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Cotton-hydrogel composite for improved wound healing: Antimicrobial activity and anti-inflammatory evaluation—Part 2

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Infection is one of the major risk factors for the development of chronic wounds. Antimicrobial wound dressing has been pointed out as a viable option for the prevention and treatment of wound infections. Thus, we developed a composite material based on cotton textile substrates functionalized with cyclodextrin-hydroxypropyl methyl cellulose-based hydrogel. The composites’ ability to encapsulate and release gallic acid (antimicrobial phenolic acid) was evaluated, as well as their mechanical properties and antimicrobial and anti-inflammatory capacity. All composites were able to retain gallic acid in their structure, with similar loading profile. The presence of gallic acid on composites was confirmed by FTIR and TGA. Composites storage moduli was reduced by the presence of gallic acid. The results suggest a straight relation between the swelling ability and gallic acid drug delivery profile. The drug delivery mechanism, of the developed composites, was mainly controlled by Fickian diffusion, based on the experimental data fitting to the Peppas-Sahlin model. Gallic acid antimicrobial and anti-inflammatory properties were transferred to the composite materials. According to the results, the developed composites can be applied on the prevention or treatment of chronic wounds.

KEYWORDS
anti-inflammatory, antimicrobial, chronic wound, composite wound dressing, gallic acid, infection

1 INTRODUCTION

Wound dressings’ research has been set by the insights on wound healing process and by patients’ demands. Nowadays, the patients have high expectations regarding the wound dressings. They must accelerate cicatrization, with minimal pain and best cosmetic and functional results.1,2 This is especially important in the case of chronic or nonhealing wounds. Chronic wounds represent a silent epidemic affecting a large fraction of the world’s population. They are a burgeoning cost in the health care system, aggravated by demographic developments.3 Chronic wounds can be triggered by repeated ischemia-reperfusion injury, altered cellular and systemic response, tissue hypoxia, or microorganisms.4,5 Among these, microorganism colonization and posterior infection are particularly significant, since virtually, all chronic wounds became contaminated, delaying the healing, causing malodorous and exudates.4 Thus, the elimination of clinically relevant infections is a prerequisite for efficient wound healing.6

The most common treatment for infected chronic wounds is the systemic administration of broad-spectrum antibiotics.7 Their indiscriminate use affects skin flora and induce multiresistant strains.8 Topical treatment with antimicrobial agents has been pointed out as a good alternative to antibiotic therapy. Topical delivery ensures antimicrobial agent release at the infection site, reducing the concentration need to control the infection and, consequently, the systemic absorption and toxicity.4,6

Antimicrobial wound dressings have been often used to protect the wound from new injury, dehydration, and microorganism deposition.2 Several materials (textiles, hydrogels, foams, and films) have been developed to deliver antimicrobial agents to infected wounds.10 Although, they still show inadequate release profile, characterized by an initial burst that leads to local toxicity owed high antimicrobial concentration, followed by a prolonged release of antimicrobial agent below required concentration, selecting the resistant microorganisms.9
In a previous work,\textsuperscript{11} our research group optimized the synthesis of a composite wound dressing obtained through the combination of cotton textile substrates and cyclodextrin-hydroxypropyl methyl cellulose-based hydrogel. The developed composite should gather the suitable properties of textiles wound dressing, mechanical stability and biocompatibility, and the hydrogels drug delivery and surface properties.\textsuperscript{7,11,12} In order to complement the previous work, composites’ ability to encapsulate and release small antimicrobial molecules was evaluated, as well as their mechanical properties. The composites’ antimicrobial and anti-inflammatory activities were also assessed.

2 | MATERIALS AND METHODS

2.1 | Materials

Gallic acid (3,4,5-trihydroxybenzoic acid) was provided by Merck, and β-cyclodextrin (βCD, 1135 g mol\(^{-1}\)) and 2-hydroxypropyl-β-cyclodextrin (HPβCD, 1309 g mol\(^{-1}\)) were acquired from AppliChem. Hydroxypropyl methylcellulose (HPMC) Methocel K4 M (84 200 g mol\(^{-1}\); normal viscosity, 400 mPa s) was purchased from VWR Prolab; 1,4-butanediol diglycidyl ether (BDGE, 50%-60% v/v) was obtained from Acros Organics. Purified water, obtained by reverse osmosis (MiliQ, Mikipore, Madrid, Spain) with a resistivity above 18.2 MX cm, was used. RAW264.7 cells were purchased from ECACC (European Collection of Authenticated Cell Culture, Salisbury, UK), lipopolysaccharide (LPS) from Sigma, and DMEM medium from HyClone. The Griess Reagent System Kit was purchased from Promega and dexamethasone from Sigma. The textile substrates used were as follows: cotton (COT) 100% tubular mesh with 150 g m\(^{-2}\), washed according to ISO 105-C01, sterile nonwoven (NW) 100% cotton with 54 g m\(^{-2}\), and sterilized gauze (GZ) 100% cotton with 24 g m\(^{-2}\).

2.2 | Composites synthesis

The optimal conditions to synthesize cotton-hydrogel composites were described in a previous work.\textsuperscript{11} The composites used in this work were obtained by one-step cross-linking procedure. A polymeric solution was prepared by dissolving the CDs (0.22 mol L\(^{-1}\) of βCD or 0.19 mol L\(^{-1}\) of HPβCD) and HPMC (5.95 × 10\(^{-3}\) mol L\(^{-1}\)) in NaOH (5 mL 0.2M). Then they were transferred to petri dishes, and the cross-linking agent (0.54 mol L\(^{-1}\) of BDGE) was added. After homogenization, the textile substracts (0.30 g of COT, NW, or GZ) were immersed on the solutions. The cross-linking procedure was performed at 50°C for 20 hours. To remove the chemical residues, the composites were washed with water (12 h), HCl (10mM) for an additional 12 hours, and kept in water for 7 days, at 25± 1°C. After, they were dried at 40 ± 1°C for 24 hours and stored in a desiccator.

2.3 | Gallic acid encapsulation and release

Gallic acid solution (2.3 × 10\(^{-2}\) mol L\(^{-1}\), in methanol 2% v/v) was dissolved in H\(_3\)PO\(_4\)/NaOH buffer (pH 3 ± 0.5).\textsuperscript{13} Dry composite samples (30 ± 3 mg) were immersed in 5 mL of gallic acid solution at 25± 1°C and 60 rpm. The amount of phenolic acid in the solution was assessed by UV-vis spectrophotometry, until the absorbance values stabilize. The gallic acid loading was calculated based on the variation of gallic acid in the initial solution and at the equilibrium.

The gallic acid release, from composites, was performed using load dry samples. The samples (30 ± 3 mg) were immersed in 5 mL of synthetic sweat solution (SSS, 0.5 g of l-histidine monohydrochloride monohydrate, 5 g of sodium chloride, and 2.2 g of sodium dihydrogen orthophosphate dihydrate, pH 5 ± 0.5). The samples were maintained at 25± 1°C, and the absorbance of the released medium was monitored until stabilization. A comparison of goodness-of-fit to the Peppas-Sahlin model was carried out by fitting the model to the experimental data. Optimal parameters were found through the software DDSolver.\textsuperscript{14}

In both cases, loading and release, the absorbance was measured at the gallic acid λ\(_{\text{max}}\) (259 nm for pH 3, ε 2.30 M cm\(^{-1}\) or 261 nm for pH 5, ε 4.27 M cm\(^{-1}\)). The gallic acid concentration was calculated based on the calibration curve, previously determined. All the absorption measurements were recorded on a Jasco V560 spectrometer, using a 1 cm quartz cuvette.

2.4 | Physic-chemical characterization

Fourier transform infrared spectroscopy (FTIR-ATR) spectra were obtained by analyzing samples (0.5 ± 0.05 g) of each composite with or without gallic acid, COT-βCD-HPMC, NW-βCD-HPMC, GZ-βCD-HPMC, COT-HPβCD-HPMC, NW-HPβCD-HPMC, and GZ-HPβCD-HPMC. The spectra were recorded between 400 and 4000 cm\(^{-1}\), in FTIR Shimadzu IRAffinity.

The thermal properties of the developed composites were characterized by differential scanning calorimetry (DSC) and thermogravimetry (TGA). DSC measurements were carried in liquid nitrogen atmosphere using DSC-822e instrument (Mettler Toledo). The calibration was made with indium as standard. Samples were weighed (2.5 ± 0.2 mg) and sealed in aluminum pans. Then they were heated from 25°C to 350°C, at a scanning rate of 10°C min\(^{-1}\). Data were treated using LAB Mettler star SW 8.1 software (Mettler-Toledo International Inc, Swiss). Composite samples’ thermal behavior was, also, investigated employing the simultaneous thermogravimetry-differential scanning calorimetry (TG-DSC) technique using a TA Instruments model Hitachi STA7200. The samples (mass approx. 10 mg) were heated (20°C to 600°C) in a standard alumina sample pan. All experiments were performed under dynamic nitrogen at a flow rate of 0.1 dm\(^3\) min\(^{-1}\) using a heating rate of 10°C min\(^{-1}\) per run. Degradation onset temperature was assigned at the temperature at which 90% mass remained.

2.5 | Mechanical properties

Strain-to-break tensile testing was performed using the tensile film geometry on a TA Instruments Hitachi DMA7100. Samples were cut into rectangular cuboids, measured, and clamped to the DMA geometry. A controlled extension force of 5 N min\(^{-1}\) was applied to the sample until the sample broke or the instrument reached maximal
extension; experiments were conducted at room temperature. At least three replicate measurements of each samples were conducted.

2.6 | Composites biological properties assessment

2.6.1 | Antimicrobial properties

The composites' antibacterial activity was tested against two bacteria: Staphylococcus aureus (ATCC 6538) and Escherichia coli (ATCC 25922). The absorption method described in JIS L 1902:2008 was used, with the following alterations. An inoculum was prepared in 20.0 ± 0.1 mL of TSB (Tryptic Soy Broth, Merck) and incubated for a period of 18 hours at 37 ± 1°C under agitation (120 rpm). Subsequently, the bacterial concentration was adjusted to 3 × 10⁸ cells mL⁻¹ via absorbance readings and based on a corresponding calibration curve. An aliquot of the above suspension (400 μL) was added to 20 mL of TSB and incubated for 3.0 hours at 37 ± 1°C. The bacterial concentration was again measured, and 3 × 10⁵ cells mL⁻¹ were obtained using a 20-fold dilution of TSB (in distilled water). The 50 μL of this inoculum was then added to each sample (1 ± 0.01 g). Samples were incubated for 8 or 24 hours at 37 ± 1°C. Subsequently, 5 mL of physiological saline solution (8.5 g of NaCl and 2.0 g of non-ionic surfactant Tween 20 [Sigma Chemical Co] per litter) were added to the samples, which were then vortexed five times for 5 seconds. The number of living bacteria was assessed by the serial dilution plate count method. All assays were performed in triplicate and repeated three times.

2.6.2 | Anti-inflammatory properties

The anti-inflammatory properties of the developed composites was evaluated in vitro, using the murine macrophage (RAW 264.7) cell line, according to the method previously described by Sobral and coworkers. The cells were cultured in DMEM medium and incubated at 37 ± 1°C under 5% CO₂, in humidified air. For each experiment, cells were detached with a cell scraper and seeded at 1.5 × 10⁵ cells per well in a 96-well plate. The plate was incubated overnight to allow their attachment. The samples were kept in DMEM for 24 hours. Then the extracted solution of the samples was added to each well, and plate was incubated for 1 hour. The following step was stimulation with LPS (1 μg mL⁻¹) for 24 hours. The effect of all tested samples in the absence of LPS was also evaluated. The cells were incubated again for 24 hours. A Griess Reagent System Kit was used to nitric oxide (NO) determination, which contains sulfanilamide, N-1-naphthylethenediamine dihydrochloride (NED), and nitrite solutions. The cell culture supernatant (100 mL) was transferred to the plate, in duplicate, and mixed with sulfanilamide and NED solutions. The nitrite produced was determined by measuring the optical density at 540 nm, in the microplate reader, and was compared with the standard calibration curve (y = 0.0063x + 0.1262, R² = 0.9994). The results were expressed in percentages of inhibition of NO production in comparison with the negative control (100%). Dexamethasone (Sigma-Aldrich, St Louis, MO, USA) was used as positive control (IC50 = 15.69 ± 1.35 μg/mL).

3 | RESULTS AND DISCUSSION

Nowadays, consumers are more aware of natural products and eco-friendly procedures’ benefits. Products obtained from natural sources are biocompatible and biodegradable, opposing to most synthetic products. The search for goods with high biocompatibility and reduced side-effects is paramount for the wound dressing market. Thus, we developed a composite wound dressing based on products from natural sources, cotton, cyclodextrins, and cellulose. The cotton still is a standard dressing for wound management, since it offers mechanical support and gas and water permeability. Cellulose, as the main component of cotton, is nontoxic, homeostatic, nonallergic, and biocompatible. Cyclodextrins are biocompatibility cyclic oligosaccharides and approved by FDA. They are able to protect and modulate the release of a wide range of molecules. An antimicrobial agent, gallic acid, was also used in the present work to add the ability to prevent and control infection to the composite. Gallic acid antimicrobial activity, biocompatibility, and incorporation on a cyclodextrin-cellulose–based hydrogel were already proved in other works.

3.1 | Gallic acid loading

All the composites were able to retain gallic acid in their structure, with similar loading profile. Gallic acid loading equilibrium was reached after 360 minutes for the composites (COT-HPMC-CD, NW-HPMC-CD, and GZ-HPMC-CD) and immediately for the nonfunctionalized textile substrates (COT, NW, and GZ). The cyclodextrin used had the same influence on the three substrates. The composites obtained with HPMC-βCD were able to retain more gallic acid than the ones with HPMC-βCD. These results are in accordance with the polymeric network and composites swelling behavior. Since in previous works, it was observed that the HPMC-βCD composites could accommodate more water molecules than HPMC-βCD and gallic acid had more affinity to form inclusion complexes with HPβCD, than with βCD. Regardless the cyclodextrin used, the GZ composites showed more affinity to gallic acid (Figure 1). They retained 256 mg GA/g composite (HPMC-βCD) and 388 mg GA/g composite (HPMC-HPβCD).

The hydrogel network assembled on the cotton fibers was the manly responsible for the composite ability to retain gallic acid. The loading profile was, also, influenced by the cross-linking degree of the polymeric network, cyclodextrins available to form inclusion complex with the phenolic compound, and gallic acid affinity to all network compounds.

3.1.1 | Physic-chemical characterization

Figure 2 shows the FTIR spectrum of the composites with or without gallic acid. The FTIR spectrums of the composites COT-HPMC-CD,
NW-HPMC-CD, and GZ-HPMC-CD were analyzed in the first part of this work. On gallic acid FTIR spectrum (Figure 2, black), there is a broad peak from the aromatic and carboxylic O–H stretching at 3483 and 3256 cm$^{-1}$. At 2924 cm$^{-1}$ was identified the peak correspondent to hydrogens bonds stretching between the phenolic groups. The intense peaks at 1659 and 1605 cm$^{-1}$ result from the conjugate acids C=O stretching. The combination of OCC asymmetric stretching and OH bend is observed through to the peaks at 1425 and 1264 cm$^{-1}$. The aromatic ring stretching is detected at 1432 cm$^{-1}$, and the CO stretching is displayed at 1218 cm$^{-1}$. The peaks observed at 1029 cm$^{-1}$ result from the out-of-plane aromatic bending. The composites with gallic acid had similar profiles to the materials without gallic acid. However, they display the appearance of two peaks at 1659 and 1605 cm$^{-1}$, identified as C–O stretching from the gallic acid conjugate acids, confirming the presence of gallic acid on the composite materials.

Figure 3 (black) displays the gallic acid DSC spectrum. Since the phenolic acid is a pure substance, only an endothermic sharp peak was observed. The gallic acid did not induce any changes on the composites’ thermal properties detected by DSC. All composites showed two endothermic peaks associated with the water lose (between 100°C and 150°C) and the cyclodextrins degradations near 300°C. Therefore, composites were subject to TGA analysis to evaluate their weight change with the temperature increase. From Figure 4 and Table 1, it is possible to observe that the presence of gallic acid induced changes on composites thermal behavior, detected by TGA. Regardless the cyclodextrin or textile substrate used, the gallic acid decreased the degradation temperature (Tg). Gallic acid maximum weight lost temperature occur near 275°C, as consequence of it crystalline melting. Hence, the composites with gallic acid Tg increment confirms the presence of gallic acid on the network. Gallic acid presence was, also, proved by the weight loss. The remaining weight percentage after decomposition was superior for the composites with gallic acid. Similar effect on the thermal behavior of fibers loaded with gallic acid was described by Neo and coworkers. They found that gallic acid shifts the loaded fibers Tg to a lower temperature, when compared with the pure gallic acid as result of a change on the phenolic compound physical state.

### 3.2 Composites’ mechanical properties

Even though hydrogels gather suitable properties for wound treatment, they lack mechanical stability, reducing their performance on the wound site. Considering this drawback, the mechanical stiffness of the developed composites was assessed by DMA (Table 2).

The storage moduli ($E'$) represents the stiffness of a viscoelastic material and is proportional to the energy stored during a loading cycle. Table 2 lists the $E'$ of the controls (COT, NW, and GZ) and composites with and without gallic acid at 25 ± 1°C (storage temperature) and 36± 1°C (end-use temperature). The temperature had no effect on the textile substrates' stiffness. However, the increase of temperature reduced the stiffness of the composites. The gallic acid encapsulation, also, reduced the $E'$ of the composites.

### 3.3 In vitro gallic acid release

Composites ability to release gallic acid was assessed using a synthetic sweat solution, to mimic the conditions found on the skin surface. All
composites were capable of sustaining the gallic acid release for 48 hours. It was observed a faster release during the first 360 minutes, followed by a plateau until 48 hours. The phenolic acid migration from the composite surface to the solution was responsible for the initial burst. The slower release, overserved after 6 hours, was a consequence of the inclusion complexes gallic acid-cyclodextrin dissociation.

The gallic acid quantity released from composites (Figure 5) was in accordance with the loading results (Figure 1). The textile substrate released almost all the gallic acid loaded (COT 71%, NW 71%, and

### Table 1: TGA values of the composite materials with or without gallic acid

<table>
<thead>
<tr>
<th></th>
<th>COT</th>
<th>G, %</th>
<th>NW</th>
<th>Tp, °C</th>
<th>Tp, °C</th>
<th>GZ</th>
<th>Tp, °C</th>
<th>G, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPMC-βCD</td>
<td>372</td>
<td>13.26</td>
<td>376</td>
<td>10.06</td>
<td>380</td>
<td>12.81</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPMC-βCD/GA</td>
<td>344</td>
<td>17.76</td>
<td>364</td>
<td>13.65</td>
<td>370</td>
<td>15.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPMC-HβCD</td>
<td>376</td>
<td>13.67</td>
<td>373</td>
<td>9.31</td>
<td>361</td>
<td>12.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPMC-HβCD/GA</td>
<td>338</td>
<td>15.88</td>
<td>362</td>
<td>18.30</td>
<td>320</td>
<td>18.27</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The composites functionalized with HPMC-βCD released 39% and 64% (w/w) of the gallic acid retained on the network.

Composites drug delivery capacity is highly dependent on the hydrogel network swelling and release properties. Thus, their drug delivery capacity is set by the following process: solvent transport into the polymeric matrix, polymer swelling, solute diffusion, and erosion/relaxation of the swollen polymer. Furthermore, previous studies had proven that the use of cyclodextrins on the polymeric hydrogels benefits their drug delivery properties.

In this case, the inclusion complex strength, between the cyclodextrins and gallic acid, had a major influence on the drug delivery capacity of the composites. The inclusion complexes HPMβCD-gallic acid are more stable than the ones obtained with βCD. Therefore, despite HPMC-HPβCD composites accommodating more gallic acid, the amount released was similar to HPMC-βCD composites.

For a more comprehensive characterization of the release profiles, the data were analyzed by fitting to Peppas-Sahlin model (Equation 1), since it have been successfully applied to HPMC and cyclodextrin based hydrogel drug delivery systems. This release kinetics model considers the diffusional and relaxational mechanism associated with the anomalous drug release process of hydrogels. Considering the right side of Equation 1, the first term calculates the molecular diffusion of the drug as consequence of the chemical potential gradient, Fickian contribution (F). The second term represents the Case II relaxation contribution (R), set by the stress and state-transition of swelling-controlled drug release.

\[
\frac{M_t}{M_\infty} = k_D t^m + k_R t^{2m}.
\] (1)

where \(M_t\) and \(M_\infty\) are the absolute cumulative amount of drug released at time (t, minute) and infinite time and \(k_D\) (diffusional constant), \(k_R\), and \(m\) are constants.

A good fitting was observed between the experimental data and the Peppas-Sahlin model, since the \(r^2\) values were superior to 0.90 for all the composites (Table 3). Moreover, \(k_D\) values were always superior to \(k_R\), meaning that the drug release mechanics of the composites was control by Fickian diffusion. The functionalization with hydrogels significantly influence the release profiles of the textiles substrates, improving it. The diffusional constants obtained were superior for the composites, comparative with the control substrates, regardless the cyclodextrins used. The predominance of Fickian diffusion as drug delivery mechanism on cyclodextrin-based hydrogels was also described in several works.

The same behavior was, also, observed on composites obtained by the conjugation of hydrogel networks and textile substrates. For instance, Cirillo and coworkers developed a cotton-gauze-hydrogel composite electroresponsive drug delivery system to be used as wound dressing. By fitting the experimental data to the Peppas-Sahlin model, they conclude that the release rate was faster when high voltages were applied and Fickian diffusion was the prevalent mechanism of drug delivery.
3.4 | Composites biological properties

3.4.1 | Antimicrobial capacity

The presence of microorganism on the wound bed may delay the healing process, giving rise to chronic wounds. Gallic acid is a simple phenolic acid with good applicability as antibacterial agent against bacteria, commonly isolated from infected wounds. It was already used, with success, to add antimicrobial properties to hydrogels networks.24,26,47 Thus, the antimicrobial activity of the developed composites was investigated (Figure 6). The textile substrates (COT, NW, or GZ), the functionalized substrates with HPMC-βCD, or HPMC-HPβCD prevented the bacteria growth. Otherwise, the composite materials loaded with gallic acid destroyed all bacteria, independently the contact time. According to the results obtained, it was verified that the gallic acid’s antibacterial activity was preserved after incorporation within the composite material.

Even though natural antimicrobial agents have been described as a viable alternative to antibiotics on the chronic wound treatment, just a few paper are available reporting the use of antimicrobial agents to functionalize cotton-hydrogel composites. Nevertheless, they support the above results regarding the maintenance of the natural agents’ antimicrobial activity after their encapsulation by textile-hydrogel composites.48,49 For instance, Xu and coworkers were able to incorporate a berberine nanosuspension in a poly(N-isopropylacrylamide)/alginate copolymer hydrogel-grafted fabrics. Based on their results, the developed composite was able to sustain the release of berberine, and it kept their biological properties (antimicrobial effect, promotion of granulation and capillary formation, wound closure, moisture regulation, and hemostasis).48 Similar results were described using a cotton fabric modified with a chitosan-poly(N-isopropylacrylamide) interpenetrating polymer network hydrogel.49 The chitosan antimicrobial activity was present on the cotton modified fabric, as well as the thermosensitive behavior of the hydrogel network.49

3.4.2 | Anti-inflammatory properties

Chronic wounds result from a breakdown on the healing process, caused by prolonged inflammation stage. Infection has been described as the major cause of chronic wounds, specially the presence of biofilms. The persistent of infection stimulate the constant influx of immune cells, extending the proinflammatory cytokine cascade and rise the proteases concentration on the wound bed. The inhibition of protease fails, leading to the destruction of the extracellular matrix and growth factors, as well as their receptors. This prevents the wound from moving forward to the proliferative phase and attracts more inflammatory cells, intensifying the inflammatory cycle.3,50-52

The production reactive nitrogen species is a consequence of the inducible nitric oxide synthase (iNOS) activity, an inflammatory mediator. NO (nitric oxide) has been described as an important mediator of acute and chronic inflammation.53 Thus, NO has been used as parameter to assess in vitro the anti-inflammatory capacity of molecules and materials.54

Therefore, the anti-inflammatory properties of the developed composites were assessed by the quantification of (NO) production inhibition. The loaded composites (HPMC-βCD/GA and HPMC-HPβCD/GA), regardless the subtracted used, were able to prevent 60% of the NO production by the cells RAW 264.7 (Figure 7). Otherwise, the controls and composites without GA, only, prevent between 10% and 30% of the NO production.

Gallic acid, besides its antimicrobial activity, has been described as a strong anti-inflammatory agent.23,55 A direct relation between the concentration of gallic acid and the inhibition of NO production in LPS/IFN-γ-activated macrophages was, also, demonstrated by Wang and coworkers.56 Accordingly, gallic acid was able to migrate from the cyclodextrins cavity and hydrogel network to the medium, without losing biological properties, based on the obtained results.

![FIGURE 6](Image 227x31 to 540x149)

**FIGURE 6** Quantitative analysis of antibacterial activity of the gallic acid loaded composites by direct contact, for 8 or 24 hours, against (A) *Staphylococcus aureus* and (B) *Escherichia coli*, 5 × 10^5 cells mL^-1_.

- COT-βCD, NW-βCD, GZ-βCD, COT-HPβCD, NW-HPβCD, GZ-HPβCD,
- COT-βCD/GA, NW-βCD/GA, GZ-βCD/GA, COT-HPβCD/GA, NW-HPβCD/GA, and GZ-HPβCD/GA [Colour figure can be viewed at wileyonlinelibrary.com]

![FIGURE 7](Image 458x741 to 516x761)

**FIGURE 7** Composites’ anti-inflammatory activity by quantification of the NO (nitric oxide) inhibition production percentage. COT, NW, or GZ (black), composites functionalized with HPMC-βCD (fill dark grey), HPMC-βCD/GA (no fill dark grey), and composites with HPMC-HPβCD (fill light grey) and HPMC-HPβCD/GA (no fill light grey)
CONCLUSIONS

In a previous work, the synthesis optimization and physicochemical characterization of composite wound dressings obtained by the cross-linking between cotton textile substrates and cyclodextrin-cellulose based hydrogels were performed. It was proved that the functionalization of the textile substrates improved their applicability as wound dressing.

Regarding the present work, encapsulation of antimicrobial gallic acid by the developed materials gave rise to a composite wound dressing with enhanced biological properties. Gallic acid was successfully loaded into the composite materials, and its presence was proven by FTIR and TGA composite profiles. The phenolic acid release was improved by the presence of cyclodextrins and was mainly driven by Fickian diffusion. The antimicrobial and anti-inflammatory properties of gallic acid were not compromised after its incorporation on the composites network.

Thus, it is possible to synthetize composite wound dressings using natural compounds and eco-friendly procedures and transfer the antimicrobial and anti-inflammatory properties gallic acid to it. This work presents a valuable route towards the functionalization of cotton improving its application as wound dressing.

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