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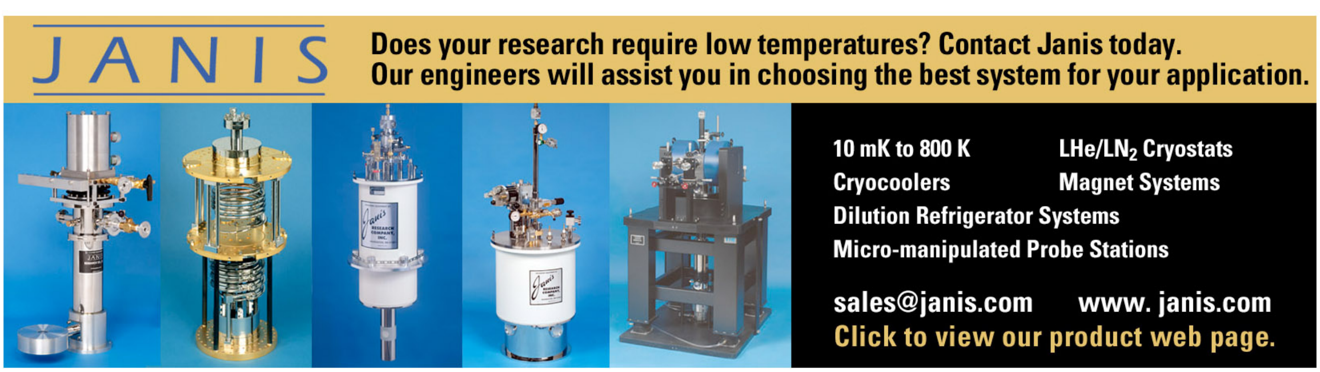

REVIEW OF SCIENTIFIC INSTRUMENTS **81**, 106104 (2010)

Note: Mechanical study of micromachined polydimethylsiloxane elastic microposts

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This paper reports the detailed statistical measurement of Young's modulus (E) and spring constant of micromachined three-dimensional polydimethylsiloxane microposts with various sizes using atomic force microscope. The paper also describes the design and fabrication of these microposts. The micropost array was fabricated with a height to diameter aspect ratio of up to 10. We have found that posts with different sizes have different E values, and posts that are cured at room temperature have smaller Young's modulus than the ones that are cured at 65 °C for the same duration.
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Many research groups have previously designed and fabricated high-density elastomeric micropost arrays for measuring the traction forces developed by living cells.[1–7] Cells attach and spread across the top surface of the regularly ordered microposts and since each micropost is discrete, it detects the cell traction forces independently at the site where it contacts the cell. This technology has been used to study cardiac fibroblasts and smooth muscle cells. The results typically show that cells exert significantly larger contractile forces at the edge of the cell compared to the nonmarginal interior.[1–3,8–10] The estimation of the traction force at each micropost requires the accurate measurements of its spring constant and deflection. Microposts are typically made using polydimethylsiloxane (PDMS) because it is biocompatible and its mechanical properties can be tuned in physiologically relevant ranges. The Young's modulus of PDMS microposts depends on the curing temperature, the mixing ratio of silicone base to the curing agent, and the mixing of PDMS curing agent with solvent such as hexane.[11] The previously reported E ranges from 0.3 to 3 MPa,[1–5,12] where E was determined by several techniques: one group determined it by using large dimension micropost with a diameter and height of 500 μm and 3.75 mm, respectively. They then used these values to calculate the traction forces of cells cultured on top of a small dimension micropost array.[12] A second group calibrated a glass micropipette by measuring the weight of small crystal of p-nitrophenol and then used it to calibrate micropost via piezoelectric manipulator.[3] These microposts were fabricated with relatively low aspect ratio up to 4.[1–5] This might be due to fabrication difficulty.

The objective of this paper is to report the fabrication of high aspect ratio micropost (up to 10) and to provide detailed statistical measurements of their Young's modulus using various micropost's dimensions that are cured at room temperature or 65 °C. The high aspect ratio micropost can be used to measure the traction forces of cells with weaker forces, and they also can improve the sensitivity of the traction force measurement. The device is designed with array of high density three-dimensional (3D) microposts (100×100) made of flexible material (silicone elastomers) with known physical and chemical properties. The 3D flexible environment will allow the study of the subcellular distribution of traction forces exerted by cells on the micropost array.[1–5] These microposts are treated as cylindrical cantilever beams: one end is fixed to the substrate and the other end is free. When a force with unknown value is applied to the free end and parallel to the substrate, the micropost will bend. In biological applications, this force is attributed to the relaxation or contraction associated with changes in a cell's contractile machinery. Because the cell is cultured and attached to the top of the microposts, these changes in cell mechanical behavior are apparent as directional deflections of the fabricated micropost. In the linear regime, the micropost behaves similar to a spring such that the deflection is directly proportional to the applied force. Hence the traction forces can be quantified by determining the deflection of each post. The relationship between force, F, and free end displacement, x, for a cylindrical beam can be determined using the bending theory of cantilever beam[10,13,14] (Euler–Bernoulli beam theory),

$$F = Kx = (K_t + K)x_d, \tag{1}$$

$$K = \frac{x_d}{x - x_d}K_t = \frac{3\pi E D^4}{64L^3}, \tag{2}$$

where E, D, L, K, K_t, and x are the Young's modulus, the diameter, the height, the spring constant, and the displacement of the micropost, respectively. K_t and x_d are the spring constant and displacement of the atomic force microscope (AFM) cantilever beam. Therefore, the Young's modulus of PDMS can be calculated after measuring the spring constant of the micropost and it can then be used as a calibrated value for determining the force.

The micropost arrays were fabricated using standard microlithography and replica-molding techniques in the following sequence. (1) A photoresist layer was patterned on a cleaned 4 in. silicon wafer to form openings at locations corresponding to the microposts. (2) Silicon micromold was

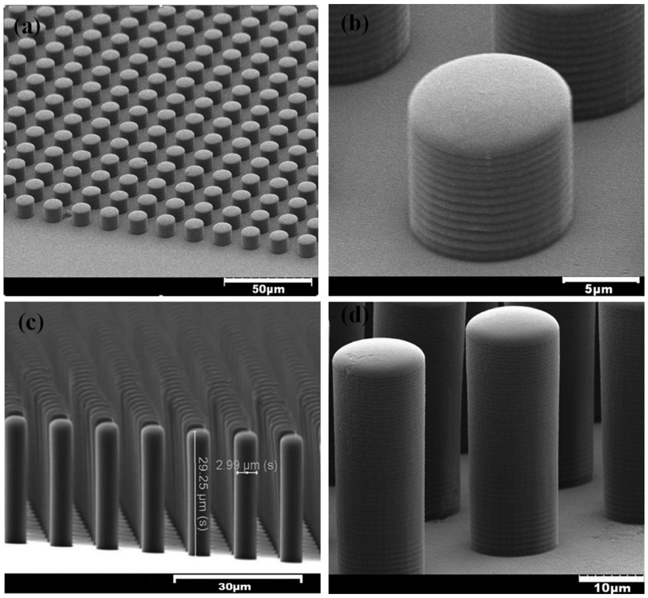

FIG. 1. Scanning electron micrographs (SEMs) of microposts with diameter, height, and spacing of [(a) and (b)] 7, 5, 7 μm; (c) 3, 30, 5 μm; and (d) 10, 30, 7 μm, respectively.

formed by etching microholes with high aspect ratio using a deep reactive ion etching system (Alcatel DRIE AMS-100). (3) The wafer was cleaned again with piranha solution and the natural oxide layer was removed using hydrofluoric acid. (4) The wafer was treated with a vapor of hexamethyldisilazane reagent in vacuum desiccators for 15 min. This step proved to be crucial for preventing the sticking of PDMS to the substrate and hence facilitates peeling off the posts from the mold after being cured. (5) The wafer was diced into 2 cm×2 cm, each containing four arrays and was placed in a Petri dish, and a PDMS, prepared by mixing resin with curing agent (10:1), was poured over them. The wafer was then placed inside a vacuum at room temperature or at 65 °C for 24 h. The long baking time is crucial for peeling off the high aspect ratio microposts. (6) After the PDMS was cured, it is peeled off manually creating the micropost arrays. The microposts were fabricated with diameter range between 3 and 10 μm, height between 5 and 70 μm, and spacing of 5–10 μm. The best achieved aspect ratio was 10. In this case the post diameter and height were 3 and 30 μm, respectively. Scanning electron micrographs (3 dimensional view) of the fabricated arrays are shown in Fig. 1.

Prior to performing the calibration using AFM, a single linear array of posts was cut from the micropost array using a vibratom. The use of single linear array of posts will help better locating and viewing single post, and preventing nearby posts from touching it during calibration, as shown in Fig. 2. This will result in more accurate measurement of force-displacement relationship. The spring constant and Young's modulus measurements were performed on microposts with small and large dimensions that were cured at room temperature or 65 °C for the same duration (24 h). The small dimension and large dimension microposts are referred to microposts with diameter (D) and height (H) of 3.3–9.2, 10–27, 16.1–26.7, and 30–42.9 μm, respectively (see Table I). Initially, the linear micropost array was fixed on the AFM stage horizontally [see Fig. 2(b)]. Then, a range of forces

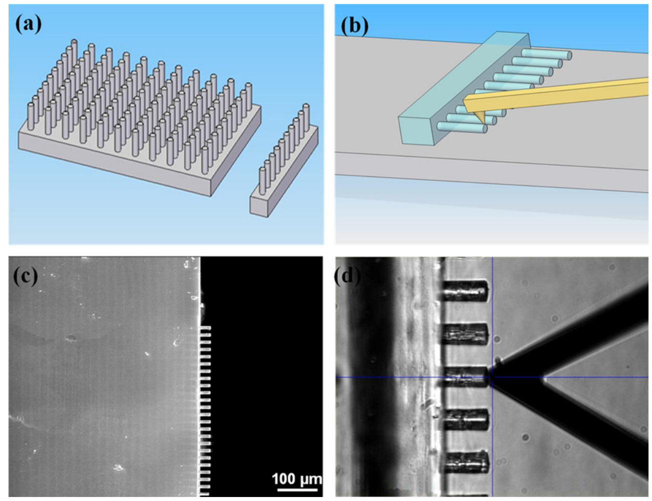

FIG. 2. (Color online) Calibration schematics of the microposts using AFM. (a) A linear array was cut from a micropost array. (b) The linear array was fixed on a glass substrate. (c) A SEM image of the linear array. (d) The cantilever tip of atomic force microscope was touching the edge of one post.

was applied on the surface of the micropost using an AFM cantilever beam with a spring constant of 0.06 N/m (Veeco Co.). The applied forces and the corresponding displacements were recorded in real time by computer and their relation was obtained by using MATLAB program. This step was repeated several times to remove the system noise and measurement error. For this analysis, we assumed the microposts have uniform material properties such that the deflection is equivalent to the corresponding traction force divided by the spring constant.

The calibration of micropost was performed as follows. First, the sensitivity of AFM cantilever beam was measured by applying force (0–60 nN) using the AFM against a glass substrate. In this case, there is no indentation during the measurement. Second, the indentation of PDMS micropost was measured by applying similar force range on PDMS substrate using AFM. The Z-travel of its base (z) includes the deflection of the cantilever beam (d) and the indentation of the PDMS micropost (σ) [Fig. 3(a)]. Third, the deflection of the micropost was determined in two steps: by applying force using AFM on its free end and by subtracting the PDMS indentation and the deflection of AFM cantilever beam from Z-travel of the cantilever beam's base (z) [Fig. 3(b)]. The deflection is given by $x=z-d-\sigma$.

The measured Young's moduli were divided into four sets of data based on micropost size and curing temperature: (1) small dimension posts that are cured at room temperature, (2) small dimension posts that are cured at 65 °C, (3) large dimension posts that are cured at room temperature,

TABLE I. Average Young's modulus (E) of PDMS micropost as a function of size and curing temperature.

Post size	Diameter (μm)	Height (μm)	No. of sample	Av. E (MPa)	Curing temp. (°C)
Small	6.0–9.2	13.0–27.0	7	0.936 ± 0.037	23
Small	3.3–9.3	10.0–15.8	6	1.378 ± 0.162	65
Large	18.5–26.2	30.0–42.7	13	0.543 ± 0.032	23
Large	16.1–26.7	30.1–42.9	14	1.090 ± 0.090	65

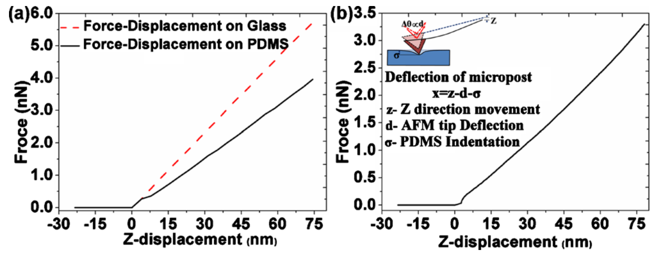

FIG. 3. (Color online) Force-displacement characteristics of the AFM cantilever beam applying forces on (a) glass and PDMS substrates. (b) A micropost (D: 24 μm, H: 41 μm) free end.

and (4) large dimension posts that are cured at 65 °C (see Table I). It is important to note that the posts were fabricated with exactly the same fabrication steps and the exact dimensions were measured using scanning electron microscope. We have then compared the measured Young's moduli of the four sets to each other as follows. The first comparison was between 20 posts with small and large dimensions that are cured at room temperature (the first and third sets). In this case, the measured Young's moduli have average values of 0.936 ± 0.037 and 0.543 ± 0.032 MPa, respectively. The difference between the two sets was 41.99%. The second comparison was between 20 posts with small and large dimensions that are cured at 65 °C (the second and fourth sets). The average measured Young's moduli were 1.378 ± 0.162 and 1.090 ± 0.09 MPa, respectively. The difference between the two sets was 20.9%. The third comparison was between large dimension microposts that are cured at room temperature or at 65 °C for the same duration (the third and fourth sets). The average measured Young's moduli of both sets of samples were 0.543 ± 0.032 and 1.090 ± 0.09 MPa, respectively. The difference was 50.18%. The fourth comparison was between small dimension microposts that are cured at room temperature or at 65 °C (first and second sets). The average measured Young's moduli of both sets of samples were 0.936 ± 0.037 and 1.378 ± 0.162 MPa, respectively. The difference is 30.08%. The p-values of all four compared data sets were much less than 0.001 which verifies that there is significant variation between compared data sets.

The difference between small and large micropost Young's moduli that are cured either at room temperature (41.99%) or 65 °C (20.09%) is significant. When the small microposts are compared to each other at two temperatures, the difference was 30.08%, which is much less than when the large microposts are compared to each other (50.18%). These results suggest that small dimension microposts are cured faster than the larger dimension post. These results also show that the microposts that are cured at 65 °C are stiffer than the ones that are cured at room temperature. They also indicate that larger microposts have smaller Young's modulus. This clearly shows that micropost dimension and curing temperature play crucial role in determining Young's modulus value. Thus, large posts cannot be used to calibrate the Young's modulus of small posts, e.g., third set. In this case, the measured Young's moduli have average values of 0.936 ± 0.037 and 0.543 ± 0.032 MPa. If small microposts are used to determine the traction forces of biological cells, then the same size posts must be calibrated. Otherwise, the use of wrong E value will increase the experiment error.

The last experiment was performed in order to evaluate the effects of AFM cantilever on the calibration results. Two types of AFM cantilever beams were used; the first has a tetrahedron tip while the second type has no tip. Both of them have the same dimensions and are made of the same material. One cantilever will touch the post by the summit of its tetrahedron tip and the other will touch the scanned post directly. The measured Young's moduli of the same post using both AFM cantilevers were 1.15 and 1.17 MPa. This clearly indicates that the AFM tip has minimal effects on the calibration results and hence can be neglected. However, it is important to note that the cantilever beam with tip is more difficult to align and exactly touch the center of the post because the tip is very small (nanometer scale) compared to the dimension of the post (micrometer scale). On the other hand, the edge of cantilever without tip can be easily aligned with the center of the micropost. The calculated spring constant can be varied between 0.608 nN/μm and 2.03 nN/nm based on the device geometry.

In conclusion, we have successfully fabricated high aspect ratio PDMS micropost array. The Young's modulus of small and large dimension microposts was measured using AFM. The low value Young's modulus of PDMS is not only found in large scale microposts but also in microposts cured at room temperature. The micropost calibration demonstrates that the posts cured at higher temperature are stiffer than posts that are cured at room temperature. It also proved that large scale PDMS and small scale PDMS microposts have different Young's moduli even if they are made of the same PDMS mixture by identical fabrication methods.

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