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Influence of heat from light curing units and dental composite polymerization on cells in vitro

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KEYWORDS
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Composite

Summary *Objectives*: The aim of the present study was to test the hypothesis that the use of a light curing unit (LCU) with high light power would result in a higher temperature and therefore a statistically significant lower number of living human gingiva fibroblasts within a pulp chamber model than the use of a light emitting diode (LED) LCU.
Materials and methods: The composites Admira, Grandio, Filtek Supreme and Filtek Z250 were polymerized with the LCUs Swiss Master Light, Optilux 501 and an LED LCU prototype in a mould on top of a pulp chamber model. The temperature was recorded within the pulp chamber with a thermocouple. The cytotoxicity of the polymerized samples was tested by using the MTT test.
Results: In general there was no considerable difference in the temperature increase within the pulp chamber model for the different LCUs and composites. There was no statistically significant difference in the cell number ($p=0.3767$) when the different LCUs were used.
Conclusions: Using a high power halogen LCU for a short time or a standard halogen or LED LCU for a longer time did not result in a considerable difference in the temperature increase or the number of living cells within a pulp chamber model. This study indicates not only that the temperature may have an effect on the living cells, but also that cells may be negatively affected by the unpolymerized composite or light of the LCUs.
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Introduction

Externally applied heat to a tooth may cause pulpal trauma of differing degrees depending on the magnitude and duration of the temperature.[1,2]

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Dental treatment often results in a temperature increase of the tooth surface and therefore the tooth pulp. The pulpal temperature increase may be caused by rotating instrument tooth preparation,[3-7] ultrasonic instrumentation,[8] laser treatment,[9-11] electrothermal debonding of ceramic brackets,[12-14] light-enhanced bleaching,[15] temporary crown and bridge materials[16-18] or the light curing of dental composites.[19,20]

The temperature increase within the pulp chamber during the light polymerization process was extensively discussed recently in cause of the introduction of plasma light curing units using a high light power.[21-23] It was concluded that high power LCUs may represent a potential hazard for the tooth depending on the light power and application time of the LCU. Since 1965, the controversially discussed threshold for a hazardous temperature increase within the pulp chamber is 5.5 and 11.1 °C which caused irreversible pulpitis in 15 and 60% of Macaca Rhesus monkey teeth.[1] A recent study on the temperature increase in teeth, however, states that a temperature increase of 11.2 °C does not damage the pulp.[24] However, another study on living dental pulp of rats[25] showed that when the temperature was kept at 46–50 °C for 30 s, stasis and thrombosis developed leading to a standstill of circulation.

Light cured dental composites are also discussed controversially in cause of their content of hazardous substances.[26-28] In particular, if composites are not cured thoroughly and do not reach a sufficient degree of monomer conversion, they can leach toxic and carcinogenic substances into the body of the patient.[26,29]

Ultraviolet (UV) light with wavelengths lower than 400 nm has negative effects on biological cells and is therefore considered to be an ocular[30] and dermatological hazard.[31] Because of this and for other reasons, around the 1980 s the wavelength of the light for the polymerization of dental composites was shifted from the short wave UV radiation to the longer waves in the visible (VIS) range (400–500 nm).[32] However, recently the negative influence of blue light on mouse fibroblasts has been published stating that blue light suppresses the cellular mitochondrial function.[33]

The primary aim of this study, therefore, was to test the hypothesis that the use of an LCU with high light power would result in a statistically significant lower number of living human gingival fibroblasts within a pulp chamber model than the use of an LED LCU. The second aim of this study was to investigate whether there is a statistically significant higher temperature increase within the pulp chamber model if a high power halogen LCU is used for 4 s compared to an standard halogen LCU or an LED LCU used for 40 s. In addition it was hypothesized that the cytotoxicity of the composite samples polymerized for 4 s with a high power halogen LCU is greater than the samples cured for 40 s with an LED LCU.

Materials and methods

Characterization of the LCUs

One light emitting diode (LED) LCU and two halogen LCUs were used (Table 1) with the recommended curing times by the manufacturer. The LED LCU was a custom-made LED LCU prototype built by the first author at the Friedrich-Schiller-University, Jena and was used for 40 s. One halogen LCU, the Optilux 501 (Kerr, Danbury, USA) was used for 40 s and a fast curing halogen LCU, the Swiss Master Light (E.M.S., Nyon, Switzerland) was used for 4 s.

The emission spectra were measured with a TIDAS diode array spectrometer (J&M, Aalen, Germany). The power was measured with a calibrated Molectron PM3 thermopile sensor and a 3Sigma power meter (Molectron, Inc., Portland, Oregon, USA). The power density of the LCUs was

Table 1 Technical details of the halogen and LED LCU used in this study.

	LED prototype	Optilux 501	Swiss Master Light
Manufacturer	First author	Kerr, Danbury, USA	E.M.S., Nyon, Switzerland
Light source	1 LED	1 Halogen bulb	1 Halogen bulb
Power of light emission (mW)	402[a]	442[a]	2812[b]
Application time (s)	40	40	4
Total energy (J)	16.1	17.7	11.3
Output face light guide tip ∅ (mm)	8.0	7.3	11.0
Area of light guide tip (cm^2)	0.50	0.42	0.95
Power density (mW/cm^2)[a]	800	1057	2960

[a] After 30 s switch-on time of the LCUs.
[b] After 3 s switch-on time of the LCU.

Table 2 Restorative materials used in this study.

	Filtek Z250	Filtek Supreme	Admira	Grandio
Manufacturer	3M Dental Products, Borken, Germany		VOCO, Cuxhaven, Germany	
LOT	6026A3.5	3910A3.5B	24829	321467
Resin type	UDMA, Bis-EMA	Bis-GMA, UDMA, TEGDMA, Bis-EMA	Ormocers/additive aliphatic and aromatic dimetha-crylates	Bis-GMA, UDMA, TEGDMA
Shade	A3.5	A3.5B	A3.5	A3.5
Filler particle size (μm)	0.01–3.5; average 0.6	0.02; 0.075	0.04; 0.7	0.04; 1–2
Recommended curing time (s)	20/40	20/40	40	20
Filler loading	68 wt%, 60 vol%	78.5 wt%, 57.7 vol%	78 wt%, 56 vol%	87 wt%, 72 vol%

determined by dividing the light output power by the area (Table 1) of the light exit window (LED) or light guide (Optilux 501, Swiss Master Light).

Composites

Four dental composites in shade A 3.5 (Table 2) were used in this study. The hybrid composite Filtek Z250 and nanofiller composite Filtek Supreme from 3M Dental Products, Borken, Germany. The organically modified ceramic (ORMOCER)[34] composite Admira and the nanofiller composite Grandio from VOCO, Cuxhaven, Germany. All four composites contain camphorquinone (CQ) as the major photoinitiator.

Cell culture

Extracted human gingival fibroblasts were maintained in DMEM growth medium (Dulbecco's Modified Eagle's Medium, Gibco, Eggenstein, Germany) supplemented with 10% FCS (fetal calf serum—PAA Laboratories, Cölbe, Germany) and 0.5% AAS (antibiotic antimycotic solution, SIGMA-Aldrich, Spruce, USA) in a humidified atmosphere at 37 °C, 5% CO_2 (carbon dioxide). For all experiments cells within passages five to eight were used.

Pulp chamber model

A three-part polytetrafluoroethylene (PTFE) in vitro pulp chamber model was build simulating the in vivo situation: tooth cavity, dentin barrier and pulp chamber (Fig. 1).[35-37] Dentin disks (500 μm thick) were cut from caries free human third molars 3-4 mm above the enamel-cementum border. The patients were in the age range from 16 to 30 years. The teeth were collected over 3 months and stored in distilled water. The disks were autoclaved and inserted into the pulp chamber model to separate the pulp chamber model into two compartments. The dentin disks remained untreated concerning, e.g. etching or the application of a bonding agent. The upper compartment (Fig. 1) simulates the tooth cavity (∅ 4 mm, 2 mm deep) and the lower compartment represents the pulp chamber containing 100 μl cell growth medium (DMEM). At the lower side of the dentin disk a polycarbonate membrane (Millipore, Billerica, USA) was in direct contact with the dentin disk (Fig. 1). Within the pulp chamber a thermocouple (type K) was implemented for monitoring the temperature (PICO TC08 data logger, PICO Technology Limited, Cambridge, UK).

Procedure cell cultures within pulp chamber model

The pulp chamber model was autoclaved, dentin disk and the polycarbonate membrane were disinfected in 70% ethanol for 20 min. Afterwards, the

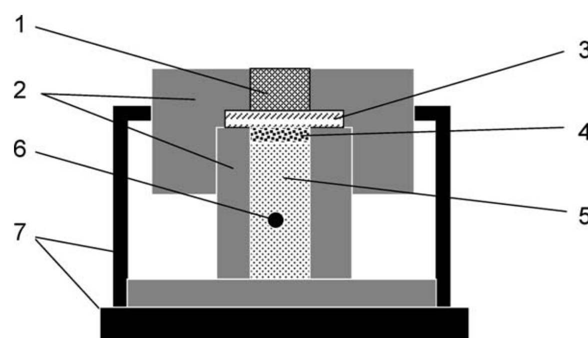

Figure 1 The composite (1) in the PTFE (2) pulp chamber model. The polycarbonate membrane (4) was in direct contact with the dentin disk (3). The human fibroblasts (4) on the polycarbonate membrane were facing the pulp chamber. Within the cell medium (5) the temperature was recorded with a thermocouple (6). The temperature was kept constant (37 °C) with a heating plate and insulation (7) around the pulp chamber model.

pulp chamber model was incubated for 60 min in cell growth medium. Subsequently, within sterile conditions, about 15,000 cells were seeded on the polycarbonate membrane (equivalent to 1200 cells/mm^2) and incubated for 24 h, 37 °C, 5% CO_2.

After removing the pulp chamber model from the incubator, the model was placed on the insulated heating plate (Fig. 1) to ensure a constant temperature of 37 °C and the thermocouple was connected to the data logger. The composite was inserted into the upper compartment of the model and covered by a Mylar strip (Moyco, Union Broach, York, USA). Finally, the light guide of the LCU was placed on the Mylar strip and the composite was irradiated for 4 s (Swiss Master Light) or 40 s (Optilux 501, LED prototype). Fourteen samples were prepared per composite and LCU, in total 168 samples.

After 5 min the polycarbonate membrane was carefully removed from the model and placed into in 24-well plates with 500 μl DMEM and 50 μl of MTT solution (Roche Diagnostics, Mannheim, Germany) per well. The membranes were incubated for 3 h, 37 °C, 5% CO. After the removal from the growth medium, the membranes were washed with PBS (phosphate buffered saline, Gibco, Eggenstein, Germany) and dried for 30 min at 37 °C. The blue formazan precipitate was extracted from the mitochondria using isopropanol, transferred to a 96-well plate and the absorption at 570 nm (OD570) was determined spectrophotometrically. The mean OD570 of control cells, which underwent the same treatment, except the insertion and polymerization of the composite, was set to represent 100%.

Cytotoxicity testing

After the polymerization, four samples of each composite and LCU were removed from the mould and inserted into a 24-well plate. Each well was filled with one sample and covered with 500 μl DMEM (10% FCS, 0.5% AAS). The extract solution was removed after 24 h. In a 96-well plate, 20,000 gingival fibroblasts were inserted per well and covered with 100 μl of the extract solution for 24 h. Nine wells were studied per sample, composite and LCU (432 measurements) and eight wells were studied without applying the extract solution, representing 100%. The cell viability was evaluated with the same procedure (MTT test) as described in the procedure for the pulp chamber model.

Statistics

For the statistical analysis, multifactor ANOVAs with the factors LCU and composite were applied to the maximum temperature, cell viability after removing the cells from the pulp chamber and cell viability for evaluating the cytotoxicity of the composites.

A Fisher's LSD test was used for the three tests to discriminate between the means and to determine homogenous groups of the temperature, cell viability after removing the cells from the pulp chamber and cell viability after the evaluation of the cytotoxicity. All statistical analyses were performed with the software Statgraphics Plus (Version 5.0) using a confidence interval of 95%.

Results

Light output characteristics of the LCUs

Fig. 2 shows the spectra of the used LCUs in this study. The integrated area of the curves corresponds to the measured light power (Table 1) of the LCUs. The Swiss Master Light shows the widest spectrum and more than six times the light power compared to the Optilux 501 or the LED prototype. All three LCUs cover the absorption or also called extinction spectrum of the photoinitiator camphorquinone.

Temperature increase within the pulp chamber model

Fig. 3a-d shows the temperature increase within the pulp chamber model measured with a thermocouple (Fig. 1). The multifactor ANOVA showed that the factors LCU ($p=0.0000$) and composite

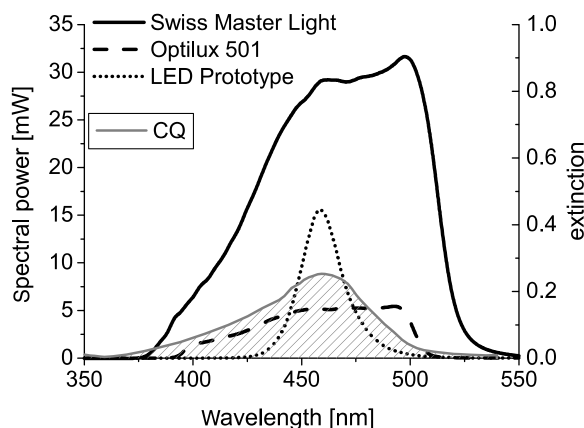

Figure 2 The spectra of the three LCUs used in the present study and the absorption/extinction spectrum of CQ. Note the broad light spectrum of the Swiss Master Light representing 2812 mW and the narrow emission spectrum of the LED LCU representing 402 mW.

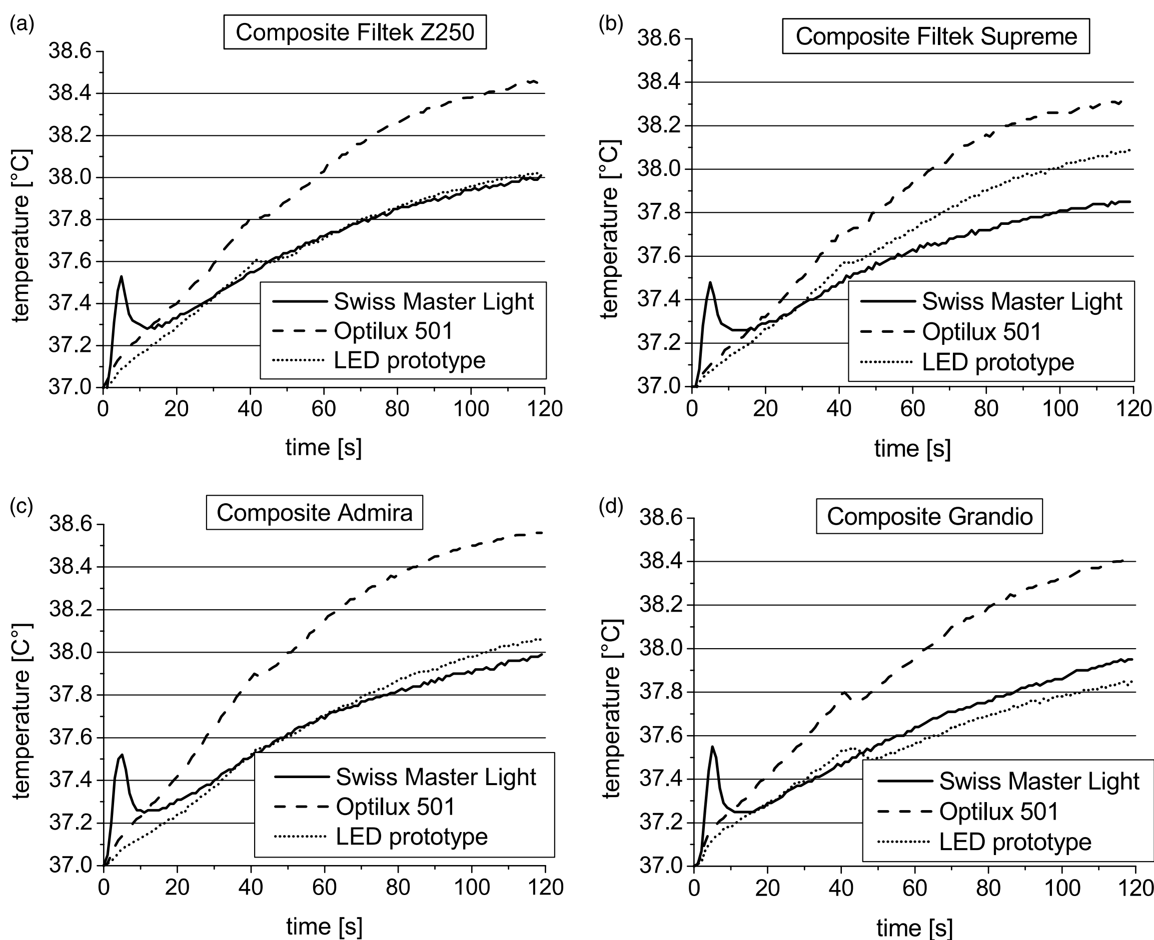

Figure 3 (a-d) The temperature development within the pulp chamber model for the different composites which were polymerized with the various LCUs. All graphs show for the Optilux 501 and LED prototype similar temperature development behaviour where the Optilux 501 causes a greater increase in temperature than the LED prototype. The Swiss Master Light causes at first a steep increase in temperature (~0.5 °C) which drops immediately after switching off the unit after 4 s.

($p=0.0322$) had a statistically significant influence on the temperature increase. Due to its higher total energy input (Table 1), the LCU Optilux 501 caused a statistically significant greater temperature increase (measured at 120 s) than the LED prototype and the Swiss Master Light.

Cell culture within pulp chamber model

Fig. 4 shows the percentage of surviving cells after the polymerization process. The graph shows the small effect of the different LCUs and the greater effect of the composite on the number of living cells. The multifactor ANOVA showed that there is no statistically significant difference in the number of surviving cells if different LCUs were used ($p=0.3767$). However, the same statistic showed that the composites ($p=0.0000$) had a statistically significant influence on the surviving cell number. The percentage of surviving cells using the composite Supreme (83%) was statistically significant lower than for all the other composites used.

Cytotoxicity testing

Fig. 5 shows the percentage of surviving cells after the cytotoxity test using the polymerized samples. The graph shows the small effect of the different LCUs and composites on the number of living cells. The multifactor ANOVA showed that there is no statistically significant difference in the number of surviving cells if different LCUs ($p=0.1730$) or composites ($p=0.1087$) are used.

Discussion

With the introduction of LED LCUs, it was claimed that the emission of blue LED LCUs is the ideal spectra

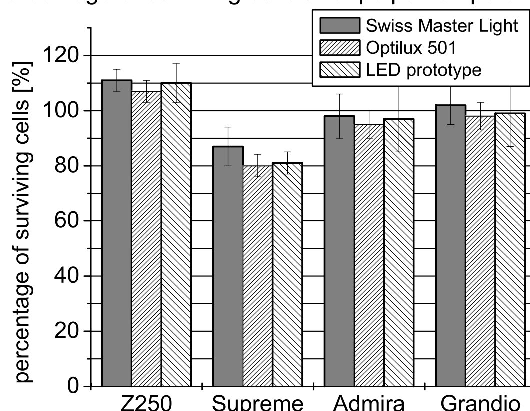

Figure 4 The number of the surviving cells within the pulp chamber model after the polymerization of the composites (Z250, Supreme, Admira, Grandio) with the Swiss Master Light, Optilux 501 and LED prototype. Note the lower percentage of the surviving cells when the composite Filtek Supreme was used.

for composites containing camphorquinone.[38] Other studies showed that when the equal light energy was irradiated the degree of monomer conversion of the composite was not statistically significant if an LED, plasma or halogen LCU was used.[39] If a composite shows a low degree of monomer conversion toxic substances may leach into the environment.[26,40] Therefore, in the present study, the toxicity test of the composite samples was used for measuring the effect of toxic substances on cells leaching from the polymerized composite into the solution. Comparing the toxicities of one composite polymerized with the different LCUs gives an indication for the curing potential of the different curing lights (halogen versus LED) and

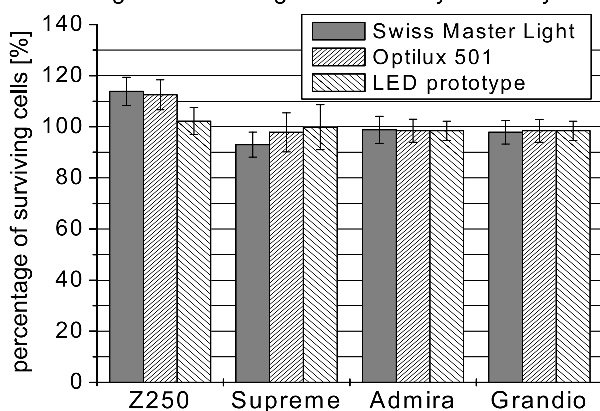

Figure 5 The number of surviving cells after exposure to the composite solutions from the composites Filtek Z250, Filtek Supreme, Admira and Grandio when testing for leaching of cytotoxic substances from the composites into the solution.

technologies (standard cure versus fast cure). The recommended curing times of the LCU manufacturers were used instead of equalling the total energy for all LCUs by adapting the curing times.

Several studies have shown that some composites contain besides camphorquinone (CQ) co-photoinitiators which cannot be activated by standard LED LCUs.[41] As a result these composites do not reach its maximum degree of monomer conversion and may leach toxic substances into the environment. In addition the polymerization process of a composite using co-photoinitiators is slowed down if irradiated by an LED instead of an halogen LCU.[42] A slower polymerization process means a delayed liberated energy from the composite and therefore a slower temperature increase within the restoration.[20] Therefore, in the present study, composites containing only camphorquinone as the photoinitiator were used.

Another influence which needs to be considered are stress proteins. Cells exposed to hyperthermia (elevated temperature) respond with an increased synthesis of proteins, also called heat shock proteins.[43] The presence of stress proteins has been shown to confer resistance to further stress. Therefore, heat shock proteins might have an impact on temperature studies using cell cultures in a natural or artificial pulp chamber. However, pre-tests to the present study showed a difference in maximum temperature less than 1 °C for the different LCUs and composites. Therefore, the influence of heat shock proteins was not observed in the present study.

The direct cell reaction to applied stress is important to consider but also the behaviour of

the cells after the applied stress because this might have a significant influence on the study results. Using the MTT test the mitochondrial activity is determined. Tetrazolium salt MTT is added to the cells and after 3–4 h of incubation formazan crystals develop in living and early apoptotic cells. Dead cells do not produce formazan crystals. However, the time after the stress and adding the tetrazolium salt MTT can have an influence on the final results. Cells might recover from the stress or stop the mitochondrial activity at all. In the present study the tetrazolium salt MTT was added directly after the removal from the dentin disk.

LCUs are commonly characterized by their power density (mW/cm^2) without defining the active light guide diameter or surface. In this study the diameters varied from 7.3 mm (Optilux 501) to 11.0 mm (Swiss Master Light). This difference appears to be small if the diameters are considered. Calculating the surface of the light guides (Table 1) shows that the Optilux 501 covers 0.42 cm^2 and the Swiss Master Light 0.95 cm^2.Therefore, the Swiss Master Light covers more than twice the area of the Optilux 501. Assuming that these two units were set to the same power density (e.g. 1000 mW/cm^2), the unit with the 11.0 mm light guide would irradiate the sample with 2.3 times the energy in comparisons to the unit with the 7.3 mm light guide. Considering that the light power which is put into a restoration correlates directly to the heat increase, scientific studies and manufacturers information should contain the power, power density and active light guide diameter to ease the comparison of different LCUs.

Discussing the temperatures reached within the pulp chamber (Fig. 3a-d), the different gradients of the temperature for the different LCUs are apparent. The characteristics of the curves are very similar for the LED prototype and the halogen unit Optilux 501, whereas the Optilux 501 causes a statistically greater temperature increase than the LED prototype due to its higher total energy (Table 1). Both LCUs cause a continuous temperature increase of the cell medium within the chamber. It is interesting to note that this warming up continued after 40 s where the LCUs were switched off. This shows that the system needs time to reaches its temperature equilibrium. The dentin disk used in the current study had a thickness of 500 μm. Dentin is known as a material having a low thermal conductivity[44] and shows therefore the characteristics of an insulating material. The thickness of 500 μm represented in the present study a deep restoration, simulating a worst case scenario. Using a thicker disk would give a better protection to the cells from the heat and light which would result in all probability in a less significant reaction of the cells to the applied stress.

The pulp chamber used in the present study is build out of PTFE in cause of its lower but similar thermal conductivity than dentin and its autoclavability. The thermal conductivity of the used PTFE is 0.25 W/m K[45] and of dentin about 0.57 W/m K[44].Therefore, both materials show very low values of thermal conductivity, which means that the heat from the composite is conducted slowly to the cells. Fig. 3a-d confirms this by the steady temperature increase within the pulp chamber after switching off the polymerization lights. A similar characteristics could be expected using a natural tooth. However, the pulp of a natural tooth would return to its initial temperature faster due to the cooling by the ambient air and the blood circulation.

The Swiss Master Light causes a steep increase in temperature (Fig. 3a-d) of about 0.5 °C within 4 s. After switching off the LCU, the temperature drops dramatically and rises again slowly due to the transfer from the heat of the mould and composite to the cell medium. The steep increase in temperature measured by the thermocouple does not only represent the warming up of the cell medium, which is proven by the steep decrease after switching off the unit. The dark thermocouple absorbs the light passing through the mould more than the translucent cell medium. Therefore, the thermocouple warms up faster than its environment.

Calculating the total energy input from the LCUs (Table 1) into the mould (light power $\times$ curing time $=$ total energy) results in the highest total energy for the Optilux 501.To compare the same total energies for the Optilux 501 and the SML, the SML needs to be used for 6.3 s. If a linear fit is applied for the first 4 s using the SML (Fig. 3a-d) a projection can be made for the SML. Inserting the 6.3 s into the linear fit, the results are for Admira (37.8 $\pm$ 0.1 °C), Grandio (37.6 $\pm$ 0.7 °C), Supreme (37.8 $\pm$ 0.4 °C) and Z250 (37.7 $\pm$ 0.2 °C). This means the temperature would increase in average 0.7 °C within 6.3 s which is well within the range of the temperature increase caused by the Optilux 501 (Fig. 3a-d).

It was hypothesized that the highest temperature would result in the lowest rate of surviving cells. As shown in Fig. 4 and by the multifactor ANOVA, there was no statistically significant difference in the number of surviving cells if the different LCUs were used. However, the number of surviving cells was statistically significantly reduced if the composite Filtek Supreme was used. There are several possible reasons for

this: the light transmission characteristics or thermal conduction of the composite; the released energy and maximum temperature during the polymerization of the composite; the amount and type of released toxic substances from the unpolymerized composite.

A different light transmission of the composite in comparison to the other composites could cause a greater light power density or a different light spectrum on the cells. The thermal conduction is strongly dependent on the composition of the composite but this effects should be negligible in comparison to the low thermal conductivity of the dentin disc. The released energy and reached, maximum polymerization temperature depends on the curing mode and the polymerization characteristics of the composite.[20] The leaching of toxic substances from unpolymerized dental composites is a well-known fact. However, the current set-up used a 0.5 mm dentin barrier between the composite and the cells which means that the toxic substances would need to diffuse through the barrier within a few minutes. All these assumptions definitely require further investigation.

After testing the cytotoxicity of the polymerized samples, no statistically significant differences could be found in the number of surviving cells for the different LCUs or composites (Fig. 5). This finding indicates that a sufficient degree of monomer conversion was reached within the composites with the different LCUs. Concerning the results from the surviving cells after the temperature increase and the results from the cytotoxicity test, the composite Filtek Supreme shows only a reduction of the cells if the composite is unpolymerized.

Conclusions

This study showed that LED, standard halogen and high-power halogen LCUs caused a temperature increase within a pulp chamber model but did not have an negative influence on human gingival fibroblasts within the cell medium.

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