Supplementary Information

Neuronal and Glial Cell Co-culture Organization and Impedance Spectroscopy on Nanocolumnar TiN Films for Lab-on-a-Chip Devices

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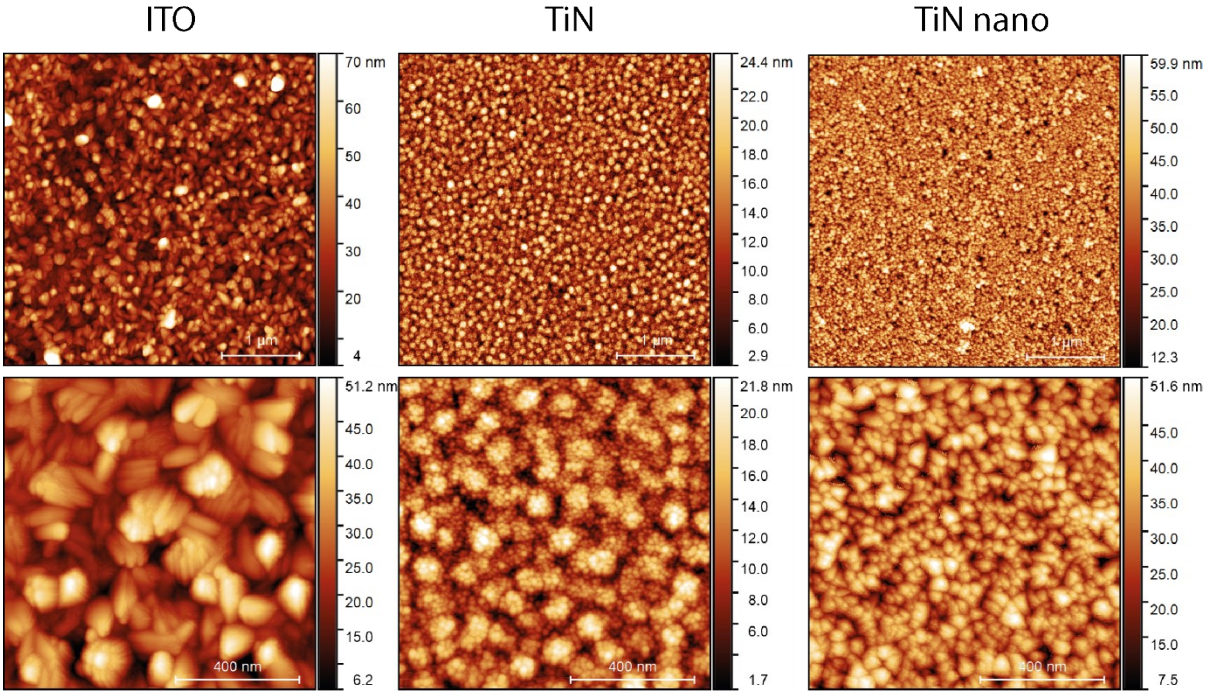
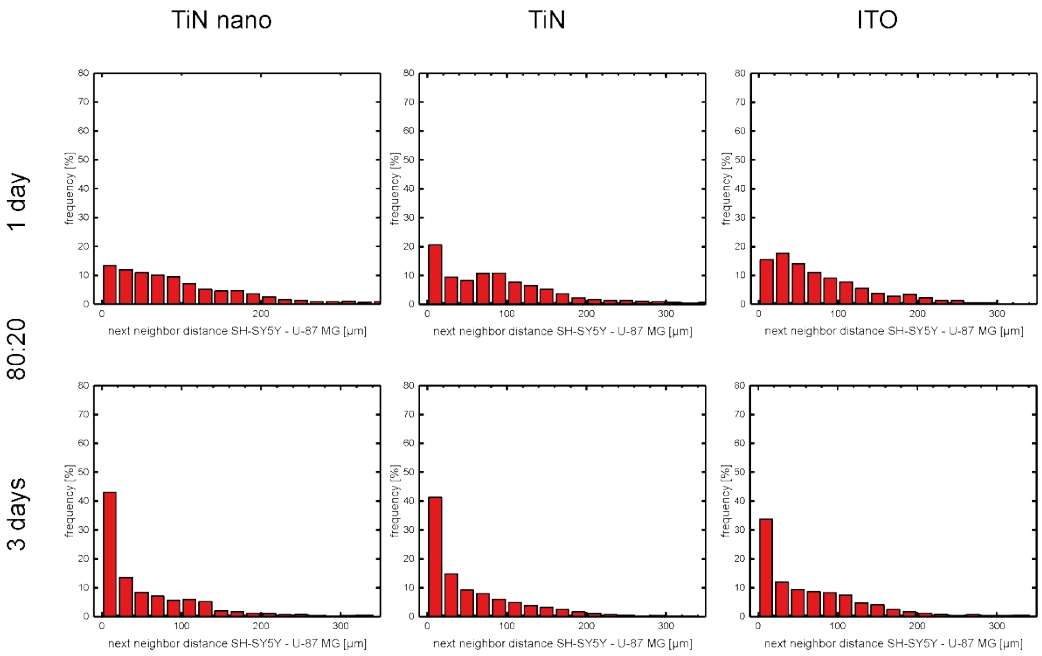
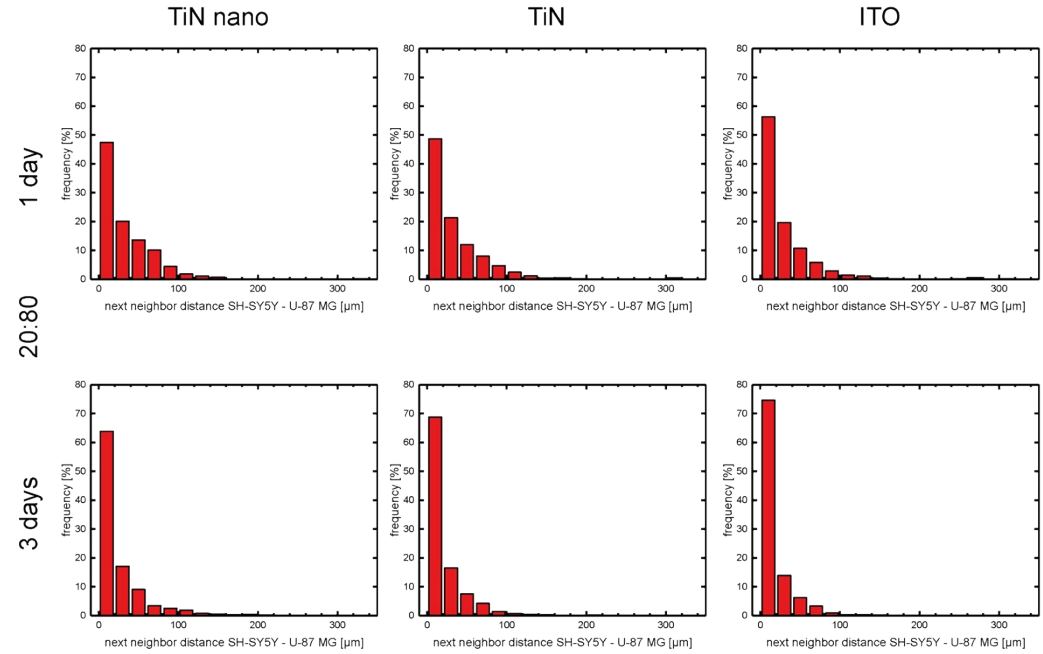


Figure S1: Atomic force microscopy-based topology characterization. The electrode materials indium tin oxide (ITO), titanium nitride (TiN), and titanium nitride with nanocolumnar structure (TiN nano) were imaged with a JPK NanoWizard 3 atomic force microscope in direct drive AC mode with a TESPAHAR cantilever (Bruker).


TiN nano
TiN
ITO
1 day
80:20
3 days
frequency [%]
next neighbor distance SH-SY5Y - U-87 MG [µm]


TiN nano
TiN
ITO
1 day
20:80
3 days
frequency [%]
next neighbor distance SH-SY5Y - U-87 MG [µm]

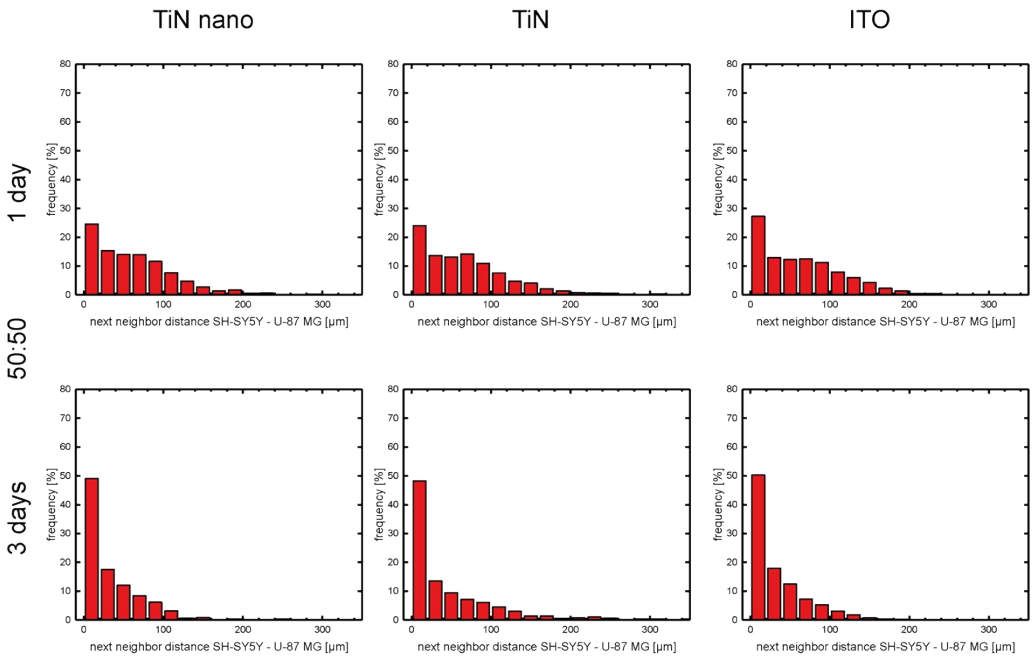


Figure S2: Histograms of center-to-center nearest neighbor distances of every neuronal cell to the next glial cell of the same data set. Cells were seeded at different ratios (80:20, 20:80, and 50:50) of SH-SY5Y to U-87 MG cells on electrode materials TiN nano, TiN, and ITO for either one or three days of culture time. Each graph represents the average of three individual samples (representing 54 experiments.

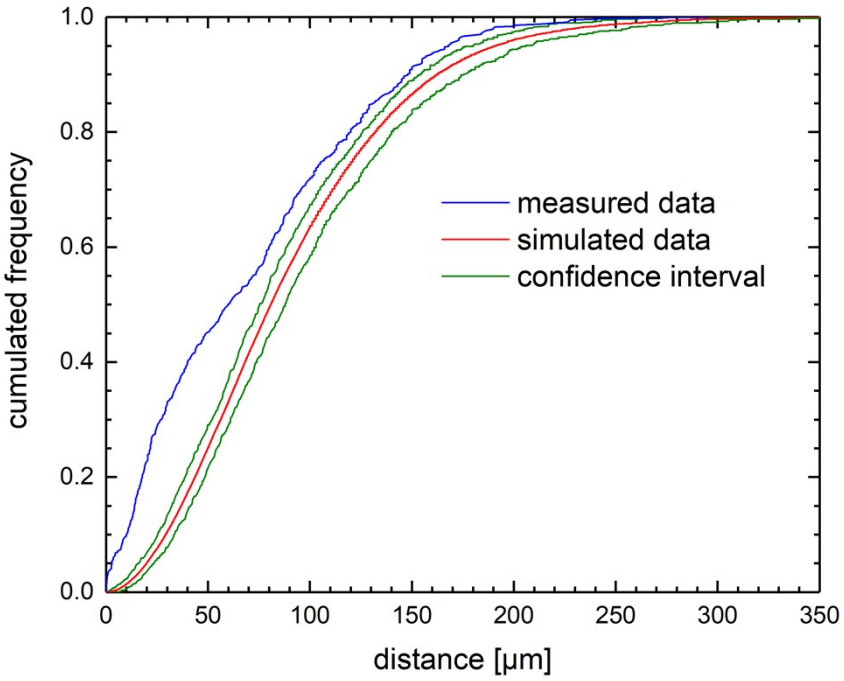


Figure S3: Cumulative distribution of center-to-center nearest neighbor distance of neuronal and glial cell nuclei. The blue curve represents the measured data, i.e. distance of nearest glial cell for every neuronal cell. We replaced the original data set of glial cell positions with 50 different simulated data sets of the same size while leaving the neuronal cell positions untouched and computed the distance of nearest glial cell for every neuronal cell (For more information about the methods see Gilles et al. [6]). The results are shown by the red curve and the 2.5 % and 97.5 % confidence interval in green. Our measured data curve is outside of the green confidence interval of randomized data meaning our results are statistically significant.

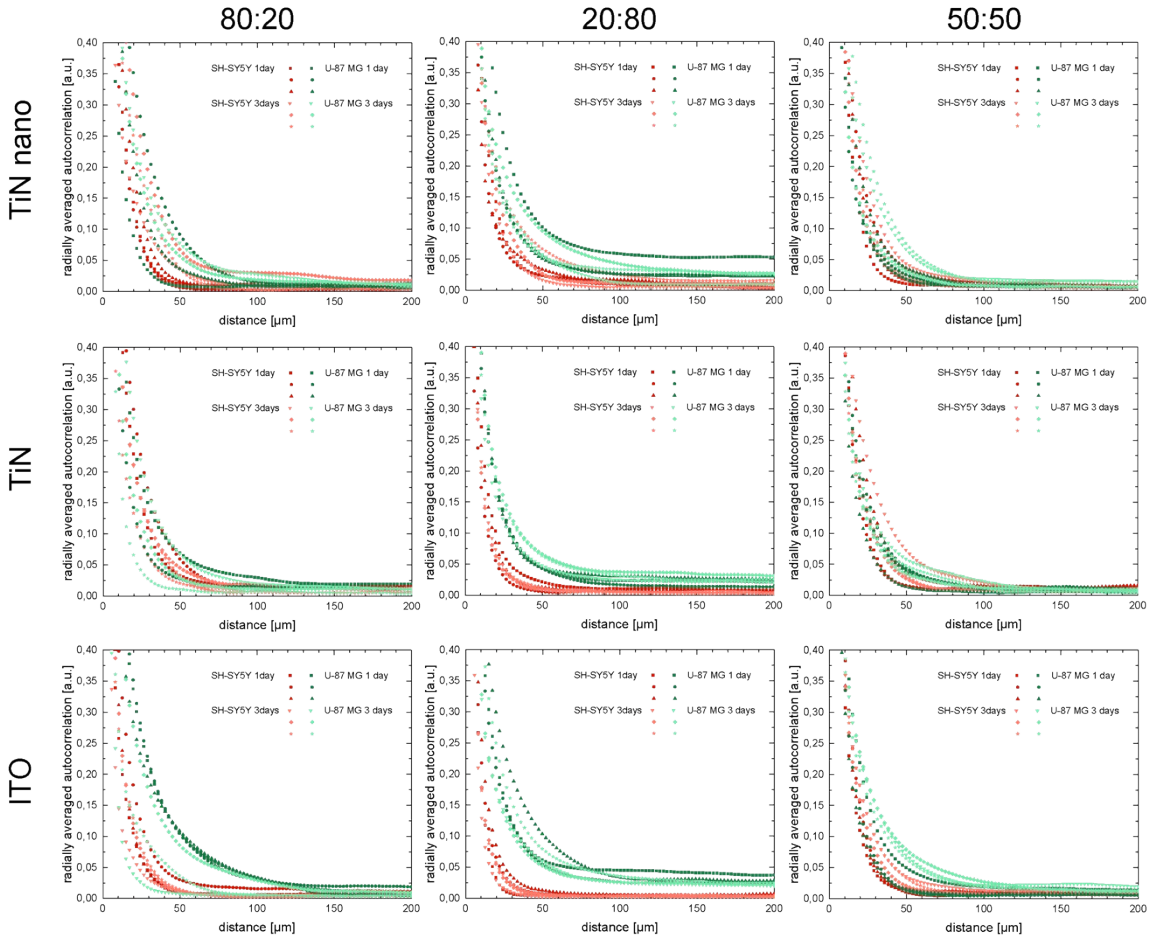


Figure S4: Radially averaged autocorrelation analysis for cell nuclei positions. Data were obtained from 54 electrode material samples (TiN nano, TiN, ITO) with varying seeding ratios of neuronal and glial cells (80:20, 20:80, and 50:50) and culture times (one day and three days). Autocorrelation curves of U-87 MG cells are shown in green and SH-SY5Y in red.

Image Analysis – Radial Autocorrelation Function:

In order to analyze the cellular network organization in terms of the nearest neighbors of each cell, the macro "Radially Averaged Autocorrelation" combined with the "Radial Profile" plugin was employed to evaluate a radially averaged two-point autocorrelation function S2 for all images as described in Abend et al.[1] Such analysis allows measurement of the average size of objects (patches of cell clusters) in conjunction with the distance between these objects as similarly shown by Baker et al. [2] and described in detail by Berryman et al.[3] Briefly, the ImageJ (Fiji distribution [4] based on ImageJ platform [5]) plugin computes the probability of finding a black pixel in increasing radial distance to an initially chosen black pixel. This process is repeated multiple times with different initial pixels. The results are radially averaged in a second step. The chosen plugin utilizes a fast Fourier transform (FFT) to reduce computation time, while simultaneously correcting for the periodicity of the FFT and finite image size, so the results do not suffer from artifacts. The results are normalized such that the value of the radially averaged autocorrelation function will always be 1 (perfect correlation) at a distance $r = 0$. It directly follows that an output value of 0 demonstrates the case of no correlation.

References

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