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Impact of high-energy electron irradiation on mechanical, structural and chemical properties of agarose hydrogels

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ABSTRACT

Due to their excellent biocompatibility and biodegradability, natural hydrogels are highly demanded biomaterials for biomedical applications such as wound dressing, tissue engineering, drug delivery or three dimensional cell culture. Highly energetic electron irradiation up to 10 MeV is a powerful and fast tool to sterilize and tailor the material's properties. In this study, electron radiation treatment of agarose hydrogels was investigated to evaluate radiation effects on physical, structural and chemical properties. The viscoelastic behavior, surface hydrophilicity and swelling behavior in a range of typical sterilization doses of 0 kGy to 30 kGy was analyzed. The mechanical properties were determined by rheology measurements and decreased by more than 20% compared to the initial moduli. The number average molecular weight between crosslinks was estimated based on rubber elasticity theory to judge on the radiation degradation. In this dose range, the number average molecular weight between crosslinks increased by more than 6%. Chemical structure was investigated by FTIR spectroscopy to evaluate the radiation resistance of agarose hydrogels. With increasing electron dose, an increasing amount of carbonyl containing species was observed. In addition, irradiation was accompanied by formation of gas cavities in the hydrogels. The gas products were specified for CO_2, CO and H_2O. Based on the radiolytic products, a radiolysis mechanism was proposed. Electron beam treatment under high pressure conditions was found to reduce gas cavity formation in the hydrogels.

1. Introduction

Agarose, a natural polysaccharide, is extracted from red algae of the class Rhodophyceae (Araki, 1966). Its basic structure consists of alternating β-D-galactopyranosyl and 3,6-anhydro-α-L-galactopyranosyl units, linked by 1→3 and 1→4 glycosidic bonds, respectively (Araki, 1966). Agarose polymers have the ability to form thermoreversible hydrogels with a comparative large sol-gel-hysteresis. Agarose forms gels upon cooling below the sol-gel-transition temperature (SGT) by a random coil-helix transition (Norton, Goodall, Austen, Morris, & Rees, 1986), when dissolved in water and heated above the gel-sol-transition temperature (GST) of about 85 °C–95 °C. The SGT is in the range of approx. 35 °C–45 °C for normal agarose. Both, the GST and SGT strongly depend on the concentration, type and the degree of methylation (Guiseley, 1970; Praiboon, Chirapart, Akakabe, Bhumibhamon, & Kajiwara, 2006). In the gel, agarose polymers form double helices that aggregate into bundles by hydrogen bonding and assemble into a three dimensional network (Arnott et al., 1974; Waki, Harvey, & Bellamy, 1982). The structure of agarose networks has been well studied in the past and tend to be isotropic with approx. 100–400 nm large pores that contain water (Pernodet, Maaloum, & Tinland, 1997). As a natural material, it shows a high degree of biocompatibility and biodegradability (Awadhiya, Kumar, Rathore, Fatma, & Verma, 2017; Hutmacher, Goh, & Teoh, 2001; Tabata et al., 2003) enabling biomedical applications, such as wound dressing (Bao, Hayashi, Li, Teramoto, & Abe, 2010), tissue engineering (Hutmacher et al., 2001; Sánchez-Salcedo, Nieto, & Vallet-Regí, 2008; Zarrintaj et al., 2018) and drug delivery (Häglund, Upadrashta, Neau, & Cutrera, 1994). Its porous structure and mechanical strength allows tuning three dimensional substrates and scaffolds for optimized cell culture and tissue growth (Ulrich, Jain, Tanner, MacKay, & Kumar, 2010). Agarose is frequently used for analytical electrophoresis of proteins and nucleic acids such as DNA and

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RNA fragments yet also in the food industry (Deszczynski, Kasapis, & Mitchell, 2003). The here presented study on high-energy electron radiation treatment of agarose and its impact on the physical, chemical and structural properties is of interest for numerous applications in biology and medicine since it allows for effective sterilization of agarose containing biomedical products while maintaining high functionality. Sterilization by ionizing radiation is often the technique of choice, since it is a precise, easy-to-handle and reagent-free tool without release of toxic byproducts (Benson, 2002; Wisotzki et al., 2016). Among the ionizing radiation techniques, electron beam treatment is highly beneficial due to large penetration depths up to several centimeters and high dose rates enabling fast sample treatment in contrast to ultraviolet- and γ-radiation techniques, respectively (Maolin, Hongfei, Yoshii, & Makuuchi, 2000; Wang et al., 2015). In response to the irradiation, the polymers can react in two different ways. Which mechanism dominates depends on the precise irradiation conditions. On the one hand, the outcome can be chain cleavage or degradation, which results in the reduction of the molecular weight of the polymer chain. However, the irradiation can also introduce covalent bonds by recombination of macroradicals between the polymer chains and therefore increase their molecular weight (Hennink & van Nostrum, 2012). These two mechanisms determine the resulting chemical, structural and physical properties of the polymer. In this study, it will be determined which process is predominant for high-energy electron irradiation of agarose. According to Ighigeanu et al. (2006), electron beam treatment sterilizes agarose hydrogels for protein electrophoresis already at doses of 6 kGy and above. In this study, mechanical and structural properties of electron irradiated agarose hydrogels with doses of up to 30 kGy are investigated since 25 kGy is a commonly accepted sterilization dose.

2. Experiment

2.1. Sample preparation

Agarose powder from Thermo Fisher Scientific (Cat. No. 16500500, Germany) was used with a gel strength of 1200 g cm^{-2} at a concentration of 1 wt.% for sample preparation. According to the supplier, the water and sulfate contents were less than 10% and 0.15%, respectively. The SGT and GST of the provided agarose was 36 °C and 90 °C, respectively. 1 wt.% agarose hydrogels were prepared in accordance to the manufacturer's instructions by dissolving the agarose powder in distilled water and boiling it in a water bath for 20 min. 10 ml of the agarose solution was filled in a plastic petri dish of 5.5 cm diameter and cooled to 6 °C for at least 24 h before irradiation. The measurements were performed by punching samples with a diameter of 10 mm out of the agarose hydrogel. The hydrogels were stored at 6 °C between measurements.

2.2. Electron beam treatment

Electron beam treatment was carried out using a 10 MeV electron accelerator (MB10-30MP; Mevex Corp., Canada) with a moving stage of 3 m/min maximum speed. The frequency of the scanning horn was 3 Hz. The pulse repetition rate of the electron beam was 180 Hz and the pulse length of the electron beam was 8 µs. Desired radiation doses were obtained using steps of 5 kGy, corresponding to former studies (Riedel et al., 2019; Wisotzki et al., 2014). The radiation doses were determined by a graphite dosimeter with an inaccuracy of 5%. To avoid radical scavenging by ambient oxygen, irradiation was performed with nitrogen as the ambient gas. Therefore, the samples were packed in sealed containers, which were filled with gaseous nitrogen. To avoid heating of the samples during irradiation, the hydrogels were cooled to room temperature by draft.

In order to decrease the gas cavity formation occurring during electron irradiation of the samples, 1 wt.% agarose hydrogels were additionally irradiated under high pressure conditions of 4 bar. The samples were irradiated in a homemade hyperbaric chamber out of polyoxymethylene material using the same irradiation conditions described above.

2.3. Rheology

Rheological properties of agarose hydrogels were determined by a MCR-300 bulk rheometer (Anton Paar, Austria) in rotational oscillation using a 10 mm-diameter parallel plate-geometry. All measurements were performed at 25 °C. Agarose samples with a diameter of 10 mm and a height of approximately 4 mm were cut out of the hydrogels beforehand. Storage G' and loss moduli G'' were examined in the strain independent region at 1% strain using frequency sweeps ranging from 0.01 Hz to 10 Hz (not shown here). The complex shear modulus was evaluated at a frequency of 1 Hz and at 1% strain. Per irradiation dose, 15 frequency sweeps were performed, i.e. five per sample on three different samples.

2.4. Swelling

Swelling experiments were performed in accordance to earlier studies on electron beam treated gelatin hydrogels (Wisotzki et al., 2014). Directly after irradiation, the samples were stored in distilled water at room temperature and weighed after 24 h. The sample weight was determined with an accuracy of 0.1 mg. After swelling, the agarose hydrogels were dried for three days at room temperature. The swelling ratio Q is determined by the inverse of the volume fraction of dry agarose in the swollen hydrogel ν and was calculated as follows (Weadock, Olson, & Silver, 1983):

$$Q = \frac{1}{\nu} \tag{1}$$

$$\nu = \frac{m_d \rho_{H_2O}}{m_d(\rho_{H_2O} - \rho_a) + m_s \rho_a} \tag{2}$$

with m_s and m_d denoting the swollen and dry weight of the samples, respectively. ρ_{H_2O} and ρ_a correspond to the densities of water, taken as 1 g cm^{-3}, and dry agarose, taken as 1.64 g cm^{-3} from literature (Laurent, 1967). For each irradiation dose, three hydrogels were examined in three different irradiation procedures, resulting in nine samples per irradiation dose.

2.5. FTIR

A Bruker FTIR (TENSOR II, Billerica, USA) equipped with a deuterated triglycine sulfate detector and a Golden Gate single-reflection diamond attenuated total reflection system (Specac, Kent, UK) was used to record the absorption spectra of agarose hydrogels. These measurements were performed on dry agarose gel. Hence, the agarose samples were air dried for three days at room temperature. The total amount of independent measurements was 48 for each treatment method, i.e. 16 measurements per sample on three different samples.

For analysis of released gas of the irradiated hydrogels, 1 wt.% agarose hydrogels irradiated with 30 kGy were stored in a desiccator directly after electron beam treatment over several days. The desiccator was cleaned beforehand using argon. A cuvette of 20 cm length and sodium chloride windows was used for collecting the gas of the desiccator. The gas was characterized using a Bruker FTIR (TENSOR II, Billerica, USA) with a deuterated triglycine sulfate detector. The experiment was repeated three times.

2.6. Gas cavity lifetime detection

Electron beam treatment is accompanied by the formation of gas cavities. The number of the gas cavities decreases with increasing time after electron irradiation. The time when no more gas cavities are

observable in the hydrogel is referred to as the gas cavity lifetime. It was recorded using a conventional camera (uEye SE, Imaging Development Systems GmbH, Obersulm, Germany). To quantify the number of cavities and thus to determine the gas cavity lifetime, images of the samples were taken every 24 h. The samples were stored at 6 °C inbetween imaging.

2.7. Contact angle

Water contact angle measurements were performed on agarose hydrogels directly after irradiation using the conventional sessile drop method. The measurements were carried out employing a Contact Angle Measuring System (G II, Krüss, Hamburg, Germany). The volume of the water droplet was 3 µl. For each sample, ten water contact angles per four different positions were determined. Three agarose samples per irradiation dose were fabricated. The experiment was independently repeated three times.

2.8. Crosslinking density

Rubber elasticity theory was used as the link between the results of the performed rheological measurements and quantifiable statements on the crosslinking density.

Within this conceptual framework agarose is pictured as a network of flexible polymers. Their behavior is dominated by the thermal energy of the system. Due to the fact that the measurements were performed in the rubbery plateau regime, an analogy can be made to the behavior of a monatomic ideal gas (Wall, 1942):

$$G^{'} = nRT \tag{3}$$

with G' denoting the storage modulus obtained from rheology measurements, n the number of active chain segments per unit volume, R corresponding to the ideal gas constant and T to the temperature.

A more sophisticated approach, as described in earlier studies on electron beam treated gelatin hydrogels (Wisotzki et al., 2014), refers the number of active chain segments n to the density of dried agarose ρ_a per number average molecular weight between crosslinks M_c, which is inversely proportional to the crosslinking density. Therefore, the volume fraction of dry agarose in the swollen hydrogel ν, as previously defined in Eq. (2), is introduced but adjusted to the volume ratio of the gels immediately after irradiation with respect to the dried agarose ν_0. The following adjustments are in accordance to earlier studies of Wisotzki et al. (2014) on electron irradiated gelatin resulting in the following formula)

$$G^{'} = \frac{\rho_a RT(\nu_0)^{1/3}}{M_c}. \tag{4}$$

Taking into account free ends in the polymer structure by adding a number average molecular weight M dependent term, one finally obtains (Flory, 1953; Wisotzki et al., 2014)

$$\frac{1}{M_c} = \frac{2}{M} + \frac{G^{'}}{\rho_a RT(\nu_0)^{1/3}} \tag{5}$$

with M as 70 kg mol^{-1} for agarose polymers (Rochas & Lahaye, 1989).

3. Results and discussion

3.1. Rheology

Polysaccharidic hydrogels such as cellulose (Driscoll et al., 2009; Leonhardt et al., 1985), starch (Teixeira, Garcia, Takinami, & del Mastro, 2018), sodium alginate (Nagasawa, Mitomo, Yoshii, & Kume, 2000; Wasikiewicz, Yoshii, Nagasawa, Wach, & Mitomo, 2005) and chitosan (Ulański & Rosiak, 1992; Wasikiewicz et al., 2005) are known to be degradable under ionizing radiation. Their viscoelastic properties are reported to decrease significantly with increasing radiation dose, see for instance (Bhat & Karim, 2009), which is correlated with an increasing molecular weight between crosslinks (Wall, 1942). In this study, agarose hydrogels are treated with 10 MeV electron irradiation to investigate the impact of the irradiation on the material properties of the gels. Viscoelastic properties of agarose hydrogels irradiated with 0 kGy to 30 kGy are exerted with rotational oscillatory rheology measurements using the parallel plate geometry. Results are shown in Fig. 1. Storage and loss moduli of unirradiated hydrogels correspond to 12.8 ± 0.2 kPa and 1.6 ± 0.8 kPa, respectively, which is in the order of magnitude of former studies on viscoelastic behavior of agarose (Fernández et al., 2008; Moritaka, Nishinari, Horiuchi, & Watase, 1980; Roberts, Earnshaw, Ferguson, & Bryant, 2011). Rheology measurements show a significant decrease of both storage and loss moduli with increasing electron dose. After irradiation with 30 kGy, the viscoelastic properties undergo a reduction of more than 20% compared to the initial moduli. A similar behavior is observed for agarose and carrageen gels regarding compression strength and strain after γ-radiation treatment (Maolin et al., 2000; Wang et al., 2015). The gel softening with a relatively constant dissipation factor tan(δ), see Fig. 1, is due to a destruction of the three dimensional network structure of agarose polymers in the hydrogel resulting in lower gel rigidity. Accordingly, liquefying of the agarose hydrogels was observed for high irradiation doses of 100 kGy and higher (not shown here). Wang et al. (2015) measured a rapid decrease in the average molecular weight for agarose hydrogels irradiated with γ-radiation up to 25 kGy, which is known to correspond to decreased rheological properties of polysaccharides (Dealy & Wissbrun, 1990; Mitchell, 1980; Normand, Lootens, Amici, Plucknett, & Aymard, 2000). Thus, a decrease in average molecular weight is expected for electron treated agarose molecules as well.

The number average molecular weight between crosslinks M_c is calculated using rubber elasticity theory, taking into account Eq. (5) and is shown in Fig. 2. Within this dose range, M_c undergoes an increase of more than 6%. Since the average mass between crosslinks increases with increasing radiation dose, polymer chain scission seems to be the predominant mechanism during electron irradiation of agarose instead of crosslinking.

3.2. Swelling

Weight monitoring during the following procedure of irradiation, subsequent swelling of the samples for 24 h and air drying for 72 h, was used to determine the volume-related swelling ratio, as given in Eq. (1). The swelling ratio, as shown in Fig. 3, is rapidly increasing at electron

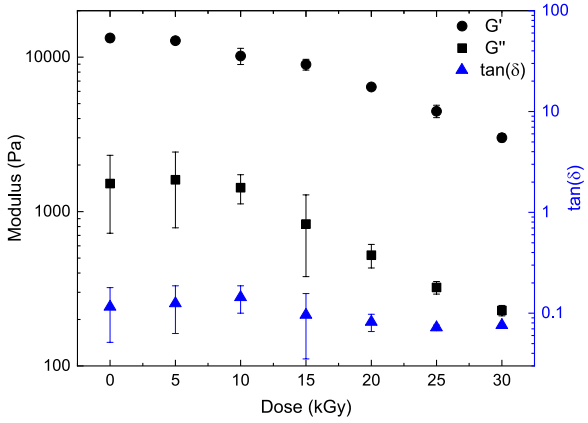

Fig. 1. Rheological characteristics; storage modulus G', loss modulus G'' and dissipation factor tan(δ) of 1 wt.% agarose hydrogels measured at a strain of 1% and a frequency of 1 Hz in dependence on electron radiation dose. Error bars indicate standard deviation.

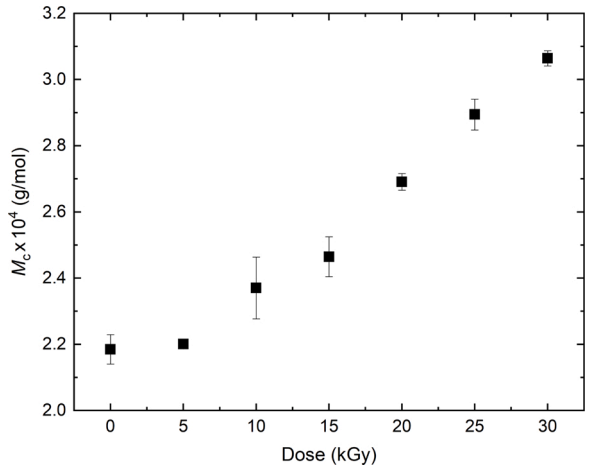

Fig. 2. Number average molecular weight between crosslinks M_c calculated using Eq. (5) vs. irradiation dose. Error bars represent the standard deviation.

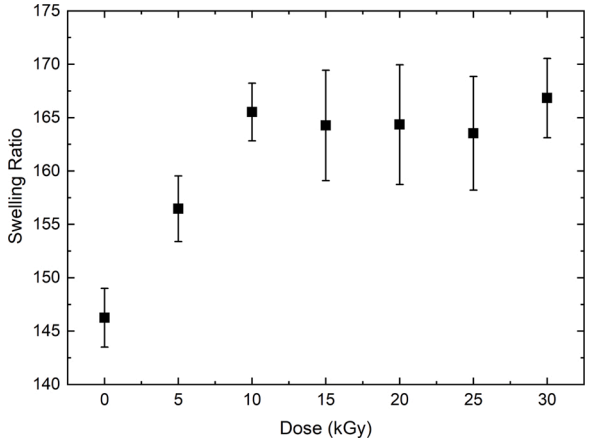

Fig. 3. Volume-related swelling ratio vs. irradiation dose after immersion in water for approx. 24 h, calculated using Eq. (1). Error bars indicate standard deviation.

doses up to 10 kGy and remains constant at further increasing doses. Higher swelling ratios correspond to an increasing mass between crosslinks, which corresponds to the former results of this study indicating that degradation is the predominant mechanism during high-energy electron beam treatment of agarose hydrogels.

3.3. Chemical structure and radiolysis

Fig. 4 illustrates the FTIR-ATR spectra of agarose treated with different irradiation doses. The bands at 771 cm^{-1} and 931 cm^{-1} are attributed to the C-O stretching vibrations of the 3,6-anhydro-galacto-pyranose (Sekkal et al., 1993). The peak at 888 cm^{-1} corresponds to C-H bending vibrations of β-anomeric galactopyranosyl units (Matsuhiro, 1996). IR bands at 1150 cm^{-1}, 1178 cm^{-1} and 1370 cm^{-1} are assigned to skeletal modes of the galactopyranose ring (Barsberg, 2010; Sekkal et al., 1993; Sekkal & Legrand, 1993). Locations and intensities of all these modes do not undergo significant alterations during electron irradiation indicating that the backbone configuration remains unaltered during electron beam treatment. The bands at 1636 cm^{-1} are assigned to the absorption of O-H bending vibrations, with significant amount originating from the remaining polymer bound water (Max & Chapados, 2009).

The strong bands at 1040 cm^{-1} to 1070 cm^{-1} are caused by the modes of C-O-C stretching of glycosidic linkage (Stanley, 1963) as well

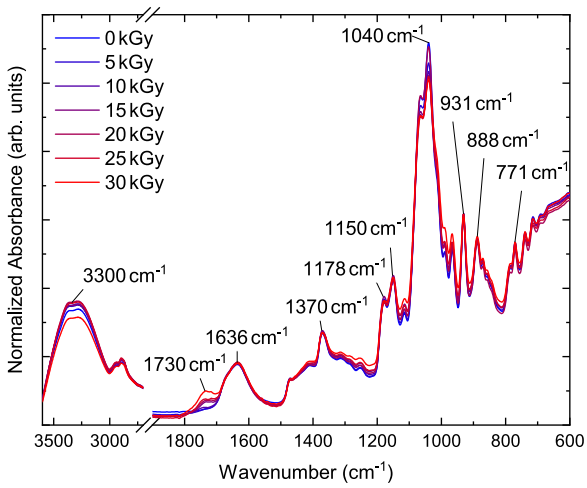

Fig. 4. FTIR-ATR spectra of dried 1 wt.% agarose irradiated with doses as indicated.

as coupling of C-O stretching and C-O-H bending from hydroxyl functions (Sekkal et al., 1993). With increasing irradiation dose, these bands decrease which can be attributed to an increasing amount of broken glycosidic bonds by radiation degradation. For higher radiation doses, an additional band at 1730 cm^{-1} occurs hinting to carbonyl C=O stretching. In literature (Wolfrom, Binkley, & McCabe, 1959), the glycosidic bond is reported to be sensitive to ionizing radiation. So far, radiation-induced scission of the glycosidic linkage is reported for numerous polysaccharides, such as cellulose (Charlesby, 1955; Wach, Mitomo, Nagasawa, & Yoshii, 2003), chitosan (Ulanski & Von Sonntag, 2000; Wasikiewicz et al., 2005) or sodium alginate (Wasikiewicz et al., 2005) and results in a reduction of the average molecular weight, as also observed in the here presented study. Von Sonntag (1980) and Von Sonntag, Bothe, Ulanski, and Adhikary (1999) reported on the radiation chemistry and reaction kinetics of neutral sugars in aqueous solution and pointed out that free $OH^{\bullet}$ and $H^{\bullet}$ radicals play an important role in the radiolysis of carbohydrates. According to their studies it is proposed that free $OH^{\bullet}$ and $H^{\bullet}$ radicals are formed by the radiolysis of water molecules and react with the C-O-C glycosidic bond by cleaving it to form carbonyl C=O groups confirming our results of increased carbonyl bands at 1730 cm^{-1}. In addition, the free $OH^{\bullet}$ and $H^{\bullet}$ radicals react with agarose bound H molecules in such a way that H_2, H_2O and agarose macroradicals form. This is in accordance with observations of γ-ray treated agarose hydrogels (Wang et al., 2015) and confirms our results of decreasing O-H stretching contributions at about 3300 cm^{-1} with increasing electron doses. Several substituents such as methyl ethers, sulfate esters and pyrucate ketals contribute to the IR spectra as well due to the extraction process from agar (Rochas, Lahaye, & Yaphe, 1986).

3.4. Gas formation

Electron beam treatment of agarose is accompanied by the formation of gas cavities in the hydrogels. Fig. 5(a) and (b) show representative images of 1 wt.% agarose hydrogels directly after irradiation with 10 kGy and 30 kGy, respectively. The amount and volume of gas cavities increases with increasing irradiation dose, whereas no cavities are observable at 0 kGy and 5 kGy. After irradiation, the gas diffuses slowly from the hydrogel, resulting in a decreasing volume of the gas cavities. A rupture remains in the network after complete diffusion. Fig. 5(c) shows phase contrast images directly taken after irradiation with 10 kGy (I.), 24 h after irradiation (II.) and 48 h after irradiation (III). In this case, after 48 h of monitoring, no further changes of the rupture were observed. This leads to irreversible destruction and might also contribute to the decrease in elastic properties observed in the rheological experiments, especially for higher doses of 30 kGy and above.

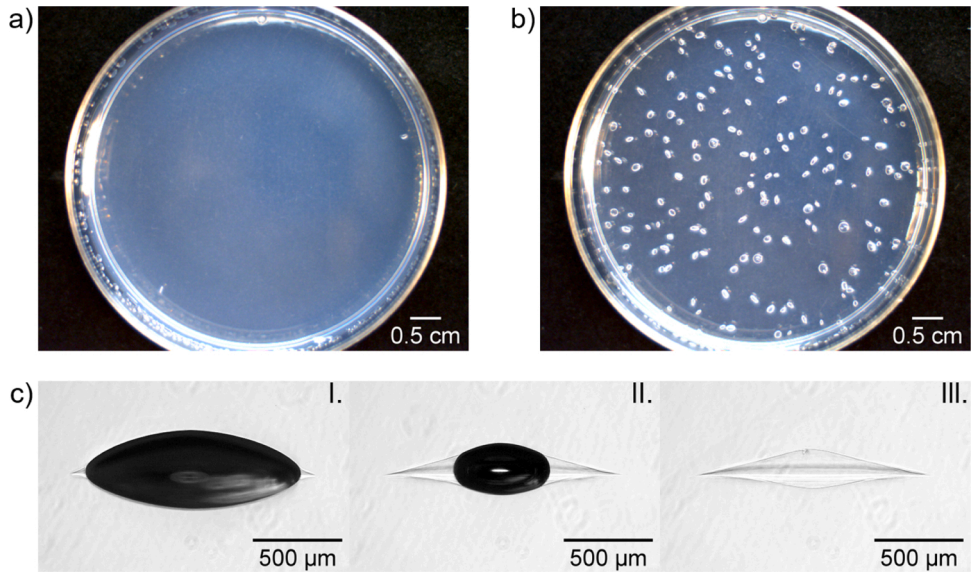

Fig. 5. Representative images of 1 wt.% agarose hydrogels irradiated with (a) 10 kGy and (b) 30 kGy. (c) Phase contrast images of a gas cavity in a 1 wt.% agarose hydrogel irradiated with 10 kGy directly after irradiation (I.), 24 h after irradiation (II.) and 48 h after irradiation (III.).

Fig. 6 illustrates the lifetime of the cavities in the hydrogels as a function of the irradiation dose. The time when no cavities are visible increases with increasing irradiation dose. This indicates, among with the previous observation of a rising number of gas cavities for a higher irradiation dose, that with an increasing dose, an increasing amount of gas is released during irradiation. No gas cavities are observable in agarose irradiated with 0 kGy and 5 kGy. Samples irradiated with 10 kGy show minimal gas cavity formation with a short lifetime of one day. With an increasing dose, the lifetime increases and finally reaches 36 days for samples irradiated with 30 kGy. The increasing error with an increasing irradiation dose may be explained by the relatively long time of storage. The gas cavity lifetime is strongly dependent on the ambient conditions like humidity and temperature, affecting the error for samples with long time of storage as well. Between the measurements, the samples were stored at 6 °C and covered by a lid to avoid drying out the hydrogels.

The determination of the formed gas was realized by FTIR measurements. For this purpose the gas of an evacuated desiccator was analyzed, which was filled with irradiated agarose samples over several days. Within this experimental setup we mainly detected CO_2 and water, as well as CO in smaller amounts, see Fig. 7. While the detection of water

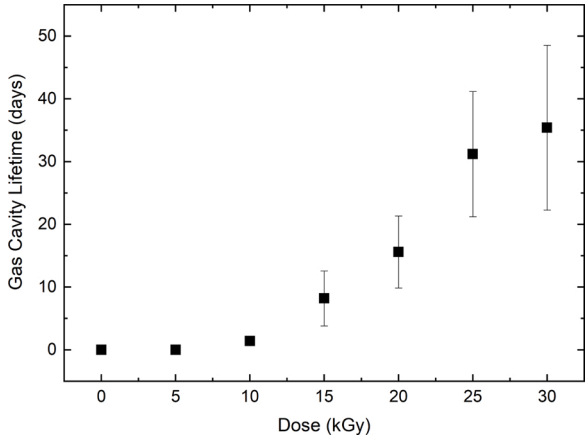

Fig. 6. Gas cavity lifetime (days after irradiation until no more gas cavities are detectable) of 1 wt.% agarose in dependency on the irradiation dose. Error bars correspond to the standard deviation.

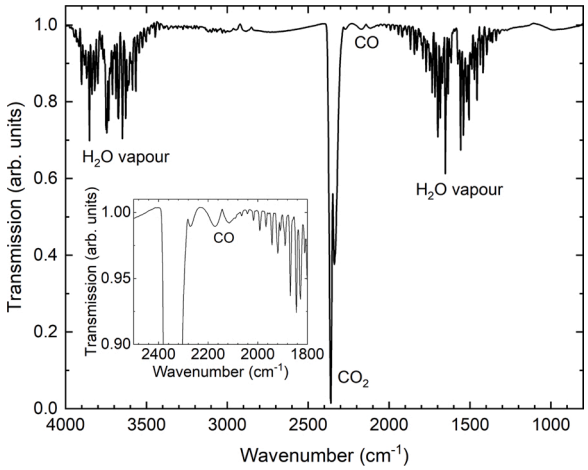

Fig. 7. FTIR measurements for the *ex situ* determination of the gas formation in 1 wt.% agarose hydrogels irradiated with 30 kGy. Therefore, the gas of an evacuated desiccator filled with the samples for more than two weeks was measured. The inset shows a detailed view of the IR bands to better illustrate the formation of CO gas.

is due to the evaporation from the hydrogel network, the presence of CO_2 and CO is attributed to gas formation during irradiation. In addition to CO_2 and CO, a small amount of H_2 and CH_4 gas release is also reported for γ-ray treated agarose (Wang et al., 2015). However, these gases are not observed in our *ex situ* experimental setup.

Harrass, Krüger, Möller, Albrecht, and Groll (2013) reported on the release of carbon dioxide and the formation of gas cavities during hydrogel crosslinking under atmospheric conditions. These authors produced polyethylene oxide-stat-propylene oxide (sPEOPO) hydrogels and observed CO_2 gas cavity formation during chemical crosslinking using N,N′-methylenebisacrylamide (MBAA) of sPEOPO molecules in water under atmospheric conditions. According to their findings, the gas cavity formation is avoided by crosslinking in an autoclave under increased pressure of 5 bar due to the increased solubility of CO_2 in water. In agreement with the study, we observe a decrease of gas cavity formation during electron beam treatment under high pressure conditions at 4 bar in a hyperbaric chamber, see Fig. 8.

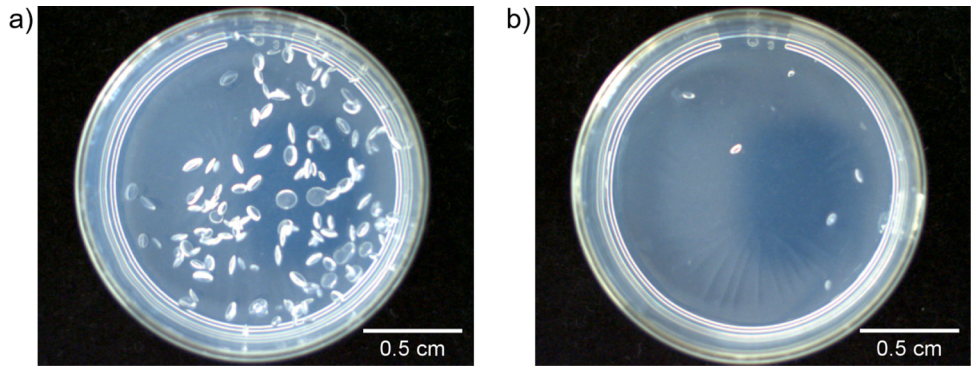

Fig. 8. Representative images of 1 wt.% agarose hydrogels irradiated with 20 kGy at (a) 1 bar and (b) 4 bar.

3.5. Hydrophilicity

Surface behavior of biomedical materials in an aqueous environment plays a specific role in the evaluation of its biocompatibility. The measurement of water contact angle is a common technique to obtain information about the surface hydrophilization and wettability of hydrogels. Fig. 9 shows water contact angle by sessile drop method in dependence on the irradiation dose. As expected for hydrophilic polymers, the untreated agarose gel-air interface exhibits a relatively low contact angle of $30.4^{\circ} \pm 1.6^{\circ}$ which is in good agreement to previous studies on 2 wt.% agarose and agar gels that are air exposed for more than 3 h (Van Oss, Zingg, Hum, & Neumann, 1977; Yasuda, Okuno, & Yasuda, 1994). First, electron beam treatment leads to larger water contact angles with a maximum of $36.6^{\circ} \pm 2.2^{\circ}$ at 5 kGy. With further increasing irradiation doses, the contact angle decreases and finally results in $22.2^{\circ} \pm 3.3^{\circ}$ at 30 kGy corresponding to a reduction of more than 25% compared to the untreated samples. Ionizing irradiation affects the pore size and surface roughness of hydrogels (Ramana Ramya, Thanigai Arul, Sathiamurthi, Asokan, & Narayana Kalkura, 2016) and leads to formation of hydrophilic groups at the sample surface, both influencing the contact angle. Hydrophilic groups are formed via radiation induced generation of macroradicals on the polymer chain resulting in polar groups, such as carbonyl, carboxyl and hydroxyl groups (Abdul-Kader, Turos, Radwan, & Kelany, 2009), which increase hydrophilicity. This behavior is similarly observed for γ-irradiated polyethylene (Costa et al., 1998) and agarose-gelatin-hydroxyapatite composite gels (Ramana Ramya et al., 2016).

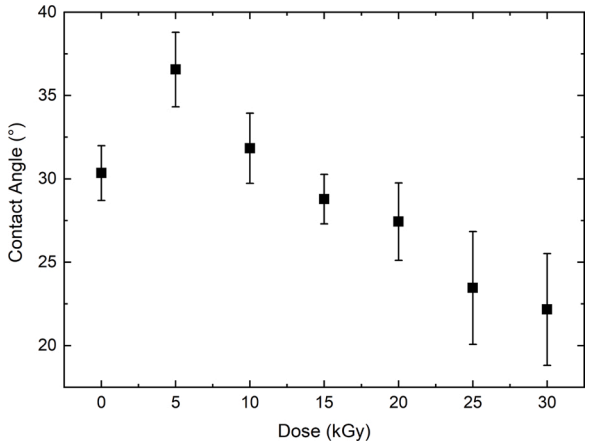

Fig. 9. Water contact angle of 1 wt.% agarose hydrogels by conventional sessile drop method depending on irradiation dose. Error bars represent the standard deviation.

4. Conclusion

The objective of this study is to evaluate the effect of high-energy electron irradiation treatment on the physical, structural and chemical properties of agarose hydrogels. Since electron beam treatment is a common sterilization method, typical sterilization doses of up to 30 kGy were investigated. In this dose range, viscoelastic properties of agarose hydrogels decreased by more than 20%, indicating a radiolytic degradation. Chemical analysis via FTIR shows a weakening of glycosidic linkage and the formation of carbonyl containing species. Formation of CO_2 and CO during irradiation was observed. This gas formation leads to the development of gas cavities within the network resulting in incurable ruptures. This network damage might additionally weaken the materials' mechanical stability. However, the formation of gas cavities can be significantly reduced by electron irradiation under high pressure conditions of 4 bar. Nevertheless, evaluation of FTIR-ATR measurements reveals no significant alterations of the agarose backbone configuration during electron beam treatment, especially for low doses and up to 30 kGy. This indicates that agarose, which is a component of many biomedical products, is radiation resilient enough to be applicable. In addition, the number average molecular weight between crosslinks was calculated using rubber elasticity theory and increases with increasing radiation dose. Considering increasing swelling ratio with increasing radiation dose and the data obtained from rheology measurements, the presented study confirms the predicted radiolytic degradation of agarose under electron irradiation. Finally, contact angle tests using the common sessile drop method demonstrate surface modification with a dose-dependent hydrophilicity.

High-energy electron irradiation is demonstrated to be a precise and powerful tool to tailor the material properties of agarose hydrogels in low doses, which are mostly applied for the sterilization and modification of biomedical products. Based on these investigations, we evaluate agarose irradiated with low doses up to 30 kGy as electron radiation resistant enough to be employed for biomedical products. The applicability of agarose in biomedicine is only limited by its radiation induced degradation at high electron doses.

Next steps will focus on the impact of the high pressure during electron irradiation on the agarose network structure and its dependency on gas cavity formation.

Author contributions

Catharina Krömmelbein: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization. Martin Mütze: Validation, Investigation, Writing - review & editing. Robert Konieczny: Investigation, Writing - review & editing. Nadja Schönherr: Investigation, Writing - review & editing. Jan Griebel: Validation, Investigation, Writing - review & editing. Wilhelm Gerdes: Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition. Stefan G. Mayr:

Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition. Stefanie Riedel: Conceptualization, Methodology, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Conflict of interest

The authors declare that there is no conflict of interest.

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