DISSOLVING MICRONEEDLES FOR INTRADERMAL DELIVERY OF AMPHOTERICIN B

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Background: Skin fungal infections are among the most prevalent infectious diseases observed in clinics. Traditional topical treatments suffer from limited permeation. Amphotericin B, an effective antifungal agent with poor solubility, was loaded into a dissolving microneedle system to treat skin fungal infections in this study.

Methods: Amphotericin B dissolving microneedles (ADM) were fabricated by a mould casting technique. The tips were cast by a drug gel mixture of amphotericin B, poly(vinylpyrrolidone) (PVP) and poly(vinyl alcohol) (PVA) (MW 9 – 10 kDa). Excess drug gel was removed after filling the cavities of the moulds. The tips were dried overnight in the microneedle moulds. The baseplates were cast using a gel containing PVP and PVA (MW 31 – 50 kDa). The ADMs were optimized and characterized in terms of height reduction rate after compression of 32 N for 30 s and insertion depth both in the Parafilm® layers (PF) and in the porcine skin. The skin deposition and dermatokinetic profiles were evaluated using Franz cell setup. The release profile of ADM tips was obtained. Thermal gravimetric analysis (TGA), differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR) and powder X-ray diffraction (PXRD) were performed for ADM tips, amphotericin B and physical mixtures. Antifungal performances of ADM were demonstrated using a disk diffusion test and in an *ex vivo* model of biofilm on the porcine skin.

Results: The ADM was an array of 16×16 needles arranged on a 0.40 cm^2 area with 850 μm needle height (600 μm pyramidal tip, 250 μm base column). Amphotericin B was located just in the tips of ADM. ADM contained 2.80 ± 0.34 mg of amphotericin B per patch. The mechanical characterisation revealed the height reduction after compression of 32 N was lower than 10%. The insertion depth of ADM in PF was between 378 and 504 μm and in porcine skin was 301.34 ± 46.86 μm. After 24 hours' application in the porcine skin, the drug deposition of ADM reached 271.40 ± 46.14 μg/cm^2. Dermatokinetic profile in the epidermis layer of the skin increased rapidly in the first 3 hours, slightly increased until 24 hours, and achieved an $AUC_{0\text{-}24}$ of 1008.0 ± 104.7 h.μg/cm^2. Dermatokinetic profile in the dermis layer displayed a similar trend and the $AUC_{0\text{-}24}$ for the dermis was 3562.0 ± 223.2 h.μg/cm^2. Amphotericin B was released completely from ADM tips in the fourth day and exhibited a 1.6 times faster dissolution rate within 4 days compared to the amphotericin B powder. The results of TGA, DSC, FTIR, and PXRD demonstrated no interaction between drugs and excipients and reduced crystallinity in ADM tips, which explained the release data. ADM showed remarkable inhibition of *Candida albicans* in the disk diffusion test with a zone area of 437.2 ± 135.4 mm^2, which equals a circle with a radius of 11.6 ± 2.6 mm. Antibiofilm activity of ADM revealed that after 24-hour treatment with ADM, the number of fungi cells inside the porcine skin was reduced from 6×10^6 CFU/mL to 2.7 ± 2.1 CFU/mL. The fungi viabilities were much reduced following 24h of administration of ADM ($p < 0.00001$). The killing rate of ADM against *Candida albicans* in the *ex vivo* porcine model reached 100%.

Conclusions: This study reports the successful incorporation of amphotericin B into bilayer dissolving polymeric microneedle arrays. The ADM was demonstrated mechanically strong and contained amphotericin B only in the tips. Moreover, it demonstrates a high drug deposition of amphotericin B *in vitro*. Dermatokinetic profiles indicated that ADM delivered amphotericin B mainly into the dermis layer and remained at a high level during a 24-hour application. The antifungal effects of ADM were shown effective both in *in vitro* agar plates and in an *ex vivo* infected porcine skin model. Overall, this research shows the promising application of ADM to combat skin fungal infections.