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ORIGINAL RESEARCH

Curing Depth of Light-activated Nanofiller containing Resin Composites

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ABSTRACT

Objective: To compare the depth of cure of nanofiller containing with conventional resin composites.

Materials and methods: Five nanofilled and nanohybrid composites were investigated and compared with a microfilled and a microhybrid reference, using the ISO 4049 scraping test, Knoop hardness (KHN) and DC profiling. Specimens from all materials (shade A3) were activated with the same LED light source for 20s. KHN (0.25N/15s) of three specimens each, produced in split molds was measured after 24 hours dark storage on sections perpendicular to the irradiated surface at 250 μm distance along the center line and two parallel lines, 0.5 mm apart, to a depth of 3 mm. Mean KHNs of the three neighboring indentations at each level were calculated. Degree of conversion (DC) was determined on specimens as mentioned above using micro-Raman spectroscopy at 125 μm distance with three measurements at each depth level.

Results: The depth of cure of all materials was >2 mm when determined according to the ISO test. KHN and DC data followed second order polynomial regression lines ($r^2 > 0.70$; $p < 0.001$). At 2 mm depth, the KHN of six resin composites was ≤80% of the top KHN whereas the DC of all materials was ≥86% of the maximum DC at the irradiated surface.

Conclusion: The ISO scraping test overemphasizes the attainable depth of cure, when compared with 80% of top KHN as arbitrarily defined curing depth. KHNs reflect the crosslink density of the polymer, whereas DC additionally includes double bond conversions not contributing to enhancement of mechanical characteristics.

Keywords: Resin composites, Nanofiller, Depth of cure, Knoop hardness, Raman spectroscopy, Degree of conversion.

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INTRODUCTION

The depth of curing of light-activated resin composite restoratives is limited by the attenuation of light passing through the material.[1] Therefore, at some depth underneath the activated free surface the composite will be poorly polymerized. Consequently, the mechanical resistance[3] is compromised and unreacted components may be leached, possibly resulting in adverse effects on biocompatibility.[2]

The depth of cure depends on the composite's monomer, filler composition and size, initiator system, color and translucency.[4-6] The activation light source, the intensity and duration of activation, as well as the distance of the light-emitting window from the target surface are determinants of the attainable depth of cure.[7-9]

The appropriate curing depth of a resin composite is not unequivocally defined and depends on the applied testing method. In the ISO 4049[10] standard for resin-based dental restorative materials the depth of cure is determined by a scraping test, where the unpolymerized soft composite is removed from a cured cylindrical specimen and the remaining height is measured. An alternative method frequently used is microhardness profiling perpendicular to the irradiated surface along the axis of beam-shaped specimens, assuming that hardness is an indication for the degree of polymerization.[11-15] Mostly, a value of 80 or 90% of the specimen's top hardness is considered the depth of adequate cure.[12,16-18] Another way to determine polymerization depth is to analyze the degree of conversion, using either FTIR or micro-Raman vibrational spectroscopy.[12,19] In particular profiling by Raman spectroscopy has the advantage that without sample preparation spectral analyses of very small sample areas can be performed for determination of the reactive double bond conversion to characterize the curing quality.[7,14,20]

Since, recently most newly introduced resin-based composites contain nanofillers the aim of this study was to determine and compare the degree of cure of nanofilled and nanohybrid composites with conventional microfilled and microhybrid types, using the ISO scraping test, Knoop hardness and DC profiling as evaluation tools on specimens of the same shade, activated with the same LED light source and same activation time.

The null hypotheses tested were that the restorative resins regardless of their different filler concepts (1) fulfill the ISO criteria for depth of cure, (2) that the KHN at 2 mm curing depth will be ≥80% of the resins' top surface hardness and (3) that KHN and DC at 2 mm depth of cure would show the same percentage decrease from their top values.

MATERIALS AND METHODS

The five nanofiller containing resin composites and the two reference materials used in this study, the microfilled DUR

Table 1: Materials tested

Materials	*Manufacturer*	*Batch no.*	*Expiry*	*Composition*		
				Monomer	*Filler*	*Vol (%)*
Durafill® VS	Heraeus Kulzer, Germany	010213	2013-07	Bis-GMA, UDMA, TEGDMA	SiO_2 (20-70 nm) and Pre-polymer <20 μm	66
Filtek™ Z250	3M ESPE, USA	N134014	2012-09	Bis-GMA, Bis-EMA, UDMA	SiO_2, ZrO_2 clusters, average 0,6 μm (0.19-3.3 μm)	60
Filtek™ Supreme XT	3M ESPE, USA	N144799	2013-02	Bis-GMA, UDMA, Bis-EMA, TEGDMA	SiO_2/SrO_2 clusters, (0.8 - 1,4 μm), SiO_2 (20 mm)	59.5
Kalore	GC, Japan	1002161	2013-02	UDMA (DuPont), DMA, UDMA	Prepolymer (SrO_2, 400 nm, lanthanoid fluoride 100 nm) Silica- and Sr-doped nanofiller	69
MI Fil	GC, Japan	1007242	2013-07	UDMA, Bis-MEPP, TEGDMA	Silica-nanofiller (16 nm), Sr-doped nanofiller (200 nm)	50
MI Flow	GC, Japan	1002121	2013-02	UDMA, Bis-MEPP, DMA	Silica- (16 nm) and Sr-doped nanofiller (700 nm), lanthanoid fluoride (100 nm)	50
Venus® Diamond	Heraeus Kulzer, Germany	010034	2013-08	TCD-DI-HEA, UDMA	Ba-Al-F-Si glass <20 μm, SiO_2 (5 nm)	64

Abbreviation: Bis-GMA: Bisphenol A diglycidylether methacrylate; UDMA: Urethane dimethacrylate; TEGDMA: 3,6-dioxaocta-methylendimethacrylate; Bis-EMA: Ethoxylated bisphenol-A dimethacrylate; DMA: Dimethacrylate; Bis-MEPP: 2,2-Bis (4-methacryloxypolyethoxyphenyl)propane; TCD-DI-HEA: (Bis-(acryloyloxymethyl)tricyclo 5.2.1.02,6 decane)

and the microhybrid Z250 are listed in Table 1, including their main compositions, as extracted from publicly available manufacturer information. All shades were A3 according to the common VITA classification.

Depth of Cure according to ISO 4049

Depth of cure was determined as specified in ISO 4049 for class 2 materials, requiring a depth of cure of 1.5 mm. For each material three specimens were produced using 20s light activation (G-Light Prima-II; GC Company, Tokyo, Japan; blue light LED with 1200 mW/cm^2 light intensity; single wavelength 430-500 nm). Immediately after curing the specimens were removed from the mold and the uncured material was removed with a plastic spatula. The height of the cured cylinder is measured and divided by two as value for the depth of cure. The data were statistically treated by one-way ANOVA and Tukey's post-hoc test ($p \leq 0.05$).

Depth of Cure by Knoop Hardness Profiling

For hardness profiling beam-shaped composite specimens (n = 3) were produced in split black Teflon molds (3 × 2 × 6 mm), placed with the 3 × 2 mm opening on a strip-covered glass plate, filled from the opposite side with slight excess, covered with another Mylar strip, and pressed flush for light activation with the LED curing device G-Light Prima-II for 20s with the light exit window in contact with the strip covered surface. Following removal from the mold, the non-polymerized part of composite was removed with a plastic spatula and the samples were dark stored for 24 hours at ambient laboratory atmosphere (23 ± 2°C, 50 ± 5% relative humidity). Prior to hardness testing the one of the samples' 3 mm wide surface was prepared by wet-grinding using ISO P1000 grit silicon carbide abrasive paper. Knoop (KHN) indentations (0.25N/15s) were produced with the micro-hardness tester HM 102 (Akashi, Mitutoyo Corp., Kanagawa, Japan) at 250 μm distance along the center line and two parallel lines, 0.5 mm apart from the center line, with the first indentations 250 μm underneath the edge of the irradiated top surface. Mean KHNs of the three neighboring indentations at each level were calculated. For each material the means at each depth level of the three specimens followed second order polynomial regression lines to describe the course of the KHN decrease.

Depth of Cure by Degree of Conversion of Double-bonds (DC)

Micro-Raman spectra of the uncured and cured materials were measured by a Raman spectrophotometer NRS-3000FL (JASCO Corporation, Tokyo, Japan) fitted with a solid-state diode laser (532 nm, 1.2 mW). The spectral resolution was approx. 1 cm^{-1}. Through the microscope objective (UMPLFL20x, NA = 0.46, Olympus) the spatial resolution was approx. 3 μm. Raman spectra were obtained in the region 1970-975 cm^{-1}. Eight accumulations throughout 5 seconds measurement were performed. This method allows to evaluate the degree of conversion (DC),

which is the percentage of vinyl functions converted to aliphatic functions by comparing the vibration band of the residual unpolymerized (meth) acrylate C = C bond at 1640 cm^{-1} against the aromatic C = C stretching band at 1610 cm^{-1} used as internal standard. The DC is calculated as follows: DC (%) = 100 [1 - ($R_{polymerized}/R_{unpolymerized}$), where R is the ratio between the peak area at 1640 cm^{-1}/ peak area at 1610 cm^{-1}. In case of the resin composite VED there is no aromatic stretching band at 1610 cm^{-1}, therefore the stretching band at 1600 cm^{-1} was used for internal calibration. Quantum-chemical calculation of the partial structure of the VED monomer's acryloyloxymethyl moiety was performed to assign the Raman signals. Assignment of Raman signals in the partial structure of VED monomer was carried out by Gaussian 09 package.[21] The partial structure (acryloyloxymethyl group) was fully optimized by B3LYP/6-31G (2d,3p) method, followed by Raman optical activity calculation with B3LYP/6-311G (2d,3p) calculation. The calculation results showed that a signal at 1610 cm^{-1} corresponded to the C = C stretching mode in the acryloyloxymethyl group. The peak at 1600 cm^{-1} did not appear in the calculation. The acryloyloxymethyl moiety is considered the reaction point for photo-polymerization; any other Raman peak out of the acryloyloxymethyl moiety has fixed intensity during the photopolymerization. Thus, the peak at 1600 cm^{-1} is suitable as internal standard.

For determination of the DC, specimens were prepared as described for KHN testing above. On each specimen spectra are registered at 0.125 mm distance along the center of the 3 mm wide beam surface and two lines, 0.5 mm apart on each side of the center line, with the first registration approximately 0.05 mm underneath the edge of the irradiated top surface of the sample. For each material the means of the three specimens' mean DCs by depth level were fitted with a second order polynomial using nonlinear regression analysis.

RESULTS

Figure 1 shows the depths of cure as determined according to the ISO 4049 method. All seven materials tested hardened in agreement with the manufacturers instructions to a depth of at least 2 mm, fulfilling the requirement of the ISO standard. Z250 showed the deepest cure, whereas FIL and VED hardened slightly deeper than the 2 mm threshold.

Figure 2 shows the relationship between KHN and depth of cure for the seven materials tested. Each point represents the mean value for the depth of cure of three specimens, calculated from the three individual KHN values of each of the specimens at the same depth underneath the irradiated surface. KHN values *vs* depth were fitted with second order

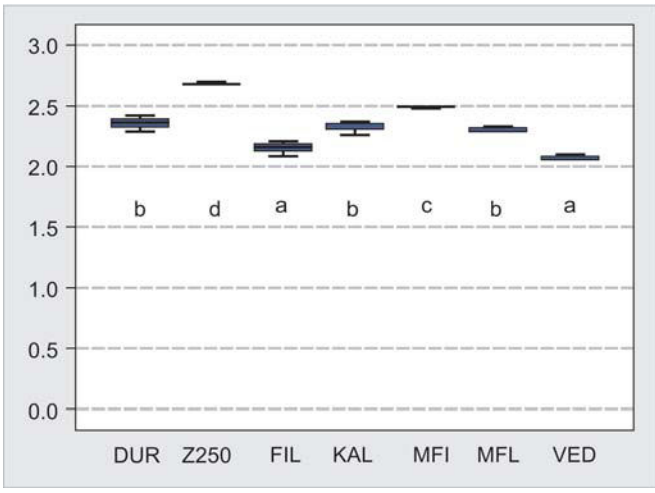

Fig. 1: Depth of cure (mm) of resin composites determined according to the procedure outlined in ISO standard 4049 (n = 3)

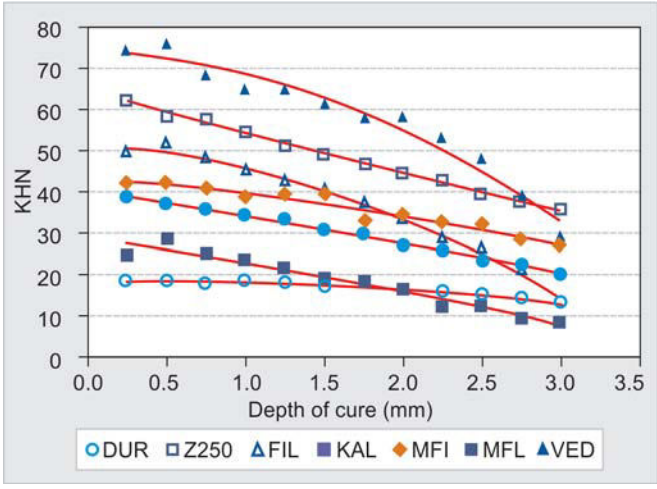

Fig. 2: Relationships between mean KHN (n = 3) and depth of cure for the resin composites investigated. Second order polynomial regression lines ($r^2 \geq 0.93$, $p < 0.001$)

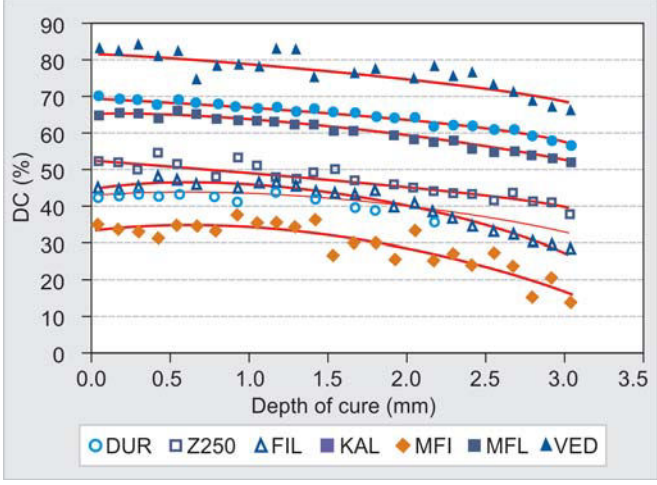

Fig. 3: Relationships between mean DC (n = 3) and depth of cure for the resin composites investigated. Second order polynomial regression lines ($r^2 \geq 0.70$, $p < 0.001$)

Table 2: Depth of cure *vs* KHN
Regression coefficients and coefficients of determination
(max. KHN = Intercept with ordinate)

	$y (KHN) = ax^2 + bx + c$ (x = depth)			r^2	% of max. KHN
	a	b	c		at 2 mm depth
DUR	–0.79	0.62	18.53	0.98	89.6
Z250	0.39	–10.85	64.32	0.99	68.7
FIL	–4.19	–0.99	73.49	0.96	74.5
KAL	–0.93	–2.66	43.08	0.93	79.0
MFI	–0.43	–5.31	39.99	0.99	69.1
MFL	–0.19	–4.32	28.47	0.96	67.0
VED	–3.44	–2.06	51.33	0.99	65.2

All coefficients of determination (r^2) are highly significant ($p < 0.001$)

Table 3: Depth of cure *vs* DC
Regression coefficients and coefficients of determination
(max. DC = Intercept with ordinate)

	$y (DC) = ax^2 + bx + c$ (x = depth)			r^2	% of max. DC
	a	b	c		at 2 mm depth
DUR	–1.94	2.57	42.81	0.76	93.9
Z250	–0.77	–1.99	52.45	0.87	86.5
FIL	–3.47	4.61	44.64	0.97	89.6
KAL	–3.48	5.17	32.57	0.83	89.0
MFI	–0.96	–1.03	69.32	0.97	91.5
MFL	–1.35	–0.44	65.48	0.97	90.4
VED	–1.17	–0.91	81.56	0.70	92.0

All coefficients of determination (r^2) are highly significant ($p < 0.001$)

polynomials using nonlinear regression analyses. The decrease in KHN for FIL, VED and MFL was more pronounced than for the other composites tested.

Table 2 summarizes the coefficients of the regression lines and the coefficients of determination ($r^2 \geq 0.93$; $p > 0.001$). Assuming that 80% of the top surface hardness, estimated as the intercept of the regression line with the ordinate axis, represents sufficient cure, only the microfilled composite DUR showed more than 2 mm curing depth.

The relationship between depth of cure and DC is shown in Figure 3. As for the presentation of the KHN data each point in the diagram represents the mean value for the depth of cure of three specimens, calculated from the three individual DC values of each of the specimens at the same depth underneath the irradiated surface. For FIL and KAL a noticeable decrease in DC beyond the 2 mm threshold depth is seen. Scattering of the DC values around the regression line was pronounced for DUR, KAL and VED, as also demonstrated by the comparatively smaller coefficients of determination (Table 3). At 2 mm depth all resin composites exhibited high degrees of conversion, between 86.5 and 93.9%.

DISCUSSION

The present investigation has shown that the three different evaluation methods for determination of the depth of cure of resin composites yielded different results. The threshold value of 2 mm curing depth after 20 seconds LED light activation was selected, since manufacturers of the composites tested claim that 20 seconds light activation of their shade A3 products would result in safe curing of 2 mm thick layers. Layer thicknesses of 2 mm are often recommended when incremental filling and curing techniques are required. As the energy of the light source has some effect on curing depth, a high intensity LED curing unit (1200 mW/cm^2) was selected. This curing device is according to the manufacturer suitable for curing of all light-activated resin composites. A previous publication mentioned that curing lights with an intensity of ≥300 mW/cm^2 effectively cure most resin-based composite materials.[22]

All resin composites tested fulfilled by far the requirements of 1.5 mm minimum depth of cure of the ISO 4049 standard. However, a number of publications conclude that scraping test data generally overemphasize depth of cure, as determined with alternative more sensitive methods, such as hardness or DC profiling.[11,12,23]

Hardness profiling has been frequently used as an indirect indicator for the degree of curing and to draw a more detailed picture of the mechanical resistance of resin composites at different curing depths.[12-14,24-26] In contrast to the present findings, where six of the seven evaluated resin composites achieved less that 80% of the top surface hardness at 2 mm depth, other reports confirm satisfactory

hardness of resin-based composites within a given depth up to 2 mm.[18] This difference might be related to the different curing units and techniques and the different composite materials used. Generally, the top of the specimen, i.e. the restoration surface, receives the highest light energy and will thus, probably also develop the highest physical properties, whereas deeper layers are affected by the reduced transmission of light through the resin composite.[1] An important limiting factor for the depth of cure is light scattering, that is related to the filler particle size. Light scattering is maximized when the filler size is about half the wavelength emitted by the curing device.[28] The hardness profiles for FIL and Z250, composites with almost the same monomer composition, yet different filler size at similar filler loading, show higher KHN for FIL to a depth of almost 3 mm. This difference between the two products might be related to the filler size, which for the microhybrid Z250 is 0.19 to 3.3 μm, in contrast to FIL including nanofiller clusters (0.8-1.4 μm) and discrete nanofillers of 20 nm.[28,29]

Indentation hardness is a nonintrinsic material property that reflects the resistance of a material to deformation under highly localized load. Hardness figures reflect both the degree of polymerization, the filler size and loading, and the quality of coupling between filler and polymer. In highly filler-loaded composites or materials with rather large prepolymer particles the filler effect on hardness is more pronounced than in composites with lower filler loading. Therefore, determination of the degree of conversion of double bonds is considered more adequate for assessment of depth of cure. Presumably, as a result of the decrease in light transmission during activation the materials tested showed a decrease in DC *vs* depth. As with KHN data second order polynomials showed the best regression fit, confirming findings reported by Leloup et al.[7] In particular FIL and KAL, yet also to some extent VED showed a steeper decrease in DC beyond 2 mm layer thickness than the alternative materials. When the percentage of the maximum degree of conversion was calculated from the regression equations for a composite depth of 2 mm, the results varied between 86.5 and 93.9%, whereas the corresponding figures for the KHN were between 65.2 and 89.6%. Thus, the depth of cure determined by micro-Raman and KHN have in common a gradual decay up to 2 mm depth and a more rapid decay at deeper layers.[14]

It has to be kept in mind that KHN and DC measurements reflect different changes in the polymer during and after curing. KHN responds mainly to the crosslinking density of the polymer, whereas DC, measuring the amount of conversion of carbon double bonds to single bonds, summarizes several events, such as chain elongation, reaction with initiator radicals and crosslinking.

The DC of VED was remarkably high. Similar high values for the degree of conversion were reported by Cerutti et al[30] and Boaro et al[31] using Raman and FTIR spectroscopy respectively. The main crosslinker included in VED is TCD-DI-HEA is a high molecular mass urethane diacrylate. It may be speculated that VED cures comparatively slowly, leading to many double bond conversions by chain elongation and crosslinking before finally, due to immobilization of the system, no further reaction between double bonds and remaining radicals is possible.[32] On the other hand, the top surface hardness of VED (KHN 51) is comparatively low. A possible explanation is that Venus Diamond due to the tricyclodecane structure of the TCD monomer might have a low E-modulus and thus a low KHN.[32]

The first null hypothesis that the resin composites tested would fulfill the ISO 4049 requirements for depth of cure was accepted. The second hypothesis that the KHN at 2 mm curing depth would be ≥80% of their top surface hardness is partially rejected, since only the microfilled DUR achieved more that 80% of the irradiated surface KHN. The third hypothesis was rejected, since the percentage reduction in DC at 2 mm layer thickness was much less than the KHN decrease.

CONCLUSION

Within the limitations of the present study, the following conclusions were drawn:

1. Depth of cure values of resin composites determined with different testing methods, are not comparable. With the simple scraping test according to ISO 4049 depth of cure is measured, whereas Knoop hardness or DC profiling are indirect measures were arbitrarily defined percentage figures of the top surface properties serve as indicator for depth of cure.
2. Hardness and DC depth profiles followed second order polynomial regression lines. The slopes and intercepts of the regression lines were material dependent.
   The DC profiling results showed that at 2 mm depth all tested materials achieved high degrees of conversion (≈ 90% of the top surface DC). In contrast, when 80% of the top hardness was defined as the clinically relevant depth of cure, only the microfilled reference fulfilled this requirement.
3. KHN reflects the crosslink density of the polymer, DC includes apart from crosslink density also the amount of conversion of carbon double bonds that will not contribute to enhancement of mechanical characteristics.

Nevertheless, denoting 80 or 90% of the top surface hardness or DC as indirect threshold measure for depth of cure is disputable.

4. Generally, based on the KHN results of this trial, an extension of activation time beyond the mostly recommended 20 seconds duration is desirable. Longer irradiation is indispensible for darker shades and when the distance of the curing light from the resin composite is increased.

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