Bubble wrap for optical trapping and cell culturing

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Abstract: In this paper, we demonstrate that the bubbles of bubble wrap make ideal trapping chambers for integration with low-cost optical manipulation. The interior of the bubbles is sterile and gas permeable, allowing for the bubbles to be used to store and culture cells, while the flat side of the bubble wrap is of sufficient optical quality to allow for optical trapping inside the bubbles. Through the use of a 100 W bulb to cure hanging droplets of PDMS, a low-cost optical trapping system was constructed. Effector T cells were cultured in bubble wrap for 8 days and then trapped with the PDMS droplet based optical manipulation. These techniques further demonstrate the opportunities for biophysical analysis afforded through repurposing common materials in resource-limited settings.

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1. Introduction

There is currently a small revolution going on in the field of microfluidics. Developments to try and enable simple storage of devices along with the development of facile device implementation have led to the new field of paper microfluidics [1–5] - a field which becomes ever more sophisticated, new implementations now incorporating surface enhanced Raman spectroscopy (SERS) substrates within them [6]. The drive to develop technology capable of analysing the outputs from such devices has led to innovations in the area of image processing and analysis on mobile phones [7], along with new low cost, or low-tech, imaging techniques [8–11] making use of, for example, holographic microscopy. Other areas that have benefited from leveraging consumer technology and simple home-made techniques include miniature dermascopes [12], centrifuges made from egg beaters [13], not to mention the huge potential of 3D printing in such areas, spanning chemical reactionware [14] to conventional printing of chips [15].

One of the main drivers for such work is to democratise, to some extent, the complex, resource intensive, equipment found in well-established labs in wealthy countries. It brings the high-tech to everyone in small convenient, low cost, packages. These find use in teaching labs, spanning schools and universities, but also to researchers in developing countries, as well as offer the opportunity to deploy such tools in areas that might be off-grid, without access to computers, cold storage or mechanisms for equipment sterilisation.

Samples and reagents for analysis are mainly kept in solid containers (multiwell plates, cell culture flasks, etc), which are convenient in well-funded labs but are of little use in resource-limited settings. These containers can be difficult to sterilise and dispose of; expensive and therefore unaffordable; and brittle, thus capable of generating ”sharps”. Whitesides et al showed that bubble wrap could be repurposed as containers to store liquid samples, perform bioanalysis and culture bacteria [16]. Bubble wrap is an attractive material for repurposing as it is very inexpensive and readily available throughout the world. Bubble wrap is easily disposed of by burning, it is very flexible and it’s easy to cut (thus not yielding sharps). Moreover, inside the...
bubbles is a sterile environment surrounded by a gas permeable plastic, allowing for the culture and storage of cells and bacteria [16].

A low-cost alternative to many optical components was introduced through the development of elastomeric optics using polydimethylsiloxane (PDMS) [17]. PDMS is an ideal material to fabricate lenses from due to its high transparency (> 95%) within the visible wavelength range and high refractive index (n = 1.47 - 1.55). Specialised high temperature equipment is negated by the low moulding temperature (< 100°C) of PDMS, making it easy to work with in the lab [17]. Elastomeric optics have been used in an increasing number of novel applications such as a rubber microscope [18] and biologically inspired wide field lenses [19]. Additionally, lenses based on hanging droplets of PDMS have been fabricated and used in a low-cost digital dermascope [12] and low-cost optical traps [20].

Here, we demonstrate the potential of using bubble wrap and PDMS droplet lenses as a basis of a low-cost and low-tech approach to optical trapping for the biophysical sciences. We show that the flat side of bubble wrap is of sufficient optical quality that the bubbles can be used as an optical trapping chamber. The sterile and gas permeable properties of bubble wrap allowed us to culture primary murine effector T cells in the bubbles, before optically trapping them. PDMS droplet lenses were then used to replace the high numerical aperture objective, common to optical tweezers, to optically trap the bubble wrap cultured cells. This further reduces the cost of our optical manipulation system making it beneficial in resource-limited regions. Moreover it enables direct cell culturing in the trapping chamber: the norm would be to culture in a vessel away from the trapping chamber and then transfer cells. Our setup removes this extra step which may damage cells, and may facilitate the handling of pathogens in a more straightforward manner; it also enables the simple study of cell-cell interactions during cell growth. In more general applications, bubble wrap could be a plausible cheap trapping chamber for airborne particles, offering a compact chamber in which the environment is highly controllable.

2. Experimental methods

2.1. Cell culture in bubble wrap

To generate effector T cells, splenocytes from wild type mice were activated for 2 days with 0.5 µg/ml anti-CD3 (clone 2C11, R&D Systems) together with 20 ng/ml IL-2 (R&D Systems). The bubble wrap was quickly cleaned with a spray of 70% ethanol/30% water before injecting cells into the 1 cm diameter bubbles, using a 1 ml syringe (BD Plastipak) with attached 26 G 23 needle (Terumo Neolus). Clear nail polish (obtained from local pharmacist) was used to seal the puncture. Due to the differing volume of air in each bubble, cells were injected until the pressure in the bubble rounded out the shape of the plastic, approx. 200 – 300 µl.

After 2 days, cells were removed from bubble wrap, washed free of activating agent and then maintained in 20 ng/ml IL-2 for 6 more days. Throughout the culture, cells were passaged every 2 days to approx. 1 x 10^6 cells per ml. Each time cells were split, they were injected into a different bubble and the puncture sealed with clear nail polish.

Sterile growth medium was injected into bubbles as a control experiment, in order to verify the sterility of the bubble wrap. Both the cell containing bubble wrap and the control bubble wrap were incubated at 37°C with 5% CO₂ throughout the culture duration.

2.2. Fabricating the trapping lens with a hanging droplet

Small objects can be magnified with liquid droplets [21, 22]. When the interfacial energies (liquid, air and solid surface) reach an equilibrium with gravity, a hanging droplet is formed [22, 23]. Through the repeated addition and curing of hanging droplets of PDMS, Lee et al. formed low-cost elastomer lenses [12], which were then shown to be suitable as the basis of
low-cost optical manipulation [20], making use of such lenses for the microscope objective and the condenser lens.

We formed the trapping lens from hanging droplets of PDMS, following steps similar to those outlined by Lee et al [12], Fig. 1. PDMS monomer and hardener was mixed at a ratio of 10 : 1 v/v (Sylgard 184 Silicone Elastomer kit, Dow Corning, Coventry, UK) and then placed in a vacuum desiccator until all bubbles were removed. Having carefully filled a 1 ml syringe with PDMS, pressure was applied until a single drop (<100 µL) fell from the syringe onto a microscope coverslip, thickness no. 1. The microscope coverslip was immediately inverted and suspended (∼20 mm) above a sheet of tin foil on the lab bench. Any excess PDMS falls from the slide until single drips are formed.

A 100 W incandescent light bulb was lowered to ∼20 mm above the microscope slide, to provide heat for the curing step, while the tin foil reflected the heat back towards the droplet lens. Temperature was crudely controlled by raising and lowering the light bulb, with temperatures in excess of 100°C easily obtainable. "Oven" temperature was maintained at between 70 - 80°C while the droplet lenses set, taking up to 15 minutes. The numerical aperture (NA) of the lens was increased, and focal length decreased, by layering the droplets of PDMS one on top of the other, with each droplet cured before the next was applied. The eventual droplet shape at the apex of the lens resembles that of a parabola, with a curvature that increases with layer number, due to the maximum surface tension that the droplet can hold before falling.

2.3. Low-cost optical trap set-up

Our experimental system for the droplet lens based optical trap, shown in Fig. 2, makes use of a 1.5 W (maximum output) 532 nm solid-state laser (Model: Ventus, Laser Quantum) as the trapping source. No expansion optics were used before the droplet lens in order to keep the system as simple as possible. A 50 : 50 beam splitter was used to reflect the trapping light up to the PDMS droplet lens and transmit white light illumination. Trapped objects were imaged from above with a Mitutoyo 0.55 NA 100x long working distance objective and CCD camera.

We appreciate that the use of a high quality laser as used here suggests this is not a particularly low cost system. However we have reported trapping with ∼40mW [20], and more recent (unpublished) developments of the system have shown trapping with a minimum of 20mW power. This puts the system well within the range of a high power laser pointer or equivalent low cost laser diode.
Fig. 2. Experimental system for optical manipulation based on PDMS droplet lens. OBJ = Mitutoyo 0.55 NA 100x long working distance objective, CCD = CCD camera, filter = Thorlabs FD1M subtractive dichroic colour filter, Magenta. Inset (a) shows a representative image of bubble wrap being used for cell culture. In this case, however, yellow food colouring has been used to enhance contrast between filled and empty bubbles. An in focus optically trapped cell (trapped by a standard set of optical tweezers and high NA objective) among untapped, out of focus, cells in bubble wrap is shown in inset (b). A video of the high NA trapped cell in bubble wrap is available in the supplementary information, see Visualization 1.

Illumination of the sample was achieved by shining a lamp into polystyrene foam packaging, perpendicular to the imaging path, Fig. 2. This produced diffuse light, which was sufficient to illuminate the sample without saturating the camera.

3. Results and discussion

3.1. Trapping in bubble wrap

Initial experiments for trapping in bubble wrap were performed on a standard set of optical tweezers, in order to test the optical quality of the flat side of the bubble wrap. A 1.5 W (maximum output) 1064 nm solid-state laser (Model: Ventus, Laser Quantum), after going through standard expansion and relay optics, was focussed with a Nikon 1.25 NA 100x oil objective to form the optical trap, in a standard inverted microscope fashion.

Various sizes of silica beads (1.01 µm - 5.2 µm) were trapped inside the bubble wrap, which was placed in direct contact with the objective, with no noticeable loss in trapping performance. However, the flexibility of bubble wrap caused it to sag across the sample holder, pulled down by the surface tension of the immersion oil. This could be fixed by either placing the bubble wrap on a coverslip or by pulling the bubble wrap taut and fixing it to the sample holder with tape, before it came in contact with the oil.
3.2. Trapping cultured cells in bubble wrap

After 8 days of culturing in bubble wrap, the control bubbles with sterile growth medium showed no bacterial growth, confirming the observations made by Whitesides et al that the interior of the bubbles is sterile [16]. Bubbles containing effector T cells showed growth and division of the cells, proving that it was not a lack of oxygen diffusion that prevented the growth of bacteria in the bubbles.

Optical trapping of cells in bubble wrap was possible by taking the above precautions of ensuring the bubble wrap was pulled taut across the objective. When trapping cells, it was necessary to prepare them by moving the cells to a new bubble in the bubble wrap. This was due to the majority of effector T cells settling and sticking to the surface of the bubble with more force than could reasonably be overcome by optical tweezers. Bubble wrap can, therefore, be used to not only store and culture cells, due to their sterility and gas permeability, but also can be used as optical trapping chambers. This allows for straightforward integration with optical tweezers systems for analysis of possible bacterial or cellular growth. A video of a trapped cell in bubble wrap is provided in the online supplementary information, see Visualization 1.
3.3. Trapping with droplet lens in bubble wrap

To further reduce the cost of our system, we moved to an optical manipulation set-up based on droplet lenses, Fig. 2 [20]. The trapping sample was injected into the bubble wrap and placed above a droplet lens of focal length $0.9 \pm 0.2$ mm, giving an estimated effective NA of $0.7 \pm 0.17$, the obtained experimental airy disk is shown in Fig. 3. Trapped cell height, as with silica sphere height, was observed to change as a function of power, indicating two dimensional trapping.

Imaging of the sample was from above, via a Mitutoyo 0.55 NA 100x long working distance objective, due to the unknown back focal plane properties of the droplet lens. We found that lensing effects caused by the trapping sample and the irregular surface of the hemispherical part of the bubble wrap significantly distorted the image, when viewed from above. Therefore, when preparing a trapping sample, the bubble was fully inflated with air in an attempt to smooth the surface. Additionally, to circumvent lensing effects caused by droplets of the trapping sample, enough liquid to just cover the lower surface of the bubble wrap was used. This caused the sample to spread and form a meniscus, rather than round up due to surface tension. The trapped object was then imaged through the centre of the bubble wrap and meniscus, where the surfaces are relatively flat, Fig. 4. Optically trapped cells could, therefore, be imaged in the bubble wrap but with a decrease in image quality, Fig. 5 and Visualization 2.

4. Conclusion

Bubble wrap has been shown to be an ideal candidate for a low-cost alternative to cell culture flasks. Being both sterile and gas permeable, bubble wrap allows for the storage and culturing of micro-organisms, while the flat side of the bubble wrap is of sufficient optical quality to allow for optical trapping. Low-cost chambers for optical trapping would be redundant without low-cost optical manipulation. By using an optical trapping system based on droplet lenses, we were able to significantly reduce the cost of our overall system. Such systems could find use in pedagogical settings, where funds are often limited yet laboratory grade equipment is required, and other resource limited regions. With a little more engineering, the PDMS lens optical trap
should be able to be integrated into a package that would sit on a mobile phone, allowing a highly portable and miniaturised system than could run from batteries or solar cell. Further work is needed to integrate appropriate measurement technology, such as positional detection to enable robust trap characterisation to be carried out, but it would seem only a matter of time before robust optical traps that are largely home-made become a reality. Here we have shown that the new age of innovative low cost microfluidics can be extended to optofluidic components, and that these integrate with methods for the analysis of cells.

Integration with other microfluidic systems to create simple optofluidic [24] devices should be straightforward due to the trapping system being made from PDMS. Therefore, this work contributes to the ever growing field of disposable, point-of-care diagnostic devices.

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