

Ex-vivo determination of antifungal activity of a new prescription non-steroidal facial cream against *Malassezia furfur* in human skin explants

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INTRODUCTION

Malassezia furfur (MF) is a lipophilic (lipid-dependent) fungus that is part of normal human skin flora that grow on the sebaceous areas of human skin, including the face, scalp, and upper trunk. Although part of the normal human skin flora, uncontrolled MF proliferation in some patients leads to development of skin diseases including tinea versicolor, pityrosporum folliculitis, and seborrheic dermatitis (SD). The objective of this study was to examine the anti-fungal properties of a new non-steroidal facial cream (NSFC) in human organotypic skin cultures (hOSCs) inoculated with MF in an ex-vivo model. This model was developed to mimic SD conditions in order to evaluate the antifungal properties of an NSFC product containing zinc PCA, piroctone olamine, dihydroavenanthramide, biosaccharide gum-2 and stearyl glycyrrhinate.

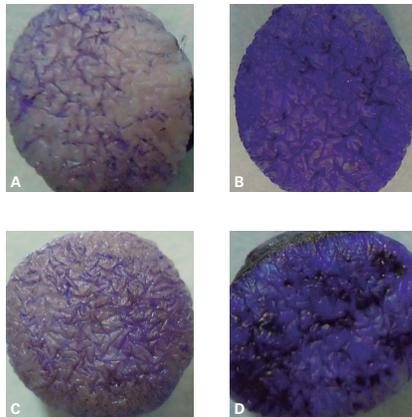


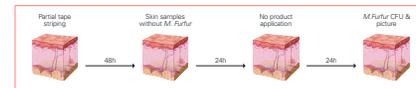
Figure 1. Skin samples images stained with violet crystal. A) Control B) *M. Furfur* C) *M. Furfur* + NSFC D) *M. Furfur* + Vaseline

METHODS

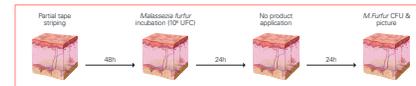
Human organotypic skin cultures hOSCs were obtained from abdominal skin removed during cosmetic surgery. The explants were altered by partial elimination of stratum corneum to facilitate colonization and stabilization of the MF (Figure 1).

MF suspension was placed on the skin surface and incubated for 24 hours under conditions that are optimal for MF growth. 24 hours post initial MF inoculation, NSFC was topically applied on skin explants (2 mg/cm²). On control skin explants, inoculated in the same way, no product was applied. A sham control group was treated with a neutral cream without known antifungal properties. Growth of MF was monitored by quantifying MF Colony Forming Units (CFUs) in a sample removed from skin surface. The quantification of CFUs was carried out by recovering fungal microorganisms from skin explants and subsequent plating them following the serial dilution method to determine the number of CFUs (Figure 2).

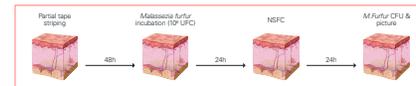
Negative control. No *M. Furfur* No NSFC (5 skin replicates)



Malassezia inoculation with No NSFC (5 skin replicates)



Malassezia inoculation with NSFC (5 skin replicates)



Malassezia inoculation with Vaseline (5 skin replicates)

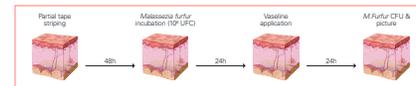


Figure 2. Study design ex-vivo model with Human skin. NSFC: Non-steroidal facial cream

RESULTS

In the altered skin explants, inoculation with MF led to successful colonization as indicated by the significant increase in MF CFUs compared to baseline: a 2-fold increase at 24 hours. The topical application of NSFC significantly reduced ($p < 0.05$) the number of MF CFUs by 90% compared to the untreated control group. The sham control treated with neutral cream did not lead to a significant reduction of the MF population (15% decrease in CFUs) (Figure 3).

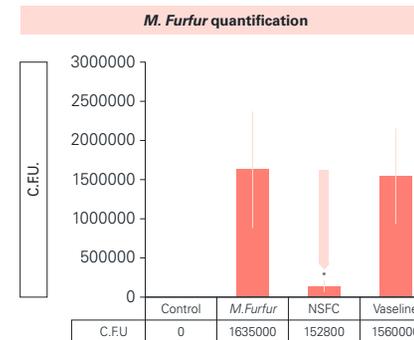


Figure 3. *M. furfur* population and response to NSFC application. Significant decrease in *M. Furfur* colonies in skin treated with NSFC. (*) $p < 0.05$

CONCLUSIONS

In this ex-vivo model, the topical application of a new NSFC significantly reduced the MF CFU count. These findings demonstrate the antifungal properties of this NSFC, specifically for MF, a key contributing fungus in Seborrheic Dermatitis.

REFERENCIAS:

- Gupta AK., et al. Seborrheic dermatitis of the scalp: etiology and treatment. J Drugs Dermatol. 2004;3(2):155-8.
- Barac A., et al. Presence, species distribution, and density of *Malassezia* yeast in patients with seborrheic dermatitis - a community-based case-control study and review of literature. Mycoses. 2015;58(2):69-75.
- Tajima M., et al. Molecular analysis of *Malassezia* microflora in seborrheic dermatitis patients: comparison with other diseases and healthy subjects. J Invest Dermatol. 2008 Feb;128(2):345-51. Epub 2007 Aug 2.