Antibacterial and antifungal activity of functionalized cotton fabric with nanocomposite based on silver nanoparticles and carboxymethyl chitosan

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ABSTRACT

Cotton is the most widely used natural fiber for textiles; however, the capacity of cotton fibers to absorb large amounts of moisture, retain oxygen and have a high specific surface area makes them more prone to microbial contamination, becoming an appropriate medium for the growth of bacteria and fungi. In recent years, the incorporation of silver nanoparticles in textile products is becoming widely used due to its broad-spectrum antibacterial activity and low toxicity towards mammalian cells. In this work the antibacterial and antifungal activity of the nanocomposite based on silver nanoparticles and carboxymethyl chitosan (NPsAg-CMQ) against *E. coli*, *S. aureus* and *C. albicans* was evaluated by the well diffusion method. The antibacterial activity against *E. coli* and *S. aureus* was also evaluated by the qualitative method of inhibition zone and the quantitative method of colony counting. Likewise,, the antifungal activity of the functionalized fabric against *C. albicans* and *A. niger* was determined by inhibition zone method and the antifungal activity method GB / T 24346-2009, respectively. The results showed an excellent antibacterial activity of the nanocomposite against *E. coli* and *S. aureus* and a good antifungal activity against *C. albicans*. Likewise, the functionalized fabric showed 100% antibacterial activity against *E. coli* and *S. aureus* and a good antifungal activity against *C. albicans* and *A. niger*. Our results indicate that our fabric can be used in garments for hospital use to contribute in the reduction of nosocomial infections.

KEYWORDS: Nanocomposite, functionalized fabric, antimicrobial, antifungal, *E. coli, S. aureus, C. albicans, A. niger*

INTRODUCTION

Cotton is the most widely used natural fiber for textiles due to its softness, low price, easy mass production and is particularly suitable for the manufacture of medical products, health care products and hygiene (A. Hebeish, El-Naggar, et al., 2011)(Shahid-Ul-Islam, Shahid, & Mohammad, 2013). However, cotton fibers absorb large amounts of moisture, retain oxygen and have a high specific surface area (Y. Gao & Cranston, 2008), makes them more prone to microbial contamination, becoming an appropriate medium for the growth of bacteria and fungi (Cheung, Ho, Lau, Cardona, & Hui, 2009). In this sense, cotton fibers with antimicrobial properties have attracted considerable attention due to their potential application in several fields such as health and medicine (Moritz & Geszke-Moritz, 2013). It has been reported the use of biocides such as triclosan, quaternary ammonium compounds or organosilicons, among others (Rajendran, Radhai, Kotresh, & Csiszar, 2013). However, these antimicrobial agents often produce highly toxic or undesirable byproducts (Falletta et al., 2007).

In recent years, the incorporation of silver nanoparticles in textile products is being widely used due to its broad spectrum antibacterial activity and low toxicity towards mammalian cells (A. Hebeish, Abdel-Mohdy, et al., 2011), (Ashayer-Soltani, Hunt, & Thomas, 2015) (Gorjanc, Kovač, & Gorenšek, 2012), (Ravindra, Murali Mohan, Narayana Reddy, & Mohana Raju, 2010). The antimicrobial properties of silver nanoparticles are size dependent because silver nanoparticles of different sizes have different surface / volume ratios, which produces different antibacterial efficiency during their interaction with microorganisms (Vigneshwaran, Kathe, Varadarajan, Nachane, & Balasubramanya, 2007), (Liu, Dai, Fu, & Hsu, 2010), (Perera et al., 2013), (Wu et al., 2018). Instability of silver nanoparticles has been reported because a variation in the size of the nanoparticles when they are applied directly on textiles can cause agglomeration of the nanoparticles, resulting in a decrease in the antimicrobial effect (Emam, Saleh, Nagy, & Zahran, 2015).

It has been described that the use of stabilizing agents, such as natural polymers, to form nanocomposites, significantly improves the stability of the nanoparticles (El-Rafie, Ahmed, & Zahran, 2014). Polymer-based inorganic nanocomposites combine the unique mechanical, optical and electrical properties of organic, inorganic and nanomaterial compounds. The polymer chains of these nanocomposites can contain reactive groups and in combination with the inorganic antimicrobial agents have exceptional advantages such as exhibiting synergistic antimicrobial effects, improving the adhesion to the substrates, avoiding agglomeration and improving the stability of silver nanoparticles inside the polymer matrix. (Bao, Feng, Wang, Ma, & Tian, 2017), (Y. Gao & Cranston, 2008).

In this work the antibacterial and antifungal activity of the nanocomposite based on silver nanoparticles and carboxymethyl chitosan (NPsAg-CMQ) against *E. coli*, *S. aureus* and *C. albicans* was evaluated by the well diffusion method. The antibacterial activity against *E. coli* and *S. aureus* was also evaluated by the qualitative method of inhibition zone and the quantitative method of colony counting. Likewise, the antifungal activity of the functionalized fabric against *C. albicans* and *A. niger* was determined by the inhibition zone method and the standard method of antifungal activity GB / T 24346-2009, respectively.

MATERIAL AND METHODS

Reagents and Materials

The following reagents were used: potassium dihydrogen phosphate (pa ≥99%) (Merck Millipore, USA), sodium hydroxide (pa ≥99%) (Merck Millipore, USA), trypticase soy agar (TSA) (Liofilchem, Italy), Trypticase soy broth (TSB) (Liofilchem, Italy), *Escherichia coli* (ATCC 25922) (Merck Millipore, USA), *Staphylococcus aureus* (ATCC 25923) (Merck Millipore, USA).
Candida albicans and *Aspergillus niger* were provided by the Microbiology Laboratory of the Universidad Nacional de San Agustin (UNSA). The nanocomposite material (NPsAg-CMQ) was provided by the LAPCI-NANO laboratory of the Universidad Nacional de San Agustin (UNSA).

Antibacterial Activity of the Nanocomposite (NPsAg-CMQ)

The antibacterial activity was evaluated by the standard well diffusion method. Inoculate of the bacteria *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) were prepared at a concentration of 1.02 x 10^3 CFU/mL and 1.36 x 10^4 CFU/mL, respectively. Then, 20 µL of the inoculum was measured and plated uniformly on the surface of the Mueller Hinton agar (MH). Then 3 wells of 7 mm diameter were made, distributed equidistantly in the petri dish and 20 µL of the nanocomposite was placed (NPsAg-CMQ). The plates were then incubated for 24 hours at 37°C, then the inhibition zone was measured around the well using a vernier caliper (Balandin, G.V.; Suvorov, O.A.; Shaburova, L.N.; Podkopaev, D.O.; Frolova, Yu.V.; Ermolaeva, 2015).

Antibacterial Activity of Cotton Fabric Functionalized with the Nanocomposite NPsAg-CMQ

Inhibition Zone Method

It was evaluated by qualitative zone inhibition method. Initially, inoculate of the bacteria *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) were prepared at a concentration of 1.02 x 10^3

CFU/mL and 1.36 x 10^4 CFU/mL, respectively. Then, 20 µL of the inoculum was measured and plated uniformly in petri dishes with Mueller Hinton agar (MH) and allowed to dry 10 minutes. On the surface of the agar, disks (previously sterilized by UV) of the control and functionalized fabric of 0.8 cm diameter were placed. The plates were then incubated for 24 hours at 37°C, then the inhibition zone was measured around the fabric using a vernier caliper (Balamurugan, Saravanan, & Soga, 2017) .

Colony Counting Method

The antibacterial effect of the fabric functionalized with nanocomposite NPsAg-CMQ was tested using the standard quantitative method ASTM E-2149-10 against *E. coli* and *S. aureus*. For this, a fabric sample, weighing 1,000 ± 0.001 g, was cut into small pieces with a size of about 1 × 1 cm^2, which were sterilized by UV radiation for 30 min. Then, these fabric pieces were immersed in a 250 mL bottle with 50 mL of 0.3 mM dihydrogen phosphate buffer solution, pH = 7.2 containing 1.5 to 3.0 x10^5 CFU/mL of bacteria.

The bottle was shaken at 150 rpm in a shaker at 37°C, for 1 h. From each incubated sample, 1 mL of solution was taken and diluted to 10^{-1}, 10^{-2}, and 10^{-3} and then seeded on an agar plate. All plates were incubated at 37°C for 24 h, and the colonies formed were counted with the naked eye. The percentage of bacterial reduction was determined as follows:

Bacterial reduction in CFU (%) = $x = \frac{\mathrm{B-A}}{\mathrm{B}} * 100$

where:

A = Colony forming units (CFU) / mL for the bottle at the end time, after one hour contact

B = Colony forming units (CFU) / mL for the bottle at time zero, after one minute of contact.

Antifungal Activity of the Nanocomposite NPsAg-CMQ

The antifungal activity of the nanocomposite was evaluated by the standard well diffusion method. An inoculum of *C. albicans* (ATCC 10231) was prepared at a concentration of 1.06 x 10^7 CFU/mL. Then, 20 µL of the inoculum was measured and plated uniformly on the surface of the potato dextrose agar (PDA). Then 3 wells of 7 mm diameter were made equidistantly distributed in the petri dish; where 20 µL of the nanocomposite (NPsAg-CMQ) was placed. The plates were then incubated for 24 hours at 37 ° C, after which time the zone of inhibition was measured around the well using a vernier caliper (Balandin, G.V.; Suvorov, O.A.; Shaburova, L.N.; Podkopaev, D.O.; Frolova, Yu.V.; Ermolaeva, 2015) .

Antifungal Activity of Cotton Fabric Functionalized with the Nanocomposite NPsAg-CMQ

Antifungal activity was evaluated by qualitative zone inhibition method. Initially an inoculum of *C. albicans* of 1.06 x 10^7 CFU/mL was prepared. It was seeded in PDA medium and then proceeded in the same way as in the case of *E. coli* and *S. aureus* described above.

Antifungal Activity of the Cotton Fabric Functionalized with the Nanocomposite NPsAg-CMQ against a Filamentous Fungus

The antifungal activity of the control cotton fabric and the functionalized fabric was evaluated against an isolated strain of *A. niger* according to the standard method of antifungal activity GB / T 24346-2009 (D. Gao et al., 2019a).

RESULTS AND DISCUSSION

Antibacterial Activity of the Nanocomposite NPsAg-CMQ

Figure 1 shows the results of inhibition of antibacterial activity by the well diffusion method against *E. coli* and *S. aureus*. There is an average inhibition halo of 5 mm for *E. coli* and 8 mm for *S. aureus*. These results show that the nanocomposite has an excellent antibacterial activity against these two types of gram-negative and gram-positive bacteria, respectively.

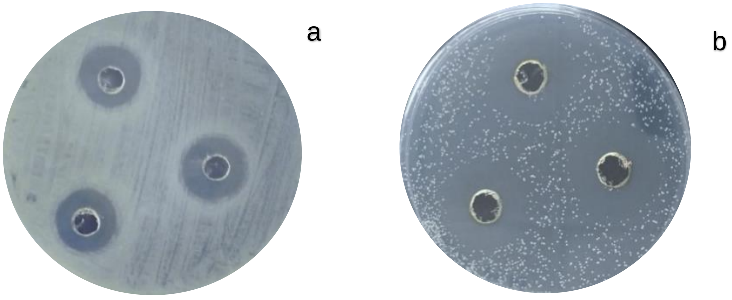

Figure 1. Antibacterial activity of the nanocomposite NPsAg-CMQ against *E. coli* (a) and *S. aureus* (b)

Antibacterial activity of the cotton fabric functionalized with the nanocomposite NPsAg CMQ

The antibacterial activity of the fabric functionalized with the nanocomposite NPsAg-CMQ was evaluated qualitatively by the zone of inhibition method and quantitatively by the colony counting method, according to the ASTM E-2149-10 technical standard.

Figure 2 shows the antibacterial activity of the control and textile fabric functionalized with the nanocomposite. The textile functionalized with nanocomposite shows an average zone of inhibition of 1.5 mm and 2 mm around the textile for *E. coli* and *S. aureus*, respectively. This form of inhibition clearly indicates that the textile functionalized with the nanocomposite possesses an antibacterial activity due to the action of the nanocomposite on the bacterial cells.

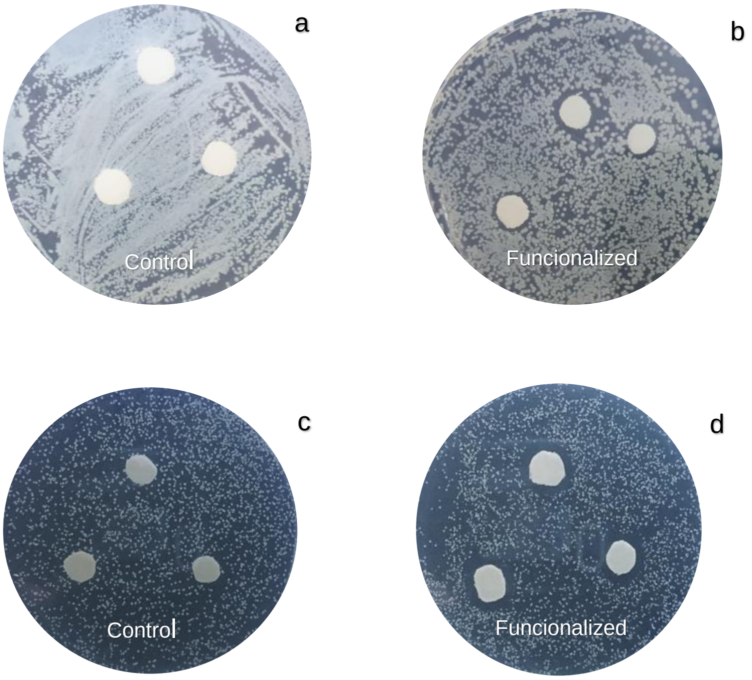

Figure 2. Antibacterial activity of the control and functionalized fabric against *E. coli* (a-b) y *S. aureus* (c-d)

The results in Table 1 and Figure 3 showed a bacterial reduction of 0% in the control fabric against *E. coli* and *S. aureus*. In contrast, the functionalized fabric exhibited a 100% bacterial reduction in both bacterial strains, showing the excellent antibacterial activity of the functionalized fabric compared to the control.

Table 1. Antibacterial reduction of control fabric and fabric functionalized with nanocomposite NPsAg-CMQ

Sample	Antibacterial activity			
	E. coli		*S. aureus*	
	Bacteria UFC/mL	% reduction	Bacteria UFC/mL	% reduction
Control	107500	No reduction	73167	No reduction
Functionalized	0	100	0	100

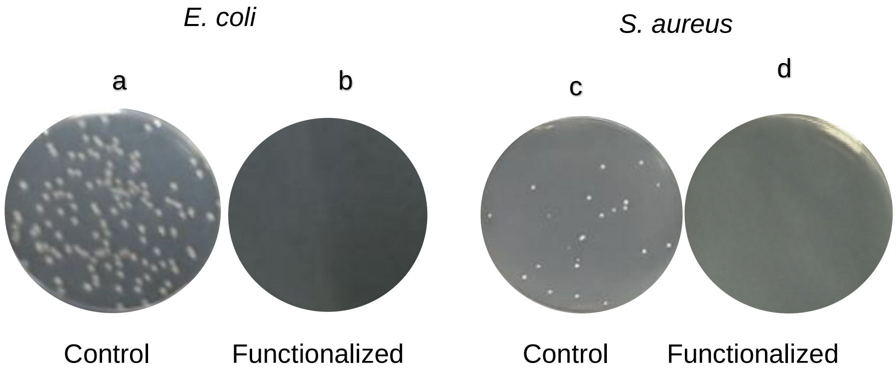

Figure 3. Antibacterial activity of the control and functionalized fabric against *E. coli* (a-b) y *S. aureus* (c-d)

Our antibacterial activity results are superior to those reported previously where 80-90% bacterial reduction was achieved (A. Hebeish, El-Naggar, et al., 2011) (El-Rafie, Shaheen, Mohamed, & Hebeish, 2012) (D. Gao et al., 2019b) (Wu et al., 2018). This demonstrates that the nanocomposite that we used (NPsAg-CMQ) has a better antibacterial activity, which was previously observed by another research group (Zea Alvarez JL., 2016). Similarly, these results for bacterial reduction correlate with Paszkiewicz *et al*. (Paszkiewicz et al., 2016), where they used bimetallic silver and copper nanoparticles. The bactericidal mechanism of the silver nanoparticles is only partially known and the antibacterial effect reported for the functionalized fabric could be explained based on the following mechanisms; the Ag+ ions formed from the oxidation of zero-valent silver (Agº) interact with the sulfur of the proteins present in the bacterial cell membrane or intracellularly, which affects the viability of the bacterial cell. It has also been proposed that the silver / silver ion (AgNPs / Ag +) nanoparticles can act with the molecules of phosphorus present in DNA producing an inactivation of DNA replication (A. Hebeish, El-Naggar, et al., 2011) (Raza et al., 2015) (Sathishkumar et al., 2009) (Ali Hebeish, Hashem, El-hady, & Sharaf, 2013) (Yang & Li, 2013) .

The release of Ag+ from the AgNPs can also catalyze the production of oxygen radicals that oxidize the molecular structure of the bacteria. Such a mechanism does not need a direct contact between the antimicrobial agent Ag+ and the bacterium, because the active oxygen produced diffuses from the textile to the surrounding environment. . (A. Hebeish, El-Naggar, et al., 2011) (A. Hebeish, El-Shafei, Sharaf, & Zaghloul, 2014).

Antifungal activity of the Nanocomposite NPsAg-CMQ

Figure 4 shows the results of inhibition of antifungal activity by the well diffusion method against *C. albicans*. An average inhibition halo of 1.5 mm is observed, this result shows that the nanocomposite has an antifungal activity against *C. albicans*.

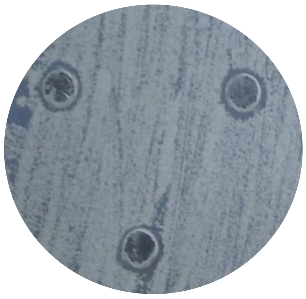

Figure 4. Antifungal activity of nanocomposite NPsAg-CMQ against *C. albicans*.

Antifungal activity of the cotton fabric functionalized with the nanocomposite (NPsAg-CMQ)

Figure 5 shows the antifungal activity of the control and textile fabric functionalized with the nanocomposite. The functionalized fabric shows an average zone of inhibition of 2 mm against *C. albicans*, which clearly indicates that the fabric functionalized with the nanocomposite possesses antifungal activity.

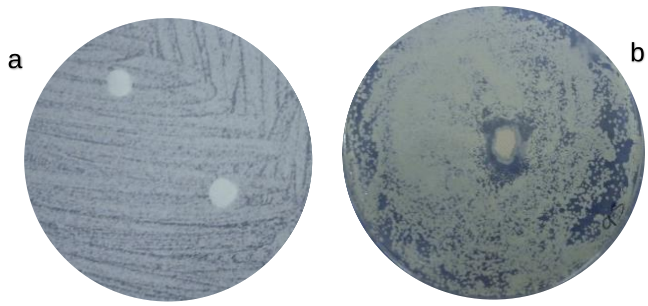

Figure 5. Antifungal activity of control (a) and functionalized (b) fabric against C. albicans.

The antifungal activity of the control cotton and the functionalized fabric was evaluated against an isolated strain of *Aspergillus niger* according to the standard method GB / T 24346-2009 (D. Gao et al., 2019a) (Yu et al., 2015). Figure 6 shows the results of the antifungal activity of the control cotton and functionalized fabric with the nanocomposite, it can be clearly seen that the control fabric (Figure 6a) is covered by the filamentous fungus, indicating that this fabric does not show resistance to the growth of *A. niger*. However, the textile functionalized with the nanocomposite (Figure 6b) shows a greater surface area without growth of the fungus.

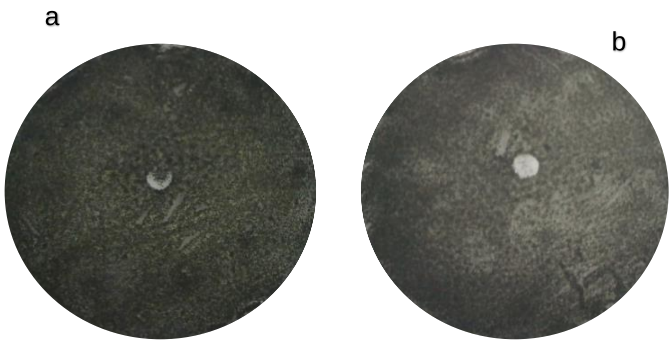

Figure 6. Antifungal activity of control (a) and functionalized (b) fabric against *A. niger*

D. Gao *et al.*, reported similar results, with the difference that they worked with a functionalized fabric with a nanocomposite based on P(DMDAAC-AGE)/Ag/ZnO, which presented an ability to inhibit the growth of *A. flavus* up to 5 days (D. Gao et al., 2019b). However, our functionalized textile showed a growth inhibition for up to 7 days, which shows a better antifungal effect.

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