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Experimental study on the use of a chlorhexidine-loaded carboxymethylcellulose gel as antibacterial coating for hernia repair meshes

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Abstract

Purpose Biomaterials with an antimicrobial coating could avoid mesh-associated infection following hernia repair. This study assesses the use of a chlorhexidine-loaded carboxymethylcellulose gel in a model of *Staphylococcus aureus* mesh infection. **Methods** A 1% carboxymethylcellulose gel containing 0.05% chlorhexidine was prepared and tested in vitro and in vivo. The in vitro tests were antibacterial activity (*S. aureus*; agar diffusion test) and gel cytotoxicity compared to aqueous 0.05% chlorhexidine (fibroblasts; alamarBlue). For the in vivo study, partial abdominal wall defects (5 × 2 cm) were created in New Zealand white rabbits (n = 15) and inoculated with 0.25 mL of *S. aureus* (10^6 CFU/mL). Defects were repaired with a lightweight polypropylene mesh (Optilene) without coating (n = 3) or coated with a carboxymethylcellulose gel (n = 6) or chlorhexidine-loaded carboxymethylcellulose gel (n = 6). Fourteen days after surgery, bacterial adhesion to the implant (sonication, immunohistochemistry), host tissue incorporation (light microscopy) and macrophage reaction (immunohistochemistry) were examined. **Results** Carboxymethylcellulose significantly reduced the toxicity of chlorhexidine ($p < 0.001$) without limiting its antibacterial activity. While control and gel-coated implants were intensely contaminated, the chlorhexidine-gel-coated meshes showed a bacteria-free surface, and only one specimen showed infection signs. The macrophage reaction in this last group was reduced compared to the control ($p < 0.05$) and gel groups. **Conclusions** When incorporated in the carboxymethylcellulose gel, chlorhexidine showed reduced toxicity yet maintained its bactericidal effect at the surgery site. Our findings suggest that this antibacterial gel-coated polypropylene meshes for hernia repair prevent bacterial adhesion to the mesh surface and have no detrimental effects on wound repair.

Keywords Antimicrobial coating · Carboxymethylcellulose · Chlorhexidine · Hernia · Mesh coating · Polypropylene

Introduction

Hernia repair using a prosthetic material is a common general surgery procedure [1]. As a possible complication of this type of surgery, infection has serious repercussions including a risk of implant failure [2]. The presence of microorganisms on the implanted prosthetic material and surrounding host tissue has been associated with increased rates of mortality and morbidity, and often another surgery for debridement of the implant zone or even recovery of the infected mesh is required [3]. Infection also lengthens hospital stay and has social, clinical, and economic impacts.

In hernia repair surgery, the incidence of infection varies according to the surgical technique employed, the size and complexity of the hernia, the prosthetic material implanted, surgery duration, the extent of tissue dissection, the use of drainage tubes and patient comorbidities [4]. According to recent estimates, infection rates are lower than 1% for the repair of a ventral hernia [5] or around 3–4% for an inguinal hernia [6]. For incisional hernia repair, reported rates range from 1 to 3% for laparoscopic repair to 13–15% for an open surgery procedure [5, 7].

In clinical practice, preoperative antibiotic prophylaxis is a routine strategy used to minimize the infection risk. Antibiotics are generally administered via the systemic route, but it is also possible to submerge the prosthetic material in antibiotic solution before its implant [8], and even use antiseptic agents for intraoperative irrigation [9]. The utilization of antiseptics for preventing mesh infections is receiving increasing attention. In general terms, these agents can act by altering the synthesis of DNA, RNA, and proteins, destabilizing bacterial membrane structure and function, or inhibiting metabolic pathways that are essential for the survival of the microorganism [10]. Owing to their non-specific mechanisms of action, antiseptics show wide-spectrum antimicrobial activity against Gram-positive and -negative bacteria along with a low risk of inducing resistance [3].

One of the most used antiseptics in clinical practice is chlorhexidine. This agent is mainly used for skin disinfection and to clean surgical devices such as catheters [11], though it has also been employed in coatings for biomedical devices [12]. In prior work, we noted that immersing a prosthetic material in a low concentration aqueous chlorhexidine solution (0.05% v/v) immediately before its implant significantly reduced bacterial adhesion to the mesh surface [13]. Although the antiseptic was applied in aqueous solution, with the drawback that it rapidly spreads through the host tissue, the results obtained were promising.

Based on these findings, we hypothesize that coating the mesh with a more viscous compound may slow down the drug spreading compared to aqueous solutions, in turn increasing the antiseptic's contact time with the implanted device and surrounding tissues. A bioactive gel-like compound would homogeneously coat the mesh, providing a local and accurate antimicrobial action at the implant site. On this account, in the present study we developed a carboxymethylcellulose gel as the vehicle for the delivery of chlorhexidine. This gel loaded with the antiseptic was used to coat a reticular lightweight polypropylene mesh and its antimicrobial efficacy and biocompatibility were then assessed in an in vitro and in vivo experimental model of prosthetic *Staphylococcus aureus* infection.

Materials and methods

Bacterial inocula

The bacterial strain used was *S. aureus* (Sa) ATCC25923 (Spanish Type Culture Collection; CECT, Valencia, Spain). Bacteria were thawed, plated onto lysogeny broth (LB) agar plates and incubated for 24 h at 37 °C. A colony was then obtained and transferred into 25 mL of LB. Following overnight incubation at 37 °C, the bacterial culture was used to prepare the target inoculum containing 1.25–1.50 × 10^6 CFU/ mL. Sterile 0.9% saline was used to dilute the bacterial suspension. The number of viable bacteria in the inoculum was determined by the spot plaque method.

Antibacterial gel

The polysaccharide carboxymethylcellulose sodium (CMC) was selected for the elaboration of the gel coating, given its wide use in pharmaceutical industry as excipient and drug carrier. To prepare the bioactive antimicrobial gel, a sterile solution of 0.05% v/v chlorhexidine gluconate, CHX (Santa Cruz Biotechnology, TX, USA) in ultrapure water was prepared. This solution was thickened by the gradual addition of 1% w/v CMC (Sigma-Aldrich, St. Louis, MO, USA), with continuous stirring until a homogeneous viscous solution had formed. Besides this antimicrobial gel, a non-antibacterial control gel was prepared as sterile 1% w/v CMC in ultrapure water. The gels were prepared the day before the in vitro/in vivo assays and stored protected from light at 4 °C.

In vitro study

Agar well diffusion test

The efficacy of the antibacterial gel was tested in vitro using an agar well diffusion test. In brief, a 10^6 CFU/mL Sa suspension was used to spread lawns onto 10

LB agar plates. Next, circular wells (8 mm in diameter, 4 mm in depth) were punched in the middle of each plate and subsequently filled with 100 µL of the corresponding control or antimicrobial gel (*n* = 5 each). The plates were incubated for 3 days at 37 °C, and pictures of all the plates were taken every 24 h. Zones of inhibition (ZOI) were recorded by measuring two perpendicular diameters on each plate using ImageJ picture processing and analyzing software (National Health Institute, USA; https ://image j.nih.gov/ij/). Results were expressed as the mean ZOI (mm) per time point.

Cell viability

A cell viability assay by means of the alamarBlue test was conducted to determine whether the vehiculization of CHX in a CMC gel would reduce the cytotoxicity of this antiseptic compared to an aqueous solution containing the same CHX concentration (0.05%). AlamarBlue is a colorimetric reagent commonly used to measure cell proliferation and cytotoxicity following exposure to chemicals or drugs; cells exerting a proliferative status will reduce this reagent, provoking a colorimetric change in the culture medium measurable by spectrophotometry. Rabbit skin fibroblasts were cultured in a controlled humid atmosphere (37 °C, 5% C O_2), as described elsewhere [14]. Cells were plated into six-well plates (2.5×10^5 cells/well) and cultured in low-glucose Dulbecco's modified Eagle medium (DMEM; Life Technologies Corporation, Carlsbad, CA, USA), containing 10% of the following: (1) no treatment, (2) 1% CMC, (3) 0.05% aqueous CHX, or (4) 0.05% CHX vehiculized in a 1% CMC gel (*n* = 6 each). Following a 24-h incubation period, the culture media were replaced by fresh DMEM containing 10% alamarBlue reagent (Bio-Rad Laboratories Inc., Hercules, CA, USA). After 5 h of incubation at 37 °C, several 100 µL aliquots were collected from each well and their absorbance (OD_{570}, OD_{600}) read with an iMark microplate absorbance reader (Bio-Rad Laboratories Inc.). Collected data were analyzed with an online software provided by the manufacturer (https ://www.bio-rad-antibodies.com/colori met ric-calcul ato r- fluor ometr ic-alama rblue .html). Results were expressed as mean percentage cell viability.

In vivo preclinical study

Prosthetic material and study groups

The biomaterial used in this study was Optilene Mesh Elastic, Op (B. Braun, Melsungen, Germany), a reticular lightweight polypropylene mesh designed for the repair of abdominal wall defects. Under sterile conditions, the mesh was cut into 5 × 2 cm pieces. Before implant, randomly selected mesh fragments were coated by immersion in either the CHX-free or CHX-loaded gel for 5 min, while

other mesh fragments were implanted without prior coating to establish the experimental groups:

- Control (*n* = 3): Op meshes without a gel coating.
- Gel (*n* = 6): Op meshes coated with 1% CMC gel.
- CHX-gel (*n* = 6): Op meshes coated with 1% CMC gel loaded with 0.05% CHX.

Experimental animals

Fifteen male New Zealand white rabbits weighing approximately 3000 g were randomly assigned to the three study groups. The study was carried out in strict accordance with the Guide for the Care and Use of Laboratory Animals of the National and European Institutes of Health (Spanish law 32/2007, Spanish Royal Decree 1201/2005, European Directive 2010/63/UE and European Convention of the Council of Europe ETS123). The number of animals included in the study was minimized according to 3R's criteria (replacement, reduction, refinement). The study protocol was approved by the Committee on the Ethics of Animal Experiments of the University of Alcalá, Madrid, Spain. All procedures were performed at the University's Animal Research Center. This center is registered at the Directorate General for Agriculture of the Ministry of Economy and Technology Innovation of the Community of Madrid (ES280050001165), indicating that all its facilities legally cover the needs and requirements of this type of research.

Surgical technique and experimental design

For the in vivo step of this study, partial hernia surgical defects were created in the anterior abdominal wall (right lateral side) of the animals. This partial defect is a validated model of acute hernia with mesh repair in preperitoneal position [15]. In this model, the external oblique fascia together with the external and internal oblique muscles are removed, and the mesh is implanted over the parietal peritoneum. Scarce fibers of the transverse muscle remain on the right longitudinal margin of the defect, without penetrating the defect area. Animals received 0.05 mg/kg buprenorphine (Buprecare; Divasa Farmavic, Spain) 1 h before and 3 days after surgery to minimize pain. Anesthesia was induced via the intramuscular administration of 70 mg/kg ketamine hydrochloride (Ketolar; Parke-Davis, Spain), 1.5 mg/kg diazepam (Valium; Roche, Spain) and 1.5 mg/kg chlorpromazine (Largactil; Rhône-Poulenc, Spain). Using a sterile surgical technique, partial defects (5 × 2 cm) were created and subsequently inoculated with 0.25 mL Sa 10^6 CFU/mL. Defects were repaired with the corresponding mesh, which was secured to the defect margins by a running polypropylene 4/0 suture interrupted only at the implant corners. The skin was then closed over the implants by placing a running polypropylene 3/0 suture. Throughout the study,

the rabbits were weighed daily and visually inspected for signs of surgical incision dehiscence, fistula or seroma formation, surgical site infection and/or other complications. After 14 postoperative days, the animals were sedated with up to 20 mg/kg of xylazine (Rompun; Bayer, Leverkusen, Germany) and then euthanized in a CO_2 chamber with increasing concentrations of CO_2, according to the guidelines for the euthanasia of experimental animals.

Visual inspection and sample collection

Following euthanasia, the tissue reaction at the implant site was visually assessed for signs associated with infection such as skin necrosis/fistula, edema or seroma, purulent exudate, vascularization, and host tissue incorporation. Once inspected, the mesh implants plus surrounding host tissue were retrieved and cut into four sections perpendicular to the longest edge (approximate mesh dimensions in each tissue block 1 × 2 cm) for microbiological, morphological, and immunohistochemical studies.

Efficacy of the antimicrobial gel coating

Through sonication techniques, bacterial adhesion to the mesh surface was quantified according to a procedure described previously [13]. Two tissue blocks corresponding to the lateral and central sides of the implant in each animal were individually transferred to glass tubes containing 20 mL of sterile Neutralizing Pharmacopoeia Diluent (8.5 g NaCl, 2.5 mL Tween-80, 0.35 g lecithin, 997.5 mL distilled water). Under sterile conditions, the mesh was harvested from the tissue and its surface was gently scraped with a scalpel blade to ease bacterial release from the deeper areas of the implant. The tissue, scraped mesh, and scalpel blade were immersed again in the glass tube and subjected to a 10-min sonication pulse at 40 kHz, using a Bransonic 3800-CPXH ultrasonic cleaning bath (Branson Ultrasonics, Connecticut, USA), followed by 1 min of vortexing. Using the supernatant from each tube, five 1/10 serial dilutions in sterile 0.9% saline were prepared and 100 µL of each dilution was seeded onto LB agar plates. Following 24 h of incubation at 37 °C, plates were counted by two blinded observers to determine viable CFU per mesh fragment via the spot plaque method. Apart from this quantification, bacterial clearance was calculated as the percentage of animals in each group showing no bacteria in the lateral and central tissue blocks.

Morphological analyses

The remaining two tissue blocks of each specimen were used for light microscopy (LM) evaluation of the implants in terms of tissue incorporation and biocompatibility. The tissue was fixed in F13 solution (60% ethanol, 20% methanol, 7% polyethylene glycol, and 13% distilled water) and embedded in

paraffin. Tissue sections (5 µm thick) were sliced, stained with hematoxylin–eosin and Masson's trichrome (Goldner-Gabe), and examined under blinded conditions with a Zeiss Axiophot light microscope (Carl Zeiss, Oberkochen, Germany).

Immunohistochemistry

Immunohistochemical techniques were performed to (1) visualize the bacterial adhesion to the implant surface and (2) assess the macrophage reaction to the implants. Both bacteria and macrophages were immunohistochemically detected in paraffin-embedded sections of tissue samples. These sections were incubated with the monoclonal antibodies against Sa (ab37644; Abcam, Cambridge, UK; 1:500) and rabbit macrophages RAM-11 (M-633; Dako, Glostrup, Denmark; 1:50), in the alkaline phosphatase-labeled avidin–biotin method. Nuclei were counterstained for 5 min in acid hematoxylin. The presence of bacteria was visually evaluated. Labeled macrophages were quantified by two blinded observers performing counts in 10 LM fields (magnification 200 ×) per tissue sample. The results were expressed as the percentage of positively stained cells out of the total number of cell nuclei per field.

Statistical analyses

The data collected in the different experiments were compared among the different study groups using the Mann–Whitney *U* test. Data recorded from the in vitro experiments were provided as mean ± standard error of the mean, and those collected with the in vivo preclinical step also included median, minimum, maximum, and interquartile range. All statistical tests were performed using the GraphPad Prism 5 computer package (La Jolla, CA, USA) for Windows. Significance was set at $p < 0.05$.

Results

In vitro study

Efficacy of the CHX-loaded gel

The results of the agar well diffusion test confirmed the antibacterial activity of the CHX-loaded gel. Wide zones of inhibition (ZOI) were observed and these were stable over time, showing a similar size after 24 h (25.34 ± 0.20 mm), 48 h (25.35 ± 0.20 mm) and 72 h (25.19 ± 0.21 mm) of inoculation. The bactericidal activity of CHX was also confirmed, as no colonies grew inside the ZOIs at any of the mentioned time points. As expected, the CHX-free gel showed no ZOI.

Cell viability

Non-treated fibroblasts in the control group and fibroblasts cultured in the presence of the CHX-free gel showed the typical elongated shape and multilayered growth pattern that characterizes this cell type (Fig. 1a, b). On the contrary, fibroblasts treated with 0.05% aqueous CHX displayed a polygonal to stellate morphology, and the cultures contained numerous apoptotic or dead cells (Fig. 1c). However, when treated with the CHX-loaded gel, fibroblasts kept their elongated shape and their culture morphology was similar to that observed in the control group (Fig. 1d). Although apoptotic and dead cells were detected in both the CHXtreated groups, these cells were mainly recorded in the wells containing the aqueous solution. The results of our alamarBlue colorimetric assay (Fig. 1e) indicated the similar viability of the untreated fibroblasts (99.15 ± 1.43%) and the fibroblasts cultured in the presence of the CHX-free gel (89.28 ± 2.37%). Highest cytotoxicity values were recorded for the aqueous CHX treatment (9.85 ± 0.37%), while fibroblasts treated with the CHX-loaded gel showed higher viability rates (67.18 ± 3.10%). Differences in cell viability were significant for the two control groups compared to the CHX-loaded gel ($p < 0.05$) and aqueous CHX ($p < 0.001$), as well as between the CHX-loaded gel and the aqueous CHX treatment ($p < 0.001$).

Table 1 Weight changes produced over time

Weight gain (%)			
	Control	Gel	CHX-gel
Minimum	−7.35	−5.20	2.55
25% percentile	−7.35	−3.35	2.89
Median	−6.90	1.34	5.44
75% percentile	5.87	5.18	7.11
Maximum	5.87	6.27	7.35
Mean	−2.79	0.97	5.13
Standard error of the mean	4.33	1.78	0.82

Data provided represent the median, minimum, maximum, interquartile range, mean and standard error or the mean values, recorded in the different study groups

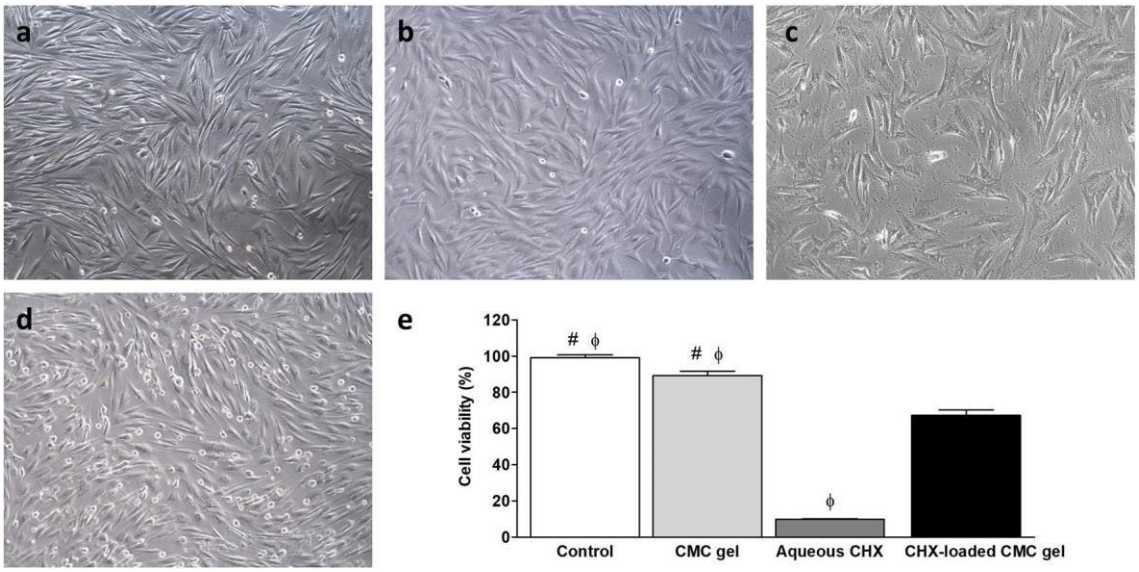

Fig. 1 Viability of cultured fibroblasts following 24 h of exposure tion of 0.05% CHX (× 100). **d** Fibroblasts treated with the CMC gel to the different treatments. **a** Phase contrast LM micrograph of non- loaded with 0.05% CHX (× 100). **e** Statistical assessment of cell viatreated control fibroblasts (magnification × 100). **b** Fibroblasts treated bility (Mann–Whitney *U* test); #: vs. CHX-loaded gel (*$p < 0.05$); ϕ : with the CMC gel (× 100). **c** Fibroblasts treated with an aqueous solu- vs. aqueous CHX (***$p < 0.001$)

Table 2 Bacterial loads recovered after sonication of lateral and central tissue fragments collected from all the implants

Bacterial yields (CFU per mesh fragment)			
	Control	Gel	CHX-gel
Lateral fragments			
Minimum	8.20×10^{3}	0	0
25% percentile	8.20×10^{3}	1.68×10^{4}	0
Median	1.14×10^{6}	1.08×10^{5}	0
75% percentile	2.06×10^{6}	1.01×10^{7}	1.50×10^{2}
Maximum	2.06×10^{6}	2.44×10^{7}	6.00×10^{2}
Mean	1.07×10^{6}	5.00×10^{6}	1.00×10^{2}
Standard error of the mean	5.93×10^{5}	3.98×10^{6}	1.00×10^{2}
Central fragments			
Minimum	6.00×10^{2}	0	0
25% percentile	6.00×10^{2}	1.50×10^{2}	0
Median	3.30×10^{5}	380×10^{4}	0
75% percentile	8.60×10^{5}	5.51×10^{6}	0
Maximum	3.97×10^{5}	1.80×10^{7}	0
Mean	8.60×10^{5}	3.24×10^{6}	0
Standard error of the mean	2.50×10^{5}	2.96×10^{6}	0
Bacterial clearance (%)	0% (0/3)	16.67% (1/6)	83.30% (5/6)

Data provided represent the median, minimum, maximum, interquartile range, mean and standard error or the mean values, recorded in the different study groups. Bacterial clearance was calculated as the percentage of implants in each group returning sterile cultures in both the lateral and central fragments

Macroscopic observations

Following euthanasia, the implant surface plus surrounding tissue were meticulously inspected to assess the response of the meshes to bacterial infection (Fig. 2). Implants in the control group showed a purulent exudate covering most of the suture line and approximately half of the implant surface. Similar observations were made in the Gel group. In these implants various amounts of exudate were observed, and two of the meshes were fully contaminated. Contrary to these observations, the CHX-gel group implants showed a clean surface, and the purulent exudate appeared as small remains restricted to the suture line. All meshes were surrounded by a fibrous capsule, which was thinner for the CHX-gel compared to the control and Gel meshes. Further, vascularization of the neoformed tissue was less intense in the CHX-gel meshes in comparison with the other groups. The implants appeared fully integrated within the host tissue with the exception of two implants: one in the control group and the other in the Gel group. These two implants showed partial tissue incorporation and were the most infected specimens in their respective groups.

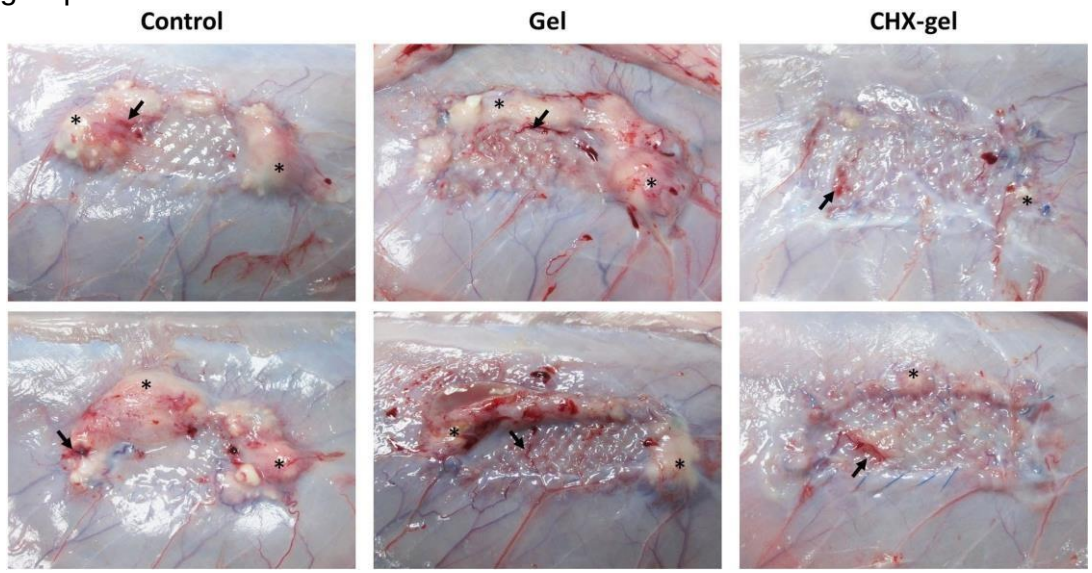

Fig. 2 Macroscopic observations of the different implants follow- gel groups, while in the CHX-gel-coated implants, dispersed exudate ing 14 days of bacterial challenge with *S. aureus*. Two representative remains were restricted to the suture line. In the control and CHXpictures per study group are shown. The build-up of purulent exudate free gel implants (Gel), neovascularization (arrow) was more intense over the implants (asterisk) was remarkable in both the control and throughout the implant compared to the CHX-gel group

Bacterial adhesion to the implant surface

Our assessment of bacterial adhesion to the surface of the different implants revealed the intense antimicrobial activity of the CHX-loaded gel coating. In this study group, null Sa adhesion was recorded in all the central tissue fragments sonicated, although one sample taken from the lateral fragments yielded positive counts. In contrast, both the control and Gel groups returned high bacterial counts. In these groups, all animals displayed bacterial adhesion to the lateral and central tissue fragments, with the exception of one animal in the Gel group for which cultures were sterile. On average, counts recorded for the lateral tissue fragments were higher than those for the central tissues (Table 2). Data arising from the lateral and central tissue fragments were statistically evaluated in an independent fashion. In both cases, bacterial adhesion was quantified as similar in the control group and Gel groups, while counts were significantly higher compared to the CHX-gel group ($p < 0.05$).

Histological findings

Histological findings were consistent with our macroscopic observations. Thus, implants in the control and Gel groups (Figs. 3, 4) appeared integrated within a dense neoformed connective tissue infiltrating the mesh pores, with extracellular matrix fibers surrounding the mesh filaments in a concentric fashion. Within this neoformed tissue, there were numerous purulent deposits and different-sized abscesses that were flanked by barriers of inflammatory cells and contained bacteria, cell debris and detritus. The presence of these structures impaired mesh integration within the host tissue. Contrary to these observations, the CHX-gel group (Fig. 5) displayed a looser connective tissue that infiltrated the mesh pores, fully integrating the mesh into the neoformed tissue. There were no abscesses embedded in this tissue. Immunohistochemical evaluation of the different implants allowed for visualization of live bacteria in areas of the tissue close to abscesses and mesh filaments in both the control and Gel groups, while no bacteria could be observed in the CHX-gel implants.

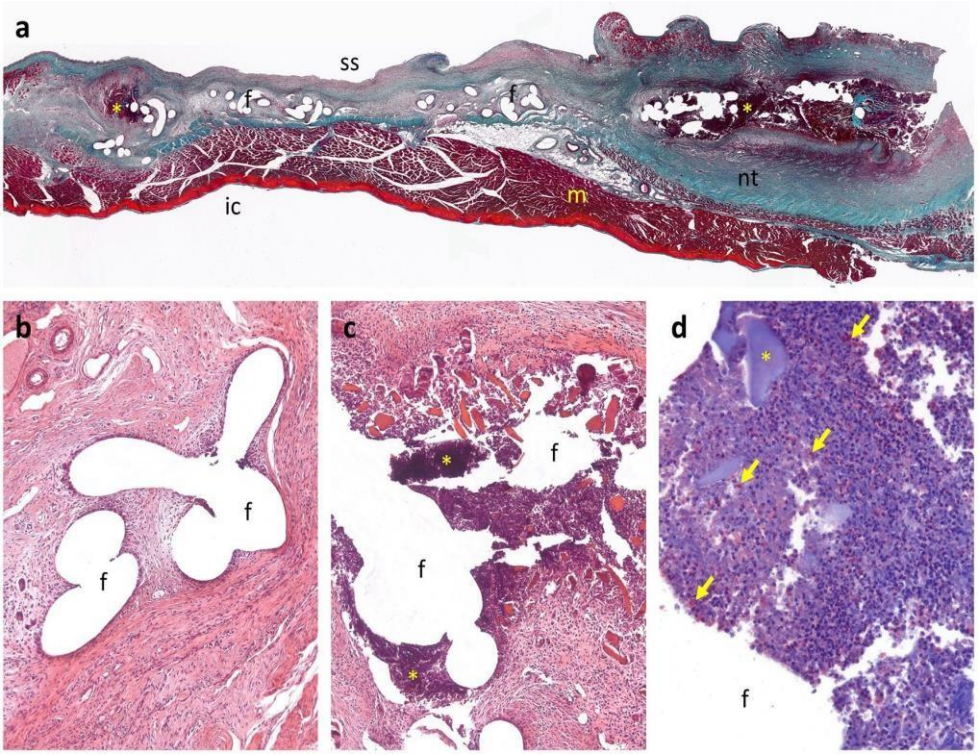

Fig. 3 Host tissue integration of the uncoated control implants. **a** Panoramic composition of the implant showing several abscesses (asterisk) embedded in the neoformed connective tissue (Masson's trichrome, × 50). **b** Detail of a mesh adequately integrated within zones of neoformed host tissue free of abscesses (hematoxylin–eosin, × 100). **c** Disrupted mesh integration in areas close to abscesses (hematoxylin–eosin, × 100). **d** The presence of bacteria (arrow) in the neoformed tissue (Sa immunolabeling, × 320). *f* mesh filaments, *ic* intraperitoneal cavity, *m* muscle, *nt* neoformed tissue, *ss* subcutaneous side

Macrophage reaction

By RAM-11 immunolabeling, we were able to observe the presence of isolated macrophages and multinucleated giant foreign body cells in all the study groups. These cells were found mainly around the Op filaments and in areas of neoformed tissue adjacent to the mesh filaments (Fig. 6a–c). Quantification of RAM-11-positive cells (Fig. 6d; Table 3) revealed a higher macrophage response in the control group (16.06 ± 1.73%), followed by the groups Gel (11.36 ± 1.87%) and CHX-gel (7.64 ± 0.80%). The percentage of RAM-
11-positive cells in the CHX-gel group was significantly lower compared with the untreated control group ($p < 0.05$).

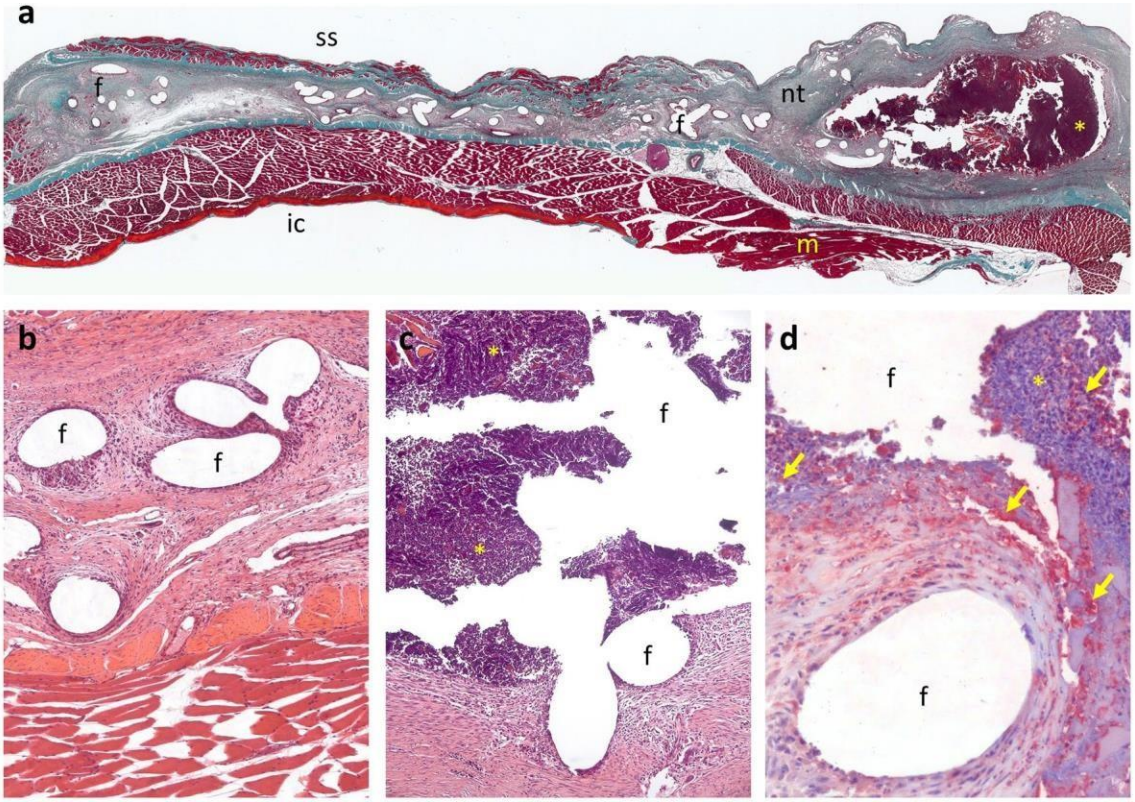

Fig. 4 Host tissue integration of the CHX-free gel-coated implants. Histological findings were similar to observations in the control implants. **a** Panoramic composition (Masson's trichrome, × 50). **b**⁋ **c** Mesh integration was impaired in neoformed tissue zones with abscesses (asterisk) (hematoxylin–eosin, × 100). **d** The presence of bacteria (arrow) surrounding mesh filaments (Sa immunolabeling, × 320). *f* mesh filaments, *ic* intraperitoneal cavity, *m* muscle, *nt* neoformed tissue, *ss* subcutaneous side

Discussion

In hernia repair surgery, the prosthetic material can be prophylactically treated by immersion in an antimicrobial solution before its implant. Typically, the antimicrobial solutions used were antibiotics [8], though recently the use of antiseptics for this purpose has been gaining popularity mainly because of their fewer side effects than antibiotics [16]. During the past few years several antiseptics were used to coat meshes [17, 18], and the efficacy shown by some has enabled their use in the design of biomedical devices of clinical use. CHX is a noteworthy example as it was the antiseptic used in the development of the first hernia repair mesh with antimicrobial properties approved by the *US Food and Drug Administration* (*FDA*) [19].

This antiseptic has a major complication arising from its toxicity. Thus, data exist indicating that CHX exerts some degree of cytotoxicity even when used at

very low concentrations [20], which could limit is applicability. An ideal vehicle for this and other antimicrobials should reduce the toxicity of the agent while preserving its bactericidal activity [21]. In this regard, we hypothesized that the polysaccharide CMC could be an adequate carrier for CHX. This compound has wide applications in the pharmaceutical industry as excipient, because of its great biocompatibility and stability as a drug administration vehicle both by the oral or topical route [22]. Further, anticancer therapy studies have shown that CMC is able to protect host tissue against the toxic effects of the drug it carries [23]. This last finding aligns with our observations when comparing the toxicity of CHX vehiculized in the gel or applied in aqueous solution, suggesting a protective effect of CMC from this undesired CHX toxicity.

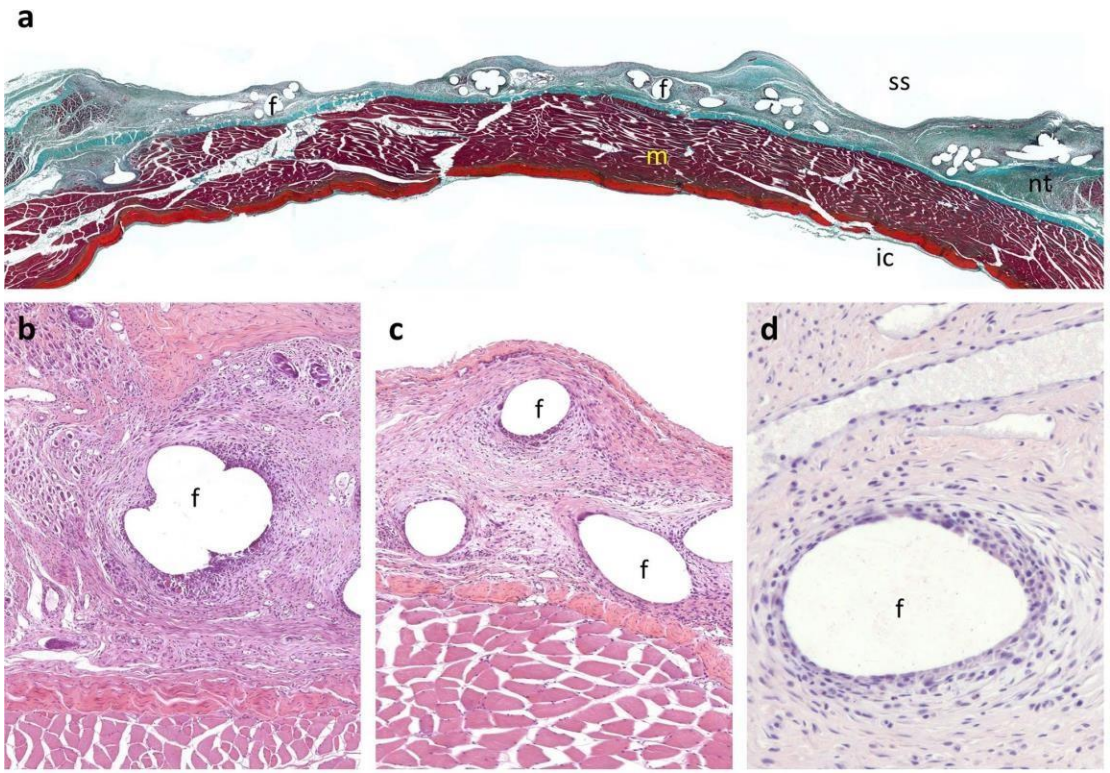

Fig. 5 Host tissue integration of the CHX-gel-coated implants. **a** Panoramic composition of the implant with no abscesses embedded in the neoformed tissue (Masson's trichrome, × 50). **b**⁞ **c** Adequate mesh integration within host tissue throughout the implant (hematoxylin– eosin, × 100). **d** No living bacteria were detected in the neoformed connective tissue (Sa immunolabeling, × 320). *f* mesh filaments, *ic* intraperitoneal cavity, *m* muscle, *nt* neoformed tissue, *ss* subcutaneous side

The lower cell toxicity recorded in vitro correlated with a reduced in vivo macrophage response observed in the CHXgel group meshes, indicating the adequate biocompatibility of the coating. In the absence of antiseptic, the CMC gel also induced a diminished macrophage reaction compared to the control implants. However, as this coating was antimicrobial-free, bacteria were able to colonize the implant thus disrupting the host tissue integration process.

The bacterial clearance achieved by the antiseptic was also high. In effect, of the 12 tissue fragments used to quantify bacterial adhesion to the surface, only one showed positive counts (6.00×10^2 CFU). This load was 4–5 orders of magnitude lower than the mean counts obtained for the control and Gel groups. Despite these significant differences, any bacteria found in this implant site must be considered a risk factor. Indeed, it has been shown that in the presence of a foreign body, a bacterial load of around 10^2 CFU is sufficient to trigger a prosthetic infection [24]. It should be mentioned that the tissue piece in question was a lateral implant fragment and, therefore, had suture material along three of its edges. Studies have shown that sutures at the interface between a mesh and host tissue are a good niche for bacteria promoting infection persistence [25]. Our observations are consistent with this idea, as we noted that the sutured margins were the most susceptible to bacterial infection and should be taken into account when designing and assessing the performance of an antimicrobial coating.

One of the drawbacks of immersing prosthetic meshes in antimicrobial solutions is that usually these are aqueous solutions that will rapidly spread throughout the host tissue determining their reduced efficacy and diminished action time of the agent [19]. The CMC gel used here to vehiculize CHX has a higher density than the conventional aqueous solution used and so the coating procedure was easier. Although not having quantitative data to confirm this hypothesis, our observations regarding the gel consistency and the lack of gel dropping following mesh impregnation lead us to believe that this coating could extend the time interval before the drug is disseminated over the host tissue compared to aqueous solutions. In this sense, the development of a CMC gel as carrier for CHX allowed us to get improved results over those obtained previously [13].

The main limitation of our proposed strategy is that CHX is found completely dissolved in the gel. For this reason, the exact concentration of the agent released over time could not be determined and neither could its release velocity be controlled. A way of resolving this limitation would be to encapsulate the CHX in a releasing particle before its incorporation in the CMC gel. This would allow for the more controlled release of the antiseptic and so optimize its efficacy, as Liu et al. recently demonstrated with the development of an antimicrobial hydrogel carrying nanostructured silver sulfadiazine suspensions [26].

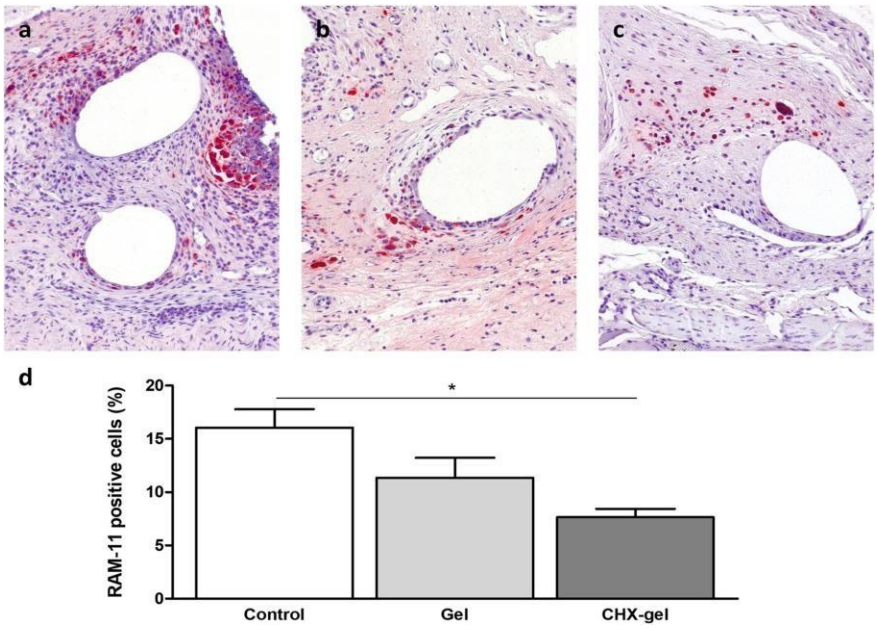

Fig. 6 Macrophage reaction. RAM-11 immunostaining (× 200) of the **a** control, **b** Gel, and **c** CHX-gel-coated implants revealing the presence and distribution of labeled macrophages (arrow) throughout the neoformed tissue. **d** Statistical assessment of RAM-11-positive cells quantified in the different study groups (Mann–Whitney *U* test). The CHX-gel-coated implants showed significantly lower numbers of macrophages compared to the control group (*$p < 0.05$)

Table 3 Quantification of the macrophage reaction observed following 14 days of the bacterial inoculation, determined as the percentage of RAM-11-positive cells

RAM-11-positive cells (%)			
	Control	Gel	CHX-gel
Minimum	13.31	5.71	4.26
25% percentile	13.31	6.03	6.18
Median	15.64	12.35	8.15
75% percentile	19.24	15.29	8.78
Maximum	19.24	16.86	10.11
Mean	16.06	11.36	7.64
Standard error of the mean	1.73	1.87	0.80

Data provided represent the median, minimum, maximum, interquartile range, mean and standard error or the mean values, recorded in the different study groups

In summary, the results shown here are highly promising and easily translatable to clinical practice, although it needs to be emphasized that greater sample sizes would be necessary to get stronger outcomes. The use of a CMC gel loaded with CHX as mesh coating suggests applications for this antimicrobial gel as a prophylactic tool to avoid infection following hernia repair surgery. Further studies comprising larger number of animals, other antimicrobials, and meshes with different architecture or chemical composition, will provide valuable data to ascertain the future implementation of bioactive CMC-based gels in human clinics. The overall conclusions of this study are:

- The use of CMC as an administration vehicle reduces the toxicity of the antiseptic CHX and helps its application.
- The prophylactic coating of a hernia repair mesh with an antimicrobial gel containing CHX avoids bacterial adhesion to the mesh surface, providing local and adequate bactericidal action in the surgical field.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Ethical approval was agreed by the Ethical Committee of the Institution.

Human and animal rights This aticle does not contain any studies with human participants. The study involving animals has been performed in accordance with the ethical standards of the Declaration of Helsinki and its later amendments.

Informed consent No human patients were included in this study.

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