Characterization of Biocompatible Parylene-C Coatings for BIOMEMS Applications

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Summary

Biological Micro-Electro-Mechanical Systems (BioMEMS) is one of the forefronts of research for adapting MEMS to meet demands for biological applications. The immense popularity of BioMEMS lies in the promise of rapid, accurate diagnostic results while requiring an exponentially lower sample volume which are achieved by replicating laboratory functionalities onto packaged miniaturized devices. These devices are usually fabricated using more than one material not necessarily bio-compatible which requires additional processing steps before they can be used for biological applications. A favorable solution is depositing a biocompatible coating. Further, improvements in our everyday life can already be felt from existing BioMEMS devices ranging from home pregnancy test [1] or lower volume blood analysis cartridge system [2], to prototype devices that can preemptively check for signs of osteoporosis [3] within minutes. However, other applications such as bio-device for testing blood glucose level demands quicker results in seconds with high accuracy for saving patients with diabetes from suffering a coma. The ever growing demands for more functionalities and/or higher efficiency and throughput can be met by high aspect ratio microstructures (HARMS) [4] and surface modification/ functionalization. Higher height-to-width aspect ratio allows greater number of structures to be fabricated per area which provides tighter packaging of devices and further reducing the sizes of BioMEMS devices [5, 6, 7, 8, 9]. Furthermore, HARMS increase the surface area of devices over common aspect ratio structures (2-5) which allows for higher throughput. In addition, modification of surface energy and roughness of HARMS can functionalize the device by tailoring the aforementioned parameters to promote and/ or prevent cells/proteins adhesion.

The power of miniaturization and the inclusion of HARMS as highlighted by the given examples are beneficial for BioMEMS; however, the fabrication of these devices using the state-of-the-art microfabrication technology usually involves more than one material [4,10]. In order to not add any extra parameters, bio-researchers ideally yearn for devices of a single material surface that will have minimum interactions with the sample and its chemicals. An optimal solution is to use a biocompatible, highly conformable, and pinhole-free coating uniformly deposit on HARMS and therefore allowing samples and/or chemicals to interact with only the coating surface. Parylene, traditionally known in MEMS for possessing barrier property, is such a coating material which possesses all the above properties as well as being chemically inert, lightweight, transparent, of high dielectric strength and many other properties that are favorable for various BioMEMS applications [6,17].

In the present study Parylene film will be deposited on different HARMS substrates typically for BioMEMS applications and will be characterized in regard to conformability, pinhole free property, surface modification, and biocompatibility with respect to biological cells.

Deposition of Parylene

Parylene uses a room temperature chemical vapor deposition (CVD) technique to deposit uniform films onto a wide range of substrates. Unlike other deposition methods (e.g. dipping, spraying, or spin-on), CVD technique avoid an intermediate liquid stage thus producing uniform, smooth, stress-free coating that is able to penetrate crevices of any-shaped devices [11]. The Parylene deposition process begins with solid dimers vaporizing (100-175°C, 1 torr) into a gaseous state (Figure 1A), pyrolyzes (~680°C, 0.5 torr) into monomers (Figure 1B), and finally polymerizes (<40°C, 0.1 torr) onto sample surfaces (Figure 1C) .

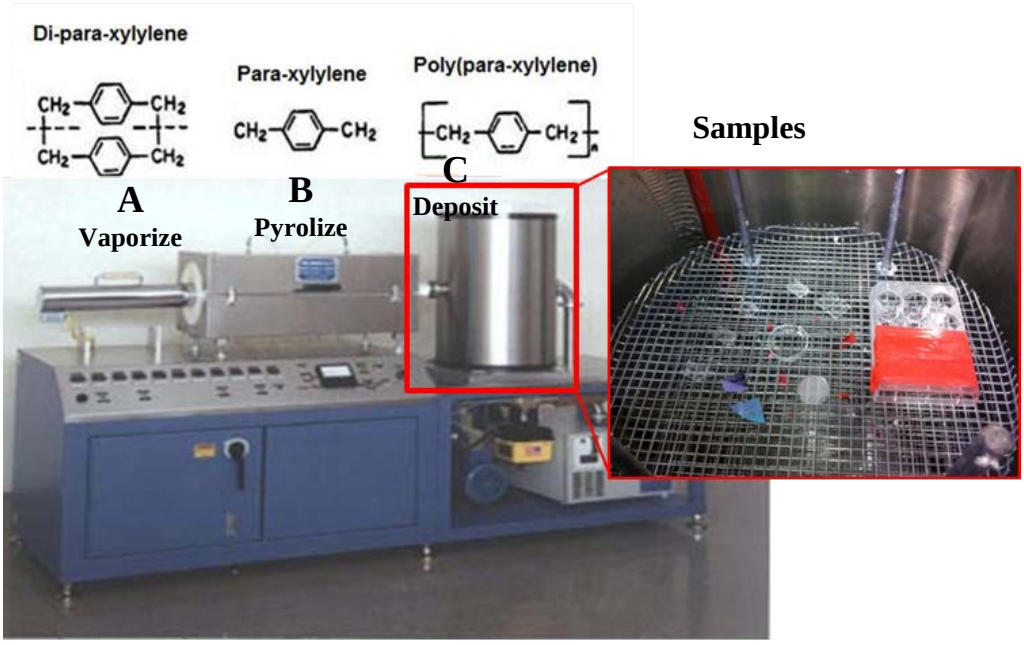

Figure 1: Deposition process system mainly composes of 3 stages: 1) vaporization, 2) pyrolysis, and 3) deposition. A) In the vaporization chamber, solid dimers are heated (~100-175° C, 1 mm Hg) until they are vaporized into gases. B) The extreme temperature (~680° C, .5 mm Hg) in the pyrolysis chamber cleaves the gaseous dimers into monomers. C) Finally, the monomers deposit on substrates and polymerize at room temperature (25° C, .1 mm Hg) in the deposition chamber. [11]

Materials and Sample Preparations

The substrate materials used in this research included flat, non-structured surfaces as well as microfabricated HARMS composed of polymethylmethacrylate (PMMA), polycarbonate (PC), and nickel (Figure 2). Samples were cleaned inside the cleanroom prior to Parylene deposition. Cross-sections for scanning electron microscope (SEM) analyses were prepared by incasing parylene-coated samples using a resin and hardener (Buehler® Expoxicure™ Resin No. 20-8130, Buehler® Expoxicure™ Hardener No. 20-8132). After allowing the resin and hardener to dry, the preserved samples were manually polished (Hyprez® Lapping Systems) successively in 1µm slurry using 400 grit, 600 grit, 800 grit, and finally cloth papers. Cross sections were also obtained by dicing the samples using a dicing wheel cooled with cooling solution without casting in resin.

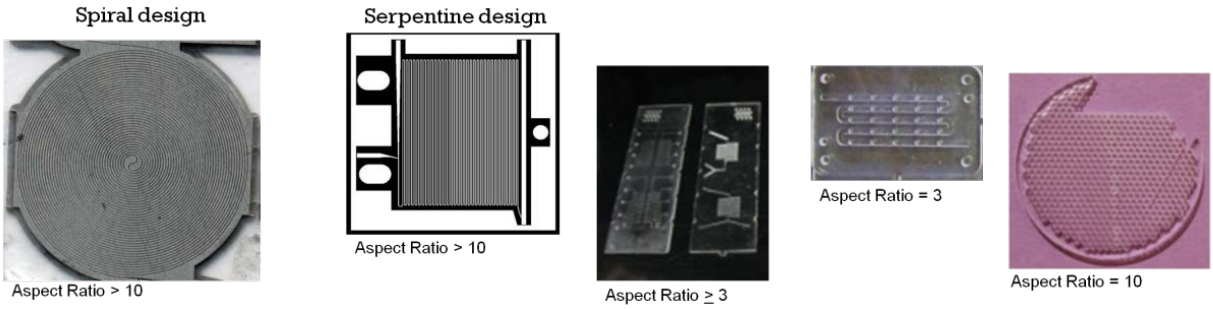

Figure 2: Samples with high aspect ratio microstructures fabricated and characterized in this study.

Preparations for Live Cell Study

Biological characterization of Parylene as deposited as well as surface modified was performed using HeLa cells. Biological interactions are examined using fluorescent spectroscopy and cell health indicator assay to evaluate cellular adhesion which can be used to analyze cytotoxicity, cell growth, and spreading and proliferation. HeLa epithelial cells were cultured in Dulbecoo's Modification of Eagle Medium (DMEM) in addition with 3% fetal bovine serum and maintained in an incubator at 37°C with 5% CO_2 atmosphere. Live cells are cultured on sterilized samples with area of 4 cm^2 (Figure 3), which were kept in 35mm petri dishes and incubated for 18 hours.

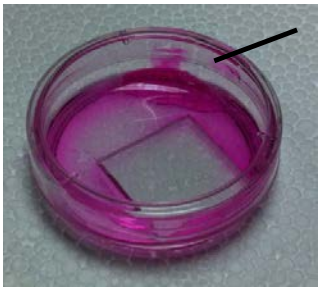

Figure 3:

Cell adhesion setup. HeLa cells are cultured on substrates (PMMA, PC, parylene) are housed within a 35mm polystyrene dish and incubated for 18 hours

Cell health and proliferation analysis was performed using a cell health indicator assay (alamarBlue®, Invitrogen™). AlamarBlue® assay (Figure 4) contains a non-fluorescent indicator dye, resazurin, which is converted to bright red–fluorescent resorufin via the reduction reactions of metabolically active cells, and the amount of fluorescence produced is proportional to the number of living cells.

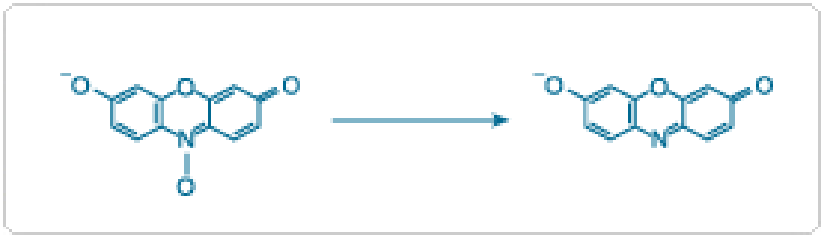

Figure 4: Reduction of resazurin, a non-fluorescent compound in alamarBlue®, to bright-fed fluorescent compound resorufin by metabolically active cells. [12]

Conformable, Pinhole-Free Coating

The conformability of Parylene-C coating was studied by examining the cross sections of parylene-deposited microstructures with aspect ratios (AR) ranging from 1:1 to 13:1. One micron thick Parylene was deposited on these samples, and they were cross-sectioned by mounting as

described above in *Materials and Sample Preparations* section. SEM micrographs in Figure 5 show the cross sections of Ni HARMS (depth 500µm and width 45µm) where 1µm Parylene coating deposited on the HARMS is seen. The red boxes in Figure 5A show that the Parylene coating is conformably following the rough contour of the microchannels, which is clearly seen in the close-up images, along the top (Figure 5B), the bottom (Figure 5C), and the sidewalls (Figures 5B and 5C) of the nickel HARMS. Also images in Figures 5B and 5C clearly prove that Parylene coating has uniform thickness of ~1µm all along the surfaces of the Ni channels. The conformability and uniformity achieved in Parylene coating is as expected [11, 13, 14] due to its unique deposition process already discussed. The results show that at 37mTorr vacuum Parylene monomers uniformly penetrate structures with extreme topology and are homogeneously adsorb over the surface of the HARMS channels. The monomers simultaneously polymerize on substrate surfaces and the thin film continues growing uniformly one molecule at a time. Parylene deposition is unlike other processes such as liquid or spray-on techniques that are more line-of-sights and cause bridging and pooling on microstructures [**Error! Bookmark not defined.**]. Similar results were observed in all the other channels that are spaced 150µm apart.

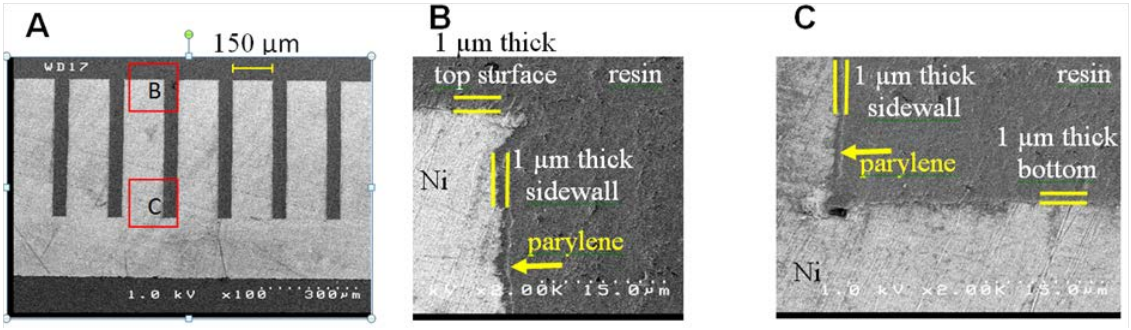

Figure 5: SEM images of cross-sectioned HARMS samples used to analyze quality of Parylene deposition. A) Overview of a Ni HARM structure with channels 500um/45um depth/width and 150µm spacing prepared by mounting and manual polishing. B) 2,000X magnification of the top of the Ni HARM showcasing uniform deposition of ~1um Parylene along top surface and sidewall. C) 2,000X magnification of the bottom of the Ni HARM shows the same uniform thickness of ~1um along the bottom surface and sidewall.

In addition to conformable deposition, pinhole properties are also examined. For this a Parylene film of ~1µm thickness was deposited onto a PMMA substrate and the SEM micrographs shown in Figure 6 virtually prove that the films are pinhole-free. High magnification SEM images of the coated surface (Figure 6A) and 4x zoom-in view [inset] show no pinholes down to 100nm diameter. Furthermore, very high resolution images (Figure 6B and 4x zoom-in [inset]), from mySEM® (NovelX), a low voltage SEM that is able to image 3D surface morphology due to its quad detector, demonstrate that parylene-coated surfaces are free of pinholes down to sub-10nm. The lack of pinholes can be explained with the single molecule chain growth of Parylene during vapor deposition and the present work demonstrates that his mechanism is compatible with PMMA, PC, and Ni HARMS substrates. Although pinholes were not observed in any of the Parylene coated samples, various sub-micron sized (~250-500nm) spots are present on the surface (Figure 6B), too. The size range, sparse distribution, and non-circular geometry of the spots indicate that they likely originate from defects on the substrate surface for example naturally occurring from imperfections, process defects, and dust particles or residues that attached to the sample surface during transportation from the cleanroom to the deposition chamber and/or inside the chamber. The non-circular defect shape (Figure 6B inset) suggests that it is not a pinhole as pinholes are expected to be of well-defined circular shape.

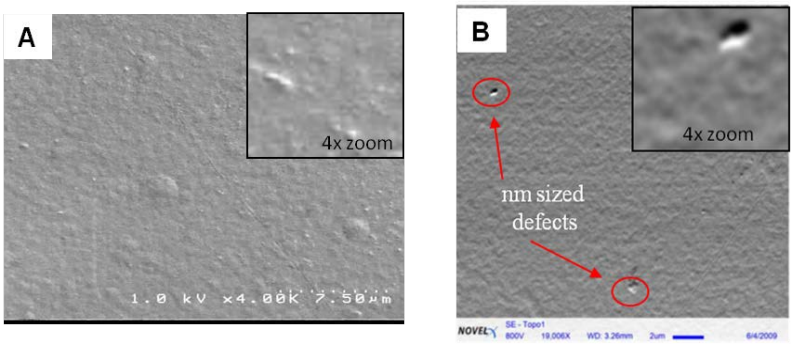

Figure 6: A) SEM imaging shows no evidence of sub-micron sized pinholes. B) Higher resolution imaging by a low voltage, quad-detectors mySEM® (with capability to show 3-D features) confirms no pinholes were detected on parylene films of ~1 µm thick.

Furthermore, pinhole-free coating is demonstrated by depositing 3µm thick Parylene coating to protect only the left half of a PMMA mesh (Figure 7A) HARMS with AR=10 patterned by x-ray lithography with walls between hexagons measuring 1500 µm tall and 150 µm wide. Acetone is a known organic solvent that aggressively dissolves PMMA and many other polymers [15]; therefore, the whole mesh is soaked in an acetone bath for 2.5 hours, and the result is documented with a camera. As can be seen in Figure 7B, the right unprotected half of PMMA mesh walls was completely dissolved. However, the other half of the mesh protected by Parylene was intact and no visual defects or damages including pinholes caused by acetone attack of the PMMA were observed (Figure 7B). Higher resolution SEM imaging, conducted for closer inspection of the interface of parylene-degraded PMMA surfaces (Figure 7C), discloses PMMA sidewalls with wavy, rough features indicating an acetone attack of the uncoated areas of the mesh. In the same image, no surface damages are observed on the chemically inert Parylene coating that is protecting the underlying PMMA.

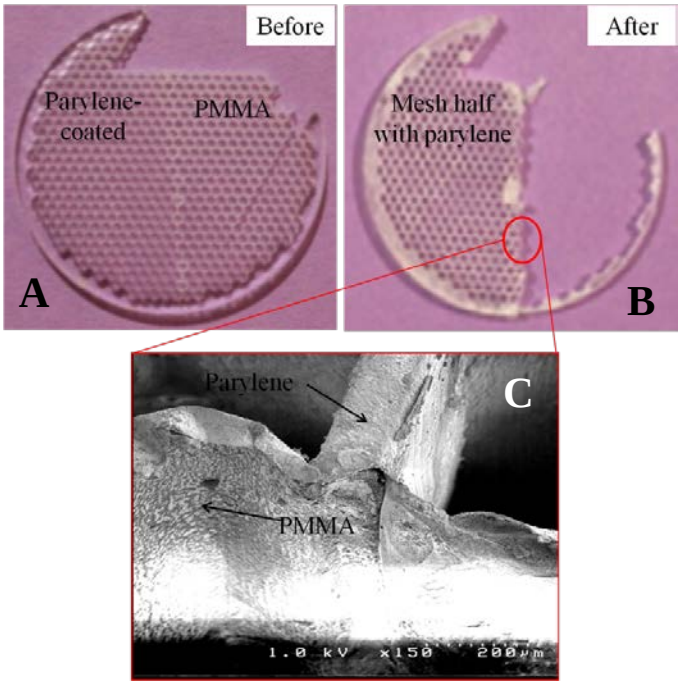

Figure 7:

A) PMMA mesh was coated with 3 µm thick Parylene and was soaked in acetone for 2.5 hours.

B) The non-coated areas were completely dissolved by acetone.

C) SEM image showing no damage to Parylene and the coated PMMA

Cell Adhesion and Quantification

Cell adhesion and quantification are used to study biological interactions on Parylene surfaces. AlamarBlue®, as detailed in section *Preparations for Live Cell Study*, is a cell health indicator assay that measures cellular activities on an entire surface. Since cell metabolism is only active after cells has adhered to a surface and begin spreading, alamarBlue® can measure these activities and indirectly be used to quantify cell adhesion. The graph in Figure 8 details the number of cells adhering to surfaces after 18 hours of incubation time. Pristine Parylene has the lowest number of cell adhering to the surface with 7744 $\pm$ 322 cells out of initially 20,000 cells. The number of adherent cells significantly ($p>0.05$, t-test) increases to 18510 $\pm$ 1576 cells which are greater than a two-fold increase after surface modification with oxygen plasma.

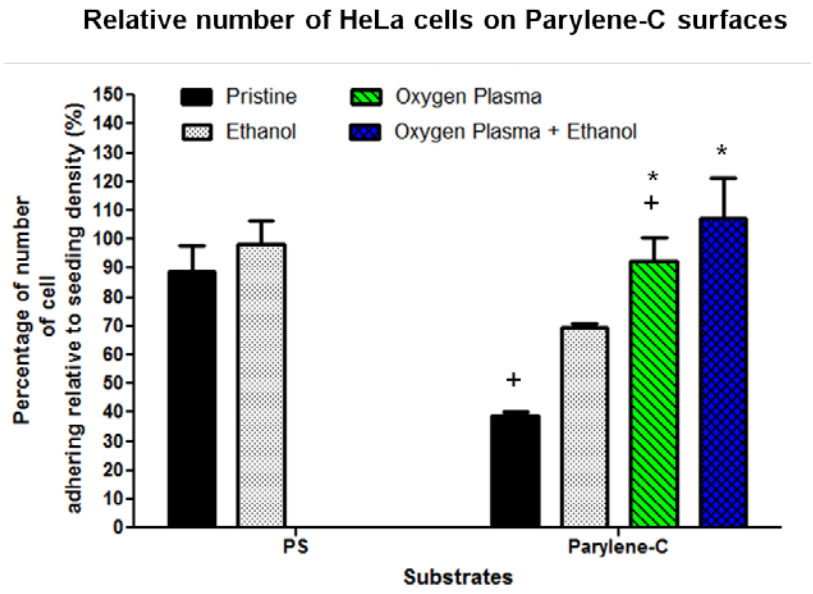

Figure 8:

Percentage of the number of cells adhering to pristine and modified parylene surfaces. The (+) denotes significant difference ($p<0.05$) between pristine and oxygen plasma treated parylene-C surface (t-test). The (*) denotes no significant difference ($P>0.05$) between oxygen plasma and oxygen plasma plus 75% ethanol treated parylene-C surfaces (t-test).

An even larger number of cells, 21485 $\pm$ 2731, were seen on O_2 plasma plus 75% ethanol treated samples but the results were not significant ($p>0.05$, t-test). Cell number for O_2 plasma plus ethanol modified surfaces exceed the initial seeding of 20,000 cells because there is at least a 10-15% error when counting with a haemocytometer. The cell adhesion results are similar to previous study [14, 16] and those observed by Chang *et al* (2007) [5]. The authors' results show at least 70-folds increase in cell adhesion for AML-12 hepatocyte and a 2-folds increase for NIH-3T3 fibroblast between pristine and oxygen plasma modified Parylene surfaces. This study's results and the findings reported by Chang *et al* (2007) [5] demonstrate pristine Parylene surface significantly limit cell adhesion. Additionally, oxygen plasma significantly promotes cell adhesion with magnitude dependent on cell line and surface modification processes and equipments. The result demonstrates Parylene as a suitable, biocompatible coating for better control of cell adhesion within BioMEMS devices.

Conclusion

In this study, CVD deposited Parylene-C coatings were successfully applied to conformably cover microfluidic structures with aspect ratios larger than 10 and thickness down to ~1µm with virtually no pinholes smaller than 100nm. Cell adhesion studies showed that pristine Parylene-C yielded the least cell adhesion and is non-cytotoxic. This is advantageous in particular for micro-devices used in cell applications as pristine Parylene will reduce the chances of cell agglomeration and clogging within microfluidic channels. On the other hand, surface

modification using O_2 plasma promotes HeLa cell adhesion by at least 2-fold suggesting local activation for example in reaction chambers to increase the capturing of cells. In conclusion, Parylene-C biocompatible coatings can homogeneously cover high aspect ratio BioMEMS structures. There deposition after completion of the regular MEMS fabrication requires no compromising for the actual processes and ensures outstanding process compatibility.

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