VARIABLE-HEIGHT MICROFLUIDIC CHANNELS FOR ACCURATE IMMOBILIZATION OF *C. ELEGANS* WORMS BY USING A SINGLE DRY ETCHING STEP

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ABSTRACT

We present an aspect ratio dependent etching (ARDE) method, utilizing the Bosch dry etching process of silicon, to create the optimal silicon master microstructures for replication of polydimethylsiloxane (PDMS) microchannels that allow immobilizing *C. elegans* worms at all sizes during their full life cycle. We take advantage of the etching speed change due to a locally changing etching load, by using different openings on the wafer surface and tune it into our favor to create the ideal three-dimensional microchannel shape with only one microfabrication step.

KEYWORDS: *C. elegans*, Bosch process, Aspect ratio dependent etching (ARDE)

INTRODUCTION

C. elegans is known to be a highly mobile model organism in biomedical research, which requires to be temporarily immobilized for imaging and manipulation. Previous confinement approaches of these nematodes either included single or multiple layers of SU8 fabrication methods, which are quite labor-intensive and inaccurate [1, 2]. To our knowledge, no one has demonstrated a successful way to fine-tune the Bosch process [3] and create variable-height three-dimensional channels or structures benefiting from ARDE.

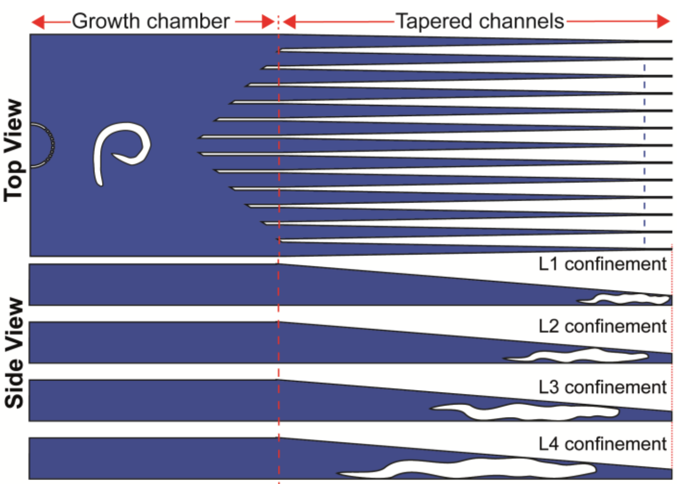

Figure 1: Schematic illustration of the confinement method for all larval stages of C. elegans, including the side and top views. We have previously observed that a single layer SU8 fabrication method prohibited early larval stages (10-15 µm in diameter) unfolded worm immobilization, as the channels had to be designed deep enough also for immobilizing later larval stages (40-60 µm in diameter). Aspect ratio dependent etching (ARDE) enables an ideal three-dimensional confinement of all larval stages so that worms do not entangle during immobilization.

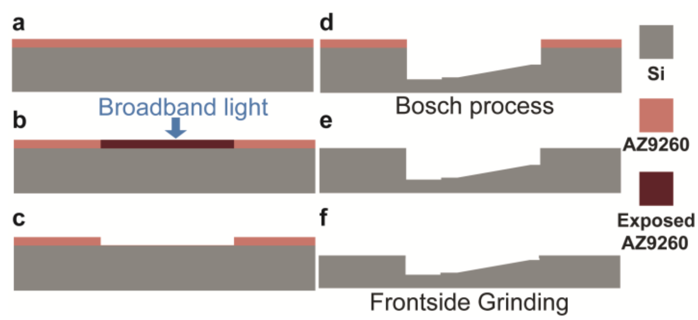

Figure 2: Schematic diagram for microfabrication of variable-height three- dimensional confinement channel master structures in silicon. (a) A Si wafer is spin-coated with positive photoresist. (b) The layout is exposed through a Cr mask. (c) The exposed design is developed. (d) The patterned photoresist and the Si wafer are inserted in a dry etchant tool to initiate the Bosch process and hence, the ARDE. (e) The positive photoresist is stripped to provide surface profiling with Scanning Electron Microscopy (SEM). (f) Once the desired surface profile is reached, the excessive Si depth is removed by front-side grinding of the wafer.

EXPERIMENTAL

We have previously observed that a single layer SU8 fabrication method prohibited early larval stages (10-15 µm in diameter) unfolded worm immobilization, as the channels had to be designed deep enough also for immobilizing later larval stages (40-60 µm in diameter). In this work, we propose a fabrication method to create perfectly tapered channels in all dimensions to introduce a paramount immobilization feature to our microfluidic platform (Figure 1). Our novel fabrication method is easy-to-implement, repeatable and can be varied for other possible surface structuring applications. A positive photoresist layer was spun on a Si wafer, exposed and developed (Figure 2). Hereafter, we utilize the Bosch process, which enables the wider parts of the channels to be etched deeper than the narrower parts. To benefit from ARDE, we had to etch deeper than our target depth. The excessive etching depth was subsequently removed by front-side surface grinding. We have characterized the effect of three different etching times on the etching profile of our channels (Figure 3). The results indicated that we were obliged to remove approximately 100 µm of Si from the front side of our wafers via grinding. The final surface scan demonstrated that we can achieve our desired profile by utilizing the ARDE of the Bosch process. The final version of our wafer includes 80 µm-wide growth chambers that are large enough to let the worms and larvae freely move and tapered channel profiles, starting from 60 µm in height and narrowing down to 10 µm height, to successfully confine nematodes at all larval stages. In order to invert our design, the silicon etching step is followed by PDMS casting to establish the pattern of our Si wafer to PDMS chips.

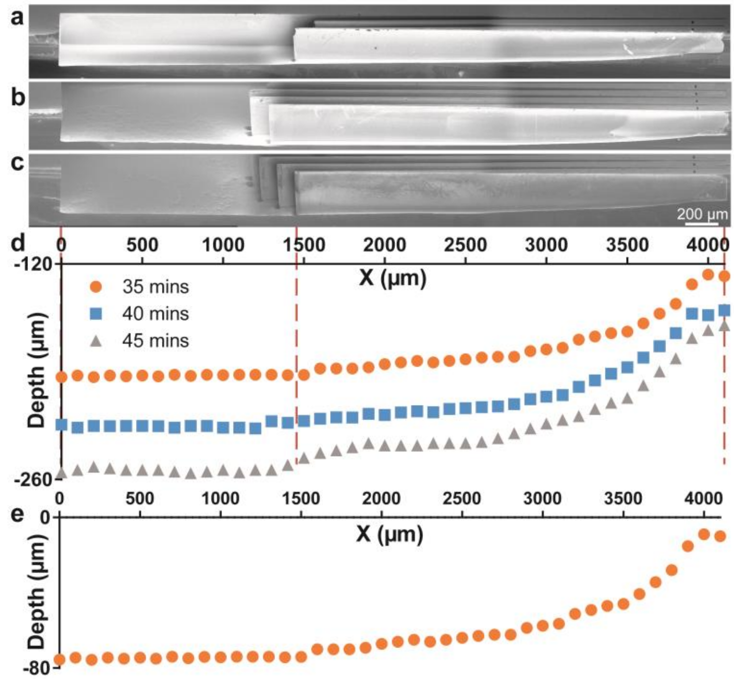

Figure 3: SEM images of the confinement channels and the resultant surface profile of the etched Si wafers. The resultant SEM stitched images obtained after (a) 35 min, (b) 40 min and (c) 45 min etching are shown. (d) The initial depth profile of three different etching times illustrates that the etching depth is deeper than it is required. (e) The excessive Si depth is removed by utilizing front-side grinding and hence the final surface profile is reached.

RESULTS AND DISCUSSION

A common drawback of the Bosch process, ARDE, is demonstrated to be quite convenient for fabrication of three-dimensional structures. By optimizing, passivation and etchant gas dose, etching time and the layout of the design, three-dimensional structures can easily be fabricated regarding the application and the surface profile. We successfully managed to reach our desired surface profile within 35 minutes of Bosch process etching.

CONCLUSION

ARDE with Bosch process facilitates the creation of optimal immobilization channels for *C. elegans* at all larval stages of development.

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