Regulate the human skin elasticity by weak electric field

NT_180

Se Jik Han[1,2]; Minjoo Noh[3]; Jihui Jang[3]; Jun Bae Lee[3]; Kyung Sook Kim[2*]

[1]Department of biomedical engineering, Graduate school, Kyung Hee University, Seoul, Korea;
[2]Department of biomedical engineering, College of medicine, Kyung Hee University, Seoul, Korea;
[3]Department of innovation, Innovation Lab, Cosmax R&I Center, Gyeonggi do, Republic of Korea
[*]To whom correspondence should be addressed. Email: moosou94@khu.ac.kr

Introduction:

The elasticity of human skin has been suggested as an indicator of health and aging. Skin elasticity indicates skin's ability to stretch and snap back to its original shape. Skin loses elasticity by aging or external stress, such as sun exposure, air pollution, poor nutrition, and smoking [1,2]. Various skincare products (collagen supplements, retinol, etc.) have been developed to improve or restore skin elasticity. Electric field (EF) stimulation has been used to change biological functions (proliferation, regeneration, etc.) of cells and tissues [3–8]. Previous studies have confirmed that EF stimulation affects cell elasticity and increases collagen production in skin tissues [4,9]. Therefore, EF stimulation is suggested as non-invasive skin therapy.

This study investigated the elasticity change of human dermal fibroblast (HDF) and artificial skin induced by weak EF stimulation using Atomic Force Microscopy (AFM) and rheometer, respectively. The effects of EF on the expression level of elasticity-related proteins were also analyzed, including collagen type Ⅰ & Ⅶ, fibrillin, and elastin.

Materials & Methods:

I. Electric field stimulation

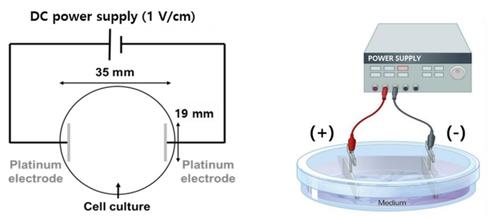

Fig. 1 Schematic of EF system

- The platinum electrode was immersed directly in cell medium to apply an electric field stimulus.
- **EF** – Strength : 100 mV/mm
  Time : 0 ~ 1,800 second
- Experiments was observed after 24 hours of stimulation.

II. Western blotting analyses

- Analysis of expression level of skin elasticity-related proteins (collagen type Ⅰ & Ⅶ, elastin, and fibrillin)
- F-actin & G-actin.

III. Elasticity of cell and artificial skin

- Cell was fix by 4%paraformaldehyde.
- To measure the elasticity of HDF, press the cell with an AFM tip as shown in Fig. 2A. The force distance (FD) curve was measured to extract the elasticity of the cell.
- The elasticity of the artificial skin was measured using a Rheometer. Fig. 2B.

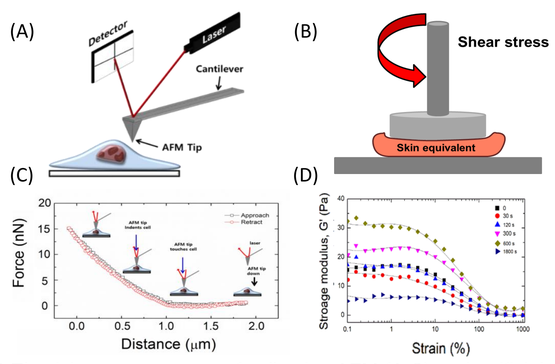

Fig. 2 Elasticity measurement of cell using AFM (A,C) and artificial tissue using rheometer (B,D)

IV. Collagen in tissue

- **Thickness** – The artificial skin was stained with Sirius Red stain following standard procedures for histological analysis.
- **Content** – Quantitatively analyze the change in collagen by dissolving artificial skin using a total collagen assay kit.

Results & Discussion:

I. The expression levels of biomarkers of cellular elasticity

- The mRNA expression level of procollagen type Ⅰ increased in response to the EF in both cells. (Fig. 3A)
- Collagen type Ⅶ decreased by EF in young HDF, while it increased after 30 sec in old HDF. (Fig. 3B)
- In young HDF, fibrillin and elastin increased by EF stimulation up to 60 sec and decreased at 120 sec.
- In contrast, in the old HDF, fibrillin and elastin did not show significant changes and were slightly decreased compared to the control group.

Fig. 3 Expression levels of elasticity-related protein collagen type Ⅰ (A), collagen type Ⅶ(B), fibrillin (C), and elastin (D)

II. Change of cellular elasticity

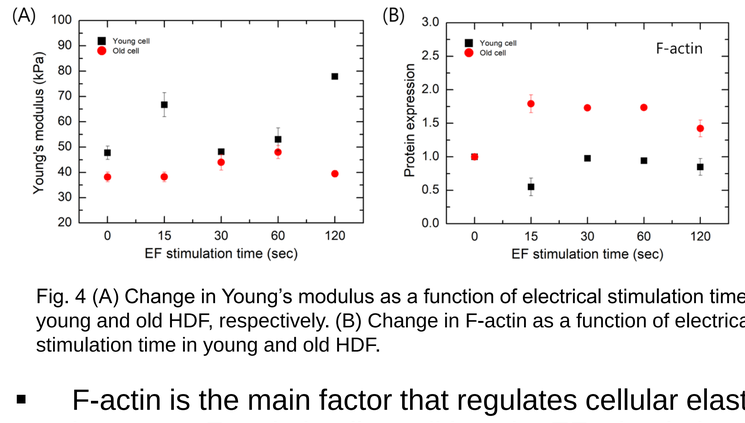

Fig. 4 (A) Change in Young's modulus as a function of electrical stimulation time in young and old HDF, respectively. (B) Change in F-actin as a function of electrical stimulation time in young and old HDF.

- Young HDF increased Young's modulus at 15 and 120 sec, and at 30 and 60 sec, they were similar to the control group. (Fig. 4A)
- In the old HDF, the Young's modulus gradually increased up to 60 sec by EF stimulation and decreased at 120 sec.
- F-actin is the main factor that regulates cellular elasticity. We confirmed that old HDF increases F-actin in all conditions by EF stimulation.
- However, the young HDF showed no significant change or decreased compared to the control group (Fig. 4B)

III. Alteration of artificial skin collagen

- The Thickness change of artificial skin by EF stimulation was investigated by histological analysis.
- When an EF was supplied for 600 sec, the thickness increased on the 1 and 5 days after stimulation.
- However, at 1800 sec, thickness was similar to the control group (Fig. 5A)
- The total amount of collagen in the artificial skin showed a slight change induced by EF stimulation (Fig. 5B)

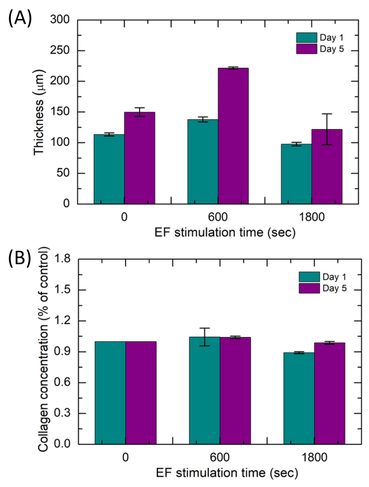

Fig. 5 (A) Thickness of artificial skin and (B) total collagen concentration as a function of EF stimulation time.

IV. Elasticity of artificial skin

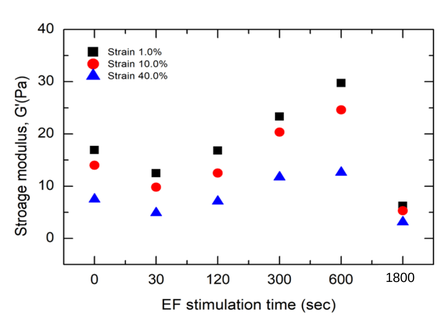

Fig. 6 Storage modulus(G') of artificial skin as a function of EF stimulation time at specific strain of 1.0, 10.0, and 40.0%.

- EF stimulation changed the viscoelastic properties of artificial skin.
- The viscoelasticity of artificial skin increased in all conditions up to 600 sec of EF stimulation.
- However, at 1,800 sec, the viscoelasticity decreased in all conditions (Fig. 6).

Conclusions:

The cellular elasticity of HDF was changed by EF, and the change showed dependency on the cell ages. F-actin contents were also changed by EF stimulation; however, the changes of F-actin showed no relation with the cellular elasticity. The thickness and content of collagen in the artificial skin were affected by EF stimulation, which resulted in viscoelastic changes of the skin.

Aknowledgments:

This research was supported by the Creative Industry Professional Development Program through the Ministry of Trade and Industry & Energy, Republic of Korea (Project No: 20002725), and Kyung Hee University (KHU-20181046).

References:

1. Kohl, E., Steinbauer, J., Landthaler, M., and Szeimies, R. M. (2011) Skin ageing. J. Eur. Acad. Dermatology Venereol 25(8):873-84.
2. Sibilla, S., Godfrey, M., Brewer, S., Budh-Raja, A., and Genovese, L. (2015) An overview of the beneficial effects of hydrolysed collagen as a nutraceutical on skin properties: Scientific background and clinical studies. Open Nutraceuticals J 8: 29-42.
3. Sheikh, A. Q., Taghian, T., Hemingway, B., Cho, H., Kogan, A. B., and Narmoneva, D. A. (2013) Regulation of endothelial MAPK/ERK signalling and capillary morphogenesis by lowamplitude electric field. J. R. Soc. Interface 10(78): 20120548.
4. Titushkin, I. and Cho, M. (2009) Regulation of cell cytoskeleton and membrane mechanics by electric field: Role of linker proteins. Biophys. J 96(2):717-28.
5. Hernández-Bule, M. L., Paíno, C. L., Trillo, M. Á ., and Ú beda, A. (2014) Electric stimulation at 448 kHz promotes proliferation of human mesenchymal stem cells. Cell. Physiol. Biochem 34(5):1741-55.
6. Robinson, K. R. and Messerli, M. A. (2003) Left/right, up/down: The role of endogenous electrical fields as directional signals in development, repair and invasion. BioEssays 25(8):759-66.
7. Jaffe, L. F. and Vanable, J. W. (1984) Electric fields and wound healing. Clin. Dermatol 2(3):34-44.
8. Wang, E. T. and Zhao, M. (2010) Regulation of tissue repair and regeneration by electric fields. Chinese J. Traumatol. - English Ed 13(1):55-61.
9. Golberg, A., Khan, S., Belov, V., Quinn, K. P., Albadawi, H., Felix Broelsch, G., Watkins, M. T., Georgakoudi, I., Papisov, M., Mihm, M. C., Austen, W. G., and Yarmush, M. L. (2015) Skin rejuvenation with non-invasive pulsed electric fields. Sci.Rep 5(1):10187.