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Antibiotic loaded microspheres as antimicrobial delivery systems for medical applications

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ABSTRACT

In this paper, we present the preparation and antibiotic loading of polymeric microspheres, composed of copolymers derived from fatty acid/amino acid components, as new polymeric platforms for antibiotic delivery systems. New polymeric materials were used to prepare microspheres with and without immobilized model antibiotics (streptomycin, chloramphenicol and amphotericin B) by a W/O/W double-emulsion/solvent evaporation method, in which chloroform and poly(vinyl alcohol) are used as the solvent and emulsifier, respectively. The antimicrobial activity of the microspheres was tested against Gram-positive *S. aureus*, Gram-negative *E. coli* bacteria, and *C. albicans*, as a representative of a fungus. The new polymeric microspheres are particularly effective carriers for streptomycin, exhibiting antibacterial activity against all tested microorganisms.

1. Introduction

Bacteria are the most numerous form of life on Earth and are responsible for a variety of infections, ranging from cutaneous to life-threatening infections, such as pneumonia, endocarditis, septicemia, osteomyelitis, and other metastatic complications [1].

Implant-associated infections are a main cause of postoperative complications, because the introduction of an implant into human body is always associated with damage of tissue continuity [2]. Any exposed surface of medical devices may be subject to microbial colonization. The presence of water, electrolytes, and organic substances give impetus for microbial growth and further colonization of the material surfaces *in vitro* and then *in vivo*. Subsequently, bacteria may form biofilms resistant to antimicrobial agents and the removal of the infected device is often the only possible clinical solution [3,4]. Therefore, the most effective approach to eliminate infection is to use a system comprising both implant and antibiotic [2].

Conventional methods of treating bacterial infections include oral or intravenous injection/administration of antibiotics [3]. Unfortunately, systemic antimicrobial treatments lead to high concentration of the antibiotics in the bloodstream causing a higher incidence of side effects. These treatments also result in higher patient costs and reduced patient comfort [5].

In the last few decades, a large number of studies have focused on developing novel and more efficient systems to combat implant-associated infections [1]. The best way to prevent implant-associated infections seems to be local antibiotic treatment, where a higher concentration of medication can be introduced to the target site, for example using antibiotic-loaded bone cements [6–8], poly(methyl methacrylate) beads [8], antibiotic-impregnated collagen sponges [9,10], or antibiotic-immobilized/loaded microspheres [2,5,11,12].

Polymeric nano- and microcarriers made of natural and synthetic polymers have shown several advantages in drug delivery, such as high stability both *in vitro* and *in vivo*, good biocompatibility, and multifunctionality. This potential has been explored for encapsulating antibiotics in polymeric particles, which allows (i) enhanced dissolution rate of sparingly soluble or hydrophobic drugs, (ii) surface functionalization allowing targeted delivery and immune system evasion, thus prolonging the *in vivo* therapeutic half- life, as well as (iii) controlled and sustained drug release. Taken together, these features translate to reduced dosing frequency and improved therapeutic efficiency [1,13]. Ultimately, polymeric micro/nanoparticulate system that is capable of delivering antibiotics in a locally applied and extended-release manner for patients receiving implants would facilitate efficient prevention and treatment of infections often occurring in transplant patients. Thus, local application of encapsulated antibiotics directly to the surgical sites could provide a non-oral, non-intravenous, and controlled time-release treatment of implant-associated infections.

Various polymers have been used as antibiotic carriers, because they can effectively deliver the drug to a target site and thus increase the therapeutic benefit, while minimizing side effects [14,15]. For local delivery of antibiotics, the most studied has been poly(lactic-*co*-glycolic acid) (PLGA), which has been used to encapsulate a broad spectrum of

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antibiotics such as: gentamicin against *Brucella melitensis*, *Mycobacterium*, *Legionella*, and *Listeria* [15–17]; rifampicin and capreomycin against *P. aeruginosa*, *S. aureus* and *M. tuberculosis* [18–21]; vancomycin against *S. aureus* and *P. aeruginosa* [22]; and fusidic acid (FA) against *S. aureus* and *S. epidermidis* [23].

No less important is the potential bioactive and/or antibacterial nature of polymers used as a matrix for antibiotic carriers. In the literature, a great number of amphiphilic systems for drug delivery, including polyesters and polypeptides which form hydrophobic blocks and polyethers, polypeptides, and polysaccharides which form hydrophilic blocks can be found. Hydrophobic blocks based on fatty acids have been used in the design of linear amphiphilic copolymers capable of self-assembly into nano- and microstructures [24]. Fatty acids are attracting attention as potential therapeutic and antimicrobial agents due to their potency, broad spectrum of activity, and lack of classical resistance mechanisms [25]. In particular, long-chain unsaturated fatty acids are bactericidal to important pathogenic microorganisms, including methicillin-resistant *Staphylococcus aureus*, *Helicobacter pylori*, and *Mycobacteria*. These antibacterial actions of fatty acids are usually attributed to long-chain unsaturated fatty acids, including oleic acid, linoleic acid, and linolenic acid, while long-chain saturated fatty acids, including palmitic acid and stearic acid, are less active [25,26].

An ideal antibiotic delivery system should be non-reactive in the body and stable for as long as antimicrobial therapy is desired. In order to preserve the stability of the carrier, amino acids such as derivatives of tyrosine and polyethers, *i.e.* poly(ethylene glycol) (PEG), can be used.

Derivatives of tyrosine possess amide and ester bonds which are potentially hydrolysable. Under physiological conditions *in vitro*, it has been shown that only the ester bonds were hydrolyzed, while the amide bond is stable to hydrolysis during incubation in aqueous media (at 37 °C in pH 7.4) that do not contain enzymes [27]. The insensitivity to hydrolysis of amide bonds is a guarantee of the long-term physical and chemical stability of the resulting nano- and micro-vehicle.

Poly(ethylene glycol) is commonly used as the shell-forming material due to its excellent inherent properties of strong hydration, weak antigenicity, and low toxicity. As such, the PEG shell could inhibit protein adsorption, reduce reticuloendothelial system (RES) uptake, and increase the blood circulation time of these carriers [28–31].

The combination of the above-mentioned units, namely fatty acids, amino acids and poly(ethylene glycol), in one polymeric system has already demonstrated amphiphilic properties and the ability to self-assemble in aqueous environment [32–36].

The aim of this study was the preparation of microspheres with and without immobilized model antibiotics (streptomycin, chloramphenicol and amphotericin B) using these poly(ester-amide)-PEGs (PEAE) as a polymeric matrix for antibiotic carriers. The PEAE microspheres with and without immobilized model antibiotics were prepared by a simple water-oil-water (W/O/W) double-emulsion/solvent evaporation method, in which chloroform and poly(vinyl alcohol) are used as the solvent and emulsifier, respectively. The antimicrobial activity of the polymeric microcarriers with and without immobilized model antibiotics was evaluated *via S. aureus*, *E. coli* and *C. albicans* growth and viability assessment.

2. Materials and methods

2.1. Polymers

New amphiphilic copolymers were prepared using derivatives of tyrosine *i.e.* desaminotyrosyl-tyrosine ethyl ester (DTE) and desaminotyrosyl-tyrosine hexyl ester (DTH) (the New Jersey Center for Biomaterials, NJ, USA) and dimer fatty acid: a hydrogenated dilinoleic acid (DLA), trade name Pripol 1009, molecular mass ~570 $g \cdot mol^{-1}$ (C36) (kindly provided by Croda, The Netherlands). 1-Ethyl-3-(3-dimethyllaminopropyl) carbodiimide hydrochloride (EDC·HCl) was obtained from GenScript Biology CRO for Drug Discovery. Other chemicals such as poly(ethylene glycol) methyl ether of molecular mass 5000 g/mol (mPEG5000) and 4-dimethylaminopyridine (DMAP), were obtained from Aldrich Chemicals. All solvents of HPLC grade were used without further purification. Synthesis of polymers were carried out in dichloromethane (DCM) solution, at room temperature, in an inert gas atmosphere. The chemical structures of PEAE copolymers were evaluated by Fourier transform infrared (FTIR) and nuclear magnetic resonance (NMR) spectroscopy in previous work [32]. The chemical structure of amphiphilic poly(ester-amide)-PEG copolymers is shown in Fig. 1.

2.2. Preparation of microspheres with immobilized antibiotics

The formation of the microspheres (Fig. 2.) was carried out in a simple double emulsification process. An aqueous solutions of antibiotics was emulsified in an organic polymer solution (10 mg in 1 ml of chloroform), forming the primary (water/oil) emulsion. The primary emulsion was then dosed as 100 μl aliquots through a pipette tip into 20 ml of a gently mixed (about 400 rpm) solution of 4% polyvinyl alcohol (PVA, Aldrich Chemicals) in distilled water, forming the secondary (water/oil/water) emulsion. After dosing the whole volume of the primary emulsion, chloroform was removed by evaporation in a fume hood for 8 h. After evaporation of the solvent, the resulting microspheres were dialyzed for 6 h, changing the water twice during the process. After dialysis, the resulting emulsion was lyophilized using a laboratory freeze dryer (Alpha 1–2 LD plus). Commercial grade streptomycin (10 mg/ml), chloramphenicol (2 mg/ml), and amphotericin B (250 μg/ml) (Sigma, USA) were used. Due to the different MBC (minimal bactericidal

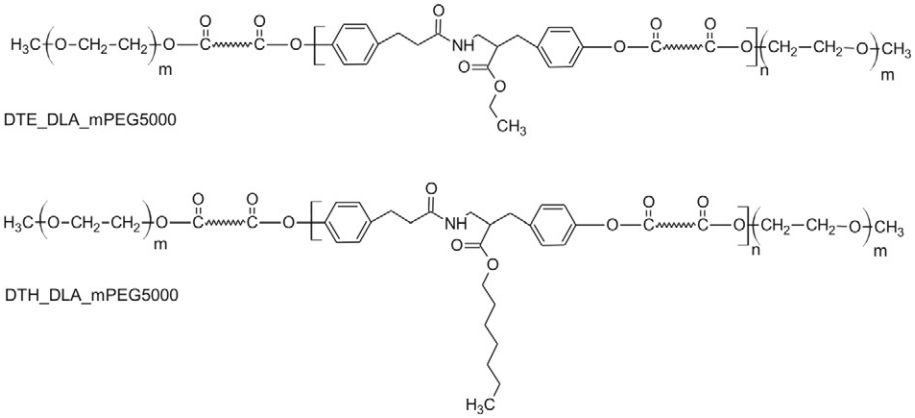

Fig. 1. Chemical structure of amphiphilic poly(ester-amide)-PEG copolymers.