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Antibiotic-containing polymers for localized, sustained drug delivery

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Abstract

Many currently used antibiotics suffer from issues such as systemic toxicity, short half-life, and increased susceptibility to bacterial resistance. Although most antibiotic classes are administered systemically through oral or intravenous routes, a more efficient delivery system is needed. This review discusses the chemical conjugation of antibiotics to polymers, achieved by forming covalent bonds between antibiotics and a pre-existing polymer or by developing novel antibiotic-containing polymers. Through conjugating antibiotics to polymers, unique polymer properties can be taken advantage of. These polymeric antibiotics display controlled, sustained drug release and vary in antibiotic class type, synthetic method, polymer composition, bond lability, and antibacterial activity. The polymer synthesis, characterization, drug release, and antibacterial activities, if applicable, will be presented to offer a detailed overview of each system.

Keywords

polymer conjugates; controlled release; sustained release; localized delivery antibiotics; biocompatible

1. Introduction

1.1. Antibiotics for treating infections

Conventional methods of antibiotic delivery involve systemic administration, often via the oral or intravenous routes, to treat a myriad of bacterial infections. While many common, non-life-threatening bacterial infections can be readily treated with an antibiotic course, issues arise when bacteria are not responsive or when the infection is serious[1]. Separately, implant-related infections are also a serious health issue that complicate already difficult, complex surgical procedures; biofilm formation at the implant site can cause implant failure and infection, leading to secondary surgery to remove the afflicted implant. Nearly 1 million

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implant-associated infections occur every year, and traditional, systemic antibiotic delivery is less efficacious in many cases [2]; an estimated 1000 times the antibiotic dose can be necessary to completely eradicate the biofilm [3]. Whether a common infection or implant-related infection, the increasing prevalence of multi-drug resistant bacteria, such as methicillin resistant *Staphylococcus aureus* (MRSA), is a notable challenge in treatment and prevention [4-7]. Over-prescription of broad-spectrum antibiotics (e.g., treating a viral infection with antibiotics) only exacerbates the resistant bacteria problem [8]. Considering the rise of resistance, the development of new antimicrobial delivery systems with improved biocidal efficacy is urgently required.

1.2. Need for improved antibiotic drug delivery systems

More efficient and effective drug delivery systems improving on conventional therapies (i.e., oral, intravenous routes) is crucial for microorganism eradication related to bacterial infections. Effective antibiotic release at concentrations above the bacteria's minimum inhibitory concentration (MIC) is a necessary condition to protect against infection; to treat current infections, the antibiotic concentration must be above the minimum bactericidal concentration (MBC). Improving pharmacokinetic and pharmacodynamic profiles, overcoming short-half life issues, and using localized delivery whenever possible could lower bacterial resistance incidence [9]. Local, controlled antibiotic release leads to lower dosing, decreased toxicity, extended release, and avoidance of systemic exposure [9, 10]. By localizing the drug at the specific infection sites, such as in implant-related infections, antibiotics specific for that strain can be administered at high dosage without surpassing the systemic toxicity, thereby lowering side effects and preventing resistance [2]. Additionally, avoiding systemic administration would increase patient compliance as well; oftentimes, patients who are prescribed oral antibiotics do not finish the entire course, breeding resistant bacteria. Particularly for implant-related infections, the ability for clinicians to locally administer a week-long antibiotic treatment would be a significant achievement. The advantage of a controlled, sustained release system is clear; this desired treatment is possible through polymeric delivery systems.

1.3 Controlled release of bioactives from polymers

The chemical conjugation of drug molecules to polymers offers numerous advantages for simple, small molecule delivery; the unique polymer properties allow for sustained and controlled release of bioactives [11, 12]. Additionally, the bioactive release rate can change based on the bonds that link the drug to the polymer (e.g., ester, amide, urethane, etc.) [11-13], formulation (e.g., powder, hydrogel, coating, microsphere) [14-16], and polymer chemical composition (e.g., non-bioactive backbone or "linker" molecule) [17, 18]. Through simple chemical modifications, the bioactive release rate can potentially be fine-tuned from days to many months, depending on the desired application and need. By covalently linking the drug, higher drug loading is achieved compared to physical incorporation [19]. Two methods of realizing this goal will be discussed, and each method has its advantages Section 2 focuses on drug conjugation to already-made polymers, whereas Section 3 describes synthesizing a monomer that contains the antibiotic and subsequently polymerizing it. This review focuses on the chemical conjugation of known antibiotic molecules with polymers; physical incorporation (e.g., admixtures, encapsulation) will not be discussed. Additionally,

we will not discuss all small, novel molecules that display antibacterial activity or polymers with inherent bioactivity (e.g., cationic, antimicrobial peptides) but instead known antibiotics. Antibiotic classes, including beta-lactams, fluoroquinolones, aminoglycosides, and sulfonamides, will be detailed herein (Scheme 1). These antibiotics are coupled to a wide range of polymers through hydrolytically labile (e.g., esters), enzymatically labile (e.g., amides), and non-labile bonds. The systems presented in this review have the potential to improve antibiotic delivery and reduce incidence of bacterial resistance.

2. Chemical Conjugation of Antibiotics to Polymers

The chemical conjugation of antibiotics to an existing polymer is described in this section. (Scheme 2) As shown in Scheme 2, a covalent bond is formed between an existing and drug molecule. Although this approach often leads to lower drug loading, it is possible to add several different drugs and/or targeting moieties to the polymer backbone. Examples of these types systems are demonstrated below and have been organized based on antibiotic class.

2.1. Beta lactams

Penicillin, one of the oldest beta-lactam antibiotics discovered, has been a target drug for many researchers. [20]. Penicillin V has been used as a ligand and conjugated using hydrolytically degradable ester linkages via carbodiimide coupling to prepare a water-soluble poly(ether-urethane) derived from poly(ethylene glycol) (PEG) and L-lysine [21]. Initially, PEG of varying molecular weights was coupled to both amino groups of lysine through urethane linkages, resulting in a block copolymer containing a free carboxylic acid allowing for further functionalization. Through dicyclohexylcarbodiimide (DCC) coupling, ethanolamine or ethylene diamine was attached to the copolymer via ester or amide bonds, leaving a free hydroxyl or amine, respectively. Penicillin was attached to the ethanolamine derivative via DCC coupling to form a hydrolysable ester bond (Figure 1a). Drug release in phosphate buffered saline (PBS) at 25°C was monitored spectrophotometrically; over 95% of the drug was released in the first 24 hrs. To elucidate the antibacterial efficacy of the polymer-released drug, five different strains of microorganisms were tested, including Klebsiella pneumonae, Escherichia coli, Staphylococcus aureus, Streptococcuspyogenes group A, and *Enterococcus faecalis*. Inhibited bacterial growth was observed for the latter three strains for both free drug and the polymer-bound drug, thus demonstrating potential as a drug delivery system superior to the status quo

Highly branched macromolecules, or dendrimers, have also been investigated as beta-lactam delivery system due to of their unique structures. The carboxylic acid moiety of penicillin V was modified with amino- or hydroxyl-terminated PEG through carbodiimide coupling then subsequently reacted with polyamidoamine (PAMAM) dendrimer to form penicillin V-conjugated PEG-PAMAM star polymers with either amide or ester bonds linking the antibiotic to PEG [22]. NMR and FT-IR spectroscopies characterized the polymer-drug dendrimer system. To determine antimicrobial efficacy, the ester-containing dendrimer (Figure 1b) was placed in dilute *S. aureus* broth and incubated for 24 hrs. The ester bonds hydrolyzed and released free penicillin that showed activity similar to unmodified penicillin,

demonstrating that highly branched polymers can be used for controlled antibiotic delivery. [22]

Polytetrafluoroethylene has been studied as a non-reactive biomaterial for implant surfaces. Polymer surface modifications of penicillin to expanded polytetrafluoroethylene (ePTFE) were carried out to prevent *S. aureus* growth on PTFE biomaterials [23, 24]. ePTFE surfaces were first activated with plasma then reacted with maleic anhydride (MA). To further modify the surface, the cyclic anhydride was hydrolyzed to yield a dicarboxylic acid and converted to a diacyl chloride with thionyl chloride. Equimolar amounts of 200 and 600 molecular weight PEG were was added as spacers before penicillin was coupled to PEG's hydroxyl terminus via DCC reaction in the presence of 4-(dimethylamino)pyridine (DMAP) (Figure 1c). To evaluate the modified surfaces, attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy and scanning electron microscopy (SEM) were used. Antibacterial activity was determined by incubating samples in broth containing a Gram-positive (*S. aureus*) or Gram-negative (*P. aeruginosa*) bacterium at 37°C for 3-4 hrs, then measuring the absorbance at 600 nm with a UV-vis spectrophotometer. All intermediates in the reaction sequence were tested as well as ePTFE-penicillin without activation or PEG spacer; the ePTFE-MA-PEG-penicillin product exhibited significant antibacterial activity (80% absorbance reduction) against *S. aureus* whereas ePTFE, MA-PTFE, PEG-MA-PTFE, and penicillin-ePTFE displayed negligible activity. Minimal activity was observed for all species against *P. aeruginosa*, a bacterial strain much less susceptible to beta-lactam antibiotics such as penicillin. The authors showed that a non-reactive surface such as ePTFE can be activated with plasma, PEGylated and decorated with an antibiotic to yield an active surface.

Cephradine was chemically conjugated to poly(lactic acid) (PLA) oligomers to overcome the hydrophilicity issues of the antibiotic; with very hydrophilic drugs, release occurs rapidly [25]. The terminal carboxylic acid groups of PLA oligomers were reacted with thionyl chloride to form the acyl chloride. Sodium cephradinate was stirred in aqueous pH 9 solution before addition of dichloromethane (DCM) and tetrabutyl ammonium bromide at 13,500 rpm. OligoPLA chloride was then added to antibiotic solution to conjugate the drug to PLA via amide linkages (Figure 1d). Size exclusion chromatography (SEC) was used to determine molecular weight. Polymers were incubated at 37°C in PBS for 40 days and media was analyzed by high performance liquid chromatography (HPLC) at different time points. The ultimate degradation products were determined to be a lactic acid dimer bound to cephradine; minimal free drug amounts were detected due to the amide bond stability under the test conditions. The degradation products exhibited antibacterial activity (methods not presented) against *S. aureus*, but were less active compared to free cephradine. Although the parent drug was not released (according to researchers' HPLC data) via hydrolysis, this method provides a novel platform to conjugate cephradine to PLA via hydrolytically sensitive linkages.

2.2. Fluoroquinolones

Members of the fluoroquinolone antibiotics class can be an effective treatment against many Gram-positive and Gram-negative bacterial infections. The broader spectrum capability of

fluoroquinolones and decreased bacterial resistance to enzymes that render beta-lactams ineffective leads to fluoroquinolones as being the most-studied antibiotic class with respect to polymer chemistry. One such antibiotic, norfloxacin, has a short half-life (3.5 hrs) [26] and has been investigated by multiple research groups.

Norfloxacin (NOR) was covalently linked to dextran with one of two tetrapeptide linkers, gly-phe-ala-leu or gly-phe-leu-gly, that are susceptible to degradation under lysosomal conditions [27]. The drug was coupled to one of the tetrapeptide linkers through two methods: reaction of NOR with pentafluorophenyl-activated peptides and pyridine or using trimethylsilyl-activated NOR with subsequent reaction with pentafluorophenyl-activated peptides. In all approaches, an amide bond links the drug to the peptide. 4-Nitrophenyl chloroformate was used to activate dextran before reaction with peptide-drug was and then attached to dextran via amide linkage. (Figure 2a); Upon enzymatic degradation using cathepsin B at pH 5.5, gly-NOR (25% after 7 hrs) and leu-NOR (80% after 7 hrs) were released. Notably, free drug was not released from either system under these conditions.

To improve upon the aforementioned system, a methoxy-terminated gly-phe-gly-gly peptide linker was used [28]. Here, NOR was covalently bound to the α-carbon of the peptide's terminal glycine residue. The peptide-drug was then conjugated to chloroformate-activated mannosylated dextran (Figure 2b). Free drug was released during both hydrolytic (PBS) and enzymatic (cathepsin B) degradation; 27% drug released in 24 hrs at pH 7.4 and 40% released at pH 5.5. In the presence of enzymes, 65% drug was released in the same time period. This system's efficacy was tested *in vivo* using mice infected with *Mycobacterium tuberculosis*. After 13 days following infection, animals were sacrificed and the bacteria in the liver, lungs, and spleen counted. The mannosylated dextran-drug conjugate displayed higher efficacy against the bacteria than NOR alone. Moreover, this system displayed effectiveness on par with isoniazide (an anti-tuberculosis drug) in the lungs, spleen, and liver.

In another example, NOR was conjugated to a poly(L-lysine citramide) carrier [29] using three different methodologies with 1) a lysine spacer, 2) an ethyl carbamate spacer, and 3) no spacer between drug and backbone. Firstly, dibenzyloxycarbonyl-L-lysine was coupled to NOR via DCC reaction; subsequently, the product was reacted with HBr to remove the protecting group and generate the hydrobromide which was then reacted with poly(L-lysine citramide imide) and dialyzed to generate the final product in which an amide bond links the drug to the polymer backbone with a lysine spacer. In the next conjugate, NOR was reacted with bromoethylchloroformate in the presence of 1,8-bis-dimethylaminonaphtalene, then poly(L-lysine citramide) to yield a polymer with a carbamate linkage between drug and backbone (Figure 2c). Molecular weights, as determined by SEC in aqueous medium, were 20 kDa for both the polymer without spacer and the polymer with lysine spacer, and 13 kDa for the carbamate spacer. Additionally, drug content ranged between 8-12 % by mass for all systems. Drug release from all three systems was determined at pH 7.4 and 4.5, all at 55°C to increase degradation rates. The conjugate with the carbamate linker released the highest free drug amount after 30 days (25% for pH 4.5, 8% for pH 7.4), as determined by HPLC. The other polymers released less than 5% NOR after the same time period due to the hydrolytic stability of amide bonds.

A NOR polyester prodrug was developed via ring-opening of cyclic esters [30]. Lactic acid (LA) and caprolactone (CL) homopolymers and copolymers were synthesized using glycerol (GL), penthaerythritol (PET), or PEG as initiators to yield hydroxyl-terminated oligoesters with two, three, or four arms, respectively (Figure 2d). This reaction was achieved by heating monomer, initiator, and catalyst (stannous octoate) in a flask then precipitating into methanol. NOR was then conjugated to the polyester free hydroxyl end groups through direct esterification. The molecular weight of the oligomers prior to coupling with drug was determined to be 1400-2500 Da by matrix-assisted laser desorption/ionization mass spectrometry (MALDI). While *in vitro* degradation studies were not yet performed, the polyester will likely undergo hydrolysis to yield free drug.

Similarly, ciprofloxacin (CIP) [31] and ofloxacin (OFL) [32] were conjugated to the hydroxyl ends of polyesters with two, three, four, or six arms. The polyesters were synthesized using CL, rac-lactide or L-lactide with GL, PET, dipenthaerythritol (DPET), or PEG as initiators to afford the respective arm lengths using the same method as described in the previous article. (Figure 2e). Fluoroquinolone dissolved in DCM was then slowly added to a polymer solution containing either pyridine for CIP-conjugate or DCC and DMAP for OFL-conjugate to yield the final products, ranging from 2-9 mol% drug content and 6-11 kDa (SEC). Degradation was performed on the polymer at 37°C in aqueous buffer (pH 1, 4, and 7.4 for CIP and pH 7 for OFL) and drug release quantified by UV-vis spectrophotometry. For all conjugates, the drug release rate was PEG > GL > PET > DEPT and rac-lactide > L-lactide > CL for monomer and initiators, respectively. The combination of PEG, the most hydrophilic of initiators used, and rac-lactide, the least crystalline of monomers used, leads to this system displaying the fastest release rate; rac-lactide initiated by PEG released 84% ofloxacin and 29% ciprofloxacin after 35 days at neutral pH. For CIP, lowering the pH to 1 increased drug release to 37% for the same system.

Similarly, NOR was conjugated to a polymer backbone to yield polyurethane conjugates [33]. Oligomers of CL and lactide terminated with hydroxyl groups were synthesized using creatinine as an initiator. These two oligoesters and oligo(ethylene adipate) diol were reacted separately with 1,6-hexane diisocyanate (HDI) to afford three different polymer precursors (Figure 2f). This reaction was achieved through oligomer addition to HDI (1:2 ratio) followed by heating at 65°C before dibutyltin dilaurate catalyst was added. Finally, NOR in dimethylsulfoxide (DMSO) was added dropwise to the mixture at ratio of 1:1. Molecular weights ranged from 23 kDa to 41 kDa, as determined by viscosity measurements. Degradation studies were performed in PBS buffer (pH 1 and 7.4) at 37°C. The lactic acid-NOR polyurethane conjugate displayed the fastest release with 18% and 10% drug release from the pH 1 and 7.4 media, respectively, after 21 days. The CL system released the slowest with 10% and 5% drug release after the same time period.

NOR was reacted with diisocyanatododecane (DDI) to yield a monomer that was subsequently polymerized with PCL-diol to afford a polyurethane using two different procedures [34]. The polymer molecular weight was increased by adding a diol or diamine to extend the chain. In the first procedure, PCL (Mw 2000 Da) and DDI in DMSO were heated to 60°C and dibutyltindilaurate was added. After 3 hrs, NOR was added and reacted for 22 hrs before chain extension with 1,4-butanediol (BDO), then terminated with

methanol. In the second method, NOR and DDI in DMSO at 60°C and dibutyltindilaurate was added. After 1 hr, PCL was added and reacted for 24 hrs. Ethylenediamine (EDA) or BDO was added as chain extender then terminated with methanol. Reaction stoichiometry for DDI/PCL/NOR/BDO and DDI/PCL/NOR/EDAStoichiometry were 2.6/1.8/0.6/0.2 and 2.6/1.6/0.6/0.4, respectively. Polymers were purified by precipitating into ether:water and washed in a Soxhlet extractor. Mw were determined by SEC before chain extension to be 18-22 kDa; after chain extension by BPO, Mw increased to 35 kDa and 41 kDa for methods 1 and 2, respectively. For EDA, Mw increased to 70 kDa.; however, in all cases, Mn changes negligibly. Thus, a large number of high molecular weight chains reacted during the chain extension. While the overall drug content was analyzed by UV-vis spectrophotometry and elemental analysis (4-5 wt%), no degradation or antimicrobial studies were performed.

In another example using polyurethanes, CIP was used [35, 36]. PCL (Mw 2000) and either 1,6-hexane diisocyanate (HDI) or DDI with dibutyltin dilaurate were combined to generate the prepolymer solution, after which ciprofloxacin was added to the reaction with triethylamine (Figure 2g). Reaction continued for 24 hrs at 60°C before dissolution in methanol and precipitated into water. Polymer Mw ranged from 20-24 kDa and 1.6 PDI. Hollow glass tubes coated with the polymers were prepared by dip-coating into dimethylacetamide solution containing polymer (10% w/w). Degradation studies were performed with cholesterol esterase in buffer and in PBS alone at pH 7.0 without enzymes with drug release quantified by HPLC. Approximately 0.4 μg ciprofloxacin was released after 28 days, twice the amount of drug released without the enzymes present. The enzyme-incubated samples demonstrated antibacterial activity against *P. aeruginosa*; the degradation media exhibited the same minimum inhibitory concentration (MIC) as free ciprofloxacin.

Levofloxacin (LEVO) was attached as a pendant group to epoxy-functionalized polydimethysiloxane (PDMS) through a platinum-catalyzed reaction [37]. PDMS was investigated as a potential means to provide localized drug release from a cytocompatible polymer on a biomedical implant. Firstly, PDMS was functionalized with epoxy groups by reacting poly(methylhydro-*co*-dimethyl)siloxane with allyl glycidyl ether in the presence of platinum-divinyltetramethyldisiloxane as catalyst to form EP-PDMS. The product was subsequently reacted with LEVO in methanol to generate PDMS with pendant epoxy and levofloxacin moieties (LEVO-EP-PDMS), linked by ester bonds (Figure 2h). Polymer coatings were prepared by exposing polymer to diethylenetriamine in methanol or chloroform, then curing at 50°C for 1 day. As comparison, EP-PDMS containing admixed LEVO at same weight percentage as tethered LEVO polymer and EP-PDMS alone were also made into coatings as controls. Differential scanning calorimetry (DSC) demonstrated that LEVO-EP-PDMS had a Tg of −45°C, 40°C higher than PDMS without drug covalently attached; nonetheless, the Tg is low enough to exhibit the rubbery desired properties. SEC revealed that EP-PDMS had a 7kDa Mw and 2.1 PDI, while LEVO-EP-PDMS exhibited lower Mw of 2 kDa and 1.4 PDI. The polymer's antimicrobial was determined by calculating the zone of inhibition against *E. coli*. The slow release of LEVO inhibited bacterial growth, with antimicrobial activity observed at the polymer surface after 28 days.

2.3. Others (Aminoglycosides, Sulfonamides, etc.)

Due to the structural complexity (e.g., multiple reactive functional groups) of some antibiotic classes, these molecules are conjugated to polymers far less often in literature. A polymeric prodrug of streptomycin, an aminoglycoside antibiotic, was developed for macrophage targeting and pH-sensitive release [38]. Ideally, the conjugate would be targeted toward certain cells, then the drug released in the lysosomes. Glycine hydrazide was synthesized by DCC coupling of benzyloxycarbonylglycine and *t*-butylcarbazate and subsequent benzyl group hydrogenolysis with palladium to afford a free amine. Glycine hydrazide was then reacted with 4-nitrochloroformate-activated dextran (Mw 40 kDa) in 1:1 DMSO:pyridine, then the *t*-butyl group removed with trifluoroacetictrifluroacetic acid (TFA). Finally, the aldehyde group in streptomycin reacted with the hydrazide of the dextran derivative in citrate buffer at pH 5 to yield the conjugate without the targeting group, with drug content of 3.8 mol%. As a targeting moiety, a mannose derivative with a terminal amine, identified by human mononuclear phagocyte receptors, was developed. First, D-mannopyranose was peracetylated with acetic anhydride in pyridine, then reacted with phosphorus tribromide in water formed the acetylglycosyl halide. This halide was reacted with 6-(benzyloxycarbonyl)aminohexanol and mercury(II) cyanide, and the benzyl protecting group subsequently removed through hydrogenolysis to afford the final product, an amine-terminated mannose derivative attached to dextran. Glycine hydrazide and streptomycin were coupled to the activated dextran using the same methodology as above (Figure 3). Release studies were performed at 37°C at physiological pH (7.4) and lysosomal pH (5.2), as hydrazone bonds are more labile at lower pH values. After 8 hrs, >50% drug was released at pH 5.2 and 30% released at pH 7.4 as determined by HPLC. To determine cell cytotoxicity, bovine erythrocytes were incubated in PBS with polymer, and the released hemoglobin was spectrophotometrically measured; less than 2% lysis occurred with the streptomycin conjugates.

Acriflavine, a topical antiseptic, was covalently attached to two different copolymers: poly(methyl methacrylate-*co*-maleic anhydride) [39] and poly(styrene-*co*-maleic anhydride) (PS-MA) [40]. Acriflavine was coupled to PS-MA in DMF and triethylamine for 12 hours then isolated by pouring over water and filtering. SEC indicated the polymer Mw to be 1.7 kDa. Release studies were performed in 10 mM PBS at 37°C at six different pH values, ranging from 6.6 to 8.0; the antibiotic controlled release, quantified by UV-vis spectrophotometry, occurred over 10 days at physiological conditions and was pH-dependent (i.e., increased pH increases release rate). Antimicrobial activity was determined with the bioassay method on agar plates using *B. subtilis* a Gram-positive bacteria. Bacterial inhibition increased from 40% after 4 hrs to 80% after 166 hrs, owing to the controlled drug release from the polymer.

3. Polymerization of Antibiotics

Thus far, chemical conjugation to pre-made polymers has been discussed. This section describes another approach: synthesizing an antibiotic-containing precursor, then polymerizing that molecule (Scheme 3). While this approach is used less often, it is an innovative approach to achieve higher drug loadings. In particular, bioactive-containing

poly(anhydride-esters) have gained considerable attention in the past decade as promising drug delivery systems owing to their uniquely high drug loadings and chemical control over sustained drug release profiles.[41]

3.1. Beta lactams

Polyacrylate nanoparticles containing N-thiolated beta-lactams covalently attached have been synthesized [42]. The beta-lactams were reacted with acryloyl chloride or mono-2-acryloyloxyethyl succinate to generate the monomers which subsequently underwent emulsion polymerization. The monomer (1 % w/w) dissolved in butyl acrylate and styrene (7:3 ratio) at 70°C was dispersed in aqueous sodium dodecylsulfate with stirring to form a suspension. Finally, polymerization was initiated by addition of potassium persulfate, stirred for 6-8 hrs, and purified by centrifugation, yielding in polyacrylate nanoparticles with ester-linked drug molecules. Nanoparticles were characterized by SEM, atomic force microscopy (AFM), transmission electron microscopy (TEM), and dynamic light scattering (DLS) to determine particle size and zetasizer to determine surface charge. Average particle sizes were 30-50 nm and zeta potential between −50 and −70 mV, indicating an anionic charge and high particle stability. Nanoparticle and monomer MICs were evaluated in agar against *S. aureus* and MRSA; nanoparticles were placed in a well plate with liquid agar, solidified, then exposed to bacteria, and incubated for 24 hrs at 37°C. Both the monomers and the nanoparticles displayed activity against both bacterial strains, with the latter showing higher activity, perhaps due to increased bioavailability. Polymers were deemed non-cytotoxic against human dermal fibroblast cells. Penicillin G-acrylate nanoparticles were also synthesized and characterized using the same methods (Figure 4a) [43]. Antibacterial testing indicated that the nanoparticles were not as effective as free drug against *S. aureus* but vastly more effective against MRSA. Penicillin binding to polymer nanoparticles effectively protects the drug and its activity against degradation by the beta-lactamase released by MRSA. *In vivo* studies were also performed on these nanoparticles using mice to assess nanoparticle toxicity by inflammatory response upon systemic and topical application [44]. Systemic delivery involved intraperitoneal injection of nanoparticle emulsions (ranging from 0.25-5%) twice daily for eight days; TNF-alpha and IL-6 concentrations in blood were determined at day 8 and day 14. For topical studies, nanoparticle emulsions were administered twice or thrice daily for eight days on an induced skin abrasion. As the cytokine levels were similar throughout both studies, the mice did not develop an inflammatory response from the nanoparticle injections. However, solid material was observed in the abdomen of the treated mice. Nonetheless, these nanoparticles have relatively low toxicity and efficacious antibacterial activity whether administered systemically or topically.

A poly(anhydride-amide) (PAA) containing ampicillin in the backbone has been synthesized as potential antibacterial coatings on medical devices (Figure 4b) [45]. The primary amine of ampicillin reacted with sebacoyl chloride in the presence of pyridine and DMF for 2 hrs. The dicarboxylic acid product was isolated by precipitation into acidic (pH 2) water and filtered. To synthesize the polymer, the diacid was suspended in DCM and reacted for 2 hrs with triphosgene as coupling agent and triethylamine as base. Polymer was isolated via precipitation into diethyl ether and filtration. GPC determined Mw to be 30 kDa and PDI to

be 1.2, while DSC identified Tg as 120°C. To make PAA coatings, polymer was dissolved in DMF and pipetted onto medical grade stainless steel coupons, then dried for a few hours under vacuum at 70°C to evaporate solvent. Drug release from coatings was performed in pH 7.4 PBS at 37°C. HPLC analysis revealed only diacid was released, as amide bonds are stable under these conditions. Because only labile anhydride bonds needed to be broken, all diacid was released within 3 hrs. Antibacterial studies using *S. aureus* were performed using the disk diffusion method. Mueller-Hinton agar was inoculated with bacteria, then PAA- and diacid-coated discs were placed on top; the plates were incubated for 24 hrs at 37°C. Both the diacid and polymer coatings showed nearly identical zones of inhibition; thus either the diacid has antibacterial activity itself, or the enzymes excreted by the bacteria cleaved the amide bonds to release free drug.

3.2. Fluoroquinolones

NOR was reacted with a methacrylate to produce an acryl monomer that subsequently underwent a free radical polymerization to form the polymer with NOR chemically incorporated into the backbone [46]. This synthesis was first carried out by reacting NOR with glycidyl methacrylate in DMF for 24 hrs at 40°C to yield the precursor methacrylate quinolone (MQ). MQ was subsequently treated to a free radical polymerization with 2.5% AIBN as initiator in DMF for 24 hrs at 40°C to afford polyquinolone (PQ, Figure 5a) that was isolated via precipitation in acetone-water. GPC determined the Mw and PDI to be 142 kDa and 2.7, respectively. After analysis, PQ was blended with low-density polyethylene (LDPE), PCL, maleated polypropylene (PPMA), and poly(butylene succinate) (PBSuc) by melting at temperatures up to 200°C. Tensile property testing performed on blends showed that incorporation of 1, 2.5, and 5% PQ into all blends decreased the percent strain at maximum load; yet, that PQ and PCL were observed to be the most compatible blends. The antimicrobial activity of PQ alone and PQ blends was evaluated against *E. coli*, *S. aureus*, *B. subtilis*, and *M. luteus* in PBS at physiological temperature via the shake flask test. In this method, bacterial culture is put into contact with the antibacterial compound in dilute PBS at 37°C for 24 hrs. Then, the bacteria number is counted after incubation at 37°C for 24 hrs. The antimicrobial activity of MQ, PQ, and all blends were evaluated using the aforementioned method. MQ and PQ displayed 100% reduction in bacteria while >98% cell reduction was observed in all blends for all strains at 5% PQ.

Another methacrylate monomer containing NOR was synthesized and polymerized via free radical polymerization [47]. NOR was reacted with 3-(acryloyloxy)-2-hydroxypropyl methacrylate (AHM) in DMF at 40°C for 24 hrs to yield AHM-NOR, precipitated into water, extracted with DCM, and then precipitated into diethyl ether. An AHM-NOR homopolymer (Figure 5b) was synthesized by initiation with 4.5% AIBN in DMF at 40°C for 24 hrs, followed by precipitation into water-acetone, filtration, and precipitation from DMF into ethanol. Mw and PDI were determined to be 26 kDa and 1.7, respectively. A copolymer of AHM-NOR (Figure 5c) with PEG methyl ether methacrylate (MPEGMA), was also synthesized using the same method with a 1:3 molar ratio of AHM-NOR to MPEGMA; product was isolated by precipitation into diethyl ether. The copolymer Mw was 62 kDa and PDI of 1.8. DSC and TGA revealed that neither polymer exhibited a Tg or Tm; Td values were about 280°C for polymers and 200°C for monomer. The polymers'

antimicrobial activity against *E. coli* and *S. aureus* was evaluated with the shake flask test. Specifically, antimicrobial agent (25 mg/mL) was mixed with bacteria then incubated at 37°C for 24 hrs after which a 100 μL sample was taken and spread on an agar plate and incubated under the same conditions. The monomer, homopolymer, and copolymer all showed 100% reduction for both bacterial strains.

3.3. Others (Aminoglycosides, Sulfonamides, etc.)

Gentamicin, an aminoglycoside, was chemically incorporated into a hyperbranched polymer with multiple cationic functionalities [48]. Gentamycin sulfate was dissolved in saturated sodium bicarbonate and reacted with *N,N′*-methylenebisarcylamide (1.5 eq) at 60°C for 3 days and dialyzed against water, yielding a branched polymer linked by glycoside and amide bonds (Figure 6a). To eliminate the terminal vinyl groups and decrease toxicity, they were end-capped by reaction with diethylamine. Using 1.5 MBA equivalents the final product's Mw was 5400 Da by SEC. Release studies were performed at pH 5.5 and 7.5 PBS at 37°C and degradation was observed by 1H-NMR spectroscopy. At physiological pH, no change in spectrum was observed after 51 days, but at pH 5.5, a chemical shift corresponding to the degradation of glycosidic and amide linkages was noted after only 5 days. Thus, the polymer breaks down in the slightly acidic lysosome conditions, but without UV-vis or HPLC data, release was not quantified. Antibacterial efficacy was determined by incubating the polymer (5 mg/mL) in LB (Luria-Bertani) broth containing *E. coli* for 12 hrs; using optical density measurements, 86% of bacteria were killed. Although the authors perform *in vitro* transfection efficiency and antitumor evaluation, these studies will not be discussed as it is beyond the scope of this the review.

Sulfamethoxazole (SMX), a sulfonamide antibiotic, was incorporated into a tetrapolymer for targeting and pH-sensitive capabilities [49]. SMX was added to *N*-methacryloylglycylglycine 4-nitrophenyl ester (MA-GG-ONp) in DMF and heated to 110°C for 24 hrs to yield MA-GG-SMX. MA-GG-SMXwas then reacted with MM-GG-ONp, *N*-(2-hydroxypropyl)methacrylamide (HMPA), and methacrylic acid (MAA) with AIBN with UV irradiation for 24 hrs at 50°C. The polymer was isolated via dissolution in methanol and precipitation into diethyl ether to yield poly(MMA-co-HMPA-co-MA-GG-SMX-co-MA-GG-ONp), where the drug is linked by an amide bond (Figure 6b). By NMR spectroscopy, the monomer molar ratios were 13:2.5:1:1.5, and SEC elucidated the Mw to be 38 kDa and PDI 2.5. TEM analysis revealed that the tetrapolymer self-assembled into a micelle with diameters ranging from 50 to 150 nm. Fluorescence spectroscopy, using pyrene as a probe, determined the critical micelle concentration (CMC) to be 1 mg/mL. The polymer also exhibited pH-dependent behavior; at pH 3.5, the polymer solution's transparency was reduced by 75%. At these lower pH values, the MAA carboxyl groups became protonated and intrapolymer interactions overcame intramolecular H-bonding, causing micelle collapse. At 7.4 pH values and higher, the MAA groups were deprotonated, leading to the sulfonamide protected in the center of the micelle. Antibacterial activity was evaluated in broth against *E. coli* by measuring changes in optical density after incubating for 18 hrs at 37°C. The polymer reduced bacterial viability by 10-20% from pH 4-8. However, adding 2% by volume DMSO to the media reduced viability to 50-60%; this increase is likely due to greater solubility and increased exposure of sulfonamide to the bacteria. Overall,

bioactivity increased with decreasing pH. To add an antibody for targeting purposes, the polymer was further modified. *N*-Boc-protected 1,4-diaminobutane was reacted with maleic anhydride in acetone and isolated via extraction after 1 hr. Product was then heated with sodium acetate in acetic anhydride at 100°C for 1 hr and extracted, after which the Boc groups were removed with TFA to yield *N*-(4-aminobutyl)maleimide (AMI). AMI was reacted with poly(MMA-co-HMPA-co-MA-GG-SMX-co-MA-GG-ONp) in DMSO at 80°C for 24 hrs and purified by dissolution in methanol and precipitation in diethyl ether to yield polymer with maleic moiety. The molar ratios of monomers were 17:4:1:1.65, and the Mw and PDI were 16 kDa and 1.9, respectively. The maleimide linker allowed for easy conjugation of antibody fragments (Figure 6c), Fab′ and $F(ab')_2$, as shown by solid-phase immunoassay experiments. Overall, these polymers displayed favorable pH-responsive properties; at physiological pH, the sulfonamide groups are protected in the micelle core and low antibacterial activity was exhibited. Upon exposure to lower pH environments (i.e., entry into lysosomes), the micelle collapsed and antibacterial activity markedly increased.

Salicylic acid (SA), an active metabolite of aspirin, is an anti-inflammatory and antipyretic that also exhibits antibacterial properties. Poly(anhydride-esters) (PAEs) containing SA have been synthesized and studied specifically for anti-biofilm activity. SA dissolved in tetrahydrofuran (THF) in the presence of pyridine is reacted with adipoyl chloride to yield a dicarboxylic acid containing two molecules of SA with an adipic acid linker molecule. The diacid is precipitated into acidic water and filtered. Acetylation with acetic anhydride and exposure to vacuum and 180°C with stirring for 4-8 hrs, then subsequent dissolution in DCM and precipitation in diethyl ether yields poly(salicylic acid adipate) (pSAA) (Figure 6d) [18, 50]. GPC determined Mw and PDI to be 18 kDa and 2,6, respectively. The polymer would maintain structural integrity *in vivo*, as DSC determined the Tg to be 59°C. Degradation studies were performed at pH 7 on polymer discs and drug release quantified by HPLC. The polymer biodegrades into SA and adipic acid; all drug was released after 90 days.[51] This polymer's ability to prevent *P. aeruginosa* biofilm formation was evaluated [52]. pSAA disks were placed in medium and inoculated with *P. aeruginosa*. For three days, every 8 hrs, media was removed and replaced with the same amount of fresh media. Each day, the number of viable cells was measured using a live/dead assay. As a control, a structurally-similar poly(anhydride-ether) that does *not* release SA was used. pSAA prevented biofilm formation five orders-of-magnitude better than the inactive polymer, even with comparable degradation rates. Additionally, *in vivo* degradation characteristics were elucidated through implantation of discs into mice. The pSAA was 80% in tact after four weeks, as determined by histology. Thus, pSAA prevented biofilm formation *in vitro* and invasion of inflammation-related cells *in vivo*. Additionally, pSAA ability to prevent *Salmonella enterica* biofilms was elucidated [53]. pSAA was dissolved in DCM and cast onto glass cover slips, then allowed to dry at ambient conditions and under vacuum for 12 hrs each. *S. enterica* was inoculated into used to inoculate brain heart infusion (BHI) broth, which was then incubated overnight at 37°C and the optical density spectrophotometrically determined. pSAA-coated coverslips and plain coverslips were incubated with the bacteria for 40 hrs, then washed with saline; biofilm cells and planktonic cells were separated and the optical densities determined. Planktonic cell counts were high for both treated and untreated coverslips, as the SA concentration released from the polymer was kept sublethal. However,

the presence of pSAA significantly reduced biofilm formation. SA released from the polymer did not affect biofilm formation by altering cell attachment capability, but instead through another mechanism.

Ferulic acid (FA) is a naturally-occurring phenolic compound that exhibits antimicrobial, properties [54], among many other relevant properties. FA has been chemically incorporated into a PAE backbone for controlled release and sustained bioactivity [55]. This goal was achieved through first synthesizing *tert*-butyl-protect FA where Meldrum's acid and *tert*-butanol were refluxed in toluene for 5 hrs, then vanillin added, followed by pyridine and piperidine as catalysts. Reaction mixture was heated at 75°C for 24 hrs, then washed to yield *tert*-butyl FA, which was subsequently dissolved in DMF in the presence of sodium hydride and reacted with adipoyl chloride to yield precursor protected with *tert*-butyl groups. TFA was used to remove *tert*-butyl groups to afford diacid that underwent solution polymerization in DCM with triethylamine as base and triphosgene as coupling agent to form PAE (Figure 6e) with 81% drug loading by mass. The Mw and PDI were determined to be 22 kDa and 1.7, respectively while DSC elucidated Tg to be 82°C. Drug release from polymer discs in PBS at pH 7.4 and 37°C was quantified via HPLC; 6% of total drug was released after 30 days. To ensure FA released from the polymer has the same antibacterial activity as free FA, a broth dilution assay using *E. coli* was performed. Polymer degradation products and free FA of the same concentration were separately dissolved in BHI media with 0.5% DMSO, then exposed to *E coli* in BHI broth and incubated for 24 hrs at 37°C. Both samples showed similar significant antibacterial activity.

4. Concluding remarks

While patients are habituated with taking daily medications when diagnosed with bacterial infections, treatment is often halted once symptoms are resolved; incomplete antibiotic dosing regimen significantly increases the development of bacteria resistance. Polymeric antibiotics as localized, controlled antimicrobial delivery systems may allow sufficiently high concentrations and extended release mechanism to effectively eradicate resistant organisms. Polymers can also protect the antibiotics from degradation by resistant bacterial enzymes[43] or by stabilizing a sensitive molecule[55]. Thus, the formulation of antibiotic-containing polymers can play a major role in protecting antibiotics from resistant bacteria and the enzymes that they produce. A multitude of antibiotic classes have been conjugated to polymers - ranging from biodegradable polyesters to non-degradable, yet still active, polyacrylates. Researchers have made significant strides since the discovery of antibiotics to not only increase patient compliance, but to also decrease side effects and other issues associated with antibiotic treatments. Future directions include fine-tuning drug release to ensure that the greatest therapeutic effect with the lowest side effects is achieved. The chemical incorporation of more complex antibiotic classes, such as macrolides, tetracyclines, and aminoglycosides also warrants future research, as very few examples are found in current literature. Development of additional hydrolytically degradable polymers (e.g., polyanhydrides, polyesters) with non-toxic degradation products will allow for less potential adverse reactions. Finally, combination therapies involving in the use of antibiotic-containing polymers in conjunction with other drug classes (e.g., anti-inflammatory,

analgesic) to fight infection in addition to inflammation and pain can be potentially efficacious for surgical procedures and implantations.

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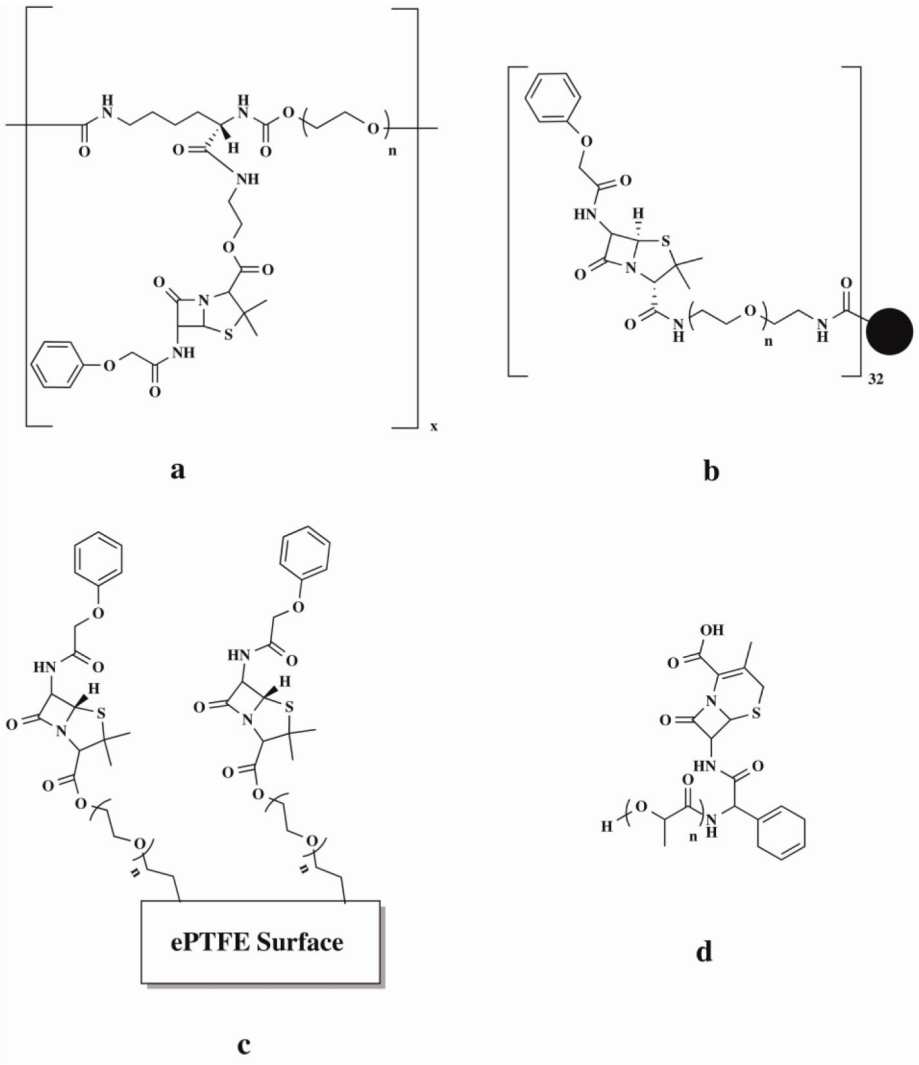


Figure 1. Penicillin V conjugate linked through ester (a), penicillin-containing PAMAM-PEG dendrimer (b), penicillin conjugated to ePTFE via PEG (c), cephradine conjugated to PLA oligomers (d)

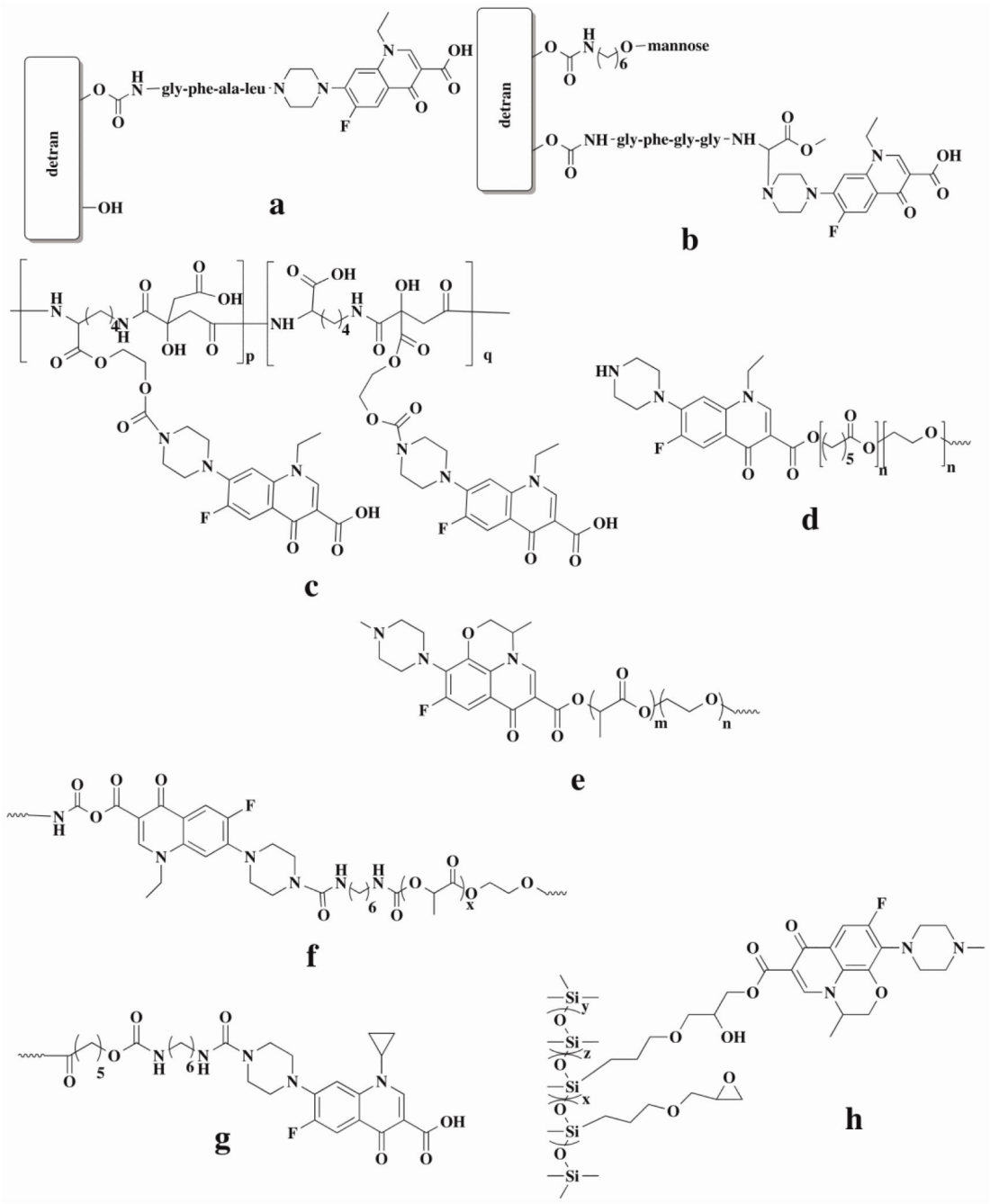


Figure 2. Dextran-peptide norfloxacin conjugate (a), mannosylated dextran-peptide norfloxacin conjugate (b), poly(L-lysine citramide imide) derivative with carbamate linking norfloxacin (c), PEG-PCL-norfloxacin polyester fragment (d), PEG-PLA-ofloxacin polyester fragment (e), PEG-PLA-norfloxacin polyurethane fragment (f), PCL-ciprofloxacin polyurethane (g), polydimethylsiloxane-epoxy-levofloxacin conjugate (h)

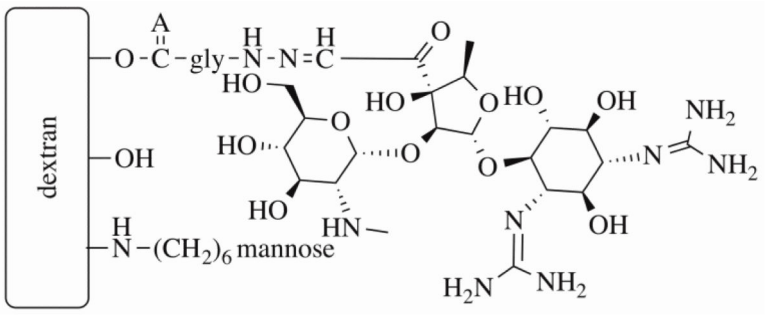


Figure 3. Streptomycin conjugate with mannose targeting group

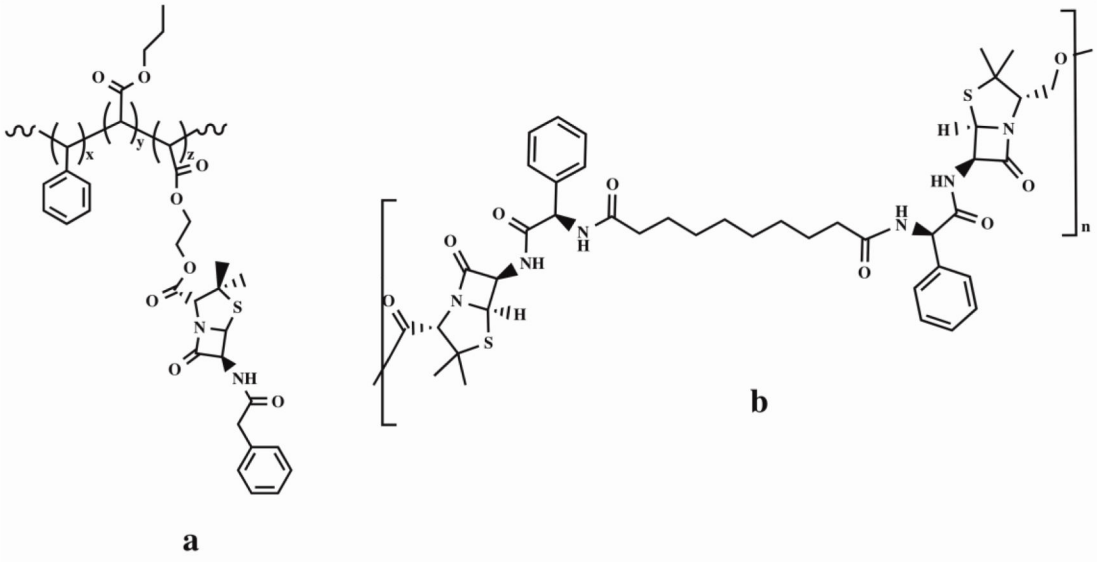


Figure 4. Penicillin G-conjugated nanoparticle repeat unit (a), ampicillin sebacic poly(anhydride-amide) (b)

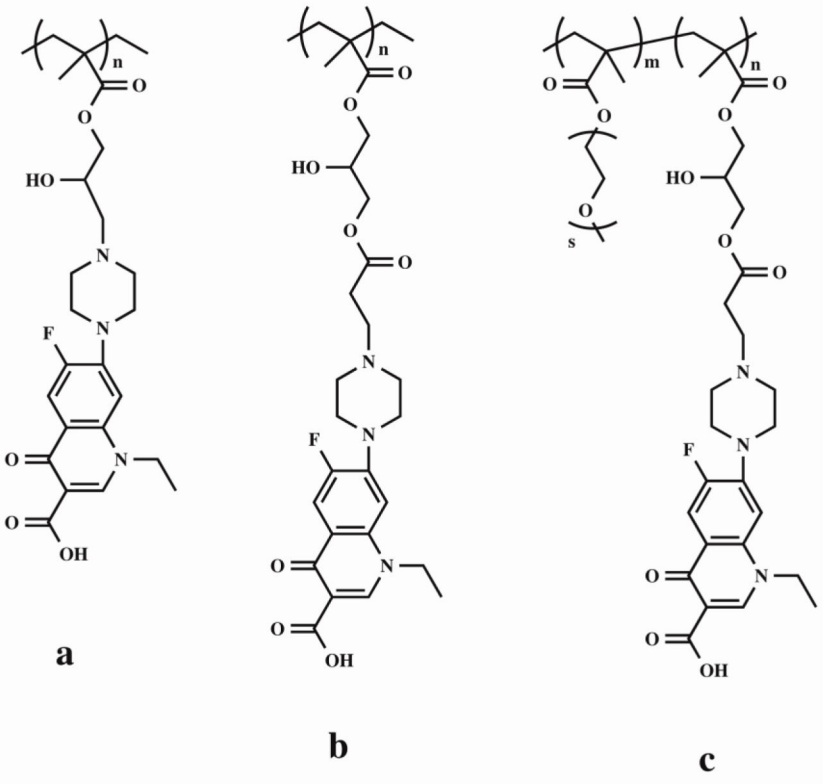


Figure 5. Norfloxacin glycidyl polymethacrylate (a), norfloxacin polymethacrylate homopolymer (b), norfloxacin-MPEGMA copolymer (c)

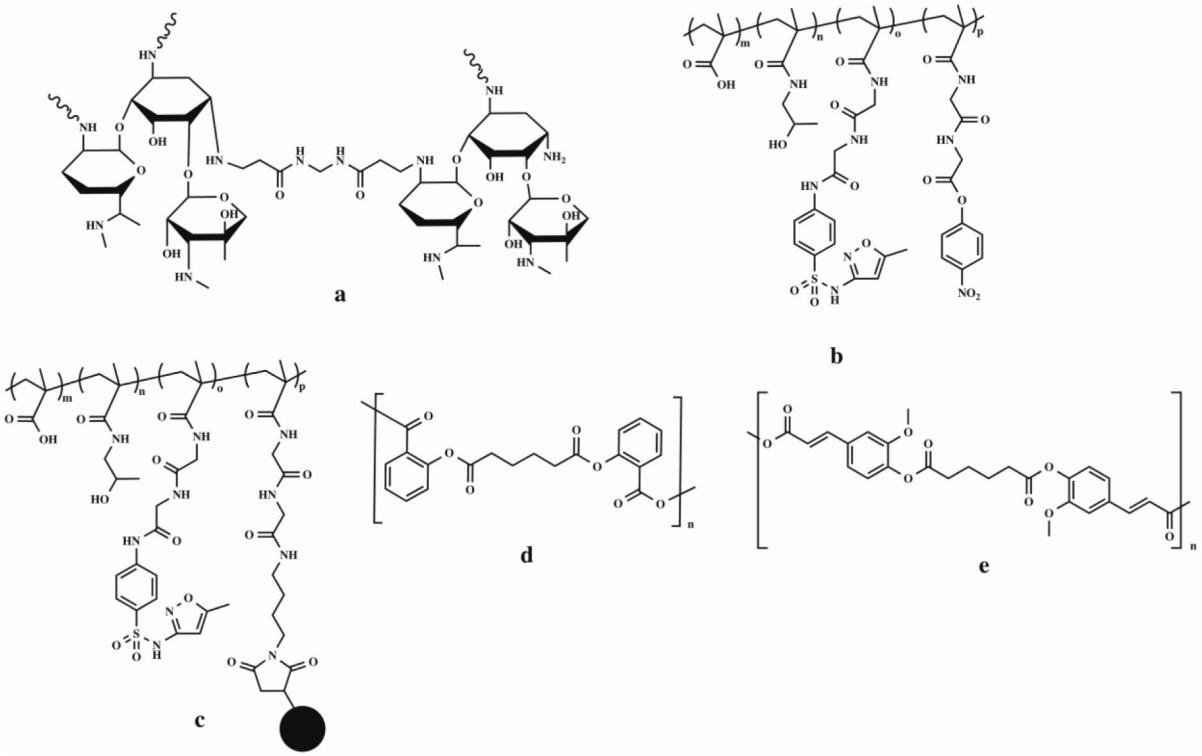
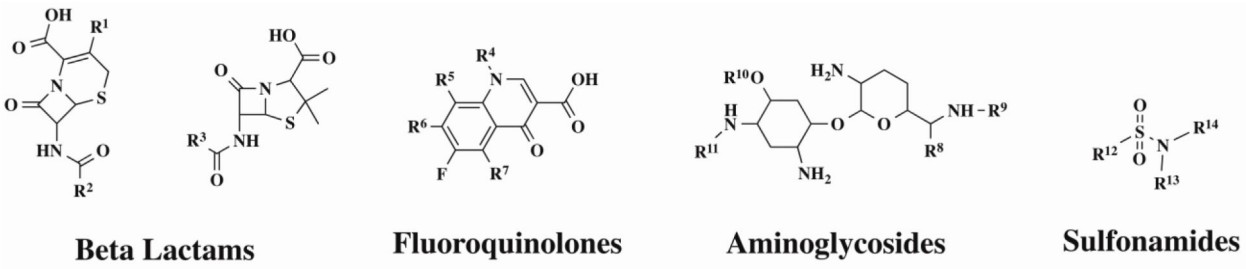
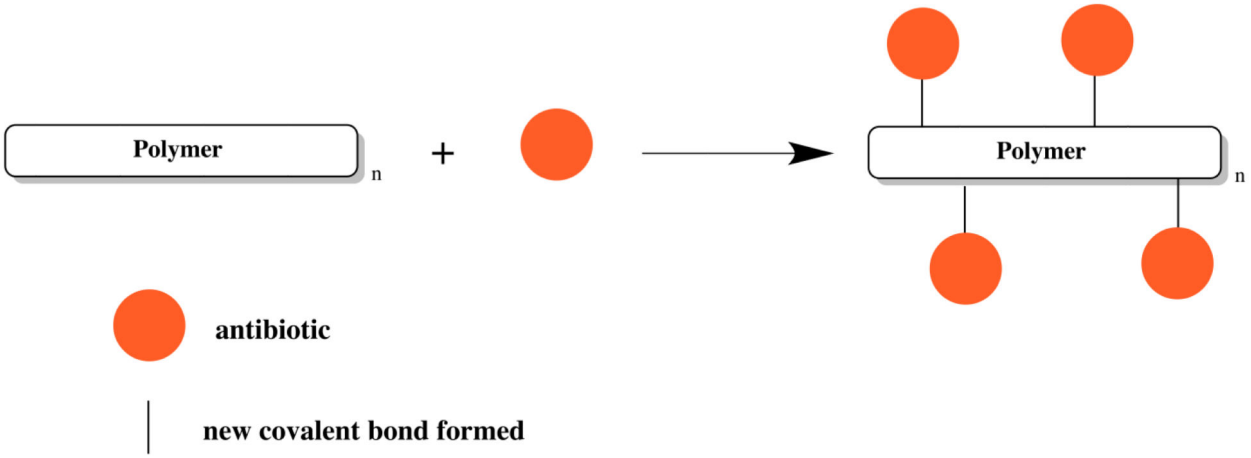


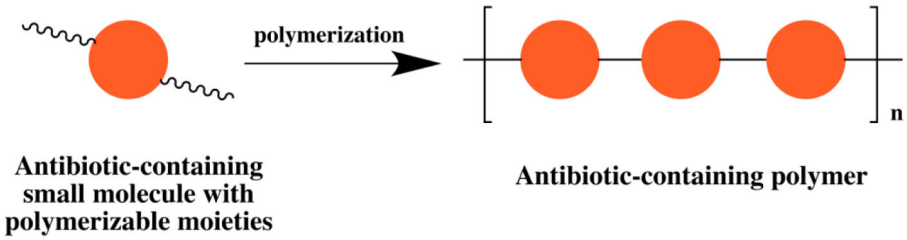
Figure 6. Gentamycin hyperbranchedhyperbrached polymer segment (a), Sulfamethoxazole tetrapolymer with 4-nitrophenyl group (b), Sulfamethoxazole tetrapolymertatrapolymer with targeting antibody attached (c), salicylic acid adipic poly(anhydride-ester) (d), ferulic acid adipic poly(anhydride-ester) (e)



Scheme 1. General structures of antibiotic classes discussed in this review



Scheme 2. Example of conjugating an antibiotic to a pre-made polymer through pendant group attachment



Scheme 3. Example of polymerizing a small molecule that contains an antibiotic