Development of a 3D Printing Strategy for Completely Polymeric Neural Interfaces Fabrication

Ciro Zinno
The BioRobotics Institute,
Department of Excellence in Robotics & AI
Scuola Superiore Sant'Anna
Pisa, Italy
ciro.zinno@santannapisa.it

Ilaria Cedrola
The BioRobotics Institute,
Department of Excellence in Robotics & AI
Scuola Superiore Sant'Anna
Pisa, Italy
ilaria.cedrola@santannapisa.it

Alice Giannotti
The BioRobotics Institute,
Department of Excellence in Robotics & AI
Scuola Superiore Sant'Anna
Pisa, Italy
alice.giannotti@santannapisa.it

Eugenio Redolfi Riva
The BioRobotics Institute,
Department of Excellence in Robotics & AI
Scuola Superiore Sant'Anna
Pisa, Italy
eugenio.redolfiriva@santannapisa.it

Silvestro Micera
The BioRobotics Institute, Department of Excellence in Robotics & AI
Scuola Superiore Sant'Anna
Pisa, Italy
Translational Neural Engineering Lab
Ecole Polytechnique Federale de Lausanne
Lausanne, Switzerland
silvestro.micera@santannapisa.it

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***Abstract*—The fabrication of neural interfaces (NIs) typically relies nowadays on the implementation of complex, expensive, and time-consuming photolithographic processes. Metals and polymers are the materials currently used to fabricate NIs. Conductive polymers could be an alternative to metals to enhance the biocompatibility of the devices. Additive manufacturing techniques provide an easier and low-cost approach to process and finely tuning the geometrical and morphological features of polymers. Here, we propose a 3D printing strategy for the fabrication of completely polymeric neural interfaces, based on extrusion printing. The materials have been chosen to enhance the biocompatibility of the devices. PDMS has been chosen as insulating substrate, while a PEDOT:PSS-based ink has been selected for the conductive component. Morphological, mechanical, and rheological analyses on the inks have been carried out and a first prototype of a neural interface has been fabricated. The PDMS has a Young Modulus of 600 kPa, in the same order of magnitude as peripheral nerves, with a thickness of 160 µm. The PEDOT:PSS inks fabricated present a shear thinning behavior, ideal for an extrusion printing process This approach could represent a valuable alternative to photolithography and an innovative method for the fabrication of NIs, due to the high degree of customization, ease of implementation, low-cost and flexibility in materials choice.**

Keywords—neural electrodes, additive manufacturing, biocompatibility, polymeric, neural interfaces

I. INTRODUCTION

NIs are devices employed in bioelectronic medicine to communicate with the central and peripheral nervous systems. Typically, these systems are fabricated using photolithographic techniques, that allow high geometrical precision. However, they require multiple complex steps to be implemented, impeding very expensive facilities, materials, and machinery. Moreover, the material choice is highly limited by the need to use harsh reagents during manufacturing [1], [2]. Furthermore, photolithographic techniques require the use of evaporated or sputtered metals to fabricate the conductive elements, which have reported to be disadvantageous for long-terms applications due to the different physiochemical properties respect to the native tissue To address these drawbacks, additive manufacturing (AM) techniques have raised wide interest in the last decades in various fields of Biomedical Sciences, including Neuroengineering. The main advantages of AM are the possibility to provide high customization with no cost, ease of implementation, and an overall cost reduction compared to photolithography. As examples, it is possible to fabricate devices adaptable to various nerve morphologies, as bifurcations or fascicles with irregular diameter along the longitudinal axis [3]. Wearable sensors and electrodes have been realized using AM approaches, in particular, extrusion and inkjet printing, and stereolithography [4]. However, when these devices are implanted *in vivo*, a consistent foreign body response (FBR) occurs: fibroblasts and macrophages trigger a biochemical cascade leading to the implant encapsulation by fibrotic tissue and chronic inflammation process harmful for the native tissue [5]. One of the reasons for such intense FBR is also the physiochemical mismatch between the implant and the surrounding tissues. For this reason, biocompatible and soft materials should be selected for NIs fabrication [2]. Typically, the insulating element of a neural interface is a polymeric substrate (polyimide, parylene C, PDMS), while the conductive element is composed of metals. Great efforts have been spent by several research groups in developing conductive polymers for many applications, in particular for neuroengineering [6]–[8], with a particular focus on PEDOT:PSS-based hydrogels and compounds. The interest in conductive polymers for NIs is also due to their processability with various AM techniques, such as extrusion-based 3D printing. Here, we present and characterize a novel fabrication process based on an extrusion 3D printing strategy, to realize a fully polymeric neural electrode. The insulating component is composed of PDMS, a widely used polymer in the field of neural interfaces thanks to its properties of being chemically inert and mechanically soft. The conductive element is realized with a custom metal-free PEDOT:PSS-based ink, appropriately tuned for being processed with 3D printing.

II. Material and Methods

A. Polymeric Inks Preparation

PDMS has been chosen for the realization of the insulating substrate (Sylgard 184, Dow Corning Corporation, Germany). The base monomer and the cross-linker agent were thoroughly mixed inside the printing syringe of the 3D printer, with an 80:20 ratio. Then, the solution was placed in a vacuum chamber for 15 min to remove air bubbles and kept in the fridge at 4°C before use. The conductive component has been realized using an adapted version of the PEDOT:PSS-based ink proposed in [8]. Pristine PEDOT:PSS (Clevios PH100, Heraeus Electronic Materials, Germany) was poured into a custom cylindrical mold made of polytetrafluoroethylene (PTFE), with aluminum plates on both the top and bottom sides. Then, the material was frozen in a fridge at -80°C. After the freezing process, the PEDOT:PSS was removed from the mold and freeze-dried for 72h. Then, the freeze-dried PEDOT:PSS was cut and re-dispersed (5% wt.) in a solution of deionized water and dimethyl sulfoxide (DMSO) with an 80:20 v/v. ratio. Ionic additives–assisted stretchability and electrical conductivity (STEC) enhancers were also added to the water-DMSO solution (1-10% wt.). The compound was then automatically mixed in a mixer (SimplyMix, IGT Testing Systems, Netherlands) at 100 rounds per minute for 4 min, to guarantee the homogeneity of the solution, with a proper dispersion of PEDOT:PSS fibrils in the liquid components. The ink was then kept in the fridge at 4°C before use.

B. Printing Process

A rectangular shape (50x15 mm) was selected to realize a prototype of a neural electrode. Different nominal heights were tested (h=[0.1:0.1:0.5] mm), to find a tradeoff between low thickness and manageability of the device. For the conductive tracks, three lines for each rectangle were designed (45x0.15 mm). The digital file was realized using a CAD software (AutoCAD 2022, Autodesk, USA). The extrusion printing process (3D Bioplotter, Envisiontec, USA) was carried out with variable printing velocity (PDMS: v_{PDMS} =[7.5:2.5:12.5] mm/s, PEDOT:PSS: v_{PEDOT}=[6:1:12] mm/s) and pressure (PDMS: P_{PDMS}=[2.5:0.5:3.5] bar, PEDOT: P_{PEDOT}=[0.6:0.2:1.2] bar). A 100 µm diameter nozzle was used, with the syringe temperature set to 25°C. To accelerate the polymerization process and avoid splashing of the PDMS, the platform temperature was set to 80°C. This temperature is also useful for the annealing process of the PEDOT:PSS ink, to let the solvent evaporate and increase percolating networks, thus increasing the conductivity. The device was printed as follows: (i) the first layer of PDMS was deposited onto the printing platform; (ii) after 1 min, PEDOT:PSS-ink traces were deposited on the PDMS layer, then (iii) another PDMS layer was deposited on top, encapsulating the conductive traces and exposing only some spots (active sites). The fabrication and printing processes are summarized in Figure 1.

C. Materials Characterization

The samples were analyzed with an optical microscope (Hirox, JPN) to evaluate the thickness in the x and y direction (Figure 2). The sample was kept on cylindrical support fixed on the microscope plate, and the x and y sections were measured. Morphological analysis of the internal structure of the

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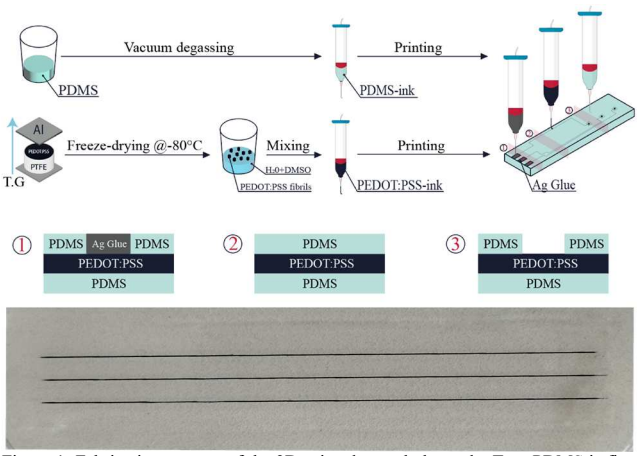

Figure 1: Fabrication process of the 3D printed neural electrode. Top: PDMS is first degassed and then it is ready for the printing process. Pristine PEDOT:PSS is freeze-dried in a custom mold, and then re-dispersed and mixed in a solution of water and DMSO. The light-blue line represents the direction of the thermal gradient. Silver glue can be deposited on some spots on the electrode to connect it with external electronics. Bottom: prototype of the 3D printed NI.

lyophilized PEDOT:PSS compound was carried out, to understand if the fabrication process allowed to obtain a fibrillar directional structure. The cylindrical samples were cut into longitudinal sections, and these sections were then imaged. To evaluate the mechanical properties of the PDMS insulating substrate for different printing parameters the ASTM D882 Standard Test Method for Tensile Properties of Thin Plastic Sheeting was followed. To perform the tensile tests, the samples were realized with a rectangular shape of 100x10 mm. The tests were carried out using a tensile machine (Instron, USA), imposing a displacement rate of 5 mm/min (Figure 3). Stress vs. strain curves were obtained from these tests, and they were used to calculate the Young Modulus as the slope of the first linear part of the curve. For PDMS tensile tests, a range of 0-40% strain was selected for the linear behavior of the material [9]. Young Modulus computations were carried out in Matlab (Mathworks, USA). PEDOT:PSS-based inks were characterized rheologically to assess their printability. A rotational rheometer was used (Anton Paar, Austria) with a 25 mm diameter steel parallel plate configuration (Figure 4). Printability was assessed by analyzing the apparent viscosity, measured as a function of the shear rate by steady-state flow tests with a logarithmic sweep of the shear rate (0.01–100 s^{-1}). For storage (G') and loss (G'') modulus calculation, a logarithmic sweep of shear stress (1–1000 Pa) at 1 Hz shear frequency was imposed. All the tests were conducted at 25°C.

III. Results

A. Printing Process

The first parameter to be optimized is the nominal height of the samples. Velocity and pressure were kept constant during this phase (v_{PDMS}=10 mm/s, P_{PDMS}=3 bar), and multiple layers of rectangular-shaped PDMS substrates were printed. Samples with a nominal height of less than 0.2 mm were difficult to

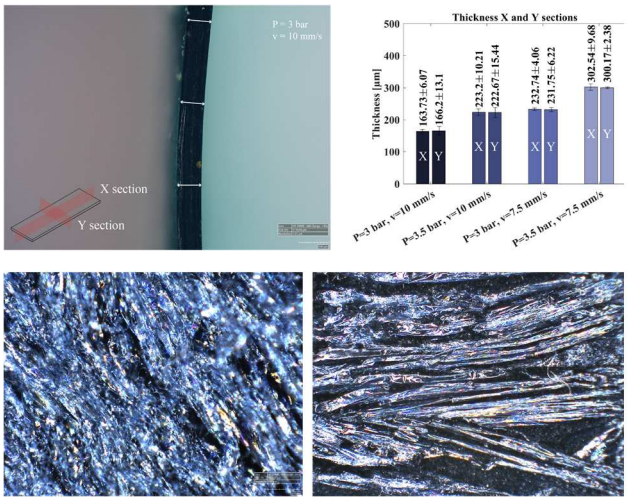

Figure 2: Morphological characterization of the materials. Top row: PDMS. Left: section of a PDMS substrate with white arrows highlighting the measuring point. Right: thickness measurements in the X and Y direction with different printing parameters. Values are expressed as mean ± standard deviation (n=5) Bottom row: PEDOT:PSS. It is possible to see a fibrillar structure, with a directional alignment given by the imposed thermal gradient

handle because of their low thickness, which led to a self-folding behavior when detaching the substrates from the printing platform. When the nominal height was set to 0.4 and 0.5 mm, often we observed a splashing behavior, with sample contours not properly defined. Thus, a value of 0.2 mm was selected for the nominal height. Then, different values of velocity and pressure of printing were tested for PDMS and PEDOT:PSS-based inks. For PDMS, the aim was to obtain a trade-off between manageability and thickness; for PEDOT:PSS the aim was the realization of a continuous track with good adhesion to the PDMS substrate. Within the selected velocity ranges for PEDOT:PSS inks, a continuous line without interruptions was obtained with v_{PEDOT}=6 mm/s. Higher values of v_{PEDOT} caused holes and discontinuity along the printed track during the printing process. Regarding pressure values, a P_{PEDOT} =0.8 bar was selected for PEDOT:PSS-DMSO inks. When the STEC enhancer was added (1-10% wt.) this value was raised to P_{PEDOT}=1.2 bar, since the STEC increases the viscosity of the ink, thus avoiding clogging at the nozzle.

B. *Materials Characterization*

The results of the morphological characterization of the PDMS substrates are shown in Figure 2. Thickness values are shown in the y direction as mean ± standard deviation (n=5 sections). The nominal height was selected from the optimization of the printing process as h=0.2 mm. Since all the printing parameters combinations led to manageable substrates, we selected the one with the lowest thickness for further testing (v_{PDMS}=10 mm/s, P_{PDMS}=3 bar), measuring 163.73 ± 6.07 µm in the x direction and 166.2 ± 13.07 µm in the y direction. Optical microscopy of PEDOT:PSS reveals the polymer structural organization, composed of microfibers and lamellae aligned to the direction of the thermal gradient (Figure 1) and resulted by the longitudinal nucleation of water crystal during the freezing process that conferred a particular orientation to polymer chains, as previously reported with other polymers [10], [11]. PDMS samples obtained with different printing parameter combinations have been mechanically tested, performing tensile tests. The Young Modulus has been obtained from the stress vs

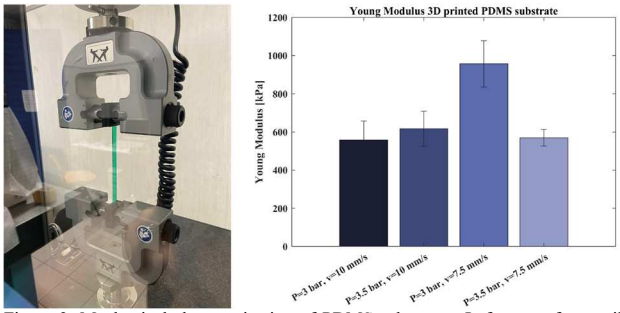

Figure 3: Mechanical characterization of PDMS substrates. Left: setup for tensile tests: the substrate is kept fixed between two grips with distances imposed by ASTM D882. Right: Young Modulus for PDMS substrates obtained with different printing parameters.

strain curves as the slope of the linear strain range (0-40%). The results are shown in (n=5). Increasing the v_{PDMS} the Young Modulus decreases from 1000 kPa to 600 kPa (Figure 3), while increasing the P_{PDMS} this effect on the Young Modulus is not reproduced. For rheological tests, since all of them have been conducted applying logarithmic frequency sweeps of shear rate and shear stress, all the curves are represented in semilogarithmic axes (Figure 4). In it is possible to see the different textures of PEDOT:PSS inks with DMSO and different STEC concentrations (1-10% wt.). Increasing STEC concentration the ink starts becoming more elastic and less liquid. This phenomenon is confirmed by a marked increase in the storage modulus G' (from 750 Pa to 2250 Pa), representing the elastic component of the compound [8]. Apparent viscosity curves show a shear thinning behavior, with viscosity decreasing with increasing shear rate.

IV. Discussion

A. *Polymeric Inks Preparation*

The material choice and combination of the custom mold used for the preparation of the PEDOT:PSS ink is useful to obtain an anisotropic thermal gradient in the structure, thanks to the directional ice crystal nucleation during the freezing process (Figure 1). This feature could improve the conductivity of the compound, as an anisotropically-oriented polymer chain would increase the percolation pathways of the structure [8]. Future experiments will be conducted to verify this hypothesis. The addition of STEC enhancers is a method to further increase the stretchability and conductivity of the ink as previously reported [7] and could be advantageous not only for the electrical properties of the NI but also during the *in vivo* implantation procedure, as compliant substrates can better resist to mechanical deformation without breakage. This will be explored in both acute and chronic studies, to evaluate the long-term behavior of the devices both from an electrical and mechanical point of view.

B. *Printing Process*

The extrusion printing process used in this work guarantees ease of implementation and flexibility in terms of printing parameters and materials choice (Figure 1). However, using a 100 µm diameter nozzle results in low spatial resolution compared to other 3D printing approaches and photolithography. This is particularly limiting in designing the size and the shape of the active sites of the electrodes since

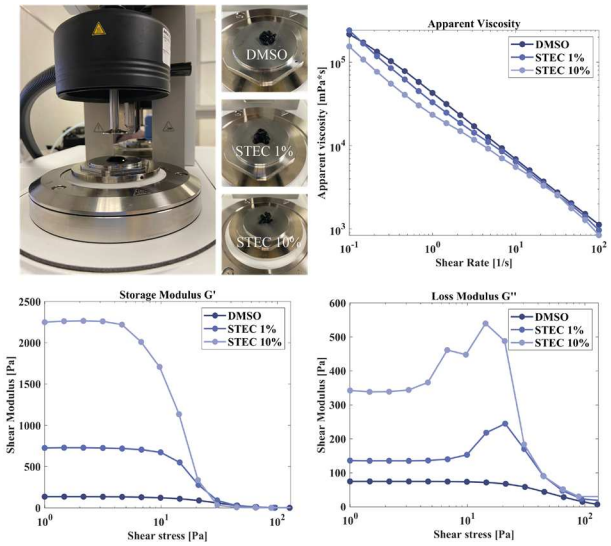

Figure 4: Rheological characterization of PEDOT:PSS inks. Top-Left: setup for rheological measurements. The three panels show the appearance of the three different ink formulations, with varying STEC concentrations. Top-right, bottom-left, bottom-right: rheological measurements; apparent viscosity shows a shear thinning behavior, ideal for extrusion printing; the storage and loss modulus indicate elastic and viscous behavior of the inks.

bigger active sites limit the selectivity of the electrodes. In future studies smaller nozzles will be used (50-30 µm), considering a modification to the inks' formulation to avoid clogging. Adhesion between the PDMS and the PEDOT:PSS tracks should be deeply analyzed both *in vitro* and *in vivo*, to evaluate how the swelling of the PEDOT:PSS ink could affect the overall device, and if this could lead to the detachment of the two components.

C. Materials Characterization

The thickness of the PDMS samples is similar in the x and y direction, highlighting the homogeneity of the substrates. The lowest thickness has been selected to reduce the mechanical mismatch between the device and the nerve as much as possible. Further AFM analysis will be carried out to quantitatively evaluate the roughness of the PDMS substrate and of the PEDOT:PSS active site. Notably, it would also be interesting to assess how different printing parameters could tune surface roughness, as it is known to influence neural cell adhesion [12]. The internal structure of the freeze-dried PEDOT:PSS ink shows a directional alignment of the microfibers and lamellae along the direction of the thermal gradient. As analyzed in [7], [8], to enhance PEDOT:PSS conductivity it is important to achieve good connectivity between PEDOT-rich domains, which can be obtained with an anisotropic polymer chains arrangement. The Young Modulus of 3D printed PDMS structure for all printing parameters except v_{PDMS}=7.5 mm/s, P_{PDMS}=3 bar, is close to 600 kPa, and it is comparable to the values reported for the epineurium of peripheral nerves [13], which are the target for these devices. This occurrence demonstrates the ability of 3D printing to finely tune the mechanical properties of our device. The shear thinning behavior highlighted from the apparent viscosity curves is the typical and desired behavior for printable inks. The values of G' and G'' are close to similar formulations in literature [8]. The differences could be due to different fabrication processes, which could lead to modification of the morphology, thus to different rheological properties. The addition of the STEC enhancers at higher concentrations (10% wt.) results in a more elastic, but more difficult to print formulation, due to the formation of a lumpy paste. The STEC concentration will be optimized in future studies to guarantee a tradeoff between the stretchability and printability of the ink.

V. Conclusion

We presented a 3D-printed fully polymeric prototype of NI. The insulating and conductive materials have been morphologically characterized. Mechanical tests have been carried out on the PDMS substrate, identifying the best printing parameters to obtain a structure similar to peripheral nerves. The chosen PEDOT:PSS formulation guaranteed printability and tuning of rheological properties with the inclusion of STEC enhancers. The printing process is straightforward to implement and allowed to process of highly biocompatible and soft materials. Future experiments will be conducted to characterize the electrochemical performance of the device and to finely tune structural parameters to fabricate a completely polymeric NI.

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