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Acrylic polymer-grafted polypropylene sutures for covalent immobilization or reversible adsorption of vancomycin

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ABSTRACT

Glycidyl methacrylate (GMA) and acrylic acid (AAc) were separately grafted onto polypropylene (PP) monofilament sutures by means of pre-irradiation using a ^{60}Co γ-source, with the purpose of loading vancomycin *via* (i) covalent immobilization through the glycidyl groups of GMA and (ii) ionic interaction with AAc moieties. The effect of absorbed radiation dose, monomer concentration, temperature and reaction time on the grafting degree was evaluated in detail. GMA grafting ranged from 25% to 800% while the grafting yield of AAc onto PP could be tuned between 9% and 454%, at doses from 5 to 50 kGy and a dose rate 13.7 kGy/h. Grafting of GMA or AAc decreased the decomposition temperature and made the sutures swellable to a certain extent. GMA grafting led to a continuous, smooth and thick coating, which was suitable for immobilization of up to 1.9 μg vancomycin per gram. The immobilized vancomycin enabled a reduction in the *Staphylococcus aureus* CFU adhered to the suture surface. On the other hand, dried AAc-functionalized sutures exhibited a rough and cracked surface which was responsible for a minor increase in the coefficient of friction. PP-g-AAc sutures exhibited pH-dependent swelling and remarkably high capability to host vancomycin (up to 109.9 mg/g), particularly those with an intermediate degree of grafting. Some AAc-functionalized sutures were shown able to inhibit bacterial growth after successive challenges with fresh lawns. Therefore, tuning the yield of grafting of GMA or AAc may enable the preparation of drug-suture combination products that retain or release, respectively, antimicrobial agents.

1. Introduction

Surgical site infections are responsible for long stays in the hospitals and, if the antimicrobial therapy fails, may force another surgery. This is not an uncommon situation (more than 5% patients undergoing surgery in developed countries) and notably increases the mortality risk and the healthcare costs (National Institute for Health and Clinical Excellence, 2008; Rozzelle et al., 2008). The causes of the infections are quite diverse, but the suture itself may play a relevant role. In fact, sutures are since recently considered as implants, with a hard or non-shedding surface that serves as substrates for microorganisms proliferation and may lead to an increase in the virulence, as the microorganisms can associate forming resistant structured communities (biofilm) (Henry-Stanley et al., 2010; Leaper et al., 2010). Moreover, the microorganisms grown on the sutures hinder wound healing and can migrate to adjacent tissues and even spread in the blood stream. Once the biofilm is established, the infection is difficult to manage; thus, preventative strategies are mandatory. Antibacterial sutures have been pointed out as interesting alternatives to prophylaxis with systemic antibiotics, overall improving the quality of life of the patients. Sutures impregnated or coated with antibacterial agents can reduce bacterial adherence and colonization, and also contribute to reduce surgical site infections after prosthetic and contaminated surgery (Leaper et al., 2010). Cephalosporin-containing (Smolianskaia et al., 1994) and neomycin-impregnated (Rodeheaver et al., 1983) suture materials implanted into tissues contaminated with *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas mirabilis*, or *Pseudomonas aeruginosa* have been shown

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able to reduce bacterial numbers. Clinical studies have mainly focused on triclosan-coated sutures, proving significant decreases in infection rate (Mingmalairak, 2011). Nevertheless, concerns on antimicrobial resistance and human safety (Aiello et al., 2011; Yazdankhah et al., 2006) prompt the search for alternatives, such as other antibiotics/antiseptics or microbicidal peptides, polymers or silver particles (Augustine and Rajarathinam, 2012; Li et al., 2012; Matl et al., 2009).

There are several approaches to incorporate active substances to implantable devices, which can be also applied to sutures: compounding, soaking in drug solutions of swelling solvents, coating with erodible layers containing dispersed drugs, covalent conjugation of the drug to the surface, and grafting of polymers that can reversibly interact with the active substances (Alvarez-Lorenzo and Concheiro, 2013; Scaffaro et al., 2013; Shanmugasundaram et al., 2011; Thipparaboina et al., 2013). The loading is quite limited in the first two cases; namely, in the compounding the presence of the active components can exert deleterious effects on the performance of the implant, while the impregnation by soaking is poorly efficient due to the slow diffusion into the material and the lack of affinity of most drugs for the surface chemical groups of the implants (Alvarez-Lorenzo et al., 2010). By contrast, covalent conjugation enables a precise control of the density of the antimicrobial agent on the surface and prevents leakage to surrounding tissues and therefore allow for continued bioactivity of in-dwelling devices (Hickok and Shapiro, 2012). Covalent conjugation requires functional groups on the surface of the material suitable for the binding of active substances, and has been largely evaluated to immobilize enzymes (Goddard and Hotchkiss, 2007). For example, versatile glycidyl groups that react with molecules bearing hydroxyl, amine or carboxylic acid moieties can be readily introduced by means of grafting of glycidyl methacrylate (GMA) or allylglycidyl ether using ionizing radiations or plasma (Nava-Ortiz et al., 2010; Schofield et al., 2006; Thierry et al., 2008). Another approach is the grafting of three-dimensional networks on the implant surface for reversible interaction with the drug. If the polymer network is stimuli-responsive, the changes in mesh size can also contribute to regulate the loading and the release processes (Alvarez-Lorenzo et al., 2010). Methacrylic acid-grafted silk sutures have been shown useful for the loading of 8-hydroxy quinoline hydrochloride (Singh and Tyagi, 1989), while 1-vinylimidazole-, acrylonitrile-, or acrylic acid (AAc)-grafted polypropylene (PP) monofilaments could uptake ciprofloxacin and tetracycline hydrochloride (Gupta et al., 2008; Saxena et al., 2011). Interpenetrating networks of AAc and N-isopropylacrylamide grafted to PP and polyethylene enabled the uptake of vancomycin (Ruiz et al., 2008). These medicated materials showed an excellent performance *in vitro* against a variety of microorganisms.

The aim of this work was to functionalize PP sutures with GMA and AAc, in separate, applying γ-ray irradiation, under various experimental conditions, in order to load vancomycin according to two different approaches: (i) covalent immobilization through the glycidyl groups of GMA and (ii) ionic interaction with AAc moieties. The grafted sutures were characterized in detail regarding their structure, physical properties and antimicrobial activity. Vancomycin is efficient against the most frequently isolated pathogens from surgical site infections, namely *S. aureus* (including methicillin-resistant strains) and coagulase-negative staphylococci (Jones, 2006). This antimicrobial agent has been previously immobilized onto titanium surfaces and bone allografts by means of wet chemistry functionalization with amine-rich layers (Antoci et al., 2008; Ketonis et al., 2010). To the best of our knowledge the only attempts to immobilize vancomycin using GMA refer to silica capillaries functionalized with epoxy groups to be applied as chiral stationary phases for capillary electrochromatography; the epoxy groups were converted to aldehyde and vancomycin was immobilized *via* reductive amination of the aldehyde groups (Kornysova et al., 2001). Radiation processing of sutures may be advantageous over these former methods, as it can be applied at larger scale and no catalysts or additives are needed to initiate the reaction (Alvarez-Lorenzo et al., 2010). Having surfaces with GMA or AAc may help to face up to a wide variety of prophylactic or therapeutic needs, depending on the convenience of having vancomycin remaining on the suture surface or migrating to surrounding tissue.

2. Materials and methods

2.1. Materials

Acrylic acid (AAc), glycidyl methacrylate (GMA) and sodium periodate were from Aldrich Chemical Co. (St. Louis MO, USA). The monomers were purified by vacuum distillation. Monofilament polypropylene (PP) suture (Prolene 4-0, 1.5 Ph. Eur.) was from Ethicon (Johnson & Johnson Medical Limited, Livingston, UK). Ethanol, boric acid, citric acid and trisodium orthophosphate-12 H_2O were from Baker (Mexico City, Mexico). Sodium hydroxide and sodium dihydrogen phosphate monohydrate were supplied by Merck (Darmstadt, Germany). Vancomycin hydrochloride was supplied by Fagron (Barcelona, Spain). Sodium cyanoborohydride was from Fluka (USA). Methanol was from VWR (Leuven, Belgium). Potassium dihydrogen phosphate was supplied by Panreac Quimica (Barcelona, Spain). Purified water (resistivity >18.2 MΩ cm) was obtained by reverse osmosis (MilliQ®, Millipore, Madrid, Spain).

2.2. Radiation-grafting of GMA and AAc

PP sutures were exposed to ^{60}Co γ-source (Gammabeam 651 PT, MDS Nordion) in the presence of air at room temperature, at a dose rate of 13.7 kGy/h, and doses from 5 to 50 kGy. The pre-irradiated sutures were placed in glass ampoules which contained a solution of GMA (10–40% in methanol:water, 8:2, v/v) or of AAc (20–50% in water) to obtain PP-g-GMA or PP-g-AAc, respectively. The ampoules were filled with argon to remove air and afterwards sealed and heated at temperatures from 40 to 70 °C for different reaction times (between 30 and 360 min). To extract the residual monomer and homopolymer that could be formed and occluded in the suture during the grafting, the samples were soaked in water for 3 h and then in ethanol (replacing the medium twice) for 20 h more, followed by drying under vacuum to constant weight. The grafting yield was calculated using the equation:

$$Yg\,(\%) = 100[(W_g - W_o)/W_o]$$

where W_o and W_g represent the weights of the pristine and the grafted suture, respectively. Diameters of pristine and grafted sutures were recorded with the use of a digital caliper (Mitutoyo, Japan). Pristine and functionalized sutures were directly observed using a Scanning Electron Microscope EVO LS 15, Zeiss (Germany) with an elemental analysis tip.

2.3. FTIR-ATR spectra

IR-ATR spectra of sutures and vancomycin hydrochloride powder were recorded using a Varian 670 IR spectrophotometer (ATR mode, Varian Inc., Santa Clara, CA, USA).

2.4. Thermal analysis

Thermal decomposition of dried samples was monitored in nitrogen atmosphere between 25 and 600 °C at a heating rate of 10 °C/min using a TGA Q50 (TA Instruments, New Castle, DE). Char yield was estimated as the weight percentage remaining after