Microneedles potentiate skin cell rejuvenation via modulation of mitochondria and epigenetic activities

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Introduction

- Microneedles have been applied to deliver genes, drugs, and vaccines through human skin for more than two decades due largely to the fact that skin permeability of those agents increases[1].
- Studies have demonstrated that repeated application of microneedles does not alter skin appearance or barrier function and causes no measurable disturbance of serum biomarkers of infection, inflammation, or immunity in mice *in vivo*, suggesting the safety of this fashionable cosmetic approach[2].
- Microneedles aim at skin cell rejuvenation, yet the underlying molecular mechanisms of microneedles' action remain to be fully elucidated[3].
- Cultured human keratinocytes (HaCaT cells) were treated with various commercially available microneedles. Subsequently, confocal microscopy, Western blot, and RNA sequencing were applied for cellular analysis

Collectively, we conclude that microneedles modulate mitochondria and epigenetic activities, leading to skin cell rejuvenation.

Methodology

- **Cell culture and treatment:** Human keratinocytes (HaCaT cells) were cultured in DMEM on glass cover slips in 6 and 12 well plates and treated with various microneedle pins (16, 36, or Nano) for Western blot or Confocal microscopy. These cells were maintained in a CO_2 incubator at 37°C.
- **Western blot:** Cells were cultured in 6 well plates and treated under their respective treatments and lysed under RIPA buffer. The lysates ran through 4-20% SDS PAGE. After wet transfer, membranes were blotted with primary and secondary antibodies. The proteins were then detected under Li-COR Odyssey imaging system.
- **Confocal microscopy:** Cells on cover slips in 6 or 12 well slides of treated cells were first fixed with formaldehyde. Next, the cells were permeabilized with methanol, blocked, and then stained with primary and secondary antibodies. Later, the cells were observed under a Carl Zeiss LSM 700 confocal microscope.
- **RNA Sequencing:** Cells treated with Nano microneedle pins and the control were RNA sequenced to determine differentially expressed genes involved in growth and mitochondrial function

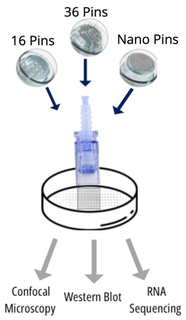

Fig. 1: A schematic representation of the experimental design of treatment to HaCaT cells via 16, 36, or Nano Pins.

Results

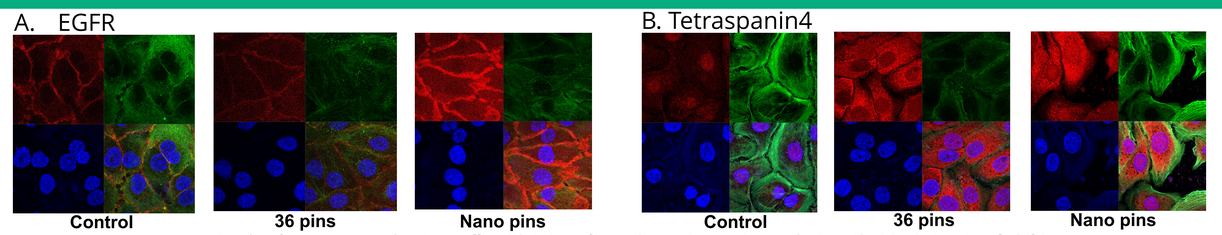

Fig. 2: Confocal microscopy of HaCaT cells with integral membrane transcription factors that test structural ability.

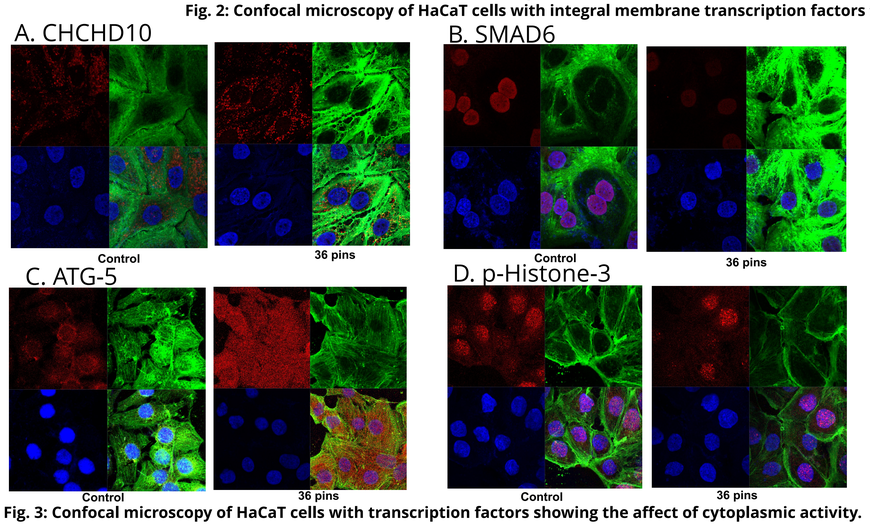

Fig. 3: Confocal microscopy of HaCaT cells with transcription factors showing the affect of cytoplasmic activity.

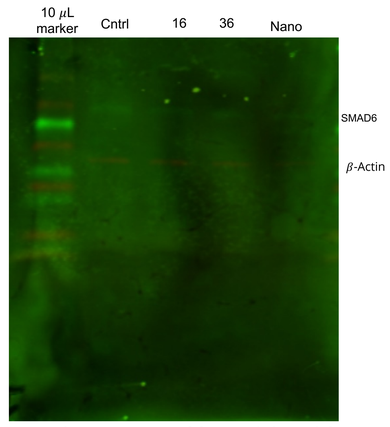

Fig. 4: Western blot activity with SMAD6 showing mitochondrial activity is upregulated after treatment.

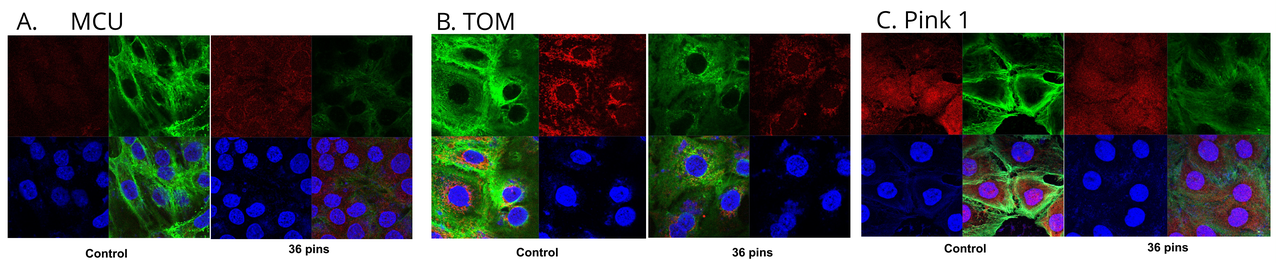

Fig. 5: Confocal microscopy of HaCaT cells with mitochondrial transcription factors shows the effect on mitochondrial activity.

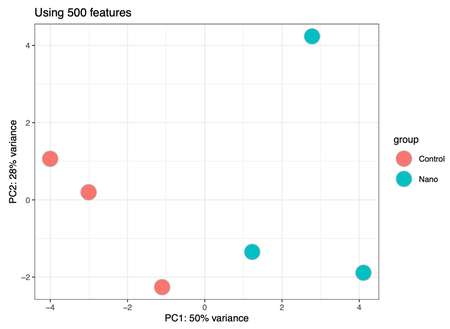

Fig. 6: Principal component analysis of control and nano samples of HaCaT cells. The separation shows sequence differentials.

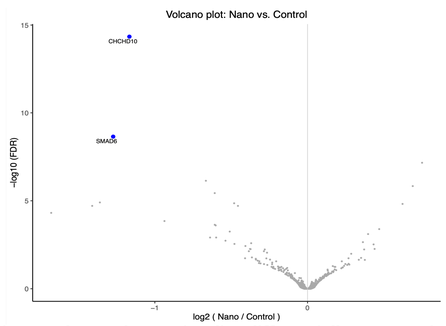

Fig. 7: Volcano plot to visualize differentially expressed genes. SMAD6 and CHCHD10, two genes associated with growth and mitochondrial function.

Acknowledgements

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Conclusion

Our results showed that microneedle treatment induced EGFR clustering on the cell membrane. Treatment also showed upregulation of Tetraspanin 4. Additionally, confocal microscopy data indicated that microneedles downregulated mitochondrial proteins, TOM20 and PINK1, and upregulated MCU. This verifies that mitochondrial activity for energy production is vital for reproduction and further growth. Furthermore, we interestingly found that microneedles induced ATG12 translocation from the cytoplasm to the nucleus. Finally, RNA sequencing data indicated that microneedles downregulated SMAD6 despite temporarily upregulating CHCHD10, which prompts further experimentation. The nuclear activity indicates nuclear translocation that is shifting energy into the nucleus to gather genetic information for further replication of cells to promote rejuvenation. Further research will delineate the cell signaling pathways affected by microneedles in human skin cells.

References

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