STABLE, ELECTRON-BEAM SUBLIMATED, NANOSTRUCTURED SILICON DIOXIDE ON POLYCARBONATE AND STAINLESS-STEEL AS A BIOADHERENT DIELECTRIC TOWARDS NEURAL MICROPHYSIOLOGICAL SYSTEMS

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ABSTRACT

Towards the development of biosensors, novel material combinations often impart challenges in microfabrication and process integration, e.g., depositing traditional materials using established processing techniques on new substrate materials. Silicon dioxide, for example, has been widely used in MicroElectroMechanical Systems (MEMS) fabrication but is rarely used in devices in combination with polymers and steel alloys. Herein, we establish an avenue improving the adherence of such an oxide material to polycarbonate and 316L stainless steel via polydopamine mediated chemistry to construct a stable coating that is indicative of its dual purpose as both a dielectric material for microelectrodes and a cell adhesion promoter. This fabrication method is a significant step towards novel BioMEMS devices and integrated biological systems currently under development.

KEYWORDS

Silicon Dioxide (SiO_2), Nanostructuring, Polydopamine (PDA), Biological Interfaces, Microphysiological Systems.

INTRODUCTION

As the field of *in vitro* diagnostics advances **[1]**, ***innovations in constituent materials*** are required to enable necessary functional metrics. For realizing neural Microphysiological Systems (MPS) with integrated sensing/stimulation modalities **[2,3]**, ***insulating dielectric materials for enhanced cellular growth*** are of great importance **[4]**. However, material incompatibility often prevents many potential applications **[5]**. Silicon dioxide (SiO_2) as a dielectric material has been widely studied and utilized in traditional MEMS fabrication **[4,6]**, and its inherent inert/porous nature has continually motivated the utilization of SiO_2 as the functional surface for many BioMEMS devices. However, SiO_2 is no exception in that challenges exist to adapt its utilization towards non-traditional substrates and MPS integration, such as those incorporating polymers and metal alloys. Generally, SiO_2 has been deposited through thermal growth, plasma-enhanced deposition processes or precursor-mediated synthesis, and often occurs well outside temperatures/conditions polymers can withstand **[7]**. Additionally, mismatch in functional groups leads to superficial adhesion to polymers and metal alloys which is potentially disrupted by humidity/hydration-induced swelling **[8]**. Thus, strategies must be employed to enhance the adhesion of SiO_2 by utilizing established intermediary compounds. Ideally, said strategies should not interfere with desired optical properties of polymers, and be scalable for device integration in polymer-metal based microfabrication processes. ***Solution-based polydopamine (PDA) treatments*** **[9]** have been previously utilized to provide a linker molecule for such disparate materials. PDA, along with electron-beam sublimation of SiO_2 provides a ***novel, low-temperature avenue*** for stable, bio-adherent dielectric coatings which

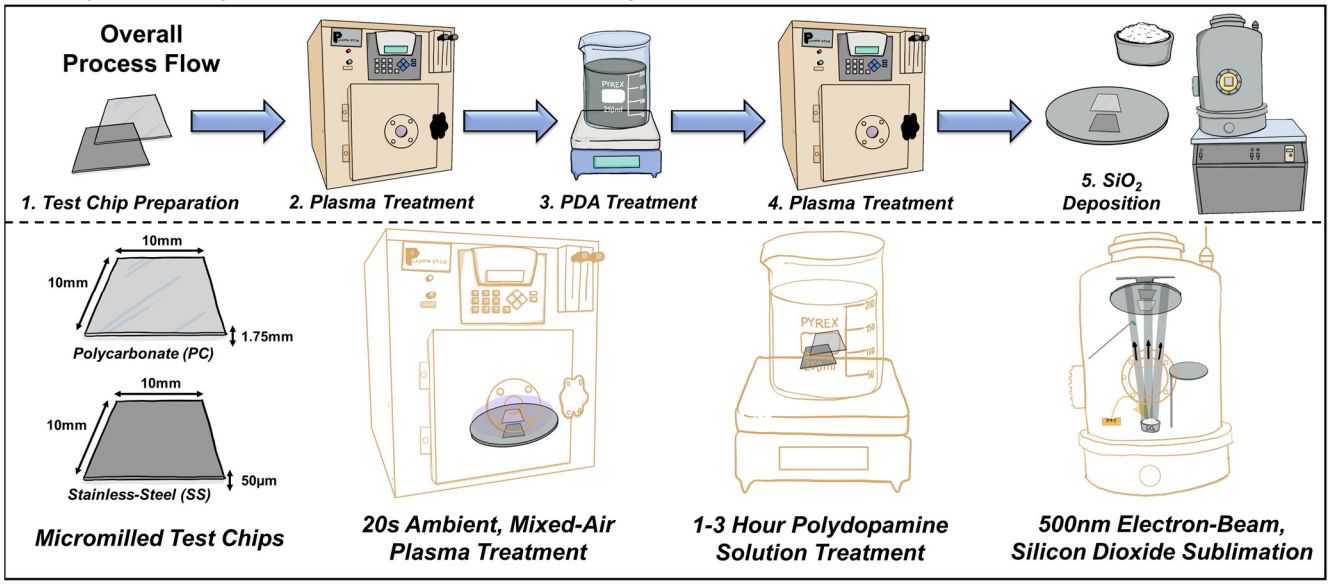

Figure 1: *Schematic representation of the overall treatment process flow for sample testing. First, test chips are micromilled from their respective substrates. The upper section describes the general treatment scheme, while the lower partition details each step. Samples were first treated in ambient, mixed-air plasma for 20s. Next, the samples were placed in a continuously stirring PDA-EDC solution for the designated 1-3 hours. The samples were washed three times in DI water and then dried with N_2 gas, before being treated again with ambient, mixed-air plasma for 20s. Prepared samples were stored in a vacuum chamber for transportation to the e-beam system. Once the samples were loaded, the chamber was pumped to an appropriate vacuum, and 500nm of SiO_2 was deposited via e-beam induced sublimation. Samples were subsequently stored under vacuum conditions until required for testing.*

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additionally enable robust neural spheroid growth. Previously, MPS approaches utilizing accessible material/insulation strategies, which retain optical clarity (e.g., PDMS) have been explored **[3,10,11]**. Herein we ***explore an electron-beam sublimation of nanoporous silicon dioxide (SiO_2) technology*** onto polycarbonate (PC) substrate and 316L stainless-steel (**SS**) microelectrode materials **[11]** using PDA to stabilize adhesion. We subsequently characterize this method and validate its future potential for 3D Microelectrode Array (3D MEA)-based neural MPS.

MATERIALS AND METHODS

10mm^2 (length by width) test chips were micromilled from bulk sheets of 1.75mm thick PC and 50µm thick SS. These were subsequently cleaned in 70% isopropyl alcohol (IPA). A stirring solution of PDA (Sigma Aldrich, USA) was prepared as per Azim et al. **[9]**, utilizing a stock 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) solution (Sigma Aldrich, USA) as a cross-linking enhancer. Test chips were plasma treated (Plasma Etch Inc., USA) in mixed air plasma for 20s, and then submerged in the solution for 1-3h for coating. Next, test chips were rinsed three times in DI water, dried with N_2 gas and stored under vacuum. PDA-coated chips were plasma treated again (20s) prior to electron-beam deposition of 500nm SiO_2 (Thermionics, USA; **Fig. 1**). Additional test chip treatments were prepared for analysis, control, and comparison. To simulate microelectrode dielectric stability in cell culture incubator conditions, coated SS chips were subjected to an incubator environment (>95% humidity) between 4-10 weeks, before scotch-tape peel test analysis of the adhesion was performed. ImageJ software (National Institutes of Health, USA) was utilized for image-based quantification of tape-peeled areas. Scanning electron microscopy (SEM) (Zeiss, Germany) and optical imaging (iPhone XS; Apple Inc., USA) were performed for surface feature comparison. Energy Dispersive X-ray Spectroscopy (EDS) (Zeiss, Germany) confirmation of SiO_2 composition was additionally performed during SEM imaging. X-ray Photoelectron Spectroscopy (XPS; Physical Electronics, USA) was utilized for compositional and chemical analysis of the plasma-treated PDA-PC. Water contact angle (WCA) measurements were obtained (DataPhysics Instruments, USA; OCA 15EC mode, SCA 20 module) to assess the relative surface energies at selected functionalization states. Ultraviolet-Visible spectroscopy (Agilent, USA) was performed to assess transparency of the PDA/SiO_2 coated PC substrates. Scotch-tap peel tests were performed by uniformly pressing the tape on to each sample, and then peeling with consistent force. Human iPSC-nociceptors (Anatomic Inc., USA) and Embryonic Stem Cell-derived – Spinal Cord Dorsal Horn (ESC-SCDH Ashton Lab, University of Wisconsin, USA) spheroids were plated within polyethylene glycol dimethacrylate (PEGDMA) channels on glass-control and SiO_2-coated polymer substrates with 3D SS electrodes. Calcein AM staining visualized organoid growth, 2-weeks after completion of terminal differentiation.

RESULTS AND DISCUSSIONS

Polycarbonate substrates and stainless-steel microelectrodes form the basis of novel 3D MEA-based neural MPS collaboratively under development between the University of Central Florida, Tulane University, and the University of Wisconsin **[11]**. In early trials of adhering SiO_2 to PC, layers of porous and flaking SiO_2 film were observed under SEM (**Fig. 2A-C,** without the addition of PDA). Additionally, serpentine "bubbles", partially released through surface incompatibility of 500nm thick SiO_2 with PC, were present which denoted delamination due to inherent stress within the thin film. However, these experiments confirmed the nanoporous nature of the oxide film, which indicated potential suitability for cellular applications. Previous published data **[10]** and internal studies confirmed 500nm SiO_2 films were sufficient as both a suitable dielectric and a stable insulation layer for 3D MEAs.

Fig. 2D-F illustrate the addition of PDA treatment before SiO_2 deposition on PC and SS substrates. As the PDA treatment time increases, a gradual darkening can be observed (**Fig. 2**, inset images), and the PDA clusters change in both size and uniformity. The 1-hour PDA treatment is shown in **Fig. 2D**, and the polydisperse topography observed may have contributed to film delamination during PDA-SiO_2 peel-tests through an inconsistent distribution of inherent forces within the combined thin film. In **Fig. 2E,** the 2-hour PDA treatment demonstrated a monodisperse topography, through

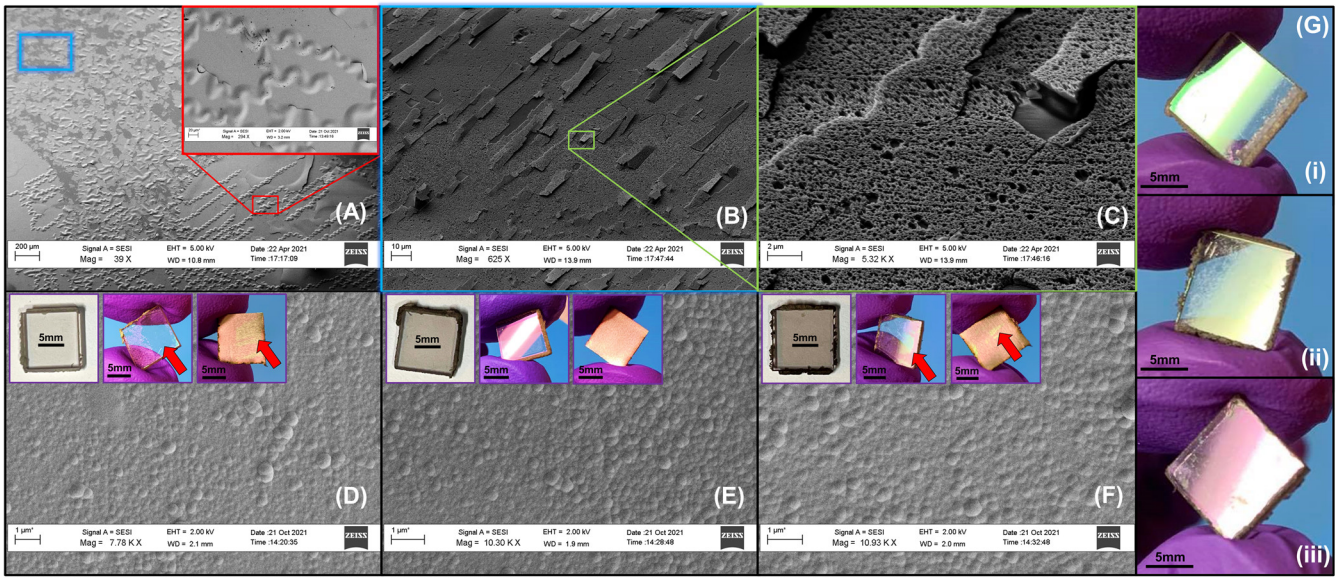
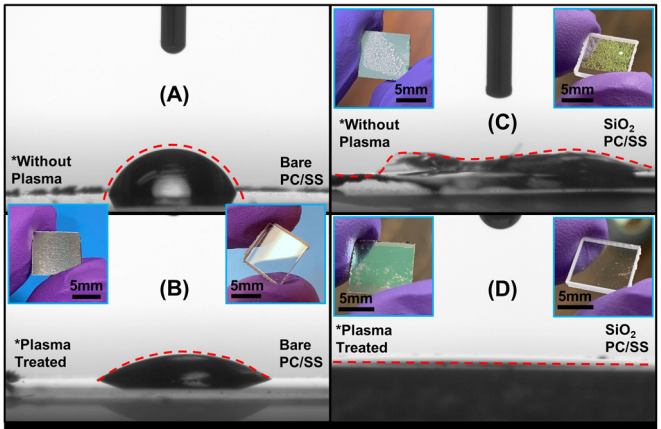


Figure 2: *SEM and optical images of SiO_2 adhesion testing. (A) SEM image of SiO_2 on PC without treatment. Inset details serpentine "bubbles," indicative of delamination. (B) Enhanced region of blue box from (A), illustrating flaking of SiO_2. (C) Enhanced region of (B), showing the nanoporous nature of the SiO_2. (D-F) Images detailing the addition of PDA treatment before SiO_2 deposition. Inset images show the minor darkening of the PC after PDA treatment, and tape-peel tests of both PC and SS (left to right). (D) 1-hour PDA treatment. (E) 2-hour PDA treatment. (F) 3-hour PDA treatment. (G) Lower concentration PDA treatment with SiO_2, illustrating color shifts from 1-hour (i), 2-hours (ii), and 3-hours (iii), that depict thin-film light interference phenomenon.*



(E) Water Contact Angle (Young Laplace; θ)

Sample	Untreated	Plasma Treated	Untreated / SiO_2	Plasma / SiO_2	1Hr PDA	2Hr PDA	3Hr PDA
Polycarbonate	83.3 +/- 1.87	37.6 +/- 7.04	Delaminated	0	36.6 +/- 0.196	40.8 +/-2.94	*13.9 +/- 0.10
Stainless-Steel	86.4 +/- 12.2	36.1 +/- 2.48	Delaminated	0	45.5 +/- 1.93	40.6 +/- 4.02	40.6 +/- 0.05

Figure 3: *WCA measurements (N=3) for substrate treatment combinations. Inset images illustrate the samples utilized for measurement. (A-B) Confirmation of plasma treatments effects to increase surface energy, as (A) demonstrates a relatively hydrophobic surface, and (B) demonstrates a sharp shirt towards a more hydrophilic state. (C-D) WCA values for SiO_2 coated bare PC/SS, with and without plasma treatment. (C) Without plasma treatment, the samples optically have an uneven and cracked appearance, and upon contact with water are immediately* ***delaminated****. (D) With plasma treatment, the SiO_2 film is more consistent, and the WCA values are both* ***0*** *indicating a high surface energy. (E) Table containing WCA values and standard error margins. The PC value denoted with an (*), is due to its lower-than-expected WCA value; to be further investigated.*

which the most robust SiO_2 adhesion was observed. Tape residue can be observed (**Fig. 2**, insets) showing a highly adherent SiO_2 film, which again is likely due to an even dispersion of stresses within the thin film, coupled with plasma-enhanced binding of the oxide to the PDA across the surface. The 3-hour PDA treatment is showcased in **Fig. 2F**, which contains a polydisperse topography, containing larger PDA groupings. This once again contributed to poorer adhesion but was observed to be more robust than the 1-hour treatment. These results **overall indicate that the 2-hour PDA-SiO_2 treatment is ideal** and merits further characterization and usage. **Fig. 2G** additionally depicts how lower concentrations of the PDA treatment from 1-3h (**Fig. 2**i-iii, respectively), combined with SiO_2, demonstrate thin film light interference phenomenon with color shifts which might be further investigated in future works.

WCA measurements were first obtained to illustrate the necessity of plasma pre-treatments before PDA and SiO_2 adhesion. **Fig. 3** contains sample images from the WCA test which were utilized to calculate the Young-Laplace WCA, as well as representative images to illustrate the samples utilized in measurements. **Figs. 3A&B** serve as baseline confirmation of plasma treatments efficacy to increase surface energy on bare PC/SS as controls. The average WCA values of **83.3° +/- 1.87** (PC) and **86.4° +/- 12.2** (SS) in **Fig. 3A** show that the initial surfaces have lower surface energies (inversely related with higher WCA), denoting a hydrophobic state. This is contrasted with **Fig. 3B**, which shows that with plasma treated samples, the WCA values decrease to **37.6° +/- 7.04** (PC) and **36.1° +/- 2.48** (SS) which indicates higher surface energies, more suitable for the establishment of binding interfaces. In **Fig. 3C&D,** the next sample stage is depicted for SiO_2 coated PC and SS, varying only the usage of plasma treatment. Without plasma treatment, the films are immediately delaminated from both PC and SS, and optically have an irregular and cracked appearance (**Fig. 3C**). When plasma treatment is utilized prior to SiO_2 deposition, the SiO_2 film is stable longer, and presents a more uniform appearance (**Fig. 3D**). The WCA values of **0** indicate a very high surface energy, and excellent hydrophilicity. However, this film still degrades within hours of exposure to ambient humidity/water without PDA. The 1-hour PDA coating on PC and SS substrates demonstrated WCA values of **36.6° +/- 0.196** and **45.5° +/- 1.93**, respectively (**Fig. 3E**). The 2-hour PDA coated samples demonstrated values of **40.8° +/- 2.94** and **40.6° +/- 4.02**, respectively. The 3-hour coated samples demonstrated values of **13.9° +/- 0.10** and **40.6° +/- 0.05**, respectively. Further characterization of PC values is required to account for apparent inconsistencies with the moderate incremental decrease in WCA across PDA treatments.

To further motivate choice of the stable 2-hour PDA-SiO_2 coating, additional material characterizations were performed (**Fig. 4**). EDS analysis was performed on the e-beam deposited SiO_2, to confirm the retention of the deposited oxide composition (relative atomic percentages of **29.83% (Si)** and **69.60% (O), Fig. 4A**). Next, the ultraviolet-visible spectrum of different stages of coated PC substrates was tested, to ensure the usability of the selected coating time/method for transmitted light biological imaging applications (**Fig. 4B**). In the visual light range (400-700nm), while plain PC has ~90% optical clarity, the unstable, poorly adherent SiO_2 illustrates an irregular, anti-reflective type signature. Optical clarity was observed to decrease no more than ~5% transmittance with an hour increase in PDA treatment time, as expected from the increased observed darkening of the PC. Interestingly, corresponding PDA treatment times coupled with the 500nm of stable SiO_2 deposition demonstrated a moderate improvement of ~3% transmittance on average, in addition to an anti-reflective signature shift, which can be observed in the inset (**Fig. 4B**). With regards to coated SS

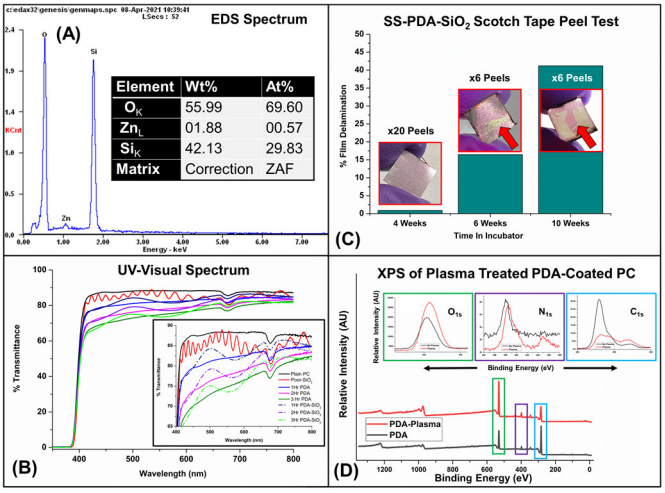


Figure 4: *Sample characterizations (N=3) of selected PDA and SiO_2 film samples. These results further motivate usage of the stable 2-hour PDA/SiO_2 coating. (A) EDS analysis of e-beam deposited SiO_2, confirming the retention of the deposited oxide composition. (B) Ultraviolet-Visible spectrum of different stages of coated PC substrates. (C) Scotch-tape peel tests, for 2-hour SS-PDA/SiO_2 films, after* ***4, 6, and 10 weeks*** *in a cell culture incubator. Inset images illustrate representative samples. (D) XPS spectra of crucial PDA interface, before and after plasma treatment.*

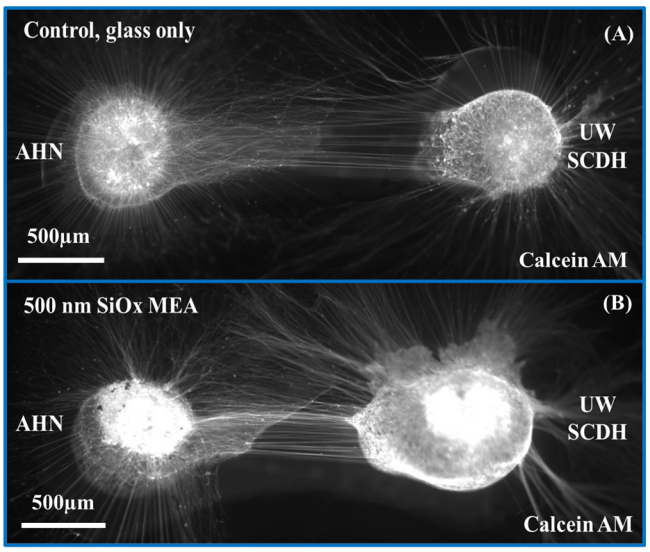


Figure 5: *Fluorescent Calcein AM staining of IPSC (Induced Pluripotent Stem Cell)-derived nociceptor and Embryonic Stem Cell (ESC)-derived spinal cord dorsal horn (SCDH) spheroid cultures within PEG channels, 2-weeks after terminal differentiation. (A) Growth on glass substrates for control, demonstrating robust growth of axonal projections, important for neuronal communication. (B) Growth on PC-SS substrates with functional SiO_2 surfaces, demonstrating differential but comparable growth and integration.*

samples, scotch-tape peel tests were performed on 2-hour SS-PDA-SiO_2 films, after **4, 6, and 10 weeks** in a cell culture incubator (**Fig. 4C**). The inset images included illustrate representative samples of the batches tested. After **>20 peel tests**, the 4-week samples proved highly stable, with the removal of **0.89% +/- 0.935** SiO_2 (i.e., **more than 99% SiO_2 was intact**). After 6-weeks, **6 peel tests** began to remove **16.5% +/- 7.49%** of the film. After 10-weeks, **6 peel tests** removed **41.2% +/- 37.3%** of the film and represents the highest variability among the measurements. Importantly, only adherence of the film and not its integrity was affected, indicating further that the chosen PDA-SiO_2 combination helped to alleviate the stress in the film, even when long-term hydration disrupted the binding to the substrate. Of note, only physically peeling the film prompted oxide removal at 10-weeks. In **Fig. 4D**, XPS spectra of crucial PDA interface was characterized, both before and after plasma treatments. Literature has established that PDA natively binds organic/inorganic surfaces through catechol groups **[12]**, which would indicate an ability for bond formation. However, treatment with mixed-air plasma was experimentally found necessary at all interfaces, resulting in significantly altered C_{1s} and N_{1s} binding energies, and suggesting higher Oxygen content binding sites. To demonstrate biocompatibility of manufactured substrates, nociceptor-SCDH spheroids were cultured atop PC/SS-PDA-SiO_2 substates in a 3D dual-hydrogel format for 2-weeks and stained with Calcein AM to show viability (**Fig. 5**). Excellent neurite outgrowth was observed, lending credence to the end-goal use within neural MPS.

CONCLUSIONS

In this work, we demonstrated a novel methodology for the realization of a stable SiO_2 dielectric and adhesion promoter functional layer on PC and SS. Due to the incompatibility of these materials with traditional SiO_2 deposition processes, a custom PDA process was utilized. We found that a 2-hour PDA treatment, coupled with mixed air plasma preparations, led to a stable 500nm SiO_2 definition on both materials. The SiO_2 coated SS additionally demonstrated excellent adhesion retention for 6-weeks in a cell culture incubator environment. We also characterized the compositional, optical, and chemical properties of the designed film and demonstrated excellent growth when incorporated within a novel, human neural 3D MPS.

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