Involvement of Electrical Stimulation in Skin Wound Healing Processes

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Introduction: Endogenous electrical fields (EF) in human body play critical roles including the electrical activation of the nervous system and muscles. Human body also generates transepithelial electrical potentials (TEP) ranging between 10 and 60 mV at various locations[1,2]. This TEP is involved in wound healing by promoting cell migration from wound edges[3]. Injured epidermis is characterized by a short circuit of the TEP, giving rise to a measurable DC current efflux between 1 and 10 μA/cm^2 and an estimated current density up to 300 μA/cm^2 near the edge of the wound[3,4,5]. Such wound current corresponds to a relatively steady local EF between 40 and 200 mV/mm. This EF persists till the complete wound re-epithelialization[4,6,7,8]. During the process of skin wound healing, epithelial cells are actively involved in the re-epithelialization[3,9]. It is also important to consider the role of fibroblasts in the healing and remodeling processes; these may be modulated by EF. Thus, the aim of the present study was to evaluate the effect of electrical stimulation (ES) on human skin fibroblast behaviors and activities.

Materials and Methods: Normal human skin fibroblasts were obtained from ScienCell Research Laboratories (Carlsbad, CA, USA). The fibroblasts were cultured in Dulbecco's modified Eagle's medium, then used at passages 4 and 5 to investigate the effect of ES on wound healing. Cells were cultured onto the heparin-bioactivated conductive PPy/PLLA membranes that have been connected to a DC constant potential source through external electrodes. Two potential intensities were tested in this study, which were 50 and 200 mV/mm. The cells were exposed to ES for 2, 4 or 6 h, and were further cultured for 24 h prior to analyses. Sham ES-exposed control groups followed the same conditions except exposure to ES. After each culture period, the cell viability/growth was investigated by measuring the lactate dehydrogenase (LDH) activity in culture supernatant using an LDH cytotoxicity assay (Promega, Madison, WI, USA). This was supported by evaluating cell viability through trypan blue exclusion assay[10]**.** The involvement of ES in wound healing was investigated by cell migration/monolayer wound repair assay as previously described[11]. This was supported by the measurement of fibroblast growth factors (FGF1 and FGF2) secretion by the electrically stimulated fibroblasts. Finally, we investigated the effect of ES on fibroblast activities to contract collagen gel matrix and on the expression of a high level of α-SMA.

Results: A bioactivated PPy/HE/PLLA membrane was produced showing low percolation threshold of conductivity at only 5% of PPy. This membrane was semi-conductive (10^{-3} S/cm) and showed no toxic effect towards normal human fibroblasts. Indeed, measurement of LDH demonstrated that no necrotic effect was induced in electrically (50 or 200 mV/mm) stimulated fibroblasts as compared to the controls. This was confirmed by the high cell viability showing

good cell adhesion and proliferation. It is important to note that, ES promoted FGF1 and FGF2 secretion by fibroblasts. ELISA measurement showed that the levels of FGF1 and FGF2 were grater in the ES-exposed fibroblasts as compared to that in the controls, confirming the contribution of ES to cell growth and wound healing. To further confirm the role of ES in wound healing we performed a cell migration assay on wound created on a monolayer culture. Our results showed (Fig. 1) that ES promoted cell migration from both scratched edges to cover the cell free space. This cell migration was dependent on the ES-exposure time because that the fibroblasts exposed to ES for 6 h migrated faster than the control, the 2 h exposed and even the 4 h exposed cells. Using a collagen contraction assay, we were able to demonstrate that the fibroblasts that had been exposed to ES recorded greater gel contraction as compared to the non-ES exposed cells. This effect was more significant at 200 mV than at 50 mV. The contractile capacity of the ES-exposed fibroblasts is due to the high α-SMA level expressed by these cells.

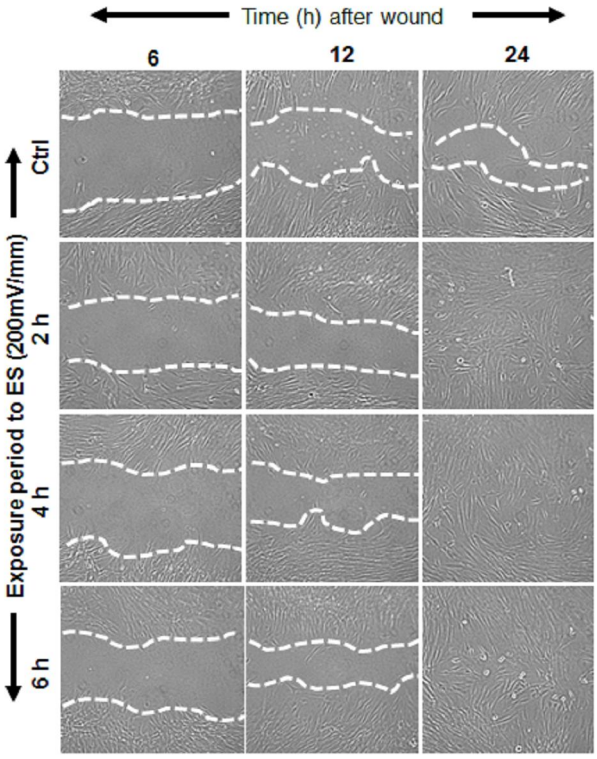

Fig. 1: Fibroblast migration after exposure to ES

Conclusions: Conductive polymer based ES promotes fibroblast adhesion and growth. This is mediated by an increase in FGF1 and FGF2 secretion. ES-exposed fibroblasts migrate well and were more able to contract a collagen gel through the expression of a high level of α-SMA. Altogether, data demonstrated that conductive polymer meditated ES promoted wound healing through a possible mechanism that implicated FGF1 and FHG2. *(This study was financially supported by a CIHR operating grant).*

References

1. Foulds IS, Barker AT. 1983 Br J Dermatol 109:515-22.
2. Zipse DW. 1993 Ieee Trans Industrial Appl 29:447-58.
3. Zhao M. 2009 Cell Dev Biol 20:674-82.
4. Barker AT, Jaffe LF, Vanable JW Jr. 1982 Am J Physiol Regul Integr Comp Physiol 242:358-66.
5. Zhao M, Song B, Pu J, Wada T, Reid B et al., 2006 Nature 442:457–60.
6. McGinnis ME, Vanable JW Jr. 1986 Dev Biol 116:184-93.
7. Chiang M, Robinson KR, Vanable JW Jr. 1992 Exp Eye Res 54:999-1003.
8. Sta Iglesia DD, Vanable JW Jr. 1998 Wound Repair Regen 6:531-42.
9. Guo A, Song B, Reid B, Gu Y et al., 2010 J Invest Dermatol 130(9):2320-7.
10. Semlali A, Jacques E, Rouabhia M, Milot J, Laviolette M et al., 2010 Allergy 65:1438-45.
11. Semlali A, Chakir J, Rouabhia M. 2011 J Toxicol Environ Health A 74(13):848-62.