Laser-guided direct writing for applications in biotechnology

David J. Odde and Michael J. Renn

Laser-induced optical forces can be used to guide and deposit 100 nm – 10 μm-diameter particles onto solid surfaces in a process we call 'laser-guided direct writing'. Nearly any particulate material, including both biological and electronic materials, can be manipulated and deposited on surfaces with micrometer accuracy. Potential applications include three-dimensional cell patterning for tissue engineering, hybrid biological–electronic-device construction, and biochip-array fabrication.

Laser-induced optical forces arising from the scattering of light by microscopic particles are widely used for the noncontact manipulation of biological particles. Arthur Ashkin, a pioneer in optical-force-based particle manipulation, first applied optical forces to levitate aerosol droplets and dielectric spheres[1]; with co-workers, he also later demonstrated the optical manipulation of a variety of biological and inorganic materials in aqueous suspension[2–5]. Optical forces are now commonly used to provide noncontact manipulation of cells, subcellular components and biomolecule-coated particles in a configuration known as an 'optical trap' or 'laser tweezers'[6–10].

Despite its ability to provide the control of cell position necessary for surface patterning, optical trapping has not been applied extensively to microfabrication. Its unpopularity for microfabrication is largely because using an optical trap to deposit particles on surfaces is tedious, requiring repeated cycles of particle capture in the fluid phase, transport through the fluid, deposition on a solid surface and release. Furthermore, the small trapping volume of conventional optical traps severely limits the number of particles that can be manipulated at one time.

Given these limitations, optical forces do not seem to be well suited to both the micrometer-scale positioning accuracy and the high-throughput deposition rates that direct-write microfabrication demands. However, by simply changing the laser-beam focus to be more like that originally used by Ashkin, we have found that optical forces can be used to manipulate thousands of particles simultaneously and to deposit them in a continuous stream onto surfaces with micrometer accuracy[11,12]. In addition, we have found that hollow-core optical fibers can aid optical-force-mediated particle guidance[11,13,14], creating new opportunities for the laser-guided direct writing of a wide variety of materials.

Physical basis of particle manipulation by optical forces

The model for particle–light interaction depicted in Fig. 1 was first proposed by Ashkin and is the working model for the ray-optics regime in which the particle is larger than the wavelength of the light[1,15]. The key physical property defining the interaction between the light and the particle is the refractive index of the particle relative to that of the surrounding fluid: the larger the difference, the stronger the interaction. When a particle interacts with the light, it is simultaneously pulled to the center of the beam, where the intensity is maximal, and pushed axially in the direction the beam is travelling. Using a weakly focused laser beam (i.e. a low-numerical-aperture focusing lens), Buican *et al.* demonstrated the guidance of living cells in an aqueous fluid for cell sorting[16]. If an appropriate target surface is placed in the light path then particles can be continuously guided and deposited in a steady stream onto the target surface (Fig. 2).

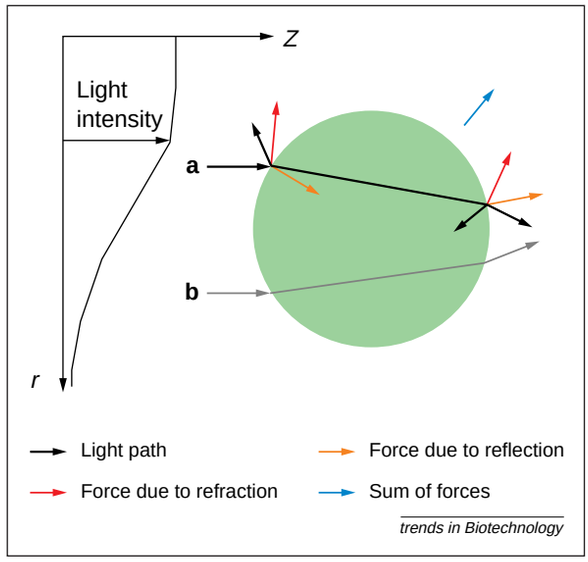


Figure 1

Optical forces on a dielectric particle. Laser light [whose intensity (*Z*) is shown varying across space (*r*) in the graph] is reflected and refracted at each interface, resulting in a redirection of the light. Photons have momentum and so their redirection by interacting with the particle results in a corresponding momentum transfer to the particle. The net result of the interactions from ray **a** is to push the particle along the beam axis and to pull the particle radially inwards. By symmetry, ray **b** pushes the particle axially and pushes the particle radially outward. However, ray **a** is stronger than ray **b**, so it overcomes the radial force directed outward. In the absence of other forces, the particle is simultaneously pulled radially inward and pushed axially in the direction of the laser beam.

D. J. Odde (odde@lenti.med.umn.edu) is at the Department of Biomedical Engineering, University of Minnesota, Minneapolis, MN 55455, USA. M. J. Renn (mrenn@mtu.edu) is at the Department of Physics, Michigan Technological University, 1400 Townsend Drive, Houghton, MI 49931, USA. M. J. Renn is currently at Optomec Design Company, Albuquerque, NM, USA.

0167-7799/99/$ – see front matter PII: S0167-7799(99)01355-4

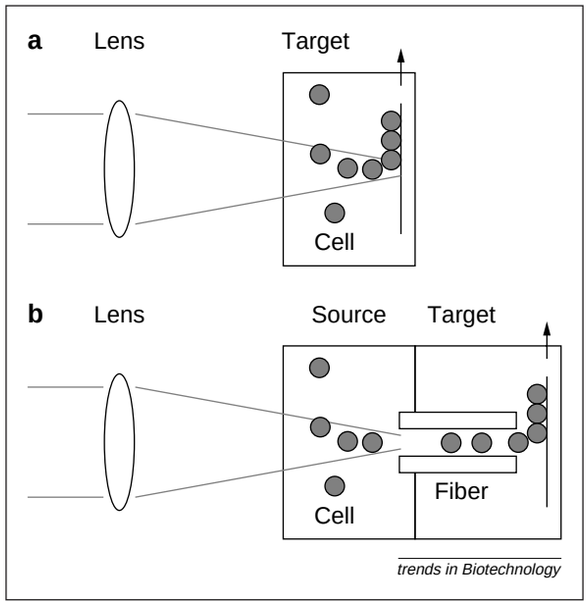


Figure 2

Laser-guided-direct-writing system. (**a**) Laser light is focused weakly into a suspension of particles. The particles are propelled by the light through the fluid and deposited on a target surface. Moving the target relative to the laser beam results in a line of particles being drawn. (**b**) Light is coupled into a hollow optical fiber and particles are carried through the fiber to the target surface. The process can be observed in real time by light microscopy.

Such an approach to deposition, which we call 'laser-guided direct writing', has been demonstrated with a variety of organic and inorganic particles in both gas and liquid phases[12,13], and with living cells in culture medium[11]. In the gas phase, particle fluxes as high as 10 kHz and placement precision below 1 μm have been achieved. The laser-guided-direct writing approach to optical manipulation stands in contrast to optical trapping: optical trapping uses a high-numerical-aperture lens to focus the beam strongly, which provides axial trapping so that a particle becomes trapped in three dimensions near the lens' focal point; by contrast, laser-guided direct writing uses a low-numerical-aperture lens to focus the beam weakly and to provide axial pushing (Fig. 1).

Hollow optical fibers

Light can be coupled into hollow optical fibers, which allows the transmission of a high-intensity beam over millimeter to centimeter distances (Fig. 2b). Although most optical fibers have a solid core, hollow-core fibers (developed for infrared-light-based communication) permit the transmission of both light and particles. The fiber's guiding geometry offers several advantages over free-space guidance.

First, natural convective fluid motion is often large enough to overwhelm the optical forces, making free-space guiding difficult. It is therefore particularly important to design chambers that suppress convective fluid motion. Hollow optical fibers alleviate this problem because the fiber interior provides a quiescent environment that is shielded from the surroundings; the outside of the fiber can be exposed to air currents (or even a vacuum) and the particles within are not disturbed. Second, laser light can be guided for several centimeters within the hollow region of the fiber. This allows particles to be transported over longer distances than is possible with tightly focused beams, and particle placement is accomplished by simply pointing the fiber's tip towards the substrate. Third, the intensity profile inside the fiber is well defined, with the intensity being a maximum at the radial center and zero at the fiber wall[17]. The intensity gradient draws particles toward the radial center of the fiber and keeps them from adhering to the fiber walls (Fig. 1). Fourth, the fiber also allows the source and deposition regions to be isolated from each other, ensuring that the directly written patterns are not corrupted by spontaneous particle adhesion to the target surface. Fifth, fibers from several source chambers can potentially be coupled to the same chamber for co-deposition of multiple materials.

Laser-guided direct writing

Whether it is accomplished with or without a fiber, laser-guided direct writing has many advantages over existing methods for surface patterning (Box 1). Most importantly, nearly any material in either liquid or aerosol suspension can be captured and deposited as long as the particle's index of refraction is greater than that of the surrounding fluid, and other forces (e.g. convection, gravity) are weaker than the optical forces (typically in the piconewton range). Potentially, many material types can be co-deposited on a single substrate, which will allow the simultaneous deposition of both electronic and biological particles.

Box 1. Advantages of laser-guided direct writing over other techniques

Optical trapping
Laser-guided direct writing allows particles to be captured continuously from the surrounding fluid and directed onto the substrate.

Photolithography
Laser-guided direct writing adds material to the surface rather than etching material away, and does not require harsh or corrosive chemicals.

Robotic micromanipulator-based deposition, ink jetting and screen printing
Particles are strongly localized within the laser beam and the deposition accuracy can be below 1 μm.

Potential applications in biotechnology

Tissue engineering

The long-term preservation of tissue-specific function is important if engineered tissue is to compensate successfully for organ failure. A number of studies have demonstrated the importance of three-dimensional (3D) structure on the behavior of cells in culture. For example, hepatocytes cultured as a monolayer lose many of their liver-specific functions within a few days. However, when these same cells are overlaid with a collagen gel to mimic the 3D structure of the liver, they retain many of their liver-specific functions for weeks in culture[18]. Therefore, the ability to organize cells spatially into well-defined 3D arrays that closely mimic the native tissue architecture can potentially help in the fabrication of engineered tissue.

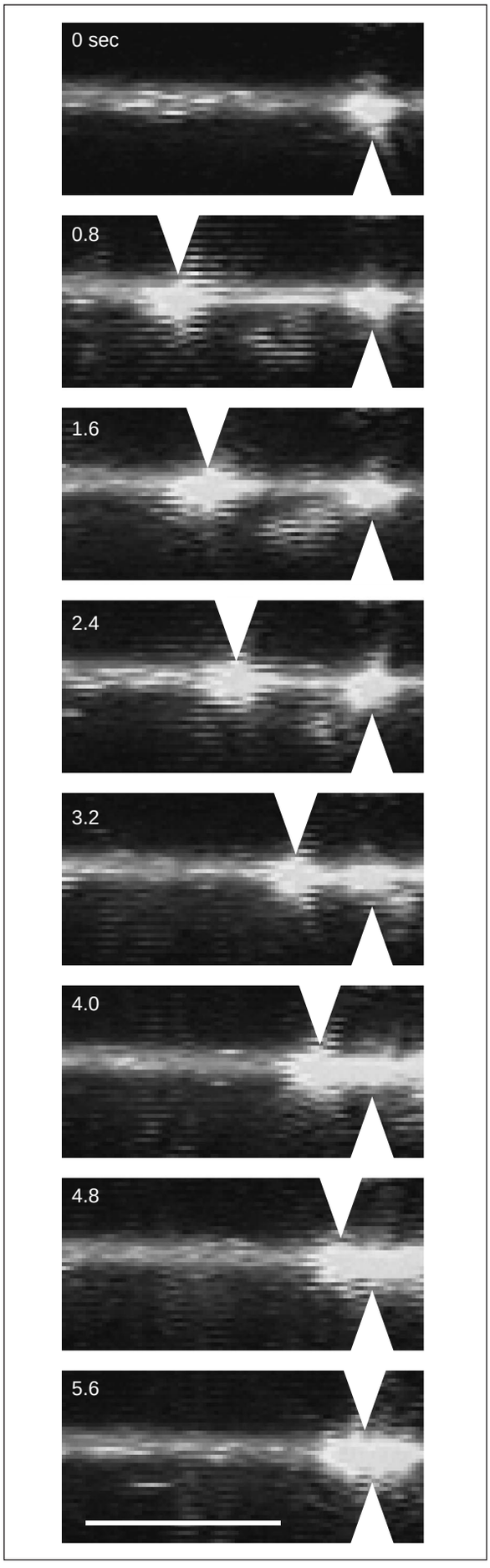


Figure 3
Guiding and depositing a spinal-cord cell. In the first frame, a single embryonic-chick spinal-cord cell (indicated by the upward arrowhead) has already been deposited on a glass target surface. In the second frame, a second cell (indicated by the downward arrowhead) is captured by the beam and is subsequently propelled from left to right (along the direction of the beam) through the fluid medium and deposited on the surface. Cells were visualized by scattered laser light, scale bar = 50 μm.

Laser-guided direct writing may offer this ability. In initial studies with embryonic-chick spinal-cord cells, we found that individual cells (diameter = 9 μm) could be guided by a 450 mW near-infrared laser beam and deposited in arbitrarily defined arrays onto a glass target surface[11] (Fig. 3). Importantly, cells that were exposed to the light remained viable and developed normal-appearing neurites. As depicted in Fig. 4, laser-guided direct writing potentially allows the 3D patterning of cells using multiple cell types with cell placement at arbitrarily selected positions.

Integration of electronic and biological materials in devices

Using a laser-guided-direct-writing system almost identical to that depicted in Fig. 2b, Renn *et al.* directly wrote clusters and lines of inorganic materials onto glass surfaces[12,13]. Previous work has also shown that atoms can be guided in an evacuated fiber[14]. Recent work has focused on the deposition of conducting and semiconducting materials for electronics fabrication and rapid prototyping.

As an example of the types of pattern that can be generated, Fig. 5 shows micrometer-scale lines of aluminum oxide, an electrical insulator, written directly onto a surface to form the shape of a ladder. The advantage of the laser-guided-direct-writing system is that a single system can be used to fabricate both electronic and biological materials, including living cells. The range of materials that we have successfully guided is broad and includes metals (e.g. 100-nm gold spheres), semiconductors, polymers, animal cells, diatoms, bacteria

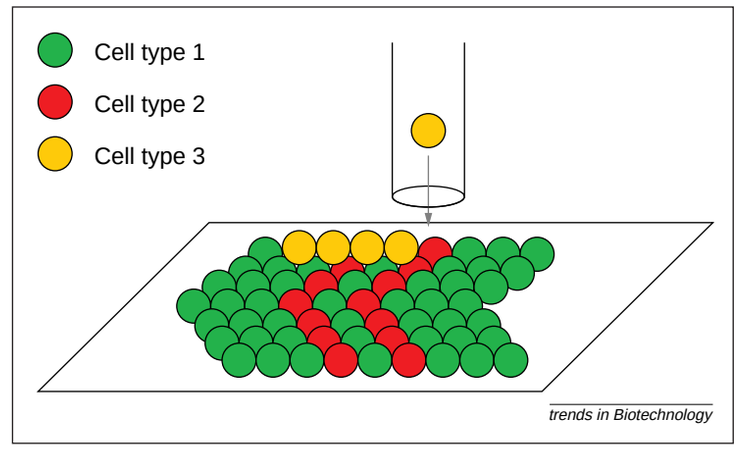


Figure 4
Schematic illustration of three-dimensional (3D) patterning of multiple cell types by laser-guided direct writing. Potentially, multiple cell types can be placed at arbitrary positions with micrometer precision in an attempt to recapitulate the complex 3D cellular organization of native tissues. Transport can be accomplished with or without a hollow optical fiber, depending on the degree of natural convection and the spatial separation required between the source of cells and the target.

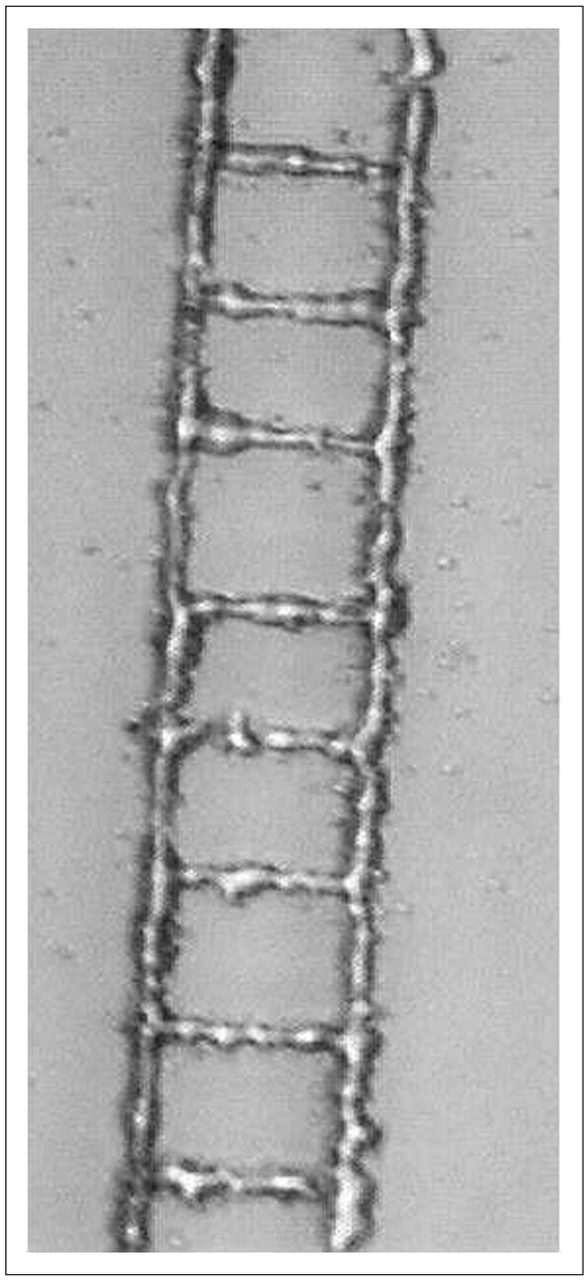


Figure 5

An optical micrograph of aluminum nitrate deposited on a glass substrate. A 500 mW, 532 nm laser was coupled into a 20 μm inner diameter, 6 mm long hollow-core fiber. The patterns were built up by the capture and deposition of aluminum-nitrate droplets (1 μm diameter) while moving the substrate near the exit of the fiber. The smallest line width is 10 μm and, in air, the deposition rate can exceed 10 kHz.

and microtubules. Also, the choice of substrate surface material is wide, as long as it is not damaged or modified by the impinging laser light. The use of a single, flexible system allows rapid prototyping with a wide range of materials integrated into a single functional device.

Microarray fabrication

In addition to the deposition of solid and semisolid particles suspended in a liquid phase, liquid droplets suspended in a gas phase can also be deposited[13]. Microarray patterns on arbitrary surfaces can be generated by depositing aerosols of liquid droplets containing

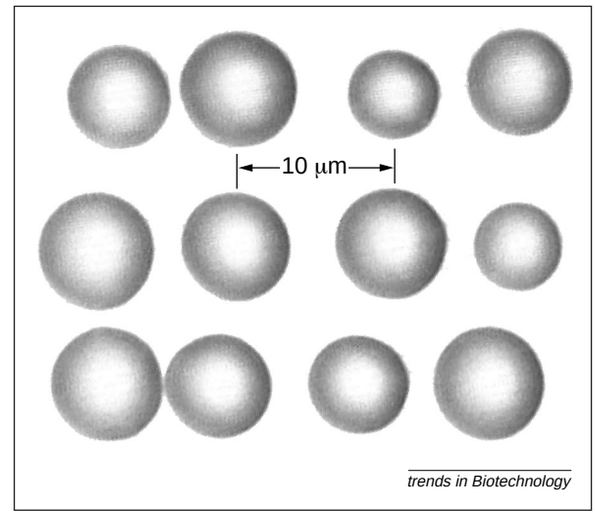


Figure 6

Array of glycerol droplets on a glass slide. The ~7 μm diameter deposits are built up from the multiple deposition of 1 μm aerosol droplets. A wide variety of biomolecules can be dissolved in glycerol (or other solvents) and then delivered to substrates as micrometer-scale droplets. The approach may provide a novel method to make biomolecule microarrays.

biomolecules such as proteins or nucleic acids. These droplets can be as small as 1 μm in diameter, and larger deposits can be generated on the surface by simply allowing several droplets to coalesce into a single droplet on the surface before moving the laser beam.

These spot sizes are one or two orders of magnitudes smaller than those typically generated by current methods (e.g. mechanical microspotting[19] or microjet printing[20]). For example, a 10 000-address microarray using microspotting requires about 3 cm^2, while laser-guided direct writing would require about 1 mm^2 assuming a 10 μm spot size (Fig. 6). In addition, preliminary deposition experiments have yielded droplet-deposition rates in excess of 10 kHz, while typical deposition rates for microspotting are less than 1 Hz. The droplet size (~1 fl) is orders of magnitude smaller than current dispensing techniques allow (~1 nl) and may lead to dramatically reduced reagent consumption.

Fundamental biological research

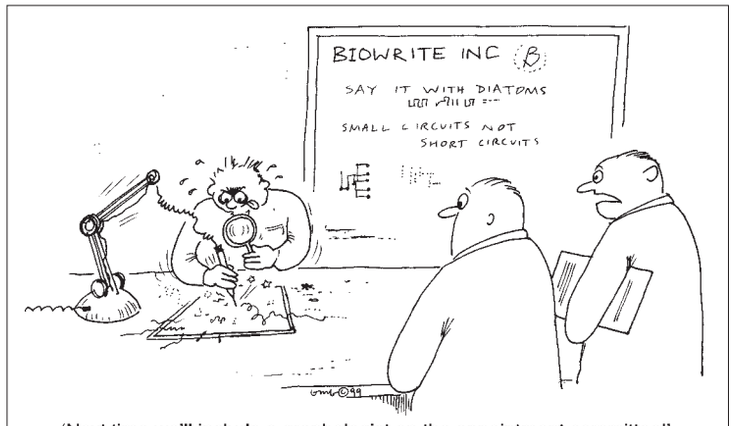
Optical trapping has been used successfully to elucidate fundamental cellular and molecular phenomena, such as the discrete 8 nm steps that the molecular motor kinesin takes as it advances along microtubules[21]. The laser-guided-direct-writing system is fundamentally different from optical trapping in that it provides propulsion along the beam axis instead of trapping. However, by simultaneously coupling light into both ends of a hollow optical fiber, a trap can be set up inside the fiber[13]. The trap-in-the-fiber can be set up at a lower cost than conventional optical traps because it does not require a high-numerical-aperture lens. It can also serve as an efficient microfluidic mixer, with one particle or droplet brought in from one end of the fiber and another from the opposite end of the fiber; the two particles collide in the middle and are held fixed for observation for as long as desired. One can conduct femtoliter-scale chemical reactions in droplets manipulated without any direct contact to a surface.

Conclusions

Laser-guided direct writing is an emerging technology for the high-throughput deposition of micrometer and submicrometer-sized particles. It is a simple system that can be set up at low cost and will deposit nearly any material with micrometer-scale accuracy. Multiple applications are anticipated in tissue engineering, hybrid electronic–biological devices, biochip-array fabrication and basic scientific research.

References

1 Ashkin, A. (1970) *Phys. Rev. Lett.* 24, 156–159
2 Ashkin, A. and Dziedzic, J. M. (1987) *Science* 235, 1517–1520
3 Ashkin, A., Dziedzic, J. M. and Yamane, T. (1987) *Nature* 330, 769–771
4 Ashkin, A., Schutze, K., Dziedzic, J. M., Eutenauer, U. and Schliwa, M. (1990) *Nature* 348, 346–348
5 Ashkin, A. and Dziedzic, J. M. (1971) *Appl. Phys. Lett.* 19, 283–285
6 Berns, M. W., Wright, W. H. and Steubing, R. W. (1991) *Int. Rev. Cytol.* 129, 1–44
7 Svoboda, K. and Block, S. M. (1994) *Annu. Rev. Biophys. Biomol. Struct.* 23, 247–285
8 Berns, M. W. (1998) *Sci. Am.* 278 (4), 62–67
9 Simmons, R. M. and Finer, J. T. (1993) *Curr. Biol.* 3, 309–311
10 Kuo, S. C. and Sheetz, M. P. (1992) *Trends Cell Biol.* 2, 116–118
11 Odde, D. J. and Renn, M. J. (1998) *Ann. Biomed. Eng.* 26, S141
12 Renn, M. J. and Pastel, R. (1998) *J. Vacuum Sci. Technol.* B16, 3859–3863
13 Renn, M. J., Pastel, R. and Lewandowski, H. (1999) *Phys. Rev. Lett.* 82, 1574–1577



'Next time we'll include a graphologist on the appointment committee!'

14 Renn, M. J. *et al.* (1995) *Phys. Rev. Lett.* 75, 3253–3256
15 Ashkin, A. (1992) *Biophys. J.* 61, 569–582
16 Buican, T. N. *et al.* (1987) *Appl. Optics* 26, 5311–5316
17 Marcatili, E. A. J. and Schmeltzer, R. A. (1964) *Bell Syst. Tech. J.* July, 1783–1809
18 Dunn, J. C. Y., Yarmush, M. L., Koebe, H. G. and Tompkins, R. G. (1989) *FASEB J.* 3, 174–177
19 Iyer, V. R. *et al.* (1999) *Science* 283, 83–87
20 Hayes, D. J., Wallace, D. B., Boldman, M. T. and Marusak, R. M. (1993) *Microcircuits Electron. Packag.* 16, 173–180
21 Svoboda, K., Schmidt, C. F., Schnapp, B. J. and Block, S. M. (1993) *Nature* 365, 721–727

Colloidal gas aphrons: potential applications in biotechnology

Paula Jauregi and Julie Varley

Colloidal gas aphrons are microbubbles encapsulated by surfactant multilayers. They provide a large interfacial area to adsorb charged and/or hydrophobic molecules; the extent and mechanism of the adsorption depends on the surfactant multilayer. The physical properties of colloidal gas aphrons have recently been characterized for a range of surfactants in order to find the best systems for particular applications. A range of exciting biotechnology applications has been identified, including the recovery of cells, proteins and other biological molecules, and the enhancement of gas transfer in bioreactors and bioremediation.

Colloidal gas aphrons (CGAs) were first described by Sebba[1] as microfoams with colloidal properties. The term colloidal was used because of the small size of the bubbles (10–100 μm in diameter), even though the dimensions are not truly in the colloidal range [which is approximately 1 nm – 1 μm (Ref. 2)].

P. Jauregi is at the Delft University of Technology, Faculty of Applied Sciences, Kluyver Institute for Biotechnology, Julianalaan 67, 2628 BC Delft, The Netherlands. J. Varley is at the Department of Food Science and Technology, The University of Reading, PO Box 226, Whiteknights, Reading, UK RG6 6AP.

In a later study, Sebba defined CGAs as microbubbles created by intense stirring (5000–10 000 rpm) of a surfactant solution[3]. The intense stirring of the surfactant solution causes gas entrainment and microbubble formation. He also described a mixing system required for the formation of CGAs that was composed of a horizontal disc capable of rotating at very high speeds; baffles were necessary in order to achieve the required mixing regime[4]. The generation of CGAs has since also been reported for both nonionic and ionic surfactants[5–8].

Sebba postulated (without any direct scientific evidence) that these microbubbles are different from conventional

 0167-7799/99/$ – see front matter PII: S0167-7799(99)01363-3