Research Article

Synthesis and Performance of Hybrid Hydrogels Loaded with Methylene Blue and Its Use for Antimicrobial Photodynamic Inactivation

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Received 8 October 2020; Revised 29 October 2020; Accepted 31 October 2020; Published 23 November 2020

Academic Editor: Manuela Curcio

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Development and characterization of hybrid hydrogels loaded with methylene blue, which are designed to apply for photodynamic therapy, are presented. Hybrid hydrogels were synthesized by grafting polyacrylamide onto dextran/dextran sulfate sodium salt using N,N′-methylene-bis-acrylamide as a cross-linker. The differences in microstructure of synthesized hydrogels were proved by scanning electron microscopy. FTIR spectra testify that the chemical nature of hydrogel components affects the hydrogel hydrophilicity. The swelling properties of hydrogels in water and absorption/desorption hydrogels’ ability towards methylene blue were studied. It was shown that dye sorption was dependent on the hydrogel type. The hydrogel based on dextran and polyacrylamide revealed the highest ability to release absorbed dye. The bactericidal effect of this hydrogel loaded with methylene blue and activated by red light in suspension and solid medium of S. aureus was tested. The increase of bactericidal activity of hybrid hydrogel was dependent on radiation doses.

1. Introduction

In recent years, hydrogels have received increasing attention as a possible means of wound therapy [1]. These porous networks as new drug carriers for photosensitive drugs have increased research attention because of its water solubility, high stability, and biocompatibility. Hydrogels are water-swellable materials having a three-dimensional polymeric network possessing both the mechanical properties of solids and diffusive transport of liquids. They have microscopic pores and elasticity, which provide their water-holding and swelling capacity. These properties made hydrogels promising materials for biomedical applications. Recently, polysaccharide-based hydrogels have got attraction in drug delivery system and dressing materials due to their extraordinary swelling properties, biodegradability, biocompatibility, and nontoxicity [2].

Bacterial infection usually occurs in burns, wounds, and lots of surgical operations. Microorganisms play an essential role in the development and maintenance of pathologies, and their removal during the wound healing or biomechanical preparation is important to the success of treatment. Hydrogels have been successfully used for antimicrobial drug delivery [3, 4]. In this case, reported freeze-dried chitosan/polyvinylpyrrolidone (PVP) hydrogel with the incorporated antibiotic amoxicillin and on carboxymethylcellulose -dextran hydrogel loaded by amphotericin could be useful for treatment of local antifungal infections, with or without concurrent systemic therapy. However, antibiotic resistance is a problem in dermatology [5]. It was shown that after long-term antimicrobial therapy, skin microbiota become resistant to the antibiotics used during the treatment, as well as to different groups of therapeutics drugs [6].

Increasing antibiotic resistance among pathogenic microbes and viruses is one of the most challenging issues in current medical research. One novel approach for the elimination of bacteria on different surfaces is to use a light-activated antimicrobial agent, which, when excited with light of an appropriate wavelength, namely, with red light,
generate singlet oxygen and/or radicals [7, 8]. Light-activated antimicrobial agents are used to treat or prevent infectious diseases and this process is known as photodynamic therapy (PDT). Antimicrobial photodynamic therapy provides an excellent alternative to antibiotics. Elaboration of known sensitizers and development of novel-efficient sensitizers are promising to highly produce reactive oxygen species, especially the most destructive hydroxyl radical. The next-generation materials for PDT underscore the importance for sensitizers delivery to the generation efficacy of reactive oxygen species which is a key to PDT success. [9]. To date, there is no fundamental report of bacterial resistance to reactive oxygen species, which highlights antimicrobial PDT as a promising method that can become a lead therapy in an antibiotic-sensitive and multiresistant bacteria treatment [10]. However, the use of a-PDT for the bactericidal effect requires that many variables be taken into account, including light parameters, photosensitizers (PS), and PS-drug delivery systems [11]. The optical photosensitizers should be actuated by red light at 630 nm and 700 nm wavelengths. Kashef et al. [12] studied the photodynamic inactivation influence on clinical resistant strains of E. coli using photosensitizer methylene blue (MB). MB (50 μg/mL) with laser light of red (163.8/cm²) is capable of reducing 37.6% in the number of drug-resistant E. coli (the initial number of bacteria was 104-105 CFU/mL). George and Kishen [13] reported that 10 mmol/L (MB), when irradiated for 20 minutes with a 30 mW laser, kills 30% of fibroblasts.

Recently, we have reported a class of hybrid dextran-polyacrylamide hydrogels [14]. These hydrogels can be designed to have optimal structure, good mechanical properties, shape stability, and softness similar to that of the soft surrounding tissue. It was shown that synthesized hydrogels exhibited the advantages in comparison with hydrogels based on linear polyacrylamide. In the present work, we focused on the creation and comparative study of the hydrogels with various structures and chemical nature. The hydrogels were loaded with light-activated photosensitizer methylene blue and their PDT microbial inactivation was studied.

2. Materials and Methods

2.1. Materials. Acrylamide (AA), cerium (IV) ammonium nitrate (CAN), and N, N-methylene-bis-acrylamide (MBA) were obtained from Aldrich. Dextran and dextran sulfate sodium salt were purchased from Fluka with Mw = 5 x 10⁵ g mol⁻¹ (designated as D500 and DSS500 throughout). Dextran samples, N, N-methylene-bis-acrylamide, and cerium salt were used without further purification. Acrylamide was twice recrystallized from chloroform and dried under vacuum at room temperature for 24 h. Methylene blue spectroscopic grades used in sorption studies were purchased from Fluka. Double-distilled water was used for polymerizations, swelling, and absorption experiments.

2.2. Hydrogel Synthesis. Cross-linked poly(acrylamide) and dextran-graft-poly(acrylamide) hydrogels were prepared by free radical cross-linking (co)polymerization of AA and D500/DSS500 in distilled water using CAN as an initiator. MBA was used as a cross-linking agent. Dextran (D500) or dextran sulfate sodium salt (DSS500) 0.02 mmol was dissolved in 25 mL water, and the solution was purged by argon bubbling for 25 min. Then, cerium (IV) ammonium nitrate (0.03 mmol mL⁻¹) was added, and after 2 min, acrylamide (0.2 mol) and N, N'-methylene-bis-acrylamide (0.2 w/w monomer) were added to the solution too. After bubbling argon for 2 min, the reaction mixture was left at room temperature for 2 h. The produced samples were washed several times with water to remove the uncross-linked polymer and unreacted monomers from hydrogels. The extracted gels were dried in a vacuum oven at 40°C to constant weight. The produced hydrogels were characterized by FTIR. The obtained hydrogels were marked as PAA, D500-PAA, and DSS500-PAA.

2.3. Swelling Measurements. The swelling behaviors of dried hydrogel samples were carried out by immersion in distilled water at room temperature. The gels were gently wiped and weighed to determine the water absorbed at various time intervals. The swelling studies were carried out until equilibrium in swelling was reached. Swelling measurements were done two or three times. The swelling ratio was calculated using the following equation:

\[ S_e\% = \frac{m_t - m_0}{m_0} \times 100, \]

where \( m_t \) is the weight of swollen hydrogel at time \( t \) and \( m_0 \) is the weight of the dry gel at time 0.

The equilibrium degree of swelling \( S_e \) after hydrogels had swollen to equilibrium in the swelling media was calculated using the following equation:

\[ S_{eq}\% = \frac{m_{eq} - m_0}{m_0} \times 100, \]

where \( m_{eq} \) is the weight of the swollen hydrogel sample at equilibrium.

The water absorbed by hydrogels is quantitatively represented by the equilibrium water content (EWC) [15]:

\[ \text{EWC}\% = \frac{m_{eq} - m_0}{m_{eq}} \times 100. \]

2.4. Characterization Techniques. The Fourier transform infrared (FTIR) spectra of the prepared hydrogels were recorded on a MAGNA 550 FTIR spectrophotometer (Nicolet Instruments Corporation, USA), using KBr pellets. Scanning electron microscopy (SEM) analysis was carried out using SEM Model Stereoscan 440 (LEO), Cambridge, UK instrument. The cryogenically fractured film in liquid nitrogen was mounted vertically on the SEM stub by silver adhesive paste. The specimens were sputter-coated with gold to avoid electrostatic charges and to improve image resolution before being examined by the electron microscopy.
2.5. Preparation of the Dye Solutions. Methylene blue ($\lambda_{\text{max}}$: 663 nm, molecular formula: C$_{16}$H$_{18}$N$_3$SCl, nature: basic blue, and molecular weight: 319 g mol$^{-1}$) was used as an adsorbate and was not purified prior to use. An accurately weighed quantity of the dye (0.1 g) was dissolved in double-distilled water to prepare a 0.1 g L$^{-1}$ stock solution. Experimental solutions of various concentrations were prepared by further dilutions of the stock solution. The absorbance of these dilute solutions was measured on a UV-visible spectrophotometer. Standard curves were prepared by plotting the absorbance values against the concentrations.

2.6. Absorption/Desorption Experiments. Prior to the dye absorption, hydrogels were immersed in water for at least 24 h to achieve the swelling equilibrium.

In absorption experiments, the swollen cubic hydrogel samples with edge 1 cm (1 g) were immersed in 29 mL dye solutions of a concentration of 0.7 $\times$ 10$^{-5}$ mol L$^{-1}$. The samples were removed from the dye solution every 15 minutes during the first hour and then, in 30, 60 minutes, and 24 hours, respectively. Absorption/desorption experiments were carried out at room temperature in cylindrical glass vessels by using batch conditions.

In 24 hours of dye absorption, the hydrogels were immersed in 4 mL of distilled water. At certain times, they were removed from the water. The experiments were made at room temperature. The absorption/desorption of dye was monitored through the analysis of the immersion solutions. The change in solution absorbance was monitored using a UV-vis spectrophotometer ($\lambda_{\text{max}}$ = 663 nm).

All of the experiments were performed in triplicate, and the average was used in this work. Dependence of absorption/desorption capacities on time was determined, and absorption/desorption kinetics was investigated in detail.

The amounts of absorbed dye per unit volume of absorbent [16] at time $t$ ($Q_t,$ mol cm$^{-3}$) and at equilibrium ($Q_{eq}$, mol cm$^{-3}$) were calculated by using the following expressions:

$$Q_t = \frac{(C_0 - C_t) \times V_1}{V_2},$$

$$Q_{eq} = \frac{(C_0 - C_{eq}) \times V_1}{V_2},$$

where $C_0$ and $C_{eq}$ are the initial and equilibrium concentrations of dye (mol L$^{-1}$), respectively, and $C_t$ is dye concentration at time $t$; $V_1$ is the volume of the solution added (L) and $V_2$ is the volume of swollen polymer (cm$^3$).

2.7. UV-Vis Spectroscopy. UV-visible spectra of dye solutions were recorded by using a Lambda 35 UV-Vis spectrophotometer (PerkinElmer, CA) in the absorbance mode (range 200–1000 nm), at the absorption maximum of the methylene blue ($\lambda_{\text{max}}$ = 668 nm). In this case, the greater the absorbance, the higher the dye concentration. The concentration of dye in solution did not exceed 0.25 $\times$ 10$^{-3}$ wt. %, to prevent dimerization of the dye and the appearance of an additional maximum of absorption. All optical spectra were acquired using quartz cuvettes with 1 cm path length.

2.8. Dynamic Study of Swelling and Dye Absorption/Desorption. To examine the swelling and absorption/desorption process, kinetic models were used [17]. The equations were expressed as follows:

The pseudo-first-order equation for swelling is

$$\ln(m_{eq} - m_t) = \ln m_{eq} - k_t,$$

and for the absorption/desorption process:

$$\ln(Q_{eq} - Q_t) = \ln Q_{eq} - k_t,$$

where $k$ (min$^{-1}$) is the rate constant swelling or absorption/desorption process.

2.9. Antibacterial Studies. LIKA-Led (Photonics Plus, Cherkasy, Ukraine) apparatus with laser emitters with a wavelength ($\lambda_{eq}$) of 660 nm was used. An irradiation with light from a 100 mW laser for up to 20, 30, and 40 min results in an energy density of 21 J/cm$^3$, 31.5 J/cm$^3$, and 42.1 J/cm$^3$, respectively.

The antimicrobial activity of methylene blue activated by red light was studied in the suspension of S. aureus (10$^5$ CFU mL$^{-1}$). The suspension of S. aureus (10$^5$ CFU mL$^{-1}$) was prepared in agar Muller-Hinton medium. 5 mL aliquots of the suspension were placed in tubes, and then, 5 $\mu$L methylene blue solution of 0.1 wt. % was added into suspension and incubated at 37°C throughout the experiment. As a result, dye concentration in the suspension of 10$^{-4}$ % was obtained. Both the suspension of S. aureus and suspension of S. aureus with methylene blue were irradiated with light (660 nm) from a 100 mW laser for up to 3 min, resulting in an energy density of 0–18 J/cm$^2$. Each test was compared to the control because every 1–3 h there was a multiplication of bacteria and a doubling of their number. The bactericidal effect was evaluated in percentage of CFU deaths relative to the control sample.

The antimicrobial activity of hydrogels loaded with methylene blue (0.0005 wt. %) in the suspension of S. aureus (10$^5$ CFU mL$^{-1}$) was studied. The hydrogel samples were placed into bacteria suspension in a mass ratio of hydrogel: suspension = 1:4. After 120 min, the equilibrium concentration of MB in the solution was equal to 0.0001 ± 0.00002% wt. %. Further irradiation with light (660 nm, 100 mW) was carried out. The bactericidal effect was evaluated in percentage of CFU deaths relative to the control sample.

Determination of colony-forming units (CFU) in bacterial suspensions was performed in the Goryaev chamber after staining an aliquot of the suspension with acridine orange with a final dye concentration of 0.001 wt. % [18]. CFU was determined using luminescence acridine orange at a wavelength of 530 nm.

A disk diffusion method was applied to study the antibacterial activity of the hydrogels on solid medium. Wild strains of Staphylococcus aureus were used as Gram-positive bacteria models in the test. Wild strains of bacteria were
obtained on an elective medium “yolk-salt agar” [19]. The sensitivity of the selected strains to the action of light and methylene blue was carried out on solid medium. A suspension of the bacteria (of approximately 10^5 CFU mL^{-1}) was prepared to a particular standard and then spread evenly onto Muller-Hinton agar on a Petri dish. Then, the Petri dish was divided into four sectors: for control (1), irradiation with light (2), for hydrogel saturated with methylene blue (3), and for hydrogel saturated with methylene blue and irradiated with light (4). The hydrogel samples and their composites with MB were cut to 5 mm side squares, placed on Petri dishes with agar. Then, sector (2) and sector (4) were irradiated with light (\lambda_{ex} = 660 nm). Then, the agar plates were placed in an incubating oven at 37 °C and left for 24 h.

The antimicrobial activity of MB-loaded hydrogels was assessed by analyzing the diameter of growth retardation [20]. All experiments were performed in triplicate and the mean average values were reported.

3. Results and Discussion

3.1. Preparation and Analysis of Cross-Linked Poly(acrylamide) and Dextran-Graft-Poly(acrylamide) Hydrogels. As was described in our previous papers [14], the hydrogel networks were synthesized via cross-linking free radical graft polymerization of AA to dextran backbone using MBA as a cross-linking agent. The cerium (IV) was used as an initiator of graft polymerization. The schematic representation of this process is demonstrated in Figure 1.

AA was grafted onto dextran (D500 or DSS500) to obtain branched macromolecules with various internal structures: star-like and brush-like, respectively (Figure 2). It was reported that dextran in nonionic form keeps the conformation of macrocoil in the grafting process. This conformation is stabilized by some internal cross-links after the addition of Ce (IV)—the initiator of radical copolymerization dextran with PAA [21]. The mechanism of Ce (IV) initiation involves the formation of chelate complex with OH group of dextran molecule. The decomposing of chelate complex generates free radicals and provokes the grafting of polyacrylamide chains to dextran as well as partial cross-linking of dextran macromolecule. Dextran sulfate is a strong polyelectrolyte. As it was mentioned by the manufacturer, DSS500 has 2-3 SO_3Na groups per dextran monolink. It means that DSS in water solution has fully expanded conforming and grafting process leads to the formation of brush-like structure [22, 23].

It should be noted that grafting on dextran resulted in star-like molecule formation, and in the case of dextran sulfate, brush-like molecule formed. After cross-linking of these macromolecules, the different structures of hybrid hydrogels could be obtained.

Additionally by the variation of amount of cross-linking agent added into reaction during grafting process of PAA to dextran and dextran sodium sulfate, the hybrid hydrogels with different cross-linking density could be obtained.

3.2. FTIR Analysis. FTIR spectra of the PAA, D500-PAA, and DSS500-PAA are shown in Figure 4. The broad absorption from 3600 to 2850 cm^{-1} corresponds to the stretching vibration of the hydrogen-bonded OH groups of dextran and dextran sulfate [25, 26]. The absorption maximum of water lies at 3400 cm^{-1}. The band from 2980 to 2880 cm^{-1} corresponds to the stretching vibrations of methyle and methylene groups (CH and CH_2 stretching modes) which are in all studied hydrogel [27]. All these bands largely overlap. Thus, this region of FTIR spectra cannot be used for comparative analysis of studied hydrogels.

For comparative experiments, the hydrogel based on individual PAA was synthesized. The microstructure of cross-linked hydrogels was examined by scanning electron microscope. Figure 3 demonstrates that studied hydrogels differ in the shape of pores. However, the mesh size does not exceed 1000 nm for all hydrogels. The hydrogels based on graft copolymers reveal the cone-shaped pores, and the pore structure differs in comparison with PAA hydrogel. The pores are more expanded for hydrogels based on DSS500-PAA. Thus, SEM results confirm a possibility to affect the internal hydrogel structure by means of grafting PAA to dextran or dextran sulfate.

The concentration of MBA for changing polymers mesh size and cross-linking density were varied for all synthesized hydrogels. These parameters affect the mechanical and swelling properties of hydrogels [14, 24].
The characteristic peaks of the PAA component for all hydrogels are registered at 1665 cm\(^{-1}\) (\(\nu\) (C = O), amide I) and 1615 cm\(^{-1}\) (\(\delta\) (N-H), amide II). The peak at 1450 cm\(^{-1}\) can be assigned to stretching vibrations in functional amide groups (\(\nu\) (C-N), amide III) [28].

Analysis of this region can give some information on the organization of PAA chains in the hydrogels. As it was mentioned above, the content of dextran or dextran sulfate component in the hydrogels is about 2.5% w/w. Thus, polysaccharide component plays the role of structure-forming component for the hydrogel synthesis process determining the route of PAA chain growth. The FTIR spectra analysis can prove that PAA chains do not form H-bond with dextran or dextran sulfate backbone for D500-PAA and DSS500-PAA hydrogels, as the position of amide I and amide II characteristic peaks for hybrid hydrogels are not shifted in comparison with linear PAA. This result proves that dextran is a structure-forming component of synthesized cross-linked samples.

Only the region below 1400 cm\(^{-1}\) is specific for dextrans [26]. The band from 1480 to 1130 cm\(^{-1}\) contains five peaks, which are located at 1460, 1410, 1350, 1325, and 1132 cm\(^{-1}\). This region is known to contain the vibrations of the in-plane bending modes of associated and monomeric alcohols and of CH and CH\(_2\) groups. For aliphatic alcohols, the bands of 1410 and 1350 cm\(^{-1}\) were assigned to in-plane deformation vibrations of hydrogen-bonded alcohols ("association bands"); the corresponding "monomeric" bands were thought to lie between 1330 and 1200 cm\(^{-1}\). The 1410-cm\(^{-1}\) vibration was assigned to both the deformation of C-OH groups and the deformation of CH and CH\(_2\) groups.

The strong 1267 peak may assume a high intensity in samples with a higher degree of hydration, such as ionized samples. In the FTIR spectrum of DSS500-PAA (in the form of sodium salt), the presence of the sulfo group (SO\(_2\)) can be determined by the presence of absorption bands at about 1267 cm\(^{-1}\) and 988 cm\(^{-1}\) originating from \(v_{as}\) (S = O) and \(v_s\) (S = O) vibrations, respectively.

3.3. Swelling Studies. The swelling behavior is an important characteristic of the hydrogels. Hence, dynamic swelling behavior of synthesized hydrogels in distilled water was studied and the results are shown in Figure 5.

As seen from Figure 5, on the initial stage, swelling occurs quickly for all samples, and then increase of swelling ratio with time depends on hydrogel chemical structure. On
the whole, swelling reaches a critical point after about 20 h. This value of percentage swelling may be named equilibrium swelling percentage (Seq). The swelling studies were carried out until equilibrium in swelling was reached.

The swelling parameters calculated from equations (2) and (3) are presented in Table 1. The data analysis represented in Table 1 indicates that mesh architecture modeling by PAA grafting onto dextran coil in noncharged or charged (sulfonated) form influences water absorption. The values of swelling ability at equilibrium state (Seq) for cross-linked D500-PAA samples are higher than the values for cross-linked PAA. It can be explained by the various mesh structures of hydrogels and the chemical nature of hydrogels. As can be seen in Figure 3, the pores in the hydrogels based on D500-PAA have cone shape. The enhanced swelling capacity of D500-PAA in comparison with PAA hydrogel is caused by the hydroxyl groups of dextran that can form hydrogen bonds with water molecules and improve the hydrophilicity of copolymer.

DSS500-PAA hydrogel containing dextran sulfate sodium salt (strong anionic polyelectrolyte) contributes to the enhanced swelling capacity of this hydrogel in comparison with D500-PAA one. With the swelling, Na\(^+\) ions move outside of the polymers more easily and the hydrophilic SO\(_3^−\) groups attract more water molecules. As a result, the water absorption rate of this hydrogel enhanced greatly (Figure 5 and Table 1).

### Table 1: Equilibrium swelling ratio (Seq), equilibrium water content (EWC), and rate constant of swelling (k) of the hydrogels.

| Sample    | Seq (%) | EWC (%) | k (min\(^{-1}\)) |
|-----------|---------|---------|-----------------|
| PAA       | 1530    | 94      | 0.5175          |
| D500-PAA  | 1833    | 94.6    | 0.6268          |
| DSS500-PAA| 2008    | 95.1    | 0.6771          |

3.4. Absorption/Desorption Studies. Methylene blue (Figure 6) is a pharmacological drug commonly applied as a photosensitizer in PDT applications due to the high quantum yield of singlet oxygen generation and the relatively low toxicity. The therapy is based on the energy (absorbed as light via the photosensitizer) transferred to the production of the high reactive singlet oxygen. As seen from Figure 6, between 550 nm and 800 nm, free MB has two characteristic absorbance bands, located at 664 nm and 600 nm, respectively. MB has the potential to treat a variety of cancers and non-cancerous diseases, with low toxicity and no side effects [29].

Our aim was to prepare the hydrogels loaded with MB having antibacterial efficacy. The absorption/desorption studies of hydrogels with various structures towards MB are presented in Figure 7.

At dye absorption/desorption studies, the effect of contact time on the absorption of MB into the prepared hydrogel at an initial dye concentration of 0.7 × 10\(^{-5}\) mol L\(^{-1}\) from 0 to 200 min was investigated. As depicted in Figure 7, the absorption increases with the increasing contact time, and rapid absorption of MB was observed during the initial 50 min.

The amount of absorbed MB increased rapidly in the early stage and then increased more slowly over extended periods, gradually approaching absorption equilibrium. This is due to the availability of active binding sites on the sorbent at the initial stage. With gradual occupancy of binding sites, the sorption became slower in the later stage. A contact time equal to 100 min was sufficient to reach equilibrium.

The concentrations of methylene blue in solution \(C_{MB}\) and the amounts of absorbed dye per unit volume of the hydrogel at equilibrium state \(Q_{eq}\) in 24 h of absorption are shown in Table 2.

As seen from Table 2, at the same initial dye concentration in the solution, the hydrogels show different \(Q_{eq}\) values. This phenomenon could be explained through the dye-gel interactions and gel morphology. Dye sorption, as it turned out, depends on the type of hydrogel. Presented in the hydrogel DSS500-PAA, the sodium sulfogroups SO\(_3^−\) dissociate to SO\(_3^−\) form, increasing the number of fixed ionized groups. This generates electrostatic repulsion forces among the adjacent ionized groups of polymer networks, inducing an expansion of the polymer chains within the hydrogel structure. In this logic, the formation of anionic complex between the positive charged MB molecules and the hydrogel networks is provided, increasing the dye absorption. These interactions occur more precisely between the charged dye groups and the charged groups of the polymer networks.

At the same time, after desorption of MB out of hydrogels, significant differences were observed (Table 2). This is clearly demonstrated in Figure 8, where photos of colored hydrogel samples after desorption in equilibrium state are shown.

An ionic complex between the positive charged MB molecules and the hydrogel DSS500-PAA networks leads to a significant decrease of released dye molecular. Unexpectedly, D500-PAA hydrogel which possesses lower absorption properties than PAA is able to release about 32%
amount of absorbed dye that is higher compared to PAA. This property may be useful in the delivery of light-activated antimicrobial agents in photodynamic therapy and provides the creation and maintenance of therapeutic concentrations of the active substance in the localization of the hydrogel.

3.5. Antibacterial Studies

3.5.1. Antibacterial Activity of Light in the Bacterial Suspension. The study of the antibacterial efficacy of red light (660 nm) against S. aureus in the bacterial suspension
indicates no bactericidal action (Figure 9). This is due to the low energy of light quanta and the absence of photosensitizer targets in bacterial cells. In contrast to ultraviolet or blue light with a wavelength of 380 nm and 490 nm, the red light with a wavelength of 660 nm is gentler for living tissues and is therefore more desirable for wound therapy. Nevertheless, infrared irradiation penetrates deeper into the body tissue than other types of light energy, which causes heating of the entire skin and subcutaneous tissue. The therapeutic effect of infrared radiation is determined by the mechanism of its physiological action, which is to accelerate the reversal of inflammatory processes and increase tissue regeneration, local resistance, and anti-infective protection.

3.5.2. Antibacterial Activity of Light-Activated MB in the Bacterial Suspension. It was also found that methylene blue at a concentration of 0.0001 wt. % in the bacterial suspension does not show bactericidal properties relative to the bacterial strains (Figure 9). Then, red light irradiation and MB of 0.0001 wt. % were combined and caused a 20% reduction in CFU with a small radiation dose of about 2 J/cm^3. An increase of energy density to 6 J/cm^3 causes a 60% inactivation of CFU. Further increase in the radiation dose does not contribute to the growth of bactericidal activity. This may be due to the adaptation of S. aureus culture to the conditions created, or the lack of oxygen or dye in solution.

3.5.3. Antibacterial Activity of Hydrogel Loaded with MB in the Bacterial Suspension. The study of the diffusion of methylene blue out of the hydrogel into the solution has showed the gradual release of the dye into the environment in a short time. This allows for a long time to provide the required concentration of the active substance in the suspension of pathogenic bacteria. Considering the obtained results, the bactericidal effect of hydrogel materials loaded with methylene blue in combination with visible light irradiation was investigated. D500-PAA of the lowest cross-linking density and highest ability to release of absorbed dye was chosen as MB-container. First of all, it was shown that individual components such as pure hydrogel, light irradiation at 6 J/cm^3, and methylene blue do not possess antibacterial ability in bacterial suspension (Figure 9). At the same time, complex action of hydrogel composite D500-PAA/MB and light irradiation resulted in a loss of 60% of the initial amount of CFU, with the incubation of the hydrogel in a solution of 40 min.

Moreover, it should be noted that the time of reaching the maximum bactericidal effect of MB in the solution is naturally higher than that contained in the hydrogel; it testifies that hydrogels loaded by MB possess a prolongation of dye activity.

3.5.4. Antibacterial Activity of Hydrogels Loaded with MB on Solid Medium. The above-described results of antibacterial studies in bacterial suspension indicate the antibacterial efficacy of composites based on cross-linked hydrogels loaded with methylene blue and activated by red light irradiation. Since such composites are promising materials for hydrogel dressings, it was interesting to study their antibacterial properties on the surface. Antibacterial efficacy is known to be caused by several factors such as the nature of the light-activated antimicrobial agent, their diffusion rate and concentration, hydrogel nature, and bacterial strains.

The bactericidal activity of hydrogel D500-PAA loaded with MB at low concentrations of $1 \times 10^{-3}$ and $1 \times 10^{-2}$ wt. %
against wild *Staphylococcus aureus* strains was investigated by the disc diffusion method (Figure 10). As seen from Figure 10, irradiation of hydrogel loaded with MB at $1 \times 10^{-3}$ wt. % with different light energy densities (660 nm) does not lead to appreciable bactericidal activity. Increasing the concentration of MB to $1 \times 10^{-3}$ wt. % increases the bactericidal activity and the increase depends on radiation doses.

Thus, the hydrogel composites loaded with methylene blue as light-activated antimicrobial agent and irradiated with red light (660 nm) possess bactericidal effect both in suspension and on solid media. It makes them promising materials for wound dressings and application for photodynamic therapy.

4. Conclusions

Hydrogels with various structures based on linear polyacrylamide, star-like copolymer dextran-graft-polyacrylamide, and brush-like dextran sulfate-graft-polyacrylamide have been synthesized. It was shown that the nature of polymer and types of internal mesh structure affect the resultant hydrogel properties. SEM study confirms the difference in the internal hydrogel structure. It was shown that D500-PAA and DSS500-PAA hydrogels have a cone-shaped pore structure and higher swelling capacity in comparison with PAA hydrogels. Moreover, DSS500-PAA cross-linked hydrogels possess the highest swelling capacity.

Obtained hybrid cross-linked hydrogels are considered as promising materials of a new generation for biomedical application. Methylene blue was used as a universal model of light-activated antimicrobial agent. Absorption/desorption process of dye into/out of the hydrogels was studied. The release of absorbed MB out of hydrogel was higher for the D500-PAA sample.

It was demonstrated that irradiation of wild strains of *S. aureus* with light at a wavelength of 660 nm has not antibacterial effect. Antibacterial activity of D500-PAA hydrogel composites loaded with methylene blue as light-activated antimicrobial agent and irradiated with red light was tested in suspension and solid medium against wild strains of *S. aureus*. The high antimicrobial efficacy was registered for both experiments. Thus, prepared hydrogel composites can be used as promising materials for wound dressings and photodynamic therapy.

Data Availability

The data on dynamic sorption/desorption studies used to support the findings of this study are available within the article. The data on antibacterial studies used to support the findings of this study are available from the corresponding author upon request and included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

References

[1] Q. Chai, Y. Jiao, and X. Yu, “Hydrogels for biomedical applications: their characteristics and the mechanisms behind them,” *Gels*, vol. 3, no. 1, 2017.

[2] R. Parhi, “Cross-linked hydrogel for pharmaceutical applications: a review,” *Advanced Pharmaceutical Bulletin*, vol. 7, no. 4, pp. 515–530, 2017.

[3] A. Smith, “Biofilms and antibiotic therapy: is there a role for combating bacterial resistance by the use of novel drug delivery systems?” *Advanced Drug Delivery Reviews*, vol. 57, no. 10, pp. 1539–1550, 2005.

[4] S. P. Hudson, R. Langer, G. R. Fink, and D. S. Kohane, “Injectable in situ cross-linking hydrogels for local antifungal therapy,” *Biomaterials*, vol. 31, no. 6, pp. 1444–1452, 2010.

[5] T. Dai, Y.-Y. Huang, and M. R. Hamblin, “Photodynamic therapy for localized infections-State of the art,” *Photodiagnosis and Photodynamic Therapy*, vol. 6, no. 3–4, pp. 170–188, 2009.

[6] S. B. Levy and B. Marshall, “Antibacterial resistance worldwide: causes, challenges and responses,” *Nature Medicine*, vol. 10, pp. 122–129, 2004.

[7] P. S. Zolfaghari, S. Packr, M. Singer et al., “In vivo killing of *Staphylococcus aureus* using a light-activated antimicrobial agent,” *BMC Microbiology*, vol. 927 pages, 2009.

[8] M. Wilson, T. Burns, and J. Pratten, “Killing of *Streptococcus sanguis* in biofilms using a light-activated antimicrobial agent,” *Journal of Antimicrobial Chemotherapy*, vol. 37, no. 2, pp. 377–381, 1996.

[9] H. Mahmoudi, A. Bahador, M. Pourhajibagher, and M. Y. Alikhani, “Antimicrobial photodynamic therapy: an effective alternative approach to control bacterial infections,” *Journal of Lasers in Medical Sciences*, vol. 9, no. 3, pp. 154–160, 2018.

[10] B. Khurana, P. Gierlich, A. Meindl, L. C. Gomes-da-Silva, and M. O. Senge, “Hydrogels: soft matters in photomedicine,” *Photochemical & Photobiological Sciences*, vol. 18, no. 11, p. 2613, 2019.
[11] N. S. Soukos, P. S.-Y. Chen, J. T. Morris et al., “Photodynamic therapy for endodontic disinfection,” Journal of Endodontics, vol. 32, no. 10, pp. 979–984, 2006.

[12] N. Kashef, G. Ravaei Sharif Abadi, and G. E. Djavid, “Phototoxicity of phenothiazinium dyes against methicillin-resistant Staphylococcus aureus and multi-drug resistant Escherichia coli,” Photodiagnosis and Photodynamic Therapy, vol. 9, no. 1, pp. 11–15, 2012.

[13] S. George and A. Kishen, “Advanced noninvasive light-activated disinfection: assessment of cytotoxicity on fibroblast versus antimicrobial activity against Enterococcus faecalis,” Journal of Endodontics, vol. 33, no. 5, pp. 599–602, 2007.

[14] O. Nadtoka, N. Kutsevol, V. Krysa, and B. Krysa, “Hybrid polyacrylamide hydrogels: synthesis, properties and prospects of application,” Molecular Crystals and Liquid Crystals, vol. 672, no. 1, pp. 1–10, 2018.

[15] D. Saraydhn, E. Karadag, Y. Işıkver, N. Şahiner, and O. Güven, “The influence of preparation methods on the swelling and network properties of acrylamide hydrogels with cross-linkers,” Journal of Macromolecular Science. Part A, vol. 41, no. 4, pp. 419–431, 2004.

[16] A. G. Ibrahim, F. A. Hai, H. A. Wahab, and H. Mahmoud, “Synthesis, characterization, swelling studies and dye removal of chemically crosslinked acrylic acid/acylamide/N,N-dimethyl acrylamide hydrogels,” American Journal of Applied Chemistry, vol. 4, no. 6, pp. 221–234, 2016.

[17] C.-W. Wong, J. P. Barford, G. Chen, and G. McKay, “Kinetics and equilibrium studies for the removal of cadmium ions by ion exchange resin,” Journal of Environmental Chemical Engineering, vol. 2, no. 1, pp. 698–707, 2014.

[18] C. Camacho-Fernández, D. Hervás, A. Rivas-Sendra, M. P. Marin, and J. M. Seguí-Simarro, “Comparison of six different methods to calculate cell densities,” Plant Methods, vol. 14, p. 30, 2018.

[19] L. M. Carantonis and M. S. Spink, “A selective salt egg agar medium for pathogenic staphylococci,” The Journal of Pathology and Bacteriology, vol. 86, no. 1, pp. 217–220, 1963.

[20] M. Lehtopolku, P. Kotilainen, P. Puukka et al., “Inaccuracy of the disk diffusion method compared with the agar dilution method for susceptibility testing of Campylobacter spp,” Journal of Clinical Microbiology, vol. 50, no. 1, pp. 52–56, 2012.

[21] N. Kutsevol, T. Bezugla, M. Bezuglyi, and M. Rawiso, “Branched dextran-graft-polyacrylamide copolymers as perspective materials for nanotechnology,” Macromolecular Symposia, vol. 317-318, no. 1, pp. 82–90, 2012.

[22] N. Kutsevol and T. Bezugla, “Insuflence of structural peculiarities of dextran sulphate-g-polyacrylamide on flocculation phenomenon,” Ecological Chemistry and Engineering, vol. 2, no. 2, pp. 251–256, 2012.

[23] M. Bezuglyi, N. Kutsevol, M. Rawiso, and T. Bezugla, “Water-soluble branched copolymers dextran-polyacrylamide and their anionic derivates as matrices for metal nanoparticles in-situ synthesis,” Chemik, vol. 66, no. 8, pp. 862–867, 2012.

[24] O. Nadotoka, N. Kutsevol, A. Onanko, and V. Neimash, “Mechanical and thermal characteristics of irradiation cross-linked hydrogels,” Nanochemistry, Biotechnology, Nanomaterials, and their Applications, pp. 205–214, Springer, Berlin, Germany, 2018.

[25] A. N. J. Heyn, “The infrared absorption spectrum of dextran and its bound water,” Biopolymers, vol. 13, no. 3, pp. 475–506, 1974.

[26] M. Cakić, S. Glišić, G. Nikolić et al., “Synthesis, characterization and antimicrobial activity of dextran sulphate stabilized silver nanoparticles,” Journal of Molecular Structure, vol. 1110, pp. 156–161, 2016.

[27] J. Coates, “Interpretation of infrared spectra, a practical approach,” Encyclopedia of Analytical Chemistry: Applications, Theory and Instrumentation, pp. 1–23, Wiley, Hoboken, NJ, USA, 2016.

[28] Z. Ye, X. Qin, N. Lai, Q. Peng, X. Li, and C. Li, “Synthesis and performance of an acrylamide copolymer containing nano-SiO2 as enhanced oil recovery chemical,” Journal of Chemistry, vol. 2013, Article ID 437309, 10 pages, 2013.

[29] J. P. Tardivo, A. Del Giglio, C. S. de Oliveira et al., “Methylene blue in photodynamic therapy: from basic mechanisms to clinical applications,” Photodiagnosis and Photodynamic Therapy, vol. 2, no. 3, pp. 175–191, 2005.