Innovative 3D skin and plant models to effectively monitor Piezo1 and Piezo2 driven mechano-transduction

Tito, Annalisa[1*]; Iacopino, Sergio[1]; De Lucia, Adriana[1]; Arciello, Stefania[1]; Carotenuto, Gennaro[1]; Vitale, Alessandra[1]; Niespolo, Chiara[1]; Tortora, Assunta[1]; Zappelli, Claudia[2]; Fogliano, Vincenzo[1,3]

1 Arterra Bioscience spa, Napoli, Italy;
2 Vitalab srl, Napoli, Italy;
3 University of Wageningen, Wageningen, Netherlands.

* Annalisa Tito, via Benedetto Brin 69, +390816584411, annalisa@arterrabio.it

Abstract

Background: Mechanosensitive Piezo1 and Piezo2 receptors sense and translate mechanical signals into cell stimuli in mammals having a pivotal role in many fundamental biological processes. Piezo receptors are also present in plants, where they are involved in root growth and mechanotransduction. The aim of this work was the development of innovative systems based on skin and plant 3D models enabling the screening and identification of natural modulators of Piezo receptors.

Methods: *In vitro* grown plants, skin explants and cell lines were used as model systems. Root penetration assay, skin and neurons co-culture experiments, immunofluorescence, and calcium influx assay in mammalian cell lines expressing Piezo receptors were performed to study the mechanisms underpinning Piezo modulation.

Results: Two screening models, based on human skin and *in vitro* plants were set up. Data showed they effectively allow to study the biology of Piezo mechanoreceptors in their native environment. Piezo1 overexpressing mammalian cell lines were used to identify a natural extract from plant cells of *Oenothera biennis* (ObHEx) able to increase Piezo1-mediated Ca^{2+} entry in absence of mechanical stimulation. Data suggested ObHEx is able to activate Piezo1 channels, and this is connected to the previously demonstrated capacity to modulate the actomyosin-based traction forces.

Conclusion: The model of reinnervated skin here developed has the potential to study the mechanisms behind the mechanosensitive cation channels in mammals while the plant model is suitable for the screening of Piezo regulators. Our data showed ObHEx is able to regulate Piezo1 activation, thus opening the possibility to develop ingredients targeting channel function in the skin.

Keywords: Mechano-sensation; mechano-transduction; Piezo receptor; skin model; plant model; *Oenothera biennis*

Introduction.

The perception of mechanical stimuli, followed by sensitive and fast signal transduction, is a ubiquitarian cell system. This conversion process is usually called mechanotransduction and it is mediated by mechanosensitive (MS) ion channels, highly conserved evolutionary proteins used by organisms to perceive and react to the conditions of the surrounding environment [1]. Among MS channels, a pivotal role is played by Piezo receptors, a family of evolutionally conserved ion channels using alternative gating models to convert different forms of mechanical force into electrical signals mediated by calcium influx [2]. In animals, functional expression of the mechanosensitive Piezo1 has been described in a variety of organs and non-neural tissues. Its expression is very high in endothelium, lung, bladder, and skin [3];while Piezo2 is predominantly expressed in sensory systems, including proprioceptive mechanosensors and Merkel cells [3,4]. Two force transduction and gating models have been proposed to understand the gating mechanism of MS channels: the membrane tension model and the tether model [5]. The membrane tension model proposes that the gating of the channels comes from force applied to the lipid bilayer tension. In the tether model, force is transmitted through a tether connecting the channel with a component of the extracellular matrix or intracellular cytoskeleton. Ellefsen and colleagues [6] revealed that Piezo1-dependent calcium signals is triggered by the traction forces generated by activated cytoskeleton myosin II previous phosphorylated by Myosin Light Chain Kinase (MYLK).

Many studies have demonstrated the prominent role of Piezo channels in various mammalian organs, whereas the study of Piezo channels in skin physiology is in an early stage. The discovery of Yoda1 and Jedi 1/2 as specific agonists of Piezo1 channels, shed new light on the possibility of exogenous activation of Piezo1 channels, independently from mechanical stimuli [7]. To date, no agonists of Piezo2 channels have been found, and to study their mechanotransduction processes, a physical stimulus must be used.
In plants, one gene homologous to animal Piezo has been identified and in the model species, *Arabidopsis thaliana*. It regulates the ability of roots to perceive obstacles during their growth in fact in *Arabidopsis* plants knock out for Piezo gene, the roots lost the ability to penetrate compact soil, generating a precise and measurable phenotype [8,9].
The purpose of this work was the development of new screening platforms aimed at the identification of natural compounds able to regulate Piezo receptors. We generated different systems based on reinnervated skin explants, plants, and mammalian cell lines and we identified a potential regulator of Piezo. These models will be used to understand the mechanisms underpinning skin mechanosensitivity and unleash the potential of natural compounds in Piezo-driven mechanosensation.

Materials and Methods.
Plant materials and growth conditions: The Columbia-0 (Col-0) ecotype of Arabidopsis thaliana, purchased from the Nottingham Arabidopsis Stock Center (NASC) was used as a wild-type background in all experiments. AtPiezo loss-of-function mutant line of Arabidopsis thaliana (SAIL_856_B11 piezo mutant) was purchased from the Nottingham Arabidopsis Stock Center (NASC). Seeds were vernalized for two days at 4°C in dark and subsequently germinated in a growth chamber at 22 °C with a 16 h light/8 h dark photoperiod. The presence of the T-DNA in the piezo line was monitored by PCR. In the text, the mutant line is referred to as *pzo1*.
***In vitro* plant rooting:** In order to mimic different levels of soil hardness, approximately 90 mL of half-strength MS medium supplemented with different agar concentrations, was poured into 120-mm × 120-mm × 15-mm plates. After pouring the first layer of medium, a portion of 4 cm was excised with a sharp sterile blade from one plate side and replaced with 7 ml of MS medium supplemented with 0.8% of agar creating a barrier that divides a thin layer of MS medium from a thicker and higher-concentrated agar's medium [9]. Arabidopsis seeds were surface sterilized with 70% ethanol for 1 min, 1% sodium hypochlorite solution for 8 min, and lastly washed five times with sterile distilled water. Seeds were plated on the 0.8% agar concentration MS medium, 2 cm above the barrier. Finally, seeds were vernalized at 4° C in the dark for 48 h and then geminated vertically. The rooting ability of plants was determined by visual examination of media containing increasing concentrations of agar. Plants with a reduced rooting penetration ability show root tips that are unable of penetrating the agar barrier or grow above the barrier. By means of a laser scanner and visual counting, we measured the percentage of root penetration on different agar media.
Cell culture and skin explants: Human Umbilical Vein Endothelial Cells (HUVEC) were grown in Endothelial Growth Medium (EBM), and Chinese Hamster Ovary cells (CHO) were grown in DMEM/F12 plus 10% FBS. Human neuroblastoma cell line and immortalized keratinocytes (HaCaT) were grown in Dulbecco's Modified Eagle Medium (DMEM) containing 10% FBS. All cell cultures were maintained at 37°C in 5% CO_2 humidified air. Skin explants were obtained from healthy female donors of different ages (21-44 years old) following breast surgery and cut using an 8mm biopsy punch. Tissues were kept in air-liquid conditions at 37°C in 5% CO2 humidified air. All donors had given their written informed consent for the use of the skin tissues, according to the Declaration of Helsinki.

Neuronal differentiation and co-culture with skin explants: Neuroblastoma cells were seeded in a 48-well plate at a density of approximately 25,000 cells per well. The next day, cells were washed in PBS and treated with 10μM retinoic acid (RA) in DMEM supplemented with 1% of iron-fortified calf serum. Medium-plus 10μM RA was changed every 2 days. 5 days post RA, the medium was changed, increasing the concentration of serum to 2%. On day 5 of neuronal differentiation, 8mm skin punches were added on the top of differentiated neurons and left them for an additional 10 days in direct contact at 37°C in 5% CO_2 humidified air. The medium was changed every 2-3 days. On day 10 of co-culture, skin punches were removed and processed for histological sectioning, as described below.

Transient transfection and calcium influx assay: 1 x 10^4 CHO cells were seeded in a 96 well plate and transfected with 50ng of human Halo Piezo1 plasmid using the reagent X-tremeGENE™ HP DNA Transfection Reagent. 48 h after transfection the cells were screened for calcium influx assay. The cells were incubated with 2,5μM Fluo3/AM for 45' at 37°C. Cells were then washed with HBSS and agonists/antagonists were added. Fluorescence was immediately read using the VICTOR Nivo microplate reader (PerkinElmer) (excitation

480/30nm, emission 530/30nm) every second for 1 minute. Results were analyzed by calculating the area under the curve (AUC). The same protocol was used for HUVEC and neuroblastoma cell lines.

Gene expression analysis of Piezo1 and Piezo2: Total RNA was extracted using GenElute Mammalian Total RNA Miniprep kit (Sigma-Aldrich) and cDNA was generated using Revert Aid First Strand cDNA synthesis kit (Thermo Fisher Scientific), according to the manufacturer's instructions. Gene expression analysis was carried out using gene-specific primers and the QuantumRNA 18S internal standard.

***Ex vivo* analysis:** ImmunoHistoFluorescence (IHF) of Piezo1, Piezo 2, PGP 9.5, beta-III-tubulin and cytokeratin20 was carried out on skin sections. After each experiment, skin punches were fixed in 4% paraformaldehyde (PFA), incubated first in 15% sucrose, then in 30% sucrose and finally embedded in OCT freezing compound. 5-20µm sections were obtained using the CM1520 cryostat (Leica Microsystems). We performed both longitudinal (all skin layers) and transverse (derma and epidermal sheets) cuts. Slides with cryosections were hydrated for 30 minutes in PBS and blocked using a solution of 6% BSA, 5% goat serum, 20 mM MgCl2 and 0.2% Tween at room temperature for 1 hour. Slides were then incubated with primary antibodies overnight at 4°C and with secondary antibodies (Alexa-Fluor, Thermo Fisher Scientific) at room temperature for 1 hour. Sections were then washed in PBS and mounted using a solution of PBS and glycerol. Imaging was performed using a Leica fluorescence microscope and images were processed using ImageJ software.

Statistical Analysis: All experiments were performed at least three times in triplicate. Graphs and statistical analysis were generated using Excel (Microsoft). The bars represent the standard deviations while asterisks indicate significant variations (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$), according to Student's t-test.

Results.

1. **Set up of plant model to screen Piezo modulators**

Piezo knock-out Arabidopsis plant roots lose the ability to penetrate in hard medium [9] therefore this phenotype can be used as an *in vitro* model for the screening of Piezo regulators. To confirm the lack of AtPiezo in the mutant line and to verify the localization of AtPiezo in wild-type plants we analysed the expression of AtPiezo in different tissues of both plant lines as reported in figure1.

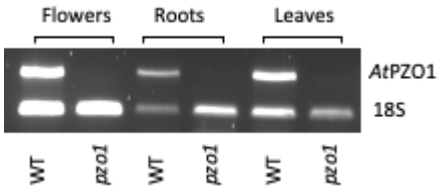

Figure 1. Gene expression analysis showing AtPiezo expression pattern in wild type and pzo1 mutant Arabidopsis plant tissues. Representative gel electrophoresis of semiquantitative RT-PCR amplicons.

The results of semiquantitative RT-PCR demonstrated that AtPiezo was expressed in flowers, roots, and leaves in wild-type plants whereas no expression was detected in the same tissues of mutant plants. To set up the screening system these plants were grown vertically on agar plates at different concentrations and the root penetrating ability was monitored as pictured in figure 2. In presence of 1% agar, the roots of mutant plants have a slightly reduced (not significant) penetration ability, however, using a 2% agar barrier the mutant plant roots showed a significantly reduced penetration ability., At 2% agar concentration the penetration of wild-type plants was 21.8% and it dropped to 5.7% in pzo1 plants. This decrease of about 75% compared to WT was in line with the results published by Fang et al., (2021) and confirmed the importance of Piezo for plant root penetration ability [8]. Preliminary experiments indicated that this plant phenotype screening platform is sensitive to chemicals or natural compounds placed in the agar medium (data not shown) and therefore it can be used to screen Piezo regulators in a living organism.

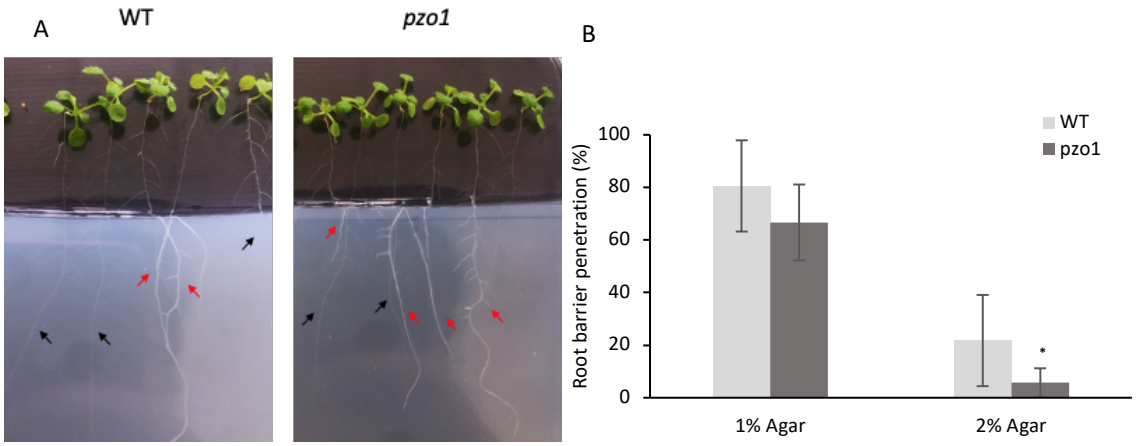

Figure 2. Analysis of root penetration ability into hard media in WT and pzo1 mutants. A) Representative images of Arabidopsis roots length in WT and pzo1 mutant growing on a 2% agar media. Black arrows indicate fully agar penetrated roots while red arrows are those not penetrated or grown above the barrier. **B)** Quantitative analysis of the effect of agar concentrations on root penetration ability of WT and *pzo1* mutant. In the graph is reported the average of three independent experiments each of them performed with 15 individual plants for condition. The statistical analysis was performed as described in material and methods.

2. Set up of *ex vivo* cultured innervated skin explants to study Piezo-mediated mechanosensation

The expression and localization of endogenous Piezo1 and Piezo2 were investigated in skin explants by using Immunofluorescence staining. As shown in figure 3A**,** we detected Piezo1 along with the epidermis, specifically in the keratinocytes of the stratum granulosum. Piezo2 was less abundant and was mostly expressed in the basal layer of the epidermis, where it colocalizes with the marker cytokeratin20, which specifically stains Merkel cells (figure 3 B). These results were consistent with published data, reporting that Piezo1 belongs to the non-sensory tissue, whereas Piezo2 locates at sensory nerve endings [4].

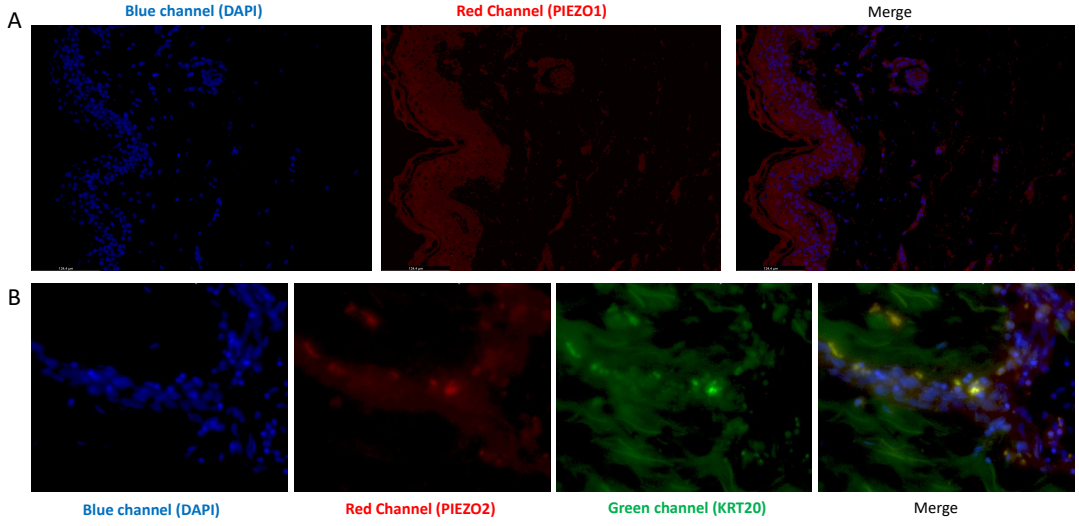

Figure 3. Localization of Piezo1 and Piezo2 in human skin explants by immunofluorescence. Panels A Longitudinal skin sections stained for PIEZO1 (red) and nuclei (blue), **Panel B** Longitudinal skin sections stained for PIEZO2 (red) and KRT20 (green) in epidermal sheet**.**

These skin explants were used to establish a model of re-innervated skin by co-culturing them with neuroblastoma cells, previously differentiated in sensory-like neurons [10]. As shown in figure 4A-B we obtained a full neuronal differentiation: cells on day 0 were characterized by a round shape and the presence of a few projections, while on day 5 they showed extensive and elongated neuritis projections. As shown in figure 4C-D the phenotype was confirmed by immunofluorescence staining of beta-III-tubulin one of the earliest markers of neuronal differentiation.

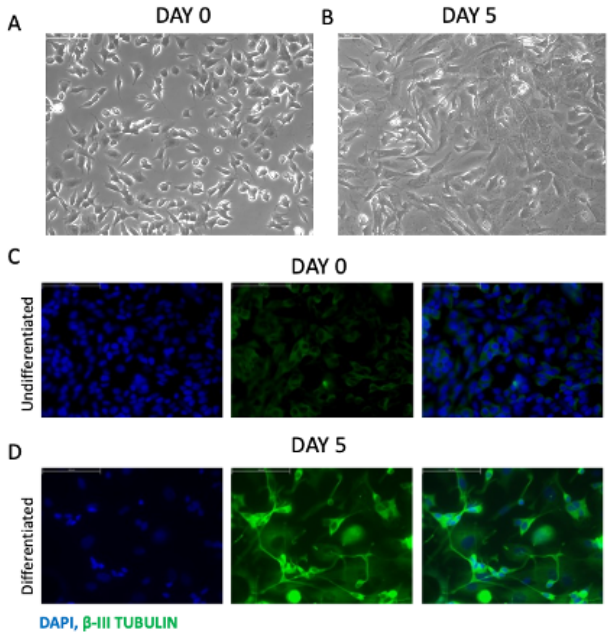

Figure 4. Analysis of neuronal differentiation on Retinoic acid-treated neuroblastoma cells. Bright-field images of undifferentiated (A) and differentiated (B) cells, showing morphological differences (40X magnification). Anti-β-III-tubulin (green) immunofluorescence staining of undifferentiated (C) and differentiated cells (D), revealing elongated neuritis projections (20X magnification).

Once the *in vitro* differentiation of neuroblastoma cells was established, a co-culture with cultured skin explants was performed by placing skin punches on the top of differentiated cells in direct contact for 10 days. The immunofluorescence staining of beta-III-tubulin and PGP9.5 of the derma section is reported in Figure 5: the presence of small nerve fibers within the derma sections is clearly visible as co-localized red and green dots. The absence of blue stained nuclei (DAPI) in the merge image indicates that only nerve fibers reached the skin. This *ex vivo* model of re-innervated human skin, using neuroblastoma-derived neurons can be used to study the mechanosensitive receptor signal transduction following specific skin treatment.

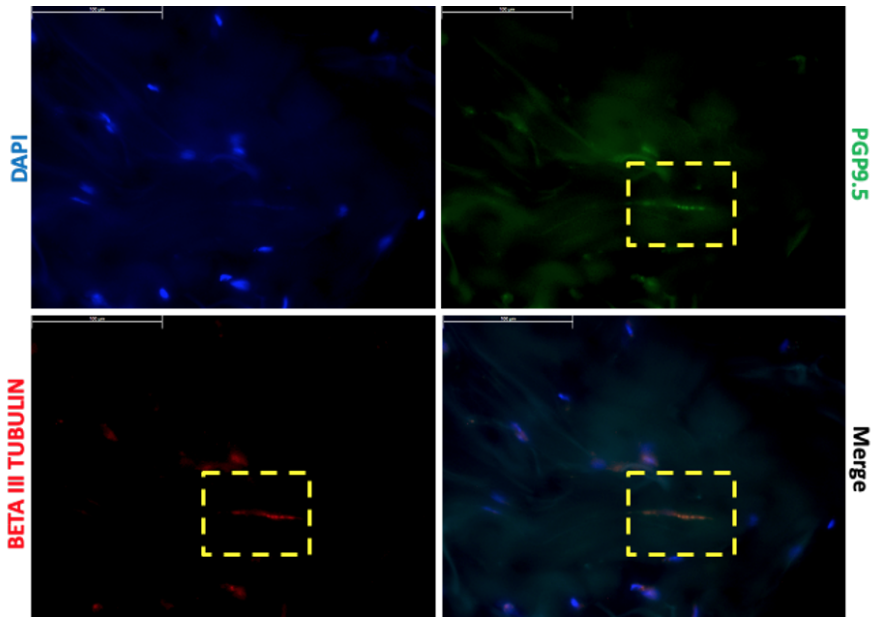

Figure 5. 3D human re-innervated skin explant. Immunofluorescence analysis of human skin explant co-cultured with neurons for 10 days show the integration of a nerve network in the skin. Double immunofluorescence for neuronal markers β-III-TUBULIN (red dots) and PGP9.5 (green dots).

3. Set up of Piezo1 overexpressing cell line as selective screening tool for Piezo1 modulator

CHO cells were transiently transfected with a vector expressing human Piezo1 and their modulation by Yoda1, a specific agonist of Piezo1, and Gsmtx4, a non-specific mechanoreceptor inhibitor was investigated [11]. Moreover, we also tested a hydrophilic extract of *Oenothera biennis* cell cultures (ObHEx) which we demonstrated as able to stimulate the MYosin Light chain Kinase (MYLK) mediated cell contraction [12,13]. As reported in figure 6, activation by 4 µM Yoda1, the specific agonist of Piezo1, triggered the intracellular calcium influx resulting in a significant 30% increase compared to untreated cells. To determine the specificity of this effect, we measured the inhibition of the Yoda1-induced Ca^{2+} entry when cells were co-treated with GsMTx4, a mechanoreceptor inhibitor [11]. CHO cells transfected with a control plasmid did not respond to Yoda1 thus confirming the reliability of the cellular system. ObHEx at 0.02% increased by 40% the entrance of Ca^{2+} entry while control cells were completely insensitive to this treatment. As observed for Yoda-1 also the ObHEx effect was reversed by 2.5 µM GsMTx4. These data support the hypothesis that ObHEx can regulate Piezo1 channel activation, but the mechanism remains unclear.

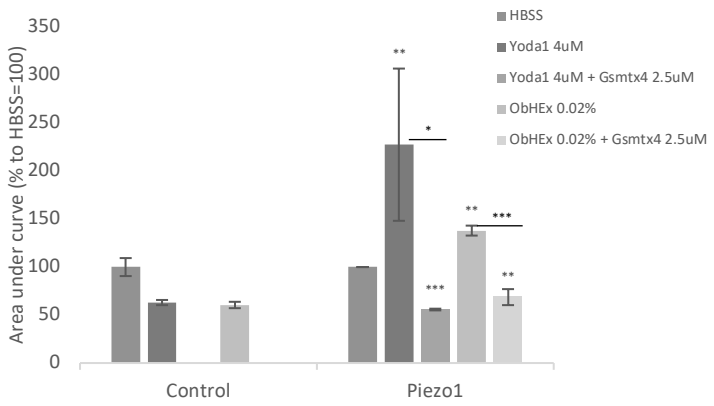

Figure 6. Ca^{2+} influx measurement in cells overexpressing Human Piezo1. Intracellular Ca^{2+} measurements in CHO transfected cells exposed to 4µM Yoda1 or ObHEx alone or in presence of Gsmtx 2.5µM. Control samples were treated with HBSS medium alone.

To dive deeper into the mechanism leading to Ca^{2+} influx inside the cells, we performed Calcium influx assay in HUVEC and neuroblastoma cells in which we have previously demonstrated the endogenous expression of Piezo1 and Piezo2 (data not shown). In these cells beside to Yoda and ObHEx we also tested the effect of calyculin, a known MYLK activator as we have previously shown that ObHEx activated the myosin II-mediated cellular traction forces, by activating the phosphorylation of MYLK [12].
The data of these experiments were summarised in figure 7 (HUVEC cell) and figure 8 (neuroblastoma cells). Again. in both cell lines, Yoda1 significantly increased calcium influx and the same effect was obtained by calyculin thus suggesting the connection between MYLK and Piezo1. To confirm this connection, we used two selective inhibitors namely Dooku1 for Yoda1-activated Piezo1 and ML7 for MYLK pathway and in both cases the effect was specifically reverted.
Interestingly, the same effect was observed when the induction of Ca^{2+} influx was triggered by ObHEx: both Dooku1 and ML7 were able to reduce it.These data support the hypothesis that ObHEx effectively regulates Piezo1 activation through traction forces generated by Myosin II phosphorylation by MYLK.

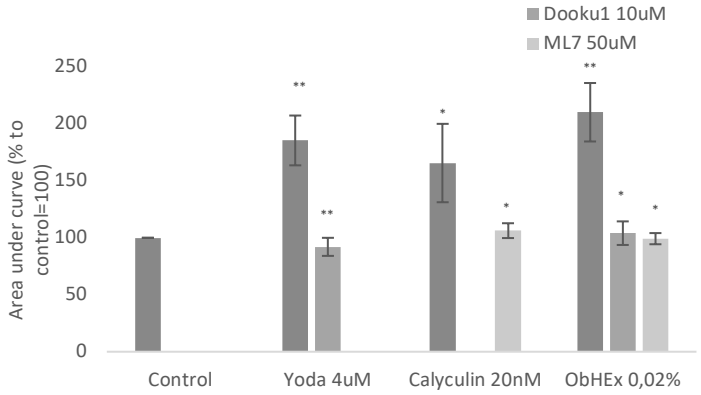

Figure 7. Effect of ObHEx on Piezo-induced Ca2+ entry in HUVECs cells. Intracellular Ca^{2+} measurements in HUVEC endogenously expressing Piezo1 channels treated with Yoda 4µM, Calyculin 20nM or ObHEx 0,02% alone or in presence of specific inhibitors.

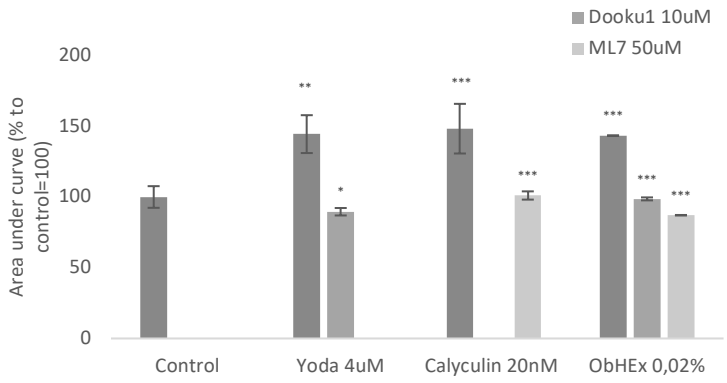

Figure 8. Effect of ObHEx on Piezo-induced Ca^{2+} entry in neuroblastoma cells. Intracellular Ca^{2+} measurements in neuroblastoma cells expressing Piezo1 channels treated with Yoda 4µM, Calyculin 20nM or ObHEx 0,02 % alone or in presence of specific inhibitors.

Discussion

All organisms can perceive and should adapt to mechanical forces. Touch, pressure or shear stress constantly stimulate all forms of life, and they are critical for a variety of biological processes. Mechanosensitive ion channels are the major sensors and effectors of mechanical stimuli, converting them into appropriate cell signals [14,15].Plants and animals share the same cellular and molecular mechanisms involved in mechanotransduction, including Piezo family receptors [3,16]. In animals, mechanical forces impact most biological events, both physiological and pathological, while in plants they mainly affect morphogenesis and root formation [14,17,18].

In this paper we developed three biological platforms for the study of Piezo mechanoreceptors. Data obtained in the plant system highlights the potential of this model to screen natural compounds associated with Piezo activity in living organisms. Loss-of-function mutant of *AtPiezo* shows impaired ability to penetrate harder agar media compared to wild-type plants. The treatment of wild-type and mutant plants during growing with different compounds or extract mixtures will allow us to observe phenotype differences. In particular, the root penetration assay can be a robust and inexpensive method for Piezo-modulator screening.

The second system we set up to investigate Piezo-mediated mechanotransduction is an *ex vivo* system based on human skin explants. They contain all the layers of the skin and can be easily manipulated to set up *ex vivo* assay[19]. Unfortunately, due to the surgery procedure, only a few, cut-off nerve endings are retained within the tissue and no endogenous nerve fibers can be detected in our cultured skin explants. For this reason, in this paper a skin re-innervation procedure was successfully implemented by using neurons differentiated from a neuroblastoma cell line. This is a particularly remarkable achievement as to date, re-innervation of skin has been obtained only using primary cells, such as iPSCs-derived neurons and rat dorsal root ganglia which are much more difficult to handle and not suitable to set up a screening platform [20–22]. Our model can be used to study the activation of Piezo receptors, with particular focus on Piezo2, which we detected in skin Merkel cells, in accordance with published data [23].

To specifically investigate the mechanisms underpinning the Ca^{2+} influx in different cell types we used Piezo 1 overexpressing cell lines and a cell-based fluorescence assay to detect Ca^{2+} influx mediated by Piezo gating. So far, the induction of Piezo channels has been mostly investigated by using either mechanical means or treatment with Yoda1, a chemical which is the first specific activator of Piezo1 [24]. To our knowledge there is no evidence of natural products able to regulate the function of Piezo. Here we identified, an extract from *Oenothera biennis* cell culture (ObHEx) as potential modulator of the Piezo1 channel. Data showed ObHEx treatment in both endothelial and neuronal cells significantly increased intracellular flux of Ca^{2+} ions and the effect was abolished when cells were co-treated with GsMTx4 or Dooku. Based on this observation we speculated that as ObHEx elicits MYLK activation [12], and MYLK-mediated Myosin II phosphorylation is able to trigger Piezo1 [6] this kinase should also play a key role in the activation mechanism induced by ObHEx. This hypothesis was confirmed by experiments in which the MYLK pathway was inhibited by calyculin We propose ObHEx as a natural regulator of Piezo1 channels and actomyosin-based traction forces. In fact, we have evidence that ObHEx potentially modulates upstream signalling mechanisms involved both in the phosphorylation of Myosin II by MYLK and the activation of Piezo1 channels.

These screening platforms are robust and reliable tools to investigate, without using animal models functional and biological mechanisms underpinning Piezo channels modulation. The future goal is to investigate the potential of natural extracts of cosmetic interest to modulate skin mechanosensation.

Conclusion

The role of Piezo receptors in mechanobiology was investigated by means of three novel and reliable platforms that allow the screening of bioactive molecules and compounds able to regulate Piezo-driven mechanotransduction. One system is based on Arabidopsis plants where we can study Piezo regulation by exploiting different root penetration phenotypes. Our long-term goal will be the implementation of plant-based platform with the generation of transgenic lines expressing the human PIEZO genes in plant PIEZO mutants. The second platform is based on reinnervated skin explants that serve as a platform to investigate the biology of Piezo mechanoreceptors in their native environment. Using the third platform based on cell lines overexpressing Piezo1, we discovered a hydrophilic extract of *Oenothera biennis* cell cultures (ObHEx), previously characterized as *in vitro* regulator of actomyosin networks, also regulates Piezo1-driven mechanotransduction.

Acknowledgements
We are very grateful to all the staff members of Arterra Bioscience Spa and VitaLab Srl contributing to this research project. We thank Professor Ivan Conte from the University of Naples Federico II for his precious suggestions.

Conflict of Interest Statement
NONE.

References

1. Katta S, Krieg M, Goodman MB. Feeling force: physical and physiological principles enabling sensory mechanotransduction. *Annu Rev Cell Dev Biol*. 2015;31:347–371.

2. Coste B, Mathur J, Schmidt M, et al. Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels. *Science*. 2010;330:55–60.

3. Fang X-Z, Zhou T, Xu J-Q, et al. Structure, kinetic properties and biological function of mechanosensitive Piezo channels. *Cell & Bioscience*. 2021;11:13.

4. Bagriantsev SN, Gracheva EO, Gallagher PG. Piezo proteins: regulators of mechanosensation and other cellular processes. *J Biol Chem*. 2014;289:31673–31681.

5. Douguet D, Patel A, Xu A, Vanhoutte PM, Honoré E. Piezo Ion Channels in Cardiovascular Mechanobiology. *Trends Pharmacol Sci*. 2019;40:956–970.

6. Ellefsen KL, Holt JR, Chang AC, et al. Myosin-II mediated traction forces evoke localized Piezo1-dependent Ca2+ flickers. *Commun Biol*. 2019;2:298.

7. Xiao B. Levering Mechanically Activated Piezo Channels for Potential Pharmacological Intervention. *Annu Rev Pharmacol Toxicol*. 2020;60:195–218.

8. Fang X, Liu B, Shao Q, et al. AtPiezo Plays an Important Role in Root Cap Mechanotransduction. *International Journal of Molecular Sciences*. 2021;22:467.

9. Mousavi SAR, Dubin AE, Zeng W-Z, et al. PIEZO ion channel is required for root mechanotransduction in Arabidopsis thaliana. *Proc Natl Acad Sci U S A*. 2021;118:e2102188118.

10. Shipley MM, Mangold CA, Szpara ML. Differentiation of the SH-SY5Y Human Neuroblastoma Cell Line. *J Vis Exp*. 2016;53193. doi:10.3791/53193.

11. Gnanasambandam R, Gottlieb PA, Sachs F. The Kinetics and the Permeation Properties of Piezo Channels. *Curr Top Membr*. 2017;79:275–307.

12. Ceccacci S, De Lucia A, Tito A, et al. An Oenothera biennis Cell Cultures Extract Endowed with Skin Anti-Ageing Activity Improves Cell Mechanical Properties. *Metabolites*. 2021;11:527.

13. Tito A, De Lucia A, Tortora A, Falanga D, Zappelli C, Angelillo M, Ceccaci S, Monti MC, Ausanio G, Di Cicco C, Apone F, Colucci MG. An hydrosoluble extract from Oenothera biennis cell culture produces skin anti- ageing effects by improving cell mechanical properties. The proceeding of the 27th IFSCC congress 2021, Mexico.

14. Marshall KL, Lumpkin EA. The Molecular Basis of Mechanosensory Transduction. *Adv Exp Med Biol*. 2012;739:142–155.

15. Peyronnet R, Tran D, Girault T, Frachisse J-M. Mechanosensitive channels: feeling tension in a world under pressure. *Frontiers in Plant Science*. 2014;5.

16. Radin I, Richardson RA, Coomey JH, et al. Plant PIEZO homologs modulate vacuole morphology during tip growth. *Science*. 2021;373:586–590.

17. Chalfie M. Neurosensory mechanotransduction. *Nat Rev Mol Cell Biol*. 2009;10:44–52.

18. Monshausen GB, Bibikova TN, Weisenseel MH, Gilroy S. Ca2+ Regulates Reactive Oxygen Species Production and pH during Mechanosensing in Arabidopsis Roots. *Plant Cell*. 2009;21:2341–2356.

19. Human skin explant model for the investigation of topical therapeutics | Scientific Reports. Available at: https://www.nature.com/articles/s41598-020-78292-4. Accessed June 30, 2022.

20. Lebonvallet N, Boulais N, Le Gall C, et al. Effects of the re-innervation of organotypic skin explants on the epidermis. *Experimental Dermatology*. 2012;21:156–158.

21. Chéret J, Piccini I, Gherardini J, Ponce L, Bertolini M, Paus R. Sensory Reinnervation of Human Skin by Human Neural Stem Cell–Derived Peripheral Neurons Ex Vivo. *J Invest Dermatol*. 2022;142:257-261.e5.

22. Chéret J, Ponce L, Le Gall-Ianotto C, Bertolini M, Paus R. Re-innervation of human skin by rat dorsal root ganglia permits to study interactions between sensory nerve fibres and native human dermal mast cells ex vivo. *Exp Dermatol*. 2021;30:418–420.

23. Woo S-H, Ranade S, Weyer AD, et al. Piezo2 is required for Merkel-cell mechanotransduction. *Nature*. 2014;509:622–626.

24. Syeda R, Xu J, Dubin AE, et al. Chemical activation of the mechanotransduction channel Piezo1. *Elife*. 2015;4.