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Cryogenic Etching of Silicon: An Alternative Method For Fabrication of Vertical Microcantilever Master Molds

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Abstract

This paper examines the use of deep reactive ion etching (DRIE) of silicon with fluorine high-density plasmas at cryogenic temperatures to produce silicon master molds for vertical microcantilever arrays used for controlling substrate stiffness for culturing living cells. The resultant profiles achieved depend on the rate of deposition and etching of a SiO_xF_y polymer, which serves as a passivation layer on the sidewalls of the etched structures in relation to areas that have not been passivated with the polymer. We look at how optimal tuning of two parameters, the O_2 flow rate and the capacitively coupled plasma (CCP) power, determine the etch profile. All other pertinent parameters are kept constant. We examine the etch profiles produced using e-beam resist as the main etch mask, with holes having diameters of 750 nm, 1 μm , and 2 μm .

Index Terms

cryogenic deep reactive ion etching; deep reactive ion etching; PDMS; MEMS; BioMEMS; vertical microcantilever arrays

I. Introduction

The use of plasma etching techniques for fabrication of devices in silicon has gained widespread use. The semiconductor and microelectronics industry has been the driving force

behind this trend. More recently plasma etching has found applications in the microelectromechanical systems (MEMS) and biological microelectromechanical systems (BioMEMS) industry. There are some differences between its use in the integrated circuit (IC) industry and the more recent MEMS and BioMEMS applications. Feature sizes are normally in the submicron region and are actually getting smaller for IC fabrication, while structures for MEMS and BioMEMS are normally a few microns wide. On the other hand, while the etch depths are only a few microns in ICs, etch depths can reach several hundred microns in MEMS and BioMEMS fabrication. Some of the requirements in reactive ion etching for MEMS and BioMEMS include generation of high aspect-ratio structures without sacrificing etch rate unduly, maintaining high selectivity of photoresist or etch mask relative to silicon and precise control of sidewall profile. In some applications a smooth surface (average roughness less than 200 nm) is required, as is the case for molds used for polymer hot embossing processes [1], [2] or for microneedles used in drug delivery [3].

Anisotropic reactive ion etching involves the delicate balance between passivation of the sidewalls and etching of the bottom of the structures. The etch is mainly produced by bombardment of ions from the plasma discharge. Lrmer and Schilp [4] first developed this etch technique at Bosch and hence it is also commonly referred to as the “Bosch process.” This technique involves alternating etch and passivation steps in a continuous cycle to achieve the high aspect-ratio structures. Both steps are done at room temperature. This technique was later co-developed and marketed by Surface Technology Systems plc (UK) and Alcatel Vacuum Technology (France) on their inductively coupled plasma (ICP) tools [5]. Some of the earliest products using this technology were surface micromachined devices such as the accelerometer for airbag deployment [6]. Another notable application is the fabrication of gyroscopes used in cars for stability control [7]. Typical etch rates are about 2 $\mu\text{m}/\text{min}$, though rates as high as 10 $\mu\text{m}/\text{min}$ are achievable today [5].

An alternative technique introduced by Tachi *et al.* [8] involves etching substrates at cryogenic temperatures, also using fluorine-based high-density plasmas. The main chemical reactions that occur in reactive ion etching are those due to spontaneous etching and those due to ion-assisted reactions [8], [9]. The spontaneous reactions which occur on both the sidewalls and the bottom account for the isotropic etch. To produce anisotropic etches the spontaneous reaction has to be slowed considerably. Tachi *et al.* accomplished this by controlling the substrate temperature, the rationale being that cooler temperatures will reduce the reaction probability or the incident flux of radicals on the sidewalls. It must be noted that the original cryogenic technique they described did not include the use of oxygen (O_2) in the plasma though its use had previously been reported by other labs [10].

Other researchers in this area [11]–[13] combined the Tachi group’s original technique with that of Zhang *et al.* [10] and were able to use O_2 in a very sensitive method for control of the anisotropic etch of silicon (Si). In SF_6/O_2 high-density plasma etching, the decomposition of SF_6 into free fluorine (F) radicals is responsible for the isotropic component of the etch cycle by formation of volatile components which include SiF_4 [9], [14], [15]. Other decomposition byproducts include ions such as SF_5^+ [9], [14], which are able to etch SiO_xF_y (ion assisted etching) though this occurs mainly at the bottom of the structures. Combining the two techniques to produce the desired etch profiles requires tuning of various parameters to achieve the right balance between etching of unpassivated silicon, sidewall passivation, and etch of the bottom of the structures. There are surprisingly few articles addressing optimization of sidewall profile in cryogenic etching [9]. A recent study has optimized cryogenic etch process parameters for making high aspect-ratio submicron (300 – 700 nm) trenches. They achieve aspect-ratios $>10:1$. Their optimized process is used for making integrated rib waveguides for photonics applications [16]. However, even when an appropriate set of parameters has been determined, they are not necessarily applicable to all

designs, and the process must be tuned for each specific mask layout and final desired etch profile.

Vertical microcantilever arrays have been used to study cell mechanobiology and traction forces and provide a platform for investigating cellular biomechanics *in vitro* [17]–[22]. One advantage they provide over other traditional methods, for example use of flexible continuous sheets, is that deflections are independent of each other. The original techniques for making these vertical microcantilever arrays have involved using photolithography on silicon wafers followed by single [23] or double casting of polydimethylsiloxane (PDMS) (soft lithography) to yield the final microcantilever arrays. The double casting results in the use of a secondary master mold for casting the final arrays. Another method to produce vertical microcantilever arrays has been reported. This method uses LIGA (German acronym for lithography-electroplating-injection-molding) for making the master molds [24]. However the LIGA technique requires a synchrotron radiation source which is not readily available. In addition, the other methods are more compatible with MEMS and other cleanroom microfabrication processes. However, LIGA technology still offers some advantages which are not surpassed by any other technology. It still produces the highest aspect-ratio structures in metal and has extremely good surface roughness. Hence there are still some applications for which LIGA is the technology of choice. The motivation for pursuing the cryogenic etching approach is to overcome the limitations of the original soft lithography technique which uses SU-8, a thick polymer photoresist for making the master molds for the microcantilever arrays. Though this method has proven to be simple, we have experienced some limitations in fabricating PDMS microcantilever arrays that we and other groups have previously reported [17], [19].

This publication describes a cryogenic etching technique used to create master molds for vertical microcantilever arrays in silicon. We investigate how two main parameters, the oxygen flow rate and the radio frequency (RF) excited capacitively coupled plasma power, affect the profile characteristics. The wafer temperature and other pertinent parameters, *e.g.*, SF₆ flow rate, are kept constant. Other labs [18], [25] have reported the use of the Bosch process for making similar master molds in silicon. However, unless process parameters are carefully optimized, it appears that dry reactive ion etching (DRIE) can produce scalloping on the sidewalls of the silicon master mold, making separation of the PDMS mold very difficult, if not impossible. The scalloping on the sidewalls introduces a surface roughness and scallop depths can range from 50 to 300 nm [26]. This in turn can severely compromise removal of replica-cast PDMS microcantilevers. Second, microcantilevers produced in this manner are not entirely cylindrical and therefore cannot be considered to behave like the simple cantilever beams. Indeed, another group has shown that such microcantilevers appear grossly scalloped under scanning electron microscopy and that their bending mechanics are significantly more complex than simple vertical cantilever beams [27]. We have used the Bosch process at standard laboratory temperature to produce master molds at the University of Michigan Lurie Nanofabrication Facility. Fig. 1 shows the scalloping effect, which occurs primarily because the cycling of the etch and passivation steps does not occur at the same time as compared to the cryogenic etch process. Both techniques require extensive fine-tuning of etch parameters to obtain the desired sidewall profile, and we were unsuccessful at optimization attempts to minimize scalloping. One advantage of the cryogenic etching technique over the Bosch process is that the etch parameters do not have to be as carefully optimized to obtain smooth sidewalls. We report our optimization results for etching cylindrical holes in the micron range with aspect-ratio < 5:1 and with different pitches.

II. Materials and Methods

For our cryogenic etching experiments, the circle patterns for the microcantilever arrays were originally designed using the free shareware computer-aided design (CAD) tool Layouteditor™. The design files were converted into a format compatible with a JEOL JBX-9300 100 kV electron beam lithography system. Silicon wafers were spin coated with ZEP 520A electron beam resist at two spin speeds, 6000 revolutions per minute (rpm) and 2000 rpm, yielding two nominal thicknesses. The wafers were baked on a contact hot plate at 180 °C for two minutes. The wafers were then loaded into a JEOL system and exposed. Designed patterns were arrays of circles with diameters of 750 nm, 1 μm, and 2 μm with edge-to-edge spacings of 1, 2 and 4 μm. Each array was designed to be approximately 2 mm². A typical four-inch wafer had 12 such individual arrays and the e-beam system took an average of eight hours to write 24 individual arrays. The exposed wafers were developed in xylenes for 40 seconds, rinsed in isopropyl alcohol (IPA) and dried under a stream of nitrogen. The fourinch wafers were either cleaved into four quarters or etched as full wafers. An Oxford PlasmaLab 100 DRIE/RIE system capable of Bosch process etching, cryogenic processing, and standard RIE dry etching of silicon, was used for the etch step. The wafers were maintained at -110°C in all cases. The SF₆ flow rate during the etch was maintained at 80 standard cubic centimeters per minute (sccm). The ICP power was maintained at 1250 W and the CCP and O₂ sccm rates were varied. For initial etch runs the wafers were cleaved and examined using a FEI Nova 600 scanning electron microscope/focused ion beam system to determine etch depth and profile. The photoresist thickness before and after the etch was determined using a Filmetrics F50 profilometer and a Horiba Jobin Yvon MM-16 ellipsometer. The e-beam resist was then stripped using acetone and the wafers were descummed in a plasma cleaner.

The silicon master molds were later oxidized in a plasma cleaner for 30 seconds, then silanized with (Tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-trichlorosilane (United Chemical Technologies; Bristol, PA) under partial vacuum overnight. To test if the master molds could be used successfully to make the microcantilever arrays, liquid PDMS (10:1 ratio of monomer to curing agent mixed using the AR-1000 Thinky™ mixer) was poured on the surface of the silanized silicon master mold, degassed in a desiccator using a vacuum pump (Leybold™ Trivac D2.5E capable of achieving a vacuum pressure of 5×10^{-4} mbar), and cured at two different temperatures (65 and 110°C) for 20 hours. The cured elastomer was peeled from the PDMS mold to yield a vertical microcantilever array.

Culture wells in PDMS were made from punching blocks of PDMS with a 4 mm × 4 mm square punch. Prior to seeding, the microcantilevers were pre-treated with normal culture medium overnight. These wells were placed over each individual array and mesenchymal stem cells (C57Bl/6-TgN(ACTbEGFP)1Os) [28] at an original concentration of 10⁵/mL were seeded into these wells at 40 μL per well, giving a final concentration of about 4000 cells/well. Cells were cultured in complete culture medium (CCM) which consisted of 10.0% fetal calf serum (FCS) and 10% horse serum in MEM with L-glutamine and penicillin/streptomycin for 7 days. Osteogenic differentiation media (ODM) was prepared by taking 192 mL (CCM) and adding 10 nM dexamethasone (200 μL of 1:100 dilution of 1 mM stock solution in deionized (DI) water), 20 mM β-glycerol phosphate (8 mL of 0.5 M stock in (CCM), and 50 μM L-ascorbic acid 2-phosphate (50 mM stock solution in DI water)) [29]. Adipogenic differentiation media (ADM) was prepared from 200 mL of CCM with 0.5 μM dexamethasone (100 μL of 1 mM stock in DI water), 0.5 μM isobutylmethylxanthine (20 μL of 5 mM stock in methanol) and 50 μM indomethacin (333 μL of 30 mM stock in methanol) [29]. Control experiments were conducted by incubating cells in CCM on the microcantilevers, on flat pieces of PDMS, and also in normal plastic tissue culture plates. Additionally, cells were also cultured on flat PDMS and in plastic

tissue culture dishes in adipogenic and osteogenic differentiation media. Cells were incubated at 37 °C with 5% CO₂ in the appropriate differentiation or control media, which was changed every three to four days. Cells were harvested 7, 10, 15, and 18 days after the initial 7 days of culture and fixed prior to staining to assess differentiation. All differentiation reagents were purchased from Sigma (St. Louis, MO).

Prior to staining, the incubated cells were rinsed with PBS and then incubated with neutral buffered formalin (NBF) for one hour at room temperature. Cells were then rinsed with either DI water (ODM) or PBS (ADM). Cells were then incubated for 20 minutes at room temperature with Alizarin Red S for detection of osteogenesis or Oil Red O for detection of adipogenesis [29], [30]. Cells were rinsed again with DI water or PBS and visualized by light microscopy.

III. Results and Discussion

The primary concern regarding the etch process was to obtain smooth sidewalls to facilitate mold separation. Fig. 3 shows an SEM image of an initial etch sample. The etch parameters were 14 sccm of O₂ with 30 W of CCP power. The etch time was one minute. As can be seen from this image, the scalloping from the Bosch process that was evident in Fig. 1 does not occur. For the image shown in Fig. 1(b) the average scallop depth was 180 nm measured over a length of 1.5 μ m.

One factor that affects the etch rate in cryogenic etching is the amount of silicon load (etchable area) to be etched on the target wafer. In both cases where either a quarter of or the entire four-inch wafer is etched, this load remained approximately the same for three separate 2 mm² or 12 separate 2 mm² arrays. In both cases the actual load (measured by subtracting the entire wafer area from area that was etched) was approximately 5%. Even though the silicon loads are approximately the same, there was still a noticeable variation in the etch rates between the three different diameters, with the smallest feature size showing the slowest etch rate. SEM images of a cleaved wafer with this effect are shown in Fig. 4. The parameters for this etch are 15 sccm of O₂ with 4 W CCP power and an etch time of two minutes and thirty seconds. The SEM images are taken with a tilt of 30°, resulting in a 50% foreshortening of the images so that the actual depth is twice that shown in the figure (Fig 4). Fig. 4(a) and 4(b) show the profile for two different hole sizes with original design diameters of 750 nm and 1 μ m. As can be seen from the measured depths of 1.68 μ m (3.36 μ m actual) and 2.03 μ m (4.06 μ m actual), respectively, the smaller feature size shows a slightly slower etch rate than the larger one.

The etch mask used in all cases was the ZEP 520A electron beam resist. The desired etch depth for the deepest etches was 8 μ m, and thus a higher selectivity mask, such as chromium, was not required. The nominal thickness for the resist at two spin speeds of 6000 rpm and 2000 rpm was ~220 and ~374 nm, respectively. Fig. 5(a) shows a plot of nominal resist left against etch time for seven different etches. The thickness of the resist was measured using the Filmetrics F50 profilometer. In all cases the etch was done with the following parameters: CCP power of 10 W, and 13 sccm of O₂, for wafers with initial resist thickness of ~220 nm (6000 rpm). Fig. 5(b) shows a plot for the wafers with initial resist thickness of ~374 nm (2000 rpm). The goodness of fit when each measurement was taken is also included in the plots. A linear fit to the data is also plotted in the two figures (Fig. 5(a) and Fig. 5(b)). The slope for the case with a spin speed of 6000 rpm is 1.79. This represents an approximate etch rate of 1.79 nm/sec. The slope for the case with a spin speed of 2000 rpm is 1.94, representing an etch rate of 1.94 nm/sec, in close agreement with that for the spin speed of 6000 rpm. When we used an etch rate determined for these settings to be about 2.3 μ m/min, the selectivity of the silicon relative to the resist was about 20:1.

The next most critical factor after smoothness of the sidewalls was the sidewall profile itself. The target was to minimize either positive or negative taper and to produce almost vertical sidewalls. The start position for test runs was a one-minute etch using 14 sccm of O₂ and 4 W of CCP power. Fig. 6(a) and 6(b) show SEM images of the profiles obtained for these parameters. As can be seen, there was a significant positive taper and an acute narrowing at the end of the etch for the 750 nm. The etch rate for these settings was about 2.6 $\mu\text{m}/\text{min}$.

Increasing the CCP power to 30 W and keeping all the other etch parameters the same (14 sccm of O₂) produced almost vertical sidewalls; however, the etch rate increased for the resist. Hence the selectivity was reduced considerably, and with these settings it would not be possible to reach some of the target etch depths. Fig. 7(a) shows an SEM image of the result for an etch using these etch parameters.

The 30 W CCP power was obviously suitable if our target maximum etch depth was less than about 5 μm . Etching with 4 W of CCP power with 15 sccm of O₂ produced a much better result. There is still a noticeable positive taper, as can be seen in the SEM image in Fig. 7(b). The time for the etch was two minutes. Reducing the O₂ flow rate from 15 sccm to 14 sccm and using 4 W of CCP power with an etch time of 2 min did not produce a significant change, as can be seen in Fig. 7(c). De Boer *et al.* [9] showed in their trend diagrams and tables that increasing the O₂ sccm while keeping the CCP power constant would shift the sidewall profile from negative to positive. We thus reduced the O₂ sccm further to 13 sccm, kept the power at 4 W, and increased the etch time to three minutes. As predicted, the positive taper was reduced, but there was a noticeable taper at the bottom of the etch (Fig. 7(d)).

Our final trial used 13 sccm of O₂ but the CCP power was increased from 4 W to 10 W. The wafer was etched for two minutes and thirty seconds. Fig. 7(e) shows an SEM image of the etch profile that was obtained for this etch. As can be seen, the sidewalls are effectively vertical. The resist (nominal 220 nm for spin speed of 6000 rpm) was totally etched in this case.

Due to the long exposure times required by the e-beam lithographic system, we attempted fabrication using a contact mask aligner with broadband (I-line) wavelength. A vacuum was applied between the mask and the wafer during the exposure. In this mode, it was difficult to produce the 750 nm features reliably and there were slight defects in the 1 μm features. The 2 μm features were much better but were not comparable to those produced by e-beam, as can be seen in Fig. 8(a) and 8(b). The resist used in this case was SPR 955CM. Fig. 9(a) and 9(b) show SEM images of etches produced from samples of these molds. The etch parameters were 13 sccm of O₂ with 10 W of CCP power for three minutes. The slight difference in etch rate due to feature size is again evident here. Table I is a summary of etch process parameters used.

While we expect some variation in etch depth, on the order of 5–10%, across the entire four inch wafer, the overall impact of these variations on the mechanical properties of our microcantilevers will be negligible compared to the systematic variations of cantilever diameter. Therefore, we conducted experiments to estimate the percentage variation in etch depth across a wafer. Based on the average depth of a 2 minute cryo etch, measurements taken in the center of the wafer versus those taken around the perimeter, the variation is approximately 1.4%. The maximum difference observed is 2.2%. The features measured were 40 $\mu\text{m} \times 4 \text{ mm}$ long trenches spaced at 6 mm intervals along their length and 4 mm intervals side to side. The field that was etched and used in experiments was approximately 2 mm² and hence variations in uniformity would be very small and should not impact experiments. These microcantilever arrays, well matched to the mechanical properties of

cells and their environment, will provide biologists with a means to quantify cellular forces by measuring the cantilever deflections or to control cell fate by providing a particular substrate stiffness. The ability to replica cast multiple arrays from a single substrate produced by cryogenic reactive ion etching will allow these arrays to be produced at modest cost and used by individuals who need not be experienced in either electron beam lithography or advanced fabrication.

The PDMS devices that were cast on the silicon master molds were much easier to separate than the ones etched using the Bosch process. We initially assumed that if the molds did not have at least some degree of positive taper, it would not be possible to separate the cured PDMS from the silicon molds even after the salinization process. Initial trials used molds which had taper (etches with parameters of 14 sccm of O₂, 4 W CCP, and etch time of two minutes thirty seconds) and those with the vertical sidewalls (etches with parameters of 13 sccm of O₂, 10 W CCP, and etch time of two minutes, thirty seconds). That assumption proved to be unwarranted. In both cases, the cured PDMS came off relatively easily.

Fig. 10 and Fig. 11 show SEM images of PDMS microcantilevers from the silicon master molds. As can be seen, it was possible to obtain microcantilevers with various dimensions for both the positive sidewall silicon molds and the straight sidewall molds. The microcantilevers do show some defects, which are especially pronounced in the 750 nm devices. The more pronounced defects in the 750 nm example occur because at this dimension the PDMS polymer is unable to replicate the molds precisely. Another explanation is that nanoscopic to microscopic air bubbles could have been trapped in the holes. What was most surprising was the fact that the 750 nm feature size with the positive side taper seemed to show more damage than its straight sidewall counterpart. It is currently not clear why this happened. This cannot be due to surface roughness differences as both types of molds show the same smooth surface after etching (Fig. 4(a), Fig. 7(e)). A more plausible explanation could be differences in contact area or damage caused by the nanoscopic to microscopic air bubbles that are trapped in the molds, which have been shown to cause damage to PDMS molds [31]. A more interesting fact was that the microcantilevers which had the closest pitches ($\sim 2 \mu\text{m}$ center-to-center or $\sim 1 \mu\text{m}$ edge-to-edge) seemed to clump together, just as in the case of the microcantilevers from the Bosch process silicon molds. Thus, though the cryogenic etching had solved the problem of the ease of separation of the mold, it did not entirely solve the damage to the separated PDMS microcantilevers, which had also been observed in the Bosch process case as shown in Fig. 12. This effect may be due to electrostatic charges that build on the surfaces of the PDMS during separation and cause the attractive forces. Another explanation could be the stiction-friction problem that has been reported by others [32], [33].

As mentioned in our Materials and Methods section, we cured the PDMS at two different temperatures. The cure temperature of 65°C had been successfully used in previous trials for the two steps of the soft lithography process [19]. Tan *et al.* [17], however, used two different temperatures for the two different steps, 65°C and 110°C. Our first attempts at molding PDMS from the silicon master were done at 65°C. Subsequent trials using 110°C seemed to solve the problem of the microcantilevers sticking to each other after mold separation. It must be mentioned that the PDMS was separated from the silicon mold immediately after removal from the curing ovens without allowing the PDMS and the mold to cool to room temperature. There was significantly less damage in this case. Fig. 13(a) and 13(b) show SEM images of microcantilevers that are about 1 μm in diameter with 1 μm edge-to-edge spacing and about 5 μm tall. It is still not clear why these procedures were effective. The manufacturer-recommended curing times and temperatures for the PDMS type used for experiments (Sylgard 184, Dow Corning; Midland, MI) are 48 hours at 25°C (room temperature), 45 minutes at 100°C, 20 minutes at 125°C and 10 minutes at 150°C. Different

groups including our own lab have also reported using different curing temperatures within this range for the same mixing ratio (10:1 curing agent to monomer) [17], [19], [34]. A recent study examined the relationship between curing temperature and the physical properties of PDMS samples for this same type and similar mixing ratio [31]. It reports an increase in compression modulus and percentage shrinkage with an increase in cure temperature and a decrease in elongation at break, tensile strength and tear strength. It is quite possible that a combination of an increase in shrinkage and compression (bulk) modulus may account for the observed results. Another report on a physical model for stiction in MEMS devices shows that stiction forces decrease with increasing temperature and this could also account for the observed behavior [35].

The vertical microcantilevers produced using the methods described above were used to investigate the response of mesenchymal stem cells when cultured in different types of differentiation medium on arrays with different average shear stiffness. In this study, we examined whether microcantilever arrays designed to have different effective shear stiffness would potentiate differentiation of mesenchymal stem cells down adipogenic and osteogenic pathways cultured with inductive medium. The arrays consist of individual microcantilevers arranged in three distinct spatial densities and have two different heights, thus providing six different surfaces for attachment. An example of the initial design parameters that were used is shown in Fig. 14. The final designs consisted of arrays of three spatial densities (edge-to-edge spacings of 1, 2 and 4 μm with two distinct heights of 2 μm and 5 μm and diameters of 1 μm , representing approximate average shear stiffness between 5 and 15 KPa based on our calculation. Fig. 15 shows cells fixed and stained with Oil Red O after seven days of culture in medium known to cause differentiation down the adipogenic pathway. Complete data have been presented in [36].

IV. Conclusion

We examined the use of cryogenic etching of silicon at -110°C as an alternative method to make master molds for vertical microcantilever arrays. Two critical factors that affect the etch process, O_2 flow rate and CCP, were varied to achieve the desired sidewall profile. The SF_6 flow rate during the etch was maintained at 80 standard cubic centimeters per minute (sccm). Using the cryogenic etch process alleviated the scalloping effects that we observed when we used the Bosch process in an attempt to fabricate the master molds and produced very smooth side walls. We observed that using a CCP power of 4 W at O_2 flow rates of between 13 and 15 sccm generally produced a positive sidewall profile which was not desirable. Using a higher CCP power (30 W) at similar O_2 flow rates produced vertical sidewalls. However because of the selectivity of the e-beam resist used, we were unable to reach target etch depths with these parameters. Final etch parameters of 10 W of CCP power and O_2 flow rate of 13 sccm produced vertical side walls. These parameters also allowed us to reach target etch depths. In all cases the ICP power, etch temperature and SF_6 flow rate were kept constant at 1250 W, -110°C and 80 sccm respectively. Experiments on etch uniformity across the wafer showed an average variation of 1.4% from the center of a wafer to the perimeter with a maximum variation of 2.2%. We observed that curing the PDMS mold at 110°C instead of 65°C seems to alleviate certain defects that could possibly be due to the stiction-friction problem that can occur in soft lithographic microfabrication.

Our optimized etching process provides an important reference for fabrication of silicon master molds for cylindrical vertical microcantilevers with aspect-ratios of around 5:1 and with feature sizes in the 750 nm to 2 μm range. It does not require the use of an additional hard mask such as chromium or gold and uses just the e-beam resist (ZEP 520A) with selectivity of about 20:1 and etch rates of about 2.3 $\mu\text{m}/\text{min}$. This reduces the complexity of the process.

Our process has allowed us to produce denser arrays of microcantilever arrays with different average shear stiffness. This provides an important tool that can be used for experiments in cellular biomechanics [22]. The ability to fabricate precisely controlled arrays of microcantilevers with diameters on the order of 1 μm will extend the range of shear stiffnesses that can be employed while studying substrate-dependent cell differentiation, motility and growth, or cancer invasion. For example, the rigidity of polyacrylamide gels used for studying cancer invasion has ranged from soft (360 Pa) to hard (3300 Pa) [37]. We show that we can produce microcantilever arrays that are sufficiently small and closely spaced that they can be used to support the differentiation of mesenchymal stem cells down the adipogenic pathway. Our 1 μm diameter posts provide a surface with a shear stiffness in the range from 1 to 20 kPa; but the range could be adjusted by altering the post diameter.

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References

1. Zhao YJ, Cui TH. Fabrication of high-aspect-ratio polymer-based electrostatic comb drives using the hot embossing technique. *Journal Of Micromechanics and Microengineering*. May; 2003 vol. 13(no. 3):430–435.
2. Elders, J.; Jansen, H.; Elwenspoek, M.; Ehrfeld, W. DEEMO: a new technology for the fabrication of microstructures. In: Jansen, H., editor. *Proceedings IEEE Micro Electro Mechanical Systems, MEMS '95*. Amsterdam, Netherlands: 1995. p. 238-243.
3. Ji J, Tay FEH, Miao JM, Iliescu C. Microfabricated microneedle with porous tip for drug delivery. *J. Micromech. Microeng.* May; 2006 vol. 16(no. 5):958–964.
4. Laermer, F.; Schilp, A. Method of anisotropically etching silicon. German Pat., DE 4 241 045 granted on 26 May 1994 (US Pat. 5 501 893 granted on 26 March 1996).
5. Laermer F, Urban A. Challenges, developments and applications of silicon deep reactive ion etching. *Microelectronic Engineering*. Jun.2003 vol. 67–68:349–355. [Online]. Available: <http://www.sciencedirect.com/science/article/B6V0W-47x71M0-5/2/f0a3701ae8d5ed3212ce5e590d7ab8b9>.
6. Offenberger, M.; Larmer, F.; Elsner, B.; Munzel, H.; Riethmuller, W. Novel process for a monolithic integrated accelerometer. In: Larmer, F., editor. *The 8th International Conference on Solid-State Sensors and Actuators, 1995 and Eurosensors IX. Transducers '95*. Vol. vol. 1. Stockholm, Sweden: 1995. p. 589-592.
7. Lutz, M.; Golderer, W.; Gerstenmeier, J.; Marek, J.; Maihofer, B.; Mahler, S.; Munzel, H.; Bischof, U. A precision yaw rate sensor in silicon micromachining. In: Golderer, W., editor. *International Conference on Solid State Sensors and Actuators, TRANSDUCERS '97*. Vol. vol. 2. Chicago, USA: 1997. p. 847-850.
8. Tachi S, Tsujimoto K, Okudaira S. Low-temperature reactive ion etching and microwave plasma etching of silicon. *Appl. Phys. Lett.* Feb; 1988 vol. 52(no. 8):616–618. [Online]. Available: <http://link.aip.org/link/APL/52/616/1>.
9. de Boer M, Gardeniers J, Jansen H, Smulders E, Gilde M-J, Roelofs G, Sasserath J, Elwenspoek M. Guidelines for etching silicon mems structures using fluorine high-density plasmas at cryogenic temperatures. *J. Microelectromech. Syst.* 2002; vol. 11(no. 4):385–401.
10. Zhang M, Li JZ, Adesida I, Wolf ED. Reactive ion etching for sub-micron structures of refractory-metal silicides and polycides. *Journal Of Vacuum Science & Technology B*. 1983; vol. 1(no. 4): 1037–1042.

11. Francou M, Danel JS, Peccoud L. Deep and fast plasmaetching for silicon micromachining. *Sensors And Actuators A Physical*. Jan; 1995 vol. 46(no. 1–3):17–21.
12. Bartha JW, Greschner J, Puech M, Maquin P. Low-temperature etching of Si in high-density plasma using Sf_6/O_2 . *Microelectronic Engineering*. Feb; 1995 vol. 27(no. 1–4):453–456.
13. Zijlstra T, van der Drift E, de Dood MJA, Snoeks E, Polman A. Fabrication of two-dimensional photonic crystal waveguides for 1.5 μm in silicon by deep anisotropic dry etching. *Journal Of Vacuum Science & Technology B*. Nov; 1999 vol. 17(no. 6):2734–2739.
14. Dagostino R, Flamm DL. Plasma-etching of Si and SiO_2 in $\text{Sf}_6\text{-O}_2$ mixtures. *Journal Of Applied Physics*. 1981; vol. 52(no. 1):162–167.
15. Flamm DL. Mechanisms of silicon etching in fluorinecontaining and chlorine-containing plasmas. *Pure And Applied Chemistry*. Sep; 1990 vol. 62(no. 9):1709–1720.
16. Pruessner MW, Rabinovich WS, Stievater TH, Park D, Baldwin JW. Cryogenic etch process development for profile control of high aspect-ratio submicron silicon trenches. *Journal of Vacuum Science & Technology B: Microelectronics and Nanometer Structures*. Jan; 2007 vol. 25(no. 1):21–28. [Online]. Available: <http://link.aip.org/link/?JVSB/25/21/1>.
17. Tan J, Tien J, Pirone D, Gray D, Bhadriraju K, Chen C. Cells lying on a bed of microneedles: An approach to isolate mechanical force. *Proc. Natl. Acad. Sci*. 2003; vol. 100(no. 4):1484–1489. [PubMed: 12552122]
18. du Roure O, Saez A, Buguin A, Austin RH, Chavrier P, Silberzan P, Ladoux B. Force mapping in epithelial cell migration. *Proc. Natl. Acad. Sci*. Feb; 2005 vol. 102(no. 7):2390–2395. [Online]. Available: <http://www.pnas.org/cgi/content/abstract/102/7/2390>. [PubMed: 15695588]
19. Addae-Mensah KA, Kassebaum NJ, Bowers MJ II, Reiserer RS, Rosenthal SJ, Moore PE, Wikswo JP. A flexible, quantum dot-labeled cantilever post array for studying cellular microforces. *Sensors and Actuators A: Physical*. May; 2007 vol. 136(no. 1):385–397. [Online]. Available: <http://www.sciencedirect.com/science/article/B6THG-4MT5JYB-1/2/412c0ca23fefde2ac94ca751795b3332>.
20. Addae-Mensah KA, Reiserer RS, Wikswo JP. Poly(vinyl alcohol) as a structure release layer for the microfabrication of polymer composite structures. *J. Micromech. Microeng*. 2007; vol. 17(no. 7):N41–N46.
21. Sniadecki NJ, Anguelouch A, Yang MT, Lamb CM, Liu Z, Kirschner SB, Liu Y, Reich DH, Chen CS. From the cover: Magnetic microposts as an approach to apply forces to living cells. *Proc. Natl. Acad. Sci*. Sep; 2007 vol. 104(no. 37):14 553–14 558. [Online]. Available: <http://www.pnas.org/cgi/content/abstract/104/37/14553>.
22. Addae-Mensah KA, Wikswo JP. Measurement techniques for cellular biomechanics in vitro. *Experimental Biology and Medicine*. Jul; 2008 vol. 233(no. 7):792–809. [Online]. Available: <http://www.ebmonline.org/cgi/content/abstract/233/7/792>. [PubMed: 18445766]
23. Lim J-M, Byun S, Chung S, Park TH, Seo J-M, Joo C-K, Chung H, Cho D-i. Retinal pigment epithelial cell behavior is modulated by alterations in focal cell-substrate contacts. *Invest. Ophthalmol. Vis. Sci*. Nov; 2004 vol. 45(no. 11):4210–4216. [Online]. Available: <http://www.iovs.org/cgi/content/abstract/45/11/4210>. [PubMed: 15505077]
24. Kim K, Park S, Lee B, Manohara H, Desta Y, Murphy M, Ahn C. Rapid replication of polymeric and metallic high aspect ratio microstructures using pdms and liga technology. *Microsystem Technologies*. Nov; 2002 vol. V9(no. 1):5–10. [Online]. Available: <http://dx.doi.org/10.1007/s00542-002-0194-6>.
25. du Roure O, Dequidt C, Richert A, Austin RH, Buguin A, Chavrier P, Silberzan P, Ladoux B. Microfabricated arrays of elastomeric posts to study cellular mechanics. *Proc.SPIE*. Jan; 2004 vol. 5345(no. 1):26–34.
26. Ayon AA, Braff R, Lin CC, Sawin HH, Schmidt MA. Characterization of a time multiplexed inductively coupled plasma etcher. *J. Electrochem. Soc*. Jan; 1999 vol. 146(no. 1):339–349. [Online]. Available: <http://link.aip.org/link/?JES/146/339/1>.
27. Zhao Y, Zhang X. Cellular mechanics study in cardiac myocytes using PDMS pillars array. *Sensors and Actuators A: Physical*. Jan; 2006 vol. 125(no. 2):398–404. [Online]. Available: <http://www.sciencedirect.com/science/article/B6THG-4H74M25-1/2/739c5089deabce103c7d3d5c2fbd4690>.

28. Peister A, Mellad JA, Larson BL, Hall BM, Gibson LF, Prockop DJ. Adult stem cells from bone marrow (mscs) isolated from different strains of inbred mice vary in surface epitopes, rates of proliferation, and differentiation potential. *Blood*. Mar; 2004 vol. 103(no. 5):1662–1668. [PubMed: 14592819]
29. Reger, R.; Tucker, A.; Wolfe, M. Mesenchymal Stem Cells: Methods and Protocols, ser. Methods in Molecular Biology. Prockop, D.; Phinney, D.; Bunnell, B., editors. Vol. vol. 449. Humana Press; 2008.
30. Chamberlain G, Fox J, Ashton B, Middleton J. Concise review: Mesenchymal stem cells: Their phenotype, differentiation capacity, immunological features, and potential for homing. *Stem Cells*. Nov; 2007 vol. 25(no. 11):2739–2749. [Online]. Available: <http://stemcells.alphamedpress.org/cgi/content/abstract/25/11/2739>. [PubMed: 17656645]
31. Ye X, Liu H, Ding Y, Li H, Lu B. Research on the cast molding process for high quality pdms molds. *Microelectronic Engineering*. Mar; 2009 vol. 86(no. 3):310–313. [Online]. Available: <http://www.sciencedirect.com/science/article/B6V0W-4TSD9BH-1/2/8bba047dea264318d53b7fcd51526e7f>.
32. Wu D, Fang N, Sun C, Zhang X. Stiction problems in releasing of 3d microstructures and its solution. *Sensors and Actuators A: Physical*. Mar; 2006 vol. 128(no. 1):109–115. [Online]. Available: <http://www.sciencedirect.com/science/article/B6THG-4J3NY62-8/2/6e86325564ec2d73eec425e00e044269>.
33. Srinivasan U, Houston M, Howe R, Maboudian R. Alkyltrichlorosilane-based self-assembled monolayer films for stiction reduction in silicon micromachines. *J. Microelectromech. Syst.* 1998; vol. 7(no. 2):252–260.
34. Xia Y, Whitesides GM. Soft lithography. *Annual Review of Materials Science*. 1998; vol. 28(no. 1):153–184. [Online]. Available: <http://arjournals.annualreviews.org/doi/abs/10.1146/annurev.matsci>.
35. van Spengen WM, Puers R, Wolf ID. A physical model to predict stiction in MEMS. *Journal of Micromechanics and Microengineering*. 2002; vol. 12(no. 5):702–713.
36. Addae-Mensah, KA. Ph.D. dissertation. Nashville, TN: Vanderbilt University; Dec. 2008 A microfabricated microcantilever array: A platform for investigation of cellular biomechanics and microforces in vitro.
37. Alexander NR, Branch KM, Parekh A, Clark ES, Lwueke LC, Guelcher SA, Weaver AM. Extracellular matrix rigidity promotes invadopodia activity. *Current Biology*. Sep; 2008 vol. 18(no. 17):1295–1299. [PubMed: 18718759]

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Fig. 1.

(a) Scanning electron micrograph (SEM) of silicon wafer with photoresist layer etched using the Bosch process at laboratory temperature. (b) Image at higher magnification showing scalloping. (c) PDMS vertical microcantilever array peeled from one silicon master mold showing both clumping and damaged microcantilevers.

Fig. 2.

Fabrication steps for making the microcantilever master molds and the casting of PDMS for making the microcantilever arrays. (a) Spin e-beam resist at 2000 or 6000 rpm. (b) E-beam exposure to define patterns. (c) Develop exposed wafer in xylenes for 180 seconds. (d) Perform cryogenic etch to define features in silicon and strip e-beam resist with acetone. (e) Pour PDMS on cleaned wafer, degas in vacuum and cure in oven. (f) Peel cured PDMS from silicon wafer to obtain device.

Fig. 3.
SEM image of a cleaved silicon wafer etched using the cryogenic etching technique. Notice the smoothness of the sidewalls. The scalloping which occurs during the Bosch process does not occur.

(a)

(b)

Fig. 4.

Difference in etch rate. Etch profile showing depth of about 1.68 μm (3.36 μm actual depth corrected for viewing angle) for circular features with original design diameter of 750 nm (a) and that showing depth of about 2.03 μm (4.06 μm actual) for circular features with original design diameter of 1 μm (b).

(a)

(b)

Fig. 5. E-beam resist etch rates for wafers spun with e-beam resist at speed of (a) 6000 rpm and (b) 2000 rpm.

(a)

(b)

Fig. 6. Initial test run at 4 W showing depth of about 1.28 μm (2.36 μm actual) for circular feature with original design diameter of (a) 750 nm and (b) 1 μm .

Fig. 7.

The effect of CCP power and O₂ flow rate on the etch profile (a) The effect of increasing the CCP power from 4 W to 30 W at 14 sccm of O₂. This changes the profile from a positive to almost straight sidewalls. The cusp on the right edges is due to the angle of the cleavage plane for the wafer. The left edges show best the sidewall profile. (b) At 4 W of CCP power with 15 sccm of O₂ the positive taper is not pronounced after a two-minute etch although the ideal vertical sidewall profile is not achieved. (c) Reducing the oxygen flow to 14 sccm of O₂ and keeping the CCP power at 4 W improves the positive taper slightly over that of 4 W CCP at 15 sccm of O₂. (d) A further reduction in O₂ flow rate to 13 sccm improves the positive taper. (e) At 10 W of CCP power with 13 sccm of O₂ the sidewall profile becomes almost vertical. This is for a two-minute thirty-second etch.

(a)

(b)

Fig. 8. E-beam and contact lithography. SEM images showing (a) smooth edges from e-beam lithography and (b) slightly rough and deformed edges from contact lithography.

(a)

(b)

Fig. 9.

Etch profiles from contact lithography. Etch profile showing depth of about 1.68 μm (3.36 μm actual) for circular feature with original design diameter of (a) 750 nm and (b) showing depth of about 2.03 μm (4.06 μm actual) for circular feature with original design diameter of 1 μm .

(a) 2 μm with 4 μm pitch(b) 1 μm with 3 μm pitch(c) 750 nm with 4.75 μm pitch**Fig. 10.**

PDMS microcantilevers from vertical sidewall molds. Devices were cured at 65 ° C.

(a) 2 μm with 4 μm pitch(b) 1 μm with 3 μm pitch(c) 750 nm with 4.75 μm pitch**Fig. 11.**

PDMS microcantilevers from tapered sidewall molds. Devices were cured at 65 ° C. The 750 nm sample with tapered sidewalls show more damage than the straight ones.

Fig. 12. Damage and stiction from some molds. Damage observed with the closest spaced microcantilevers when the molds are separated. This effect is not noticeable at higher pitches. Device was cured at 65°C.

(a)

(b)

Fig. 13. PDMS molding results at 110 °C (a) PDMS microcantilevers from almost vertical sidewall molds with diameters of 1 μm edge-to-edge spacing of 1 μm and about 5 μm tall. (b) The same microcantilevers at a lower magnification.

(a)

(b)

Fig. 14.

Design parameters. (a) Design parameters showing variation of height and diameter with spatial density of arrays for a particular shear stiffness. (b) Variation of height with shear stiffness at a spatial density of 1 μm edge-to-edge spacing

Fig. 15.

Image showing mesenchymal stem cells cultured on microcantilevers with 1 μm diameter and 2 μm center-to-center spacing and in medium known to cause differentiation down the adipogenic pathway. Cells were fixed and stained with Oil Red O after seven days of culture and oil droplets that were formed are clearly visible.

TABLE ISummary of etch parameters ¹

CCP power (W)	O ₂ flow rate (sccm)	Etch time	Figure
30	14	1 min	3
4	15	2 min 30 secs	4
4	14	1 min	6
30	14	2 min	7(a)
4	15	2 min	7(b)
4	14	2 min	7(c)
4	13	3 min	7(d)
10	13	2 min 30 secs	7(e)
10	13	3 min	8 ²

¹ICP power, etch temperature and SF₆ flow rate were kept constant at 1250 W, -110°C and 80 sccm respectively

²From contact lithography