PROMETEUS

preterm brain-oxygenation and metabolic eu-sensing

D2.2 - Sensor biocompatibility results

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Version: F: final; D: draft; RD: revised draft	F
Date:	23.01.2024

European Innovation Council

Funded by the European Union

This project has received funding from the European Union's Horizon Europe research and innovation programme under grant agreement No 101099093

Grant Agreement	101099093
Acronym	Prometeus
Project full title	Preterm Brain-Oxygenation and Metabolic EU-Sensing: Feed the Brain

Deliverable	**D2.2**
Deliverable Name	**Sensor biocompatibility results**
Nature of deliverable	R: Document, report (excluding the periodic and final reports)
Dissemination level	PU (Public – fully open automatically posted online on the Project Results platforms)
Scheduled delivery date	31/01/2024
Actual delivery date	23/01/2024

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History of Changes

Revision	Date (dd/mm/yyyy)	Author	Changes	Status (Draft/In-review/ Submitted)
v 1	08/01/2024	Berta Ben Shachar, PhD		Draft
v 2	15.01.2024	Berta Ben Shachar, PhD	Page 3	Draft

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1. Cytotoxicity, irritability and sensitization study results for the sensor chip and patch components.

Disclaimer: The following tests were outsourced to a certified Testing Facility, as part of regulatory requirements for development of company's FirsQ Continuous Metabolic Monitoring (CMM) wearable device, and were sponsored by Qulab Medical. These tests are not part of the ethics approval requirements as submitted in deliverable D2.1 or any other deliverables planned for 'Preterm Brain-Oxygenation and Metabolic EU-Sensing: Feed the Brain – Prometeus' grant, and are shared in this report for informative purposes only. Any additional in-vitro biocompatibility tests that will be required and experiments that will be initiated within the scope of the PROMETEUS project will be pre-approved and supervised by Qulab Medical and their results will be reported/shared accordingly. All portions of this study performed adhered to the study protocol and local Standard Operating Procedures (SOPs), and they were conducted in compliance with the most recent version of the Good Laboratory Practice (GLP) regulation(s) and appropriate standard(s). Full reports will be provided upon request.

1.1. Irritation study in rabbits – report summary:

The test article was composed of disassembled parts of continuous metabolic monitoring device (CMM), including silicon chips glued to stainless steel, white patch of skin adhesive, double-sided sticker for the device, printed circuit boards (PCBs), plastic parts and electrical connectors. Lot No. is QU-001. Intracutaneous reactivity test in rabbits was carried out according to ISO 10993-10.

The test articles were aseptically sampled and extracted at an extraction ratio of 6cm^2/mL in saline and corn oil at (50±2) °C for (72±2) hours.

A total of 3 male New Zealand White rabbits of age approximately 4~5 months and weight 2.7 to 2.9 kg were administered with 0.2 mL doses of each test article extract into five separate sites on the left side of the back of each rabbit. Similarly, each control vehicle was injected on the right side of the back of each rabbit. The injection sites were observed immediately post-injection (within 5 mins) and at 24, 48 and 72 hours post injection and were scored at 24-, 48-, and 72-hour time points. Clinical symptoms except skin reactions were observed at each time point post injection.

The final test article score is 0 (Saline) and 0 (Corn Oil). No abnormal clinical symptoms except skin reactions were observed at all time points.

Under the conditions of this study, the difference between the test extracts and their respective control mean scores were less than 1.0. The test article met the requirement of the test, not causing any observable local dermal irritant effects.

1.2. Cytotoxicity – report summary

The objective of this assay was to evaluate the cytotoxic potential of the test article towards a specified mammalian cell line Balb/c 3T3 cells (abbreviated as 3T3) when exposed to the extract of the test article.

The test article was extracted in DMEM extraction medium for 24 hours in an incubator adjusted to 37±1°C, with a ratio of 6 cm^2/mL. The negative control article was extracted using the ratio of 3 cm^2/mL at the same conditions. The positive control article was extracted using the ratio of 6 cm^2/mL at the same conditions.

The HDPE and test article were tested neat (100%) and at 56.2%, 31.6%, 17.7%, 10.0%, 5.6%, 3.1% and 1.8% dilutions. The positive control (ZDBC) was tested at neat (100%) and at 68.0%, 34.0%, 32.0%, 30.0%, 28.0%, 14.0% and 7.0% dilutions. The SLS positive control was tested neat (150 µg/mL) and at 56.2 %, 32.0%, 30.0%, 28.0%, 24.0%, 12.0% and 6% dilutions. Each sample was treated with a cell culture plate.

The OD_{540} of vehicle controls were all ≥0.3. The %diff of the left (2nd column) and the right (11th column) mean OD_{540} of the vehicle controls was within 15% from the mean of all controls. The viability of the top dose of negative control (neat extracts) was 95.0%, higher than 70%. The viability of positive control (ZDBC) ranged from 0.2% to 102.4%, and there were four test concentrations (28.0%, 30.0%, 32.0% and 34.0%) ranging from 10% to 90%, the viability of the highest concentration of positive control (neat extracts) was 0.4%, less than 70%. Calculated IC_{50} values based on above data, IC_{50} for ZDBC being 31.7%, met the criterion. The viability of positive control (SLS) ranged from 1.4% to 95.9%, and there were five test concentrations (12.0%, 24.0%, 28.0%, 30.0% and 32.0%) ranging from 10% to 90%, the viability of the highest concentration of positive control (150 µg/mL) was 1.8%, less than 70%. Calculated IC_{50} based on above data, IC_{50} for SLS being 33.8% (equal to µg/mL), met the criterion.

The results showed that the %viability of the top dose of test article (100%) was 94.8%, higher than 70%, showing non-cytotoxicity.

1.3. Sensitization – report summary

The test article was composed of disassembled parts of a continuous metabolic monitoring device (CMM), including silicon chips glued to stainless steel support, white patch skin adhesive, double-sided sticker for the device, printed circuit boards (PCBs), plastic parts and electrical connectors. Lot No. is QU-001. Test article was evaluated for its potential sensitization capacity in a guinea pig sensitization maximization test. The study was conducted in accordance with ISO 10993-10.

The test article was sampled under aseptic conditions and extracted with the concurrent extraction vehicle at an extraction ratio of 6 cm^2/mL at 50±2 °C for 72±2 hours. The extraction vehicles were saline and corn oil.

A total of 34 guinea pigs, approximately 4~5 weeks old and weighing 345.05 ~ 450.39 g at initiation of dosing, were arbitrarily assigned to 4 groups (Negative control: 6/group; Test: 11/group).

All animals were intradermally and topically inducted, and then received a challenge patch. Each challenge site was scored at approximately 24±2 h and 48±2 h following challenge. The observation was conducted daily during the in-life phase on all animals. The body weight was measured prior to the first dosing and on the day of termination for all animals.

The dermal reactions following challenge showed:

The positive sensitization rate to all control animals was 0%.

The positive sensitization rate to all test animals was 0%.

No test article clinical abnormalities were observed, and no abnormal body weight change were detected in animals throughout the study.

Under the conditions of this study, the saline and corn oil test article extracts did not show evidence of causing any delayed dermal contact sensitization response in the guinea pigs. The test article was not considered a sensitizer in the guinea pig sensitization maximization test.

2. 14-day dummy- CMM patch implantation study in pig

Aims:

1. Evaluation of the efficacy and safety of FirstQ applicator mechanism.
2. Evaluating dermal irritation and subdermal effects (inflammatory and/or fibrosis response) following Qulab's FirstQ patch implantation in pig's skin for 24 hours and 14 days, as compared to the effect of a dummy patch (without μProbes), Freestyle Libre CGM and a pristine skin control.
3. Evaluating skin recovery following 14 days of patch wear, following for 1 to 2 days post removal.

Experimental Design:

- Single, young (10-15kg) domestic pig.
- Number of applicators -3
    - μProbes: Stainless steel supports 1.5mm
- Number of dummy patches (without μProbs)- 2 – Negative control
- Number of commercial CGMs- single FreeStyle Libre – positive control

Results

Observation of the pig wearing the sensors throughout the entire 14-days of wear did not reveal any inconvenience or unusual behavior. To secure the sensors from falling off, due to extremely active behavior of the pig, additional tape (brown) was mounted on top of the implanted sensors (Fig.1 – day 1).

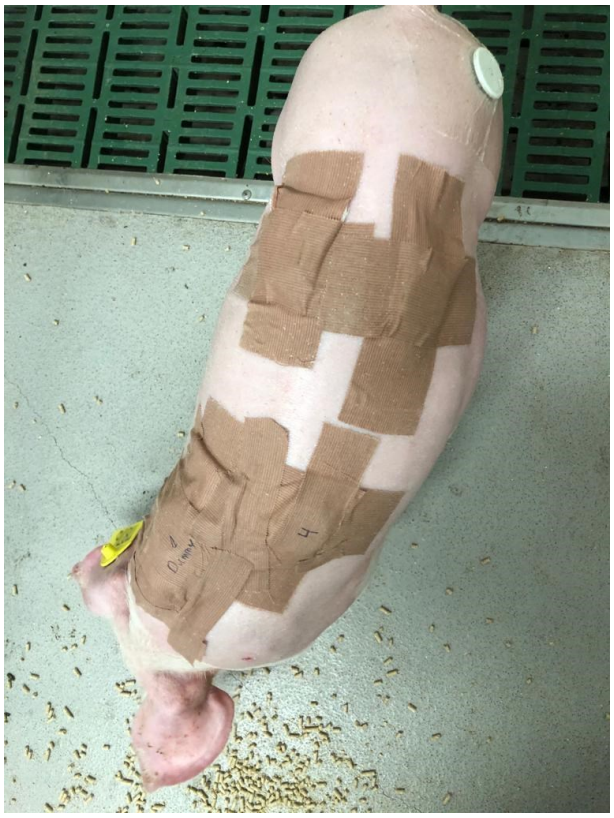


Fig. 1 Pig at day-1after sensors mounting (Fig. 1)

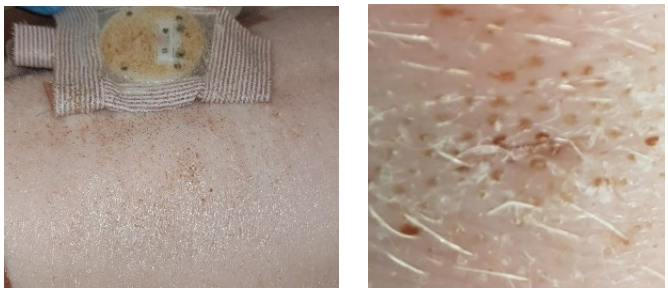


Fig. 2 Pig's skin after FirstQ patch removal upon 14 days of wear

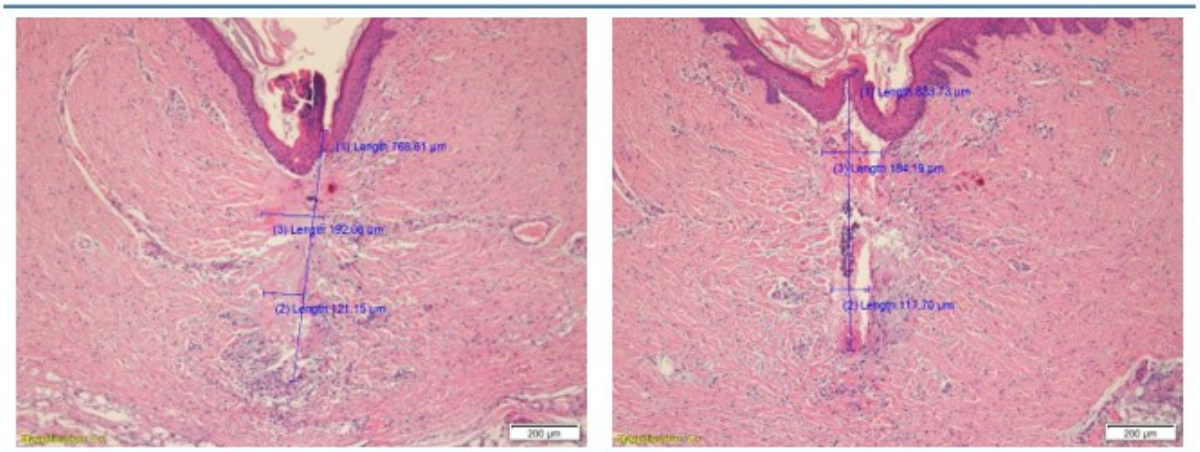


Figure 1: Sample# 1-1, Very clean needle canal with minimal inflammatory reaction. X4, H&E.

Figure 2: Sample# 1-2, Very clean needle canal with minimal inflammatory reaction. X4, H&E.

Fig.3 Histological analysis of skin biopsies taken 1-day upon Sample#1 (patch #2) removal, following 14-days wear

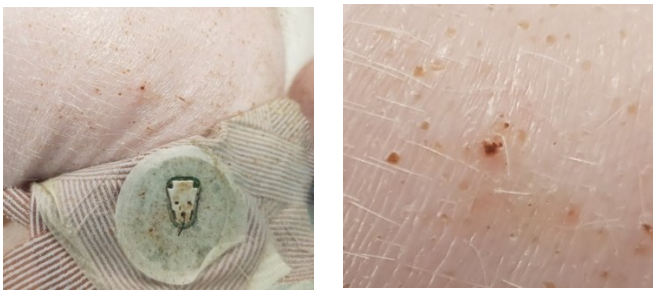


Fig. 4 Pig's skin after Freestyle Libre CGM removal following 14 days of wear

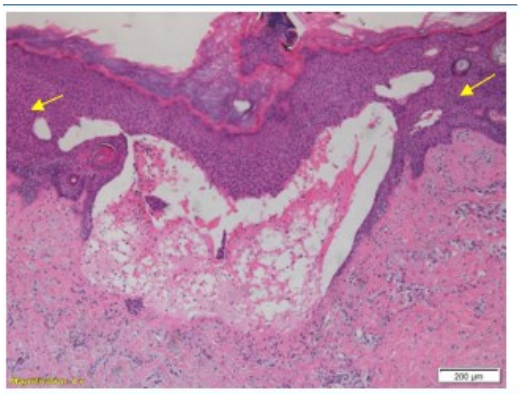


Figure 4: Sample# 4-1, Very strong proliferation of the epidermis (arrow) and only partial canal. X4, H&E.

Fig. 5 Histological analysis of skin biopsies after 14-days wear of Freestyle Libre commercial CGM, taken 1-day upon its removal

Histological analysis of a biopsy from one representative patch, taken after 14-days continuous wear, presented a clean 0.8mm needle canal with no observable inflammatory reaction (Fig. 3). These results support the 0-edema and 0-erythema scored by topical observation of the pig's skin (Fig. 2). Although topical observation of pig's skin area around the FreeStyle CGM scored 0-erythma and 0- edema (Fig. 4), the histological analysis revealed extensive epidermal proliferation which correlated with only partial canal resolved from the 5mm sensor filament (Fig. 5, note: the insertion of the sensing filament is driven by 7mm stainless needle which instantly retracts back to the applicator, leaving only a 5mm flexible filament within the dermis).

3. ETO-Sterilization and batch release: Pig skin penetration results, showing overall device safety.

Aims:

3.1. Mechanical and safety *in-vivo* (pig model) validation for ETO-sterilization and batch release protocol of FirstQ Sensor Patch (CMM) and mechanical applicator prototypes, specifically designed for clinical studies.

3.2. Evaluation of FirstQ applicator mechanism efficiency and FirstQ sensor patch safety in terms of (1) microprobes' insertion depth and (2) confirming microprobes' integrity upon patch removal.

3.3. Evaluating dermal irritation and subdermal effects (inflammatory and/or fibrotic response) following FirstQ patch implantation and 4-hours wear, in pig's skin.

Experimental Design:

- Single (87kg) domestic female pig was used.
- Number of Mechanical Applicators employed for mounting: two
- Number of FirstQ Patches mounted - 8 at different locations on a back
    - μProbes: silicon sensors on stainless steel supports (length:1.5mm)
    - Silicon μProbes without sensing elements (enzyme, electronics, Bluetooth signal transmitter)

Study KPIs:

(1) Validating prototype safety - all silicon microprobes should be successfully recovered unbroken from the tissue. Successful prototype safety validation will be achieved if all microprobes appear unbroken upon microscopic inspection after their detachment from the skin, we answer 'no' to question # 3 "Silicon is broken and part of it is detached from the metal support and is missing, raising suspicion that silicon remains left in the tissue. yes/no."

(2) At least 70% of the samples will present an average penetration depth higher or equal to 800µm.

(3) Establish post implantation analysis of the microprobes to evaluate their penetration depth based on intradermal tissue traces

Results:

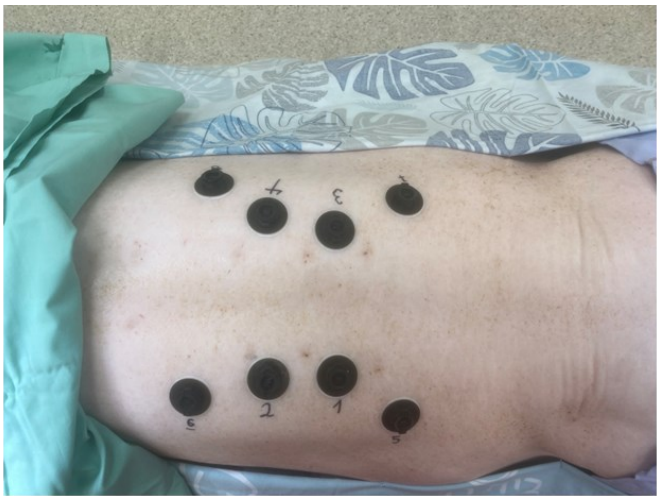
a. b.

Fig. 6 Assembly, sterilization and packing lot release for First In Men (FIM) studies and *in-vivo* validation in pig: a. FirstQ Patch and b. FirstQ Applicator

ETO sterilization, and single batch release of mechanical applicator and sensor CMM-patch was successfully completed (Fig. 6). Six-month shelf life was established for this batch to allow for completion of the FIM safety study. Microbiology, sterilization validation and shelf-life reports are available upon request.

Mechanical and safety validation of the patch applicator and FirstQ patch were further established *in-vivo* in a pig model. As depicted below (Fig. 7), eight FirstQ patches (carrying microprobes w/o sensing elements) were mounted using FirstQ applicator, followed by 2-hour skin implantation. At the end of the 2-hour wear period, patches were carefully removed and skin biopsies at the

microprobes' penetration sites were taken for histological analysis (Fig 8.). In parallel to histological analysis, all recovered microprobes were subjected to microscopic inspection to verify 1) microprobe integrity and 2) microprobe penetration depth marks in the form of tissue and interstitial fluid residues on their surface (Fig. 9).

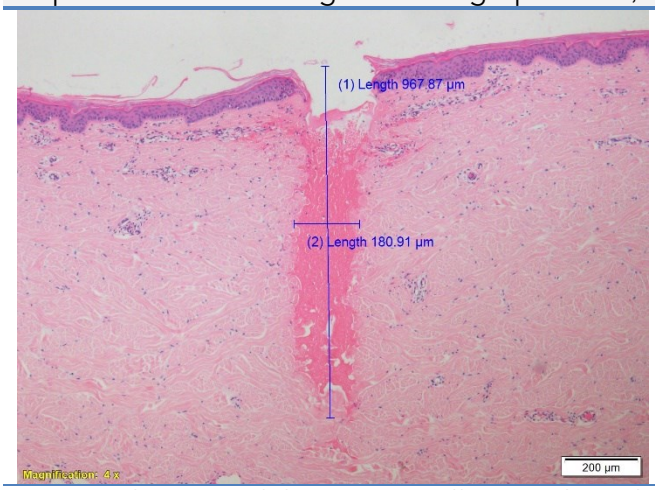


a: Patches mounted on pig at different body locations

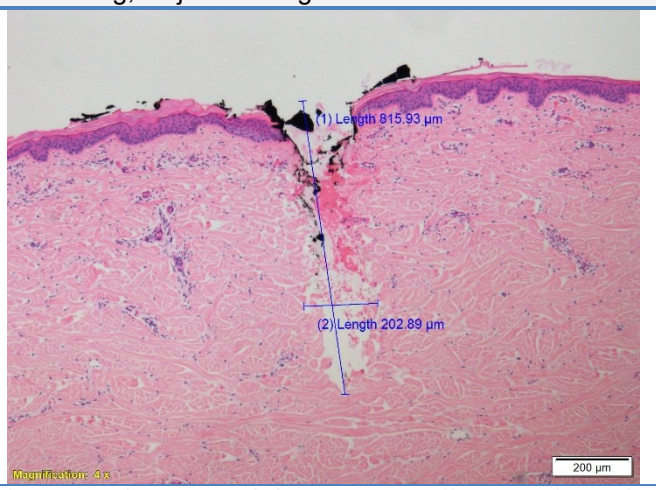
b: Patch #4, microprobes penetration marks on pig skin upon patch removal

Fig. 7 a.FirstQ sensor patch mounted on pig skin at different locations, b. representative sensor patch #4 before and after its removal on pig's skin

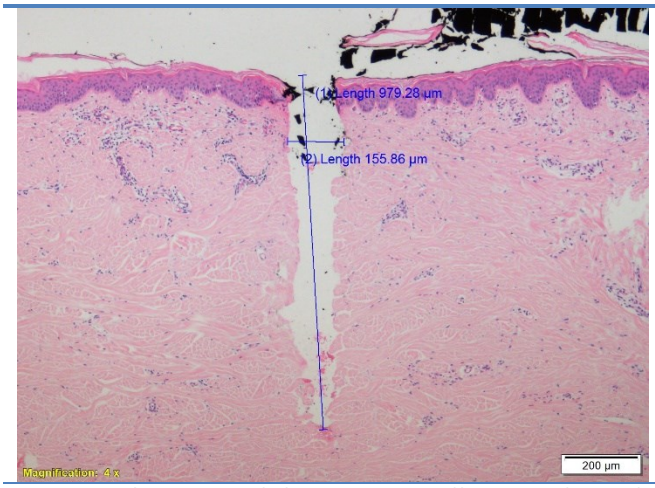
Representative Histological Photographs: Skin; H&E staining; Objective magnification X4



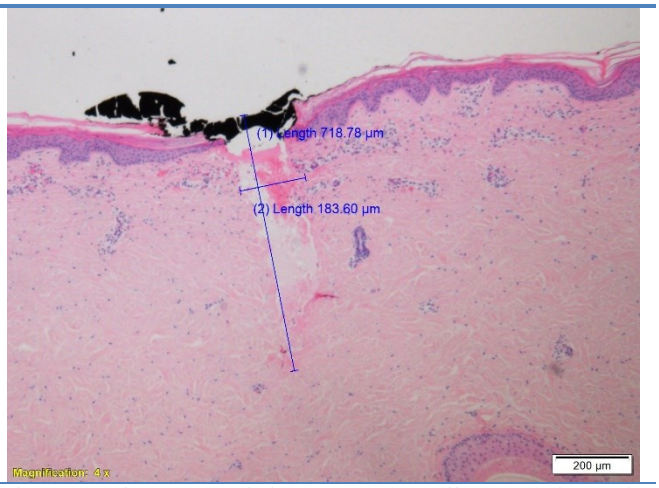
a: Sample #1, slide #2, needle canal with measurements. Note that there is no ink here X4, H&E.



b: Sample #2, slide #1, needle canal with measurements. X4, H&E.



c: Sample #4, slide #3, needle canal with measurements. X4, H&E.



d: Sample #5, slide #18, needle canal with measurements. X4, H&E.

Fig. 8 Photographs from Histological analysis of skin biopsies taken from four representative CMM sensors mounted on pig skin.

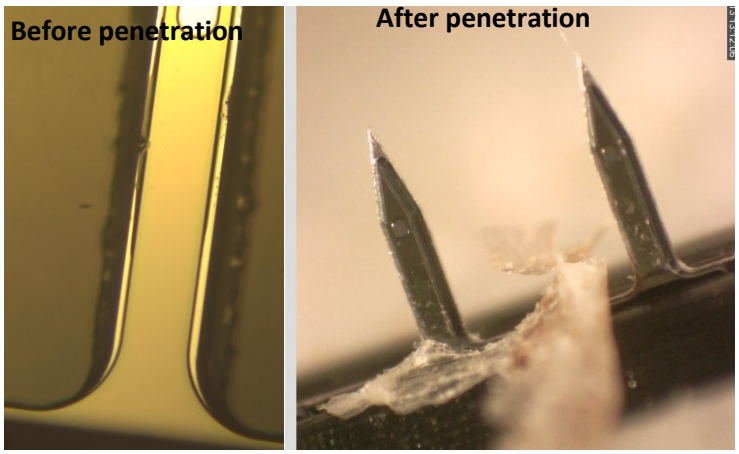


Figure 9: Representative microprobe patch after safely inserted and removed from pig skin, leaving visible tissue residues (x20)

Visual microprobe sensor inspection post skin removal, has indicated 70-100% penetration of all microprobes into the skin tissue. No damage to all silicon microprobes and their stainless supports was recorded. Histological analysis correlates with the visual observation of the microprobes' penetration depth, with >0.8mm measured dermis canal.

All of the KPIs for this study were successfully achieved, and overall safety of the FirstQ CMM sensor has been confirmed, allowing its further compliance for clinical studies in humans.