Dissolving microneedle for intradermal delivery of amphotericin B

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Abstract

Amphotericin B, a biopharmaceutics classification system (BCS) IV drug, is a highly potent antifungal agent with minimal resistance. Unfortunately, the topical delivery of amphotericin B is difficult due to its limited solubility and poor permeation profile. Thus, an efficient intradermal delivery system is highly sought after for the treatment of prevalent skin fungal infections. The aim of this study is, therefore, to explore the potential of a novel dissolving polymeric microneedle patch to deliver amphotericin B intradermally. The patch (16 × 16 needles, 850 µm height) was cast using aqueous blends of poly(vinyl alcohol) and poly(vinyl pyrrolidone) and contained drugs only in the tips with an amount of 2.80 ± 0.34 mg. This patched showed sufficient mechanical properties to withstand compression and reached an insertion depth of 301.34 ± 46.86 µm in the porcine skin. Moreover, after 24 hours' application, it demonstrates a high drug deposition of 271.40 ± 46.14 µg/cm^2 amphotericin B *in vitro*. Dermatokinetic profiles indicated that this patch delivered amphotericin B mainly into the dermis layer and achieved an AUC_{0-24} for the dermis was 3562.0 ± 223.2 h·µg/cm^2 during a 24-hour application. Furthermore, the antifungal effects of the patches were demonstrated effective against *Candida albicans* both in *in vitro* agar plates and in an *ex vivo* infected porcine skin model. Accordingly, this dissolving microneedle patch containing amphotericin B could be promising to combat skin fungal infections. Moving on, the system will be tested in an animal model.

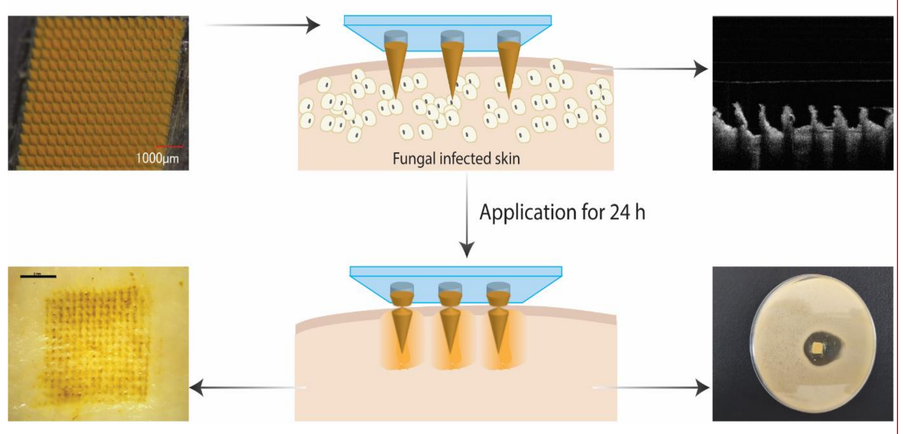
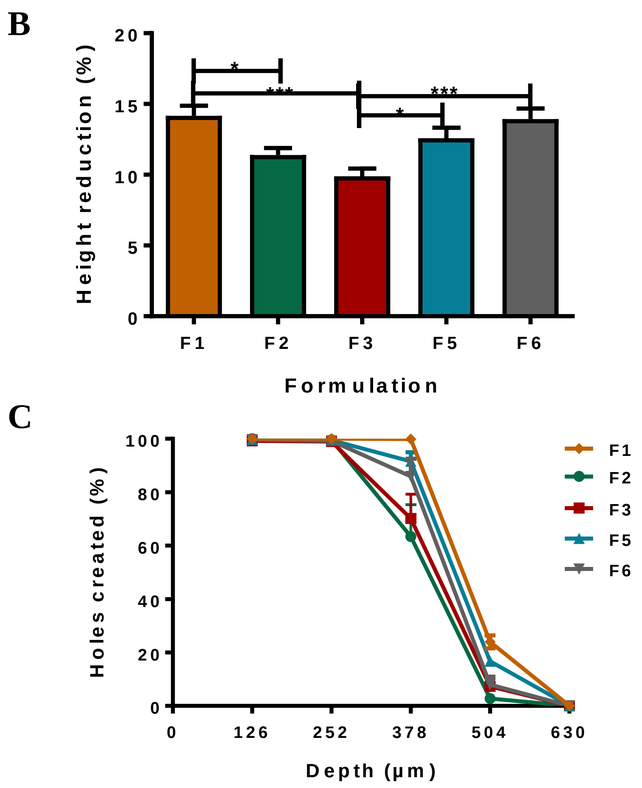


Figure 1. A dissolving microneedle system was developed to deliver amphotericin B for treating fungal infected skin. The system could deliver great amount of amphotericin B in the skin in the dermal tissues and showed antifungal effects.

A

Formulation code	Composition% (w/w) Amphotericin B	PVP	PVA	Patch morphology	Drug content (mg/patch)
F1	20	10	10		1.58 ± 0.17
F2	30	10	10		2.37 ± 0.46
F3	40	10	10		2.80 ± 0.34
F4	50	10	10		—
F5	40	20	0		3.06 ± 0.64
F6	40	0	20		2.99 ± 0.21



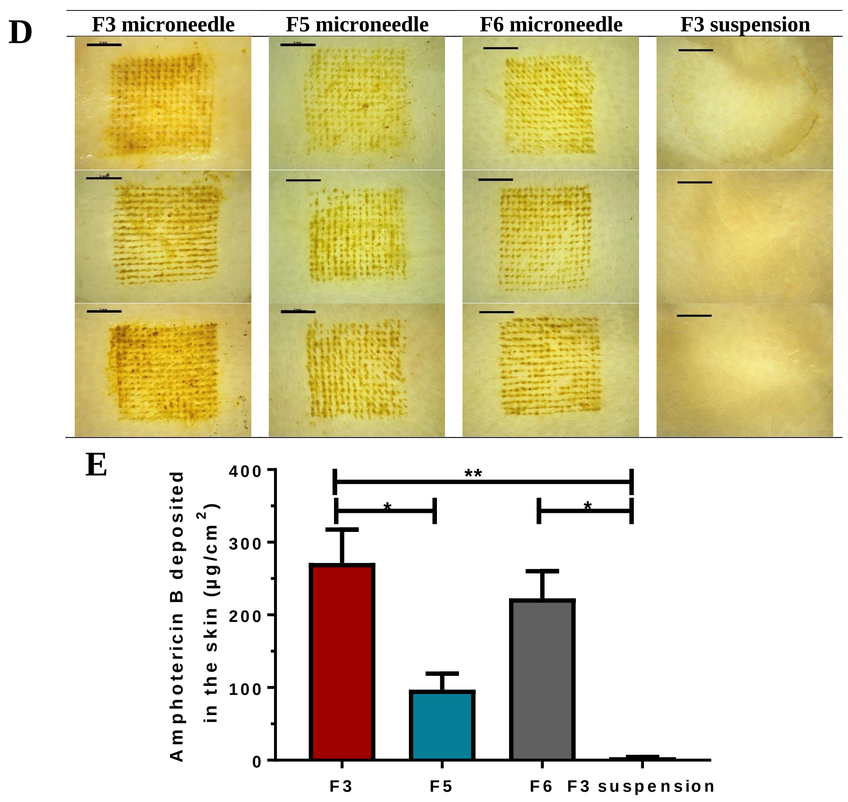


Figure 2. Formulation optimization.
A. The formulation used to fabricate the microneedle tips, the patches formulated and the drug content of the fabricated patches.
B. The height reduction of the fabricated patches after applying a compression force of 32 N. (means + SD, n = 90; *p < 0.05, ***p < 0.001).
C. Percentage of holes created in Parafilm® M layers after applying an insertion force of 32 N per array for all the fabricated patches. (means ± SD, n = 3).
D. Skin deposition images after applying F3, F5, F6 microneedle patches and F3 suspension in the porcine skin for 24 h (n = 3). Scale bar = 2 mm.
E. Skin deposition results of F3, F5, F6 microneedle patches and F3 suspension in the porcine skin. (means + SD, n = 4; *p < 0.05, **p < 0.01).
So, F3 microneedle patches were taken into further investigation.

Figure 3. Formulation characterisation of F3 microneedle patches. *Ex vivo* dermatokinetic profiles in the epidermis (A) and in the dermis (B) of amphotericin B after applying F3 microneedle patches in the skin. (Means ± SD, n = 4).
C. Representative optical coherence tomography (OCT) picture immediately after the application of F3 microneedle patches applied to the porcine skin. Scale bar = 1 mm.
D. *Ex vivo* multiphoton fluorescence images of the porcine skin after applying F3 microneedle patches for 24 h. Three-dimensional image for visualising the distribution of amphotericin B inside the skin tissue.

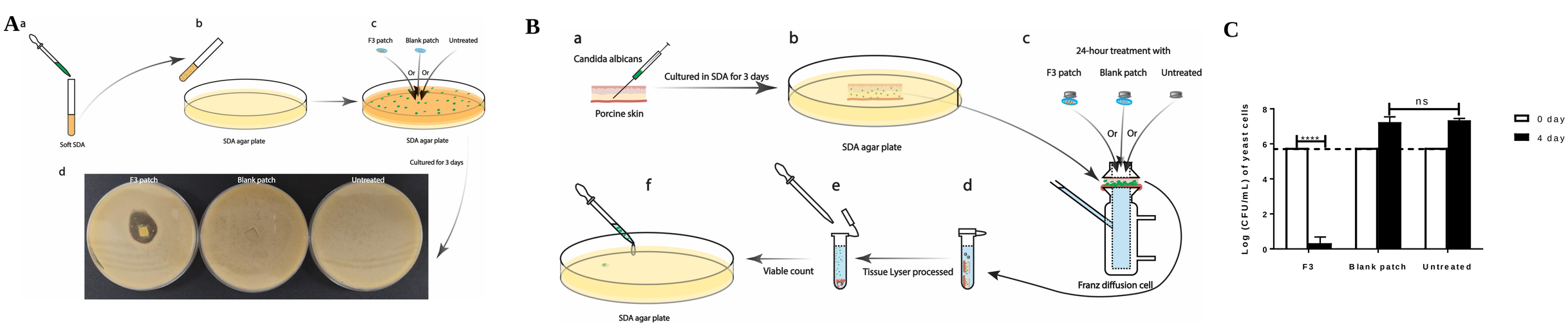


Figure 4 Antifungal performance of F3 microneedle patches.
A. Disk diffusion tests were performed as shown in step a, b, and c. After 3 days' culture, the growth of fungi results of F3 microneedle patches, Blank patches and untreated were shown in d, respectively.
B. Ex vivo antibiofilm studies in *Candida albicans* infected porcine models. (a) An aliquot of 100 µL of *Candida albicans* culture ((6 × 10^6 CFU/mL) was injected subcutaneously to a well-trimmed, disinfected porcine skin. (b) The infected porcine skin was cultured for 3 days on SDA plates. (c) The infected skins were glued on a Franz cell donor compartment and were treated with F3 microneedle patches containing amphotericin, or blank patches without amphotericin B or with only a metal weight. (d) After 24-hour treatment, the skin was collected and soaked in 1 mL of sterile PBS. (e) The skin samples were processed and crashed using a Tissue Lyser to harvest the fungi cells inside. (f) Viable counts were performed for the skin sample mixture.
C. The viable counts results of the F3 microneedle patches, blank patches and untreated group on Day 0 (the injected amount) and Day 4. (Means + SD, n = 3; ****p < 0.0001, ns: no significant difference)