ISIS@MACH ITALIA

Science and Technology Facilities Council
ISIS Neutron and Muon Source

Experiment Proposal

Experiment number GP2023030

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Experiment title	Confocal based analysis of mechanosensation in an ex vivo model of re-innervated human skin
MRF Instrument	**Confocal Microscope 2**
Access Route	Direct Access
Science Areas	Biology and Bio-materials
Sponsored Grant	None
Grant Title	-
Start Date	-
Similar Submission?	-
Industrial Links	Arterra Bioscience Spa

Days requested: 4
Previous GP Number: -
DOI: -
Sponsor: -
Grant Number: -
Finish Date: -

Non-Technical Abstract The skin is the largest organ of the human body and it functions also as a “sensory” tissue. Together with the sensory neurons of the skin, mechanoreceptors give us the ability to perceive touch, temperature, pain and other mechanical forces, translating them into chemical signals. To this aim, we have developed a skin explant model re-innervated by human sensory-like neurons. Wee propose to functionally validate our model, with focus on Piezo-mediated mechanotransduction. Piezo receptors are increasingly being recognised as the major mechanical “sensors” of the human body. They open in response to mechanical stimuli, triggering the flux of monovalent cations (Ca2+, K+ and Na+) within the cell, regulating essential pathways. Since the role of Piezo has not been explored in re-innervated human skin, this proposal could have a great impact on the field.

Publications -

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IM@IT E-platform: No

Instruments	**Days Requested:**
Access Route	**Previous RB Number:**
Science Areas	**DOI:**
Sponsored Grant	**Sponsor:**
Grant Title	**Grant Number:**
Start Date	**Finish Date:**
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Sample record sheet

Principal contact Dr Chiara Niespolo, Arterra Bioscience Spa, ITALY
MRF Instrument **Confocal Microscope 2** **Days Requested:** 4
Special requirements:

SAMPLE

Material	Histology cryosection of re-innervated skin (Ex vivo model of skin explant re-innervated by sensory-like neurons)	-	-
Formula	-	-	-
Forms	Solid		
Volume	cc		
Weight	mg		
Container or substrate	Cell culture plate	-	-
Storage Requirements	-	-	-

SAMPLE ENVIROMENT

Temperature Range	37 C - RT K	-	-
Pressure Range	- mbar	-	-
Magnetic field range	- T	-	-
Standard equipment	Do Not Know	-	-
Special equipment	Cell culture plate	-	-

SAFETY

Prep lab needed	Yes	-	-
Sample Prep Hazards	-	-	-
Special equip. reqs	-	-	-
Sensitivity to air	No	-	-
Sensitivity to vapour	No	-	-
Experiment Hazards	-	-	-
Equipment Hazards	-	-	-
Biological hazards	No, the sample is treated in order to be devitalized (embedded and frozen)	-	-
Radioactive Hazards	-	-	-
Additional Hazards	-	-	-
Additional Details	-	-	-
Sample will be	Disposed of by instrument scientist	-	-

Confocal based analysis of mechanosensation in an ex vivo model of re-innervated human skin

1. Background and Context

The skin is the largest organ of the human body and it functions not only as a protective barrier from the external environment but also as a “sensory” tissue. In fact, all the layers of the skin contain specialised sensory fibers able to detect mechanical, thermal and nociceptive stimuli. Cutaneous nerve fibers are often associated with the so-called “mechanoreceptors”. These are ion channels anchored to the plasma membrane of cells, responsible for the passage of ions from one side of the membrane to another. Ions play an important role in stimulus-response reactions of cells as a second messenger and their diffusion into and out of the cells regulates critical biological events in a broad range of tissues and organisms. Together with the sensory neurons of the skin, mechanoreceptors give us the ability to perceive touch, temperature, pain and other mechanical forces, translating them into chemical signals. Therefore, it is crucial to have an experimental model that closely resembles the biological reality. To this aim, we have developed a skin explant model re-innervated by human sensory-like neurons. In fact, ex vivo cultured human skin is usually denervated, mostly due to surgical practices. Despite being a valid, alternative model to the animal in vivo testing, denervated skin cannot be used to fully unravel the mechanisms of mechanosensation. Here we propose to functionally validate our model, with focus on Piezo-mediated mechanotransduction. Recently discovered and worth a Nobel Prize in 2021, Piezo receptors are increasingly being recognised as the major mechanical “sensors”of the human body. They open in response to mechanical stimuli, triggering the flux of monovalent cations (Ca^{2+}, K^{+} and Na^{+}) within the cell, regulating essential pathways. Since the role of Piezo has not been explored in re-innervated human skin, the current proposed research could have a great impact on the field. In fact, screening for Piezo regulators could help to lay the foundations of new therapeutic strategies for the treatment of hypersensitive skin and pruritus, for example. This project is part of a wider industrial research programme which this year has received significant funding from MISE.

2. Proposed experiment

The aim of the proposed project is to determine whether our *ex vivo* re-innervated skin model is functional, with respect to Piezo-mediated mechanosensation and calcium signalling. To facilitate this, we will take advantage of a genetically modified neuronal cell line, stably expressing a calcium reporter, GCaMP6m. The latter is a genetically encoded fluorescent Ca^{++}indicator consisting of the calmodulin-binding peptide M13, a circularly permuted green fluorescent protein (GFP) and calmodulin. The gene is under the control of the Synapsin 1 promoter that drives its expression specifically in neuron cells. When calcium enters the cell, the fusion calcium indicator undergoes a conformational change and GFP fluorescence is activated from its quenced status. These cells are co-cultured with human excised skin. Human excised skin is obtained from female donors who undergo plastic and reconstructive surgery. Despite the sample being a surgery waste, all donors submit an informed consent form and agree to the donation. We are currently in the process of submitting an application to the Research Ethics Committee of the University Federico II of Naples (pending request). The skin/neurons co-culture is maintained for 10 days, changing culture medium every 2-3 days. Therefore, we aim at stimulating the outermost layer of the skin (epidermis) to trigger mechanoreceptors, specifically Piezo receptors, both in the derma and in the neurons below, thus activating the calcium signaling. Stimulation of mechanoreceptors can be performed chemically and mechanically. The GFP signal can be detected either via live imaging or in cryo-embedded tissue. Considering the challenges of the project, we think that ISIS@MACH ITALIA represents the most suited facility to realise it. Specifically, the instrument needed is the *Confocal Laser 2* (Laser Scanning Confocal Microscope Leica TCS SP8), which will enable monitoring the GFP signal in the tissue, and to reconstruct its 3D spatial distribution. Two strategies will be followed to apply a mechanical stimulus to the tissues and to monitor their response: i) selected regions of the tissue will be mechanically stressed by means of an AFM tip, controlling the force, depth and frequency of the stress, and then the specimen will be immediately transferred to the Confocal microscope in order to detect the fluorescence of the GFP protein, in response to the Ca^{++} release; ii) the re-

innervated tissue will be placed in between two glass slides and a static weight will be applied over the top slide, while performing the experiment (this approach has been already applied in the paper https://doi.org/10.1002/adma.201804600 by authors belonging to ISIS@MACH ITALIA Team), thus allowing for the live acquisition of the fluorescence signal coming from the activated GFP. The results obtained through this analysis will provide us with unique information about the functionality of our model. This is fundamental for both basic research and future cosmeceutical/clinical testing (i.e. ipersensitive skin, itching). In fact, when the mechanisms of mechanoreceptors' activation are more fully understood, our *ex vivo* model will be used to screen for molecules, within our in-house set of plant-derived extracts, capable of altering such pathways.

3. Summary of previous experimental proposals or characterisation

In our lab we have previously characterised a human neuronal cell line (SHSY5Y) and demonstrated that it expresses Piezo receptors and it also displays sensory-like properties (i.e. responsiveness to capsaicin and expression of sensory markers, such as TRPV1). Preliminary immunofluorescence data also showed that after 10 days of co-culture between skin and neurons we were able to detect nerve fibers within the derma section, the part of the model which is in direct contact with the neuron cells. However, so far, we were not able to establish whether the skin is in functional communication with the neurons below, due to the lack of sophisticated instruments.

4. Justification of experimental proposals request

Confocal Laser 2 (Laser Scanning Confocal Microscope Leica TCS SP8) is the ideal instrument to obtain 3D chemical mapping of complex systems like those mentioned in this proposal. It has an inverted microscope which perfectly fits for cellular imaging. The excitation and the emission wavelengths can be chosen to maximise the signal according to the fluorescence characteristics of GFP. Given the number of samples and conditions we have planned to test, we are requesting 4 days at the Medium Range Facility to access the instruments we need.