# Nanostructured cellulose materials: adsorption of antibiotics onto cellulose fibers functionalized with glycidylmethacrylate for the manufacturing of antibacterial fabrics

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## ABSTRACT

In this work we report how cotton fibers can be permanently functionalized with glycidylmethacrylate (GMA) by means of the Fenton's reaction and used for manufacturing biomedical textiles having significant capability to adsorb amoxicillin and vancomycin, two different antibiotics largely used in the clinical activity. The pristine cellulose fibers have no adsorption capability. The adsorption properties of GMA modified cotton fibers are due to their surface nanostructured by the whole of GMA appendages. The reversible adsorption capacity can be modulated by chemical treatments after the grafting of GMA on the cellulose backbone, through the epoxide ring opening by addition of water or primary diamines having different aliphatic chain length (2 to 6 C atoms). Such materials can find suitable application as wounds dressing or more in general for the topical administration of drugs.

***Keywords***: cellulose, glycidylmethacrylate, amoxicillin, vancomycin, antibacterial textiles

## 1 INTRODUCTION

Textiles are daily used in any hospital and medical centre. The management of the medical textiles is an important issue not only from an economical point of view but also for the health of the patients and for the safety of the medical and paramedical staff. On the other side, it is well recognized that textiles are an excellent substrate for bacterial and fungal growth under appropriate moisture and temperature conditions. In a clinical setting, they can be an important source of bacteria [1]. In the last years more attention has been devoted toward textiles with antimicrobial properties which could be used in all that situations where a high level of protection against dangerous microorganisms is necessary [2]. In all these and analogous situations the adoption of textiles properly functionalized in order to provide them antimicrobial capabilities is highly requested. The preparation of special cellulose fibers and textiles for medicine and healthcare has been attempted through different approaches and materials. In this work, which follows the granting of an international patent, we present the application of cellulose fabrics functionalized by GMA grafting through the Fenton's reaction for the adsorption of two widely used antibiotics, amoxicillin and vancomycin, see Figures 1 and 2 [3].

With regard to the biocompatibility of the materials described in this work we can say that GMA as well as other acrylic and methacrylic monomers are already in use in different biomedical applications [4].

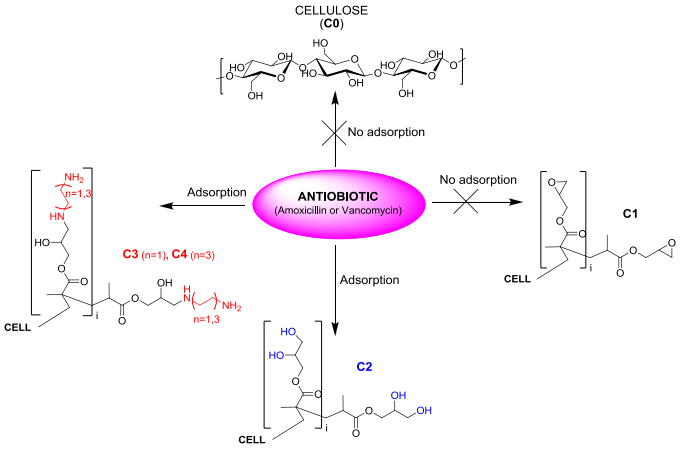


Figure 1: Nanostructured cellulose materials structures and properties.

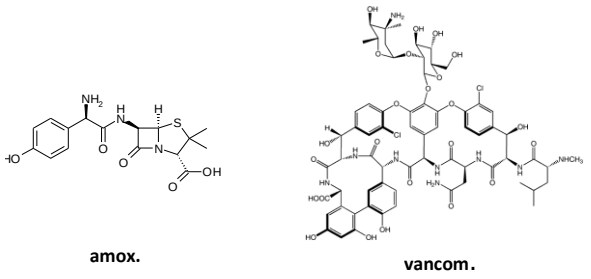


Figure 2: Amoxicillin and vancomycin formula.

## 2 RESULTS

### 2.1 Preparation

The cotton fabrics used in this work (C0) are common gauzes for sanitary purposes with a size of about 10cm×10cm and a weight of about 0.5g.

The materials C1 and C2 were prepared according to the procedure reported in reference [5].

The preparation of C3 and C4 was pursued by putting about 5g of C1 (MS~0.3-0.4) in 150mL of DMF at 70°C for 30min under stirring. Then about 5g of ethylendiamine (ED) or hexamethylenediamine (HMD) were added leaving the mixture under reaction over night at 70°C. The solid material was then removed from the flask and carefully washed with hot water and finally with hot acetone (apparatus similar to the Soxhlet extractor) in order to remove the unreacted ED or HMD molecules. The samples were finally dried in oven at 80°C.

### 2.2 Characterisation

The characterisation of all the materials was performed by FT-IR and $^{13}$C CP/MAS spectroscopy techniques [5,6]. This last one is a particularly effective technique for the characterisation of cellulosic materials not only in order to follow, step by step, any functionalisation process but also in order to check any modification of the morphological structure of the cellulose fibers. Herein we refer mainly to this technique. In Fig.3 we report the $^{13}$C CP/MAS spectra of all the materials under consideration starting from the pristine cotton gauze, C0.

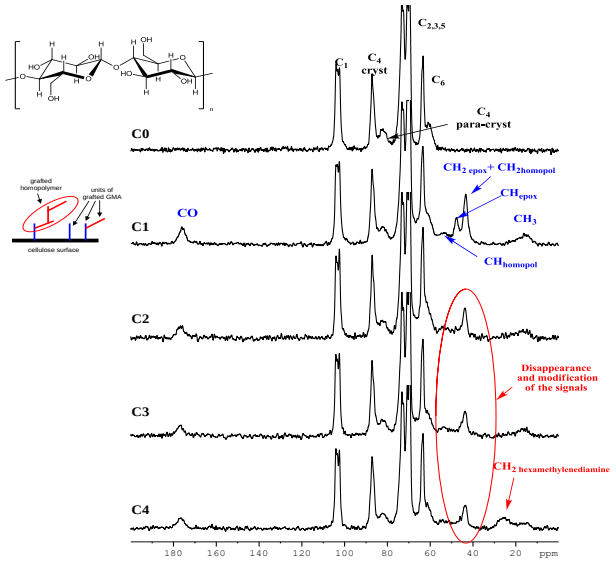


Figure 3: $^{13}$C CP/MAS spectra of C0-C4 gauzes.

### 2.3 Adsorption of antibiotics

All the adsorption experiments were performed in batch conditions. Aqueous solutions of the two antibiotics shown in Figure 2 at different concentrations (ranging from $1.0\times10^{-4}$M to $4.0\times10^{-3}$M for the amoxicillin solutions and from $2.0\times10^{-4}$M to $1.0\times10^{-3}$M for the vancomycin solutions) were prepared diluting stock solutions with deionised water in a 50 mL flask. Then 20 mL of the final solutions were pipetted into a 25mL conical flask and about 150mg of C0, C1, C2, C3 and C4 were introduced. All the flasks were shaken at 100rpm in a thermostatic bath (Julabo SW22) at the temperature of 25°C for a variable time. The concentration of the solutions was determined by a UV-spectrophotometer (Jasco V-650). The molar absorptivity of the amoxicillin was found to be $M^{-1}cm^{-1}$ at 272.5nm while for the vancomycin $M^{-1}cm^{-1}$ at 280.5nm.

The adsorption capacity was evaluated by equation 1:

$$Q = \frac{(C_0 - C)V}{m} \qquad (1)$$

where $C_0$ and C are, respectively, the initial and the final concentration (M), V is the volume of the solution (L) and m is the mass of adsorbent (g).

Table 1 reports the adsorption capacity for C2, C3 and C4 versus amoxicillin and vancomycin.

| Substrate | Q amoxicillin mol $g^{-1}$ | Q vancomycin mol $g^{-1}$ |
|---|---|---|
| C2 | $4.763\times10^{-5}$ | $8.905\times10^{-6}$ |
| C3 | $2.378\times10^{-4}$ | $1.920\times10^{-5}$ |
| C4 | $4.149\times10^{-4}$ | $2.610\times10^{-5}$ |

Table 1: Adsorption capacity for C2, C3 and C4 versus amoxicillin and vancomycin.

### 2.4 Microbiological tests

Microbiological inhibition tests were carried out following a procedure similar to the Kirby-Bauer (KB) antimicrobial susceptibility testing methodology [7].

The KB method consists of the:

- Spreading a culture broth, with the presence of bacteria in standardised concentration, on Petri dishes with culture medium constituted by agar added with substances favouring bacteria growth (Mueller-Hinton medium);
- Depositing, on the culture medium, a disc of absorbent paper impregnated with an antibiotic;
- Observing the presence/absence of bacterial growth inhibition halos around the disks with antibiotic substance and measuring the halos diameter;

The presence of a bacterial growth inhibition halo and its size gives information about sensitivity of the microorganism toward the tested antibiotic.

In order to test the applicability of our materials in the biomedical field for the manufacturing of antimicrobial fabrics amoxicillin plus clavulanic acid (amoxicillin alone has low efficiency) and vancomycin were adsorbed on both the pristine cotton C0, C1 and C2 by soaking the materials (gauzes of about 10x10cm of size MS~0.5 and a weight ranging from 500mg up to 800mg) in $1\times10^{-3}$M aqueous solutions of both antibiotics (40mL) for 6h at 25°C. The materials were then extensively washed with water (4×50mL) in order to remove the excess of antibiotic solution retained by the fibers and dried in air for 24h before using them.

The experimentation was carried out using standardised bacterial strains (0.5McFarland) of Staphyloccocus aureus (S. aureus) ATCC 29213 (Gram+) and of Escherichia coli (E. coli) ATCC 2592 (Gram-). For each test, disks having a diameter of about 1cm were taken from each gauze, deposited on the Petri dishes containing the Mueller-Hinton medium and incubated for 24h at 35°C.

In Fig.4 we report for convenience one of the inhibition tests on S.aureus with C0, C1 and C2 treated with vancomycin.

C0 C1 C2

Figure 4: Kirby-Bauer antimicrobial susceptibility test.

The formation of the inhibition halo around C2 impregnated with vancomycin is clearly evident.

| Samples | n. Tests | n. negative | n. positive |
|---|---|---|---|
| C0+amox/clav. acid versus E.coli | 11 | 11 | 0 |
| C0+vancom. versus S. aureus | 4 | 3 | 1 |
| C1+ amox/clav. acid versus E.coli | 8 | 8 | 0 |
| C1+vancom. versus S. aureus | 8 | 6 | 2 |
| C2+ amox/clav acid versus E.coli | 8 | 0 | 8 |
| C2+vancom. versus S. aureus | 8 | 1 | 7 |

Table 2: Kirby-Bauer antimicrobial susceptibility test.

Table 2 reports the results obtained for the inhibition of S.Aureus and E.coli with C0, C1 and C2 impregnated with vancomycin and amoxicillin plus clavulanic acid, respectively.

Specimens of C2, 8 + 8 samples, treated with vancomycin followed or not followed by washing treatment, were also used for ex-vivo experiments, using C0 gauzes (8 samples) without antibiotic treatment as blank. The study was performed applying colonies of S.aureus onto previously accurately cleaned forearm skin surface of 8 health volunteers. Two S.aureus colonies were applied on the proximal and distal site of the forearm skin of all subjects. Each skin-infected area was then covered with numerated gauzes. The experiment was designed as a double blind experiment, so the operators were unable to identify the different materials. Gauzes were removed 24 hours after their skin application and then placed into a nutrient broth for 24 hours at 37°C.

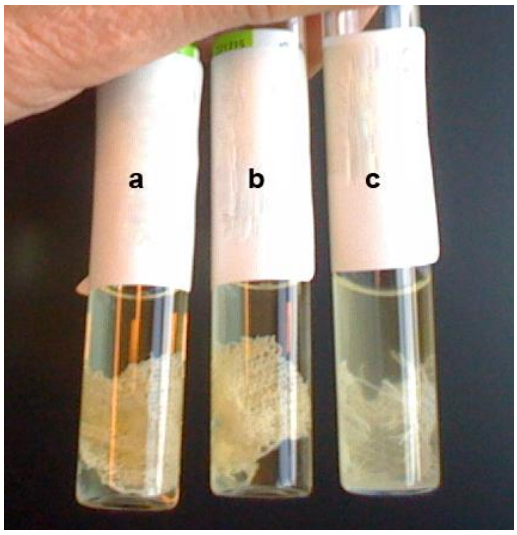


Figure 5: Ex vivo experiments with C0 (c) and C2 (a and b) against S. aureus

Fig. 5 is related to specimens used for the ex-vivo experiments on one volunteer. In particular the photo shows the complete inhibition of S.aureus growth, resulting a whole clear broth, by using C2 samples treated with vancomycin, with (Fig.5a) or without (Fig.5b) washing treatment. The pristine gauzes C0 which is normally used in the clinical activity (without antibiotic) is completely colonized by the S.aureus as evidenced by the turbidity of the growing medium (Fig.5c).

## 3 DISCUSSION

The C1 preparation is the starting and essential point of this work. It is based on the trasformation of a linear polymer like cellulose C0 in a branched polymer C1 where the GMA appendages form a whole which can be described as a surface nanostructure, see Figure 1. The chemical stability of this nanostructure is exceptional as GMA is linked to cellulose by a strong carbon-carbon covalent bond

and the glycidyl ester is actually very difficult to hydrolise. Glycidyl groups make the C1 surface hydrophobic and suitable to adsorb non polar molecules by electrostatic interaction. Glycidyl group can be transformed in glycerol affording C2 and in glycerol derivatives affording C3 and C4 by epoxide ring opening with water and ammines, respectively, see Figure 1. C2-C4 surfaces expose hydrophilic appendages and are much more hydrophilic than cellulose itself where the glucose OH groups interact each other to form those strong hydrogen bonds between cellulose chains responsible of many cellulose properties. C2-C4 surfaces are suitable to adsorb polar molecules by electrostatic interaction.

$^{13}C$ CP/MAS spectroscopy technique allows to follow all the preparation steps from C0 to C1, and then to C2, C3 and C4, see Figure 3. Even without entering in the details, it is easy to see that the cellulose profile of C0 does not change at all in the other spectra, while the GMA profile is superimposed and step by step modified. These observations allows to describe C1-C4 as modified cellulose, furthermore the maintainance of the cellulose morphology evinced by the constant ratio between crystalline and paracrystalline forms strongly supports the hypothesis of a surface modification. These characterisation aspects are crucial for our purposes, as we do not need a huge modification of the cellulose properties.

In order to study the applicability of C1-C4 for the manufacturing of antimicrobial textiles, we used amoxicillin and vancomycin as test molecules in a series of adsorption experiments, see Fig. 2. Amoxicillin, a wide spectrum β-lactam antibiotic which inhibits the bacterial cell wall synthesis, is administrated to the patients either orally or topically. It is effective against a wide number of Gram-positive and Gram-negative bacteria. Notheworthy, synergic effects for amoxicillin are obtained when it is administered with clavulanic acid. Vancomycin is a glycopeptide antibiotic used in the treatment of infections caused by Gram-positive bacteria like as Staphylococcus aureus and it is considered as "last resort" drug. It was observed that both C0 and C1 had a null adsorption capacity toward the amoxicillin and the vancomycin. Instead, C2, C3 and C4 showed a significant and different ability to catch both the antibiotic molecules from their aqueous solutions as shown by Table 1. These results agree with the favorauble electrostatic interaction between hydrophilic surface and polar molecules like amoxicillin and vancomycin. The fact that C3 and C4 are more efficient than C2 is not surprising considering that C3 and C4 are characterized by the presence of aliphatic chains of different length with a terminal hydrophilic primary amino group. Such branches are free to move around their grafting points and are able to adsorb a higher number of antibiotic molecules compared to the short hydrophilic branches of C2. In particular, this effect is much more evident in the case of bulky molecules like as the vancomycin.

The results above reported found confirmation through the microbiological inhibition tests. For the moment the experiments were performed only by considering C0, C1 and C2. In Tab.2 we report the results for Kirby-Bauer antimicrobial susceptibility test. According to the previous considerations the pristine cotton gauzes C0 and the C1 specimens gave negative antimicrobial activity for both antibiotics in the majority of the tests due to their negligible capability to adsorb the drugs and retain them after the washing procedure. Some positive results that were obtained by using the vancomycin could be ascribed to a not fully effective removal of the drug from the fibers during the washing operations. Instead, C2 specimens gave positive antimicrobial action in the majority of the tests. It means that even after extensive washing with water (that causes the release of a certain amount of antibiotic) a significant quantity of drug is still retained onto the material.

The ex-vivo experiments illustrated by Figure 5 not only confirm the previous results, but open the way to the development of C2 for making gauzes for specific topic effects and for transdermal administration of drugs. It is not difficult to imagine the positive feedback of the use of these gauzes in hospital and for home care.

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