Statistical Analysis Plan

Full study title: *Monitoring wound status using multi-parameter optical fibre sensors*

Short title/acronym: *OFFSWM*

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Version Number	Date	Author	Description of Changes

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[A] Sample Size

[A.1] Sample Size Estimation / Power Calculation (as per protocol) [Protocol Page. 42]

[A.1.1] This is a feasibility study and so is not powered for statistical testing. 10 participants will provide a sample size of up to 200 measurements for each OFSSWM parameter (humidity, NH3, CO2, temperature) at each visit.

[A.2] Sample Size After Trial Completed

[A.2.1] 10 participants will have provided measurements for each parameter using both OFSSWM and conventional device at each visit (baseline and 3 further visits).

[A.2.2] OFSSWM device: Measurements (humidity, NH3, CO2, temperature) are taken from the ambient and from the wound surface. There are 3 fibres measuring each parameter. In total, there will be up to 300 measurements (up to 100 per fibre) collected for each parameter in the wound at each visit. Please see section [D.4.1] for more details on how measurements are collected for OFFSWM.

[A.2.3] Conventional device: All parameters (humidity, CO2 and temperature) are measured at the same time every second for 8 minutes with 1 second sampling time. The conventional device failed to collect measurements for NH3 so only humidity, CO2 and temperature have been collected. There are expected to be up to 481 measurements for each parameter at every visit in each environment (ambient, wound and healthy skin).

[A.3] Final Sample Size

[A.3.1] OFSSWM device: Final expected sample is up to 600 measurements (300 each for ambient and wound) for each parameter (humidity, NH3, CO2, temperature) at each visit.

[A.3.2] Conventional device: Final expected sample is up to 1443 measurements (ambient, wound and healthy skin combined) for each parameter (humidity, CO2 and temperature) at each visit.

[B] Randomisation

[B.1.1] Randomisation will not be employed for this study. All patients will be measured firstly using the optical sensor, and then using the conventional measurements.

[C] Interim Analysis

[C.1] Justification for Interim Analysis

[C.1.1] No interim analyses are planned

[C.2] Definition of Endpoints used in Interim Analysis

[C.2.1] Not applicable

[C.3] Statistical Methods for Interim Analysis

[C.3.1] Not applicable

[D] Final Statistical Analysis

[D.1] Summary of Baseline Data [Protocol Page. 42]

[D.1.1] Descriptive statistics will be presented to summarise the distribution of baseline variables. The continuous baseline variables (e.g. Age, eGFR, HbA1c, ulcer area, % surface area slough, pain VAS score) will be reported with means & standard deviation, if shown to be normally distributed, using a combined skewness and kurtosis test, otherwise will be reported with medians, Interquartile Ranges (IQR).. The categorical variables (e.g. sex, type of diabetes, concomitant medication, ulcer infection) will be reported with frequencies & percentages.

[D.1.2] A Consolidated Standards of Reporting Trials (CONSORT) flow diagram will be produced with the number of patients eligible, screened, recruited, reasons for not participating, number of patients with completed visits.

[D.2] Definition of Primary Endpoint [Protocol Page. 26]

The primary endpoint is the feasibility of the trial which contains the following outcomes:

[D.2.1] Total number of eligible patients; Number and percentage of patients who are eligible that have consented; Recruitment rate per month defined as the total number of participants recruited divided by the recruitment period in months; Reasons for not participating.

[D.2.2] Non-completion rates and reasons. The following will be looked at:

1. Percentage of patients not completing a visit with reasons
2. Percentage of patients where not all measurements have been taken (both OFSSWM and conventional) at each visit including all environments (ambient, wound, healthy skin) with reasons.

[D.2.3] Feasibility of taking measurements for each optical probe parameter from a wound (OFSSWM only). This will be assessed by describing the following:

(1) The best optical probe position with regard to the surface of the wound at each visit

(2) Number of failures to get any readings per parameter at each visit. Failure is defined when OFSSWM measurements could not be obtained and/or measurements are out of range. For example, due to fibre or sensitive film damage, motion artefacts, or opto-electronic unit malfunction.

(3) Reasons for signals being distorted at each visit

[D.2.4] To investigate whether GCMS (Gas chromatography/mass spectrometry) of discarded wound dressings to measure parameters using OFSSWM is possible. This is defined as the proportion of wound dressings which have measured parameters successfully. Success is defined as the close relation between the measurements from conventional devices/GCMS data to the OFSSWM results.

[D.3] Statistical Methods for Primary Analysis [Protocol Page. 26]

Descriptive statistics will be presented to summarise feasibility as follows:

[D.3.1] Total number of eligible patients will be presented. Frequency and percentage of participants who are eligible that have consented will be calculated with a 95% confidence interval (CI). Reasons for not participating will be presented as frequencies and percentages. The recruitment rate will be presented as a rate per month as defined in [D.2]. This will be conducted in the Full Analysis Set (FAS) as described in [E.1].

[D.3.2] Non-completion rate and reasons will be presented as a frequency and percentage at each visit as defined in [D.2]. This will be conducted in the Intention to Treat (ITT) population as described in [E.1].

[D.3.3] Feasibility of taking measurements for each optical probe parameter from a wound will be described as frequencies and percentages as described in [D.2] per fibre at each visit. This will be conducted in the ITT population as described in [E.1].

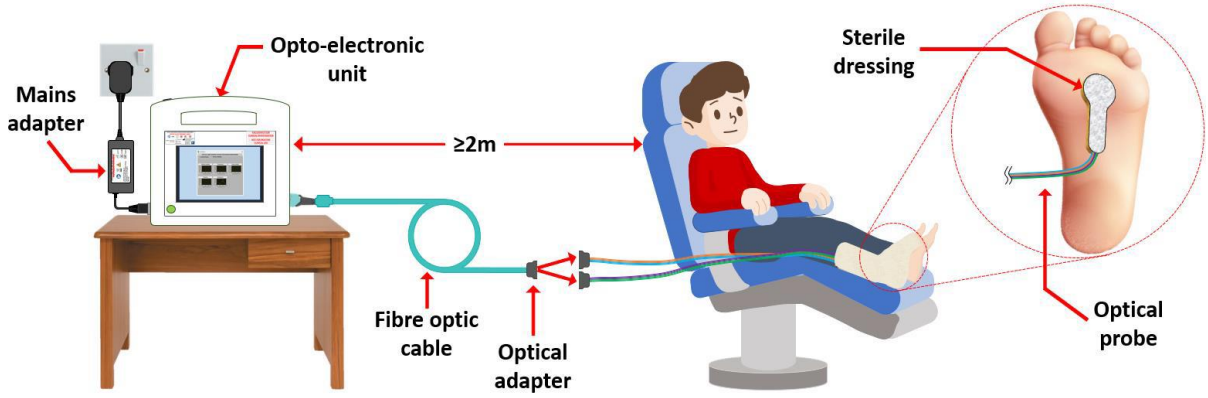
[D.3.4] The frequency and percentage of wound dressing which have measured parameters successfully will be presented at each visit for each fibre. This will be conducted in the ITT population as described in [E.1].

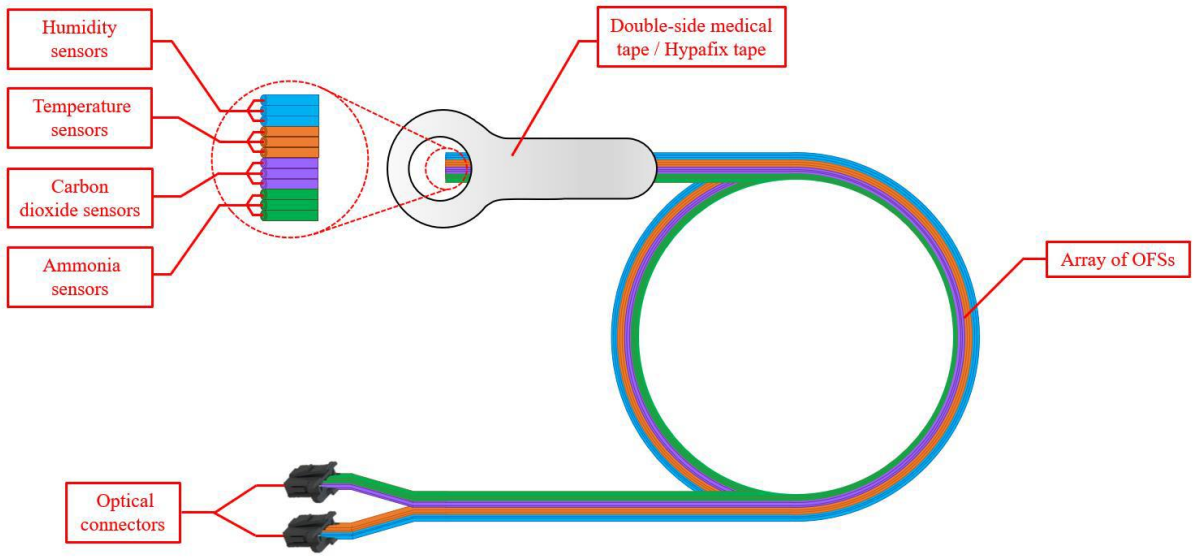
[D.4] **Definition of Secondary Endpoints** [Protocol Page. 26]

[D.4.1] ***Time series per parameter***: Quantitative sensor data will be recorded; the OFSSWM and the conventional devices will record 4 parameters (humidity, NH3, CO2, and temperature) that are potentially associated with wound healing. OFSSWM and conventional device measurements are taken at 4 visits (baseline and then every 2 weeks).

OFS SWM

The OFSSWM consists of 3 main parts namely: Opto-electronic unit; optical probe; and sterile dressing. The optical probe is based on an array of 12 optical fibre sensors (OFS). The OFSs are divided in 4 groups of 3 fibres each to monitor parameters such as carbon dioxide, relative humidity, temperature, and ammonia. The optical probe has 2 ports, each containing 2 OFS groups, as shown in the figure below.





The protocol followed to obtain measurements using the OFSSWM parts was the following:

Ambient measurements are taken using the relative humidity/temperature port from the optical probe for 10 minutes. One measurement is recorded per second cycling through each fibre in turn:

t=1 (second): Relative Humidity fibre 1 reading

t=2 (second): Relative Humidity fibre 2 reading

t=3 (second): Relative Humidity fibre 3 reading

t=4 (second): Temperature fibre 1 reading

t=5 (second): Temperature fibre 2 reading

t=6 (second): Temperature fibre 3 reading

t=7 (second): Relative Humidity fibre 1 reading

….

up until t=600 seconds (10 minutes)

Therefore, the measurements in ambient for each outcome will consist of three sets of readings (1 set per fibre) where each set is a time series of length with readings taken once every 6 seconds and offset by 1 second.

The Relative Humidity/Temperature port is then removed and replaced with the NH3/CO2 sensors attached for 10 mins. Similarly, each of the CO2/NH3 measurements also consist of a set of three time series of length 100, with each reading measured every 6 seconds and each fibre series offset in the same way as above.

After completion of measurements using the CO2/NH3 port from the optical probe in ambient, the optical probe is placed on the wound surface to start measurements from the wound. The same process as before is repeated, however, the initial wound measurements are obtained using the CO2/NH3 port. After 10 min this port is replaced with the relative humidity/temperature port to continue measurements from the wound surface.

Conventional

Conventional device measurements are taken after the OFSSWM measurements. All parameters are measured at the same time every second for up to 10 minutes. This is first completed in the ambient environment then repeated in the wound then healthy skin.

[D.4.2] ***OFSSWM device and conventional parameter measurements:*** For the OFSSWM measurements, at each visit, the average measure of each parameter will be taken for each fibre for each parameter (CO2, humidity and temperature). For example, for baseline measurements of CO2, there will be 3 fibres measuring CO2, firstly the average of each fibre will be taken, then the average of the 3 fibre averages will be taken for a patient.

For the conventional device measurements, at each visit, the average of each parameter will be taken for each parameter (CO2, humidity and temperature).

The averages taken above will be calculated for ambient and wound measurements and utilised to compare parameter measurements between OFSSWM device and conventional device at each visit.

[D.4.3] ***Wound size:*** This is measured as per normal clinical care with Silhouette wound assessment camera at each visit.

[D.4.4] ***Incidence of secondary infection***: This is defined as the proportion of patients who experience a secondary infection at each visit.

[D.4.5] ***Pain in the area of the ulcer:*** This is assessed by patient completed 100mm Visual Analogue Scale (VAS) at each visit.

[D.4.6] ***Wound healing status:*** Comparisons of all parameters between wound healing status (healer vs non-healer) at each visit for both OFSWM and conventional devices. The averages of the parameters as calculated as per [D.4.2] will be used for this analysis. A wound healer is defined as having 50% area reduction over 4 weeks (by visit 3) or completely healed by 8 weeks. This will only be completed for wound measurements.

[D.4.7] ***Infection status:*** Comparisons of all parameters between whether the wound is infected or not at each visit for both OFSSWM and conventional devices. The averages of the parameters as calculated as per [D.4.2] will be used for this analysis. Having a wound infection will be defined as having mild, moderate or severe infection at each timepoint (binary outcome). This will only be completed for wound measurements.

[D.5] **Statistical Methods for Secondary Analyses** [Protocol Page. 42]

[D.5.1] ***Time series per parameter***: For conventional device measurements, the time series will be presented graphically per parameter (CO2, humidity and temperature) at each visit using line graphs for ambient, wound and healthy skin separately. This will be repeated for the OFSSWM measurements except all 3 fibres for each parameter (CO2, humidity, NH3 and temperature) will be presented on the same graph.

[D.5.2] ***Comparison between OFSSWM device and conventional parameter measurements:*** All average values of each parameter as calculated in [D.4.2] across all visits will be pooled together. The mean (SD), median (IQR) of the average of each parameter for each visit will also be presented for both OFSSWM and conventional devices. The distribution of the average values of the parameters will be tested using the Skewness and Kurtosis tests.

If there is a normal distribution, the mean difference of the average values for each parameter between conventional and OFSSWM device will be calculated with the standard deviation and 95% CI. A paired T-Test will be conducted to test the mean difference with a 5% significance level. This will also be presented graphically using Bland-Altman plots.

If the average values of the parameters are not normally distributed, the median, interquartile range (IQR) will be presented. A Wilcoxon Signed-Rank Test will be conducted to test the mean difference with a 5% significance level.

The above will be done for the ambient and wound measurements.

[D.5.3] ***Wound size:*** This will be presented with means, standard deviation, medians, interquartile ranges.. This will be presented at each visit.

[D.5.4] ***Incidence of secondary infection***: The incidence of secondary infection will be presented as a percentage with a 95% CI at each visit.

[D.5.5] ***Pain in the area of the ulcer:*** This will be presented with means, standard deviation, medians, interquartile Ranges. This will be presented at each visit.

[D.5.6] ***Wound healing status:*** The mean, standard deviation, median, interquartile range will be presented for all parameters by wound healers and non-healers. This will be described for both OFSSWM and conventional devices at each visit. No statistical tests will be conducted. This will only be completed for wound measurements.

[D.5.7] ***Infection status:*** The mean, standard deviation, median, interquartile range will be presented for all parameters by whether wound is infected or not infected at each visit. This will be described for both OFSSWM and conventional devices. No statistical tests will be conducted. This will only be completed for wound measurements.

[D.6] Statistical Methods for Sub-group Analyses

[D.6.1] No sub-group analyses are planned.

[D.7] Statistical Methods for Sensitivity Analyses

[D.7.1] No sensitivity analyses are planned.

[D.8] Definition of Safety Endpoints [Protocol Page. 27]

[D.8.1] AEs, Serious Adverse Events (SAEs), Serious Adverse Device Effect (SADE) and unexpected adverse device effects will be reported during the study, including major and minor amputations and hospitalisation, results of physical examinations, vital signs (pulse rate, BP), and special investigations such as ECGs, clinical laboratory data and results of scans.

[D.9] Statistical Methods for Safety Endpoints

[D.9.1] Total number of any AEs, SAEs, SADEs and unexpected adverse device effects will be presented along with the number of patients who have at least one occurrence. All AEs, SAEs, SADEs and unexpected adverse device effects will be provided as a listing. This will be conducted in the Safety Set (SS) as described in [E.1].

[E] Analysis Groups and Missing Data

[E.1] Definition of Analysis Groups [Protocol Page. 43]

[E.1.1] Full Analysis Set (FAS): All participants who were eligible to take part in the study.

[E.1.2] Intention To Treat (ITT): All participants who have consented to take part in the study.

[E.1.3] Safety Set (SS): All participants who have attended at least one visit.

[E.2] Procedure for Accounting for Missing, Unused, and Spurious Data

[E.2.1] As this is a feasibility study and exploratory, no methods will be employed to handle any missing data. If a patient withdraws before all visits have been attended, then data up until the date of withdrawal will still be used in the final analysis.

[F] Unplanned Analyses

[F.1] Unplanned Analyses Requested by the CI

[F.1.1] None

[F.2] Unplanned Analyses Requested by the Sponsor

[F.2.1] None

[F.3] Unplanned Analyses Requested by the Journal Reviewer

[F.3.1] None

[G] Comments

[G.1.1] Data will be received in Excel spreadsheets.

[G.1.2] STATA will be utilised to conduct analyses in this SAP.