Bacterial Aggregates Establish at the Edges of Acute Epidermal Wounds

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Objective: The bacterial composition and distribution were evaluated in acute standardized epidermal wounds and uninjured skin by a molecular *in situ* technology benchmarked to conventional culturing. This was done to reveal whether bacterial biofilm is present in acute wounds.

Approach: On the buttock of 26 healthy volunteers, 28 suction blisters were made and de-roofed. Four wounds were biopsied immediately after wounding, whereas the remaining 24 wounds were treated daily with sterile deionized water and covered with a moisture-retaining dressing. On day 4 post-wounding, swabs were obtained for culturing from the wounds and adjacent skin, and the wounds including adjacent skin were excised. Tissue sections were stained with peptide nucleic acid (PNA) fluorescence *in situ* hybridization (FISH) probes, counterstained by 4',6-diamidino-2-phenylindole, and evaluated by confocal laser scanning microscopy (CLSM).

Results: No bacterial aggregates were detected at day 0. At day 4, coagulase-negative staphylococci (CoNS) were the sole bacteria identified by CLSM/PNA-FISH and culturing. CoNS was isolated from 78% of the wound swabs and 48% of the skin swabs. Bacterial aggregates (5–150 μ m) were detected by PNA-FISH/CLSM in the split stratum corneum and fibrin deposits at the wound edges and in the stratum corneum and the hair follicles of the adjacent skin. The bacterial aggregates were more common ($p=0.0084$) and larger ($p=0.0083$) at wound edges than in the adjacent skin.

Innovation: Bacterial aggregates can establish in all wound types and may have clinical significance in acute wounds.

Conclusion: Bacterial aggregates were observed at the edges of acute epidermal wounds, indicating initiated establishment of a biofilm.

Keywords: bacterial aggregates, biofilm, acute wounds, standardized epidermal wounds, confocal microscopy, PNA-FISH

INTRODUCTION

THE HUMAN SKIN acts as a physical barrier toward the environment and is naturally colonized by an assembly of commensal microorganisms.^{1,2} Thus, the incessant shedding of the corneocytes prevents massive microbial colonization.^{2–4} However, environmental changes may lead to a microbial imbalance, resulting in the predominance of pathogenic species.⁵ Interestingly, the skin as an organ itself seems to discriminate between harmless and pathogenic microorganisms.³ Characterization of the skin microbiota in specific habitats provides important knowledge regarding the equilibrium between beneficial colonization and infection.¹

Skin injury initiates an ordered sequence of wound-healing events.⁶ Even minor skin traumas can develop into an infected wound, unless the opportunistic pathogens are eradicated efficiently.⁷ To reinstate regular wound repair, barriers to healing must be outbalanced.^{6,7} A factor that delays the healing process is bacterial biofilm,⁸ defined as a coherent cluster of microbial sessile cells that are extremely tolerant toward antibiotics and host defense mechanisms⁹ and difficult to diagnose.¹⁰

Traditional methods for the identification of microorganisms in clinical microbiology are based on the ability of bacteria to grow and form colonies on commercial media.¹¹ The efficiency of this technique is limited with regard to slow-growing, fastidious, or dormant microorganisms,^{1,2,12} as optimal nutrition and growth conditions are not always met in the microbiological laboratory.¹³ Further, the majority of microorganisms in nature are considered viable but not capable of being cultured.⁴ Indeed, it has been estimated that less than 1% of bacterial species are cultivable.^{1,2,12} In the Human Microbiome project, the diversity and ratio of species were found to be considerably higher than previously recognized by the use of molecular methods.¹⁴ Therefore, a combination of modern molecular approaches is more appropriate to diagnose biofilm-associated infections.¹⁵

Confocal laser scanning microscopy (CLSM) in combination with peptide nucleic acid (PNA)–fluorescence *in situ* hybridization (FISH) staining is a useful technique for the detection of bacterial biofilms in clinical tissue samples.¹⁶ The PNA probes target specific 16S sequences of the bacterial ribosome and have high hybridization affinity to rRNA.¹⁷ CLSM/PNA-FISH has been successfully applied in our previous studies,^{18–21} providing the advantages of visualization of bacterial distribution in high resolution. Despite the limitations in using sequential sectioning,^{22,23} the method

is powerful for investigating spatial dispersal of bacterial aggregates. In addition, full-thickness skin biopsies allow insight of the spatial three-dimensional bacterial distribution on and within the skin, as well as the diversity of the microbiota in the various compartments of the skin architecture, for example, hair follicles.²¹

The human skin microbiota is assumed to be heterogeneously distributed²⁴ and naturally distributed as aggregates, as this is the most common growth form of bacteria.²⁵ In this study, it was hypothesized that the microbiota of both healthy and acute injured skin are distributed as bacterial aggregates. To further investigate the importance of skin microbiota in relation to infection and wound healing, the bacterial distribution in human skin was examined in healthy individuals.

CLINICAL PROBLEM ADDRESSED

Knowledge of the initial development, quantity, and spatial distribution of bacterial aggregates in acute wounds will promote the understanding of the progress of infected acute and chronic wounds.

MATERIALS AND METHODS

Ethical approval, participants, and study design

This trial was approved by the Regional Committee on Biomedical Research Ethics for the Capital Region of Denmark (H-6-2014-001).

Healthy non-smoking volunteers between 18 and 65 years old were recruited via www.forsoegsperson.dk and were included after them providing written consent. The volunteers were remunerated. The culture results and biopsies in this study originated from a randomized, double-blind clinical trial (ZINGEL) registered at ClinicalTrials.gov (NCT02116725).²⁶ In the three-arm trial, one wound was made on each buttock of the participants. Two of the three treatments (zinc sulfate shower gel, placebo shower gel, or sterile deionized water = control) were allocated to the wounds in each participant.²⁶ The 24 participants who received control treatment in addition to the 2 participants from whom the two wounds were biopsied on day 0 were included in this study between March 17 and May 13, 2014.

The 16 men and 10 women were 27 ± 6 years and had a body mass index of 24 ± 4 kg/m² (mean $\pm$ SD). Six participants had skin type I, five had type II, eight had type III, six had skin type IV, and one had skin type V.

Wounding and treatment

Suction blisters (10 mm in diameter) were raised on the buttocks, 1/3 of the distance from the sacral

bone to the femoral tuberculum major, using heated chambers (40°C) kept under a constant vacuum of 380 mm Hg for 67 ± 12 min (NP-4; Electronic Diversities, Finksburg, MD).²⁷ The epidermal blister roof was excised with a sterile scalpel. Four wounds in two participants were biopsied just after wounding (day 0) and served as controls. The wounds and surrounding skin of the remaining 24 participants were treated once at days 0–3 for 2 min with 1 mL of sterile deionized water (Fresenius Kabi, Bad Homburg von der Höhe, Germany) and subsequently rinsed with 50 mL tap water (38°C). Wounds and surrounding skin were covered with a bacterial-proof transparent island dressing composed of a central non-adherent absorbent viscose pad (5×4.5 cm) on a vapor-permeable film with polyacrylate adhesive (Mepore®; Mölnlycke, Göteborg, Sweden).²⁸ The adjacent skin served as an internal control skin to the wounds.

Cultivation

Specimens were obtained from the adjacent skin (10 mm from wound edge) and the wound bed by rotating a saline-saturated swab (eSwab™; Copan, Brescia, Italy) three times clockwise and three times counterclockwise (Supplementary Fig. S1a, b; Supplementary Data are available online at www.liebertpub.com/wound). The swabs were kept at 4°C for maximally 24 h and plated by using the three sector streak method on agar plates containing 5% horse blood (Statens Serum Institut, Copenhagen, Denmark) and on agar plates containing modified Conradi-Drigalski diagnostic substrate selective for Gram-negative rods (Department of Clinical Microbiology, Herlev Hospital, Herlev, Denmark). The plates were incubated aerobically at 35°C for 24 h, and the bacterial growth was semi-quantified in ascending order: no growth (0), colonies in the first, second, or third sector of the agar plate as +, ++, or +++. Coagulase-negative staphylococci (CoNS) were identified by the Staphaurex® agglutination test (Thermo Fisher Scientific, Waltham, MA) and confirmed by matrix-assisted laser desorption-ionization-time of flight (MALDI-TOF) mass spectrometry (Bruker, Billerica, MA) in duplicates.

Harvesting of biopsies

The biopsies were obtained by using aseptic techniques, and the wound and surrounding skin was disinfected with chlorhexidine acetate (2 mg/mL)-cetrimide (1 mg/mL). The biopsy area was infiltrated with a local anesthetic (Carbocain® 10 mg/mL) containing epinephrine (5 µg/mL). Wounds were excised (15×3 mm) with a sterile scalpel (#11), including 3 mm of the adjacent skin. A surgical adhesive drape with 6×8 cm aperture (Barrier®; Mölnlycke) was

applied. The excision was closed with continuous resorbable 4-0 poliglecaprone 25 suture (Monocryl®; Ethicon, Johnson & Johnson International, Brussels, Belgium). No post-operative infections were observed using this procedure. One participant with skin type I presented keloids of 2.5 cm in diameter 2 years post-operatively. The scars responded to intralesional (0.75 mL) glucocorticoid injections (Kenalog® 40 mg/mL) with flattening within 6 weeks.

Biopsy fixation and preparation, PNA-FISH, and hematoxylin and eosin staining

Skin biopsies were fixed in 4% buffered paraformaldehyde (pH 7.4) at 4°C overnight and embedded in paraffin. Three consecutive tissue sections (4 µm) were cut perpendicular to the surface, deparaffinized, and stained with PNA-FISH-TexasRed-conjugated 16S rRNA probes.²⁹ One section from each sample was stained with a universal bacterial (BacUni) probe, and the two sequential sections were stained with a probe specifically recognizing CoNS (AdvanDx, Woburn, MA). The sections were counterstained with 3 µM 4',6-diamidino-2-phenylindole (DAPI) (Life Technologies, Eugene, OR), mounted (Prolong Gold, Life technologies), and cover-slipped (Marienfeld, Lauda-Königshofen, Germany). In addition, a PNA-FISH-stained section from each sample was, after examination, un-sealed with acetone, rinsed in lukewarm water, stained with hematoxylin and eosin (H&E), dehydrated in 99% ethanol, and mounted (Pertex; Histolab, Göteborg, Sweden).

CLSM and slide scanning

The PNA-FISH/DAPI-stained slides were examined by CLSM (Axio Imager.Z2, LSM710 CLSM; Zeiss, Jena, Germany) and the complementary 3D reconstruction software (Zen Black 2010, version 6.0; Zeiss). Fluorescence images were obtained with the settings described by Ring *et al.*²⁰ In addition, the entire fluorescence and H&E-stained sections were scanned by using a digital slide scanner (Axio scan. Z.1, Zeiss) with a 20×/0.95 objective (Zeiss).

Bacterial aggregates exceeding 5 µm in diameter were classified as a biofilm.²⁵ The aggregates were semi-quantified according to diameter: 5–10, 10–50, or >50 µm.²¹ Thus, single bacterial cells or very small bacterial aggregates (<5 µm) were disregarded as biofilm. Subsequently, the aggregate diameters were measured by interactive microscopy image analysis software (Imaris 8×64 version 8.1.2; Bitplane, Zürich, Switzerland). The aggregate measurement refers to the largest aggregate diameter from each participant in each location: stratum corneum, including hair follicle infundibulum, hair follicle funnel, and wound edge. The semi-quantification and the

measurements of the bacterial aggregates were performed blinded by one investigator (Lene Bay) without prior knowledge of the culturing results. In addition, the bacterial morphology was classified as cocci,²¹ and the activity of the cells was rated by their rRNA contents according to the intensity of the fluorescence.³⁰ Bright red or pink fluorescent bacteria with a high content of rRNA were considered active in growth, and purple or blue bacteria with a low rRNA content were considered dormant.³⁰ The recent guideline in fluorescence microscopy was applied to avoid misinterpretation of auto-fluorescent granules as bacterial aggregates.¹⁶

Statistical analysis

The frequency distribution of variables was analyzed on the semiquantified aggregate sizes by using Fischer's exact test. A one-way ANOVA Kruskal-Wallis nonparametric test was performed on the measured biofilm aggregates (diameter) in relation to the respective location: stratum corneum, including hair follicle infundibulum, hair follicle funnel, and wound edge. The Mann-Whitney two-tailed U test using Gaussian approximation was applied on the bacterial aggregate measurements between groups depending on location. The criterion for statistical significance was $p \leq 0.05$. The analysis was performed by using the Prism 5.02 software (GraphPad Software, La Jolla, CA). Sensitivity and specificity of CLSM/PNA-FISH and culturing were calculated and expressed as percentages for ease of interpretation by using MedCalc Statistic Software (MedCalc Software, Ostend, Belgium).

RESULTS

Histopathological investigation

Inflammation increased slightly from day 0 to day 4, with a dermal infiltrate consisting mostly of lymphocytes and occasionally of eosinophil granulocytes. Neutrophil granulocytes were observed at the surface of the wounds. The wound edge was defined by the transition from normal epidermis to parakeratotic and hyperplastic epidermis. The mean length of neoepidermis from the respective wound edge was 0 mm day 0 ($n=4$) and 1.15 ± 0.41 mm (mean $\pm$ SD) day 4 ($n=23$).

Cultivation

The isolated bacteria were CoNS identified by the Staphaurex agglutination test and MALDI-TOF mass spectrometry. No other species were isolated. CoNS were recovered from 78% of the swabbed wounds and from 48% of the swabbed adjacent skin (Table 1). The bacterial growth was

Table 1. The Recovery of Viable Coagulase-Negative Staphylococci by Culturing and Detection of Coagulase-Negative Staphylococci Aggregates by Confocal Laser Scanning Microscopy Per Sample in Adjacent Healthy Skin and Acute Epidermal Wound on Day 0 and 4

	Culturing		Confocal microscopy		
	Day 0 $n=4$	Day 4 $n=23$, n (%)		Day 0 $n=4$, n (%)	Day 4 $n=24$, n (%)
Skin	n/a	11 (48)	Stratum corneum	0	3 (12.5)
			Hair follicles	0	3 (12.5)
Wound	n/a	18 (78)	Wound edge	0	16 (67)
			Wound bed	0	0

No culturing was done from day 0 wounds or skin.
n/a, not applicable; n , number of wounds.

semiquantified and correlated well with the semiquantified bacterial aggregates (Table 2).

CLSM/PNA-FISH

At day 0, only single scattered bacteria or very small bacterial aggregates ($<5 \mu\text{m}$) were observed at the adjacent skin. In the 4-day-old wounds, bacterial aggregates were observed in 18 of the 24 biopsies (75%). The aggregates were localized at the wound edges in 16 of these 18 biopsies, yet none were observed in the wound bed. The aggregates were associated with split stratum corneum and induced fibrin deposits (Fig. 1 and Table 1) in relation to the wound edge. Overall, aggregates of $10\text{--}50 \mu\text{m}$ in diameter were found in 58% of the examined biopsies. Notably, large aggregates ($50\text{--}150 \mu\text{m}$) were exclusively present at wound edges (Fig. 1, 3 and Table 1).

In skin adjacent to the wounds (Fig. 2), 3 of the 24 biopsies (12.5%) contained small bacterial aggregates ($5\text{--}10 \mu\text{m}$) in the stratum corneum, and a further 3 biopsies (12.5%) contained medium-sized bacterial aggregates ($10\text{--}50 \mu\text{m}$) in the hair follicles (Fig. 3 and Table 1).

The bacterial aggregates resembled cocci, and all aggregate-containing sections were identified as CoNS by the specific PNA probe. In the CoNS/

Table 2. Correlation Between Coagulase-Negative Staphylococci Growth and Aggregate Size

Bacterial growth $n=23$		Bacterial aggregate diameter $n=24$	
0	5 (22%)	$<5 \mu\text{m}$	6 (25%)
+	1 (4%)	$5\text{--}10 \mu\text{m}$	4 (17%)
++	12 (52%)	$10\text{--}50 \mu\text{m}$	9 (38%)
+++	5 (22%)	$>50 \mu\text{m}$	5 (21%)

The numbers refer to the highest score per sample.
 n , number of wounds (one wound/participant).

FIG. 1. Bacterial aggregates establish at the wound edge. Skin sections stained by (a) hematoxylin and eosin showing the split stratum corneum and fibrin residue (black arrow) at the border of the wound bed (black bracket a) and the adjacent covered healthy skin (black bracket a). The same skin wound stained by fluorescent dyes (b) showing the location of the detail photo (white square) of (c) Bacterial aggregates (white arrows c) appear pink due to the red conjugated PNA probe and DAPI (blue). Nucleated eukaryotes are stained blue (black arrow c). Erythrocytes appear yellow (white arrow b) and surrounding tissues green due to auto-fluorescence. DAPI, 4',6-diamidino-2-phenylindole; PNA, peptide nucleic acid.

DAPI-stained sections, only specific probe-stained bacteria were observed. The CLSM/PNA-FISH method achieved 94% sensitivity and 83% specificity when compared with the results obtained by culturing.

The growth activity level of the bacteria was evaluated in the universal probe-stained sections. Actively growing bacteria were primarily found at the wound edge, whereas dormant bacteria were often found within hair follicles and stratum corneum of the adjacent skin. Occasionally, the bacterial aggregates appeared in different activity stages, both within the same aggregates and as separated active or dormant aggregates (Fig. 2).

The bacterial aggregates were significantly ($p=0.0084$) more common at the wound edge than in adjacent skin. An analysis of variance showed a significant difference ($p=0.0355$) within aggregate

sizes (diameter) found at the wound edge, compared with the ones found in the stratum corneum and hair follicles of the adjacent skin. Bacterial aggregates were significantly larger at the wound edge ($p=0.0083$) and in hair follicles ($p=0.0357$) than in the stratum corneum of the adjacent skin.

DISCUSSION

The spatiotemporal distribution of bacterial aggregates in acute wounds has previously not been investigated. Our CLSM/PNA-FISH analysis of full-thickness skin biopsies from acute epidermal wounds provides important information on the distribution of the microbiota in the epidermal and dermal compartments.²¹ We found that pronounced bacterial aggregates accumulated in opportunistic niches, such as keratin residues from split stratum corneum and fibrin deposits at the

FIG. 2. Bacterial aggregates in adjacent skin covered by occlusive dressing. Bacterial aggregates indicated by arrows in a hair follicle (**a**) and on stratum corneum (**b**). Bacteria were stained by a universal bacterial-conjugated Texas Red (red) CoNS PNA probe and by DAPI (blue) and in combination they appear blue/purple due to low growth activity. Nucleated eukaryotes are stained blue, erythrocytes are yellow, and surrounding tissues appear green. CoNS, coagulase-negative staphylococci.

edges of acute wounds. These niches, which presumably are inaccessible for immune cells, provide favorable conditions for bacterial aggregates to establish and grow. They most likely arise when the blister roof is excised. However, such niches may also arise in naturally occurring clinical wounds.

The findings of bacterial aggregates at the edges of the acute wounds promote an awareness of the possible presence of aggregates generally in all wounds. Nonetheless, bacterial biofilms have been documented in a higher prevalence in chronic wounds (78%)³¹ than in acute wounds (6%),³² which indicates an important impact of the pres-

ence of biofilms in chronic wounds. A direct comparison of the prevalence figures is not possible, because visualization of biofilms in acute wounds is not standard practice. Our view is that bacterial aggregates are present in all wound types, but they possibly play a more significant role in chronic wounds.

Recent *in vivo* experiments have shown that bacterial biofilms are physical barriers to re-epithelialization.¹³ Biofilms contribute to impaired wound healing and persistent inflammation in the wound bed of chronic wounds.^{2,7,19,32–35} Bacterial aggregates within the wound bed develop if

FIG. 3. Distribution of the bacterial aggregates. (**a**) Distribution of aggregates according to size and location in wounds and adjacent skin post-wounding day 4 in 24 healthy volunteers. One symbol represents the largest aggregate diameter in each location per biopsy. In some biopsies, aggregates were observed in several locations. The dotted lines define the grouping of the aggregate sizes. (**b**) The aggregates diameter was measured in µm at the widest dimension.

vascularization is impaired, disabling the inflammatory cells to be recruited.⁷ Acute wounds of otherwise healthy humans are normally well vascularized,³¹ enabling inflammatory cells to be recruited appropriately and enabling development of bacterial aggregates only in tissue residues. This was confirmed by the absence of bacterial aggregates in the wound bed of the acute epidermal wounds and the displayed undisturbed epithelialization. The early time point at which the biopsies were harvested and the participants being healthy with assumed normal oxygenation, blood perfusion, and immune response contributed to this confirmation. Therefore, the detection of biofilms at an early time point is important when choosing treatment.³⁶

In the healthy covered adjacent skin, the microbiota was observed after four days as small- or medium-sized bacterial aggregates demonstrating normal distribution of the microbiota in healthy covered skin. Further, the growth activity of the bacteria on the adjacent skin was low to medium, assessed by fluorescence intensity,³⁰ which may reflect the activity level of commensal microorganisms when the microenvironment has been changed. The adjacent skin received the same treatment and was covered with the same non-adherent, occlusive dressing as the wounds, thereby serving as intern control skin to the wounds. Despite cleansing and disinfection, some commensal bacteria seem to persist, enabling them to colonize and establish bacterial aggregates during the 4 days. The introduction of a wound and the add-on of a dressing may be a more favorable situation. The lack of aggregates in the healthy non-covered adjacent skin (day 0) compared with the proliferation of small- to medium-sized aggregates at the adjacent skin (day 4) could be related to use of the dressing. Accordingly, occlusion changes the environment *per se*,³ and the skin trauma interrupts the equilibrium between skin and microbiota.²⁴ Thus, the prominent aggregates established at the wound edge cannot be explained by the dressing alone but are rather related to the opportunistic niche of the wound. Bacteria were observed more often in the adjacent skin by cultivation than by CLSM/PNA-FISH. This may be due to cultivation being based on colony-forming units in the full swabbed area, whereas merely three sections per sample were analyzed by CLSM/PNA-FISH. In addition, wounds were swabbed and disinfected before obtaining the biopsies and bacteria may have been removed. Moreover, single bacteria or bacterial aggregates <5 μm were excluded in the CLSM/PNA-FISH, but included by the culturing method.

Previous microbiological findings of buttock skin include *Propionibacterium spp.*, *Proteobacteria spp.*, *Corynebacterium spp.*,^{1,24} and *Micrococcaceae spp.*³⁷ The group of species detected in this study was similar to previous studies describing CoNS as the most abundant group of bacteria.^{4,38} The sole presence of CoNS indicates that the conditions may have favored these rapidly growing species. CoNS are considered a group of opportunistic pathogens³⁸ central in skin infections and wounds,⁴ and the presence of CoNS during wound closure is associated with wound dehiscence.³⁹

Previous studies on the cutaneous microbiota are based mainly on culturing and less on molecular, culture-independent methods. In healthy volunteers, cultured cyanoacrylate skin surface biopsies,³⁷ as well as 16S sequenced swabs^{12,40} and tape-stripped skin,²⁴ have been used to analyze the distribution of microorganisms in skin. Eighty-five percentage of the bacteria were detected within the six apical layers of stratum corneum of the volar forearm skin, of which 25% originated from hair follicles.³⁷ In comparison, we recently reported that 35% of bacterial aggregates were found in the stratum corneum and 65% in hair follicles of the axilla.²¹ On day 4 in the adjacent covered buttock skin, we discovered an even distribution of the bacterial aggregates between the stratum corneum and hair follicles. A caveat was that bacterial aggregates were only found in the adjacent skin in a few samples. The divergent findings may be ascribed to different conditions, anatomic locations, and disinfecting agents. Regardless of the ratio, hair follicles are significant reservoirs of the skin microbiota³⁷ and have to be taken into consideration during disinfection.

This study had some limitations. The daily cleaning and disinfection before obtaining the biopsies might have interrupted the distribution of the bacterial aggregates and could be a drawback of the CLSM/PNA-FISH method. In addition, two balanced groups would have been preferred. Later time points than day 4 may have revealed the influence of the bacterial aggregates in the wounds. Further, it was a disadvantage that the cultivation did not represent the microbiota in the deeper layers of the skin.¹² The growth activity of the bacterial aggregates was evaluated in the sections stained with the universal (BacUni) probe to limit misinterpretation. This is caused by the difficulty in distinguishing whether bacteria stained by the specific CoNS probe are not fluorescent due to low 16S rRNA content (low activity) in the bacteria or because they are not CoNS. If the BacUni and the specific CoNS probe, each with a unique fluor-

ophore, were added on the same section, both the growth activity and the species specificity could be validated simultaneously.

In conclusion, measurement of bacterial aggregates provides novel information of the distribution of the microbiota in healthy and in acute traumatized skin. Bacterial aggregates of CoNS were detected in association with split stratum corneum and fibrin residues at the edges of acute epidermal wounds. These opportunistic niches, inaccessible for immune cells, provided the ideal niche for the development of bacterial aggregates. In comparison, the microbiota of the healthy adjacent skin was heterogeneously distributed as small aggregates that were embedded mainly in stratum corneum and hair follicles.

INNOVATION

Various factors affect the wound-healing process. Impaired wound healing is due to hypoxia, prolonged inflammation, and incomplete microbial eradication. Specific bacteria species impact the healing process by disturbing the closure. In particular, the spatial distribution and the quantity of bacterial aggregates have clinical significance in the development of infectious wounds.

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KEY FINDINGS

- Bacterial aggregates establish at the edge of acute epidermal wounds in split stratum corneum and fibrin.
- Bacterial aggregates are larger and more frequent at the edge of acute wounds than in healthy skin.
- No bacterial aggregates were detected in the wound bed of the acute wounds.

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AUTHOR DISCLOSURE AND GHOSTWRITING

No competing financial interests exist. The content of this article was expressly written by the authors listed. No ghostwriters were used to write this article.

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Abbreviations and Acronyms

BacUni	= universal bacterial
CLSM	= confocal laser scanning microscopy
CoNS	= coagulase-negative staphylococci
DAPI	= 4',6-diamidino-2-phenylindole
FISH	= fluorescence <i>in situ</i> hybridization
H&E	= hematoxylin and eosin
MALDI-TOF	= matrix-assisted laser desorption-ionization-time of flight
PNA	= peptide nucleic acid