Emulating microcurrent devices for the next dimension in facial sculpting

Cristina Carreno; Lipotec S.A.U. (The Lubrizol Corporation)

Carreno, Cristina PhD[1]; Valerio-Santiago, Mauricio PhD[1]; García, Consuelo[1]; Subirós, Ramón PhD[1]; Soley, Albert PhD[1]; Almiñana, Núria PhD[1]; Delgado, Raquel PhD[1].

[1]Lipotec S.A.U. (The Lubrizol Corporation), 08850 Gava, Barcelona, Spain

Introduction of research

At-home microcurrent devices are gaining in popularity thanks to consumer interest in non-surgical medical aesthetic treatments. They primarily combat signs of aging, like facial sagging, with impressive results (1-2). This is due, in part, to electrical stimulation in the range of microcurrents that can boost cellular metabolism and matrix production (3). However, the biological mechanism of action of electrical stimulation on the dermal layer as it relates to skin appearance remains relatively unknown. In this line, the role of MBNL1 in the contraction of the dermal layer and its role in the myofibroblast phenotype appearance were identified as unique changes that occur in the skin after electrical stimulation (4-5).

Inspired by technological advances of at-home devices, the objective of the study was to first, understand the specific biological changes induced by electrical stimulation that occur in the dermal layer of the skin. The second was to develop an active ingredient that could mimic the cellular and macroscopic changes brought on by a microcurrent device to deliver global facial lifting effects as a novel topical anti-aging solution, demonstrated *in vitro* and *in vivo*.

Methodology

The effect of electrical stimulation in the range of microcurrents was examined in primary human dermal fibroblasts, comparing it against the activity of the novel active ingredient. For that, markers of myofibroblasts phenotype, namely MBNL1 and EDA-fibronectin (EDA-Fn) (6-8) were assessed using immunostaining and quantification by confocal microscopy. Additionally, the novel active ingredient was assessed in its capacity of contraction of a 3D collagen gel, in which cells were embedded, and compared with the effect obtained by the electrical stimulation.

Clinical benefits were evaluated in a double-blind, half-face design study in a group of males and females (40-65 y.o) using a cream containing 2% of the active ingredient or a placebo cream twice per day over 28 days. An additional group used a commercially available microcurrent device once a day over the same period for efficacy comparison. Macrophotographs of the face were taken and analyzed with a specialized software (CameraScan) at the initial time and at the end of the study. Anti-wrinkle efficacy was evaluated by the calculation of the coefficient of visibility of wrinkles after 7 days of treatment. Lifting measurements were also evaluated by means of image analysis: the eyelid lifting effect was calculated by the measurement of the distance of a vertical line from the middle point of the baseline of the upper eyelid to the first fold in the upper eyelid; the eyebrow lifting effect was quantified measuring the distance of a diagonal line starting at the middle point of the baseline of the upper eyelid to the upper point in the

eyebrow arc, and the cheek lifting effect was evaluated by determining the extent and direction of the skin displacement which took place at the end of the treatments (LiveViz® Infinity, Quantificare).

Results

The effect of electrical stimulation on the increase of MBNL1 protein levels in human dermal fibroblasts, was evaluated by immunostaining. The active ingredient increased MBNL1 levels in a similar way than the electrical stimulation, in a dose dependent manner. Figure 1A shows representative images of MBNL1 immunostaining. When dermal fibroblasts, previously exposed to electrical stimulation or active ingredient treatment, were embedded in a collagen gel, the diameter of the collagen gel was reduced after 24 hours, indicating an increased ability of the cells in providing extracellular matrix contraction (Figure 1B). Finally, EDA-Fn levels were evaluated by immunofluorescence after digestion of the collagen gels. As seen in Figure 1C, the active ingredient treatment increased EDA-Fn, in a similar way than the cells stimulated with electrical signals. In conclusion, these results demonstrate that the electrical stimulation in the range of microcurrents promotes the formation of myofibroblasts, enhancing their contractile capacity, and that the new active ingredient mimics the biological mechanisms triggered by electrical stimulation in the dermal layer of the skin.

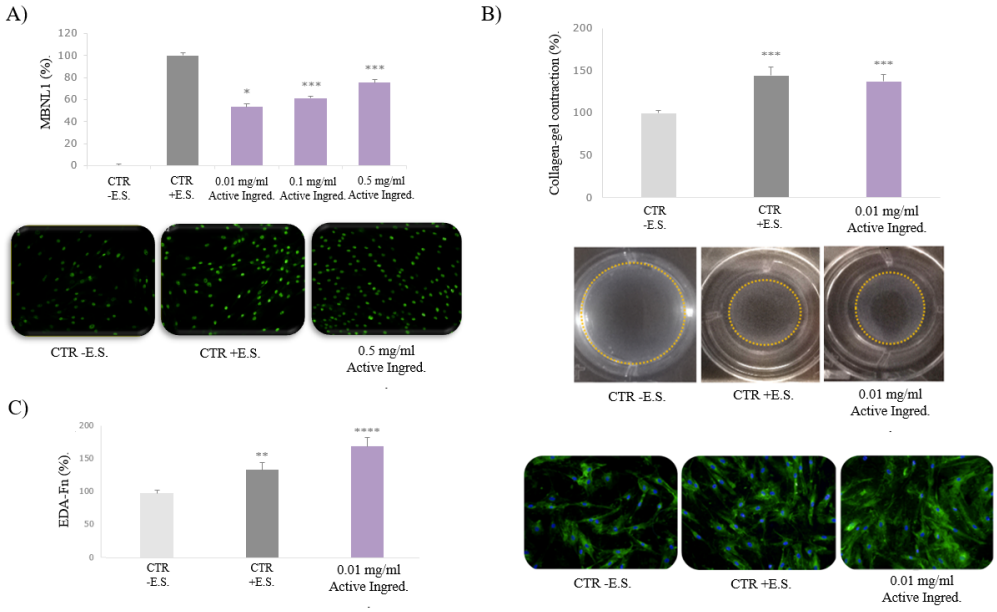

Figure 1. A) *Upper panel*: % MBNL1 levels in human fibroblasts after electrical stimulation (CTR +E.S.) and after active ingredient treatment at different concentrations. *Lower panel*: representative images showing MBNL1 protein expression. **B)** *Upper panel*: % of collagen-gel contraction after electrical stimulation (CTR +E.S.) and active ingredient treatment. *Lower panel*: representative images showing the average collagen gel contraction. **C)** *Left panel*: quantification of EDA-Fn levels in human dermal fibroblasts after electrical stimulation (CTR +E.S.) and active ingredient treatment. *Right panel*: representative images of EDA-Fn staining in human dermal fibroblasts. Statistical significance: (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001), using an unpaired T-test. CT-E.S.; non-treated control experiment not exposed to electrical stimulation.

In the clinical study, volunteers showed a reduction of the visibility of wrinkles on the cross-feet region of -5.9%, similar to the effect obtained for the group applying a microcurrents device (Figure 2A). An eyelid lifting effect was observed after 28 days of the active ingredient application (Figure 2B), with an increase of the vertical distance by 6.1%, which was comparable to the effect obtained by using the microcurrents device. As shown in Figure 2C, treatment with the active cream also induced a reduction of eyebrows sagging, leading to a significant eyebrow lifting effect comparable to microcurrents application. Finally, a

cheek lifting effect was determined and depicted by arrows indicating the extent and direction of skin displacement occurred after 28 days of active or placebo cream application (Figure 2D).

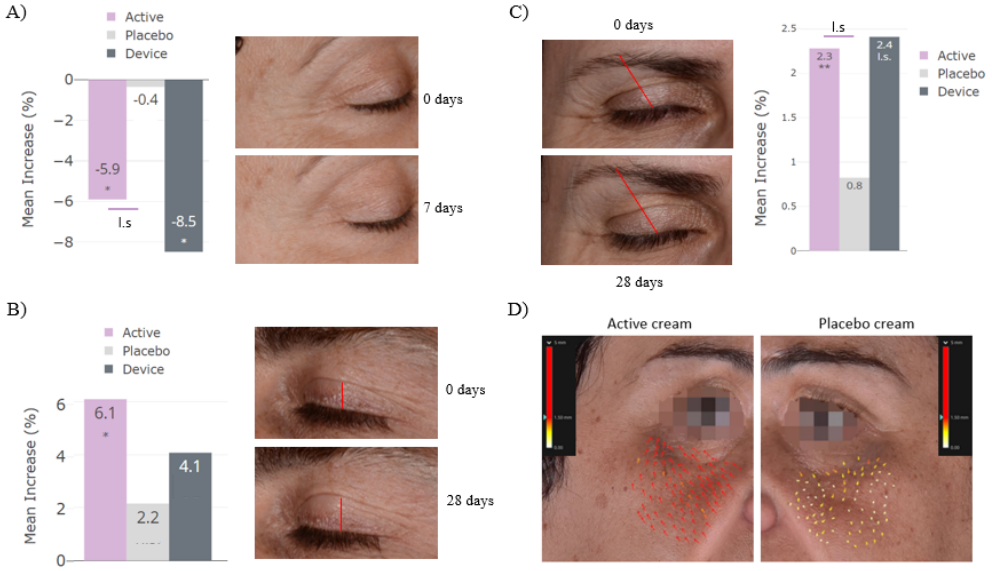

Figure 2. A) *Left*: quantification of coefficient of visibility of wrinkles in the cross-feet region after 7 days. *Right:* representative image showing the anti-wrinkle effect after 7 days of active cream application. **B)** *Left:* quantification of the eyelid lifting effect after 28 days. *Right*: representative image showing the eyelid lifting effect after 28 days of active cream treatment. **C)** *Left:* representative image showing the eyebrow lifting effect after 28 days of active cream treatment. *Right*: quantification of the eyebrow lifting effect after 28 days. **D)** Representative image showing the cheek lifting effect obtained after 28 days of active or placebo cream treatment, showed as multiple arrows indicating the extent and direction of skin displacement. Statistical significance: (l.s $p<0.1$, *$p<0.05$, **$p<0.01$), using a paired T-test.

Conclusion

There is a demand for cosmetic solutions to generate visibly noticeable and fast results. In response, beauty devices, like microcurrents, have grown in popularity, but accessibility due to high price and potential side effects limits their acceptance. In this study, we have investigated the impact of external microcurrent stimulation on fibroblast functionality and have validated the role of MBNL1 and EDA-fibronectin in the contraction of the dermal layer. Further, we were able to develop a novel active ingredient that could mimic these biological processes to generate comparable effects *in vitro* and *in vivo*. The active ingredient has been shown to induce the contraction of a 3D collagen gel and clinically, generate a facial lifting effect in the eyebrow, eyelid, and cheek, as well as visibly reducing the appearance of wrinkles, to generate device-like skin benefits.

References

1. Saniee F., H. Ghafarian Shirazi H.R., Khademi Kalantari K. *et al*., *Life Science J*. **9(3),** 1184-1189 *(2012)*.
2. Saniee F., Khademi Kalantari K., Yazdanpanah P. *et al., Journal of Jahrom University of Medical Sciences*. **10(2)**, 8-15 *(2012)*.
3. Golberg A., Khan S., Belov V. *et al., Sci Rep.* **5**, 10187 *(2015)*.
4. Jennings J., Chen D., Feldman D., *Bioelectromagnetics*. **29(5)**, 394-405 *(2008)*.
5. Rouabhia M., Park H., Meng S. *et al.*, *PLoS One*. **8(8)**, e71660 *(2013)*.
6. Davis J., Salomonis N., Ghearing N. *et al., Nat Commun.* **6**, 10084 *(2015)*.
7. Serini G., Bochaton-Piallat M.L., Ropraz P. *et al., J Cell Biol*. **142(3)**, 873-881 *(1998)*.
8. Kohan M., Muro A.F., White E.S., Berkman N., *FASEB J*. **24(11)**, 4503-4512 *(2010)*.

About the speaker

Dr. Cristina Carreño is the Global Business Development Director of Lipotec™ Active Ingredients at Lubrizol.

She received her PhD in Organic Chemistry from the University of Barcelona in 1997, where she specialized in both peptide and combinatorial chemistry. For nine years she was in charge of the Peptide Synthesis Facility at the University of Barcelona, after which she joined the research company of Lipotec Group managing the discovery of new compounds for the pharmaceutical and cosmetic industry. Since 2008 she also took responsibility for the New Business Development area in the company for active ingredients on a global basis.

Dr. Carreño is the author of several patents in the field of new cosmetic actives.