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Functionalised nanoscale coatings using layer-by-layer assembly for imparting antibacterial properties to polylactide-co-glycolide surfaces

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ABSTRACT

In order to achieve high local biological activity and reduce the risk of side effects of antibiotics in the treatment of periodontal and bone infections, a localised and temporally controlled delivery system is desirable. The aim of this research was to develop a functionalised and resorbable surface to contact soft tissues to improve the antibacterial behaviour during the first week after its implantation in the treatment of periodontal and bone infections. Solvent-cast poly(D,L-lactide-co-glycolide acid) (PLGA) films were aminolysed and then modified by Layer-by-Layer technique to obtain a nano-layered coating using poly(sodium4-styrenesulfonate) (PSS) and poly(allylamine hydrochloride) (PAH) as polyelectrolytes. The water-soluble antibiotic, metronidazole (MET), was incorporated from the ninth layer. Infrared spectroscopy showed that the PSS and PAH absorption bands increased with the layer number. The contact angle values had a regular alternate behaviour from the ninth layer. X-ray Photoelectron Spectroscopy evidenced two distinct peaks, N_{1s} and S_{2p}, indicating PAH and PSS had been introduced. Atomic Force Microscopy showed the presence of polyelectrolytes on the surface with a measured roughness about 10 nm after 20 layers' deposition. The drug release was monitored by Ultraviolet–visible spectroscopy showing 80% loaded-drug delivery in 14 days. Finally, the biocompatibility was evaluated *in vitro* with L929 mouse fibroblasts and the antibacterial properties were demonstrated successfully against the keystone periodontal bacteria *Porphyromonas gingivalis*, which has an influence on implant failure, without compromising *in vitro* biocompatibility. In this study, PLGA was successfully modified to obtain a localised and temporally controlled drug delivery system, demonstrating the potential value of LbL as a coating technology for the manufacture of medical devices with advanced functional properties.

1. Introduction

The use of antimicrobial biomaterials is becoming increasingly important in medicine and dentistry, in which the elimination of bacteria and device-associated biofilms is essential for effective treatment [1,2]. For instance, the conventional treatment of periodontitis by scaling and root planing is advantageously accompanied by the adjuvant administration of antibiotics, which can be applied by systemic or local administration [3]. Compared to systemic drug delivery, local administration in periodontology is considered to be more effective, since the pathogen-specific drug can be placed directly in the periodontal pocket achieving effective concentrations for a sufficiently long period of time. In addition, the risk of undesired side effects caused by high systemic doses or resistance development can be reduced [3,4]. It is therefore beneficial to use local delivery systems that control the release of their agents and guarantee lasting drug concentrations in the pocket in spite of high sulcular fluid rates [3]. Among antibiotics, metronidazole (MET) is highly effective for the management of anaerobic infections, such as intra-abdominal and gynaecologic infections, septicemia, endocarditis, bone and joint infections, central nervous system infections, respiratory tract infections, skin and skin-structure infections. Moreover, MET is largely used for treating infections by anaerobic bacteria associated with periodontal diseases due to the low minimum inhibitory concentration it requires [5]. For treatment of mixed aerobic and anaerobic infections, metronidazole should be used in combination with other antibacterial agents that are appropriate for the treatment of the aerobic infection, because MET is ineffective against aerobic bacteria [6,7].

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For treating periodontitis, a number of resorbable drug delivery systems were developed during recent decades, such as drug loaded hydroxypropyl cellulose films [8], or drug carrying gels such as Elyzol® (Dumex GmbH, Bad Vilbel, Germany) dental gel, based on melted glycerol mono-oleate [9,10]. However, for these systems, the periodontal milieu often poses the major problem that the required period of drug exposure (7–10 days) cannot be achieved [11]. Also in the field of periodontal surgery, as in the transplantation of a mucous membrane [12], resorbability of the scaffold material is important to avoid inflammatory effects and surgical removal. Therefore, the use of films or membranes represents a promising approach in periodontal treatment to promote tissue regeneration by avoiding migration of epithelial cells into the periodontal pocket and incorporating antibiotics to inhibit the related bacteria growth [13]. Different barrier membranes with a graded functionalised structure are proposed in the literature in order to obtain tuned mechanical and degradation characteristics [14,15]. Bottino et al. [16] have proposed multilayered membranes consisting of three layers: an inner layer based on poly(DL-lactide-co-ε-caprolactone) (PLCL) and two outer functionalised layers, based on ternary polymeric blends (gelatin, PLCL and PLA) in contact with the surrounding tissues. The outer layer, in contact with bone, was loaded with nano-hydroxyapatite to enhance bone regeneration; while the other layer, in contact with epithelial tissue, was functionalised with metronidazole, incorporated in the layer bulk. Interestingly, the direct incorporation of antibiotics to these membranes has given promising results in terms of inhibition of bacteria growth [7,17,18]. However, although the antibacterial effects of these drugs are well known, recent findings have warned of the potentially toxic effects of these highly concentrated antibiotic drugs on dental pulp stem cells and dental pulp fibroblasts [19,20]. Furthermore, the direct drug release from polymeric substrates undergoes bulk degradation, modifying dramatically the physico-chemical properties of the device, mainly in terms of mechanical properties [21].

Therefore, the purpose of this research was to propose an innovative localised and controlled delivery system in the treatment of dental and periodontal infections in order to: (1) reduce the released antimicrobials amount to the gum surface, preventing both bacteria resistance and drug-related systemic side effects, and (2) preserve the physico-chemical properties of the barrier membrane without incorporating the antibiotic into the bulk of the material. Specifically, in this study, a periodontal biodegradable membrane was coated by Layer-by-Layer (LbL) technique to obtain discrete nanoscale layers to incorporate and to control the release of the antibiotic drug with minimal interaction with the biomaterial substrate.

LbL assembly, firstly introduced by Decher [22], is an alternative surface modification technique to Langmuir Blodgett deposition and Self-Assembled Monolayers (SAMs). LbL electrostatic assembly of charged polymers has been widely used as a versatile technique for the formation of multilayered thin films with tailored structure and composition in a wide range of electrical, magnetic, biological and optical applications [23]. LbL assembly is based on the alternating exposure of a charged substrate to solutions of positively and negatively charged polyelectrolytes. A rinsing step is included between the two previously described adsorption processes, to remove excess as well as to prevent cross-contamination of the polyelectrolyte solutions [24]. The LbL technique allows fine control of the coating properties and the obtainment of homogeneous multi-layered structures. It is also applicable to substrates of any shape, it is environmentally-friendly and it allows room temperature processing and low-cost manufacturing [25]. Polyelectrolyte multilayered films have therefore been considered for biomedical application such as capsules for drug delivery [26,27], immunosensing [28], regenerative neurobiology [29], antibacterial and anti fungal protection [30,31]. Despite its potential, LbL has not yet been translated for routine application in the manufacture of medical devices, and further research is required to demonstrate its practical value. In this study, nano-structured multilayered coatings were deposited on resorbable dense membranes based on a biocompatible and biodegradable synthetic polymer (poly(D,L-lactide-co-glycolide acid), PLGA) using the LbL method. The antibiotic drug was incorporated into the polyelectrolyte layers, based on poly(sodium4-styrenesulfonate) (PSS) and poly(allylamine hydrochloride) (PAH), after optimisation of the process to obtain appropriate drug release kinetics. The nano-coating was characterised by morphological, physico-chemical and biological analyses in order to evaluate the multilayered correct deposition, and the biological and antibacterial behaviour.

2. Material and methods

2.1. Materials

Poly(D,L-lactide-co-glycolide) (PLGA; lactide:glicolide (75:25), Mw = 66,000–107,000), Poly(sodium4-styrenesulfonate) (PSS average Mw ~ 70,000; powder), Metronidazole (MET; Mw: 171.15), and Ethylenediamine (ED) were purchased from Sigma–Aldrich, UK. Poly(allylamine hydrochloride) (PAH) was purchased from Alfa Aesar, UK. As solvent, acetone (99.8%) was purchased from Fisher Scientific, UK. All materials and chemicals were used as received without any additional purification.

2.2. PLGA film preparation and functionalisation

The compact film was prepared by a solvent casting process. Briefly, PLGA was dissolved in 20 mL of acetone (3% w/v) at room temperature by vigorous stirring, then cast on Petri dishes (φ = 8 cm, Duroplan) and dried under fume hood for 48 h.

Aminolysis. PLGA compact films were aminolysed by immersion in 0.05 M ED solution and allowed to react at 20 °C for 15 min to obtain a positive charge on the surface by $-NH_2-$ grafting. Treated films were washed three times with ice cold water, followed by soaking for a further hour in fresh water on ice, dried in a oven at 37 °C for 12 h, and, finally, stored in a desiccator over silica gel until use.

Layer-by-Layer. The deposition of PSS/PAH multilayered films (shown in Fig. 1) was carried out at room temperature. PSS and PAH solutions were prepared as 5 mg/mL solutions in 0.1 M NaCl and the pH was adjusted to 5.5. Prior to LbL deposition all the polymer solutions were filtered through 0.45 μm Polyvinylidene fluoride (PVDF) membrane (Fisher). The ζ-potentials of the polyelectrolytes solutions was measured by laser Doppler electrophoresis (Zetasizer Nano, Malvern instrument, USA). Aminolysed PLGA films (size 5 × 5 cm with a thickness ~180 μm) were first immersed in PSS solution (5 mL) and left in incubation for 15 min. Then, they were rinsed with 0.1 M NaCl water solution at pH 5.5 for 5 min. The, PLGA films were finally immersed in PAH solution (5 mL) and left in incubation for 15 min, following the same rinsing procedure. The procedure was repeated to obtain 20 layers (10 bilayers of PSS/PAH). The antibiotic drug, metronidazole, was incorporated into the nano-layers from the ninth layer following the same process described above, dissolving 0.1% w/v MET in PSS and PAH solutions. At the end of the LbL procedure, the samples were rinsed with deionised water at pH 5.5 for 10 min. The films were dried in an oven at 37 °C and then stored at 3 °C.

2.3. Physico-chemical characterisation

Surface composition was determined by X-ray Photoelectron Spectroscopy (XPS) on Theta Probe (Thermo Scientific, East