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Efficacy of silver coated surgical sutures on bacterial contamination, cellular response and wound healing

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ABSTRACT

The resistance demonstrated by many microorganisms towards conventional antibiotics has stimulated the interest in alternative antimicrobial agents and in novel approaches for prevention of infections. Silver, a natural braod-spectrum antimicrobial agent known since antiquity, has been widely employed in biomedical field due to its recognized antibacterial, antifungal and antiviral properties. In this work, antibacterial silver coatings were deposited on absorbable surgical sutures through the *in situ* photo-chemical deposition of silver clusters. Scanning electron microscopy (SEM), Energy dispersive X-ray spectroscopy (EDX) and thermo-gravimetric analysis (TGA) were performed in order to investigate the presence and distribution of the silver clusters on the substrate. The amounts of silver deposited and released by the silver treated sutures were calculated through Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS), and the results were related to the biodegradation of the material. The microbiological properties and the potential cytotoxicity of the silver-treated sutures were investigated in relation with hydrolysis experiments, in order to determine the effect of the degradation on antibacterial properties and biocompatibility.

1. Introduction

Surgical sutures are sterile filaments used to close wounds and provide support during the healing process. Some of the most important features associated to an ideal suture are biocompatibility, reduction of tissue reaction, sterilization and low cost [1]. Synthetic absorbable sutures are found to be non-toxic and non-irritating materials, suitable for sutures and other surgical implants [2]. The healing of a surgical wound involves matrix formation that bridges the margins of the wound, supports cells and regenerating vasculature, and restores the resistance of the tissues to functional stress [3]. A variety of absorbable and non-absorbable materials, ranging from natural or synthetic polymers produced in braided and/or monofilament form, are commonly used for suturing. Sutures may be classified into several groups according to their physical and biochemical characteristics. One criterium is the absorbability of the suture from the healing tissues that can occur by enzymatic proteolysis for materials of animal origin (*e.g.* catgut) or hydrolysis for synthetic sutures (*e.g.* such as polyglycolic acid, polyglactin 910).

Generally, absorbable sutures do not require removal. The sutures that undergo rapid degradation in the tissues lose their tensile strength within 60 days and can be considered "absorbable sutures" [4]. Polymorphonuclear cells and other macrophages will digest the suture material after their degradation by hydrolysis [5,6]. Commonly used bioabsorbable suture materials that have shown desirable properties in relation to tissue reactions are gut, polyglycolic acid (PGA), polyglactin (PG), and chromic gut (CG) [7]. The appropriate suture material should be selected according to tensile strength, tissue biocompatibility and resorption rates [8]. Clinically, most absorbable sutures are made of biodegradable linear aliphatic polyesters, such as polyglycolic acid and its derivates [9]. Polyglycolic acid is absorbed by the body through a mechanism of biodegradation and it is a polymer of glycolic acid prepared by the polymerization of glycolide. Polyglactin is a synthetic, absorbable, braided suture consisting of 90% glycolide and 10% L-lactide [10,11].

New sutures have been developed over the past years to improve their physical properties and biological activity. In this sense, the use of modified sutures coated with antimicrobial agents has been extensively accepted as a valuable tool for the reduction of wound infections with demonstrated safety and tolerability [12]. Wounds infections can be classified as either acute (*e.g.*, traumatic, surgical and burns) or chronic (*e.g.*, leg ulcers and pressure ulcers). Recently, the use of

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modified sutures has been applied to improve tissue integrity, healing, immune response and to minimize microbials infections [13,14].

Surgical wounds are frequently complicated by infections of varying degrees of precociousness and severity, giving rise to a range of clinical problems and prolonging hospitalisation with increased healthcare costs. [15]. Because of the increased resistance of bacteria to bactericides and antibiotics and the toxicity of some antimicrobial agents, there is a great interest in finding ways to formulate new safe and cost-effective biocidal materials. Previous studies have shown that antimicrobial formulations in the form of metal nanoparticles could be used as effective bactericidal materials [16]. Recently, highly reactive metal oxide nanoparticles were presented as a new generation of bactericidal materials against Gram-positive and Gram-negative bacteria. In particular, it is well known that silver ions and silver-based compounds are highly toxic to microorganisms. Silver ions have been used as antibacterial component in ion exchange fibers and in coatings of medical devices [17]; in the last years, silver based products have been developed for traditional and innovative applications, such as for production of wound dressings and biocompatible antibacterial polymers.

In this study, absorbable "Polyglactin 910" sutures, commonly used in surgical practice in wounds and mucous membranes, were treated with silver by using a technology based on the *in situ* synthesis and deposition of silver particles [18].

Morphological analysis was performed in order to evaluate the distribution of the silver particles deposited on the surface of the sample by scanning electron microscopy (SEM). The quantification of silver deposited on the suture was determined by X-ray spectroscopy energy dispersive (EDX) and thermogravimetric analysis (TGA). The stability and durability of silver coating was tested using kinetic degradation, and the silver release was quantified by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The antibacterial activity of the silver treated sutures was measured on *Staphylococcus aureus* and *Escherichia coli* by qualitative analyses, such as agar diffusion tests, and quantitative assays, such as the serial dilutions method and spectrophotometric assay. The potential cytotoxicity was investigated by viability and cell proliferation assays through direct and indirect contact methods in 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and fluorescent Live/Dead assay. The influence of the silver coating on the wound healing process was also studied by the scratch assay.

2. Materials and method

2.1. Materials

Absorbable multifilament polyglactin 910 PGLA sutures (Atramat Italia) were purchased from a local pharmacy. Chemicals and reagents such as silver nitrate, methanol, deionized water and biological media were purchased from Sigma Aldrich.

2.2. Silver treatment on PGLA surgical sutures

PGLA surgical sutures were treated with silver according to a silver deposition technology based on the *in situ* photoreduction of a silver solution. The impregnating silver solution was prepared by mixing 0.5 wt/vol.% of silver nitrate in 5 v/vol.% of methanol and deionized water under magnetic stirring at room temperature. Methanol was used as reducing agent in the photo-reduction process. The samples were impregnated in the silver solution for 5 min and, then, exposed to UV lamp for 15 min (Jelosil, $\lambda = 365$ nm, distance 20 cm). After the treatment, the samples were washed with deionized water to remove the unreacted salt.

2.3. Morphological and microanalytical characterization

Morphology of the silver coating and distribution of the silver clusters on the braided multifilament sutures PGLA were analyzed in triplicate by SEM microscopy (Zeiss EVO). Microanalysis was performed through EDX (Bruker) to assess and quantify the presence of silver deposited on the sample.

2.4. Thermogravimetric analysis (TGA)

TGA was performed using a METTLER TOLEDO operating in nitrogen gas in order to calculate the amount of silver on the sutures. The samples were heated with a rate of 10 °C/min from 20° to 1200 °C. The solid residue represents the incombustible silver coating after the burning of the organic fraction. The samples were tested in triplicate.

2.5. Degradation kinetics

Degradation kinetics of absorbable sutures were evaluated in phosphate buffered saline solution (PBS, pH 7.4) at 37 °C. In triplicate, treated and untreated sutures were incubated into 50 mL PBS solution at 37 °C for 4 weeks and their degradation rate was evaluated at defined time points (1, 7, 14, 21 and 30 days). Small amounts of eluate were extracted and stored at 4 °C for further chemical and biological characterizations. Moreover, both treated and untreated sutures were extracted and stored for chemical, antibacterial and biological characterizations [19].

2.6. Measurement of silver concentration in sutures extracts

Silver release from the silver coated sutures was evaluated through a static degradation experiment, performed according to the method described above (paragraph 2.5). Silver concentration was measured using inductively coupled plasma mass spectrometry analysis (ICP-MS, iCAP Q Thermo Scientific) using ICP grade reagents. In triplicate, samples were acidified with 1% nitric acid to create an acid matrix diluted 1:40. Before analysis, samples were filtered through a 0.45 μm filter.

2.7. Sutures digestion and silver quantification

In triplicate, sutures (0.2 g) were digested into 9 mL nitric acid (69%) at 180 °C for 5 min. Deionized water was used to obtain 1% nitric acid as matrix. Silver quantification was performed using ICP-MS and reported as the percentage of silver deposited on the silver treated sutures.

2.8. Microbiological characterization

The antibacterial activity of the silver treated sutures was tested on *S. aureus* (S1, inoculating bacterial density 3×10^8 CFU/mL) and *E. coli* (DH5(α), inoculating bacterial density 5.2×10^9 CFU/mL) through agar diffusion tests, according to Standard "SNV 195920-1992" [20]. One hundred μL of bacterial suspension were plated on nutrient agar; then, the samples were placed over bacteria and incubated at 37 °C for 24 h. The antibacterial capability was evaluated by the width of inhibition area of bacterial growth, with levels defined between "insufficient" (agar plate completely covered by bacteria) and "good" (inhibition area ≥1 mm) antibacterial capability. The antibacterial capability of the silver treated sutures was also quantified through quantitative tests performed according to the serial dilution method. The *in vitro* assay for testing antibacterial efficacy of silver treated Polyglactin 910 sutures has been previously reported [21,22]. The silver treated and untreated samples were introduced in 5 mL of nutrient broth inoculated with *S. aureus* and *E. coli* (1.5×10^5 colony forming units CFU/mL) and incubated at 37 °C in a shaking incubator for 12 h. Then, serial dilutions were performed and 100 μL of each dilution were spread plated on agar plates and incubated at 37 °C for 24 h. The bacterial colonies (CFU) number grown on the agar plates after incubation was determined and the antibacterial efficacy of the specimen (ABE in %) was calculated according to the following Eq. (1) [23]:

$$\mathrm{ABE}(\%) = (\mathrm{Vc}-\mathrm{Vt})/\mathrm{Vc} \times 100 \tag{1}$$