The *SPaCE* swab: Point of care sensor for simple and rapid detection of acute wound infection

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Supporting Information

PROTOCOL

SPaCE Pilot: Pilot study to investigate the use of SPaCE phospholipid vesicle technology: a point of care tool to aid diagnosis of clinically relevant burn wound infection

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University Hospitals Bristol NHS Foundation Trust is the research sponsor for this study.

Funder:
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This protocol describes **SPaCE** and provides information about procedures for entering participants. Every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the trial. Problems relating to this trial should be referred, in the first instance, to the Chief Investigator.

This trial will adhere to the principles outlined in the NHS Research Governance Framework for Health and Social Care (2nd Edition). It will be conducted in compliance with the protocol, the Data Protection Act, GDPR and other regulatory requirements as appropriate.

Abbreviations

UHB	University Hospitals Bristol NHS Foundation Trust
SWCBC	South West Children's Burns Centre
QVH	Queen Victoria Hospital NHS Foundation Trust
NBT	North Bristol NHS Trust
AE	Adverse Event
SAE	Serious Adverse Event
SOP	Standard Operating Procedure
MRC	Medical Research Council
WHO	World Health Organisation
MC&S	Microscopy, Culture and Sensitivity

Keywords: Burn, infection, diagnosis, colonisation, development, wound exudate, adult burns, child, paediatric burns, scalds, nanotechnology, point of care device, swab, phospholipid, vesicles, fluorescent dye.

Table of Contents

1. Introduction

The objective of this study is to evaluate the technology that is intended to be incorporated into a *SPaCE-swab* sensor kit. The kit is intended to be a low cost, fast, near-to-patient method of assessing the infection state of a wound. It would rapidly indicate wound colonisation (onset of infection) by the four principal microbial wound pathogens: ***S****taphylococcus aureus,* ***P****seudomonas* ***a****eruginosa,* ***C****andida* species, and ***E****nterococcus faecalis* (abbreviated to *SPaCE* for the purposes of this application*)*.

It is important to note that:

1. This study will NOT be altering the current patient care pathway;
2. The SPaCE swab will **not** be used by clinicians to determine treatment: interpretation will be carried out by the scientific team at the University of Bath
3. The data from the study will be used to *evaluate* whether this In Vitro Diagnostic (IVD) medical device *should be developed* and validated for CE marking. As such this product will be registered with the MHRA as an In Vitro Diagnostic (IVD) medical device.

The underpinning technology - similar to that used in the *Smartwound* dressing (*EVIDEnT* study, IRAS project number: 206022) - uses the lipid vesicles to detect microbially-secreted virulence factors (when *quorum sensing* in the bacterial colony triggers toxin production). These virulence factors and toxins, discharged at the point of toxin release (*PTR*) when a wound is on the threshold of developing a full-blown infection, lyse the vesicles, releasing fluorescent dye which is easily detectable by eye (Figure S-1a and S-1b).

The kit would be utilised when wounds are swabbed, as part of standard care. The swab would be placed in our patented vesicle gel, left for a minimum of 60 minutes and observed for any colour change (Figure S-2). A bright green colour means that virulence factors and toxins associated with *SPaCE* pathogens have been detected, and clinically significant infection is taking hold at the PTR.

The medium-term plan beyond the development of the *SPaCE-swab sensor kit* would be to integrate these vesicles into a wound dressing, thus creating an *in situ* detection system for *SPaCE* pathogens at the PTR. However, such a dressing would be a Class IIb medical device, with significant technical and regulatory hurdles prior to initial patient use. Recent studies at the University of Bath have shown that a different approach to PTR detection could be used: the wound can be swabbed, the swab immersed in the vesicle suspension, and the result observed in around 1 hour.

This project if successful could lead to a near-to-patient, low cost and rapid wound swab sensing kit, which could be used immediately in secondary care settings and has the potential for long-term use in primary care settings and in low-resource settings.

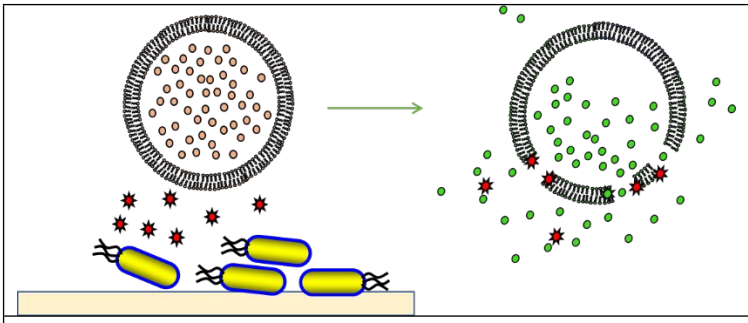


Figure S-1a: Schematic of phospholipid vesicle activation by bacterial virulence factors at the PTR.

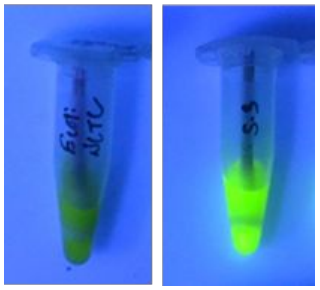


Figure S-1b: Non-activated (left) and activated (right) vesicles in Eppendorf tube.

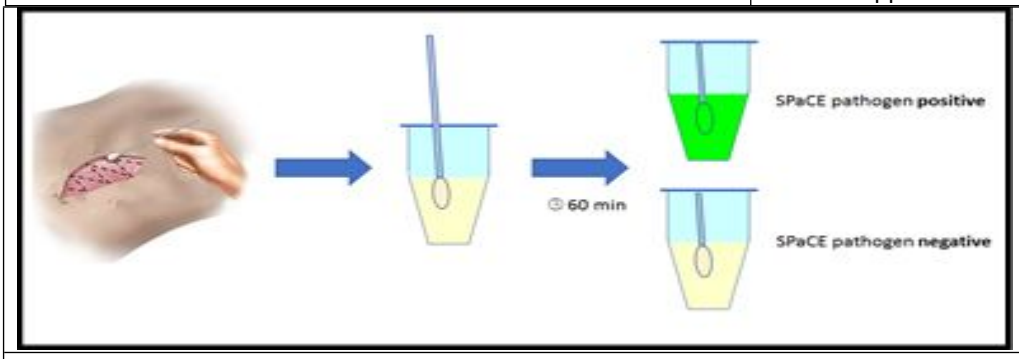


Figure S-2: Schematic of wound pathogen sensing methodology

2. Study Aim and Objectives

2.1 Study Aim:

To test the ability of the SPaCE point of care nano-vesicle technology to detect clinically relevant burn wound infection and investigate the clinical and patient acceptability of carrying out a larger trial.

2.2 Study Objectives:

Primary Objective

- Undertake a pilot study to assess the ability of the SPaCE technology in aiding diagnosis through colour-change, to indicate clinically important burn wound infection (e.g. need for antibiotics, delayed healing, erythema and unexpected need for surgical intervention).

Secondary Objectives:

- Investigate practicalities of sample collection and viewing technology 'switch-on' / colour change in clinical setting, including standardisation of photography.
- Agreement of the technology result with retrospective clinical decision of wound infection.
- Agreement of the technology 'switch-on' with hospital microbiology results.

3. Study design

3.1 Recruitment

Patients with burn injuries presenting to the South West Children's Burn Centre at University Hospitals Bristol NHS Foundation Trust, North Bristol NHS Trust and Queen Victoria Hospital will be invited to take part. Patients with suspected burn wound infection (suspected infection arm) and without suspected burn wound infection (control arm) will be invited to participate.

If a patient fulfils the inclusion criteria (section 3.2), the adult, child and/or parents will be approached by a member of either the research team, or GCP-compliant member of the burns clinical team, who will provide them with age-appropriate literature and discuss recruitment to the study. Participation in the study is voluntary. Informed consent will be received from patients and parents once they have received adequate information as explained in section 3.3.

We aim to recruit 20 patients in the control arm and 20 patients in the suspected infection arm across the three sites.

True wound infection will be defined with a reference standard at note review follow up, to be agreed prior to the start of the pilot study.

3.2 Participant entry

3.2.1 Inclusion criteria

Control group

- Patients with burns of all types and sizes
- 1month – 100 years of age
- At least 24 hours after injury
- No signs of wound infection
- Not being, or have been, treated with antibiotics within 7 days

Suspected infection group

- Patients who present with a suspected burn wound infection. This must include at least 2 of the following:

- Patient pyrexia above 38°C
- Redness surrounding wound

- Increased pain at wound site
- Increased temperature at wound site compared to normal skin
- Evidence of purulent discharge at the wound site.
- Adult aged 17-100 years/child over 8years
- Not on antibiotics for more than 24hours

3.2.2 Exclusion Criteria

- Adult without mental capacity to consent
- Patients on antibiotics for greater than 24 hours

3.2.3 Control becomes Non-control

If a control patient comes back to hospital with a suspected infection within 24 hours after recruitment to the control group, this will be recorded on the CRF as a suspected infection in a control subject. These patients' control SPaCE swab results will be excluded from the study group. Samples collected after the time that infection was suspected may be included within the suspected infection group if the patient re-consents to the study.

3.3 Consent

Eligible patients will be offered age-appropriate written information and the opportunity to ask any study-related questions before signing a written consent form. For children under the age of 16, a parent will consent on behalf of their child as well as assent from the child, where appropriate. Time between the study team being made aware of these patients and being able to give information will be short due to the trauma nature of the speciality. We will ensure that patients and parents feel fully informed of the study procedures before receiving consent.

3.4 Pilot study pathway (flow diagram shown in appendices 1)

There will be two patient groups: a control group without signs of burn wound infection (recruited at least 24 hours after burn injury) and a group of those with a suspected infection as per inclusion criteria (section 3.2) (retrospectively confirmed against the agreed reference standard at follow up).

Once screening and consent has been completed the existing wound dressing will be removed as per the clinical management plan and the following procedures carried out

- Photograph of wound once dressing removed
- All samples will be taken using a standard method using even pressure and moved in an 'Essen' spiral
- The first SPaCE swab will be taken prior to cleaning (swab to be pre-moistened with saline) and placed into vials containing the SPaCE technology medium. The swab included in the SPaCE pack is CE marked and being used as its intended purpose.
- Wound cleaned as per local standard clinical care, but with a final saline clean prior to swab
- Clinical MC&S swab taken of wound
- Second SPaCE swab taken and placed into a second vial containing the SPaCE technology medium
- Routine clinical wound management
- The SPaCE vials and swab will be incubated at room temperature in the clinical area where the patient is being treated and the room temperature recorded.
- A minimum of 60 minutes after the swabs have been inserted into the SPaCE vesicle solution, a member of the research team will take a photograph of the vial next to a standardised colour scale. All sites will be issued with a camera set to fixed focus and aperture. SPaCE vials will be

photographed in standard lighting conditions as described in the photographic SOP using the camera, light source and imaging frame provided by University of Bath. SPaCE vials will be imaged alongside the colour gradient chart containing the participant number and date of image.

- Once the SPaCE vial has been photographed, the vial and swab will be disposed of in clinical waste as per hospital policy.
- A member of the clinical team will record their interpretation of switch on/off from the vials on the CRF before disposal, using the provided 'on/off' indicative scale. This will **not** be used in, or affect the clinical decision making process.

Demographic and routinely collected clinical data (including, but not limited to, patient and injury details, microbiology culture of wound swabs and blood, laboratory tests for markers of inflammation, and infection and clinical observations) will also be recorded.

Laboratory staff at Bath University will be blinded to all clinical data; they will only receive photographs of SPaCE vials with a corresponding patient identification number. The study team at Bath University will make their decision using photographs to determine if the technology was activated (switch-on).

There will be no blinding at hospital sites, but clinical treatment decisions will be made independently and will be irrespective of SPaCE results.

Patients will be followed-up if they re-present to the Burns service within 3 weeks. A final review of notes at 3 weeks will involve collection of information on any further treatment after they presented to the service and a retrospective diagnosis of wound infection against the agreed reference standard.

3.5 Clinical follow-up

The participant will be followed up by the research staff at each site through a notes review, for up to 3 weeks after recruitment or until the wound has healed.

- Retrospective clinical diagnosis on infection within 1 week of recruitment. (see section 3.6)
- Alternatively, clarification of control status i.e. if patient was originally planned to be a control and then developed an episode of suspected infection.
- Observation of the clinical course for up to 3 weeks post recruitment.

3.6 Retrospective clinical diagnosis on infection

An agreement of clinical diagnosis on infection will be made, independently, by 2 senior clinicians (a third, if there is disagreement), from review of clinical microbiology results, clinical symptoms and wound photography. The clinicians making these decisions are not to be members of the research staff involved in recruitment. This decision will be made within a week of recruitment to the study to allow time for results to be back and 2 clinicians to have assessed the clinical documentation.

3.7 Data management

Screening logs will be kept at each participating site. Paper data collection forms (CRFs) will be stored in a locked cabinet in each site's research office. Source data will be stored in accordance with the NHS code of confidentiality.

Research staff will ensure that the participants' anonymity is maintained through protective and secure handling and storage of patient information at the trial centres (as relevant). The participants will only be identified by a patient ID number on the CRF and database. All documents will be stored securely and only accessible by trial staff and authorised personnel. Data will be collected and retained in accordance with the Data Protection Act 2018 and The General Data Protection Regulation 2016.

Data identified by the participant's unique study number will be entered directly by each clinical site into the secured database.

Study documents (paper and electronic) will be retained in a secure location, during and after the trial has finished. All essential documents, including patient records and other source documents will be retained for a period of 25 years following the end of the study. Where study related information is documented in the hard copy medical records – those records will be identified by a 'Do not destroy before dd/mm/yyyy' label where date is 25 years after the last patient's last visit. Where electronic records are in use, trust policy will be followed.

4. Data collection and analysis, sample size, statistical analysis and results

4.1 Data collection and analysis

We anticipate the recruitment period to be 9 months

Study data will be stored in an encrypted password protected database built by the investigators within a Redcap database which meets all international data standards. No identifiable personal data will be stored within the database and investigators will only have access to their own site's data.

Since this is a pilot study and patient numbers are low we will not use statistical analysis other than simple correlation analysis of data:

Control group: Number of SPaCE sensor positives and negatives recorded in this population; Clinical microbiology results.

Infection group: Number of SPaCE pathogen positives and negatives in this population; Elevated Clinical microbiology results.

We will examine clinical microbiology (swab test) results for all patients and look for any correlation with identified micro-organisms and the SPaCE test result

Where available, we will record WBC and CRP levels.

We will collect demographic data and burn injury details, time to healing and outcomes in addition to screening criteria. Data from the clinical record, including temperature, blood results and any other relevant results, will be analysed using descriptive statistics; relationships between variables will be investigated using correlation matrices. Analysis will be carried out using the R statistical language and be reported in various ways, including tabular data and charts.

4.2 Sample size

We aim to recruit 20 patients in the control arm and 20 patients in the suspected infection arm.

4.3 Results

Results will be presented as a flow diagram of participants, as shown in the STARD guidance in appendix 3.

5. Safety Reporting

5.1 Adverse events (AE)

As the primary outcome from this study refers to burns wound infections, recognised complications of burn injury, such as wound infection, systemic response, surgical interventions and sepsis, do not need to be reported as adverse events.

An ***adverse event*** is any untoward medical occurrence in a subject to whom a medicinal product/medical device/intervention has been administered, including occurrences which are not necessarily caused by, or related to, that product.

5.2 Serious adverse events or reactions (SAE)

An ***adverse event, adverse reaction*** is defined as serious if it:

(a) results in death, (b) is life-threatening, (c) requires hospitalisation or prolongation of existing hospitalisation, (d) results in persistent or significant disability or incapacity, or (e) consists of a congenital anomaly or birth defect.

5.3 Reporting Procedures

Adverse events will be recorded and reported in accordance with University Hospitals Bristol's Research Safety Reporting SOP. It has been agreed with the sponsor that 'wound infection', among other recognised complications from burn injury listed above, does not need to be reported as an adverse event, as this is one of the main inclusion criteria for the study.

5.3.1 Non serious AEs

All such events should be recorded other than recognised complications related to burn injury and infection (see section 5.1).

5.3.2 Serious AEs

Serious Adverse Events must be reported by the local investigators to the CI and sponsor within 24 hours of the research team becoming aware of the event

All SAEs should be reported to the Research Ethics Committee where in the opinion of the Chief Investigator, the event was:

- "Related" – that is, possibly, probably or definitely resulted from administration of any of the research procedures, and
- "Unexpected" – that is, the type of event is not listed in the protocol as an expected occurrence.

6. Ethical and regulatory considerations

6.1 Review by an NHS Research Ethics Committee and Health Research Authority

The research will be performed subject to a favourable opinion from an NHS REC and Health Research Authority (HRA) and local site capacity and capability confirmation. Ethics review of the protocol for the trial and other trial related essential documents (e.g. PIL and consent form)

will be carried out by a UK NHS REC. Any subsequent amendments to these documents will be authorised by the sponsor and then submitted to the REC and HRA for approval prior to implementation.

6.2 Amendments to protocol

Any amendments to the trial documents must be approved by the sponsor prior to submission to the HRA and REC.

6.3 Research Governance

This study will be conducted in accordance with:

- The principles of Good Clinical Practice, as set out in the International Conference for Harmonisation of Good Clinical Practice (ICH GCP) guidelines
- The UK Policy Framework for Health and Social Care Research.

6.4 Consent

All participants will be required to give written informed consent. This process, including the information about the trial given to patients in advance of consent and recruitment, is described above in section 3.3.

6.5 Confidentiality

All data and samples will be pseudo anonymised and labelled with the allocated study number, along with storage details. Once testing and photography is completed, the sample will be destroyed.

6.6 Indemnity

This is an NHS-sponsored research study. For NHS-sponsored research, HSG (96)48 reference no. 2 refers. If there is negligent harm during the clinical trial when the NHS body owes a duty of care to the person harmed, NHS Indemnity covers NHS staff, medical academic staff with honorary contracts, and those conducting the trial.

NHS Indemnity does not offer no-fault compensation and is unable to agree in advance to pay compensation for non-negligent harm.

Ex-gratia payments may be considered in the case of a claim.

6.7 Monitoring and Audit

The study will be monitored in accordance with University Hospitals Bristol's Monitoring SOP. All trial-related documents will be made available on request for monitoring and audit by UH Bristol, the relevant Research Ethics Committee, and for any other regulatory authorities.

6.8 Trial Management

The day-to-day management of the trial will be coordinated by Children's Burns Research Centre Team with Dr. Amber Young as CI.

7. Publication policy

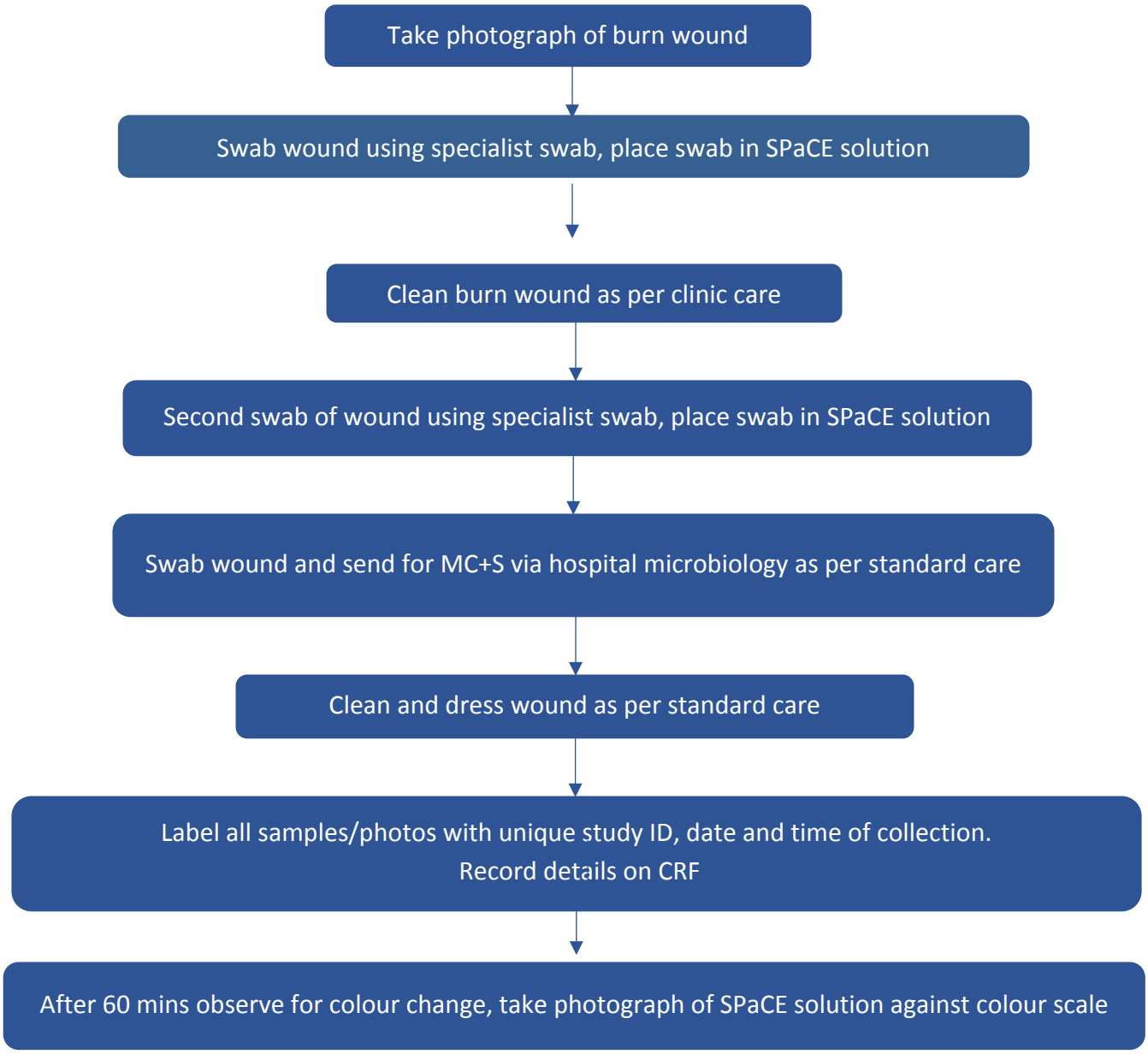
Peer reviewed scientific journals, internal reports and conference presentations.

Appendix 1: SPaCE Study Pathway

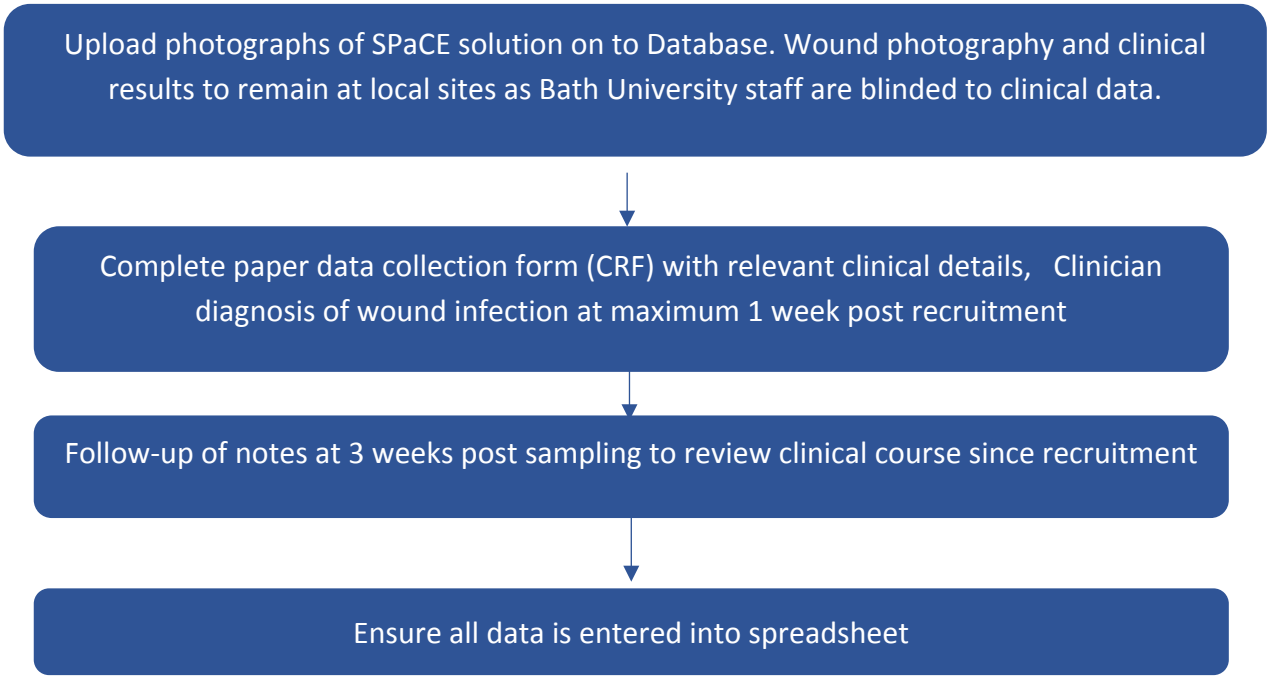
Screening and Consent

Patient attends burns clinic/ward -> identified to research team -> Screened for inclusion/exclusion criteria -> If eligible patient consent to study and given study ID

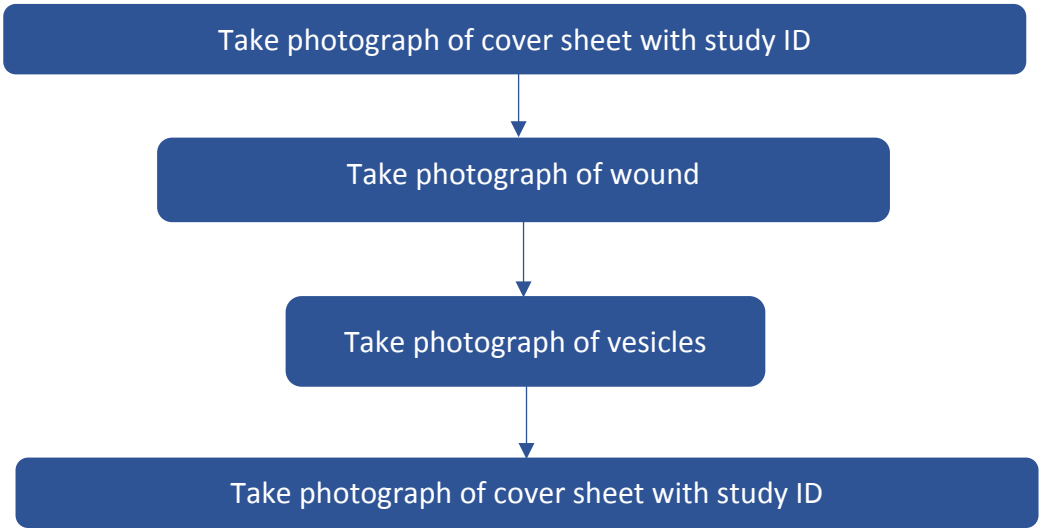
Study Activities



Data collection and follow up



Photography process



Appendix 2: STARD diagram Result Analysis

Prototypical STARD diagram to report flow of participants through the study