Printed 3D Electrode Arrays with Micrometer-Scale Lateral Resolution for Extracellular Recording of Action Potentials

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1. Introduction

2D microelectrode arrays (MEAs) have been used in a number of scientific studies encompassing monitoring of environmental and biological systems with high spatiotemporal resolution.\(^1\)\(^-\)\(^5\) In the field of bioelectronics, these devices have been used as platforms to better understand complex cell-to-cell communication in vitro as well as in vivo.\(^6\)\(^-\)\(^10\) In general, such devices are produced using microfabrication techniques allowing well-defined high-resolution features. In particular, high-density MEAs based on complementary metal-oxide semiconductor (CMOS) technologies have emerged within the last years, allowing the simultaneous recording of several thousands of electrodes in parallel.\(^11\)\(^,\)\(^12\) However, cleanroom processing of new MEA designs can be costly or require long development times, which is not ideal for rapid prototyping. Alternative fabrication methods such as additive manufacturing have come to fruition enabling overall lower material waste and faster production times for low-volume novel designs, using a broad range of different materials.\(^13\)\(^-\)\(^15\) In particular, inkjet technology has become of considerable interest for fabricating MEAs due to its maskless design flexibility and printing capabilities of dielectric and conducting materials.\(^16\)\(^-\)\(^18\) Such devices have allowed the production of MEAs for extracellular recording of action potentials using a variety of different substrates including soft materials such as polydimethylsiloxane (PDMS) or even hydrogels.\(^19\)

While 2D MEAs allow the monitoring of planar networks, recording from the inside of brain tissue or slices requires the application of 3D electrode systems. 3D recording devices have already been used for several decades as neurological probes in vivo.\(^20\)\(^-\)\(^23\) Concerning in vitro experiments, 3D MEAs have been applied for studies on acute brain slices and dissociated cell cultures.\(^24\)\(^-\)\(^26\) With the rise of more complex in vitro model systems, which go beyond simple 2D-cell cultures, such systems also gain importance in the field of organ-on-a-chip models.\(^27\)\(^-\)\(^29\) Conventionally, 3D microelectrode devices are produced using micro and nanofabrication.\(^10\) However, these approaches are elaborate and typically have limitations concerning the type of materials that can be employed. Furthermore, it would be advantageous to modify existing 2D-technology in a postprocess to enable 3D recordings and stimulation. These issues can be addressed using additive manufacturing approaches such as inkjet printing. Yet, presently, drop-on-demand inkjet technology still encounters challenges in obtaining the desired resolution achieved with cleanroom-fabricated 3D MEAs for single-cell recording and stimulation. Several factors influence the possibility of inkjet printing 3D microstructures, such as the ink’s rheological properties as well
as the solvent evaporation. So far, printed micropillars with a diameter of 22 µm have been fabricated using a commercial inkjet printer. In addition, micropillars with an aspect ratio greater than 50 have been realized by using an in situ simultaneous laser annealing system. However, the lateral dimensions of these inkjet-printed structures are still in the range of several tens of micrometers and thus too large for individually addressing neurons with cellular or subcellular resolution.

An alternative to classical inkjet printing is based on electrohydrodynamic printing, which has become of considerable interest due to its capability of focusing even smaller volumes of fluid via electrostatic forces for different ejection modes. Electrohydrodynamic inkjet printing of nanoparticles in femtoliter droplets has been reported for specialized applications in micro and nanotechnology. For example, electrohydrodynamic inkjet systems can produce elaborate sub-micrometer, conductive 3D structures such as straight and tilted pillars, helices, walls, and bridges. If integrated with individually addressable MEAs, these structures might be of interest for cell–chip coupling devices and biosensors.

In this paper, we demonstrate the fabrication of a functional 62-channel 3D MEA using electrohydrodynamic inkjet printing. The 3D pillar-shaped microelectrodes are printed and sintered to obtain a diameter of less than 3 µm, well below the size found in conventional MEAs. Growth rate and pillar shape were investigated in addition to the electrochemical properties of the 3D microelectrodes.

2. Results

2.1. Printing Process

An electrohydrodynamic inkjet printer was used to form 3D microelectrodes. This printing device uses a mount in which a glass pipette with a thin nozzle is held above a three-axis motor stage where the substrates are laid. By applying a pulsed voltage waveform to the glass pipette, an electrostatic force is applied to the nozzle opening resulting in the jetting of femtoliter droplets of gold nanoparticle (AuNP) ink onto the substrate. As in any other inkjet printing method, the waveform is a crucial aspect which determines the size and reliability of jetting individual droplets with negligible satellites. An inadequate waveform can otherwise lead to positional inaccuracies for the main droplet and change the overall volumetric precision when forming 3D structures.

To fabricate micrometer-sized pillars, a nozzle diameter of 3 µm and a substrate–nozzle distance of 20 µm was chosen to allow an accurate deposition of nanoparticle ink. Preliminary tests shown in Figures S1 and S2 in the Supporting Information determined the applied waveform for all later experiments, i.e., a ramp waveform starting from 20 to 140 V, applied for 1 ms over the set target area (see the Experimental Section for details). An exemplary printed micropillar with the chosen waveform was imaged using a scanning electron microscope in Figure 1.

In this paper, different printing protocols were employed to produce parameterized pillar arrays for morphological studies as well as functional 3D microelectrode devices. To allow for continuous printing, all protocols were based on a recursive print cycle to prevent the nozzle from drying during the print.

2.2. Pillar Growth

2.2.1. Constant Nozzle–Substrate Distance

In a first investigation, we aimed to understand the initial growth and formation of electrohydrodynamic inkjet-printed pillars to better quantify their overall morphology. For potential applications of 3D MEAs in electrophysiology, both the height and the width of the individual pillars play an important role. The height determines the potential penetration depth of the microelectrode in the target tissue, while the width defines the lateral resolution. We therefore investigated pillar height and width in dependence of the printing process. For the pillar width, we distinguish between the value at half the pillar height and directly at the tip (see the Experimental Section).

As a first analysis the substrate–nozzle height was held constant at 20 µm. Figure 2a (blue data points) shows the printed pillar height with respect to the number of deposited droplets. As expected, we observe an increase in pillar height with rising droplet number. After the formation of an initial base during the first 5 droplets, the pillars exhibit an approximately linear growth rate of 0.82 ± 0.05 µm per droplet. However, in this particular printing configuration, the growth of the pillars stagnated around 25 droplets of AuNP ink. This is to be expected as the pillars were approaching the nozzle tip. In contrast to the pillar height, the width of the pillars only showed a slight dependence on droplet number. During initial pillar formation within the first 5 droplets, the width of the pillars was ≈4.5 µm as shown in Figure 2b. After continual deposition, this value...
decreased to a rather stable width of \( \approx 3 \) \( \mu \)m, consistent with the observed constant growth rate. In comparison, the width seen at the tip of the micropillars decreased with increasing droplet number. This is of interest to the pillars’ growth, as the tip directly interacts with the next droplet, which can change the pillars’ overall morphology. As shown in Figure 2c, we saw a pillar tip width decreasing to 1.5 \( \mu \)m at 30 droplets. This decrease is most likely due to a change in the distance between the printing nozzle and the tip during pillar growth. One hypothesis is that smaller droplets are deposited once the pillar tip starts to approach the nozzle. This could be caused by a partial clogging of the nozzle due to the close proximity of the micropillar. As the nozzle jets the ink on top of a very close structure, the nozzle tip can be wetted along with the micropillar. The excess ink will continuously dry increasing the concentration of gold nanoparticles at the meniscus of the nozzle and altering the nozzle’s jetting performance until the nozzle is clogged. Furthermore, one might envision that a nozzle can be damaged when impacting against the micropillars. However, typically this would result in a larger nozzle diameter and was not observed in our experiments.

Once the micropillars were sintered, a notable decrease in the height of the printed structures was perceived, as shown in the 3D-rendered images in Figure 3. This decrease amounts to 50–80% of its original value (Figure 2d). The large variance seen for small droplet numbers can be explained by instabilities during the initial pillar formation, which results in a variation of height profiles. Apart from the pillar height, the pillar width was also affected by the sintering process. We can see that after sintering, the pillar width tends toward 2 \( \mu \)m for higher numbers of deposited droplets and the tip width tends toward 1 \( \mu \)m.

The decrease in the lateral dimensions after sintering, shown in

Figure 2. Pillar analysis for a fixed nozzle height of \( \approx 20 \) \( \mu \)m above the substrate. a) The absolute height of the micropillars before (blue) and after (red) sintering. b) The pillar width and (c) the pillar tip width before (blue) and after (red) sintering. d) The difference in height between sintered and unsintered pillars seen in (a). For each data point, the average and standard deviation were calculated for 10 pillars.

Figure 3. 3D-rendered images of the printed micropillars for varying numbers of deposited ink droplets. a–f) Unsintered pillars of 5, 10, 15, 20, 25, and 30 droplets, respectively. In comparison, (g–l) show the same pillars in their sintered states. The pillar heights are given from (a–l) as 3.3, 8.0, 12.7, 16.9, 19.0, 18.4, 2.0, 5.4, 9.2, 12.2, 14.5, and 13.8 \( \mu \)m, respectively.
Figure 2c,d, is in the order of 75% of the original value, which is similar to the decrease in height described above. Therefore, we assume that during sintering the entire micropillar shrinks isotropically by a factor of ≈25%. The shrinking can be attributed to the breaking of the nanoparticles’ insulating organic shell and the formation of a more compact structure.

2.2.2. Adaptive Nozzle–Substrate Distance

To print pillars with a higher aspect ratio, we investigated the possibility of further increasing the height of the pillars. In this experiment, we started the printing process at a nozzle–substrate distance of 20 µm. This distance was increased by 1 µm every pass after the 15th droplet to maintain a consistent jetting regime with respect to the nozzle–micropillar distance. As a result, a continuous linear growth of the pillar was observed with a growth rate of 0.75 ± 0.01 µm per droplet as shown in Figure 4a. This value is in agreement with that observed in the linear regime in Figure 2a. As such, the pillars’ growth can be maintained linearly if the distance between the nozzle and the pillar remains constant after a preliminary pillar is formed. Again, we see a slight effect on the printed pillar width with increasing number of droplets as shown in Figure 4b,c in blue. As described in Section 2.2.1, this might be caused by the ejection of smaller droplets due to a gradual clogging of the nozzle. However, in this case, we assume that the drying is primarily caused by the print’s run time (≈16 min) leading to a small but progressive change in droplet volume. This aspect should be considered for the fabrication of very high aspect ratio pillars with pillar heights above 100 µm. As observed in the previous experiment, the height of the pillars was reduced by a factor of ≈25% after thermal curing (compare Figure 4d), which is also evident in the corresponding 3D images in Figure 5.

To better understand how the pillar formation occurred through the continuous deposition of gold nanoparticle ink, we evaluated the gravitational versus the surface tension forces, which is typically expressed by the Bond number. The following equation was derived (see the Supporting Information for more detail)

$$B_o = \frac{(\rho_{\text{ink}} - \rho_{\text{air}})g}{4\pi\chi_{\text{Au}}\rho_{\text{Au}}} \left(\frac{3\rho_{\text{Au}}V_{\text{Au}}}{\rho_{\text{ink}}}\right)$$

(1)

where $\rho_{\text{ink}}$ is the density of the gold nanoparticle ink (1794.6 kg m$^{-3}$), $\rho_{\text{air}}$ is the density of air (1.2 kg m$^{-3}$), $g$ is the gravitational acceleration (9.8 m s$^{-2}$), $V_{\text{Au}}$ is the deposited volume of gold nanoparticle ink after solvent evaporation (5.6 ± 0.2 × 10$^{-18}$ m$^3$) as evaluated from the pillar growth (see the Supporting Information), $\rho_{\text{Au}}$ is the density of gold (19 320 kg m$^{-3}$), $\chi_{\text{Au}}$ is the weight content of gold in the ink droplet (0.5, i.e., 50%), and $\gamma$ is the surface tension of the ink (25 ± 5 × 10$^{-3}$ N m$^{-1}$). The resulting Bond number was 7 ± 1 × 10$^{-6}$, indicating that surface tension has an ≈5 orders of magnitude stronger influence than gravity. The back-calculated volume for a droplet of ink was 121 ± 4 fL, which equates to a droplet radius of 3.1 ± 0.1 µm (see the Supporting Information). It is envisioned that each droplet wets the tip of the 3D

Figure 4. Pillar analysis for a stepped nozzle starting at ≈20 µm above the substrate. a) The absolute height of the micropillars with a linear fit before (blue) and after the sintering process (red). b) The pillar width and (c) the pillar tip width for unsintered (blue) and sintered micropillars (red). d) The difference in height between sintered and unsintered pillars. For each data point, the average and standard deviation was calculated for 12 pillars.
structure, however, due to the small volume, the ink’s solvent can be assumed to evaporate almost instantaneously, resulting in a thin layer of gold nanoparticles added to the top of the pillar.

In particular for pillars exhibiting a high aspect ratio, we were interested in the vertical alignment before and after sintering. As can be seen in the example illustrated in Figure 6, the sintered micropillar showed a tilt (compare Figure 6a,b). We measured the tilt of all the micropillars for both sintered and unsintered states (Figure S4, Supporting Information). The tilt angle of sintered pillars does not seem to rely on the pillar height as can be seen in the individual histogram plots of Figure S5 in the Supporting Information. The pooled data in Figure 7 shows a median of 1° and 9° of unsintered and sintered pillars, respectively. To exclude a possible systematic error due to print conditions, we verified that the pillars were not tilted in the same direction (data not shown). We hypothesize that the tilting of micropillars was caused by an inhomogeneous sintering of nanoparticles, which induced varying degrees of stress and changed the pillars’ morphology.

This is supported by previous research showing that the sintering temperature and time can greatly vary the internal structure of nanoparticle-based 3D microstructures.[44]

2.3. Functionality of Printed Microelectrode Pillar Arrays

For potential recording or stimulation applications in bioelectronics, the electrode–electrolyte interface plays an important role. Often it is characterized by its impedance, which is typically governed by a capacitive behavior. To quantify this capacitance for our sintered micropillars, we printed AuNP micropillars onto a microelectrode array as illustrated in Figure S6 and Video S1 in the Supporting Information. Figure 8 shows optical and 3D-rendered images of the printed microstructures on an MEA with 8 µm diameter electrodes. As we can see from Figure 8b, the deposition of gold was limited to the microdisk opening defining the maximum base diameter of our micropillar.

In the case of the 3D micropillar electrode, the active surface area incorporates the planar microelectrode with the addition of the pillar surface. Profilometric data were taken of each microelectrode pillar as shown in Figure S7 in the Supporting Information. The data suggested a cylindrical addition to the overall surface area, which would follow the below equation

\[ A_{\text{pillar}} = \pi r_1^2 + 2\pi r_2 h \]  

(2)

where \( r_1 \) is the radius of the planar microelectrode, and \( r_2 \) and \( h \) are the radius and height of the micropillar, respectively.

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**Figure 5.** 3D-rendered images of the printed micropillars for varying numbers of deposited ink droplets. Pillars of 20, 40, 60, 80, and 100 droplets are shown in (a–e) before sintering and (f–j) after sintering, respectively. All 3D images had their z-scale reduced by a factor of two for better clarity. a–j) The pillar heights were 14.5, 29.7, 46.6, 61.8, 74.4, 11.1, 22.5, 35.3, 46.5, and 55.5 µm, respectively.

**Figure 6.** Optical images taken with a 150× objective can be seen for (a) unsintered and (b) sintered states of the same micropillar of 100 droplets. c) A profile along the pillar’s tilt for unsintered (blue) and sintered (red) with a tilt of 3.3° and 19.3°, respectively. Superimposed profiles measured perpendicular to the pillar’s axial direction (d) before and (e) after sintering, respectively. In both (d) and (e), the pillar diameter is in the range of ~3 µm.

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Impedance spectroscopy measurements were conducted on eight micropillar electrodes. Figure 8d shows the absolute impedance (blue) versus the frequency in a double-logarithmic plot. The corresponding phase in degrees is shown by the red trace. We observe an average impedance value at 1 kHz of 2.2 MΩ. The double-layer, which forms between the gold micropillar electrode and an electrolyte solution does not behave as an ideal capacitor, as evident by the deviations from a phase angle of $-90^\circ$. We used the circuit shown in Figure 9 with a constant-phase element for fitting the data (fits shown in Figure S8, Supporting Information). The constant-phase element can be explained in terms of laterally distributed time constants at the electrode–electrolyte interface caused by local variations of the surface. Based on this assumption we can quantify the effective capacitance, with the following equation\[^{45,46}\]

$$C_{\text{eff}} = \frac{Q}{\alpha} \left( R_e^{-1} + R_t^{-1} \right)^{\alpha-1}$$  \hspace{1cm} (3)

where $Q$ and $\alpha$ define the constant phase element, $R_e$ is the ohmic impedance, and $R_t$ is the charge-transfer impedance. From the acquired data and using Equation (3), we obtain a value alpha of 0.87 $\pm$ 0.01 and an effective capacitance of 22 $\pm$ 3 pF. If we calculate the surface area of the 3D microelectrode with an 8 $\mu$m planar base diameter, a height of 14.4 $\pm$ 1.0 $\mu$m, and a pillar diameter of 1.5 $\pm$ 0.3 $\mu$m, we obtain a total surface area of 116 $\pm$ 14 $\mu$m$^2$. The specific capacitance of the micropillars was thus in the range of 19 $\pm$ 3 $\times$ 10$^{-2}$ F m$^{-2}$, which is roughly a factor of two lower than previously observed values for printed planar gold electrodes but still in line with typical results for gold electrodes reported in literature.\[^{18}\] A comparison of the absolute impedance and phase of 3D microelectrodes and 2D microelectrodes are shown in Figure S9 in the Supporting Information.

To investigate the biocompatibility of the printed 3D MEAs, HL-1 cells were cultivated on the chips for 5 days in vitro for the detection of extracellular action potentials. This cardiac muscle cell line exhibits pacemaker activity and conduction of action potential waves, which can be recorded once a confluent layer is grown.\[^{47–49}\] The activity of the cells was either spontaneous or excited by the addition of norepinephrine and stopped by the addition of sodium dodecyl sulfate (SDS) after a few minutes of recording. HL-1 action potentials were measured amperometrically exploiting the double-layer capacitance formed at the electrode–electrolyte interface. To exclude the detection of electrochemical activity such as the redox-active molecule norepinephrine, a bias potential of 0 V versus Ag/AgCl (3 m NaCl) was applied. This proof-of-principle investigation was conducted over 4 samples where action potential amplitudes ranging between 50 and 120 pA were observed on all 4 chips.

![Figure 7](image-url)  
**Figure 7.** Histogram showing the angle deviation from the normal of the same 60 pillars before and after sintering shown in Figure 4a (20–100 droplets of AuNP ink). The angle was measured from 90% to 10% of the maximum height of the pillar as shown in Figure S4 in the Supporting Information.

![Figure 8](image-url)  
**Figure 8.** a) Optical image of the microelectrode array (MEA) with printed micropillars, imaged with a 5× objective. b) A pillar printed on an 8 $\mu$m opening of the MEA imaged with a 150× objective and (c) the same pillar in 3D as obtained from profilometric data with its corresponding z-scale bar. The pillar width and height are $\sim$2 and 15 $\mu$m, respectively. Finally, (d) impedance measurements of 8 electrode openings with printed micropillars in a Bode plot.
An exemplary trace, along with an optical image of the confluent HL-1 layer is shown in Figure 10. Similar action potentials signals were perceived on the other samples after 5 days in vitro shown in Figure S10 in the Supporting Information.

3. Discussion

Overall, the presented approach allows for the versatile production of functional printed gold microelectrode arrays in terms of lateral and vertical resolution. The lateral extension of the micropillar electrodes lies in the range of micrometers and is well below what is currently achievable with classical inkjet technologies. In contrast to classical microfabrication techniques, which typically depend on the structuring of silicon probes, the printed 3D microelectrodes rely only on additive manufacturing. Thus, it is possible to use this approach in a postprocess for the modification of planar 2D-device technology. For example, future devices could be printed on CMOS-MEA platforms to enable high-density recording and stimulation in 3D geometries. Nevertheless, electrohydrodynamic inkjet printing still faces challenges such as sensitivity to substrate roughness, nonuniformity, as well as nozzle clogging, which can affect the reproducibility of the prints. In addition, this technique is difficult to implement in high-throughput applications for low-cost sensor production, such as roll-to-roll manufacturing. The strength of this approach therefore has to be seen rather in rapid prototyping of new designs and postproduction of high-resolution 3D features on existing planar recording and stimulation technologies. Future investigations should focus on alternative curing procedures such as infrared, photonic, or selective laser sintering to further reduce the thermal load for postprocessing of the printed devices.[31,50,51] In addition, applications in bioelectronics will in particular benefit from high-resolution 3D printing or electrodeposition of alternative materials such as polystyrene sulfonate-doped poly(3,4-ethyleneoxythiophene) (PEDOT:PSS) to further reduce the impedance of the electrode–electrolyte interface.[52–56]

4. Conclusion

In this paper, we have demonstrated the direct fabrication of high-resolution 3D microelectrode arrays using electrohydrodynamic inkjet printing. The individually addressable pillar electrodes exhibited a diameter of less than 3 μm after sintering, which is significantly smaller than what can be achieved with standard inkjet printing technologies. We have printed pillar electrodes with an aspect ratio of up to ≈25 and characterized the printing process as well as geometrical properties of the 3D electrodes using laser profilometry before and after sintering. We have performed impedance spectroscopy in phosphate-buffered saline to evaluate the electrical surface properties. Action-potential recordings from HL-1 cells were performed to assess the functionality of the 3D microelectrode arrays, which is important for potential bioelectronic applications. Overall, we believe that the presented approach offers a cost-effective alternative for rapid prototyping of 3D microelectrode arrays with versatile geometries, which could potentially be used for extracellular recording and stimulation in tissues or 3D model cell cultures.

5. Experimental Section

Electrohydrodynamic Inkjet Printing: All prints were performed using a commercially available super inkjet printer (Model SJJ-S050, SJJ Technology Inc., Tsukuba, Japan) at room temperature. A glass capillary with a nozzle diameter of 1.3–1.8 μm was used to eject AuNP ink (CAu-2000, SJJ Technology, Tsukuba, Japan) onto two different substrates. The first substrate consisted of a glass slide (Cat. No. 7204, Muto Pure Chemicals, Tokyo, Japan) with a dimension of 76 × 26 × 1.3 mm². The glass slide was cleaned using a 1:1 ratio of 96% sulfuric acid (Kanto Chemical, Tokyo, Japan) and 31% hydrogen peroxide (Santoku Chemical Industries, Tokyo, Japan) for 10 min. Before and after the pretreatment, the glass slide was thoroughly rinsed with deionized water and finally dried using pressurized air. The second substrate was a cleanroom fabricated Pt microelectrode array with 62 working electrodes with an opening dimension of 8 μm. A detailed fabrication process of the chip has been described previously.[8] The jetting parameters used for all experiments were software controlled, using a ramp waveform starting from 20 to 140 V and applied for 1 ms over the desired target area. The nozzle ejected a droplet at a frequency of 3.3 and 5 Hz for Sections 2.2 and 2.3, respectively. The chuck had a velocity of 1.5 mm s⁻¹ and an acceleration of 10 mm s⁻² between each deposited droplet.

Ink and Substrate Properties: The AuNP ink had a viscosity of 10 mPa s at 23 °C and a surface tension of 25 ± 5 mN m⁻¹. Contact angle measurements (LSB-B100, NiCK Corporation) were carried out on static drops of deionized water and ink on the cleaned glass. The data are shown in Figure S11 in the Supporting Information. Both, the deionized water and ink gave contact angles of 5.0 ± 0.5° and 17.6 ± 2.4°, respectively, calculated using i2win software (NiCK Corporation).

Sintering: In order to sinter the micropillars, the samples were placed in a high-temperature furnace (glass substrates: Model HT 16/16 Nabertherm, Lilienthal, Germany; MEA substrates: DO-300A, AS ONE,
Japan) at room temperature. The oven was set to 250 °C for 2 h and the samples were left to cool to room temperature.

Optical Micropillar Characterization: The printed pillars were measured using a 3D laser scanning confocal microscope (VK-X250, Keyence, Osaka, Japan) in combination with a 150× objective (150×/0.95 CF Plan Apo OFN25, Nikon, Japan). Each micropillar was independently measured, with a low and high laser intensity (double-scan feature) to better evaluate the pillars' morphology. Depending on the steepness and tilt of the pillar, the scan limits were adapted to better determine the width of the 3D structure. The laser’s brightness and neutral-density filter were automatically calibrated by setting the upper and lower limits of the scan and using the auto gain function. All measurements were scanned with a z-pitch of 80 nm, on a vibration-dampened table (Vision IsoStation, Newport, USA) to reduce external interferences.

Constant Nozzle–Substrate Distance Data Analysis: All data files were imported in MATLAB for data processing. Background areas were manually defined and then fitted using a 2D polynomial of order one in x and y. After subtraction of this fit, a median filter of kernel size 3 × 3 was applied to remove remaining high-frequency noise. For reference, a false-color plot was made as shown in Figure S12a in the Supporting Information. The pillars' height was defined by using the absolute maximum of the z-data. The center of the pillar was manually assigned and vertical and horizontal profiles were taken to evaluate the width and tip width of the pillars (compare Figure S12b,c, Supporting Information). The width of the pillar was taken as the next data point below or equal to half the pillar's maximum height for both the x- and y-profiles. Similarly, the tip width of the pillar was extracted as the following data point below or equal to 500 nm of the maximum height of each profile. The pillars' tip width and width were defined as the averaged values between both profiles. These measurements are shown in Figure S12b,c in the Supporting Information in red. The linear fit was calculated for the measured heights of the unsintered micropillars between 5 and 25 droplets, with an assigned confidence interval of 95%. A total of 60 pillars were evaluated and were compared before and after sintering. The pillars’ mean and standard deviation in height, pillar width, and tip width were evaluated from 10 samples fabricated with different droplet numbers of 5, 10, 15, 20, 25, and 30.

Adaptive Nozzle–Substrate Distance Data Analysis: The same analysis procedure as described in the preceding paragraph was used apart from the pillar width analysis. Axial and radial profile cuts of the pillars were manually taken (MultiFileAnalyzer, Keyence) and imported into MATLAB. From these profiles, the pillars' width was displayed as shown in Figure 6. A single profile exhibiting the overall pillars' morphology was used to measure the micropillar width. The measurements evaluated in Figure 4c were taken as the first data point under or equal to half of the corresponding micropillars' maximum height. The tilt angle of each micropillar was calculated as arctan of the distance covered by the tilted pillar, divided by the corresponding height of the pillar in the range of 90–10% of the absolute maximum. These measurements are shown in Figure S4 in the Supporting Information. The calculated linear growth for the unsintered micropillars between 0 and 100 splats had a set y-intercept at 0 μm with an assigned confidence interval of 95%. The pillars’ mean and standard deviation in height, pillar width, and tip width were evaluated from 12 samples fabricated with 20, 40, 60, 80, and 100 droplets of deposited AuNP ink.

Electrochemical Characterization: Impedance spectroscopy measurements were carried out using a potentiostat (VSP-300, BioLogic Science Instruments, Seyssinet-Pariset, France) in a two-electrode configuration. A platinum mesh was used as a combined counter and reference electrode and the sintered 3D microelectrodes were used as working electrodes. Phosphate-buffered saline (P4417, Sigma-Aldrich) was used as the electrolyte solution. The model used to quantify...
the electrode–electrolyte interface assumed lateral variations of the interface causing distributed time constants. The equivalent circuit uses a constant phase element (Q and α) in parallel with a charge-transfer impedance (Rt) and an Ohmic impedance (Rs) in series. The effective capacitance was then back-calculated using Equation (3). The surface area per printed 3D microelectrode was calculated by measuring the width and height of the 3D structure (shown in Figure S7, Supporting Information) and using a radius of 4 µm for the microelectrode opening. The standard deviation of the surface area and specific capacitance of the 3D microelectrodes were calculated from the measured height, diameter, and back-calculated capacitance using propagation of uncertainty assuming independent variables.

Scanning Electron Microscopy: The printed sample was first sputtered with gold using a high-vacuum coating system (5 × 10⁻⁴ bar, 40 s, 40 mA, approx. film thickness 10 nm; BAL-TEC Med 020, LabMakelaar Benelux BV, The Netherlands). The sample was fixed to the substrate holder and conductive carbon cement and paste (Leit-C-Plast, Neubauer Chemikalien, Germany and N 650 Planocarbon, Plano GmbH, Germany, respectively) were applied to limit charge accumulation. The sample was placed into a scanning electron microscope (JSM-6060LV, JEOL, Japan) and images of the micropillars were taken with an acceleration voltage of 20 kV at a magnification of 6000× under an angle of 45°.

Cell Culture Chemicals and Materials: Claycomb medium, ethylenediaminetetraacetic acid (EDTA), fibroconnectin, gelatin, norepinephrine bitartrate, and trypsin were bought from Sigma-Aldrich (St. Louis, USA). Fetal bovine serum, l-glutamine, and penicillin/streptomycin were purchased from ThermoFisher Scientific (Waltham, USA). 2-Propanol (≥99.5%), ascorbic acid, and ethanol (≥99.5%) were purchased from Carl Roth (Karlsruhe, Germany). Deionized water was taken from an Ultra Clear purification system (Evoqua Water Technologies, Barsbüttel, Germany).

HL-1 Cell Culture: HL-1 cells were cultured in Claycomb medium supplemented with fetal bovine serum (10%), l-glutamine (2 × 10⁻³ M), penicillin/streptomycin (100 U mL⁻¹ and 100 µg mL⁻¹), and norepinephrine (0.1 × 10⁻³ M). The cells were placed in a humidified incubator (CB2120 CO₂, Binder, Germany) at 37 °C in 5% CO₂ atmosphere. Upon reaching confluency and displaying mechanical contractions, the cells were detached via incubation in a protease solution (0.05% Trypsin-EDTA) and used for experiments. The 3D MEA chips were plasma treated prior to cell seeding (O₂, 0.8 mbar, 304 W, 5 min, Diener Femto, Diener electronic, Ebhausen, Germany) and sterilized by dipping into 2-propanol. Once dry, the chips were incubated with a solution of gelatin (0.2 mg mL⁻¹) and fibroconnectin (5 µg mL⁻¹) for 1 h at 37 °C. The protein solution was aspirated, and the chips were rinsed with Dulbecco’s phosphate buffered saline (D8537, Sigma-Aldrich). The cells were then seeded onto the chips for confluency after 5 days in vitro (DIV5). Optical evaluation of the HL-1 cell confluency was performed using a 5× objective with an inverted microscope (Axiovert 40 CFL, Carl Zeiss, Germany).

Action-Potential Recording: Extracellular signals were observed amperometrically on DIV5 with a custom-built 64-channel amplifier in a grounded Faraday cage adopted from the previous research. The amplifier system delivered an output signal of 1 mV pA⁻¹ using a 1 GΩ feedback resistor and had an active bandwidth ranging between 1 mHz and 3.4 kHz. The measurement was performed in relation to a single (shared) Ag/AgCl reference electrode (3 M NaCl) (RE-6, BASI, USA). The medium was exchanged 1 h prior to the experiment, with an initial volume of 1 mL on the chip. In addition, 5 × 10⁻³ M norepinephrine solution (dissolved in 33.6 × 10⁻³ M ascorbic acid) was freshly prepared. HL-1 cells were chemically stimulated by step-wise addition of 5 µL of 5 × 10⁻³ M norepinephrine to the chip’s medium until activity was perceived. Finally, the cell signal was stopped by adding 100 µL of 1 M SDS to the medium to confirm the cellular origin of the signals. The total measurement time of the experiment was ≈5 min. The signal analysis was performed in MATLAB where each of the 64 channels was individually examined. A first order polynomial fit was subtracted from each channel to remove baseline fluctuations. Spikes with a minimum peak prominence of 60 pA were flagged and then averaged along with their standard deviation over a defined time interval.

Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

3D microelectrode arrays, additive manufacturing, bioelectronics, electrohydrodynamic inkjet printing, gold nanoparticles

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[1] M. Kudera, H. A. O. Hill, P. J. Dobson, P. A. Leigh, W. S. McIntire, Sensors 2001, 1, 18.
[2] A. Stett, U. Egert, E. Guenther, F. Hofmann, T. Meyer, W. Nisch, H. Haemmerle, Anal. Bioanal. Chem. 2003, 377, 486.
[3] A. Natarajan, P. Molnar, K. Sieverdes, A. Jamshidi, J. J. Hickman, Toxicol. In Vitro 2006, 20, 375.
[4] F. Davis, S. P. J. Higson, Environ. Sci.: Processes Impacts 2013, 15, 1477.
[5] K. J. Krause, A. Yakushenko, B. Wolfrum, Anal. Chem. 2015, 87, 7321.
[6] A. Blau, C. Ziegler, M. Heyer, F. Endres, G. Schweitzgelbe, T. Matthies, T. Stieglitz, J.-U. Meyer, W. Göpel, Biosens. Bioelectron. 1997, 12, 883.
[7] J. Viventi, D.-H. Kim, L. Vigeland, E. S. Frechette, J. A. Blanco, Y.-S. Kim, A. E. Avrin, V. R. Tiruvadi, S.-W. Hwang, A. C. Vanleer, D. F. Wulsin, K. Davis, C. E. Gelber, L. Palmer, J. Van der Spiegel, J. Wu, J. Xiao, Y. Huang, D. Contreras, J. A. Rogers, B. Litt, Nat. Neurosci. 2011, 14, 1599.
[8] A. Yakushenko, E. Käthelhön, B. Wolfrum, Anal. Chem. 2013, 85, 5483.
[9] D. Kireev, S. Seyock, M. Ernst, V. Maybeck, B. Wolfrum, A. Offenhaüsser, Biosensors 2016, 7, 1.
[10] W. Lee, D. Kim, N. Matsuhisa, M. Nagase, M. Sekino, G. G. Malliaras, T. Yokota, T. Someya, Proc. Natl. Acad. Sci. USA 2017, 114, 10554.
[11] F. Heer, S. Hafizovic, W. Franks, A. Blau, C. Ziegler, A. Hierlemann, IEEE J. Solid-State Circuits 2006, 41, 1620.
[12] M. Ballini, J. Müller, P. Livi, Y. Chen, U. Frey, A. Stettler, A. Shadmani, V. Visvamy, I. L. Jones, D. Jäckel, M. Radivojevic, M. K. Lewandowska, W. Gong, M. Fiscella, D. J. Bakkum, F. Heer, A. Hierlemann, IEEE J. Solid-State Circuits 2014, 49, 2705.
[13] Y. Huang, M. C. Leu, J. Mazumder, A. Donmez, J. Manuf. Sci. Eng. 2015, 137, 014001.
