

MULTI-MATERIAL PALETTE FOR 3D MICROELECTRODE ARRAYS FOR A VARIETY OF 3D ELECTROGENIC MICROPHYSIOLOGICAL SYSTEMS

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ABSTRACT

Herein we report on a multi-material palette towards the microfabrication of 3D Microelectrodes Arrays (MEAs) for integration with a variety of 3D electrogenic Microphysiological Systems (MPS). Our goal is to fabricate 3D MEAs with six microelectrodes by utilizing materials such as polycarbonate (PC), polymethyl methacrylate (PMMA), and polysulfone (PS). We created a reliable microfabrication process by combining laser micromachining, laser-induced breakdown spectroscopy (LIBS), 3D needle assembly, SU-8 coatings and micromilling/ microdrilling techniques. The 3D MEAs demonstrate impedance characteristics similar to commercial MEAs. Additionally, all material combinations showed outstanding biocompatibility for applicability in 3D neuronal and cardiac studies.

KEYWORDS

Hybrid Microfabrication, 3D Microelectrode Arrays, 3D Electrogenic Microphysiological Systems, Biocompatibility.

INTRODUCTION

In vitro neural or cardiac studies have emerged as a significant area of research in areas like neurotoxicity, cardiotoxicity, and pharmacology [1,2]. Micro Physiological Systems utilize complex human cellular models in a microchip format that create precise biological signatures in a benchtop setting. Using advanced devices such as 3D MEAs, researchers have been able to monitor the electrical activity of MPSs in a controlled environment [3]. These biosystems have allowed for a greater understanding of neural, cardiac functions, identification of abnormal activity, examination of the effects of drugs on neural or cardiac activity, and exploration of underlying mechanisms of neurological and cardiac diseases. In this study, we characterize hybrid microfabrication technologies for pick-and-place development of application-specific 3D MEAs.

MATERIAL AND METHODS

For device fabrication, a 30x30x1.75mm PC, 30x30x2mm PMMA, and 30x30x2mm PS substrates were micromilled/microdrilled with electrode/connector locations (Figure 1). Stainless Steel microneedles of height 1.90mm were magnetically inserted to define ~100-150µm 3D microelectrodes and Ag-ink cast for metallization from micromilled traces on the backside. SU-8 was spun cast in 2D/3D at 3,000 rpm as insulation material. Male connectors were inserted for interface connectivity. For SU-8 recording site definition on 3D microelectrodes a green laser micromachining process was utilized: 100% power, 50Hz, 6,000 burst and 30% output. Alternatively, a **Laser Induced Breakdown Spectroscopy (LIBS)** process [4] was used. Figure 2 depicts LIBS, confocal and SEM images of a single 3D MEA (PC as a sample) which indicate a layer of the SU-8 layer measuring ~2.9µm in thickness in 2D/3D. A culture region in the central part of the device was defined using PDMS to attach a transparent polymer (PET-G) ring. Device and systems characterizations including SEM, Confocal Microscopy, BODE Impedance Spectroscopy, and Biocompatibility all followed prior work [3].

RESULTS

Material Palette

Table I depicts optimized micromilling/microdrilling conditions for all three polymers: PC known for its high impact resistance and transparency; PMMA exhibits an excellent optical clarity, scratch resistance and weatherability [5,6]; PS offers a unique combination of high temperature resistance, chemical resistance, and mechanical strength [7]. All three offer 3D MPS developers great options for the microfabrication of 3D MEAs.

Impedance Spectroscopy

Full spectrum impedance sweeps are shown in Figure 3 A-C. Classic MEA definition is depicted both for 3D micro-tower and smaller 30µm x 30µm electrodes clearly separated from fully insulated MEAs (PC n=6, PMMA n=6, and PS n=4). Fully insulated electrodes report an impedance >2MΩ, >69kΩ, >629MΩ. Once laser ablated to define recording sites, the 3D microelectrodes depicted a lower impedance ~15.9kΩ, and ~76kΩ @ 1kHz (PC n=6 and PS n=5) as expected.

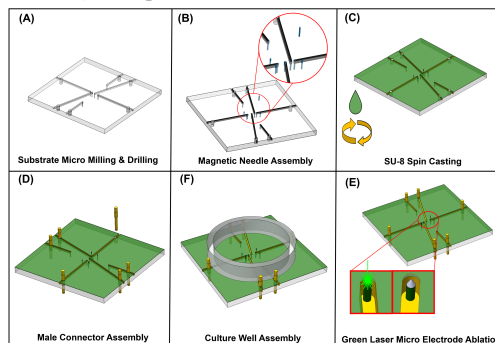


Figure 1: Microfabrication process schematics. (A) Micromilling & drilling of PC, PMMA, or PS substrate for 3D MEA. (B) Magnetic insertion of SS microneedles & Ag-ink cast for metallization. (C-D) SU-8 spin casting & insertion of male connectors with Ag-ink cast for metallization. (E) Green laser micromachining/LIBS process for the SU-8 recording site definition on 3D microelectrodes.

		Polycarbonate	Polymethyl Methacrylate	Polysulfone
		PC	PMMA	PS
Needle Electrodes MDB-0.20mm Drill Bit	Velocity mm/s	20	20	20
	RPM	20,000	20,000	20,000
	Depth of Cut (mm)	2.1 (2 times)	2.1 2.2 2.3	2.1 2.2 2.3
	Rapid Plane (in/min)	10	10	10
TRACES MILL-T4	Velocity mm/s	20	20	20
	RPM	20,000	20,000	20,000
	Depth of Cut (mm)	0.5	0.5	0.5
	Rapid Plane (in/min)	10	10	20
Male Connectors MDB-1.00mm Drill Bit	Velocity mm/s	20	20	20
	RPM	20,000	20,000	20,000
	Depth of Cut (mm)	2.1	2.3	2.1
	Rapid Plane (in/min)	10	10	20
Outline CR-0620-F2 62.0 MIL	Velocity mm/s	25	25	25
	RPM	20,000	20,000	20,000
	Depth of Cut (mm)	2.1	2.2	2.2
	Rapid Plane (in/min)	20	20	20

Table I: Optimized Micromilling & Microdrilling parameters used for the hybrid microfabrication of the 3D MEAs.

Biocompatibility Assay

Figure 4 A-P depicts a detailed investigation carried out in a 24-well multiwell plate to examine the biocompatibility of materials used in the microfabrication process. All materials were

observed to support excellent cell growth, and fluorescent imaging of live/dead NIH-3T3 cells on all constituent materials showed strong viability at 4 days *in vitro*.

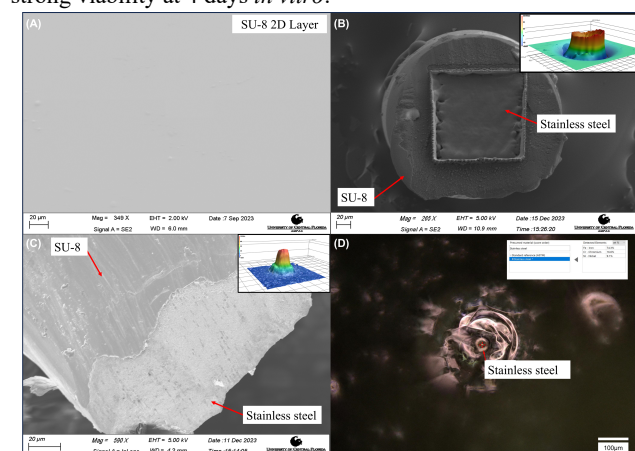


Figure 2: (A) 2D SEM image of the uniform SU-8 layer measuring ~2.9 μm in thickness. (B-C) SEM images of cross sectioned 3D microelectrodes with laser ablation with a height of 130 μm and 458 μm on the correspond inset. (D) LIBS image of 3D microelectrode showing the composition of the microelectrode with 74.3% of iron, 16.6% Chromium and 9.1% of Nickel characteristic of Stainless Steel and no SU-8.

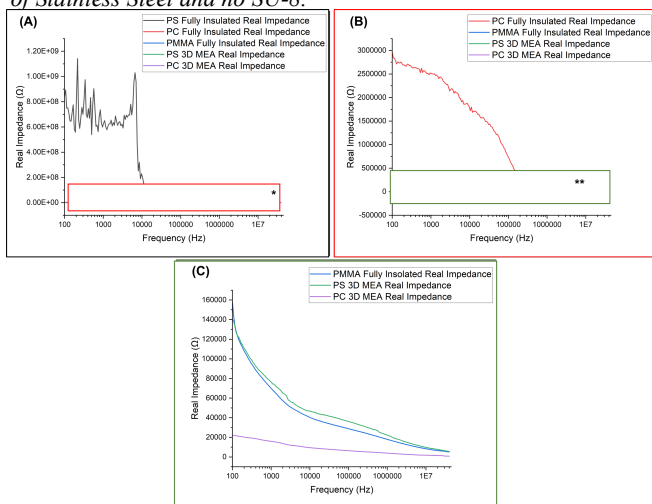


Figure 3: Electrical impedance response. (A, B, C) show sweeps of high impedance values depicting fully insulated 3D microelectrodes and after green laser micromachining/LIBS process definition of smaller 3D microelectrodes showing lower impedance values. Work on PMMA is ongoing since full insulation has not yet been achieved.

CONCLUSION

The development of a multi-material palette for 3D Microelectrode Arrays expands the scope of materials available to biologists for the integration of 3D electrogenic microphysiological systems atop these devices. By utilizing commonly used microfluidic materials polycarbonate; polymethyl methacrylate, and polysulfone, this work provides possibilities for new sensor integration besides fluid flow for complex 3D cardiac and neural systems. We have depicted the efficacy of the hybrid microfabrication process with thorough characterizations of process conditions, biocompatibility tests, impedance spectroscopy, and SEM/confocal imaging. These results show promise for the future development of *in vitro* neurotoxicity, cardiotoxicity, pharmacology assays using electrogenic MPSs.

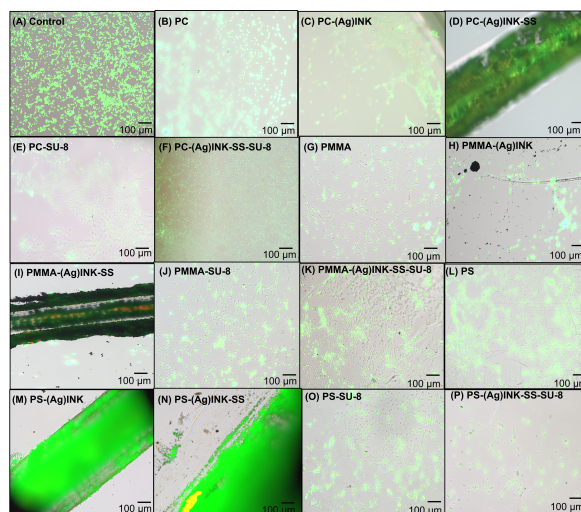


Figure 4: Material biocompatibility assay results. NIH 3T3 cells were cultured in a 24 well plate to perform the biocompatibility assay with the different material combination. Live/Dead fluorescence confocal image of cells at DIV04 (A) cell control, (B, G, L) PC-PMMA-PS, (C, H, M) PC-PMMA-PS-Silver ink, (D, I, N) PC-PMMA-PS-Silver ink-SS, (E, J, O) PC-PMMA-PS-SU-8, (F, K, P) PC-PMMA-PS-Silver ink-SS & SU-8 showcased excellent cell growth and showed good viability. The florescent stain green for living cells (Calcein) and a red staining (Propidium Iodide) indicates dead cells.

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