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Research paper

Sutures modified by silver-loaded montmorillonite with antibacterial properties

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ABSTRACT

Montmorillonite (Mt) was modified via cation-exchange using silver nitrate ($AgNO_3$) as a precursor to exchange the Na^+ in the Mt interlayer space and produce (Ag^+Mt). The twice dipping and twice rolling method was subsequently used to further strengthen sutures (Ag^+Mt/sutures). When the blood and tissue compatibility of the Ag^+Mt/sutures prepared by different processes was assessed, the Ag^+Mt/sutures were discovered to exhibit good blood and tissue biocompatibilities. Subsequently, when the antibacterial properties were examined, the Ag^+Mt/sutures were found to inhibit bacterial growth by 99 ± 0.9% for both Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus*.

1. Introduction

Surgical sutures that remain unabsorbed cannot inhibit bacteria, and the sutured incisions are susceptible to infection by microorganisms, such as bacteria, viruses, and mold, during the healing process (Alberti et al., 2011; Gao and Cranston, 2008; Horan et al., 1992; Katz et al., 1981; Uff et al., 1995). Therefore, a course of antibiotics following the sutures is necessary (Ford et al., 2005; Justinger et al., 2009; Mueller and Krebsbach, 2008; Panacek et al., 2006). In recent years, there have been tremendous developments in the technology used at home for preparing antibacterial agents, following extensive fundamental research. Currently, natural, organic and inorganic antibacterial agents are available in the market (El-tahlawy et al., 2005; Goldstein, 1987). Unfortunately, some of these agents are toxic or not very effective, which makes them unsuitable for applications in health foods, purification and textiles. However, inorganic antibacterials are non-toxic, do not develop tolerance and can significantly reduce many bacterial infections (Lim and Hudson, 2004; Liu and Bai, 2005; Renaud et al., 2005; Shameli et al., 2010).

Among the various inorganic antibacterial agents, silver metals and ions have long been known to possess strong inhibitory and bactericidal effects as well as a broad spectrum of antimicrobial activities. Furthermore, inorganic antibacterial agents do not exhibit any adverse effects on the human body during the application process (Dastjerdi and Montazer, 2010; Dastjerdi et al., 2009; Gunal et al., 2012; Stobie et al., 2008; Yuranova et al., 2003). Previous studies have shown that silver ions have a higher bactericidal capacity than elemental silver (Feng et al., 2000). Silver ions supported on inorganic mineral materials present a strong, sustained release antibacterial activity (Rivera-Garza et al., 2000; Rosa-Gomez et al., 2008; Shameli et al., 2011). Montmorillonite (Mt), an inorganic mineral, not only has the advantage of lamellar structure, high specific surface area and high cation exchange capacity (Brigatti et al., 2013), but also is heat resistant and possesses excellent acidic and alkaline properties. Therefore, Mt is an ideal carrier material (Addy et al., 2012; Ugochukwu et al., 2014). More importantly, Mt does not demonstrate any adverse effects in animals or humans. Mt has a good adsorption capacity for bacteria and is a suitable pharmaceutical carrier (Rong et al., 2008).

The Ag-loaded Mt antibacterial agent (Ag^+Mt) was generated by cation-exchange, using silver nitrate ($AgNO_3$) as a precursor to promote exchange in the Mt interlayer space. The effects of the preparation process on the antibacterial ability of the sutures were extensively investigated.

2. Experiments

2.1. Chemicals and materials

Analytical grade $AgNO_3$ and Mt were kindly provided by the Sanding Technology Company in Zhejiang Province, China. General unabsorbed surgical sutures used in the study were 4-0 black and were obtained from the Medical Suture Needle Factory in Shanghai, China.

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2.2. Methods

2.2.1. Preparation of the Ag^+Mt antimicrobial agent

A 2.0 mass% solution of stock sodium montmorillonite (Na^+Mt) was purified at 30 °C for 4 h using magnetic stirring. Subsequently, the slurry was poured into a beaker, sealed with plastic wrap and allowed to stand for 10–12 h. The liquid supernatant was then submitted to high-speed (8000 rpm) centrifugation. The resulting liquid supernatant was discarded, and the solid product was dried at 80 °C and then ground to pass through a 60-mesh sieve. Ag^+Mt was synthesized at different mass percentages using $AgNO_3$ solution and Mt. The mixtures were cation exchanged under fixed conditions. Following centrifugation, the solid was repeatedly washed with distilled water to remove free Ag^+ and dried overnight at 80 °C.

2.2.2. The Ag^+Mt suture

The Ag^+Mt powder prepared as an antibacterial agent was added to 25 mL deionized water and stirred at room temperature until homogeneous. Next, the clipped suture was immersed in deionized water (80 °C) for 10 min, removed and dried naturally. The pretreated suture was fully immersed in the antibacterial liquid consisting of Ag^+Mt, condensate of aliphatic alcohol and epoxyethane (penetrating agent JFC) and 5% sodium alginate at 45 °C for 30 min. Subsequently, the pretreated suture was removed and completed using the dip-rolling method (roll-over of 90%), prebaked at 80 °C for 5 min, baked at 120 °C for 3 min, washed using cold water and dried at 60 °C for 2 h. The preparation procedure for the Ag^+Mt/sutures is depicted in Fig. 1.

2.3. Characterization

2.3.1. X-ray diffraction

The products were structured and analyzed by powder X-ray diffraction (XRD) in a vacuum. XRD analysis was performed using a Rigaku D/MAX-IIIC X-ray diffractometer (Tokyo, Japan) with monochromated Cu K_α radiation ($\lambda = 1.5418$ Å, 40 kV, 40 mA) and operated by using a step scan program (steps of 0.02° from 2–30°).

2.3.2. Scanning electron microscopy

Scanning electron microscopy (SEM), energy dispersive spectrometry (EDS) and mapping were performed using a FEI Quanta 200 SEM (Philips FEI, Eindhoven, Netherlands model Quanta 200) at an accelerating voltage of 20 kV.

2.3.3. Antibacterial performance

The antibacterial properties of the antibacterial sutures and the surgical sutures were analyzed using Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus*, with unfinished sutures as the control group and the finished ones as the experimental group. The following procedures were adhered to the analysis: 1) material sterilization; 2) sample inoculation; 3) vaccination immediately after elution; 4) vaccination after culture; 5) training after elution; 6) colony number determination; 7) result calculations. In detail, a representative sample was selected from the control group and from each experimental group and cut into a 1 × 1 cm piece as a sample for sterilization (step 1). Precisely 3 mL of the tested broth (bacterial concentration of 1.4×10^5 cfu/mL) was then inoculated into each of the sterile vials containing the sample to ensure that the bacilli did not stick to the wall of the bottle (step 2). The bottles were capped tightly, and the vials were then incubated at 37 °C at 220 rpm for 16 to 18 h, with several oscillations to wash the bacteria (step 3). Following a 10-fold serial dilution of the inoculated broth, a 100 μL aliquot of the bacterial dispersion from each dilution series was transferred into a solid beef extract peptone medium (step 4). The dispersion was uniformly coated using a coating stick, incubated without shaking to allow absorption for approximately 20 min at room temperature and then placed in an inverted culture incubator at 37 °C for 16 to 18 h (step 5). The inhibition rate was calculated as follows (Duran et al., 2007):

$$R(\%) = [(A-B)/A] \times 100\%$$

where R is the inhibition rate. A, the control group, was inoculated and cultured for 16 to 18 h, after which the average number of bacterial colonies was counted. B, the experimental group, received the treatment, and the average number of bacterial colonies was measured after 16 to 18 h.

2.3.4. Hemolysis tests

The hemolytic activity of the Ag^+Mt/sutures was determined as previously reported (Lequin et al., 2006; Travis et al., 2000). Briefly, erythrocytes (6×10^8 cells) from healthy human blood were washed three times with 0.9% NaCl and incubated with 1.0 g of the Ag^+Mt/sutures at 37 °C for 1 h. After centrifugation, the absorbance of the sample dispersion was measured at 450 nm. For negative and positive controls, erythrocytes in PBS (A_{blank}) and in 0.1% Triton X-100 (A_{triton}) were used. The percentage of hemolysis was calculated according to the equation:

$$\text{Hemolysis} = \left[\left(A_{sample} - A_{blank}\right)/\left(A_{triton} - A_{blank}\right)\right] \times 100\%.$$

2.3.5. In vitro cytotoxicity tests

Cytotoxicity of the Ag^+Mt/sutures was measured against human endothelial cell meridians by the Mt assay. The cells were seeded in a 96-well plate at a density of 1×10^4 cells $well^{-1}$ and incubated in complete DMEM (Dulbecco modified eagle medium) containing 10% hyclone fetal bovine serum (a high glucose DMEM) at 37 °C in 5% CO_2 for 24 h prior to the start of the assay. Subsequently, the culture medium was removed and replaced with 100 μL of the medium containing the

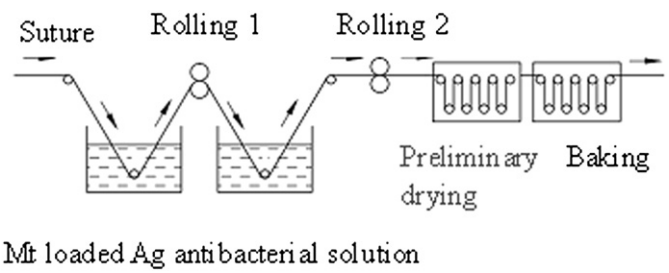

Fig. 1. Procedure for the antibacterial finishing of the suture.

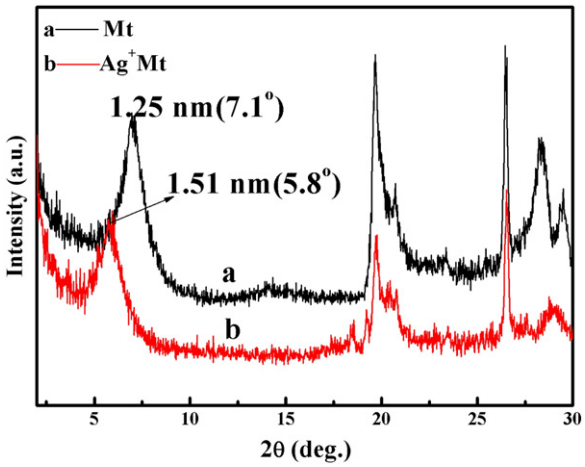

Fig. 2. XRD patterns of Mt (a) and Ag^+Mt (b).