Communications to the Editor

Patterned Hexagonal Arrays of Living Cells in Sol−Gel Silica Films

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The formation of templated sol−gel materials possessing long-range order has attracted much recent attention. In general, templates are arranged into an ordered array by external mechanical force or self-assembly and the formation of the surrounded framework is established by the polymerization of inorganic or organic monomers. In the nanoscale regime, molecular additives lead to a microporous zeolite structure while larger mesoporous materials have been prepared by using surfactants or emulsion droplets as templates.[1−6] Latex and silica spheres or block-copolymers can also serve as templates and result in an ordered porous structure with a pore size up to a few microns.[7−15] Although patterns at micron or even submicron scales can be made by photolithography, they are costly, time-consuming, and generally incompatible with living cells and their associated biochemical functionality. Utilization of natural self-organization such as a preorganized bacterial superstructure[16,17] or, in our case, cell aggregates provides a fast and inexpensive resource.

The mild conditions (i.e., pH and temperature) of the sol−gel process provide a way to immobilize microorganisms while retaining their bio-functionality in silica matrixes. After immobilization, the cells can be lyophilized or pyrolyzed to remove some or all of the organic material and leave behind voids in the silicate matrix. Alternatively, intact cells at known positions in an addressable array in the matrix can find applications in screening genomic libraries. Both dead and living cells are of interest; living cells retain all of their biofunctionality whereas dead cells may still retain active biomolecules that have specific applicability.[18] The demonstrated stability of yeast cells in the silicate sol[19−22] and their roughly spherical shape make them good candidates as templates for patterning.

Dip-coating in conjunction with surfactant-templating has been applied to the production of continuous, macroscopic films as thin as ∼0.1 μm that contain long-range mesostructural order.[23] The decrease in the dimensionality of the film during drying and shrinkage could cause particles that are initially contained in the sol to pack in regular arrays. Spherical particles minimize their volume when they pack in hexagonal arrays. In this communication we show that when the particles are living cells of uniform diameter, the simple method of film pulling produces a two-dimensional hexagonal array of ordered cells in a silica film.

Monodispersity of the templates has a direct impact on the quality of ordered materials. For this study, efforts were made to grow yeast cells with as uniform a size distribution as possible.[24] Exposing cells to nutrient starvation blocks cells at a specific point in the early G_1 phase prior to formation of a bud and drives them into G_0. Cells that have progressed beyond this point in the cell cycle will complete the cell cycle then enter G_0 where cells are generally unbudded spheres. The films were prepared by dip-coating microscope slides with a mixture of sol[25] and yeast solution (1:1 vol) at a pulling rate of 1.9 mm/s. The backside of the slide was in constant contact with the wall of the reservoir to stabilize the movement.

Hexagonal close-packed arrays of cells (Figure 1) readily assemble as a result of dip coating. Typical aggregates contain more than 500 cells. Defects in the long-range structure, shown in the center and the upper left of the photograph, are caused by a nonuniformity in cell size. Scanning electron microscopy (SEM) shows that no hexagonal deformation of cells due to compression by the adjacent cells occurs (Figure 2). The imbedded cells appear to be almost perfect hemispheres in an ordered monolayer array.

The immobilized cells in the silicate matrix are not killed by the dip-coating process. When films containing immobilized cells are placed on filter paper soaked with YPD followed by 3 h of incubation at 30 °C, budded cells are observed. Both fresh films and dry films that are stored in air at room temperature for up to 3 days contain a large portion of viable cells.

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(24) The yeast strain used for these experiments was W303-1A (MAT**a** *his3 leu2 trp1 ura3 ade2 can1*). W303-1A cells were grown for 1 week in 50 mL of YPD (1% Yeast Extract, 2% Peptone, 2% dextrose) in an orbital shaker set at 30 °C and 300 rpm. After 1 week of incubation, the cells had utilized the nutrients in the media and had arrested as uniformly round unbudded cells (G_0 phase of the cell cycle). The saturated culture in YPD contained about $5-10 \times 10^8$ yeast cells/mL.
(25) The sol was prepared by refluxing 10.00 g of tetraethyl orthosilicate (TEOS), 8.86 g of ethanol, 13.83 g of distilled water (molar ratio of 1:4:16) and 200 μLof 2 N HCl at ca. 70 °C for an hour followed by aging for a minimum of 4 h.

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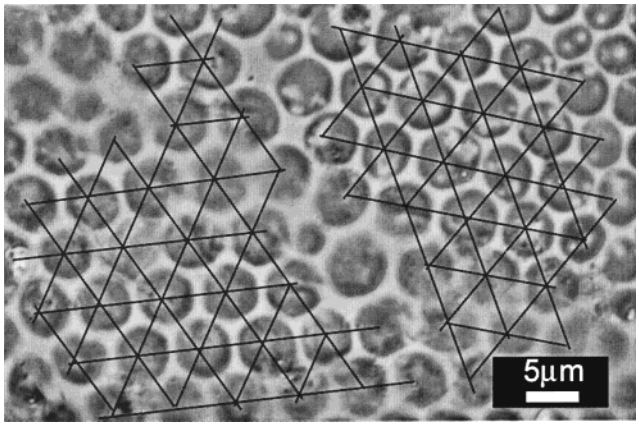

Figure 1. Photograph at 1000× magnification of a silicate film containing a monolayer of hexagonal close packed yeast cells. The ordered regions are indicated by lines; defects caused by the presence of smaller cells are found in the center and upper left of the image.

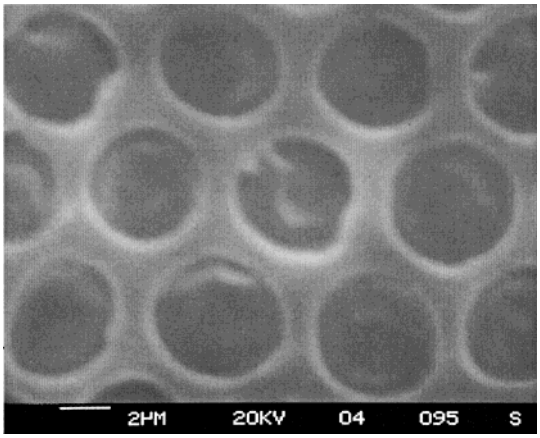

Figure 2. Scanning electron microscopy image of the silica films templated by spherical cells.

Atomic force microscopy (AFM) reveals that the positions originally occupied by the cells are depressions or craters. The AFM tip likely damages the imbedded cells during scanning; only craters are found even in the fresh silicate films with viable cells. The depth of the craters is about the same as the cell radius and indicates that the cells are only partly imbedded in the silica film.

The formation of the two-dimensional hexagonal close-packed structure is probably the result of the shrinkage of the film during formation and the shear forces that occur during film-pulling. Aggregation and compaction to minimize the volume occupied by the cells lead to a hexagonal close-packing array. In the case of surfactant/inorganic composite materials, the assembly process is thought to be governed by electrostatic complementarity between the ions in solution; the molecules with opposite charges interact directly while the interaction between similarly charged molecules is mediated by counterions.[26] The negatively charged silicate matrix (pH >2) probably interacts with cell membranes in a similar manner and establishes the interstitial framework. The shear force between the back of the slide and the reservoir may contribute to the monolayer assembly since the largest structures are found at the bottom of the backside of the slides.

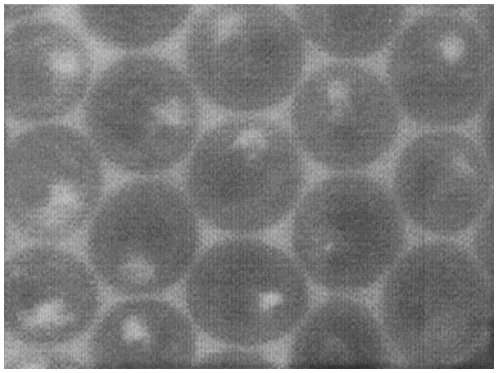

Figure 3. Photograph of the luminescence of cells stained with DAPI taken at 1000× magnification.

Accessibility of the cells in the silicate films is required if they are to be used as reactors, catalysts, or sensors. The porous silicate framework is well-known to allow molecular diffusion to occur; substrates for encapsulated enzymes readily diffuse and the solid material can be used to make biosensors.[27−30] To demonstrate the accessibility of the cells in the silicate film, DAPI,[31] a DNA-specific fluorescent molecule with a 20-fold increase in its fluorescence quantum yield when bound to DNA, is used as a probe.[32] The air-dried films are treated with the staining solution for 15 min and then washed with distilled water. In Figure 3, the luminescence inside the templated structure shows that the cell functionalities are sustained (even in the dead cells) and are accessible to external reagents.

A potential important application of the one-step self-assembly process is to use the ordered two-dimensional array of immobilized living yeast cells to express a library of gene products. The addressability with a resolution of microns would allow a specific cell containing the gene product of interest to be identified and manipulated. Using this system, it will be possible to screen libraries based on color or fluorescence. For example, a library of coding sequence representing an entire genome fused to green fluorescent protein (GFP) could be screened by fluorescence. A genomic library could also be screened based on the expression of a reporter gene, such as β-galactosidase,[33] which produces an easily detectable color change. This approach can be potentially automated to eliminate massive gene screening and selection processes.

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