NOVEL METHOD FOR MEASURING THE HEALING PROCESS IN CELL CULTURES

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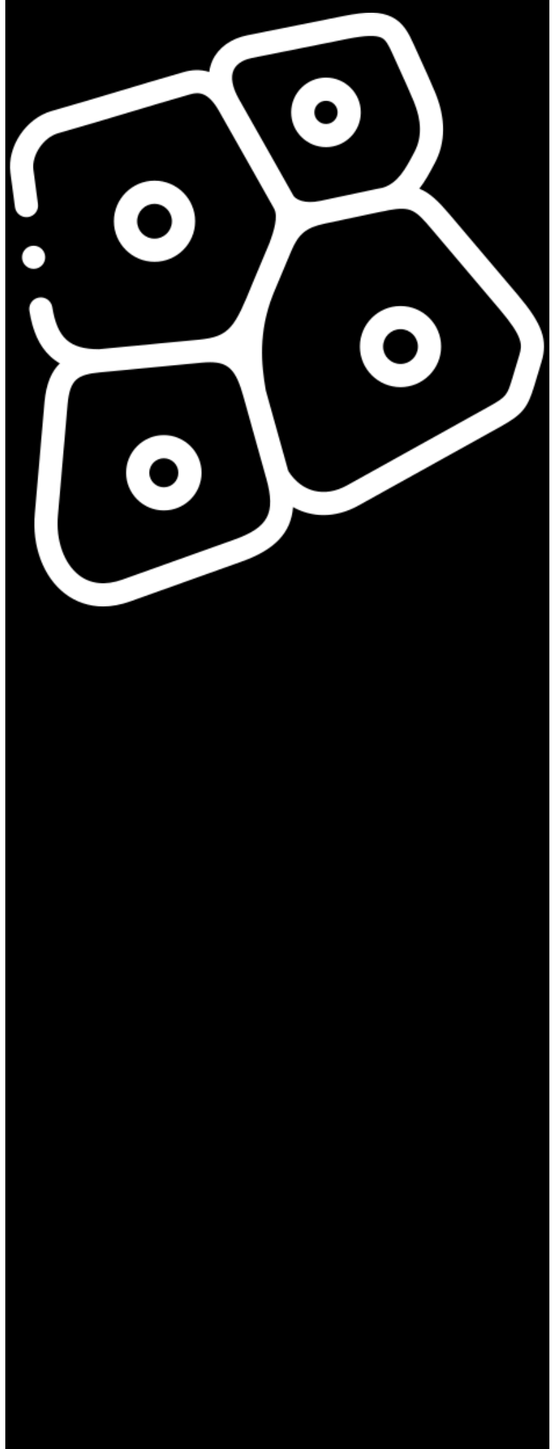
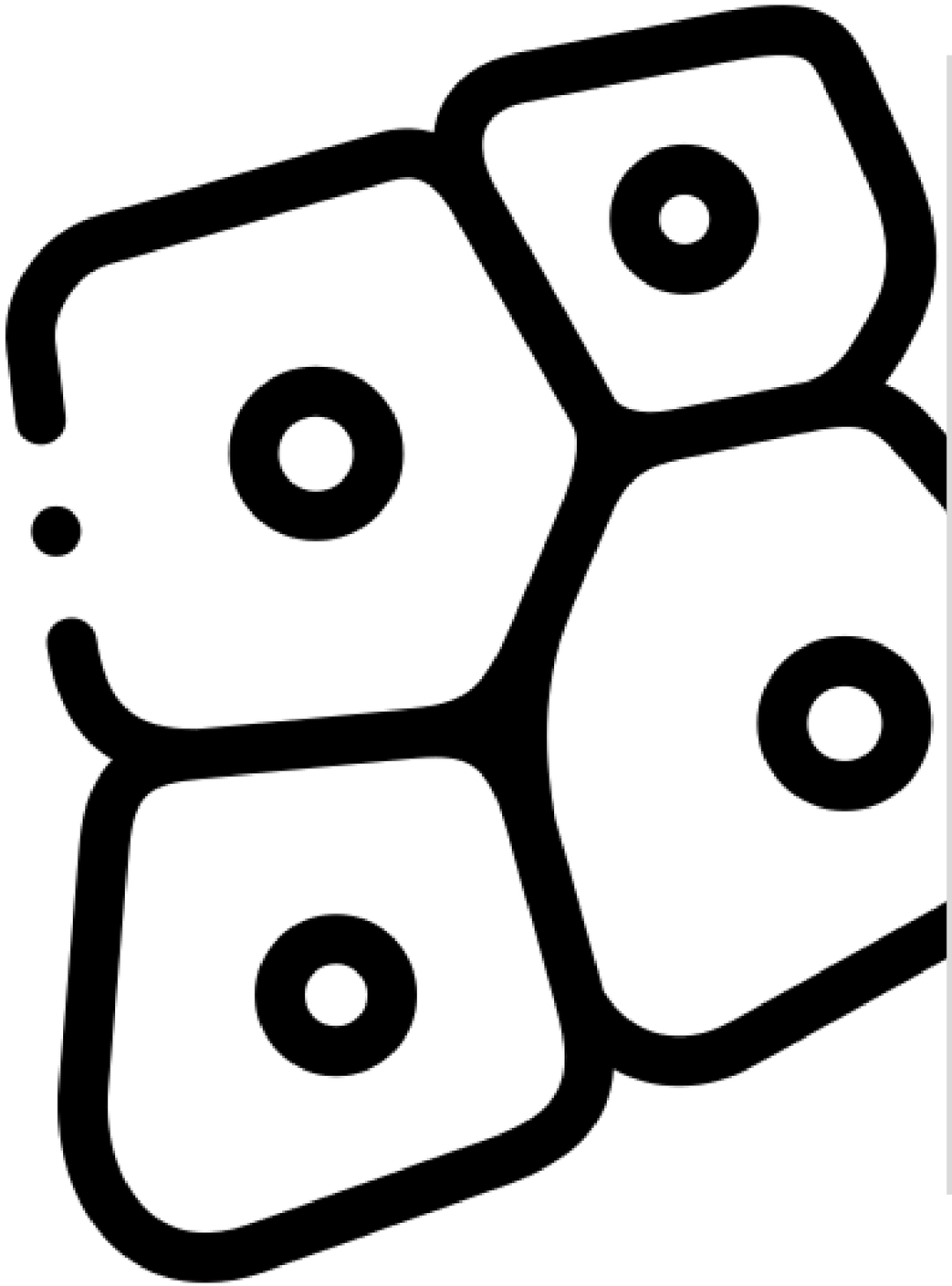
Objetives

- Present a healing prediction and estimation method based on ECIS technique and Mesoscopic model for wound-healing assays.
- Apply the method to electrical wound-healing assays performed with MDCK type II cells.
- Compare results of method and validate it using optical microscopy to monitor of the healing process [*in process*].



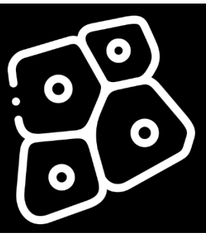


Wound-healing assays

(WHA)

- Adherent cells cultured on biocompatible substrates multiply, migrate and spread to cover available surface, until reaching confluence. Cells form adherent contacts with surface and intercellular junctions, composing a cell monolayer.
- Cell monolayer can be easily broken. Surface region of a wound is free of cells. During healing process, cells migrate and reproduce from edge towards available surface.
- In WHA, cell monolayer is deliberately wounded, and healing process is monitored.
- Cell migration and proliferation mechanisms can be studied with WHA, relevant to understand biological processes such as metastasis, tissue repair and embryogenesis.

Wound-healing assays



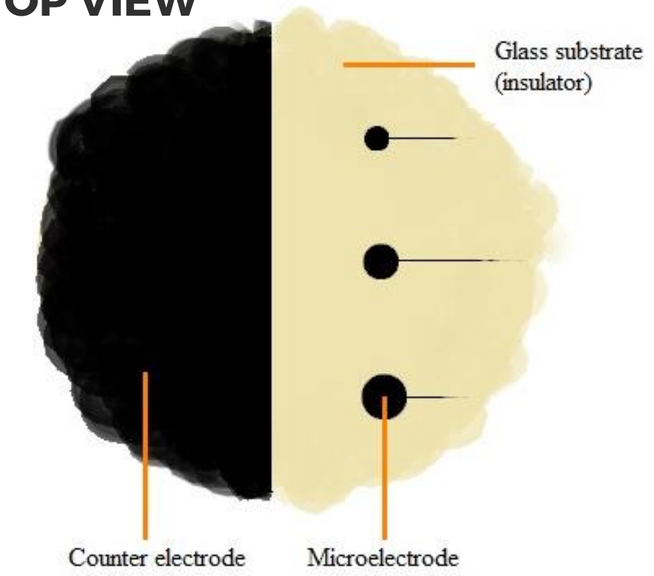
	MECHANICAL	ELECTRICAL
SOME DETAILS...	Most popular type of assay.	More recent*, based on Electric Cell-substrate Impedance Sensing (ECIS) technique. Arises as an alternative or complementary method to monitor healing. *[C. Keese et al, (2004). *PNAS* 101, 1554 (2004)]
WOUND PRODUCTION	Mechanically, i.e. by scraping off a portion of cell monolayer.	Electrically, exciting system for a few seconds with electric alternating current of high amplitude and frequency, which produces severe electroporation in cells above microelectrode and, therefore, their death.
HEALING MONITORING	Optically, by time-lapse microscopy.	Electrically, non-intrusively, by measuring time evolution of electrical impedance.
PROS & CONS	**Limited by precision that can be achieved in mechanical wounding, cannot plan its size.** Estimation of healing speed affected by image taking and processing methods. **Microphotographs taken** out of controlled atmosphere (**out** of optimal environment)	**Wound area is equal to microscope area.** We developed a non-intrusive method for predicting and estimating healing as function of time.

Electric Cell-substrate Impedance Sensing

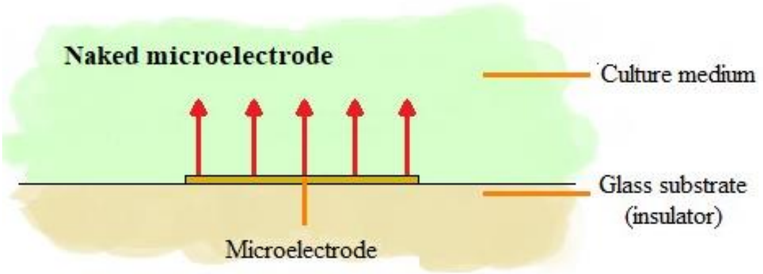
(ECIS)

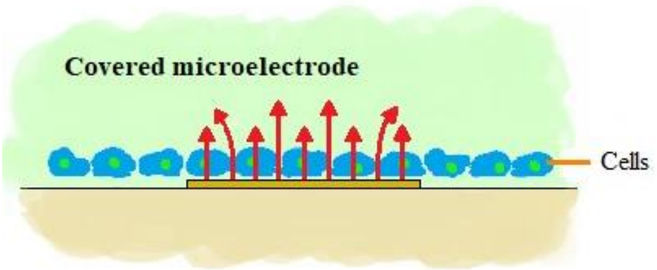
- The system is excited with alternating current, and the impedances of the same naked and cell-covered biocompatible microelectrode are measured for a range of frequencies.
- Allows to quantify morphological and functional properties of cells, fitting models to impedance measurements.

TOP VIEW



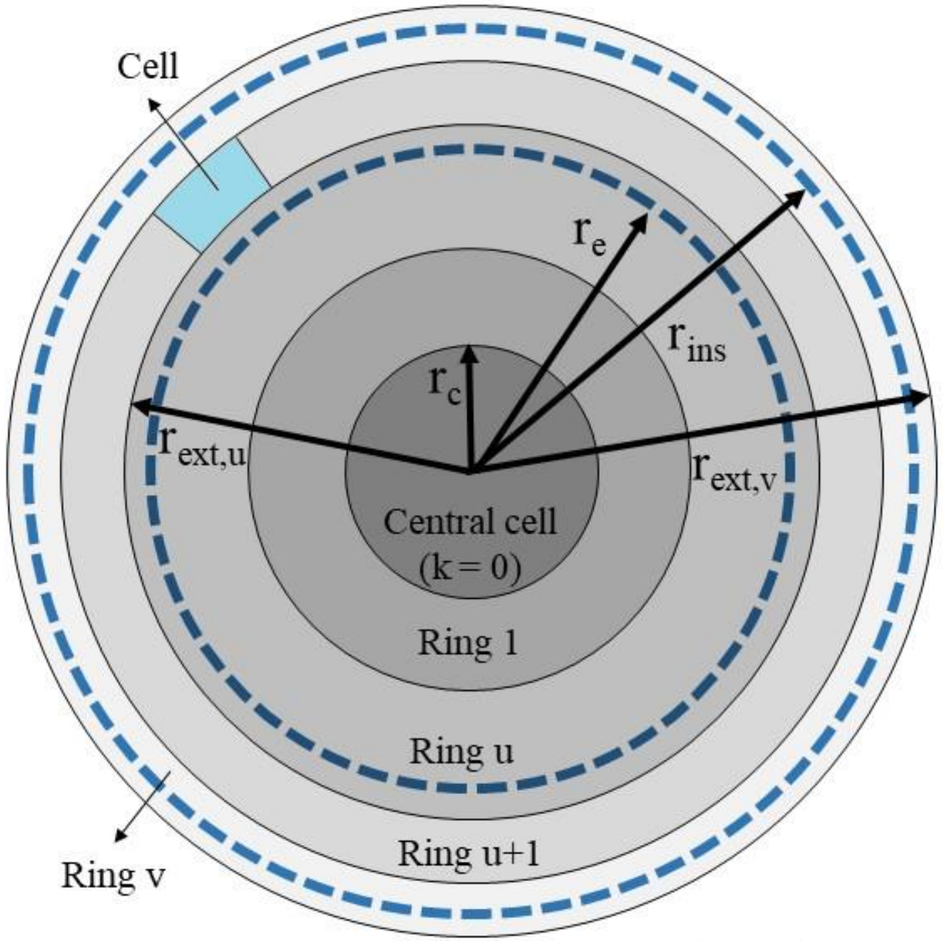
SIDE VIEW





Mesoscopic Model
(MM)

TOP VIEW



--- Limit of microelectrode

--- Limit of insulator

- Describes electrical response of a monolayer formed by a central cell surrounded by cell rings, electrically coupled to each other, covering a finite microelectrode and a finite insulator.
- First in allowing local properties modeling.
- Geometry compatible with WHA.

[A.C. Buchini Labayen et al. (2022). *PRE* 105(4), 044401]

Work flow

CELL CULTURE 1

Culturing MDCK type II cells on biocompatible microelectrode surface

Measuring impedance of the covered microlectrode

WOUNDING 2

Exciting system for a few seconds with electric alternating current of high amplitude and frequency

HEALING MONITORING 3

as in electrical assays

Monitoring electrical impedance over time

as in mechanical assays

Taking microphotographs

Doing time-lapse microscopy

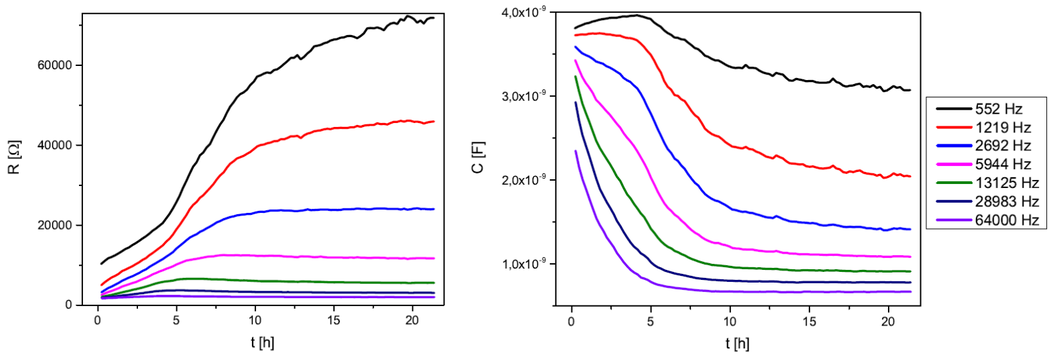
DATA ANALYSIS 4

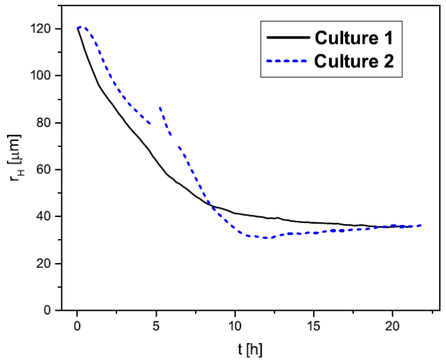
1. Fitting MM to experimental data
2. Calculating impedance when all rings are dead and then reviving one by one
3. Creating a curve of impedance (R and C) vs healing radius, and making a polynomial fit of them
4. Fitting polynomial functions to experimental data of healing process, to obtain healing radius in function of time

1. Detecting microelectrode and wound in each image, using an algorithm that splits color channels, selects green (better contrast), makes binary masks for contouring each element and applies filters to enhance results
2. Calculating quantity of pixels of each segment, and percentage of microelectrode surface of wound
3. Calculating healing radius in function of time from a time-lapse

Results

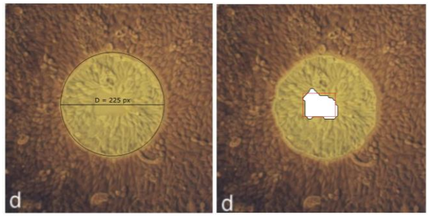
ELECTRICAL MONITORING OF HEALING





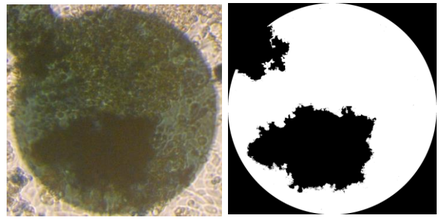
- Different cultures show similar healing rates under similar conditions, but speed of healing depends on growing conditions.
- Healing is not complete, A central area of microelectrode remains without healing (~ 8% of the total area of the microelectrode), also observed in microphotographs.

OPTICAL MONITORING OF HEALING



Images of healed culture taken from reference*

Wounded remaining area occupies ~ 7% of the total area of the microelectrode.

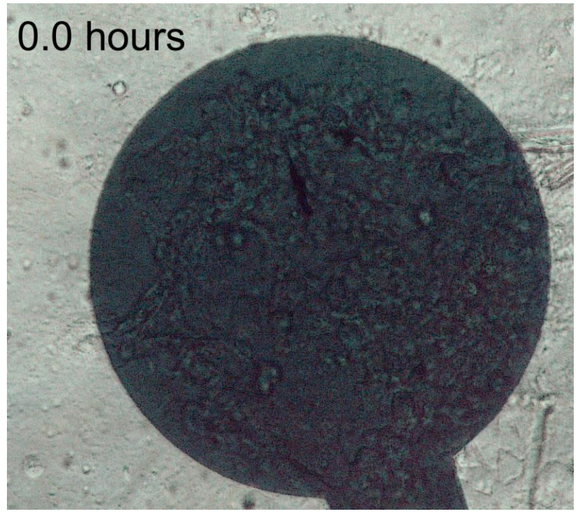


First image of healed culture taken in lab

Taken with inverted microscope and camera, out of controlled atmosphere

Wounded remaining area bigger than expected (~ 20% of the total area of the microelectrode).

Current image set taken in lab



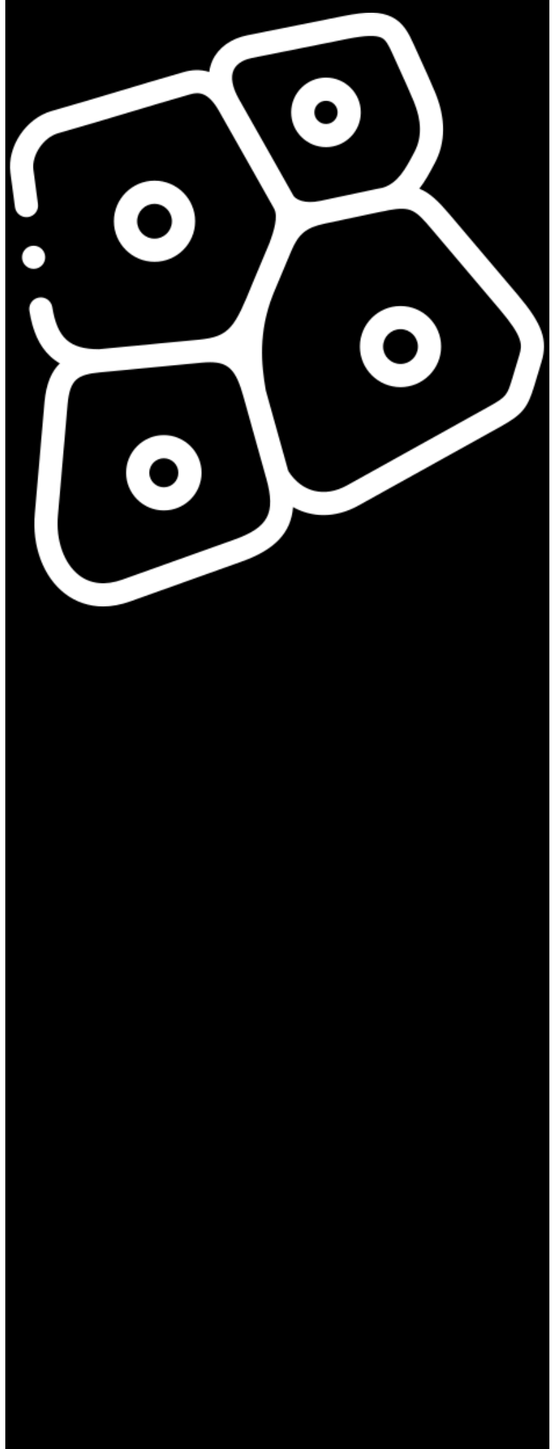
in uncontrolled atmosphere

- Taken using new inverted microscope with incorporated camera, out of controlled atmosphere (out of optimal environment).
- Algorithm developed to process microphotographs. →
- Challenges in doing time-lapse of complete healing process, and to simultaneously monitor it electrically and optically under similar conditions.

*[C. Keese et al, (2004). *PNAS* 101, 1554 (2004)]

Conclusions

- Developed a method based on ECIS and Mesoscopic model to predict healing radius as a function of time in WHA, and to estimate the speed of the healing process in cell cultures.
- Being validated using a tradicional technique of monitoring healing process (optical microscopy), promising partial results.



Thank you!

(Gracias! Obrigada!)

Questions?

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