LAB-ON-A-CHIP DEVICES WITH NANOPHASE SURFACE TOPOGRAPHY FOR NEURAL ELECTROPHYSIOLOGICAL APPLICATIONS

By

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ABSTRACT

In this study, a novel class of neural electrophysiological devices featuring vertically aligned three-dimensional conducting nanostructures was designed and fabricated. This class of device features enhanced *(i)* cell-biomaterial interaction (resulting from the coating of the device nanostructured electrodes with poly-D-lysine and laminin, and from the structured topography of these electrodes), in combination with enhanced *(ii)* signal selectivity (originating from a spatial resolution ranging between $1.20*10^{-3}$ channels$/\mu m^2$ and 1.68 channels$/\mu m^2$, and a temporal resolution of 2 μs/sample) and *(iii)* signal discrimination (emerging from the signal-to-noise ratio for a cell generating an action potential, relative to its noise background level at rest, measured equal to 12.64 dB).

The deflection of the three-dimensional electrode tips generated by neurons adhering on the hierarchically-structured substrates over multiple weeks was used to transduce the elastoplastic force, energy and power generated by a neuron to adhere on a substrate. These dimensions were found to be a strong function of scale and were therefore measured at three different spatial resolutions – *(i)* at the focal adhesive level (invariant focal adhesive force of 16.5 μN, strain energy between 10 pJ and 15 pJ and strain power ranging from 175 aW after the first day of culture, to approximately 10 aW measured 20 days after cell seeding), *(ii)* at the dendritic levels (focal adhesive force varying between a minimum of 20 μN to almost 45 μN, strain energy varying between 40 pJ and less than 10 pJ, while the strain power varies between a maximum of 425 aW after the first day of culture, to less than 25 aW, measured 20 days after cell seeding occurred) and *(iii)* at the aggregate cellular level (adhesive force varying between a minimum of about 50 μN to 750 μN, adhesive strain energy between 35 pJ and 750 pJ measured at day 20, maximal strain power one day after neuron seeding, equal to 2.8 fW [1], and subsequently decreased to a minimum of approximately 100 aW measured at day 12 after seeding).

[1] 1 fW = 10^{-15} W; 1 aW = 10^{-18} W

These results suggest a relative invariance of force and energy at the focal adhesive level, while at the dendritic and cellular levels both force and energy are found to vary greatly during the weeks of the experiment; at the cellular level, force and energy reach a maximum during the last day of the experiment, indicating a likely increase for these two dimensions for longer time intervals, therefore leading to conclude that at this level adhesive force and energy are a strong function of cell diversification – specifically cellular increase in area and production of dendritic extroflections or arborizations. Conversely, the strain power was maximal for all the levels of analysis, at day one after seeding. This factor seems to suggest that the high power expenditure of the cell during the initial stages of development is necessary both to anchor the spherically-shaped and intrinsically unstable cell on the substrate, as well as to differentiate the spherical geometry – typically a very stable, low energy configuration – into a diversified, polar morphology, with considerably higher surface and associated energy.

The fabricated device was interfaced with appropriate signal preamplification, amplification, data acquisition hardware and software, to record voltage signals over time for extracellular action potentials as well as resting potentials generated by neuronal cells grown *in vitro*. The substrate was appropriately coated with poly-D-lysine and laminin to enhance cell-biomaterial interaction at the interface level. Individual action potentials were successfully detected, subsequent to a short train of electrical stimulation.

The voltage for an action potential relative to the resting potential measured for the same cell was then used to measure the electrical signal emanating from the cell at sub-second timescale, and was then analyzed in form of information theory, using the Kullback-Leibler definition of divergence of the distribution from a reference measure – aptly derived with few modifications from Shannon's definition of entropy – to calculate the information entropy of an action potential. The aggregate information entropy for an action potential measured using the equipment designed and fabricated in this thesis was found to be equal to 15.56 bytes, portraying a sharp difference between a slow phase and a fast phase – respectively identified as slow K^+ response and fast K^+ response in this thesis.

Finally, the experimental results gathered in this thesis are analyzed in combination with the literature to determine the thermodynamic energy balance for a neuron generating an action potential at sub-second timescale. Under the assumption of a constant capacitance, the electrical energy expense for an action potential is determined to be equal to $3.44*10^{-11}$ J, and the corresponding electrical power is calculated equal to $1.91*10^{-10}$ W. Correspondingly, the derived value for the electrical energy during a resting potential is found to be equal to $1.0*10^{-11}$ J and the corresponding electrical power expended by a cell to generate a resting potential is calculated to be equal to $5.55*10^{-11}$ W. From the literature the average of the aggregate biochemical and metabolic energy for an action and a resting potential are determined to be respectively equal to $1.83*10^{-11}$ J and $7.89*10^{-12}$ J. The second principle of thermodynamics is then applied to estimate the entropy variation during the generation of an action potential – calculated equal to $5.18*10^{-14} J/K$ – and during a period of resting potential – calculated equal to $6.79*10^{-15} J/K$. These results can correspondingly be represented in terms of entropy rates and are found to be equal to $2.87*10^{-13} J/s\,K$ for an action potential and to $3.77*10^{-14} J/s\,K$ for a resting potential, leading to conclude that the emanation of an action potential by a neuron is a physically irreversible phenomenon. Additional conclusions based on statistical thermodynamics are made to determine a lower limit for the frequency of occurrence of a macrostate (the *Wahrscheinlichkeit*), corresponding to the generation of an action potential.

In conclusion, this body of work analyzes the development of a new class of hierarchically-integrated neural electrophysiological devices featuring three-dimensional nanostructured electrodes. These devices, which feature enhanced cell-biomaterial interaction, signal selectivity and discrimination, have been successfully deployed to integrate a discussion on neuronal electrophysiology based on *(a)* mechanics, *(b)* information theory and *(c)* thermodynamics.