RAPID COST-EFFECTIVE PLOTTER CUT STENCIL MASKS FOR THE DEPOSITION OF ORGANIC AND INORGANIC MATERIALS AND THE CREATION OF A NEW ANTIBIOTIC DIFFUSION TEST FOR MINIMUM LETHAL CONCENTRATION DETECTION

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ABSTRACT

The use of plotter cutters in stencil mask prototyping is underutilized but has several advantages over traditional MEMS techniques. In this paper we investigate the use of a conventional plotter cutter as a highly effective benchtop tool for rapid prototyping of stencil masks in the ***sub-100µm range*** and characterize patterned layers of different organic/inorganic materials. Furthermore, a ***diagnostic monitoring application in healthcare and a potential replacement of Standard Kirby-Bauer (KB) Diffusion Antibiotic Resistance Tests*** **[1, 2]** allowing for three times the amount of Antibiotics to be tested within the same nutrient agar petridish are demonstrated.

KEYWORDS

Stencil Mask, Antibiotic Testing, Kirby Bauer, Plotter Cutter, Minimal Inhibitory Concentration

INTRODUCTION

Traditionally MEMS stencils have been largely fabricated using laser micromachining, CNC milling, DRIE, and other photolithographic approaches due to their allowances for great precision and ease of workflow integration. However, with lengthier processing times, high cost and accessibility concerns, these technologies have become much less appealing **[3,4]**. Technologies such as plotter cutters and electronic cutters **[5]** with extreme process optimization could replace older stencil masking technologies. These tools have shown to be more effective in microfluidic and biosensor applications in recent years **[6,7]**. Usage of these stencil masks can be extrapolated to a myriad of applications such as electrode and microenvironment patterning for the manipulation and selective localization of bacterial and eukaryotic cells. Such localization is imperative to the study of cellular behavior such as morphology, response to pharmaceutical drugs in critical disease states such as cancer **[8,9]**. The capabilities demonstrated in this paper showcase how the Silhouette Cameo 4 tool provides precision with a multitude of swift cuts leading to the rapid prototyping of material layers for accurate diagnosis, analysis treatment or acute action. We additionally utilized the stencil masks for varied deposition of organic and inorganic materials such as Cr, Au, SiO_2, gelatin and bacterial patterning and testing using *Escherichia coli*.

MATERIALS AND METHODS

Kapton® Films (75µm thickness) were cut using *Silhouette Cameo 4* plotter in dimensions of 2mm down to 250µm utilizing "Single Line Cut", "Hammer Head" and "Serpentine" designs along with a 2mm design additionally cut for Interdigitated Electrodes fabrication (IDE). Kapton® masks were attached to glass slides, and Cr/Au (50/25 nm) was deposited using E-beam deposition (**Fig. 1**). Separately 10 wt% gelatin was cast through the masks on the glass slides. For bacterial patterning experiments, Kapton® stencils were placed on the surfaces of nutrient agar plates. Cultures of *E. Coli* K-12 bacteria were inoculated within the patterns of the masks, placed

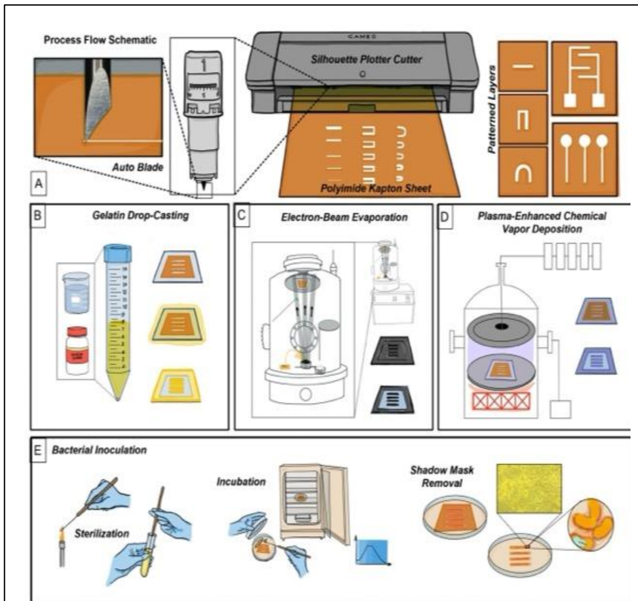

Fig. 1. *Process flow schematic: A) Silhouette Plotter Cutter along with fabricated designs that were evaluated in this study (right). B) Schematic of the casting process for 10 wt % gelatin. C) E-beam evaporation. D) Plasma Enhance Chemical Vapor Deposition (PECVD). E) Culturing of E. Coli K-12.*

in an incubator for 48 hours at 37ºC and removed. Antibiotics Streptomycin Sulfate (5-20µg), Oxytetracycline Hydrochloride (15-60µg) and Kanamycin Sulfate (15-60µg) were utilized in the new diffusion test. Impedance measurements, SEM and Keyence Confocal imaging were used to characterize the stenciled patterns.

RESULTS

The key cutting feature of the plotter cutter is shown in **Fig. 2**. The blade has an ellipsoidal curvature, length of 1400.19µm and a tip of ~ 26µm with a flat surface rather than having a radius of curvature creating a micron-scale flat feature when cutting through materials

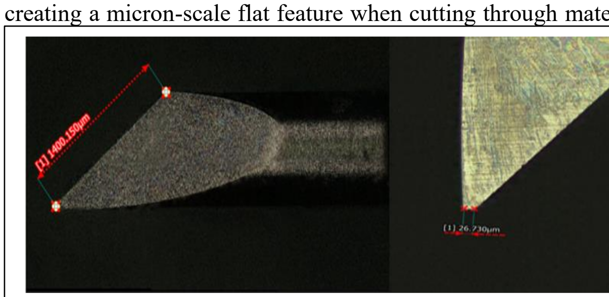

Fig. 2. *Laser confocal scanning images of the AutoBlade from the plotter cutter showcasing ellipsoidal curvature on the left and tip dimensions on the right. The blade characterization helps with assessment of cutting features on specific materials.*

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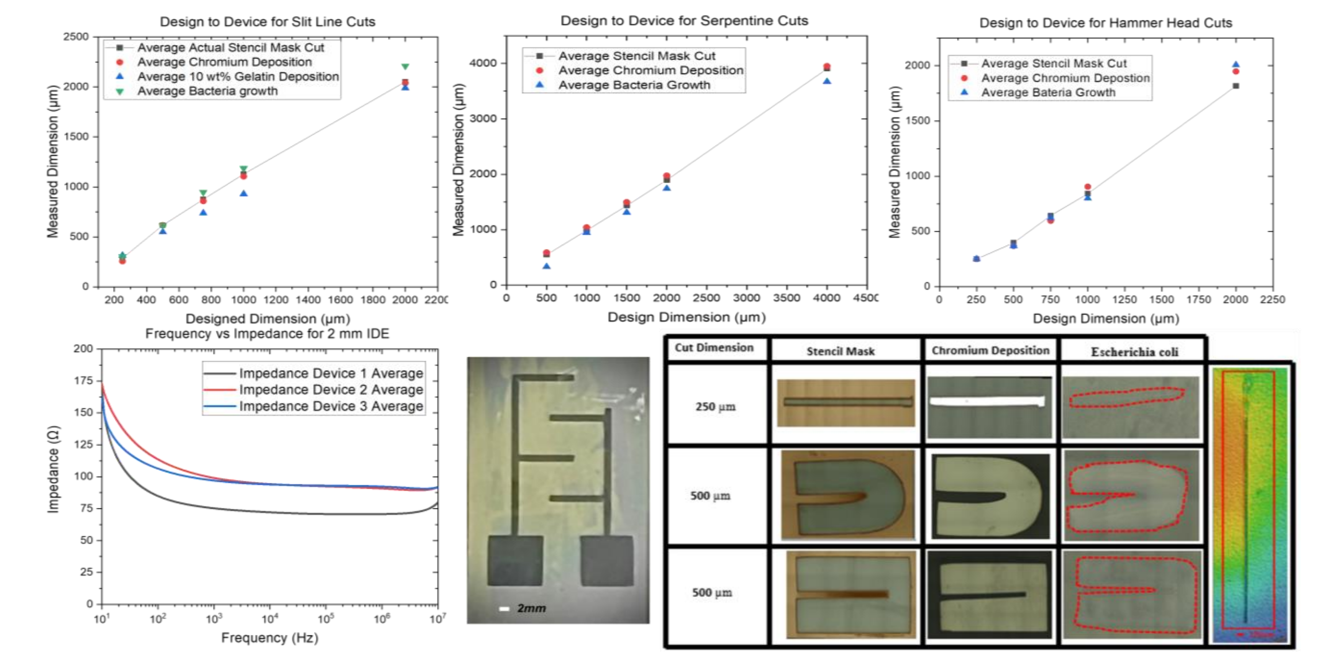

Fig. 3. Design to device images and measurement for all stencil mask designs - Slit Line Cuts, Serpentine Cuts, Hammer Head Cuts (Top), along with the impedance characteristics of the 2mm IDE device (Bottom Left). Image of the Cr/Au deposited IDE and the smallest feature size that was micromachined at a sub-100µm cut (Bottom Right).

analogous to Kapton ®. **Fig. 3** depicts design to device measurement with representative images of patterned features along with the impedance characteristics of the IDE. The design to device followed expected trends of reduced feature sizes producing increased errors with the ***average errors for patterns determined to be 10.46%***. Additionally, an ***optimized sub-100 µm cut*** was demonstrated with the slit line pattern for the first time to our knowledge. The 2mm metal IDE demonstrated ideal microelectrode behavior with an ***1kHz impedance value of 75-100 Ohms*** (**Fig. 3**).

Further all stencil antibiotic tests showed reduced length measurements of zone of inhibition for all concentrations in the three types of antibiotics used. Average ***zone of inhibition length reductions from 69.4 – 87.5%*** were observed (**Fig. 4**) compared to the standard KB method **[1, 2]**. These suggest increased throughput of KB tests to measurement of minimum inhibitory concentration in a localized fashion to potentially lower limits of detection using our novel diffusion test methodology.

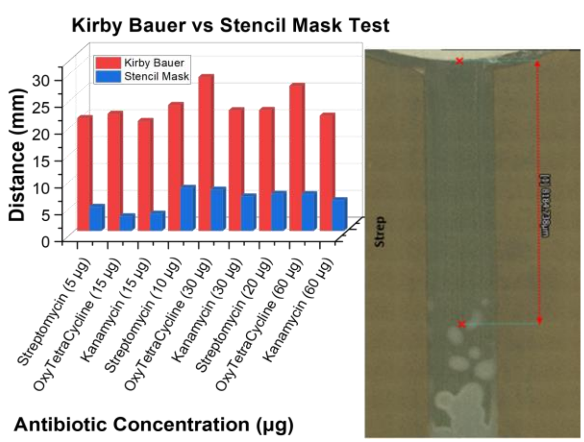

Fig. 4: Standard Kirby Bauer vs Stencil Mask Test. Inhibition measurements for Antibiotics Streptomycin Sulfate test as a representative image.

CONCLUSIONS

Kapton® films (75) µm were best suited for our application as they are thin enough to cut in 1 pass and prevent tearing while cutting. The ***effective parameters*** for cutting through the Kapton® were determined as: ***F=22-26 N; Speed= 1mm/s with fabrication time in seconds***. Design to device measurements showcase an ***average error of 10.46%*** with expected trends with smaller dimensions patterns. Expected IDE impedance performance and a novel diffusion test for minimum lethal concentration detection of bacteria were additionally demonstrated.

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