Pulsed Electrical Stimulation Activates Fibroblast Migration, Collagen Contraction, α-smooth muscle actin expression and Smad 2/3 phosphorylation

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Statement of purpose: Skin wound healing is characterized by the involvement of multiple physiological events where growth factors, cytokines, cell migration, tissues contraction take place (1, 2). During these evens fibroblasts play an important role in granulation tissue formation and wound repair (3). On the other hand, the interplay between these physiological and biochemical events with the endogenous electrical field (EF) has been recognized as a potentially important but still unveiling biological cue that accompanies the onset of wounds until re-epithelialization (4). However, the material platform and electrical stimulation (ES) methodology are under development. The mechanistic pathway of how cells reacting to ES remains primitive. The aim of this study was to investigate the healing characteristics and the underlying signaling pathway of human dermal fibroblasts under the influence of pulsed electrical stimulation (PES).

Materials and Methods: A conductive textile made of electrically conducting polypyrrole (PPy) coated on woven polyethylene terephthalate (PET) micro-fibres was used as a cell culture scaffold to deliver four different PES regimes to human skin fibroblasts. After 24 hours PES exposure, cells were harvested for the following assays. 1) Monolayer wound healing/cell migration; 2) Gel contraction; 3) Fibroblast growth factor (FGF1 and FGF2) gene expression and protein secretion; 4) The expression of α-smooth muscle actin (α-SMA), a marker of myo-fibroblast; and finally, 5) Smad signaling pathway.

Results: In cell monolayer scratching and migration assay mimicking wound healing, the fibroblasts previously experienced PES recorded higher migration speed in comparison with the non-stimulated cells, as evidenced by the narrower separation between wound edges at different culture times up to cell confluence. The gel contraction assay showed that the PES regimes rendered the cells more contractile compared with the non-electrically treated fibroblasts. The enhanced cell migration was supported by a significant ($p < 0.05$) increase in FGF2 secretion by the PES-exposed fibroblasts. The more contractile property was supported by the upregulated transcription of the messenger RNA of α-SMA which was further confirmed by the IHC staining of the stress fibres. Interestingly, the PES regimes were found to modulate the fibroblast activities through Smad signaling pathway. Indeed, the PES increased total Smad2 and Smad3, compared to the control. This was paralleled by an increase in phosphorylated Smad2 and Smad3. This was supported by the translocation of Smad2/3 dimer from the cytoplasm to the nucleus of the PES treated fibroblasts.

Conclusions: This study demonstrated that the PES regimes used in this experiment induced fibroblast growth and migration through growth factor secretion. Fibroblasts to myo-fibroblasts trans-differentiation was confirmed by the high expression of α-SMA in the electrically stimulated cell populations, confirming the shift of fibroblast to myo-fibroblasts. The effect of PES involves Smad2/3 signaling pathway.

References:

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