Fabrication of Silicon Micro Tips for Microbial Cell Lysis Applications

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One of the emerging fields of research with rapid and extensive use in medicine, research and industry is the single cell genomics12. Recent technical advances now allow us to use RNA-Seq to profile single mammalian cells. Large population of cells in complex tissues such as the human brain or bone marrow are characterized using the single cell sequeuncing to identify different constituent cell types and their functions. However, these technologies have thus far failed to translate to single microbial cells. Their small cell size (~1 µm), limited RNA quantity and strong cell wall make it very hard to perform lysis of single microbial cells needed to access the genomic contents. Importantly, microbial cell walls are composed of a wide variety of polysaccarydes, lipoproteins, etc. Currently, there are no rapid lysing or the profiling techniques with high throughput that can be applied to wide vaiety of microbes, including fungi, bacteria, etc. We are developing a microfluidic and micro electro mechanical system (MEMS) based platform which can puncture the cell walls or cell lysis for single microbial cells.

Our goal in this paper is to demonstrate a process flow for the fabrication of silicon tips using KOH based wet etching and Cryo or Bosch based dry etching approaches. Fabricating KOH based tips is relatively a simple process because of silicon crystallographic orientation based etch. However, fabricating longer tips with a few or less than a micron pitch is challenging due to the fixed lateral etching. To address this, we demonstrate tighter pitch tips by using the low temperature etch process. A detailed process optimization of the tip fabrication for different pitches by different methods will be discussed.

Briefly, KOH based tips were fabricated on an oxidized silicon wafer with <100> orientation. The wafer was patterned with 4 µm squares, 5 µm pitch and then the oxide is etched by reactive ion etching technique. Further, after the resist removal and cleaning process, silicon is etched using KOH etch to form the silicon systematic pyramids (Fig.1(a)). A single pyramid can be seen in Fig. 1(b). These pyramids were used to crush the 4 µm sized polystyrene beads. The preliminary results of these experiments can be seen in Fig. 1(c) and 1(d). To crush smaller sized beads with tighter pitch another set of samples were prepared using 30 nm chrome mask with 1 µm circle and 2 µm pitch. Using the Bosch etch process the pillars were fabricated as shown in Fig. 2. Another set of chrome patterned samples were etched using the low temperature (-90 ºC) Cryo process to get the sharp tips as shown in Fig.3.

1 Whitesides, G. M., The origins and the future of microfluidics. Nature 2006, 442, 368-373

2 Thompson, A. M.; Paguirigan, A. L.; Kreutz, J. E.; Radich, J. P.; Chiu, D. T., Microfluidics for single-cell genetic analysis. Lab Chip 2014, 14, 3135-3142.

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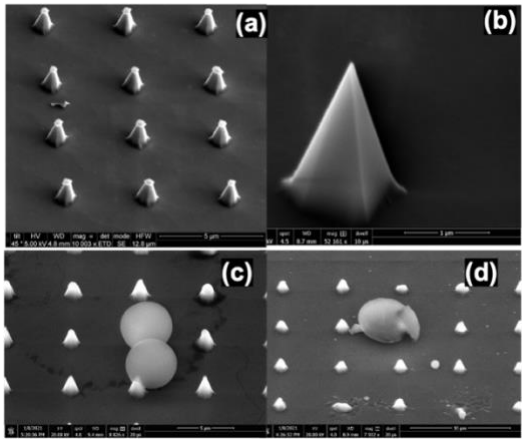

Figure 1. SEM images of the silicon tips etched with KOH. (a) array of tips with 5 µm pitch, (b) single tip, (c) polystyrene bead punctured by the sharp silicon tip, (d) polystyrene bead crushed by the silicon tips.

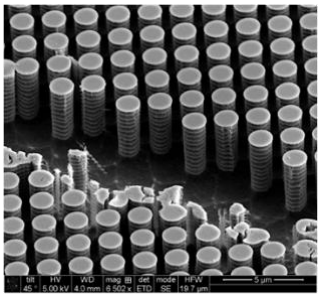

Figure 2: SEM image after Bosch etching 1 µm diameter circles with 2 µm pitch.

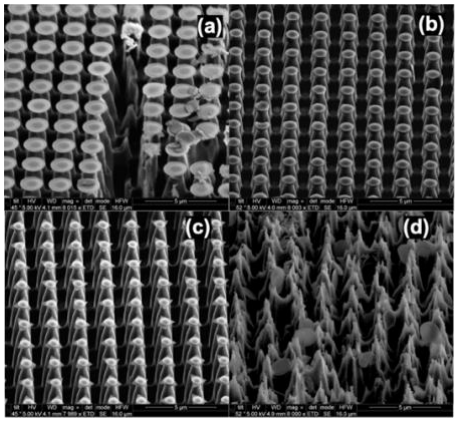

Figure 3: SEM image after Cryo etching 1 µm diameter circles with 2 µm pitch. Results after etching for (a) 5 min, (b) 7.5 min, (c) 10 min, and (d) 15 min.