

# NEW INSIGHTS ON MICROBIOME COLONIZATION IN DANDRUFF-AFFLICTED SCALP BY CONFOCAL AND ELECTRON MICROSCOPIES

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## INTRODUCTION & METHODS

Skin resident microbial or fungal species are often described as commensal or pathogenic, their interaction with skin is fundamental in disorders such as dandruff scalp. We present a characterization study by Transmission and Electron Microscopy (TEM) of the ultrastructure of stratum corneum (SC) and its associated microflora on healthy and dandruff scalps.

D-Squame samples were fixed in 2.5% glutaraldehyde with 0.7% ruthenium hexamine trichloride (RHT), post-fixed in 1% osmium tetroxide, then embedded in epoxy resins for TEM ultrastructural examination of sections counterstained with uranyl acetate and lead citrate. Swab samples were fixed in paraformaldehyde followed by specific labelling using solophenyle flavine fluorescent dye for confocal acquisitions.

## RESULTS

### 1 Quality of dandruff vs. normal SC

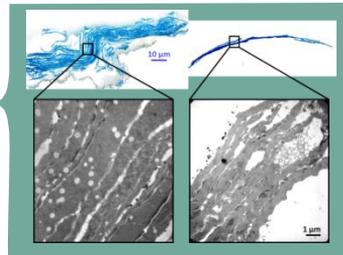


Figure 1. Thicker superficial squames on dandruff scalp (left) compared to healthy (right).

### 2 Localization of bacteria and yeasts

On healthy scalp, malassezia and bacteria are observed at the surface or in dilated interstices of stratum disjunctum (Fig. 3); but in deeper interstices of SC compactum on dandruff scalp, with frequent co-localization (Fig. 4).

6x more malassezia were observed on dandruff scalp (average over 30 fields of view of 1mm<sup>2</sup>)

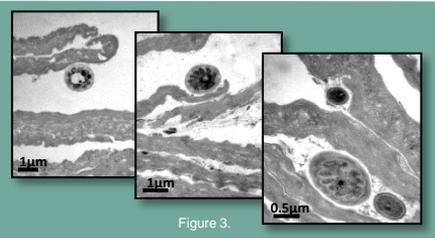


Figure 3.

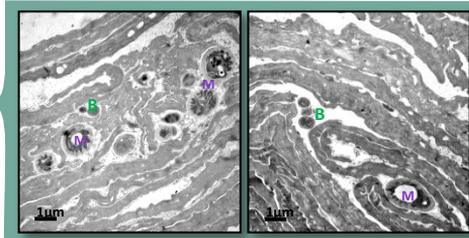
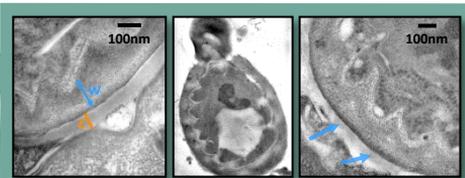


Figure 4

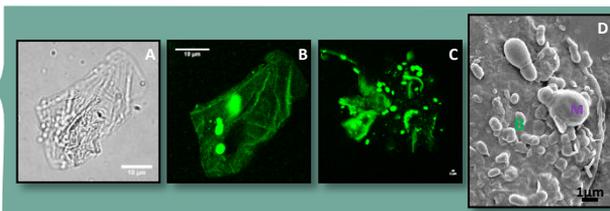
### 3 Malassezia capsule and cell wall



On healthy scalp (left), a lipid-rich capsule-like structure (c), 50-70nm thick, is observed surrounding the cell wall (w) of malassezia. The capsule is rarely seen on dandruff affected area (center), and often discontinuous and thinner (20-30nm) on healthy parts of dandruff scalps (right, blue arrows).

### 4 Specific identification

(A) Transmitted light images. (B,C) Fluorescence confocal images (63x, 1,2-NA water immersion objective,  $\lambda=488\text{nm}$ ) of swab samples with malassezia visualized on the surface of corneocytes after specific labelling using solophenyle flavine fluorescent dye. (D) Direct SEM observation on D-Squame.



## CONCLUSION

These results highlight the importance of maintaining / repairing the barrier function of the scalp in order to keep a healthy scalp. In particular, the SC cohesion and integrity, to prevent the colonization of the deep layers of the SC by micro-organisms and to prevent related pathogenous effect leading to inflammation, differentiation and keratinocyte hyper-proliferation. They also suggest addressing the lipid capsule of malassezia to limit inflammatory response from keratinocytes

The authors declare no conflict of interest