Geometrically Optimized mPAD Device for Cell Adhesion

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Abstract

Cell Adhesion is of particular interest in biomedical research as it plays a role in many critical biological functions in normal and unhealthy systems. Because of its size, fast responsive times, and electromechanical nature, MEMS naturally lends itself to the study of cell adhesion. This project will address previous literature on MEMS application in cell adhesion research and propose a novel MEMS device. In particular, two previous methods are presented: highly deformable silicone substratum that wrinkles due to adhesive forces and a tightly-packed array of micromachined cantilevers as force transducers on silicone wafers. The proposed MEMS device is an improvement on the latter method, with the geometry and positioning of the pillars optimized to maximize the deflection of the pillars, making displacements due to cell adhesion easier to detect by optical means. Additionally, the design is optimized to allow for the maximum number of pillars that can come into contact with the cell. This design feature will yield the greatest amount of individual force vectors, leading to the creation of a highly detailed vector field representation of cell adhesion forces. A MATLAB program was written to first identify the most significant design considerations. These parameters were then used to define the pillar geometry and array spacing with respect to the optimization criteria. Based on the calculated dimensions, an appropriate pillar material was selected and a microfabrication process was developed. The device will be coupled with an optical sensor capable of reading deflections in the pillars and translating these values to a force vector field.
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INTRODUCTION

Essential life functions such as development, repair, and senescence of tissues are not only driven by molecular and biochemical signaling, but also by mechanical cues between a cell and its surroundings. In complex organisms, the mechanical interplay between cells and the extracellular matrix (ECM) during cell adhesion regulates key physiologic events such as cell motility and migration, embryogenesis and metastasis, tissue and organ formation, and wound repair [3]. Cell adhesion is accomplished through the binding of ligands in the ECM to integrin receptors in the cell plasma membrane, spreading of the cells across the attachment surface, and generation of mechanical traction forces at the adhesion sites by the contraction of the actomyosin cytoskeleton. The processes of binding, spreading, and contraction are mechanochemical sensory probes in the ECM for biochemical and mechanical cues for cellular events [29].

Over the past 20 years, extensive research has led to a much greater understanding of the biochemical mechanisms that encourage cellular adhesion forces. However, there have been relatively few advances in the study of the mechanical forces of cell adhesion, in part due to the high difficulty in measuring the minute physical forces exerted by cells over very small surfaces. As a result, the temporal and spatial coordination of adhesion mechanics remains a mystery [30]. Elucidation of these interactions will help predict and control cell behavior and function on substrates. Consequently, investigation of cell adhesion on artificial materials is vital to biotechnological applications such as biosensors, biomedical implants, and tissue engineering [22].

The comparable scale of microelectromechanical systems (MEMS) and cellular interactions naturally leads to the application of MEMS in the study of cell adhesion. Colonization of cells on artificial surfaces like silicon allows for the functional coupling of living cells to microelectronic integrated circuits. The use of micro fabricated post array detectors (mPADs), in particular, enables direct measurement of adhesion forces with less complex mathematical analysis than those of continuous substrate methods.
LITERATURE REVIEW

There have been numerous attempts to isolate and measure cellular forces using a wide range of experimental setups. Initial attempts to measure cellular adhesion forces were made by placing a cell over a continuous substrate and measuring the deformations of the substrates (Figure 1). The first of these methods was the wrinkle method which used a flexible substrate that would wrinkle due to cellular adhesion forces. In this method, cells are plated onto a thin layer of polydimethylsiloxane (PDMS). The traction forces deform the substrate producing a wrinkled pattern which indicates the direction and relative magnitudes of cellular forces. Although the wrinkle method is sensitive to deformations over 1 square micrometer and to nano-Newton forces, it is impractical to use this method to quantitatively measure cellular forces as the analysis of the wrinkle pattern requires very complex mathematics. An improvement to the continuous substrate design involved using embedded fluorescent particles in a polyacrylamide gel [23]. As the gel substrate deformed due to cellular traction forces, the displacement of the embedded particles could be measured and then used to create a traction force field [1]. This method is problematic because it requires the embedded particles to be laid out in a precisely uniform array. As such, creating the substrate and accurately measuring the particle displacements is very difficult.

![Figure 1: a) keratocyte on wrinkling silicone substrate. b) stationary rat cardiac fibroblast causing distortions on a micropatterned silicone substratum with regularly spaced dots [19]](image-url)
Current methods used to measure cellular forces have been designed to avoid the complexity of using a continuous contact with the cell by instead using free-standing micropillars. Recent attempts at measuring cellular forces involved using micropost array detectors (mPADs) which are microfabricated cantilevers that bend in response to cellular forces [1]. The first of these devices used a single horizontal cantilever on a microchip. This approach could only characterize the forces applied by a single cellular region. The most recent approach has involved the use of large numbers of densely packed elastomeric posts (Figure 2). Cells would be suspended above the substrate by the tips of these posts. The advantage of this approach is that each of the pillars in the array is a discrete structure which is not affected by the deflections of neighboring posts. As such, by avoiding the complexity of a continuous contact surface, the cantilever array method only requires calculation of the cantilever stiffness and a measurement of the cantilever deflection to determine the force exerted by a cell at a specific point. A potential drawback of using the cantilever array method is that the geometry of the array structure supporting the cell may cause it to exert forces differently than if it were lying on a flat substrate [17]. However, concurrent experiments which have used the micropillar array and a single substrate have shown that the effects of the micro pillar geometry do not significantly affect cellular adhesion forces.

The most significant differences among experiments using micro pillar arrays have been in the selection of material, pillar geometry, and array spacing. These design parameters depend on which cells are used. Different cells exhibit very dramatic differences in cellular adhesion forces. For example, myocyte exerts forces of up to 100 micro Newtons
while kidney cell forces are in the nano-Newton range. As such, the geometry and the material of the array is designed to be both stiff enough to withstand a specific type of cell’s adhesion forces and to also deflect enough to be measurable. The diameter of the pillars ranged from about 1-4 micrometers while the height varied considerably from several to a hundred micrometers. In general, micro post arrays are customized for a specific type of cell, assuming the cellular forces fall within a known range of forces. As such, mPADs arrays can take on a variety of aspect ratios and pillar spacing designs. (Figure 3).

![Figure 3: Single Spaced, low aspect-ratio structure](image)

The spacing between posts is determined by the flexibility of the cantilever. The gap must be wide enough to allow unrestricted lateral deflection of the pillars. The pillars must also be spaced close enough together to hold the cell above the substrate and prevent it from falling in. Both of these criteria depend on the type of cell chosen for the experiment [22]. The problem of falling-in can be reduced by using attachment pads which increase the contact area between the cell and the pillars while not significantly affecting the cantilever stiffness (Figure 4).
Additionally, the surface area of the array that comes into contact with the cell must be treated with fibronectin laminin which will allow the cell to exert a traction force on the top of the pillars (Figure 5).

In terms of material choice, most experiments used PDMS because of its flexibility and its isotropic material properties. Monocrystalline silicon is not as widely used because it is much stiffer, much less sensitive to smaller adhesion forces, and has an anisotropic Young’s modulus.

In addition, past experiments have used different methods to measure cantilever deflection. Since the substrate is opaque, only detection methods using reflected light can be used to measure displacement [22]. The silicon substrate is highly reflective, providing high optical contrast between the cantilever tips and the surface. However, as the array features become increasingly smaller, fluorescent markers have been used to increase the contrast between the pillar tips and the substrate [29].
PROPOSAL

The proposed design will use the cantilever approach to measure the forces exerted by the membrane of Madine–Darby canine kidney cells (MDCK) on the substrate. The variables will be the geometry of the cantilevers and the special orientation of the cantilever array. Adhesive at the ends of the cantilevers will also be used to hold portions of the cell to the pillars. The adhesive will consist of a layer of protein or laminin to which the proteins in the cells will attach.

The force-sensor will be manufactured in two steps. First, a single crystal silicon micro-mold will be fabricated by patterning cylindrical cavities with deep reactive ion etching (DRIE). PDMS will then be poured into the mold to form the pillar array, cured and removed from the mold.

An important feature of the pillar design is the relatively small aspect ratio. According to some recent experiments, an aspect ratio as small as 2:1 for the pillar height to diameter can be achieved [34]. Although pillars could be manufactured in this manner before, it is now useful to have this smaller aspect ratio because of improvements in optical equipment resolution. One of the advantages of pillars with a low aspect ratio is that large numbers of them can be packed together without the displacements interfering with one another. With more pillars, a higher resolution of forces can be achieved.
GEOMETRIC ANALYSIS

A comprehensive geometric analysis will highlight the most important design considerations. mPADs detect forces on the basis of cantilever deflections and thus can be reasonably modeled as ideal springs.

The relation between the deflection at the top of the pillar and lateral force is:

\[ F(\delta) = K\delta \]  

(1)

From Equation (1), if force is kept constant, then the deflection is dependent on the spring constant or stiffness \( K \):

\[ K = \frac{3EI}{H^3} \]  

(2)

Where \( E \) is the Young’s modulus, \( I \) is the moment of inertia, and \( H \) is the height of the pillar. Since the direction of the cellular mechanical forces cannot be predicted pillars with circular cross sections are ideal, as they offer uniform deformation in all directions. Thus the moment of inertia, \( I \):

\[ I = \frac{\pi D^4}{64} \]  

(3)

\( D \) is the diameter. From Equations (2) and (3), the spring constant can be varied by adjusting the height, the cross sectional diameter, and Young’s modulus. Increasing the height decreases the spring constant, while increasing the diameter or Young’s modulus increases the spring constant. To keep the deflection within the elastic deformation range, the height will have to be reduced or the elastic modulus increased.
To ensure the pillar does not deform plastically, special consideration must be given to stress due to beam bending. The stress in a bending beam is:

$$\sigma = \frac{M y}{I} \quad (4)$$

where $M$ is the bending moment, $y$ is the location of the stress relative to the neutral axis, and $I$ is the moment of inertia. The bending moment is:

$$M = F(H - x) \quad (5)$$

where $F$ is the applied force, $H$ is the pillar height, and $x$ is the distance from the base of the pillar. There is no bending moment at the tip of the pillar and the maximum moment is at the base of the pillar. So from Equations (4) and (5) the largest stresses in a cantilever are located at the base at the farthest distance from the center:

$$\sigma = \frac{F H r}{I} \quad (6)$$

where $r$ is the pillar radius, $F$ is the applied force, $H$ is pillar height, and $I$ is the moment of inertia. Using Equation (6) the maximum force can be determined without pushing the pillar into the plastic range. The yield stress and elastic modulus of the pillars is wholly dependent on geometric variables and fabrication processes of PDMS.

Due to the PDMS fabrication process various imperfections are introduced. Through SEM analysis the pillars are noticed to have enlarged bases, and notches on the sidewalls
or scallops. These changes all contribute to change the equivalent diameter of the pillar [34].

![Figure 8: a) ideal pillar. b) scalloped surface profile. c) conical pillar shape and scallops [23]](image)

Equation (7) calculates an equivalent diameter, factoring in the manufacturing defects. For low aspect ratio pillars, such diameter corrections are very small and can safely be ignored. Though for pillars with higher aspect ratios the scallops and base diameters will change the pillar response noticeably.

\[
D = D_s + \frac{D_b - D_s}{H} y + 2q \sin\left(\frac{2\pi}{p} y\right)
\]  

(7)

In order for the pillar deflection equations to apply, the base substrate is assumed to be rigid, and distortion free. This is largely true if the cells do not touch the substrate. To reduce this and increase force vector density the adhesion area percentage (AP) is kept high.

![Figure 9: Areas Percentage of Single-spaced structures [34]](image)
Where $D$ is pillar diameter, and $L$ is center-to-center distance of neighboring pillars. For single-spaced pillars the $\text{AP} \approx 19.63\%$ [34].

To further confirm that the substrate effectively does not deform, cells were placed on the mPADs and on both structures underneath the cell, and immediately adjacent to the cell. The array was then measured for displacements. The pillars immediately adjacent to the cell showed no measurable displacements, thus confirming that the substrate is a rigid fixed constraint for the pillars [33].
DIMENSION ANALYSIS AND OPTIMIZATION

Several factors must be considered to achieve the optimal force resolution. First, the material of the mPADs must be flexible enough to cell adhesion forces so as to attain optically measurable displacements. Second, the geometry and spatial arrangement of the posts must minimize the amount of cell flow down the sides of the posts, generate a detailed vector field representation of the forces, and is manufacturable (Figure 11). A natural hypothesis is that relatively closely spaced posts will lead to minimum cell flow along the posts and greatest amount of individual force vectors since more posts can be packed per unit area.

Figure 11: (a) single spaced structure isolating cell from base substrate, distantly spaced (b) and extremely low (c) structures have higher chances of cell adhering to substrate and calling deformation. (d) structures away from the cell do not have significant displacements while (e) and (f) show dramatic displacement in structures not directly touching cell [23]

An intuitive performance criterion is then the maximization of the post packing density and minimization of the post spring constant. Denoting the diameter of the post as D and the distance between adjacent posts as L (Figure 11), the number of posts per unit area, N, can be expressed as:

\[ N \propto \frac{1}{(D + L)^2} \quad (9) \]
From optimization theory, to find the values of $D$, $L$, and $H$ that optimize the force resolution, one minimizes a cost function, $J$, related to the performance criteria of maximum post density and minimum spring constant, subject to the constraints of the system. The cost function to be minimized is thus the weighted sum of the area of the sensory field of each post and the spring constant:

$$
J = C_1 \cdot (D + L)^2 + C_2 \cdot K
$$

(10)

where $C_1$ and $C_2$ are relative weighting coefficients.

Mathematically, the optimization problem has the form:

$$
\text{min } J(x_i) \text{ subject to } g_j(x_i) \leq 0, \text{ for } j = 1, \ldots, m, \text{ and } h_k(x_i) = 0, \text{ for } k = 1, \ldots, n.
$$

where the cost function $J$, the $m$ inequality constraints $g$, and the $n$ equality constraints $h$ are functions of $N$ variables, $x = (x_1, \ldots, x_N)$. The variables of interest are $D$, $H$, and $L$. The inequality and equality constraints, detailed in the following section, include the mechanical behavior during cell adhesion, material properties of the mPADs, practical values of the physical parameters of the posts, and the resolution of the optical imaging technology.

The corresponding Lagrangean of the optimization problem is:

$$
L(x_i) = J(x_i) + \sum \lambda_j g_j (x_i) + \sum \mu_k h_k (x_i)
$$

(11)

where $\{\lambda_j\}$ and $\{\mu_k\}$ are sets of Lagrangean multipliers corresponding to the inequality and equality constraints, respectively. At the optimal value of $\{x_i^*\}$, the Lagrangean is at its minimum value corresponding to $L'(x_i^*)=0$. Using partial differentiation of $L(x_i)$ with respect to $x_i$, a system of $N$ conditions on optimality arise. Together with other Karush-Kuhn-Tucker conditions, this system of $N$ equations allows for the calculation of $\{x_i^*\}$ that minimize the cost function [non-linear programming].
Optimization Constraints:

System Dynamics:

The deflection, \( \delta \), due to the applied force \( F \), is related by Hooke’s Law (1).

For a mPAD post of diameter \( D \), height \( H \), and Young’s Modulus \( E \) is given by:

\[
K = \frac{3\pi ED^4}{64H^3}
\]  

(12)

Material Properties:

The range of Young’s modulus of PDMS is 1-2 MPa within normal curing times. A stress analysis shows that the maximum stress applied to PDMS posts without causing permanent deformation is:

\[
2GPa = \sigma_y > \frac{1}{2} \left( \frac{L \cdot D \cdot F}{\frac{1}{64} \pi \cdot D^4} \right)
\]  

(13)

The yield stress is determined to be 2GPa for PDMS [28]. The geometric dimensions of the pillar and the selected material must result in a design that is stiff enough to withstand the lateral force of cellular adhesion while not deforming plastically. Stressing the pillars only in the elastic range allows for the experiment to be repeated several times with high precision.

Physical Parameters:

From the literature review, the ranges for the practical values of mPAD height and diameter are tabulated in Table 1. The center to center distance between adjacent posts, must be at least twice the maximum displacement of the posts, \( \delta_{max} \), so that adjacent posts to not interfere with each other.
Table 1: Allowable ranges of the physical parameters of mPADS

<table>
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<tr>
<th>Parameter</th>
<th>Range</th>
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<tbody>
<tr>
<td>Height ($H$)</td>
<td>$4 \mu m - 150 \mu m$</td>
</tr>
<tr>
<td>Diameter ($D$)</td>
<td>$100 nm – 5 \mu m$</td>
</tr>
<tr>
<td>Distance between posts ($L$)</td>
<td>$&gt; 2\delta_{max}$</td>
</tr>
</tbody>
</table>

Optical Detection Limits:

The resolution of the optical imaging technology is dependent on the type of microscope and the software package used to analyze the images. In literature, the resolution is on the order of 50nm [12].

Optimization Trends:

It is insightful to plot the various relationships between the post density, the spring constant, and the geometric and spatial parameters of the posts to arrive at the factors underlying the cost function optimization. Since the packing density is inversely proportional to $(D + L)^2$, maximizing the density corresponds to minimizing $D$ and $L$ (Figure 12). As the spring constant is proportional to $\frac{D^4}{H^3}$, minimization of $K$ corresponds to minimizing $D$ while maximizing $H$ (Figure 13).
Since the distance between posts, \( L \), must exceed twice the maximum deflection, \( L \) is inversely proportional to the spring constant; minimizing the spring constant corresponds to maximizing \( L \) (Figure 14). As the spring constant is a function of \( D \) and \( H \), there exists a relationship between the geometric parameters of the mPADs:

\[
\frac{3\pi D^4}{64H^3} > \frac{2F_{\text{max}}}{L}
\]  

(14)
From these relationships, it is evident that minimizing $D$ and maximizing $H$ will minimize the cost function. However, from Equation (14), $H^3$ is upper bounded by $D^4$ for constant $L$. Thus, as $D$ is minimized, $H$ will decrease as well. The optimal value of $L$ results from balancing the criterion of maximum packing density and deflection.

**Results**

A custom optimization algorithm in MATLAB 7.2.0 (Appendix) was used to perform the optimization for MDCK cells with cell adhesion forces ranging from 1-10 nano-Newton. Using weighting coefficients $C_1$ and $C_2$ that normalized the performance criterion relative to each other, the following results were found for PDMS posts with a Young’s Modulus of 2 MPa.
Table 2: Optimal parameter values for mPAD posts with maximum deflection and density.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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<tr>
<td>Spring constant ( K )</td>
<td>( 0.0100 \text{ N/m} )</td>
</tr>
<tr>
<td>Minimum deflection ( \delta_{\text{min}} )</td>
<td>( 0.1 \text{ ( \mu )m} )</td>
</tr>
<tr>
<td>Maximum deflection ( \delta_{\text{max}} )</td>
<td>( 1 \text{ ( \mu )m} )</td>
</tr>
<tr>
<td>Diameter ( D )</td>
<td>( 1.2141 \text{ ( \mu )m} )</td>
</tr>
<tr>
<td>Height ( H )</td>
<td>( 4 \text{ ( \mu )m} )</td>
</tr>
<tr>
<td>Distance between posts ( L )</td>
<td>( 2 \text{ ( \mu )m} )</td>
</tr>
<tr>
<td>Aspect ratio</td>
<td>( 3.2945 )</td>
</tr>
</tbody>
</table>

Given the assumptions for the force range and the chosen Young’s modulus of the pillar material, the final geometric dimensions are consistent with our optimization criteria. Our determined spring constant \( K \) is comparable with previously calculated values for PDMS pillars on the same dimensional scale. The minimum deflection corresponding to the lowest sense force is well within the range of the optical sensing devices. The maximum deflection is also easily detectable and will not cause the pillars to exceed the maximum yield stress. The pillar diameter is well within the manufacturable feature size, provides ample surface contact area for cell adhesion, and is small enough to maximize pillar density. The calculated height is high enough to isolate the cells from the base substrate and is reasonable for the manufacturing process—the feature size is small enough to neglect scallops and non-uniform base to tip diameters. The distance between the pillars is large enough to allow for the maximum lateral deflection of the pillars and close enough to prevent cells from falling between the pillars and touching the substrate. The aspect ratio is characteristic of an easily manufacturable structure.
MATERIALS AND FABRICATION

Given the calculated geometries, the microfabrication process and materials were selected accordingly. As past approaches to studying cellular adhesion forces have shown, the design will use an array of pillars to detect extremely small forces. The pillars of the device are made out of PDMS, a silicone based elastomer, for its desirable physical and chemical properties. PDMS is chemically inert, thermally stable, nontoxic, and can be made to be hydrophilic which makes this material ideal for many bioMEMS applications [33]. Additionally, PDMS is highly compressible with a wide range of stiffness that can be adjusted by cure time and ratio of curing agent to polymer [17]. Since the cells under observation with our device exert forces on the nano-Newton scale, the pillars must be flexible enough to deflect a reasonable amount to be detected. In addition to having a very low elastic modulus (0.87-3.6 MPa [17]), PDMS can conform to structures on the sub-micron scale [33]. Within the visible spectrum it is transparent. PDMS is also extremely cost efficient compared to single crystal silicon at only about $50 per pound including chemical processing [17]. For our application that requires a flexible material with cell-friendly surfaces, PDMS has been demonstrated to be the ideal material.
The process involved with creating mPADs has been detailed by several groups (Figure 15). Basically, pillars can be molded on the sub-micron scale by pouring PDMS into an etched silicon template. The manufacturing process is simplified by the low aspect ratio. The silicon template is created from a silicon wafer with the deep etching Bosch Process. First, the wafer is etched using conventional photolithography and a mask with circular holes corresponding to the pillar diameter [12]. This creates a shallow array of cylindrical holes which then can be deep reactive ion etched (DRIE) with Cl₂/BCl₃ anisotropically which can be used to form the high aspect ratios. The walls of the holes are coated with a 0.3 micron passivation layer deposited with PECVD so that the pits can be selectively etched. The pits are alternatively etched and then passivated to create high aspect ratio features. Although etch selectivity is greatly improved with the Bosch process, the surface of the cylinder walls is not perfectly smooth. Rather, the process creates scalloped features which were caused by alternating between an etching gas such as sulfur hexafluoride (SF₆) and a passivation gas such as octafluorocyclobutane (C₄F₈) (Figure 16b). Unfortunately, these scallops decrease the flexibility of the pillars and
hence the sensitivity [34]. The mold should also include a shallow etched area above the array of holes for a base to be formed. After the desired etch depth is reached, the wafer is cleaned and treated with a chemical vapor that facilitates the release of cured PDMS from the mold. This treating process is called silanization and uses tridecafluoro-trichlorosilane vapor [12].

![Figure 16: a) ideal pillar. b) scalloped surface profile. c) conic pillar shape [23]](image)

PDMS is commercially available from Dow-Corning in the form of Sylgard 184 liquid silicone prepolymer. Dow Corning claims this variety of PDMS has a tensile strength of 6.2 MPa, well above the stresses caused by the cellular adhesion forces [28]. This substance can be poured over the mold and will fill the etched cavities and shallow base area. The PDMS mixture is cured overnight or for 12 hours between 65°C and 100°C which will theoretically give the pillars the desired stiffness, although this has to be refined after testing [6, 17, 29]. The PDMS structure can then be peeled back from the mold as it is extremely flexible. Although the silanization process decreases the damage caused by removing PDMS from the mold, the pillars will have a slight conic shape caused by extraction (Figure 16c). Like the scallops, this conic shape will slightly decrease the flexibility of the pillars [34]. The severity of these structural defects is greatly reduced and the pillar is much closer to the ideal model. Pillars with diameters as small as 1 to 4 µm have easily been created with this process, and theoretically PDMS can make up features as small as 100 nm if needed [17, 34]. Since the PDMS base would deform a great deal when forces were applied to the pillars because of its low elastic modulus, a single crystal silicon layer is used for reinforcement. PDMS exhibits
excellent adhesion to single crystal silicon and can be easily “soft bonded” with surface contact at room temperature and regular pressure [1]. Groups have tested cells exhibiting forces on the micro-Newton scale on structures with aspect ratios as low as 2:1 and the base substrate is undeformed in this configuration [34]. Cells do not naturally adhere to PDMS so the structure must be coated with a substance to which proteins can adhere. In order to accomplish this, the structure is oxidized and sterilized in an air-plasma that makes the PDMS surface hydrophilic and hence will absorb fibronectin [19]. Fibronectin is a type of high molecular weight protein that will bind to the receptor proteins in the cell. Submersing the structure in a fibronectin solution will promote cell adhesion to it.

In order to detect tip deflection optically, the pillars must be labeled with fluorescent markers. To do this, flat PDMS stamps are created for the top surface of each pillar. These stamps are treated with an oxygen plasma as with the rest of the structure and are incubated with fluorescently labeled fibronectin through microcontact printing for one hour [22] (Figure 17). After the stamp dries, it is placed in contact with the array surface and coated in a layer of normal fibronectin so there is a homogenous makeup of extracellular matrix protein.

Figure 17: a) Microcontact printing of fluorescent markers [19] b) labeled pillars [25]
This fabrication process will produce reasonable geometric results but the mechanical characteristics of the structure will vary (Figure 18). The Young’s modulus of PDMS has to be detected and the spring coefficient, k, has to be found through testing (Figure 19). This calibration can be achieved by manipulating the pillars with a glass micro-pipette or AFM tip of known modulus and spring constant [29].

The Madine–Darby canine kidney cells (MDCK) intended for use with our device can be obtained with a previous culturing procedure. Medium containing cells are mixed with a reconstructed soluble collagen and temporarily suspended in a CO2 independent medium [7]. The collagen-cell solution has a final cell density of 1x10^5 cells/mL. This solution is...
poured into a bottom tissue culture dish previously coated with collagen or fibronectin. The solution is incubated for one hour at 37°C and covered with an additional layer of the cell culture solution [7]. Multiple cells are obtained from the culture and can be observed simultaneously (Figure 20).

Figure 20: Multiple cells observed [29]
OPTICAL SENSING

After fabrication, the device must be calibrated for experimental use. A calibration of the mPADs is performed using SEM to determine exact pillar geometries and non-deflected pillar locations. After placing the cells on the mPADs, they are imaged using a phase-contrast microscopy with a charge-coupled device (CCD) camera. The cells are maintained at a temperature of 37°F and provided necessary nutrients. The images are collected at a steady rate for 4-6 hours [17]. To ensure good images the microscope was focused on the fluorescently labeled tops. Then either laser confocal microscopy or epifluorescence microscopy is used to identify the locations of the deflected pillars. The pictures are collected using a CCD camera, and imaging software such as Openlab [17].

![Figure 16: a) pillar tip deflections relating to force vectors b) close-up of photographed displacement](17)

The collected images were placed into a custom Matlab software package to determine the pillar locations, cell area, and cell edges. Commercial software packages are also available to determine deflection, and forces [17]. Black and white images are generated from the fluorescence images to remove background noise and determine the edges of the posts. There are several algorithms to reach this, each with different advantages and disadvantages. The most basic involves manually labeling the post edges [17]. Though it is very accurate at detecting posts, it is very tedious due to large number of posts, and was easily biased due to the user.
Figure 17: a) Original optical image was captured using a phase contrast inverted microscope. b) The estimated background intensity in pseudo-color shows the nonuniform noise. c) The position for individual structures was achieved after background deduction. d) The displacement of each structure was derived when compared with a reference array, and the displacement mapping was derived. The force mapping was consequently achieved upon multiplication with determined spring constant [23]

Other methods involve using raw fluorescence images and setting limits to remove background, then determining the post edges and finally the post center. The accuracy of this method varies depending on the exact implementation, the size of the limit area, and better determinations of the thresholds [17]. After the deflections are determined force analysis packages are run to determine forces, and produce vector plots. Various packages are available from commercial to free. One such package for MATLAB is available from Johns Hopkins [17]. Based on various microscopy methods, and image software used displacement resolutions of down to 50nm can be achieved [6].
CONCLUSION

The final design obtained from the optimization of geometric parameters, pillar density, and selected material properties was well within our confines determined by the range of cellular adhesive forces for MDCK cells and optical sensing resolution. The device design is theoretically easily manufactureable as it has a low aspect ratio and feature sizes consistent with standard PDMS applications. The success of our optimized design suggests that using mPADs to measure cellular force adhesion is the most effective and practical method available while maintaining a reasonably low cost and simplicity of design. Future advancements using this design as a basis include three-dimensionally mapped force vector fields given simultaneous improvements to analysis software.


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Aspect Ratios From a Single Template for Cellular Force Measurements." Micro Electro 
**BIOGRAPHIES**

**Richard Besen** (September 22, 1985) is a junior mechanical engineering undergraduate student at Northwestern University from North Andover, Massachusetts. He is also a DJ on the school radio station and musician. In the future, Richard hopes to start his own company with a casual dress code. Also, he has never lost a game of Connect Four.

**Albert Leung** was born outside of San Francisco, in Fremont, California. He will receive his Bachelor of Science in Mechanical Engineering from Northwestern University in 2007. Albert has previously worked for Bipolarics, the Northwestern Physics Department, and for Motorola. His interests of research are in MEMS and NEMS applications in the communications industry.

**Feng Yu**, from Lexington, Kentucky, is a Mechanical Engineering junior at Northwestern University. After graduation, June 2007, he intends to attend graduate school. After which he intends to pursue a career in the aerospace industry preferably in the field of autonomous aerial vehicles. He is currently a participant in the 2006 Design Competition at Northwestern so he can gain real world experience in the field of autonomous robotics.

**Yan Zhao** received her Bachelor of Engineering in Electrical Engineering from McGill University, Montreal, Quebec in 2006. Her research at the Haptics Laboratory focused on sensorimotor performance in the design of haptic interfaces. She is currently a doctoral student in the Department of Biomedical Engineering at Northwestern University. Her research interests include nanoscale neuroprosthetics and instrumentation for neurophysiology.
DELEGATION OF TASKS

The tasks were broken up as follows:

Abstract
Albert Leung, Yan Zhao, Rich Besen

Introduction
Yan Zhao, Albert Leung

Literature Review
Albert Leung

Proposal
Richard Besen, Albert Leung, Feng Yu, Yan Zhao

Geometry Analysis
Feng Yu

Dimensional Analysis and Optimization
Yan Zhao

Materials and Fabrication
Richard Besen

Optical Sensing
Feng Yu
DIMENSIONAL ANALYSIS AND OPTIMIZATION

MATLAB Program

%Optimization of geometric parameters of mPAD
options=optimset('MaxFunEvals',1e6,'MaxIter',1e5,'Display','iter','TolCon',1e-16);
%options=optimset('MaxFunEvals',1e6,'MaxIter',1e5,'TolCon',1e-16);

force=[1e-9,10e-9]; %canine kidney
favg=(force(1)+force(2))/2;
force=[force favg];
E=2e6; % E PDMS

%initial guess
D0=(5e-6-100e-9)/2;
H0=(150e-6-4e-6)/2;
k=(3*pi*E*D0^4)/(64*H0^3); % k=3piED^4/64H^3
davg=force(3)/k;
L0=(2*davg-H0)/2;
x0=[D0,L0,H0];

[x,FVAL,EXITFLAG,OUTPUT,LAMBDA]=fmincon(@(x)myfunc(x,force,E),x0,[],[],[],[],[],[],@(x)mycon(x,force,E),options);

function f=myfunc(x,force,E)
k=(3*pi*E*x(1)^4)/(64*x(3)^3); % k=3piED^4/64H^3
dmin=force(1)/k;
f=1e11*((x(1)+x(2))^2)+1e2*k+(dmin-20e-9);

function [c, ceq]=mycon(x,force,E)
%variables: [D, L, H]
k=(3*pi*E*x(1)^4)/(64*x(3)^3); % k=3piED^4/64H^3
dmax=force(2)/k;
dmin=force(1)/k;

c=[100e-9-x(1)
 x(1)-5e-6
 2*dmax-x(2)
 4e-6-x(3)
 x(3)-150e-6
 1.5-x(3)/x(1)
 100e-9-dmin
 (x(2)*force(2)*x(1)/2)/(pi*x(1)^4/64)-2e6 % max stress]

ceq=[];