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1 **Econazole Imprinted Textiles with Antifungal Activity**

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20 **Keywords**

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

23 **Abstract**

24 In this work, we propose pharmaceutical textiles imprinted with lipid
25 microparticles of Econazole nitrate (ECN) as a mean to improve patient
26 compliance while maintaining drug activity. Lipid microparticles were
27 prepared and characterized by laser diffraction ($3.5\pm 0.1\ \mu\text{m}$). Using an
28 optimized screen-printing method, microparticles were deposited on textiles,
29 as observed by Scanning Electron Microscopy. The drug content of textiles
30 ($97\pm 3\ \mu\text{g}/\text{cm}^2$) was reproducible and stable up to 4 months storage at
31 $25^\circ\text{C}/65\%$ Relative Humidity. Imprinted textiles exhibited a thermosensitive
32 behavior, as witnessed by a fusion temperature of 34.8°C , which enabled a
33 larger drug release at 32°C (temperature of the skin) than at room
34 temperature. *In vitro* antifungal activity of ECN textiles was compared to
35 commercial 1% (w/w) ECN cream Pevaryl[®]. ECN textiles maintained their
36 antifungal activity against a broad range of *Candida* species as well as major
37 dermatophyte species. *In vivo*, ECN textiles also preserved the antifungal
38 efficacy of ECN on cutaneous candidiasis infection in mice. *Ex vivo*
39 percutaneous absorption studies demonstrated that ECN released from
40 pharmaceutical textiles concentrated more in the upper skin layers, where the
41 fungal infections develop, as compared to dermal absorption of Pevaryl[®].

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

45

46 **1. Introduction**

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

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

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

79 Econazole Nitrate (ECN) is currently marketed for the treatment of
80 vaginal candidiasis and topical fungal infections as a cream formulation [15,
81 16]. It has demonstrated antifungal activity against *Candida* and
82 dermatophytes species [15-17]. Encapsulation of ECN in lipid particles [18],

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

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

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

106

107 **2. Experimental methods**

108 **2.1. Materials**

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

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

128 **2.2 Organisms**

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

139 **2.3. Preparation of ECN textiles**

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

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

150 **2.4. Characterization of microparticles**

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

160 Fusion temperature of the microparticles imprinted on textile was measured
161 using thermal analysis based on heat-leak-modulus (TA-HLM) [28]. With TA-
162 HLM, textile samples are wrapped around a sensor probe and heated. The
163 samples of ECN-loaded textile (2.5 x 5 cm) were analyzed at a heating rate of
164 0.8°C per second and heated from 0°C to 100°C. Measurement was repeated 3
165 times.

166 **2.5 HPLC-UV analysis**

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

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

175 The flow rate was 1.4 mL per minute. The column temperature was set to 35°C.

176 The injection volume was 20 μ L. ECN was analyzed at 220 nm. ECN retention
177 time was 8.7 minutes. The limit of quantification with this method is 9 μ g/mL.

178

179 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

180

181 **2.6 HPLC-MS/MS method**

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

191

192 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 ₂ O 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-cm² 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-cm²
 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**

208 Disks of 0.79 cm² (10 mm diameter, containing 71 µg of ECN) were cut out of
209 the imprinted textile. *In vitro* release was performed using Franz cells from
210 PermGear Inc. (Hellertown, PA, USA) with an opening of 9 mm in diameter, 5
211 mL receptor size and a thermostated jacket. Diffusion tests were carried on
212 semi-permeable polycarbonate membranes of 0.6 µm in pore size. The
213 receptor fluid composition was optimized to ensure ECN diffusion was not
214 limited by ECN solubility. Although ECN was not soluble at pH 7.4, its solubility
215 was improved in 10 mM phosphate buffer solution (PBS) with 1.37 mM of NaCl
216 at pH 4.5 with PEG-400 (70:30 v/v) [29]. Indeed, Pevaryl diffusion (20 mg) on
217 Franz cells using a semi-permeable polycarbonate membranes of 0.6 µm in
218 pore size after 6h at 32°C, ECN solution recovery was 49.5%±1.3 and
219 13.5%±1.3 with and without PEG, respectively (n=3 for each condition, data
220 not shown). The receptor fluid (10 mM phosphate buffer solution (PBS) with
221 1.37 mM of NaCl at pH 4.5 with PEG-400 (70:30 v/v)) was thermostated at 22°C
222 or 32°C and was constantly stirred at 100 rpm. Samples of 400 µL were
223 withdrawn at 30 minutes, 1, 2, 3, 4, and 6 hours, filtered and replaced with the
224 same volume of receptor fluid. Samples were diluted 1:50 in a mixture of 20%
225 acetonitrile: 80% H₂O with 0.1% FA before quantification by HPLC-MS/MS.

226 **2.10 Antifungal Disk Diffusion tests**

227 *C. albicans* SC5314 and *Candida* strains from Nantes were routinely grown at
228 30°C in yeast peptone dextrose (YPD; 1% yeast extract, 2% Bacto peptone, 2%

229 dextrose plus 2% agar for solid medium) and Sabouraud (SB) culture medium
230 respectively. *C. albicans* SC5314 were suspended in liquid YPD medium to an
231 OD₆₀₀ of 0.1, and 150 µL of the cell suspension were spread on YPD Petri dishes
232 (10 cm diameter). Disks of 0.79 cm² (10 mm diameter, containing 71 µg of ECN)
233 were cut out of the imprinted textile, and equivalent quantity of Pevaryl® (7.1
234 mg Pevaryl®, 71 µg ECN) was weighed on a filter paper disk (10 mm in
235 diameter). Placebo textiles imprinted with drug-free microparticles and
236 standard 10-µg ECN disks were used as controls. All disks were placed on the
237 YPD Petri plates. The plates were incubated at 32°C, and growth inhibition
238 diameters were measured at 18 h. This was replicated 9 times for each
239 formulation and repeated 3 times independently.

240 Other *Candida spp* isolates were suspended in sterile saline (0.85% NaCl) to
241 achieve 1 x 10⁶ cells per mL, which were deposited onto SB Petri dishes (10 cm
242 in diameter). Disks of 0.50 cm² (8 mm in diameter, containing 50 µg of ECN)
243 were cut out of the imprinted textile, and equivalent quantity of Pevaryl® (5.0
244 mg Pevaryl®, 50 µg ECN) was weighed on a filter paper disk (8 mm in
245 diameter). Placebo textiles (8 mm in diameter) and standard 10-µg ECN disks
246 were used as controls. All disks were placed on the SB Petri plates. The plates
247 were incubated at 35°C and growth inhibition diameters were measured at
248 18h. This was replicated 4 times for each formulation and repeated 2 times
249 independently.

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

258 **2.11 Ex vivo diffusion test on pig ear skin**

259 Pig ears were kindly provided by Dr. Fairbrother from the Veterinary
260 Department of Université de Montréal (Saint-Hyacinthe, QC, Canada). Ears
261 were washed with water and 1% SDS and shaved using a razor. The skin was
262 gently peeled off from the dorsal region of the ears, washed again with 1% SDS
263 and water and stored at -20°C until the next day. Skin diffusion tests were
264 performed using Franz cells as described above. The receptor fluid was
265 identical to *in vitro* release studies. It contained 10 mM PBS at pH 4.5 with 30%
266 PEG-400, to ensure ECN solubility, and was stirred at 100 rpm [29]. The
267 receptor compartments were heated to 37°C to help maintain the surface of
268 the skin at 32°C. Disks of 0.79 cm² (10 mm in diameter, containing 71 µg of
269 ECN) were cut out of the imprinted textile, and equivalent quantity of Pevaryl®
270 (7.1 mg, 71 µg ECN) was weighted on a filter paper disk (10 mm in diameter).

271 The disks were applied upside down on the surface of the skin, so that the
272 formulation was in direct contact with the skin. A small weight (5 g) was
273 applied in order to ensure contact between the formulation and the skin
274 surface. The system was dismantled after 24 hours and all the receptor fluid
275 was collected. The used textiles and filter paper disks were collected for ECN
276 quantification. The skin surface was washed with 8 mL of ethanol/water
277 (50:50), which was collected for ECN quantification. The skin was separated in
278 3 layers: stratum corneum (SC), epidermis and dermis. The SC was removed
279 using 20 strips of 1 cm² 3M tape, which was extracted with 20 mL of
280 acetonitrile. Epidermis was peeled off from the dermis after heating at 80°C for
281 10 seconds. Both epidermis and dermis were cut into pieces and were
282 suspended in 1 mL acetonitrile. All samples were sonicated for 30 min then
283 heated at 55°C for 4 hours. Liquid layers were filtered and diluted with 20%
284 acetonitrile with 0.1% FA. Samples were diluted (1:20 for epidermis, 1:200 for
285 residual formulation on filter paper disks and textiles, and 1:10 for washing,
286 SC, dermis, and receptor fluid) and quantified by HPLC-MS/MS. The Overall
287 recovery of ECN (sum of residual textile, washing, SC, epidermis, dermis) was
288 97.7% ± 5.7 (n = 9). Skins from 3 different pig ears were tested in triplicate
289 each (n = 9).

290 **2.12 Cutaneous candidiasis model in immunosuppressed mice**

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

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

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

311 **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 $90 \pm 19 \mu\text{g per cm}^2$. 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 25°C/65% RH
332 (Table 3). After 4 months, ECN content was still $97 \mu\text{g/cm}^2$, which represents
333 108% of the initial content. Altogether, the reproducibility of the production

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

336

337 Table 3: ECN content of pharmaceutical textiles upon storage at 25°C/65%RH.

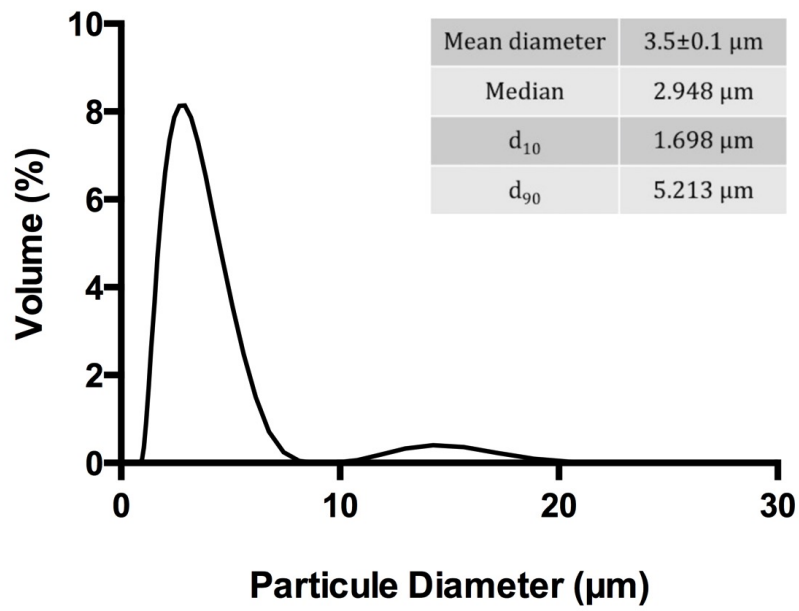
338 T₀: After screen-printing. (n = 9)

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

339

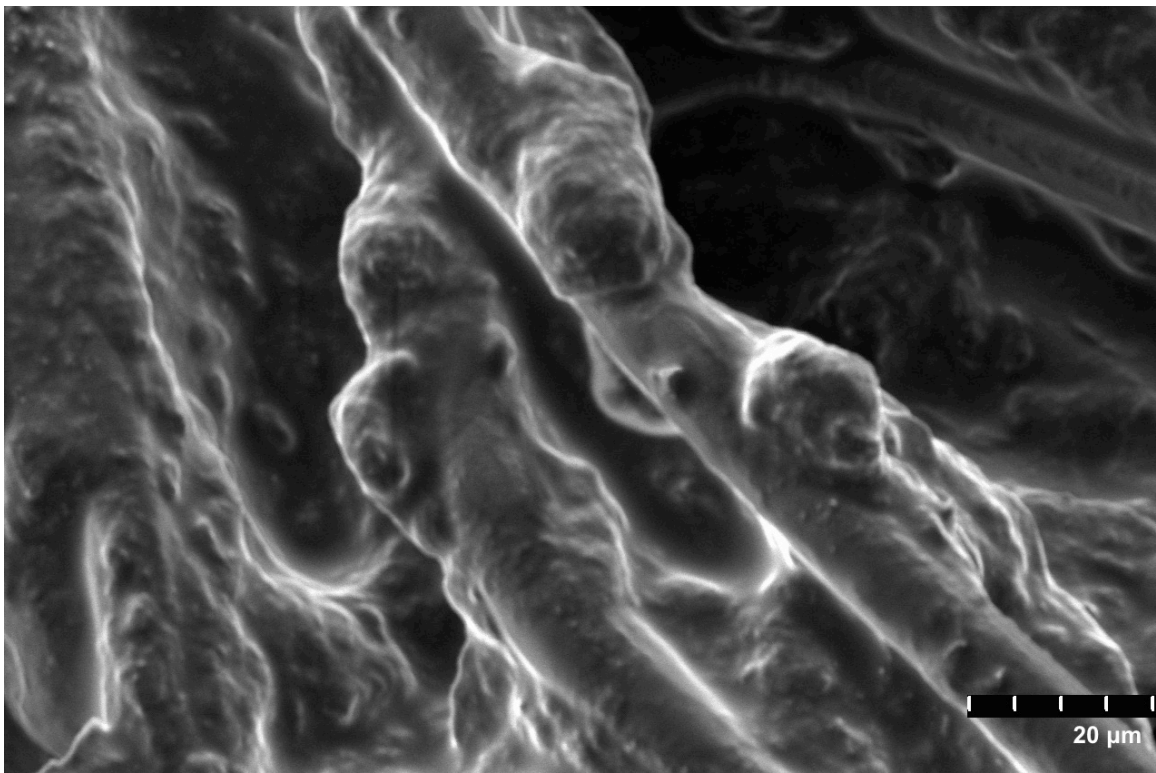
340 In a second step, physicochemical properties of the microparticles were
341 examined. Microparticle size was evaluated before deposition on textile using
342 laser diffraction (Figure 1). Particles exhibited micro-range diameter (3.5±0.1
343 µm). Pevaryl® particle size was also measured by dynamic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

351



352
353
354
355

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



356

357 Figure 2: Scanning electron microscopy image of ECN pharmaceutical textile.

358 Scale = 20 μm .

359

360 Lipid microparticles deposited on textile exhibited a fusion temperature of

361 34.8°C, as determined by TA-HLM, which confirmed their solid character at

362 room temperature (Figure 3). The latter is a method similar to differential

363 scanning calorimetry, which allows for the measurement of fusion

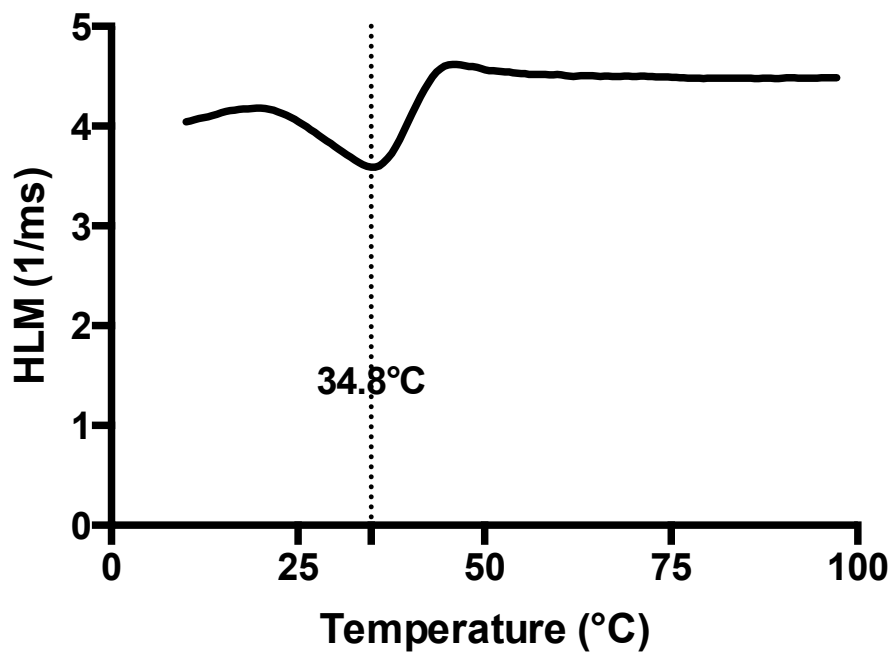
364 temperature of microparticles deposited on a textile surface [28]. This fusion

365 temperature value is crucial to allow triggered release upon contact with the

366 skin, which is at approximately 32°C, while maintaining a good stability upon

367 storage at room temperature.

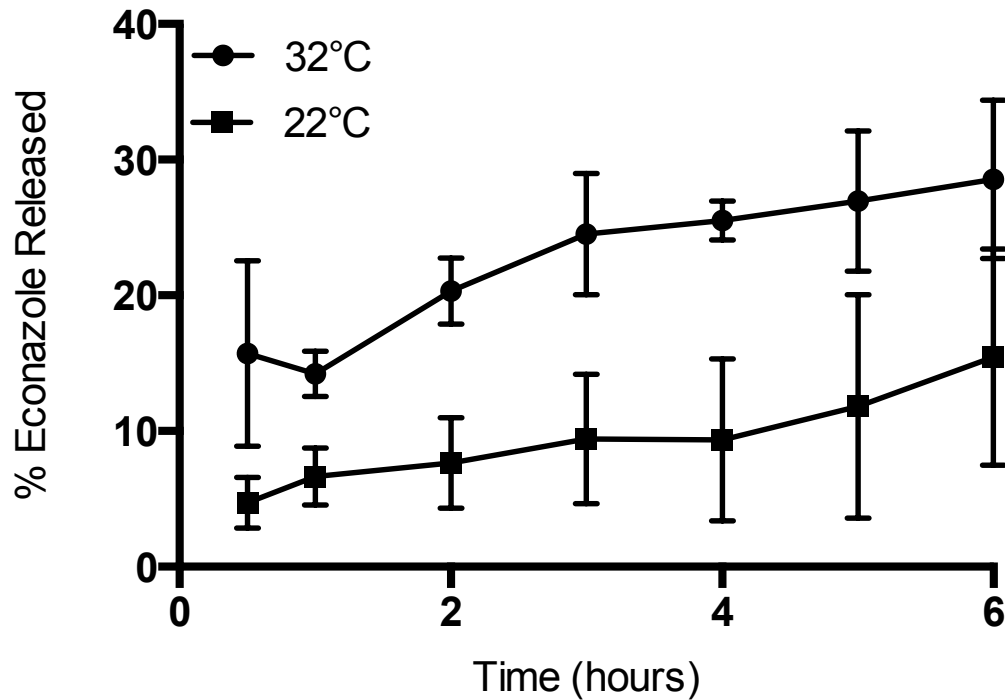
368



369

370 Figure 3: TA-HLM of ECN textile.

371
372 The thermo-sensitive behavior of the pharmaceutical textiles was further
373 confirmed by the drug release profiles obtained at room temperature and at
374 32°C in Franz diffusion cells (Figure 4). As expected, release at 32°C was higher
375 and faster than at room temperature. Indeed, the textiles released about twice
376 the amount of ECN at all time points (although no significant difference was
377 seen at 6 hours due to larger error bars). Raising the temperature to 32°C, close
378 to the fusion temperature of the microparticles, initiated the
379 fluidification/fusion of the microparticles and allowed ECN diffusion from the
380 textile. It can be noted that no increase in ECN concentration in receptor fluid
381 was observed after 24h (data not shown), indicating that the maximum release
382 was reached within 6 hours. This suggested the textiles served as a reservoir
383 for ECN, since less than $29\pm 6\%$ of the total ECN was released.



384

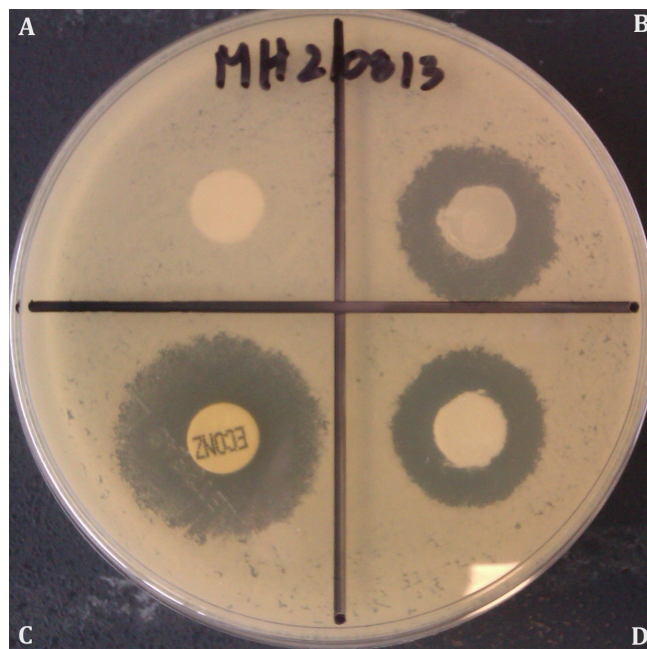
385

386 Figure 4: ECN release from pharmaceutical textiles at 22°C (black squares) and
 387 32°C (black circles) on Franz diffusion cells through polycarbonate
 388 membranes. Mean \pm standard error bars (n=4).

389

390 3.2 Antifungal activity of pharmaceutical textiles

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



397

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

402

403 Experimental conditions were first optimized with the *C. albicans* SC5314
404 strain using the antifungal disk diffusion test [30]. Figure 5 shows a
405 representative setup for the experiment; ECN imprinted textiles were
406 compared to ECN standard disks, Pevaryl® deposited on filter paper, and
407 placebo composed of textiles imprinted with a blank formulation (*i.e.* same
408 composition, without ECN). The textile formulation demonstrated an
409 inhibition zone corresponding to 81% of that of the commercial formulation
410 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® (%)
<i>C. albicans</i> (SC5314)	n=27	18.8±0.9	23.1±2.5	81
<i>C. albicans</i> (4 Nantes Isolates)	n=8	21.7±1.0	23.4±1.9	93
<i>C. lusitaniae</i>	n=4	18.5±0.7	20.3±0.4	91
<i>C. kefyr</i>	n=8	23.6±3.2	28.1±2.4	84
<i>C. glabrata</i>	n=8	18.3±5.9	19.5±4.2	92

<i>T. mentagrophytes</i>	n=14	24.8±0.7 ^a	39.8±3.2 ^a	62 ^a
<i>T. rubrum</i>	n=3	29±1.7 ^b	47±4.2 ^b	62 ^b

425 ^a IZD of textile formulation on *T. mentagrophytes* were measured after 4 days
426 at 25°C

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

428

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

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

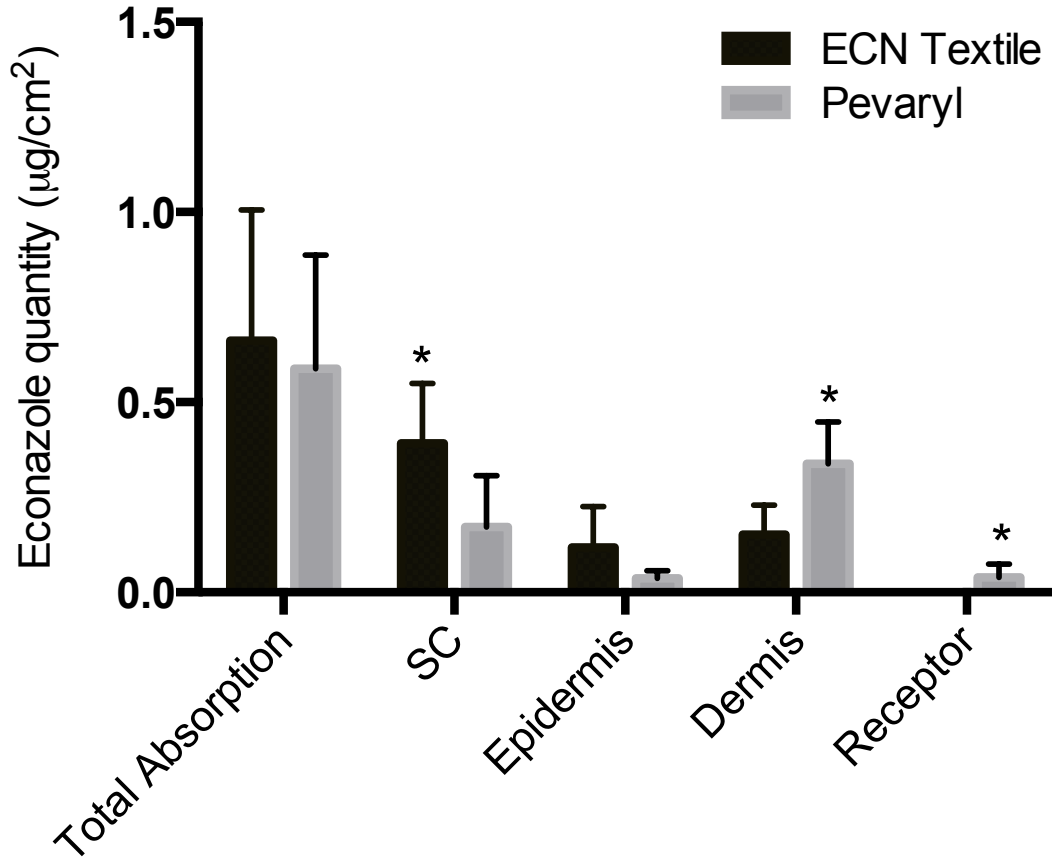
445 controlled release products like the ECN textiles but rather immediate release,
446 this newly developed technology of imprinted textiles was shown to preserve
447 the pharmaceutical activity of the antifungal drug.

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

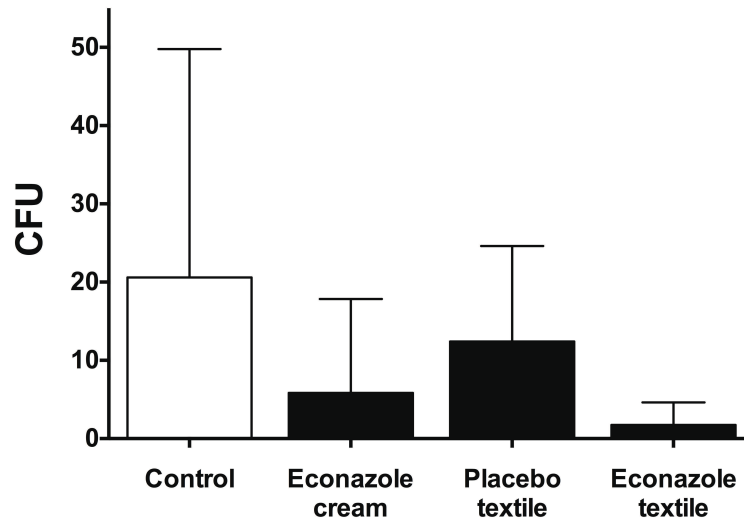
476 ECN skin distribution was slightly different between the ECN textile and the
477 commercial cream on filter paper. ECN from textiles was mainly distributed in
478 the SC and penetrated less in the epidermis and dermis, whereas ECN from the
479 commercial formulation was mainly found in the dermis 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



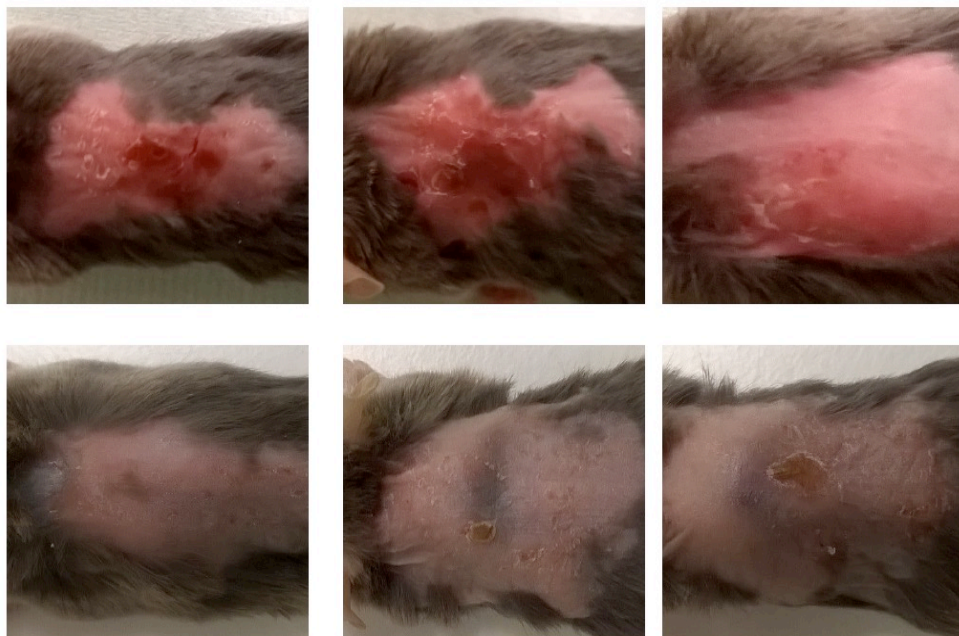
515

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

519

520

521



Econazole textile

Econazole cream

placebo

522

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

526

527 **4. Conclusion**

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

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

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548 **Conflict of interest.**

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

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