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Wound Healing Process Studied on DC Non-Homogeneous Electric Fields

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Abstract : Cell migration, wound healing and regeneration are some of the physiological phenomena in which electric fields (EFs) have proven to have an important function. Physiologically, cells experience electrical signals in the form of transmembrane potentials, ion fluxes through protein channels as well as electric fields at their surface. As soon as a wound is created, the disruption of the epithelial layers generates an electric field of ca. 40-200 mV/mm, directing cell migration towards the wound site, starting the healing process. In vitro electrotaxis, experiments have shown cells respond to DC EFs polarizing and migrating towards one of the poles (cathode or anode). A standard electrotaxis experiment consists of an electrotaxis chamber where cells are cultured, a DC power source and agar salt bridges that help delaying toxic products from the electrodes to attain the cell surface. The electric field strengths used in such an experiment are uniform and homogeneous. In contrast, the endogenous electric field strength around a wound tend to be multi-field and non-homogeneous. In this study, we present a custom device that enables electrotaxis experiments in non-homogeneous DC electric fields. Its main feature involves the replacement of conventional metallic electrodes, separated from the electrotaxis channel by agarose gel bridges, through electrolyte-filled microchannels. The connection to the DC source is made by Ag/AgCl electrodes, incased in agarose gel and placed at the end of each microfluidic channel. An SU-8 membrane closes the fluidic channels and simultaneously serves as the single connection from each of them to the central electrotaxis chamber. The electric field distribution and current density were numerically simulated with the steady-state electric conduction module from ANSYS 16.0. Simulation data confirms the application of nonhomogeneous EF of physiological strength. To validate the biocompatibility of the device cellular viability of the photoreceptor-derived 661W cell line was accessed. The cells have not shown any signs of apoptosis, damage or detachment during stimulation. Furthermore, immunofluorescence staining, namely by vinculin and actin labelling, allowed the assessment of adhesion efficiency and orientation of the cytoskeleton, respectively. Cellular motility in the presence and absence of applied DC EFs was verified. The movement of individual cells was tracked for the duration of the experiments, confirming the EF-induced, cathodal-directed motility of the studied cell line. The in vitro monolayer wound assay, or “scratch assay” is a standard protocol to quantitatively access cell migration in vitro. It encompasses the growth of a confluent cell monolayer followed by the mechanic creation of a scratch, representing a wound. Hence, wound dynamics was monitored over time and compared for control and applied the electric field to quantify cellular population motility.

Keywords : DC non-homogeneous electric fields, electrotaxis, microfluidic biochip, wound healing
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