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Preparation of Biodegradable Cationic Polycarbonates and Hydrogels through the Direct Polymerization of Quaternized Cyclic Carbonates

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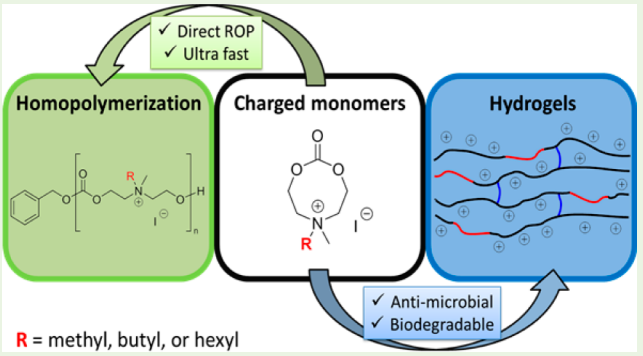
Supporting Information

ABSTRACT: Polymers exhibiting both antimicrobial and biodegradable properties are of great interest for next generation materials in healthcare. Among those, cationic polycarbonates are one of the most promising classes of materials because of their biodegradability, low toxicity, and biocompatibility. They are typically prepared by a chemical postmodification after the polymer has been synthesized. The main problem with the latter is the challenges of ensuring and verifying complete quaternization within the polymer structure. Herein, we report the first example of synthesizing and polymerizing charged aliphatic cyclic carbonates with three different alkane pendant groups (*N*-methyl, *N*-butyl, and *N*-hexyl) by ring-opening polymerization (ROP). These charged eight-membered cyclic carbonates displayed extraordinary reactivity and were even polymerizable in polar solvents (e.g., DMSO) and in catalyst free conditions that are generally unobtainable for other ring opening polymerization processes. A computational study was carried out and the findings were in agreement with the experimental data in regards to the dramatic increase in reactivity of the charged monomer over their neutral analogs. Furthermore, a series of hydrogels were prepared using the different charged eight-membered cyclic carbonates, and we found it to have a significant impact on the hydrogels' ability to swell and degrade in water. Finally, the hydrogels demonstrated antibacterial activity against *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive). These materials could be ideal candidates for biologically relevant applications where cationic structure is required.



KEYWORDS: *cyclic carbonates, antimicrobial, hydrogels, ring opening polymerization*

1. INTRODUCTION

Polycations or cationic polymers are commonly employed nowadays in many different applications such as household products (e.g., soaps and shampoos)[1] or flocculants for water purification.[2] Furthermore, they are being tested in biomedical applications such as drug delivery,[3,4] gene transfer,[5−9] and antimicrobial coatings.[10−19] Over the past decade, a considerable amount of attention in the scientific community has been given to the synthesis of cationic polymers with hydrolyzable polymer backbones. The main reason is to develop cationic polymers which are biodegradable and do degrade after doing its job depending on the application in the environment or in the body. This is usually made possible by including urethane, ester, or carbonate functional groups in the polymers' backbone, which can then readily hydrolyze under physiological conditions.[8,20−30] Among those, cationic polycarbonates are

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one of the most promising class of materials because of their fast biodegradability, low toxicity, and biocompatibility.[31]

The current method of preparing biodegradable cationic polycarbonates is via postchemical modifications made after the formation of the polymer. For example, Pascual et al., prepared cationic polymers via ring opening polymerization (ROP) of *N*-substituted eight-membered cyclic carbonates, which were then quaternized with methyl iodide.[32] After this postquaternization step, the polymers exhibited broad-spectrum antimicrobial properties against Gram-positive and Gram-negative bacteria. Similarly, Hedrick et al. prepared polycarbonate based cationic polymers by first polymerizing cyclic carbonates containing alkyl chloride side chains, which were then postfunctionalized with nitrogen containing reagents; the resulting cationic polymers were studied for antimicrobial applications.[33] The main drawback to such a process lies in the difficulty in obtaining and ensuring complete quaternization.[12,34,35] A more elegant method should rely on the "direct-polymerization" of monomers already bearing quaternary ammonium functional groups. This approach ensures a better control of the degree of quaternization in the polymer.[7,36]

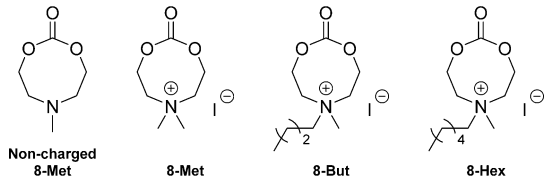
Upon evaluating the literature about the preparation of polycarbonates, they seem to be exclusively prepared via postpolymerization methods. We suspect this trend is due to the solubility of charged monomers in nonpolar solvents. Generally, polar solvents are usually overlooked for use in ring opening polymerizations (ROP) because they tend to coordinate with active species and inhibit the ROP process. Therefore, only a few examples have been presented in the literature performing the ROP in highly polar solvents such as DMSO or DMF.[37] In this work, we investigate the ring-opening polymerization of cationic N-substituted eight-membered cyclic carbonates. The final goal is to develop a process to produce cationic polycarbonate hydrogels with antimicrobial properties by a direct-polymerization approach.

2. RESULTS AND DISCUSSION

We have recently demonstrated that *N*-substituted eight-membered cyclic carbonates possess superior reactivity in comparison to 5 and 6-membered cyclic carbonates. Consequently, we envision that these monomer families could be better suited to be polymerized in polar solvents rather than five and six-membered cyclic carbonates. In this work, a series of cationic monomers were prepared with different alkyl pendant chains (methyl, butyl, and hexyl) starting from N-substituted eight-membered cyclic carbonates. Afterward, these monomers were homopolymerized and their reactivities were further studied with computational modeling. Hydrogels were prepared using the aforementioned monomers, and characterized with FTIR, water swelling, and rheology. Finally, the hydrogels were screened against both Gram-positive and Gram-negative bacteria to evaluate their use as antimicrobial agents.

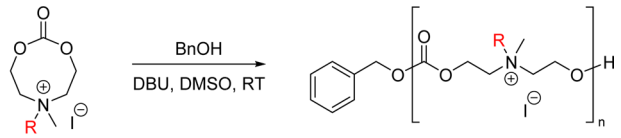
2.1. Monomer Synthesis and Polymerization. Noncharged eight-membered cyclic carbonates were synthesized using a ring closure process of diethanolamines with triphosgene, in the presence of triethylamine.[32,38,39] Three cyclic monomers with different alkyl chains were prepared. Subsequently, these monomers were quaternized with iodomethane to afford the quaternized version of the eight-membered cyclic carbonates with three different alkyl chains (i.e., **8-Met**, **8-But**, and **8-Hex**) with excellent isolated yields of ~93% (Scheme 1). The monomer structure was confirmed by ^{1}H and ^{13}C NMR spectroscopy (see the Supporting Information)

Scheme 1. Charged Aliphatic *N*-Substituted Eight-Membered Cyclic Carbonates Used in the Direct Preparation of Cationic Polycarbonates



The ability to promote the polymerization of the functionalized monomer via ROP in the presence of DBU was evaluated (Scheme 2). The organocatalyst DBU was used here based on

Scheme 2. Representative Scheme to Obtain Linear Homopolymers via ROP of Monomer 8-Met, 8-But, and 8-Hex



(8-Met, 8-But, 8-Hex)

past successful polymerizations of eight-membered cyclic carbonates.[38] The monomer **8-Met** was insoluble in many organic solvents available except for DMSO. Although DMSO is not considered the most suitable solvent to perform ROP, a series of homopolymerizations with **8-Met** at three different degrees of polymerization (DPs) (i.e., 50, 100, and 200) were carried out (Table 1, Entries 3, 4, and 5).

Table 1. Exploration of Homopolymerization of Charged Eight-Membered Cyclic Monomers via ROP

entry	monomer	target DP	DBU amt	time	% conv.	M_n by ^{1}H NMR
1	**Noncharged 8-Met**	50	5.00 mol %	1 week	34	
2	**8-Met**	50	(none)	1 day	>97	14 200
3			0.33 mol %	1 min	>97	14 900
4		100	0.33 mol %	1 min	>97	20 600
5		200	0.33 mol %	1 min	>97	39 300
6	**8-But**	200	1.00 mol %	1 min	>97	64 000
7	**8-Hex**	200	1.00 mol %	1 min	>97	71 200

After screening reaction conditions with different catalyst loadings, we found that 0.33 mol % of DBU was optimum for the **8-Met** monomer. It appeared that higher amounts of DBU allowed for different side reactions to take place (see the Supporting Information). Using ^{1}H NMR, the polymerizations were monitored by the disappearance of **8-Met**'s methylene protons adjacent to the carbonate (4.62 ppm) and their subsequent reappearance at 4.59 ppm (Figure 1). Monomer conversion was determined using relative peak integrations

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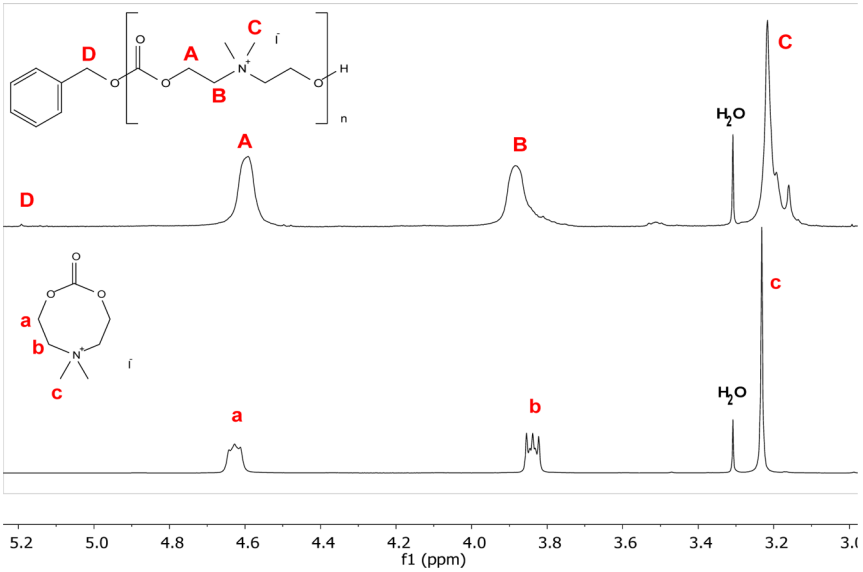

Figure 1. Homopolymerizations of **8-Met** (DP: 50) were carried out and characterized with ^{1}H NMR in d_6-DMSO. Depicted above is of the homopolymer, and below is the monomer **8-Met**.

values. Polymerization was also evidenced following the shift of the carbonyl by ^{13}C NMR from 153.26 to 152.92 ppm (see the Supporting Information). Further characterization by FTIR-ATR provided additional evidence of the formation of polycarbonate by the disappearance of the monomer's C═O stretching band at 1758 cm^{-1} and the appearance of a polycarbonate C═O band at 1751 cm^{-1}. A large new peak at 1247 cm^{-1} was also observed and identified as the C−O from the polycarbonate (see the Supporting Information).

All polymerizations listed in Table 1 for the charged monomers at different DPs reached full conversion incredibly fast, especially in comparison to the noncharged monomer. For the analogous **noncharged 8-Met** monomer, we were only able to observe 34% monomer conversion, despite using much more DBU (5.00 mol %) and longer reaction time of 1 week in DMSO (Table 1, Entry 1). Moreover, we observed that **8-Met** was reactive enough to polymerize by itself within 24 h without the presence of a catalyst, obtaining high molar masses calculated by ^{1}H NMR (Table 1, Entry 2). We attempted to characterize the polymers by SEC in various solvents (DMF, THF, and H_2O) but we were unable to obtain any data. We suspect that the polymers had either (1) degraded into smaller chains or (2) got stuck in the column. We also attempted a postpolymerization anion exchange with LiTFSI in H_2O. However, we were also unable to detect any presence of a polymer with our GPC-SEC setup.

Polymerizations of **8-But** and **8-Hex** were carried out in a similar fashion as in the previous section, see Table 1. At first, 0.33 mol % DBU was applied in each polymerization and we observed a decrease in monomer conversion with increasing length of the alkane pendant group. As a solution, DBU content was increased to 1 mol % and we were again able to realize full monomer conversions of **8-But** and **8-But** in 1 min reaction time. Polymerization were confirmed by ^{1}H and ^{13}C NMR (see the Supporting Information). We also attempted to characterize the by SEC but we were unable to obtain any data even using THF and DMF with salts (ie: LiBr and LiTFSI) at different concentrations (5 and 10 mM).

The solubility of all the cationic polycarbonates was surveyed in a range of organic solvents (Table S1). The polymers were consistently insoluble in solvents such as DCM, ACN, THF, and acetone. All synthesized polycarbonates were soluble in polar aprotic solvents such as DMF and DMSO. The solubility in water was mainly governed by the alkyl chain length. All polymers derived from **8-Met** were soluble in H_2O, however the polymers loses its solubility in water as the pendant group is extended to **8-But** and **8-Hex.**

From our homopolymerizations, we observed quite an extraordinary reactivity of the **8-Met** and the other charged monomers. We observed that **8-Met** was reactive enough to polymerize by itself within 24 h without the presence of a catalyst, obtaining high molar masses calculated by ^{1}H NMR (Supporting Information). Under the conditions used, complete monomer conversion of the **noncharged 8-Met** analog could not be obtained even when significantly more DBU was used. We have previously confirmed that the ring size matters in terms of reactivity,[40] but in this case a tremendous difference in terms of reactivity was found from charge to noncharge monomers in spite of the same ring size. Therefore, we decided to examine this system with a thorough computational study.

2.2. Computational Modeling. To gain a deeper insight into the reactivity of **8-Met**, DFT studies were carried out (the reported energy values correspond to free energies (G) and are given in kcal/mol; for further computational details, see the Supporting Information) with a special focus on the reasoning behind the high reactivity of the quaternary ammonium salts and the reactivity difference with their noncharged counterpart **8-Met**. Also, the role of DBU in enhancing the reaction rate was studied. The calculations were performed at the M062X/6-311+G(d,p) level with DMSO as the solvent (IEFPCM). Ammonium species **A-1** served as models for the experimental substrate **8-Met**. The neutral amine monomer **A-2** was also computed, and the two substrates (**A-1** and **A-2**) were taken as ground states (G = 0) of their respective energy profiles.

To understand the high reactivity of the ammonium salt **8-Met**, we compared its energy profile with that of the neutral species **A-2**. Similar to our previous mechanistic study on a related system,[2] the carbonate ring opening reaction was found to be a stepwise process (Figure 2). Thus, the initial

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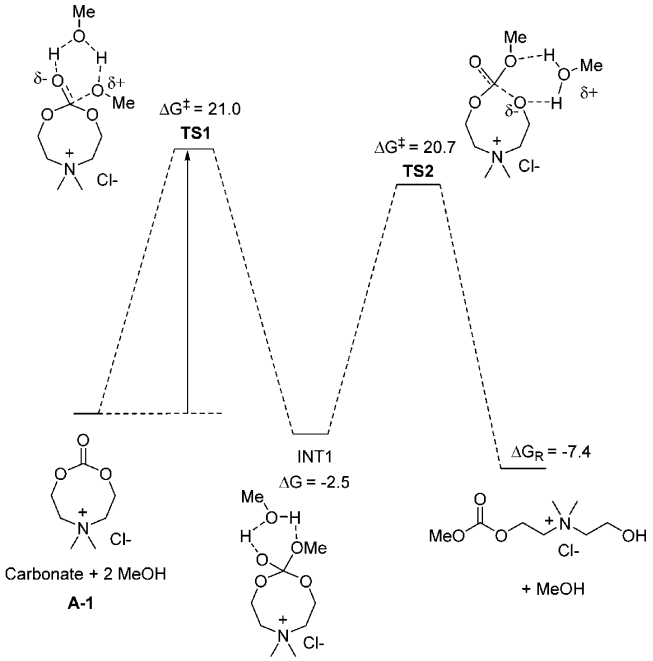

Figure 2. Reaction energy profile for substrate **A-1** and its Gibbs Free energies computed at M062*X*/6-311+G(d,p) (iefpcm, solvent = DMSO) level.

nucleophilic addition of methanol to **A-1** through **TS1** leads to the formation of the quaternized intermediate (**INT1**), followed by a ring opening (**TS2**) process to the open-chain monomeric product (Figure 2). If the calculations are performed in the presence of a single explicit molecule of methanol,[40] the activation energies are exceedingly high and unaffordable ($\Delta G^{\ddagger}$ = 41.7 kcal/mol), whereas the introduction of a second explicit molecule of methanol, like in Figure 2, seems critical for the correct solvation of the transition states, and thus, for the activation of the substrates, showing a large reduction of the activation barriers (**TS1**, $\Delta G^{\ddagger}$ = 21.0 kcal/mol). In fact, **TS1** and **TS2** are highly polar structures, with significant negative charge development around the oxygen atoms of the carbonate, especially the carbonyl group, whose double bond breaks during the initial attack. Therefore, the use of both explicit and implicit solvent systems seems to be determinant for the correct computational description of the systems, as well as for the experimental activation of the reaction. For **A-1**, the Gibbs Free energies of both steps are low and similar (**TS1**, $\Delta G^{\ddagger}$ = 21.0, **TS2**, $\Delta G^{\ddagger}$ = 20.7) (see Figure 3), contributing almost equally to the reaction rate.

On the other hand, the comparison of the cationic species N^{+}-Me_2 species (**A-1**) vs the neutral N-Me one (**A-2**) is also illustrative, because the former presents energy values in general 5−6 kcal/mol lower than the latter. This energy difference theoretically corresponds to a 1 × 10^4 times faster reaction rate, which is in fair agreement with the observed experimental rate increase for the ammonium salt. These findings confirm that the positive charge on nitrogen exerts a strong inductive effect through the carbon chain, making the remote carbonyl group highly electrophilic. Indeed, $-{}^{+}NR_3$ is known to present one of the strongest + I inductive effects.

Finally, the role of DBU was also checked, finding that in its presence the activation energy of the process is reduced in ca. 10 kcal/mol, becoming a really fast process at ambient conditions, as found experimentally. DBU binds and activates the incoming molecule of methanol, increasing its nucleophilicity, during the initial attack **TS1**.

2.3. Charged Polycarbonate Hydrogels. Because of the 3D structure of hydrogels, it is difficult to ensure total quaternization of the entirety of the hydrogel using the postquaternization method. Furthermore, it can be just as challenging to remove any unreacted quaternizing agent from the hydrogel.

In order to prepare polycarbonate hydrogels, charged monofunctional eight-membered cyclic carbonates bearing were directly copolymerized in DMSO with eight-membered dicyclic carbonate and PEG-diol, which served as a cross-linker and initiator, respectively. Again, DBU was employed to catalyze the ROP reactions (Figure 4A). The resulting hydrogels were named based on the charged eight-membered cyclic carbonate used in the synthesis (i.e., **Gel-8-Met**, **Gel-8-But**, and **Gel-8-Hex**).

The reaction was confirmed by FTIR-ATR after the removal of DMSO from the gels. Evidence of the formation of polycarbonate structure was noted by the disappearance of the monomers' C═O stretching band at 1758 cm^{-1} and the appearance of a C═O band at 1745 cm^{-1}, considered to be the polymer. Furthermore, a large new peak at 1244 cm^{-1} was identified as the C−O from the polymer (Figure 4). To further confirm the formation of 3D structures, the rheological behaviors of the gels at room temperature were also characterized using oscillatory tests (see the Supporting Information). We were able to realize the elastic modulus (G') to be greater than the viscous (G'') modulus for all the hydrogels. This $G' > G''$ behavior suggests that the gels that we obtained were covalently cross-linked. Furthermore, we continued to observe this behavior when we removed DMSO from one of the gels and reswelling it in water (100 wt %).

One particular characteristic of cationic hydrogels is their ability to swell in water without losing their three-dimensional structure due to their hydrophilicity. The level of swelling, responsiveness, and degradability are important features taken

	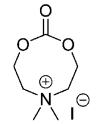	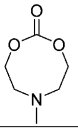
Name	**A-1**	**A-2**
TS1	ΔG‡ = 21.0	ΔG‡ = 26.6
INT1	ΔG = -2.5	ΔG = 3.1
TS2	ΔG‡ = 20.7	ΔG‡ = 23.5
Product	ΔG = -7.4	ΔG = -5.7

Figure 3. Comparison of the Gibbs Free energy values for the three different substrates, computed at M062*X*/6-311+G(d,p) (iefpcm, solvent = DMSO) level.

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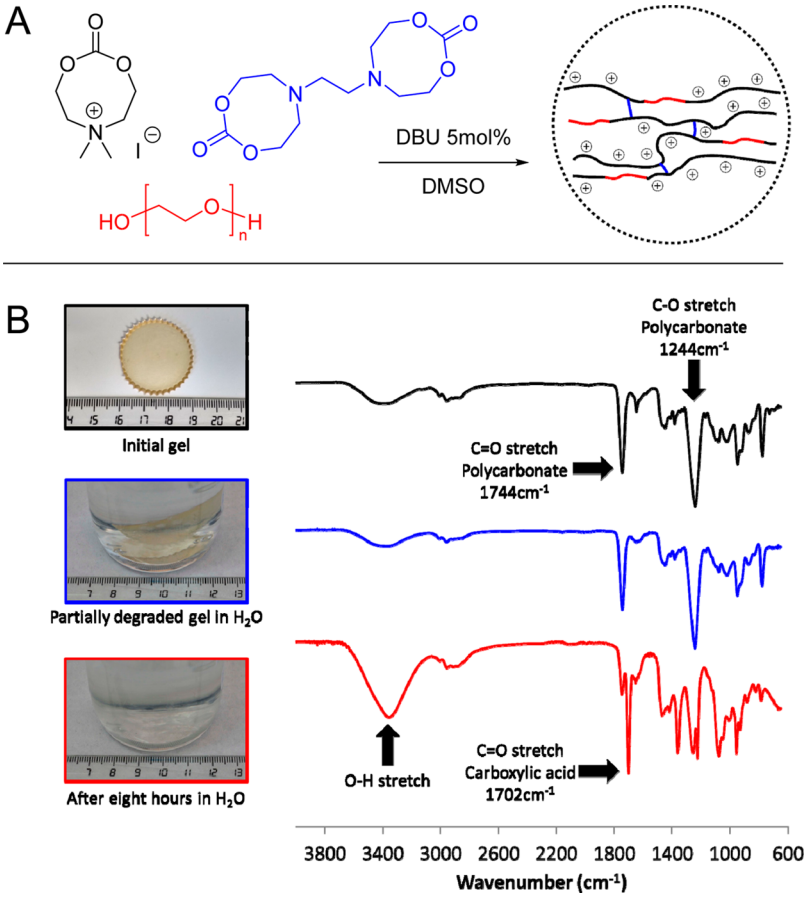

Figure 4. (A) Representative scheme used to create the polycarbonate ion gels using the **8-Met** monomer (black), bis(eight-membered dicyclic carbonate) (blue), and PEG (red). (B) We used FTIR-ATR to characterize the **Gel-8-Met** in its initial state (black) and after leaving the gel in water after 8 h at room temperature (red); an additional intermediary scan was also performed (blue). Polycarbonate signals at 1744 and 1244 cm^{-1} decreased with time in water, and carboxylic acid signal at 1702 cm^{-1} was observed upon the degradation of the polycarbonate.

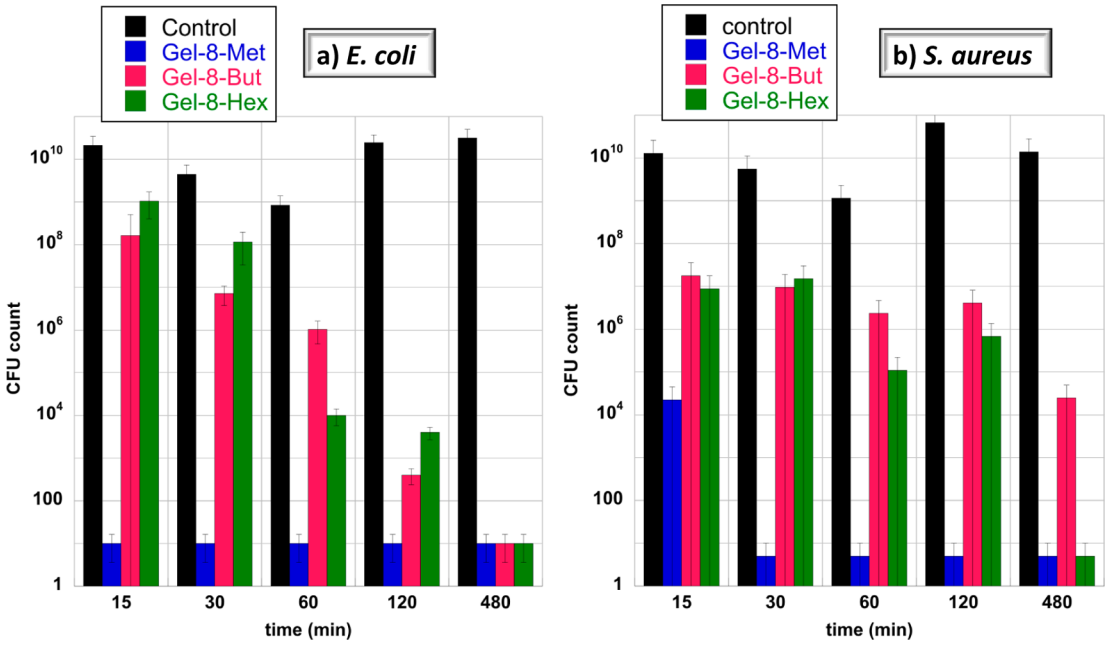

Figure 5. Antibacterial efficacy of the hydrogels measured at different times (15 and 30 min and 1, 2, and 8 h) against *E. coli* (left) and *S. aureus* (right). The CFU/mL values were calculated from counting the number of colonies on agar plates when different dilutions were plated.

into account when designing these hydrogels. Thus, the swelling behaviors of the gels were studied by submerging dried pieces of gels in water. The "swelling behavior" was influenced by the 'R' pendant group of the functional

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monocyclic carbonate used to create the hydrogels. As expected, lengthening the alkyl chain from methyl to hexyl increases the hydrophobicity, and consequently its ability to swell water was reduced from 600 wt % to 55 wt % (Table S2). Aliphatic polycarbonates are known to rapidly hydrolyze in the presence of water.[32] Thus, the degradation in water at 25 °C of the gels with different alkyl chains was evaluated. The gels' "time to degradation" was significantly prolonged from 8 h to 5 days by using monomers with longer alkane chains, (methyl < butyl ≪ hexyl) (Table S2). In our opinion, this is because the swelling decreased with longer alkane pendant groups, and water would have more difficulty degrading the carbonate linkage. The degraded gels were characterized by FTIR-ATR and were compared to its pristine initial state, shown in Figure 4. Upon degradation, the signals pertaining to the carbonate linkages decreased in intensity, whereas the carboxylic acid signal (C═O stretch, 1702 cm^{-1}) grew.[32] With these results, we can expect to engineer gels with a blend of the available monomers to achieve specific swelling and longevity in water properties.

2.4. Antibacterial and Hemolysis Study. The antimicrobial properties of the hydrogels were assessed against two frequent pathogens: *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive). These microbes are common pathogens that often manifest on dermal wounds and are typically treated by topical application of antibiotics. Hydrogels composed of the **Gel-8-Met**, **Gel-8-But**, and **Gel-8-Hex** monomers were used in this screening.

All hydrogels in the study significantly reduced the bacteria population after 1 h (Figure 5). **Gel-8-But**, the sample showing the least antibacterial activity against *E. coli*, was still able to reduce the number of viable bacteria by 2 orders of magnitude (from 1×10^8 to 1×10^6 CFU/mL) within 1 h. **Gel-8-Hex** showed stronger bactericidal activity against *E. coli*, with a reduction of approximately 5 orders of magnitude (from 1×10^9 to 1×10^4 CFU/ml). **Gel-8-Met** displayed the strongest antibacterial activity against *E. coli* of the three hydrogels, and was able to kill all the bacteria in the solution within 15 min. After 8 h of incubation, no bacterial growth of *E. coli* was detected for any of the hydrogels. The hydrogels also displayed bactericidal activity against *S. aureus*, although to a lesser degree compared to the *E. coli* trials. Similarly, the **Gel-8-Met** displayed the strongest antibacterial, followed by **Gel-8-Hex** and then **Gel-8-But**. The **Gel-8-Met** reduced the bacteria count from 10^4 to ∼7 CFU/ml in 30 min of incubation. The least active antibacterial gel, **Gel-8-But**, was able to reduce the *S. aureus* amount by about 1 order of magnitude within 1 h. This gel was able to further reduce by another 2 orders of magnitude after 8 h. Some studies have shown the killing efficiency is usually increases with increasing the alkyl chain length. In this particular case, the shorter the alkyl-chain displayed higher antibacterial activity.[19] In addition, considering that the activity was monitored relative to the weight of the material, the **Gel-8-Met** sample has the highest amount of cationic groups. In our opinion, the **Gel-8-Met** antibacterial efficiency can be explained because it has much higher solubility in aqueous media, which facilitates a faster contact with bacteria and thus enhancing its killing efficiency. In addition the Gel-8-Met possess higher cationic groups per weight enhancing the antimicrobial behavior. In comparison with works of Pascual et al.,[32] the gels in the presented work appear to exhibit faster and higher bactericidal. The gels in the aforementioned work were unable to completely eliminate both *S. aureus* and *E. coli* after an incubation period of 18 h, where as our **Gel-8-Met** successfully suppressed both bacteria within 30 min.

To get a better understanding of the antimicrobial mechanism scanning electron microscopy (SEM) images were taken before and after treating the microbes with the hydrogels. As shown in Figure 6, the surfaces of *E. coli* cells after hydrogel treatment for 8 h are highly distorted suggesting that bacteria were killed via membrane disruption.

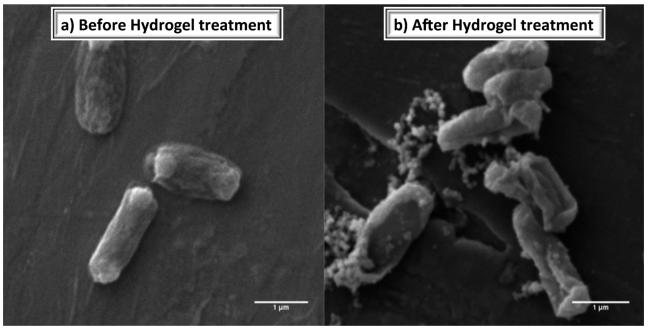

Figure 6. Scanning electron microscopy (SEM) images of *E. coli* were taken before (left) and after (right) 8 h of treatment with **Gel-8-Met**; scale bars corresponds to 1 μm. The micrograph after the hydrogel treatment suggests that the bacteria underwent membrane disruption.

Hemolysis studies were carried for the hydrogels, after 1 h approximately 80% of the red blood cells underwent lysis. The hydrogels that we prepared exhibited higher hemolysis than those prepared by Pascual et al.[32] probably because of the higher amounts of quaternized ammonium functional groups present within the gels. We believe that in the future, new hydrogels could be prepared by using a mixture of both charged and noncharged monomers to get a proper compromise between antimicrobial activity and hemolysis.

3. CONCLUSION

In this paper, we report the first example of synthesizing and the ROP of cationic aliphatic cyclic carbonates with three different alkane pendant groups (*N*-methyl, *N*-butyl, and *N*-hexyl). The charged monomers showed very high reactivity, and were even polymerizable in polar solvents (e.g., DMSO) and in catalyst-free conditions that are generally unfavorable for ROP processes. Complete monomer conversion in our polymerizations could be realized within a minute. A thorough computational study showed that the cationic cyclic monomers were indeed orders of magnitude more reactive than their noncharged analogs. This was in agreement to the experimental studies performed within this article. Polycarbonate gels were also prepared from the aforementioned monomers. The swelling and the biodegradability of the gels appeared to be highly dependent on the nature of the monomeŕs pendant group. These hydrogels also demonstrated bactericidal activity against *E. coli* and *S. aureus* by disrupting the membrane of the bacteria. With further tailoring of the gel's composition, new hydrogels based on this system could be prepared for future works in biodegradable broad-spectrum antimicrobial research activities and applications.

4. EXPERIMENTAL SECTION

4.1. Materials and Equipment. ^1H and ^{13}C NMR spectra were recorded with Bruker Avance DPX 300, Bruker Avance 400, or Bruker Avance 500 spectrometers. The NMR chemical shifts were reported as

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δ in parts per million (ppm) relative to the traces of nondeuterated solvent (e.g., δ = 2.50 ppm for d_6-DMSO or δ = 7.26 for $CDCl_3$). Data were reported as chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet, br = broad), coupling constants (J) given in Hertz (Hz), and integration. Fourier transform infrared−attenuated total reflection (FTIR-ATR) spectroscopy was performed with a ThermoFisher Nicolet 6700. Gel permeation chromatography (GPC-SEC) was performed using an Agilent Technologies PL-GPC 50 Integrated GPC system, with a Shodex KD-806 M column. For the GPC, *N*,*N*-dimethylformamide with a 10 mM concentration of LiBr at 50 °C was used as the solvent and toluene as a marker. Polystyrene of different molecular weights, ranging from 2100 to 1 920 000 g mol^{-1}, were used for the calibration of the GPC.

Triethylamine (≥99%), benzyl alcohol (99.8%), *N*-methyldiethanolamine (≥99%), *N*-butyldiethanolamine (≥99%), diethanolamine (≥98.5%), N,N,N′,N′-tetrakis(2-hydroxylethyl)ethylenediamine (technical grade), lithium bromide (≥99%), methyl triflate (≥97%), and 1,8-diazabicyclo[5.4.0]undec-7-ene (≥99%) were purchased from Sigma-Aldrich and used as-is. 1,8-Bis(dimethylamino)naphthalene (99%), 1-bromohexane (≥99%), potassium carbonate (ACS grade, anhydrous), sodium iodide (≥99%), magnesium sulfate (97%), iodomethane (99%), acetonitrile (extra dry), DCM (≥99.9%), diethyl ether (≥99.8%), acetone (≥99.5%) THF (≥99%), DMSO, and DMF (GPC grade) were purchased and used as-is from Fisher Scientific. Triphosgene was purchased from TCI and used as is. Bis-(pentafluorophenyl) carbonate (97%) was purchased from Manchester Organics and was used as-is. Deuterated solvents including $CDCl_3$ and d_6-DMSO were purchased from Deutero and were used as is.

4.2. Typical Eight-Membered Cyclic Carbonate Synthesis. *6-Methyl-1,3,6-dioxazocan-2-one (**Noncharged 8-Met**).* A 1L single neck round-bottom flask was charged with *N*-methyldiethanolamine (10.0 g, 83.9 mmol), triethylamine (17.4 g, 167.8 mmol), THF (600 mL), and a large oval stir bar. The mixture was then allowed to stir and cooled to −78 °C using a liquid nitrogen/acetone bath. This was then followed by a dripwise addition of triphosgene (8.3 g, 28.8 mmol) in THF (100 mL). Once all of the triphosgene has been added, the reaction was taken out of the liquid nitrogen/acetone bath and allowed to stir for an additional 2 h at room temperature. The reaction mixture was then filtered to remove the triethylammonium chloride salt and concentrated under vacuum. Cold diethyl ether (500 mL) was then added to help precipitate the remaining salts, which were then filtered again. The remaining filtrate was concentrated under vacuum to give the final product as a yellow oil (9.24g, 76% yield). ^{1}H NMR (500 MHz, DMSO): δ = 4.13 (t, CH_2, J = 5.3 Hz, 4H), 2.73 (t, CH_2, J = 5.3 Hz, 4H), 2.41 (s, CH_3, 3H). ^{13}C NMR (101 MHz, DMSO): δ = 155.36 (C═O), 68.71 (CH_2), 55.64 (CH_2), 44.39 (CH_3).

*6-Butyl-1,3,6-dioxazocan-2-one (**Noncharged 8-But**).* Reddish yellow oil (13.8 g, 88% yield). ^{1}H NMR (300 MHz, DMSO) δ 4.09 (t, J = 5.2 Hz, 4H), 2.73 (t, J = 5.3 Hz, 4H), 2.54 (t, J = 7.1 Hz, 4H), 1.43−1.30 (m, 2H), 1.30−1.15 (m, 2H), 0.86 (t, J = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 155.32, 69.02, 56.15, 53.55, 29.19, 19.64, 13.84.

*6-Hexyl-1,3,6-dioxazocan-2-one (**Noncharged 8-Hex**).* Brownish oil (15.5 g, 86% yield). ^{1}H NMR (300 MHz, DMSO) δ 4.08 (t, J = 5.3 Hz, 4H), 2.72 (t, J = 5.3 Hz, 4H), 2.58−2.44 (m, 2H), 1.36 (s, 2H), 1.23 (s, 6H), 0.86 (t, J = 6.9 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 155.29, 69.00, 56.45, 53.55, 31.21, 26.94, 26.11, 22.08, 13.84.

4.3. Quaternization of Cyclic Carbonates with Iodomethane. *6,6-Dimethyl-2-oxo-1,3,6-dioxazocan-6-ium iodide (**8-Met**).* A 16 mL vial was charged with 6-Methyl-1,3,6-dioxazocan-2-one (0.5 g, 3.44 mmol), ACN (2 mL), and a stir bar. Iodomethane (0.585g, 4.134 mmol) was then added dripwise and the reaction continued to stir for 2 h at room temperature. The products were precipitated with ether and dried under vacuum. A fine white powdered product was collected (0.92g, 93% yield). ^{1}H NMR (300 MHz, DMSO) δ 4.62 (t, J = 4.5 Hz, 2H), 3.90−3.81 (m, 4H), 3.25 (s, 6H). ^{13}C NMR (75 MHz, DMSO) δ 153.26, 65.47, 64.72, 51.75.

*6-Butyl-6-methyl-2-oxo-1,3,6-dioxazocan-6-ium iodide (**8-But**).* Yellow solid (0.81g, 93% yield). ^{1}H NMR (400 MHz, DMSO) δ 4.62 (s, 4H), 3.91−3.77 (m, 4H), 3.54−3.47 (m, 2H), 3.19 (s, 3H), 1.76−1.65 (m, 2H), 1.33 (h, J = 7.3 Hz, 2H), 0.93 (t, J = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 153.18, 64.62, 63.73, 63.53, 48.11, 23.50, 19.17, 13.44.

*6-Hexyl-6-methyl-2-oxo-1,3,6-dioxazocan-6-ium iodide (**8-Hex**).* Dark yellow solid (0.78g, 94% yield). ^{1}H NMR (400 MHz, DMSO) δ 4.73−4.54 (m, 4H), 3.93−3.77 (m, 4H), 3.56−3.46 (m, 2H), 3.19 (s, 3H), 1.79−1.62 (m, 2H), 1.35−1.21 (m, 6H), 0.86 (t, J = 6.4 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 153.17, 64.61, 63.89, 63.68, 30.52, 25.35, 21.76, 21.46, 13.76.

4.4. General Procedure for Homopolymerization. Monomer **8-Met** (574 mg, 2 mmol, 50 equiv) and benzyl alcohol (4.33 mg, 0.04 mmol, 1 equiv) were first dissolved in dried DMSO (1.5 mL). Later, DBU (3.04 mg, 0.02 mmol) was added to the reaction. A ^{1}H NMR sample was made after 1 min to confirm the complete conversion of the monomer to product. The crude polymer solution was precipitated in excess DCM and dried under vacuum to give a white powder (Yield: 0.41g, 71%).

4.5. General Procedure for Gel Preparation. A 12 mL vial was charged with a stirbar, and **8-Met** (0.632g, 2.2 mmol), eight-membered dicyclic carbonate[2] (0.120g, 0.42 mmol), and PEG_{1500} end-capped diol (0.030g) were dissolved in 1 mL of DMSO. Next, DBU (0.018g, 0.11 mmol) was added into the reaction which was then heated to 50 °C for 10 min. The contents of the vial were then poured out into silicone molds and allowed to cool to room temperature in N_2 atmosphere overnight. To remove the DMSO and catalysts, the gel was immersed in excess DCM for 1 day at room temperature. Lastly, the gels were dried under vacuum to give a white opaque gel.

4.6. Rheology. Rheology measurements on the gels were conducted on Anton Paar Physica MCR 101 rheometer using oscillatory tests with parallel plate geometry. Angular frequency sweeps from 0.0628 s^{-1} to 314 s^{-1} at constant strain amplitude (γ = 1%) were applied at 25 °C. Later, G' and G'' values were plotted versus frequency.

4.7. Swelling Test. Dried pieces of gels (∼100 mg) were immersed in deionized H_2O at room temperature. After a certain time, the gels were removed from the water and the excess water was gently removed from surface of the gel with a paper towel. The increase in mass was followed gravimetrically. The swelling degree (S_t) was calculated using the following formula

$$S_t(\%) = \frac{m_t - m_0}{m_0} 100$$

Where m_t is the mass of the gel after time t and m_0 is the initial mass of the dried gel.

4.8. Antibacterial Assays. *Escherichia coli* cells from C41 strain were grown overnight in Luria−Bertani (LB) media at 37 °C and constant shaking of 200 rpm. Dry hydrogels were cut into 5 mm^2 squares, weighted to ensure the same amount of material for all the samples, and placed in 1.5 mL centrifuge tubes. The concentration of bacteria solution was adjusted with LB media to an O.D. = 1 at 600 nm (Corresponding to approximately 2.4 × 10^9 CFU/mL) in a Nanodrop One (ThermoScientific). Then 1 mL of bacteria solution was added to the tubes containing hydrogels and to a negative control tube without hydrogel. All tubes were incubated at room temperature with slow shaking (20 rpm), under those conditions no significant bacterial growth will occur during the experiment. At each time measured, 100 μL samples were collected and serial dilutions were prepared with LB media before being plated on LB agar plates. After overnight incubation at 37 °C, the colony-forming units (CFU) on the plates of different dilutions were counted. The CFU/mL of the samples in the presence of the hydrogels were calculated from the CFU at different dilutions. Each test was carried out in three replicates.

In order to observe bacteria damage by SEM, *E. coli* bacterial cells control or treated with **Gel-8-Met** as described above were washed with PBS twice, and fixed with glutaraldehyde (GA) 2.5% (v/v in PBS) for 2 h. After the samples were washed twice with PBS and resuspended in water. The samples were diluted 10 times and a final volume of 2 μL of each sample was deposited over a metallic cylinder for the metallization process with an Au−Pd alloy (60% Au, 40% Pd).

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Images were acquired using a JSM-6490LV SEM (JEOL, Japan) at 5 kV.

4.9. Hemolysis Assay. The hemolytic activity of the hydrogels over red blood cells was tested using rat red blood cells (rRBC) collected from the Animal Facility Unit of CIC BiomaGUNE (Spain). Red cells were washed four times in PBS by centrifugation at 2000 *g* for 10 min. The RBC solution was diluted in PBS to give a standardized absorbance value of 0.6 at 412 nm when diluted 1:100. Dry hydrogels were cut into 5 mm^2 squares, weighted to ensure the same amount of material for all the samples, and placed in 1.5 mL centrifuge tubes. 1.5 mL of rRBC solution was added to each tube, and at each incubation time 200 μL of sample was collected. All samples were centrifuged 10 min at 2000 *g* and the supernatants were diluted 1:10 in PBS to measure the absorbance at 412 nm. As a positive control, using the same standard concentration, a 100% hemolysis rRBC solution was lysed in water. As a negative control, a rRBC solution was treated without the hydrogels. The data were normalized using the positive control as 100% hemolysis.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsbiomaterials.7b00335.

Characterization (^{1}H and ^{13}C NMR and FTIR-ATR) of the monomers and polymers; rheology and swelling results of the hydrogels prepared. (PDF)

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Notes

The authors declare no competing financial interest.

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