

Fabrication of Nanocone Array using Soft Lithography Technique for Antimicrobial Application

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Article Info

Volume 83

Page Number: 1198 - 1204

Publication Issue:

May - June 2020

Abstract:

The topography structures on cicada and dragonfly wings exhibit bactericidal properties on the surface upon contact with microbes. Inspired by the naturally-occurring surface micro- and nanostructures, several researchers have investigated the fabrication of these micro- and nanoscale structures in recent years. In this study, an electron beam lithography was employed to produce the desired pattern using photoresist/silicon substrate as a master mold. The pattern was subsequently replicated on PDMS prepolymer using soft lithography technique, while FESEM and AFM were utilised for imaging purposes. Using this lithography technique, nanocones with a size dimension of 200 to 300 nm in base diameter and 150 nm in height for a $400 \times 400 \mu\text{m}^2$ write-field size were successfully fabricated in approximately 2 hours.

Keywords: Electron beam lithography, nanocone, PDMS, Soft lithography

Article History

Article Received: 11 August 2019

Revised: 18 November 2019

Accepted: 23 January 2020

Publication: 10 May 2020

I. INTRODUCTION

Certain naturally-occurring features in organisms exhibit unique functions on their surface such as self-cleaning mechanisms, anti-biofouling, and superhydrophobic coatings as well as bactericidal and anti-microbial properties [1-5]. For instance, cicada and dragonfly wings were previously reported to repel several pathogenic bacterial strains such as *B. subtilis*, *E. coli*, and *P. aeruginosa* [6-8]. Several studies have also revealed the bactericidal properties of cicada wings, in which it was shown to rupture the cell morphology of the bacteria upon attachment to its surface. Topography study of nanostructures on surface of cicada wings *Psaltoda claripennis* species and bacterial activity on structure have been done by Ivanova *et. al.*, (2012). The surface of these wings consists of an array of spherically-capped, conical nanoscale pillars with near-hexagonal symmetry. Each nanoscale pillar is approximately 200 nm in height and 100 nm in base dimension (diameter), peaked with a spherical cap of approximately 60 nm (diameter) and a pitch spacing of 170 nm (from

center to center). The unique functions of these naturally-occurring surfaces have led to several studies aimed at fabricating artificial surfaces with micro- and nanoscale structures for various biological applications [9-12]. Additionally, a review of the proposed nano-architectures for antimicrobial surfaces based on these biological features has been reported by Elbourne *et. al.*, (2017).

There are two approaches in fabricating micro and nanostructures for antimicrobial surface, either by chemical approach which includes surface polymerisation, functionalisation and derivatisation or physical approach also known as surface structure modification. To date, lithography is one of the most promising techniques for fabricating micro- and nanoscale structures for various applications [14]. Numerous studies have been reported in mimicking micro and nanostructures of antimicrobial surface using available lithography technologies [15-18]. However, this proposed technique required specific equipment operating at certain temperature and pressure process condition. Electron beam lithography (EBL) is a versatile technique that can

produce various patterns with resolutions down to a few nanometers in size which commonly used for producing the master mold. The EBL tool consists of a scanning electron microscope (SEM) and a pattern generator system with the addition of a beam blanker to control the exposure area and mechanical stage which moves to the desired position for large substrates. Additionally, an electron-beam (e-beam) is focused on an electron-sensitive resist film and changes its solubility properties, depending on the tone of the resist films [19]. Recently, EBL was applied for patterning the surfaces in biomedical applications such as the flow detector, microfluidic, and biosensor devices [20].

While, soft lithography is a quick, easy, direct process and low-cost technique which replicates micro and nanostructures from the fabricated molds. The fabricated master mold can be utilized multiple times, thus reducing the time and cost involved in the fabrication process. For instance, soft lithography can control the molecular structure of surfaces and pattern complex molecules that are biologically relevant, fabricate channel structures that are appropriate for microfluidics, and manipulate cells, as indicated in a review by Whitesides *et. al.*, (2001). Besides, the techniques in soft lithography have been used to pattern surfaces via stamping, while the molding and embossing processes have been used for fabricating microchannels structures.

Polydimethylsiloxane (PDMS) is the most versatile and commonly used molding material for nano-imprinting and soft lithography due to its various properties such as flexibility which allows a conformal contact with non-planar surfaces, high UV transparency, low surface energy, permeability to gases, biocompatibility, non-toxic nature and ease of handling. The use of PDMS has been largely driven by the development of various soft lithography techniques which include micro-contact printing, replica molding, micro-transfer molding, micro-molding for devices such as capillaries and drainage tubing, biomaterial in catheters and membrane oxygenators as well as solvent-assisted micro-molding. In addition, PDMS is commonly used in a wide variety of biological applications, in which the properties of PDMS for biomedical micro- and nanosystems were previously investigated [22]. Besides, PDMS can also be manipulated and

conformed to submicron features for the development of microstructures, thus making it a suitable platform for miniaturised biological studies [23, 24].

Hence, this study aims to fabricate an array of dot structures on silicon mold and subsequently, replicate these microstructures on PDMS micro/nanocones using the soft lithography technique. This combination lithography technique is capable in producing these structures with minimal cost, ease operation, and applicable to the biomedical application.

II. RESEARCH METHODOLOGY

The process flow for the fabrication of PDMS micro/nanopattern is shown in Fig. 1. A p-type silicon wafer with an approximate size of 1 cm² with an orientation of <100> was cleaned using acetone, methanol, and isopropanol solutions, respectively, in a sonicator for 10 min. Approximately 3.0 wt.% poly(methyl methacrylate) (PMMA) with a molecular weight of 996K (Sigma Aldrich) in chlorobenzene (Sigma Aldrich) was spun-coated on a silicon substrate at spinning speed of 4000 rpm for 60 s. The PMMA thin film was pre-baked at a temperature of 140 °C for 30 min in an oven. The process was followed by pattern writing using a scanning electron microscope (JOEL JSM 6460-LV) system integrated with an EBL Raith ELPHY Quantum system.

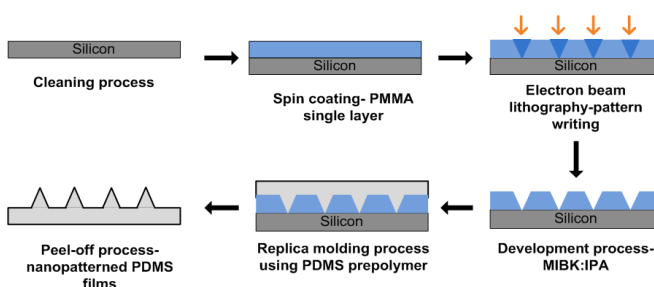


Fig. 1 Schematic illustration of the PDMS replication process flow

A dot array pattern (400 x 400 dots) with 500 nm spacing was designed using the GDSII editor software (ELPHY Quantum). For the EBL exposure setup, a write-field size of 200μm² with a magnification of 300X and a working distance of 10 mm were optimised. An e-beam was tuned until a donut-shaped pattern was observed as in Fig. 2 (a).

The e-beam dosage (pAs) as illustrates in Fig. 2 (b) for dots is given by the product of beam current and dwell time where I_{beam} is the beam current and t_{dwell} is the dwell time, refer to (1). It is defined as the number of the incoming electrons onto the surface sample during the exposure process. Table 1 lists the EBL exposure settings for the dotted-array pattern on a single PMMA layer coated on a silicon substrate (master mold). A maximum e-beam accelerating voltage of 30 keV was applied to minimise the undesired proximity effect during exposure. The exposed pattern was then developed in methyl isobutyl ketone (MIBK): isopropyl alcohol (IPA) with a ratio of 1:3 at a temperature of 23 °C for 40 s, and subsequently dipped into an IPA stopper solution to halt the developing process. In the final step of the process, the mold was rinsed with DI water and blow-dried with nitrogen gas.

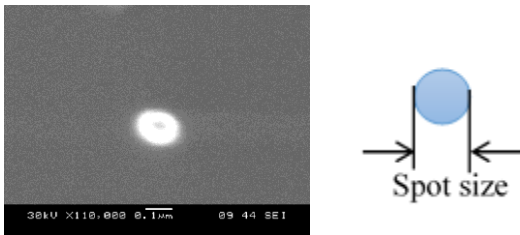


Fig. 2 A single e-beam exposure on PMMA resist/Si substrate and (b) Concept of dose for dots

$$\text{Dot dose (DD)} = I_{beam} \times t_{dwell} \quad (1)$$

Table 1 EBL setting for parameters defining the dot array

EBL parameters	Setting
Accelerating voltage	30 keV
Beam current	50 pA
Aperture spot size	40 μm
Dot dwell time	2.0 ms
Dot dose	0.1 pAs

The PDMS replication process was performed on a PMMA resist/Si master mold using the soft lithography technique previously described. The PDMS solution was prepared by mixing PDMS elastomer Sylgard 184 (Dow Corning) and a curing agent at a ratio of 10:1. The solution was stirred for 5 minutes prior to degassing in a vacuum chamber to remove the trapped air bubbles. The remaining PDMS solution was then poured onto the sample

until the PDMS film was approximately 2 mm thick and degassed again before undergoing the curing process in the oven at a temperature of 50 °C for 3 hours. The cured PDMS film was then carefully peeled off from the PMMA/Si mold.

The surface morphology characterisation of the patterns on the PMMA resist and PDMS replicates was performed by ultra-high resolution field emission scanning electron microscopy (FESEM) and atomic force microscopy (AFM). The imaging of the nanostructure was performed using FESEM (FEI Nova NanoSEM 450) at 2-5 keV under a specific magnification. The substrates were coated with a thin platinum-based film using the Quorum Q150R thin-film coater prior to viewing under the microscope. The surface topography was viewed using AFM (Dimension Edge, Bruker) in a tapping mode perpendicular to the axis of the cantilever at a frequency of 1 Hz and analysed with a nanoscope analysis software. Topography imaging scans were performed using antimony (n) doped silicon probes (MPP-11100-10; Veeco/Bruker) with a spring constant of 20-80 N/m, tips with a radius of curvature of 8 nm, and a resonance frequency between 311 to 361 kHz.

III. RESULT AND DISCUSSION

In this study, EBL exposure was utilized to fabricate a dot array pattern on PMMA/Si master mold and subsequently, replicated the pattern using a replica molding process known as soft lithography. This technique is easily performed without undergoing a pattern transfer to the silicon substrate using either the lift-off or etching process. The PMMA resist which was exposed to e-beam underwent a chain scission degradation process, in which the smaller, soluble fragments were subsequently removed by the MIBK: IPA developer solution with a ratio of 1:3. The optimization dosage of exposure process have been performed to produce a high-resolution pattern with the resist thickness fully developed and desired width. The optimized exposure dosage depends on many parameters such as beam energy, acceleration voltage, aperture size, resist material, substrate material, baking temperature, resist thickness, developer time and temperature. The EBL exposure times for patterning a 200 x 200 microns square write-field is shown in Table 2. The process took approximately 34 min and

24.80 s to complete one exposure cycle containing 160 000 dots.

Table 2 The calculation of EBL exposure time

Dwell time	5 min 17.20 s
Settling time	13 min 13.00 s
Stage time	0.00 s
Calculation time	3.00 s
Transmission time	15 min 51.60 s
Total time	34 min 24.80 s

Fig. 3 shows the FESEM image of the master mold which consists of an array of dots arranged hexagonally. Dot sizes were shown to range between 300 to 400 nm in diameter with a pitch distance of 500 nm. Additionally, it was observed that the dot sizes became larger due to the unstable beam and influence of electron scattering during the exposure process. During the beam exposure, the electrons experience a small-angle forward scattering upon bombardment onto the resist, thereby widening the primary beam size. Subsequently, a large-angle backscattering occurs after the electron-beam (e-beam) passes through the resist and penetrates the substrate. For instance, the diameter of a single e-beam exposed at a higher magnification of 100 KX and above was approximately 100 nm (refer Fig. 2). Thus, it is difficult to produce a pattern that is smaller than 100 nm in size. In addition, undesirable proximity effects which occur also broadens the effective area of exposure in the resist, thereby affecting the target feature size. It has been previously reported that the high-energy electron beams were thought to minimise the effect during exposure, in which the forward-scattering was reduced and the back-scattering area became more in-depth and broader [25].

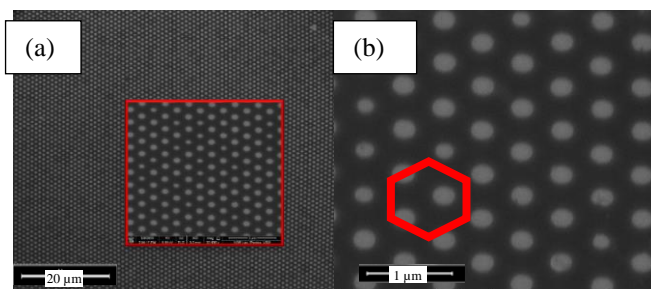


Fig. 3 FESEM image of a dot array on the master mold at magnification (a) 5KX and (b) 50KX

Fig. 4 shows the AFM images of the surface morphology and cross-sectional profiles of the dot array on the master mold. The uniform and hexagonal nano-dot array surface observed in Fig. 4 (a) was achieved using the EBL technique based on the proposed settings for the exposure parameters. The depths of penetration were measured at approximately 80 nm with the Nanoscope Analysis software as shown in Fig. 4 (c). Additionally, it was observed that the short developing times (< 40 s) led to unexposed patterning with undefined shapes, while longer developing times (> 40 s) created an uncontrolled increase of the width. Besides, the e-beam dose factor was also shown to influence the depth of penetration on PMMA to produce well-defined features consistent with a previous study by Veroli *et. al.*, (2016). Hence, further optimisation studies on the e-beam dosage, current, and dot dwell time should be performed to fabricate the desired pattern with specific size dimensions. The size of the exposed patterns varies with acceleration voltage, aperture size, resist material, substrate material, baking temperature, and resist thickness.

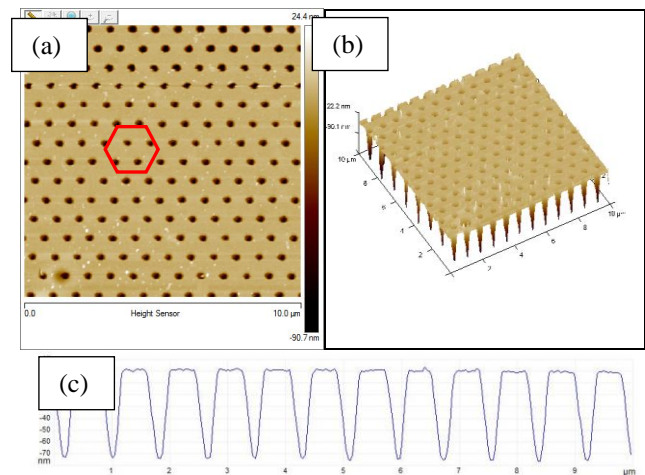


Fig. 4 Surface morphology of the dot array master mold is illustrated for the top view (a), 3D image (b) and cross-sectional profile (c)

For the replica molding process, PDMS elastomer material was selected as it was easy to fabricate, inert to cells and compatible with various biological studies [27, 28]. Fig. 5 illustrates the AFM topography image and FESEM imaging for micro/nanocone array with a feature size of approximately 150 nm in height, 200 nm in width and a pitch spacing of 500 nm. Based on the morphological comparison with the master mold, it

was evident that the fine features of the hexagonally-shaped dotted arrays of the micro/nanocone were successfully replicated from the master mold using a low curing temperature about 50 °C. It has been previously shown that lower curing temperatures and longer curing times were identified as favorable conditions for the replication of micro- and nanostructures using PDMS [29, 30].

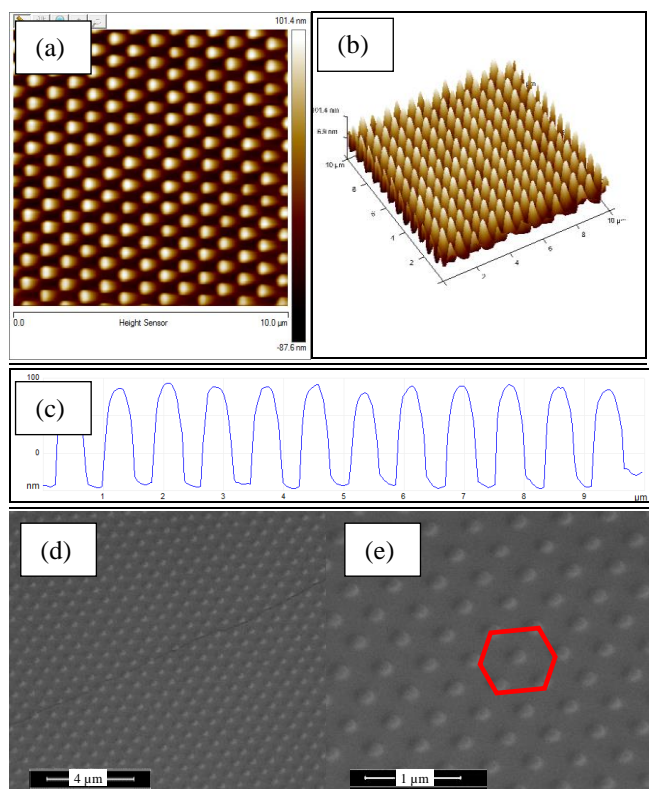


Fig. 5 Image of cone array (a) AFM topography image (10 μm) (b) AFM angle view (c) AFM cross sectional view, (d) FESEM image at magnification 20KX and (e) FESEM image at magnification 50KX

It is believed that physical surface topography modification by nanostructures creation has the potential to be employed as a bactericidal surface on biomedical devices and applications to reduce or prevent bacterial infection. The bactericidal efficiency of nanostructures against several bacteria strains has been previously studied using various shapes, sizes, densities, aspect ratios, and surface wettability of nanostructures [16, 18, 31, 32]. Nevertheless, it is necessary to further expand this study by investigating the bacterial interaction on these surfaces using a wide range of microbes. Besides, the features of the micro- and nanoscale structures such as the shape, spacing, base diameter, tip width, height, aspect ratio, and angle require

further investigations to gain an understanding of the microbe-surface interactions.

IV. CONCLUSION

This study focused on the micro- and nanolithography fabrication processes involved in creating an anti-microbial surface for biological applications. We have demonstrated the use of high-resolution EBL and easy processing soft lithography technique, in which nanocones with size dimensions of 200-300 nm in base diameter, 150 nm in height and a pitch spacing of 0.5 μm were successfully fabricated. These structures and arrays have huge potential to be employed as anti-microbial surface.

ACKNOWLEDGMENT

The authors would like to thank the staff and technicians of the Nano-Optoelectronics Research & Technology laboratory (NORLab), School of Physics, Universiti Sains Malaysia (USM) for providing the facilities for the fabrication and characterisation of nanostructures. We are also grateful to the Malaysian Ministry of Higher Education and School of Mechanical Engineering, USM for the scholarship funding provided. This work is also funded by the USM Bridging Grant (Ref no: 304.PMEKANIK.6316201).

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