TAILORING ENHANCEMENT OF SILICON DIOXIDE ADHESION TO POLYCARBONATE SUBSTRATES FOR 3D MICROELECTRODE ARRAYS (3D MEAS) AND OTHER BIOSENSORS

Surbhi Tidke[1], Omar S. Cepeda-Torres[1], Pablo Morales-Cruz[1], Brian E. Butkus[1], Andre Childs[1], and Swaminathan Rajaraman[1]*
[1]University of Central Florida, Orlando, Florida, USA

ABSTRACT

Widely employed for its affordability and design flexibility, polycarbonate (PC) encounters adhesion challenges when paired with silicon dioxide (SiO_2). The interfacial bonding between SiO_2 and PC is compromised, lacking both chemical and physical interactions. SiO_2, being a polar material with high surface energy, contrasts with PC, a nonpolar material having low surface energy **[1]**. Herein, this paper investigates enhancing the adhesion between polycarbonate and silicon dioxide using solution-based chemistries, employing (3-glycidoxypropyl) methyl diethoxysilane (GPTMS) and (3-aminopropyl) trimethoxysilane (APTMS). In the bonded-layer substrate final configuration, a sandwich layer is formed between the PC substrate and the deposited SiO_2 layer. This layer comprises a solution-based coating of GPTMS followed by the application of a trientine (trien for short), serving as an intermediate interface to improve adhesion properties. Additionally, another sandwich layer is formed using APTMS followed by the application of a bisphenol A diglycidyl ether (BADGE), serving the same purpose as trien for GPTMS. Furthermore, the study explores the biocompatibility of these adhesion promoter mediated coatings of SiO_2 atop PC towards 3D Microelectrode Arrays (MEAs) and other biosensor applications utilizing these novel silane coupling agents, GPTMS and APTMS.

KEYWORDS

Polycarbonate Adhesion, GPTMS, APTMS, Biocompatibility, 3D Microelectrode Arrays (3D MEAs), Surface Functionalization

INTRODUCTION

Polycarbonate is widely used in engineering for its excellent mechanical and optical features but lacks chemical and abrasion resistance **[2]**. Silicon dioxide is a critical material in the field of biosensors, microelectrode arrays and cell culture plates due to its excellent insulating properties, biocompatibility, and ability to form a stable interface with biological environments. For those reasons, the integration of SiO_2 onto polycarbonate substrates is particularly important for the development of flexible and durable biosensors. SiO_2 provides an inert surface, maintaining the structural integrity and biological functioning of biomolecules when anchored to SiO_2 surfaces **[3]**. The adhesion between SiO_2 films and PC's surface remains a challenge, as weak adhesion issues arise from coefficient of thermal expansion differences and contaminants from various processing steps used **[4]**. Amongst other polymers, polyesters show promise, their compatibility issues with semiconductor fabrication processes makes them less useful for microfabricated biosensors **[5]**. Our previous work documented the role of polydopamine (PDA) in facilitating adhesion between polycarbonate-SiO_2 layers, benefiting 3D Microelectrode Array (3D MEA) systems **[2]**. However, the repeatability of this coating is still under investigation especially with integration to a Microphysiological System application. Our dedication to refining adhesion promotion and scaling of the novel assay demonstrated in Didier et al. **[2]** remains a major priority.

Investigating factors such as PC surface treatment, SiO_2 deposition conditions, and interfacial structure is crucial to address PC-SiO_2 adhesion. Various methods have been proposed and investigated to enhance the adhesion of PC to SiO_2, such as plasma treatment **[6]**, UV irradiation **[7]**, chemical functionalization, and flame pyrolysis **[8]**. These methods aim to modify the surface chemistry and morphology of PC, introducing polar reactive groups, increasing surface free energy, and forming covalent or hydrogen bonds with SiO_2. Silane coupling agents, commonly employ organosilicon compounds and consist of silicon-based chemicals with a minimum of two distinct reactive groups. This characteristic allows them to form covalent bonds with both inorganic and organic substances **[9]**. The adhesion enhancement afforded to polycarbonate with SiO_2 deposition, along with sandwiched layers of chemically functionalized silane coupling agents, remain undocumented in existing literature.

This study investigates the solution-based chemical functionalization of PC for enhanced adhesion of SiO_2 material, utilizing intermediate silane coupling agents such as GPTMS and APTMS. The study also includes the application of epoxy-based adhesives, trien on GPTMS, and BADGE on APTMS, to improve the adhesion of PC-SiO_2 material and possibly enhance the stability of the silane chemistry. The function of these agents as molecular bridges is instrumental in enabling the formation of covalent bonds between the components, thus ensuring effective adhesion between PC-SiO_2 materials. We further investigated the bonding mechanism, transparency and initial biocompatibility and stability of these coatings. The biocompatibility of APTMS and GPTMS, focusing on

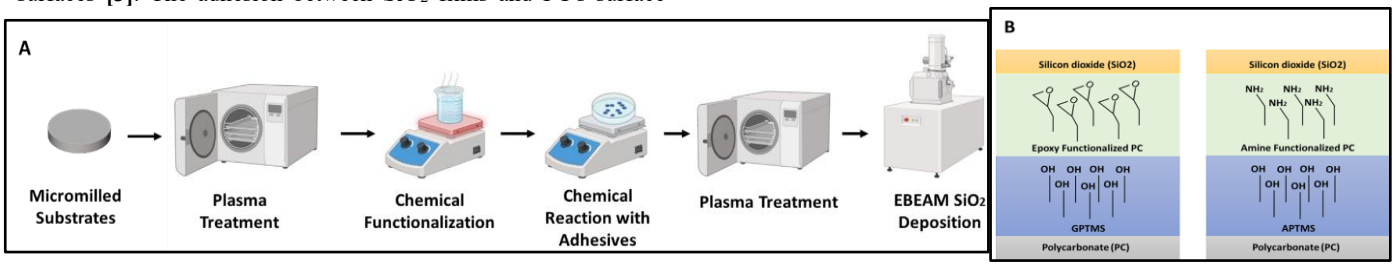

Figure 1: *(A) Schematic representation of the overall treatment process flow shows coating process starts with micromilling test chips, followed by plasma treatment. Samples are then immersed in solutions for varying durations, washed thoroughly, and treated with surface modifiers. After a final plasma treatment, they're stored under vacuum until SiO_2 deposition. (B) The schematic illustrates a sandwich layer formation between the PC substrate and SiO_2 top layer.*

978-1-940470-05-4/HH2024/$25©2024TRF
DOI 10.31438/TRF.HH2024.70

the hydrolysis-condensation kinetics of silane coupling agents and their potential for grafting onto OH-rich solid substrates such as cellulose **[10]**, resulting in a stable interface for cellular adhesion has been studied with some *in vitro* cell cultures. GPTMS has shown excellent cell growth compared to APTMS. This study can be further explored for accurate detection and analysis in various biosensing applications, while also facilitating ease in semiconductor fabrication processes. Validating its future potential in 3D MEA systems will be pivotal in harnessing its full capabilities.

MATERIALS AND METHODS

In order to test the adhesion between PC-SiO_2, 10mm² (length by width), test chips were micromilled from bulk sheets of 1.75mm thick PC. For biocompatibility, circular test chips with a diameter of 12 mm were micromilled. As shown in **Fig. 1(A),** after exposure to ambient mixed air plasma with an airflow rate of approximately 20-50 standard cubic centimeters per minute (sccm) for 20 seconds, the plasma treatment (Plasma Etch Inc., USA) introduced hydroxyl (OH) groups **[11]**. Thereafter, the adhesion test chips underwent a 12-hour reaction at 60°C, and biocompatibility samples underwent a 6-hour reaction at 60°C by submerging them into GPTMS and APTMS solutions. For adhesion testing, the samples were submerged into GPTMS and APTMS (in a 90 ml:10 ml of ethanol: coupling agent) solutions, and for biocompatibility, the samples were dipped into GPTMS and APTMS (in a 98.75 ml:1.25 ml volume ratio of ethanol: coupling agent) solutions to functionalize epoxy (C-O-C) and amine (NH_2) groups on the PC surface, respectively (**Fig. 1(B)**). The functionalized samples were thoroughly washed with alcohol and DI water. Adhesives known for their properties such as mechanical strength, durability, and ease of processing were employed atop the silane coupling agent coatings to further enhance the bonding present. For adhesion testing, trientine and BADGE (0.01 mg each) were applied to both PC-GPTMS and PC-APTMS surfaces respectively. Subsequently, the samples were heated for 3 minutes on a hot plate at 100°C followed by solvent and DI water washing. For biocompatibility testing, adhesives were not employed due to their respective limitations. BADGE, an epoxy resin compound containing bisphenol A, poses health concerns due to its potential endocrine-disrupting effects, potentially compromising cell viability and function by leaching into the culture medium **[12]**. Similarly, Trientine, primarily a copper-chelating agent used in treating Wilson's disease, may exert cytotoxic effects on cells, thus interfering with experimental outcomes in cell-based assays **[13]**. BADGE, trientine, GPTMS, and APTMS were procured from Sigma-Aldrich. Once the solution-based processing was completed for all the samples, a layer of 400 nm of SiO_2 was deposited using an e-beam deposition system (Thermionics PVL-438, USA). The samples were stored under vacuum conditions, and their chemical composition and interaction with the PC surface were characterized using X-ray photoelectron spectroscopy (Escalab 250 Xi XPS). Scanning Electron Microscopy (Zeiss ULTRA-55 FEG SEM) was performed for surface topographical analysis. Ultraviolet-Visible spectroscopy (PVL-466) assessed the transparency of the silane coupling agents and epoxy coatings applied onto PC substrates. Scotch tape peel tests were conducted ten times on samples to test adhesion by uniformly pressing the tape onto each sample, followed by consistent force during peeling. NIH3T3 cells were passaged onto a 48-well plate at 15,000 cells per well and imaged over 4 days *in vitro* **[14]**.

RESULTS AND DISCUSSION

SEM images of solution-based chemistry coatings with SiO_2 on PC substrates is depicted in **Fig. 2(A)** shows morphology of SiO_2 deposited on PC-GPTMS with plenty of protruding islands

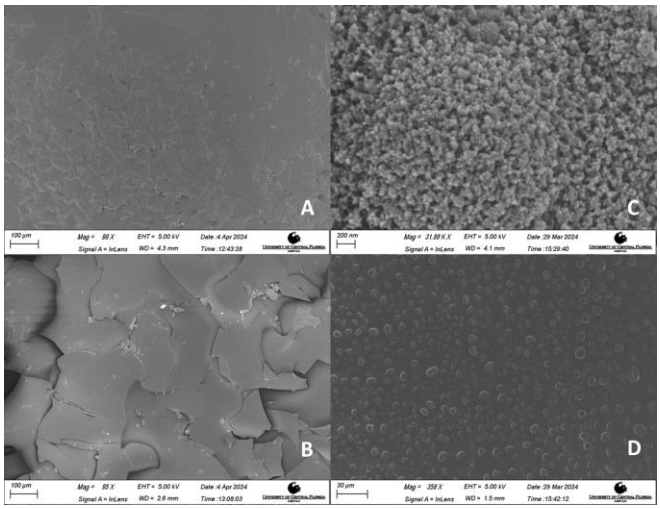

Figure 2*: SEM images showcasing the deposition of SiO_2 on various substrates: (A) PC–GPTMS, (B) PC-GPTMS-trien epoxy, (C) PC-APTMS, and (D) PC-APTMS-BADGE.*

distributed nonuniformly all over the surface. This image exhibits a comparable morphology to the Polyethylene and Polyacrylamide polymer films adhesion to SiO_2 **[15, 16]** demonstrating good adhesion between SiO_2 and polycarbonate. **Fig. 2(B)** showcases SiO_2 deposited onto PC-GPTMS-trien and appears to exhibit a fractured, brittle topography, in contrast to SiO_2 deposited on PC-GPTMS in **Fig. 2(A)**. **Fig. 2(C)** provides the details of SiO_2 deposited on PC-APTMS morphology with irregular grain structure with nanoscale topography aligning with findings in other reported papers in literature **[17]**. **Fig. 2(D)** surface texture explains the aqueous morphology of SiO_2 deposited on PC-APTMS- BADGE. These figures together suggest potential interactions between the inorganic-organic polymer matrix and the silicon dioxide film.

XPS analysis was utilized to demonstrate changes in the chemical composition of the functionalized PC surface, revealing altered binding energies subsequent to surface modification through peak fitting analysis of the XPS profiles. The plots illustrated in **Figs. 3(A-D)** depicts the C1s XPS profile of the functionalized SiO_2-PC-silane coupling agents substrates before and after chemical functionalization with adhesive components (trien and BADGE). In **Fig. 3(A)**, SiO_2-PC-GPTMS treated with trien, the binding energy of the ***C–C bond shifted from 285.1 eV, as observed in the SiO_2 deposited onto PC-GPTMS, to 285.5 eV for the C–C or C–N bond***. Similarly, the C–O bond exhibited a peak shift from 286.2 eV to 286.7 eV, indicating a change in morphology post-treatment with trien. Furthermore, a decrease in the peak area of the C–O bond, corresponding to the epoxy groups, was noted, reducing from 10.44% to 5.08%. These variations are attributed to the reaction between the epoxy rings on the PC surface and the amine groups of trien **[19]**. In **Fig. 3(B)**, SiO_2-PC-APTMS treated with BADGE resin, a chemical shift in the C1s XPS profile was observed, ***transitioning from the C–C or C–N bond at 285.5 eV to the C–C bond at 285.1 eV, accompanied by an increase in the peak area of the C–O bond at 286.2 eV***. These observed variations are attributed to the chemical cross-linking between the epoxy groups of BADGE and the amine groups present on the PC surface **[18][19]**.

XPS surface spectra depicted binding between the inorganic compound (SiO_2) and organosilicon coupling agents, as shown in **Fig. 3(C)**. For ***SiO_2-trien-PC-GPTMS, carbon (C1s) content is evidenced at 284.5 eV, oxygen (O1s) content at 531 eV, and silicon (Si 2p) in SiO_2 at 103.3 eV***. **Fig. 3(D)** illustrates ***SiO_2-BADGE-PC-***

APTMS, showing amine (N1s) at 398.1 eV alongside carbon (C1s) content at 284.5 eV, oxygen (O1s) content at 531 eV, and silicon (Si 2p) in SiO_2 at 103.3 eV.

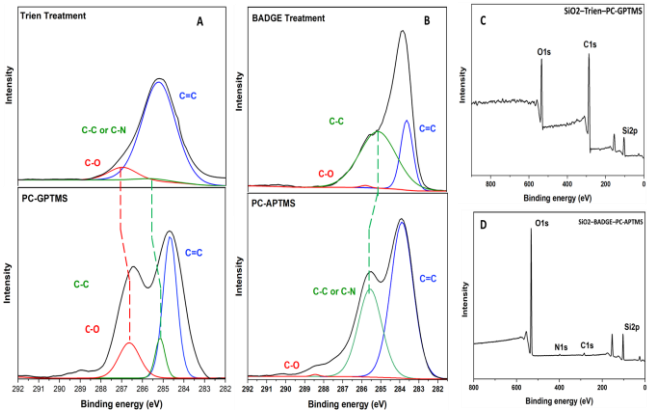

***Figure 3**: The XPS profiles depict the functionalized SiO_2-deposited PC substrates post-treatment with silane coupling agents and adhesive components. (A): PC-GPTMS and PC-GPTMS treated with trien. (B): PC-APTMS and PC-APTMS treated with BADGE resin. Comparative spectra of the respective functional PCs are included for reference.*

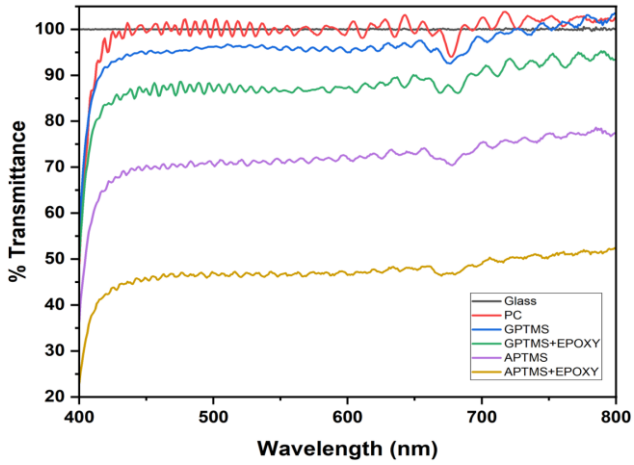

***Figure 4**: UV-Vis spectrum of micromilled PC substrate with treatments within the 400-700 nm visible range.*

Fig. 4 shows results from Ultraviolet-Visible spectroscopy analyzed with different micromilled substrate treatments. In the visual range between 400-700 nm, plain PC exhibited ~100% optical clarity. ***SiO_2-deposited GPTMS showed ~95% optical transmittance, while SiO_2-deposited PC-GPTMS treated with trien exhibited ~85% optical clarity*** measured using transmittance through the treatment surface. The spectra indicate a decrease in optical clarity for SiO_2-PC-APTMS treated with BADGE. Scotch tape peeling test presented in **Fig. 5** reveals preliminary adhesion results with samples deposited with SiO_2 for different solution-based treatments showing delamination upon using scotch tape onto the sample material. The ***PC-GPTMS showed good adhesion to SiO_2 while, PC-APTMS coating showed delamination after 10 peels of scotch tape***. The samples were compared to control SiO_2-polydopamine substrates used as the standard for our current 3D MEAs **[2]**. On the other hand, the PC-GPTMS and PC-APTMS treated with trien and BADGE epoxy group respectively, showed poor adhesion compared to other investigated samples. However, we are actively investigating various methodologies to address inherent challenges, particularly issues related to the deposition of SiO_2 coatings and its adhesion with polycarbonate.

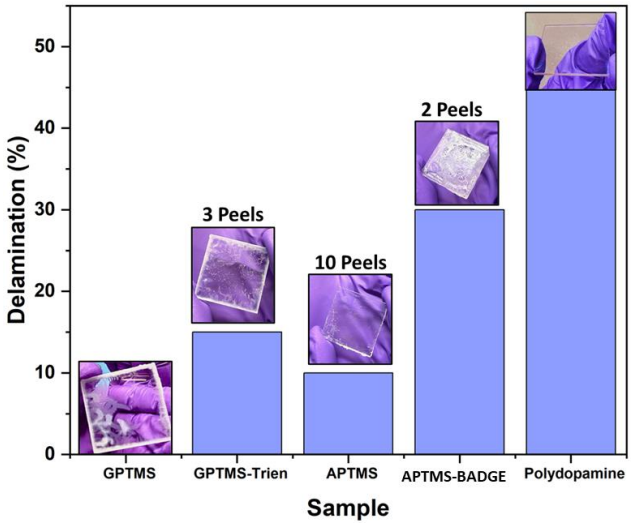

***Figure 5**: Initial scotch tape delamination test of SiO_2-deposited samples with varied solution treatments.*

A biocompatibility assay was conducted over 4 days *in vitro* to ascertain various coatings and their hospitability to cells. Live cell imaging was conducted on PC-GPTMS-SiO_2 and PC-APTMS-SiO_2 substrates along with two controls (native surface of 48 well plate and polycarbonate substrate coated in polydopamine and SiO_2). As can be observed in **Fig. 6**, ***the controls and GPTMS (6 hours) was able to host healthy NIH3T3 cells over entirety of the assay***. It appears that APTMS substrates did not support healthy cells, likely due to the increased concentration of ammonia groups and possible diffusion into the cell culture over the period of the 4 days but this phenomenon is under investigation as other researchers have shown APTMS to be a compatible coating for biocompatibility **[20]**.

CONCLUSIONS

This work demonstrates results of an investigation into the surface adhesion between PC-SiO_2 surface by introducing sandwich layer of silane coupling agent GPTMS and APTMS. Poor adhesion was observed when adhesives trien and BADGE were applied onto PC-GPTMS and PC-APTMS (SiO_2 was deposited as top layer). GPTMS shows good adhesion to SiO_2 and sufficient transmittance for inverted microscopy as well. The SEM morphology shows interesting morphologies with APTMS coated surfaces showcasing required nanoscale topographies for Microphysiological System integration atop these surfaces eventually on 3D MEAs coated with these materials. We went further and performed biocompatibility test by introducing NIH3T3 cells on 6 hours process of PC-GPTMS-SiO_2 and PC-APTMS-SiO_2. GPTMS showed excellent biocompatibility with the APTMS coating showcasing some undesired effects.

ACKNOWLEDGMENTS

The authors would like to thank the GSA at UCF and Hilton Head MEMS 2024 workshop for travel funding. Further financial support for the work comes from an NIH UH3TR003150 grant.

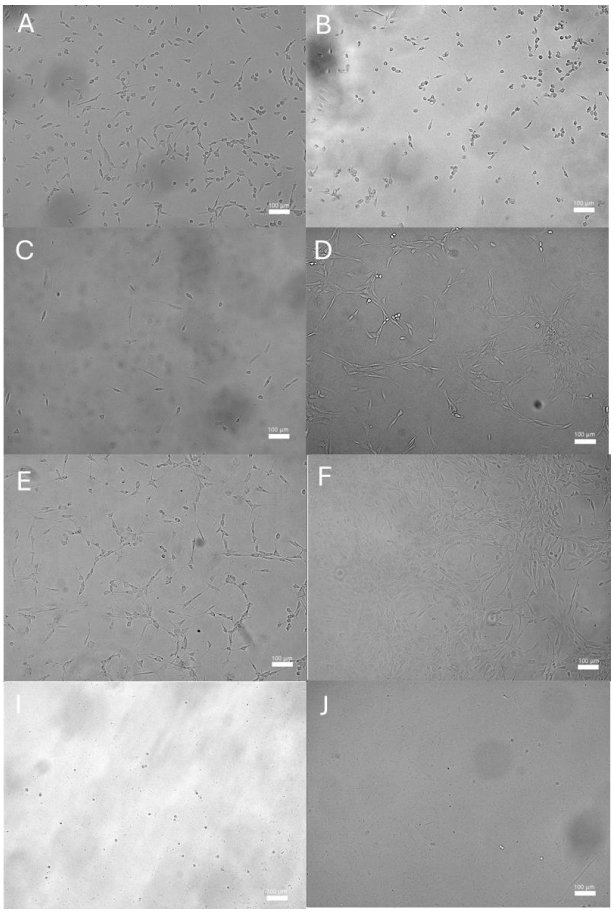

***Figure 6**: Four-day biocompatibility assay with NIH3T3. (A-B) Cells grown on native well surface (control). (A) Day 1 (D1). (B) Day 4 (D4). (C-D) Cells on a polycarbonate substrate coated in polydopamine and SiO_2. (C) Day1 (D) Day 4. (E-F) GTPMS 6 hours. (E) Day 1. (F) Day 4. (I-J) ATMPS 6 hours. (I) Day 1. (J) Day 4 (Scalebar 100µm in all images).*

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CONTACT

Swaminathan Rajaraman, Ph.D.; email: swaminathan.rajaraman@ucf.edu; tel: +1-407-823-4339;