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Preliminary study on printed microelectrode array by Aerosol Jet Printing technology

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Abstract— Nowadays, additive manufacturing (AM) is a promising process for the fabrication of devices for biomedical applications. AM offers technologies capable of respecting the main requirements of biocompatibility and safety for medical devices. The microelectrode arrays (MEAs) have become a valuable tool in the study of electrophysiological analysis of *in vitro* electrogenic cells (i.e. cardiac and neuronal cells). Common MEAs are based on microfabrication techniques produced on rigid substrates with standard metal, making them expensive tools. Aerosol-jet printing (AJP) can be an alternative fabrication strategy for reducing the costs of MEAs production. AJP allows the development of customized devices and realizes an easy and reliable patterning of conducting polymers with high resolution compared to other ink printing techniques. Here we present a novelty fabrication strategy for the realization of printed MEAs. The preliminary results demonstrate the feasibility of the Aerosol Jet Printing techniques for the fabrication of MEA with a focus on the conductive tracks and the passivation layer deposition i.e. the first and second layer of the electrode array realization.

Keywords—additive manufacturing, printed sensor, aerosol jet printing, biomedical application, microelectrode array.

I. INTRODUCTION

Recently, additive manufacturing (AM) has become a valuable tool in a variety of sectors, from industry to the medical field, thanks to scalable properties and no waste material, and a production process able to reduce cost and time. In the biomedical application [1], the use of innovative fabrication techniques, e.g. AM and printed sensors, is becoming crucial, due to the important advantages such as easy accessibility, processing of varied biocompatible materials, sustainability, and device customization. Furthermore, the possibility to print the electrodes and the sensors directly on the medical device allows the development of prototypes with high resolution, repeatability, and accuracy [2]-[3].

Microelectrode array (MEA) is a measuring device composed of several microelectrodes used to record extracellular action potential signals of electrogenic cells (i.e. neuronal and cardiac cells) and to electrically stimulate multiple sites simultaneously. Furthermore, MEA is employed *in vitro* and *in vivo* for several applications, from neurodegenerative disorders studies to implantable brain-machine interfaces [4]. The microfabrication of common MEAs allows dimensions

of 10-100 μm with thousands of microelectrodes but the process is expensive concerning time and cost production. This leads to reuse and extending the lifetime of the microelectrode with a decrease in the quality of the recorded signals [5].

Recently, printing techniques have been increasingly used in electronic devices, as a promising strategy to achieve customized and low-cost production for patterning high-resolution microstructures with complex geometries on the micrometer scale [6]. Aerosol jet printing (AJP) is a feasible technique for printing different components and sensors on a wide range of substrates. The aerosol mist is focused by a sheath gas, which surrounds the aerosol as an annular ring inside the deposition head, from the nozzle to the substrate.

Furthermore, small droplets dimensions (1-5 μm diameter) and performance achievable allow the printing of objects of micro dimension. In an AJP system, the atomizer (viscosity ranging 1-1000 cP) creates a dense mist of material (the droplets are of 1-5 microns diameter) starting from the liquid ink. Printed lines range from 10 μm to millimeters. Furthermore, AJP is capable to deposit both conductive and insulating materials. This technique provides a selective deposition both on planar and 3D structures, without using pre-patterned lithographic masks, and thus allows the adaptation of different geometries in a cost- and time-efficient manner [7]-[8]. Furthermore, printing technologies have enabled the evolution of printed and flexible electronics. Another advantage is the ease of incorporating newly emerging ink materials such as PEDOT:PSS, a conductive polymeric material that has excellent properties in terms of conductivity, processability, and stability. For medical applications, it shows good biocompatibility and chemical resistance. It is used as a coating for the metallic material due to its low impedance for electrical sensing and stimulation of cellular activity, to improve the signal-to-noise ratio (SNR) of the recorded signals and the increase of the surface area [9]-[10]. So far AJP technique was utilized for the realization of microelectrode array for detection of biocomponent e.g. dopamine and for the biosensing of other biological components[10] For what concern the realization of MEA for the recording of action potential signals, composing techniques with AJP were employed in the realization of the bioelectronic device, e.g. the combination of 3d printing stereolithography and AJP technique [11]. The novelty of this

work is the fully AJP printed technique developed for the fabrication of microelectrode array for stimulation and recording of the action potential of neuronal cells, able to reach the microscopic electrode dimension suitable for such biomedical application thanks to the resolution of the additive manufacturing technique. Furthermore, the possibility to print conductive polymer opens up to the use of more suitable material for interfacing with biological materials (e.g. cells, tissue, biological molecules). However, due to the novelty of the AJP technique adopted in the MEA fabrication, it is necessary a further investigation of the suitable ink materials adopted and their properties, the design and the fabrication process with imposed parameters, and the preliminary deposition results in order to obtain a bioelectronic device adequate to the intended use of biological investigations.

In this paper, the design and the preliminary fabrication process of a microelectrode array able to record action potential signals and to stimulate electrically the neuronal cells by AJP are proposed. In Section II, the development of MEA is discussed in detail with a focus on the conductive traces and passivation layer deposition. In Section III, the preliminary results in terms of morphological and electrical characterization are exploited in order to define the suitable design and process parameters of the AJP technique.

II. MATERIAL AND METHODS

A. Design of microelectrode array

The design and the accurate selection of the printed materials are fundamental for the proper realization of the MEA by AJP. According to the literature [5], the sensing electrode size must be comparable to the cell size (4-100 μm), while the distance between the electrodes must be greater than the cell size. The interelectrode distance was defined between 200 to 300 μm according to intrinsic AJP specifications and the electrode geometry range. The design involved the realization of an array of 4x4 electrodes of 50 μm diameter with a total distribution of the recording sensitive area of 1-2 mm^2 . The schematic drawing of the micro-electrode array (MEA) is shown in Fig. 1. The layout of the MEA was designed by using AutoCAD software, which can provide an

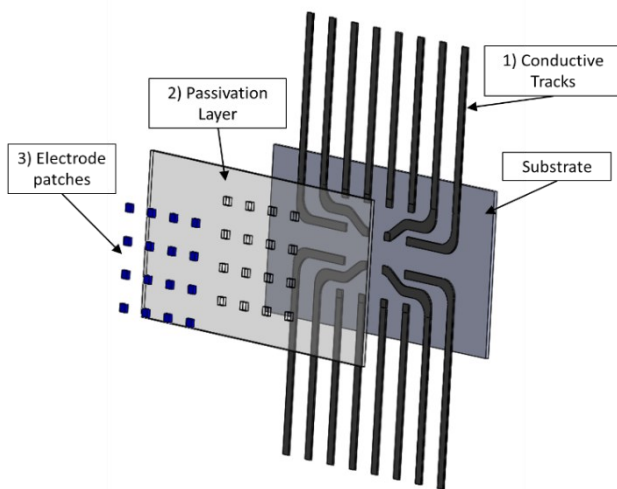


Fig. 1. Rendering of the three layers of the microelectrode array. Glass slide substrate, 1) 16 conductive tracks in silver ink, 2) the passivation layer deposit with NOA 81, and 3) 16 electrode patches in PEDOT:PSS.

output file to control the AJP printer movements during the manufacturing process. The fabrication process was designed with three steps: first, the 16 conductive tracks were printed onto a substrate. Second, the passivation layer was printed surrounding and on top of the conductive traces, leaving openings at the end of the Ag traces that correspond to the 16 electrodes. All conductive tracks were designed with a width of 50 μm , while the openings in the dielectric layer had a width and a length of 50 μm as the nominal area of electrode patches in the MEA design. The printing of the conductive layer will be repeated several times to increase the thickness and thus to reduce the resistance, since AJP is capable of depositing layer with a thickness in the range 0.1-2 μm . The number of overlapped deposition was limited to five to ensure thickness uniformity of the printed track and to avoid shortcuts between the silver paths.

B. Material selection

The glass microscope slide was adopted as a substrate for the printed MEA in order to glue a 3D printed ring to create the culture chamber. The first layer of conductive tracks was printed with Smart Aerosol Conductive Ink (S-CS61308, Genesink, France), based on silver nanoparticles, suitable for applications requiring high conductivity and high resolution at very low curing temperatures. The silver ink selection is necessary due to the need of an high conductive material able to bring the signal to the hardware for the signal recording of the action potential of neuronal cells. However, this material has no good biocompatible properties, and it is not corrosion resistant in an aqueous environment. In fact, a passivation layer with insulating and biocompatible material is added to avoid the corrosion of the silver in the electrolyte solution and the crosstalk between the several recording signals. The second layer for the passivation, as a coating to the silver track, and for the realization of a multilayer structure was obtained with an insulating ink, Norland Optical Adhesive, NOA 81 (Norland Products Inc., Cranbury, New Jersey, USA), a UV-curable polymer. It results to be hard, resilient bond with good adhesion on the glass. Besides having low conductivity, it is a transparent and biocompatible material. This ink is characterized by a viscosity of 300 cP at 25° C. The NOA 81 was selectively printed in order to leave 16 openings on the silver track ends in the center of MEA that correspond to the 16 sensing electrodes. The selectively opening for the electrode array was coated with biocompatible and conductive properties, that is needed at the interface between the silver and the cell culture, to prevent also a cytotoxic effect on the cells. The selected ink due to the AJP technique possibility to allow the deposition of conductive polymers, Poly(3,4-ethylenedioxythiophene)-poly(styrenesulfonate) (PEDOT:PSS) (Orgacon IJ-1005, Sigma-Aldrich, USA) was chosen as a surface coating (third layer) for the leaving opening at the end of the silver lines of the first layer. In this way, the printed PEDOT:PSS-based contact pads are in direct contact with the cells and the electrolyte solution of the cell culture, to obtain an increased SNR and better biocompatibility and more surface area incrementation. The last procedures ensure that only PEDOT:PSS and NOA 81 inks were exposed to the electrolyte solution.

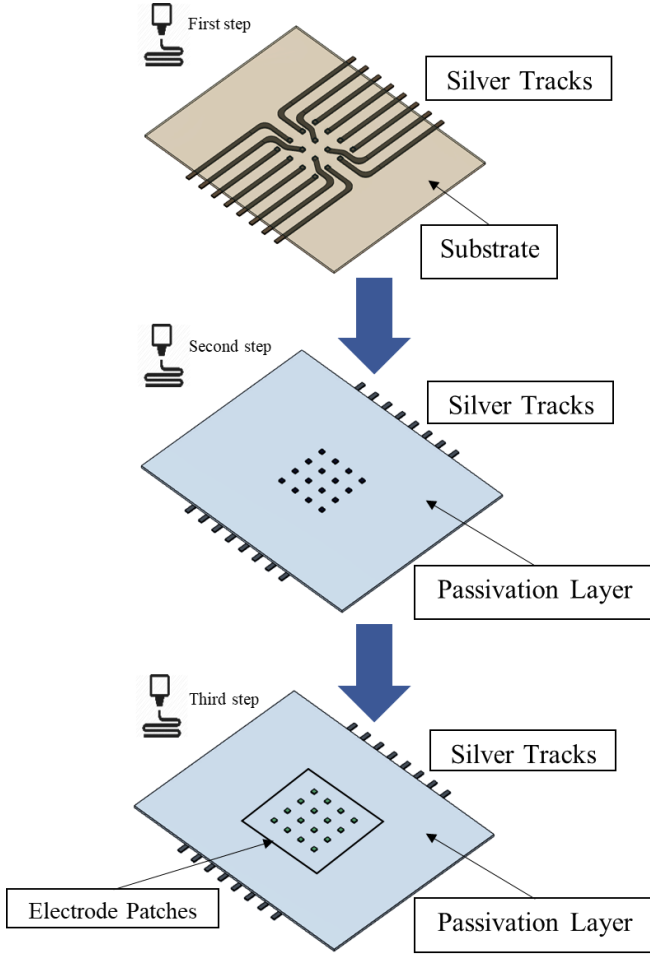


Fig. 2. Rendering of the process fabrication with Aerosol Jet Printing of the three steps. The first step was the deposition of the silver for the conductive tracks. In the second, the passivation layer was printed with NOA 81. Finally, the last step the electrode array was printed with PEDOT:PSS ink.

C. Fabrication of microelectrode array

In this work, the fabrication process of MEA was composed of three steps as shown in Fig. 2 to realize the different layers geometries of the electrode array, using AJP (AJ300, Optomec, Albuquerque, NM, USA). The AJP was used with the pneumatic atomizer mode to deposit both the silver-based ink, the insulator ink. Finally for the deposition of PEDOT:PSS patch, the ultrasonic atomizer mode was employed, due to the viscosity of the conductive polymer (7-12 cP at 22°C).

In the proposed method, for the printing of silver tracks and NOA 81 insulating layer, the temperature of the platen that allows the movement of the substrate in the X-Y direction was imposed at 60 °C to dry the ink during the printing, and the printed speed was fixed at 2 mm/s. While for the PEDOT:PSS, the temperature plate was set at 40°C and the printed speed at 1.5mm/s. The nozzle exit diameter used to print Ag traces and NOA 81 layer was 200 μm. Printing process parameters tuned to obtain the desired track width are listed in Table 1.

TABLE I. PRINTED INK PARAMETERS

Parameter	Printed Material		
	S- CS61308 Genesink	Norland Optical Adhesive 81	PEDOT:PSS
Sheat gas flow (SCCM)	100	40	30
Atomizer gas flow (SSCM)	635	1395	30
Exhaust gas flow (SCCM)	620	1365	/
Atomizer current (mA)	/	/	605
Platen Temperature (°C)	60	0	40
Printing speed (mm/s)	2	1.5	1.5

Process flows are expressed in the unit standard centimeter cube per minute (SCCM). For MEA fabrication the three steps were performed and studied: first, the silver-based conductive tracks were printed and thermally sintered at 150 °C for 1 h, recommended by the manufacturer, in an oven to sinter the ink and to reach the highest conductivity. Then the dielectric layer was printed and cured during the printing process by exposing to UV light by using the LED Spot type Panasonic ANUJ6180 (Panasonic, Osaka, Japan) and the lens ANUJ6423 as UV Curing System, characterized by a spot diameter of 3 mm, a peak UV intensity of 17,200 mW/cm² at an irradiation distance of 8 mm. In our trials, the selected power was 5% of the maximum peak intensity. Finally, the PEDOT:PSS-based patches were deposited and thermally sintered at 130 °C for 6 min, recommended by the manufacturer.

For the preliminary tests for silver ink, conductive tracks samples based on CAD drawings were printed with several overlapped depositions of ink. For each number of depositions, two samples were reproduced. While for the PEDOT:PSS patches, several deposition of the array were performed to evaluate the feasibility of printing,

D. Morphological and electrical characterization

Preliminary tests were performed for determining the geometrical characteristics of the printed layers and the resistivity of the printed tracks according to the number of deposition layers. The size of the features, i.e. thickness and width, of the printed tracks was measured by using the optical microscope and the Profilm3D (Filmetrics, San Diego, CA, US). The measurements of the resistance were performed by means of a multimeter (HP34401A, Agilent).

III. RESULTS

The optimization of the printed microelectrode array requires some preliminary tests to estimate 1) the thickness, the width, of the silver tracks, the passivation layer and the electrode array and 2) the resistivity of the silver tracks.

A. Morphological characterization

The morphological characterization of the fabricated MEA by means of optical microscopy and profilometer measurements was performed to evaluate the geometrical features of the microelectrode array. The optical microscope images are shown in Fig. 3. For the overlapped depositions of 1, 2, and 3 layers, there is no contact between the silver

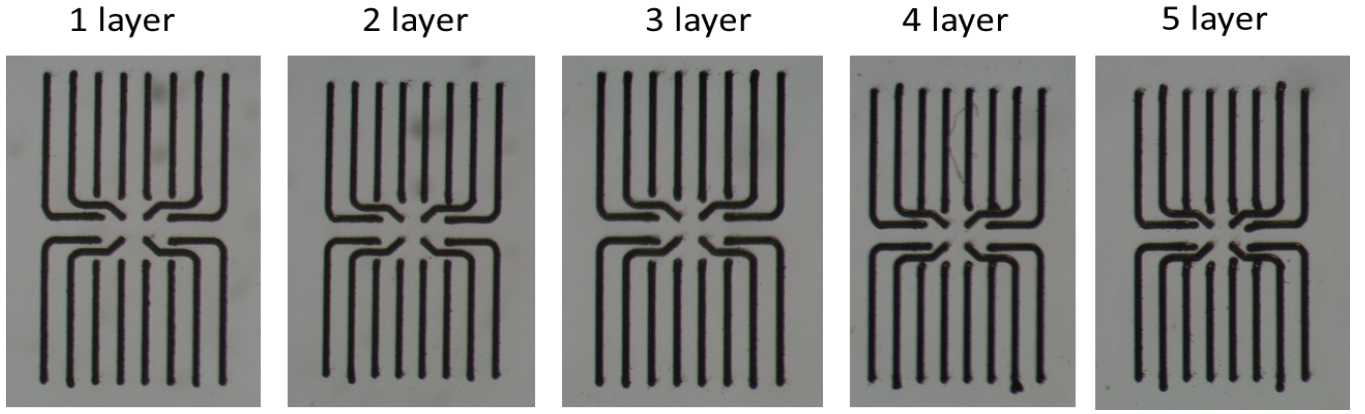


Fig. 3. Optical microscope image of the different number of overlapped deposition of silver layer.

tracks, avoiding unwanted electrical contacts. While increasing the overlapped depositions to 4 and 5 layers, the

TABLE II. GEOMETRICAL FEATURES OF THE TRACKS.

Number of layers	Geometrical features			
	Width (μm)	Standard deviation (μm)	Thickness (μm)	Standard deviation (μm)
1	67.72	± 1.33	1.44	± 0.13
2	64.57	± 0.57	3.83	± 0.57
3	68.35	± 1.83	6.26	± 0.47
4	65.24	± 1.35	8.18	± 0.46
5	67.21	± 2.60	10.76	± 0.21

silver tracks come into electrical contact and produce shortcuts between the tracks and an inadequate outcome. The profilometer images show the profile of the depositions according to the width and thickness. The images carried out with the profilometer, after the acquisition, were firstly optimized with specific filtering techniques to enhance the profile quality, and then, the geometrical features were measured. Table 2 shows the mean and deviation standard of

the width and thickness of the conductive tracks for the different overlapped depositions.

The width remains approximately the same for the different number of depositions in a range of 65-68 μm . The thickness increases with the number of depositions, as we can expect,

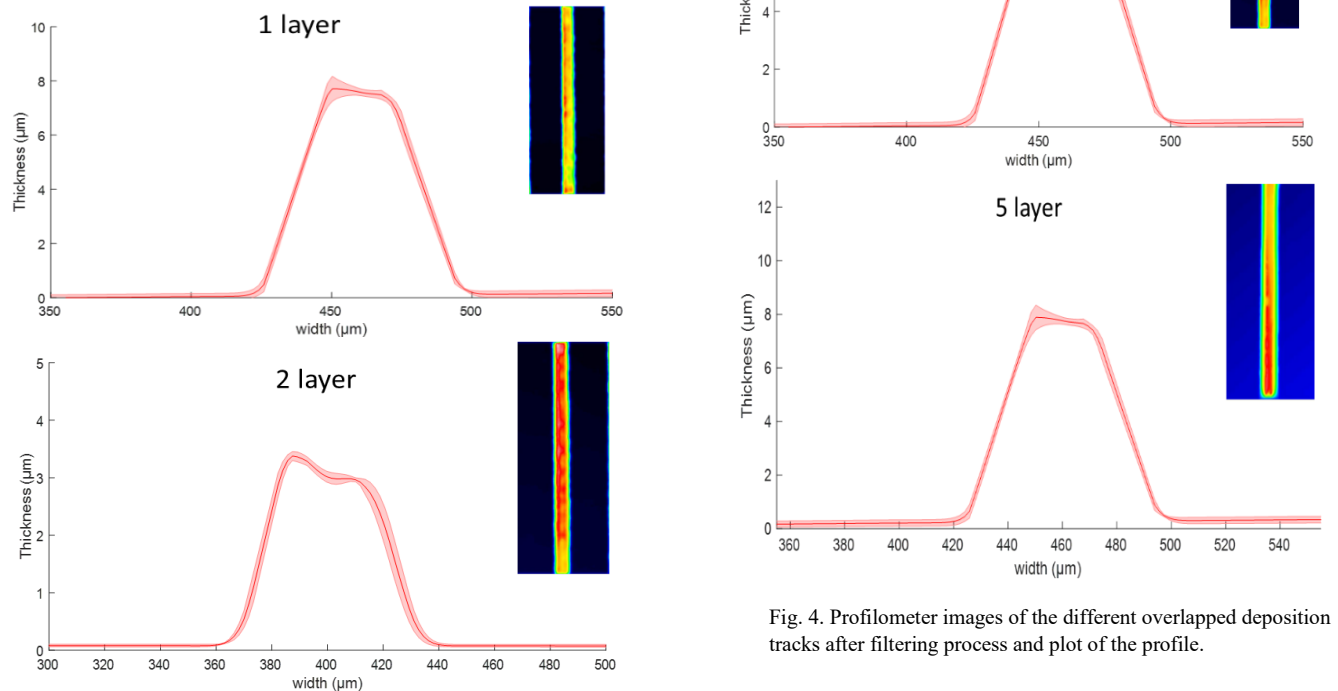


Fig. 4. Profilometer images of the different overlapped deposition tracks after filtering process and plot of the profile.

from $1.44 \pm 0.13 \mu\text{m}$ to $10.76 \pm 0.21 \mu\text{m}$. In detail, in Fig. 4 the characteristics of the different profiles of the silver tracks obtained by varying the number of overlapped depositions are shown. For the five profiles, the filtered image taken with the profilometer is shown in insets and the mean and deviation standard of the silver track thickness (y-axis) with respect to the width (x-axis) are shown. For the mean and deviation standard values, five equidistant lines were traced on the silver profiles. As it has been shown, the deposition of more layers of silver leads to an increase in thickness, however as regards the width of the track, there is not a considerable widening of the track. This is favorable as far as the technology is concerned. Despite the different deposition layers, there is no widening of the trace so that the overlapped deposition of silver layers does not lead to changes in the thickness of the trace. It means that the several layers sufficiently solidify before the following deposition.

Concerning the passivation layer deposition, the NOA 81 was printed on the conductive track with a single deposition. In Fig. 5 the profilometer acquisitions show a uniform distribution of the NOA 81 deposition with a mean thickness of $15 \mu\text{m}$ about eight measurements of an equidistant slice taken from the profilometer image, the profile changes with $\pm 2 \mu\text{m}$ from the nominal value, in the x-axis is reported the width, while in the y-axis the thickness of the NOA 81 layer. The profile variation is due to the AJP technology as additive manufacturing technology with a deposition of ink trace and moreover, the instantaneous curing yield to a non homogeneous deposition due to the variations of few micron. Such variation of the profile does not lead to a damage of the cells or reorientation due to the morphology of the substrate [12]–[14].

In the feasibility study of the PEDOT:PSS-based ink, it was printed according to the CAD design; in particular the deposition of 16 spots was realized in order to obtain the biocompatible interface between cells and silver and for the surface coating of the silver ink, avoiding the corrosion environmental for the conductive tracks. After setting the optimal parameters, several deposition were performed with different setting time. The time corresponds to the open shutter time (2-4-6 seconds of open shutter) and consequently, the quantity of extruded material for the single

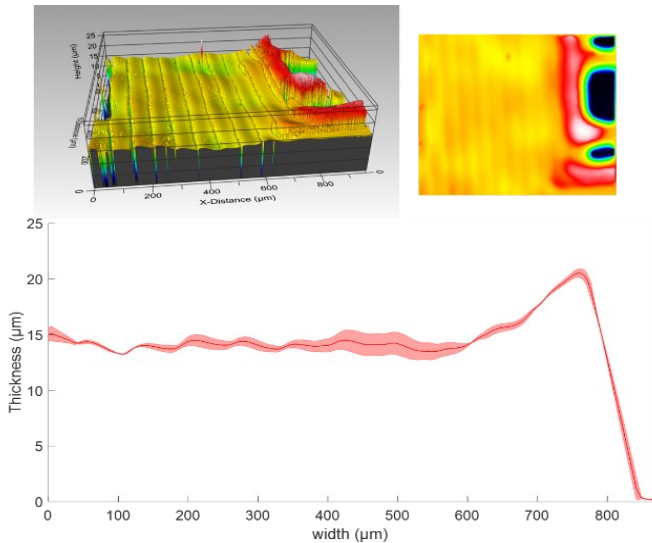


Fig. 5. Deposition of passivation layer and plot of the NOA 81 profile.

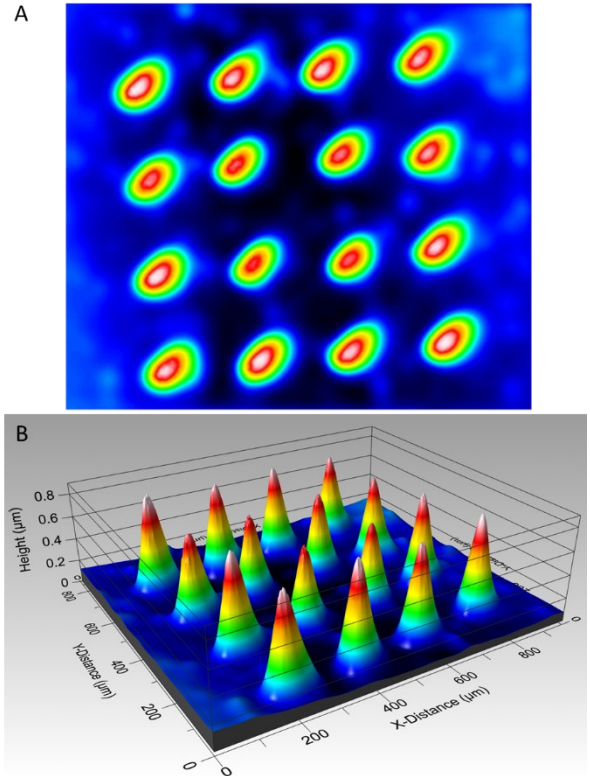


Fig. 6. Profilometer images of the 16 electrodes array printed with PEDOT:PSS-based ink.

electrode. In Fig. 6 the deposition of the 16 spots of PEDOT:PSS-based ink is shown with the profilometer, in the Fig. 6A the top views of electrode array show the results obtained from the depositions. It is shown how the spots have no shortcuts between them. While the Fig. 6B shows the three dimensional view of the printed array and the formation of micropillar with height of $0.8 \mu\text{m}$.

B. Electrical characterization

The electrical characterization of the fabricated MEA by means of a bench multimeter was performed to evaluate the electrical resistance of the printed Ag tracks of the microelectrode array, taking into account the geometrical features of the tracks. For the measurement, five lines with different overlapped depositions as samples are printed with an imposed length to measure the resistance of the silver tracks. Fig. 7 shows the mean value of the resistance and the deviation standard according to the number of overlapped depositions silver layers in the x-axis. The resistance, as expected, changed considering the number of depositions. In particular, the resistance decreases proportionally with the increase in the number of layers.

The equation (1) to extract the resistivity ρ from the measured resistance R is:

$$\rho = R \cdot (w \cdot t) / l \quad (1)$$

where w is the average width of the tracks, t is the average thickness and l is the length of the measured silver track resistivity of the silver tracks. The mean value derived from the equation (1) for the S-CS61308 Genesink is $5.7 \mu\Omega \cdot \text{cm}^2$

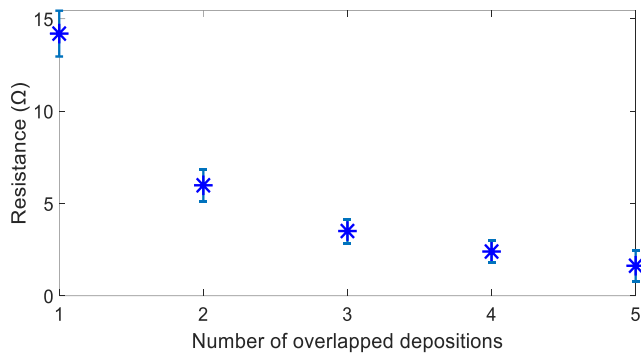


Fig. 7. Plot of the measurement resistance value for different overlapped deposition.

and is in agreement with the manufacturer datasheet, with slightly higher value.

In conclusion, the preliminary results obtained by the printing of the three layer, specifically the conductive tracks, the passivation layer, and the sensing electrode array confirmed the possibility to achieve the suitable designed geometrical features and the optimization of the AJP process. In particular, the three overlapped depositions of silver ink result to be a proper compromise between the average width and thickness of the tracks that avoid unwanted electrical contact and the resistance obtained from the measurement. Despite the overlapped deposition, the width remains constant, allowing to increase the thickness and consequently, increase the conductivity of the material, in order to obtain a better signal quality.

A single layer of NOA 81 is sufficient to create an insulating layer able to shield the recording signals and avoid the corrosion of silver paths. The substrate topography resulting from the deposition by AJP technique does not lead to a cell damage or reorientation of the neuronal cells according to the literature. Finally, the PEDOT:PSS showed an adequate feasibility in the production of the recording electrode array for the action potential signals. In order to create an more biocompatible interface with the biological environmental and increase the SNR thanks to the employment of a conductive polymer.

IV. CONCLUSION

A printed microelectrode array was proposed as an alternative to the traditional microfabrication methods, by using Aerosol Jet Printing, a novel additive manufacturing technology. The possibility to deposit directly on the substrate a micropattern of sensors ensures the customization of the device, low cost and quick fabrication. In this work, a preliminary study of the design of the printed MEA and the selection of suitable materials for the biomedical application was developed. In order to realize a bioelectronic device by fully AJP printed for the stimulation and recording of action potentials signals

of neuronal cells. The design of the MEA and the optimization of the parameters of the AJP machine lead to the realization of the three main layer of the printed MEA, in particular, the overlapped depositions of silver tracks for the conductive lines, the NOA 81 as the passivation and coating layer and finally, the PEDOT:PSS-based ink for the biological interface and the sensing electrode array.

The future developments will concern the full characterization of the three layers of the printed MEA, and the subsequent testing of the whole device for action potential recording and electrical stimulation of neuronal cells for the study of *in vitro* neurodegenerative disorders. Finally, the printed MEA will be added to an automated device for cell culture.

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