1. Introduction

Onychomycosis is a disease at a prevalence of 10% with *T. rubrum* (*Trichophyton rubrum*) accounting for 98% of the infections (Gupta et al., 2020). Currently, a variety of treatments are present, but due to the low efficacy, the relapse rate stays high (25%-30%) (Aggarwal et al., 2020). As a result, the development of new drugs continues. To avoid the systemic toxicity and associated side effects on the gastrointestinal and hepatic system, a preference for topical treatments over oral therapies is shown (Aggarwal et al., 2020). However, the low rate of drug penetration remains a huge obstacle for topical medicines. Maintaining the hydration of the nail plate is one of the means to increase drug permeation and is being done by applying antifungal lacquers at this present time (Baran and Kaoukhov, 2005). Likewise, this can be achieved by directly printing a film onto the nails using a nail printer. This technology has been available for cosmetic purposes since 2018. In spite of the fact that it is not commonly seen in nail salons, its ability to create high-quality images with a resolution of 2400 dpi is highly praised by individual nail artists. Nail printers capable of 3D printing have revealed new possibilities of topical treatment which will be explored in this project. A novel design of topical medicine comprising double layers is proposed and its efficacy will be quantified using two separate experiments.

2. Aims and Objectives

A novel design of topical medicine will be made possible and its efficacy will be tested. First of all, the printability of all drug components with different textures will be analysed. A reasonable formulation will be accomplished by adjusting the chemical compositions of the drug to be printed. After that, the medicine will be optimised by investigating the relationship between the number of layers printed and the inhibition effect. Graphic statistical analysis is expected. Finally, the efficacy of the medicine will be tested on infected nail plate models. Statistical analysis should be performed to verify if there is a significant difference between the new drug and existing antifungal lacquers.

3. Materials

Pharmaceutical antifungal gels Nagel Batrafen, Lamisil DermGel will be purchased.
Ammonio methacrylate copolymer A, Triacetin, butyl acetate, ethyl acetate, ethanol absolute will be purchased.
O2Nails V11 Printer (Cyber Nails, China), loaded with the standard SM10 Special Inkjet Cartridge (Cyber Nails, China) will be used.
T.rubrum and Sabouraud's Dextrose Agar plates will be used.
Bovine hooves/human nails will be obtained.

Formulations of commercial products involved:

Nagel Batrafen contains 0.77% (w/w) ciclopirox in a water miscible lotion base consisting of

purified water USP, cocamide DEA, octyldodecanol NF, mineral oil USP, stearyl alcohol NF, cetyl alcohol NF, polysorbate 60 NF, myristyl alcohol NF, sorbitan monostearate NF, lactic acid USP, and benzyl alcohol NF (1%) as a preservative ("Nagel Batrafen Price Comparison: Uses, Dosage, Form & Side Effects," n.d.).
Lamisil DermGel contains 1% Terbinafine gel, Isopropyl myristae, polysorbate 0, barbomer, ethanol 96%, sorbitan laurate, benzyl alcohol, sodium hydroxide, butylated hydroxytoluene, water ("Lamisil DermGel Price Comparison," n.d.).

4. Methods

4.1 Printability of gels and film:

Printability of two gels and film suspension will be calculated with the formula $Z = \frac{(\alpha\rho\gamma)^{1/2}}{\eta}$ (Fromm 1984). In which Z is the printability of the ink, α is the radius of cartridge orifice/m, ρ is the density/ kg m^{-3}, γ is the surface tension/ N m^{-1}, η is the viscosity/ Pa s.

The radius of the cartridge orifice of SM10 is measured using a micrometer screw gauge. Masses and volumes of Nagel Batrafen, Lamisil DermGel, and film suspension (Ammonio methacrylate copolymer A, Triacetin, butyl acetate, ethyl acetate, ethanol absolute) are measured thus their densities are calculated. Measurements of the surface tensions and viscosities of Nagel Batrafen, Lamisil DermGel, and film suspension (Ammonio methacrylate copolymer A, Triacetin, butyl acetate, ethyl acetate, ethanol absolute) are taken using tensiometer and viscometer respectively.
All measurements should be taken at room temperature.

Calculate printability Z and compare Z values with printable range [1 10] (Derby and Reis, 2003).
Modify components by increasing the portion of ethanol if Z is too large.
Plot a graph indicating changes in printability when ethanol percentage increases (printability against ethanol percentage).

4.2 Diffusion susceptibility test on discs and pre-infected nail plates

Preparation of SDA Petri Dish:
Weight 26 g of SDA with analytical balance;
Place them into a 500 mL Duran bottle with 400 mL distilled water;
Tightly seal the bottle and shaken well for even suspension;
Autoclave the solution at 121°C for 2 hours;
Cool it down and pour (mix it well before pouring) it into sterilised Petri dishes in a microbiological safety cabinet to maintain sterility;
Once the Petri dishes had solidified at room temperature, use parafilm to seal them and

stored at 4°C.

Preparation of inoculum:
T. rubrum isolates were sub-cultured on Sabouraud's Dextrose Agar plates for 7 days under the incubation temperature of 32 degrees.
For the inoculum preparation around 5-10 ml of sterile saline solution was then pipetted onto the fungal colonies on the plate. The surface of the agar was then scrapped under saline solution using a sterile loop to form a suspension of dermatophyte hyphae and conidial. A syringe was then used to draw the fungal suspension and to press the suspension through an autoclaved stainless steel filter with Whatman's filter paper 40 (diameter 110 mm) attached. (This was to remove all dermatophyte hyphae from and ensure that only micro-conidia were left in the suspension)
The suspension was vortexed for 15 seconds before turbidity checks were carried out by using a UV spectrometer. The target absorbance was 0.15-0.17 au, a similar value that was given by McFarlands solution. More saline was added until the solution meets the target reading. The final concentration obtained was from 0.4 x 104 to 5 x 104 colony forming units (CFU)/ml.

4.2.1 Disc Diffusion Test

Prior to the experiment, all discs required for disc diffusion experiment were placed in a small Duran bottle and autoclaved at 121°C for 2 hours. Then 10 layers of antifungal gels are printed onto 3 discs followed by printing of the film to 2 out of the 3 discs. This is to simulate the effect on actual nails illustrated by Fig. 1. Methanol was used as a solvent control. After that, discs are allowed to dry for 10 minutes. During the experiment, discs were placed on a sterile empty petri dish and were prepared in a safety cabinet.

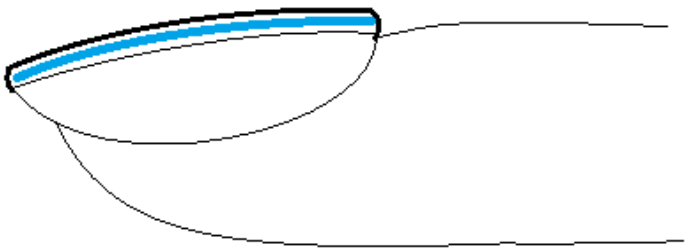

Fig.1 An occlusive film covers the antifungal gel in direct contact with infected nails to minimise drug loss and keep the nail plate hydrated.

100 µL of fungal inoculum was loaded onto the SDA petri dish. The inoculum was spread by a sterile disposable spreader to evenly distribute the inoculum on the petri dish.
Use sterile tweezers to transfer the discs onto the petri dish as shown in Fig. 2

The petri dish was then sealed using parafilm and was incubated at 32°C.

Repeat the process with 2 more layers of gel printed every time until 20 layers in total are printed.
Repeat the process with 1 more layer of film printed every time until 10 layers in total are printed.
Results for drug testing for disc diffusion were obtained by measuring the radius of the zone of inhibition after 24 hours after incubation. Graphs of radius against the number of gel layers and film layers will be plotted respectively. Statistical analysis will be performed afterward.

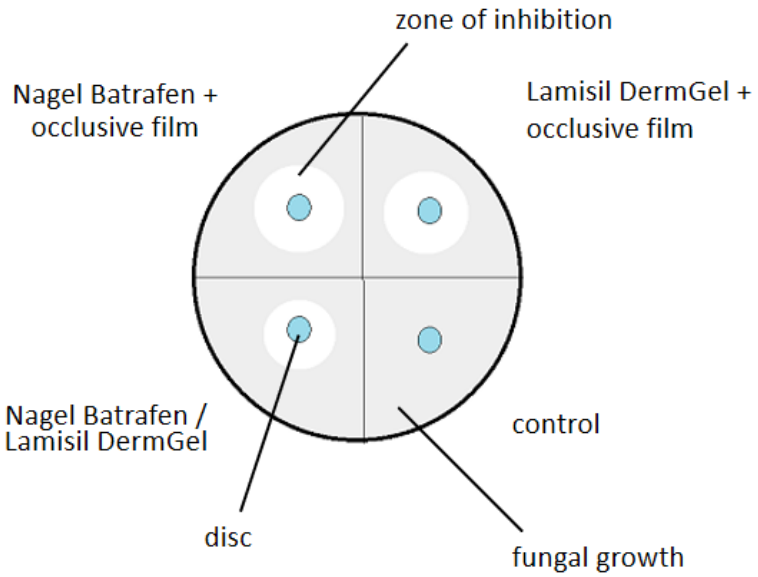

Fig.2 Display of disc diffusion test

4.2.2 Infected nail plate model diffusion test

Preparation of bovine hooves/nail cadaver:
Nails or nail substitutes are cut into squares (1.5cm x 1.5cm), submerged overnight in water, and then sliced to a thickness of 0.5 mm (Lusiana et al., 2013). Then nails plate models are pre-infected with *T. rubrum*. A sterile cotton swab was dipped onto culture, and afterwards, the Petri dish containing SDA was streaked evenly over the entire surface in three directions. The plate was allowed to dry for 15 min. 4 sterile nail plate models are then placed onto each Sabouraud's Dextrose Agar plate and the assembly is incubated for 7 days under the incubation temperature of 32 degrees.

After 7 days, infected nail models are carefully isolated using spatula and forceps. Then the optimal number of layers of antifungal gels are printed onto 3 nail plate models followed by printing of the optimal number of layers of the film to 2 out of the 3 nail plate models. This is to simulate the treatment effect on actual nails illustrated by Fig. 1. 5 µL of methanol was used as a solvent control. After that, infected nail plates with treatment are allowed to dry for 10 minutes before being transferred onto a fresh SDA plate.

Results are recorded according to how many percentages of the nail plate border has observable fungal growth (Fig.3). Data would be recorded every 24 hours over a week.

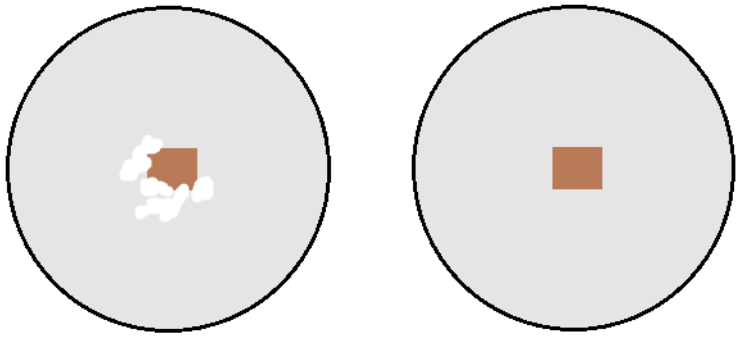

Fig.3 70% infected (left) vs 0% infected (right)

Statistical analysis should be performed to verify the efficacy of the drugs.

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