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# Antibacterial protection of suture material by chlorhexidine-functionalized polyelectrolyte multilayer films

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**Abstract** The formation of bacterial biofilms on the surface of implanted materials is a critical factor that may lead to chronic microbial infection and tissue necrosis. In the present study we analysed the stability of polyelectrolyte multilayer (ML) films on suture materials and the antibacterial effect obtained with chlorhexidine (CHX)-functionalized films built on different types of suture materials such as silk, polyester and copolymer of glycolide and L-lactide. The comparison of *Escherichia coli* culture on glass coverslips and glass coverslips with ML and CHX showed at 24 h an inhibition of the bacterial relative luminescence (40.68%, $P < 0.5$) and at 48 h (99.46%, $P < 0.001$). In another way, simple soaking of suture material overnight in CHX digluconate 20% without polyelectrolyte films did not at all protect sutures from bacterial colonization but CHX-functionalized polyelectrolyte films, made from poly-L-glutamic acid and poly-L-lysine, inhibited *Escherichia coli* proliferation.

## 1 Introduction

Bacterial species are capable of colonizing different surfaces and proliferating on them, forming adherent biofilms [1, 2]. This could represent a major problem for implanted materials such as sutures, dental implants, and catheters [3–8]. Experimental and clinical data indicate that most wound infections begin around material left within the wound, and that the incidence of late suture complications such as local abscesses is directly related to the degree of contamination at the time of material placement [9–16].

Different strategies to circumvent this problem have been developed, including surface modifications of materials by simple coating with antibacterial agents [17, 18]. As an alternative, a new surface coating method, the layer-by-layer (LBL) self-assembly technique, is proposed here to confer antibacterial properties to suture materials. This technique, based on sequential adsorption on a substrate, of oppositely charged compounds such as synthetic polyelectrolytes, biopolymers or inorganic particles represents one of the most well-established methods for constructing thin biofunctional films [19–22]. Functionalization of these films by inserting charged active molecules (like growth factors, proteins [23–29] or DNA [30–32]) during the film build-up has been developed for different biological applications, i.e.: biosensor fields [33, 34], cell signaling control [35, 36], anti-coagulant coatings [37] and anti-adhesive surfaces [38].

Chlorhexidine (CHX) has been successfully used for many years in the local treatment of periodontal diseases and in oral surgery due to its antiseptic activity on oral bacterial flora [39–41].

Our assumption was that the incorporation of CHX in polyelectrolyte films could result in antibacterial effects, thus minimizing inflammatory complications induced by suture materials. In this study, we functionalized LBL films with CHX digluconate 20% (w/v) built on the surface of different suture materials such as silk, polyester and a copolymer of glycolide and L-lactide. The LBL coatings were made from homopolypeptides, namely poly-L-glutamic acid (PGA) and poly-L-lysine (PLL), and CHX was

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adsorbed on top of PGA-ending architectures. The coatings were analyzed in terms of stability and antibacterial effect.

## 2 Materials and methods

### 2.1 Polyelectrolyte film preparation

Poly(ethylenimine) (PEI, average molecular mass: 750,000 g $mol^{-1}$, P3143 lot number: 093K0098), poly(sodium4-styrenesulfonate) (PSS, molecular mass: 70,000 g $mol^{-1}$), poly(allylamine hydrochloride) (PAH, molecular mass: 70,000 g $mol^{-1}$), poly(L-glutamic acid) (PGA, molecular mass determined by viscosimetry: 54,800 g $mol^{-1}$), and poly(L-lysine) (PLL, molecular mass determined by viscosimetry, 23,400 g $mol^{-1}$) were purchased from Sigma (St. Quentin Fallavier, France) and were used as received to build up layer-by-layer (LBLs) polyelectrolyte films. PEI, PSS, and PAH solutions were prepared at a concentration of 5 mg $ml^{-1}$ and used to build precursor films on the top of which PGA and PLL layers were further deposited. Solutions of PGA and PLL were prepared at a concentration of 1 mg $ml^{-1}$.

$PLL^{-FITC}$ (molecular mass, 23,400 g $mol^{-1}$, Sigma) was used to check if the build-up of the polyelectrolyte ML films indeed occurs at the surface of the investigated suture materials. All the polyelectrolytes were dissolved in a 0.15 M NaCl solution without any additional pH adjustment. All solutions were prepared from Milli Q water ($\rho = 18.2$ MΩ cm). The pH of the PSS, PAH, PGA and PLL solutions was comprised between 5 and 6 whereas the PEI solution used to adsorb a precursor layer on the surface of the suture materials, on the glass cover slips or on the ZnSe crystal had a pH close to 10.

Chlorhexidine digluconate at 20% (w/v) was used directly from the commercial solution (Pierre Fabre Médicament, Parc Industriel de la Chartreuse, Castres, France), which contained also different excipients like ethanol, glycerol, levomenthol and E124. CHX has a positive charge at pH 5–6, pH at which our experiments were performed, and was hence adsorbed on a negatively charged layer i.e. PGA during the film build-up.

Polyelectrolyte ML films were deposited on two types of substrates:

i. glass coverslips, 14 mm in diameter (VWR International, Strasbourg, France), were pre-treated with 10 mM sodium dodecyl sulfate (SDS) followed by 0.12N HCl for 15 min at 100°C in 24 wells plates (Nunc, VWR) and water-rinsed three times after treatment. Three hundred microliters of each polyelectrolyte solution has been deposited alternately on coverslips during 10 min, each deposition being followed by three rinses (of 5 min each) with 0.15 M NaCl.

ii. suture materials were pre-treated with a solution of ethanol–KOH 1N (50%/50%) for 30 min, followed by three rinses with a 0.15 M NaCl solution and dipped in the polyelectrolyte solutions.

Three different types of suture materials were used:

- non-absorbable natural surgical suture (Silkam®) made from braided or twisted silk fibrils (polypeptide chains) dyed black with sulfur Black 1 C.I. 53185 or hematein (Logwood-21 CFR73.1410) and coated with pure beeswax or refined paraffin wax (Aesculap AG & Co. KG D-78532 Tuttlingen, Switzerland);
- non-absorbable synthetic surgical suture (Ethibond® excel) composed of polyester (ethylene terephthalate) and coated with polybutylate (Ethicon TSA 81002, 92787 Issy les Moulineaux, France);
- absorbable synthetic surgical suture (Vicryl® rapide) composed of a copolymer made from 90% glycolide and 10% L-lactide (polyglactin 910) and coated with a copolymer composed of 90% caprolactone and 10% glycolide followed by a mixture composed of equal parts of copolymer of glycolide and L-lactide (polyglactin 370) and calcium stearate (Ethicon TSA 81002, 92787 Issy les Moulineaux, France).

The basic architecture of the films built on suture material and glass coverslips was PEI-$(PSS\text{-}PAH)_2$-$(PGA\text{-}PLL)_3$. The (PSS-PAH) part acted as an anchoring film whereas the PGA-PLL part was considered as the bioactive part of the architecture. In the physicochemical conditions of the build up (0.15 M, NaCl, pH 6) these films have a thickness around 40 nm as measured by optical waveguide lightmode spectroscopy [24].

The CHX-functionalized architecture was: PEI-$(PSS\text{-}PAH)_2$-$(PGA\text{-}PLL)_2$-PGA-CHX.

### 2.2 Infrared spectroscopy in the attenuated total reflexion mode

Films were deposited on a trapezoidal ZnSe crystal by flowing each polyelectrolyte solution as well as the rinsing NaCl solution during 10 min over the crystal surface by means of a peristaltic pump.

For these experiments the polyelectrolytes were dissolved in 0.15 M NaCl solutions prepared from $D_2O$ instead of $H_2O$ in order to be able to measure the amide I band characteristic of the PGA-PLL films. The crystal was mounted in a 500 µl flow cell which was made tight. The Infrared spectra were acquired by averaging 512 single beam spectra in the attenuated totally reflected mode (ATR) at a resolution of 2 $cm^{-1}$ with an Equinox 55 spectrometer (Bruker, Wissembourg, France) using a liquid nitrogen-cooled mercury cadmium telluride detector.

The obtained spectra were displayed in the form of difference spectra between the spectrum of a given architecture on the surface and the spectrum corresponding to the PEI layer.

### 2.3 Stability of LBL films on suture materials

The mechanic resistance of films built with $PLL^{-FITC}$ on suture materials was tested by crossing rat mucous membrane and forceps according to the procedure described by Bariol et al. [42] and observed by fluorescence microscopy.

### 2.4 Bacterial seeding

*Escherichia coli* D22 cells were transformed by electroporation with a plasmid bearing green fluorescent protein (GFP), gene *mut3*, under the control of the *Salmonella enterica* serovar Typhimurium ribosomal protein promoter. The transformed bacteria expressing GFP were then grown aerobically in Luria-Bertani broth (LB) medium at 37°C and selected with ampicillin (1 µl/ml of LB). GFP-expressing bacteria were harvested in mid exponential phase, diluted to an optical density of 0.01 at a wavelength of 600 nm in LB and plated on the coatings built on suture materials or glass coverslips. After 24 and 48 h of incubation under agitation at 40 rpm at 37°C, the bacterial growth was measured with a homogeneous single reagent method (BacTiter-Glo™ reagent). Experiments were done in 24-well plates (Nunc), with 1,500 µl of bacterial growth medium deposited in each well. The number of bacterial cells approximately deposited was about $10^6$ ($10^6$ bacteria in 1,500 µl LB). To avoid the uncertainties in the results due to the high level of evaporation from the wells, peripheral wells were always filled with 1,500 µl of LB medium (they also served as controls for contamination) and tubs of water were placed on the device.

After the time according to the experiments, suture materials were rinsed three times with PBS and the bacteria adsorbed on sutures materials were visualized using an inverted fluorescence microscope (Nikon, Eclipse TE200) and photographed using a digital camera (DXM-1200, Nikon).

### 2.5 Bacterial viability assay

A homogeneous single reagent method was used for determining the number of viable bacterial cells in culture, based on quantification of the produced ATP which is an indicator of metabolically active cells.

This procedure involves adding a single reagent (BacTiter-Glo™ Reagent) directly to bacterial cells cultures and measuring relative luminescence.

The system uses a proprietary thermostable luciferase to enable extraction of ATP from bacterial cells and to support a stable luminescent signal. The luminescent signal is proportional to the amount of ATP present, which is directly proportional to the number of cells present in the culture medium. These experiments were done with films built up on glass coverslips. All steps of this protocol were performed at room temperature (22–25°C). A volume V of BacTiter-Glo™ reagent was added to an equal volume V of cell culture medium. Three kinds of coverslips were used: unmodified glass, glass covered with an unmodified LBL film (glass and ML) and glass with LBL film functionalized with CHX digluconate.

## 3 Results

### 3.1 Proof of concept: possibility to confer antibacterial activity to solid substrates by covering them with CHX impregnated polyelectrolyte ML films

#### *3.1.1 Buildup of two dimensional functionalized films*

The peak labeled 1 corresponds to the ionized carboxylic acid groups of PGA (1,580 $cm^{-1}$) whereas the peaks labeled 2 (1,035 $cm^{-1}$) and 3 (1,007 $cm^{-1}$) correspond to the sulfonate groups of PSS. The peaks labeled 4 and 5

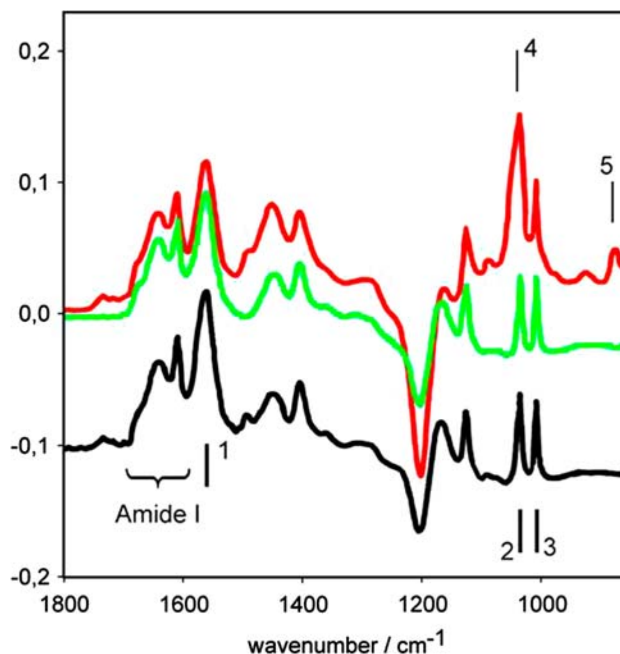


**Fig. 1** Infra red spectra obtained in the ATR mode of a PEI-(PSS-PAH)$_2$-(PGA-PLL)$_2$-PGA LBL; film (Black), of the same film after adsorption of CHX digluconate solution and rinse with NaCl solution (Red) and after application of an additional PGA layer (Green). This last spectrum has been downshifted for the convenience of the representation

correspond to the molecules present in the CHX digluconate solution.

The adsorption of CHX on PGA-ending films was followed by means of infra red spectroscopy in the attenuated total reflexion mode [43]. The Infrared difference spectra clearly show that molecules present in the solution of CHX digluconate 20% do adsorb on the PEI-(PSS-PAH)$_2$-(PGA-PLL)$_2$-PGA ML and are still present after rinsing the surface with a solution of 0.15 M NaCl (Fig. 1). However these molecules are quantitatively desorbed from the surface when it is put again in contact with a PGA containing solution. We have selected polyelectrolyte MLs ending with negatively charged PGA owing to the positive charge carried by CHX in order to increase the film load in the active molecule.

This suggests that the CHX molecules present in the solution of CHX digluconate 20% interact with the deprotonated carboxylic groups of PGA (seen at around 1,580 cm$^{-1}$ in the ATR spectra, peak 1 in Fig. 1) which allows their adsorption on a PGA terminating layer. However the charge of the CHX molecules is too small to allow these molecules to be embedded between two PGA layers. The spectre of deprotonated carboxylic groups of PGA not change during the interactions with CHX. This spectre disappears only during the H$^+$ binding with these groups. Indeed, these binding would have entailed the loss of the negative charges and consequently the binding of the CHX molecules.

### 3.1.2 *Antibacterial activity of CHX-two dimensional functionalized films*

Figure 2a shows the luminescence signal of time *Escherichia coli,* as measured with the bacterial viability assay, obtained 24 and 48 h after bacterial seeding on unmodified glass or coverslips, glass and ML (covered with a PEI-(PSS-PAH)$_2$-(PGA-PLL)$_2$-PGA film), and glass and ML and CHX digluconate 20%. At 24 h, luminescence levels were significantly different ($P < 0.5$) on glass and ML and CHX in comparison with coverslips. This difference increased at 48 h since no detectable luminescence were observed on glass and ML and CHX. Antibacterial activity was only effective with CHX-functionalized coverslips, making the demonstration of the validity of the approach. In another way, Fig. 2b shows *Escherichia coli* growth inhibition with CHX. This inhibition at 24 h is 45% and 100% at 48 h in the case of glass and ML and CHX. Having shown the possibility to impregnate PEI-(PSS-PAH)$_2$-(PGA-PLL)$_2$-PGA polyelectrolyte ML films with CHX and the efficiency of this functionalized coating as antibacterial coatings, we now wish to demonstrate that such architectures can be deposited and remain functional on different kinds of suture materials.

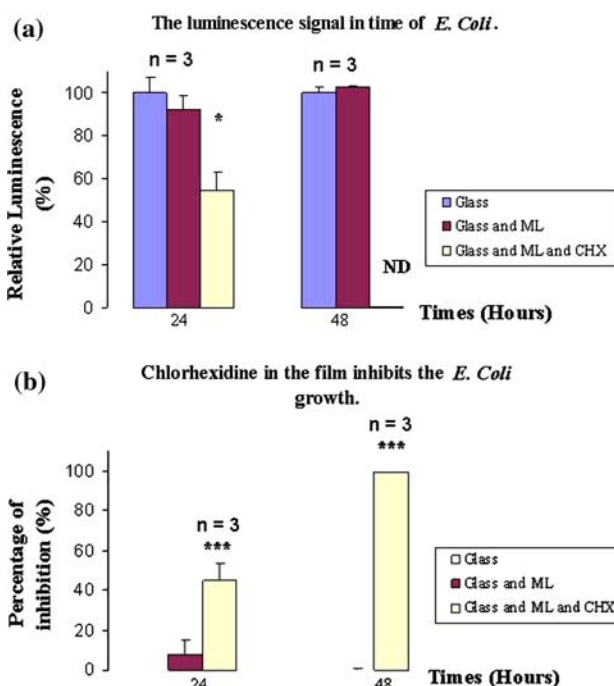


**Fig. 2** (**a**) Relative luminescence signal 24 and 48 h after bacterial seeding on glass (purple histogram), glass and ML (brown histogram) and glass and ML and CHX (yellow histogram). All the data of luminescent signal are normalized with regard to the data of the luminescent signal on the glass. Signals represent the mean of three replicates for each measurement ($n = 3$), * = ($P < 0.5$), *** = ($P < 0.001$). (**b**) Chlorhexidine in the film inhibits the *Escherichia coli* growth. All the values are normalized with regard to the values of the culture on the glass. The values of the culture of *Escherichia coli* on the glass represent 0% of inhibition. The percentage of inhibition at time $t = 24$ h is 8% on glass and ML (brown histogram) and 45% on glass and ML and CHX (yellow histogram). At time $t = 48$ h, 100% of growth inhibition of *Escherichia coli* was observed on glass and ML and CHX. Signals represent the mean of three replicates for each measurement ($n = 3$), *** = ($P < 0.001$)

## 3.2 Functionalization of suture materials

### 3.2.1 *Buildup and stability of LBL films on suture materials*

PEI-(PSS-PAH)$_2$-(PGA-PLL)$_2$-PGA-films containing PLL$^{-FITC}$ were built up on suture materials as described in materials and methods section. Figure 3 shows that all the suture materials tested constitute excellent supports for LBL films adhesion, and that established coatings resist after crossing mucous membrane and after crossing forceps.

### 3.2.2 *CHX-functionalized coatings effect on bacterial cultures*

The different suture materials were modified with PEI-(PSS-PAH)$_2$-(PGA-PLL)$_2$-PGA coatings either without or

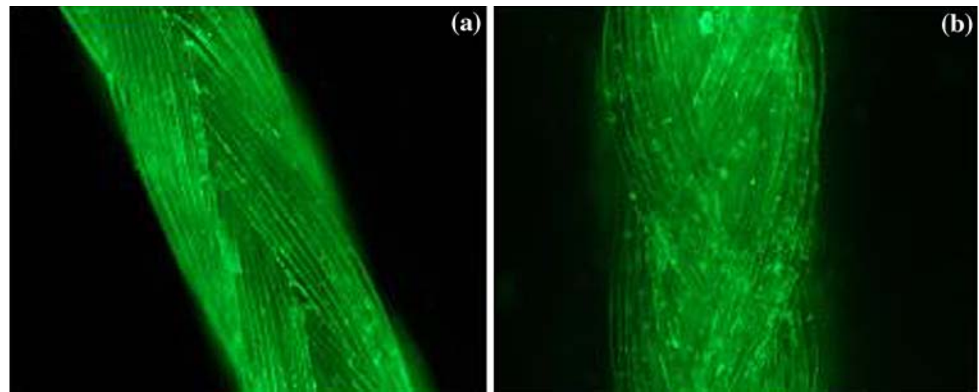


**Fig. 3** Qualitative evaluation of the mechanical resistance of polyelectrolyte ML films deposited on: (**a**) LBL film-covered polyester suture after crossing mucous membrane; (**b**) LBL film-covered polyester suture after crossing forceps

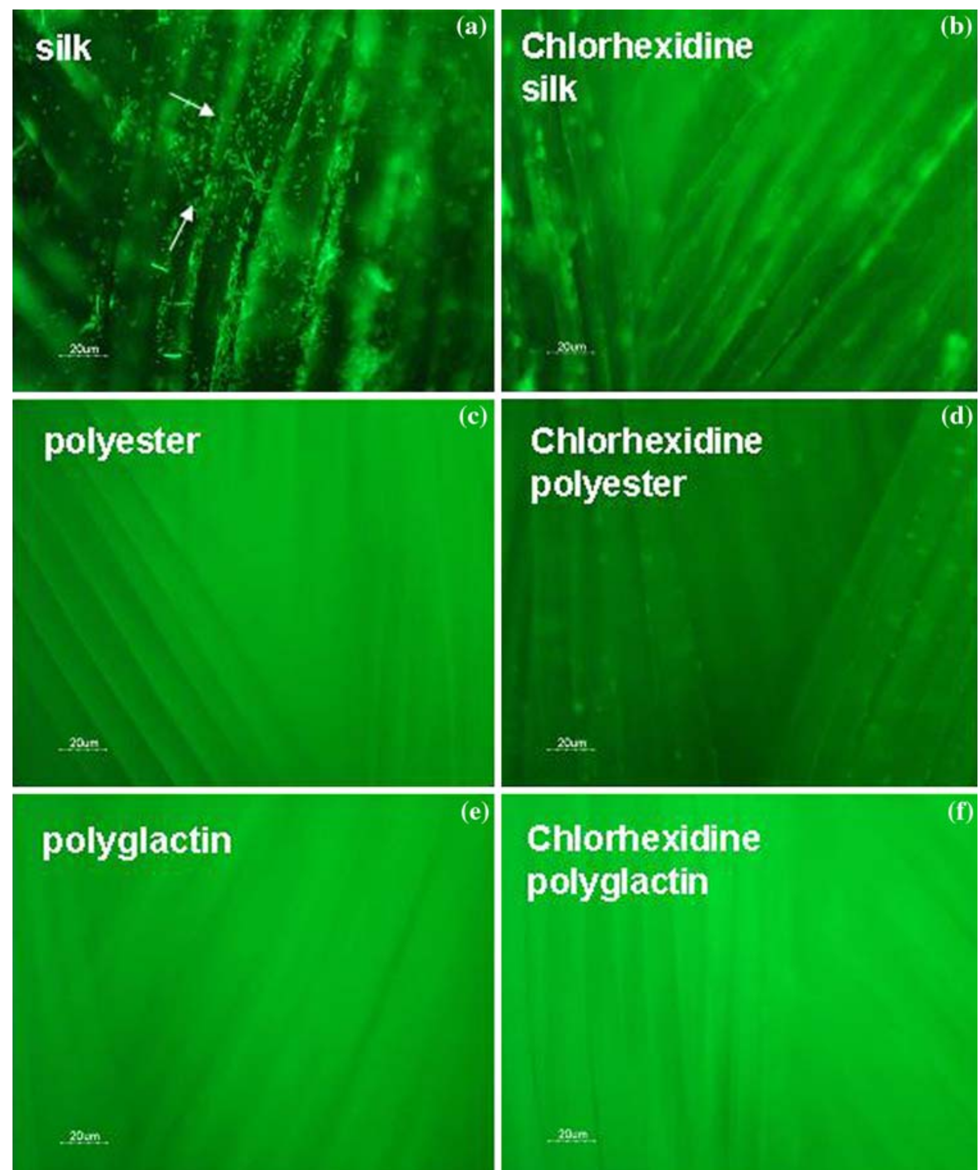


**Fig. 4** Antibacterial effect of CHX incorporated into PEI-$(PSS\text{-}PAH)_2$-$(PGA\text{-}PLL)_2$-PGA polyelectrolyte ML films, 24 h after bacterial seeding. In the absence of CHX, significant bacterial colonization on (**a**) silk suture, and no bacterial colonization neither on (**c**) polyester nor on (**e**) polyglactin. No bacterial proliferation in the presence of CHX on (**b**) silk suture, on (**d**) polyester and on (**f**) polyglactin. Pictures represent the similar observations of one of three experiments which were made

with CHX. In this latter case, after 24 hours, no bacterial proliferation was observed either on the silk, polyester or polyglactin suture (Fig. 4). Moreover, 7 days after bacterial seeding of the bacteria, one observed that CHX-coatings remain effective on every suture material (Fig. 5). In the control experiments, in the absence of CHX, the silk suture was abundantly colonized after 24 h whereas polyester and polyglactin suture were not. However, after 7 days, in the

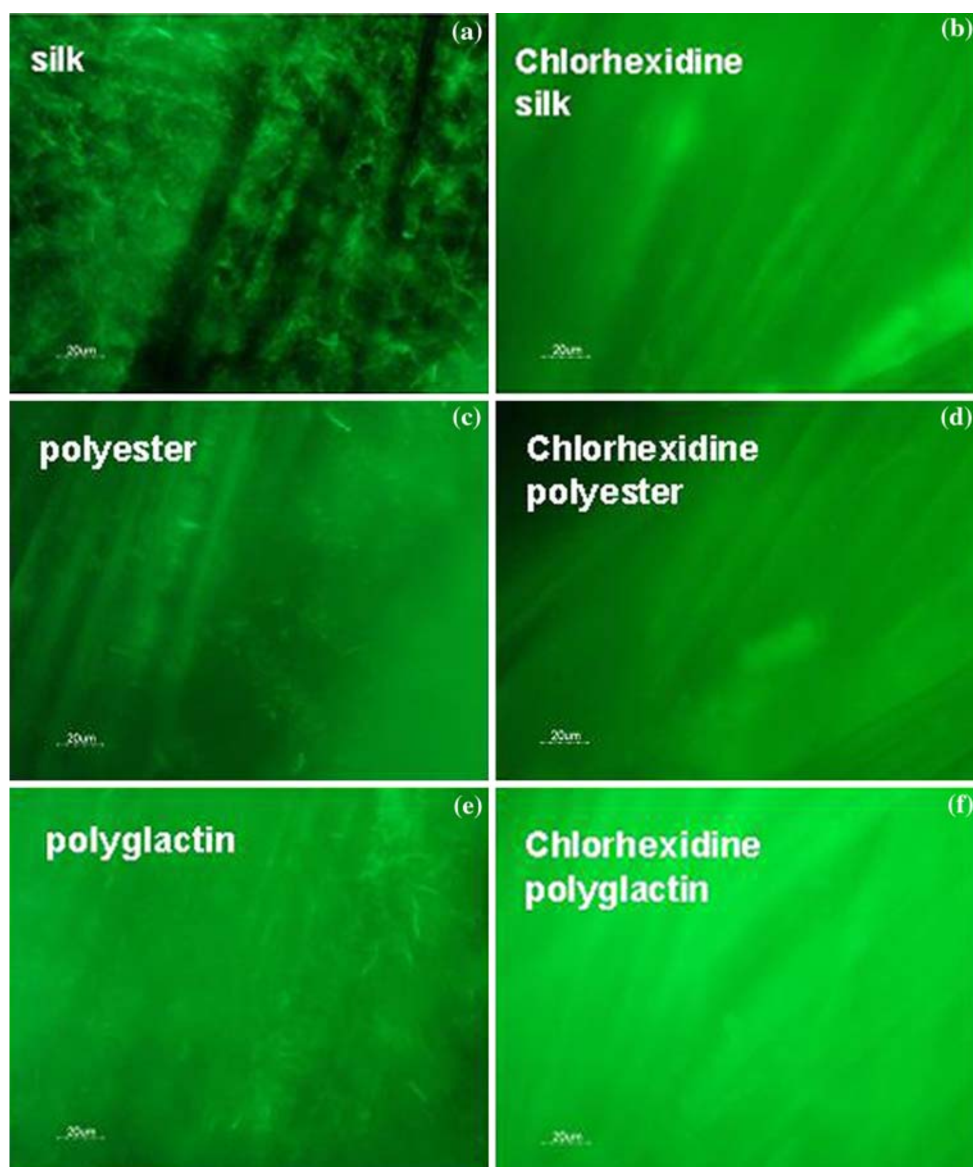


**Fig. 5** Antibacterial effect of CHX incorporated into polyelectrolyte films, 7 days after bacterial seeding: (**a**, **c**, **e**) bacterial colonization without CHX on silk suture (**a**), on polyester (**c**) and on polyglactin (**e**); no bacterial proliferation in the presence of CHX adsorbed on PEI-(PSS-PAH)$_2$-(PGA-PLL)$_2$-PGA polyelectrolyte films on every type of material suture (**b**, **d**, **f**). Pictures represent the similar observations of one of three experiments which were made

absence of CHX, the three types of sutures are equally colonized.

The luminescent signal is proportional to the amount of ATP, which is directly proportional to the number of cells present in the culture medium. At 24 h, luminescence signal in the media containing these suture materials were not detected showing that if bacteria were present in the culture medium, their number were not enough to produce the amount of ATP detectable with a homogeneous single reagent method. These observations were the same in the medium culture at day 7 where bacteria did not adhere on suture materials.

## 4 Discussion

The probability of postoperative surgical site infection is influenced by selected risk factors present at the time of the operation. Particularly, the role of suture material as a medium for wound contamination and infection due to the affinity of bacterial species for the material has been brought into question.

As a consequence, surgical site infections can be associated with severe morbidity, especially in high-risk patients. Measures for reducing the risk of surgical site infection include surgical technique, appropriate antimicrobial prophylaxis, and adjunctive strategies for reducing wound contamination and promoting wound healing. In this study, the susceptibility of suture materials, especially silk, being colonized with *Escherichia coli* has been confirmed in vitro.

This phenomenon was previously described by Akiyama et al. [6] who used *Staphylococcus aureus* to contaminate wounds previously sealed with silk. The authors observed the presence of glycocalyx around the bacteria after 1 h,

and glycocalyx adhering to the sutures 3 h after contamination. We show here that *Escherichia coli* colonization is less extensive on polyglactin 910 suture than on silk suture. Indeed, the microbial colonization seems to be highly variable, depending on the specific microbial species on the suture structure, and on the chemical composition of the device [44, 45].

Particularly, suture structure composition and tissue reactivity influence infectivity, with braided suture material being more vulnerable to microbial contamination than monofilament devices [46, 47]. One advantage of non-absorbable suture materials is that they elicit little tissue reaction [38]. Silk suture is a non-absorbable material. Unfortunately, the braided nature of silk suture allows surface debris and bacterial accumulation, resulting in inflammation of the area surrounding the wound [39]. Nylon suture, another non-absorbable material, is less prone to harbour bacteria or to cause wound reaction because of its monofilament strand with no interstices [14, 39]. However, as with all non-absorbable materials, nylon suture must also be manually removed. Sutures can be used in the re-approximation of tissues separated by surgical or accidental trauma, the promotion of primary healing, or the control of haemorrhage [40]. Retarded healing may result from inflammatory reactions caused by the suture materials themselves.

Therefore, tissue reaction is one of the crucial factors in choosing the best material for the task at hand from a wide variety of suture materials. In this study we hypothesized that the risk of infection would be decreased by creating an antibacterial environment within and immediately adjacent to the suture infrastructure. Indeed, if the surgical suture is implicated as the cause of a wound infection, then an antibacterial coating should nearly eliminate the possibility of the suture material becoming a vector of infection.

In this strategy, the suture material becomes an active part of the wound aseptic process. We have shown in this study that antibacterial molecules can be associated to suture material by modifying their surface with polyelectrolyte ML films which are reservoirs for the active molecules, CHX digluconate from a 20% (v/v) solution. CHX digluconate is a chemical antiseptic that combats both Gram-positive and Gram-negative microbes, and is both bacteriostatic and bacteriocidal. It was previously shown that LBL films could be functionalized with bioactive molecules [23–32]. Here, the role of these films is very important since simple soaking of suture material overnight in CHX digluconate 20% (v/v) solution without polyelectrolyte films did not protect sutures from bacterial colonization. After 24 h however, the observation of suture material with CHX-functionalized films clearly shows that no bacterial proliferation occurred either on the silk, polyester or polyglactin 910 sutures. Similar results were reported in a recent study [17] which shows the efficacy of a triclosan-coating on polyglactin 910 suture. The authors observed, in vitro, a reduction in Gram-positive and Gram-negative bacterial adherence to the triclosan-coated braided suture. They concluded that suture treated with triclosan provides an effective strategy for reducing per-operative surgical morbidity. Other studies [18] have shown, in vivo, the effectiveness of this approach against Gram-positive species and bacteriostatic effect against *Escherichia coli*.

## 5 Conclusion

In the present study, we show the effectiveness in vitro of a new type of suture material surface functionalization. The same strategy could be applied to other medical objectives, including the modification of catheter and surgical device surfaces. Nevertheless, it is now important to quantify the amount of adsorbed CHX and to rate its availability. Indeed, the advantage in polyelectrolyte LBL films is seen in their ability to allow the loading of active molecules at different levels of the architecture in order to increase their amount, and the duration of the desired biological response.

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