Supplementary information

S1. Surface characterisation of the pMEAs

The surface morphology of the printed layers was observed by means of scanning electron microscopy (SEM). Particularly, the interfaces between the polyimide (Kapton) substrate, the PEDOT:PSS-based electrode, and the SU-8 dielectric layer were investigated (Figure S1). The zoom image in figure S1b shows that both the electrode and the dielectric layer have a homogeneous surface that is smoothly adjusted on the substrate. Especially, the PEDOT:PSS layer appears as a second-skin, owing to the small amount of material that was inkjet printed.

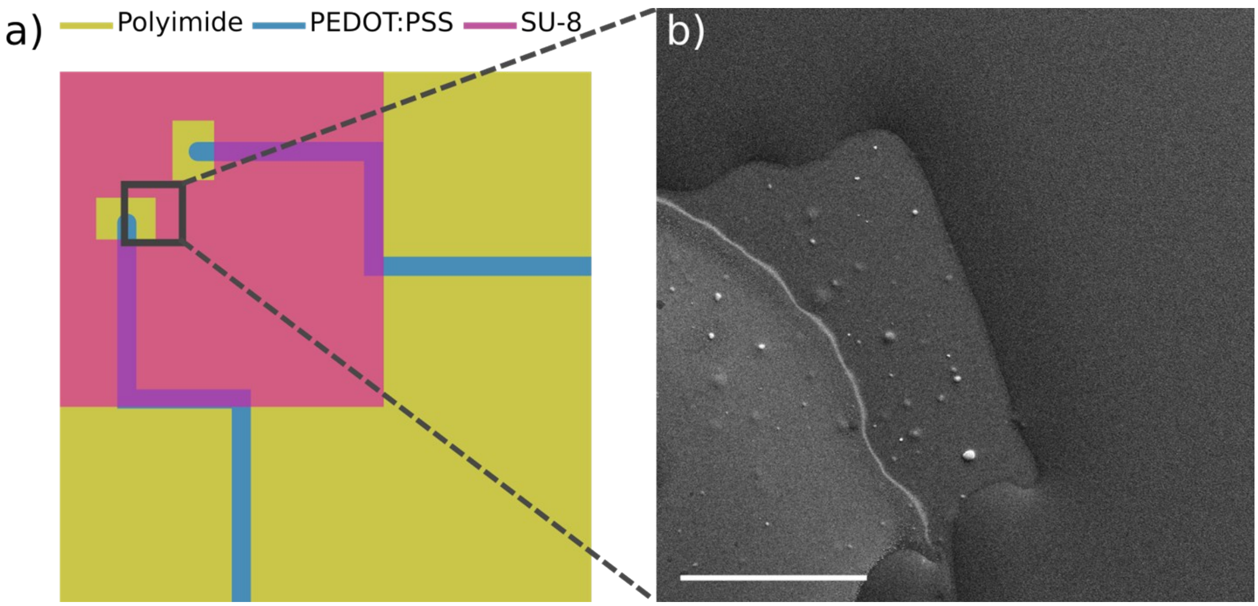

Figure S1. Surface characterisation of pMEAs. a) Section of the pMEAs design with highlighted the area of the three interfaces analysed: the polyamide substrate, the PEDOT:PSS electrode and the SU-8 dielectric layer. b) SEM image of the three interfaces of interest (scale bar 200 µm).

S2. Long-term stability of PEDOT:PSS electrodes in solution

We performed impedance measurements of PEDOT:PSS-based electrodes before and after using the pMEAs for cell culture (1 week experiment) and then after having kept the devices submerged in cell culture media for 2 more weeks. Figure S2a shows that the mean impedance recordings increased slightly during the observation period. The subsequent representations (Figure S2b-d) reveal that the main variation was observed after the first week experiment, with cell culturing, while during the next two weeks changes were no larger than the standard deviation. The small magnitude of these changes indicates that the electrodes did not suffer a significant degradation after use or after being exposed to the cell culture solution for an extended period of time.

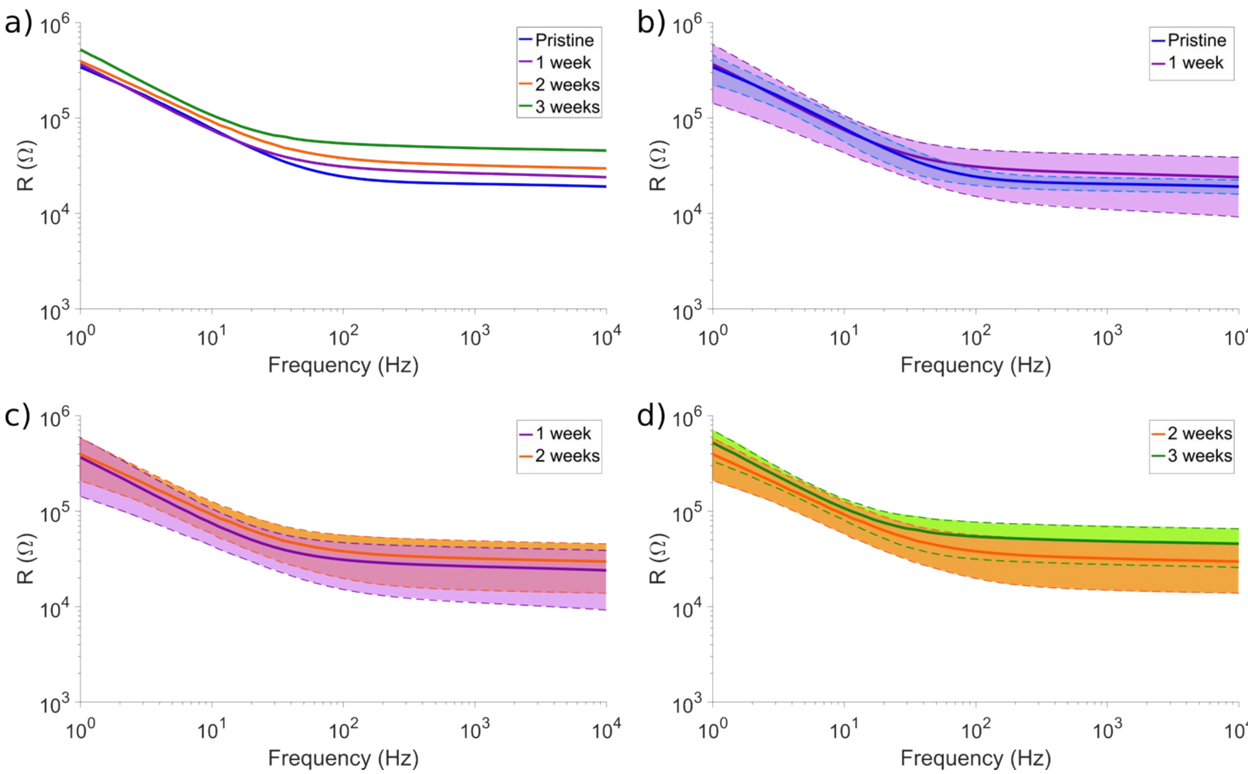

Figure S2. Impedance evolution over time. a) Mean values of the recorded impedance from pristine devices to devices submerged in culture media for 3 weeks. b) Comparison between pristine devices (blue) and same devices cultured with cells after 1 week (purple), with mean value and standard deviation. c) Comparison between devices cultured with cells after 1 week (purple) and same devices submerged in culture media for 2 weeks (orange), with mean value and standard deviation. d) Comparison between devices submerged in culture media for 2 weeks (orange) and same devices submerged in culture media for 3 weeks (green), with mean value and standard deviation.

S3. Transparency of the pMEAs

In order to confirm that the devices fabricated were transparent we used an inverted microscope to image HL-1 cells on the surface of the pMEAs.

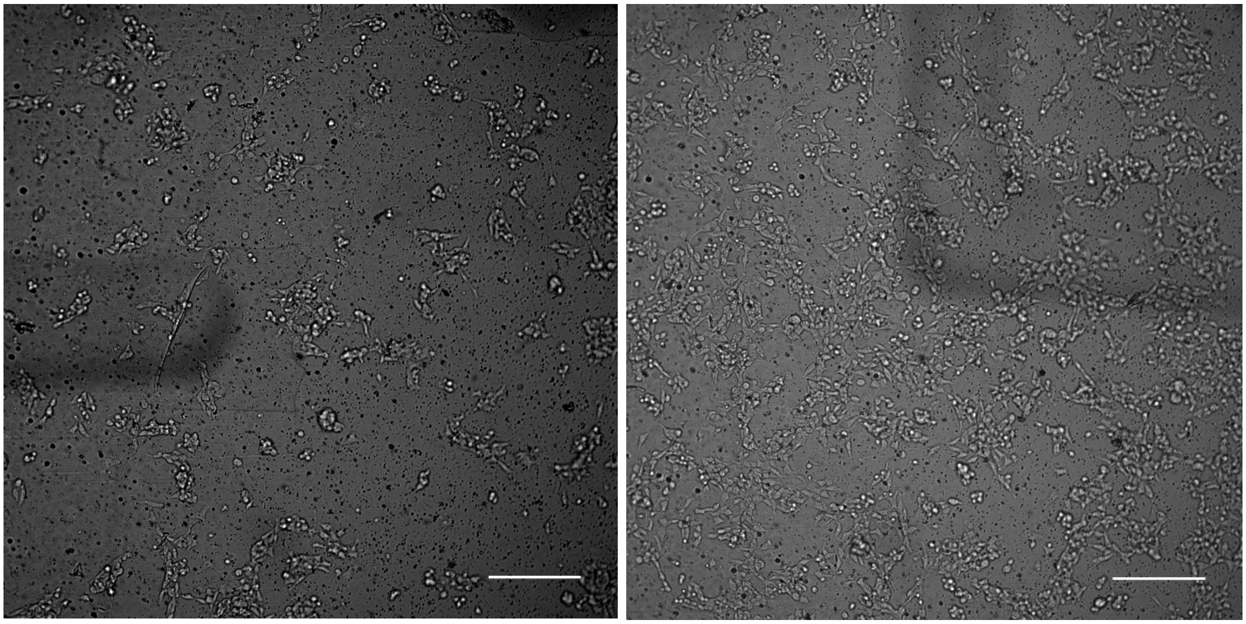

Figure S3. Bright-field images of HL-1 cells on the surface of the pMEA at 1 DIV obtained with an inverted microscope. The cells, PEDOT:PSS tracks and SU-8 openings are highlighted for increased visibility (scale bar 200 μm).

S4. Waveforms of HL-1 action potentials

The APs were acquired in one-minute recordings from 22 electrodes and averaged over all the detected AP for each single electrode (Figure S3). Although varied in shape, they all fit the description and observations found in the literature for HL-1 waveforms: there is first a positive peak, followed by a larger negative deflection and a positive hump after it. The diversity in the shapes of the peaks observed here stems from the different amplitude of each of these three features in each case.

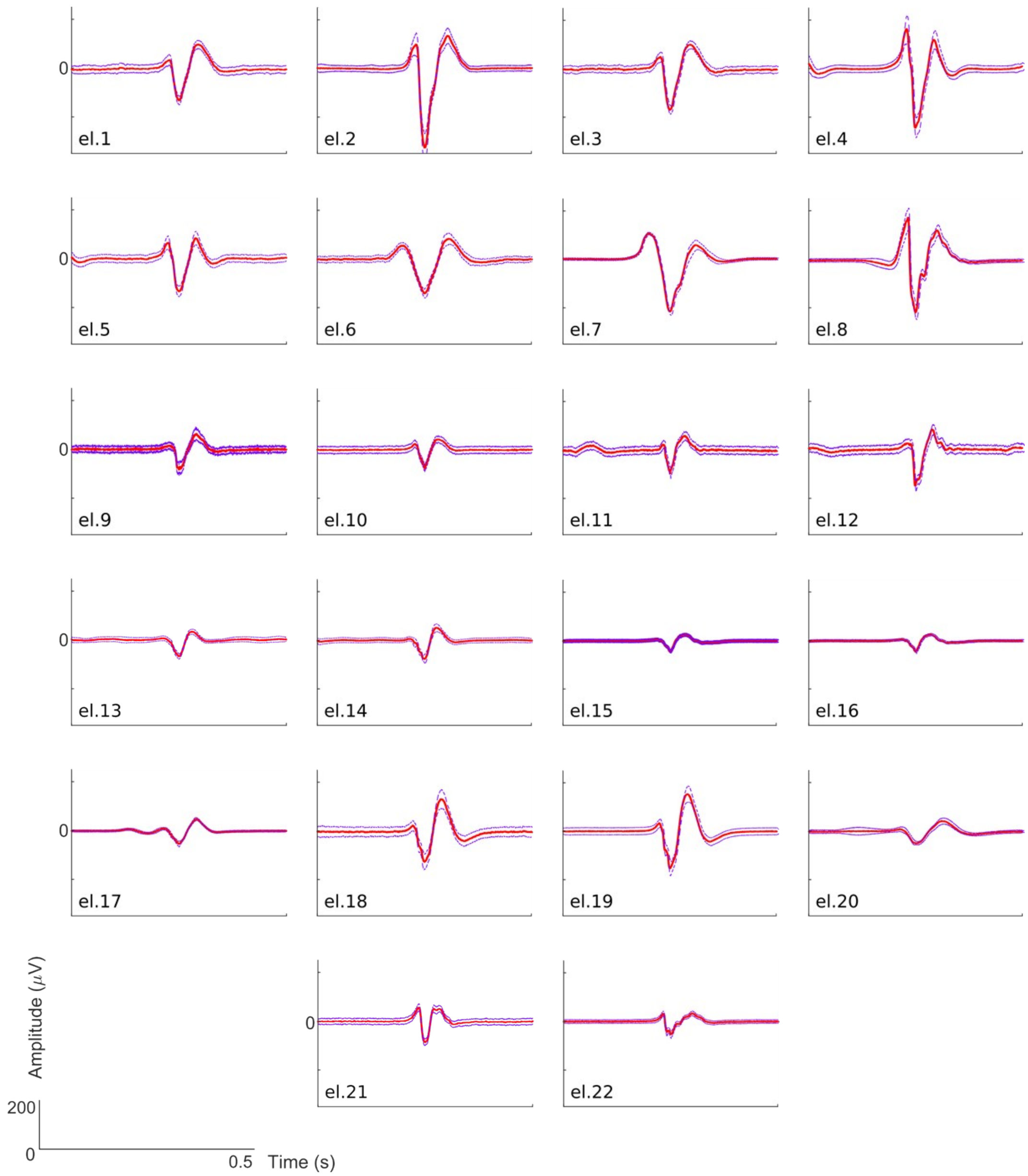

Figure S4. Average waveform of the action potentials detected in 1-minute recordings on 22 electrodes. The red line represents the average whereas the blue lines indicate the values one standard deviation above and below the mean.

S5. Variations in electrodes area and printing issues

The fabrication of pMEAs was based on inkjet printing, through the patterning of multiple layers. We adopted PEDOT:PSS-based ink for the conductive traces and SU-8 for the dielectric layer. In this process the main issues were linked to the resolution and reproducibility of the patterns, which in turn was due to the adopted printer (lab-scale printer, with a typical resolution of 50 μm). We experienced a deviation of few tens of μm in the reproducibility of the nominal value. The printing process was characterized by the patterning of the first layer of PEDOT:PSS-based ink, which was then cured in oven. Afterwards, the second layer of SU-8, designed to define the electrodes' area, was printed. These second step required the redefinition of the printing origin, thus causing a variable misalignment in the dielectric pattern. Such variability was linked to the intrinsic reproducibility of the machine. The misalignments of the SU-8 layer resulted in different areas of PEDOT:PSS being exposed in different electrodes, that caused a final distribution of the averaged electrodes' area characterised by a standard deviation of 0.02 mm^2. In figure S1 a well-aligned pattern (Figure S4a) is compared with other three where the misalignments were well visible, for electrodes printed in the parallel (Figure S1b) and perpendicular direction (Figure S4c,d) with respect to the printing orientation. In Figure S1a the design of a PEDOT:PSS trace and the SU-8 opening is reported. Here is evident that the SU-8 openings were designed bigger than the electrodes' area, having free margins on the sides of the square opening. In particular the openings (440 μm x 620 μm) left margins of 210 μm on the sides and 170 μm on the other free edge. Such foresight was taken to limit the misalignment issue. Indeed, in these examples, even if the printed SU-8 layer was deposited slightly down it didn't affect the final electrode's area.

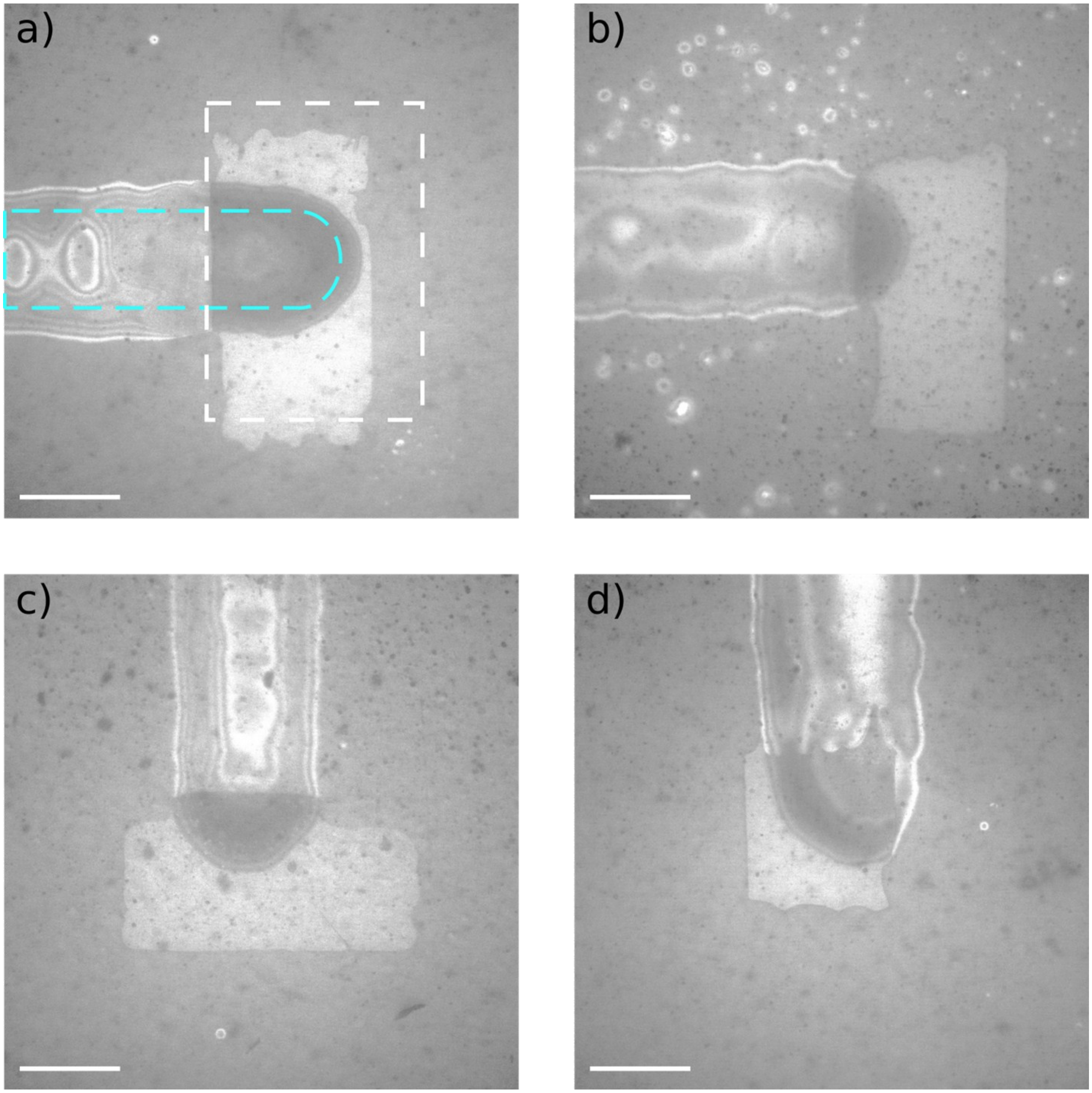

Figure S5. Printing criticality, the misalignment. a) Optical microscope image of a good printed electrode (scale bar 200 μm) with the superimposition of the intended design (light blue: the PEDOT:PSS pattern, white: the opening in the dielectric layer). b, c, d) Optical microscope images of printed electrode with misalignment issue (scale bar 200 μm).