# 3D microbiotic skin models to understand and modulate skin colonization and infection

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# Microbiote methods applied to dermocosmetic

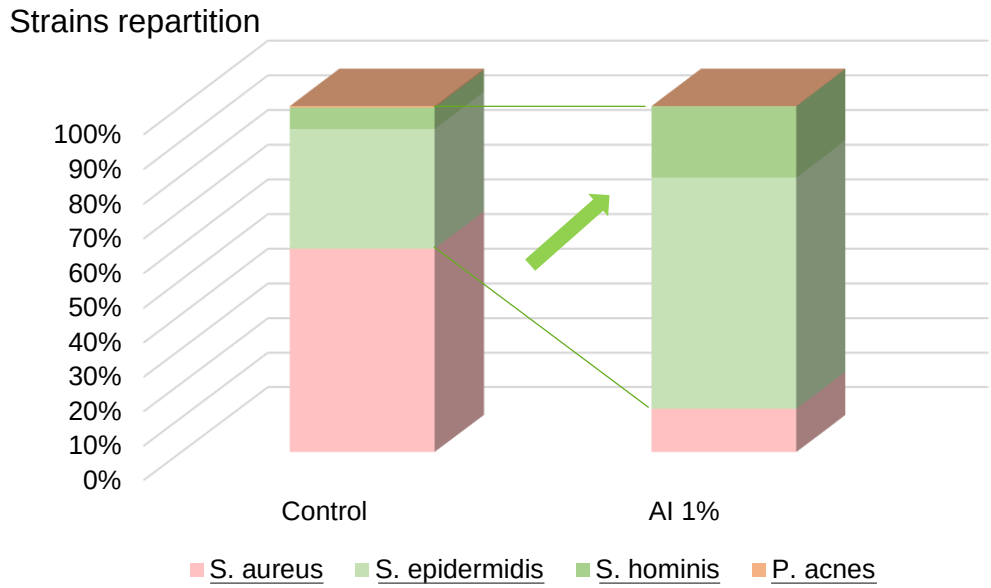
Studying skin microbiote and modulation by cosmetic active ingredients require the development of several incremental methods to evaluate:

- Several characteristics of commensal strains as growth, biofilm formation, stress impact such as UV or pollution,
- Interaction between skin cells and germs as adhesion, growth, virulence, inflammation.

# CO-CULTURES

**Materials & Methods:**

- 4 ATCC® strains are amplified using selective media combined to aerobic or anaerobic culture condition: *S. aureus* (6538™), *Staphylococcus epidermidis* (12228™), *S. hominis* (27844™) and *Propionibacterium acnes* (11827™).
- *In tubo* co-cultures of up to 4 species and qPCR quantification after 2 days interaction with or without active ingredients at 37°C .
- Evaluation of relative germs growth and modulations by active ingredient (n=3,T-Test (***) p<0.001, (*) p<0.05).




**Selection of active ingredients favoring the growth of skin benefic bacteria to the detriment of more opportunist bacteria.**



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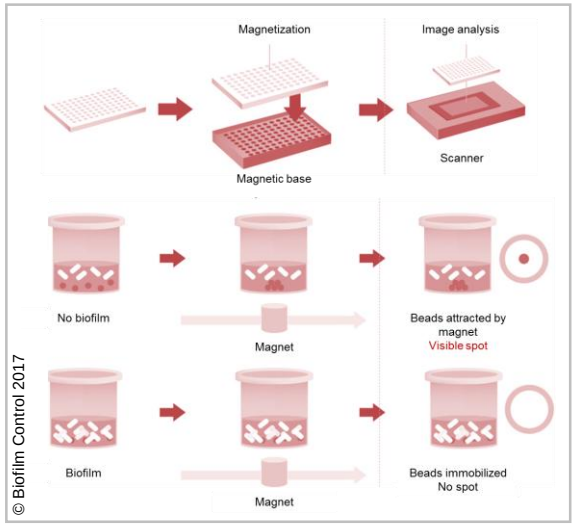
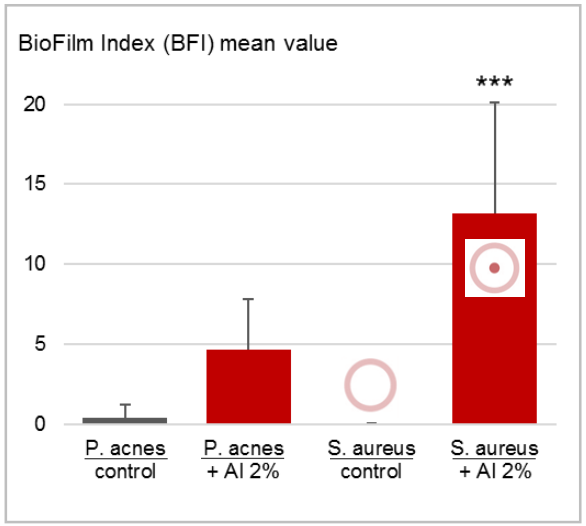
# BIOFILM FORMATION

**Materials & Methods:**

- Defined bacterial culture associated to suspension of magnetizable particles are placed in the wells of a microplate incubated for 4-6 h for the bacteria to adhere to the substrate and initiate biofilm formation.
- The microplate is placed on a block containing 96 mini-magnets for one minute before reading.
- Results are expressed as a score of 0 to 20, biofilm present to biofilm absent (BioFilm Ring Test® - n = 16, Mann-Whitney Rank Sum Test vs relative control, (***) p<0.001).




**When a biofilm is formed, the particles are embedded, no spot is visible and BioFilm Index is close to 0. Quantitative biofilm evaluation is easiest compared to agar based method.**



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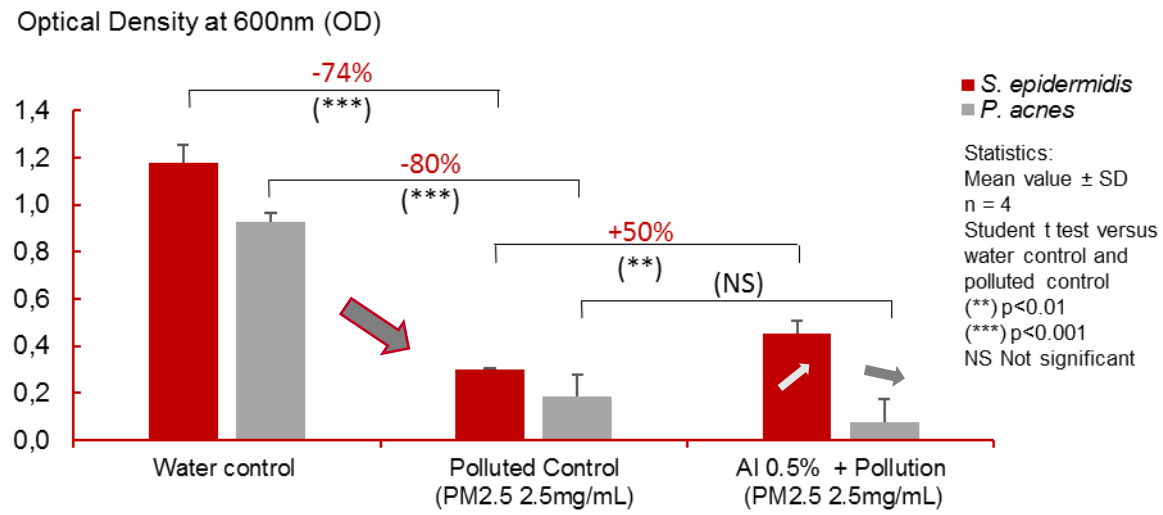
# POLLUTION IMPACT ON MICROBIOTE

**Materials & Methods:**

- ***S. epidermidis* or *P. acnes*** treated or not by 0.5% active ingredient (AI) during PM2.5 exposure:
  - SE $10^7$ ATCC® 14490™ for 24h in TSB media in aerobia or PA $10^8$ ATCC® 6919™ for 72h in Schaedler media in anaerobia.
  - Particulate matters: 1649b urban dust particles containing mainly polycyclic aromatic hydrocarbons, polychlorinated biphenyls and chlorinated pesticides.
- **Bacterial growth assessment** by spectrophotometry.

## Bacterial growth evaluation after PM exposure



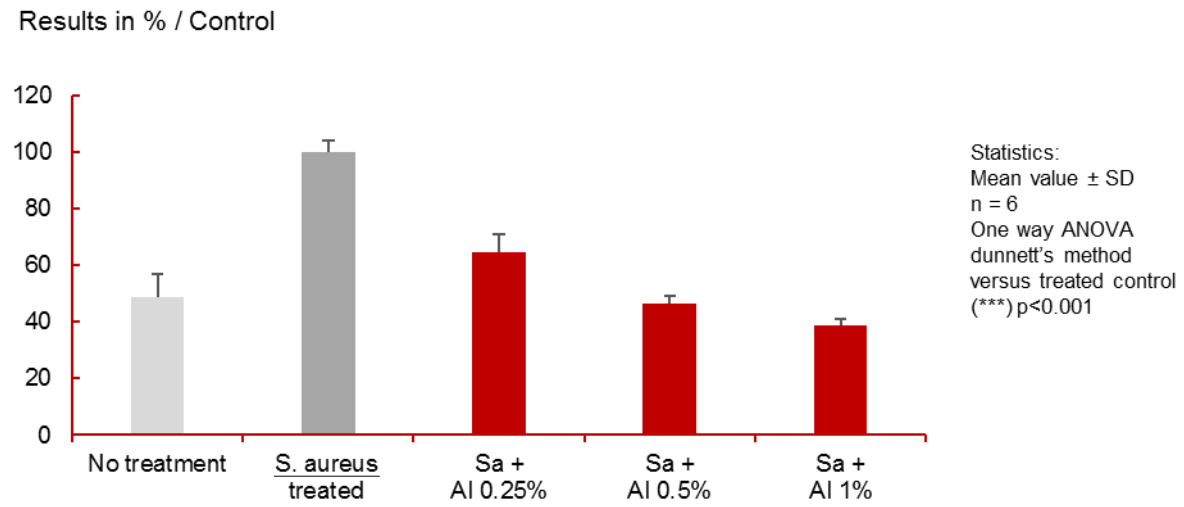

**Both germs were impacted by pollution.**
**Significant protection was obtained only in favor of *S. epidermidis* with the AI.**

# INFLAMMATION

**Materials & Methods:**

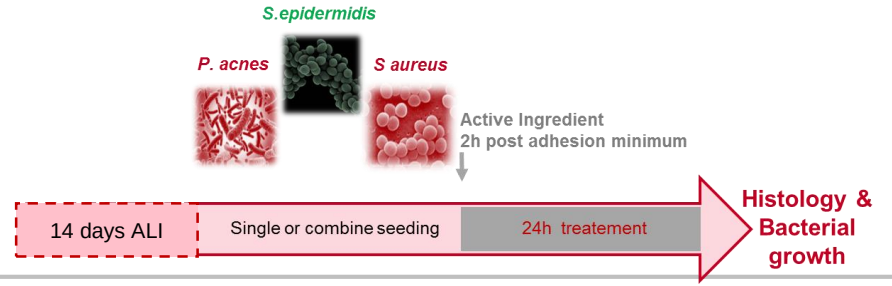
- **HaCat cell** lines grown until confluence and treated or not for 24h with or without active ingredient in dose effect.
- ***S. aureus*** (1.5 $10^7$ ATCC® 12600™) applied for 2h.
- **IL-8 quantification & cell viability** (ELISA & MTT).

## IL-8 release after *S. aureus* challenge



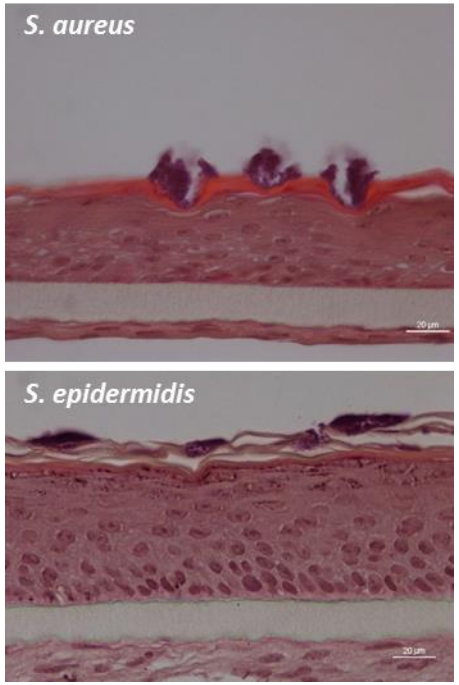

**Within 1 day *S. aureus* induced inflammation.**
**Significant protection was obtained with a specific hydrolyzed yeast extract.**

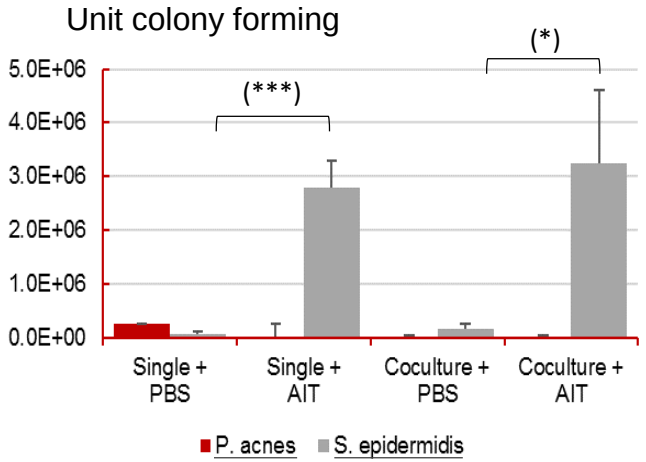
# 3D EPIDERMAL MODELS




## 3D reconstructed microbiotic epidermis

- **Epidermal reconstructions:** 19yo primary keratinocytes grown for 14 days differentiation at the air-liquid interface (ALI) on fibroblast underlaying membrane.
- ***S. epidermidis alone or in combinaison with P. acnes* or *S. aureus*** (SE $10^5$ ATCC® 12228™ & PA $10^7$ ATCC® 11828™ or SA $10^4$ CIRI SH1000).
- **Topic treatment** with active ingredient (AIT) or PBS for 1 day.
- **Histology & bacterial growth evaluation.**



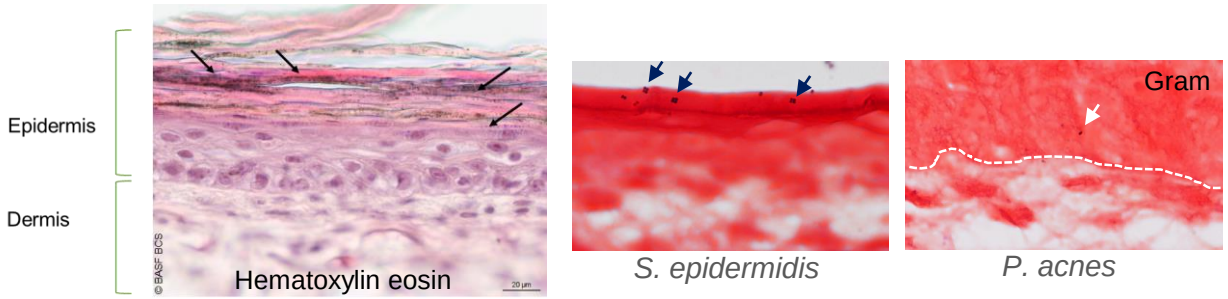

**Compared to *S. epidermidis*, *S. aureus* already damaged skin surface after 1 day exposure.**
***S. epidermidis* growth is independent of *P. acnes* and is promoted by the active ingredient.**

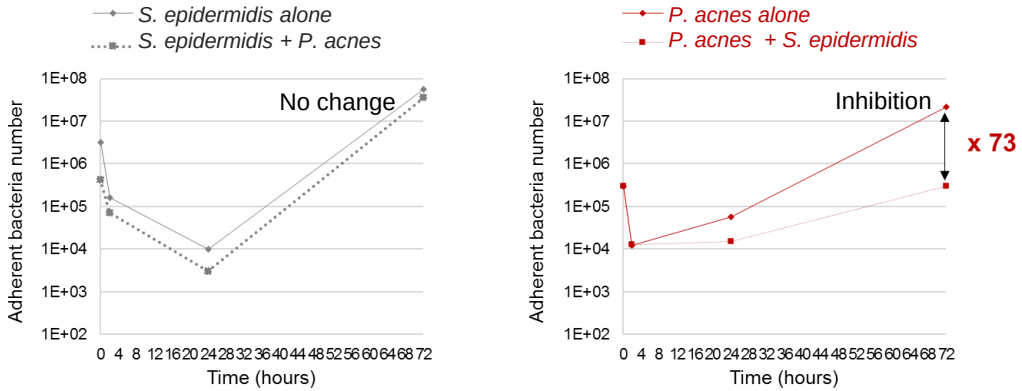
# 3D SKIN MODELS

## 3D reconstructed microbiotic skin

- **Microbiotic Mimeskin®:**
  - 61yo fibroblasts grown on a collagen based matrix for 28 days.
  - 61yo keratinocytes grown on dermal equivalents for 7 days before differentiation at the air-liquid interface for 1 or 2 weeks.
- ***S. epidermidis* and *P. acnes alone or in combination***
  - SE or PA alone at $10^7$ or combined at $10^6$ SE + $10^7$ PA SE ATCC® 14990™ & PA ATCC® 6919™
  - Seeding after 1 or 2 weeks emersion to mimic wound healing or mature skin.
- **Histology and Bacterial growth evaluation.**

## Results with seeding at 1 week post emersion




***S. epidermidis* is mainly at the skin surface whereas *P. acnes* is seen deeper within the skin layers due to its anaerobic growth preference. *P. acnes* did not impact *S. epidermidis* whereas *S. epidermidis* inhibited *P. acnes* growth.**



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