

A FIXED SKIN BIOPSY PROTOCOL FOR SINGLE-CELL RNA SEQUENCING ANALYSIS OF LARGE SAMPLES

Background

We are part of a large research consortium (SEAWave) that aims to provide a comprehensive study of the impact of 5G on health. We are leading the work package studying the effects of 5G FR2, the next-gen 5G frequencies, on humans, while other groups will perform studies on cells and animals. In this study, we have volunteers with:

- 1, healthy skin
 - 2, inflamed skin
 - 3, cancer-prone skin
 - 4, dermatoporosis skin
- Those participants are exposed to precise doses of 5G generating 168 skin samples for scRNA seq.

2, FIXATION

Always keep the skin samples at low temperature, place the skin immediately into the freshly prepared fixation buffer on a pre-chilled glass Petri dish on ice.



Using two disposable scalpels, start chopping the skin into very small pieces in a crisscross motion in the fixation buffer.

Incubation time is crucial. After a few tests, we found that the optimal incubation time for fixation is between 19-20 hours.

5, PURIFICATION

FACS Fluorescence-Activated Cell Sorting can remove all the debris created in the previous process and keep all cell types for scRNA-seq analysis.

4, DISSOCIATION

4,2 Washing Wash the skin tissue on the cell strainer at least three times to dissociate and recover the maximal number of cells as shown in the following figures.

3, DIGESTION

Add 10 ml of Liberase TL solution to the samples in a 50 ml tube to increase O2. Incubate in a 37°C water bath for 1 hour, then store at 4°C overnight.

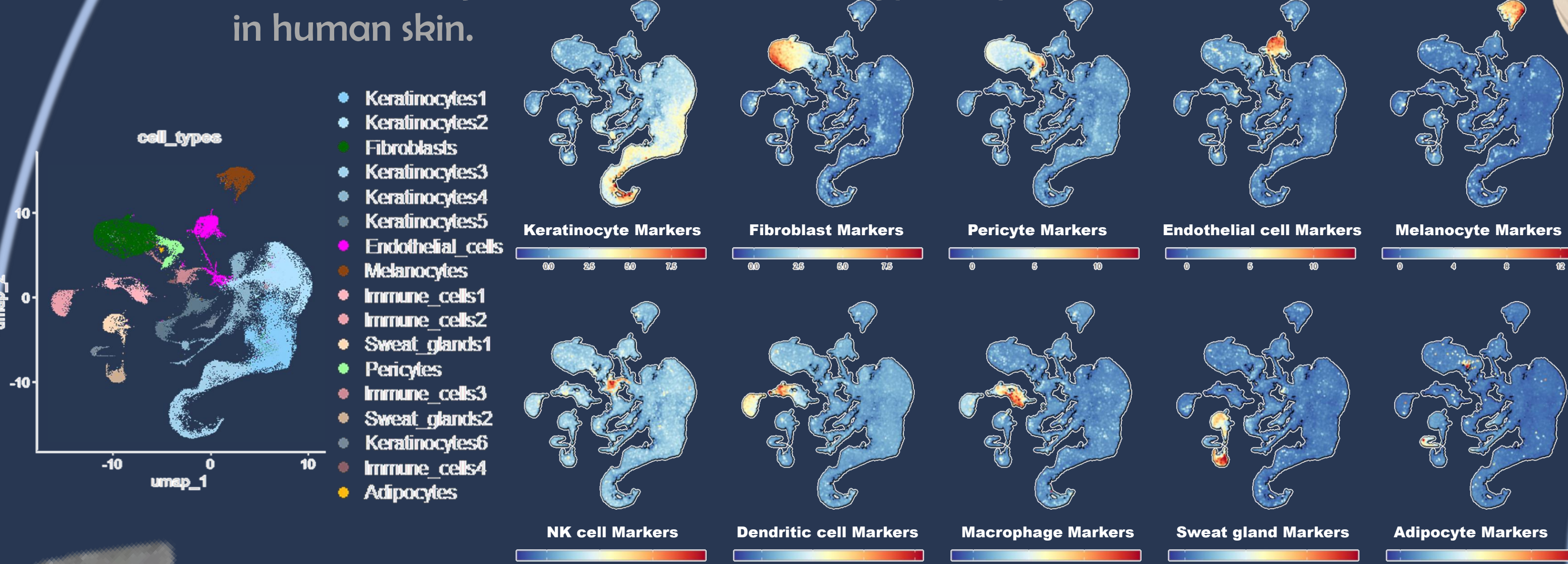
4,1 Mechanical Force

Place a 70 µm cell strainer on a 50 ml tube and pour the cells over it. Use the back of a 1 ml syringe plunger to push undissociated tissue through the strainer to maximize cell collection.

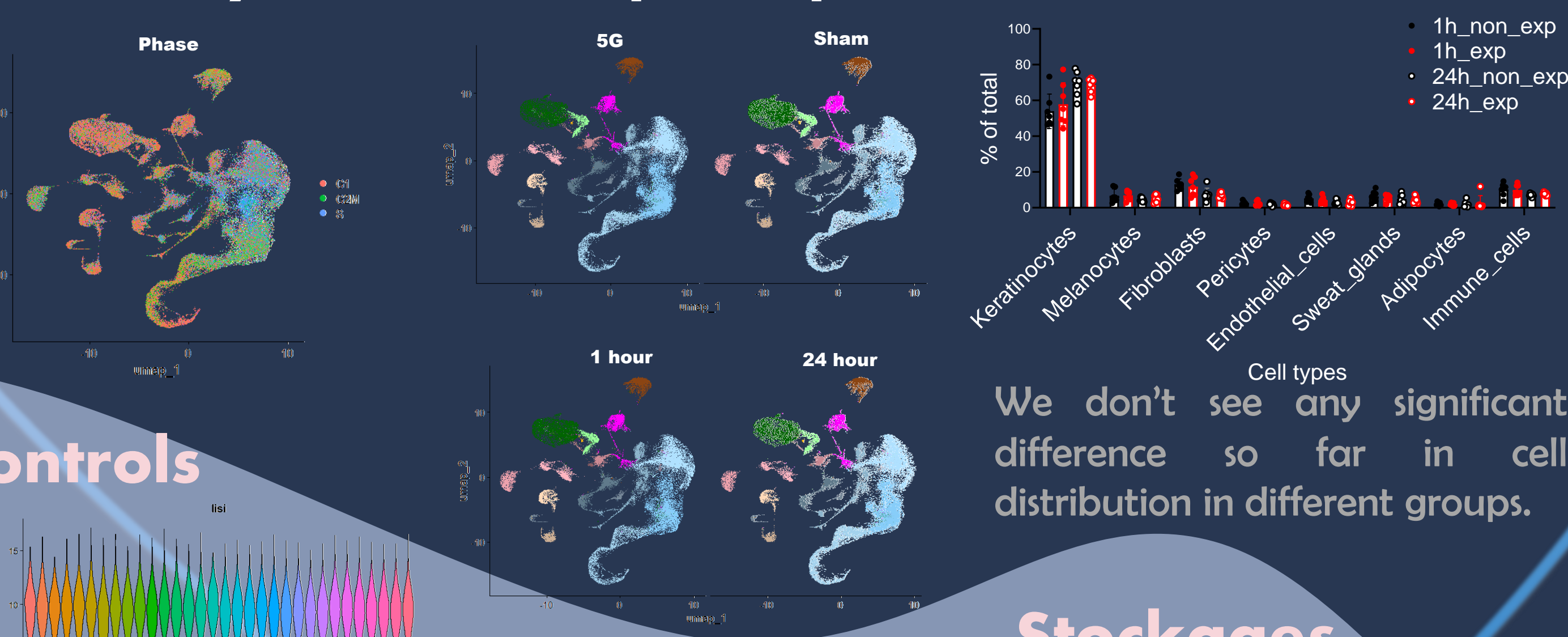
RESULT

1, Cell Type Determination

We successfully to identify all the cell types expected in human skin.



2, Cell Cycle and Group Comparison



Our Objectives?

Our study examines whether 5G induces slight genetic signaling changes. Traditional scRNA-seq methods are insufficient for large batches due to the need for high reproducibility and minimal batch effects.

We have developed an innovative protocol for large-scale analysis of fixed skin samples using scRNA-seq. This unbiased and sensitive method will analyze 168 samples, making it ideal for studying 5G effects at a single-cell level.

1, PUNCH EXCISION

Exposition system emit 5G waves on each arm in a very localized manner, with a double-blind setup. Skin biopsies, 7.5 mm in size, are taken 1 hour and 24 hours after exposure, under local anesthesia. The biopsies are swiftly placed in a cooled medium.

Tests reveal that No. 15 disposable scalpels are optimal for turning skin into foam.



400%

SEQUENCE UP TO 40 SAMPLES. REDUCE BIAS.

SAFE

HIDE PATIENT PERSONAL GENETIC DATA AND KEEP SAFETY.

SPECIFIC

THE PROBES INCREASE SPECIFICITY, SENSITIVITY, REDUCE BACKGROUND NOISES.

Advantages

the 10x Chromium Fixed RNA Profiling differs to the classic sc-RNA seq and has a lot of advantages.

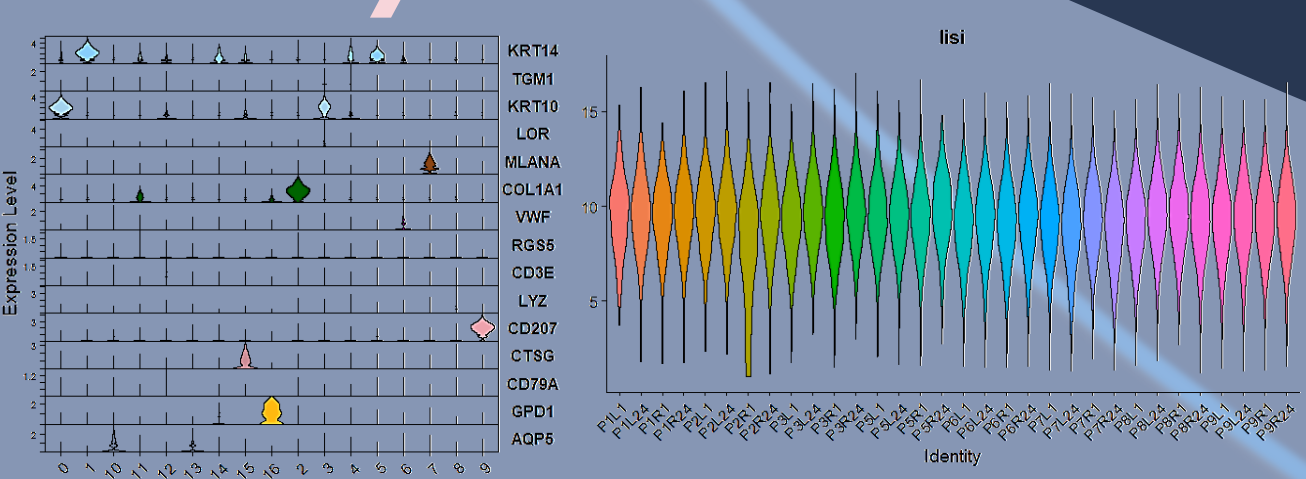
DISCUSSION

Our data show that fixed-skin can be used for single-cell RNA analysis.

Our protocol increases reproducibility, at the acceptable cost of the number of live cells and read per cells, when compared to a fresh tissue protocol. UMI (unique molecule amplifier) numbers, which is a more important readout than read per cells, is within the range or UMIs for other published fixed-tissues such as liver, kidney and brain.

Further optimization is ongoing, with the hope to provide a solution that increases UMIs.

Quality Controls



10x Sequencing Protocol



Stockages

Fixed samples can be stored for: Short Term Storage

4°C Quenching buffer
Enhancer (0,1 Volume)
50% Glycerol (0,1 Volume)

Long Term Storage

-80°C Quenching buffer
Enhancer (0,1 Volume)

Addition order:

- 2, Enhancer heated at 65 °C
- 3, 50% Glycerol

1, Quenching buffer



Founded by the European Union

SEAWave

CHUV Centre hospitalier universitaire vaudois

Zhouxing Su1, Christine Pich-Bavastro1, Olivier Gaide1
1 Lausanne University Hospital, University of Lausanne, Lausanne, Switzerland