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RESEARCH PAPER

# Drug-Free Chitosan Coated Poly(isobutylcyanoacrylate) Nanoparticles Are Active Against *Trichomonas vaginalis* and Non-Toxic Towards Pig Vaginal Mucosa

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## ABSTRACT

***Purpose*** The present work reports a non-conventional therapeutic strategy based on the use of vaginally-applied formulations for the treatment of trichomoniasis due to *Trichomonas vaginalis* without adding a drug.

***Methods*** The formulations were based on a thermosensitive pluronic® F127 hydrogel containing mucoadhesive poly(isobutylcyanoacrylate) nanoparticles coated with a mixture of chitosan and thiolated chitosan (75/25 wt%). The nanoparticles were obtained by anionic emulsion polymerization of isobutylcyanoacrylate. The anti-*T. vaginalis* activity of the formulations was evaluated *in vitro*.

***Results*** Chitosan-coated nanoparticles showed a strong anti-*T. vaginalis* activity at 100 μg/mL independently on the proportion of thiolated chitosan. No anti-*T. vaginalis* activity was reported neither with chitosan-uncoated poly(isobutylcyanoacrylate) nanoparticles nor with chitosan used as a solution. These results suggest that the anti-*T. vaginalis* activity was related to poly(isobutylcyanoacrylate) nanoparticles but only when they are coated with chitosan. Histological analysis of *ex vivo* pig vaginal mucosa in contact with pluronic® F127 hydrogel containing poly(isobutylcyanoacrylate) nanoparticles coated with the mixture chitosan/thiolated chitosan (75/25 wt%) did not reveal any toxicity.

***Conclusion*** This study demonstrated that poly(isobutylcyanoacrylate) nanoparticles coated with chitosan were active against *T. vaginalis* without adding a drug. Besides their anti-*T. vaginalis* activity, the formulations are non-toxic towards pig vaginal mucosa.

**KEY WORDS** histotoxicity · nanoparticles · poly(isobutylcyanoacrylate) · thermosensitive hydrogel · *Trichomonas vaginalis*

## ABBREVIATIONS

| | |
|---|---|
| HIV | Human immunodeficiency virus |
| IBCA | Isobutylcyanoacrylate |
| MTZ | Metronidazole |
| Np | Nanoparticle |
| PBS | Phosphate buffer saline |
| PIBCA | Poly(isobutylcyanoacrylate) |
| SVF | Simulated vaginal fluid |
| TBA | Thiol-butylamidine |
| TYM | Trypticase-yeast extract-maltose |

## INTRODUCTION

*Trichomonas vaginalis* is a protista parasite responsible for the urogenital trichomoniasis, one of the most common sexually transmitted disease in the world. According to the last estimations of the WHO, approximately 153 million people are infected worldwide yearly by this parasite. Urogenital trichomoniasis has important medical, social and economical implications. Reports have implicated *T. vaginalis* in the upper reproductive tract post-surgical infections, reversible infertility, premature rupture of the placental membranes, premature labor, low-birth-weight infants and neonatal morbidity and mortality (1–3). *T. vaginalis* causes discomfort and psychosocial distress in infected patients. The symptoms include a

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discharge that is usually copious and frothy, resulting in local pain and irritation. Pruritus might be also present. Symptoms often peak just after menses. As demonstrated with other sexually transmitted diseases characterized by vaginal inflammation, there is an increased rate of the Human immunodeficiency virus (HIV) acquisition associated with *T. vaginalis* (4). Certain factors such as poor personal hygiene and low socioeconomic status are associated with high incidence of the infection.

Conventional treatment of urogenital trichomoniasis relies on metronidazole (MTZ), a 5-nitroimidazole drug derived from the antibiotic azomycin. Oral delivery of MTZ is usually efficient in eliminating *T. vaginalis* parasite. However, the systemic absorption of such molecule represents a risk of allergy and favours the occurrence of drug resistance (5). Today, it is assumed that at least 5% of the new clinical cases of trichomoniasis are caused by drug resistant parasites (6). Furthermore, the use of MTZ oral tablets is contraindicated during the first trimester of pregnancy because of the highlight of a mutagenic activity in bacteria and carcinogenic activity in mice. Nevertheless, MTZ is considered as a second line of therapy in the latter stage of pregnancy (7,8). Consequently, the Food and Drug Administration in the USA had classified MTZ as a class B risk factor for pregnancy, and a possible but not confirmed risk to the foetus. The lack of approved alternative therapies justifies the development of new approaches for the treatment of trichomoniasis especially those including a local drug delivery, hence limiting the adverse effects.

In this context, here we describe a non-conventional therapeutic strategy based on the use of vaginally-applied drug-free formulation for the treatment of trichomoniasis. The formulation is based on the combination of the effects of mucoadhesive nanoparticles and a thermosensitive hydrogel. Compared to conventional therapeutic strategies, these two elements could make easier the achievement of higher formulation concentrations in the vicinity of *T. vaginalis* minimizing thus systemic absorption and unwanted side-effects. The nanoparticles are composed of poly(isobutylcyanoacrylate) (PIBCA) core coated with a mixture of chitosan and thiolated chitosan. Among the characteristics of these nanoparticles, mucoadhesive properties due to chitosan and thiolated chitosan are the most important ones when foreseen mucosal administration (9–11). The nanoparticles were included into a vehicle composed of a thermosensitive pluronic® F127 hydrogel ((ethylene oxide)$_{97}$(propylene oxide)$_{69}$(ethylene oxide)$_{97}$ block copolymers). Considering an appropriate pluronic® F127 concentration, such vehicles are fluid at room temperature facilitating their application and spreading within the vaginal cavity, while they form a highly viscous hydrogel above sol–gel transition temperature promoting prolonged contact-time with the vaginal mucosa at body temperature. An improved patient compliance is expected because of the ease of application and a reduction of the frequency of administration.

In the present work, after the design of the formulation, the anti-*T. vaginalis* activity of nanoparticles was investigated *in vitro*. A special attention was paid to the evaluation of the eventual toxicity of the formulations *ex vivo* on pig vaginal mucosa.

## MATERIALS

Pluronic® F127 (Poloxamer P407) and pluronic® F68 (Poloxamer P188) copolymers of pharmaceutical grade were from BASF ChemTrade GmbH (Ludwigshafen, Germany). MTZ, phosphate buffer saline (PBS, 0.01 M, pH 7.4 at 25°C) and all reagents used for the preparation of simulated vaginal fluid (SVF) and for chitosan thiolation were from Sigma-Aldrich (Saint-Quentin Fallavier, France). Isobutylcyanoacrylate (IBCA) was a generous gift from Henkel Biomedical (Dublin, Ireland). Traut's reagent (2-iminothiolane) was synthesized in the Department of Organic Chemistry (Biocis UMR CNRS 8076), School of Pharmacy, University of Paris-Sud (Châtenay-Malabry, France). *T. vaginalis* strain (ATCC PRA-98; Taxonomy ID: 412133) was stored in liquid nitrogen containing 6% dimethyl sulphoxide as cryoprotectant. This strain was MTZ-sensitive.

SVF was prepared as previously described by Owen and Katz (12). NaCl (3.51 g), KOH (1.4 g), $Ca(OH)_2$ (0.22 g), bovine serum albumin (0.018 g), lactic acid (2.00 g), acetic acid (1.00 g), glycerol (0.16 g), urea (0.4 g) and glucose (5.00 g) were added to 900 mL of distilled water contained in a beaker and stirred mechanically until complete dissolution. The pH of the mixture was then adjusted to 4.5 using HCl. The pH was fixed at 4.5 within the range of the normal, premenopausal vaginal pH. The final volume was adjusted to 1 L.

Water soluble chitosan was from Amicogen (Seoul, Korea). The average molecular weight was 20,000 g/mol according to the manufacturer. This chitosan was denoted Chito20. The degree of deacetylation was 92%. The inclusion of thiol groups to chitosan was carried out following the method developed by Bernkop-Schnürch *et al.* (13) and applied by Bravo-Osuna *et al.* (9), Petit *et al.* (10) and Mazzaferro *et al.* (11). Briefly, 1 g of chitosan was dissolved in 100 mL of acetic acid solution (1% w/v). The pH of the solution was adjusted to 6.5 with NaOH (1 N). Then, the Traut's reagent (2-iminothiolane) was added in a chitosan:2-iminothiolane weight ratio of 5:2. After an incubation period of 24 h at room temperature under continuous stirring, the resulting thiolated chitosan was dialysed (Spectra/Por® 3 membrane MWCO: 3,500) against different aqueous media: 8 h against 5 L of 5 mM HCl, 8 h against 5 L of 5 mM HCl containing 1% NaCl two times, 8 h against 5 L of 5 mM HCl and finally, 8 h against 5 L of 5 mM HCl (40 h in total). Dialysed products were freeze-dried (Christ Alpha 1-4 freeze-dryer. Bioblock Scientific, Illkirch, France) and stored at −20°C until use.

The resulting polymer is chitosan-4-thiol-butylamidine, denoted Chito20-TBA.

## METHODS

### Nanoparticle Preparation

Nanoparticles coated with chitosan and thiolated chitosan were prepared by anionic emulsion polymerization according to previous works (9–11,14). Briefly, 0.069 g of mixtures of chitosan and thiolated chitosan (Chito20/Chito20-TBA) at different thiol proportion (100/0, 75/25, 50/50 and 25/75 wt%) were dissolved in 5 mL of 0.2 M nitric acid in a glass tube at 40°C under vigorous magnetic stirring and argon bubbling. After 10 min, 0.25 mL of IBCA monomer were added. Argon bubbling was kept for an additional 10 min and stopped. The reaction was allowed to continue at 40°C under vigorous magnetic stirring for 50 min.

PIBCA nanoparticles uncoated with chitosan and thiolated chitosan were prepared similarly by replacing chitosan and thiolated chitosan by 1% w/v pluronic® F68 as stabilizer.

Nanoparticle suspensions were purified by dialysis using a Spectra/Por® membrane with molecular weight cut off of 100,000 g/mol, twice during 30 min, then during 60 and 90 min and once overnight against 1 L of acetic acid solution 16 μM. Purified nanoparticle dispersions were stored at 4°C until use.

Polymerization medium used as a control for the anti-*T. vaginalis* activity evaluation was prepared similarly without IBCA, without chitosan or pluronic® F68 and purified in the same conditions than the nanoparticles.

### Physico-chemical Characterization of the Nanoparticles

The mean hydrodynamic diameter of the nanoparticles and their size distribution were determined from the Z-average obtained at 20°C by quasi-elastic light scattering using Zetasizer Nanoseries (Malvern Instruments Ltd. UK). The scattered angle was fixed at 173° and 60 μL of each sample was diluted in 2 mL of acetic acid 0.16 μM (Millex, SLAP 0225, Millipore, France). Zeta potential of nanoparticles was measured using Zetasizer Nanoseries (Malvern Instruments Ltd. UK). The dilution of the suspensions (1:33 (v/v)) was performed in NaCl (1 mM). Each experiment was conducted in triplicate.

### Preparation of the Hydrogels

Hydrogels were prepared according to the so-called "cold method" (15–17). Pluronic® F127 hydrogel (20 wt%) was obtained by gradually adding under stirring the pluronic® F127 powder to water maintained at 4°C. Hydrogel containing the nanoparticles was prepared by slowly dispersing under magnetic stirring pluronic® F127 powder (20 wt%) directly in the nanoparticle dispersion maintained at a temperature of 4°C until the complete dissolution of the polymer. The nanoparticles were composed of PIBCA/(Chito20/Chito20-TBA) (75/25 wt%). The concentration of the nanoparticles into the hydrogel was 20 mg/mL.

### Evaluation of the Anti-*T. vaginalis* Activity of the Formulations

*T. vaginalis* was grown axenically at 35°C in trypticase-yeast extract-maltose (TYM) medium (18) supplemented with 10% horse serum (Gibco, France) and subcultured every 2 days (19).

Culture tubes with fresh TYM medium enriched with filtered heat-inactivated horse serum 10% alone (Control 1 denoted C1) or with the tested formulations, were inoculated with $2\times10^5$ protozoa in 3 mL. The tested formulations were PIBCA/(Chito20/Chito20-TBA) at different proportions of chitosan and thiolated chitosan (100/0, 75/25, 50/50 and 25/75 wt%). The concentration of nanoparticles was varied as 12.5, 25, 50 and 100 μg/mL.

The results were compared to *(i)* PIBCA/F68 nanoparticles, *(ii)* solutions composed of (Chito20/Chito20-TBA) mixtures at different proportions of chitosan and thiolated chitosan (100/0, 75/25, 50/50 and 25/75 wt%), *(iii)* the polymerization medium (Control 2 de noted C2). MTZ was used as a reference anti-*T. vaginalis* drug at the lethal concentration of 7 μg/mL.

The tubes were incubated for 24 h at 35°C and the number of parasites per milliliter in each tube was determined microscopically with a haemocytometer (Kova® Glasstic® Slide 10, Hycor Biomedical, United States). The experiments were run three times.

The results were estimated as the percentage of culture growth inhibition compared with control 1 (C1).

For statistical analysis, a t student's *t*-test was applied ($p=0.05$).

### *Ex Vivo* Evaluation of the Toxicity of the Formulations on Vagina Pig Mucosa

Experiments were carried out on female pigs (INRA Jouy en Josas, France) weighing between 60 and 63 kg in average. The animals were fasted for 24 h but had free access to tap water. All experiments on animals adhered to the Communities Commission Directive (DE/86/609/CEE) and were performed in conformance with the French Ministry of Agriculture Permission No. 78–16. Pigs were sacrificed by intravenous injection (20 mL) of overdosed sodium phenobarbital (Dolethal, Vetoquinol Laboratory, Lure, France) and the

vaginal mucosa was taken over a 10 cm length. The mucosa was placed in SVF and stored at −20°C immediately after sacrifice of the animal and kept at this temperature until use. It has been shown that porcine mucosa can be frozen during storage without affecting the mucus layer (20). Samples were cut in 1 cm square pieces by means of surgical scissors to obtain intact vaginal tissue and defrosted before experiments at ambient temperature in the presence of freshly prepared SVF.

The mucosa was then placed in Franz cells and the receiver compartment was filled with SVF (11.2 mL). About 0.5 mL of each formulation was uniformly deposited on the mucosa. The contact surface was 1 $cm^2$. The investigated formulation is composed of PIBCA/(Chito20/Chito20-TBA) (75/25 wt%) gelified with pluronic® F127. The controls were: SVF, pluronic® F127 and the nanoparticles without hydrogel. The concentration of the nanoparticles was 20 mg/mL and the concentration of pluronic® F127 was 20 wt%.

Franz cells were closed and put in a thermostated bath at 37°C. After 12 h of contact with the formulations, the receptor and donor liquids were removed. The tissues were fixed in FineFix (Milestone, Italy), paraffin-embedded and cut into 4 μm thick slides. Hematoxilin-eosin-saffranin staining was performed prior to histological analyses. The light micrographs of specimens were obtained with a Microscope Axiophot Zeiss (Germany) connected to a digital camera (PCO, Germany). Whole specimens were scanned with a slide scanner (Nikon Super Coolscan 8000) customized for histopathology (Groupe Régional d'études sur le cancer, Caen, France).

## RESULTS

### Nanoparticle Physico-chemical Characterization

The PIBCA/(Chito20/Chito20-TBA) nanoparticles were obtained by anionic emulsion polymerization of the monomer isobutylcyanoacrylate (Fig. 1). These nanoparticles are composed of a hydrophobic core of PIBCA while their shell is formed by a layer of a mixture of chitosan and thiolated chitosan at a different thiol proportion (100/0, 75/25, 50/50 and 25/75 wt%). Nanoparticles without chitosan were stabilized by pluronic® F68. The nanoparticle mean hydrodynamic diameter was in the range of 185–210 nm (Table I) in agreement with previous data (21). As expected, electrophoretic mobility measurements showed that chitosan-coated PIBCA nanoparticle zeta potential was positive ranging from +34.3 to +40.4 mV. It differed slightly depending on the composition of the nanoparticle shell.

### *In Vitro* Evaluation of the Anti-*T. vaginalis* Activity of the Formulations

The anti-*T. vaginalis* activity of nanoparticles composed of PIBCA/(Chito20/Chito20-TBA) (75/25 wt%) was studied after 24 h of incubation with *T. vaginalis*. The anti-*T. vaginalis* activity of the nanoparticles was compared with the activity of a reference drug, MTZ, used at a lethal concentration of 7 μg/mL.

The nanoparticles without addition of any drug exhibited a strong anti-*T. vaginalis* effect at a concentration of 100 μg/mL (Fig. 2). The composition in thiolated chitosan did not have an effect on this activity. Indeed, comparable anti-*T. vaginalis* activity was found with other tested proportions of (Chito20/Chito20-TBA) (100/0, 75/25, 50/50 and 25/75 wt%) (Fig. 2).

No anti-*T. vaginalis* activity was observed neither with chitosan-uncoated nanoparticles composed of PIBCA/F68 (Fig. 3) nor with chitosan solutions (Fig. 4). Also, no activity was observed with the polymerization medium (Figs. 3 and 4).

### *Ex Vivo* Evaluation of the Toxicity of the Formulations on Vaginal Pig Mucosa

The histotoxicity of PIBCA/(Chito20/Chito20-TBA) (75/25 wt%) nanoparticles (20 mg/mL) gelified with pluronic® F127 20 wt% was investigated *ex vivo* on pig vaginal mucosa. The results on Fig. 5a showed that the epithelium was regular with a totally preserved architecture in comparison with SVF (Fig. 5b) and pluronic® F127 20 wt% (Fig. 5c). In the presence of non-gelified nanoparticles, the epithelium had a regular architecture with a slight focal desquamation without stroma abnormalities (Fig. 5d). True ulcerations were absent from all the mucosa specimens and the underlying stroma was devoid from inflammatory cells which confirmed the absence of significant toxicity.

## DISCUSSION

The present study reports the design and the *in vitro* biological activity evaluation of vaginally-applied formulations for the treatment of urogenital trichomoniasis. These formulations are mainly composed of PIBCA nanoparticles coated with a mixture of chitosan and thiolated chitosan. Even if alkylcyanoacrylate-based nanoparticles have been studied so far for their ability to encapsulate drugs and to control their delivery and targeting to biological tissues, (22) the strategy we envisioned in the present work is different. Here, we investigated the possibility to use these nanoparticles for the treatment of infections due to *T. vaginalis* without adding any drug. The results showed, unpredictably, a strong anti-*T. vaginalis*

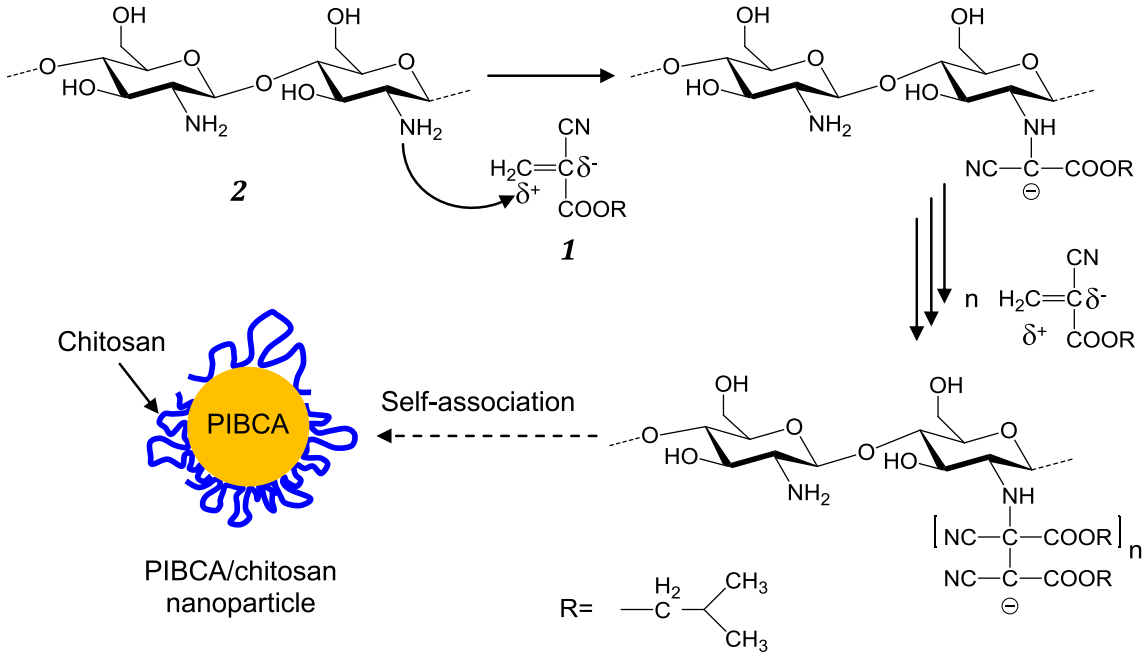


**Fig. 1** Schematic representation of isobutylcyanoacrylate monomer (*1*) polymerization by the amine groups of deacylated units of chitosan (*2*) according to anionic mechanism. PIBCA: poly(isobutylcyanoacrylate).

activity *in vitro* with chitosan-coated PIBCA nanoparticles at a concentration of 100 µg/mL (Fig. 2a). This anti-*T. vaginalis* effect is conditioned by the presence of chitosan on the PIBCA shell since no activity was observed with chitosan-uncoated PIBCA nanoparticles (Fig. 3). Previous works from the literature indicated that sialic acid is the major component of *T. vaginalis* membrane contributing to the negative charge of the protozoan (23). It could be suggested that the positively-charged shell of chitosan-coated nanoparticles was likely to interact with the negatively charged membrane of *T. vaginalis* through electrostatic interactions.

Based on these findings, one could consider also the possibility to use a solution of chitosan as anti-*T. vaginalis* agent instead of chitosan-coated nanoparticles. However, no anti-*T. vaginalis* activity of chitosan in the solution was detected whatever its concentration (from 12.5 to 100 µg/mL) (Fig. 4).

**Table I** Effect of Thiolated Chitosan Proportion on the Mean Hydrodynamic Diameter ($D_h$) and the ζ Potential of PIBCA/(Chito20/Chito20-TBA) Nanoparticles Prepared by Anionic Emulsion Polymerization. Control is Composed of PIBCA Nanoparticles Uncoated with Chitosan Stabilized with 1% w/v of Pluronic® F68. ($n = 3$)

| Composition of the Np shell (%) | | $D_h$ (nm) | ζ potential (mV) |
|---|---|---|---|
| Chito20/Chito20-TBA | 100/0 | 185 ± 2 | +39.6 ± 0.6 |
| | 75/25 | 211 ± 4 | +34.3 ± 1.0 |
| | 50/50 | 196 ± 2 | +39.0 ± 0.4 |
| | 25/75 | 192 ± 2 | +40.4 ± 0.8 |
| Pluronic® F68 | 100 | 189 ± 2 | −7.5 ± 0.3 |

These results suggest that the anti-*T. vaginalis* activity is related to PIBCA nanoparticles but only when they are coated with chitosan.

In the next step of this work, the mucoadhesive properties of chitosan to the mucosal surfaces were improved by increasing thiol proportion in the mixture Chito20/Chito20-TBA. Data from the literature suggests that the adhesive properties of thiolated chitosan were due to the interaction of thiol groups with the cysteine-rich domains of mucus (7,24). It is worth to note that the presence of thiol groups on PIBCA shell is not the main parameter controlling the anti-*T. vaginalis* activity since comparable results were obtained whatever the proportion of thiolated chitosan (Fig. 2).

The chosen nanoparticle formulation is composed of PIBCA/(Chito20/Chito20-TBA) (75/25 wt%). We have previously demonstrated that this thiol proportion was large enough to ensure good mucoadhesion in comparison with non-thiolated chitosan (10). Mucoadhesion is dominated by two major mucus components: water and mucin contents. Water content is highly similar across various mucosal surfaces and usually within the range of 90–98% (25–29). Mucin content usually ranges between 2 and 5% by weight for gastrointestinal, cervical, ocular, nasal, and lung mucus despite significant differences in glycosylation (25,26).

For the next experiments, the nanoparticles were used at a concentration of 20 mg/mL. This corresponds to the highest nanoparticle concentration that could be obtained using the anionic emulsion polymerization of IBCA

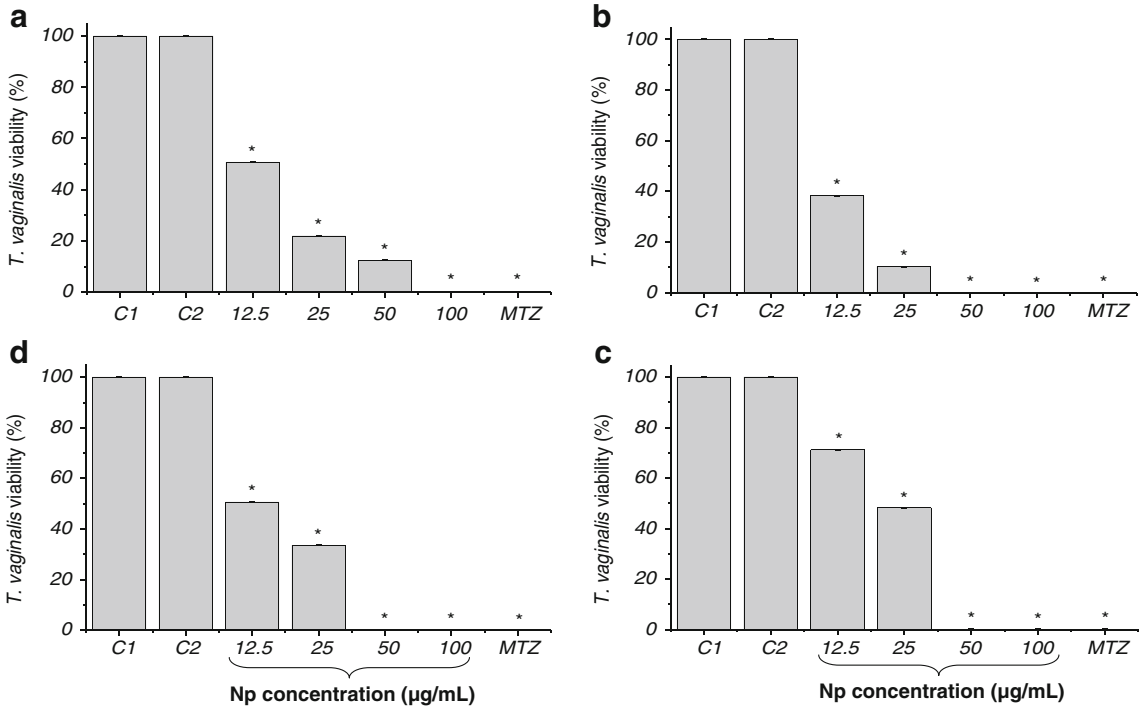


**Fig. 2** Effect of thiol content in the nanoparticle shell on viable *T. vaginalis* after 24 h of incubation. Composition of Chito20/Chito20-TBA of the tested nanoparticles was varied as: 100/0 wt% (**a**), 75/25 wt% (**b**), 50/50 wt% (**c**), 25/75 wt% (**d**). Nanoparticle concentration was varied as: 12.5, 25, 50 and 100 $\mu$g/mL. *T. vaginalis* viability was also evaluated in culture medium without nanoparticles (C1) and in the polymerization medium without nanoparticles (C2). MTZ was used as a reference anti-*T. vaginalis* drug at the lethal concentration of 7 $\mu$g/mL. Data are the mean of three determinations ± sd. * significant *versus* control $p < 0.05$.

monomer in the presence of Chito20/Chito20-TBA. The nanoparticles were then dispersed in a thermosensitive pluronic® F127 hydrogel. Acting as a vehicle for the nanoparticles, this hydrogel makes easier the application of the formulations in the vaginal cavity. Once gelified due to temperature increase, the hydrogel-containing nanoparticles would develop mucoadhesive characteristics which are highly desirable for facilitating the presence of the formulation on the vaginal mucosa. In this way, high local nanoparticle concentrations could be maintained at the vaginal epithelium surface. An improved patient compliance is expected because of a reduction of the frequency of administration as well as the use of lower dosages of anti-*T. vaginalis* formulations.

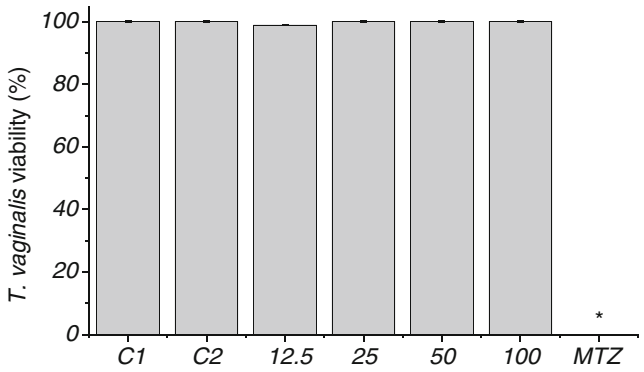


**Fig. 3** Effect of PIBCA/F68 nanoparticles uncoated with chitosan on *T. vaginalis* viability. Nanoparticle concentration was varied as: 12.5, 25, 50 and 100 $\mu$g/mL. *T. vaginalis* viability was also evaluated in culture medium without nanoparticles (C1) and in the polymerization medium without nanoparticles (C2). MTZ was used as a reference anti-*T. vaginalis* drug at the lethal concentration of 7 $\mu$g/mL. Data are the mean of three determinations ± sd. * significant *versus* control $p < 0.05$.

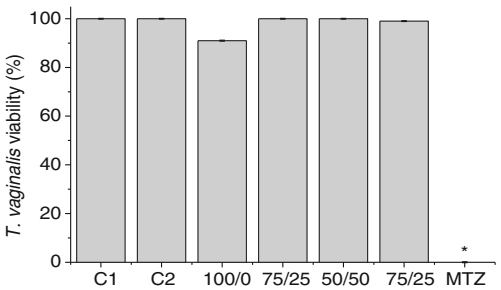


**Fig. 4** Effect of thiolated chitosan proportion in solutions composed of (Chito20/Chito20-TBA) (100/0, 75/25, 50/50 and 25/75 wt%) on *T. vaginalis* viability. The formulations were tested at a concentration of 12.5 $\mu$g/mL. Same results were obtained with the other concentrations (25, 50 and 100 $\mu$g/mL). *T. vaginalis* viability was also evaluated in culture medium without nanoparticles (C1) and in the polymerization medium without nanoparticles (C2). MTZ was used as a reference anti-*T. vaginalis* drug at the lethal concentration of 7 $\mu$g/mL. Data are the mean of three determinations ± sd. * significant *versus* control $p < 0.05$.

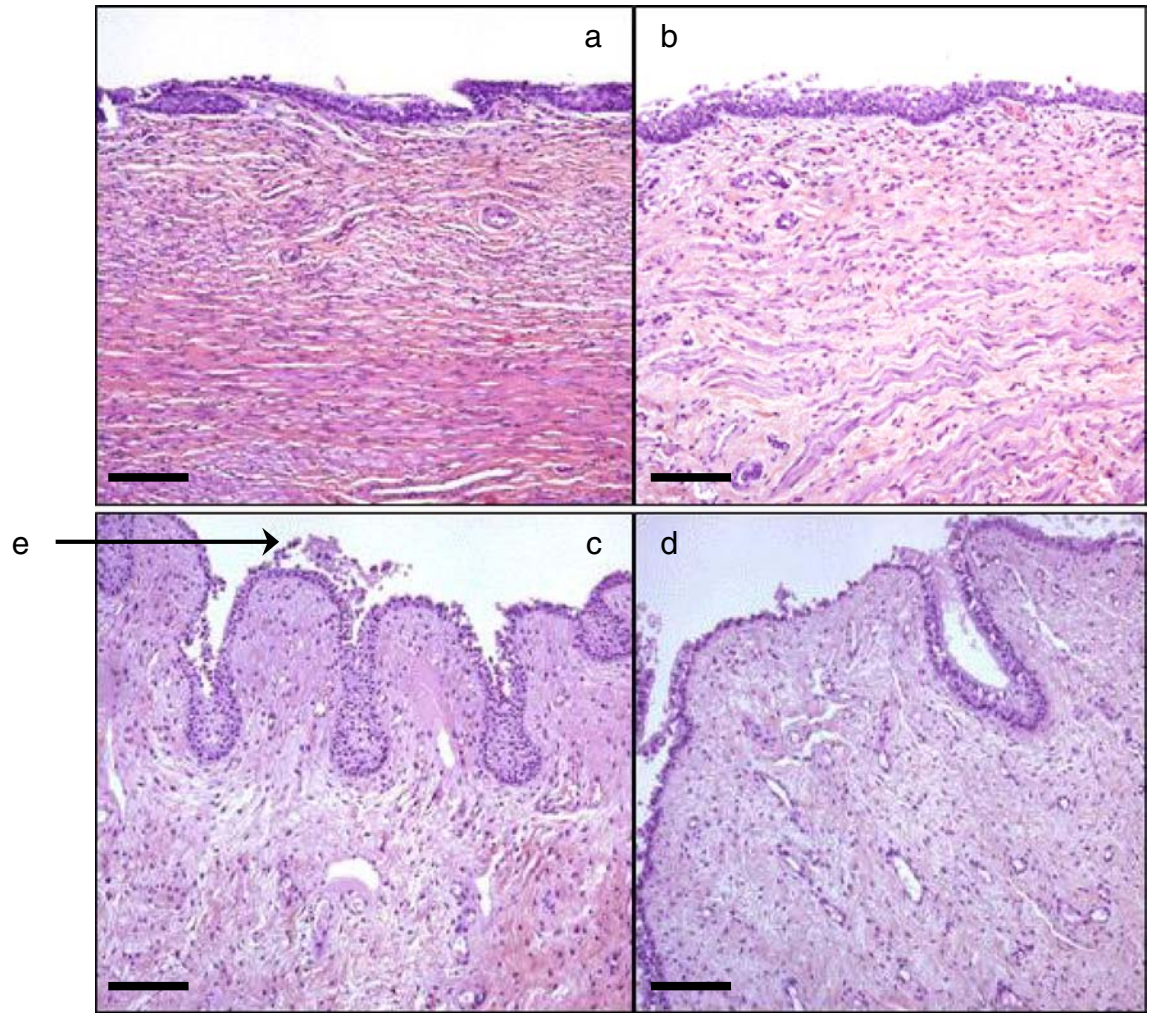


**Fig. 5** Histology images of pig vaginal mucosa after 12 h in contact with (**a**) nanoparticles composed of PIBCA/(Chito20/Chito20-TBA) (75/25 wt%) gelified with F127 20 wt%, (**b**) SVF, (**c**) F127 20 wt% and (**d**) non-gelified nanoparticles PIBCA/Chito20/Chito20-TBA (75/25 wt%). The concentration of nanoparticles was 20 mg/mL. The scale bar corresponds to 0.02 cm. (Original magnification ×100). (**e**) slight focal desquamation without stroma abnormalities.

Although active against *T. vaginalis*, our formulation should have low toxicity after application on the vaginal mucosa. In this context, histotoxicity of pluronic® F127 hydrogel-containing PIBCA/(Chito20/Chito20-TBA) (75/25 wt%) nanoparticles assessed using pig vaginal mucosa showed the absence of ulceration or inflammatory signs (Fig. 5). Noteworthy, among the larger experimental animals, the pig has the advantage of being remarkably similar to human in terms of anatomy, physiology, metabolism and histology. Many research works have reported that excellent correlation was found between human and porcine vaginal tissues (20,30). Previous data on the histotoxicity of the PIBCA-based nanoparticles also demonstrated the absence of inflammation or ulceration of small intestine and the colon mice sections collected after repetitive oral *in vivo* administration of PIBCA/(Chito20/Chito20-TBA) 75/25 wt% nanoparticles (31).

## CONCLUSIONS

This work has demonstrated that PIBCA nanoparticles coated with chitosan were active against *T. vaginalis* without the addition of any drug. The anti-*T. vaginalis* activity was related to PIBCA nanoparticles but only when they are coated with chitosan. The anti-*T. vaginalis* activity of the nanoparticles is rather controlled by the presence of chitosan than thiol groups on the nanoparticle shell. The nanoparticles contained into thermosensitive pluronic® F127 hydrogel did not show any toxicity *ex vivo* on pig vaginal mucosa. These encouraging results prompt to perform *in vivo* experiments on trichomoniasis animal models.

## ACKNOWLEDGMENTS AND DISCLOSURES

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