# Chemical Recognition in Cell-Imprinted Polymers

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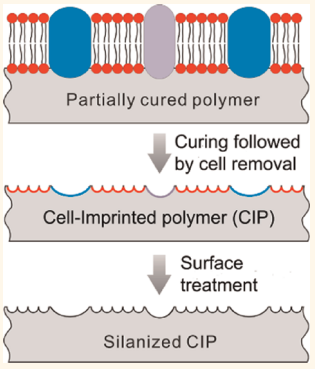
**ABSTRACT** A glass slide covered with bacteria is pressed into another glass slide coated with partially cured polydimethylsiloxane (PDMS). The PDMS is hardened and the cells are removed to create a textured surface whose indentations preferentially capture the same type of bacteria when a mixture of bacteria is flowed over it. Overcoating the cell-imprinted PDMS with methylsilane groups causes the resulting surface to lose much of its ability to preferentially capture the imprinted bacteria, although the shapes of the imprints, measured by atomic force field microscopy, are shown to be hardly affected. We interpret this behavior as strong evidence that chemical recognition plays a dominant role in cell sorting with cell-imprinted PDMS polymer films.




**KEYWORDS:** PDMS · self-assembly · bacteria sorting

The history of what is called "molecular imprinting" seems to be a bit convoluted.[1,2] It seems to have been based on the mistaken idea Pauling[3] put forward to explain how antibodies are made, which involves a protein antibody self-assembling about an antigen template. Pauling suggested to his postdoctoral student Dickey that he prepare substrate-selective adsorbents. Dickey precipitated a mixture of silica gel and the dye methyl orange, which when washed out of the silica gel caused the silica gel to show an increased affinity for the dye template.[4] Many studies followed, but the selectivity was not particularly good and seemed to degrade with time,[5] and there was a problem of reproducibility. The next significant advance in molecular imprinting was made in 1972 by Wulff and Sarhan[6] who used synthetic organic polymers to trap, covalently bond with, and imprint molecules. Independently, another important advance was made in 1981 by Arshady and Mosbach[7] who demonstrated a noncovalent bonding approach to create an imprint of the template molecule within the polymer matrix. By the early 1990s the field of molecular imprinting of polymers was rapidly growing.[1,2] Further successes involved the application of polymer imprinting to objects having a larger and more varied surface area, such as viruses, bacteria, and cells.[8,9] In molecular imprinting, the molecules to be imprinted are first allowed to form a complex with the polymerizable entities, which are subsequently cured; after removing the template molecules specific recognition sites are left in the polymer, which are complementary in size and shape to the analyte.[1,2] One question that has remained not completely settled is how the recognition works. It seems to be a combination of physical shape selectivity and chemical recognition. This work investigates this question for cells and reaches the conclusion that chemical recognition plays the dominant role when polydimethylsiloxane (PDMS) is used as the polymeric cross-linking network.

One way to learn about what makes the polymer imprinting mechanism selective is to investigate its use to resolve a racemic mixture of enantiomers. In a series of experiments, Wulff and co-workers[1] polymerized a series of similar monomers with various arrangements of the functional groups or with different spatial properties and determined the resolving power for various sugar racemates when the template sugar enantiomer was removed. It was concluded that "the arrangement of the functional groups in the cavity is the decisive factor for the selectivity, while the shape of the cavity is somewhat less important." The approach of Wulff and co-workers involves the creation of covalent bonds between the molecule template and the polymer network, which are subsequently cleaved. Mosbach and coworkers followed a noncovalent approach which was argued to be more versatile.[2] For example, Ramström, Andersson, and Mosbach[10] demonstrated higher selectivity for carboxylic acids by using a judiciously

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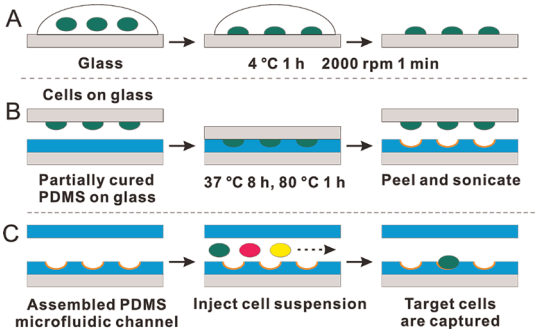


**Figure 1. Schematic diagram of the cell imprinting process and its application for cell sorting: (A) preparation of the template; (B) polymer imprinting with the cell template; and (C) cell sorting with the CIP microfluidic chip.**

chosen mixture of monomers. Thus, the importance of chemical recognition cannot be denied, but it remains unclear from past studies how chemical recognition compares with physical shape selectivity for larger objects such as viruses, bacteria, and cells. The goal of this study is to settle this matter in the case of cell-imprinted PDMS, which may be taken as typical of other polymeric networks.

## RESULTS

In our experiment, bacteria were selected to be representative of the two Gram-staining groups; in each group, two species similar in shape and size were included. Among them, the two Gram-positive coccus, *Staphylococcus epidermidis* (*S. epidermidis*) and *Staphylococcus aureus* (*S. aureus*) are grape-like in shape with diameters of ~800 nm, whereas the Gram-negative bacillus, *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae (K. pneumoniae)* are rod-shaped, ~2.0 $\mu$m long and ~0.5 $\mu$m in diameter.[11]

Figure 1 presents the process flow of the experiment we carried out. We prepared a cell-imprinted polymer (CIP) using a surface imprinting method described previously.[8] Buffered cell suspensions of each type of bacteria were spread respectively on glass slides and kept at 4 °C for 1 h. The buffer was then removed by spinning at 2000 rpm to avoid salt crystal formation, leaving the attached cells on the glass substrate, which was then used as the template stamp. We pressed the stamp into a partially cured PDMS membrane and let the polymer cure at 37 °C for 8 h, followed by 80 °C for 1 h. The PDMS kit we used contains a mixture of vinyl-terminated PDMS oligomers, cross-linkers of polysiloxanes with vinyl and hydrogen groups, and residual ingredients including octamethylcyclotetrasiloxane, benzene, toluene, and ethylbenzene.[12] During the curing under mild temperature, these precursors formed complexes with the cell surface and adjust their positions to reach the lowest energy. The resulting arrangement of the polymer networks was fixed by curing at 80 °C. After that, we peeled off the stamp and removed possible cell residue on the PDMS surface by sonication. Using fluorescently labeled template cells we verified that no residue remained on the CIP surface. The success of this removal procedure can mainly be attributed to the inertness of the PDMS surface.

**TABLE 1. Hydrophobicity and Nonspecific Affinity of Native and Silanized PDMS Surfaces[a]**

| substrate | advancing water contact angle | captured cell number (mm$^2$) |
|---|---|---|
| native PDMS | 104 ± 1 | 3500 ± 200 |
| alkylsilane group modified PDMS | 105 ± 1 | 2400 ± 400 |
| anilinesilane group modified PDMS | 42 ± 2 | 61000 ± 700 |
| fluorosilane group modified PDMS | 107 ± 1 | 400 ± 100 |

[a] Advancing contact angle of water and nonspecific cell adhesion test results of native PDMS and PDMS modified using methyltrichlorosilane, aminophenyltrimethoxysilane, and (tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-trichlorosilane, which introduced alkylsilane, anilinesilane, and fluorosilane groups, respectively. Nonspecific affinity to cells was measured with the microfluidic method similar to the cell sorting experiment, using 20-$\mu$L injections. The uncertainty in the advancing water contact angle and the captured cell number is one standard deviation based on four measurements.

To compare CIPs with and without chemical recognition, we modified some of the CIP microfluidic chips with different silanes. Silanization was carried out in a desiccator immediately after $O_2$ plasma treatment. The vapor of different silanes link to the hydroxyl groups generated on the PDMS by $O_2$ plasma, forming a uniform monolayer of desired groups on the PDMS surface.[13] We found that the silanization could produce surfaces with varying degrees of hydrophobicity and nonspecific cell affinity (Table 1). Among the silanes investigated (shown in Figure 2) methyltrichlorosilane produced a surface most closely resembling unmodified PDMS. We chose to use this silane in all subsequent experiments.

The morphology of the cavities on this surface are essentially preserved, as the coating thickness is calculated to be on the order of 1 nm. This assertion was verified using atomic force field microscopy (AFM), as shown in Figure 3. Therefore, we believe the modification process using methyltrichlorosilane achieved our goal of removing chemical recognition while maintaining the physical size of the imprints.

We compared the recognition performance of the CIPs before and after silanization (see Table 2). Two groups of bacteria were tested as representatives of Gram-positive and Gram-negative bacteria. In each group, two species similar in shape and size were included for testing. The cell capturing experiment was carried out in microfluidic channels. We covered the CIP surface with an array of 30-$\mu$m-deep microchannels, which assist the cells in the suspension to make better contact with the CIP surface. The cell

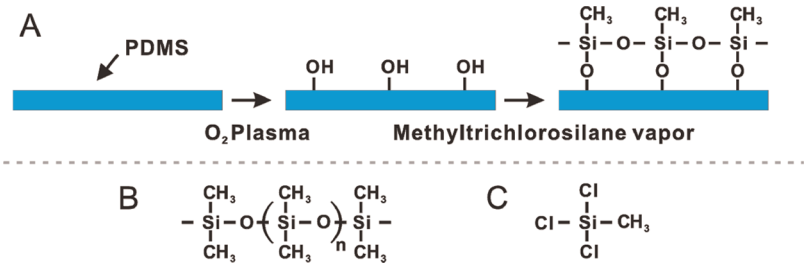


Figure 2. Silanization of the CIP chip: (A) the chemical process; (B) the structure of PDMS; and (C) the structure of the most effective silane used.

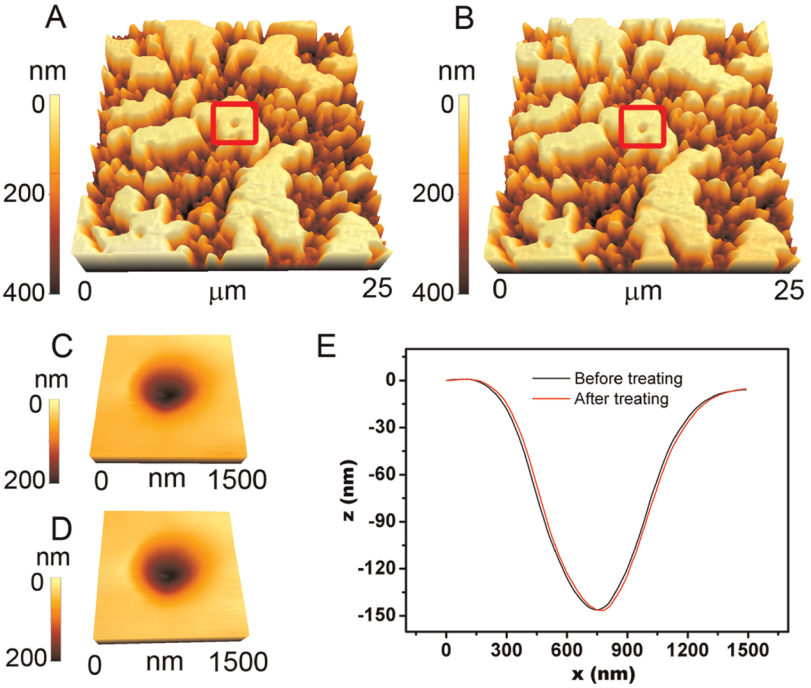


Figure 3. AFM images of a same location on the PDMS surface containing imprints of *Staphylococcus aureus*: (A) before silanization, and (B) after silanization. Close-ups of the areas marked with red squares in panels A and B are shown in panels C and D and the profiles of these areas are presented in panel E.

**TABLE 2. Selectivity of Capturing Templated Bacteria with the Cell-Imprinted Surfaces before and after Silanization[a]**

| | ratio of template imprinted to average of other imprinted | |
|---|---|---|
| bacteria | unmodified | modified |
| *E. coli* | 1.8 ± 0.5 | 1.1 ± 0.1 |
| *K. pneumoniae* | 1.8 ± 0.4 | 1.3 ± 0.1 |
| *S. epidermidis* | 2.0 ± 0.3 | 1.3 ± 0.3 |
| *S. aureus* | 2.0 ± 0.4 | 1.2 ± 0.2 |

[a] The calculation is based on the ratio of numbers of bacteria captured on their imprints to the average number captured on the imprints of the other bacteria. The uncertainty represents one standard deviation obtained from four replicate measurements.

suspensions were flowed over the imprints. For each test, we injected a 50 $\mu$L cell suspension ($OD_{600}$ = 0.2) at a flow velocity of ~0.2 mm/s, then rinsed the channel with 50 $\mu$L of phosphate buffered saline (PBS) at the same flow rate to avoid random settling of suspended cells on the CIP surface. For visualization, the cells were stained with CellTracker Orange, a fluorescent dye that labels the cell's interior while leaving the cell membrane unaffected.[14] Then, the imprinted surfaces were inspected under a confocal microscope.

Figure 4A shows the selectivity achieved for the unmodified CIPs. We find that the selectivity between the Gram-positive and Gram-negative groups is stronger than the selectivity between the cells in a same group. The two Gram-positive species used are sphere-shaped, whereas the two Gram-negative species are rod-shaped. In addition, some selectivity was also observed between the closely related species in the same Gram groups. However, after silanization (Figure 4B), both the intergroup and the intragroup selectivities are significantly decreased (see Table 2).

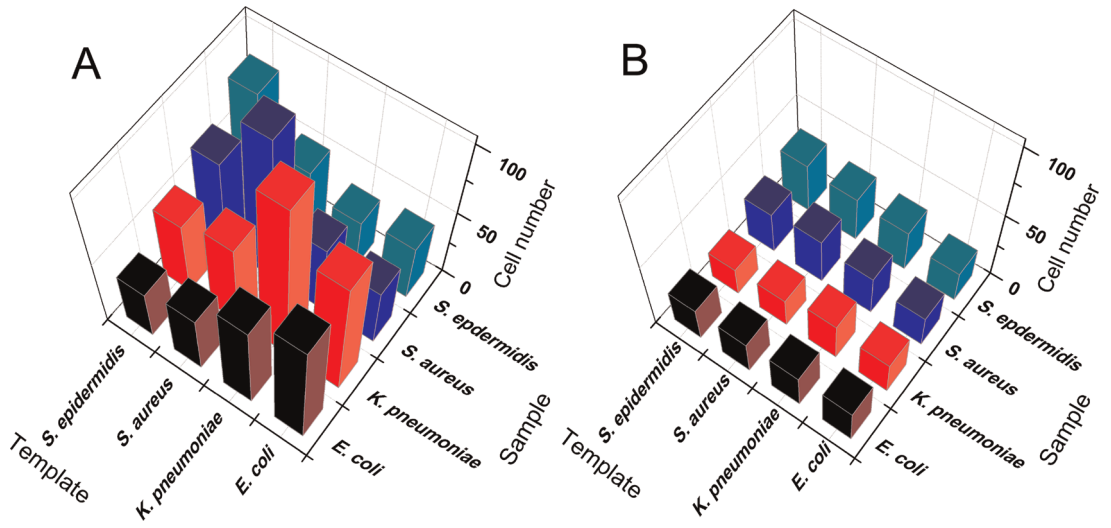


**Figure 4. Numbers of different cells captured on CIPs imprinted by different bacteria for (A) unmodified CIPs and (B) the corresponding silanized CIPs.**

## CONCLUSIONS

As discussed above, silanization hardly affects the surface morphology (on the order of 1 nm change), but it does convert the chemical properties of the surface to be like native PDMS. We believe that Figure 4 presents strong evidence that not only is chemical recognition important but it represents the dominant mechanism for cell selectivity. Nevertheless, as evidenced by the weak intergroup selectivity remaining after silanization, we also believe that physical shape selection is occurring, but to a minor extent. Examination of Table 2 shows that CIPs made with one type of bacteria template are able to select the same bacteria in a mixture with a selectivity that is about a factor of 2 greater than for other bacteria. But silanization of the CIP surface reduces this selectivity to almost no preferential selection. Although it may take more than one sorting cycle to capture and release a nearly pure bacterial strain, the dominant role of chemical recognition seems clearly established by this study.

## EXPERIMENTAL METHODS

**Materials.** *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 33495), *Staphylococcus aureus* (ATCC 29213), and *Staphylococcus epidermidis* (ATCC 12228) were obtained from Niaz Banaei, Medical Center, School of Medicine, Stanford University. PDMS was obtained from RS Hughes. CellTracker Orange was purchased from Invitrogen and used following the protocol given by the vendor. All other chemicals were purchased in highest or analytical grade from Sigma Aldrich or VWR.

**Cell Handling.** All cell strains were cultured on LB agar plate at 37 °C in an incubator. Before the experiment, fresh culture was harvested and rinsed using PBS by centrifuging at 1200 G and 4 °C. $OD_{600}$ was used to measure the cell density in a suspension. For visualization, we stained the cells with CellTracker Orange following the protocol suggested by the vendor.

**Template Preparation.** A 10 $\mu$L cell suspension (approximately $10^9$ cells/mL) was spread on the surface of microscope slides and kept at 4 °C for 1 h. After the cells settled onto the glass surface, the excess solvent was removed by spinning the slide at 2000 rpm for 1 min.

**Stamp Fabrication.** Optimization of the imprinting protocol was conducted and discussed in our previous work.[8] Briefly, we diluted a PDMS curing mixture (monomer/cross-linker = 10:1) using cyclohexane to a volume ratio of 2:1, and spin-coated this solution onto a microscope slide (30 s at 1500 rpm). After precuring the PDMS at 80 °C for 4 min, we pressed the template stamp into the prepolymer and kept the stack at 37 °C for 8 h, followed by curing at 80 °C for 1 h. After that, we peeled off the template slide and cleaned the imprinted polymer film by submerging it in a Petri dish filled with distilled water and sonicating for 5 min.

**Surface Silanization.** The imprinted PDMS slides were treated with a plasma cleaner (Harrick PDC-32G) using 18-W coil power for 10 s, at an air pressure of 200 mTorr. After that, the slides were immediately put into a desiccator, with a test tube containing 10 $\mu$L of silane in it and sealed for 3 h. Finally, the slides were cleaned by sonicating in water for 1 min. The substrates were then inspected with a scanning probe microscope (XE-70, Park Systems) using noncontact AFM mode.

**Cell Sorting.** PDMS chips containing an array of microchannels were fabricated *via* standard soft lithography. Each channel was 30-$\mu$m in height and 100-$\mu$m in width. The total volume of the channels was about 1 $\mu$L. The chip was reversibly bonded to the imprinted substrate by the adhesion between PDMS surfaces without a heating or plasma treating process. A pipet tip was inserted into the inlet of the channel as a reservoir and was filled with cell suspension. A syringe was connected to the outlet of the channel, to draw the cell suspension through the channel *via* negative pressure. For each test, a 50 $\mu$L cell suspension ($OD_{600}$ = 0.2) was infused at a flow velocity of 0.2 mm/s. Then 50 $\mu$L of PBS was used to rinse the channel at the same flow rate. The imprinted area of the chip was inspected under a confocal microscope (TCS SP2, Leica).

*Conflict of Interest:* The authors declare no competing financial interest.

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