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Keywords

Econazole nitrate; fungal infection, topical administration; thermo-responsive formulation; lipid microparticles; candida.

Abstract

In this work, we propose pharmaceutical textiles imprinted with lipid microparticles of Econazole nitrate (ECN) as a mean to improve patient compliance while maintaining drug activity. Lipid microparticles were prepared and characterized by laser diffraction (3.5±0.1 µm). Using an optimized screen-printing method, microparticles were deposited on textiles, as observed by Scanning Electron Microscopy. The drug content of textiles (97±3 µg/cm^2) was reproducible and stable up to 4 months storage at 25°C/65% Relative Humidity. Imprinted textiles exhibited a thermosensitive behavior, as witnessed by a fusion temperature of 34.8°C, which enabled a larger drug release at 32°C (temperature of the skin) than at room temperature. *In vitro* antifungal activity of ECN textiles was compared to commercial 1% (*w/w*) ECN cream Pevaryl®. ECN textiles maintained their antifungal activity against a broad range of *Candida* species as well as major dermatophyte species. I*n vivo,* ECN textiles also preserved the antifungal efficacy of ECN on cutaneous candidiasis infection in mice. *Ex vivo* percutaneous absorption studies demonstrated that ECN released from pharmaceutical textiles concentrated more in the upper skin layers, where the fungal infections develop, as compared to dermal absorption of Pevaryl®.

Overall, these results showed that this technology is promising to develop pharmaceutical garments textiles for the treatment of superficial fungal infections.

1. Introduction

Textile is a material that has been purposed to clothing for centuries. In recent years, the combined efforts of chemists, textile engineers and cosmetologists resulted in the development of biofunctional textiles that bring additional functions to garments than simple warmth and body protection. Also called cosmetotextiles, such textiles are defined as textile items containing substance or mixture that release their active compounds when in contact with the human body [1]. Firstly focused on improved comfort, cosmetotextiles have since then been developed for slimming, moisturizing, and perfuming [2]. Innovative technologies have been incorporated into such fabrics, such as microencapsulated substances [1, 3] or phase change materials that help the thermoregulation of the body [4]. Rapidly, various biofunctional textiles have been envisioned for the delivery of topical bioactive molecules, since the close and prolonged contact of fabric with the skin could make cloth an easy drug delivery system. Silver nanoparticles [5] and chitosan [6] were used as preservatives for antibacterial clothing. Fabrics with antioxidant properties

were developed by incorporation of vitamin E [7] or gallic acid [8]. Some clinical indications have also been examined, such as venous insufficiency using aescin supported textiles [9] and atopic dermatitis with zinc oxide functionalized textiles [10]. Such examples show the evolution of cosmeto-textiles to pharmaceutical textiles, offering more than an improved comfort, but also a treatment for various skin diseases.

In particular, superficial fungal diseases are common worldwide and their incidence continues to increase. In 2010, they were the 4th most prevalent disease in the world, affecting more than 948 million people worldwide [11]. As compared to bacteria, fungal topical infections are longer in duration and require weeks and even months of fastidious treatment. Patient compliance would be greatly improved if a regular piece of textile (such as bandage or socks) could be used instead of applying a cream daily. Antifungal textiles have been prepared by soaking the fabric into a solution of antifungals [12, 13], and promising clinical results have been obtained from a sock prototype to treat tinea pedis [14]. However, fabrication technology and controlled release of antifungal agents still need to be improved.

Econazole Nitrate (ECN) is currently marketed for the treatment of vaginal candidiasis and topical fungal infections as a cream formulation [15, 16]. It has demonstrated antifungal activity against *Candida* and dermatophytes species [15-17]. Encapsulation of ECN in lipid particles [18],

microspheres [19], and micelles [20] has been reported to improve cutaneous efficacy of ECN. More precisely, comparing micro- and nano- solid lipid particles, nanoparticles were shown to improve transdermal administration whereas microparticles enhanced skin deposition [21]. Moreover, the lipid composition favored a good biocompatibility of the particles and improved skin penetration of the drug [22].

In this work, a novel ECN formulation on textile support was tested as a proof of concept for the treatment of topical fungal infections. The formulation is based on proprietary lipid microparticles exhibiting thermosensitivity in order to release the drug on contact with the skin [23]. Deposition of the microparticles on textile is achieved using an in-house modified screen-printing technique. The latter is a simple method where the microparticles are passed through a mesh with predefined openings to control the amount and the topology of the deposit [24, 25]. This method allows for a physical uniform deposit of the microparticles at specific areas on textiles without addition of chemical binders. The solid microparticles (Dermotex®) and deposition method (On2™) are technologies proprietary to Biomod Concepts Inc., and have been used by the company to produce intelligent cosmetic textiles [23]. The objective of this study is to evaluate the potential of a pharmaceutical textile, namely a microparticle formulation of ECN deposited on textile. Its *in vitro* antifungal activity, percutaneous absorption, and *in vivo* pharmaceutical

efficacy on a superficial fungal infection were compared to the commercial 1% (*w/w*) ECN cream Pevaryl®.

2. Experimental methods

2.1. Materials

ECN-loaded microparticles on textile and all placebo textile formulations were provided by Biomod Concepts Inc. (Ste-Julie, QC, Canada) and prepared according to their patented technology [23]. Laya™ textiles were provided by Biomod Concepts (Sainte-Julie, QC, Canada). ECN was purchased from AK Scientific (Union City, CA, USA, Lot# TC24717). Pevaryl® 1% (*w/w*) ECN formulation was purchased from Johnson & Johnson (France, Lot # DDB3400) and its generic version from Mylan Pharmaceuticals (Saint-Priest, France). Miconazole Nitrate was purchased from AK Scientific (Union City, CA, USA, Lot# TC25782). ECN standard disks were purchased from Rosco (Neo-sensitabs 10 µg disks, Denmark, Lot #1201-1). Prednisolone acetate was purchased from Sanofi Aventis (Paris, France) Polyethylene Glycol 400 (PEG-400) was purchased from Medisca Inc. (Montreal, QC, Canada). Sodium dodecyl sulfate (SDS) and semi-permeable polycarbonate membranes (Nucleopore Track-Etch Membrane, pores of 0.6 µm, 25 mm in diameter) were purchased from Sigma-Aldrich (Oakville, ON, Canada). Tape used for tape stripping was purchased from 3M tape (St-Paul, MN, USA). All samples were filtered using

PTFE filters purchased from Fisher Scientific (EMD Millipore Millex, pores 0.45 µm, 13 mm in diameter, Ottawa, ON, Canada). All solvents (HPLC grade) were bought from Fisher Scientific (Ottawa, ON, Canada).

2.2 Organisms

Candida albicans strain SC5314 was originally isolated from a patient with disseminated candidiasis, and served as reference for the *C. albicans* genome sequencing project [26, 27]. Thirteen clinical isolates of *Candida spp.* and *C. albicans* (CAAL93, CAAL121, CAAL123, CAAL124, CAAL294), *C. kefyr* (CAKE3, CAKE4), *C. krusei* (CAKR1, CAKR3), *C. glabrata* (CAGL1,CAGL5), and *C. lusitaniae* (CALU1, CALU2) were obtained from the Department of Parasitology and Medical Mycology, EA1155, at the University of Nantes, France. *Trichophyton rubrum* (n=2) and *T. mentagrophytes* (n=2) were obtained from the Laboratory of Parasitology and Medical Mycology at the Centre Hospitalier Universitaire of Nantes.

2.3. Preparation of ECN textiles

Intelligent textiles imprinted with ECN-loaded microparticles were prepared by Biomod Concepts Inc. using their patented technology [23]. Briefly, ECN lipid microparticles (1% *w/w*) were prepared under high shear using FDA-approved ingredients. The microparticles formulation was then applied onto textile surface using an adapted screen-printing method optimized for the microparticles deposition. A stencil with openings of more than 400 µm was

used to apply the microparticles on 21.6 x 27.9 cm pieces of a polyester non-woven textile provided by Biomod Concepts Inc. ECN imprinted textiles were kept at 22°C in sealed aluminum/acrylonitrile-coated packagings until analysis.

2.4. Characterization of microparticles

One hundred milligram (100 mg) of the ECN-loaded microparticles preparation used for screen-printing was diluted in 5 mL of milliQ water and analyzed for article size distribution at 22°C by laser diffraction (LS 13 320, Beckman Coulter, Mississauga, ON, Canada). Pevaryl® particle size was measured by dynamic light scattering (Zetasizer Nano ZS, Malvern, Worcestershire, UK) using the automatic algorithm mode. Samples were prepared by diluting 100 mg of Pevaryl® in 5 mL of MilliQ water, position 4.65 and attenuator at 8. Measurements were recorded 3 times for each formulation.

Fusion temperature of the microparticles imprinted on textile was measured using thermal analysis based on heat-leak-modulus (TA-HLM) [28]. With TA-HLM, textile samples are wrapped around a sensor probe and heated. The samples of ECN-loaded textile (2.5 x 5 cm) were analyzed at a heating rate of 0.8°C per second and heated from 0°C to 100°C. Measurement was repeated 3 times.

2.5 HPLC-UV analysis

High-performance liquid chromatography (HPLC) with ultraviolet (UV)-analysis was used for stability and quantification of samples.

The HPLC-UV system (Agilent 1100 Series, Mississauga, ON, Canada) consisted in a degasser, dual pumps, auto-sampler, column heater and photo-diode array detector. A C18 column (25 x 4.6 mm, 5 µm packing, Zorbax-C18, Agilent, Santa Clara, CA, USA) was used with a matching pre-column (Agilent Zorbax C18). Mobile phase was composed of methanol and water using the gradient detailed in Table 1.

The flow rate was 1.4 mL per minute. The column temperature was set to 35°C. The injection volume was 20 µL. ECN was analyzed at 220 nm. ECN retention time was 8.7 minutes. The limit of quantification with this method is 9 µg/mL.

Table 1: Gradient of solvents in the HPLC-UV system

Time (min)	Water (%)	Methanol (%)
0	43	57
3	28	72
6	2	98
10	2	98
13	43	57
15	43	57

2.6 HPLC-MS/MS method

HPLC-Mass spectrometry (MS)/MS was used for *in vitro* release and *ex vivo* experiments on pig skin, which presented lower concentrations of ECN than the limit of quantification (LOQ) of HPLC-UV method. An Agilent 1100 series HPLC (Mississauga, ON, Canada) was coupled to a 4000Q TRAP™ (AB Sciex, Concord, ON, Canada) hybrid triple-quadrupole/linear ion trap MS. All the parameters can be found in Table 2. Each sample was injected twice. HPLC-MS/MS method was developed and validated for ECN, using miconazole nitrate as an internal standard. No matrix effect was found with any components of the skin.

Table 2: LC-MS/MS parameters

HPLC	Agilent 1100 series			
MS/MS	AB Sciex 4000 Qtrap			
Software	Analyst® (version 1.6.2)			
Ionisation	Turbo electrospray, positive ionization (ESI)			
Scan mode	Multiple reaction monitoring (MRM)			
Analyte parameters	**Compounds**	**DP (V)**	**MRM**	**CE (eV)**
Test molecule	Econazole	90	381 > 125	40
Internal Standard	Miconazole	90	417 > 161	40
Source parameters	Gas temp (°C)	650		
	Gas flow (L/min)	50		
	Curtain gas (psi)	25		
	Capillary (V)	5500		
Mobile phase	Composition	A: 0.1% Formic Acid (FA)+ H_2O B: 0.1 % Formic Acid + Acetonitrile: Isopropanol (80/20)		
	Gradient	15 to 97% of phase B in 1.5 min, then stay at 97% until 2.2 min, decrease to		

		15% at 2.3 min and stay at 15% of phase B until 3.5 min
Flow rate	0.7 mL·min^{-1}	
Column temperature	45°C	
Injection volume	2 µL	
Injection temperature	5°C	
Column	Luna C8 column (30 x 2.0 mm, 5 µm, Phenomenex, Torrance, CA, USA)	

193

194 **2.7 Quantification of ECN Textile**

195 One-cm^2 pieces of ECN imprinted textile were sampled from the center region
196 of randomly selected textile sheets for a good statistical analysis. Ten out of the
197 30 sheets imprinted for this study were sampled and analyzed. To extract ECN
198 from the fabric, textile samples were suspended in 1 mL of methanol, sonicated
199 for 30 minutes and heated at 55°C for 4 hours. After cooling down, 500 µL of
200 the extraction solution was sampled, filtered, and quantified by HPLC-UV.

201 **2.8 Stability**

202 Three 15 x 15 cm sheets of imprinted ECN textile were placed in a stability
203 chamber (25°C/65% relative humidity (RH)). At each time point, three 1-cm^2
204 pieces were samples per sheet from the center area of the textile for analysis.
205 The sample preparation and quantification methods were as described above
206 (section 2.7). The stability was monitored up to 4 months.

207 **2.9 *In vitro* release**

Disks of 0.79 cm^2 (10 mm diameter, containing 71 µg of ECN) were cut out of the imprinted textile. *In vitro* release was performed using Franz cells from PermGear Inc. (Hellertown, PA, USA) with an opening of 9 mm in diameter, 5 mL receptor size and a thermostated jacket. Diffusion tests were carried on semi-permeable polycarbonate membranes of 0.6 µm in pore size. The receptor fluid composition was optimized to ensure ECN diffusion was not limited by ECN solubility. Although ECN was not soluble at pH 7.4, its solubility was improved in 10 mM phosphate buffer solution (PBS) with 1.37 mM of NaCl at pH 4.5 with PEG-400 (70:30 *v/v*) [29]. Indeed, Pevaryl diffusion (20 mg) on Frantz cells using a semi-permeable polycarbonate membranes of 0.6 µm in pore size after 6h at 32°C, ECN solution recovery was 49.5%±1.3 and 13.5%±1.3 with and without PEG, respectively (n=3 for each condition, data not shown). The receptor fluid (10 mM phosphate buffer solution (PBS) with 1.37 mM of NaCl at pH 4.5 with PEG-400 (70:30 *v/v*)) was thermostated at 22°C or 32°C and was constantly stirred at 100 rpm. Samples of 400 µL were withdrawn at 30 minutes, 1, 2, 3, 4, and 6 hours, filtered and replaced with the same volume of receptor fluid. Samples were diluted 1:50 in a mixture of 20% acetonitrile: 80% H_2O with 0.1% FA before quantification by HPLC-MS/MS.

2.10 Antifungal Disk Diffusion tests

C. albicans SC5314 and *Candida* strains from Nantes were routinely grown at 30°C in yeast peptone dextrose (YPD; 1% yeast extract, 2% Bacto peptone, 2%

dextrose plus 2% agar for solid medium) and Sabouraud (SB) culture medium respectively. *C. albicans* SC5314 were suspended in liquid YPD medium to an OD_{600} of 0.1, and 150 µL of the cell suspension were spread on YPD Petri dishes (10 cm diameter). Disks of 0.79 cm^2 (10 mm diameter, containing 71 µg of ECN) were cut out of the imprinted textile, and equivalent quantity of Pevaryl® (7.1 mg Pevaryl®, 71 µg ECN) was weighed on a filter paper disk (10 mm in diameter). Placebo textiles imprinted with drug-free microparticles and standard 10-µg ECN disks were used as controls. All disks were placed on the YPD Petri plates. The plates were incubated at 32°C, and growth inhibition diameters were measured at 18 h. This was replicated 9 times for each formulation and repeated 3 times independently.

Other *Candida spp* isolates were suspended in sterile saline (0.85% NaCl) to achieve 1 x 10^6 cells per mL, which were deposited onto SB Petri dishes (10 cm in diameter). Disks of 0.50 cm^2 (8 mm in diameter, containing 50 µg of ECN) were cut out of the imprinted textile, and equivalent quantity of Pevaryl® (5.0 mg Pevaryl®, 50 µg ECN) was weighed on a filter paper disk (8 mm in diameter). Placebo textiles (8 mm in diameter) and standard 10-µg ECN disks were used as controls. All disks were placed on the SB Petri plates. The plates were incubated at 35°C and growth inhibition diameters were measured at 18h. This was replicated 4 times for each formulation and repeated 2 times independently.

Trichophyton rubrum and *T. mentagrophytes* were grown on SB culture medium. *Trichophyton* species were suspended in sterile saline (0.85% NaCl) with 0.1% SDS to achieve 1 x 10^6 fungal cells per mL, and the cells deposited on SB petri dishes (10 cm in diameter). The plates were incubated at 25°C, and growth inhibition diameters were measured after 4 days for *T. mentagrophytes* and after 7 days for *T. rubrum*. This was replicated 3 times for each formulation and species. This was repeated 3 times independently for *T. mentagrophytes* and once for *T. rubrum.*

2.11 *Ex vivo* diffusion test on pig ear skin

Pig ears were kindly provided by Dr. Fairbrother from the Veterinary Department of Université de Montréal (Saint-Hyacinthe, QC, Canada). Ears were washed with water and 1% SDS and shaved using a razor. The skin was gently peeled off from the dorsal region of the ears, washed again with 1% SDS and water and stored at -20°C until the next day. Skin diffusion tests were performed using Franz cells as described above. The receptor fluid was identical to *in vitro* release studies. It contained 10 mM PBS at pH 4.5 with 30% PEG-400, to ensure ECN solubility, and was stirred at 100 rpm [29]. The receptor compartments were heated to 37°C to help maintain the surface of the skin at 32°C. Disks of 0.79 cm^2 (10 mm in diameter, containing 71 µg of ECN) were cut out of the imprinted textile, and equivalent quantity of Pevaryl® (7.1 mg, 71 µg ECN) was weighted on a filter paper disk (10 mm in diameter).

The disks were applied upside down on the surface of the skin, so that the formulation was in direct contact with the skin. A small weight (5 g) was applied in order to ensure contact between the formulation and the skin surface. The system was dismounted after 24 hours and all the receptor fluid was collected. The used textiles and filter paper disks were collected for ECN quantification. The skin surface was washed with 8 mL of ethanol/water (50:50), which was collected for ECN quantification. The skin was separated in 3 layers: stratum corneum (SC), epidermis and dermis. The SC was removed using 20 strips of 1 cm^2 3M tape, which was extracted with 20 mL of acetonitrile. Epidermis was peeled off from the dermis after heating at 80°C for 10 seconds. Both epidermis and dermis were cut into pieces and were suspended in 1 mL acetonitrile. All samples were sonicated for 30 min then heated at 55°C for 4 hours. Liquid layers were filtered and diluted with 20% acetonitrile with 0.1% FA. Samples were diluted (1:20 for epidermis, 1:200 for residual formulation on filter paper disks and textiles, and 1:10 for washing, SC, dermis, and receptor fluid) and quantified by HPLC-MS/MS. The Overall recovery of ECN (sum of residual textile, washing, SC, epidermis, dermis) was 97.7% ± 5.7 (n = 9). Skins from 3 different pig ears were tested in triplicate each (n = 9).

2.12 Cutaneous candidiasis model in immunosuppressed mice

Female mice were treated with prednisolone acetate on the day before and on the day after inoculation. Hairs on the back of anesthetized mice were plucked by hand to make a hairless square. The skin was then slightly abraded using sandpaper and *Candida albicans* inoculum (25 µL at 3. 10^9 yeast/mL) was applied. Mice were then randomly distributed into 4 groups of 6 animals. At day 3 post-infection, treatment was applied topically on the skin lesion once daily during 5 consecutive days. A first group was treated with the reference drug, Mylan ECN cream at a dose of 50 µg of ECN per lesion, a second one with a disk of ECN textile at a dose of 50 µg of ECN per lesion, a third one with a placebo textile, and a fourth one was untreated and served as control group of the disease.

In order to evaluate the infection level, microbiological studies were undertaken. Skin specimens from infected locus were taken with a biopsy punch at day 9 post-infection. Each sample (half of the biopsy) was homogenized in saline solution with a tissue grinder. Dilutions were inoculated on Sabouraud-chloramphenicol-gentamicin agar plates. After a 48-h incubation time at 35°C, the number of yeast colonies was counted.

The procedure was approved by the ethical committee of Pays de la Loire, France with the agreement D44015 for the Unité Thérapeutique Experimentale, Faculté de médecine, Nantes.

2.13 Statistical Analysis

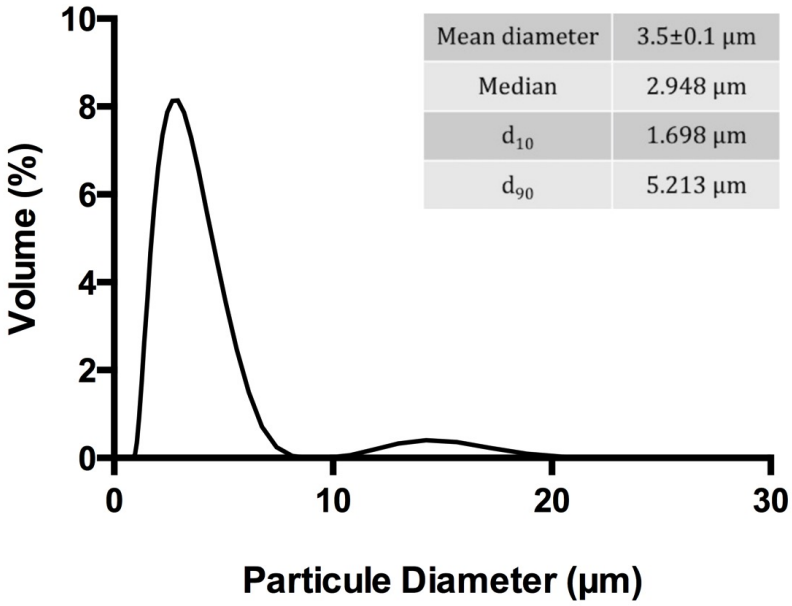
312 Statistical analysis was executed by means of Graph Pad® 6.0c (Prism
313 Software, San Diego, CA, USA). Multiple *t*-test was used with corrected *p*-value
314 using the sidak-bonferroni assuming unequal variance method for *ex vivo* pig
315 skin diffusion tests. All *p*-values ≤ 0.05 were considered to be significant.
316
317 **3. Results and discussion**
318
319 **3.1 Physical properties of pharmaceutical textiles**
320
321 ECN-loaded lipid microparticles (1% *w/w*) were prepared under high shear
322 and deposited on textile using a screen-printing method as previously
323 described [23]. The pharmaceutical textiles were first characterized for their
324 ECN content. ECN was extracted from textile samples and analyzed by HPLC.
325 ECN content was measured to be 90±19 µg per cm^2. The uniformity and the
326 homogeneity of deposition of ECN-loaded microparticles were assessed from
327 10 different sheets of textile and 3 different areas per sheet and was
328 determined to be less than 10% of the mean ECN value (data not shown).
329 Moreover, the ECN content was monitored as indicator of the chemical stability
330 of the pharmaceutical textiles. Stability was monitored each week during 6
331 weeks and again after 16 weeks using a stability chamber at 25C°C/65% RH
332 (Table 3). After 4 months, ECN content was still 97 µg/cm^2, which represents
333 108% of the initial content. Altogether, the reproducibility of the production

process and the stability over several months demonstrated the potential of the pharmaceutical textiles as new therapeutic products.

Table 3: ECN content of pharmaceutical textiles upon storage at 25°C/65%RH. T_0: After screen-printing. (n = 9)

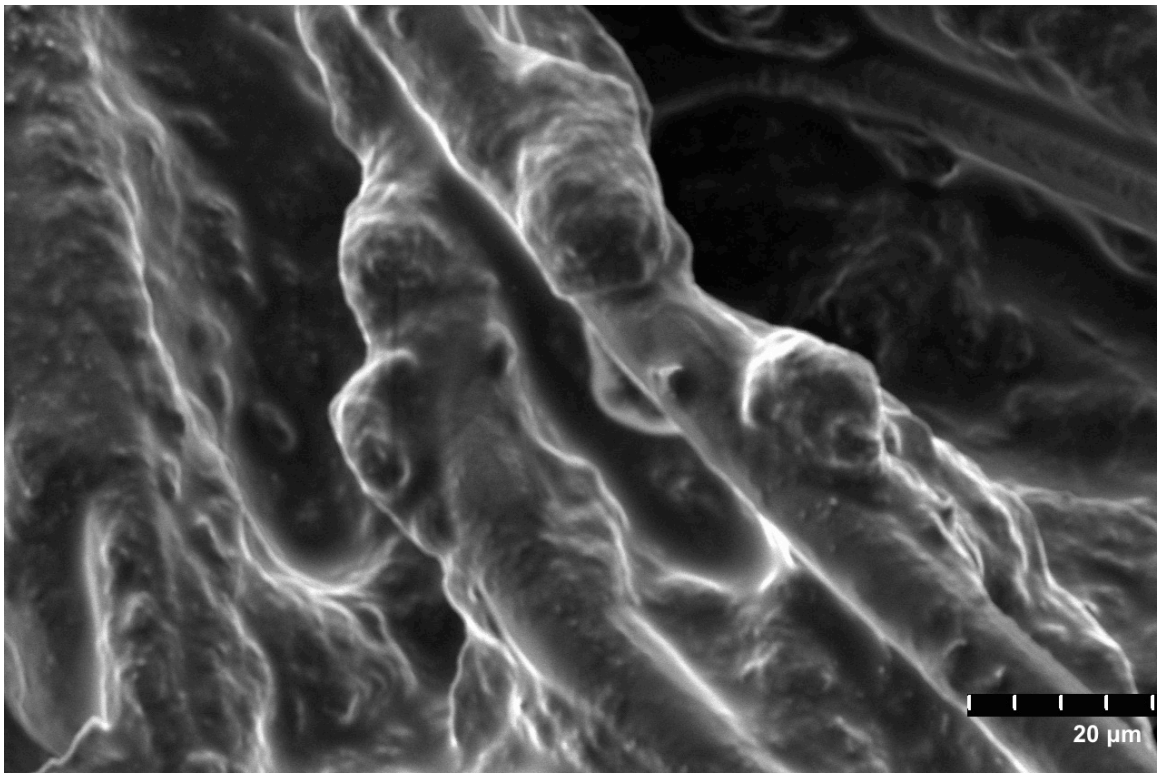
Time in weeks	T_0	T_1	T_2	T_3	T_4	T_6	T_{16}
ECN Textile content (µg/cm^2)	90±19	99±10	105±11	100±20	96±9	84±2	97±3

In a second step, physicochemical properties of the microparticles were examined. Microparticle size was evaluated before deposition on textile using laser diffraction (Figure 1). Particles exhibited micro-range diameter (3.5±0.1 µm). Pevaryl® particle size was also measured by dynamic light scattering and indicated a mean particle size of 348 nm with a polydispersity of 0.3. After screen-printing, ECN textiles were observed by scanning electron microscopy for their size and morphology. Figure 2 shows the presence of microparticles deposited on the textile fibers along with a film surrounding the microparticles. The film could be microparticles that partially melted or fused during the deposition process, probably due to the low fusion temperature of microparticles.



352
353
354 Figure 1: ECN microparticles size distribution by laser diffraction (n=9).

355



356

Figure 2: Scanning electron microscopy image of ECN pharmaceutical textile. Scale = 20 μm.

Lipid microparticles deposited on textile exhibited a fusion temperature of 34.8°C, as determined by TA-HLM, which confirmed their solid character at room temperature (Figure 3). The latter is a method similar to differential scanning calorimetry, which allows for the measurement of fusion temperature of microparticles deposited on a textile surface [28]. This fusion temperature value is crucial to allow triggered release upon contact with the skin, which is at approximately 32°C, while maintaining a good stability upon storage at room temperature.

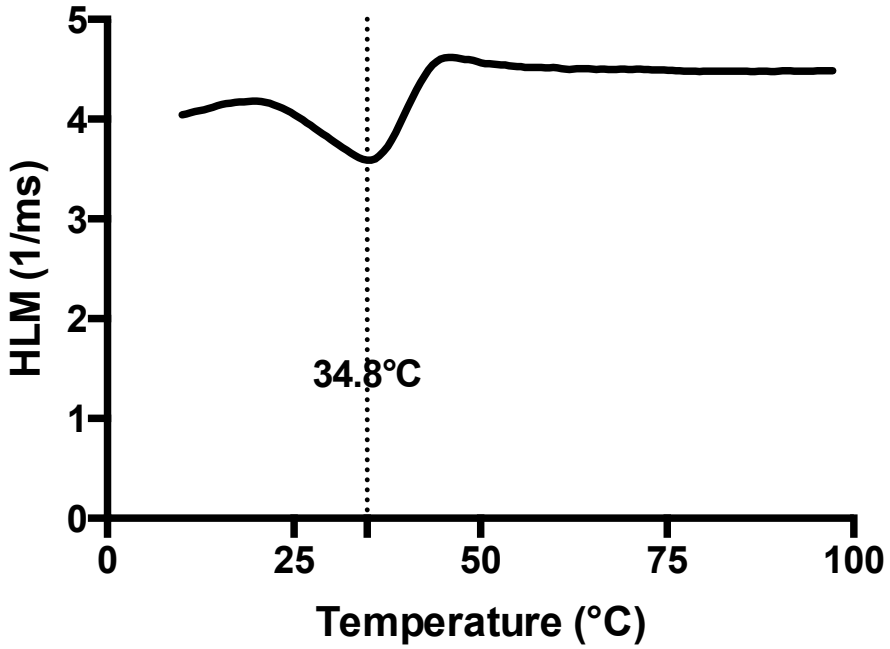


Figure 3: TA-HLM of ECN textile.

The thermo-sensitive behavior of the pharmaceutical textiles was further confirmed by the drug release profiles obtained at room temperature and at 32°C in Franz diffusion cells (Figure 4). As expected, release at 32°C was higher and faster than at room temperature. Indeed, the textiles released about twice the amount of ECN at all time points (although no significant difference was seen at 6 hours due to larger error bars). Raising the temperature to 32°C, close to the fusion temperature of the microparticles, initiated the fluidification/fusion of the microparticles and allowed ECN diffusion from the textile. It can be noted that no increase in ECN concentration in receptor fluid was observed after 24h (data not shown), indicating that the maximum release was reached within 6 hours. This suggested the textiles served as a reservoir for ECN, since less than 29±6% of the total ECN was released.

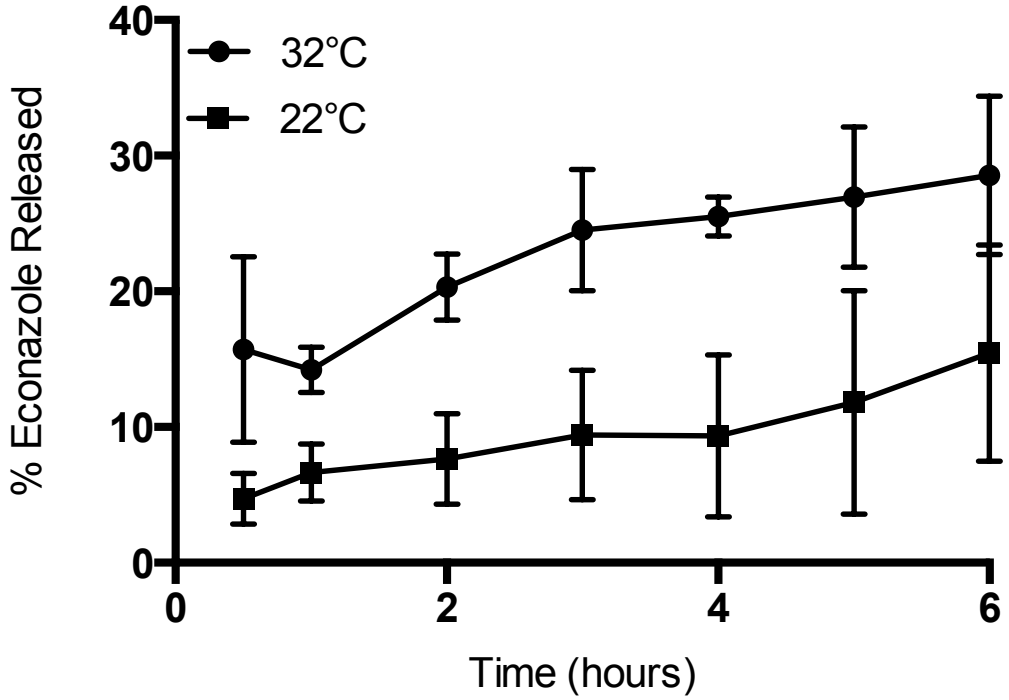


Figure 4: ECN release from pharmaceutical textiles at 22°C (black squares) and 32°C (black circles) on Franz diffusion cells through polycarbonate membranes. Mean ± standard error bars (n=4).

3.2 Antifungal activity of pharmaceutical textiles

Once their thermo-sensitive behavior was verified, the intelligent textiles were examined to ensure that the developed technology was able to preserve the pharmaceutical activity of the drug. Antifungal activity of ECN textiles was compared to Pevaryl®, a commercial formulation of ECN. Pevaryl® is a 1% (*w/w*) ECN cream indicated to treat *Candida* and dermatophytes superficial infections [15, 16].

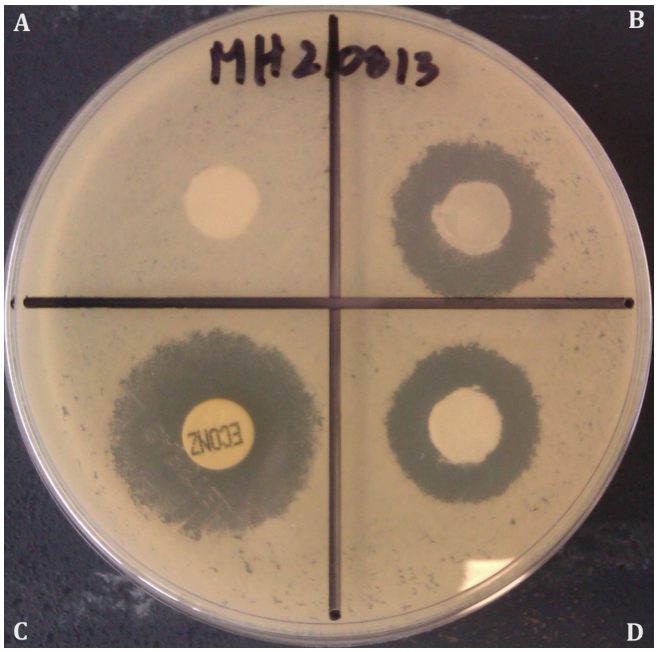


Figure 5: Antifungal disk diffusion test on *C. albicans* SC5314 containing textile placebo (imprinted with blank formulation) (A), Pevaryl® formulation (71 µg ECN) on a filter paper (B), soluble ECN 10 µg standard disks (C), and ECN textile formulation (71 µg ECN) (D).

Experimental conditions were first optimized with the *C. albicans SC5314* strain using the antifungal disk diffusion test [30]. Figure 5 shows a representative setup for the experiment; ECN imprinted textiles were compared to ECN standard disks, Pevaryl® deposited on filter paper, and placebo composed of textiles imprinted with a blank formulation (*i.e.* same composition, without ECN). The textile formulation demonstrated an inhibition zone corresponding to 81% of that of the commercial formulation on SC5314. The same experiment was repeated on a combination of 4 other *C.*

411 *albicans* strains and the ECN textiles exhibited an inhibition of 93% (Table 4).
412 These results showed that ECN maintained a roughly comparable activity on
413 textile as compared to the cream formulation. The slightly lower activity may
414 be due to the lower diffusion of ECN from the textile support. Pevaryl® might
415 also exhibit a slightly better efficacy because of its nanometer particle size
416 range, which increases surface area and facilitates diffusion, resulting in
417 increased drug activity [30]. These results were confirmed by assessing other
418 *Candida* species. ECN activity of ECN textiles reached 91%, 84% and 91% of
419 the activity of the commercial formulation on *C. lusitaniae, C. kefyr* and *C.*
420 *glabrata*, respectively (Table 4). The activity was less important for *C. krusei*,
421 which often demonstrates an intrinsic resistance to azole drugs [31].
422
423 Table 4: Inhibition zone diameter (IZD) of ECN textile and Pevaryl® on
424 *Candida spp* and *Trichophyton* species after 18 hours at 32°C

Strain	Replicata	IZD of ECN Textile (mm)	IZD of Pevaryl® (mm)	Ratio Textile/ Pevaryl® (%)
C. albicans (SC5314)	n=27	18.8±0.9	23.1±2.5	81
C. albicans (4 Nantes Isolates)	n=8	21.7±1.0	23.4±1.9	93
C. lusitaniae	n=4	18.5±0.7	20.3±0.4	91
C. kefyr	n=8	23.6±3.2	28.1±2.4	84
C. glabrata	n=8	18.3±5.9	19.5±4.2	92

T. mentagrophytes	n=14	24.8±0.7[a]	39.8±3.2[a]	62[a]
T. rubrum	n=3	29±1.7[b]	47±4.2[b]	62[b]

[a] IZD of textile formulation on *T. mentagrophytes* were measured after 4 days at 25°C

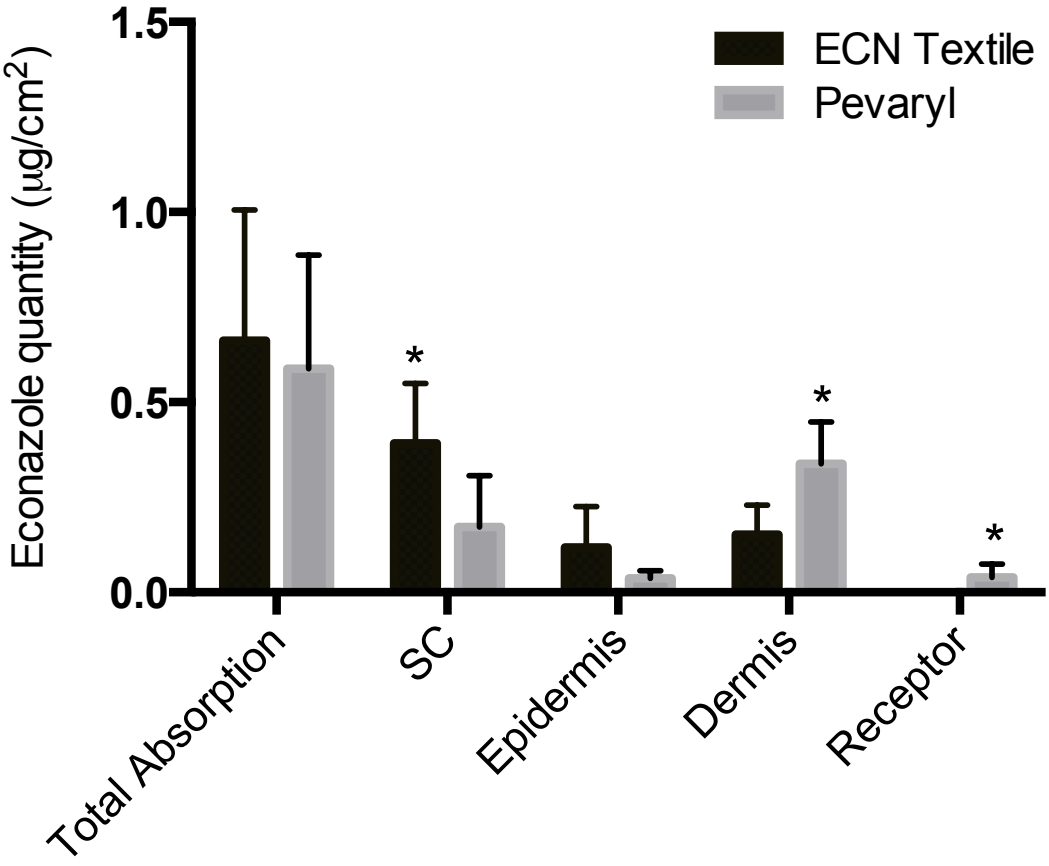
[b] IZD of textile formulation on *T. rubrum* were measured after 7 days at 25°C.

Finally, inhibition of dermatophytes growth was examined on the two most prevalent species in fungal skin infections, namely *Trichophyton mentagrophytes* and *T. rubrum* [12, 13] (Table 3). In these latter tests, the activity of ECN reached 62% of the commercial formulation activity. This lower result could be explained by the temperature (25°C) at which the experiment was conducted. This temperature was selected for dermatophytes to grow several days *in vitro*, but is lower than the fusion temperature of the microparticles. At this temperature, ECN has a limited release, as shown in figure 3, so that the effective concentration of ECN might have been lower than the commercial formulation. Although the experimental conditions were not adapted for the pharmaceutical textiles, similar results were obtained by Hammer's et *al.* They concluded that *T. mentagrophytes* and *T. rubrum* were less susceptible than *C. albicans* when exposed to antifungal textiles [13].

Overall, these results demonstrate that ECN textiles maintain their antifungal activity against all azole-susceptible *Candida* species tested *in vitro*. Although *in vitro* disk diffusion tests are not designed to test the long-term efficacy of

445 controlled release products like the ECN textiles but rather immediate release,
446 this newly developed technology of imprinted textiles was shown to preserve
447 the pharmaceutical activity of the antifungal drug.
448
449 **3.3 Percutaneous absorption of ECN**
450 The impact of the lipid microparticle formulation on percutaneous absorption
451 was examined *ex vivo* by comparing the ECN textile to the commercial
452 formulation. Pig skin has been reported as a good model for skin percutaneous
453 absorption [32]. Commercial formulation was weighed on a filter paper to
454 provide the same ECN amount as ECN textile. Both ECN formulations were
455 applied upside down on pig skin for 24 h with the receptor fluid temperature
456 maintained at 37°C in the Franz diffusion cells. ECN was quantified by HPLC-
457 MS/MS in the different layers of the skin: SC, epidermis, dermis, and receptor
458 compartment (Figure 6). Overall percutaneous absorption was similar for both
459 formulations, showing that both systems diffused similar quantities of ECN.
460 This was in agreement with the comparable antifungal activity of both
461 treatments on *Candida* species (Table 4). Pevaryl's diffusion may be slightly
462 underestimated due to some absorption of ECN on the paper support.
463 Nevertheless, this partition would not have significantly impacted the total
464 release of econazole, which was less than 1% of the ECN loading. Other stimuli,
465 like friction, might further enhance drug delivery upon contact with the skin.

466 Nevertheless, this reservoir effect of the textile was already observed for other
467 cosmetotextiles [8].



468

469
470 Figure 6: ECN content after 24 hour in Franz cells diffusion test on pig skin
471 using ECN textile (black bars) and Pevaryl® on filter paper (grey bars).
472 Receptor compartment was set at 37°C. ECN quantification was done by LC-
473 MS/MS. Mean ± standard deviation (n=4). Asterisks indicate statistically
474 significant values ($p < 0.05$).

475

476 ECN skin distribution was slightly different between the ECN textile and the
477 commercial cream on filter paper. ECN from textiles was mainly distributed in
478 the SC and penetrated less in the epidermis and dermis, whereas ECN from the
479 commercial formulation was mainly found in the dermis and was even
480 quantifiable in the receptor compartment (Figure 6). This difference might be
481 attributed to the particle size of both formulations. Indeed, Pevaryl® was
482 determined to be a nano suspension whereas the particles deposited on the
483 textiles were in the micron range (Figures 1&2). Nanometer formulations have
484 been reported to penetrate deeply into the dermis, and have also been used for
485 transcutaneous absorption of drugs [21]. In our case, the micrometer particles
486 were designed to concentrate into the upper layer of the skin, where the fungal
487 infections develop. Moreover, their lipid composition is thought to improve
488 their affinity for the skin tissues, resulting in a higher concentration of ECN in
489 the SC than the commercial formulation. This suggests that the pharmaceutical
490 textiles allowed for targeted delivery of active drug in the upper skin layers, as
491 observed for cosmetotextiles using microspheres [8, 21]. No ECN was
492 measured in the receptor compartment with the ECN textile, indicating that
493 systemic exposure was limited as compared to Pevaryl®, which helps limiting
494 undesirable effects.
495
496 **3.4** ***Activity in a cutaneous candidiasis murine model***

497 The antifungal activity of ECN textiles was evaluated by challenging animals
498 with superficial fungal infection induced by *C. albicans*. The clinical strain used
499 for the experimental murine model was confirmed to be responsive to
500 econazole by the diffusion test (IZD = 25 mm and 31 mm for ECN textile and
501 commercial cream, respectively). ECN cream was applied daily, and textiles
502 (placebo or ECN) were replaced daily for 5 days. After 5 days of treatment,
503 effective infection by *C. albicans* was observed in the control group by
504 retroculture of biopsy samples (Figure 7). In contrast, the treatment with the
505 ECN textiles led to an important reduction of the yeast cutaneous burden,
506 which was also observed in the group treated with the ECN cream. This study
507 also highlighted that the textiles alone helped reducing of the burden.
508 However, no statistical significance could be concluded from this experiment,
509 since few mice from the control group were cured without any treatment,
510 probably because of incomplete immunosuppression. Nevertheless, wound
511 observation before and after treatment revealed that ECN textiles were well
512 tolerated and helped reducing the fungal burden without causing any irritation
513 (Figure 8).
514

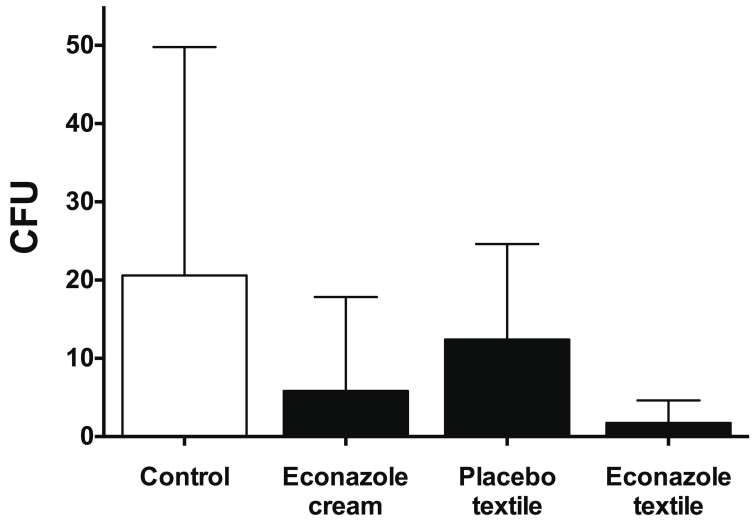


Figure 7. *In vivo* antifungal efficacy of pharmaceutical textiles, commercial cream and placebo textiles. Evaluated by quantification of the fungal burden (CFU) in skin biopsy samples. Mean ± standard deviation (n=6).

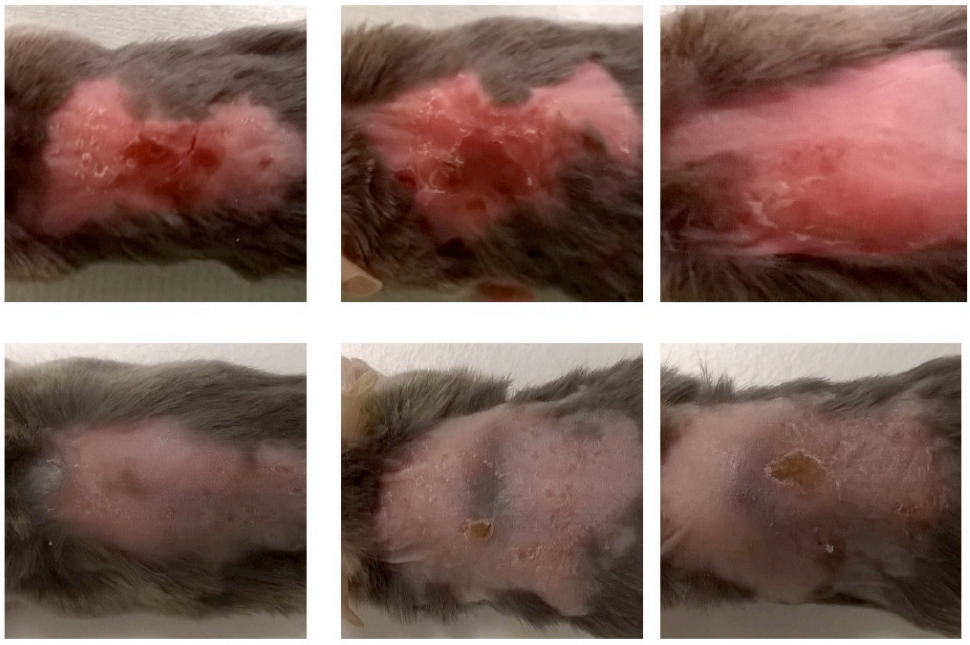


Figure 8: Representative cutaneous pathology pictures before (upper pannel) and after treatment with ECN textiles, ECN cream or placebo textile (bottom pannel).

4. Conclusion

This study was aimed at assessing pharmaceutical textiles using ECN as a model drug for skin diseases. The technology of pharmaceutical textiles lies in a dual innovation. Firstly, the thermo-sensitive microparticle formulation ensured stability during storage and triggered thermo-sensitive release upon contact with skin. The lipid microparticles allowed skin diffusion and drug distribution within the upper layers of the skin, which is optimal to treat superficial fungal infections and to prevent from systemic absorption.

Secondly, the adapted screen-printing method adapted to the microparticle drug formulation was able preserved the pharmaceutical activity of the drug. ECN efficacy was maintained *in vitro* on a broad range of fungi strains and *in vivo*, ECN textiles enabled high therapeutic efficacy against cutaneous candidiasis in mice. Overall, these results revealed the potential this technology to develop pharmaceutical textiles for the treatment of superficial infections. Such textiles could be developed in bandages or socks, which, through ease of use, would improve patient compliance.

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Conflict of interest.

This work has been partially founded by Biomod Concepts Inc. The patented technology belongs to Karine Théberge and Biomod Concepts Inc. All the analyses and characterizations have been performed at the University of Montreal or Nantes, independently from the company.

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