32nd IFSCC Congress
London 19-22 September 2022

Cutibacterium acnes-derived extracellular vesicles promote acne-like phenotypes in human keratinocytes and sebocytes

385

SEPPIC | A company Air Liquide HEALTHCARE

BIZE Cécile[1*] ; VINCENT Gaëlle[1] ; MARTIN Patricia[2] ; FONTANIÉ Maxime[2]

[1] *Seppic Research & Innovation, 127 chemin de la poudrerie, BP 90128, 81105 Castres, France*

[2] *Vibiosphen, 516 Rue Pierre et Marie Curie, 31670 Labège, France*

[*] *BIZE Cécile, Seppic, 127 chemin de la poudrerie – BP 90128, 81105 Castres, France*
cecile.bize@airliquide.com

INTRODUCTION

Among the multiple commensal microorganisms present in the healthy skin flora, *Cutibacterium acnes (C. acnes)* is a ubiquitous Gram-positive aerotolerant anaerobic bacterium belonging to the *Actinobacteria phylum*, that predominantly resides deep within the sebaceous follicle, in contact with keratinocytes. Like mammalian cells, in addition to soluble factors, most Gram-negative and -positive bacteria release extracellular vesicles (EVs) which can be involved in the intercellular communication within or between living organisms.

In this context, we examined whether *C. acnes* (phylotype IA1, DSM1897) secretes EVs and whether these EVs can be involved in the development of acne vulgaris.

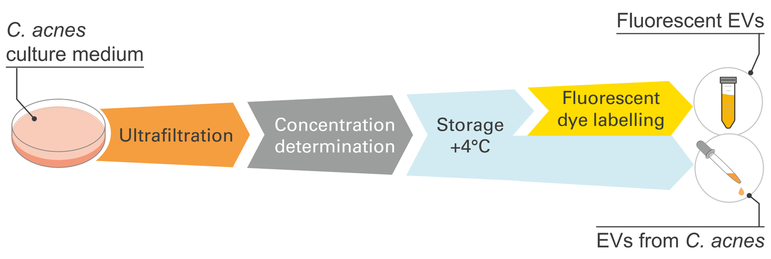
Materials & Methods

• EVs production

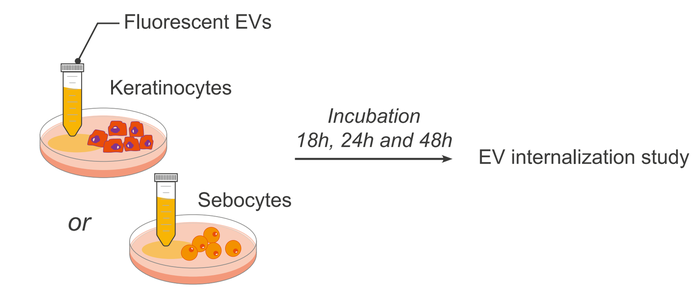
EVs from *C. acnes* (DSM1897) strain were isolated by ultrafiltration and stored at +4°C until use.

The concentration of EVs was estimated using protein concentration determination with the BCA assay.

A fraction of the EVs was labeled with the lipophilic fluorescent dye Vybrant DiI cell-labeling for 30 min at 37 °C [1].

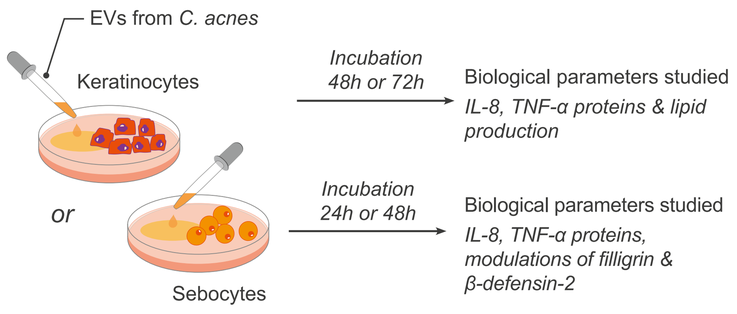


• Internalization of EVs



Fluorescent EVs were added in the culture medium of keratinocytes or sebocytes, and EV internalization was studied by fluorescent microscopy after 18h, 24h and 48h of incubation.

• Evaluation of EVs effects on keratinocytes and sebocytes

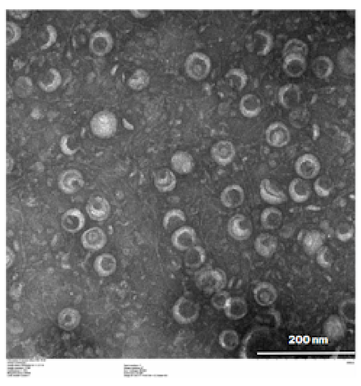


EVs from *C. acnes* were added in the culture medium of primary human keratinocytes for 48h or 72h or sebocytes for 24h or 48h.

Production of IL-8 and TNF-α proteins in culture supernatants, as well as lipid production (Bodipy staining) were evaluated on sebocytes. On keratinocytes, production of IL-8 and TNF-α proteins in culture supernatants were also studied. Modulations of filaggrin (ELISA and immunofluorescence) and β-defensin-2 (ELISA) in cell lysate were determined.

Results & Discussion

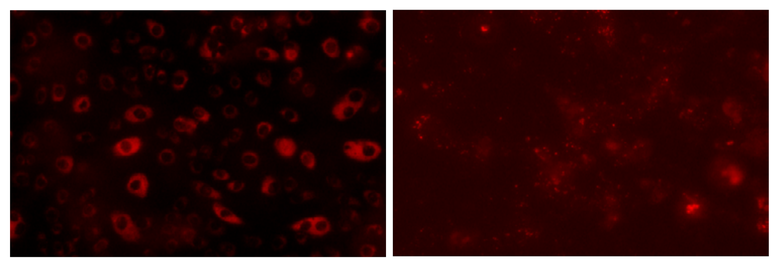
C. acnes secretes EVs



Representative TEM image of EVs from *C. acnes*. (Scale bar, 200 nm).

The size of *C. acnes*-derived EVs was in the range of 30-100 nm, which is in agreement with literature [2, 3].

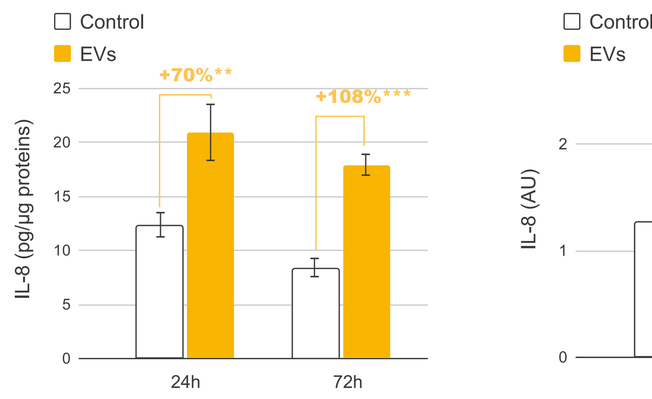
C. acnes-derived EVs internalization in human epidermal keratinocytes and sebocytes



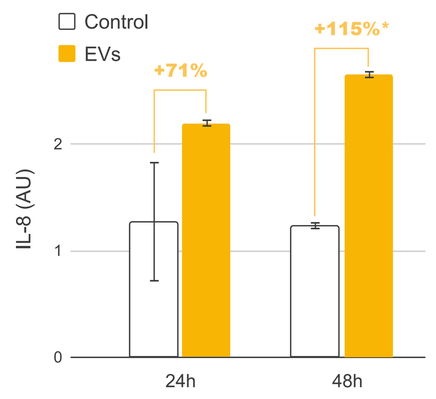
Isolated EVs were labeled with DiI (red) and used to treat both keratinocytes (on the left) and sebocytes (on the right) for 48h (magnification x10).

EVs were located in the perinuclear area, indicating that EVs were endocytosed into cells.

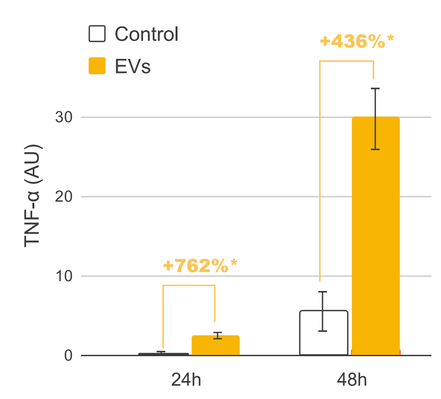
EVs increase the expression of proinflammatory cytokines in both human keratinocytes and sebocytes



▲ Secreted protein levels of IL-8 in keratinocytes treated with *C. acnes*-derived EVs (protein normalization)



▲ Secreted protein levels of IL-8 in sebocytes treated with *C. acnes*-derived EVs (Hoechst normalization)



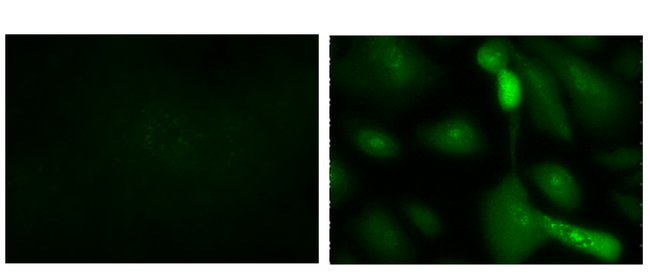
▲ Secreted protein levels of TNF-α in sebocytes treated with *C. acnes*-derived EVs (Hoechst normalization)

TNF-α production was not affected by EVs treatment on keratinocytes.

EVs derived from *C. acnes* upregulate the expression of proinflammatory cytokines in the human epidermis (keratinocytes and sebocytes), thereby participating in inflammatory responses.

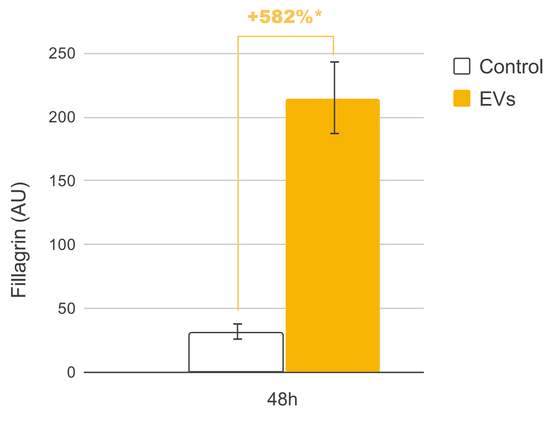
Student's t-test
LS: limit of significance p<0.1
* p<0.05; ** p<0.01; *** p<0.001

EVs induce dysregulation of epidermal differentiation



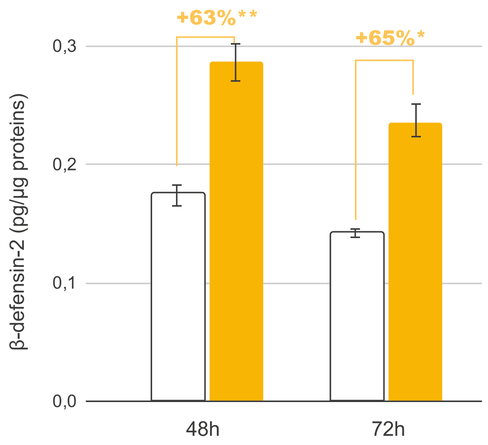
Control | *C. acnes*-derived EVs

Filaggrin expression of keratinocytes exposed to EVs from *C. acnes* (immunofluorescence) ▶



EVs significantly upregulated the expression of filaggrin. Thus *C. acnes* derived-EVs can induce keratinization.

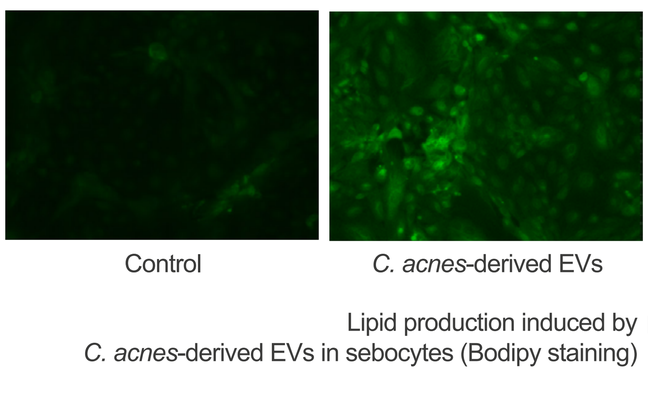
C. acnes-derived EVs induce AMPs production in keratinocytes



◀ β-defensin-2 expression by keratinocytes exposed to EVs from *C. acnes* (ELISA)

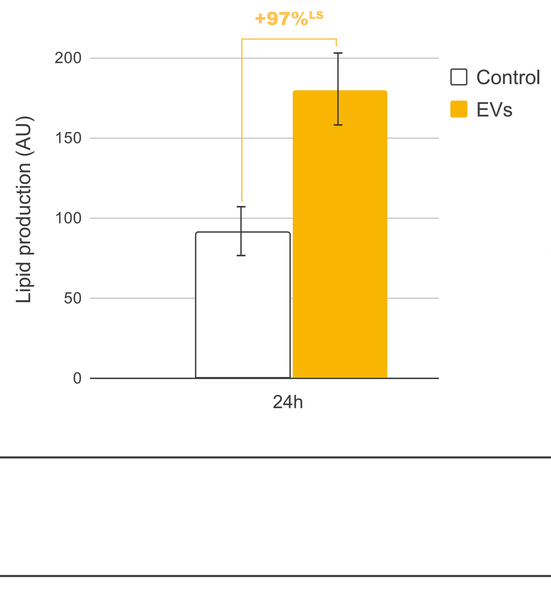
EVs incubation stimulated β-defensin 2 production in keratinocytes.

C. acnes-derived EVs induce lipid production in sebocytes



Control | *C. acnes*-derived EVs

Lipid production induced by *C. acnes*-derived EVs in sebocytes (Bodipy staining) ▶



EVs from *C. acnes* induced lipid production in sebocytes.

CONCLUSION

In summary, our study suggests that lipid bilayer-enclosed and nanosized *C. acnes*-derived EVs efficiently induce not only inflammatory responses but also epidermal hyperkeratinization and sebum production, that are, acne-like phenotypes.

C. acnes-derived EVs induced acne-like phenotypes in primary human keratinocytes, such as increased secretion of inflammatory cytokines and dysregulated epidermal differentiation. Indeed, EVs significantly induced inflammatory cytokine IL-8 production and dysregulated epidermal differentiation by increasing filaggrin protein expression. Moreover, EVs stimulated the production of antimicrobial peptides (β-defensin 2) by keratinocytes. EVs also stimulated the production of IL-8 and TNF-α on human sebocytes derived from iPS. This inflammation induced by *C. acnes*-derived EVs is a typical component of acne. Finally, *C. acnes*-derived EVs induced sebum production.

References

1. Choi EJ et al. (2018) J. Invest. Dermatol., 138, 1371.

2. Jeon J et al., (2017) Proteomics Clin Appl. 11.

3. Chudzik A, et al., (2022) Int. J. Mol. Sci. 23, 5797.