Electrical stimulation through PPy/HE/PLLA conductive membrane improved wound healing properties of diabetic fibroblasts

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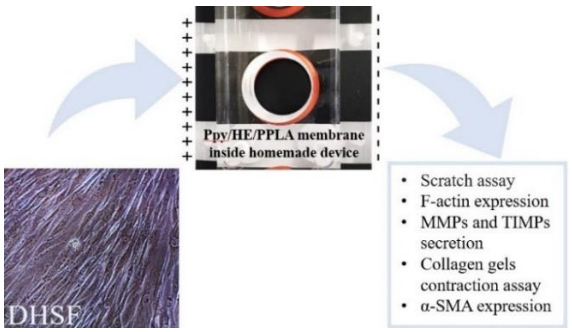
Statement of Purpose: The global diabetes prevalence was estimated to 9.3% in 2019 to 10.2% by 2030 and 10.9% by 2045.[1] Diabetic foot ulcer (DFU) is a common problem among the diabetic population. DFU is challenging to health care systems and is one of the most costly health problems.[2] DFU is mostly characterized by a prolonged chronic inflammatory phase, the impaired proliferation of keratinocytes and fibroblasts, and a decrease in the tensile strength of skin wound repair.[3] Considering the key roles of fibroblasts and keratinocytes in wound healing, it is important to find a way to promote cell proliferation in DFU. Previous studies demonstrated that electrical stimulation (ES) promoted the proliferation of fibroblasts extracted from skin of healthy donnors.[4] In our recent study, we used a PPy/HE/PLLA membrane to expose the diabetic human skin fibroblast (DHSF) to ES for the first time.The PPy/HE/PLLA membrane had a stable conductivity and showed a biocompatibility with the DHSF. We demonstrated that a low intensity of ES promoted the proliferation of the DHSF.[5]The purpose of the present study was to investigate the effects of ES on the migration of diabetic fibroblasts, their capacity to contract a collagen scaffold, and the secretion of the proteolytic enzymes involved in wound healing.

Methods: The DHSF were isolated from the skin tissues collected from 8 diabetic patients (61 to 80 years old) following the leg amputation surgery. The tissue collection was appoved by the Ethical Committee of the CHU-Université Laval and the patients gave the informed-consent. The ES was dilivered through the conductive PPy/HE/PLLA membranes synthesized in the lab. The DHSF at passage 4 and 5 were seeded on the membrane and subjected to ES at 20 or 40 mV/mm for 6h and 24h. The ability of the DHSF to migrate toward the wound in a monolayer scratch assay was investigated by the Hoechst 33342 staning for cell nuclei and the staining of F-actin by phalloidin (P5282, Sigma- Aldrich). Also, the ability of the DHSF to modulate extracellular matrix (ECM) was investigated by measuring the secretion of MMPs (Matrix Metalloproteinases) and TIMPs (Tissue Inhibitors of Metalloproteinases) using a Human MMP and TIMP Array Panel kit (Millipore, St. Charles, MO, USA). The capacity of the fibroblasts to contract wound and the presence of myofibroblasts were studied using a fibsoblast-populated collagen gel and by staining α-smooth muscle actin (α-SMA). All the exprements were repeted 3 times and the statistical significance of the differences between the non-ES (control) and ES (test) values was determined by One-Way ANOVA; *p* values were declared significant at ≤0.05 .



(Figure1: Extraction of DHSF, expose to ES, and cell behavior analyzes.)

Results: The exposure to ES increased the production of F-actin at the edges of each cell, indicating upregulated migration. This was confirmed by the reduced wound size in the cell monolyer scratch assay in the electrically stimulated culture as compared to the non-stimulated one. Indeed, the non-ES DHSF monolayer showed about 50% open wound after a 48 h culture, while the fibroblasts exposed to 20 mV/mm for 6 and 24 h were able to migrate and cover 75% and 81% of the wound area, respectively. Similar effect was also observed at 40 mV/mm. The Cytokine Array test revealed that the ES has a significant effect on MMPs and TIMPs secretions. Indeed, following the exposure to ES at 20 mV/mm for 6 h, the MMP2 and MMP-7 increased more than about 50% and 30% respectively ($p<0.001$). After a 24h incubation, the collagen gel contraction was at miximum. The collagen contraction could be explained by the presence of α-SMA positive cells as demonstrated by the immunohistochemical staining.

Conclusion: The presence of the F-actin as a crucial protein in cell migration at the edge of the DHSF after ES and the results of the cell monolayer scratch assay demonstrated that the ES accelerated the migration of the DHSF toward the wound. Also, ES by affecting the level of MMPs and TIMPs contributed to ECM remodeling. Moreover, the collagen gel contraction and the presence of the myofibroblasts highlighted the posibility of restoration of the functionality of the DHSF in wound contraction following ES.

References: 1- Saeedi P. Diabetes Res. Clin. Pract.2019;1: 107843 **2**-Mariam TG.J. Diabetes Res. 2017; 16 **3**-Sharifian Z. Laser Med Sci. 2014; 29:1227-35. **3**-Lerman, Oren Z. *Am J Pathol*. 2003;162: 303-312. **4**- Rouabhia M, PloS One. 2013; 8:e71660. **5**-Abedin-Do A. Biology. 2021;10:641.