USZ Universitäts Spital Zürich

Clinical Study Protocol

EFFECTS OF PHOTODYNAMIC THERAPY ON THE HUMAN INGUINAL SKIN MICROBIOME TO IMPROVE ANTISEPTIC EFFECT

SHORT TITLE: Effect of photodynamic treatment on skin microbiome (PHOMIC)
Single center study

Study Type:	Health-related intervention
Study Categorization:	Other Clinical Trial Category *A*
Study Registration:	*Bundesamtes für Gesundheit: www.kofam.ch. SNCTP000003386* *International trial registry ClinicalTrials.gov (clinicaltrials.gov)* ***NCT04067843***
Study Identifier:	*SNCTP000003386* *Sponsor study Identifier: PHOMIC*
Sponsor-Investigator and Principal Investigator:	*PD Dr. med. Yvonne Achermann* University Zurich *Division of Infectious Diseases and Hospital Hygiene* *Yvonne Achermann, MD* *University Hospital Zurich* *Rämistrasse 100, 8091 Zürich* *Switzerland* Phone: *044 255 34 02* E-Mail: yvonne.achermann@usz.ch
Study Intervention:	*Effects of photodynamic treatment on skin microbiome*
Protocol Version and Date:	*V04, 14.1.2020*

SIGNATURE PAGE

Study number	*SNCTP000003386* *Bundesamtes für Gesundheit: www.kofam.ch.* *International trial registry ClinicalTrials.gov (clinicaltrials.gov)*: *NCT04067843*
Study Title	*Effects of photodynamic therapy on the human inguinal skin microbiome to improve antiseptic effect*

Sponsor-Investigator (Principal Investigator):

This clinical trial protocol was subject to critical review and has been approved by the Sponsor-Investigator. The information herein is consistent with

- the current risk/benefit evaluation of the intervention,
- the moral, ethical and scientific principles governing clinical research as set out in the current version of the Declaration of Helsinki, Good Clinical Practice.

PD Dr. med. Yvonne Achermann

Zürich, 14.1.2020 _____ _____

Place/Date Signature

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STUDY SYNOPSIS

Sponsor / Sponsor-Investigator	*PD Dr. med. Yvonne Achermann*
Study Title:	***Effects of photodynamic therapy on the human inguinal skin microbiome to improve antiseptic Effect – a pilot study***
Short Title / Study ID:	*Effects of photodynamic treatment on skin microbiome (PHOMIC)*
Protocol Version and Date:	*v04, 14.1.2020*
Trial registration:	*Bundesamtes für Gesundheit: www.kofam.ch. SNCTP000003386* *International trial registry ClinicalTrials.gov (clinicaltrials.gov) NCT04067843*
Study category and Rationale	Other clinical study Category A
Background and Rationale:	Periprosthetic joint infections are a feared complication after orthopedic surgery in particular in our increasing elderly population. These infections are usually difficult to treat, because microorganisms persist in biofilms on the orthopedic implant surface. Therefore, it would be desirable to prevent these infections. It is hypothesized that bacteria from the skin surface or dermis - such as *Staphylococcus aureus*, coagulase-negative staphylococci, or *Cutibacterium* sp. - are transmitted into the periimplant tissue during surgery. In a ongoing interdisciplinary study with the Orthopedic University Hospital Balgrist (data in preparation for publication), we see that common skin antisepsis preparation is not effective to eliminate skin bacteria before surgery because they persist in sebaceous or sweat glands. Photodynamic treatment (PDT) has recently gained attention in the treatment of acne, a disease of the pilosebaceous unit, in which also *Cutibacterium acnes* is implicated. The PDT works here on the one hand through a long-lasting destruction of the sebaceous glands, and on the other hand due to anti-inflammatory and antimicrobial effects.
Objective(s):	The overarching aim of this research project is to prevent orthopedic implant-associated infections. This study aims to investigate if photodynamic therapy has an effect on bacterial skin colonization and decrease number of colonizing bacteria associated with sebaceous and sweat glands in order to improve skin antisepsis strategies for the prevention of surgical site infections.

Outcome(s):	Primary outcome 1. **Immediate effect** of photodynamic treatment in combination with surgical antisepsis on bacterial skin colonization 1.1. To quantitatively evaluate bacterial density and species before photodynamic treatment and after photodynamic treatment and antisepsis skin preparation 2. **Long-term effect** on bacterial skin colonization in the groin after photodynamic treatment 2.1. To quantitatively evaluate bacterial density and species before and after photodynamic treatment (7 and 21 days) 2.2. To evaluate changes of sebaceous and sweat glands after photodynamic treatment on skin biopsies Secondary outcome 3. To evaluate **phylogenetic similarity of the microbiota** on the dermis before and three weeks following photodynamic treatment
Study design:	Pilotstudy, open label, single center
Inclusion / Exclusion criteria:	**Inclusion criteria** Healthy male and female participants ≥ 18 years who • volunteer for the pilot study in which a routine photodynamic treatment in the Department of Dermatology will be applied and effect of skin colonization will be analyzed, and • an informed consent is signed by the participant (after information about the project). **Exclusion criteria** • Pregnant and lacting women • Participants with inability to follow the procedures of the study, e.g. due to language problems, psychological disorders, dementia, etc., • Participants taking antibiotics in the 14 days prior to the photodynamic treatment or until follow-up at 21 days • Participants who received oral retinoid therapy within the last 6 months • Participants who received anti-inflammatory agents as NSAR within the 14 days prior and after the PDT • Participants taking any photosensitizing drugs within 4 weeks prior to the photodynamic treatment (PDT) • Participants who had a history of photosensitivity disorder • Fitzpatrick's skin phototype V-VI
Study Intervention:	• Photosensitizer application, followed by fluorescence photography and photodynamic therapy (PHT) (1x) over 15 minutes at the dermatology ward • Skin swabs • Skin biopsies in only 5 participants

Reference Intervention:	From a current prospective study at the University Hospital Balgrist, we have the reference database of colonizing bacteria in the groin without photodynamic treatment. In addition, we will take skin swabs before and after skin antisepsis without antisepsis in 10 participants.
Number of Participants with Rationale:	Aim: 20 volunteers There will be only descriptive statistics to see if numbers of colonizing bacteria are decreased with photodynamic treatment to plan a clinical trial in patients getting a hip joint arthroplasty and previous antisepsis.
Study Duration:	1 year

Study Schedule:	• Project start (FPFV): July 2019
	  ○ First participant: July 2019
	  ○ Last participant: June 2020
	• Project end (LPLV): July 2020

Investigator(s):	Yvonne Achermann, MD Division of Infectious Diseases and Hospital Hygiene University Hospital Zurich Rämistrasse 100, 8091 Zürich Email: yvonne.achermann@usz.ch Tel: 044 255 34 02 Dr. med Laurence Imhof Department of Dermatology University Hospital of Zurich, Zurich University Hospital Zurich Rämistrasse 100, 8091 Zürich Email: laurence.imhof@usz.ch Tel:+41 44 255 36 11
Study Centre(s):	Single-center
Statistical Considerations:	Categorical data will be tested for differences using Fisher's exact or chi-squared tests, as appropriate, whereas continuous variables will be tested using Wilcoxon rank sum tests.
GCP Statement:	This study will be conducted in compliance with the protocol, the current version of the Declaration of Helsinki, the ICH-GCP as well as all national legal and regulatory requirements.

LIST OF ABBREVIATIONS

AE	Adverse Event
ClinO	Clinical Trial Ordinance (KlinV)
CRF	Case Report Form
eCRF	Electronic Case Report Form
GCP	Good Clinical Practice
ICH	International Council on Harmonization
ISF	Investigator Site File
PI	Principal Investigator
SAE	Serious Adverse Event
SDV	Source Data Verification
SNCTP	Swiss National Clinical Trial Portal
SOP	Standard Operating Procedure
TMF	Trial Master File
PJI	Periprosthetic Joint Infection
C. avidum	*Cutibacterium avidum*
CNS	Cogulase-negative staphylococci
Sp.	Species
CEC	Competent Ethics Committee
PDT	Photodynamic therapy
MAL	Methyl aminolevulinate
NSAR	Non-steroidal anti-inflammatory drugs

1 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

1.1 Sponsor, Sponsor-Investigator (Principal Investigator)

Division of Infectious Diseases and Hospital Hygiene
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1.2 *Investigator for Intervention: Photodynamic treatment*

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1.3 *Study design and statistician (Biometrician)*

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1.4 *Study Nurse*

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University Zurich

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1.6 *Monitoring Institution*

Since we only measure microbiological data from skin samples, we think, that an interne monitoring would be adequate. See chapter 12.3.

ETHICAL AND REGULATOR ASPECTS

Before this study will be conducted, the protocol, the proposed participant information and consent form as well as other study-specific documents will be submitted to a properly constituted Competent Ethics Committee (CEC) in agreement with local legal requirements, for formal approval.

The decision of the CEC concerning the conduct of the study will be made in writing to the Sponsor-Investigator before commencement of this study. The clinical study can only begin once approval from the CEC has been received.

1.7 Study Registration

The study will be registered in the Swiss Federal Complementary Database („Portal“) (Registry of ongoing Research Projects (RoPS)) and in the international trial registry ClinicalTrials.gov (clinicaltrials.gov).

1.8 Categorization of the Study

Category A: The health-related study intervention (PTD, taking biopsies) entails only minimal risks and burdens.

1.9 Competent Ethics Committee (CEC)

Approval from the appropriate constituted Competent Ethics Committee is sought for the clinical trial. The reporting duties and allowed time frame are respected. No substantial amendments are made to the protocol without prior CEC approval, except where necessary to eliminate apparent immediate hazards to study participants.Premature study end or interruption of the study is reported within 15 days. The regular end of the study is reported to the CEC within 90 days, the final study report shall be submitted within one year after study end. Amendments are reported according to chapter 2.9.

1.10 Ethical Conduct of the Study

The study will be carried out in accordance with principles enunciated in the current version of the Declaration of Helsinki, the guidelines of Good Clinical Practice (GCP) issued by ICH, and Swiss competent authority's requirements.

CEC will receive annual safety and interim reports and be informed about non-substantial amendments, the course of the study, and the study stop/ end in agreement with local requirements.

1.11 Declaration of Interest

No conflict of interest

1.12 Participant Information and Informed Consent

The investigator must explain to each Participant the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits and any discomfort it may entail. Each participant must be informed that the participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment.

The participant must be informed that his/her medical records may be examined by authorized individuals other than their treating physician.

All participants for this study will be provided a participant information sheet and a consent form describing this study and providing sufficient information for participants to make an informed decision about their participation in this study.

The participant information sheet and the consent form will be submitted with the protocol for review and approval for the study by the CEC. The formal consent of a participant, using the approved consent form, must be obtained before that participant is submitted to any study procedure.

The participant should read and consider the statement before signing and dating the informed consent form, and should be given a copy of the signed document. The consent form must also be signed and dated by the investigator (or his designee) and it will be retained as part of the study records.

1.13 Participant Privacy and Confidentiality

The investigators are liable to treat the entire information related to the study and the compiled data strictly confidentially. Any passing-on of information to persons that are not directly involved in the study must be approved by the owner of the information.

Data generation, transmission, archiving and analysis of personal data within this study, strictly follows the current Swiss legal requirements for data protection. Prerequisite is the voluntary approval of the Participant given by signing the informed consent prior start of participation of the clinical trial.

Individual participant medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited. Participant's confidentiality will be further ensured by utilizing participant identification code numbers to correspond to treatment data in the computer files.

Such medical information may be given to the participant's personal physician or to other appropriate medical personnel responsible for the participant's welfare, if the patient has given his/her written consent to do so.

Data generated as a result of this study are to be available for inspection on request by the monitors and by the CEC.

1.14 Early Termination of the Study

The Sponsor-Investigator may discontinue the study prematurely according to certain circumstances:

- ethical concerns,
- insufficient participant recruitment,
- when the safety of the participants is doubtful or at risk, respectively,
- alterations in accepted clinical practice that make the continuation of a clinical trial unwise,
- early evidence of benefit or harm of the experimental intervention

1.15 Protocol Amendments

Substantial amendments (significant changes) are only implemented after approval of the CEC.

Significant changes to be authorized by the CEC are the following:

- changes affecting the participants' safety and health, or their rights and obligations;
- changes to the protocol, and in particular changes based on new scientific knowledge which concern the trial design, the method of investigation, the endpoints or the form of statistical analysis;
- a change of trial site, or conducting the clinical trial at an additional site; or
- a change of sponsor, coordinating investigator or investigator responsible at a trial site.

Under emergency circumstances, deviations from the protocol to protect the rights, safety and well-being of human participants may proceed without prior approval of the sponsor and the CEC. Such deviations shall be documented and reported to the sponsor and the CEC as soon as possible.
All Non-substantial amendments are communicated to the CEC within the Annual Safety Report (ASR).

2 INTRODUCTION

2.1 Background and Rationale

The use of orthopedic implants has been steadily increasing over the last 50 years. Despite considerable technical progress, including perioperative skin disinfection and antibiotic prophylaxis, implant-associated infections remain a feared complication. Infection rates up to 2% after primary joint arthroplasties (Tande and Patel 2014) have been described. National data published by Swissnoso – the National Center for Infection Prevention –reported an infection rate of 1.2% in hip arthroplasties (Vergleichsbericht 2015/2016). Considering an infection rate of up to 1.2%, 400 surgeries with primary hip arthroplasty implantation at the Orthopedic Department of

the Balgrist University Hospital (data of 2015) would result in five periprosthetic joint infections (PJI) per year, which is medically highly relevant regarding morbidity, mortality and estimated costs of 225'000 CHF in total.

Because bacteria grow and persist in biofilms on the implant surface such infections are difficult to eradicate. Definite cure of biofilm infections requires adequate surgical debridement and prolonged treatment with an antimicrobial agent resulting in high health-care costs (Tande and Patel 2014). Most commonly isolated microorganisms in implant-associated infections are staphylococci, streptococci, enterococci, Gram-negative bacteria, and anaerobic bacteria such as *Cutibacterium acnes/avidum* (formerly *Propionibacterium acnes/avidum*) (Tande and Patel 2014).

The majority of implant-associated infections are acquired during surgery. It is hypothesized that the same bacteria that colonize the skin surface contaminate the orthopedic implant and cause acute or chronic infections (Pulido, Ghanem et al. 2008). Prevention of implant-associated infections is key to avoid re-operations and prolonged antibiotic treatment. Current preoperative prevention strategies are multifaceted with the focus on skin antisepsis and single shot of an antibiotic within 30-60 minutes before surgery (Bratzler, Dellinger et al. 2013, Dumville, McFarlane et al. 2015). Preoperative skin disinfection is performed with alcoholic povidone-iodine (PVI) or chlorhexidine gluconate (CHG) prior to implantation as a standard skin preparation (Dumville, McFarlane et al. 2013, Berrios-Torres, Umscheid et al. 2017). Povidone iodine oxidizes cell constituents and inactivates proteins by iodinating them. Regarding the skin commensal *Cutibacterium acnes*, it could be shown that despite administration of standard preoperative prophylaxis before shoulder arthroplasty surgeries, *C. acnes* could be detected in different sample types (in dermis, fascia, synovium, and glenoid tissue of shoulder) of 3 out of 10 patients without any signs of infection (Matsen, Russ et al. 2015). There is an increasing number of studies reporting the presence of bacteria in deep tissue, which is commonly considered as sterile. A recent study by Lee et al. found viable *C. acnes* in the dermal tissue in seven out of 10 male volunteers after surface skin antisepsis (Lee, Pottinger et al. 2014). All these study results have led to the assumption that not only the superficial skin bacteria but also bacteria in the dermis may find the way to deeper structures and infect an implant.

We hypothesize that local photodynamic treatment (PDT) is able to reduce persistent skin colonizing bacteria through the destruction of the sebaceous and sweat glands as well through bactericidal effects and thus improve skin antisepsis before surgical incision and implantation of foreign material leading to a lower rate of surgical site infections. The aim of this pilotstudy in maximum 20 participants within a year is to evaluate the effect of PDT on colonizing bacteria immediately before surgical skin antisepsis and after it (1 and 3 weeks later). If we see promising results in this pilotstudy, we will plan a prospective clinical trial in patients undergoing hip arthroplasty surgery.

2.2 Study Intervention and Indication

Study invervention	Indication
PDT	- Bactericidal effect on skin bacteria - Destruction of sebaceous and sweat glands
Skin swabs	- To measure bacterial growth of colonizing bacteria
Skin biopsies in 5 participants	- To measure growth of persistent bacteria in

	sebaceous and sweat glands (Microbiology) - To analyze skin/dermis structure including size of sebaceous and sweat glands (dermatohistopathology)

2.3 Clinical Evidence to Date

Photodynamic treatment (PDT) has recently gained attention in the treatment of acne, a disease of the pilosebaceous unit, in which also *C. acnes* is implicated. The PDT works here on the one hand through a longlasting destruction of the sebaceous glands, and on the other hand due to anti-inflammatory and antimicrobial effects (Sakamoto, Lopes et al. 2010). In following studies, photodynamic therapy not only validated its efficient activity against bacteria involved in acne such as *C. acnes*, it showed also activity against various other classes of microorganisms (Awad, Tovmasyan et al. 2016). The mechanism of action of the PDT is a photochemical reaction through the generation of reactive oxygen species in the presence of oxygen, mainly excited singlet oxygen, by a non-toxic-photosensitizer reacting with visible light. Photodynamic treatment is routinely performed among others for non-melanoma skin cancer and therapy-refractory inflammatory dermatoses at the Department of Dermatology at the University Hospital Zurich. The treatment enjoys great popularity as it is well tolerated, can be repeated arbitrarily often, and gives excellent cosmetic results.

2.4 Justification of Study Intervention

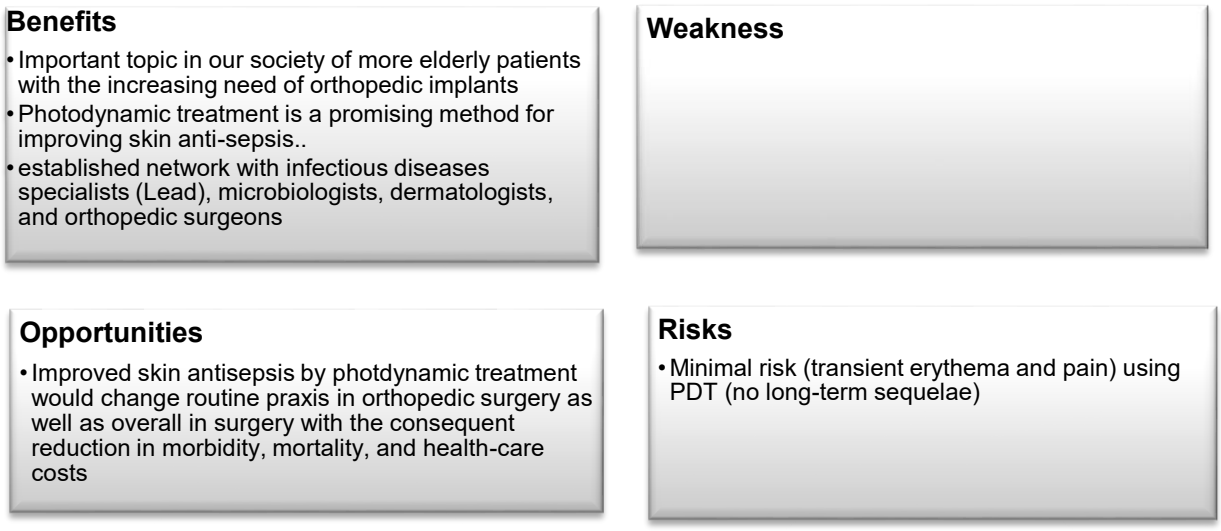
Skin swabs and small skin biopsies have only minimal risks and the PDT enjoys great popularity as it is well tolerated. Therefore, we select this promising treatment to investigate an innovative strategy to improve skin antisepsis in a pilotstudy. If we see that we have sterile cultures after PDT and skin antisepsis we will plan a larger prospective study based on these data.

2.5 *Explanation for Choice of Comparator Intervention*

Not applicable, study without PDT is currently ongoing (study acronym DESCAV, ethical number 2018-00783) at the Orthopedic University Hospital Balgrist. The results of this prospective study will be used as reference for this pilot study results.

2.6 Risk / Benefits

In the following, we describe benefits, weakness, opportunities, and risks of this study.



Possible side effects of the PDT treatment are pain during PDT in the area of treatment, erythema and edema during and after light treatment (Borgia, Giuffrida et al. 2018). These side effects are self-limiting.

Since our study interventions (see above) have minimal risks for participants, it is reasonable to conduct this pilotstudy before starting a larger clinical trial with patients undergoing hip arthroplasty surgery.

2.7 Study Population

Twenty healthy male and female volunteers' ≥ 18 years will be recruited for this clinical study. The study will be approved by the local ethical committee and conducted with a study nurse (Daniela Egli) and a doctoral thesis student (Isabel Waldmann) in collaboration with the Clinic for Dermatology at the University Hospital Zurich, with the Institute of Medical Microbiology at the University of Zurich, and with the Orthopedic University Hospital Balgrist in Zurich. An informed consent will be obtained from each participant. We will exclude pregnant and lactating women, participants taking antibiotics in the 14 days prior to the photodynamic treatment or until follow-up at 21 days, participants who received oral retinoid therapy within the last 6 months, participants who received anti-inflammatory agents within the 14 days prior and after the photodynamic treatment, participants taking any photosensitizing drugs within 4 weeks prior to the photodynamic treatment, participants who had a history of photosensitivity disorder or having a fitzpatrick's skin phototype V-V (16)

We will not include vulnerable participants

3 STUDY OBJECTIVES

3.1 Overall Objective

The overarching aim of this research project is to prevent orthopedic implant-associated infections. The specific aim is to evaluate the effect of photodynamic treatment on colonizing bacteria immediately before surgical skin antisepsis (aim 1) and 3 weeks later (aim 2). In addition in aim 3, we will evaluate phylogenetic similarity of same bacterial species before and after photodynamic treatment if they persist.

3.2 Primary Objective

Here we use skin swabs from the groin before and after Photodynamic treatment. For all these swabs, we will use the scratching method using a scalpel as already applied in a previous study (ethical approval BASEC-Nr 2016-01017). The skin swabs will be sent to the microbiology laboratory of the University of Zurich for bacterial analysis.
We distinguish between an immediate (day 0) and long-term effect (3 weeks) investigating the skin microbiome after PDT.
In Detail:

1. Immediate effect of photodynamic treatment in combination with surgical antisepsis on bacterial skin colonization
   - 1.1. To quantitatively evaluate bacterial density and species before photodynamic treatment and after photodynamic treatment and antisepsis skin preparation
     - 1.1.1. **Parameter**: growth of bacteria (yes/no), number and identification of bacteria (species name), semiquantitative amount of bacteria
2. Long-term effect on bacterial skin colonization in the groin after photodynamic treatment
   - 2.1. To quantitatively evaluate bacterial density and species before and after 7 and 21 days of photodynamic treatment
     - 2.1.1. **Parameter**: growth of bacteria (yes/no), number and identification of bacteria (species name), semiquantitative amount of bacteria
   - 2.2. To evaluate changes of sebaceous and sweat glands after photodynamic treatment on skin biopsies
   - 2.3. **Parameters**: Number and size of glands

3.3 Secondary Objectives

To evaluate phylogenetic similarity of the microbiota on the dermis before and one and three weeks following photodynamic treatment

3.4 Safety Objectives

Evaluation of adverse events

4 STUDY OUTCOMES

4.1 Primary Outcome

Outcome 1: Immediate effect (Same day) of photodynamic treatment in combination with surgical antisepsis on bacterial skin colonization

- Skin swabs:
  - Bacterial growth, density, and species before and immediately after PTD with standard bacterial cultures, MALDI-TOF for bacterial identification, semiquantitative amount of bacteria with dilution bacterial plates, molecular analysis using e.g RNA seq, whole genome sequencing
- Skin biopsies
  - Changes of sebaceous and sweat glands after PDT (Histopathology with immunostaining)

Outcome 2. Long-term effect (1 and 3 weeks after PDT treatment) on bacterial skin colonization in the groin after photodynamic treatment

- Skin swabs:
  - Bacterial growth, density, and species before and immediately after PTD with standard bacterial cultures, MALDI-TOF for bacterial identification, semiquantitative amount of bacteria with dilution bacterial plates, molecular analysis using e.g RNA seq, whole genome sequencing
- Skin biopsies
  - Changes of sebaceous and sweat glands after PDT (Histopathology with immunostaining)

4.2 Secondary Outcomes

Outcome 3: To evaluate phylogenetic similarity of same bacterial species before and after PTD based on the core genome analysis using whole genome sequencing

- Phylogenetic analysis using whole genome sequencing of bacterial species

4.3 Safety Outcomes

Every PDT is documented as in routine clinics on a standardized protocol (see appendix 1).

Clinical signs and symptoms after PDT as adverse events. We will distinguish between:

- Mild redness and pain as to be expected (yes/no)
- Advanced redness and pain more than expected (yes/no): adverse event.
  - “More than expected” is defined, if patients needs to take more than 1 painkiller/day
- Other signs and symptoms not expected (yes/no)

5 STUDY DESIGN AND COURSE OF STUDY

5.1 General Study Design and Justification of the Design

This is a pilotstudy of healthy volunteers, open label, and single center. If we see promising results in this pilotstudy, we will plan a prospective clinical trial in patients undergoing hip arthroplasty surgery.
The intended procedures are photodynamic treatment (PDT) on the first day followed by skin swabbing and biopsies for control of skin microbiome of treated area immediately after the PDT (outcome 1) and by controlling 3 weeks after PDT (outcome 2).
We think that this study design with healthy volunteers is adequate before starting a clinical trial.

5.2 Study Duration and Study Schedule

The expected duration of participant's participation is 35 days from first study visit to the last follow-up time to report side effects of photodynamic treatment. The screening visit can be any time before but at least 1 week before the first study visit. The study schedule is indicated below.

Figure 1. Flow chart

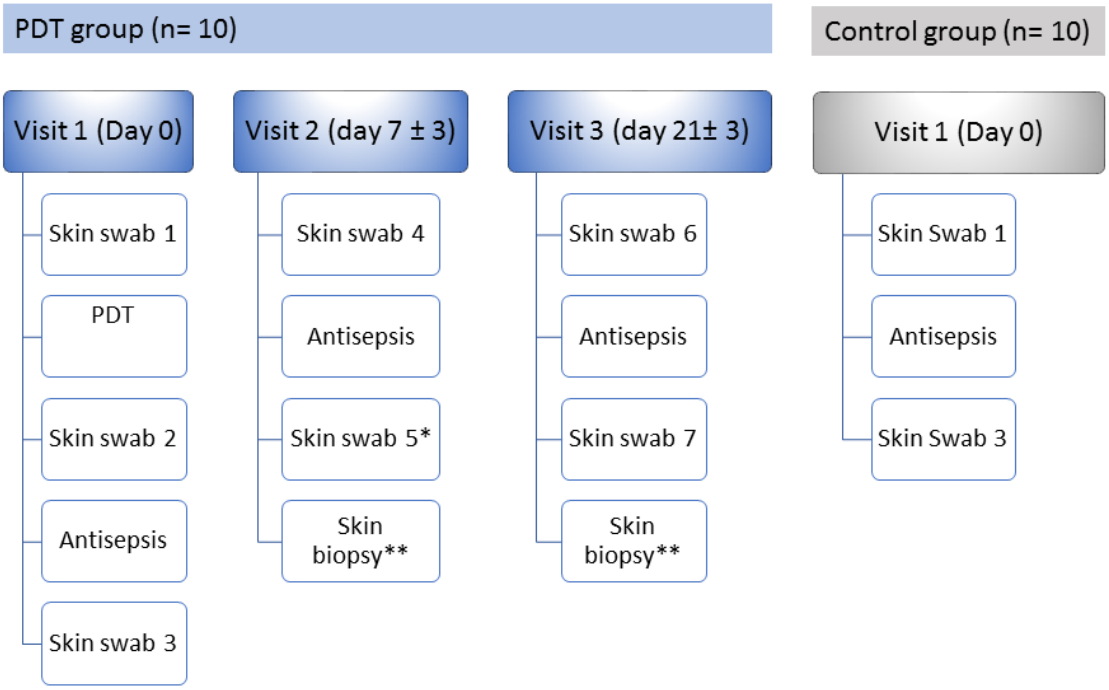


Fig.1. Study design of the PDT versus the control group
* Only performed in 8 out of 10; ** only performed in 4 participants

At the day of inclusion, the voluntary participant will be informed and asked for signing the informed consent by Dr. Yvonne Achermann when fulfilling in- and exclusion criteria. This visit and the last visit (day 35 of the study) will be at the outpatient clinic for Infectious Diseases at the University Hospital Zurich. Demographic data (age, sex, current medications, and underlying diseases) will be noted.

Appointments for the photodynamic treatment (day 0) and the control visits (day 7 and 21) at the Department of Dermatology with Dr. L. Imhof or at the Infectious Diseases department will be made

Study schedule:

On day 0 with Dr. Imhof and Dr. Achermann, a skin scraping of the groin will be performed and sent for microbiological culture. Then, 5% topical methyl aminolevulinate (MAL) as the photosensitizer will be applied for 3 hours under occlusion in the right groin of the patient. After the incubation, the photodynamic process will be started by irradiation of the area with 40J/cm2 red light from a 633nm emitting LED during 13 minutes.

Immediately after the MAL-PDT, skin antisepsis will be performed. Antisepsis of the skin is performed with a povidone-iodine/alcohol solution (Betaseptic®, Mundipharma, Limburg, Germany) and repeated three times for a total duration of 3 minutes to imitate standard surgical antisepsis before a hip arthroplasty surgery. Skin scraping will be repeated after the 3rd antisepsis and 7 and 21 days later (study visit day 7 and 21) for bacterial cultures.

As a control group, we will take skin swabs before and after skin antisepsis without PDT in 10 participants.

On day 35 (±7 days) of the study, volunteers will be seen for the last study visit to document any adverse effects and to inform about the individual study result.

Substudy in 5 volunteers:

In the course of the study a total of three small skin biopsies are taken (3mm diameter each) from 5 volunteers. The time and locations of the performed biopsies will be

1. from the right groin immediately after the MAL-PDT or at day 7
2. from untreated control area in the left groin after the MAL-PDT (day 0 or 7)
3. from the MAL-PDT area on day 21 as the control after treatment.

5.3 Methods of Minimizing Bias

Excluded bias in our collective

- No selected participants
- Both sex can be included
- Any age

Potential bias in our collective:

- Since we will search for healthy volunteers, the microbiome analysis might be slightly different to the population undergoing a hip arthroplasty surgery in an orthopedic hospital.

6 STUDY POPULATION

Twenty healthy male and female volunteers' ≥ 18 years will be recruited for this clinical study. With the results of an ongoing trial investigating the effect of skin antisepsis before hip arthroplasty, we have a perfect comparator intervention group without the PDT intervention. An expected enrolment goal is 2-4 participants per month.

6.1 Eligibility Criteria

6.1.1 Inclusion Criteria

Patients fulfilling all of the following inclusion criteria may be enrolled in the study

Inclusion criteria:

Healthy male and female participants ≥ 18 years who

- *volunteer for the pilot study in which a routine photodynamic treatment in the Department of Dermatology will be applied and effect of skin colonization will be analyzed, and*
- *an informed consent is signed by the participant (after information about the project).*

6.1.2 Exclusion Criteria

The presence of any one of the following exclusion criteria will lead to exclusion of the participant:

Exclusion criteria:

- Pregnant and lacting women

- Participants inability to follow the procedures of the study, e.g. due to language problems, psychological disorders, dementia, etc.,
- Participants taking antibiotics in the 14 days prior to the photodynamic treatment or until follow-up at 14 days
- Participants who received oral retinoid therapy within the last 6 months
- Participants who received anti-inflammatory agents as NSAR within the 14 days prior and after the PDT
- Participants taking any photosensitizing drugs within 4 weeks prior to the photodynamic treatment (PDT)
- Participants who had a history of photosensitivity disorder
- Fitzpatrick's skin phototype V-VI

6.2 Recruitment and Screening

Volunteers for this pilot study will be recruited by the Division of Infectious Diseases of the University Hospital Zurich with a flyer (appendix 2) as a recruitment tool (on pinboard at the University Hospital Zurich and the University Hospital Balgrist). Written informed consent will be taken under supervision of PD Dr. Y. Achermann.
The participants will be informed in writing and verbal on:

- The nature, purpose and duration of, and procedure for the research project;
- Their right to withhold or to revoke their consent at any time without giving reasons;
- Their right to receive information at any time in response to further questions relating to the research project;
- Their right to be informed of results concerning their health, and their right for such information or to designate a person who is to take this decision for them;
- Measures to protect the biological material and the personal data

All participants will have enough time to decide whether to participate or not (at least 1 week).
The formal consent of a participant will be obtained before the participant is submitted to any study procedure. The consent form will be signed and dated by the investigator or his designee at the same time as the participant sign. A copy of the signed informed consent will be given to the study participant. The form will be retained as part of the study records in the patient file.

There will be a financial compensation (value CHF 50.00) to the participants.

6.3 Criteria for Withdrawal/ Discontinuation of Participants

A participant must be withdrawn from the study if

- safety reasons of PDT (other side effects than expected, or more intense known side effects).
- participant consent withdrawal

We need a minimal 10 to 15 participants to show the proof of principle of our study. Since we ask for 20 participant we can ask for 5 other participants if we had to exclude them.

A participants who discontinued the study will be follow-up at least once after PDT treatment (3 weeks) to ask for side effects. If no intervention was done, no follow-up is needed.

7 STUDY INTERVENTION

7.1 General Information

The following flowchart shows all steps described in the study plan:

7.1.1 Study Intervention

Study Intervention(s) A)

Who	List of interventions
PD Dr. med. Yvonne Achermann	Skin antisepsis, taking swabs in 20 participants
Dr. med. Laurence Imhof	Photodynamic treatment (see details below) in 20 participants Skin biopsies (substudy) in 5 participants

7.1.2 Control Intervention

No control intervention in this study

7.2 Administration of Study Intervention

7.2.1 Study Intervention

Details of study Intervention	Justification
1. Inguinal skin scraping before photodynamic treatment	Baseline microbiome data
2. Natural bacterial porphyrin fluorescence photography	Baseline fluorescence photography
3. Photosensitizer application, followed by fluorescence photography Photodynamic therapy (PHT) (1x) over 15 minutes at the dermatology ward	Treatment to kill bacteria and to reduce glands in order to reduce colonization bacteria
4. Skin antisepsis in the groin, skin scraping after antisepsis	To measure bacterial colonization to know the immediate effect of PDT
5. Punch biopsy[1] specimen of the MAL-PDT area (immediately after PDT) and of an untreated area as a control	To analyze size and structure of glands in the skin
6. Inguinal skin scraping and punch biopsy of the MAL-PDT area after 7 and 21 days	To measure bacterial colonization to know the long-term effect of PDT

[1] only in 5 participants

7.2.2 Modification of Interventions

Trial is not modifiable

7.3 Compliance with Intervention

There will be no compliance problem of the study participant since the intervention takes place at visits 2 and 3. No intervention by the participant is needed except reporting of adverse effects.

7.4 Data Collection and Follow-up for Withdrawn Participants

Not applicable

7.5 Concomitant Intervention(s)

Since we only include participants for our analysis who do not take antibiotics or NSAR in the 14 days prior to the photodynamic treatment or until follow-up at 14 days, we will inform on that issue at the screening and at each study visit. We will record it in our eCRF.

The reason for this exclusion criteria is the impact on the bacterial skin flora and on inflammation in skin histopathology.

8 STUDY PROCEDURES

8.1 Study Flow Chart/Table of Study Procedures and Assessments

Flow-chart see chapter 5.2.

Study plan: Effects of photodynamic therapy on the human inguinal skin microbiome to improve antiseptic effect

Study tasks	**Screening**	**Intervention Period**			**Follow-up**
Visits	**1**	**2**	**3**	**4**	**5**
	at least -7	0	7±3	21±7	35±7
7. Participant information and informed consent	√				
8. Inclusion- and exclusion criteria	√				
9. Demographic data	√				
10. Inguinal skin scraping before photodynamic treatment		√			
11. Natural bacterial porphyrin fluorescence photography		√	√	√	√
12. **Study intervention**: Photosensititer application, followed by fluorescence photography Photodynamic therapy (PHT) (1x) over 15 minutes at the dermatology ward		√			
13. **Primary outcome measure**: Skin antisepsis in the groin, skin scraping after antisepsis		√			
14. **Secondary outcome measure**: Punch biopsy[1] specimen of the MAL-PDT area (immediately after PDT) and of an untreated area as a control		√		√	
15. **Primary and secondary outcome measure:** Inguinal skin scraping and punch biopsy[1] of the MAL-PDT area			√	√	
16. (serious) adverse events		√	√	√	√
17. End of study information of study results					√

[1] optional, only in 5 volunteers

Skin scrapping

For all skin swabs, we will apply the scratching method using a scalpel as described previously (17) and illustrated below in Figure 2. The swabs and the biopsies will be analyzed for bacterial growth. The remaining sample will be immediately frozen at -80C° for later molecular analysis such as metagenomics.

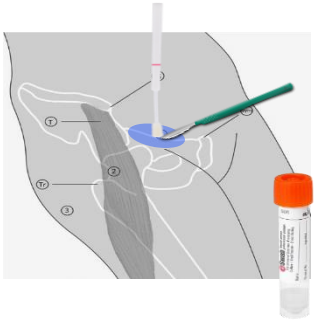


Figure 2. Specimen collection in the groin of the hip before disinfection. Skin scrapings will be removed with sterile blades and transferred to ESwab culture swabs (Copan).
T, musculus tensor fascia latae; Tr, trochanter

Photodynamic treatment

This will be done by the dermatologist Dr. med. L. Imhof. She will apply 5% topical methyl aminolevulinate (MAL) as the photosensitizer for 3 hours under occlusion in the right groin of the participant. She will then start the photodynamic process by irradiation of the area with 40J/cm2 red light from a 633 nm emitting LED during 13 minutes.

Skin antisepsis

After the MAL- photodynamic treatment, we will perform skin antisepsis as routinely done in clinics three times with a povidone-iodine/alcohol solution (Betaseptic®, Mundipharma, Limburg, Germany) for a total duration of 3 minutes to imitate standard surgical antisepsis before a hip arthroplasty surgery. We will perform skin scraping (see above) before and after the 3rd antisepsis and 21 days later for bacterial cultures.

Skin Biopsies

This will be done by the dermatologist Dr. med. L. Imhof in 5 volunteers. A small piece of skin (3 mm) will be taken and transferred to a sterile tube for microbiological culture and histopathology. The skin biopsies will be taken after a local anesthesia. The wound will be closed with one stitch.

8.2 Assessments of Outcomes

8.2.1 Assessment of Primary Outcome

What	***When***	***How***
Bacterial density and species of skin swabs and biopsies	Visit 2: immediate after intervention And Visit 3	Microbiological diagnostic methods (see below)
Histopathology	Visit 2 (day 0) and Visit 3 (after 21 days)	Routine dermato-histopathology

Microbiology

In short, the patient's sample swabs will be streaked out onto Columbia sheep blood agar plate without antibiotics (bioMérieux, Mary-l'Etoile, France), colistin-nalidixic acid (CNA) blood agar (bioMérieux) plate for aerobic cultivation and a Brucella plate (in-house sheep blood agar plates with hemin and vitamin K1) for anaerobic cultivation using GENbag (bioMérieux). Final identification will be done by matrix-assisted laser desorption ionization-time of flight mass spectrometry spectrometry (MALDI-TOF MS) using a Bruker MALDI Biotyper. The amount of bacteria will be semiquantitiative described as +, ++, +++ based on growth on agar plates. All cultured microorganisms will be stored in skim milk at -80C° for potential subsequent analysis by whole genome sequencing for analyzing the secondary outcome.

Histopathology
This part is to determine changes of eccrine gland lobules as a reduction in size, destruction or marked atrophy with molecular and immunofluorescence methods. In addition we will investigate the skin inflammation (type of immune cells, fibrosis).
The participants of this substudy have to sign a separate informed consent document for taking the skin biopsies.

8.2.2 Assessment of Secondary Outcomes

For all persistent isolated microorganisms after PTD and antisepsis preparation of the skin will be subjected to whole genome sequencing for phylogenetic analysis based on core genome single nucleotide polymorphisms. To do so, chromosomal DNA of isolated bacteria will be extracted using the DNeasy UltraClean Microbial Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. DNA library preparation is performed using the QIASeq FX Kit (QIAGEN). Pooled libraries will be sequenced on an Illumina MiSeq (paired-end sequencing, 2 x 150 bp reads, 50-fold coverage). Raw sequencing data (FASTQ) will be processed using trimmomatic (41). Trimmed sequencing reads are assembled to CONTIGS using SPAdes (42). Core genome single nucleotid polymorphisms (SNPs) are determined by analyzing CONTIGS with parSNP (43). Concatenated SNPs are transformed into a multi-alignment file which eventually serves for the calculation of a phylogenetic tree by maximum likelihood (44). Whole genome sequencing, assembly and phylogenetic comparisons will be done at the Institute of Medical Microbiology (IMM) at the University of Zurich.

What	***When***	***How***
Whole Genome Sequencing/Phylogeny	After termination of all study participants	In collaboration with the Institute for Clinical Microbiology with a lot of experience in this field

8.2.3 Assessment of Safety Outcomes

8.2.3.1 Serious Adverse Events

Recording of serious adverse event (SAE) information time of onset, duration, resolution, action to be taken, assessment of intensity, and relationship with study intervention.

8.2.4 Assessments in Participants who prematurely Stop the Study

Participants who are withdrawn from the study prematurely due to adverse event, will be followed-up until adverse-effects disappeared. Follow up may include but is not limited to physical examination, laboratory tests, vital signs, telephone calls. Outcomes and resolution of events will be recorded in the Case Report Forms. In case of lost to follow up, efforts will be made to contact the patient or to ascertain the vital status of the participant.

8.3 Procedures at Each Visit

8.3.1 Screening Visit = Visit 1

Screening visit, Day – at least -7 days before the study start

- Information of the study and all necessary study visits
- Check for In- and Exclusion criteria

- Enrollment if agree to participate in study
- Enter basic demographic parameters in electronic CRF (age, sex, current medications, and underlying diseases)
- Scheduling of all study visits

Duration: ca. 30 minutes
Location: Division of Infectious Diseases, University Hospital Zurich

Study schedule:

Substudy in 5 volunteers:

In the course of the study a total of three small skin biopsies are taken (3mm diameter each) from 5 volunteers. The time and locations of the performed biopsies will be

1. from the right groin immediately after the MAL-PDT or at day 7
2. from untreated control area in the left groin after the MAL-PDT(day 0 or 7)
3. from the MAL-PDT area on day 21 as the control after treatment.

8.3.2 Visit 2 = Day 1 of the study

On day 1 with Dr. Imhof and Dr. Achermann, a skin scraping of the groin will be performed and sent for microbiological culture. Then, 5% topical methyl aminolevulinate (MAL) as the photosensitizer will be applied for 3 hours under occlusion in the right groin of the patient. After the incubation, the photodynamic process will be started by irradiation of the area with 40J/cm2 red light from a 633nm emitting LED during 13 minutes.

Immediately after the MAL-PDT, skin antisepsis will be performed. Antisepsis of the skin is performed with a povidone-iodine/alcohol solution (Betaseptic®, Mundipharma, Limburg, Germany) and repeated three times for a total duration of 3 minutes to imitate standard surgical antisepsis before a hip arthroplasty surgery.

For all inguinal swabs, we will use the scratching method using a scalpel as already applied in a previous study (ethical approval BASEC-Nr 2016-01017). The swabs will be sent to the microbiology laboratory of the University of Zurich for bacterial analysis (analysis under supervision of Prof. R. Zbinden).

Skin biopsies will be done as a routine procedure at the Department of dermatology.
In short:

- Inguinal skin scraping before PDT
- Natural bacterial porphyrin fluorescence photography
- Study intervention: Photosensitizer application for 3 hours
- PDT for 15 minutes
- Inguinal skin scraping
- Skin antisepsis with povidone/iodine (3xtimes for 1 minute)
- Inguinal skin scraping
- Punch biopsies in 5 participants
- Documentation of adverse effects

Duration: 4 hours
Location: Department of dermatology, University Hospital Zurich

8.3.3 Visit 3 (day 7±3)

Skin scraping after skin antisepsis will be repeated 7 days later and analyzed for bacterial cultures.

In short:

- Inguinal skin scraping

- Skin antisepsis
- Inguinal skin scraping
- Punch biopsies in 5 participants
- Documentation of adverse effects

Duration: 30 - 60 minutes
Location: Department of dermatology or Infectious Diseases, University Hospital Zurich

8.3.4 Visit 4 (day 21±7)

Skin scraping after skin antisepsis will be repeated 21 days later (study visit day 21) and analyzed for bacterial cultures.

In short:

- Inguinal skin scraping
- Skin antisepsis
- Inguinal skin scraping
- Punch biopsies in 5 participants
- Documentation of adverse effects

Duration: 30 -60 minutes
Location: Department of dermatology or Infectious Diseases, University Hospital Zurich

8.3.5 Visit 5 = End of Study Visit (day 35±7)

On day 35 (±7 days) of the study, volunteers will be seen for the last study visit to ask for side effects and to inform about the individual study result.
In short:

- End of study
- Documentation of adverse effects
- Information of the individual study results

Duration: 30 minutes
Location: Division of Infectious Diseases, University Hospital Zurich

9 SAFETY

During the entire duration of the study, all serious adverse events (SAEs) that may be causally related to the study intervention are collected and documented in source documents. Reportable events are recorded in the case report form (CRF). Study duration encompassed the time from when the participant signs the informed consent until the last protocol-specific procedure has been completed, including a safety follow-up period.

In order to guarantee participants' safety and health, further adverse events which must be documented or reported are to be designated in the protocol or at the request of the responsible CEC.

9.1 Definitions

Adverse events
Adverse events (AEs) are defined as any untoward medical occurrence in a patient or clinical investigation participant after the intervention and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any favorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the intervention, whether or not related to the intervention. An AE may also consist of a new disease,

an exacerbation of a pre-existing illness or condition, a recurrence of an intermittent illness or condition, a set of related signs or symptoms, or a single sign or symptom.

Serious Adverse Event

A serious adverse event is defined as any event which:

- requires inpatient treatment not envisaged in the protocol or extends a current
- hospital stay;
- results in permanent or significant incapacity or disability;
- is life-threatening or results in death; or
- causes a congenital anomaly or birth defect.

9.2 Recording and Assessment of Serious Adverse Events

The investigator has the responsibility for SAE identification, documentation, and assessing the causal relationship study intervention.

All SAEs will be fully documented in the appropriate eCRF. For each SAE, the investigator will provide the onset, duration, treatment required, outcome and action taken with regard to the study intervention.

The assessment by the investigator with regard to the study intervention relation is done according to the following definitions:

Unrelated	• The event started in no temporal relationship to the medical intervention applied and • The event can be definitely explained by underlying diseases or other situations.
Related	• The event started in a plausible temporal relationship to the medical intervention applied and • The event cannot be definitely explained by underlying diseases or other situations.

9.3 Reporting of Serious Adverse Events

If, in the course of a clinical trial, serious adverse events occur in participants in Switzerland, and it cannot be excluded that the events are attributable to the intervention under investigation, the investigator must report these events:

- to the CEC **within 15 days**.

Safety and protective measures

If immediate safety and protective measures have to be taken during the conduct of this clinical trial, the investigator must notify the CEC of these measures, and of the circumstances necessitating them, **within 7 days**.

Annual Safety Report

All SAEs will be summed up in the **annual safety report (ASR)** and submitted to the CEC. ASR shall contain:

- A summary of events including severity and causal relationship to the intervention and on the safety of participants.

- The accompanying letter provided with the Annual Safety Report should contain a short summary of the status of the clinical trial in Switzerland (number of centers open/closed, number of patients recruited/recruitment closed, and number of SAEs).

9.4 Follow up of (Serious) Adverse Events

Participants terminating the study (either regularly or prematurely) with

- reported ongoing SAE, or
- any ongoing AEs of laboratory values or of vital signs being beyond the alert limit

will return for a follow-up investigation. This visit will take place up to 30 days after terminating the treatment period. Follow-up information on the outcome will be recorded on the respective SAE page in the eCRF.

10 STATISTICAL METHODS

Categorical data will be tested for differences using Fisher's exact or chi-squared tests, as appropriate, whereas continuous variables will be tested using Wilcoxon rank sum tests.

10.1 Hypothesis

We hypothesize that photodynamic treatment improves skin antisepsis by reduction of persistent skin colonizing bacteria through the destruction of the sebaceous and sweat glands and by its bactericidal effects.

10.2 Determination of Sample Size

Aim: 20 volunteers to show the effect of PDT in healthy volunteers.

10.3 Planned Analyses

- As a pilot study, we plan to do descriptive statistics only.
- Categorical date will be tested for differences using Fisher's exact or chi-squared tests.
- Continuous variables will be tested using Wilcoxon rank sum tests.
- Data will be analyzed using Stata® version 14.2 (Stata Corporation, College Station, TX). Two-tailed P-values <0.05 are considered statistically significant.

10.3.1 Datasets to be Analyzed, Analysis Populations

All Study participants that terminated the study (n = max 20 volunteers)

10.3.2 Primary Analysis

Descriptive comparisons of microbiological data before and after PDT by Dr. Achermann, Dr. Kuster, and Dr. Zbinden.

10.3.3 Secondary Analyses

Statistical analysis of microbiological data using whole-genome analysis: will be done by Dr. Achermann in collaboration with the Institute of Medical Microbiology.
These analysis will be done after termination of all study participants

10.3.4 Interim Analyses

No interim analyses is needed for this small study design.

10.3.5 Safety Analysis

Analysis of SAE as standard (number of adverse and serious adverse events)

10.3.6 Deviation(s) from the Original Statistical Plan

Not applicable.

10.4 Handling of Missing Data and Drop-Outs

Missing outcome data will be handled as missing. No imputation methods will be used.

11 ELIGIBILITY OF THE PROJECT SITE(S)

PD Dr. med. Yvonne Achermann as the project leader is an Infectious Disease attending physician with a lot of experience in the field of orthopedic infections working at the University Hospital of Zurich and the Orthopedic University Hospital of Balgrist in Zurich. She will be responsible for the study design/protocol, perform skin swabs and and analyze microbiological cultures in collaboration with the institute for microbiology (IMM, with Prof. R. Zbinden).

Dr. med. Laurence Imhof is the main investigator at the Department of Dermatology of the University Hospital Zurich. There she is the head of Laser Medicine, Photodermatology and Radiation Therapy. She has a lot of experience in photodynamic treatment and will therefore peform photodynamic treatment as indicated in the study plan.

PD Dr. med. Stefan Kuster is a specialist in Infection Prevention and Control and Hospital Epidemiology (master of science in Clinical Epidemiology and Healthcare Research) and supervises the Swissnoso Surgical Site Infection Surveillance program at the University Hospital Zurich. He will be involved in the study design.

PD Dr. med. Yvonne Achermann, Dr. med. Laurence Imhof, and PD Dr. med. Stefan Kuster are trained in Good Clinical Practice.

Prof. Dr. med. Reinhard Zbinden provides the infrastructure for examining the skin swabs.

12 DATA QUALITY ASSURANCE AND CONTROL

The Sponsor-Investigator is implementing and maintaining quality assurance and quality control systems with written SOPs and Working Instructions to ensure that trials are conducted and data are generated, documented (record), and reported in compliance with the protocol, GCP, and applicable regulatory requirement(s).

12.1 DATA HANDLING AND RECORD KEEPING

The study will strictly follow the protocol. If any changes become necessary, they must be laid down in an amendment to the protocol. All amendments of the protocol must be signed by the Sponsor-Investigator and if essential submitted to CEC.

12.1.1 Case Report Forms

The investigators will use electronic case report forms eCRF, one for each enrolled study participant, to be filled in with all relevant data pertaining to the participant during the study. All participants who either entered the study or were considered not-eligible or were eligible but not enrolled into the study additionally have to be documented on a screening log. The investigator will document the participation of each study participant on the Enrolment Log.

For studies with electronic CRF:

For data and query management, monitoring, reporting and coding an internet-based secure data base Redcap® developed in agreement to the Good Clinical Practice (GCP) guidelines will be used for this study. It is the responsibility of the investigator to assure that all data in the course of the study will be entered completely and correctly in the respective data base. Corrections in the eCRF may only be done by the investigator or by other authorized persons. In case of corrections the original data entries will be archived in the system and can be made visible. For all data entries and corrections date, time of day and person who is performing the entries will be generated automatically.

CRFs/eCRFs will be kept current to reflect participant status at each phase during the course of study. Participants must not to be identified in the eCRF by name. Appropriate coded identification (e.g. Participant Number) will be used.

We assure that any authorized person, who may perform data entries and changes in the eCRF, can be identified. A list with signatures and initials of all authorized persons will be filed in the study site file and the trial master file, respectively.

The investigators assure to perform a complete and accurate documentation of the participant data in the eCRF. All data entered into the eCRF must also be available in the individual participant file either as print-outs or as notes taken by either the investigator or another responsible person assigned by the investigator.

Essential documents must be retained for at least 10 years after the regular end or a premature termination of the respective study (KlinV Art. 45).

Any patient files and source data will be archived for the longest possible period of time according to the feasibility of the investigational site, e.g. hospital, institution or private practice.

12.1.2 Specification of Source Documents

The following documents are considered source data, including but not limited to:

- SAE worksheets
- Nurse records, records of clinical coordinators, and

Source data must be available at the site to document the existence of the study participants and substantiate the integrity of study data collected. Source data must include the original documents relating to the study, as well as the medical treatment and medical history of the participant.

The following information (at least but not limited to) should be included in the source documents:

- Demographic data (age, sex)
- Inclusion and Exclusion Criteria details
- Participation in study and signed and dated Informed Consent Forms
- Visit dates
- Microbiological data (identification of bacteria)
- SAEs (related) and concomitant medication
- Reason for premature discontinuation

12.1.3 Record Keeping / Archiving

All study data will be archived for a minimum of 10 years after study termination or premature termination of the clinical trial (at the Division of Infectious Diseases, University Hospital Zurich).

12.2 Data Management

All basic demographic data, all microbiological and histopathological results will be manually entered in an automatically secured online processing system (REDCap® electronic data capture system). The PI will check and validate data on a regular basis for consistency.
All study data are encrypted in REDCap®. The principal investigator is responsible for data collection and will keep the screening log, which guarantees the confidentiality of data by the use of participant ID numbers. The participant ID numbers are automatically assigned in consecutive ascending form by the REDCap® system. For the purpose of fulfilling their task, the principal investigator grants the necessary access authorization in the form of user log-in and password to the study staff. Thus, the data cannot be altered in any way by unauthorized persons. In the REDCap® system, all relevant processing operations are documented in a user-specific manner in order to ensure traceability. This is done by means of registration software, which records who has edited which data at which time.

Whole genome sequencing data will be generated at the Institute of Medical Microbiology, University of Zurich. Data will be organized as follows: i) sequencing runs: YYMMDD_M04885_Run#_000000000-FlowCellID ii) samples: Sample#_S#_L001_[R1/R2]_001.fastq.gz. Data will be stored and managed on the Server of the Institute of Medical Microbiology. For analyses data will be copied to a LINUX-based workstation.

All patients have to give their written consent to the project. These informed consents documents (IC) will be stored according to ethical regulations.

Health related personal data captured during this project from participants are strictly confidential and disclosure to third parties is prohibited; coding will safeguard participants' confidentiality. Confidentiality will be ensured by using coded data. The code will be protected against unauthorized access and will be stored appropriately. Data will be stored for up to 10 years after publishing the study as requested by most journals.

- *Patient's and microbiological/histopathological data*:
    - Yvonne Achermann will be responsible for basic characteristics of the volunteers of this pilot study. After the last study visit, data will be encoded by the PI and safely stored at the Investigator Site File.
    - Clinical and microbiological data will be saved in the electronical database Redcap (see above)
    - The collected project data may be subject to inspection by the CEC.

- *Handling of isolated bacteria of the scrapped skin samples*:
  The sub-cultivated bacterial samples will be stored and labeled with an encoded name at -80°C at the bacteriology laboratory of the Division of Infectious Diseases and Hospital Epidemiology (responsible Yvonne Achermann). The code for the bacterial strains with correspondence to patient data will be stored at the Investigator Site File.

- *Handling of the skin tissue samples:*
  - If the study patients agree, the remaining material from their skin samples will be kept for further research purposes in the Dermatological Biobank. If a study patient opposes the storage of skin sampling, they will be destroyed after the analyses planned as part of the study
  - Study patients receive a separate information leaflet for this purpose with the corresponding declaration of consent .The collection and storage of samples in the Dermatologische Biobank as well as the quality and safety standards are laid down in the regulations Dermatological Biobank USZ from the 1st of October 2017 (Dermatologische Biobank EK 647) and become in this research project accordingly implemented

12.3 Routine Monitoring

Since we only measure microbiological data from skin samples, we think, that an interne monitoring would be adequate. This would include:

- Microbiological study part (cultivation procedures) under supervision of Prof. Zbinden. Regular control of study result by a microbiologist not involved in the study (to be determined)
- Data entry will be done by the doctorand and by the study nurse by a 4-eyes control. Control of data entry, informed consents, and SAE will be done by the sponsor and by Stefan Kuster (biometrician) on a regular base (4-eyes principle).

12.4 Audits and Inspections

A quality assurance audit/inspection of this study may be conducted by the CEC. The quality assurance auditor/inspector will have access to all medical records, the investigator's study related files and correspondence, and the informed consent documentation that is relevant to this clinical study.

The investigator will allow the persons being responsible for the audit or the inspection to have access to the source data/documents and to answer any questions arising. All involved parties will keep the patient data strictly confidential.

12.5 Confidentiality, Data Protection

Direct access to source documents will be permitted for purposes of monitoring, audits and inspections.

12.6 *Storage of Biological Material and Related Health Data*

Data will be stored for up to 10 years after publishing the study as requested by most journals.

If the study patients agree, the remaining material from their skin samples will be kept for further research purposes in the Dermatological Biobank. If a study patient opposes the storage of skin sampling, they will be destroyed after the analyses planned as part of the study.
Study patients receive a separate information leaflet for this purpose with the corresponding declaration of consent. The collection and storage of samples in the Dermatologische Biobank as well as the quality and safety standards are laid down in the regulations Dermatological Biobank USZ from the 1st of October 2017 (Dermatologische Biobank EK 647) and become in this research project accordingly implemented

13 PUBLICATION AND DISSEMINATION POLICY

After the statistical analysis of this trial the sponsor will make every endeavor to publish the data in a medical journal.
Data of this study can only be published if Stefan Kuster, Reinhard Zbinden, Laurence Imhof, and Yvonne Achermann agree on the data presentation, analysis, and authors list.

☞ ***Commitment for study coordination, ethical protocol, generate microbiological data, data entry, study analysis****: Division of Infectious Diseases and Hospital Epidemiology, USZ (Dr. Achermann, Dr. Kuster, Steven Maurer under supervision of YA and SM)*
☞ ***Commitment for performing photodynamic treatment****: Department of Dermatology, University Hospital Zurich (Dr. med. Laurence Imhof)*
☞ ***Commitment for bacterial culture, bacterial identification, storage****: Institut of Medical Microbiology, UZH (Prof. Zbinden)*
The Principal Investigator will perform the study in accordance with the current protocol version. The Principal Investigator will ensure that any investigators and any other staff comply with the terms of the Protocol and this agreement.

14 FUNDING AND SUPPORT

14.1 Funding

This study will be financed by the sponsor.

External funding is provided by the Vontobel foundation for salary (study nurse and PhD student (CHF 76’034) and by the Galderma company for the PDT treatment and microbiological/histolological examinations (CHF 11’323).

14.2 Other Support

15 INSURANCE

Insurance is covered by “Versicherung für klinische Versuche und nichtklinische Versuche“ by Zürich Versicherungs-Gesellschaft AG (Policy no.: 14.970.888).

Any damage developed in relation to study participation is covered by this insurance. So as not to forfeit their insurance cover, the participants themselves must strictly follow the instructions of

the study personnel. Participants must not be involved in any other medical treatment without permission of the principal investigator (emergency excluded). Medical emergency treatment must be reported immediately to the investigator. The investigator must also be informed instantly, in the event of health problems or other damages during or after the course of study treatment.

The investigator will allow delegates of the insurance company to have access to the source data/documents as necessary to clarify a case of damage related to study participation. All involved parties will keep the patient data strictly confidential.

A copy of the insurance certificate will be placed in the Investigator's Site File.

16 REFERENCES

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17 APPENDICES

Appendix 1: Documentation of PDT as in routine clinics at the clinic for dermatology

Aufklärung über Therapie und Wichtigkeit des UV-Schutzes.

Einzeichnen der Felder und Start PDT am

Felder:

Feld 1-

Heute Feld:

Crème und Einwirkungszeit: Metvix 3h

Maschine:
Aktilite Dosis: 37J/cm2 Abstand: 6 cm
Waldmann PDT 1200L Dosis: 80 J/cm2 Abstand: 100 mW/cm2

Gabe von 1g Paracetamol (Dafalgan) 1h vor Therapiebeginn

Behandlung nach der Therapie:
Ialugen Plus-Verband für 24 h
Ialugen Plus dem Patienten mitgegeben zur Nachbehandlung

Appendix 2. Flyer

Auswirkungen der Lichttherapie auf Hautbakterien

Probandinnen und Probanden gesucht

Für eine wissenschaftliche Studie suchen wir Frauen und Männer mit Mindestalter 18 Jahre für die Untersuchung der Hautbakterien vor und nach einer Lichttherapie.

1) **Ziel der Studie:** Es ist bekannt und natürlich, dass Bakterien die ganze Haut – inklusive Schweiss- und Talgdrüsen - kolonisieren. Vor Operationen wird die Haut desinfiziert um diese Bakterien abzutöten. Leider ist diese Präventionsmassnahme nicht 100% effektiv. Die Gefahr besteht, dass noch vorhandene Bakterien mit dem chirurgischen Schnitt in die Tiefe vorschleppt werden und dort eine Infektion verursachen kann. Aus diesem Grund müssen die gegenwärtigen Desinfektionsprozesse hinterfragt und verbessert werden. Mit dieser Studie am Universitätsspital Zürich untersuchen wir, ob die Lichttherapie Hautbakterien abtöten und dieTalg- und Schweissdrüsen in der Haut verkleinern kann. Diese Therapie ist auf der Klinik für Dermatologie etabliert, wird gut vertragen und es entstehen keine Folgeschäden.

Ablauf: Die Studienteilnahme beinhaltet 4 Termine innerhalb von 5 Wochen. Diese finden entweder in der Klinik für Infektionskrankheiten oder der Klinik für Dermatologie statt. Der erste Termin dauert ca. 20 Minuten und dient der Studienaufklärung und Abklärung zur Teilnahme. Der zweite Termin dauert 4 Stunden und beinhaltet die Lichttherapie mit Hautabstrichen vor und nachher. Die dritte und vierte Visite dauert zwischen 15 und 30 Minuten zur Kontrolle der Hautbakterien und zum Studienabschluss.

Alle Daten werden vertraulich behandelt. Für die Versuchspersonen ergibt sich kein direkter medizinischer Nutzen. Es gibt eine Entschädigung von CHF 50.00 für die Teilnahme. Alle Untersuchungen sind kostenlos.

Wir würden uns freuen, wenn Sie an einer Studienteilnahme interessiert sind. Sie können gerne per Email oder telefonisch mit PD Dr. Yvonne Achermann Kontakt aufnehmen:

yvonne.achermann@usz.ch;
Tel: +41 44 255 34 02 direkt oder übers Sekretariat (044 255 33 22)

Bitte nehmen Sie zur Kenntnis, dass ihre Daten bei Zustandekommen eines Kontakts registriert werden. Sollten Sie später nicht einer Teilnahme interessiert sein, werden ihre Daten unverzüglich gelöscht.

Dieses Projekt ist organisiert durch:
PD Dr. med. Yvonne Achermann, Klinik für Infektionskrankheiten, Universitätsspital Zürich und
Dr. med. Laurence Imhof, Klinik für Dermatologie, Universitätsspital Zürich