CHAPTER 4

Comparison of *ex vivo* methods to determine early degradation of dental resin composites after intra-oral wear

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Abstract

Identification of sensitive methods to detect early degradation of composite resins either after *in vitro* experiments or intra-oral wear is important as the use of more sensitive methods will allow shorter experimentation times to reach statistically significant and relevant conclusions. The aim of this study was to compare the results of the different conventional methods and more surface-sensitive techniques as applied in the previous chapters of this thesis to investigate the early degradation of dental resin composites after 30 days of intra-oral wear during different conditions of oral health application. 16 volunteers used an acrylic palatal appliance with four discs of different resin composites inserted. During wear, three regimes of manual brushing were applied: brushing with a fluoridated toothpaste, with water, and absence of brushing. Resin composites included two direct and two indirect composites. Early composite degradation was evaluated using two conventional methods (hardness and surface roughness measurements) and two less conventional surface-sensitive techniques to calculate filler exposure (measurement of equilibrium, advancing and receding contact angles and XPS analysis). The dynamic range over which the results of the different methods and techniques ranged was largest for the roughness measurements, followed by contact angle measurements and XPS analyses. Moreover, filler exposure calculated from measured contact angles correlated with surface roughness data. In conclusion, early degradation can best be studied by surface roughness measurements or analysis of equilibrium, advancing and receding water contact angles as they most sensitively reflect changes at a surface and their results for composite degradation confirm each other. XPS is also suitable for surface analysis of early composite degradation, but its results partially reflect the bulk matrix making it slightly less suitable. Hardness, though suitable to provide information on bulk matrix degradation, is unsuitable for measuring early composite degradation that is still confined to the surface.

Comparison of *ex vivo* methods to determine early degradation of dental resin composites after intra-oral wear

Introduction

Studies on the degradation of dental resin composites are mostly done *in vitro* and sometimes *in vivo*, with *ex vivo* evaluation of the composites after intra-oral wear. *In vitro* designs usually involve a single factor to mimic the degradative oral environment (Blumer et al. 2015, Cebe et al. 2015, Jongsma and Kleverlaan 2015, Silva et al. 2017). In the oral cavity, composite degradation is quite complicated and influenced by an orchestra of mechanical, physical, chemical and biological factors (Williams and Zong 1994, Göpferich 1996, Ilie and Hickel 2009, Delaviz et al. 2014).

Investigating the material changes after either *in vitro* exposure to single factors or intra-oral wear to demonstrate actual, multi-factorial degradation of dental composites, requires time periods of at least weeks to most often months if not years. Conventional methods to study composite degradation comprise hardness measurements (Freund and Munksgaard 1990), evaluation of material loss (Ferracane 1994), surface morphology or roughness measurements (Fúcio et al. 2008, Beyth et al. 2008). In the previous chapters of this thesis, we have evaluated degradation after 30 days of intra-oral wear of different composite types under different intra-oral conditions by a variety of conventional and less conventional techniques, that are relatively seldom used for the evaluation of composite degradation. However, these less conventional techniques (the contact angle and X-ray Photoelectron Spectroscopy (XPS) based techniques) were chosen because of their extreme surface sensitivity as compared to the more conventional methods applied to study composite degradation. Considering that degradation of composites starts at the surface, it is felt that contact angle and XPS based techniques might yield a higher sensitivity to detect the early onset of composite degradation. Contact angles typically reflect the composition of the outermost molecular surface, but do not yield any chemical information, as does XPS (Tavana et al. 2004). XPS however, though extremely surface-sensitive in the range of several nanometers, has a

larger depth of information than reflected by contact angles (Ionescu et al. 2012). In the past, surface modeling based on contact angle measurements and XPS analysis has been used to demonstrate degradation of composite surfaces after artificial ageing in distilled water (180 days) and in citric acid (14 days) (Rinastiti et al. 2011) and after 14 days exposure to biofilms *in vitro* (Rinastiti et al. 2010a). Although both techniques demonstrated surface degradation including increased filler exposure, filler exposure at the composite surfaces obtained from both techniques did not numerically correspond (Rinastiti et al. 2010b).

The aim of this study was to compare the results of the different conventional methods and more surface-sensitive techniques as applied in the previous chapters of this thesis to investigate the early degradation of dental resin composites after 30 days of intra-oral wear during different conditions of oral health application.

Materials and methods

Resin composites

Two direct (Beautifil II, BT and Filtek Z350 XT, FL) and two indirect (Lava Ultimate CAD/CAM, LV and Estenia C&B, ES) resin composites with a difference in the filler content determined by manufactures (BT: 83.3%; FL: 78.5%; LV: 80%; ES: 92%) were selected for this study. Chemical composition and sample preparation for each resin composite was described previously (Chapters 2 and 3). Sample discs (2 mm thickness and 5 mm diameter) were made of the different composites according to the manufacturer's instructions and one sample of each composite was inserted to a removable palatal appliance.

Volunteers and oral hygiene regimes

The study was setup as an *ex vivo* within-volunteer comparison, with permission from the Medical and Health Research Ethics Committee (MHREC) Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia (Ref: KE/FK/1125/EC). Data from fifteen healthy volunteers between 18-30 years of age were

analyzed. The exclusion/inclusion criteria and the volunteer enrollment and exclusion from analysis have been described previously (Chapters 2 and 3).

Appliances were provided with metal clasps in the molar or pre-molar region to create stable retention in the oral cavity. Three different brushing regimes of the appliance with the four composites were used, no brushing, brushing with water or brushing with toothpaste (Colgate Total®, Colgate-Palmolive company, Guangzhou, China), twice a day for 2-3 min (Chapters 2 and 3). Absence of brushing only pertained to the palatal device and volunteers were instructed to remove their appliance and rinse it only under flowing water. Further after removal of the device, volunteers were allowed to brush their teeth with toothpaste according to their daily oral hygiene routine.

Filler exposure prior to and after intra-oral wear of the composites from X-ray photoelectron spectroscopy (XPS)

To determine filler exposure prior to and after intra-oral wear and the chemical composition of the composites, XPS was carried out with 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. Binding energies were determined by setting the binding energy of the C_{1s} component due to C-C bonds at 284.8 eV. A survey scan was made with a 1000 x 250 μm spot at a resolution 150 eV in the range of 1-1200 eV, after which narrow scans were made of the C_{1s} (280-300 eV) electron binding peak. The C_{1s} peak was decomposed in 4 components (1: C-C at 284.8 eV, 2: C-N/C-O at 286.3 eV, 3: C=O/O-C-O at 288.1 eV and 4: O-C=O at 289.5 eV) with a full width at half maximum set at 1.4 eV, whereas the O_{1s} was decomposed in 2 components (C=O at 531.5 eV and C-O/C-O-C at 532.7 eV) with a full width at half maximum set at 1.7 eV.

SiO_2 was the most predominant component in all filler particles and used for calculation of the filler exposure at the surface, using the following approach

$$\%O_{filler} = 2 \times \%Si_{measured} \quad (1)$$

hence

$$\{O_{matrix}/C_{matrix}\}_{experimental} = (\%O_{measured} - 2 \times \%Si_{measured})/\%C_{measured} \quad (2)$$

$$\{O_{matrix}/C_{matrix}\}_{experimental} = \%matrix_{exposed} \times \{O_{matrix}/C_{matrix}\}_{theoretical} \times 100\% \quad (3)$$

from which it can be calculated that

$$\%filler_{exposed} = 100\% - \%matrix_{exposed}$$
$$= 100\% - [\{O_{matrix}/C_{matrix}\}_{experimental} / \{O_{matrix}/C_{matrix}\}_{theoretical}] \times 100\% \quad (4)$$

in which $\%Si_{measured}$, $\%O_{measured}$, $\%C_{measured}$ are directly measured using XPS, $\%O_{filler}$ is the percentage oxygen due to silica filler particles calculated, $\{O_{matrix}/C_{matrix}\}_{theoretical}$ is the O/C ratio that can be calculated theoretically from the molecular composition of methacrylates and urethanes in the composites (taken here as 0.35) (Chapter 2).

Filler exposure prior to and after intra-oral wear of the composites from contact angles

Water contact angles represent an extremely sensitive way to monitor changes in surface composition of a material and can be applied to quantitate the fractional exposure of filler particles at a composite surface (Rinastiti et al. 2010b). Because of their extreme surface sensitivity, contact angles were chosen to compare filler exposure prior to and after intra-oral wear. Samples prior to wear were analyzed immediately after preparation, while samples after intra-oral wear and biofilm removal were further cleaned by sonication five times for 1 min in demineralized water and dried in air. Next, water droplets of 1.5-2 µL ultra-pure water were placed on the composite surface with a microsyringe. While keeping the syringe in the droplet, the droplet volume was increased to yield advancing contact angles while subsequently reducing the volume to create receding angles. Contact angles were measured from the droplet contours after black-white thresholding using a customized instrument connected with a camera. After release of the syringe from the droplet and the

formation of a sessile droplet, equilibrium contact angles were measured from the droplet contour.

The different types of water contact angles allow to calculate the fractional surface exposure by filler particles at the composite surface. The equilibrium contact angle, determined in part by the matrix and the filler particles exposed, can be expressed according to Cassie and Baxter (1944)

$$\cos \theta_E = (1 - f_{filler}) \times \cos \theta_A + f_{filler} \times \cos \theta_R \quad (5)$$

where "θ_E" is the equilibrium water contact angle, "θ_A" the advancing angle reflecting the more hydrophobic matrix, "θ_R" the receding angle reflecting hydrophilic silica particles and "f_{filler}" the fractional surface exposure of filler particles at the composite surface (Chapter 3).

Hardness prior to and after intra-oral wear of the composites

Hardness of the composites prior to and after intra-oral wear was evaluated on a Shimatzu instrument (Tokyo, Japan) using a Vickers diamond tip, a pyramidal diamond indenter with a facing angle of 136 degrees. Three indentations were made on different positions on each sample disc under a 981 mN load applying a dwell time of 10 s, leaving an indentation of the diamond tip on the sample surface. The indentation lengths were measured with 40X optical microscope.

Roughness prior to and after intra-oral wear of the composites

Surface roughnesses of the composite surfaces prior to and after intra-oral wear were measured by means of optical profilometry (Proscan 2000, Scantron, Taunton, England). Specimens were scanned with 300 Hz speed in two different directions (5 x 5 mm), and the mean R_a surface roughness was calculated. R_a is arithmetical mean deviation of the assessed surface profile which we used as a roughness parameter in this study.

Correlation analysis

Linear regression lines were used to depict a relationship between two output parameters of the different methods and techniques applied. Pearson's correlation coefficients of determination (R^2) and 95% confidence intervals were calculated to indicate the strength of the relationship.

Results

Filler exposures determined from XPS and contact angle measurements are compared in Fig. 1. Both techniques yield a large dynamic range (defined as the difference between the largest and smallest value obtained divided by the median) over which filler exposure is calculated to spread by a factor of 1.34 and 1.58 with respect to the median, obtained by the XPS (38%) and contact angle (36%) technique. However, there is no significant correlation between filler exposure calculated from XPS and contact angles. Therefore it must be concluded that both techniques measure "a different sort of filler exposure". This can be understood from the fact that XPS measures deeper into a surface than do contact angles and therewith the percentage filler exposure calculated from XPS data will reflect more of the bulk than filler exposure calculated from contact angles.

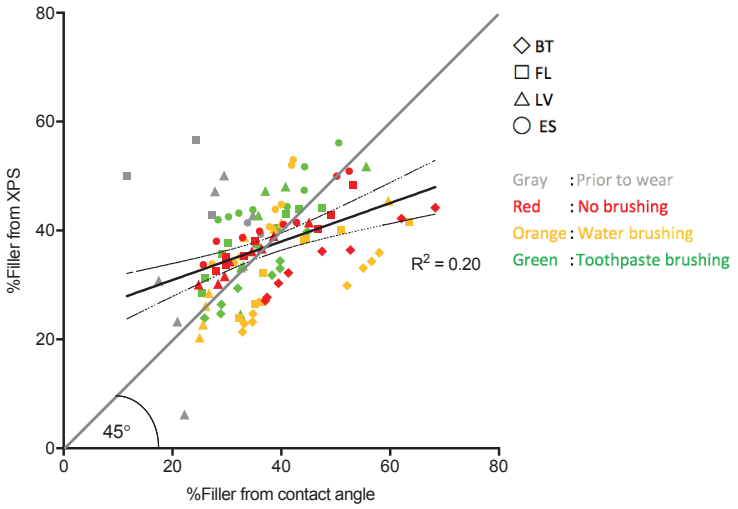

Figure 1 %Filler exposure calculated from XPS analysis, as a function of %filler exposure calculated from contact angle measurements for the four composites prior to and after intra-oral wear for the three brushing regimes. Each data point represents one of the composite types under one particular regime of oral health care in each of the fifteen volunteers that participated in this study. The solid grey line represents the line of identity, while the dotted lines indicate the 95% confidence interval.

Hardness of the composites prior to and after intra-oral wear is shown in Fig. 2 as a function of the percentage filler exposure calculated from contact angles and XPS data. The dynamic range with respect to the median (26.1 µm) is relatively small (0.54) as compared with XPS analysis and contact angle measurements. Hardness is a typical bulk property which explains the complete lack of relationship between hardness and filler exposure at the outermost composite surface calculated from water contact angles. The decrease in indentation length, i.e. an increase in composite hardness with the percentage filler exposed as calculated from XPS data, confirms that XPS probes more of the bulk of the composite than contact angles, although the correlation coefficient between filler exposure by XPS and hardness is low.

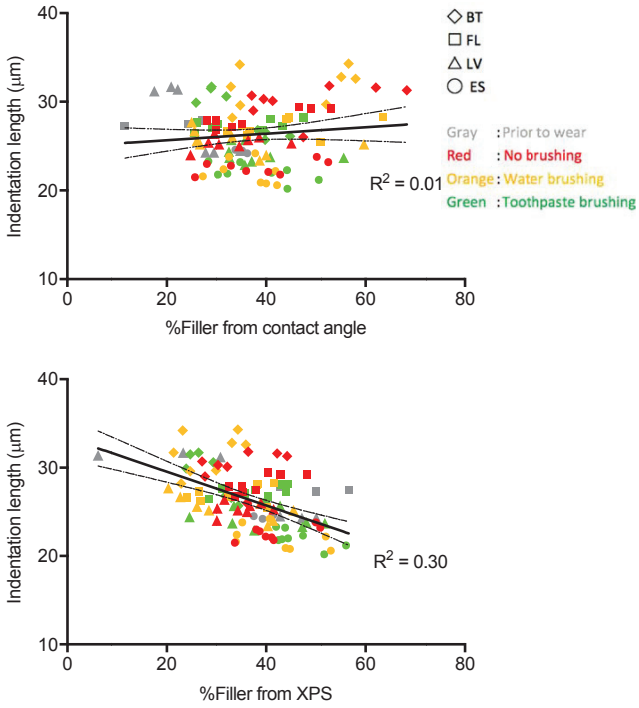

Figure 2 Indentation length as a measure of composite hardness as a function of the %filler exposure, calculated contact angle measurement (top graph) and XPS analyses (bottom graph). Each data point represents one of the composite types under one particular regime of oral health care in each of the fifteen volunteers that participated in this study, while the dotted lines indicate the 95% confidence interval.

Opposite to hardness, roughness is a true surface property and by consequence increasing roughness corresponds at a fair level of correlation with the percentage filler exposure calculated from contact angles, but not with filler exposure calculated from deeper-probing XPS data (Fig. 3). Its dynamic range with respect to the median (0.8 µm) is relatively large (5.00), especially when compared with the one of hardness measurements.

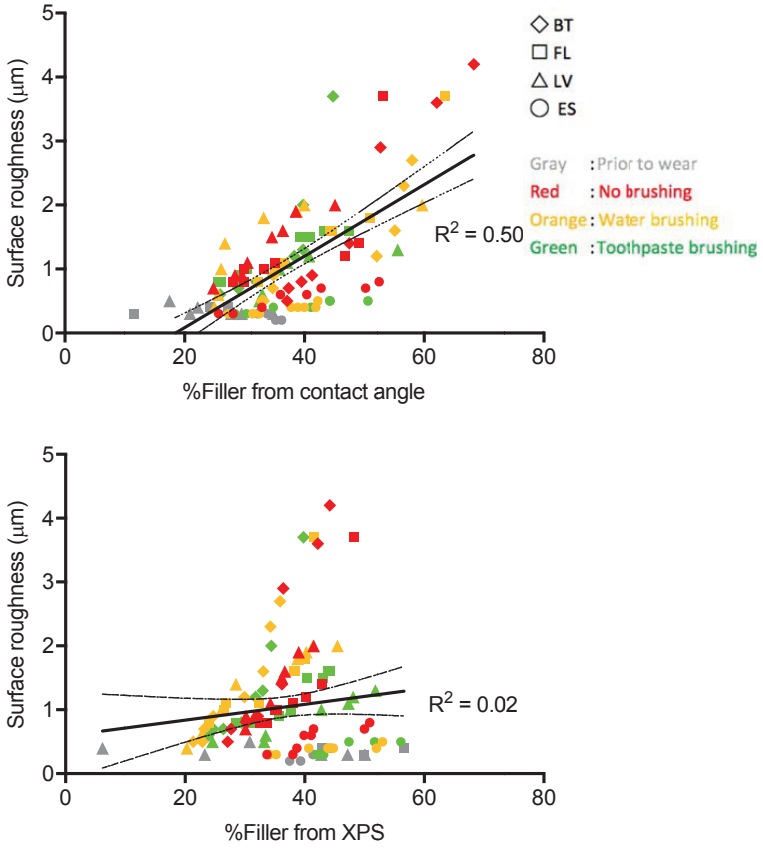

Figure 3 Surface roughness as a function of the %filler exposed, calculated from contact angle measurements (top graph) and XPS analysis (bottom graph). Each data point represents one of the composite types under one particular regime of oral health care in each of the fifteen volunteers that participated in this study, while the dotted lines indicate the 95% confidence interval.

Discussion

Identification of sensitive methods to detect early degradation of composite resins either after *in vitro* experiments or intra-oral wear is important as the use of more sensitive methods will allow shorter experimentation times to reach statistically significant and relevant conclusions. The (near-)surface region plays a significant role in the degradation of dental resin composites through adsorption and absorption of oral fluid components (Eliades et al. 2003) and mechanical wear (Göpferich 1996). Whereas the onset of degradation is a surface phenomenon, absorption of enzymes from saliva, food and beverage components into the composite will eventually lead to softening of the bulk matrix (Finer and Santerre 2004). Thus degradation will first become obvious from altered surface properties of a composite, followed later by changes in bulk properties.

Conventional hardness or indentation length measurements (Santerre et al. 2001) to demonstrate composite degradation will reflect degradation of the bulk matrix and accordingly, in our 30 days studies of intra-oral wear, show only a very small dynamic range, with no correlation with the results of surface-sensitive techniques based on contact angles and XPS data. Thus it can be concluded that hardness is not suitable to demonstrate early degradation as a surface phenomenon. This conclusion is supported by the observation that hardness did not correlate with results of any of the more surface-sensitive techniques applied in this study (Fig. 2).

Surface roughness constitutes another conventional parameter employed to study composite degradation. Its dynamic range observed in this study is quite high and results correlate with contact angle based evaluations (Fig. 3) and accordingly surface roughness measurements can be classified to reflect surface changes as an early indicator of composite degradation.

Two surface-sensitive techniques were applied to study early degradation, but in line with previous observations (Rinastiti et al. 2010b), both techniques indicated increased filler exposure upon early degradation but results did not correspond numerically (Fig. 1). Calculation of filler exposure from measured contact angles with water relies on differences in wettability of the relatively

hydrophobic matrix *versus* the relatively hydrophilic filler particles, which makes the measurement of equilibrium, advancing and receding contact angles, highly sensitive to changes at the surface. This explains why its results compare best with surface roughness data on composite degradation, although roughness as such can interfere with the application of the Cassie and Baxter equation (Eq. 5) to derive filler exposure. Filler exposure from XPS relies on the analysis of photoelectrons released from a material under the influence of X-ray irradiation. Although X-rays can penetrate a material up to several micrometers, the mean electron free path of ejected photoelectrons is about 3-10 nm, which makes the technique a surface-sensitive technique, but not to the extent of contact angle measurements. This is confirmed here by a slightly smaller dynamic range in filler exposure calculated using XPS data as compared to filler exposures derived from water contact angles and a lack of correlation between both types of filler exposures derived.

In conclusion, early degradation can best be studied by surface roughness measurements or analysis of equilibrium, advancing and receding water contact angles as they best reflect changes at a surface and their results for composite degradation confirm each other. XPS is also suitable for surface analysis of early composite degradation, but its results will partially reflect the bulk matrix. Hardness, though suitable to provide information on bulk matrix degradation, is unsuitable for measuring early composite degradation that is still confined to the surface.

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