Case Report
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# Studying microbiote competition and skin interaction using organotypic 3d skin models

## Abstract

The skin microbiome plays an important role in maintaining healthy skin. Microbiome dysbiosis is known to lead to skin conditions like dermatitis and acne. Although it is relatively easy to study effect of ingredients on individual micro-organisms, it is hard to extrapolate these results to a complex skin system harboring multiple micro-organisms. In this study, we characterized the best conditions for the introduction of Staphylococcus epidermidis and Propionibacterium acnes in organotypic skin models to evaluate bacteria competition and regulation by active ingredients. The colonization and growth of the two species were analysed using microscopic visualization and Colony Forming Unit assay after bacteria recovery from artificial tissues. We showed that Staphylococcus epidermidis and Propionibacterium acnes could both efficiently colonize reconstructed skin models under specific conditions, and react to active ingredients treatment. To our knowledge, this is the first study that evidences multi germ interactions in 3D skin models, which is suitable to promote active ingredient selection for a healthier micro flora balance.

**Keywords:** skin equivalent, microbiote, propionibacterium acnes, staphylococcus epidermidis

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## Introduction

The skin surface varies topographically owing to regional differences in skin anatomy and, according to culture-based studies, these regions are known to support distinct sets of microorganisms.[1] Regions with higher temperature and humidity encourage growth of Gram-negative bacilli, coryneforms and *Staphylococcus aureus*, whereas areas with high density of sebaceous glands encourage growth of lipophilic microorganisms such as *Malassezia spp* or facultative anaerobes such as *Propionibacterium acnes*. Because humans sebum contain much more quantities of triglyceride than other mammals to support skin protection, *Propionibacterium acnes* is particularly present in greater abundance in human skin. When skin homeostasis is disturbed, commensal bacteria could become pathogens and could contribute to tissue damage as for acne and also rosacea,[2] dandruff,[3] atopic dermatitis[4] or wound healing.[5]

Until now, epidermal or skin equivalent models were developed to study skin contamination by germs seeded separately.[6] None were jointly seeded with 2 different germs. To study bacteria co-regulation and sensitivity to ingredients of dermo-cosmetic interest for acne, we propose here to integrate both beneficial aerobic *Staphylococcus epidermidis* (SE) and opportunistic detrimental aero tolerant *Propionibacterium acnes* (PA) in a 3D organotypic skin model.

## Skin equivalent models

The best conditions were first determined for the introduction of SE and PA in a skin equivalent model.[7] Briefly, fibroblasts (61 yo donor) were seeded onto a collagen-glycosaminoglycan-chitosan matrix and grown for 28 days at 37˚C in a 5% $CO_2$ atmosphere. Keratinocytes (61yo donor) were grown on the dermal equivalent for 7 days of submerged culture, before elevation at the air-liquid interface for differentiation during one or two weeks. In parallel, *Propionibacterium acnes* ATCC 6919 (LGC standard, France) were first amplified at 37˚C in anaerobic conditions (Gaspack) in Brain Heart Infusion (Biomerieux, France) whereas *Staphylococcus epidermidis* ATCC 14990 (LGC standard, France) were grown on Tryptic Soy Broth in aerobic conditions (Becton Dickinson, Germany). Skin equivalents were infected by SE or PA separately at $10^7$ and combined at $10^6$SE+$10^7$PA. Infection was performed after 1 week epidermis emersion (stage 1) to mimic wound healing and anoxic growing condition for bacteria or 2 weeks of emersion (stage 2). After 2hours, the samples were rinsed with PBS (Life Technologies, UK) to eliminate non-adherent bacteria and then cultured up to 3 days post-infection. The bacterial adhesion and development efficiency were estimated using the colony forming unit assay after the recovery of bacteria from skin equivalents at 2, 24 and 72hours post-infection. The skin morphology and the presence of bacteria were visualized by microscopy after hematoxylin eosin and Gram staining. Once determined, the best infected epidermal conditions were reproduced on polycarbonate membrane with fibroblast on the over side. 0.1% of active ingredients were systemically applied during emersion to evaluated modifications of adhesion and proliferation of both strains at 3days' post-infection.

## Results

At 3 days post infection, microscopic visualization revealed no harmful impact on reconstructed skin due to the colonization by the 2 bacteria present within the superficial epidermis layers (Figure 1A). SE is mainly present at the surface of the skin whereas PA is seen deeper within the skin layers due to its anaerobic growth preference. When seeded separately after 1 week emersion, despite some difference in adhesion and growth recovery, both germs can colonize the skin and grow over 3 days (Figure 1B). When seeded at 2-weeks emersion to mimic a more mature and healthy skin condition, only PA could not grow properly being more exposed to air.

When seeded jointly after 1 week emersion at a 10/1 ratio in favour of PA to compensate the growth capacities difference between the 2 strains, PA did not impact SE growth while SE clearly inhibited the development of PA (Figure 1B).

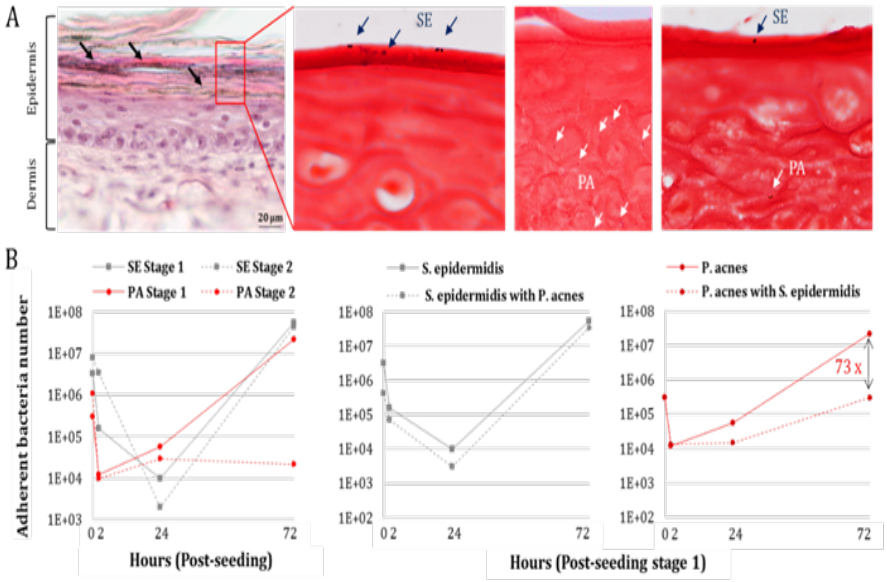


**Figure 1** A. Infected skin equivalent morphology. B. Adhesion and growth of the 2 strains seeded separately at 1 or 2 weeks of post emersion or seeded jointly at 1 week post emersion.

## Discussion

For the first time this study shows cutaneous interactions between two bacterial strains grown on 3D reconstructed skin models. The experimental conditions developed allow to evidence the regulation of *Propionibacterium acnes* by *Staphylococcus epidermidis* known to contribute to the skin defense thanks to phenol-soluble modulins, antimicrobial peptides and free fatty acids production or immuno-priming. Moreover, this 3D model is a useful tool to select active ingredients which promote a healthier microflora balance and to better understand their mechanisms of action. Future evolutions of this model will integrate more virulent *Propionibacterium acnes* ribotypes known to be involved in physiological acne, new germs combinations (*Staphylococcus aureus+epidermidis* to mimic atopic dermatitis). Epidermal differentiation, lipid profile and inflammatory markers will be studied to better understand skin defense mechanisms.

## Acknowledgements

None.

## Conflict of interest

The author declares no conflict of interest.

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