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Short communication

Synthesis and antibacterial activity evaluation of a novel cotton fiber (*Gossypium barbadense*) ampicillin derivative

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ABSTRACT

We prepared cellulose cotton fibers containing ampicillin moieties and evaluated their antibacterial activity. In spite of recent progress in experimental and clinical medicine, the problem of chronic wounds treatment remains to be solved. In fact conventional methods are based on solutions of antibiotics and antiseptics and ointment bandages but the efficacy of this method is low and so the idea to use modified cotton gauzes would have to prevent infections insorgence during wounds healing. Ampicillin, a large spectrum antibiotic, was covalently coupled to cellulose backbone of hydrophilic cotton fibers by a heterogeneous synthesis to produce a functionalized biopolymer with a satisfactory degree of substitution (DS) and antibacterial activity. The obtained biopolymer was characterized by infrared spectroscopy (FT-IR). Finally, the antibacterial activity in inhibiting microorganism growth in Petri dishes, was evaluated. The results suggested that these biomaterials posses an excellent "in vitro" antibacterial activity and so they can be efficiently employed in biomedical fields for chronic wounds management to ensure a valid protection against infections and contaminations. Biopolymers so functionalized were found to be very efficient to contrast sensible bacteria growth.

1. Introduction

Wound dressing before the 1960s were considered to be only the so-called passive products having a minimal role in the healing process. The pioneering researches in the last decade introduced the concept of an active involvement of a wound dressing in establishing and maintaining an optimal environment for wound repair (Değim, 2008). The new advances in wound healing resulted in the development of dressing from traditional passive materials to functional active dressing which, through the interaction with the wounds they cover, create and maintain a moist and healing environment (Huang & Yang, 2008). An ideal wound dressing should protect the wound from bacterial infection, provide a moist and healing environment, and be biocompatible (Lou, 2008). Recently blends made by natural polymers such as starch, cellulose, chitin, chitosan, cotton, gelatin, alginate and dextran have been reported for the development of wounds dressings (Edwards, Howley, & Cohen, 2004; Kim et al., 2007; Lin et al., 2007; Mi et al., 2001; Muzzarelli et al., 2005). New fibrous and wound dressing media have been developed to encourage wound occlusion, exudate transport and drug dispensation on demand with much reduced distress to the patient (Miraftaba, Qiaoa, Kennedyb, Ananda, & Groocockc, 2003). However, natural fibers are presently under investigation as materials for the controlled release of bioactive molecules to contrast the progression of infection in chronic wounds management. On the other hand, literature reports on biomaterials in which the antibacterial molecules were simply absorbed on the polymer surface by the adsorption methodology (Adamopoulos et al., 2007) and not covalently grafted. In this way only medicated soaked gauzes have been prepared (Denkbas, Öztürk, Özdemir, Kekeci, & Agalar, 2004; Zilberman & Elsner, 2008).

Cellulose is the most important constituent of natural fibers and it can be functionalized with bioactive molecules using its primary hydroxylic groups; natural fibers are biocompatible, biodegradable and non-toxic so they can be used to cover infected wounds. In the present work, ampicillin moieties have been covalently linked to cotton fibers to produce a functionalized biomaterial that is able to protect wounds from infections thanks to the presence of the active molecules. This study showed that the designed systems preserve the antibiotic activity of ampicillin moieties also compared with the non-functionalized cotton dishes. The synthesis of this biomaterial has been conducted under mild conditions using thionyl chloride in dry THF. The evaluation of antibacterial activity has been demonstrated by "in vitro" tests using Petri dishes of the type "Mueller–Hinton Agar". Furthermore Infrared Spectroscopy showed the presence of the covalent bond with ampicillin and the degree of substitution was determined by saponification and then volumetric analysis.

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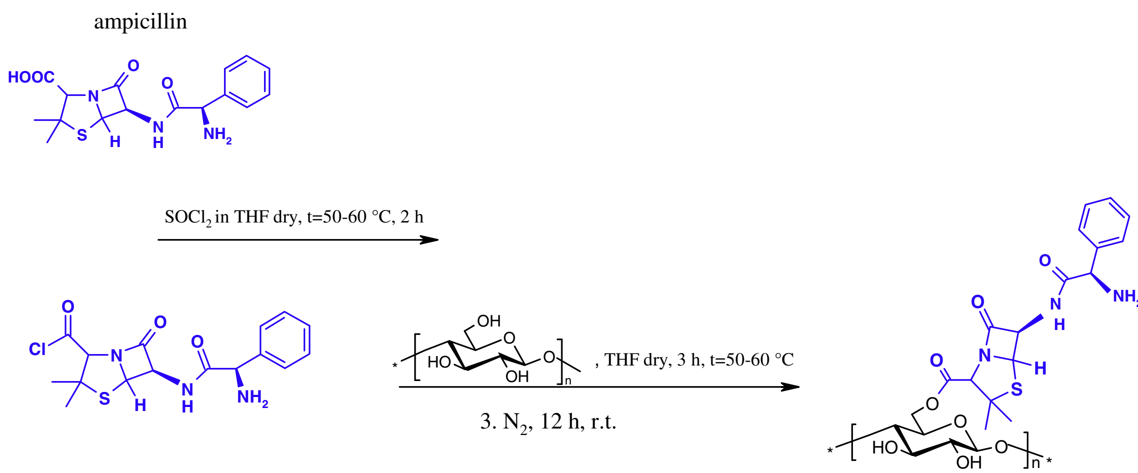

Scheme 1. Synthetic route to the cotton fiber ampicillin derivative.

2. Cellulose functionalization

Functionalized biopolymer was obtained by heterogeneous synthesis (Klemm, Philipp, Heinze, Heinze, & Wagenknecht, 1998) (Scheme 1) of ampicillin acylic chloride with primary hydroxylic groups of glucose units to form an ester bond. Briefly, ampicillin (0.00827 mmol, 2.89 g) was dissolved in dry THF and then was added thionyl chloride (0.009 mmol, 0.6 mL) in little excess and the reaction mixture was allowed to react for two hours at 50–60 °C with magnetic stirring and under N_2 reflux. After that, a sample of cellulosic cotton fiber (0.14 g) was added and the reaction was conducted for three hours in the same conditions. After five hours, reaction was left overnight at room temperature under magnetic stirring. The so obtained product was, finally, washed with a sodium bicarbonate saturated aqueous solution until neutrality and with acetonitrile. Finally functionalized cotton was dried under vacuum (Cassano et al., 2007). Cotton fiber ampicillin derivative yield = 0.2361 g.

3. Characterization

FT-IR spectra, realized by Jasco 4200 using KBr disks, made by compressing the powder obtained by grinding the fibers with KBr, confirmed ampicillin linkage to the fibers. In fact, we observed a detectable modification of hydrophilic cotton spectrum especially in the wavenumber range between 1600 and 2000 cm^{-1} (Fig. 1a and c). There is a new band at 1740 cm^{-1} which confirms ester linkage formation (Fig. 1c). Anyway experimental evidences suggested that functionalized cotton fibers, after alkaline hydrolysis, give back hydrophilic cotton and ampicillin sodium salt as showed in Figs. 1a, b, 2a and b. In particular, Fig. 2a displays some strong bands, around 1650, 1500 and 850 cm^{-1}, with an higher intensity than analogous bands in ampicillin sodium salt spectrum, attributable to NaOH, used for previous alkaline hydrolysis.

4. Determination of the degree of substitution of functionalized cotton fibers

The substitution degree was determined by volumetric analysis dispersing a sample of 50 mg of ester derivative in 5 mL of 0.25 M ethanolic sodium hydroxide solution under reflux for 17 h. The dosing, in return of the excess of soda, was realized by titration with 0.1 N HCl (first equivalent point) (Trombino et al., 2008). The moles of chloride acid used between the first and second equivalence correspond to the moles of free esters. The degree of substitution (DS) was determined by the Eq. (1). In this equation $MM_{glucose\ unit}$ is the molecular mass of a glucose unit; g_{sample} is the weight of the sample; $n_{free\ ester}$ is the number of moles of free ester; $MM_{free\ ester}$ is the molecular mass of free ester; and MM_{H2O} is the molecular mass of water. DS for functionalized cotton is 0.63, a very interesting value considering the difficulties due to the proper nature of cotton fiber and to the heterogeneous strategy of synthesis.

$$DS = \frac{MM_{glucose\ unit}}{\left(\frac{g_{sample}}{n_{free\ ester}}\right) - MM_{free\ ester} - MM_{H2O}} \quad (1)$$

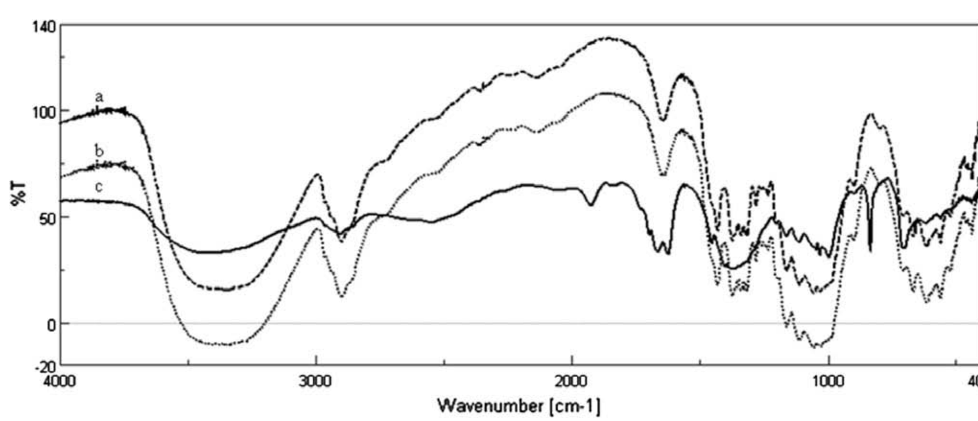

Fig. 1. IR analysis of (a) hydrophilic cotton, (b) cotton after alkaline hydrolysis, (c) ampicillin derivatized cotton.

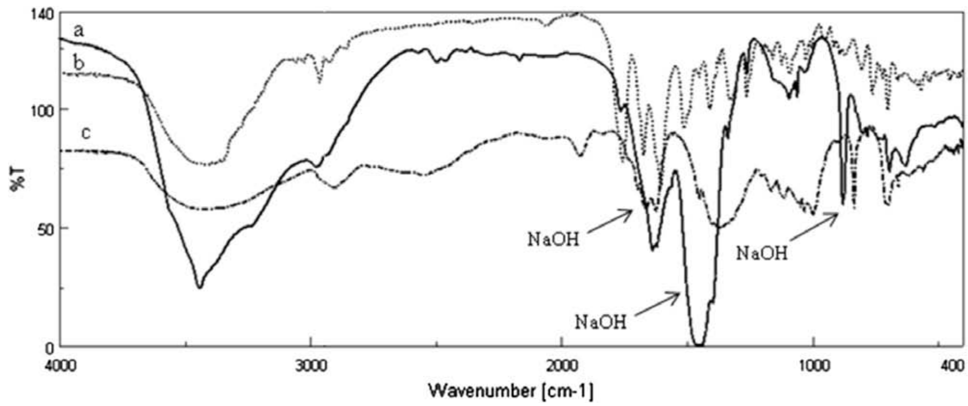

Fig. 2. IR analysis of (a) ampicillin sodium salt, (b) ampicillin sodium salt after alkaline hydrolysis, (c) ampicillin derivatized cotton.

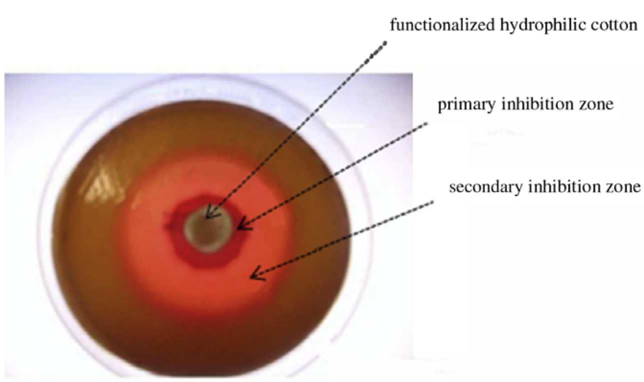

Fig. 3. Antibiogram picture of *Streptococcus faecalis* growth inhibition after treatment with functionalized cotton disks.

5. Antimicrobial testing

The antimicrobial testing was done in accord with accepted biological practices to test antibacterial efficacy. A pressed functionalized cotton disk, suitably sterilized at high temperatures, similarly to the disks used for a normal antibiogram (Jawetz, Melnick, & Adelberg's, 2008) has been used. On Petri dishes was inoculated a sensible bacterium, *Streptococcus faecalis*, at a concentration of 0.51 McFarland. After 18–24 incubation hours in a thermostat at 37 °C to encourage the growth of bacterial lawn, microrganisms form a primary inhibition zone in which death is total (100%) and a secondary zone in which bacterial hemolysis is less pronounced (Fig. 3). This shows that ampicillin, although covalently attached to the glucose units of cellulose in cotton fiber, preserves its antibacterial activity against a sensible microorganism. For this test has been used a solid culture soil of the type Mueller–Hinton agar containing agar, casein idrolisate, soluble stark.

6. Conclusion

Antibacterial cotton fibers were successfully prepared introducing ampicillin moieties onto cellulose backbone. An ''in vitro'' test was used to assess their antibacterial activity. In fact this test shows a positive behavior of ampicillin in inhibition of bacteria proliferation. The results suggested that these biomaterials posses an excellent antibacterial activity and so this synthetic strategy can be used to obtain versatile biopolymers that could be efficiently employed in biomedical fields for chronic wounds management to ensure a valid protection against infections and contaminations.

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