PCCA EctoSeal P2G™

TECHNICAL REPORT

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.

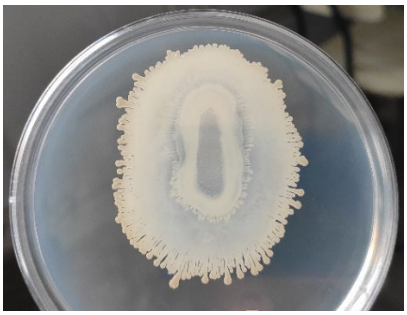

Figure 1. *Candida albicans growing on sabouraud dextrose agar medium. Stock photo ID1563059965.*

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.

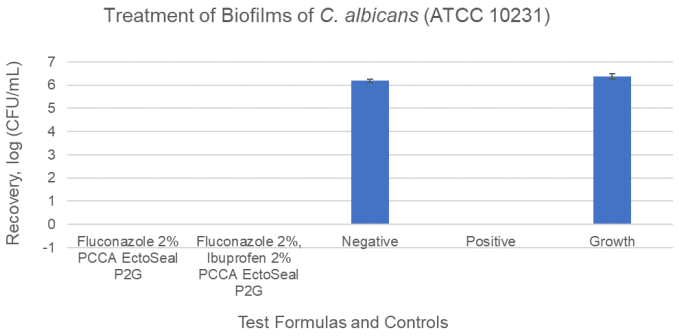

Figure 2. *Enumeration for surviving microorganisms after treating biofilms of C.albicans with the fluconazole in EctoSeal test formulas (1,2) and test controls (negative, positive and growth).*

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.

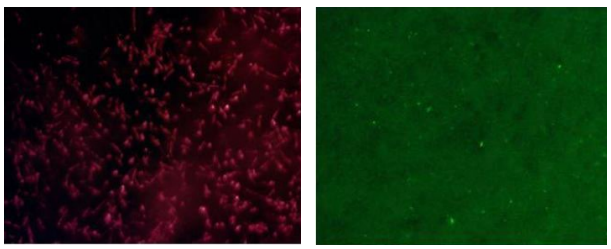

Figure 3. *Representative images of stained biofilms using an inverted fluorescent microscope: red staining (10x magnification) indicates dead cells whereas green staining (4x magnification) indicates live cells.*