAR- application and cohesive failure in the substrate after SC-application.

After IAR-application, shear will cause the old and newly adhered composite resin to slide over each other, causing adhesive failure. After SC-application, silica-coated particles may become partially incorporated in the old composite and stick into the newly adhered composite resin. Shear will then cause cohesive failure in the old composite resin, and likely not in the newly adhered composite because the interfacial region of the old composite is weakened by aging.

3 Recommendations for future research

Based on my experiences, there are a number of recommendations for future research:

1) In the present thesis, we focused on surface roughness and filler particle exposure as determinants for repair bond strengths. Therewith we have neglected the influence of the degree of conversion. Correlations have been suggested between the degree of conversion and the aging of composite resins,[5] and composite-to-composite repair bond strengths. Incomplete conversion yields C=C bonds on the surface due to unreacted methacrylate groups,[6] that are reduced with time, thereby reducing the potential for bonding of new resin material.[7]

    Using FT-Raman spectroscopy (RFS 100/S, Bruker Optics Inc, Ettlingen, Germany) we determined the degree of conversion, i.e. the percentage of vinyl functions converted. The degree of conversion was determined from the ratio of the peak heights of vinyl C=C bonds (1638 cm^{-1}) with the one of aromatic carbons (1608 cm^{-1}), used for normalization. The ratio between the peaks at 1638 cm^{-1} and 1608 cm^{-1} are subsequently compared according to[8]

$$DC(\%) = 100 \times \left[1 - \left({R_{cured}} / {R_{uncured}} \right) \right]$$

    where R indicates the ratio of the peak heights at 1638 cm^{-1} and 1608 cm^{-1} in cured and uncured specimens, and DC denotes the degree of conversion.

    The results of this brief pilot study are summarized in Table 5 and show that the degree of conversion indeed increases during all aging conditions, therewith reducing the possibilities to obtain good chemical bonding during relayering.

Table 5 Degree of conversion (%) for three different composite resins after different aging conditions.

Composite resin	Non-aged	Thermocycling	Water storage	Citric acid immersion	Biofilm exposure *in vitro*	*In situ*
AS	56.4	76.7	72.6	69.7	62.2	67.0
G	55.1	75.0	75.8	66.3	87.7	90.1
FS	48.6	56.6	59.3	87.7	83.4	81.5

*TE could not be measured

Fig. 4 presents the degree of conversion as a function of the mean bond strength and percentage cohesive failure in the substrate, observed throughout this study as a function of the degree of conversion.

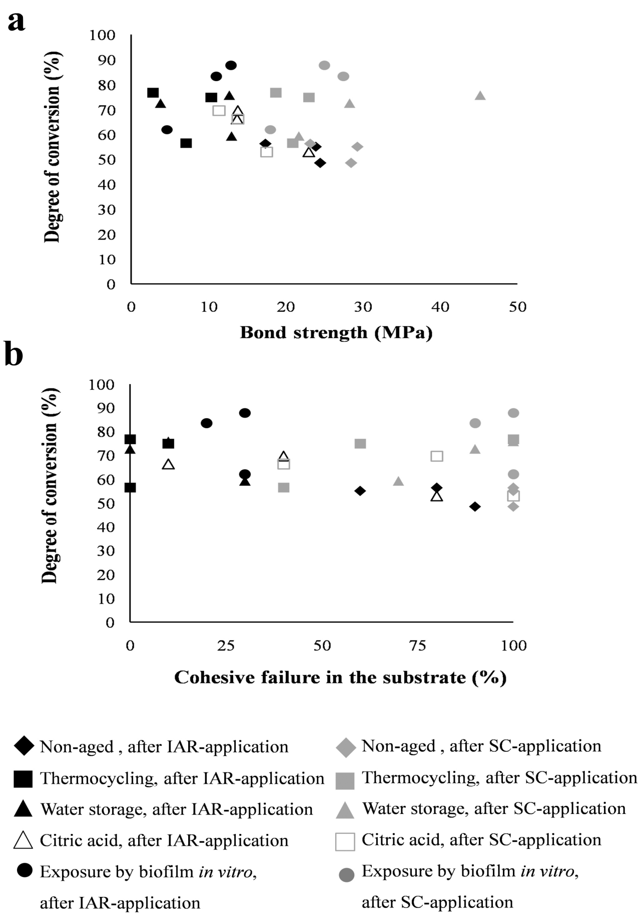

Fig. 4 Degree of conversion directly after aging as a function of the mean bond strength (a) and the percentage cohesive failure in the substrate (b) after different surface conditionings.

From Fig. 4 it can be concluded that there is no correlation between the degree of conversion and composite-to-composite bonding, neither on the basis of bond strength nor failure type. This confirms the conclusion from chapter 4, that there is no significant difference in immediate repair of composite resins polymerized with and without Mylar strip. Thus, mechanical interlocking may be at least equally important in composite-to-composite repair than direct chemical interactions. However, more research is needed to verify our conclusions.

2) The quality of our *in situ* gold standard needs to be improved and we propose to extend the number of volunteers to 10-20 volunteers for each group. In addition, a more realistic intra-oral aging condition may be created by allowing the volunteers to brush with specific dentifrices and mouthrinses and register or control their diet. In addition, effects of Western versus South-east Asian diets on composite resin surfaces may be compared.
3) Composite resin surface degradation is a multi-factorial process and it would be worthwhile to combine thermocyling, water storage and citric acid immersion in a single *in vitro* aging model.

4 Conclusions of this study and clinical relevance

Dentistry has become less invasive and at the same time more cost-effective over the years, in part due to the availability of new polymeric materials. Clinical results indicate failure of composite restorations over time. Alternatively, immediate repair may be required for a variety of reasons given in chapter 4. Relayering of composite resins is a good option for the repair of defective composite restorations, within the philosophy of minimal invasive repair. Unfortunately, no consensus exists on how to condition (non-)aged composite surfaces before relayering, neither is the exact condition of aged composite surfaces known.

The general conclusions of this study are that exposure to biofilms *in vitro* simulates intra-oral aging best, while also in general, SC-application is preferred for relayering in the repair of composite restoration. Composite restorations with a high filler particle exposure at the surface will reduce the effect of SC-application. Although *in vitro* aging gave variable effects on filler exposure for different composite types, *in situ* aging never yielded elevated filler particle exposure of any composite type. Moreover, since in daily clinical practice the dentist is unaware of the type of composite employed and can not detect the state of the composite surface by eye, use of SC-application is recommended for the repair of composite restorations.

References

1. Kakaboura A, Fragouli M, Rahiotis C, Silikas N (2007) Evaluation of surfacecharacteristics of dental composites using profilometry, scanning electron, atomic force microscopy and gloss-meter. *J Mat Sci Mater Med* 18:155-163.
2. Ferracane JL (2006) Hygroscopic and hydrolytic effects in dental polymer networks. *Dent Mater* 22:211-222.
3. Gale MS, Darvell BW (1999) Thermal cycling procedures for laboratory testing of dental restorations. *J Dent* 27:89-99.
4. Tezvegril A, Lassila LVJ, Vallittu PK (2003) Composite-composite repair bond strength: effect of different adhesion primers. *J Dent* 31:521-525.
5. Ferracane JL, Mitchem JC, Condon JR, Todd R (1997) Wear and marginal breakdown of composites with various degrees of cure. *J Dent Res* 76:1508-1516.
6. Özcan M, Alander P, Vallittu PK, Huysmans MC, Kalk W (2005) Effect of three surface conditioning methods to improve bond strength of particulate filler resin composites. *J Mat Sci Mater Med* 16:21-27.
7. Roulet JF (1987) Degradation of dental polymers. Karger, Basel, Germany.
8. da Silva E, Poskus L, Guimaraes J, Barcellos A, Fellows C (2008) Influence of light polymerization modes on degree of conversion and crosslink density of dental composites. *J Mat Sci Mater Med* 19:1027-1032.
9. Gauthier MA, Stangel I, Ellis TH, Zhu XX (2005) A new method for quantifying the intensity of the C=C band of dimethacrylate dental monomers in their FTIR and Raman spectra. *Biomaterials* 26:6440-6448.

Summary

The development of new aesthetic materials enables the dentist to treat patients based on the concept of minimal invasive dentistry. In **chapter 1** we explain the concept of minimal invasive dentistry, which involves preservation of tooth structure by repair of existing restorations through relayering (composite-to-composite-bonding). Improvements of composite resins and problems occurring in composite restorations due to the influence of dynamic oral conditions are described. A composite restoration ages over time and failure frequently occurs. The success of relayering depends on the strength of composite-to-composite bonding. The first aim of this study was to find the best model for simulating intra-oral aging of composite resins. The second aim was to determine the best possible conditioning method for composite-to-composite repair based on the bond strength analyses.

Chapter 2 includes a comprehensive review of biofilm formation on dental restorative and implant materials. Biomaterials are indispensable for restoration of oral function, but prone to biofilm formation, impacting oral health. Oral bacteria adhere to hydrophobic and hydrophilic surfaces, but due to fluctuating shear, little biofilm accumulates on hydrophobic surfaces *in vivo*. More biofilm accumulates on rough than on smooth surfaces. Oral biofilms are multispecies, and on acrylic *Candida albicans* can be found. Biofilms on gold and amalgam *in vivo* are thick and fully covering, but little viable. Oppositely, biofilms on ceramics are thin and highly viable. Biofilms on composite resins and glass ionomer cements cause surface deterioration, which enhances biofilm formation again. Residual monomer release from composite resins influences biofilm growth *in vitro*, but effects *in vivo* are less pronounced, probably due to the large volume of saliva into which compounds are released and its continuous refreshment. Similarly, conflicting results have been reported on effects of fluoride release from glass ionomer cements. Therefore, new preventive measures include surface modification of existing materials, that have a more lasting effect on biofilm formation.

The most common methods to evaluate the bond strength are shear and microtensile bond strength measurement, but both modes of evaluation have never been directly compared. Therefore, in **chapter 3,** shear and microtensile failure in composite-to-composite-bonding are compared. Disk-shaped and rectangular-blocks of a nanohybrid and nanofilled composite resin were prepared for shear and microtensile measurements, respectively. Half of all specimens were aged using thermocycling. Non-aged and thermocycled specimens were conditioned by intermediate-adhesive-resin-application (IAR-application) or silica-coating and silanization followed by IAR-application (SC-application). Composites resins, of the same kind as their substrate, were adhered onto the substrates and shear or microtensile forces applied to the interface. The results showed significant differences between shear and microtensile failure stresses, and effects of conditioning, aging and composite types were observed. Mean failure shear stresses (7 - 28 MPa) were significantly lower than microtensile ones (32 - 53 MPa), regardless of aging or conditioning, with average standard deviations approaching 50%. The dependability of the bonds, indicated by its Weibull modulus, was similarly low in shear and microtensile modes. Failures after shear were more frequently cohesive than after application of a tensile force, except in thermocycled composite resins after IAR-application. Based on the study in this chapter, we draw the conclusion that clinically, restorations are mainly exposed to shear and shear evaluations are to be preferred over microtensile ones, also since Weibull moduli are similar for both modes. Hence, considering the high percentages of cohesive failure in shear combined with the low Weibull moduli, this study indicates that the least dependable link in composite-to-composite-bonding is the composite resin itself and not the adhesive interface, with the exception of bonding created after thermocycling by IAR-application.

Composite-to-composite repair can be done either on the fresh or aged composite resins. **Chapter 4** evaluated immediate repair bond strengths and failure types of composite resins with and without surface conditioning and characterized the interacting composite surfaces by their surface composition and roughness. In this study, microhybrid, nanohybrid and nanofilled composite resins were photo-polymerized and assigned to four groups: 1) No conditioning (Control), 2) No conditioning, polymerized against a Mylar strip (Control, with strip), 3) Intermediate adhesive resin application (IAR), 4) Chair side silica coating, silanization and intermediate resin application (SC). Composite resins, similar as their substrates, were adhered onto the substrates. Shear force was applied to the interface in a universal testing machine and failure types were evaluated under light microscopy. Surface characterization was done by contact angle measurements, X-ray Photoelectron Spectroscopy, Scanning Electron and Atomic Force Microscopy. We found significant effects of the composite type and surface conditioning. Conditioning the composite resins with their IARs did not result in significant improvements in bond strength compared to the control (bond strengths between 14.5 and 20.0 MPa). SC increased the bond strength in all composite resins except TE by on average 8.9 MPa, while in all composite resins the surface roughness increased from 7 to 384 µm. Failure types in this group were exclusively cohesive. Physico-chemical modeling of the composite surfaces showed that the surfaces were dominated by the resin matrix, with a major increase in silica-coverage after SC for all composite resins. Thus, it can be concluded that intermediate adhesive resin conditioning did not improve the composite-to-composite immediate repair strength. Silica coating and silanization followed by its corresponding IAR, strongly increased repair bond strengths and provided exclusively cohesive failures in the substrate in all composite resins.

The study in c**hapter 5** evaluates the effects of aging on repair bond-strengths of microhybrid, nanohybrid and nanofilled composite resins and characterizes the interacting surfaces after aging. Disc-shaped composite specimens were assigned to one of three aging conditions: 1) Thermocycling (5000x, 5-55°C), 2) Storage in water at 37°C for six months, or 3) Immersion in citric-acid at 37°C, pH 3 for one week and a non-aged control. Two surface conditionings were selected: intermediate adhesive resin application (IAR-application) and chair-side silica-coating followed by silanization and its specific IAR-application (SC-application). Composite resins, of the same kind as their substrate, were adhered onto the substrates and repair shear bond-strengths were determined, followed by failure type evaluation. Elemental surface compositions were determined by X-ray Photoelectron Spectroscopy and surface roughnesses analyzed using Scanning Electron and Atomic Force Microscopy. Surface roughness increased in all composite resins after aging, but filler particle exposure at the surface only increased after thermocycling and citric acid immersion. Composite type, surface conditioning and aging method significantly influenced the repair bond-strengths ($p < 0.05$, three-way ANOVA) with the least severe effects of water storage. Repair bond-strengths in aged composite resins after IAR-application were always lower in non-aged ones, while SC-application led to higher bond strengths than IAR-application after thermocycling and water storage. In addition, SC-application led to more cohesive failures than after IAR-application, regardless the aging method.

Another factor that may inlfiuence intra-oral aging is the presence of an adhering biofilm on composite surface. Therefore in **chapter 6,** we evaluate the effect of biofilm on composite resin degradation. In this study, mixed oral species biofilms adhering on composite surfaces increased their roughness and decreased filler exposure, probably due to matrix swelling, except for a microhybrid composite resin. Mean failure shear stresses after intermediate adhesive resin application were significantly lower after aging by exposure to biofilms than prior to aging. Silica-coating maintained the same failure stress levels in non-aged and aged composite resins. Weibull moduli were generally low, indicating a low dependability of the bonds. Failure modes were predominantly cohesive after silica-coating, while intermediate adhesive resin application yielded more adhesive failure. In conclusion, silica-coating is to be preferred in the repair of composite resins that have been exposed to oral biofilms.

In **chapter 7,** we present an *in situ* pilot study, evaluating the effect of intra-oral aging on the degradation of composite surfaces. Seven volunteers wore palatal appliances with composite specimens inserted for 180 days. The appliance, including the composite specimens, was cleaned with water twice per day. Fresh composite specimens were prepared as a control group. All composite resins were polymerized against a Mylar strip. Composite surfaces were evaluated by their elemental surface compositions (X-ray photoelectron spectroscopy), surface roughnesses (profilometry) and surface morphologies (scanning electron microscopy) prior to and after intra-oral aging. Intra-oral aging increased the surface roughness significantly by a factor of 2 to 5. Due to matrix swelling, filler particle exposure was decreased significantly except for the nanohybrid composite resin. It is concluded that in general aging in the oral environment increased the surface roughness and decreased filler particle exposure of composite resins.

In **chapter 8**, the general discussion of this thesis, we summarize the data collected in chapters 4 to 7. Based on the "gold standard" (intra-oral aging), it is concluded that exposure to biofilm *in vitro* is the best method to simulate intra-oral aging. Consequently it is argued that in general SC-application is the best conditioning method to be used in relayering of composite restorations. Finally, recommendations for future research are given and the clinical relevance of our findings discussed.

Samenvatting

De ontwikkeling van nieuwe esthetische materialen stelt de tandarts in staat om de patiënt op basis van het concept van de minimaal invasieve tandheelkunde te behandelen. In **hoofdstuk 1** leggen we het concept van de minimaal invasieve tandheelkunde uit. Als onderdeel van dit concept, dat streeft naar behoud van zoveel mogelijk eigen tandweefsel, kan reparatie van bestaande restauraties door middel van het opnieuw opbrengen van een composiet laag op een defecte restauratie (composiet aan composiet hechting). Verbeteringen van composiet harsen en problemen die zich voordoen bij composietrestauraties, te wijten aan diverse invloeden van het mond milieu, worden beschreven. Een composiet restauratie veroudert in de loop van de tijd, waardoor vaak defecten in de restauratie ontstaan. Het succes van herstel van een restauratie d.m.v. het opnieuw aanbrengen van een composiet op een defecte composiet restauratie, hangt af van de sterkte van de hechting tussen de bestaande en nieuw aangebrachte composiet lagen. Het eerste doel van deze studie was om het beste model te vinden voor het simuleren van veroudering van composieten in de mond. Het tweede doel was het bepalen van de behandelmethode die de sterkst mogelijke hechting tussen composieten bewerkstelligt.

Hoofdstuk 2 bevat een uitvoerig overzicht van de literatuur over biofilmvorming op tandheelkundige implantaat en restauratie materialen. Enerzijds zijn biomaterialen onmisbaar voor functieherstel in de mond. Anderzijds zijn biomaterialen gevoelig voor biofilmvorming, wat nadelige gevolgen heeft voor de mondgezondheid. Mondbacteriën hechten zich aan hydrofobe en hydrofiele oppervlakken, maar als gevolg van fluctuerende vloeistof stromen langs de oppervlakken, hecht er weinig biofilm *in vivo* op hydrofobe oppervlakken. Meer biofilm verzamelt zich op ruwe dan op gladde oppervlakken. Biofilms in de mond bestaan uit veel verschillende bacteriesoorten, daarnaast kan op acryl *Candida albicans* worden gevonden. *In vivo* biofilms op goud en amalgaam zijn dik en bedekken deze materialen volledig, ze bestaan echter maar uit weinig levende organismen. Daarentegen zijn biofilms op keramiek dun en bevatten zeer veel levende organismen. Biofilms op composieten en glasionomeer cementen tasten de oppervlakte integriteit van deze materialen aan, waardoor biofilmvorming op deze materialen wordt vergemakkelijkt. Hoewel het vrijkomen van monomeren uit composiet *in vitro* de groei van biofilms beïnvloedt, zijn de effecten *in vivo* beduidend minder uitgesproken. Vermoedelijk hangt dat samen met de relatief grote hoeveelheid speeksel waarin monomeren vrijkomen en worden afgevoerd. Bij resultaten van studies naar de effecten van fluoride vrijlating uit glasionomeer cement doet zich vermoedelijk datzelfde fenomeen voor. Vandaar dat nieuwe maatregelen ter preventie van biofilmvorming zich voornamelijk richten op aanpassingen aan het oppervlak van materialen, dit heeft immers vermoedelijk wel effect op de biofilmvorming.

De meest voorkomende methoden ter evaluatie van de hechtsterkte tussen materialen zijn afschuif- en trekkracht meting, maar beide vormen van evaluatie zijn nog nooit rechtstreeks met elkaar vergeleken. Daarom zijn in **hoofdstuk 3** m.b.v. afschuif- en trekkracht metingen de hechtsterkte van composiet aan composiet met elkaar vergeleken. Schijfvormige en rechthoekige blokken van een nanohybride en nanogevulde composiet werden opgesteld voor respectievelijk afschuif- en trekkracht hechtsterkte metingen. Veroudering van de helft van alle exemplaren werden met behulp van temperatuur wisselingen gesimuleerd. Niet verouderde en composieten die verouderd waren met temperatuur wisselingen werden voorbehandeld met een hechtlaag van hars (IAR-applicatie) of een silica-coating en silaan behandeling gevolgd door een IAR-applicatie (SC-applicatie). Composieten, van dezelfde soort als hun substraat, werden aangebracht op de substraten en afschuif- en trekkracht metingen werden aan de raakvlakken verricht. De resultaten toonden significante verschillen in hechtsterkte tussen de afschuif- en trekkracht metingen. Daarnaast werden er verschillen in hechtsterkte ten gevolge van verschillen in voorbehandeling, veroudering en type composiet geobserveerdDe gemiddelde afschuifkracht waarbij de binding tussen composieten het begaf (7 tot 28 MPa) was significant lager dan de trekkracht waarbij de binding tussen composieten het begaf (32 tot 53 MPa), ongeacht de conditionering of veroudering, met een gemiddelde standaarddeviatie van bijna 50%. De betrouwbaarheid van de binding, aangegeven door zijn Weibull modulus, was laag voor zowel trek- als afschuifkracht metingen. Loslatingen vonden vaker in het materiaal plaats wanneer het werd blootgesteld aan een afschuifkracht dan na blootstelling aan een trekkracht, behalve in composiet verouderd door temperatuur wisselingen en na IAR-applicatie. Gebaseerd op de studie in dit hoofdstuk trekken we de conclusie dat klinisch restauraties voornamelijk worden blootgesteld aan schuifkrachten en dat hechtsterkte metingen d.m.v. afschuifkracht de voorkeur hebben boven hechtsterkte metingen d.m.v. trekkracht metingen, mede gezien het feit dat de Weibull moduli gelijk zijn voor beide methoden. Gelet op de hoge percentages van loslatingen in afschuifkracht metingen, in combinatie met de lage Weibull moduli, geeft deze studie aan dat de minst betrouwbare schakel in composiet-op-composiet binding het composiet hars zelf is, en niet het raakvlak tussen de composieten, met uitzondering van de binding die gemaakt is na veroudering door temparatuur wisselingen m.b.v. IAR-applicatie.

Reparatie van composiet restauraties door het aanbrengen van een nieuwe laag composiet, kan plaatsvinden op verouderde of nog niet verouderd composiet. In **hoofdstuk 4** wordt de hechtsterkte geëvalueerd die ontstaat onmiddellijk na het aanbrengen van een nieuwe laag composiet, en worden eveneens de breukvlakken geëvalueerd. Dit gebeurde voor composieten met en zonder oppervlakte behandeling, tevens werden de raakvlakken tussen de composieten gekarakteriseerd door hun oppervlakte samenstelling en oppervlakte ruwheid in kaart te brengen. In deze studie werden microhybride, nanohybride en nanogevulde composiet harsen gepolymeriseerd d.m.v. belichting en verdeeld over vier groepen: 1) geen voorbehandeling (Controle), 2) geen voorbehandeling, gepolymeriseerd tegen een strook Mylar (Controle, met strip), 3) hecht hars toepassing (IAR), 4) silica coating, silaan behandeling en toepassing van een hechtlaag van hars (SC) aan de stoel. Composieten, gelijk aan hun substraten, werden gehecht aan de substraten. Afschuifkrachten werd getest op het hechtoppervlak m.b.v. een gestandaardiseerde machine en de breukvlakken werden geëvalueerd m.b.v. licht microscopie. Oppervlakte karakterisering vond plaats door randhoek metingen, röntgen foto-elektron spectroscopie, elektronen microscopie en atomaire kracht microscopie. We vonden significante effecten tussen de verschillende composieten en oppervlakte verouderings processen op de hechtsterkte. Voorbehandeling van composiet harsen met hun IAR's resulteerden niet in significante verbeteringen in de hechtsterkte ten opzichte van de controle (hechtsterke tussen 14,5 en 20,0 MPa). SC verhoogde de hechtsterkte in alle composieten, behalve TE, met gemiddeld 8,9 MPa, terwijl in alle composieten de oppervlakteruwheid steeg van 7 tot 384 micrometer. In deze groep vonden loslatingen uitsluitend plaats door het ontstaan van defecten in het materiaal zelf, niet in de hechting tussen composieten. Fysisch-chemische modellering van de composiet oppervlakken liet zien dat de oppervlakken werden gedomineerd door de hars matrix, met een grote toename in silica-dekking na SC voor alle composieten. Derhalve kan worden geconcludeerd dat het toepassen van een hechtlaag van hars de composiet-op-composiet hechtsterkte niet verbetert. Silica coating en daarna een silaan behandeling gevolgd door de bijbehorende IAR, geeft een sterk toegenomen hechtsterke voor alle composieten, zoveel zelfs dat defecten in het composiet ontstonden voordat er defecten tussen de composieten plaats vond.

De studie in **hoofdstuk 5** evalueert de effecten van veroudering op de reparatie hechtsterktes van microhybride, nanohybride en nanogevulde composieten en karakteriseert de hechtoppervlakken na veroudering. Schijfvormige composiet blokjes werden toegewezen aan een van de drie verouderingssimulaties: 1) temperatuur wisselingen (5000x, 5-55 ° C), 2) Opslag in water bij 37 °C gedurende zes maanden, of 3) Onderdompeling in citroenzuur bij 37 °C, pH 3 voor één week of toegewezen aan een niet aan veroudering blootgestelde controle groep. Twee oppervlakte voorbehandelingen werden geselecteerd: de toepassing van een hechtlaag van hars (IAR-applicatie) en silica-coating, gevolgd door silaan behandeling en zijn specifieke IAR-toepassing (SC-toepassing) aan de stoel. Composieten, dezelfde soort als hun substraat, werden gehecht aan hun substraten en reparatie hechtsterktes werden bepaald, gevolgd door evaluatie van de reden van loslating. Oppervlakte samenstelling werd bepaald door röntgen foto-elektron spectroscopie en oppervlakte ruwheid werd geanalyseerd met behulp van elektronen microscopie en atomaire kracht microscopie. Oppervlakteruwheid steeg in alle composieten na veroudering, maar blootstelling van vulmiddel deeltjes aan het oppervlak was alleen maar toegenomen na temperatuur wisselingen en onderdompeling in citroenzuur. Composiet type, oppervlakte voorbehandeling en verouderingsmethode beïnvloedden de reparatie hechtsterke significant ($p < 0,05$, three-way ANOVA), opslag in water had het minste invloed op de hechtsterkte. Reparatie hechtsterktes tussen de verouderde composieten na IAR-toepassing waren altijd lager dan hechtsterktes tussen niet-verouderde composieten, terwijl SC-applicatie tot hogere hechtsterktes leidde dan IAR-applicatie na veroudering door temperatuur wisselingen en opslag in water. Daarnaast leidde SC-applicatie, meer dan na IAR-toepassing, tot loslatingen door gebreken in het materiaal zelf, ongeacht de verouderingsmethode.

Een andere factor die de veroudering van composieten in de mond beïnvloedt is de aanwezigheid van een biofilm op het composiet oppervlak. Daarom wordt in **hoofdstuk 6** het effect van biofilm op de composiet degradatie geëvalueerd. Biofilms, bestaande uit diverse soorten mondbacteriën, hechtten zich aan composietoppervlakken en verhoogden hun ruwheid en verminderden vulmiddel blootstelling. Vermoedelijk gebeurde dit laatste als gevolg van matrix zwelling, uitzondering daarop vormt een microhybride composiet. Gemiddelde maximale afschuifkrachten na de toepassing van een hechtlaag van hars waren significant lager na veroudering door blootstelling aan biofilms dan voorafgaand aan veroudering. Gemiddelde maximale afschuifkrachtenna toepassing van silica-coating waren gelijk voor verouderde en niet-verouderde composieten. Weibull moduli waren over het algemeen laag, hetgeen wijst op een lage betrouwbaarheid van de bindingen. Loslatingen waren overwegend het gevolg van defecten in het materiaal bij toepassing van silica-coating, terwijl de toepassing van een hechtlaag van hars meer defecten in de binding tussen materialen liet zien. Kortom, silica-coating geniet de voorkeur bij de reparatie van composiet restauraties die zijn blootgesteld aan orale biofilms.

In **hoofdstuk 7** presenteren we een *in situ* pilot-studie, waarbij het effect van intra-orale veroudering op de afbraak van composietoppervlakken wordt gepresenteerd. Zeven vrijwilligers droegen 180 dagen lang houders aan het verhemelte met composiet blokjes.De houder, met het composiet, werd tweemaal daags schoongemaakt met water. Nieuwe composieten dienden als een controlegroep. Alle composieten werden gepolymeriseerd tegen een Mylar-strip. Composiet oppervlakken werden beoordeeld op de elementaire samenstelling van hun oppervlak (röntgen foto-elektron spectroscopie), oppervlakte ruwheden (profilometrie) en oppervlakte morfologie (scanning elektronen microscopie) vóór en na intra-orale veroudering. Intra-orale veroudering verhoogde de oppervlakteruwheid significant met een factor 2 tot 5. Door matrix zwelling, was het percentage vuldeeltjes aan het oppervlak significant afgenomen, met uitzondering van het nanohybride composiet. Geconcludeerd wordt dat in het algemeen veroudering in de mond zorgt voor verhoogde oppervlakte ruwheid en een verminderde vuldeeltjes blootstelling van composieten.

In **hoofdstuk 8,** de algemene discussie van dit proefschrift, vatten we de gegevens uit de hoofdstukken 4 tot 7 samen. Op basis van de "gouden standaard" (intra-orale veroudering), wordt geconcludeerd dat de blootstelling aan een biofilm *in vitro* de beste methode is om veroudering in de mond te simuleren. Bijgevolg wordt betoogd dat in het algemeen SC-toepassing de beste voorbehandelingsmethode is wanneer men defecten in een bestaande composiet restauratie met composiet wil repareren. Tenslotte worden aanbevelingen gedaan voor toekomstig onderzoek en wordt de klinische relevantie van onze bevindingen besproken.

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Curriculum Vitae

Margareta Rinastiti was born in Yogyakarta, Indonesia at April 19th, 1974. She got her Bachelor degree in Dentistry from Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta, Indonesia in 1997 and graduated as a dentist in 1999. She started her academic career at Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta in 1999 as part-timer teaching staff in Department of Orthodontic until 2000. In 2000 to 2001, she worked as a dentist in Community Health Center in Bantul, Yogyakarta. In 2001 she started to study in Biomaterial sciences at Graduate School, Universitas Gadjah Mada that was funded by Directorate of Higher Education, Ministry of Education, Republic of Indonesia and obtained her master degree in 2003. At the same time, in 2002 – 2003 she worked as part-timer teaching staff at Department of Conservative Dentistry and at Department of Oral Biology in 2003 - 2005. From 2005 until now, she is appointed as a permanent lecturer and researcher at Department of Conservative Dentistry. In 2006, she undertook sandwich PhD program in the Department of BioMedical Engineering**,** University Medical Center Groningen, The Netherlands.