PCCA RESEARCH & DEVELOPMENT

Sm
Science Manual

PCCA EctoSeal P2G™

Index

Evaluation of the Safety and Toxicological Profile on Reconstructed Human Epidermis

Introduction:

The epidermis is the outermost skin layer and it is increasingly used as a route of drug administration. Topical compounded medications must be non-toxic and non-irritant to the skin and, therefore, it is important to guarantee the safety of the bases used in compounding. The aim of this study was to evaluate the safety and toxicological profile of EctoSeal P2G™, in comparison to Poloxamer, using a 3-dimensional (3D) *in vitro* model of reconstructed human epidermis: EpiDerm™ by MatTek Corporation (Ashland, MA), a highly differentiated 3D model which consists of human-derived epidermal keratinocytes, cultured and differentiated to resemble the human epidermis.

Methodology:

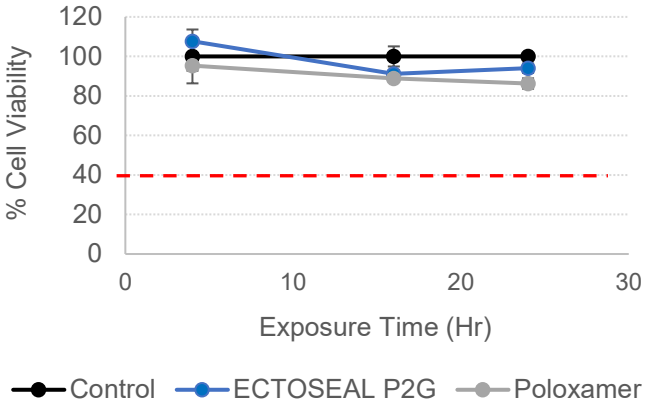
Upon receipt of the MTT-100 kit R, the EPI-200 cells (Lot 39169) were maintained in the supplied culture media and stored in accordance to the manufacturer's protocol until the initiation of the study. Following preparation of the cells, the EpiDerm™ tissues were treated in triplicate with 100 µL of the test product EctoSeal P2G 20% and another set of tissues were treated with Poloxamer 20% for 4, 16 and 24 hours. A triplicate set of EpiDerm™ tissues was also left untreated to serve as negative control. Following the exposure period, the dosing solutions were removed and the cells were analyzed for cell viability by the MTT Effective Time 50 (ET_{50}) assay, which consists of measuring the reduction of MTT (3-[4,5-dimethylthiazol-2yl]-2,5-diphenyltetrazolium bromide) by the cells. Succinate dehydrogenase enzymes within the mitochondria of viable cells have the ability to reduce soluble yellow tetrazonium salt of MTT to an insoluble purple formazan derivative. MTT is therefore an indicator of cell viability as the tissues are evaluated for their ability to reduce soluble-MTT (yellow) to formazan-MTT (purple).

Results and Discussion:

Viability of the epidermis tissue cells following exposure to the test products is represented by the absorbance of the respective extracts and expressed in percentage relative to the negative control, as follows: % Cell Viability=100 x [OD(test product) / OD(negative control)]. The greater the absorbency of the extracts, the greater the amount of MTT reduced by succinate dehydrogenase and, as a result, the higher the percent cell viability within the tissue.

Upon 24 hours of study, the viability of the cells exposed to EctoSeal P2G was superior to 90%. Similarly, the viability of the cells exposed to Poloxamer, a surfactant widely used in wound care, was superior to 85%, as demonstrated in the Table and Figure below.

Exposure Time (Hr)	**% Cell Viability**		
	Control (mean ± SD)	EctoSeal P2G (mean ± SD)	Poloxamer (mean ± SD)
4	100.00±13.63	107.68±1.03	95.34±2.58
16	100.00±5.09	91.18±1.99	88.89±1.40
24	100.00±1.18	94.02±2.42	86.29±2.63



The toxic exposure time (ET_{50}) is the time when cell viability is reduced to 50%, which is represented by a red dashed line in the Figure above. The general guideline for correlation of *in vitro* and *in vivo* results states that products with an ET_{50} of 24 hours are expected to be non-irritant. According to the results obtained, the ET_{50} of both EctoSeal P2G and Poloxamer is superior to 24 hours and, therefore, both products have a good safety and toxicological profile.

In conclusion, the proprietary compounding base EctoSeal P2G does not cause toxicity to the epidermis tissue. As a result, compounded medicines including this new proprietary compounding base may be applied to the skin without causing any toxicity to the epidermis tissue.

In Vitro Drug Release of Metronidazole 2% Topical Hydrogel (EctoSeal P2G) and Poloxamer Gel

Introduction:

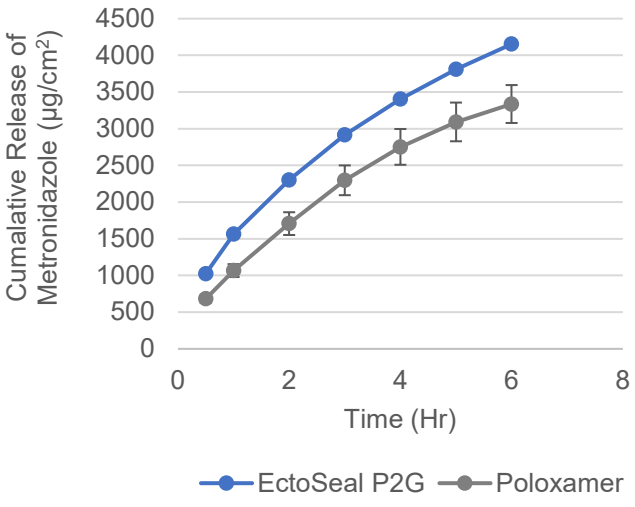
The *in vitro* drug release is a performance test for topical drug products used to measure the release rate of active pharmaceutical ingredients (APIs) from semisolid dosage forms. It is important to test the *in vitro* drug release of newly developed products (e.g., EctoSeal P2G) to ensure performance and comparability to a product of reference (e.g., Poloxamer Gel). This test is not intended though to predict *in vivo* performance, as opposed to the skin percutaneous absorption studies, since the primary factor that impacts bioavailability and clinical performance is skin permeation. However, this test can detect *in vitro* changes, as a result of formulation differences, that may correspond to altered *in vivo* performance of the dosage form. For this reason, its main purpose and use is comparison testing in which any difference in delivery rate is undesirable. This test is required by the FDA to determine the acceptability of minor processes and/or formulation changes in commercially approved semisolid dosage forms. The Unites States Pharmacopoeia (USP) recognizes different apparatus for the *in vitro* drug release test in the monograph <1724> Semisolid Drug Products – Performance Tests. The aim of this study was to evaluate and compare the *in vitro* drug release of Metronidazole 2% Topical Hydrogel (EctoSeal P2G) (PCCA Formula 14774) and Metronidazole 2% in Poloxamer 407 22% Gel. Poloxamer Gel is a well-established and referenced base used in wound management therapy, whereas PCCA EctoSeal P2G is a newly developed base with superior properties.

Methodology:

The *in vitro* drug release test was performed using the Franz Diffusion System (PermeGear, Inc.) using vertical diffusion cells (VDC) composed of 6-cell units. Each VDC cell assembly consisted of two chambers (donor and receptor chambers) separated by a membrane and held together by a clamp. The test samples (600 mg) sat on a synthetic, inert, highly permeable support membrane, intended to keep the test samples and receptor medium separate. A heating water circulator was used to maintain the temperature controlled at 37°C ± 1.00°C. The 6-cell units operated together at one time (i.e., single run). The receptor medium samples were collected at 0.5, 1, 2, 3, 4, 5 and 6 hours (Hr) by stopping the stirrer, withdrawing 1 mL of sample, and replacing the same volume with stock receptor medium.

Results and Discussion:

For each cell unit, the amount of metronidazole released (µg/cm^2) was determined at each sampling time, and the cumulative amount of metronidazole released was plotted *versus* time (Hr). Metronidazole 2% exhibited a similar *in vitro* release profile from both Topical Hydrogel (EctoSeal P2G) and Poloxamer Gel throughout the study period of 6 hours. The amount released from Topical Hydrogel (EctoSeal P2G) was slightly higher at all time points in comparison to Poloxamer Gel. By the end of the study, a total of 7,350.70 ± 49.88 µg/cm^2 (61.26%) and 5,905.56 ± 457.17 µg/cm^2 (49.21%) of metronidazole had been released from Topical Hydrogel (EctoSeal P2G) and Poloxamer Gel, respectively.



This comparative study was not designed to evaluate any statistical differences between the two bases. Instead, it is able to provide qualitative insights on the drug release performance of the bases.

In conclusion, the *in vitro* drug release test has demonstrated that metronidazole 2% has comparable release profiles when incorporated in the well-established Poloxamer Gel *versus* the newly developed PCCA EctoSeal P2G.

In Vitro Evaluation of Wound Healing by Phenytoin 2% and Misoprostol 0.0024% Topical Hydrogel (EctoSeal P2G) and Poloxamer Gel

Introduction & Methodology:

Re-epithelialization is a process in wound healing that involves the migration of keratinocytes (cells within the epidermal layer of the skin) from the edge, towards the center of the wound, to form a thin layer of cells over the exposed area. The rate at which keratinocyte migration occurs is important in wound healing as it is the body's first attempt at restoring the protective skin layer. Delays in this healing process may result in wound infections and hypertrophic skin scarring. The purpose of this study was to assess the ability of the test formulations Phenytoin 2% and Misoprostol 0.0024% Topical Hydrogel (EctoSeal P2G) (PCCA Formula 14811), and Phenytoin 2% and Misoprostol 0.0024% in Poloxamer Gel, to facilitate keratinocyte migration by evaluating *in vitro* the process of re-epithelialization, using primary human keratinocytes.

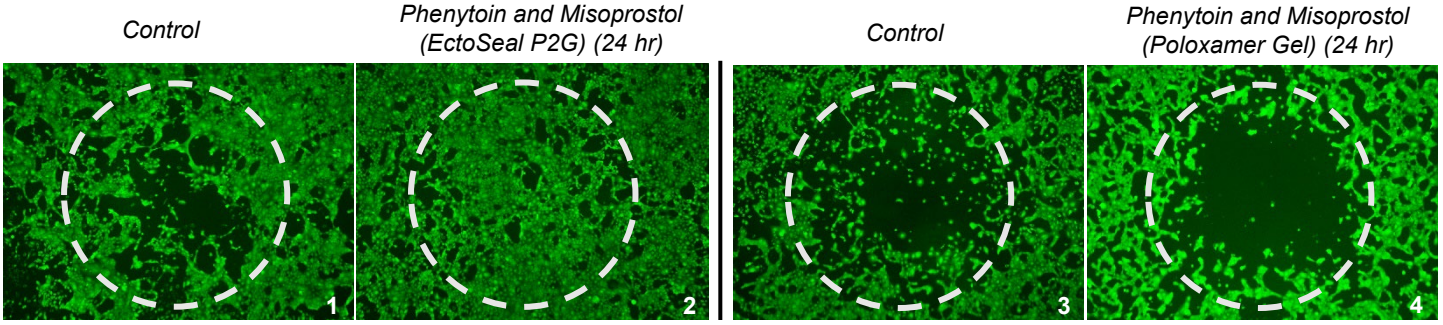
The *in vitro* evaluation was performed using the Oris™ cell migration assay kit (Platypus Technologies, Inc.), which consists of a 96-well plate, cell seeding stoppers that inhibit the spread of cells into the migration zone (center of the wells), and a black mask that allows for detection of cell migration. The cells were treated with the test formulations for 24 hours and then stained with Calcein AM, a non-fluorescent dye that is converted to green fluorescent calcein by viable cells. Fluorescence was detected using the CLARIOstar® plate reader (BMG Labtech) with Stars software for analysis at 483/14 excitation and 530/30 emission wavelength.

Results and Discussion:

The abilities of the test formulations to facilitate migration of primary human keratinocytes into the migration zone was quantified based on the green fluorescence detected by the plate reader, which is expressed in terms of Relative Fluorescence Unit (RFU). Phenytoin 2% and Misoprostol 0.0024% Topical Hydrogel (EctoSeal P2G) at 24 hours showed a mean change of 70.62% from control, which means that the topical hydrogel significantly promoted cell migration. In the contrary, Phenytoin and Misoprostol in Poloxamer Gel did not increase cell migration (negative mean change) (Table 1 and Figures 1-4). Keratinocyte migration is part of the re-epithelialization process in wound healing and, therefore, the EctoSeal formula is likely to have greater wound healing abilities than the corresponding formula for Poloxamer Gel.

Table 1. *Mean Relative Fluorescence Units (RFU) ± SD and mean change (%) ± SD for Phenytoin and Misoprostol (EctoSeal P2G), and Phenytoin and Misoprostol (Poloxamer Gel), from control following 24 hours post-application.*

Test formulations	Mean RFU ± SD	Mean change (%) ± SD	*p*-value
Phenytoin and Misoprostol (EctoSeal P2G)	121,381 ± 30,784	70.62 ± 43.27	6.95E-06
Phenytoin and Misoprostol (Poloxamer Gel)	87,362 ± 28,507	-29.99 ± 22.85	0.0007



Figures 1-4. *Keratinocyte migration (green fluorescence) for Phenytoin and Misoprostol (EctoSeal P2G) (1-2), and Phenytoin and Misoprostol (Poloxamer Gel) (3-4), from control following 24 hours post-application.*

In Vivo Evaluation of Wound Healing by Phenytoin 2% and Misoprostol 0.0024% Topical Hydrogel (EctoSeal P2G) and Poloxamer Gel

Introduction:

Chronic wounds are a major clinical problem that leads to considerable morbidity and mortality worldwide. Newly developed products are subjected to both *in vitro* and *in vivo* studies to ensure safety and efficacy in wound management. A commonly used *in vivo* test is the diabetic mice wound healing model (BKS-db), including both control group and test group(s).

Methodology:

The *in vivo* evaluation test was conducted by GemPharmatech Co., Ltd, following ethics approval: certification number 511214900020707; animal protocol GPTAP20230810-4; project number PO-GJC052023078305-01. The test products Phenytoin 2% and Misoprostol 0.0024% Topical Hydrogel (EctoSeal P2G) (PCCA Formula 14811) (A), and Phenytoin 2% and Misoprostol 0.0024% in Poloxamer Gel (B) were provided by PCCA. C57BL/6J/BKS-db male diabetic mice (n=12) were divided in 3 groups (n=4) according to blood glucose and body weight: control group, test group A and test group B. All mice were anesthetized and the hair on their dorsal skin was shaved. Two full-thickness excisional skin wounds were made to the back of each mouse using an 8-mm biopsy punch. Subsequently, 300 mg of the test product A and B were applied daily on the skin wounds of the mice in the test groups A and B, respectively, for a total of 9 days. The photos below show the skin wounds on days 0, 3 and 9. The wound areas were measured, normalized as percentage of day 0 and expressed as mean ± standard deviation (mean ± SD). The statistical analysis was performed using a T-test in which $p < 0.05$ is considered statistically significant.

Results and Discussion:

The mice were successfully treated for 9 days in the test groups A and B, as shown in Figures 4-6 and 7-9, respectively. The mice in the control group (Figures 1-3) developed a slower wound healing response, in comparison to the treated mice. The test group A [Phenytoin and Misoprostol Topical Hydrogel (EctoSeal P2G)] – demonstrated a more effective treatment response in comparison to the test group B (Phenytoin and Misoprostol in Poloxamer Gel). Table 1 shows a lower mean percentage of relative wound area at day 9 for the test group A (49.67%), when compared to the test group B (71.54%) and the control group (75.44%). These mean percentage results obtained are consistent with Figures 1-9.

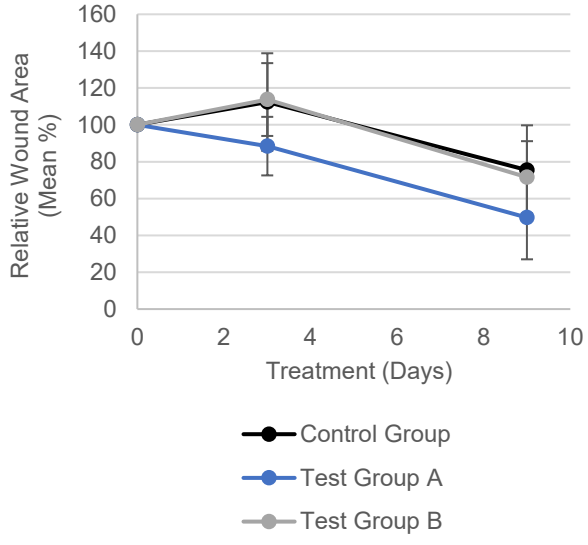
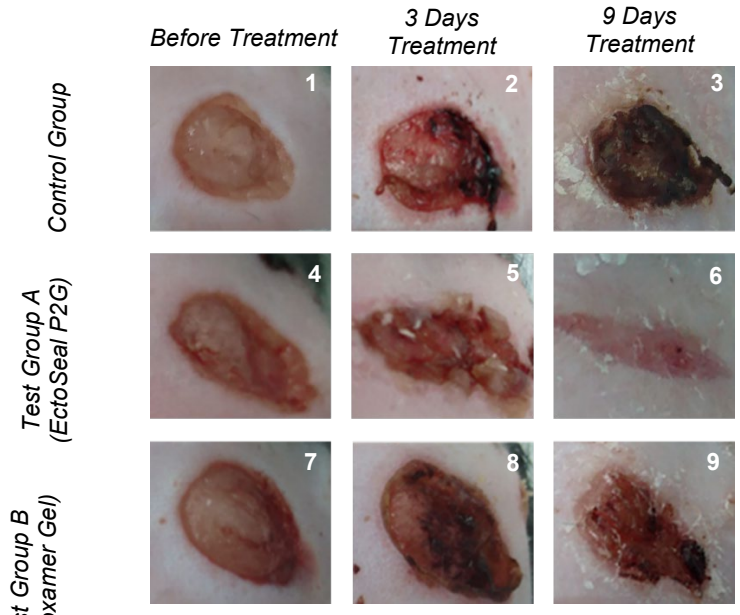


Table 1. *Mean percentage of relative wound area for the mice in the control group and test groups A and B, for a total of 9 days.*



Figures 1-9. *Digital photographs of the wound areas for selected mice in the control group and test groups A and B, at day 0 (before treatment) and days 3 and 9 of treatment.*

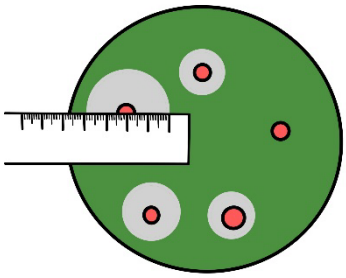
Zone of Inhibition by Clindamycin 2% Topical Hydrogel (EctoSeal P2G) Against *Staphylococcus aureus*

Introduction:

The zone of inhibition (ZOI) test (also referred to as Kirby-Bauer test) is a microbiology, culture-based disk diffusion assay used to determine the susceptibility or resistance of pathogenic bacteria to antibacterial agents. In this study, the antibacterial clindamycin HCl 2% was incorporated in the newly developed base, PCCA EctoSeal P2G, and its effectiveness was tested *in vitro* against the gram-positive bacteria *Staphylococcus aureus* (*S. aureus*).

Methodology:

A total of 30 Mueller-Hinton Agar (MHA) plates were inoculated to create a confluent lawn of *S. aureus*. A sterile disk was placed on the center of the plates, and it was impregnated with either Clindamycin HCl 2% (alone) or Clindamycin 2% Topical Hydrogel (EctoSeal P2G 20%), at the following concentrations: 100% (neat), 75% dilution, 50% dilution and 25% dilution (3 replicates each). Two plates were used as positive control (no disk) and negative control (disk impregnated with sterile water), for each concentration. All plates were incubated at 30-35°C for 18-24 hours and the presence of a ZOI was investigated to determine the *in vitro* effectiveness of the antibacterial agent clindamycin against *S. aureus*. The ZOI is a clear circular area around the disk where there is no bacterial growth.



Figures 1 and 2. *Illustration of the ZOI antimicrobial susceptibility test. Stock photos ID 688079770 and 2340799933.*

Results and Discussion:

Following incubation, the MHA plate for the positive control displayed confluent growth across the entirety of each plate, whereas the MHA plate for the negative control showed no visible inhibition of growth by the disk impregnated with sterile water, as expected. In the contrary, the MHA plates for both Clindamycin HCl 2% (alone) and Clindamycin 2% Topical Hydrogel (EctoSeal P2G 20%) displayed a visible ZOI against *S. aureus* at 100% (neat), 75% dilution, 50% dilution and 25% dilution (all replicates). The ZOI was consistent in size throughout the dilution series, with little change in diameter. For Clindamycin 2% Topical Hydrogel (EctoSeal P2G 20%), the average ZOI diameter for the 100% (neat) was 45.6 mm; for the 75% dilution was 45.4 mm; for the 50% dilution was 45.3 mm; and for the 25% dilution was 46.3 mm, as shown in the table below.

	Clindamycin 2% Topical Hydrogel (EctoSeal P2G 20%)			
	100%	**75% dilution**	**50% dilution**	**25% dilution**
Zone of Inhibition (ZOI) (mm)	46.4 44.2 46.3	45.3 44.8 46.2	46.3 46.6 42.9	47.6 45.2 46.1
Inocula Count (CFU/mL)	$2.4x10^8$	$2.4x10^8$	$2.4x10^8$	$2.4x10^8$

It is concluded that the Clindamycin HCl 2% (alone) and Clindamycin 2% Topical Hydrogel (EctoSeal P2G 20%) yielded a comparable ZOI and, therefore, both formulations are capable of inhibiting the growth of *S. aureus.*

Nail Drug Permeation of Topical Formulations Including Fluconazole and Ibuprofen (EctoSeal P2G)

Topical formulations are ideal to treat fungal infections of the nails but drug permeation is limited which leads to poor treatment efficacy. In this study, the drug uptake (permeation) of topical formulations including fluconazole and ibuprofen was evaluated. Fluconazole showed greater drug uptake from the EctoSeal nail hydrogels than the DMSO nail solution, which suggests that PCCA EctoSeal P2G is a potential compounding base for the delivery of topical nail formulations.

Introduction:

Disorders of the nail are a common occurrence, particularly fungal infections (onychomycosis) which are often characterized by nail brittleness, discoloration and thickening (Figure 1). Topical treatments are ideal because the active pharmaceutical ingredients (APIs) are concentrated externally at the site of infection, thus avoiding the systemic effects and drug interactions of the oral treatments. However, topical formulations have low efficacy due to the poor permeation of drugs through the nails.[1] As such, there is a lot of research being conducted to enhance nail drug permeation.[2] The purpose of this study is to evaluate the transungual (through the nails) drug uptake (permeation) of topical formulations including fluconazole and ibuprofen in PCCA EctoSeal P2G.[3] These APIs are commonly indicated in the treatment of onychomycosis, and the associated inflammation and pain that can be presented in these infections. Fluconazole has antifungal properties and ibuprofen has anti-inflammatory and analgesic effects, which provides a synergistic approach to the topical treatment.

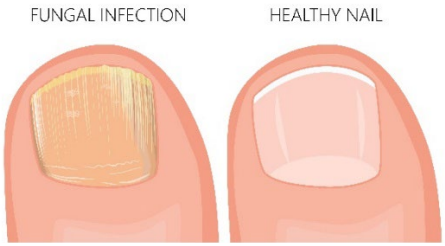


Figure 1. *Illustration of toe nail fungal infection. Stock vector ID 1474800791.*

Methodology:

The transungual drug uptake experiment consisted of collecting nail clippings from volunteers, exposing the nail clippings to the test formulations, then analyzing the samples to quantitatively determine the amount of fluconazole and ibuprofen that penetrated the nails.

Materials (test formulations):

1. Fluconazole 2%/Ibuprofen 2% Topical Nail Hydrogel (EctoSeal P2G™) (PCCA Formula 15341) (Table 1).

Rx	
Fluconazole	2 g
Ibuprofen	2 g
Benzyl Alcohol	1.5 g
DMSO	2 g
Base C	5 g
Base, PCCA EctoSeal P2G™ Powder	15 g
Purified Water	q.s. 100 g

Table 1. *Fluconazole 2%/Ibuprofen 2% Topical Nail Hydrogel (EctoSeal P2G™): PCCA Formula 15341.*

2. Fluconazole 2%Topical Nail Hydrogel (EctoSeal P2G™) (PCCA Formula 15340).

3. Fluconazole 2%/Ibuprofen 2%/Dimethyl Sulfoxide (DMSO) Nail Solution (PCCA Formula 12663).

Informed consent was obtained from 5 volunteers who met the eligibility criteria and were willing to participate in this study. All volunteers were female, Asian and Caucasian, aged between 28 and 55 years old. The collected nail clippings were cleaned and immersed in Di water for 1 hr. After drying out with Kimwipes®, the test formulations were applied onto the nail clippings and put into moisturized box to avoid drying. After 48 hrs, the nail clippings were washed with water and methanol to remove any of the remaining fluconazole and ibuprofen. The clippings were immersed in liquid nitrogen and then struck with a hammer to reduce the nails to a fine powder. The pulverized nail powder was suspended in methanol overnight to extract fluconazole and ibuprofen from the nails. Followed by filtration with PVDF filter, fluconazole and ibuprofen concentrations were measured using Ultra-Performance Liquid Chromatography (UPLC). The amount of fluconazole and ibuprofen retained in the nail plate were presented as mass (µg) per mg of nails.

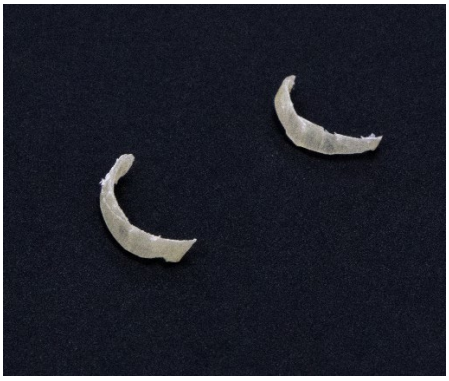


Figure 2. *Nail without EctoSeal Preparation.*

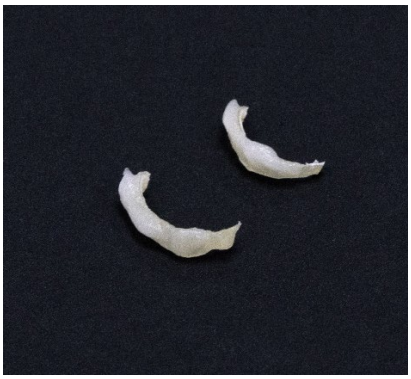


Figure 3. *Nail with EctoSeal Preparation.*

Results and Discussion:

The transungual drug uptake experiment showed that fluconazole penetrated well into the nail plate across the three test formulations. As shown in Figure 1, fluconazole displayed similar nail drug permeation in both topical hydrogels formulated with PCCA EctoSeal P2G which means that ibuprofen did not interfere with the drug uptake. When the topical nail hydrogel is compared with the nail solution, it is concluded that EctoSeal P2G facilitated greater transungual drug uptake than DMSO. Although preliminary, results from this study indicate that PCCA EctoSeal P2G is a potential compounding base for the delivery of topical nail formulations.

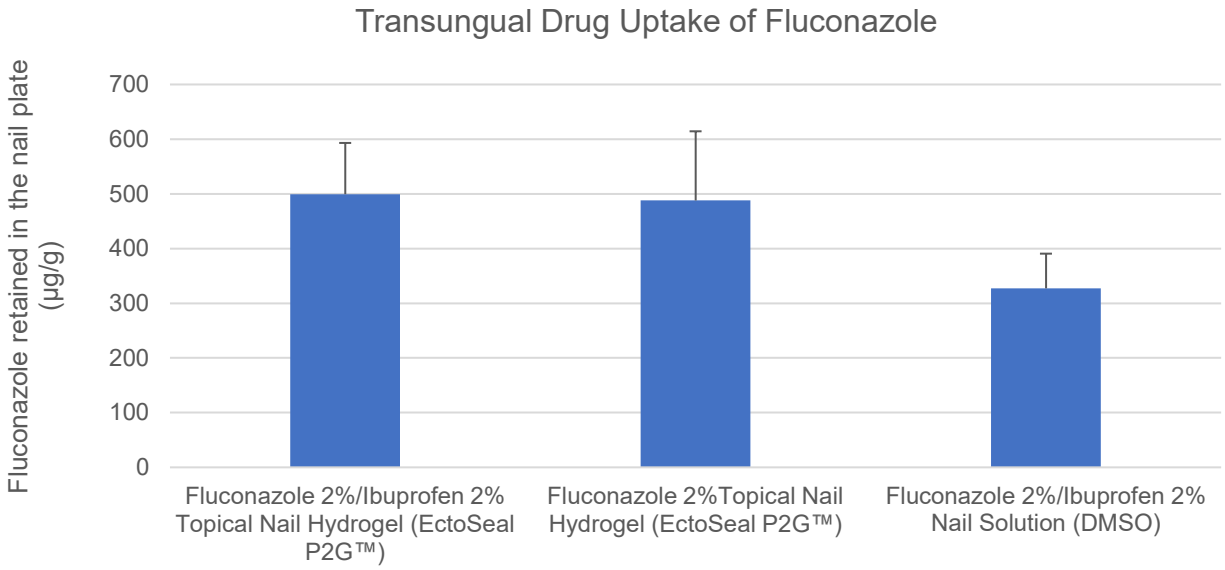


Figure 1.
Transungual drug uptake of fluconazole across the three test formulations (PCCA Formulas 15341, 15340 and 12663).

References:

1. Pollard TD, Bonetti M, Day A, et al. Printing Drugs onto Nails for Effective Treatment of Onychomycosis. Pharmaceutics. 2022;14(2):448. Published 2022 Feb 19.
2. Kerai LV, Bardés J, Hilton S, Murdan S. Two strategies to enhance ungual drug permeation from UV-cured films: Incomplete polymerisation to increase drug release and incorporation of chemical enhancers. Eur J Pharm Sci. 2018;123:217-227.
3. PCCA (2023) PCCA EctoSeal P2G™ Powder (30-5217). Available at: https://www.pccarx.com/products/PCCAECTOSEALP2G%E2%84%A2POWDER/30-5217/PROPRIETARYBASES

In Vitro Antibiofilm Efficacy of Topical Formulations Against Biofilms of *Candida albicans*

Introduction:

Candida albicans is one of most important fungal pathogens in chronic wounds due to its ability to generate biofilms, as shown in Figure 1, and an escalating resistance to current antifungals. Biofilms are communities of microorganisms (e.g., fungi) encapsulated in an extracellular matrix produced by themselves. The process of biofilm formation commences by the approach and attachment of microorganisms to a surface. Antibiofilm properties of antifungal topical formulations are critical to ensure the efficacy of the treatment.

The purpose of this study is to evaluate the antibiofilm efficacy of fluconazole topical compounded formulations including PCCA EctoSeal P2G.

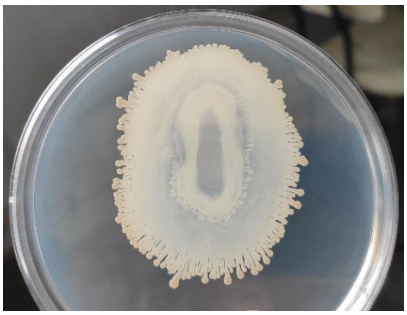


Figure 1. *Candida albicans growing on sabouraud dextrose agar medium. Stock photo ID1563059965.*

Methodology:

An *in vitro* 96-well, plate-based biofilm model was used for the antibiofilm evaluation, which included both enumeration and imaging. Pre-formed 48-hr old biofilms of *Candida albicans* ATCC 10231 (test organism) were exposed to the test formulas for 24 hr. Following neutralization steps, surviving microorganisms were recovered and enumerated. Test controls were as follows: isopropanol 70% (positive control), PBS (negative control) and untreated biofilms (growth control). The test formulas included the antifungal agent fluconazole 2%, as displayed in Table 1. Six sample replicates were used for the enumeration of the treatments and controls. Two sample replicates were used for the imaging testing.

	Formula Description	PCCA Formula
1	Fluconazole 2% PCCA EctoSeal P2G	15340
2	Fluconazole 2%, Ibuprofen 2% PCCA EctoSeal P2G	15341

Table 1. *Test formulas including the antifungal agent.*

Results & Discussion:

The enumeration testing shows that there were no countable colonies of *C.albicans* (ATCC 10231) from the positive control (isopropanol 70%), as expected (Figure 2). When there are no countable colonies, it means that the survival of the corresponding microorganisms is below the limit of detection of the equipment. As shown in Figure 2, there were no surviving microorganisms either from the fluconazole in EctoSeal test formulas (1,2). This is evidence to support that the antifungal topical formulations successfully eliminated the biofilms of *C.albicans*. In the contrary, there were >6 CFU/mL countable colonies of *C.albicans* for both the negative control (PBS) and the growth control (untreated biofilms), which indicates that the experiment was well designed and conducted.

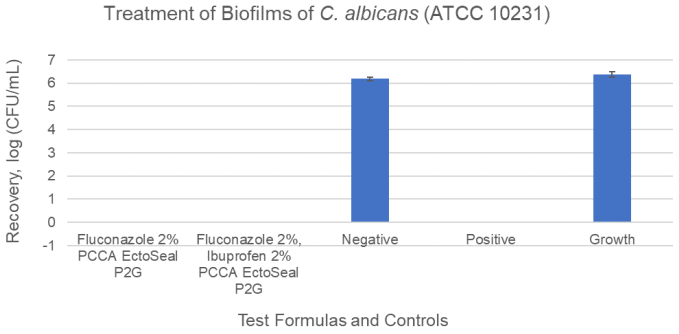


Figure 2. *Enumeration for surviving microorganisms after treating biofilms of C.albicans with the fluconazole in EctoSeal test formulas (1,2) and test controls (negative, positive and growth).*

The imaging testing shows images of the stained biofilms (Figure 3). Red staining refers to dead cells, as in the biofilms treated with the fluconazole in EctoSeal antifungal topical formulations. Green staining refers to live cells, as in the biofilms of the negative and growth controls.

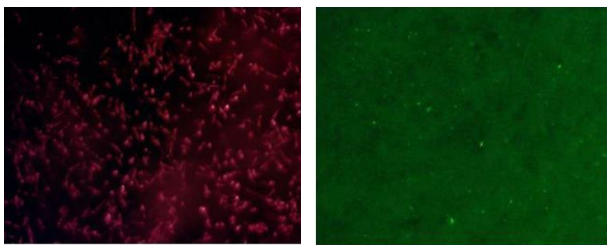


Figure 3. *Representative images of stained biofilms using an inverted fluorescent microscope: red staining (10x magnification) indicates dead cells whereas green staining (4x magnification) indicates live cells.*