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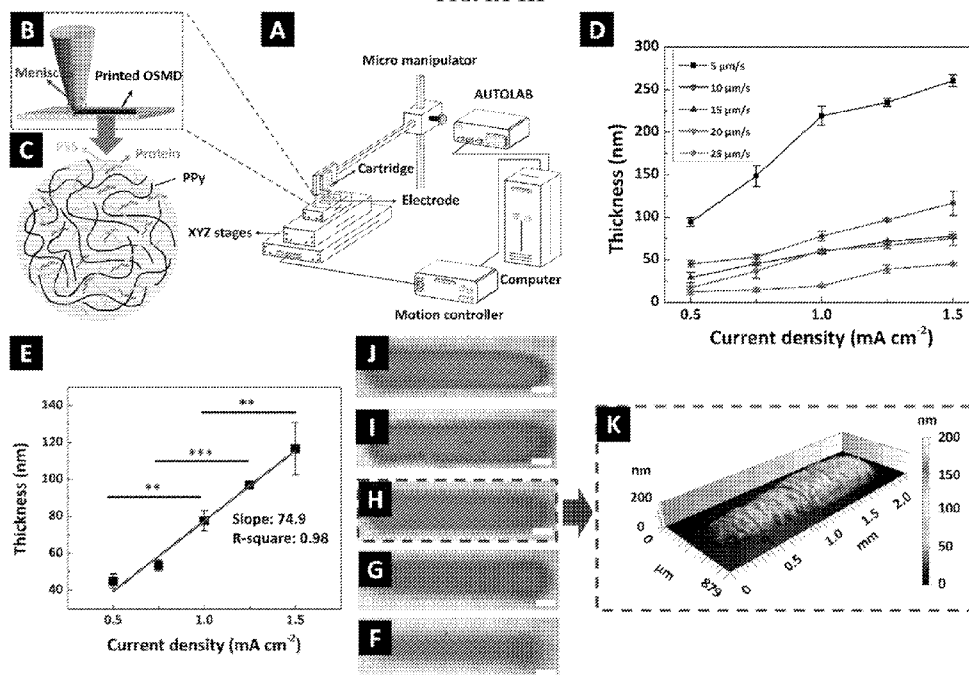
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(54) Title: SOFT DIRECT 3D PRINTING OF ORGANIC SEMICONDUCTORS

FIG. 1A-1K



(57) **Abstract:** Organic semiconductor microelectronic devices (OSMDs) can be fabricated through direct 3D printing using a gel-pen technique. The technique utilizes in situ electrochemical deposition of the meniscus provided by a wet hydrogel cartridge at the electrode interface. Bioactive proteins such as laminin, fibronectin and glucose oxidase can be incorporated in the gel and entrapped within the OSMDs.

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SOFT DIRECT 3D PRINTING OF ORGANIC SEMICONDUCTORS

BACKGROUND

[0001] This application claims priority to U.S. Provisional Patent Application Serial No. 63/449,445, entitled "Soft Direct 3D Printing of Organic Semiconductors," the entire contents of which are hereby incorporated by reference.

[0002] This disclosure pertains to systems and methods for fabrication of microelectronic devices.

[0003] The necessity for developing advanced microelectronic devices has grown immensely in a variety of scientific / technological fields such as flexible electronics, micro / nano electromechanical systems, organic bioelectronics and biosensing. Choice of functional material coupled with employment of advanced fabrication techniques constitute key elements for successful demonstration of next-generation microelectronics. Most recently, Organic Semiconductors (OSs) have emerged as ideal material candidates for construction of soft and conductive microstructures. OS is an umbrella term, referring to molecules / polymers with electroactive properties, stemming from π -conjugated (alternative single and double) bonds in their chemical structure. If doped properly with a counterion in their bulk structure, OSs can exhibit a wide range of electrical conductivity from semiconductors to even close-metallic levels. OSs offer many attractive properties such as mixed ionic / electronic conductivity, mechanical softness, and biocompatibility, which make them appealing in printed circuitry, optoelectronics, and neural interfaces. Among OSs, conjugated polymers such polypyrrole (PPy) and poly(3,4-ethylenedioxythiophene) (PEDOT) have garnered tremendous attention owing to their superior conductivity, outstanding chemical stability, and excellent biocompatibility. Such OS platforms have been widely used for applications such as neural electrodes, solar cells, and chemical / biological sensors.

[0004] One of the most fascinating features of OSs is their facile processability and ease of fabrication. To date, construction of organic electronics in micron-scale have mostly relied on conventional printing / patterning techniques such as aerosol jet printing, inkjet printing, screen printing, photolithography, electrochemical patterning (through selective removal / deposition), and dip-pen nanolithography. The challenges associated with these approaches include minimal control over geometry, the need for sophisticated / expensive instruments, and multi-step / labor-intensive fabrication procedures.

SUMMARY

[0005] The present disclosure relates generally to systems and methods for direct writing of organic semiconductor microelectronic devices. There has been a growing interest towards direct writing of conjugated polymer OS structures using various 3D printing techniques such as nozzle printing and direct writing based on ion-conductance microscopy tips. In direct writing techniques, typically a meniscus (liquid bridge) is formed at the interface between the tip of the 3D printer and the surface, and as the tip moves based on the pre-defined geometry, the meniscus is pulled and the 3D structure is directly constructed in a layer-by-layer fashion.

[0006] In particular, the present disclosure relates to a gel-pen 3D printing technique that can be utilized to fabricate conductive and bioactive organic semiconductor microelectronic devices (OSMDs) for a variety of applications, including electronic circuitry, biosensing and bioelectronics. Aspects of the present disclosure relate to in-house 3D printer for direct writing of OSMDs made of any suitable organic semiconductor material, including but not limited to polypyrrole (PPy), poly(aniline) (PANI) or poly(3,4-ethylenedioxythiophene) (PEDOT). Embodiments of this technique, generally referred to as gel-pen, use a meniscus-guided wet hydrogel cartridge to directly electrodeposit the precursor solution, i.e. monomer and dopant, at the electrode interface which leads to creation of OS structures. Fabrication and characterization of OSMDs with various geometries and arbitrary shapes have been successfully demonstrated. Moreover, by using the hydrogel cartridges as reservoirs for biomolecules such as laminin and glucose oxidase, these bioactive proteins can be entrapped within the produced OSMDs, and their biological activity and functionality have been further assessed.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] FIG. 1 shows (A) a schematic of an experimental setup of an exemplary 3D printer, according to preferred embodiments described herein, (B) a close-up illustration at the electrode interface of an exemplary 3D printer according to preferred embodiments, (C) exemplary deposited structures including 3D mesh of organic semiconductor, dopant, and bioactive agent, (D) thickness measurement at various set of current densities (0.5 to 1.5 mA cm^{-2}) and stage velocities ($5 \text{ } \mu\text{m s}^{-1}$, $10 \text{ } \mu\text{m s}^{-1}$, $15 \text{ } \mu\text{m s}^{-1}$, $20 \text{ } \mu\text{m s}^{-1}$, and $25 \text{ } \mu\text{m s}^{-1}$), (E) thickness of deposited lines with respect to current density at stage velocity of $10 \text{ } \mu\text{m s}^{-1}$, optical micrographs of deposited lines at stage velocity of $10 \text{ } \mu\text{m s}^{-1}$ and various current densities

of (F) 0.5 mA cm^{-2} , (G) 0.75 mA cm^{-2} , (H) 1 mA cm^{-2} , (I) 1.25 mA cm^{-2} , and (J) 1.5 mA cm^{-2} , and (K) 3D render of a deposited line at stage velocity of $10 \text{ } \mu\text{m s}^{-1}$ and current density of 1 mA cm^{-2} , obtained from MCM.

[0008] FIG. 2 shows images of exemplary fabricated OSMDs prepared using embodiments of gel-pen 3D printing method, including (A)-(B) array of capacitors, (C)-(D) array of square mazes, (E)-(F) array of six-pointed stars, (G)-(H) spiral, (I)-(J) brain electrode array, (K)-(L) concentric circles, (M)-(N) multi-line electrode, and (O) resistor array fabricated on thin and flexible gold-coated PDMS film.

[0009] FIG. 3 shows optical micrographs of Ppy lines deposited using embodiments of gel-pen 3D printing method at (A) 2-pass, (B) 4-pass, (C) 6-pass, (D) 8-pass, and (E) 10-pass, MCM cross-sectional 3D render of lines deposited at (F) 2-pass, (G) 4-pass, (H) 6-pass, (I) 8-pass, and (J) 10-pass, (K) thickness measurement of deposited lines at various passes, (L) width of the deposited lines at various passes, and 3D printed shapes fabricated at 4-pass, including (M) a circle and (N) a triangle.

[0010] FIG. 4 shows micrographs of deposited lines at current density of 1 mA cm^{-2} and stage velocity of $10 \text{ } \mu\text{m s}^{-1}$ using (A) $20 \text{ } \mu\text{l}$ pipette tips, (B) $300 \text{ } \mu\text{l}$ pipette tips, (C) 1 ml pipette tips, and (D) 10 ml pipette tips, (E) line thickness with respect to opening diameter of pipette tips, (F) line width with respect to opening diameter of pipette tips, micrographs of deposited lines at current density of 1 mA cm^{-2} and adjusted stage velocity of (G) $10 \text{ } \mu\text{m s}^{-1}$ for $20 \text{ } \mu\text{l}$ pipette tips, (H) $16 \text{ } \mu\text{m s}^{-1}$ for $300 \text{ } \mu\text{l}$ pipette tips, (I), $22 \text{ } \mu\text{m s}^{-1}$ for 1 ml pipette tips, and (J) $53 \text{ } \mu\text{m s}^{-1}$ for 10 ml pipette tips, and (K) line width with respect to opening diameter of pipette tip at current density of 1 mA cm^{-2} and adjusted stage velocities.

[0011] FIG. 5 shows (A) illustration of exemplary etching procedure, images of a resistor array (B) before etching and (C)-(D) after etching, images of a capacitor array (E) before etching and (F)-(G) after etching, (H) current-voltage sweep of the resistor, (I) hysteresis loop for capacitor, (J) impedance magnitude of electrodes over a frequency range, and (K) cyclic voltammetry of electrodes in a potential sweep in the range of $-0.4 \text{ V} - 0.8 \text{ V}$.

[0012] FIG. 6 shows (A) a schematic for a proposed detection mechanism of glucose at low activation potential of 300 mV vs. Ag/AgCl reference electrode, where OS acts as a mediator in electron transfer pathway, (B) amperometric current response of GOx-OS biosensors and OS control structures, (C) calibration curve of biosensors showed an operating range within $0.1\text{-}25 \text{ mM}$ for glucose concentration and dynamic range of $0\text{-}1.25 \text{ } \mu\text{A}$ for current

response at both activation potentials, (D) linear range of biosensors between 0.1 and 1.5 mM glucose concentrations at both activation potentials, (E) longevity (sensitivity loss) of biosensors at both activation potentials over 30 days, (F) electroactivity (change in charge storage capacity) of biosensors at both activation potentials for simulated time frames, and (G) amperometric current response of a GOx-OS biosensor upon sequential addition of (1) glucose (0.5 mM), (2) acetaminophen (0.2 mM), (3) ibuprofen (0.2 mM), (4) ascorbic acid (0.2 mM), and (5) urea (0.2 mM) at activation potential of 300 mV vs. Ag/AgCl.

[0013] FIG. 7 shows (A) MTT assay characterization of bioactive OSMDs prepared using embodiments of gel-pen 3D printing method showing viability, attachment and proliferation of HUVECs on electrodes, biocompatibility assessment of OSMDs on splenic cells after 7 days incubation including (B) cellular viability (normalized with respect to control) and (C) activated T-cells / B-cells assessed by flow cytometry, (D) bright field and (E) epifluorescence micrographs of endothelial cells fixed and stained after 48 h for laminin-incorporated lines (LM-OSMDs) and lines without laminin, and (F) quantification of cellular density for LM-OSMDs and OSMDs.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

[0014] The present disclosure relates to techniques for direct writing of organic semiconductor microelectronic devices (OSMDs).

[0015] Preferred embodiments disclosed herein relate to a straightforward and versatile 3D printing technique, referred to as gel-pen, for fabrication of microelectronic devices, particularly OSMDs. Exemplary embodiments are based on in-situ electrochemical deposition of the meniscus provided by a hydrogel cartridge at the electrode interface. Fabrication and characterization of OSMDs with arbitrary shapes and geometries have been successfully demonstrated via gel-pen 3D printer. Bioactive proteins such as laminin and glucose oxidase are preferably incorporated in the gel and entrapped within the OSMDs. High-performance glucose biosensors based on OSMDs exhibited high sensitivity, low limit of detection, fast response time, high specificity, and longevity. Bioactive OSMDs are biocompatible and support cell adhesion and proliferation. Conductive and bioactive OSMDs hold great promise for a host of applications, including flexible electronics, bioelectronics, and biosensing.

[0016] Preferred embodiments herein relate to a 3D printing technique utilizing gel-pen methodology for fabrication of OSMDs. This approach is based on highly localized and in-situ electrodeposition of the solution precursor (containing monomer, dopant, and protein)

which is delivered by a gel cartridge at the electrode interface. Preferred examples of gel in the gel cartridge include hydrogels such as agarose as well as any synthetic or natural hydrogels. Preferred examples of monomer include monomers of any suitable organic semiconductor (OS) material, such as pyrrole, aniline, or 3,4-ethylenedioxythiophene. Preferred examples of dopant include any suitable negatively charged dopant or ions, such as perchlorate or polystyrene sulfonate. Preferred examples of protein include any suitable proteins or biomolecules, such as laminin fragments, fibronectin fragments, or glucose oxidase. Preferred processing parameters are utilized. Various OSMDs have been designed, fabricated and characterized. 3D microstructures can be prepared through layer-by-layer deposition. Additional embodiments of this method incorporate GOx in the gel cartridge and allow for fabrication of high-performance glucose biosensors and bioactive microdevices. Biosensors show high sensitivity, fast response time, excellent specificity, and reproducibility. The longevity and electroactivity of biosensors improves significantly by utilizing low activation potentials, such as 300 mV. Moreover, immobilization of LM in OSMDs significantly improves cellular attachment and proliferation. These conductive, biocompatible and bioactive OSMDs based on gel-pen 3D printing can be widely used in various applications such as flexible electronics, organic bioelectronics, and wearable biosensors.

EXAMPLES.

MATERIALS AND METHODS

[0017] *Materials:* Pyrrole (Py) ($M_w = 67.09$ g/mol), gold etchant (nickel compatible), D-(+)-glucose, Urea (ACS reagent, 99.0-100.5%), MTT solution, Laminin from Engelbreth-Holm-Swarm murine sarcoma basement membrane (L2020 – 1 MG), and glucose oxidase (GOx, type X-S from *Aspergillus niger*) were purchased from Sigma Aldrich. Poly(sodium-p-styrenesulfonate) (PSS) (average $M_w = 70$ kD) was purchased from Alfa Aesar. Agarose ITM (biotechnology grade), phosphate-buffered saline tablets (100 ml-biotechnology grade), and SylgardTM 184 silicone elastomer (polydimethylsiloxane (PDMS), Electron Microscopy Science) were ordered from VWR. CEP 200 (MICRO-CHROME ETCHANT (chromium etchant) was purchased from HTA Enterprises (Microchrome Technology Division). Pipette tips (EptIPS, various sizes) purchased from Eppendorf. Acetaminophen, Ibuprofen, and Ascorbic acid were donated to the lab. Oregon Green 488 Phalloidin and DAPI were purchased from Invitrogen. FITC Anti-Mouse CD3 Antibody and PE Anti-Mouse CD69 Antibody were purchased from BiLegend. APC Cy7 Rat Anti-Mouse CD45R Antibody and FITC Rat Anti-Mouse CD86 Antibody were purchased from BD Biosciences.

[0018] *PDMS film fabrication:* A blend of 1:10 curing agent: PDMS base elastomer (mass ratio) was prepared and well mixed, followed by degassing for 1 hr. The mixture was then poured in a glass mold, spin-coated at a speed of 1000 rpm for 5 s, followed by heat treatment in an oven at 60°C for 2 hr. Thin, flexible PDMS films (thickness of 0.2 mm) were then detached from the glass.

[0019] *Preparation of conductive electrodes:* Custom gold electrodes were fabricated on silicon wafers / PDMS films, using an in-house electron beam evaporative deposition system. A 20 nm layer of chromium was deposited first to improve adhesion of the 100 nm gold layer onto the silicon wafer. Electrodes were then individually cut by hand to approximately 3 cm × 3 cm.

[0020] *Preparation of gel cartridges:* A 2 wt% mixture of agarose powder and Py:PSS (0.2 M Py and 0.2 M PSS) solution in deionized water (DIW) was prepared. The mixture was heated up to near-boiling temperature and shaken constantly until agarose became completely soluble. The gel was then injected into the pipette tip by a syringe. Five minutes at room temperature was sufficient to solidify the hydrogel. The cartridge tip was dipped in a separate container of Py:PSS solution (less than 5 seconds) prior to use. In case of protein incorporation, GOx (concentration: 2 kU ml⁻¹) / LM (concentration 100 µg ml⁻¹) solution was directly incorporated at the tip of the cartridge and incubate for 1 h so that it was absorbed by the gel.

[0021] *Gel-pen 3D printing:* Electrodes were secured on top of a sub-micron three-dimensional translation stages (XMS100, XMS50, and GTS30V, Newport). The stages were connected to a computer controller (Newport XPS-Q4). The gel cartridge was mounted on a high-precision manually-articulated three-axis micromanipulator (World Precision Instruments Taurus-L), and a meniscus was set up at the interface of electrode and gel cartridge. As the motorized 3D stages translated the movement of electrode underneath the gel cartridge (controlled by uFab Software (Newport)), AUTOLAB instrument (PGSTAT 128N, USA METROHM) supplied electrical current through the gel and into the electrode in galvanostatic mode (NOVA 2.1 software was used to adjust deposition parameters). Amperage was calculated based on applied current density (in the range of 0.5-1.5 mA cm⁻²) and the surface area of the cartridge gel tip, i.e. opening diameter of the utilized off-the-shelf pipette tip. A digital magnifier (Dino-Lite Edge) was also used to monitor the gel-pen 3D printing on a computer via Dino Capture 2.0 software. All depositions were performed on a vibration isolation table (63-series, TMC) with a built-in and enclosed Faraday cage. The humidity level

was monitored and kept at 50% using a humidifier.

[0022] *Materials Confocal Microscopy*: In order to characterize the morphology of the created structures, a confocal laser scanning microscope (Observer with an Laser Scanning Module (LSM) 800, Zeiss Germany) was used in combination with ConfoMap data analysis software. Scans were performed in a tile-by-tile fashion at 50X magnification, using a raster-scanning laser at 405nm. Tiles were 162 μ m square and sufficient tiles were scanned to capture the ridge and the bare gold on either side of the ridge.

[0023] *Imaging*: Optical micrographs were captured using an upright microscope (Imager Z1, Zeiss Germany) and stereo microscope (Discovery. V8, ZEISS Germany). ZEN-pro Axiovision digital processing software (Zeiss Germany) was used to analyze the images.

[0024] *Etching*: To remove gold and chromium, electrodes were briefly dipped in gold etchant solution for 10 s, followed by chromium etchant solution for 5 s. Etching times were optimized. Higher etchant exposure would damage OSMDs structure, while lower etching time would not etch the entire gold / chromium layer from the silicon wafer electrode.

[0025] *Electrical characterization*: Semiconductor Device Parameter Analyzer (B1500A, Keysight Technologies Inc) was used for electrical measurements. Briefly, probe tips (Signatone, diameter 1 μ m) of two electrodes connected to Source Measurement Unit (SMU-8, Keysight Technologies Inc) were placed on two sides of the OSMD. Probe movement was thoroughly adjusted using micromanipulators and a stereo microscope. I-V sweep was performed by applying a voltage in the range of -3 V to 3 V (increasing step: 50 mV), and current was automatically recorded. EasyEXPERT group+ software (resident GUI-based, Keysight Technologies Inc.) was used to analyze the data. For capacitance measurements, hysteresis loop was obtained by performing double I-V curve in the range of -3 V to 3 V and a scan rate of 2 V s⁻¹.

[0026] *Electrochemical characterization*: Both electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) were conducted using Autolab PGSTAT 302N (Metrohm USA Inc.) and Nova Frequency Response Analyzer software (version 2.1, Metrohm USA Inc.) in potentiostatic mode. A solution of 0.1 M PBS (pH= 7.4) was used as the electrolyte. A three-electrode configuration including Ag/AgCl reference electrode, platinum foil counter electrode, and OSMDs were immersed in the PBS solution. In EIS, a

sinusoidal AC signal with 10 mV rms amplitude was imposed to measure the impedance over a frequency range of 1-10⁵ Hz. For CV staircase analysis, the potential of the working electrode was swept across -0.8 V to 0.4 V range with respect to the Ag/AgCl reference electrode at a constant scan rate of 0.1 V s⁻¹. In each experiment, CV was repeated three times and the third cycle was used to plot CV graphs.

[0027] *Amperometric Detection:* BioStat™ (ESA Biosciences, Inc.) was used to record the current response at polarization potentials of 300 mV and 700 mV vs. Ag/AgCl, which was applied to the biosensor in a stirred solution of 20 ml PBS (1X, pH= 7.4, T=37°C). Once the background current was stabilized, successive injections of glucose solution were added (cumulative concentration ranging from 0.1 mM to 25 mM). The size of GOx-OS electrode was 2 mm × 2 mm × 2 μm (length × width) which was deposited by 10 ml gel cartridge at current density and stage velocity of 1 mA cm⁻² and 10 μm s⁻¹ on platinum foil. In the three-cell configuration, a Pt wire counter electrode and an Ag/AgCl reference electrode were used. Resulting current measurements in the linear range were used to calculate the sensitivity and limit of detection (LOD) of the biosensor. The reproducibility of the biosensors was investigated by measuring the current generated by 0.5 mM glucose in 20 ml PBS by using three different glucose biosensors. Each biosensor was tested by three replicates of analysis. The total mean value was calculated, and the relative standard deviation (RSD) provided the analytical precision.

[0028] *Longevity of biosensors:* GOx-OS biosensors were fabricated and amperometric detection of the linear range was performed at the specified days over the course of one month. After biosensing, the biosensors were kept at 4°C in PBS. The biosensing took approximately 30 min.

[0029] *Electroactivity loss of biosensors:* GOx-OS biosensors were fabricated and were immediately used for CV measurements. First, the biosensors were subjected to activation potentials of 300 mV or 700 mV vs. Ag/AgCl by the BioStat™ system in a stirred solution of 20 ml PBS (1X, pH= 7.4, T=37°C) for the simulated time frames and were then transferred to electrochemical vial for CV measurements.

[0030] *MTT assay:* After 24, 48, 72 h of seeding 25000 Human Umbilical Vein Endothelial Cells (HUVEC) per well, 20 μl of 5 mg ml⁻¹ MTT solution was added to each well and incubated for 4 hours at 37°C. After incubation, medium was aspirated, and formazan

crystals were solubilized by 0.04 N HCl in isopropyl alcohol and absorbance was measured at 570 nm wavelength after using a reference wavelength of 630 nm in Elx808 Bio Tek plate reader. Wells with only media were used to subtract background and the viability % was obtained by normalizing the optical density values to undertreated control samples at 24 h.

[0031] *Spleen Cell Culture and Flow Cytometry*: Spleen cells were isolated from 3-month-old C57/B6 mice. Briefly, spleen was dissected and minced through a 70-micron sieve and red blood cells were lysed using RBC lysis buffer. After washing and counting cells using Cellometer (Nexcelom Bioscience), spleen cells were cultured in RPMI 1640 media with 10% fetal bovine serum either without (control) or with exposure to OS composite polymer. After 7 days of exposure, the immune cell numbers were counted and analyzed by flow cytometry using the following antibodies, FITC Anti-Mouse CD3 Antibody and PE Anti-Mouse CD69 Antibody, APC Cy7 Rat Anti-Mouse CD45R Antibody, and FITC Rat Anti-Mouse CD86 Antibody.

[0032] *Endothelial Cell Culture and Microscopy*: First, 5 by 5 array of connected cubes with dimensions of $100\ \mu\text{m} \times 100\ \mu\text{m} \times 2\ \mu\text{m}$ (length $\times$ width $\times$ height) were 3D printed via MPL, providing a total area of $500\ \mu\text{m} \times 500\ \mu\text{m}$. The microstructures were fabricated using composite polymer resin with or without incorporated laminin. Following MPL fabrication, samples were soaked in ethanol for 2 hr to make sure DMSO is completely removed from the structure. Primary mouse endothelial cells were isolated from glomeruli of H-2K^b-tsA58 mice (Jax Stock # 032619) as previously described.^[84] After trypsinization, 10^6 cells were seeded onto a 60 mm dish containing a glass coverslip with MLP-microstructures. Cells were cultured for 48 hr and cell adherence was assayed by fixing the cells in 4% paraformaldehyde for 20 min followed by permeabilization using 0.1% Triton X-100 for 5 min; F-actin was stained using Oregon Green 488 phalloidin for 60 min. After washing in PBS, cell nuclei were DAPI stained, and coverslips were mounted on glass slides ZEN-pro Axiovision digital processing software was used to analyze the images and fluorescence quantification. Following imaging by epifluorescence microscopy, Adobe Photoshop was used to adjust image brightness/contrast.

[0033] *Statistical Analysis*: Statistical analysis was carried out using OriginPro software (Northampton, MA). *P*-values are calculated by one-way ANOVA followed by Tukey Post-Hoc test. Data are presented as mean $\pm$ standard error of mean (SEM) and/or mean $\pm$ standard deviation (SD). Symbols ** and *** represent significance level of $p < 0.05$ and

$p < 0.001$, respectively and n.s. for no significance.

[0034] FIGs. 1A-1K show an exemplary gel-pen 3D printing setup, according to preferred embodiments disclosed herein, and optimization of processing parameters. FIG. 1A shows an exemplary experimental setup of a 3D printer, including XYY 3D stages connected to a motion controller, cartridge gel tip mounted on a micro-manipulator, gold electrode, and a computer or processor in communication with an electrochemical workstation such as an AUTOLAB potentiostat. FIG. 1B shows a close-up illustration at the electrode interface. The gel cartridge delivers the solution precursor containing monomer, dopant and protein to the electrode surface through a liquid bridge (meniscus). In the illustrated embodiment, a wet hydrogel cartridge comes into contact with the electrode surface, in this case a gold-coated silicon wafer / thin and flexible polydimethylsiloxane (PDMS) sheet, and delivers solution precursor, i.e. monomer (pyrrole) and dopant (polystyrene sulfonate (PSS)), at the interface in form of a liquid bridge (meniscus). The cartridge shown in this example is a pipette tip containing 2 wt% agarose hydrogel and the solution precursor. The pipette tip serves as an inexpensive and versatile container for the gel, prevents fast gel dehydration in ambient environment, and most importantly, provides a consistent and controlled route for direct writing without the need for constant resupply of solution. As the 3D stages move based on the pre-defined geometric design, a constant electrical current is applied between the conductive electrode and the hydrogel cartridge using the potentiostat (e.g. AUTOLAB PGSTAT 128N (USA METROHM)), which leads to in-situ electropolymerization of monomer at the site of contact. As the stages move based on the geometry of the structure the AUTOLAB delivers current at the electrode surface, therefore a trail of monomer is instantly electrodeposited, leading to fabrication of organic semiconductor microelectronic devices (OSMD).

[0035] In this approach, simultaneous electrodeposition at selective regions guided by the gel cartridge and 3D movement of stages result in construction of OSMDs. FIG. 1C shows an exemplary illustration of the deposited structures, which in this embodiment are a 3D mesh of Ppy (organic semiconductor), PSS (dopant), and bioactive agent (protein). Taking advantage of the biocompatible and wet nature of the gel cartridge, bioactive proteins such as laminin and glucose oxidase can be incorporated into the gel and entrapped within the OSMD matrix to further demonstrate their potential in biomedical applications. To characterize structural properties of OSMDs and optimize the processing parameters, lines were fabricated with an exemplary gel-pen 3D printing setup. Optimal humidity for deposition was determined

to be 50 % since the gel cartridges stayed hydrated and fresh for deposition of OSMDs and longevity of a single cartridge (maximum deposited length) was found to be 30 mm. In preferred embodiments, a 2 wt% agarose hydrogel can be used to produce a thin and consistent meniscus for deposition.

[0036] The structural properties of deposited OSMDs can be adjusted by changing the applied current density and stage motion velocity. The preferred operating ranges for current density and stage velocity are $0.5 - 1.5 \text{ mA cm}^{-2}$ and $5 - 25 \text{ } \mu\text{m s}^{-1}$, respectively. To investigate the effect of these parameters, 2 mm-long lines were deposited at various stage velocities and current densities and Materials Confocal Microscopy (MCM) was utilized to assess the topography and thickness of lines. FIG. 1D shows the combined correlation between stage velocity and current density with thickness of the deposited lines. FIG. 1D shows thickness measurement at various sets of current densities (ranging from 0.5 to 1.5 mA cm^{-2}) and stage velocities ($5 \text{ } \mu\text{m s}^{-1}$, $10 \text{ } \mu\text{m s}^{-1}$, $15 \text{ } \mu\text{m s}^{-1}$, $20 \text{ } \mu\text{m s}^{-1}$, and $25 \text{ } \mu\text{m s}^{-1}$, respectively). As shown, a combination of slower speeds and higher current densities produced OSMDs with higher thickness values. For example, the thickness increased significantly from $12.3 \pm 3.4 \text{ nm}$ at current density of 0.5 mA cm^{-2} and stage velocity of $25 \text{ } \mu\text{m s}^{-1}$ to $260.5 \pm 7 \text{ nm}$ at current density of 1.5 mA cm^{-2} and stage velocity of $5 \text{ } \mu\text{m s}^{-1}$. It was also observed that the deposited line width varies as the stage velocity and current density changes.

[0037] The optimized set of parameters ideally minimizes the operation time while depositing higher amount of OS with stable and high-quality morphology. FIG. 1E shows thickness of the deposited lines with respect to current density at stage velocity of $10 \text{ } \mu\text{m s}^{-1}$. At stage velocity of $10 \text{ } \mu\text{m s}^{-1}$ there was an excellent linear correlation between line thickness and current density ($R^2=0.98$), demonstrating the predictability and consistency of the fabrication. Moreover, this velocity resulted in deposition of smooth lines at almost all current densities. FIG. 1F-1J show optical micrographs of deposited lines at stage velocity of $10 \text{ } \mu\text{m s}^{-1}$ and various current densities of 0.5 mA cm^{-2} (F), 0.75 mA cm^{-2} (G), 1 mA cm^{-2} (H), 1.25 mA cm^{-2} (I), and 1.5 mA cm^{-2} (J). Preferred, optimal parameters are 1 mA cm^{-2} (current density) and $10 \text{ } \mu\text{m s}^{-1}$ (stage velocity), which result in deposition of consistent and high-quality lines for gel-pen 3D printing. The average thickness and width of deposited lines at this set of parameters were measured to be $77.6 \pm 5.5 \text{ nm}$ and $488.7 \pm 16 \text{ } \mu\text{m}$, respectively. FIG. 1K shows a 3D render of a deposited line at optimized parameters, i.e. stage velocity of $10 \text{ } \mu\text{m s}^{-1}$ and current density of 1 mA cm^{-2} , obtained from MCM.

[0038] FIG. 2 shows exemplary fabrication of various OSMDs based on gel-pen 3D printing and optimized processing parameters, as discussed above. FIG. 2A-2B show an array of capacitors, FIG. 2C-2D show an array of square mazes, FIG. 2E-2F show an array of six-pointed stars, FIG. 2G-2H show spirals, FIG. 2I-2J show a brain electrode array, FIG. 2K-2L show concentric circles, FIG. 2M-2N show multi-line electrodes, starting with deposition of 4 adjacent lines and ending with a single deposited line. FIG. 2O shows an image of a resistor array fabricated on thin and flexible gold-coated PDMS film. FIGs. 2A, C, E, G, I, K, M, and O were imaged using a camera, while FIGs. 2B, D, F, H, J, L, and N are stereo micrographs (Scale bars: 1 mm). All structures were fabricated using 20 μ l pipette tip gel cartridges (2 wt% hydrogel concentration) and optimized parameters, i.e. current density of 1 mA cm⁻² and stage velocity of 10 μ m s⁻¹.

[0039] Layer-by-layer deposition at the optimum parameters was also used to fabricate 3D OSMDs. FIG. 3 shows examples of multi-pass deposition based on gel-pen 3D printing. The motion stages were programmed to move forward and backward along the same geometry to deposit multi-pass OSMDs. FIGs. 3A-3E show optical micrographs of Ppy lines fabricated at 2-pass (A), 4-pass (B), 6-pass (C), 8-pass (D), and 10-pass (E) (scale bars: 500 μ m), along with their corresponding MCM cross-sectional 3D render of lines deposited shown in FIGs. 3F-3J. FIG. 3K shows thickness measurement of deposited lines at various passes and FIG. 3L shows width of the deposited lines at various passes. MCM analysis showed that the thickness grows linearly as the number of passes increases ($R^2=0.97$), and on average, each pass added 64.8 nm to the deposited structure. Moreover, it was observed that the line width increases from 488.7 ± 16.5 μ m at single-pass to 972.6 ± 41.9 μ m at 10-pass. FIGs. 3M and 3N show a circle and triangle fabricated at 4-pass with gel-pen 3D printing setup, respectively (scale bars: 500 μ m). Structures were fabricated using 20 μ l pipette tip gel cartridges (2 wt% hydrogel concentration) and optimized parameters, e.g. current density of 1 mA cm⁻² and stage velocity of 10 μ m s⁻¹.

[0040] In most meniscus-guided 3D printing techniques, line width is limited to the size of the depositing tip. Preferred embodiments described herein include a straightforward route to fabricate various line widths using off-the-shelf pipette tips with different openings as the gel cartridge, referred to as tip-swapping. FIGs. 4A, 4B, 4C, and 4D show deposited lines using pipette tips with opening diameters of 375 μ m (regular 20 μ l pipette tip), 600 μ m (regular 300 μ l pipette tip), 835 μ m (regular 1 ml pipette tip), and 2 mm (regular 10 ml pipette tip),

respectively. The lines were deposited using optimized parameters, i.e. current density of 1 mA cm^{-2} and stage velocity of $10 \text{ } \mu\text{m s}^{-1}$ (scale bars: $500 \text{ } \mu\text{m}$).) FIG. 4E shows line thickness with respect to opening diameter of pipette tips and FIG. 4F shows line width with respect to opening diameter of pipette tips. In FIG. 4E and 4F, current density and stage velocity were set at 1 mA cm^{-2} and $10 \text{ } \mu\text{m s}^{-1}$, respectively. As shown in FIGs. 4E and 4F, the relation between line thickness / width and opening diameter of the tip is a good linear fit. FIGs. 4G-4J show micrographs of deposited lines at current density of 1 mA cm^{-2} and, in FIG. 4G, adjusted stage velocity of $10 \text{ } \mu\text{m s}^{-1}$ for $20 \text{ } \mu\text{l}$ pipette tips. Processing parameters had been originally optimized for tips with opening diameter of $375 \text{ } \mu\text{m}$ (regular $20 \text{ } \mu\text{l}$ pipette tip). To further demonstrate the versatility of tip-swapping, the velocity was adjusted for larger tips based on the cartridge residence time, i.e. ratio of opening diameters, which was calculated to be $16 \text{ } \mu\text{m s}^{-1}$ for $300 \text{ } \mu\text{l}$ pipette tip (FIG. 4H), $22 \text{ } \mu\text{m s}^{-1}$ for $1000 \text{ } \mu\text{l}$ pipette tip (FIG. 4I), and $53 \text{ } \mu\text{m s}^{-1}$ for 10 ml pipette tip (FIG. 4J). FIG. 4K shows line width with respect to opening diameter of pipette tip at current density of 1 mA cm^{-2} and adjusted stage velocities. As expected, using the adjusted velocity and optimized current density (1 mA cm^{-2}), the fabricated lines were smooth and high-quality and the thickness stayed roughly the same, i.e. $77.6 \pm 5.5 \text{ nm}$ for $20 \text{ } \mu\text{l}$ pipette tip, $69.1 \pm 8.6 \text{ nm}$ for $300 \text{ } \mu\text{l}$ pipette tip, $55.7 \pm 11.5 \text{ nm}$ for $1000 \text{ } \mu\text{l}$ pipette tip, and $82.8 \pm 14 \text{ nm}$ for 10 ml pipette tip.

[0041] Various OSMDs were designed, fabricated and characterized. FIG. 5 shows electrical and electrochemical characterization of OSMDs. For electrical characterization, the electrodes were subjected to etchant to remove gold and chromium, and after etching, the resultant OSMDs were remained untouched on the silicon wafer. FIG. 5A shows an illustration of an exemplary etching procedure. Unexposed gold and chromium layers were removed from the surface of the electrode through etching. A resistor array is shown before etching in FIG. 5B, and after etching in FIGs. 5C and 5D. A capacitor array is also shown before etching in FIG. 5E, and after etching in FIGs. 5F and 5G. FIG. 5H shows a current-voltage sweep of the resistor. Linear fit shows the resistor behavior. The resistance and conductance were measured to be $631 \pm 71 \text{ k}\Omega$ and $1668 \pm 189 \text{ } \mu\text{S}$, respectively. FIG. 5I displays the hysteresis loop of the capacitor. The rectangular I-V shape demonstrates the capacitive behavior.

[0042] Electrochemical properties of OSMDs were characterized. $3 \text{ mm} \times 5 \text{ mm}$ OS electrodes were fabricated with gel-pen 3D printing at various current densities, i.e. 0.5 mA cm^{-2} , 1 mA cm^{-2} , and 1.5 mA cm^{-2} followed by performing electrochemical impedance

spectroscopy (EIS) and cyclic voltammetry (CV). EIS was performed to explore the impedance behavior of the OS electrodes. FIG. 5J shows the impedance magnitude of electrodes over a frequency range of $1-10^5$ Hz. Impedance magnitude decreased over the entire frequency spectrum (1 to 10^5 Hz) as current density of deposition increased in the electrodes. This impedance trend is in parallel with other related works in the literature, and can be attributed to thicker deposited OS layer at higher current densities. Specifically, at biologically relevant frequency of ≈ 1 kHz, impedance significantly decreased as the current density increased, i.e. from $1802.5 \pm 246 \Omega$ at current density of 0.5 mA cm^{-2} to $31.15 \pm 1.8 \Omega$ at current density of 1.5 mA cm^{-2} ($p < 0.001$).

[0043] Cyclic voltammetry (CV) was conducted to investigate the ion exchange between OS electrode and the electrolyte during redox reactions FIG. 5K shows cyclic voltammetry of electrodes in a potential sweep in the range of $-0.4 \text{ V} - 0.8 \text{ V}$. In FIG. 5J and 5K, $3 \text{ mm} \times 5 \text{ mm}$ electrodes were fabricated using 10 ml pipette tips at stage velocity of $10 \mu\text{m s}^{-1}$ and various current densities, i.e. 0.5 mA cm^{-2} (squares and lines), 1 mA cm^{-2} (circles and lines), and 1.5 mA cm^{-2} (triangles and lines). During CV, the voltage was swept in the range of -0.4 V to 0.6 V at a constant scan rate of 0.1 V s^{-1} . Charge storage capacity (CSC) is proportional to the area of the CV curve and is a metric of the charges of mobile carriers accumulated within the OS during current-potential sweep. It was observed that CSC increased significantly as the deposition current density increased, and CSC of electrodes were calculated to be $1.16 \pm 0.1 \text{ mC cm}^{-2}$, $12.3 \pm 0.15 \text{ mC cm}^{-2}$, and $23.164 \text{ mC cm}^{-2}$ at current densities of 0.5 mA cm^{-2} , 1 mA cm^{-2} , and 1.5 mA cm^{-2} , respectively ($p < 0.001$).

[0044] To further demonstrate the versatility of gel pen-fabricated OSMDs, glucose biosensors were developed. Immobilization / entrapment of the enzyme glucose oxidase (GOx) within the matrix electrodes has been a popular approach for construction of electrochemical sensor which work on the basis of amperometric detection of glucose. Here, GOx was entrapped within OSMDs by directly injecting GOx solution (concentration of 2 kU ml^{-1}) into the tip of the gel cartridge. GOx solution was absorbed by the gel tip after 1 hour which was then used to deposit $2 \text{ mm} \times 2 \text{ mm}$ biosensor electrodes (GOx-OS) on platinum foil. During electrodeposition, GOx was entrapped within porous structure of OSMDs by physical interactions and polymer charge balance.

[0045] It was observed that the GOx-OS biosensors exhibit a current response upon

addition of glucose (at a given concentration of 0.5 mM) at activation potentials equal to and above 300 mV, and reach a saturation limit at 700 mV. For amperometric detection, successive injections of glucose (in the operating range of 0.1 mM – 25 mM) were made to a phosphate buffered saline (PBS, pH= 7.4, $\theta= 37^{\circ}\text{C}$) containing GOx-OS biosensors and the current response was recorded at activation potentials of 300 mV and 700 mV vs. Ag/AgCl reference electrode. Fig. 6A shows a proposed detection mechanism of glucose at low activation potential of 300 mV vs. Ag/AgCl reference electrode, where OS acts as a mediator in electron transfer pathway. In amperometric biosensors, glucose is typically detected through consumption of oxygen and oxidation of hydrogen peroxide at high potential of 700 mV, which is an oxygen dependent pathway. However, relevant studies in the literature have demonstrated that OSs can serve as mediators for detection of glucose at lower activation potentials (i.e. 300 mV) via an oxygen-independent pathway. It was observed that the GOx-OS biosensor showed current response to glucose addition in an oxygen-depleted PBS media at activation potential of 300 mV, demonstrating that the detection mechanism is oxygen-independent. FIG. 6B shows amprometric current response of GOx-OS biosensors (lines demmonstrate biosensing at activation potentials of 300 mV and 700 mV vs. Ag/AgCl, respectively) and OS control structures (lines demonstrate biosensing at activation potentials of 300 mV and 700 mV vs. Ag/AgCl, respectively). OS electrodes without GOx did not exhibit current changes in response to addition of glucose at neither activation potentials (curve for 300 mV and curve for 700 mV), confirming that glucose biosensing is enzymatic. However, GOx-OS biosensors showed considerable current changes at both activation potentials (curve for 300 mV and curve for 700 mV).

[0046] Calibration curves were plotted to correlate current response to glucose concentrations. FIG. 6C shows calibration curves of biosensors showed an operating range within 0.1-25 mM for glucose concentration and dynamic range of 0-1.25 μA for current resposne at both activation potentials. FIG. 6D shows linear range of biosensors between 0.1 and 1.5 mM glucose concentrations at both activation potentials. While the glucose operating range was 0.1 mM-25 mM, biosensors showed a linear range up to 1.5 mM at both activation potentials. Using the linear range, the sensitivity and limit of detection were calculated to be $4.82 \pm 0.36 \mu\text{A mM}^{-1} \text{cm}^{-2}$ and 0.01 mM at 300 mV, and $6.3 \pm 0.44 \mu\text{A mM}^{-1} \text{cm}^{-2}$ and 0.004 mM at 700 mV. It is worth noting that both limits of detection fall below the glucose concentration in biological fluids. In addition, response time of biosensors was measured to be $11 \pm 1.7 \text{ s}$ and $10 \pm 1.2 \text{ s}$ for activation potentials of 300 mV and 700 mV, respectively. The

precision of the GOx-OS biosensor was investigated by measuring the reproducibility of biosensors which is derived from the closeness of current responses of different biosensors to a given glucose concentration. At 0.5 mM glucose, the relative standard deviation (RSD) was 12.2 %, i.e. the current response of $0.11 \pm 0.01 \mu\text{A}$, for 300 mV and 11.2 %, i.e. current response of $0.21 \pm 0.02 \mu\text{A}$ for 700 mV respectively. According to the Food and Drug Administration, the precision of biosensors should not exceed RSD of 15%. Therefore, GOx-OS biosensors can be precisely and efficiently utilized to detect glucose.

[0047] As pointed out earlier, 700 mV activation potential resulted in significantly higher sensitivity and lower limit of detection compared to 300 mV ($p < 0.05$). While applying higher activation potentials yields higher sensitivity values, utilization of lower activation potential could be advantageous since it can potentially increase the lifetime of biosensor. To that end, the longevity (change in sensitivity) of the GOx-OS biosensors was examined over a 30 day-time period. FIG. 6E shows longevity (sensitivity loss) of biosensors at both activation potentials over 30 days. Biosensors were stored in PBS at 4°C after biosensing. At 700 mV, the sensitivity of GOx-OS biosensors decreased 52.4 % (from $6.3 \pm 0.44 \mu\text{A mM}^{-1} \text{cm}^{-2}$ at day 1 to $3 \pm 0.33 \mu\text{A mM}^{-1} \text{cm}^{-2}$ at day 30). Reducing the activation potential to 300 mV improved the longevity of the biosensor and resulted in a 33.43% drop in sensitivity over the course of 30 days (from $4.82 \pm 0.36 \mu\text{A mM}^{-1} \text{cm}^{-2}$ to $3.21 \pm 0.07 \mu\text{A mM}^{-1} \text{cm}^{-2}$). Based on the reaction cascade for glucose detection it can be hypothesized that at lower activation potentials, less hydrogen peroxide is oxidized which can help retaining the bioactivity of GOx. Interestingly, after the sixth day the sensitivity value at activation potential of 300 mV outperforms that of 700 mV, further demonstrating the destructive effect of higher voltages on sensitivity of biosensors over time.

[0048] Since it was observed that activation potential plays a key part in sensitivity loss, the electrochemical behavior of the potentiostated GOx-OS biosensors were also investigated. For GOx-OS biosensors, measuring the current response for the operating range of glucose concentration took approximately 30 min, during which the biosensor was potentiostated (subjected to the activation potential). To simulate this condition, CV was conducted on 300 mV and 700 mV potentiostated GOx-OS biosensors after 0 min, 30 min, 90 min, 300 min, and 900 min, which corresponded to before applying voltage, day 1, day 3, day 10, and day 30, respectively. FIG. 6F shows electroactivity (change in charge storage capacity) of biosensors at both activation potentials for simulated time frames. In C-E, circles / lines and

squares / lines demonstrate activation potentials of 300 mV and 700 mV vs. Ag/AgCl, respectively. At activation potential of 700 mV, CSC dropped 84.7 %, i.e. from $16.53 \pm 1.08 \text{ mC cm}^{-2}$ at 0 min to $2.52 \pm 0.65 \text{ mC cm}^{-2}$ at 900 min. However, at activation potential of 300 mV, the CSC decreased 63.89 %, i.e. from $15.74 \pm 1.98 \text{ mC cm}^{-2}$ to $5.69 \pm 0.64 \text{ mC cm}^{-2}$. These results demonstrate that when subjected to higher activation potential, GOx-OS biosensors experience more loss in electrochemical properties. On a molecular level, the decrease in electroactivity can be explained by nucleophilic attack by water or anions and / or oxidation by hydrogen peroxide. This phenomena presumably causes ring opening and loss of conjunction of OS, which leads to decrease in conductivity and electroactivity. Overall, these findings suggest that smaller decrease in sensitivity of GOx-OS biosensors over time at low activation potential can be attributed to higher GOx activity retention and / or lower loss of electroactivity of the biosensor.

[0049] Another advantage of biosensing at low activation potential is the higher specificity of the biosensor. FIG. 6G shows amperometric current response of a GOx-OS biosensor upon sequential addition of (1) glucose (0.5 mM), (2) acetaminophen (0.2 mM), (3) ibuprofen (0.2 mM), (4) ascorbic acid (0.2 mM), and (5) urea (0.2 mM) at activation potential of 300 mV vs. Ag/AgCl. Although the concentration of analytes 2-5 are usually lower than glucose in blood, they could stimulate large amperometric current responses compared to glucose, which can be attributed to their fast charge transport capability. As shown, the biosensor only exhibited current response upon addition of glucose, demonstrating high specificity and anti-interference effect of the biosensor. This can be presumably associated to elimination of oxidation effect for detection at low activation potential of 300 mV. Compared to relevant amperometric biosensors in the literature, developed GOx-OS biosensors in this work offer high sensitivity, decent longevity, fast response time, and excellent specificity, which is presumably due to efficient entrapment of GOx within the OS mesh, as well as the oxygen-independent detection pathway at low activation potential of 300 mV.

[0050] To investigate whether OSMDs provide suitable substrates for cellular attachment and proliferation, MTT assay was performed by seeding endothelial cells on OSMDs, and comparing to tissue culture treated plastic wells. FIG. 7 shows fabrication / characterization of bioactive OSMDs via gel-pen. As shown, OSMDs provided an excellent surface for attachment, growth, and proliferation of cells and were comparable to the traditional tissue culture treated plastic wells, validating the biocompatibility of structures. As shown in

FIG. 7A, MTT assay shows excellent viability, attachment and proliferation of HUVECs on electrodes. Control samples (culture treated dish) and OSMD electrodes are shown in squares / lines and circles / lines, respectively (data are represented as mean $\pm$ SD, $n=5$). After 72 h of seeding, the surfaces were fully covered with cells and the rate of proliferation slowed down, however, there was no statistical difference between OSMDs and tissue culture treated plastics. For OSMDs to be used as biosensors they should not trigger immune response in the body. To investigate this, lymphocytes were cultured on OSMDs, and viability and immune cell response were measured compared to control substrates (without OSMDs) after 7 days. FIGs. 7B and 7C show biocompatibility assessment of OSMDs on splenic cells after 7 days incubation. Cellular viability (normalized with respect to control) (shown in FIG. 7B) and activated T-cells / B-cells assessed by flow cytometry (shown in FIG. 7C). OSMD electrodes and control (without structure) are shown in black and grey, respectively (data are shown in mean $\pm$ SD, $n=3$). The initial cell count was $5000000 \text{ cell ml}^{-1}$, therefore there are no error bars for B at day 0, and n.s. indicates no significance. As shown in FIG. 7B, the normalized viability was 98% on OSMDs after 7 days (with no statistical difference to control samples), indicating that the structures did not induce cell mortality. Furthermore, expression of CD69 (activation marker for T-cells) and CD86 (activation marker for B-cells) were measured by flow cytometry. It was observed that there was no significant difference in activation of T-cell and B-cells between OSMDs and control substrates, which confirmed that OSMDs do not provoke immune cell response. Together, these findings demonstrate the biocompatibility of OSMDs and support their applications in biomedical devices such as biosensors.

[0051] To further demonstrate the potential of gel-pen 3D printing in biomedical engineering applications, we functionalized the OSMDs with bioactive properties. To that end, laminin (LM) was incorporated within the gel cartridge through direct injection, which led to immobilization of LM within OSMDs during electrodeposition (LM-OSMDs). LM is a substrate-bound protein in the extra cellular matrix which is vital for cellular attachment, cell signaling, cell migration and proliferation. To investigate the bioactivity, primary endothelial cells were cultured on LM-OSMDs and OSMDs (4 mm-long lines), which were immunostained after 48 h. FIGs. 7D and 7E show bright field and epifluorescence micrographs of endothelial cells fixed and stained after 48 h with DAPI (to visualize cell nuclei) and Oregon Green 488 Phalloidin (to visualize F-actin) for laminin-incorporated lines (LM-OSMDs) and lines without laminin (OSMDs). Scale bars are 500 μm . As shown in the fluorescent micrographs, cells displayed adherence to LM-OSMDs and were well-spread, whereas for

OSMDs, there was barely any signs of attachment, and cells presented rounded morphology. Moreover, cell density was significantly higher on LM-OSMDs (85 ± 14 cells mm^{-2}) compared to OSMDs (8 ± 3 cells mm^{-2}) ($p < 0.001$). FIG. 7F shows quantification of cellular density for LM-OSMDs and OSMDs. Data shown in mean $\pm$ SEM, $n=5$, *** $p < 0.001$. These results demonstrate that incorporation of LM improve cellular adhesion and supports spreading of living cells.

WHAT IS CLAIMED IS:

1. A method for direct 3D printing of an organic semiconductor microelectronic devices (OSMD), comprising:

loading a wet hydrogel cartridge with precursor solution to form a loaded wet hydrogel cartridge, wherein the wet hydrogel cartridge comprises a pipette tip with a sized opening, and wherein the precursor solution comprises a solution of monomer and dopant;

placing the sized opening of the loaded wet hydrogel cartridge in proximity to an electrode interface;

establishing a meniscus between the precursor solution in the loaded wet hydrogel cartridge and the electrode interface;

applying a current through the precursor solution in the loaded wet hydrogel cartridge into the electrode interface to electrodeposit a portion of 3D microstructure on the electrode interface;

moving the electrode interface relative to the loaded wet hydrogel cartridge at a selected velocity while applying the current, whereby a geometric design of 3D microstructure is electrodeposited on the electrode interface as the meniscus is moved; and

etching the geometric design of 3D microstructure to create an organic semiconductor microelectronic device (OSMD).

2. The method of claim 1, wherein the monomer is pyrrole, aniline, or 3,4-ethylenedioxythiophene and the dopant is perchlorate or polystyrene sulfonate.

3. The method of claim 1, wherein the electrode interface comprises gold or chromium.

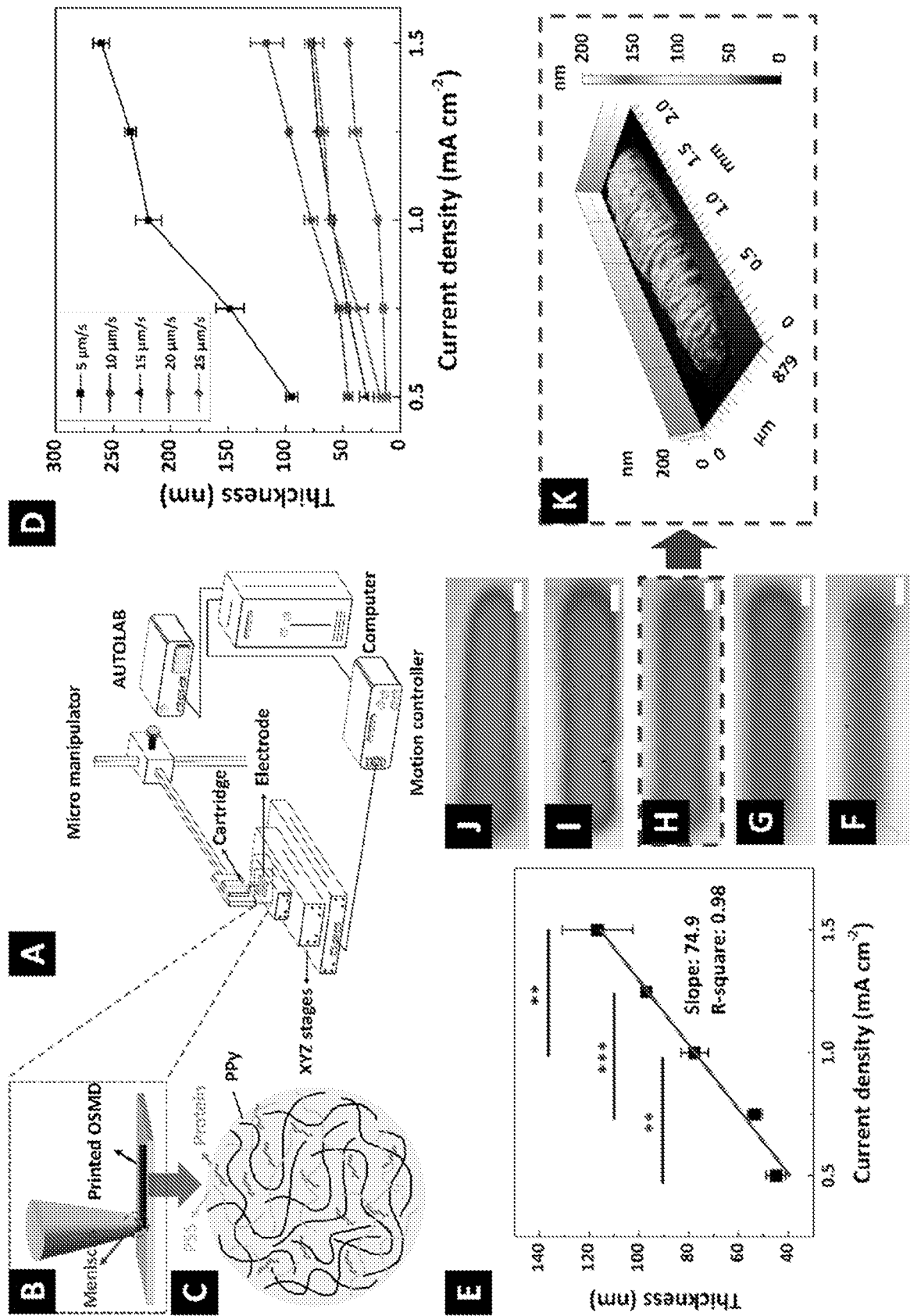
4. The method of claim 1, wherein the sized opening of the pipette tip is 375 μm to 2 mm in diameter.

5. The method of claim 1, wherein the current applied through the precursor solution has a current density of 0.5 to 1.5 mA cm^{-2} .

6. The method of claim 1, wherein the selected velocity at which the electrode interface is moved relative to the loaded wet hydrogel cartridge is 5 to 25 $\mu\text{m s}^{-1}$.
7. The method of claim 1, wherein the precursor solution further comprises protein.
8. The method of claim 7, wherein the protein is laminin, fibronectin or glucose oxidase.
9. The method of claim 1, wherein the sized opening of the pipette tip is 375 μm , the current applied through the precursor solution has a current density of 1 mA cm^{-2} , and the selected velocity at which the electrode interface is moved relative to the loaded wet hydrogel cartridge is 10 $\mu\text{m s}^{-1}$.
10. The method of claim 1, wherein the electrode interface is a gold-coated silicon wafer or a polydimethylsiloxane sheet.
11. A system for direct 3D printing of an organic semiconductor microelectronic devices (OSMD), comprising:
 - a wet hydrogel cartridge, wherein the wet hydrogel cartridge is loaded with precursor solution, wherein the wet hydrogel cartridge comprises a pipette tip with a sized opening, and wherein the precursor solution comprises a solution of monomer and dopant;
 - an electrode interface, wherein the electrode interface may be placed in proximity to the wet hydrogel cartridge;
 - an instrument for applying a current through the precursor solution in the wet hydrogel cartridge into the electrode interface to electrodeposit a portion of 3D microstructure on the electrode interface;
 - a motorized unit that moves the electrode interface relative to the loaded wet hydrogel cartridge at a selected velocity to allow a geometric design of 3D microstructure to be electrodeposited on the electrode interface during current application; and
 - a processor in communication with the motorized unit for controlling the movement of the electrode interface.

12. The system of claim 11, wherein the monomer is pyrrole, aniline, or 3,4-ethylenedioxythiophene and the dopant is perchlorate or polystyrene sulfonate.
13. The system of claim 11, wherein the electrode interface comprises gold or chromium.
14. The system of claim 11, wherein the sized opening of the pipette tip is 375 μm to 2 mm in diameter.
15. The system of claim 11, wherein the instrument applies a current having a current density of 0.5 to 1.5 mA cm^{-2} .
16. The system of claim 11, wherein the motorized unit moves the electrode interface relative to the wet hydrogel cartridge at a selected velocity of 5 to 25 $\mu\text{m s}^{-1}$.
17. The system of claim 11, wherein the precursor solution further comprises protein.
18. The system of claim 17, wherein the protein is laminin, fibronectin or glucose oxidase.
19. The system of claim 1, wherein the sized opening of the pipette tip is 375 μm , the current applied through the precursor solution has a current density of 1 mA cm^{-2} , and the selected velocity at which the electrode interface is moved relative to the wet hydrogel cartridge is 10 $\mu\text{m s}^{-1}$.
20. The system of claim 1, wherein the electrode interface is a gold-coated silicon wafer or a polydimethylsiloxane sheet.

FIG. 1A-1K



2/7

FIG. 2

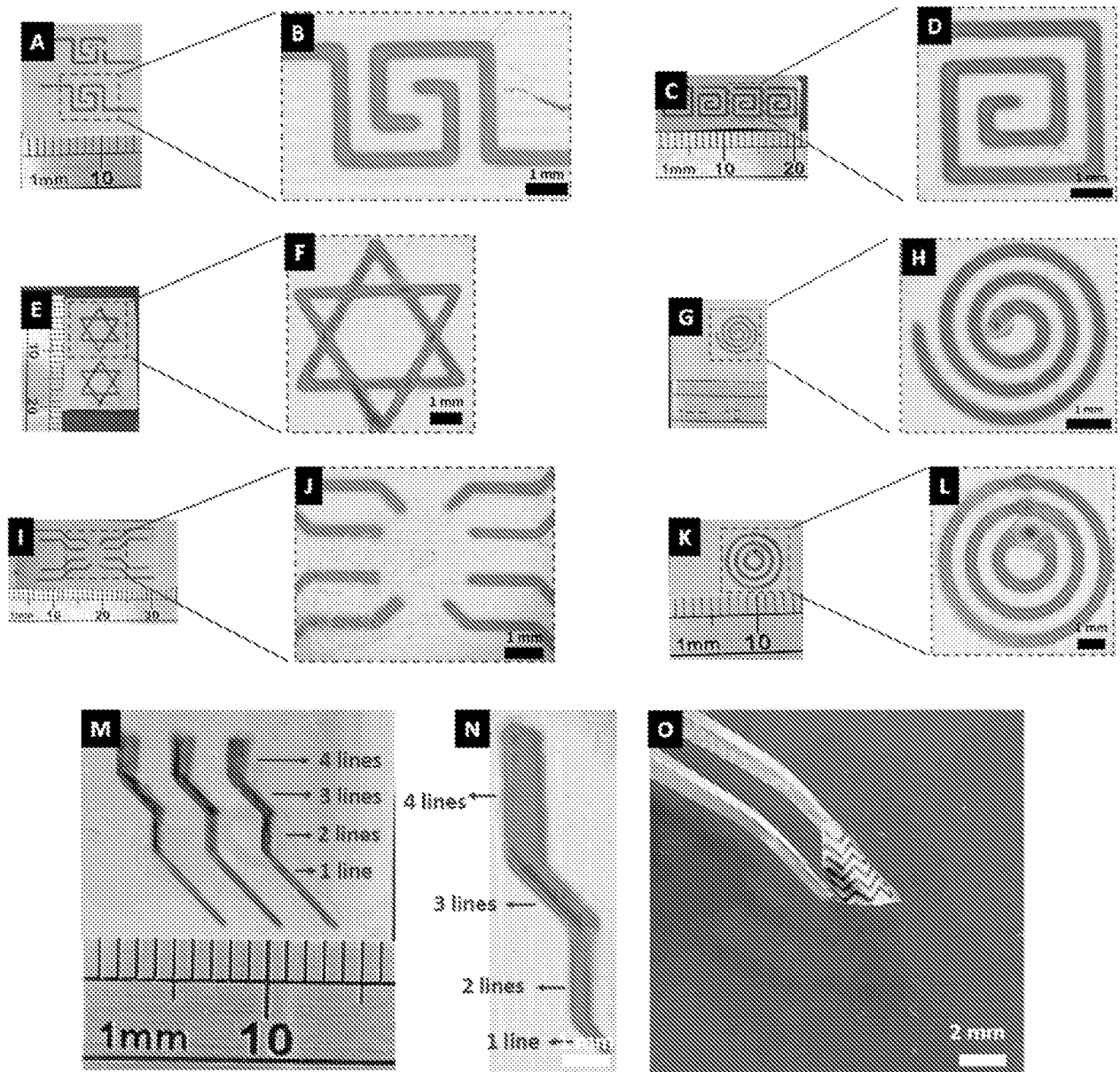


FIG. 3

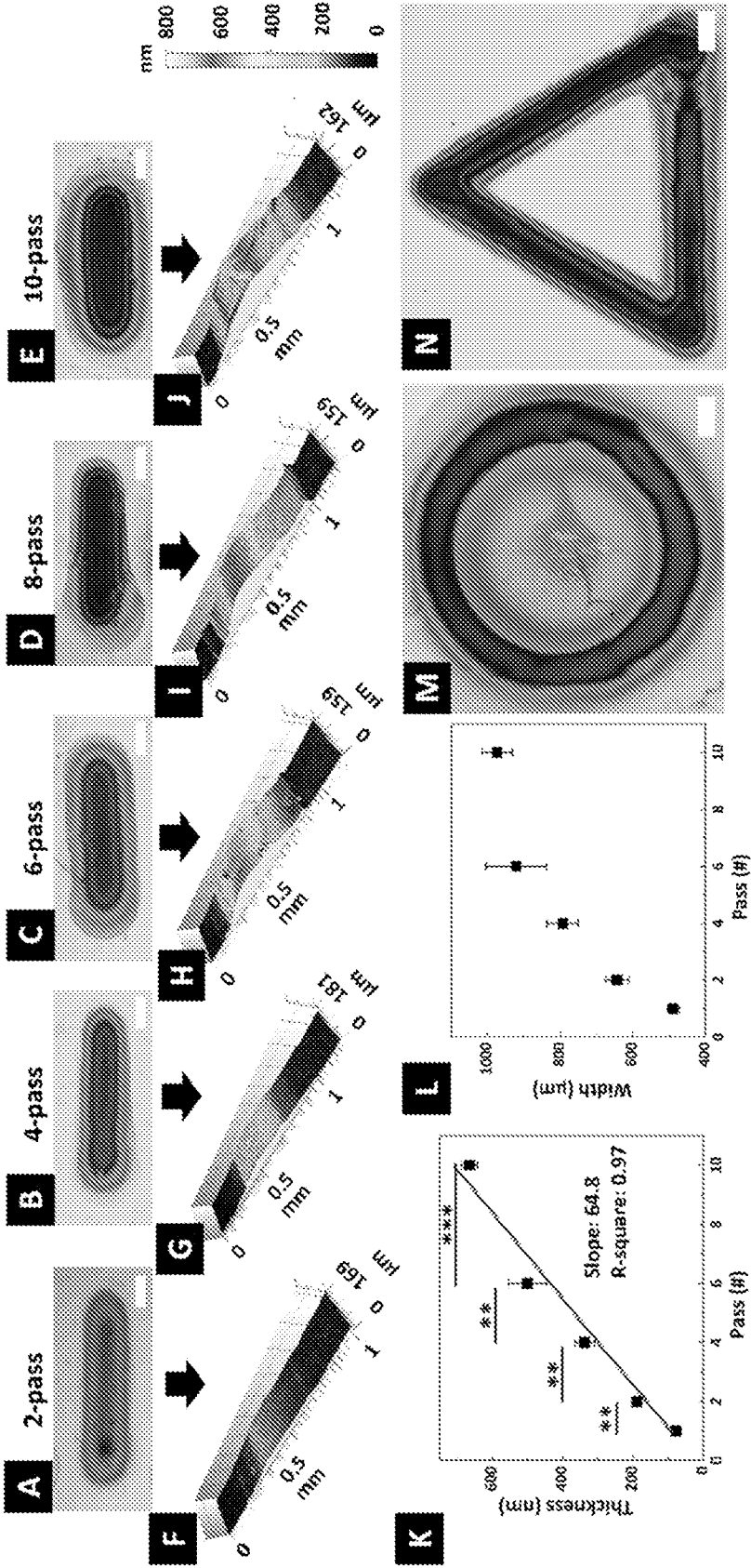


FIG. 4

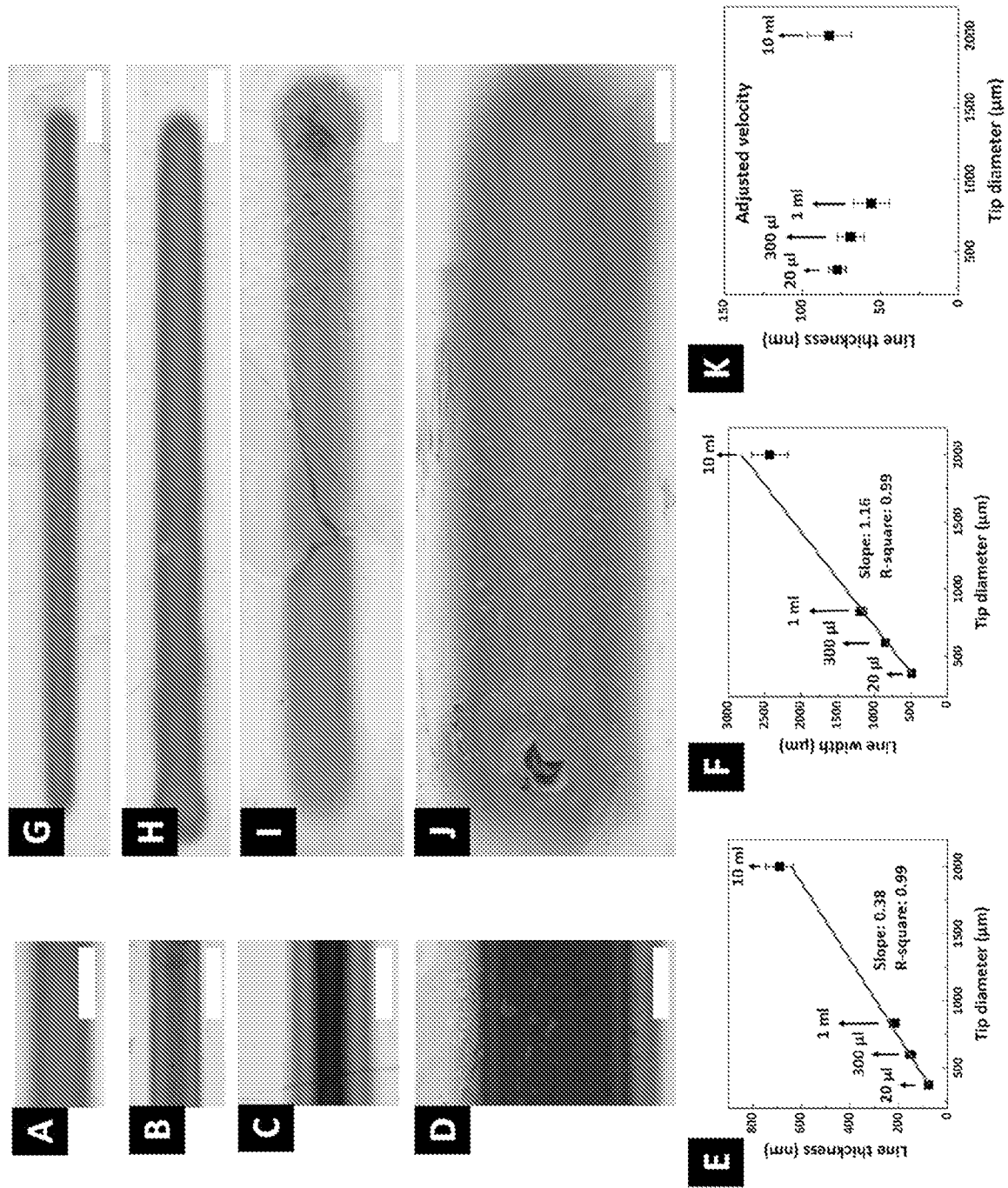


FIG. 5

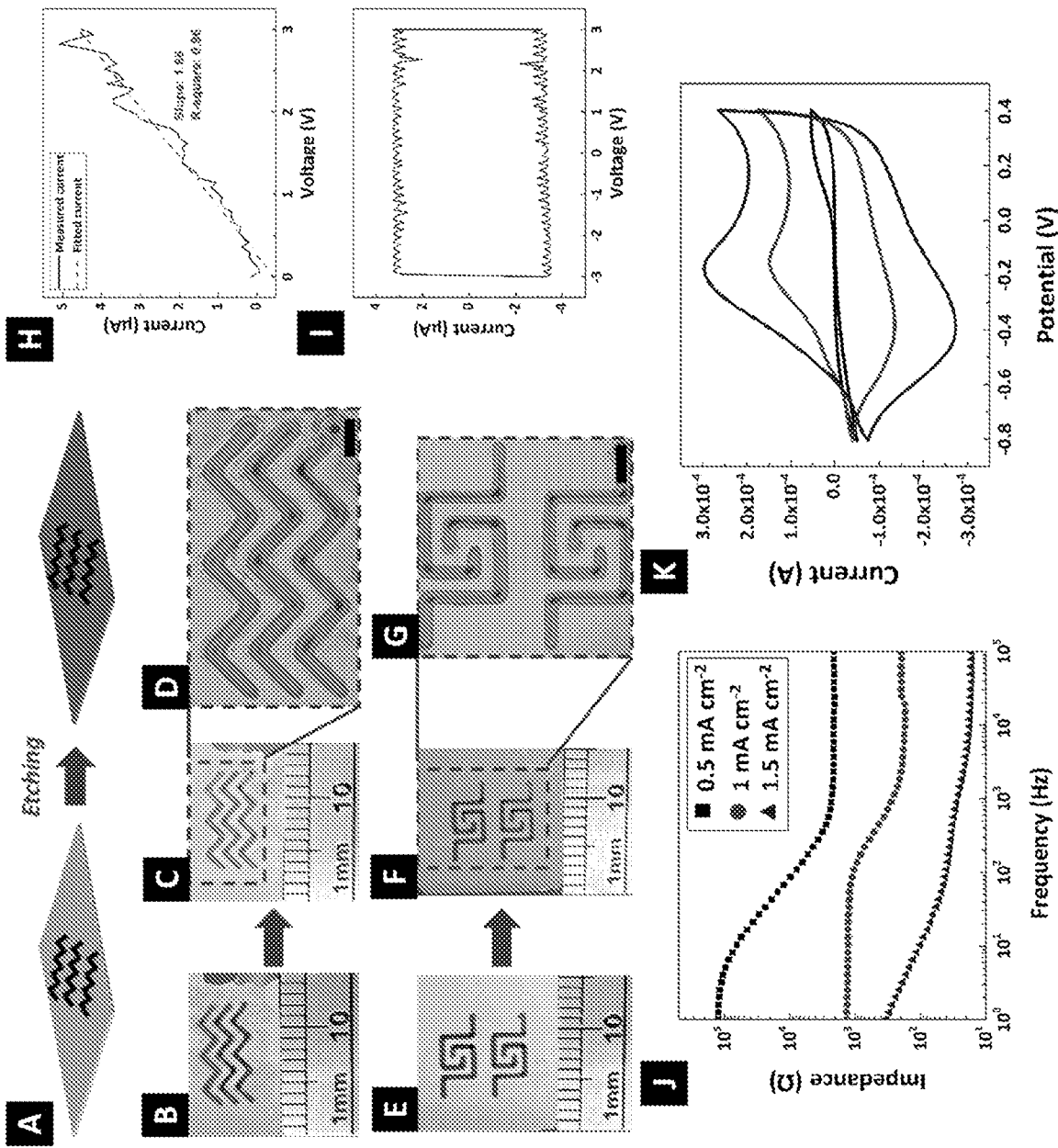


FIG. 6

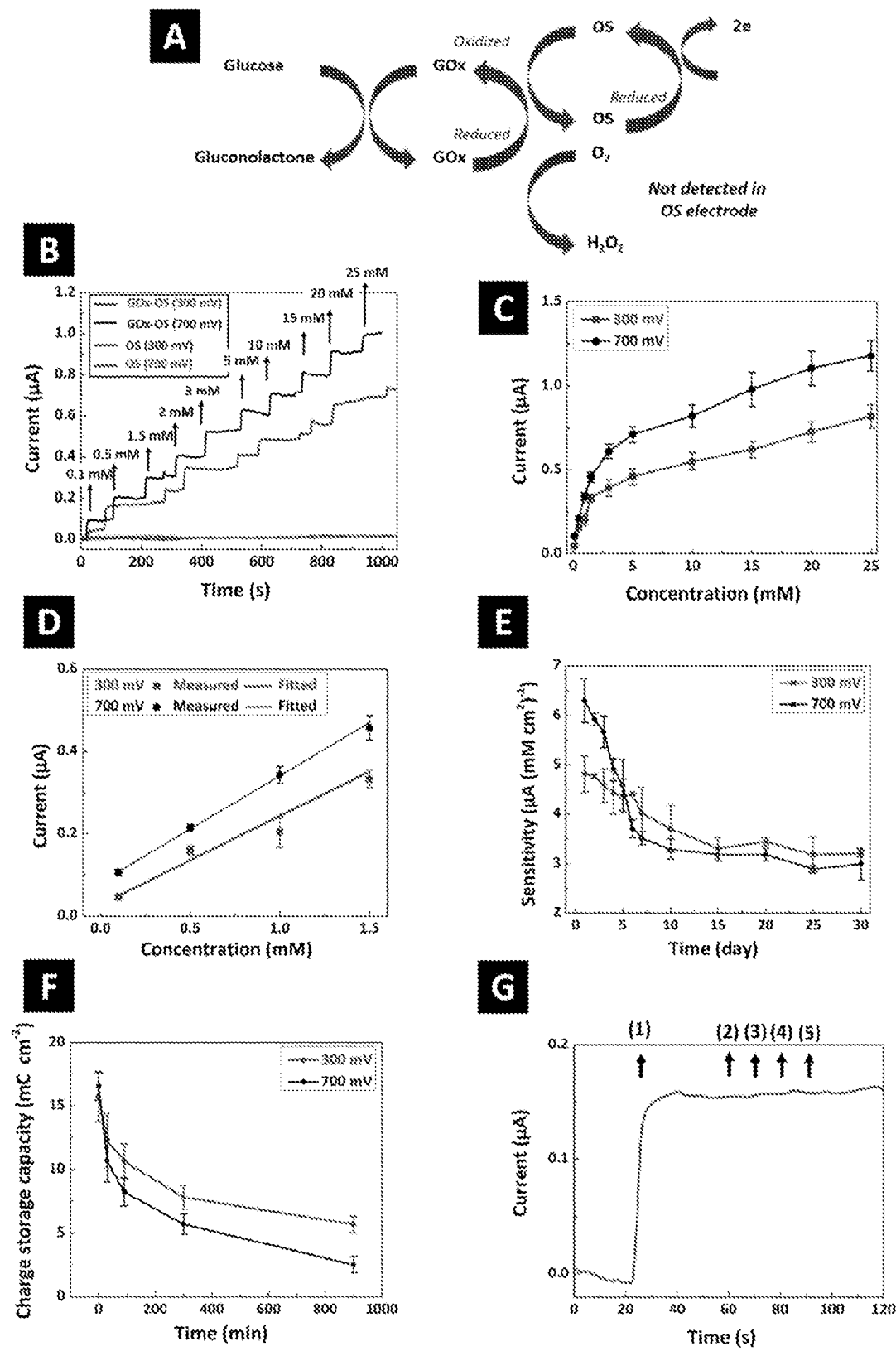
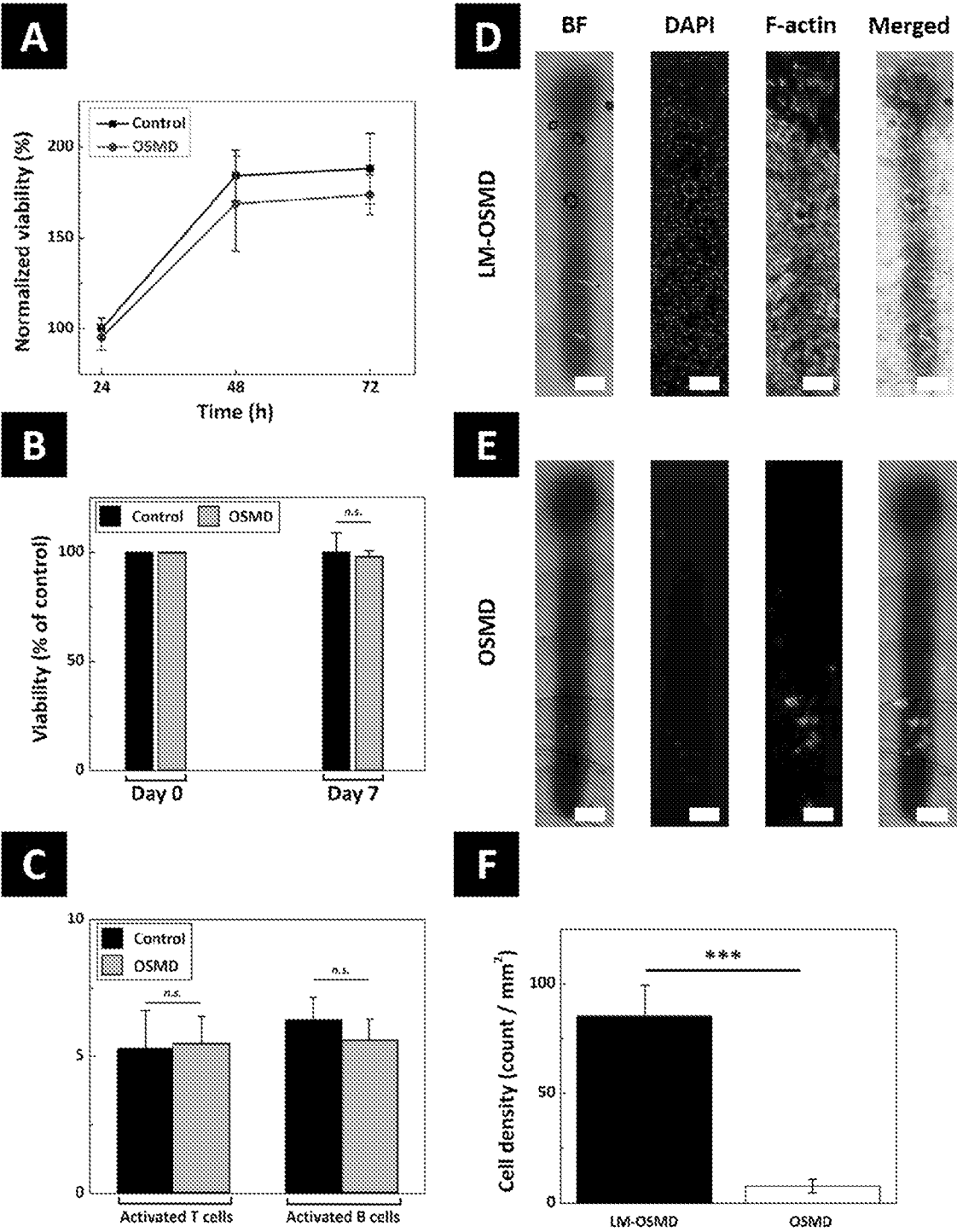


FIG. 7



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2024/018358

A. CLASSIFICATION OF SUBJECT MATTER INV. B29C64/106 H10K71/12 H10K71/13 ADD.		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) H10K B29C		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 10 179 953 B2 (PENN STATE RES FOUND [US]) 15 January 2019 (2019-01-15) column 5 - column 7; claim 1 column 10, line 32 column 8, lines 51-55 -----	1 - 20
Y	WO 2022/049582 A1 (OREN ERAN [IL]; MANSDORF NADAV [IL]) 10 March 2022 (2022-03-10) paragraphs [0021] - [0026], [0061], [0071]; figure 1 -----	1 - 20
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