Anaerobe 49 (2018) 21–29

Contents lists available at ScienceDirect

Anaerobe

journal homepage: www.elsevier.com/locate/anaerobe

Pathogenesis and toxins

Developing an *in vitro* artificial sebum model to study *Propionibacterium acnes* biofilms

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ARTICLE INFO

Article history:
Received 23 June 2017
Received in revised form
7 November 2017
Accepted 20 November 2017
Available online 22 November 2017

Handling Editor: Regina Maria Cavalcanti Pilotto Domingues

Keywords:
Propionibacterium acnes
Biofilm
Artificial sebum
Virulence factors

ABSTRACT

Aim: The aim of the present study was to develop a new model system to study *Propionibacterium acnes* biofilms. This model should be representative for the conditions encountered in the pilosebaceous unit.
Methods and results: The new model, consists of an artificial sebum pellet supported by a silicone disc. Sebum pellets were inoculated with various *P. acnes* strains isolated from both normal and acneic skin. Growth and biofilm formation was verified by conventional plating at different time points, as well as by resazurin assays and fluorescence microscopy after LIVE/DEAD staining. The artificial sebum pellets were also used in assays to measure the production of certain virulence factors implicated in the pathogenesis of acne, including lipase, protease and the presence of CAMP factors.
Conclusion: The artificial sebum model can sustain biofilm growth of *P. acnes*, as was determined by increasing CFU counts for up to 1 week after inoculation. Metabolic activity and biofilm formation were confirmed using resazurin staining and fluorescence microscopy respectively. The production of virulence factors in this model was demonstrated as well.

1. Introduction

Propionibacterium acnes (recently renamed as *Cutibacterium acnes* [1]) is an anaerobic but aerotolerant member of the skin microbiome, naturally inhabiting the human pilosebaceous units [2–5]. These pilosebaceous units consist of a hair follicle and an associated sebum gland, and hormonal imbalance can stimulate sebocyte proliferation and lead to increased sebum production. This sebum overproduction, combined with an altered follicular growth can result in an obstruction of the pilosebaceous unit, leading to the formation of microcomedones. These comedones are anaerobic and lipid-rich micro-environments that provide *P. acnes* with ideal growth conditions and an ample supply of the necessary nutrients [6–8]. When *P. acnes* colonizes the pilosebaceous unit and starts proliferating, the host immune system is stimulated, resulting in an increased inflammatory response [9,10]. Moreover, *P. acnes* can produce multiple virulence factors such as lipases, proteases and the Christie–Atkins–Munch-Petersen (CAMP) factors, that degrade host tissue and lead to further activation of the human immune system. For example, lipases break down triglycerides thereby releasing free fatty acids that act as damage associated molecular patterns [11–17].

Previous work has shown that *P. acnes* can form biofilm-like structures inside the pilosebaceous unit [5,18]. Since biofilms are typically more refractory to antibiotic treatment, it is difficult to completely eradicate the infection [19]. This could explain the need for a prolonged antibiotic treatment, and the pattern of relapse and outbreaks commonly observed in acne [20].

One of the contributing factors in the etiology of acne is an increased sebum production and this sebum is the main source of nutrients for *P. acnes*. Human sebum is normally produced through holocrine secretion by the sebocytes and contains approx. 50% triglycerides, 25% wax esters, 15% squalene, 8% free fatty acids and 2% cholesterol [21–26].

It is widely accepted that bacterial biochemistry depends on the nutrients that are present, and Yu et al. [27] recently demonstrated that the secreted proteome of *P. acnes* also varies in different growth media. Using *in vitro* models providing nutrient-poor conditions resembling the interior of the pilosebaceous unit may lead to useful insights in the biology and virulence of *P. acnes*. In the present study we developed an artificial sebum (based on the composition of synthetic sebum proposed by Wertz et al. [26]) by

Abbreviations: CTB, CellTiter-Blue; CAMP factors, Christie–Atkins–Munch-Petersen factors.

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https://doi.org/10.1016/j.anaerobe.2017.11.002
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mixing lipids with bacterial growth medium, thereby adding essential growth factors not provided by the lipids. This medium was subsequently used in an *in vitro* biofilm model to study various aspects of the biology of *P. acnes*. Using topical or oral antibiotics is a cornerstone in the treatment of acne vulgaris [28,29]. However, prolonged therapy with low doses of antibiotics has led to the emergence of antibiotic resistance since the end of the 1970's [30]. Antibiotic resistance to erythromycin and a reduced sensitivity to clindamycin, both commonly used topical antibiotics during the treatment of acne, are now found worldwide [31–33].

2. Materials and methods

2.1. Strains and culture conditions

Ten *Propionibacterium acnes* strains were tested (Table 1). Strains were cultured in reinforced clostridial medium (RCM) or on reinforced clostridial agar (RCA) (LabM, Heywood, UK). All cultures were grown at 37 °C under anaerobic conditions (generated using the Anaerogen Compact system (Oxoid, Aalst-Erembodegem, Belgium) or the Gaspak EZ system (BD, VWR, Leuven, Belgium)) [34]. Planktonic cultures were obtained by inoculating RCM with a few colonies from 3 to 5 day old pure cultures and incubating anaerobically for 24 h. Biofilms in 96-well microtiter plates (MTP, SPL Life Sciences, VWR, Leuven, Belgium) were formed starting from these 24 h old planktonic cultures. The cultures were diluted with RCM to an optical density of 1.0 at 590 nm (corresponding to approx. 2.5×10^8CFU/mL). 100 μL of this suspension was then transferred to the MTP and incubated anaerobically for 4 h to allow cells to adhere to the bottom of the wells. Subsequently, the supernatant containing the planktonic cells was removed and the wells were carefully washed with 100 μL physiological saline (PS, 0.9% NaCl, AppliChem-Panreac, Darmstadt, Germany). After removing the PS, 100 μL RCM was added and the plates were further incubated for 20 h, producing mature biofilms. *Staphylococcus aureus* LMG8224, obtained through the BCCM/LMG Bacteria Collection (Ghent, Belgium), and *Streptococcus agalactiae* (an isolate from our own collection) were used in the CAMP test. These strains were first resuscitated on Columbia agar plates containing sheep blood (BD, Erembodegem, Belgium).

2.2. Production and inoculation of sebum pellets

The artificial sebum is composed of 2 g tripalmitin, 300 mg palmitic acid, 100 mg cholesterol, 50 μg tocopherol acetate, 0.55 mL triolein, 1.437 mL jojoba oil and 0.877 mL squalene (all acquired from Sigma-Aldrich, Bornem, Belgium). Each component was chosen to represent a group of physicochemically related components found in human sebum (e.g. palmitic acid represents the free fatty acids). The components were heated to 85 °C and mixed to a homogenous oil, which was subsequently autoclaved. Next, the sterilized oil was mixed with 1 mL double concentrated RCA and 1 mL double concentrated RCM, providing other essential nutrients. While still warm, 50 μL of this artificial sebum was added to previously autoclaved medical grade silicone discs (diameter of 6 mm, thickness of 4 mm); the artificial sebum was then allowed to solidify and to adhere to the discs, forming sebum pellets.

These pellets were subsequently transferred to a 48-well MTP (Thermo-Fisher, Waltham, MA, United States). 24 h old planktonic *P. acnes* cultures in RCM were centrifuged for 5 min at 2200×*g* (Eppendorf centrifuge 5804 R, Eppendorf, Hamburg, Germany), the supernatant was discarded, and cells were resuspended in 2.5 mL phosphate buffered saline (PBS, 0.14 M NaCl, 0.003 M KCl (Merck, Overijse, Belgium), 0.0101 M Na_2HPO_4 (Sigma) and 0.0018 M KH_2PO_4 (Sigma), pH 7.4). To ensure complete removal of residual RCM, this washing step was repeated two more times. The resulting bacterial suspension was set to an optical density of 0.05 at 590 nm (corresponding to approx. 5×10^6CFU/mL) and 750 μL was used to inoculate the pellets. After 15 min of incubation at 37 °C, the suspension was aspirated and the pellets were washed with 750 μL PS, which was subsequently removed. A schematic overview of this procedure is shown in Fig. 1.

2.3. Biofilm formation on artificial sebum pellets

After 0 h, 24 h, 48 h, 72 h and 1 week of anaerobic incubation at 37 °C, the inoculated artificial sebum pellets were transferred to an Eppendorf tube containing 2 mL PBS. These tubes were then vortexed for 2 min and sonicated for 5 min (40 kHz). This process was repeated, after which a serial dilution of these suspensions was made to determine the number of CFU through conventional plating. To verify that cells were not damaged during sonication, planktonic cultures were sonicated one, two or three times and plated to estimate the cell count after the increasing sonication steps. No difference was observed in number of CFU recovered, compared to cultures that were not sonicated (data not shown). Cell viability after 1 week incubation was assessed by measuring metabolic activity, using a resazurin-based staining (CellTiter-Blue (CTB), Promega, Leiden, The Netherlands). 2.1 mL stock solution of CTB was diluted with 10.5 mL PS. Pellets were transferred to a 48-well MTP and 1 mL of this diluted CTB was added to the wells containing the pellets. The plate was covered and incubated at 37 °C for 2 h shaking at 600 rpm. Subsequently 100 μL was transferred to a 96-well MTP and fluorescence (excitation wavelength: 560 nm/emission wavelength: 590 nm) was measured using an EnVision multilabel reader (Perkin Elmer).

Biofilm formation was visualized using fluorescence microscopy as well as confocal microscopy. Pellets incubated for 7 days were stained with SYTO9 (excitation wavelength: 485 nm/emission wavelength: 530 nm) and propidium iodide (excitation wavelength: 485 nm/emission wavelength: 630 nm) (LIVE/DEAD BacLight Bacterial Viability Kit, Thermo-Fisher) (3 μL of both dyes per mL PS, staining for 10 min at room temperature). An EVOS FL Auto Imaging System fluorescence microscope (Life Technologies, Carlsbad, California, USA) with a 2×, 10× or 20× objective and appropriate filter cubes, was used to visualize both live and dead cells. For confocal imaging, a Nikon C1si confocal laser scanning microscope equipped with a Plan Apo VC 40× objective lens (Nikon) was used to visualize the live cells.

Table 1
Propionibacterium acnes strains used in the present study.

Strain	Biological origin	Type
LMG16711[T,a]	Human, acneic skin	IA_1
SK137[b]	Human, acneic skin	IA_1
SK182[b]	Human, acneic skin	IA_1
HL001PA1[b]	Human, normal skin	II
HL030PA1[b]	Human, normal skin	IB
HL030PA2[b]	Human, normal skin	IA_2
HL037PA1[b]	Human, normal skin	IA_2
HL053PA1[b]	Human, acneic skin	IA_1
HL060PA1[b]	Human, acneic skin	II
HL063PA1[b]	Human, normal skin	IA_1

T type strain.
[a] This strain was obtained from the BCCM/LMG Bacteria Collection (Gent, Belgium).
[b] These strains were obtained from BEI Resources, NIAID, NIH (Manassas, VA, United States).

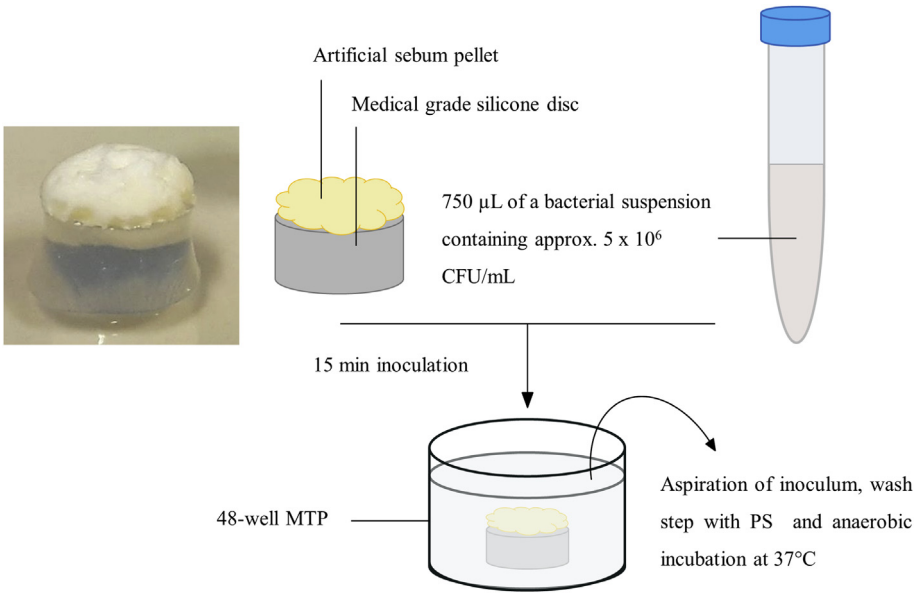

Fig. 1. Picture of the artificial sebum pellet on top of the silicone disc and schematic representation of the inoculation process used.

2.4. Assessing virulence factor production

The production of lipases and proteases by *P. acnes* grown on artificial sebum (7-day old cultures in order to have a sufficient cell count), as well as the co-hemolytic potential (production of CAMP factors) was assessed. The production of these virulence factors in biofilms grown on artificial sebum was then compared to the production in 24 h old planktonic cultures and biofilm grown - for a total of 24 h - in MTP. At those time points the *P. acnes* cells are in the mid to late exponential growth phase.

Lipase production was determined in planktonic cell cultures and biofilm cultures using the fluorogenic substrate 4-methylumbelliferyl (4-MU) oleate (Sigma) [34]. Sterile supernatant was obtained by centrifuging the supernatant of 24 h-old planktonic cultures or the collected supernatant of biofilm cultures in 96-well MTP at 4500×*g* for 10 min and filtering through 0.22 µm membranes (Millipore, Overijse, Belgium). The substrate solution was prepared by dissolving 4-MU-oleate in dimethylsulfoxide (DMSO, Sigma) to a concentration of 0.4 mg/mL DMSO. Next, 100 µL sterile supernatant and 50 µL substrate solution were combined in a black 96-well MTP (Perkin Elmer). To quantify lipase activity on the pellets, the pellets were transferred to the wells of a black 96-well MTP and 100 µL sterile RCM was added together with 50 µL of the substrate. Finally, the fluorescence (excitation wavelength: 355 nm/emission wavelength: 460 nm) was measured using an EnVision multilabel plate reader every 5 min for at least 6 h. The slope at the beginning of the reaction was then calculated and standardized for the amount of cells present.

The production of protease was determined using the EnzChek protease assay (Invitrogen, Paisley, UK) according to the manufacturer's instructions. Briefly, 100 µL supernatant from planktonic cells and biofilm cells grown in a 96-well MTP was collected as described above. The supernatant was transferred to a black 96-well MTP and 100 µL of the substrate solution (10 µg/mL) was added. The plate was incubated, protected from light, for 1 h at 37 °C and fluorescence (excitation wavelength: 485 nm/emission wavelength: 530 nm) was measured using an EnVision multilabel plate reader. To quantify protease production in biofilms grown on artificial sebum pellets, the pellets were transferred to a 2 mL Eppendorf tube together with 100 µL sterile RCM and 100 µL substrate solution. After 1 h incubation at 37 °C, the solution was transferred to a black 96-well MTP to measure the fluorescence.

The ability to produce CAMP factors was assessed using the CAMP test. To this end, *S. aureus* was streaked in the center of a Columbia agar plate containing 4% sheep blood, and *P. acnes* strains were streaked perpendicular, 1 cm from the *S. aureus* streak. As a positive control, *S. agalactiae*, a known producer of CAMP factors, was also streaked perpendicular to *S. aureus*. A clear arrowhead between the *S. aureus* streak and the one from *P. acnes* indicates the production of CAMP factors. To measure the production of CAMP factors by planktonic and sessile cells, a modification of the hemolysis assay, described by Brackman et al. [35] was used. 200 µL supernatant from planktonic cultures and MTP-grown biofilms were collected as described above. This supernatant was transferred to a 2 mL Eppendorf tube together with 200 µL supernatant of a planktonic *S. aureus* culture grown for 24 h in RCM, and 800 µL of a 4% sheep blood (Biotrading, Keerbergen, Belgium) suspension in PBS. The tubes were incubated for 24 h and 100 µL was transferred to a flat bottom 96-well MTP (SPL Life Sciences) after centrifuging at 100×*g* for 2 min. Finally, the absorbance was measured at 420 nm using an EnVision multilabel plate reader. To assess the possible production of CAMP factors in biofilms grown on artificial sebum pellets, the same experimental setup was used. However, 200 µL sterile RCM and a pellet were now added to an Eppendorf tube together with 200 µL *S. aureus* supernatant and 800 µL 4% sheep blood in PBS.

2.5. Statistics

All experiments were repeated at least three times and data were analyzed using SPSS Statistics version 23.0 (SPSS, Chicago, IL, United States). Normality of the data was determined using the Shapiro-Wilk test. Depending on the normality, the data were further analyzed using a *t*-test or Mann-Whitney *U* test, or an ANOVA test or Kruskal-Wallis test. A $p < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. P. acnes biofilm formation in the artificial sebum model

Growth of *P. acnes* on the artificial sebum pellets was assessed at different time points, using conventional plating techniques. The pellets were inoculated using a suspension containing a low cell count (approximately 5×10^6 CFU/mL) in order to observe actual cell growth and increase in CFU in contrast to survival of the inoculated cells. Pellets were then analyzed after inoculation and after 1, 2, 3 and 7 days of anaerobic incubation. For all *P. acnes* strains tested, growth was observed with the number of CFU increasing from approx. 10^4–10^5 CFU per pellet to approx. 10^7 CFU per pellet after 7 days of incubation (Supplementary Fig. 1).

Cell viability (as assessed by measuring metabolic activity) of the cells after 1 week growth on the artificial sebum pellets was measured using a resazurin staining (CTB) assay. All pellets inoculated with the strains tested generated fluorescence signals

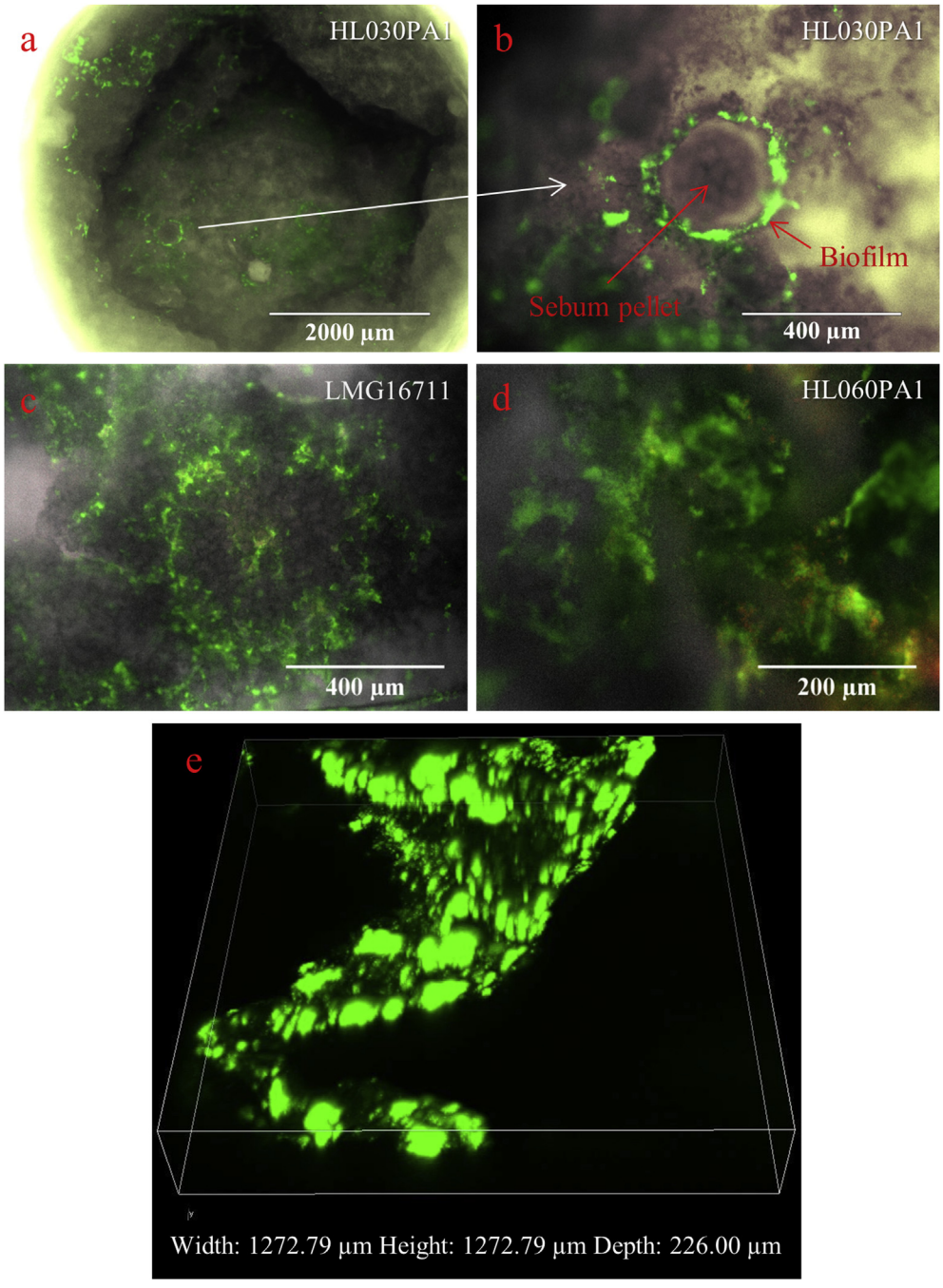

Fig. 2. Microscopy images of *P. acnes* biofilms on artificial sebum pellets following LIVE/DEAD staining (SYTO9 (excitation wavelength: 485 nm/emission wavelength: 530 nm) and propidium iodide (excitation wavelength: 485 nm/emission wavelength: 630 nm)). The grey structure is the artificial sebum pellet itself, green structures are live cells and biofilm that were produced during 1 week incubation and while red clusters are dead cells. (a–d) Fluorescence microscopy images of biofilms on the artificial sebum pellet. (a) HL030PA1 60× magnified (scale bar: 2000 µm), (b) HL030PA1 300× magnified (scale bar: 400 µm); (c) type strain LMG16711 300× magnified (scale bar: 400 µm) and (d) HL060PA1 600× magnified (scale bar: 200 µm). (e) A 3-D confocal microscopy image of *P. acnes* biofilm strain HL060PA1 on artificial sebum pellet (40× magnified), only live cells are visualized. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

significantly higher compared to sterile pellets ($p < 0.05$), thus indicating metabolic activity of the cells present after 7 days of incubation on the artificial sebum pellets (Supplementary Fig. 2).While CTB staining is a convenient alternative to plating in order to measure bacterial growth, there is no strong correlation between both methods. For example, *P. acnes* HL060PA1 generates low fluorescence signals using the CTB assay, but can reach more than 10^7 cells/pellet after 1 week of incubation. Regardless, CTB staining of biofilms grown in the artificial sebum model allows to gain insight in the metabolic state of the bacteria, which can be important when testing novel antibacterial or anti-acne components. Some components might not kill the bacteria, but interfere with other cellular processes and this could be observed using CTB staining.

Biofilm formation on the pellets was confirmed using microscopy after a LIVE/DEAD staining of the inoculated pellets (Fig. 2). Fluorescence microscopy imaging has indeed revealed the formation of biofilm like structures containing live cells (as represented by green clusters). The small fraction of red clusters, indicating dead cells, could be due to the slow-growing nature of *P. acnes*. These pictures, combined with the plating results and the viability assay, confirm bacterial growth of *P. acnes* on the artificial sebum pellets. The *in vitro* artificial sebum model, is an alternative to existing biofilm models for acne-related *P. acnes* research. Most models to study bacterial biofilms are based on microtiter plates, glass beads or use a flow cell which has a continuous supply of fresh medium [36]. However, in case of biofilm formation by *P. acnes* in acne, these methods are not particularly relevant. The human skin, the natural environment of this bacterium, is a lipid-rich environment in which free water and nutrients are limited [37]. These conditions are mimicked in the artificial sebum model, unlike most other biofilm models used in current acne-related *P. acnes* research [38–41]. In addition, this model is technically not-demanding and inexpensive, and allows the gap to be bridged between other *in vitro* models and more complex animal models [42,43].

3.2. Production of virulence factors

We determined the production of virulence factors by *P. acnes* grown in planktonic cultures (24 h), in biofilms in MTP (4 h adhesion followed by 20 h of additional incubation) and in biofilms grown on artificial sebum (incubated for 7 days). These time points were chosen in order to have cells in mid to late exponential growth and to have a sufficient number of cells to allow quantification of virulence factor production.

Using the fluorogenic substrate 4-MU-oleate, lipase production

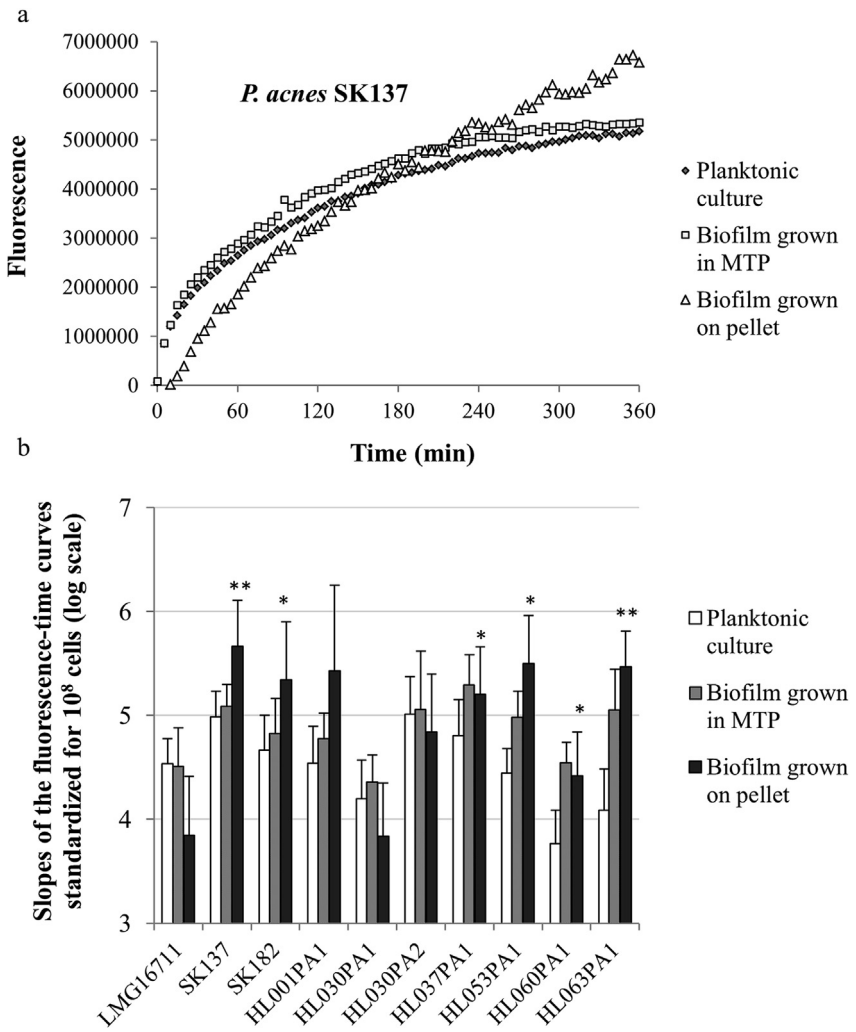

Fig. 3. (a) Fluorescence-time curves obtained with *P. acnes* SK137. The produced lipase hydrolyzes 4-MU oleate releasing the fluorophore, causing an increasing fluorescence signal over time. (b) Slopes at the start of the reaction, normalized for 10^8 cells. Mean ± SD (number of replicas n > 3). * significantly higher lipase activity on the artificial sebum pellets compared to planktonic cells, ** higher lipase activity on the artificial sebum pellets compared to planktonic cells and compared to biofilm in a MTP, one-way ANOVA.

by *P. acnes* was determined in planktonic cultures, biofilm cultures grown in a MTP and grown on artificial sebum pellets. In this assay, fluorescence is measured over time, resulting in curves (Fig. 3a) for which the slopes in the early stages of the reaction can be used as a measurement of the amount of lipase that is present. Given that the different growth conditions result in different final cell numbers, the measured slopes were normalized to 10^8 cells (Fig. 3b). For 6 out of 10 strains investigated, the lipase activity was significantly higher ($p < 0.05$) in biofilms grown on artificial sebum pellets than in planktonic cultures, while for 2 strains the lipase activity was significantly lower ($p < 0.05$). Only 2 strains had significantly higher ($p < 0.05$) standardized slopes when comparing lipase production in biofilm on the pellet vs. in a MTP.

P. acnes is one of the predominant bacterial species on the human skin, favors the lipid-rich regions of the skin, and colonizes the pilosebaceous unit. It is therefore not surprising that the genome of this bacterium encodes multiple putative lipases, including GehA and GehB [13,17]. GehA is thought to be the enzyme responsible for most of the degradation of the triglycerides found in nascent sebum [44]. Lysis of these triglycerides releases free fatty acids which are inflammatory, while GehA itself is also a strong chemotactic and has pro-inflammatory activity [45]. In addition, free fatty acids released from nascent sebum cause ductal hypercornification of the pilosebaceous unit and cause keratinocytes to adhere to each other, assisting in the formation of comedones. Additionally, these free fatty acids also promote *P. acnes* cell-cell adhesion and adhesion to the internal structures of the pilosebaceous unit, thus contributing to colonization and biofilm formation [8,17,45,46].

Protease production was assessed using the commercially available EnzChek protease kit. Fluorescence could be observed in all growth conditions, indicating the presence of protease (Fig. 4). The production of protease was compared between the different growth conditions and was either similar between groups or lower in biofilms produced on the pellet compared to in the biofilm in a MTP.

Protease production and excretion by *P. acnes* was already described by Lee et al. [11] In the present study we demonstrated the presence of protease in different growth conditions, including in biofilms formed on sebum pellets. In the pilosebaceous unit protease can supply *P. acnes* with nutrients by breaking down host tissue and it can activate the host immune response directly by inducing an immune response from both keratinocytes and sebocytes, the two most important cells that make up the pilosebaceous unit [11,12].

The production of CAMP factors, was determined using the CAMP test. All tested *P. acnes* strains formed clear arrow heads on 5% sheep blood plates in the zones where *S. aureus* and *P. acnes* converged, indicating the lysis of the sheep blood cells (Fig. 5a).

In the absence of *S. aureus*, isolated cells or supernatant from planktonic or biofilm cultures of *P. acnes* did not show hemolysis of sheep blood, even in the presence of a suitable growth medium (RCM) (data not shown). This lack of sheep blood hemolysis by *P. acnes* alone is in accordance with the results of previously published work [34] of our group, in which no hemolytic activity could be found in *P. acnes* using horse blood. Next, the ability to co-hemolyse sheep blood in combination with *S. aureus* supernatant was determined. Overall, co-hemolysis occurred in all conditions tested (Fig. 5b). For all strains tested, supernatant from planktonic cultures and from biofilm grown in MTP were able to co-hemolyse sheep blood cells causing optical densities higher than the blank (i.e. hemolysis by *S. aureus* alone). When biofilms grown on the artificial sebum pellets were tested, all but two strains, HL001PA1 and HL060PA1, were able to generate a positive co-hemolysis signal as indicated in Fig. 5b. The results for these two strains are in

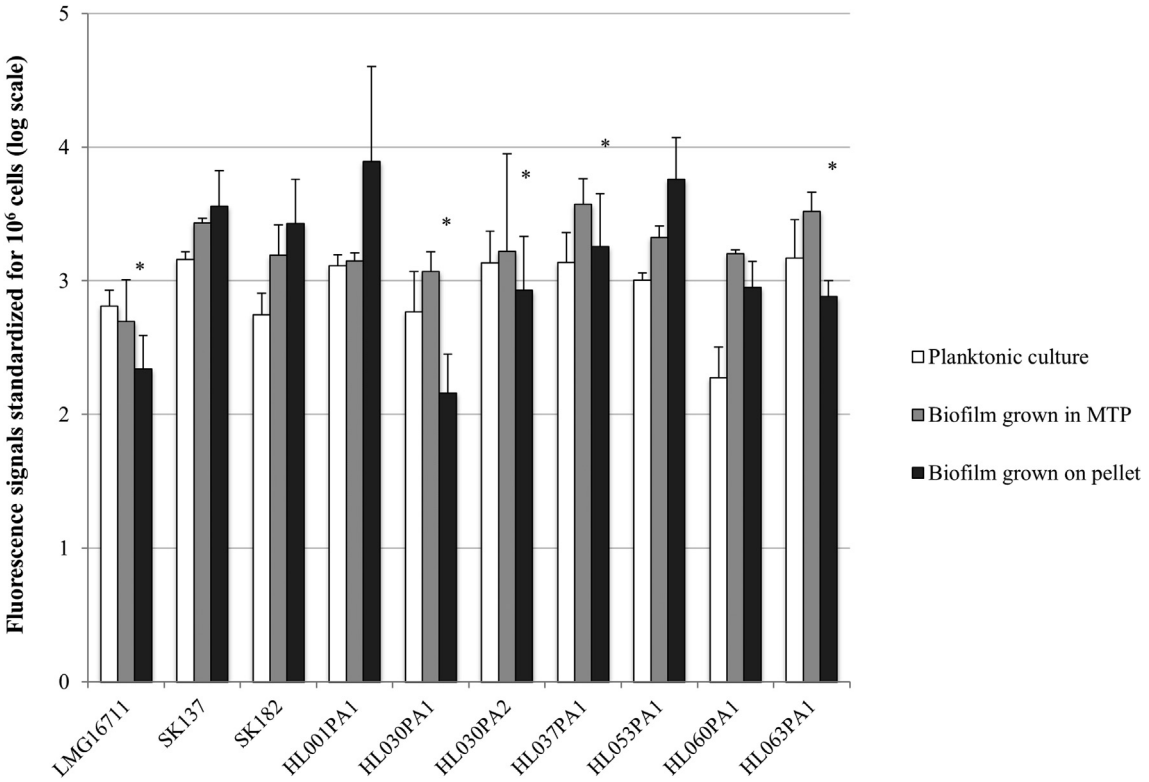

Fig. 4. Fluorescence signals obtained with the Enzchek protease assay (normalized to 10^6 cells). Bars represent averages and the error bars standard deviations (number of replicas $n > 3$). * Lower standardized signal in biofilm grown on sebum pellet, compared to the signal generated in a biofilm grown in a MTP ($p < 0.05$) as determined with the Kruskal-Wallis test.

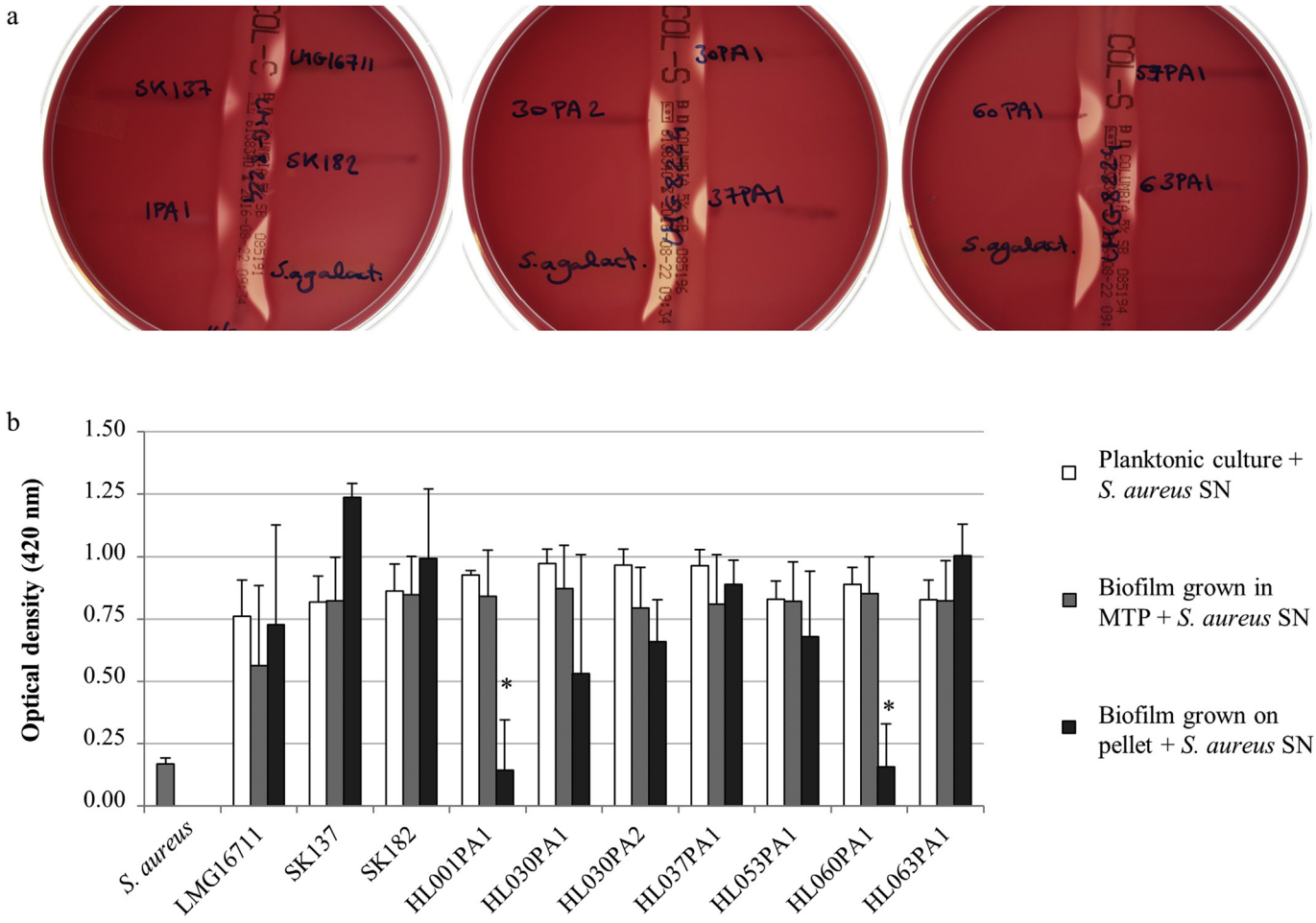

Fig. 5. (a) Co-hemolysis of sheep blood by *S. aureus* and *P. acnes*. Clear arrow heads indicate complete lysis of the red blood cells where both the *S. aureus* produced toxin and the *P. acnes* CAMP factors interact with the red blood cells. (b) Co-hemolysis by *P. acnes* in different growth conditions. * The signals that were not significantly higher than blank signals (i.e. hemolysis by *S. aureus* supernatant alone, $p > 0.05$), as determined with the *t*-test. Bars represent averages and the error bars standard deviations (number of replicas $n > 3$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

contrast with the results of the CAMP test in which all strains were able to produce CAMP factors. These results are interesting as the strains in question both belong to *P. acnes* type II, recently reclassified as *P. acnes* subsp. *defendens*, which is rarely associated with acne. This outcome shows the importance of the microenvironment on the production of certain virulence factors.

The genome of *P. acnes* contains five genes with sequence similarity to the co-hemolytic CAMP factor produced by *Streptococcus agalactiae* [13,16]. Co-hemolysis between CAMP factors and *S. aureus* supernatant is caused by a synergistic effect of the CAMP factors and an *S. aureus* toxin that acts as a sphingomyelinase. This enzyme breaks down sphingomyelin, which is an integral part of mammalian cell membranes, making them more vulnerable to the membrane pore-forming CAMP factors [47].

In the present study, we confirmed the ability of *P. acnes* to co-hemolyse sheep blood in combination with supernatant of *S. aureus*, irrespective of the mode of growth, in all but two strains. The production of these CAMP factors could be an important component in the etiology of acne. Studies have shown that these factors are both secreted and cell-associated and belong to the most abundantly secreted proteins in *P. acnes* [16,17,48]. CAMP factors can activate the human immune system directly through binding with TLR-2, or indirectly due to the combined effect with host produced sphingomyelinase, which weakens cellular lining, causing an invasion by *P. acnes* in the dermis as a result [15,49,50].

4. Conclusion

We developed a new model, based on artificial sebum, to study *P. acnes* biofilms. The artificial sebum model sustains *P. acnes* growth and biofilm formation while mimicking some key aspects of the pilosebaceous unit. We have also shown that the model can be used in a wide range of experiments ranging from microscopy to the detection of the production of certain virulence factors that might be relevant in the pathogenesis of acne.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgements

The authors would like to thank Eline Teirlinck for the help with the confocal microscope as well as the Laboratory of General Biochemistry and Physical Pharmacy (Ghent University) for providing the confocal microscope and technical assistance. We would also like to thank Lynn De Waele for the help during the optimization of the artificial sebum composition.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.anaerobe.2017.11.002.

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