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ORIGINAL ARTICLE

Carboxylated camphorquinone as visible-light photoinitiator for biomedical application: Synthesis, characterization, and application

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Carboxylated-camphorquinone;
Cytocompatibility

Abstract Camphorquinone (CQ) is by far the most widely used visible-light photoinitiator in biomedical applications. Despite the fact that CQ is well established and has a good clinical acceptance, photoinitiating systems based on CQ have some drawbacks in particular poor water solubility and low photoreactivity. Here the synthesis and testing of a new camphorquinone derivative are described. Carboxylated-camphorquinone (CQCOOH) was synthesized using ketopinic acid as the starting material. The chemical structure of CQCOOH was verified by ^{1}H-NMR, ^{1}H–^{13}C-COSY-NMR, FT-IR, mass spectroscopy, and UV–visible spectrophotometry. CQCOOH was used with an amine coinitiator and diphenyliodonium chloride (DPIC) accelerator as a visible light induced photoinitiating system for the preparation of hydrogels. CQCOOH exhibits a significantly higher photoreactivity compared to CQ with the same amine-DPIC system. Already after 5 s exposure time, the polymer crosslinked with the CQCOOH-amine-DPIC system to form a hydrogel. Furthermore, higher crosslink density and better mechanical properties are observed compared to those hydrogels prepared with the CQ-amine-DPIC system. Experiments measuring the activity or integrity of cells (MTT and LDH assay, respectively) were performed to investigate the compatibility of the photoinitiating system components with human gingival fibroblast (HGF). According to

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these experiments neither CQCOOH, L-arginine nor N-phenylglycine as amine coinitiators have toxic effects even in high concentrations. In contrast, HGF cell viability is considerably decreased at DPIC-concentrations higher than 0.25%.

1. Introduction

In recent years, photopolymerization has emerged as a valuable tool in biomedical applications because of its ability to rapidly convert a liquid monomer or macromer into 3D polymer networks under physiological conditions with temporal and spatial control. In this way biodegradable polymer hydrogels were prepared which can be used as drug delivery systems (Bertz et al., 2013; Lu and Anseth, 1999; Scott and Peppas, 1999; Wöhl-Bruhn et al., 2012a). However, for applications for example in dental surgery with direct preparation of hydrogels in situ the photopolymerization has to be carried out with visible light lamps as commonly used by dentists (Kamoun and Menzel, 2010). Photopolymerization uses light to dissociate initiator molecules into free radicals, which react with double bonds in the monomers or pre-polymers and thus crosslinking can occur. Camphorquinone (1,7,7-trimethylbicyclo[2.2.1]heptane-2,3-dione, CQ) belongs to the aliphatic α-diketones (Fig. 1) and is utilized as a photoinitiator for visible-light photocrosslinking. It is most frequently applied among the commercial photoinitiators (Alvim et al., 2007). The efficiency of this photoinitiator alone is insufficient. However, addition of e.g. tertiary amines as an electron/proton donor or as a reducing agent, gives an effective photoinitiating system (Cook, 1992; Jakubiak et al., 2003; Kamoun and Menzel, 2010). Such combinations are widely used for the crosslinking of methacrylate-based dental restorative polymers. CQ absorbs light in the UV-region at 200–300 nm and in the visible light region at 467 nm (responsible for its yellow color) (Cook, 1992; Kamoun and Menzel, 2010). Actually many publications have been devoted to the investigation of the mechanism of CQ-amine photoinitiating system. Absorption of light by CQ typically leads to the formation of two excited states: (i) the "singlet state", which does not involve reversal of electron spin, and (ii) the "triplet state" which is the one relevant to free radical formation and which has a very short half-life (Tsai and Charney, 1969).

While in the triplet state, the CQ molecule may interact with an amine molecule to generate an excited state complex, the "exciplex" (Fig. 2) (Cook, 1992; Kamoun and Menzel, 2010). In the exciplex the CQ abstracts a hydrogen atom from the tertiary amine resulting in the formation of free radicals (Stansbury, 2000). The hydrogen abstraction by triplet $^3CQ^*$ from amines, proceeds much faster compared to the reaction with the pure monomer. This is since the amines have a much lower oxidation potential compared with other hydrogen donors (Cook and Chen, 2011). Therefore, it is assumed that the CQ-amine system reaction is facilitated by the electron–proton transfer (Cook, 1992; Kamoun and Menzel, 2010; Pyszka et al., 2004).

Addition of diphenyliodonium chloride DPIC (Kamoun and Menzel, 2010, 2012; Kim and Scranton, 2004; Wang et al., 2006), or diphenyliodonium hexafloro-phosphate (DPIHP) (Guo et al., 2008) further accelerates photopolymerization, because they regenerate the dye, and produce additional active radicals (Fig. 2).

There are some disadvantages associated with the use of CQ as a visible-light photoinitiator (Yoshida and Greener, 1994; Jakubiak et al., 2001). A yellow shade of the photoinitiator (Park et al., 1999) results in a more intense color of unpolymerized filling composite and complicates the production of high-esthetic tooth colored restorations. Furthermore, an inner shielding effect (Jakubiak et al., 2001) of the colored CQ may result in unreacted photoinitiator (Janda et al., 2004). A significant lower photoreactivity of CQ was observed at higher concentrations (Schneider et al., 2008). To address these disadvantages of CQ, alternative photoinitiators such as phenyl propane-dione (PPD) or acrylphosphine dioxides (APO), which absorb light at lower wavelengths than CQ have been studied (Schneider et al., 2008; Schroeder et al., 2008; Sun and Chae, 2000). PPD showed a lower rate of polymerization without affecting the final degree of conversion compared to CQ (Ogunyinka et al., 2007).

The poor solubility of CQ in water restricts its utilization for crosslinking polymers for hydrogel formation. Thus, it is mandatory to use polar solvents e.g. DMSO or DMF mixed with water dissolve the CQ (Kamoun and Menzel, 2010). Magoshi and Matsuda (2002), Matsuda and Magoshi (2002), Nakayama et al. (2001) and Okino et al. (2002) have reported for the first time the water soluble carboxylated camphorquinone (CQCOOH) as a visible light photoinitiator, which might be

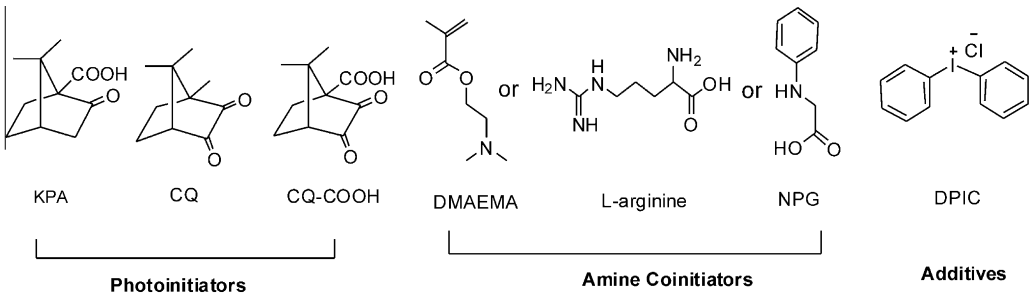

Figure 1 Chemical structures of the three-component radical photoinitiator system, photoinitiators, amine coinitiators, and iodonium salt accelerator, respectively.

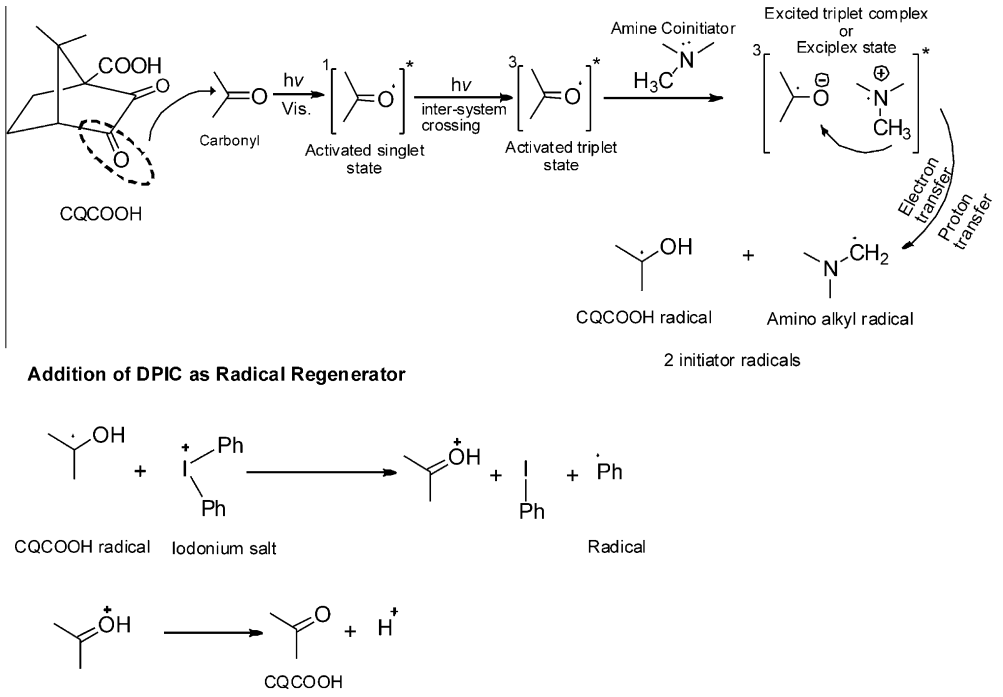

Figure 2 Reaction mechanism of three-component photoinitiator system containing photoinitiator (CQCOOH), an electron donor (amine coinitiator), and an electron acceptor or accelerator (iodonium salt).

an interesting alternative to CQ. However, no studies or further details regarding the synthesis, characterization and properties of CQCOOH as visible-light photoinitiator have been published so far.

In this contribution, we report on the synthesis and chemical characterization of water soluble carboxylated camphorquinone. Its photoreactivity and the properties as a photoinitiator used to prepare hydrogels were investigated in detail. Furthermore, the biocompatibility of all components of a photoinitiating system containing CQCOOH has been evaluated.

2. Experimental part

2.1. Materials

Dimethyl sulfoxide (DMSO, 99.7% purity) and 2-(dimethylamino)ethyl methacrylate (DMAEMA) were obtained from Acros Chemica, Geel, Belgium. N-phenylglycine (NPG), diphenyliodonium chloride (DPIC), (1S)-(+) ketopinic acid, (KPA, 99%), dimethyl sulfoxide, DMSO-d_6 grade (99.9%, atom %D), L-arginine (>98.5%, FCC), selenium dioxide (SeO_2, 99.999%), and glacial acetic acid (99.9%) were purchased from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). DL-camphorquinone (1,7,7-trimethylbicyclo[2.2.1]-heptane-2,3-dione) was purchased from Acros-Organics (New Jersey, USA). D_2O was obtained from Aldrich Chemica, USA. Tetrahydrofuran (THF) was distilled directly before use. The solution of photoinitiator and coinitiator was prepared in the dark and kept there until use on the same day. Shaking and sonicating were required to yield well mixed solutions.

A light-emitting diode lamp (LED-lamp) (Type: Bluephase, Ivoclar Vivadent Clinical, Lichtenstein) was used for irradiation with visible light and initiation of the polymerization mixture at a wavelength of 430–490 nm and a light intensity of 1000 mW/cm^2. The distance between irradiation source and sample was almost 0 cm. The irradiation time was 40 s.

2.2. Synthesis of carboxylated camphorquinone (CQCOOH)

7,7-Dimethyl-2,3-dioxobicyclo[2.2.1] heptane-1-carboxylic acid, or carboxylated camphor-quinone (CQCOOH) has been synthesized according to a modified procedure which was first published by Pande et al. (1991) and Ikemura et al. (2010). Starting material is commercial grade ketopinic acid (KPA), which is oxidized by SeO_2 in glacial acetic acid as reaction solvent. KPA (4.0 g, 22 mmol) and selenium dioxide (3.4 g, 31 mmol) were refluxed gently in acetic acid (50 mL) for at least 24 h. The suspension was filtered and the filtrate was concentrated under vacuum in a rotary evaporator. The obtained oily residues were dissolved in ethyl acetate (50 mL) and washed with distilled water ten times to remove unreacted selenium dioxide and unconverted ketopinic acid. The organic phase was dried with Na_2SO_4 and concentrated again under reduced pressure to remove ethyl acetate using a rotary evaporator. The crude oily product was re-crystallized from a mixture of ethyl acetate–hexane (1:10). The oil that separated crystallized upon cooling and 2.8 g, (i.e. a yield of 64% of the CQCOOH crystals) was isolated. For further purification column-chromatography on silica gel with ethyl acetate was performed. The product was isolated by crystallization from an ethyl acetate–hexane mixture (yield 1.75 g, i.e. 44%, Mp. 234–237 °C). The homogeneity and purity of carboxylated camphorquinone were checked by TLC in ethyl acetate–hexane mixture solution (R_f = 0.62).

^{1}H-NMR: (DMSOd$_6$, 2.5 ppm): δ/ppm: 1.15 (s, 3H, H7a), 1.18 (s, 3H, H7b), 1.60–1.67 (m, 1H, H5a), 1.89–1.97 (m, 1H, H6a), 2.19–2.28 (m, 1H, H5b), 2.48–2.55 (m, 1H, H6b), 2.79 (d, 1H, J = 5.12 Hz, H4), 12.87 (s, broad, 1H, H8, COOH).
^{13}C-NMR: (DMSOd$_6$): δ/ppm: 17.88 (C7b), 20.58 (C5), 21.99 (C7a), 26.15 (C6), 43.6 (C1), 57.44 (C4), 67.40 (C7), 169.0 (COOH), 197.24 (C2), 201.05 (C1) (Fig. 4).
FT-IR: IR (KBr)/cm^{-1}: 1780, 1760 (CO, s), 1697 (COOH, s), 1385, 1370 (CH$_3$, gem-dimethyl).
MS: $C_{10}H_{12}O_4$ (M_w 196): (EI, 70 eV)/m/z: 196 [M]$^+$ (6), 181[M]$^+$ (3), 168 [M]$^+$ (40), 140 [M]$^+$ (40), 122 [M]$^+$ (25), 96 [M]$^+$ (52), 79 [M]$^+$ (30), 68 [M]$^+$ (40), 53 [M]$^+$ (18), 44 [M]$^+$ (18), 41 [M]$^+$ (5).
UV/vis: λ_{max} 225, 460 nm.

2.3. Preparation of the polymer-photoinitiating system mixture

Hydroxyethyl starch was substituted with 2-hydroxyethyl methacrylate (HES-HEMA) by Harling et al. (2010) and Wöhl-Bruhn et al. (2012b). HES-HEMA was used as a water-soluble-polymer and has been chosen for testing and evaluating the crosslinking efficiency of the photoinitiating system. HES-HEMA polymer has been previously synthesized and characterized (Harling et al., 2010; Wöhl-Bruhn et al., 2012b) as well as has been crosslinked by CQ-amine-DPIC system in visible-light photoinitiating system (Kamoun and Menzel, 2012).

The HES-HEMA polymer-photoinitiating system mixture was prepared as follows: HES-HEMA polymer (20 wt%, DS = 0.048) was dissolved in distilled water for 2 h; the solution was then mixed thoroughly and sonicated to guarantee complete dissolution. Photoinitiator either CQ or CQCOOH (0.25 mol%) was dissolved in a water: DMSO mixture (~4:1) or water, respectively and added to the polymer solution. Subsequently the amine coinitiator (L-arginine, 0.5 mol%) was added to the polymer-photoinitiator mixture. The polymer-photoinitiating system mixture was prepared in the dark and in brown glass vials under nitrogen to avoid any premature polymerization. Finally, DPIC accelerator (0.5 wt%) was added and the mixture was gently stirred for two hours and sonicated again, to guarantee homogeneity. The solution is poured in PP casting molds and crosslinked by exposure to a visible-light emitting diode lamp (i.e. LED lamp) for a certain exposure irradiation time (the thickness of the crosslinked polymer disk is approximately 1.9 mm).

The crosslinking densities (P_x) of the HES-HEMA gel have been calculated using the equilibrium swelling theory and Flory–Rehner equation as described previously (Kamoun and Menzel, 2010).

2.4. Calculation of crosslinking density (P_x)

According to Flory–Rehner equation, the crosslinking density (P_x) can be described by the number average molecular weight between two adjacent crosslinks (M_c). M_c was calculated using simplification of the Flory–Rehner equation and compensation with the Flory–Huggins interaction parameter and equilibrium swelling theory of hydrogel. The crosslinking density is determined using a further simplified Eq. (1) by Kamoun and Menzel (2010).

$$P_x = (M_c v)^{-1} \text{mol cm}^{-3} \quad (1)$$

where v is the specific volume of dry used polymer (0.64 cm^3 g^{-1} at 20 °C for HES).

3. Characterizations

3.1. Proton nuclear magnetic resonance spectroscopy (^{1}H-NMR)

NMR-spectra were recorded on an NMR-DRX400 instrument (BRUKER, Germany, 300 MHz). Approximately 50 mg of the sample was dissolved in 0.8 mL of either D_2O, or DMSO-d$_6$. NMR spectra were analyzed by the MestRec software program.

3.2. Fourier transform infrared spectroscopy (FT-IR)

The lyophilized samples were analyzed by FT-IR on an EQUINOX 55 instrument (Bruker, Germany). 1–2 mg of the samples for IR analysis was well dried, ground to a very fine powder, mixed with infrared grade KBr (about 130–150 mg) and pressed to transparent KBr disks. The FTIR spectrum was obtained by recording 64 scans between 4000 and 400 cm^{-1} with a resolution of 2 cm^{-1}.

UV/Vis spectrophotometer, model: (Lambda L950, Perkin-Elmer, Germany) was used to detect the absorption light spectrum area.

The degree of conversion (DC%) for the double bonds was calculated from the IR-spectrum via the changes in the C=C absorbance peak intensity and using the unchanged carbonyl-ester absorbance as internal reference, which is only approximate because the conjugation with the C=C changes the peak shape. The DC was obtained by subtracting the percentage of remaining or uncrosslinked carbon double bonds (C=C %) from 100% (Yoshida and Greener, 1994). For FTIR spectra of pure HES, uncrosslinked HES-HEMA, and crosslinked HES-HEMA polymers see Fig. 1.

3.3. Oscillation rheology measurements

Rheological characterization of polymer hydrogel samples was performed with a Rheostress RS-100-HAAKE instrument (Karlsruhe, Germany). The oscillation shear flow measurement was conducted at 25 °C, using plate–plate geometry (PP20 Ti, ~2.5 cm diameter, and ~1 cm gap), and an angular frequency range from 0.1 to 10 Hz, employing the HAAKE standard application software version 2.1.

3.4. Physical properties of CQCOOH

The physical properties of different camphor photoinitiator derivatives, such as KPA, CQ, and CQCOOH, are summarized in Table 1.

Table 1 Physical properties of camphor photoinitiator derivatives (ketopinic acid (KPA), camphorquinone (CQ) and carboxylated camphorquinone (CQCOOH)).

Camphor photoinitiator derivatives			
	KPA	CQ	CQCOOH
Solubility	Water, ethanol, DMSO	DMSO, ethanol	Water, ethanol, DMSO
Color and shape	White powder	Yellow crystal	Yellow crystal
Linear formula	$C_{10}H_{14}O_3$	$C_{10}H_{14}O_2$	$C_{10}H_{12}O_4$
M wt. (g/mol)	182.22	166.22	196.1
Mp (°C)	237–239	197–203	234–237
Absorption region (λ_{max}, nm)	Only in UV at 285	UV at 254 and Vis. at 467.	UV at 225 and Vis. at 460.
Color bleaching or photo-bleaching	–	Very low	Very high

KPA has no photo-bleaching character.

3.5. Investigation of the biocompatibility of photoinitiating system ingredients

Human gingival fibroblasts (HGF, Provitro, Cat.-No.: 1210412) have been chosen for biocompatibility testing. The cells were cultured and grown in Dulbecco's minimal essential medium (DMEM) (Biochrom, Cat.-No.: FG0435) supplemented with fetal bovine serum (FCS, 10% v/v), 100 U/mL penicillin and 100 µg/mL streptomycin at 37 °C in a 5% CO_2, 95% humidity atmosphere. For the cytotoxicity assays cells were seeded at passage 9 with 5000 c/100 µl/well in 96-well plates.

3.5.1. MMT-assay

In order to determine the influence of single ingredients of the photoinitiating system (i.e. CQCOOH, the amine coinitiators, or DPIC), on cell viability, a quantitative colorimetric tetrazolium-assay (MTT, Roche, Cat.-No.: 11465007001) was used. This assay detects only living cells via cleavage of tetrazolium salts into a blue-colored formazan dye by dehydrogenase enzymes embodied in active mitochondria. Thereby the amount of intracellular converted formazan is directly proportional to cell viability.

Cells were seeded in 96-well plates at a density of 5000 c/well and incubated overnight at 37 °C and 10% CO_2. The supernatant was replaced with different dilutions of photoinitiating system ingredients in fresh medium (final concentration depended on the original proportion of the substance in 1 mL pure hydrogels, whereas dilutions ranged from: 50.0% to 0.39%). After a 24 h incubation 10 µl of MTT labeling reagent was added and after 4 h cells got disintegrated by the addition of 100 µl solubilization solution causing the formazan release overnight. The wells were evaluated on a multiwall scanning spectrophotometer (Tecan infinite F200) using a wavelength of 540 nm with reference at 650 nm. Parallel medium containing test substances was treated the same way in the absence of cells to exclude staining effects with adding substances itself.

3.5.2. LDH-assay

Lactate dehydrogenase (LDH) is an oxido-reductase which catalyzes the inter conversion of lactate and pyruvate. LDH is most often measured to evaluate the presence of tissue or cell damage. The colorimetric LDH assay is based on the reduction of the tetrazolium salt in a NADH-coupled enzymatic reaction to formazan, which is water-soluble and exhibits an absorption maximum at 492 nm.

In LDH assays the amount of membrane damaged or dead cells can be assessed by determining the amount of LDH within the cell culture medium which has been released by those cells. The amount of LDH is measured as enzymatic activity in a dye conversion reaction and is proportional to the cytotoxic effect of substances.

Cells were seeded according to MTT-assay protocol but the supernatant was replaced with FCS-reduced (1% instead of 10%) medium containing different concentrations of photoinitiating system ingredients (see above). Cells in medium without substances (low LDH activity) and in medium with 1% Triton X-100 (high LDH activity) served as controls. After overnight incubation 100 µl of supernatant has been transferred carefully in new plates and got combined with 100 µl dye mixture (LDH, Roche, Cat.-No.: 11644793001). Staining reaction took place for approximately 10 min and was stopped by adding 50 µl 1 N HCl. The incubated suspensions were evaluated on a multi-well scanning spectrophotometer (Tecan infinite F200) using a wavelength of 492 nm with reference at 650 nm. Parallel medium containing test substances was treated in the same way in the absence of cells to exclude staining effects by the added substances itself.

4. Results and discussion

7,7-Dimethyl-2,3-dioxobicyclo[2.2.1]heptane-1-carboxylic acid, or carboxylated camphorquinone (CQCOOH) showed an absorption spectrum similar to camphorquinone but with a much higher water solubility (Table 1). CQCOOH has been synthesized according to the slightly modified procedure of Pande et al. (1991) and Ikemura et al. (2010), (Fig. 3).

Some factors have been found which have a strong and direct influence on the yield of CQCOOH. Table 2 summarizes different synthesis conditions and the yields obtained. Inspecting the data in Table 2, it can be noticed that both refluxing time and the molar ratios between two reactants (KPA:Se),

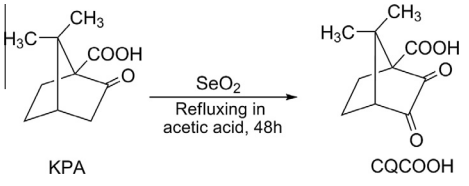

Figure 3 Synthesis scheme of carboxylated camphorquinone (CQCOOH) from ketopenic acid (KPA).

Table 2 Influence of reaction time and molar ratios of two reactants on the yield (%) of carboxylated camphorquinone.

KSA: Se	Refluxing time (h)	Yield (%)
1:1	16	15
1:1.5	16	21
1:1.5	>16–48	48
1:3	16	34

have a significant impact on the yield. The maximum yield of CQCOOH has been obtained by reaction times >16 h. Furthermore, a high molar ratio of the oxidizing agent (i.e. selenium dioxide) to ketopinic acid results in a higher yield of CQCOOH.

The chemical structure of carboxylated camphorquinone was verified by ^{1}H-NMR spectroscopy, (Fig. 4). The signal at 12.87 ppm is due to the COOH-group of CQCOOH. Further evidence for the structure of the CQCOOH is given by IR spectra. The spectrum of the CQCOOH shows two peaks for carbonyl groups. One for the ketone groups as it is found in CQ, and additionally a peak at 1697 cm^{-1} for the COOH-group, as it is found in KPA. Fig. 4 shows a contour plot of the two-dimensional data set obtained from the COSY spectroscopy of CQCOOH in DMSO-d$_6$. An unambiguous assignment of all peaks and their correlation to the proposed structure of the CQCOOH is possible.

Fig. 5 illustrates the effect of different irradiation times on the crosslink density of HES-HEMA hydrogels crosslinked with CQ and CQCOOH based photoinitiating systems, respectively. The crosslink density (P_x) has been used as an indicator for the efficiency of the crosslinking process and the photoreactivity of the photoinitiating system. P_x has been calculated using the equilibrium swelling theory and the Flory–Rehner equation as previously described (Kamoun and Menzel, 2010). At irradiation times shorter than 60 s, no crosslinked hydrogel was obtained when the CQ-based system was used. The crosslink density in this case is close to zero. Even at longer irradiation times P_x remains low for the CQ photoinitiator. In contrast for the CQCOOH-based system, crosslinked polymers were obtained at irradiation times of only 5 s. Furthermore, the crosslink densities significantly increase with irradiation time and reach a maximum after approximately 180 s. This behavior indicates that the CQCOOH-amine-DPIC system has a higher photoreactivity than the CQ-amine-DPIC system.

The decreased intensity of C=C peak (at 1650 cm^{-1}) of HES-HEMA structure in FTIR-spectrum was used as an

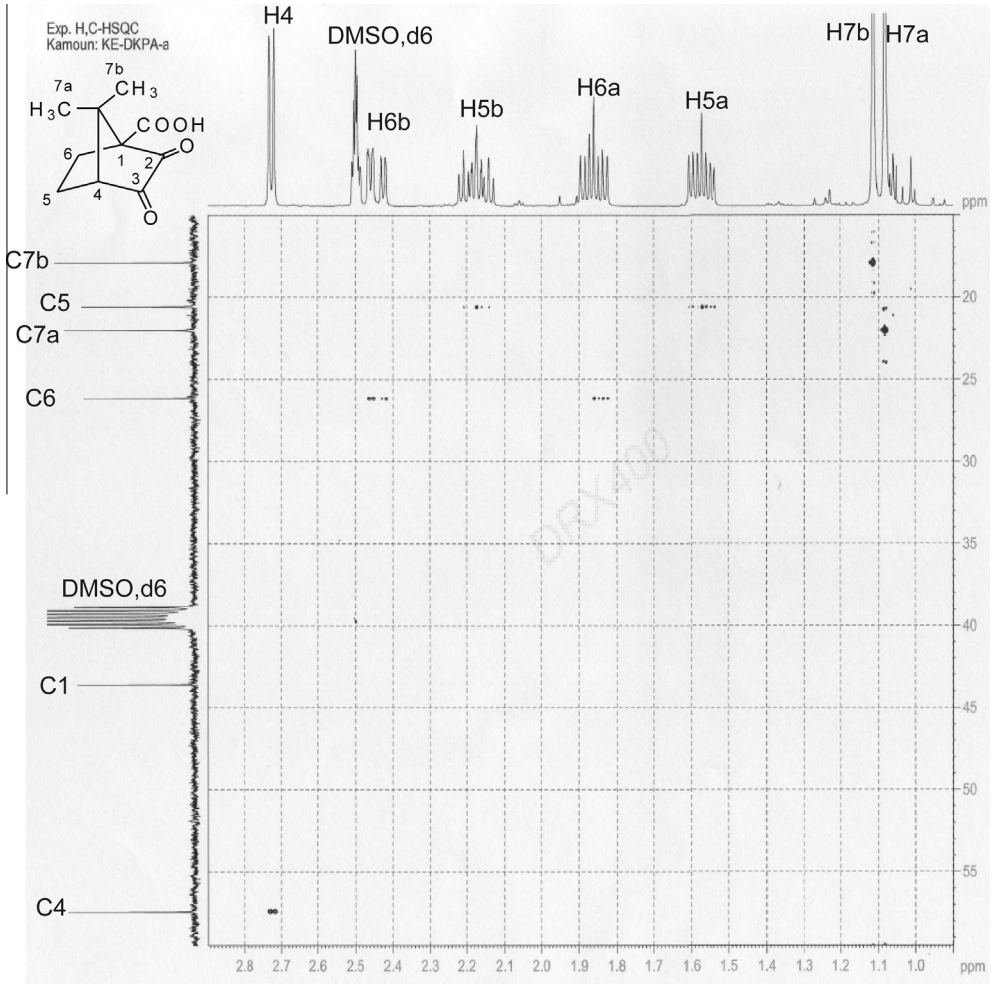

Figure 4 A plot of ^{1}H–^{13}C-COSY-NMR spectrum of carboxylated camphorquinone photoinitiator (CQCOOH).

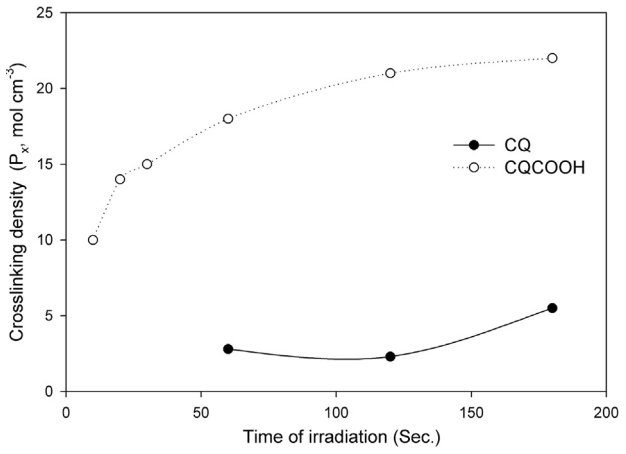

Figure 5 A plot of crosslink density values (P_x) of HES-HEMA polymer crosslinked with those of CQ (0.05 mol%) and CQCOOH (0.05 mol) systems vs. exposure of irradiation time, (HES-HEMA polymers were crosslinked by photoinitiator-L-arginine-DPIC as the photoinitiating system).

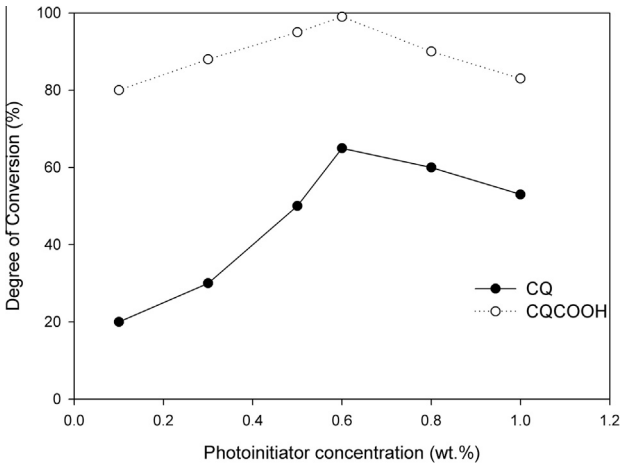

Figure 6 A plot of degree of conversion (DC%) of HES-HEMA polymer crosslinked with CQ and CQCOOH systems vs. photoinitiator concentration, (HES-HEMA polymers were crosslinked by photoinitiator-L-arginine-DPIC as the photoinitiating system).

indicator for the double bond consumption upon photocrosslinking, while carbonyl ester group peak (at 1730 cm^{-1}) remained unchanged and thus could be used as an internal standard reference. The degree of conversion (DC) has been calculated using the following equation:

$$DC = (1 - [A_{1650}/A_{1730}]\text{crosslinked}/ \times [A_{1650}/A_{1740}]\text{uncrosslinked})100. \quad (2)$$

The degrees of conversion of double bounds in the HES-HEMA polymer after crosslinking with either the CQ- or the CQCOOH-based system as function of the photoinitiator concentration are presented in Fig. 6. The distinct reduction for C=C peak after visible light irradiation (one minute in case two photoinitiating systems), was detected equally in the spectra of the back and the front side of polymer hydrogel at the highest photoinitiator levels. These results are consistent with the results reported by Kim and Chu (2009). They have reported that there are no differences in the DC between the front and the back side of dextran-methacrylate hydrogels crosslinked by riboflavin-L-arginine with visible light. Additionally, Kim (2005), found that the photoinitiator concentration has no effect on depth of cure. As shown in Fig. 6, the degree of conversion increases with increasing the photoinitiator concentration up to a maximum value at 0.6 wt%. These results are consistent with the results published by Jakubiak et al. (2007). They reported that an increasing CQ-photoinitiator concentration can cause two side reactions. The quenching of the triplet state $^3CQ^*$ by CQ molecules and/or a fast termination reaction, due to the high concentration of radicals formed in the photoreaction. Concisely, the DCs are much higher for polymers crosslinked with CQCOOH compared to those crosslinked with the CQ-system. These results are further evidence for a higher photoreactivity of CQCOOH compared to CQ.

The mechanical properties of the HES-HEMA hydrogels give further information about the crosslink density and therefore the efficiency of the tested photoinitiators. Samples have been prepared with CQ or CQCOOH and are shown in Fig. 7,

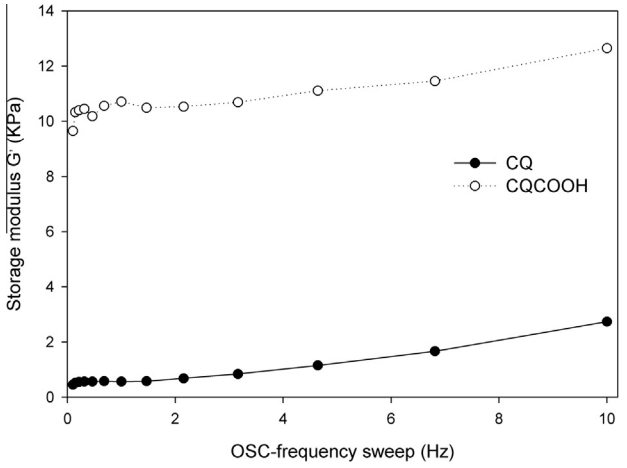

Figure 7 Effect of photoinitiator system type (CQ or CQCOOH) on the mechanical properties of obtained crosslinked polymers, (HES-HEMA polymers were crosslinked by photoinitiator-L-arginine-DPIC as the photoinitiating system).

respectively. Hydrogel samples were crosslinked under the same crosslinking conditions (i.e. 20 wt% HES-HEMA polymer, 0.25 mol% photoinitiator, 0.5 mol% of L-arginine coinitiator, 0.5 wt.% DPIC and 60 s irradiation time). Dynamic mechanical characterization of the hydrogels has been carried out by oscillation rheology measurements. This characterization allows the evaluation of the elastic (G′: storage modulus) and viscous (G″: loss modulus) components for the crosslinked polymer. Fig. 7 displays a plot of the storage modulus (G′) for HES-HEMA hydrogels crosslinked by CQ-L-arginine-DPIC or CQCOOH-L-arginine-DPIC as photoinitiating systems, respectively. It can be seen that the storage modules for the polymer crosslinked with the CQCOOH-system (~10 kPa) is much higher compared to that measured for the system crosslinked with CQ (~0.5 kPa). These results give further evidence for a better crosslinking with CQCOOH as the photoinitiator.

The higher photoreactivity of CQCOOH, which is mainly responsible for the high DC, short exposure times needed, and high storage modulus of hydrogels obtained, may be attributed to the better solubility of CQCOOH in water, which results in a more homogeneous reaction mixture. In contrast the less water-solubility of CQ might form aggregates in the reaction mixture. Within the aggregates CQ molecules are at a high local concentration, which might result in more side reactions as described by Jakubiak et al. (2007) and therefore less efficient radical crosslinking.

The components of the new photoinitiating system have to be non-toxic to find application in the production of drug delivery systems. The biocompatibility of the different compounds of the photoinitiator system was investigated via cell viability (MTT-assay) and cytotoxicity tests (LDH-assay) and is displayed in Fig. 8. For the testing increasing amounts of the photoinitiator system components were added to cell culture medium. The photoinitiator CQCOOH and the amine coinitiator L-arginine showed no effect on cell viability even at high concentrations (see MTT-assay in Fig. 8). N-phenylglycine (NPG) is another potent coinitiator, which could substitute L-arginine and was included in the testing to gain information regarding its biocompatibility. For NPG no loss of cell viability was observed. However an exceptional curve

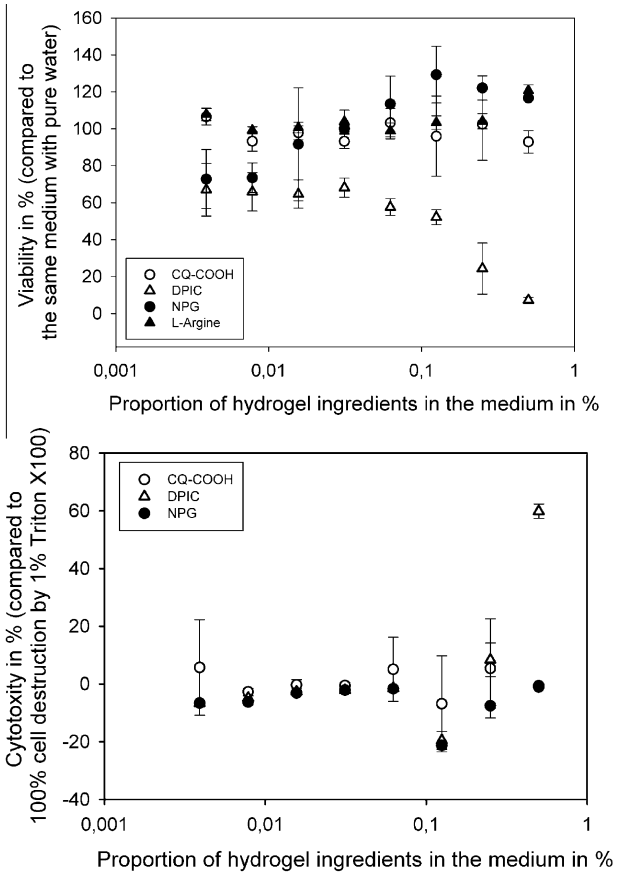

Figure 8 Cell viability test by MTT-assay (top) and cytotoxicity test by LDH-assay (bottom) with human gingival fibroblast cells (HGF) for the single ingredients of the photoinitiating system, CQCOOH (photoinitiator), NPG, L-arginine (amines coinitiators), and DPIC (accelerator).

progression at low concentration is observed. It starts with medium values, which become much better with higher concentrations indicating improved cell viability in the presence of NPG. However, because of cell morphology and controls measurements without cells we attribute this behavior to a high auto-staining activity of the samples. Thus it can be concluded that NPG has good biocompatibility at all concentrations tested. This interpretation is consistent with results of Kucybala et al. (1996) and Wang et al. (2006), who demonstrated that a CQ-NPG system is less toxic than a CQ-DMA-EMA photoinitiator system. In accordance to our results, Hern and Hubbell (1998) and Gao et al. (2003), have shown the good cytocompatibility of L-arginine, when they have used arginine to prepare RGD peptides incorporated in acrylate hydrogels for tissue engineering applications. The results of the cytotoxicity test with a LDH assay (Fig. 8) substantiate these results for CQCOOH and NPG.

In contrast, addition of the accelerator DPIC results in a significant reduction of the cell viability to approximately 60% even at the lowest concentration. The adverse effect of DPIC becomes even more severe at higher concentrations (see MTT-assay in Fig. 8). Again the LDH assay substantiates the cytotoxicity of DPIC. However, the effect here can be seen only for higher concentrations (see LDH-assay in Fig. 8). Although, Wang et al. (2006) and Guo et al. (2008), have discussed the effect of addition of DPIC and DPIHP (diphenyiodonium hexafluorophosphate) to CQ-amine system for crosslinking of HEMA-BisGMA polymer, no data related to cytocompatibility of iodonium salt compounds have been published before.

CQCOOH has been previously used for the preparation of hydrogels for biomedical applications and no adverse effects of the photoinitiator on cells have been reported (Magoshi and Matsuda, 2002; Nakayama et al., 2001; Okino et al., 2002). However, no explicit biocompatibility testing had been carried out for CQCOOH. The very good biocompatibility of CQCOOH as found here is in accordance with the good cytocompatibility of CQ which has been previously proven with HGF cells by Atsumi and co-workers (Atsumi et al., 2001, 2004). The biocompatibility testing shows that CQCOOH based systems with L-argine or NPG as coinitiators are well suited for biomedical applications. However, due to the very limited cell compatibility of the accelerator DPIC, the photoinitiating system should be optimized without accelerator or DPIC replaced by a more compatible accelerator.

5. Conclusion

In summary, we have described the synthesis and characterization of carboxylated camphorquinone (CQCOOH) as a water-soluble photoinitiating system to be used with visible light. It was shown that CQCOOH with an amine coinitiator and an accelerator gives a very reactive photoinitiator system, which in aqueous solution is more effective than a system based on the commercial and conventional camphorquinone (CQ). The high photoreactivity of carboxylated camphorquinone has been proven by a quick formation of crosslinked polymer, higher crosslink density, higher degree of double bond conversion (DC%), and better mechanical properties (high storage modulus) compared to hydrogels crosslinked with the camphorquinone system. The high photoreactivity of

CQCOOH-amine-DPIC system can be ascribed to the presence of the additional camphor group like the COOH-group which improved the solubility and with that the homogeneity and light sensitivity of the system. Carboxylated camphorquinone is highly biocompatible, no toxic effects were found by MTT and LDH assays with HGF cells. While, NPG and L-arginine as amine coinitiators are also non-toxic, the accelerator DPIC reduced cell viability apparently. Carboxylated camphorquinone as a visible-light photoinitiator has great potential to replace the conventional used camphorquinone in aqueous systems for various biomedical applications.

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References

Alvim, H.H., Alecio, A.C., Vasconcellos, W.A., Furlan, M., Oliveira, J.E., Saad, J.R., 2007. Analysis of camphorquinone in composite resins as a function of shade. Dent. Mater. 23, 1245–1249.

Atsumi, T., Iwakura, I., Fujisawa, S., Ueha, T., 2001. The production of reactive oxygen species by irradiated camphorquinone related photosensitizers and their effect on cytotoxicity. Arch. Oral Biol. 46, 391–401.

Atsumi, T., Ishihara, M., Kadoma, Y., Tonosaki, K., Fujisawa, S., 2004. Comparative radical production and cytotoxicity induced by camphorquinone and 9-fluorenone against human pulp fibroblasts. J. Oral Rehabil. 31, 1155–1164.

Bertz, A., Wöhl-Bruhn, S., Miethe, S., Tiersch, B., Koetz, J., Hust, M., Bunjes, H., Menzel, H., 2013. Encapsulation of proteins in hydrogel carrier systems for controlled drug delivery: influence of network structure and drug size on release rate. J. Biotechnol. 163, 243–249.

Cook, W.D., 1992. Photopolymerization kinetics of dimethacrylates using the camphorquinone/amine initiator system. Polymer 33, 600–609.

Cook, W.D., Chen, F., 2011. Enhanced photopolymerization of dimethacrylates with ketones, amines, and iodonium salts: the CQ system. J. Polym. Sci., Part A: Polym. Chem. 49, 5030–5041.

Gao, C., Guan, J., Zhu, Y., Shen, J., 2003. Surface immobilization of bioactive molecules on polyurethane for promotion of cytocompatibility to human endothelial cells. Macromol. Biosci. 3, 157–162.

Guo, X., Wang, Y., Spencer, P., Ye, Q., Yao, X., 2008. Effects of water content and initiator composition on photopolymerization of a model BisGMA/HEMA resin. Dent. Mater. 24, 824–831.

Harling, S., Schwoerer, A., Scheibe, K., Daniels, R., Menzel, H., 2010. A new hydrogel drug delivery system based on hydroxyethylstarch derivatives. J. Microencapsulation 27, 400–408.

Hern, D.L., Hubbell, J.A., 1998. Incorporation of adhesion peptides into nonadhesive hydrogels useful for tissue resurfacing. J. Biomed. Mater. Res. 39, 266–276.

Ikemura, K., Ichizawa, K., Jogetsu, Y., Endo, T., 2010. Synthesis of a novel camphorquinone derivative having acylphosphine oxide group, characterization by UV–VIS spectroscopy and evaluation of photopolymerization performance. Dent. Mater. J. 29, 122–131.

Jakubiak, J., Sionkowska, A., Linden, L.A., Rabek, J.F., 2001. Isothermal photo differential scanning calorimetry. Crosslinking polymerization of multifunctional monomers in presence of visible light photoinitiator. J. Therm. Anal. Calorim. 65, 435–443.

Jakubiak, J., Allonas, X., Fouassier, J.P., Sionkowaska, A., Andrzejewska, E., Linden, L.A., Rebek, J.F., 2003. Camphorquinone-amines photoinitiating systems for the initiation of free radical polymerization. Polymer 44, 5219–5226.

Jakubiak, J., Wrzyszczynski, A., Linden, L.A., Rebak, J.F., 2007. The role of amines in the champhorquinone photoinitiated polymerization of mutifunctional monomer. J. Macromol. Sci. Part A Pure Appl. Chem. 44, 239–242.

Janda, R., Roulet, J.-F., Kaminsky, M., Steffin, G., Latta, M., 2004. Color stability of resin matrix restorative materials as a function of the method of light activation. Eur. J. Oral Sci. 112, 280–285.

Kamoun, E.A., Menzel, H., 2010. Crosslinking behavior of dextran modified with hydroxyethyl methacrylate upon irradiation with visible light-effect of concentration, coinitiator type, and solvent. J. Appl. Polym. Sci. 117, 3128–3138.

Kamoun, E.A., Menzel, H., 2012. HES-HEMA nanocomposite polymer hydrogel:swelling behavior and characterization. J. Polym. Res. 19, 9851–9865.

Kim, O., 2005. Effect of photo-accelerator on the polymerization behavior of light-activated polymeric dental restorative composites. J. Ind. Eng. Chem. 11, 287–292.

Kim, S.-H., Chu, C.-C., 2009. Visible light induced dextran-methacrylate hydrogel formation using (−)-riboflavin vitamin B2 as a photoinitiator and L-arginine as a co-initiator. Fibers Polym. 10, 14–20.

Kim, D., Scranton, A.B., 2004. The role of diphenyliodonium slat (DPI) in three component photoinitiator systems containing methylene blue (MB) and an electron donor. J. Polym. Sci. Part A Polym. Chem. 42, 5863–5871.

Kucybala, Z., Pietrzak, M., Paczkowski, J., 1996. Kinetic studies of a new photointiator hybrid system based on camphorquinone-N-phenylglycine derivatives for laser polymerization of dental restorative and stereolithographic (3D) formulations. Polymer 37, 4585–4591.

Lu, S.X., Anseth, S.K., 1999. Photopolymerization of multilaminated poly (HEMA) hydrogels for controlled release. J. Controlled Release 57, 291–300.

Magoshi, T., Matsuda, T., 2002. Formation of polymerized mixed heparin/albumin surface layer and cellular adhesional responses. Biomacromolecules 3, 976–983.

Matsuda, T., Magoshi, T., 2002. Preparation of vinylated polysaccharides and photofabrication of tubular scaffolds as potential use in tissue engineering. Biomacromolecules 3, 942–950.

Nakayama, Y., Youn, K.-J., Nishi, S., Ueno, H., Matsuda, T., 2001. Development of high-performance stent: gelatinous photogel-coated stent that permits drug delivery and gene transfer. J. Biomed. Mater. Res. 57, 559–566.

Ogunyinka, A., Palin, W.N., Shortall, A.C., Marquis, P.M., 2007. Photoinitiation chemistry affects light transmission and degree of conversion of curing experimental dental resin composities. Dent. Mater. 23, 807–813.

Okino, H., Nakayama, Y., Tanaka, M., Matsuda, T., 2002. In situ hydrogelation of photocurable gelatin and drug release. J. Biomed. Mater. Res. 59, 233–245.

Pande, C.S., Bassi, K.D., Jain, N., Dhar, A., Glass, J.D., 1991. Diketopinic acid-a novel reagent for the modification of arginine. J. Biosci. (India) 16, 127–135.

Park, Y.J., Chae, K.H., Rawls, H.R., 1999. Development of a new photoinitiation system for dental light-cure composite resins. Dent. Mater. 15, 120–127.

Pyszka, I., Kucybala, Z., Paczkowski, J., 2004. Reinvestigation of the mechanism of the free radical polymerization photoinitiation process by camphorquinone-coinitiator systems: new results. Macromol. Chem. Phys. 205, 2371–2375.

Schneider, L.F.J., Pfeifer, C.S.C., Consani, S., Prahl, S.A., Ferracane, J.L., 2008. Influence of photoinitiator type on the rate of polymerization, degree of conversion, hardness and yellowing of dental resin composites. Dent. Mater. 24, 1169–1177.

Schroeder, W.F., Cook, W.D., Vallo, C.I., 2008. Photopolymerization of N,N-dimethylaminobenzyl alcohol as amine co-initiator for light-cured dental resins. Dent. Mater. 24, 686–693.

Scott, R.A., Peppas, N.A., 1999. Highly cross-linked, PEG containing copolymers for sustained solute delivery. Biomaterials 20, 1371–1380.

Stansbury, J.W., 2000. Curing dental resins and composites by photopolymerization. J. Esthet. Dent. 12, 300–308.

Sun, G.J., Chae, K.H., 2000. Properties of 2,3-butanedione and 1-phenyl-1,2-propanedione as new photosensitizer for visible light cured dental resin composites. Polymer 41, 2605–2612.

Tsai, L., Charney, E., 1969. The triplet states of alpha-dicarbonyls. J. Phys. Chem. 73, 2462–2463.

Wang, Y., Spencer, P., Yao, X., Ye, Q., 2006. Effect of coinitiator and water on the photoreactivity and photopolymerization of HEMA/camphorquinone-based reactant mixtures. J. Biomed. Mater. Res. Part A 78A, 721–728.

Wöhl-Bruhn, S., Badar, M., Bertz, A., Menzel, H., Mueller, P.P., Bunjes, H., 2012a. Comparison of in vitro and in vivo protein release from hydrogel systems. J. Controlled Release 162, 127–133.

Wöhl-Bruhn, S., Bertz, A., Harling, S., Menzel, H., Bunjes, H., 2012b. Hydroxyethyl starch-based polymers for the controlled release of biomacromolecules from hydrogel microspheres. Eur. J. Pharm. Biopharm. 81, 573–581.

Yoshida, K., Greener, E.H., 1994. Effect of photoinitiator on degree of conversion of unfilled light-cured resin. J. Dent. 22, 296–299.