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The effect of excitation laser power in Raman spectroscopic measurements of the degree of conversion of resin composites

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ABSTRACT

Objectives. To evaluate the effect of excitation laser power in Raman spectrometry by comparing the spectra and the degree of conversion (DC) values obtained using excitation powers between 300 and 1000 mW.

Methods. Five commercial and three experimental resin composites were light cured at 1200 mW/cm^2 for 10–20 s from a commercial blue-violet LED dental curing unit. Raman spectra were collected from composite specimens within 9 min after light-curing. The excitation laser (1064 nm) was focused on the spot of 0.4 mm in diameter. The following powers were used for specimen excitation (mW): 300, 400, 600, 800, and 1000. From Raman spectra, the DC values were calculated and compared among different laser powers. Also, vector-normalized Raman spectra collected using the lowest excitation power (300 mW) were compared to those collected using the maximum excitation power (1000 mW).

Results. Varying the excitation laser power between 300 and 1000 mW resulted in statistically significant differences in both the DC values and the intensity of particular spectral features. The effect of varying laser power on Raman spectra and obtained DC values was material-dependent. The DC values measured within an individual material using different laser powers varied between 3.2 and 7.2% (absolute DC difference). The spectral bands affected by variations in laser power were assigned to symmetric and asymmetric stretching of $-CH_2$ (2900-3100 cm^{-1}), symmetric stretching of aliphatic C=C (1640 cm^{-1}) and scissoring of C–H (1458 cm^{-1}).

Significance. The DC can be artificially elevated through increasing excitation laser power. This effect should be considered in Raman spectroscopic evaluations of DC in specimens during ongoing post-cure polymerization.

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1. Introduction

The degree of conversion (DC) is a fundamental characteristic of a polymerized resin composite because it affects multiple material properties: higher DC values are related to better mechanical properties [1], lower water sorption [2], lower release of potentially toxic compounds [3], and better biocompatibility [4,5]. Vibrational spectrometric methods used to evaluate the DC of dental resin composites include mid-infrared [6], near-infrared [7], and Raman spectrometry [8]. All of these methods work by quantifying the amount of double C=C bonds in uncured and cured specimens, which is then used to calculate the ratio of double bonds which were transformed into single bonds during the polymerization [9]. Although infrared and Raman spectrometry employ different physical phenomena (absorption and inelastic light scattering, respectively), both methods are used in a similar manner to measure the intensity change of the spectral band at 1640 cm^{-1} [10]. Infrared spectrometry has been traditionally used in evaluations of the DC of dental composites [11], however, Raman spectrometry is considered as an equally valid method that offers an advantage of time-savings and lower technique sensitivity due to the non-destructive and non-contact specimen manipulation [10]. The main advantage of Raman spectrometry is its ability to analyze native specimens without any preparation [8], unlike infrared spectrometry which usually involves pulverizing [12] or clamping the specimen onto the crystal for attenuated total reflectance [13].

Raman spectrometers analyze inelastically scattered irradiation using either a charge-coupled device (CCD) array or an interferometer [14]. The former type of detector is common in dispersive instruments which employ visible light excitation (typically 780, 633, 532, and 473 nm), while the latter uses near-infrared excitation (most commonly 1064 nm) and records an interferogram which is converted into a Raman spectrum using Fourier-transform (FT) [15]. Dispersive Raman spectrometers using a near-infrared excitation have also recently become available [16]. Because the intensity of Raman scattering is inversely proportional to the fourth power of excitation wavelength, shorter wavelengths offer the advantage of a better signal-to-noise ratio [15]. The drawback of excitation using shorter wavelengths (higher photon energy) is the occurrence of unwanted fluorescence which diminishes the quality of Raman spectra [11]. In most cases, fluorescence can be avoided by using near-infrared excitation, however, at the cost of signal intensity. This situation typically results in lower sensitivity, which could be partially overcome by increasing excitation power and performing a larger number of scans that are then averaged to improve the signal to noise ratio [17].

In Raman spectrometry, a small surface area of the specimen is irradiated by the excitation laser. The diameter of the irradiated spot varies more than 3 orders of magnitude, ranging from less than a micrometer [18] up to the millimeter range [19]. High energy density at the irradiated site can lead to localized specimen heating [20]. The magnitude of heating is influenced by different instrument-related factors (wavelength and power of the excitation irradiation, spot size), as well as by specimen-related factors (absorption at that particular wavelength, thermal capacity, and the capability for thermal dissipation) [21]. The power of excitation radiation in Raman spectrometers is usually adjustable, allowing the operator to find a balance between signal quality and a potentially detrimental effect of laser-induced specimen heating. The effect of laser-induced heating has been recognized and described for pharmaceutical [20] and biological materials [22], as well as in various substances for non-medical application [23–26]. However, the effect of laser-induced heating in measurements of the DC in dental resin composites has not been evaluated to date, although Raman spectrometry has been widely used for this purpose [8,10,11,17,27–33].

The purpose of the present study was to evaluate the effect of varying excitation laser power between 300 and 1000 mW on the measurement of DC in dental resin composites. The research hypotheses were that laser power would not affect: (I) DC values recorded within a short time after light-curing of resin composite specimens, nor would it have a significant effect on (II) qualitative and quantitative Raman spectral features, which reflect specimen composition.

2. Materials and methods

2.1. Material selection

To explore a possible effect of varying excitation laser power on Raman spectra and the calculated DC values, 5 commercial composites (Table 1) and 3 experimental composites (Table 2) were investigated. The commercial composites were chosen in order to include representative materials of different types of contemporary composites: a sculptable bulk-fill composite (FBF), a flowable bulk-fill composite (SDR), a conventional sculptable composite containing pre-polymerized fillers (TEC), a conventional flowable composite (TEF), and a conventional sculptable composite with very high filler load (GRA). The level of detail regarding material composition in Table 1 varies among materials, because manufacturers never provide a full disclosure of this information. The experimental composites followed formulations from previous studies [34,35] and included a flowable composite (EXP-FLO), a sculptable composite (EXP-SCU), and a composite containing 20 wt% of bioactive glass (EXP-BG). The detailed procedure for the preparation of the experimental materials is described elsewhere [34,35]. Briefly, research-grade components listed in Table 2 were blended in a dual asymmetric centrifugal mixer (Speed Mixer TM DAC 150 FVZ, Hauschild & Co. KG, Hamm, Germany) and deaerated in vacuum for 12 h.

All of the investigated composites contained a classical blue light-absorbing photoinitiator system comprised of camphorquinone and a tertiary amine, except TEC, which in addition to camphorquinone and the tertiary amine also contains the violet light-absorbing photoinitiator Lucirin-TPO [36]. The experimental composites contained 0.2% of camphorquinone and 0.8% of ethyl-4- (dimethylamino) benzoate, expressed as weight ratios in the corresponding resin systems.

2.2. Specimen preparation

Resin composite specimens were prepared in cylindrical Teflon molds of 6 mm in diameter. Clinically relevant spec-

Table 1 – Commercial composite materials – information provided by the respective manufacturers.

Material (abbreviation)	Shade LOT No. EXP	Composition	Filler load (wt%/vol%)	Filler size	Manufacturer	Recommended exposure duration (s)
Filtek Bulk Fill Posterior (FBF)	A2 N938959 2020-12-28	Zirconia, silica, ytterbium trifluoride, proprietary AUDMA, DDDMA, UDMA	77/58	4–20 nm agglomerates 0.1 μm	3M /ESPE, St. Paul, MN, USA	20
GrandioSO (GRA)	A2 1648523 2019-06	Ba-Al-borosilicate glass, SiO_2 nanofillers, Bis-GMA, TEGDMA, Bis-EMA	87/71	0.02–1 μm	Voco, Cuxhaven, Germany	20
Smart Dentin Replacement (SDR)	Universal 1705000888 2019-05-31	Ba-Al-F-borosilicate glass, Sr-Al-F-silicate glass, modified UDMA, Bis-EMA, TEGDMA	68/45	4.2 μm (average)	Dentsply, York, PA, USA	20
Tetric EvoCeram (TEC)	A2 V40834 2020-10-13	Barium glass, ytterbium trifluoride, mixed oxide, prepolymers, UDMA, Bis-GMA, Bis-EMA	76/54	40 nm–3 μm	Ivoclar Vivadent, Schaan, Liechtenstein	10
Tetric EvoFlow (TEF)	A2 V36426 2020-09-02	Barium glass, ytterbium trifluoride, mixed oxide, highly dispersed silica, prepolymers, Bis-GMA, UDMA, decandi-oldimethacrylate	58/31	40 nm–3 μm	Ivoclar Vivadent, Schaan, Liechtenstein	10

Bis-GMA: Bisphenol-A-glycidylmethacrylate; Bis-EMA: Ethoxylated bisphenol-A-dimethacrylate; UDMA: Urethane dimethacrylate; TEGDMA: Triethylene glycol dimethacrylate; DDDMA: 1,12-dodecanediol dimethacrylate; AUDMA: high molecular weight aromatic dimethacrylate.

Table 2 – Experimental composite materials.

Material (abbreviation)	Filler composition (wt%)	Resin com-position (wt%)	Filler load (wt%/vol%)	Material name used in the original publication (reference number)
Experimental-flowable (EXP-FLO)	Barium glass (40%): particle size d50/d99 (μm): 0.8/2.3, silanization 6 wt%. Silica (10%): primary particle size: 12 nm, silanization 4–6 wt%.	67% Bis-EMA 23% TEGDMA 10% HEMA	50/32	Ba40Si10 (34, 37)
Experimental-sculptable (EXP-SCU)	Barium glass (47%): particle size d50/d99 (μm): 1.0/4.0, silanization: 3.2 wt%. Silica (23%): primary particle size: 12 nm, silanization 4–6 wt%.	60% Bis-GMA 40% TEGDMA	70/48	BG-0 (13, 35)
Experimental-bioactive (EXP-BG)	Bioactive glass 45S5 (20%): particle size d50/d99 (μm): 4.0/13.0, silanization: none. Barium glass (33%): particle size d50/d99 (μm): 1.0/4.0, silanization: 3.2 wt%. Silica (17%): primary particle size: 12 nm, silanization 4–6 wt%.	60% Bis-GMA 40% TEGDMA	70/51	BG-20 (13, 35)

Bis-EMA: Ethoxylated bisphenol-A-dimethacrylate; TEGDMA: Triethylene glycol dimethacrylate; HEMA: 2-hydroxyethyl methacrylate; Bis-GMA: Bisphenol-A-glycidylmethacrylate.

imen thickness (2 mm for conventional and experimental composites; 4 mm for bulk-fill composites) and exposure time (following manufacturer recommendations for commercial composites; 20 s for experimental composites) were selected. The curing time for commercial composites, as recommended by respective manufacturers, is given in Table 1. The exposure time for experimental composite was chosen in accordance to previous studies [35,37], which indicate that 20 s results in an acceptable cure for 2-mm thick layers.

The cylindrical molds were filled with the composite paste, mold openings were covered with a polyethylene terephthalate (PET) film, and composites were light-cured through the upper opening using a light-emitting diode (LED) curing unit (Bluephase G2, Ivoclar-Vivadent, Schaan, Liechtenstein; wavelength range 380–515 nm, having a radiant exitance of 1185 mW/cm^2, as measured with integrating sphere, IS, Gigahertz-Optik GmbH, Puchheim, Germany and spectrometer HR4000, Ocean Optics, Dunedin, FL, USA). The distal end of

the curing unit light guide was centered over the mold opening, in contact with the PET film. Light-curing was performed at room temperature (22 ± 1 °C) and specimen handling was done in a dark environment. Immediately after light-curing, specimens were transferred to the sample compartment of the spectrometer. The operator was given 1 min to mount the specimen in the universal holder and make initial adjustments. Thus, the collection of Raman spectra was scheduled to start at 1 min post-cure.

2.3. *Raman spectrometry*

Raman spectra were collected using an FT-Raman spectrometer (Spectrum GX, PerkinElmer, Waltham, USA) from the bottom specimen surface in order to simulate DC measurements at the selected layer thicknesses (2 or 4 mm). At the center of the specimen, a circular spot of 0.4 mm in diameter was excited using a NdYaG laser (1064 nm). Raman spectra were recorded at a resolution of 4 cm^{-1}. Forty scans were used for the collection of each spectrum because a preliminary study showed that this number enabled an acceptable signal-to-noise ratio for the whole range of laser powers used (300–1000 mW). Because each scan lasted for 12 s, the collection of a single Raman spectrum required 8 min. Raman spectra of uncured composites (n = 5 per material) were collected using the same parameters. The following excitation laser power values were used (mW): 300, 400, 600, 800, and 1000. For each combination of material and power, 10 specimens were prepared, resulting in a total of 400 specimens (8 materials × 5 powers × 10 repetitions).

Raman spectra were processed using the Kinetics add-on for Matlab (Mathworks, Natick, Massachusetts, USA). The DC was calculated by comparing the relative change in peak heights of spectral bands at 1640 cm^{-1} (aliphatic C=C) and 1610 cm^{-1} (aromatic C=C) before and after light-curing, according to the following Equation [10]:

$$DC\,(\%) = \left(1 - \frac{(1640\,cm^{-1} / 1610\,cm^{-1})_{peak\ height\ after\ curing}}{(1640\,cm^{-1} / 1610\,cm^{-1})_{peak\ height\ before\ curing}}\right) x\,100 \quad (1)$$

2.4. *Statistical analysis*

The Shapiro–Wilk test was used to confirm that there are no significant departures from normality. Uniformity of variances among experimental groups (Levene's test) were considered to be acceptably homogeneous. To evaluate the effect of different laser excitation powers, two statistical approaches were performed.

First, the DC values that were calculated for all levels of factors *material* and *power* were compared using a two-way ANOVA with partial eta-squared statistics. This examination was followed by a one-way ANOVA and Pearson correlation analysis within individual materials in order to evaluate the effect of factor *power*. Tukey post-hoc adjustment was used for multiple comparisons. All statistical testing used a pre-set alpha of 0.05. This portion of statistical analysis was performed using software (SPSS 20, IBM, Armonk, NY, USA).

Table 3 – Results of the two-way ANOVA for degree of conversion values obtained among all laser powers and all materials (n = 400). Partial eta-squared values indicate the effect size and practical significance of each factor. Observed power level reflects the ability of statistical analysis to detect statistical significance for a given dataset.

Factor	p	Partial eta-squared	Observed power
Power	<0.001	0.154	~1.0
Material	<0.001	0.942	~1.0
Power*Material	0.012	0.125	0.99

Second, within an individual material, the vector-normalized Raman spectra that were collected using the lowest excitation power (300 mW) were compared to those collected using the maximum excitation power (1000 mW) by means of a t-test (pairwise comparison of independent groups of n = 10). This procedure was performed in order to investigate the Raman bands that were affected by change of laser excitation power. Vector normalization was performed by dividing Raman scattering intensity for each wavenumber by the spectrum "norm", which was calculated as the square root of the sum of the squared intensities of the spectrum. All testing used a pre-set alpha of 0.05. Spectra normalization and statistical testing were performed using software (Kinetics add-on package, version 1.0, for Matlab, version 7.5.0, Mathworks, Natick, MA, USA).

3. Results

3.1. *Degree of conversion as a function of laser power*

The results of the two-way ANOVA which was performed across all levels of factors *material* and *power* are shown in Table 3. Both factors exerted a highly significant influence on DC values. The factor *material* was considerably more influential compared to *power*, as indicated by partial eta-squared values. The high statistical powers identified for both factors ($\tilde{1}$.0) indicate that their effect on DC was sufficiently large to be detectable within the study design used. As a result of a significant interaction between the factors *material* and *power*, a one-way ANOVA for the factor *power* was performed within each material individually (Table 4). Statistical significance was identified in 4 out of 8 materials. For the remaining 4 materials, low statistical power was identified, suggesting that the effect size was too small to be detectable at the given sample size. Different partial eta-squared values were observed among the materials, indicating that the various materials tested showed different sensitivities to variation of laser power.

Mean DC values and the corresponding standard deviations as a function of laser power are shown in Fig. 1. Multiple comparisons following the one-way ANOVA revealed in which pairs of DC values the p-values were sufficiently small to be considered statistically significant. Pearson's correlation analysis showed a moderate but highly significant, positive correlation between the DC values and laser power in 5 out of 8 materials (Pearson's R = 0.44–0.55). Pearson's correlation analysis showed higher statistical power (yielding lower p-values)

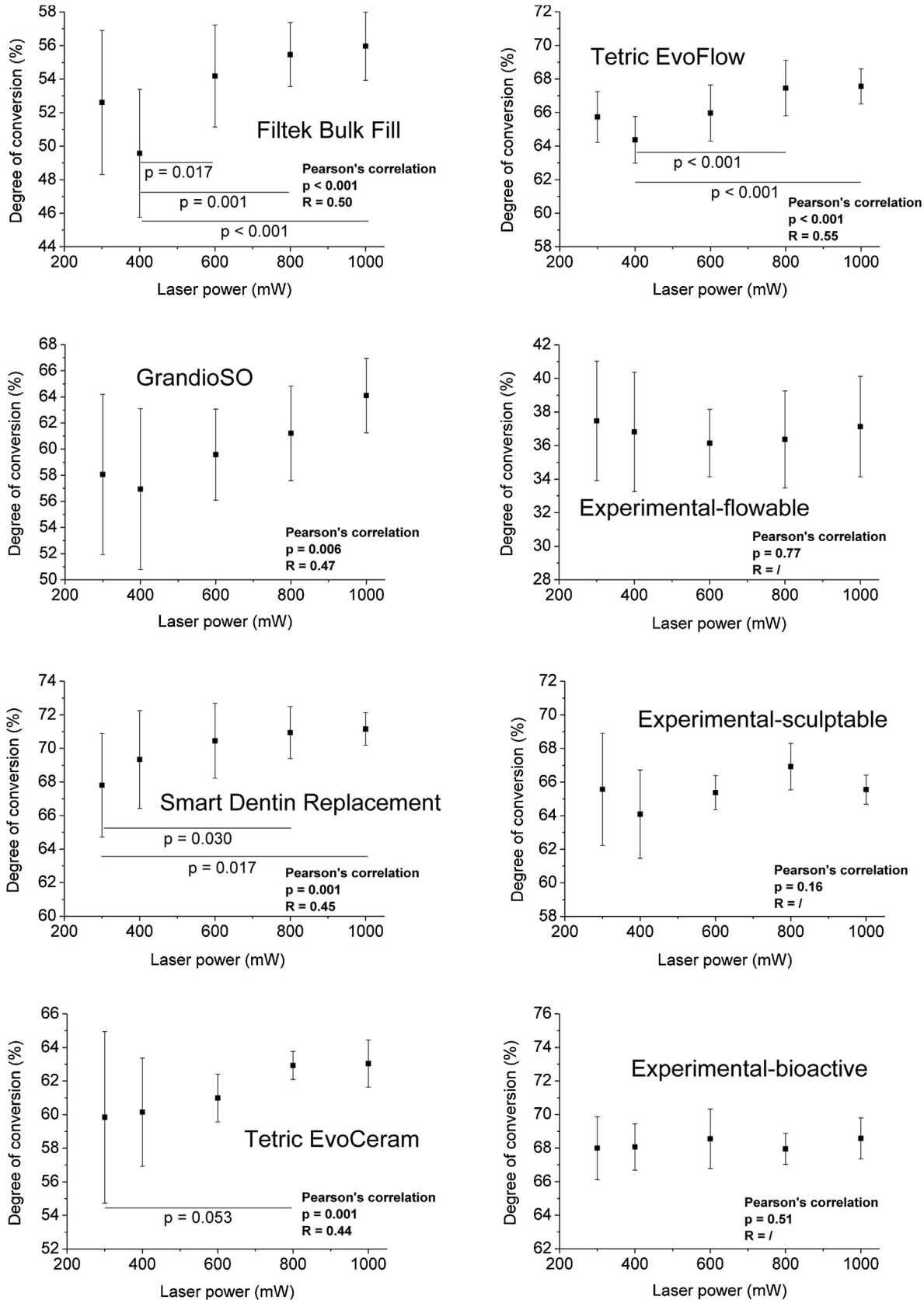

Fig. 1 – Mean values of the degree of conversion (standard deviations shown in error bars) as a function of laser power. Pairwise comparisons with p-values ≤ 0.05 are indicated. The scale at the y-axis is shifted according to different degree of conversion values among materials. For comparability, the full range of the y-axis is set to 14% for all materials, except for GrandioSO (18%). The results of Pearson's correlation analysis (p and R values) are shown.

Table 4 – Results of the one-way ANOVA for the degree of conversion values obtained among all laser powers within an individual material (n = 50 per material). Partial eta-squared values indicate the effect size and practical significance of each factor. Observed power reflects the ability of statistical analysis to detect statistical significance for a given dataset.

Material	p	Partial eta-squared	Observed power
Filtek bulk fill posterior	<0.001	0.371	0.98
GrandioSO	0.091	–	0.58
Smart dentin replacement	0.012	0.244	0.84
Tetric EvoCeram	0.018	0.229	0.80
Tetric EvoFlow	<0.001	0.419	0.99
Experimental-flowable	0.867	–	0.11
Experimental-sculptable	0.072	–	0.63
Experimental-bioactive	0.915	–	0.10

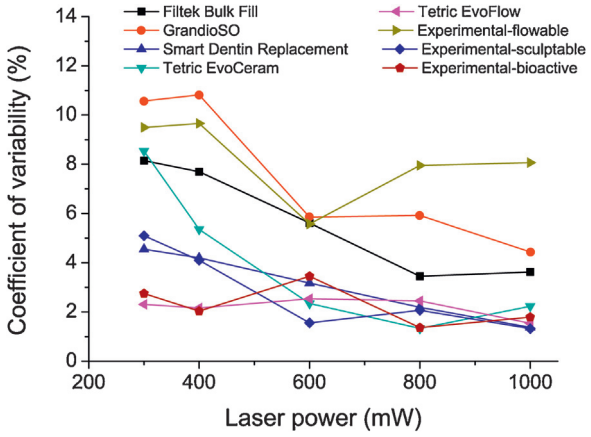

Fig. 2 – The coefficient of variability (relative standard deviation) of the degree of conversion data as a function of laser power.

than the one-way ANOVA and was able to detect the effect of laser power in GRA, in which the one-way ANOVA showed no significant effect (Table 4).

To describe the effect of the laser power on the quality of DC data, the coefficients of variability (CVs) as a function of laser power are plotted in Fig. 2. The CV (also known as relative standard deviation) was calculated as the ratio of the standard deviation and the corresponding mean value. CV values varied in the range of 1–11 % and generally decreased with the increase in laser power. However, for some materials (TEF, EXP-BG), practically no reduction in CV was identified with the increasing laser power.

3.2. *Comparison of Raman spectra recorded using 300 and 1000 mW*

Fig. 3 shows Raman spectra obtained using the lowest (300 mW) and the highest (1000 mW) laser power settings. Each of the presented spectra is an average of ten individual experimental runs. The vector-normalized average spectrum collected at 300 mW was subtracted from the vector-normalized average spectrum collected at 1000 mW and the result is shown as a blue line at the bottom of the plots. Statistically significant differences identified by t-tests are shown as red marks on that line. The statistically significant differences occurred most frequently at high wavenumbers (2900–3100 cm^{-1}), which corresponded to symmetric and asymmetric stretching of $-CH_2$. Of more practical relevance are the differences identified in spectral bands used for the DC calculation: in FBF and TEC at 1640 cm^{-1} (symmetric stretching of aliphatic C=C), and in SDR the band at 1458 cm^{-1} (scissoring of C–H). The enlarged part of Raman spectra highlighting the region between 1750–1500 cm^{-1} is shown in Fig. 4.

4. Discussion

Because varying laser power exerted a significant effect on the measured DC values in 5 out of 8 materials, the first research hypothesis was rejected.

The DC of resin composites is commonly discussed in terms of mobility of the growing polymer chain component, which is affected by numerous factors: resin chemistry (monomer structure and molecular weight [38], hydrogen bonds and other intermolecular interactions [39], the ratio of base and diluent monomers [40]), filler particle geometry, packing and total surface area [41], filler surface silanization [42], material optical properties [43], and light-curing conditions [44]. The present work did not attempt an in-depth analysis of these factors because compositional details of commercial materials remained unknown due to manufacturer's non-disclosure policies. The main idea of the present work was to include a variety of materials, apply clinically relevant curing conditions, and explore if the DC measurements at the bottom layer would be affected by varying the excitation laser power present at a fixed spot size. Although Raman spectrometry can theoretically be used for determining specimen temperature through comparison of Stokes and anti-Stokes band intensities [22], this procedure was not done because the FT-Raman spectrometer employed was capable of recording only Stokes scattering.

Because spectrometric analyses generally benefit from a higher signal intensity, the selection of excitation laser power in Raman spectrometry aims at finding the balance between the signal intensity and any possible damage to the specimen due to excessive heating. Besides crude damage caused by specimen decomposition due to burning [45,46], a more delicate laser-heating effect can be expected if the DC is measured within a short time period after light-curing. This is because the polymerization of resin composites is not completed within the period of light-curing but instead extends for approximately 24 h post-cure, due to mobility restrictions occurring in the reaction medium during the polymerization [47]. All investigated composites are known to demonstrate a significant 24 h post-cure polymerization after being light-cured under similar conditions [35,47,48] The extent of that post-cure polymerization can be influenced by small temperature changes; even the increase from 20 to 37 °C can result in post cure DC increase being 4–9 % higher in absolute terms [49]. Thus, by subjecting resin composite specimens to Raman spectrometry immediately after light-curing [8,10,17,27–30,33,50] or within a short time period after light-curing, i.e. 1 min [31] or 1 h [32], the ongoing polymerization

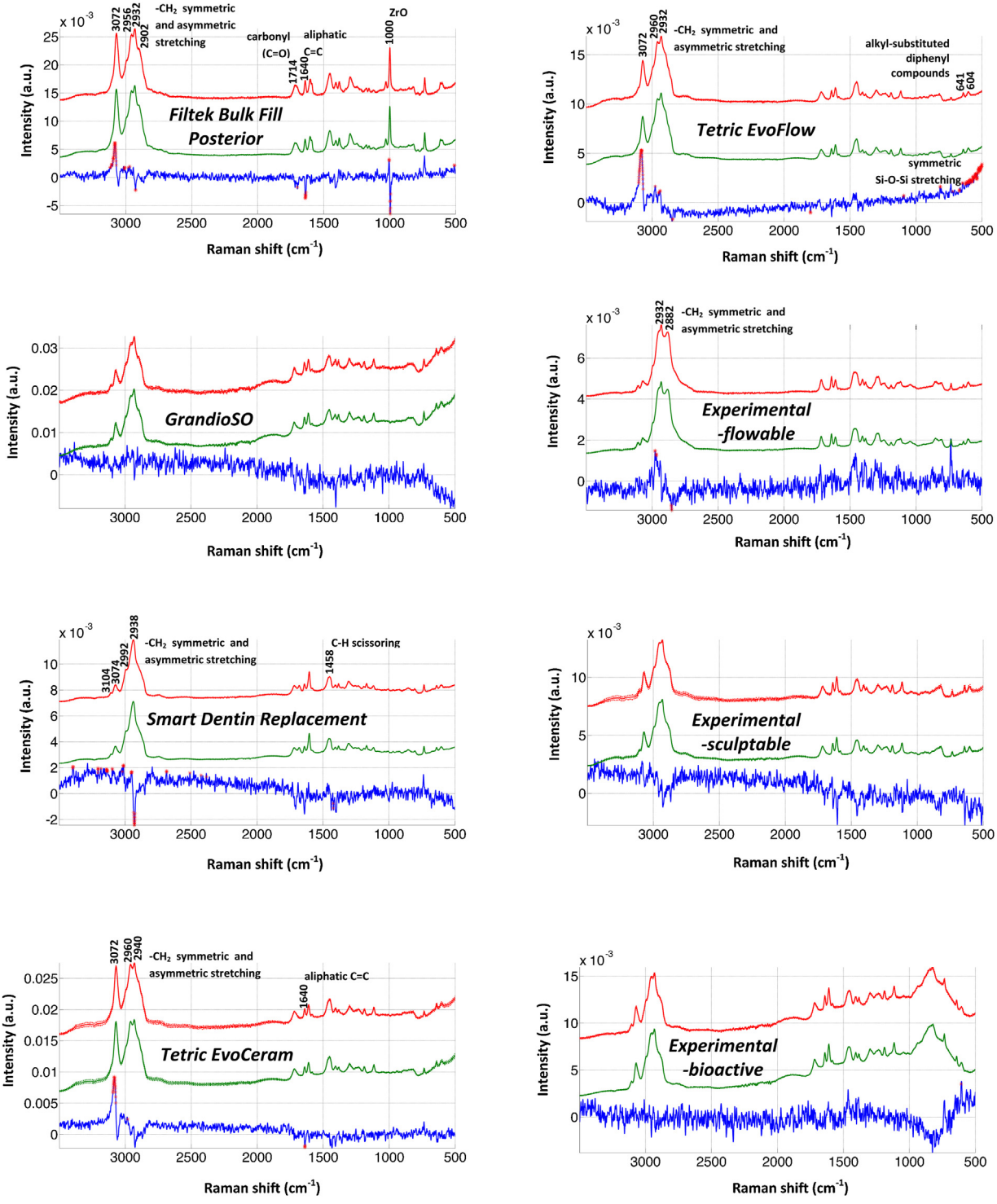

Fig. 3 – Averaged Raman spectra (n = 10) for the excitation power of 300 mW (red) and 1000 mW (green). Thin dotted lines denote one standard deviation (not distinguishable for some materials). The blue line at the bottom of the plot represents the difference between the red and the green spectra. Red marks on that line denote wavenumbers demonstrating statistically significant differences. The spectral bands whose intensities were significantly different between spectra collected at 300 mW and 1000 mW are identified and corresponding band assignments are shown. The vector-normalized spectra (red and green) are plotted on the common scale for comparability, while the difference spectrum (blue) is shown on a magnified scale. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

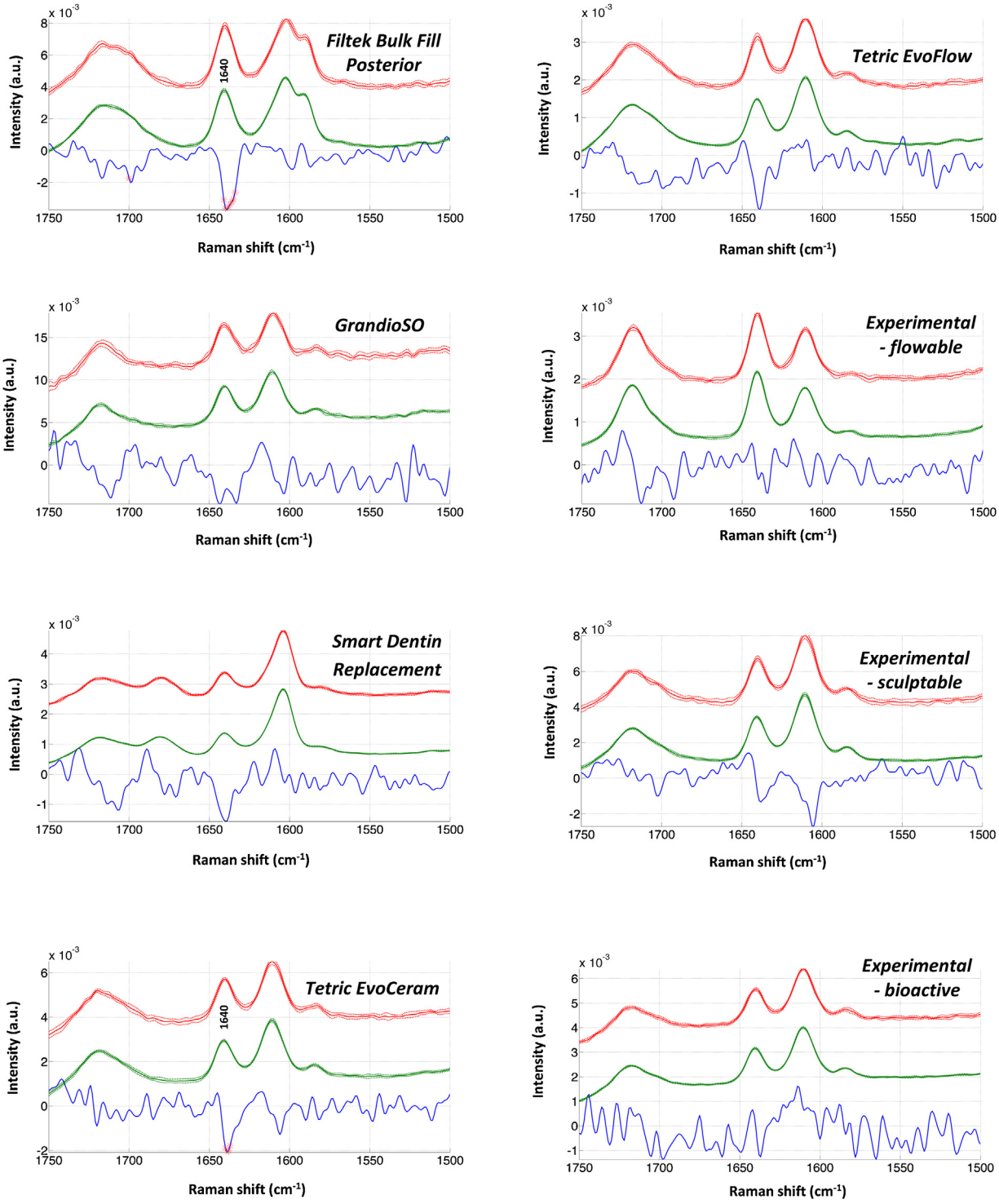

Fig. 4 – The enlarged portion of Raman spectra from Fig. 3. For the spectral region between 1750–1500 cm^{-1}, averaged Raman spectra (n = 10) for the excitation power of 300 mW (red) and 1000 mW (green) are presented. Thin dotted lines denote one standard deviation (not distinguishable for some materials). The blue line at the bottom of the plot represents the difference between the red and the green spectra. Red marks on that line denote wavenumbers demonstrating statistically significant differences. The vector-normalized spectra (red and green) are plotted on the common scale for comparability, while the difference spectrum (blue) is shown on a magnified scale. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

can be affected by the laser-induced specimen heating [51,52]. This effect was investigated in the present study by varying excitation laser power between 300 and 1000 mW. Because collection of Raman spectra for each specimen was initiated just 1 min post-cure and lasted for 8 min during testing, the measured DC values (Fig. 1) represent the time average of the slowly-increasing DC during 9 min post-cure.

It should be noted that the aforementioned effect of laser-induced heating on the DC values was not an inherent material property, but rather depended on the amount of unreacted monomer (function of curing conditions) [35] and the ability of the elevated temperature to enhance the post-cure polymerization (function of material composition) [51]. Thus, the effect size of laser-induced heating can be hypothesized to be affected by factors determining the radiant energy reaching the bottom of the composite layer at which the DC was assessed. These factors include curing light exposure duration, incremental layer thickness, and light transmittance of a particular material. In this regard, partial-eta squared values in Table 4 can be considered as a "relative sensitivity" to variation in applied laser power, for each material and conditions under which specimens were prepared. The highest sensitivity was observed in TEF, which is speculated to be due to the low filler loading and short curing light exposure duration. The low filler loading of this material enabled better resin mobility during the post-cure period [42], while the short curing light exposure duration contributed to more unreacted monomer [3]. On the other hand, EXP-FLO showed no influence of laser power on DC, despite having a filler loading comparable to that of TEF. This finding may be because more monomers in EXP-FLO have reacted due to the longer curing light exposure duration (20 s), resulting in less monomer available for post-cure polymerization and consequently the less pronounced effect of laser-induced heating. The same speculation can be applied to EXP-SCU and EXP-BG, both of which received a higher radiant energy than the commercial composites because they were exposed for 20 s in 2 mm thick layers. An additional factor contributing to the comparatively higher radiant energy at the bottom of the 2 mm thick layers in the experimental composites is their high light transmittance (6–12 % for EXP-FLO [53], 8–16 % for EXP-SCU and 6–11% for EXP-BG [35]), which can be attributed to the lack of blue-light-absorbing pigment particles [54]. In light of the aforementioned considerations, it is probable that the effect size of the laser-induced heating on the DC values for each material would differ if the exposure conditions were changed. A more pronounced effect would be expected if composite specimens were undercured, because a lower DC attained during the light-curing period usually leads to a more extensive post-cure DC increase [55].

In order to assess the magnitude of using different laser powers on observed DC values, differences between the lowest and the highest mean DC value within each material were calculated. For materials showing a statistically significant effect of laser power (in either one-way ANOVA or Pearson correlation analysis), these differences amounted to (% absolute increase) of 6.4 (FBF), 7.2 (GRA), 3.4 (SDR), 3.2 (TEC), and 3.2 (TEF). The listed values represent the range of experimental error that would be introduced to DC measurements purely by adjusting the laser power in the range of 300–1000 mW. This effect should be taken into account when using Raman spectrometry for DC measurements in non-static conditions, i.e. before the post cure polymerization has been finished. The range of laser powers investigated in the present study has practical relevance for dental research because laser power as high as 1000 mW has been used for the DC measurements by means of FT-Raman spectrometry [17,31].

The collection of each spectrum using the FT-Raman spectrometer required 8 min, which is considerably longer than the data acquisition time for dispersive Raman spectrometers. However, contemporary dispersive micro-Raman instruments usually employ much higher irradiance values. For example, the laser power of 20 mW at the excitation spot of approximately 1.5 μm [8,56] results in an irradiance 3 orders of magnitude higher than that in the present study. At this point, it is not possible to compare the amount of heat produced by the FT-Raman instrument employed in the present study to that produced by dispersive micro-Raman systems, due to differences in: (I) excitation laser wavelength (usually shorter in dispersive instruments), (II) power density (higher in dispersive instruments), and (III) data acquisition time (shorter in dispersive instruments). Because dispersive micro-Raman instruments with highly focused laser beams are frequently used for DC evaluation in resin composites [8,10,27,32,33,56], this factor remains a relevant question for further studies.

The maximum available laser power for the FT-Raman spectrometer used in this study was 1000 mW. The excitation power range of 300–1000 mW was selected because the signal-to-noise ratio deteriorated considerably for laser power below 300 mW, rendering the spectra unusable at the selected number of scans. Increasing laser power improved the signal-to-noise ratio, which was generally reflected as a lower CV of the DC data, as shown in Fig. 2. However, the relationship between laser power and CV differed considerably among materials, meaning that, for some materials, higher laser power can be successfully used to reduce the data variability, while in other products an increase in laser power had no such effect. For example, CV for TEF (around 2%) was at the low-end of calculated values and was independent of laser power, while in materials with higher initial DC values, the CV was reduced for a factor of 2 (GRA and FBF) or even for a factor of 4 (TEC). It appears that, for some composites, other important sources of data variability exist besides the signal-to-noise ratio in the Raman spectrum. However, this topic was outside the scope of the present study.

To assess a possible effect of specimen chemical changes due to laser-induced heating, the spectra collected at 300 mW and 1000 mW were compared (Fig. 3). Spectral bands having intensity values affected by laser power were identified and assigned in accordance with literature references [57,58]. At the higher laser power setting, neither new spectral bands emerged nor were the existing bands lost, indicating that no major compositional changes occurred. However, intensity differences of certain bands were identified (Fig. 3), which led to the rejection of the second research hypothesis. The magnitude of the observed differences was minor but sufficient to yield statistical significance. The differences were most frequent for the bands in the wavenumber region of 2900–3100 cm^{-1}, which are assigned to $-CH_2$ stretching. For FBF, laser power also affected the intensity of bands at 1640

and 1714 cm^{-1}, reflecting higher consumption of C=C bonds at the higher laser power and the corresponding changes due to the loss of conjugation with carbonyl (C=O) group [39]. It is interesting to note that the difference in the intensity of the aliphatic C=C band at 1640 cm^{-1} was identified only for FBF and TEC (Fig. 4), whereas the results of ANOVA and Pearson correlation analysis indicated that the laser-induced heating effect was also detectable for GRA, SDR, and TEF. This discrepancy occurred because a simple pairwise comparison of the spectra collected using laser power of 300 mW and 1000 mW had, as expected, lower statistical power than the ANOVA and Pearson correlation analysis which took into account the whole range of laser powers. An additional observation that may be relevant for DC evaluation of SDR is that a significantly lower intensity was identified at the minimum between the bands at 1458 and 1406 cm^{-1}. This finding may affect the baseline for the band at 1458 cm^{-1} (C—H scissoring), which is occasionally used as a reference in the DC evaluation of resin composites and adhesive systems which lack the phenyl ring [59]. On the other hand, the aromatic C=C band at 1610 cm^{-1}, which is regularly used as a reference in composites that contain the phenyl ring, remained unaffected by the increasing laser power and therefore its use as an internal standard remains justified. Raman scattering intensities of other spectral bands were also affected by laser power (e.g. the ZrO band at 1000 cm^{-1}), but those findings were not relevant for the DC evaluation.

Further studies should explore the effect of variations in excitation laser power for Raman spectrometers employing different excitation wavelengths and power densities.

5. Conclusion

Within the limitations imposed by the present investigation, the following conclusions may be reached:

1 Raman spectra collected using an FT-Raman spectrometer were affected by the level of excitation laser power, leading to statistically significant differences in the measured degree of conversion values.
2 The identified absolute degree of conversion differences were material-dependent and ranged from 3.2 to 7.2%, suggesting that the degree of conversion values can be artificially elevated through increasing excitation laser power. The effect of variations in laser power should be considered in Raman spectroscopic evaluations of the degree of conversion in specimens ongoing post-cure polymerization.

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