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Low-density polypropylene meshes coated with resorbable and biocompatible hydrophilic polymers as controlled release agents of antibiotics

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ABSTRACT

The application of bioactive meshes in abdominal surgery for the repair of hernias is an increasing clinical activity in a wide sector of the population. The main secondary effect is the appearance of infections from bacteria, specifically *Staphylococcus aureus* and *S. epidermidis*. This paper describes the development and application of low-density polypropylene meshes coated with a biocompatible and resorbable polymer as a controlled release system of the antibiotic vancomycin. The polymeric coating (a non-cross-linked copolymer of 2-hydroxyethyl methacrylate and 2-acrylamido-2-methylpropanesulfonic acid) has a thickness of 14–15 μm and contains 0.32 mg cm^{-2} of the antibiotic vancomycin. The in vitro experiments demonstrate the excellent inhibitory character of the coated meshes loaded with the antibiotic, following the standard protocol of inhibition of halo in agar diffusion test. This inhibitory effect is maintained for a relatively long period (at least 14 days) with a low concentration of antibiotic. The acrylic polymer system regulates the release of the antibiotic with a rate of 24 μg h^{-1}, due to its slow dissolution in the medium. Experiments in vivo, based on the implantation of coated meshes, demonstrate that the system controls the infection in the animal (rabbits) for at least 30 days. The concentration of antibiotic in the blood stream of the rabbits was below the detection limit of the analytical technique (<1–2 μg ml^{-1}), which demonstrates that the antibiotic is released in the local area of the implant and remains concentrated at the implantation site, without diffusion to the blood stream. The systems can be applied to other medical devices and implants for the application of new-generation antibiotics in a controlled release and targeted applications.

1. Introduction

Hernia repair is one of the most frequently performed surgical procedures—midline laparotomy produces incisional hernias at a rate of 2–9% [1]; the placement of a synthetic mesh is the standard technique of reinforcement. The application of a polypropylene mesh by Uscher [2] was considered one of the greatest advances in this field. Surgical techniques have progressed, through the application of advanced designs and materials such as synthetic meshes [3]. The function of meshes is to provide mechanical closure to the defect and to induce strong scar tissue with good biocompatibility and low cell adhesion [4]. There is a variety of meshes made of synthetic resorbable materials, and non-resorbable or organic material derived from human or pig tissues. Meshes of polypropylene (PP) with controlled net size have been applied extensively because of polypropylene's high biocompatibility, inert character, morphology and other properties [5]. Clinical complications as a result of the application of surgical meshes include inflammatory response, irregular or low formation of scar tissue and, most importantly, the appearance of infections; these have been estimated to occur in about 3–4% of inguinal hernias and 6–10% of incisional hernias [6]. This is relevant because of the considerable number of surgical procedures. In the USA alone, infection affects more than 30,000 patients a year with inguinal hernias and more than 3000 patients a year with incisional hernias. The more common microorganisms involved in these bacterial infections are *Staphylococcus aureus* (Sa) and *S. epidermidis* (Se), together with Gram-negative species including *Escherichia coli* and *Pseudomonas aeruginosa* [7].

The adhesion of bacteria to the surface of a biomaterial is a crucial step in the pathogenesis of infection. Some microorganisms are capable of forming a biofilm on the mesh that protects the immune system and the action of the antibiotics [8]. Once the mesh has been infected, no treatment is possible and it is necessary to

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remove the prosthesis. The administration of single-dose prophylactic systemic antibiotics is a standard procedure and has proven efficacy in surgery of implants, including orthopedic and breast prostheses [9]. In addition to the state of the patient, the infection is related to the design, structure, composition and morphology of the mesh [10], and is more frequent for microporous implants and multifilament meshes [11,12].

The infection of biomaterials for hernia repair is an increasing clinical problem, because of the noticeable increase of the repair surgery of the abdominal wall. Primary and secondary hernias are very frequent, even after the application of minimal invasive techniques. The application of biomaterials is necessary to minimize the rate of relapse [7,13].

The best way to treat the infection of an implant is to prevent the colonization of microorganisms in the early stages, avoiding the formation of biofilm [14] and the colonization of the biomaterial by pathogens [15]. An adequate method is the modification of the implant surface with a biocompatible and resorbable polymer coating for the controlled release of the antibiotic just in the site of action [16].

We consider that the application of coatings based on hydrophilic and biocompatible resorbable acrylic polymers could offer an excellent system for the control of infections on site. Our experience in the preparation of slow resorbable hydrosoluble systems based on non-cross-linked 2-hydroxyethyl methacrylate–2-acrylamido-2-methylpropanesulfonic acid (HEMA–AMPS) copolymers indicates that this system offers a good alternative for the preparation of bioactive coated meshes.

2. Materials and methods

2.1. Synthesis and characterization of polymer coating

2.1.1. Reagents

HEMA (Fluka) was exhaustively purified [17]. AMPS (Avocado) and azobis(isobutyronitrile) (AIBN, Merck) were recrystallized twice from ethanol. Vancomycin was purchased by Normon laboratories.

2.1.2. Polymer preparation

Polymerization was carried out by a free radical mechanism. A mixture of 77 mol.% HEMA (1.55 g) and 23 mol.% AMPS (0.46 g) was diluted in water:isopropanol (50:50) to obtain a final concentration of 0.3 M. After deoxygenation with N_2 for 15 min, the reaction was initiated at 50 °C using AIBN (1.5×10^{-2} M) as free-radical initiator (Fig. 1). After 24 h the solvent was removed by evaporation, and the isolated solid was redissolved in water and dialysed using MW-3500 dialysis membrane (Spectrumlabs). Finally the copolymer was freeze-dried in a TELSTAR lyoalpha lyophilizer (yield of polymer was 95.8 wt.%).

2.1.3. Polymer characterization

Structural characterization of the polymers was carried out by nuclear magnetic resonance (NMR) and Fourier transform infrared spectroscopy (FTIR). ^{1}H NMR was performed using a Varian XL-300 spectrometer (300 MHz); spectra were recorded at room temperature in deuterated water solution prepared at 10 mg ml^{-1} for ^{1}H NMR (300 MHz). Polymer compositions were determined from the ^{1}H NMR spectra by the integrated intensities of proton NMR signals assigned to the comonomeric units. Signals of the CH_3 groups of HEMA (0.98–1.2 ppm) and CH_3 groups of AMPS (1.40 ppm) (see Fig. 2).

Attenuated total reflection FTIR spectra were recorded in a Spectrum One FTIR spectrometer, Perkin-Elmer. Polymer samples were analysed without further treatment at room temperature by 32 scans and with a resolution of 4 cm^{-1}.

2.1.4. Coating of PP meshes

The synthesized polymer was loaded with the bioactive compound by rehydrating in water and adding 20% w/w vancomycin. The homogeneous solution was finally dried by lyophilization.

The meshes (Parietene®, Covidien, Mansfield, USA, low density 38 g m^{-2}) were coated by a casting technique. A solution of 5% w/w of polymer or polymer/vancomycin was prepared in ethanol. The solution was deposited onto the mesh drop by drop with a Pasteur pipette and evaporated at room temperature for 24 h. The result was the coating of the PP mesh containing a total amount of 0.32 mg cm^{-2} of antibiotic. Measurement of the coating thickness was carried out with electronic gauge equipment and finally the coated meshes were sterilized in a closed chamber with ethylene oxide at 40 °C during 20 h.

2.2. Release studies in vitro

Fragments (1 cm^2) of lightweight PP were coated with the polymer loaded with vancomycin. The coating fragments were introduced in vials with 5 ml of phosphate-buffer saline (PBS) at 37 °C. Aliquots of 1 ml were taken at different times and replaced with the same volume of fresh medium, and then analysed by high-performance liquid chromatography (HPLC), using aShimadzu SL-200 with a Shimadzu UV/vis detector and a Waters μBondpack C18 column. The flow rate was 1 ml min^{-1} and the mobile phase was a mixture of MeOH/water 80/20. Measurements were done at 278 nm. All the experiments were carried out in triplicate. A control of the polymer without vancomycin was tested after different times of treatment in PBS, to ascertain if there were detected products of biodegradation. The results indicated clearly that there was no interference with the signals of vancomycin.

2.3. Biocompatibility studies

Lightweight monofilament PP meshes were used as the experimental model. Three groups were established:

(1) mesh PP control without polymer (PP group);
(2) polymer-coated PP mesh experimental (group POL);
(3) polymer-coated PP mesh experimental with vancomycin (group VC).

2.3.1. Biocompatibility in vitro

In order to ensure the survival of cell populations in contact with different types of prosthesis (PP, POL and VC) we used cultured fibroblasts obtained from dermis from skin biopsies of experimental animals (New Zealand white rabbit). After the biopsy, the epithelial tissue was submerged in minimum essential medium (MEM) and processed under sterile conditions in a Telstar VA 30/70 laminar flow hood (Müller 220 V, 50 MHz). The dermis was carefully separated from the epidermis using two scalpel blades and was cut into small fragments (explants), which were collected and placed in a 25 cm^2 culture flask. The explants were kept in an incubator at a temperature of 37 °C, with an atmosphere of 5% CO_2, until a confluent cell population had been produced. At that time the cells were treated with trypsin–EDTA and transferred to multiwell plates (6 well). Fragments of 1 cm^2 of meshes were placed in the bottom of the wells, and were seeded with a suspension of 3×10^5 cells (2 ml per well). Thus, the prosthetic material was in contact with the cell population and its culture medium. Periodically, cells were trypsinized and counted in viable and non-viable using the method of Trypan blue stain (Gibco) in a Neubauer chamber. The cultures were maintained for 14 days.