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Antimicrobial Activity from Colistin–Heparin Lamellar-Phase Complexes for the Coating of Biomedical Devices

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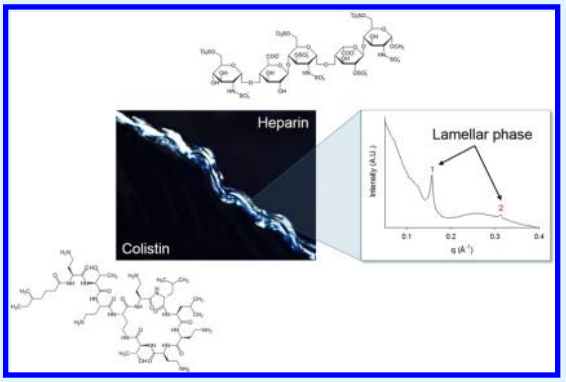
ABSTRACT: Infections arising in hospitalized patients, particularly those who have undergone surgery and are reliant on receiving treatment through biomedical devices, continue to be a rising concern. It is well-known that aqueous mixtures of oppositely charged surfactant and polymer molecules can self-assemble to form liquid crystalline structures, primarily via electrostatically driven interactions that have demonstrated great potential as tailored-release nanomaterials. Colistin is a re-emerging antibiotic used against multidrug-resistant Gram-negative bacteria. Its amphiphilic structure allows it to form micellar aggregates in solution. Thus, the aim of this study was to determine whether structured complexes form between colistin and negatively charged biopolymers, such as the highly sulfated anticoagulant, heparin. Cross-polarized light microscopy and synchrotron small-angle X-ray scattering were employed to visualize the appearance of birefringent structures and identify liquid crystalline structures, respectively, formed across the interface between solutions of colistin and heparin. A lamellar phase with a lattice parameter of ∼40 Å was formed upon contact between the oppositely charged solutions of colistin and heparin. In addition, in vitro release studies showed a slow release of colistin from the lamellar-phase gel complexes into the bulk media, and disk diffusion bioassays revealed antimicrobial activity against *Pseudomonas aeruginosa*. This system provides a novel, cost-effective, and simple approach to reducing the risk of infections by potentially applying the formulation as a coating for biomedical implants or tubing.



KEYWORDS: *colistin, heparin, lamellar phase, sustained-release, antimicrobial activity, Pseudomonas aeruginosa*

1. INTRODUCTION

There continues to be a growing concern for hospitalized patients who are at high risk of contracting infections in the lungs, urinary tract, bloodstream, and wounds. Biofilms are formed after the attachment of microorganisms onto surfaces within the body, where their metabolic activities change, producing a microenvironment that becomes a rich breeding ground for colonization of bacteria that often become resistant to treatment with antibiotics.[1−3] Common strategic approaches to combatting the formation of biofilms include (i) rendering surfaces nonadhesive to avoid attachment of microorganisms,[4,5] (ii) functionalizing surfaces that exhibit a "killing effect" upon contact of microorganisms,[6−8] or (iii) the application of biocide leaching materials onto surfaces.[9,10] Raad et al. demonstrated improved antimicrobial activity against resistant pathogens and inhibition of biofilm formation after impregnating central venous catheters (CVC) with a combination therapy of chlorhexidine (CHX) and minocycline−rifampin (M/R) compared to first-generation M/R-treated catheters, silver sulfadiazine treated catheters, CHX-treated peripherally inserted central catheters, and uncoated CVCs.[11] Islas et al. loaded the antibiotic, ciprofloxacin, into acrylic acid and poly(ethylene glycol) methacrylate grafted poly(vinyl chloride) urinary catheters, which displayed sustained release for several hours and inhibited the growth of *Escherichia coli* and *Staphylococcus aureus* and adhesion of bacteria on the catheters.[12]

A major contributing factor to emerging resistance is the inappropriate use of antibiotics (in particular, over-administering or administering more frequently than necessary). The use of novel drug delivery systems is an important strategy for reducing the risks from both of these scenarios, enabling reduced total administration to provide levels consistently above minimum inhibitory concentration (MIC), and reducing the frequency required.

It has been known for at least four decades now that interactions between oppositely charged surfactant and polymer molecules in solution often lead to the formation of liquid

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crystalline structures.[13] The type of nanostructure formed depends on the packing geometry of the molecules,[14] which can be influenced by various parameters, such as the surfactant-to-polymer molar charge ratio,[15] polymer charge density,[16−18] surfactant chain length,[19] or changes in temperature,[20] salt concentration,[21,22] and solution pH.[23−25] The most commonly encountered liquid crystalline phases in oppositely charged surfactant and polymer systems are the hexagonal[19,26−28] and lamellar[29−32] phases. The hexagonal phase consists of cylindrical micelles that are packed on a two-dimensional hexagonal lattice,[33] around which the charged polymer can wrap,[17] whereas the lamellar phase consists of parallel stacks of amphiphile bilayers,[33] separated by an ionic interlayer stabilized by the polyelectrolytes.[30−32] Liquid crystalline structures have the ability to solubilize a diversity of therapeutic agents with different physicochemical properties because they are composed of hydrophilic, hydrophobic, or amphiphilic regions or a combination of the three. Furthermore, it is also known that the existing nanostructure can control the rate of drug release from these intricate matrices.[34] Therefore, the formation of highly ordered structures in oppositely charged surfactant and polymer systems can be exploited for application as controlled-release drug delivery systems.[27,35−41]

Colistin is a re-emerging antibiotic that is effective against multidrug-resistant Gram-negative bacteria, such as *P. aeruginosa*, which is one of the leading causes of infections.[42] The commercially available forms of colistin include colistin sulfate, which is employed for oral and topical applications, whereas colistimethate sodium is mainly for parenteral use, and both of which have been delivered by inhalation.[42] Moreover, colistin has been used in both human and veterinary medicine.[43] Recently, Liu et al. discovered that the mcr-1 gene is involved in the resistance of bacteria against colistin.[44] What is of great concern is that the bacterial strains that harbor this plasmidic gene are spread worldwide; therefore, an alternative means of preventing infections from occurring is highly sought, namely in the form of a novel drug delivery system, for the reasons stated above. The amphiphilic structure of the polypeptide (Figure 1) leads to its self-assembly into micellar aggregates in aqueous media above its critical micellar concentration.[45] Protonation of the primary amine groups on colistin at physiological pH ($pK_a \approx 10$) establishes an overall positive charge. Thus, it was hypothesized that liquid crystalline structure(s) would form upon association of colistin with an oppositely charged biomaterial, such as heparin.

Heparin is a highly sulfated anionic polysaccharide, composed of repeating glucosamine and uronic acid residues (Figure 1). Heparin plays a crucial role in various processes in the body such as blood coagulation, cell adhesion, cell growth, and inflammatory responses.[46] Systems that combine antimicrobials with heparin and chitosan have been investigated for the use in coating of biomedical devices. These materials were created using the layer-by-layer method and were found to reduce bacterial adhesion.[47] Moreover, it is known that chitosan−heparin films have strong anticoagulant activity.[48] The properties demonstrated by these systems provide insight into the diverse functionality of heparin as a biomolecule, which is favorable for further exploitation in the field of drug delivery.

The specific aims of the research presented in this paper were (i) to structurally characterize the liquid crystalline phase(s) formed at the colistin−heparin solution interface, (ii) to measure the antimicrobial activity of colistin from the structured complexes against *P. aeruginosa*, and (iii) to study the diffusion of colistin from the nanostructures formed in bulk aqueous mixtures of colistin and heparin for potential application as a coating for biomedical devices.

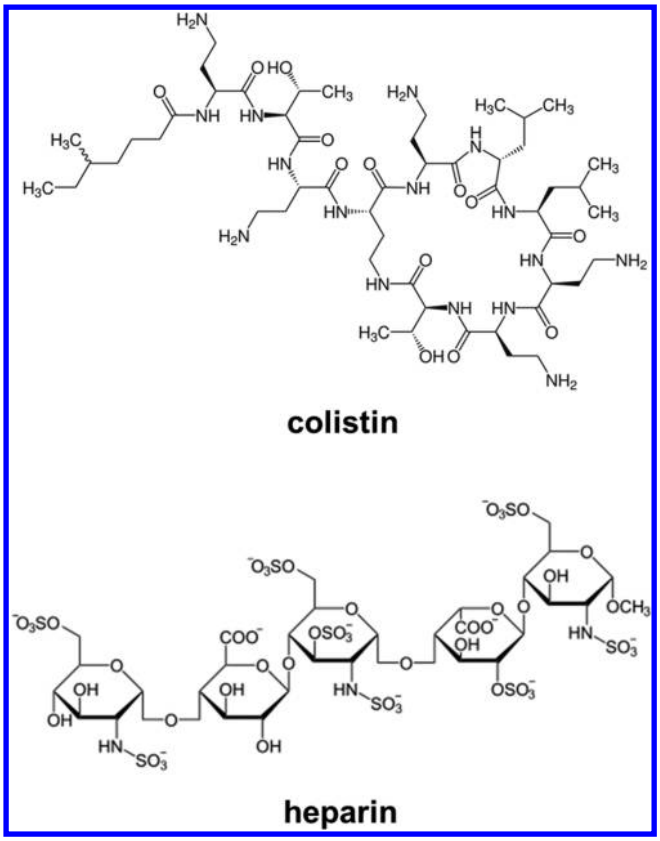

Figure 1. Chemical structures of the amphiphilic antibiotic, colistin, and the highly sulfated glycosaminoglycan anticoagulant, heparin.

2. EXPERIMENTAL SECTION

2.1. Materials. Colistin sulfate (batch no. 20120719; ≥22558 U/mg; colistin A-to-colistin B ratio = 0.3) was sourced from BioPharm (Shanghai, China), heparin sodium (porcine mucous, 35000 IU in 35 mL) was obtained from Hospira (Victoria, Australia), and sodium chloride analytical reagent was obtained from Chem-Supply (South Australia, Australia). These materials were used without further purification. Milli-Q-grade water purified through a Milli-pore system (Billerica) was used throughout this study.

2.2. Characterization of Liquid Crystalline Nanostructures. *2.2.1. Sample Preparation. Colistin−Heparin Solution Interfaces.* It has been demonstrated previously that examining interfaces created between solutions of oppositely charged surfactant and polymer molecules is a useful approach to studying the phase behavior of such systems.[41] Here, concentrated solutions of colistin and heparin were brought into contact so that molecules could interact at a well-defined interface. The system was composed of solutions of 50 wt % colistin and 5 wt % heparin, which were the theoretical concentrations calculated to achieve charge equivalence; a surfactant-to-polymer molar charge ratio above the critical aggregation concentration where coacervation and formation of liquid crystalline structure(s) generally occurs in aqueous mixtures of oppositely charged surfactants and polymers. The highly viscous solution of colistin, whose consistency resembled that of honey was loaded into an open-ended VitroCom rectangular glass tube (dimensions: 0.4 × 5.0 × 50 mm) by capillary action, and the bottom was sealed with parafilm. The solution of heparin was slowly pipetted into the top opening of the flat cell, creating an interface between the two components. The sealed sample cell was then ready for inspection under a cross-polarized light

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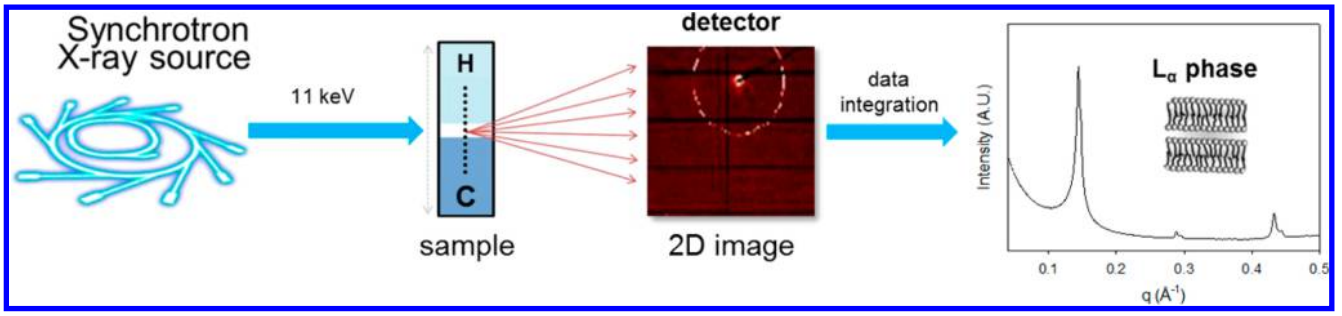
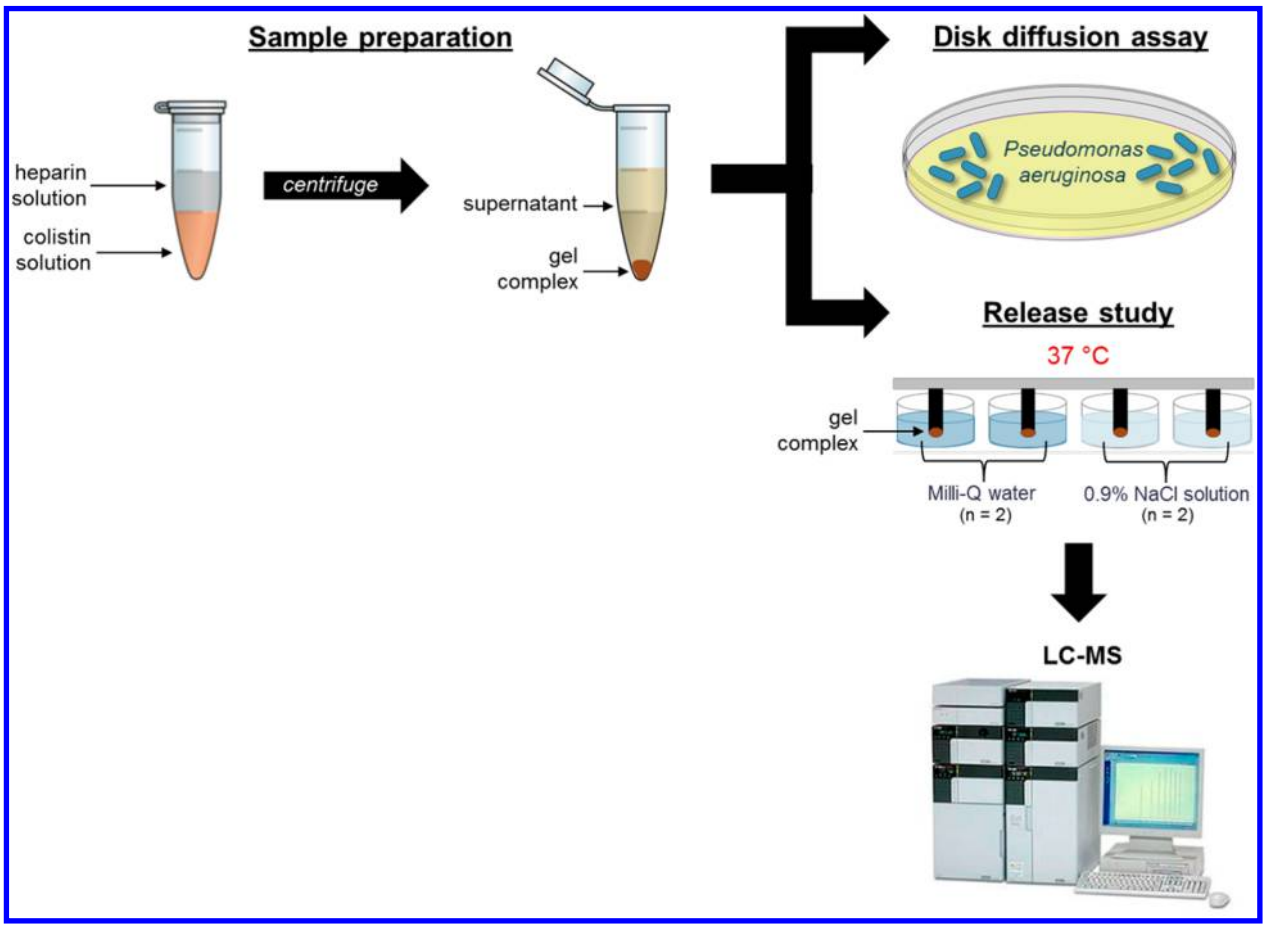


Figure 2. Illustration of how a SAXS profile can be obtained spatially across the interface created between solutions of colistin (C) and heparin (H) by conducting a "line scan" at 100 μm steps when the sample is placed in line with a synchrotron X-ray source.

Scheme 1. Illustration of the Procedures Involved in the Preparation of Bulk Aqueous Mixtures of 50 wt % Colistin and 5 wt % Heparin Solutions (50 μL of Each Solution)[a]



[a]The gel complex and supernatant produced after centrifugation were analyzed to assess their antimicrobial activity and diffusion behavior by determining their zone of inhibition on a nutrient agar plate cultured with *Pseudomonas aeruginosa* and the rate of drug release from colistin–heparin lamellar-phase complexes into various media, respectively.

microscope and spatially resolved structure analysis across the interface with synchrotron small-angle X-ray scattering (Figure 2).

Bulk Colistin–Heparin Complexes. Equal volumes of 5 wt % heparin solution were added drop-wise to solutions of 50 wt % colistin (50 μL) into preweighed 1.5 mL microcentrifuge tubes. Their masses were recorded to determine the total amount of colistin and heparin molecules present in the system. The samples were then centrifuged to force the association between colistin and heparin molecules and the settling of the coacervate formed to the bottom of the tube (Schematic 1). The pellet consisted of the colistin–heparin gel complex, and the supernatant was composed of the uncomplexed or dispersed materials and released counterions. A microspatula was used to further mix the complex prior to extraction from the tube, and the unwashed pellet was transferred onto either the nutrient agar plate for activity measurements or the sample holder to study its release behavior.

2.2.2. Cross-Polarized Light Microscopy. Cross-polarized light microscopy (CPLM) was employed to visualize the appearance of any anisotropic liquid crystalline structures exhibiting birefringence at the colistin–heparin interface using a Nikon ECLIPSE Ni–U upright microscope fitted with cross-polarizing filters and a DS-U3 digital camera control unit (Nikon) at room temperature.

2.2.3. Small-Angle X-ray Scattering. Synchrotron small-angle X-ray scattering (SAXS) was used to identify liquid crystalline structure(s) formed at the colistin–heparin interface. The sample prepared in the flat cell was mounted vertically on a remotely operated XYZ translation stage at the Australian Synchrotron SAXS/WAXS beam-

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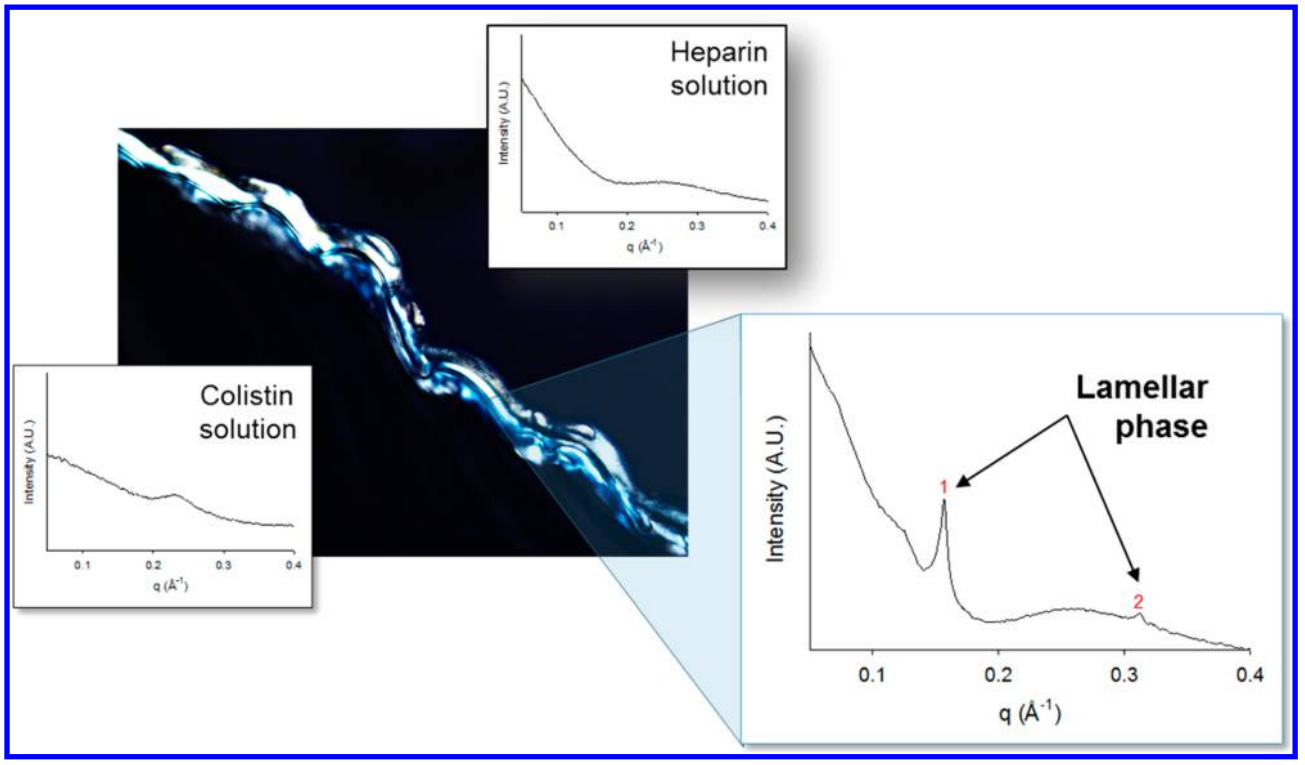

Figure 3. Formation of the lamellar phase at the interface between isotropic solutions of 50 wt % colistin and 5 wt % heparin observed under CPLM as a band exhibiting birefringence and characterized by equidistant Bragg reflections in the SAXS profile.

line[49] and exposed to an X-ray beam with a wavelength of 1.12713 Å (11 keV) with a sample to detector distance of 1034.97 mm providing a q range from $0.018 < q < 1.02$ Å^{-1}, where q is the magnitude of the scattering vector, defined as $q = 4\pi/\lambda \sin(\theta/2)$, λ being the wavelength and θ the scattering angle. Two-dimensional SAXS patterns were collected across the interface at 100 μm steps with 1 s acquisition at each position using a 1 M Pilatus detector (active area 169 × 179 mm^2 with a pixel size of 172 μm).

The two-dimensional SAXS patterns were then integrated into the one-dimensional scattering function $I(q)$ using the computer software ScatterBrain Analysis.

2.3. Disk Diffusion Assay. The disk diffusion assay measures the activity of an antibiotic against a specific strain of bacteria. Samples were placed on nutrient agar plates (made by Media Preparation Unit) cultured with *P. aeruginosa* ATCC 27853 (American Type Culture Collection) from glycerol stocks and labeled appropriately. The samples included Milli-Q water (10 μL) and 5 wt % heparin solution (10 μL) as the negative controls, an Oxoid colistin antimicrobial susceptibility disk (10 μg, ThermoScientific, Denmark) as the positive standard control, and 50 wt % colistin solution (10 μL), the gel complex (total mass produced), and the supernatant (10 μL) as the test samples. The agar plates were incubated at 37 °C for 24 h before the zones of inhibition were measured.

2.4. In Vitro Release Studies. Release studies were performed in duplicates to understand the diffusion behavior of colistin from colistin−heparin complexes and how the rate of drug release was influenced by the concentration of salt in the release medium. The total mass of the gel complex containing 21.4 ± 0.5 mg of colistin was loaded into a capped cylindrical holder (radius = 1.25 cm, height = 0.1 cm), providing a reproducible surface area (~0.05 cm^2), which was affixed onto the lid of a 24 well Corning Costar cell culture plate and submerged in the release medium, either Milli-Q water or 0.9% NaCl solution (representative of saline solution), as depicted in Schematic 1. The plate was incubated at 37 °C and continuously shaken gently for the entire duration of the release study (24 h). The total volume of the release media were collected at predetermined time intervals and were subsequently replaced with fresh drug-free media. These samples were stored in a −8 °C freezer prior to analysis by liquid chromatography mass spectrometry.

2.4.1. Colistin Assay by Liquid Chromatography−Mass Spectrometry. Liquid chromatography−mass spectrometry (LC−MS) was utilized to quantify the concentration of colistin released from nanostructured gel complexes into the aqueous media by employing the method developed by He et al.[50] Briefly, the LC−MS system used was a Shimadzu LCMS 2010 EV quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source coupled to a Shimadzu Prominence chromatography system (Kyoto, Japan). Reverse-phase high-performance liquid chromatography (RP-HPLC) analysis was conducted with a Phenosphere-NEXT C18 column (5 mm, 150 × 4.6 mm) at room temperature. An aliquot (100 μL) of each sample was injected into the RP-HPLC system. The gradient program employed was as follows: 15−50% mobile phase B over 10 min at 1 mL/min, then 50−100% mobile phase B for 15 min at 1.5 mL/min and re-equilibration to 15% mobile phase B over 5 min at 1 mL/min (mobile phase A: 0.05% TFA in Milli-Q water; mobile phase B: 0.05% TFA in acetonitrile). The eluent was infused directly into the ESI source. Mass spectra were acquired in the positive ion mode over 30 min with a scan range of 200−1800 m/z. The chromatographic system also included a photodiode array detector that was set at 214 nm.

2.4.2. Data Analysis. Calculation of the apparent diffusion coefficient, D (cm^2/s), of colistin across the colistin−heparin gel complex into the release medium was derived by using the slope of the linear curve attained when the moles of drug released per unit area, Q (mol/cm^2), was plotted against the square root of time, $t^{1/2}$ ($s^{1/2}$), and applying it to the Higuchi equation:[51]

$$Q = 2 \cdot C_0 \cdot \sqrt{\frac{D \cdot t}{\pi}} \quad (1)$$

where C_0 is the initial concentration of drug in the gel (mol/cm^3), which was inferred from the difference between the total mass of colistin incorporated into the sample mixture and the mass of colistin measured in the supernatant by LC−MS.

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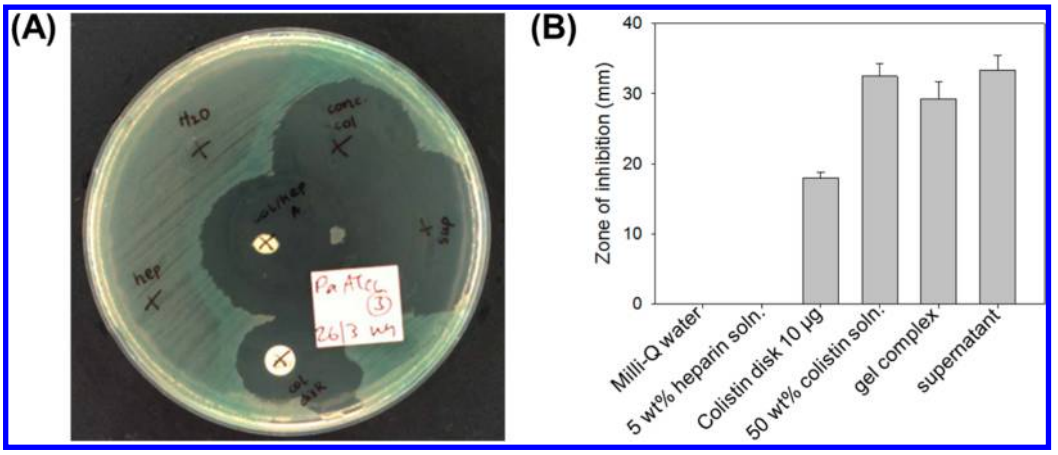

Figure 4. (A) A representative photograph of (B) zones of inhibition measured for various samples (n = 4) on a plate of nutrient agar cultured with *P. aeruginosa*. Negative controls: Milli-Q water and heparin solution. Positive control: commercially available colistin disk (10 μg). Test samples: colistin stock solution (0.5 g in 10 μL), colistin–heparin lamellar-phase gel complex (21.4 mg ± 0.6 mg), and supernatant (13.6 mg ± 0.2 mg in 10 μL).

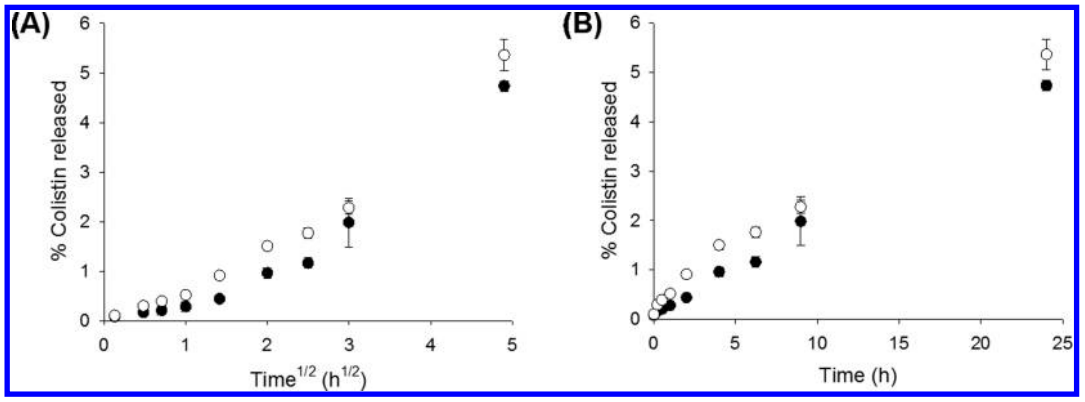

Figure 5. Cumulative percent release profile of colistin from colistin–heparin lamellar-phase gel complexes into either Milli-Q water (filled circles) or 0.9% NaCl solution (open circles) at 37 °C (n = 2) as a function of square root of time (A) or time (B).

As it is well-known that liquid crystalline systems normally display diffusion-controlled release;[34,52−54] data were plotted as percent colistin released versus time$^{1/2}$.

3. RESULTS

3.1. Formation of Ordered Structure at the Colistin–Heparin Interface. Upon contact between isotropic solutions of 50 wt % colistin and 5 wt % heparin, a band exhibiting birefringence appeared at the liquid–liquid interface when observed under a cross-polarized light microscope (Figure 3). This was indicative of the presence of an anisotropic mesophase, either a lamellar or hexagonal phase. A distorted nonlinear formation at the interface would suggest that the structure was quite mobile and seemingly less viscous than what is characteristic for hexagonal phases and lacked the angular fan-like texture usually observed for hexagonal phases.[55] Synchrotron SAXS was used to confirm that it was a lamellar phase that self-assembled at the colistin–heparin interface with a lattice parameter of ∼40 Å, as given by the emergence of Bragg reflections that were equally spaced from one another at $q \approx 0.1563$ and 0.3128 Å^{-1} (Figure 3).

3.2. Antimicrobial Activity of Colistin–Heparin Complexes Against *Pseudomonas aeruginosa*. The disk diffusion assay is a basic method commonly used to determine the minimum inhibitory concentration of antibiotics or compare the sensitivity of bacterial pathogens to antimicrobial agents. Although there have been developments in studying activity to overcome issues with diffusion through agar,[56] this approach has been used routinely to demonstrate colistin activity[57,58] and has been used here to determine whether colistin is available in an active form from these complexes. The zone of inhibition displayed by various samples were examined, which gave an indication of the ability of colistin to radially diffuse from its location on the cultured agar plate and impart its activity to kill the bacteria in the local environment. The negative controls, Milli-Q water and heparin solution, did not develop zones of inhibition (Figure 4). In contrast, the 50 wt % stock solution of colistin (0.5 g in 10 μL), the colistin–heparin gel complex (21.4 mg ± 0.6 mg), and the supernatant (13.6 ± 0.2 mg in 10 μL) demonstrated almost twice the activity as the 10 μg colistin disk (Figure 4).

It should be noted that ZOI data result from both kinetics of diffusion of the compound as well as its effectiveness and are therefore a poor way to indicate diffusion; the intention of this results is to show retention of activity, and the kinetics of release are described in the next section.

3.3. Release Behavior of Colistin from Bulk Colistin–Heparin Complexes. Release studies were conducted to support the findings obtained from the disk diffusion assay and gain more knowledge of the diffusion behavior of colistin from the complexes formed in aqueous mixtures of colistin and heparin. Milli-Q water was used as the control release medium,

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and the rate of drug release was also evaluated in 0.9% NaCl solution, which was representative of physiological saline.

The release behavior of colistin from the colistin−heparin complex in both media demonstrated a similar trend as a function of the square root of time; however, it did not completely fit the "normal" release of a passive drug (Figure 5A). Here, the thickness of the matrix shrinks as colistin is released so the rate of diffusion of colistin from the structured complex would be expected to speed up over time, especially in comparison to the diffusion coefficient of colistin at, for example, t = 1 h and t = 9 h (Table 1). Moreover, when the

Table 1. Comparison of the Diffusion Coefficient of Colistin from Colistin−Heparin Lamellar-Phase Complexes in Either Milli-Q Water or 0.9% NaCl Solutions[a]

	diffusion coefficient of colistin ($\times 10^{-10}$ cm^2 s^{-1}) n = 2	
release medium	$t \approx 1$ h	$t \approx 9$ h
Milli-Q water	0.22 ± 0.01	3.55 ± 0.21
0.9% NaCl solution	0.51 ± 0.06	3.61 ± 0.42

[a]The release study was conducted in duplicates at 37 °C.

percent colistin released was plotted against time, the release behavior closely resembled that of a zero-order release profile (Figure 5B), suggesting that an erosion process was involved.[40] The control disks and concentrated colistin solution present colistin in an immediately available form; hence, it was not appropriate to measure release from these controls that were used in the ZOI studies.

The rate of drug release exhibited in the saline solution was slightly more rapid than the release of colistin in Milli-Q water. These differences were emphasized when comparing the diffusion coefficient, D, of colistin in the various conditions (Table 1). Specifically, the apparent diffusion coefficient at $t \approx$ 1 h increased by 2-folds in the presence of salt, while there was no significant difference observed at $t \approx 9$ h between the two media.

4. DISCUSSION

4.1. Formation of the Lamellar Phase at the Colistin−Heparin Interface. Colistin is an amphiphilic molecule capable of forming micellar aggregates in aqueous media with a mean particle size of 2.07 ± 0.3 nm above its critical micellar concentration (1.5 mM; determined by air−water interfacial tension and dynamic light scattering measurements).[45] However, there have been no reports of heparin self-assembling into any type of ordered structure in solution. There was some indication of structure existing in the 50 wt % colistin solution with a broad peak appearing at $q \approx 0.2310$ Å^{-1} in the SAXS profile (Figure 3). Interestingly, when the oppositely charged solutions of colistin and heparin were brought into contact, lamellar phase was formed at the liquid−liquid interface. The amphiphilic nature of colistin favored its interaction with the highly sulfated heparin and subsequent close packing of the molecules into a highly ordered nanostructure. The lamellar phase is known to arise in other biorelevant oppositely charged surfactant and polymer systems. Mixtures of the cationic surfactant myristyltrimethylammonium bromide (MTAB) and DNA[28] and at the interface between solutions of the anionic bile salt, sodium taurodeoxycholate, and the cationic polymer, chitosan,[41] produced lamellar phases with lattice parameters of ∼49 and ∼43 Å, respectively. In the colistin and heparin system presented here, the internal dimension of the lamellar phase was significantly smaller (∼40 Å; Figure 3). This was most probably attributed to the shorter chain length of the hydrophobic component (C_7) in the chemical structure of colistin in comparison with the longer hydrocarbon tail of MTAB (C_{14}) or the bulky structure of amphiphatic bile salts, which enabled a more-condensed parallel stacking of the surfactant moieties into a bilayer structure. Because the oppositely charged polymer molecules are envisaged to situate themselves in between the stacked bilayers, the main parameters dictating the lattice parameter of the lamellar phase formed in such polymer systems, which is defined as the spacing between the midsections of the bilayer structure, would either be (i) the degree of repulsion between the like charges on the polymer backbone, which depends on the polymer charge density, or (ii) the surfactant chain length. In this case, the surfactant chain length has shown to significantly influence the internal dimensions of the lamellar phases formed.

4.2. Release of Colistin from Lamellar-Phase Colistin−Heparin Complexes to Elicit Its Antimicrobial Activity against *P. aeruginosa*. Interestingly, antimicrobial activity was observed from the lamellar phase gel complexes. In the absence of the release data, this was not necessarily expected as at charge neutralization, the colistin is bound within the coacervate, so its activity is expected to be low. The antimicrobial activity against *P. aeruginosa* displayed from both the colistin−heparin complex and the supernatant solution was only double to that of the control disk of colistin even though the initial concentration of colistin was significantly higher than what was contained in the commercially available colistin disk. This may reflect the impact of complexation both in the bulk gel and potentially in the supernatant. A further possible explanation could be that there was no correlation between the concentration and activity of the antibiotic when the minimum inhibitory concentration of colistin was surpassed (MIC_{50}: 1.0 μg/mL and MIC_{90}: 3.0 μg/mL as measured by the Kirby Bauer disk diffusion method).[59] Nonetheless, these findings highlight that colistin was able to escape from within the liquid crystalline structure into the surrounding medium and elicit its bactericidal activity.

Initially it was thought that the presence of salt in the agar plate would induce the dissociation of the lamellar phase, an effect that is known to arise when the addition of salt introduces a screening effect between oppositely charged surfactant and polymer molecules constituting the meso-phase.[21,37,60−64] If this were the case, an increase in salt concentration could facilitate the diffusion of colistin out of the gel complex. The in vitro release studies confirmed that the saline solution was not a significant contributing factor to the release of colistin into the release media, indicating strong electrostatic interactions between colistin and heparin in the complex.

The relatively slow release of colistin from the lamellar phase gel complexes, where an initial lag period was exhibited to allow for the dissociation of colistin from heparin, offers a means of delivering sustained release of an antibiotic and related antimicrobial activity. The total release of 5% in 24 h seen in Figure 5 means that a coating applied at the same surface area to volume ratio could provide up to a significantly longer period of release, possibly on the time-scale associated with the duration in which patients would require intubation after surgical procedures. The colistin and heparin system may therefore be applicable as a more favorable alternative to

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existing coatings for biomedical devices, reducing the need for frequent replacement of implants or catheter tubes in critically ill patients. Importantly in the context of reducing the potential development of resistance, such a coating has the potential to provide an effective local treatment, avoiding systemic administration and extended periods of exposure to drug below the minimum inhibitory concentration. Such drug delivery approaches to antimicrobial treatment are extremely important as the pipeline of antimicrobial drugs is exhausted, and we seek more-effective ways to deploy existing drugs.

4.3. Implications as an Antimicrobial Coating for Biomedical Devices. Various cationic molecules have previously been exploited in combination with heparin as a coating for the surfaces of medical devices, namely catheters, and also in analytical applications.[65−68] Hsu et al. developed a patented technology in which complexation of the heparin ion with an alkyl(C_{16-18}) benzyl dimethylammonium ion produced improved heparin ion coating on different types of medical devices with superior surface adhesion and higher affinity to plastic surfaces than that exhibited from the cationic surfactant alone. The complex formed was reported to be more hydrophobic and ten times less soluble in saline, which, as a consequence, improved antithrombogenic performance of the system.[65] Similarly, the association of heparin with benzylakonium[66] or a cationic block copolymer with star-shaped branching[68] also resulted in its enhanced wettability and adhesion on medical tubings, which significantly prevented leaching of heparin into the media and subsequently blood clot aggregation. In addition, these coatings offer a means of reducing the risk of catheter-related bloodstream infections. The formation of supramolecular ion complexes upon interaction between oppositely charged ions in solution was again taken advantage of by Sun et al., when a spectrophotometric method was established by the group for the detection of small amounts of heparin using crystal violet as the bioprobe.[67]

The novelty of the system presented in this paper lies in the therapeutic itself participating in the formation of highly ordered mesophases rather than being loaded into a matrix that provided "passive release", such as that previously shown with the bile salt−chitosan, sodium dodecyl sulfate−poly(diallyldimethylammonium chloride), and cetyltimethylammonium bromide−poly(acrylamide-acrylic acid) systems.[41] The viscosity of the lamellar gel phase would be important during the application process, where it is envisaged that a more freely flowing formulation would allow the delivery system to, for instance, be easily "painted" on surfaces with a brushlike tool. This can be achieved by determining the optimal colistin-to-heparin molar charge ratio in the dilute regime that produces similar outcomes reported here. In addition, heparin has been demonstrated to also reduce adhesion of bacteria and biofilm formation by attracting proteins with antimicrobial activity.[69,70] Therefore, future studies that directly measures the concentration of heparin released from these lamellar phase gel complex would also be of interest, as will the study of other negatively charged polymers such as hyaluronic acid. Furthermore, additional experiments will be designed to study the antimicrobial activity from these colistin−heparin complexes against different types and strains of bacteria.

5. CONCLUSIONS

This is the first report of an antibiotic, colistin, forming a highly ordered structure with the highly sulfated anticoagulant, heparin. Diffusion of colistin from the lamellar phase gel complexes exhibited slow release and antimicrobial activity against *P. aeruginosa*. These preliminary findings render the colistin and heparin system highly applicable as a coating for biomedical devices, such as implants or catheters, to provide sustained-release of an antibiotic to patients who are at high risk of contracting infections while hospitalized. In addition, this system also offers a platform for the exploitation of other charged therapeutics to actively participate in the formation of liquid crystalline structures with oppositely charged biomaterials for use in drug delivery.

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Notes

The authors declare no competing financial interest.

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