Multi-drug delivery systems constructed from a basic polymeric scaffold, and which have the ability to target a variety of biomedical applications, can streamline the development of nanomedicine to provide both environmental and economical relief. Herein, amphiphilic ABA-triblock copolymers are synthesized and assembled sequentially into micelles and nanogels as drug delivery systems following a thorough evaluation on advanced in vitro models to explore their potential for the treatment of cancer and bacterial infections. Short blocks of 5-methyl-5-allyloxycarbonyl-1,3-dioxan-2-one (MAC) are oligomerized from PEG6k and thereafter functionalized with dihydroxyphenylalanine (dopa)-functional thiols using thiol-ene coupling (TEC) click chemistry. The copolymers self-assemble into well-defined micelles in aqueous solution and are further formulated into nanogels via UV-induced TEC. The resulting spherical micelles and nanogels are stable nanoparticles, with sizes ranging between 100 and 200 nm. The nanogels are found to be non-toxic to a panel of cell lines and mask the toxicity of the potent drugs until their release. The nanogels would be superior to micelles for the elimination of cancer cells supported by both 2D cell culture and a 3D spheroid model. The opposite conclusion could be drawn for bacteria inhibition.

1. Introduction

Polymer-based materials have been studied extensively as drug delivery systems (DDSs) for a variety of biomedical applications, including cancer therapy,[1] antimicrobial,[2] and tissue engineering purposes.[3] Currently, the design of DDSs are mostly set toward fulfilling only one programmed task, for example, targeting one specific type of cancer or exclusively combatting bacterial infections. Indeed, common sense in the biomedical and materials science field is to develop compounds with specific functions that target specific sites for predetermined applications. In reality, the design, synthesis, and evaluation of a DDS requires tremendous efforts.[4] Emphasis should rather be placed on a modular approach, that is, starting with relatively basic and readily available materials that can be adapted to meet a multitude of biomedical applications by a simple switch of the configuration, for example, the biopolymer chitosan.[5] In this way, human, energy, and capital resources can be conserved.

To achieve this, the intended configurations should be compatible to allow for simple switching. Among the numerous polymer-based self-assembled structures, spherical micelles and nanogels are closely related because in most cases, nanogels can be regarded as crosslinked micelles.[8] The assembly of micelles from amphiphilic polymers in an aqueous environment was instrumental in the evolution of the field of drug delivery, in that the hydrophobic core of the micelle could be used to transport and conceal the toxic payload, while the hydrophilic shell allowed for aqueous solubility, protecting the cargo in the core.[7] Many self-assembled structures can arise from amphiphilic polymers, and these structures can be crosslinked to form stable materials if their constituent design is appropriate, such as micellar nanogels, which are nanosized hydrogels with crosslinked micellar structures. A well-known example is the crosslinked knedel-like nanoparticles (NPs) developed by Wooley and co-workers.[8]

Other nanogel systems built from crosslinked micelles have been reported to be pH-responsive and thermo-responsive.[9] Due to crosslinking, the nanogel behaves as a unimolecular particle and does not have a critical micelle concentration (CMC), which is the concentration threshold at which unimers assemble into micelles.[10] The absence of a CMC is advantageous in that there exists no concentration at which the micelles would become unstable and disassemble. Nanogels are therefore more likely to withstand the infinite dilution effect that occurs when the DDS is administered in vivo to large volumes of blood, where thermodynamic and kinetic stability
are crucial.[31] The difference in stability between micelles with a CMC and nanogels without, means that there are certain biomedical applications that will be more fitting for each. It is important that both micelles and nanogels can be developed from the same fundamental polymer materials with only slight modifications. Although different strategies have been discussed for the purpose of generating stimuli-responsive nanogels,[32] one of the most simple strategies used to create the nanogel configuration from micelles is through UV-initiated thiol-ene coupling (TEC) click chemistry,[33] which is highly efficient, facile, and robust—significantly streamlining the purification process.

To date, a variety of polymeric micelles and an increasing number of nanogels have been reported as DDSs.[14] Unfortunately, no comprehensive study has been conducted to investigate and compare micelles and their nanogel counterparts as DDSs. Several review articles have, however, served to discuss the advantages and limitations of micelles and nanogels in general. Limitations include a) the unattainable transformation from micelle to nanogel because of the lack of functional groups necessary for crosslinking; b) impure nanogel formation as a result of side-reactions or inefficient crosslinking and c) irrelevance in certain applications due to unpredictable degradation, toxicity, and/or biodistribution profiles.[15] No matter the reason, a detailed comparative study between micelles and nanogels is still much needed.

Herein, polyethylene glycol (PEG) based amphiphilic ABA-type triblock copolymers were synthesized and assembled into micelles that were subsequently crosslinked using TEC click chemistry to create nanogels. An allyl-functional cyclic carbonate was oligomerized from PEG, enabling post-functionalization of the allyl groups, while maintaining the stealth characteristics of PEG. These allyl-functional triblock copolymers were then partly functionalized with 3,4-dihydroxyphenyl-functional thiol (dopa-thiol) using UV-initiated TEC, resulting in amphiphilic ABA-block copolymers with dopa functionalities and allyl groups (Figure 1) to allow for a) micelle formation through self-assembly, b) the subsequent formation of nanogels via TEC in the presence of trimethylolpropane tris(3-mercaptopropionate) (TMP(SH)₃) as crosslinker, c) improvement of the stability of the micelles via hydrogen bonding from the incorporated dopa groups, d) increase in the drug-loading capacity through interactions between the aromatic dopa groups and the hydrophobic drug,[16] e) biocompatibility, through the use of nontoxic PEG, 2,2-bis(hydroxymethyl)propionic acid (bis-MPA) and nature-derived dopa constituents.[17] The resulting micelles and nanogels were assessed using advanced in vitro models and were compared as potential DDSs for cancer therapy and antibacterial applications.

2. Results and Discussion

2.1. Materials Synthesis

The general synthesis procedure of linear polymers with dopa functionalities and allyl pendant groups are shown in Figure 1. 5-Methyl-5-allyloxy carbonyl-1,3-dioxan-2-one (MAC) was successfully oligomerized from the macroinitiator, hydroxy-functional PEG (Mₙ: 6000 g mol⁻¹, PEG6k) using ring-opening polymerization (ROP) with an organocatalyst/ co-catalyst system of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and thiourea (TU). Two triblocks were synthesized, having different degrees of polymerization (DPs) of MAC, PEG-(b-MAC)₂ (triblock A, MAC DP = 3) and PEG-(b-MAC₆.5)₂ (triblock B, MAC DP = 6.5) (Table S1, Supporting Information) as calculated from ¹H NMR spectroscopy, see Figures S1 and S2, Supporting Information. The DPs were slightly lower than the targeted DPs of 5 and 10, corresponding to a monomer conversion of approximately 60–65%. The D was low for both polymerizations, with values around 1.1. The molecular weight obtained from SEC correlated well with that calculated from ¹H NMR spectroscopy, Table 1.

The functionalization of the pendant allyl ester groups of the outer blocks of the synthesized PEG-(b-MAC₆.5)₂ triblock copolymers was successfully achieved using UV-initiated TEC, supported by ¹H NMR spectroscopy (Figures S3 and S4, Supporting Information). The composition of the final polymers was found to be PEG-(b-MAC(allyl₁₂/dopa₃))₁ (Triblock A-dopa, f_allyl groups = 67%) and PEG₆k-(b-MAC(allyl₁₂/dopa₁₂))₁ (Triblock B-dopa, f_allyl groups = 50%), Table 1 and Table S2, Supporting Information.

Evaluation of the triblock copolymers with SEC showed that the modification with dopa resulted in polymers with lower Mₙ compared to the unfunctionalized polymers, Figure S5, Supporting Information. Since the analysis from ¹H NMR spectroscopy indicated that the reaction had occurred successfully to ≈50% of the allyl esters, it was concluded that the lower Mₙ could result from either two causes. The first, as a result of a decrease in the hydrodynamic volume of the triblock copolymers due to decreased solubility of the dopa-functional polymers in DMF, and the second as a result of interactions between the dopa groups and the column. This would also explain why Triblock B-dopa had a larger elution volume than Triblock A-dopa, as the former had longer MAC blocks and a greater number of dopa groups. DSC was used to analyze the thermal properties of the synthesized materials.

2.2. Thermal Properties

The T_g of the PEG6k precursor was unfortunately below the detection limit so any influence of the oligomerization of MAC could not be detected. The oligomerization did, however, have a clear impact on the crystallization of the PEG6k (Table S3, Supporting Information) where the T_m was found to decrease from 61 to 52 °C for Triblock A and from 61 to 50 °C for Triblock B. The influence of the short MAC chains also led to a reduction in the heat of melting (ΔH_m), an indication of crystallinity, which decreased from 78% to 69% for Triblock A and from 78% to 58% for Triblock B. This decrease in crystallinity is as a result of the amorphous MAC chains disrupting the crystallizability of the PEG block. The functionalization of Triblock A with dopa-thiol further decreased the crystallinity from 69% to 61%.
All four triblock copolymers were found to be well below their $T_g$ at room temperature. The $T_g$ is, however, very broad for all polymers, and in order to draw any reliable conclusions regarding the effect of DP, MAC or dopa content, more data points are necessary. Due to their semi-crystalline nature, the triblock copolymers occur in the solid phase at room temperature.
Table 1. Molecular weights (g mol$^{-1}$) and DP values of synthesized polymers from SEC and NMR.

| Sample      | Composition                  | DP$_{target}$ | DP$_{actual}$ | Dopa units$^a$ | $M_n$ $^b$ | $M_w$ $^b$ | $D_p$ $^b$ |
|-------------|------------------------------|---------------|---------------|---------------|-----------|-----------|-----------|
| PEG6k       | PEG$_6k$                     | 0             | 0             | 0             | 6000      | 6144      | 1.03      |
| Triblock A  | PEG$_6k$-(b-MAC)$_{12}$      | 5             | 3             | 0             | 7200      | 7730      | 1.09      |
| Triblock B  | PEG$_6k$-(b-MAC)$_{32}$      | 10            | 6.5           | 0             | 8800      | 8993      | 1.11      |
| Triblock A-dopa | MAC(allyl$_1$/dopa$_3$)$_2$ | –             | 3             | 2             | 8200      | 6716      | 1.07      |
| Triblock B-dopa | MAC(allyl$_{12}$/dopa$_{20}$)$_2$ | –             | 6.5           | 3.25         | 10 400    | 6313      | 1.03      |

$^a$Molecular weight and DP of MAC calculated from $^1$H NMR. For PEG6k, 6000 g mol$^{-1}$ was used as the $M_n$. $^b$Molecular weight and DP acquired using SEC in DMF calibrated with PEG standards. $^c$Number of dopa units per block of MAC.

2.3. Formation of Micelles and Nanogels

The resulting Triblock A-dopa and Triblock B-dopa were self-assembled to form micelles using the thin film method,[13c] further referred to as micelle-A and micelle-B, respectively. The resulting micelles have a hydrophilic outer layer and a hydrophobic core within. Dopa, at neutral pH, shows overall hydrophilicity, therefore facilitating hydrophobic interactions with other hydrophobic components in the core. Moreover, the catechol groups may aid in stabilizing the micelle structure and improve drug loading through interactions such as $\pi-\pi$ stacking and hydrogen bonding.[38] Nanogels were then formed from micelle-A and micelle-B by introducing a crosslinker, followed by UV-initiated TEC, further referred to as nanogel-A and nanogel-B, respectively. The disappearance of the allyl signal from $^1$H NMR spectroscopy indicated full conversion (crosslinking) of the micelles to the nanogel (Figure S6, Supporting Information). This was corroborated by the absence of a CMC value for nanogel-A and nanogel-B, while micelle-A and micelle-B had CMC values of 339 and 170 nm, respectively (Figure 2B).

2.4. Characterization as a DDS

2.4.1. Size and Morphology

The hydrodynamic sizes of the micelles and nanogel morphology were determined using DLS (Figure 2A). Both morphologies have dispersity values lower than 0.15, indicative of narrow size distributions of the NPs. The average size of the micelles were $\approx$170 nm, while that of the crosslinked micelles within the nanogel were lower, $\approx$130 nm. The sizes of the micelles and nanogels are within the range of 100–200 nm, which is considered to be optimal for achieving passive targeting to solid tumors due to the well-documented enhanced permeability and retention (EPR) effect,[19] implying that both types of NPs could be applied DDSs for cancer therapy following the same rationale as in earlier published work.[13c]

The average sizes of the micelles from DLS tend to be larger compared to nanogels formed from the same dopa polymer, likely due to aggregate formation of the micelles, rather than a single-layer micelle.[30] Additionally, this led to contracted internal spaces compared to micelles. Micelles have a slightly larger size and are not perfectly spherical, compared to nanogels, as confirmed via TEM (Figure 2B). This is likely due to the nature of the micelles which are multi-layered, and during the water evaporation stage are pulled apart during sample preparation, while unimolecular nanogels maintained their spherical shapes due to stable covalent bonds. Interestingly, dark dots were observed distributed on nanogels’ surfaces, indicating either roughness on the surfaces or higher densities in these areas of the nanogels. SEM was used to confirm that surface roughness was observed. It is postulated that the porous irregular surface may represent loops of linear PEGs while the hydrophobic dopa branches fold within the core (Figure 2B). These findings show the key differences in morphology between micelles and crosslinked nanogels. Additional SEM images can be found in Figure S8, Supporting Information.

To assess the degradability of the particles, the micelles and nanogels were respectively incubated in PBS (pH 7.4, 0.01 M) in either a cold environment (4 °C, storage temperature) or at room temperature (25 °C, ready to use temperature) for up to 4 weeks. Interestingly, their hydrodynamic sizes decreased by 5–10% regardless of temperature after 28 days (Figure 2C). This small decrease in size is not due to the complete disintegration of the NPs, as dispersity index values remained low (Figure 2D). It is more likely that the NPs self-adjusted to the best kinetically stable state in solution; a similar phenomenon was found in a dendritic nanogel system.[13c]

2.4.2. Drug Loading and Release

To further understand how the structural difference between micelles and nanogels influence their behavior in drug delivery, a model anti-cancer drug, doxorubicin (DOX), as well as an antibiotic, ciprofloxacin (CIP), were individually loaded into the hydrophobic dopa functional cores of micelles and nanogels via physical entrapment, to target cancer and bacterial infections, respectively. The loading conditions were first optimized with DOX through analysis of the loading efficacy and the particle size. The DOX to polymer weight ratios used in this study were 2:1, 1:1, 1:2, 1:4, and 1:8. It was found that the 2:1 and 1:1 ratios formed visible aggregates, and for these particles it proved difficult to remove unloaded DOX. The 1:8 ratio led to stable particles but unfortunately, the loading efficiency was quite low because much less drug is initially incorporated compared to the 1:2 and 1:4 ratios. Both of the ratios of 1:2 and 1:4 led to stable colloidal solutions and were further analyzed by DLS, where it was found that the 1:2 ratio led to a broad size distribution, expressed with a high dispersity index (Table S4, Supporting Information). Even though the 1:2 ratio led to a higher loading efficiency compared to the 1:4 ratio, it is not ideal for clinical application due to the significant increase in the particle size after drug loading (Table S4, Supporting Information). The loading ratio of 1:4 was therefore used in the following
experiments. In general, higher drug loading efficiencies were found for the Triblock B-dopa NPs compared to the Triblock A-dopa NPs, this is likely due to the increased amount of dopa functionalities which enhances both hydrophobicity through aromaticity and absorption through hydrogen bonding. All of the NPs demonstrated a much higher loading capacity for DOX compared to CIP, which may be due to CIP’s limited solubility in organic solvents leading to entrapment difficulties within the hydrophobic cores of NPs. The loading efficiencies were found to be very similar between micelle-A and nanogel-A, for both DOX and CIP loaded NPs. For micelle-B to nanogel-B, however, the loading efficiency increased significantly, likely due to the greater amount of dopa in Triblock B-dopa NPs, which cause them to be more tightly packed.

The sizes of the NPs remained below 200 nm after DOX loading, which is advantageous in enabling the accumulation of NPs at the tumor site via the EPR effect for cancer therapy. For CIP loading, however, the sizes of the NPs increased after loading where CIP-micelle-A and CIP-nanogel-A had sizes larger than 200 nm. For the purpose of antibacterial infections, which could be done by local injection or spraying on the skin surface, the EPR effect is not viable, so NPs with sizes larger than 200 nm is acceptable.

DOX release was measured at pH 7.4 at 37 °C in PBS to mimic physiological conditions. In general, DOX release was more rapid from micelles compared to nanogels, especially for micelle-A with lower dopa contents (Figure 2E), releasing ≈80% of DOX after 72 h. The other three NPs released DOX at a slower rate, leading to 50–60% DOX release within 72 h. The release rate of DOX is greater from micelles assembled from these dopa functionalized linear copolymers, compared to micelles from linear dendritic copolymers, which have stronger hydrophobic interactions.[21] On the other hand, the release rates of DOX from nanogels were quite similar for the linear copolymer micelles and the linear dendritic copolymer micelles.[13c]

Figure 2. Characterization of micelles and nanogels. A) Size and dispersity index measured by DLS. B) Morphology observed by TEM and SEM. The change of C) size and D) dispersity index of micelles and nanogels up to 28 days. E) Drug release properties of DOX loaded micelles and nanogels in PBS at 37 °C.
2.4.3. Biocompatibility

To investigate whether the dopa-functional micelles and nano-gels are suitable for drug delivery applications, the cytotoxicity of the materials was investigated. The pristine polymers, Tri-block A-dopa and Tri-block B-dopa were tested against a panel of different types of cancer cells (breast MCF-7, pancreatic PANC1, bone MG63, and glioblastoma U87) as well as non-cancerous cells (monocyte Raw 264.7 and human dermal fibroblast [hDF]) using the MTT assay, which measures the mitochondrial activity of the cells to reflect their cellular viability.12 The polymer materials were found to be non-toxic (mitochondrial activity >70%) against most cell lines up to 200 µg mL⁻¹, with only one cell line, hDF, exhibiting weak cytotoxicity at the high concentration of 200 µg mL⁻¹ as an extremity (Figure 3A). These results confirm that the materials are promising for drug delivery systems, as such high concentrations would not occur in vivo.

2.5. Biomedical Applications

2.5.1. Evaluation as Anti-Cancer DDSs

An ideal DDS for cancer treatment causes reduced side-effects because of the limited interaction of the drug to non-cancerous cells, but causes improved efficacy toward cancerous cells due to the drug’s encapsulation within the NPs.24 It was revealed that DOX-loaded NPs, in comparison to the free drug, caused significantly reduced cytotoxicity toward murine monocyte Raw 264.7 cells up to 72 h of treatment, especially visible for micelle-B, nanogel-A, and nanogel-B (Figure 2B). Monocytes and macrophages from blood are sensitive to toxic compounds like chemotherapeutics,25 so the reduced toxicity indicates adequate protection from the drug within the NPs, avoiding unnecessary exposure of drug. This is in line with the drug release results (Figure 2E), which showed that micelle-B, nanogel-A, and nanogel-B demonstrated slower drug release rates, indicating better DOX encapsulation and shielding of the NP shell. Moreover, the reduced toxicity in macrophages may enable their targeting ability to harness macrophages as a secondary DDS, targeting early stage tumors where the EPR effect does not exist.26

On the other hand, for cancer cells such as MCF-7 and PANC1, all of the DOX loaded NPs exhibited a similar or slightly improved toxicity profile compared to free DOX, while the pristine NPs (~10x concentration higher than the DOX-loaded NPs) were not cytotoxic at all. From Figure 3B and 3C it can be concluded that the DOX-loaded NPs are able to inhibit the activity of cancer cells to a similar or greater degree than free DOX, while reducing the toxicity of DOX to non-cancerous cells. This is an important point, because most literature published on micelles or nanogel-based NPs conclude similar or improved drug potency on cancer cells after drug loading, but...
very few reported systems demonstrate the protective effects of NPs toward non-cancerous cells or other healthy tissues.\cite{27} The fact that the DOX-NPs synthesized in this work enable these protective effects is highly advantageous, and draws attention to their use as effective anti-cancer drug delivery systems.

To better evaluate the potential therapeutic efficacy, the more complex 3D spheroids model of cancer cells was used. This model is considered to be a better in vitro mimic of the real environment within the body.\cite{28} In Figure 4A, consistent results of the 2D cell tests are shown, except that the activity was improved for MCF-7 in 3D format. Confocal microscopy was used to track free DOX or the DOX released from NPs (Figure 4B). It was found that both micelles and the nanogels were able to assist DOX in being internalized within the 3D spheroids, not only on the surface of 3D tumors, but also deep within the core, exhibiting overlapping nuclei signals. This reveals another key factor that may influence the therapeutic efficacy in clinical and in vivo applications. That is, that NPs may assist in drug accumulation at the tumor site due to the EPR effect, leading to increased local drug concentration. This could be a factor in allowing the drug to be taken up by the 3D tumors to reach the nuclei, where DOX can intercalate with DNA to achieve enhanced therapy.\cite{29}

The cytotoxicity analyses on cancer cells and drug tracking using the 3D model support that dopa functionalized NPs are promising DDSs for anti-cancer purposes. In this context, micelle-B, nanogel-A, and nanogel-B are superior because of the significantly reduced toxicity of DOX. However, unlike micelles that are constrained by a CMC, nanogels are not, thereby evading the fast disassembly during blood circulation, improving the possibility of accumulating at the tumor site due to the EPR effect.\cite{30} As a result, it is further concluded that nanogels formulated from linear dopa-functionalized polymers are superior to micelles for cancer treatment.

**Figure 4.** Evaluation of the therapeutic efficacy of DOX-NPs on 3D tumor model. A) Viability of cells from 3D spheroids measured by APH assay. B) Co-localization study of DOX-NPs on 3D PANC1 spheroids, DOX signal is seen as red fluorescence, with green fluorescence representing Calcein-AM labeled living cells, nuclei were stained with Hoechst 33342 and represented as the blue signal. Scale bar = 100 µm.
2.5.2. Antibacterial Evaluations

In addition to cancer treatment, an important utilization of DDSs is as an antimicrobial platform. CIP is a broad-spectrum antibiotic, widely used clinically to treat bacterial infections. CIP was loaded into micelles and nanogels similar to DOX loading, and the loading efficacy and average sizes after CIP loading were determined, as summarized in Table S4, Supporting Information. After CIP loading, the disk diffusion method was first used to investigate whether CIP is still active after encapsulation. Both gram-negative (Escherichia coli 208) and gram-positive bacteria (Staphylococcus aureus 2569) were tested and all the NPs loaded with CIP displayed a zone of inhibition on the agar plate while the pure NPs at the same dose did not (Figure 5A; Figures S9,S10, Supporting Information). To further compare the potency of CIP-NPs, the dose of CIP was...
normalized to the diameter of the zone of inhibition and was plotted as bars, shown in Figure 5B, which clearly revealed that CIP-micelles were more effective compared to CIP-nanogels.

As a quinolone antibiotic, CIP’s antibacterial activity is derived by interfering with the function of enzymes involved in DNA replication and transcription, further jeopardizing the metabolism of bacteria. Therefore, in the second step, bacterial viability was measured using the AlamarBlue assay during the exponential phase when treated with pristine NPs or CIP-NPs.[32] To further evaluate the role of micelles and the nanogel in assisting CIP delivery to combat bacteria, two CIP-resistant strains, E. coli 208 and S. aureus 7920 were also applied in comparison with non-resistant strains. In both cases, CIP and CIP-NPs were less effective in inhibiting bacterial viability in CIP-resistant strains (Figure 5C; Figure S11, Supporting Information), but the micelle morphology displayed very similar or improved efficacy compared to free CIP. Meanwhile, pristine NPs did not show any inhibition on either gram-negative or gram-positive bacteria (Figure S12, Supporting Information).

To better investigate the efficacy of NPs and CIP-NPs, their minimum inhibitory concentrations (MICs) on CIP-resistant strains were determined. MICs of pristine micelles and nanogels were calculated using the mass of those micelles and nanogels, and MICs were not present up until 1.5 mg mL$^{-1}$, indicating that none or very low antibacterial activity was found toward the two CIP-resistant strains from the AlamarBlue analyses (Figure S12, Supporting Information). MICs of CIP-NPs were calculated according to the mass of CIP encapsulated. As shown in Figure 5D, CIP-micelles demonstrated similar or even better antibacterial properties toward both E. coli 208 and S. aureus 7920 than free CIP. However, CIP-nanogels showed lower antibacterial activity toward E. coli 208 and S. aureus 7920 compared to CIP and CIP-micelles. This is likely due to the crosslinked nature of the nanogels which are able to maintain highly stable structures that entrap CIP effectively and result in less CIP reaching the bacteria within 24 h. This is further supported by comparing CIP-nanogels-B to CIP-nanogel-A, which have less crosslinking and a higher MIC. Here, the increased number of dopa groups is unlikely to have a direct influence on CIP’s efficacy, as none of the dopa-containing pristine NPs demonstrated any clear inhibition on bacteria growth over all of the assays conducted. Therefore, the antibacterial activity of CIP-NPs resulted from released CIP, with NPs only acting as vehicles. Micelles worked better than nanogels for antibiotic delivery, likely because micelles are less stable and easily disassemble in solution to release the drug that efficiently inhibits the growth of bacteria.

Indeed, antimicrobial drug resistance is an inevitable outcome of the evolutionary principle that microbes are constantly evolving, enabling them to adapt to lethal conditions.[33] That means that all known therapeutic compounds will eventually become ineffective. Therefore, besides reducing, overusing, or misusing antimicrobial drugs to slow down the development of resistance, the improvement of existing drugs is also crucial. Supported by the comprehensive assessment in this study including utilizing CIP-resistant strains, the formation of micelles formed from dopa-functionalized linear polymers is envisioned to be a promising strategy.

3. Conclusions

PEG$_{68}$-(b-MAC$_3$)$_2$ (Trblock A) and PEG-(b-MAC$_{13}$)$_2$ (Trblock B) linear triblock copolymers with dopa functionalities and allyl pendant groups were synthesized and used to formulate micelles and nanogels. Both NP morphologies have demonstrated good stability at sizes below 200 nm. Pristine polymers, micelles, and nanogels were found to be non-toxic to most tested cell lines. The micelles and nanogels were able to encapsulate and release drugs via diffusion, where the released drugs were still found to be highly functional. Interestingly, the two morphologies behaved oppositely toward cancer cell inhibition and antibacterial activity. For cancer cell inhibition, both micelles and nanogels demonstrated similar or improved efficacy toward a variety of cancer cells and were able to deliver DOX into 3D tumor models. Importantly, DOX-micelle-B and the two nanogels were able to significantly reduce the cytotoxicity of the drug to macrophage cells. It is beneficial that nanogels do not have a CMC, and are therefore considered to be more suitable than micelles for potential cancer therapy. For antibacterial evaluations, both CIP-micelle formulations demonstrated similar or improved effective compared to free CIP, while CIP-nanogels were significantly less effective, suggesting that micelles are superior to nanogels for bacteria inhibition in this study. Here, we have demonstrated that it is valuable to design a basic material platform that can be used to further develop drug delivery systems with different morphologies that are able to fulfill the various requirements of diverse biomedical applications. A unified approach to drug delivery is highly advantageous in that valuable resources can be preserved. To be clinically relevant, sophistication on the molecular level should be met with simplicity using practical means.

4. Experimental Section

Materials: All chemicals, drugs, reactants, and cell culture consumables were purchased from Sigma-Aldrich and used as received, unless otherwise stated. Anhydrous dichloromethane (DCM) and tetrahydrofuran (THF) were obtained from Merck. The UV initiator Irgacure 184 (I184) was purchased from Ciba Specialty Chemicals, Inc. Deuterium chloroform (CDCl$_3$) and deuterated methanol (MeOD) for NMR analyses were purchased from Cambridge Isotope Laboratories, Inc. Poly(ethylene glycol) ($M_w$ 6000 g mol$^{-1}$ [PEG$_{6000}$]) was freeze-dried before use and 1,8 diazabicycloundec-7-ene (DBU) was stored over molecular sieves before use to reduce traces of moisture, which would otherwise interfere with the polymerization.

Phonol red-free dulbecco’s modified Eagle’s medium (DMEM) was obtained from Gibco. Fetal bovine serum (FBS) and penicillin–streptomycin solution were purchased from Hyclone Laboratories. Mueller-Hinton broth (MHBII) was from Fluka. Hoechst 33342 and Calcein-AM were purchased from Invitrogen.

Cell lines involved in the study were purchased from ATCC (American Tissue Culture Collection) and maintained in Dulbecco’s modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS) and 100 units mL$^{-1}$ penicillin and 100 mg mL$^{-1}$ streptomycin under 5% CO$_2$ at 37°C. This included four cancer cell lines (breast cancer: MCF-7; pancreatic cancer: PANCl; osteosarcoma: MG-63; glioblastoma: U87) and two noncancerous cell lines (mouse monocyte cell Raw 264.7 and human debris fibroblast cell [hDF]).

*E. coli* 208 and *E. coli* 178 were obtained from North Carolina State University. *S. aureus* 2569 was from DSMZ, *S. aureus* 7920 was received
Materials Synthesis and Characterization: For a detailed description of the materials, material synthesis, characterization, and instrumentation please see the Supporting Information.

The synthesized materials, 5-methyl-5-allyloxycarbonyl-1,3-dioxan-2-one (MAC), thiourea co-catalyst (TU), and N-(3,4-dihydroxyphenethyl)-4-mercaptopbutanamide (dopa-thiol) were synthesized according to previously reported procedures.14,15

Micelle Formation: Micelles from Triblock A-dopa and Triblock B-dopa were prepared (DI) water. The polymer was first dissolved in a small amount of DCM (8 mg polymer in 800 µL DCM) in a glass vial, thereafter the solvent was evaporated during rotation of the vial to form a thin film on the inside of the vial. DI water (8 mL) was then added to the vial (final polymer concentration 1 mg mL⁻¹) and the solution was ultra-sonicated for 20 min.

Nanogel Formation: Nanogels were produced by crosslink micelles. Micelles from PEG6k-[(allyl1/dopa2)]₂ (Triblock A-d) (20.0 mg, 0.21 mg, 0.2 µg) were mixed with 100 µL of DMF:H2O (4:1) to allow the micelle/nanogel to disaggregate. The resulting solution was transferred onto a 96-well plate (round bottom) containing 100 µL of LB Broth (Lennox) with the desired concentration of pure NPs or CIP-NPs. After 3 h of incubation at 37 °C with shaking, 10 µL of AB agent was added into each well and were incubated for an additional 30 min. The fluorescence intensity was then recorded at the wavelength of 560/590 nm (excitation/emission). Six parallel wells were set for each sample at each concentration.

For the bacteria metabolism analysis, 5000 CFU bacteria were transferred into 96-well plates (round bottom) containing 100 µL of LB Broth (Lennox) with the desired concentration of pure NPs or CIP-NPs. After 3 h of incubation at 37 °C with shaking, 10 µL of AB agent was added into each well and were incubated for an additional 30 min. The fluorescence intensity was then recorded at the wavelength of 560/590 nm (excitation/emission). Six parallel wells were set for each sample concentration.

Spheroids Models: 3D tumor spheroids were formed using PANC1 cells as described previously.15 Briefly, PANC1 cells suspension (100 µL per well, DMEM without phenol red) supplemented with 20% methylcellulose was transferred into 96-well plates (round bottom) and incubated for 3 days to allow the formation of 3D spheroids. Thereafter, the 3D tumors were exposed to samples at the desired concentrations and the viability was assessed via the acid phosphatase assay (APH), as described previously. To track DOX distribution, samples with 2.5 µg mL⁻¹ of DOX eq. were added to the culture medium for pre-formed 3D spheroids. After 4 h of incubation, the fluorescent probes Hoechst 33342 (5 µg mL⁻¹) and Calcein-AM (5 µM) were added to stain the nuclei and living cells according to the protocols. Spheroids were washed with PBS twice before recording the fluorescent images using a Nikon Ti-S fluorescent microscope with the following filter settings (ex/em nm): blue 350/460; green 480/510; red 560/635. Data were recorded with the software Lumenera Infinity Analyze, and analyzed with Image J.

Minimum Inhibitory Concentration (MIC): A single colony of the bacteria from the agar plate was suspended into MHB II broth and incubated with shaking at 37 °C until log phase concentration. The bacteria solution was then diluted with broth to reach a concentration of 10¹⁰ CFU mL⁻¹. The compounds were diluted using the double dilution method. The bacteria solution was added into the wells with equal amounts of compound solutions, yielding a final concentration of 5 × 10⁻¹³ CFU mL⁻¹. The plates were incubated at 37 °C with shaking for 18 h and the optical density was then measured at 620 nm.

The Disk Diffusion Test: 10 µL of NP solution and CIP-NPs solution were transferred onto filter papers with diameter of 5 mm, which were then transferred onto the agar plate inoculated with bacteria. After overnight incubation, the diameters of the inhibition zones were measured and the images were recorded in triplicate for each independent set.

Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest
The authors declare no conflict of interest.
Data Availability Statement

Research data are not shared.

Keywords

antibacterial activity, block copolymers, cancer treatment, drug delivery, micelles, nanogels

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