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Acoustic Patterning of Microspheres and Microbubbles in an Acoustic Tweezers

A.L. Bernassau¹, P. MacPherson¹, J. Beeley¹, B.W. Drinkwater², D.R.S. Cumming¹

¹ School of Engineering, University of Glasgow, G12 8LT, Glasgow, UK
² Department of Mechanical Engineering, University of Bristol, Bristol, BS8 1TR, UK

Anne.Bernassau@glasgow.ac.uk, Tel: +44 (0)141 330 6690, Fax: +44 (0) 141 330 4907

Abstract - We describe the construction of an ultrasonic device capable of micro-patterning a range of microscopic particles for bioengineering applications such as targeted drug delivery. The device is formed from seven ultrasonic transducers positioned around a heptagonal cavity. By exciting two or three transducers simultaneously, lines or hexagonal shapes can be formed with microspheres, emulsions and microbubbles. Furthermore, phase control of the transducers allows patterning at any desired position in a controlled manner. The paper discusses in detail direct positioning of functionalised microspheres, emulsions and microbubbles. With the advantages of miniaturization, rapid and simple fabrication, ultrasonic tweezers is a potentially useful tool in many biomedical applications.

Keywords - Acoustic radiation pressure, particle patterning, 2D particle manipulation, sonotweezers

I. INTRODUCTION

Therapeutic ultrasound is a currently expanding field and new clinical applications are being developed constantly. Manipulation and control of drug delivery carriers using ultrasonic waves is a promising technology in the fields of medicine and biotechnology. Carriers such as functionalised microspheres (Pichot 2004), liposomes (Lasic and Papahadjopoulos 1998), emulsions (Davis et al. 1987), and ultrasound gas-filled microbubbles (Wu and Nyborg 2008) may provide additional methods for enhancing targeted drug mechanisms. They have the potential to increase binding efficiency in drug and gene therapy applications by improving delivery of active components into tissues and cells (Fechheimer et al. 1987).

Surface functionalised microspheres can be easily combined with antibodies or other adhesion ligands in order to interact with the corresponding receptors at the cell surface. They may be used for cell labelling, drug delivery, specific targeting of cells and tissue through ligand-receptor interactions for improved efficiency and specificity of the drug action (Jepson et al. 2004).
Liposomes and emulsion micro droplets are well known vectors capable of transporting hydrophobic molecules and effectively facilitate their absorption by lining cells (cell wall absorption). It has been shown that liposomes may be an alternative to ultrasound microbubbles for delivering macromolecules into cells (Silva et al. 2011; Wu et al. 2006). Oil-in-water emulsions are used to enclose oil-soluble drugs in the oil phase of the emulsions, forming lipid microspheres that can therefore serve as carriers for many oil-soluble drugs. An emulsion of soybean oil has been successfully employed in this way for drug delivery (Nakano 2000).

Ultrasound microbubbles are widely used to improve contrast in clinical and research applications of ultrasonic imaging and have recently been used in targeted drug delivery applications (Bloch et al. 2004; Suzuki et al. 2011). The bubbles are loaded with the drug delivery to the target being achieved by exploding the bubbles via ultrasound excitation at the appropriate point. Microbubbles can differ in their shell and gas core makeup. The shell may be composed of albumin, galactose, lipid or polymers. The gas core can be composed of air, or heavy gases such as perfluorocarbon or nitrogen. One or more biologically active molecules may be incorporated or encapsulated on or into the carrier shell. Properly driven to their target (e.g. a malignant region) and burst there, these drug carriers are potentially capable of increasing the concentration of the drug delivered to the desired site of action.

Spatial control of particles has been achieved with optical tweezers (Ashkin et al. 1987; Grier 2003), hydrodynamic flow (Kim et al. 2012; Swastika and Siva 2010) and dielectrophoresis (Benguigui and Lin 1984; Jen and Chen 2009; Markarian et al. 2003) methods. These methods have their limitations: low concentration, continuous flow of liquid, and the inability to manipulate particle position, respectively. Ultrasonic manipulation offers an alternative approach. Manipulation of drug carriers in acoustic standing waves has been reported previously (Lum et al. 2006; Yamakoshi and Koganezawa 2004). It has been demonstrated that acoustic radiation force can be used to localise microbubble carriers along the wall of a vessel (Dayton et al. 1997). Standing surface acoustic wave devices have been demonstrated to trap and align cells (Shi et al. 2009; Wood et al. 2009), lipids (Neumann et al. 2010) and microbubbles (Meng et al. 2011; O’Rorke et al. 2012; Rorke et al. 2012). It has been demonstrated that the trapped particles can be transported by changing the driving frequency of the transducers (Glynne-Jones et al. 2009; Kozuka et al. 1996). This method presents several challenges including unstable force resulting in movement among trapped particles. Unfortunately, these devices are either unsuitable for device miniaturisation and integration or have not been demonstrated for a controlled manipulation.
In this paper, we present a method that offers multiple decisive advantages over previous approaches, including a small device, compatibility with sterile cell culture, simplicity in experimental setup and control as well as the potential for integration with analytic sensors modules. The device creates interference patterns using travelling acoustic waves that allows a repeatable and accurate control of the transport of the trapped particles (Courtney et al. 2011). The device has already shown capability in cell patterning for tissue engineering application (Bernassau et al. 2012). Here, we will discuss patterning and manipulation of different types of drug carriers: functionalised beads, emulsions and microbubbles. These carriers could be used for “active targeting”, where a bioconjugate ligand specifically adheres to the target site (Demos et al. 1999; Klibanov et al. 1997), or through “passive targeting”, which relies on the pharmacokinetics of the carrier as determined by its size and composition to concentrate it in a region of interest.

II. STRUCTURE AND WORKING MECHANISM

The sonotweezers device was made by bonding NCE51 Noliac Ceramic lead zirconate titanate (PZT) (E.P. Electronic Components Limited, UK) plates to a flexible printed circuit board (Flexible dynamics Ltd, UK) and folding it into a heptagon (Bernassau et al. 2012). Synchronisation between channels was achieved using an arbitrary waveform generator providing four output channels (TGA12104, Aim and Thurlby Thandar Instruments, UK) allowing independent control of the amplitude, phase and frequency. The signals from the waveform generators were amplified and electronically matched by high speed buffers, BUF634T (Texas Instruments, UK).

System behaviour with a combination of two and three simultaneously excited transducers was investigated and discussed in detail elsewhere (Bernassau et al. 2011). In this paper, each excited transducer (the transducers are numbered sequentially 1 to 7) was separated by at least one inactive transducer. The combination where the activated transducers were on adjacent sides of the device was found to be less effective in trapping than the combination where at least one inactive face separates the active transducers. For the two transducer (1-3) setup, the excited transducers were separated by one inactive transducer (Fig. 1a). In the three transducer (1-3-5) setup, transducer (3) is separated by one inactive transducer on each side (Fig. 1b). In this study, only 180° phase shifts were used to move the micro beads or cells.

Fig. 1 shows the computer simulation results obtained with two and three transducers excited simultaneously showing the theoretically expected patterns. The program is based on Huygen’s principle and simulates the acoustic pressure distribution within the heptagonal cavity. In order to simplify the model, the boundaries were
assumed to be perfectly absorbing. The wave field generated by one transducer, \( g(r) \), was modelled as the sum of several simple cylindrical point sources, \( f(r) \):

\[
f(r) = A e^{-\frac{\alpha r}{2}} \cos(\alpha r - kr + \Phi)
\]

where \( A \) is the amplitude, \( \alpha \) is the damping factor, \( \lambda \) is the wavelength, and \( \Phi \) is the initial phase in degrees.

\[
g(r) = \sum_{i=1}^{\infty} f(r_i)
\]
Fig. 1. Simulation results showing the creation of standing waves in the middle of the heptagonal cavity when (a) two (b) three transducers are excited simultaneously. The energy maxima are grey, and the energy minima are white; (c), (d) show the central area of (a) and (b) respectively when magnified by 10 times. (c) clearly shows the energy minima (white) as edges that are useful for trapping material in lines, whereas (d) shows hexagonal traps.

It can be seen in Fig. 1 that the most regular standing wave patterns are situated in the middle of the heptagonal cavity, at the intersection of the travelling waves and the experiments follow this behaviour. The pattern arises from the constructive and destructive interference between two or more travelling waves. For two active
transducers a linear pattern of nodes and antinodes is formed (Fig. 1c). For three active transducers, the nodes adopt a pattern with a hexagonal shape (Fig. 1d). The force acting on the particles, $F_r$, is as (Laurell et al. 2007).

$$F_r = -\left(\frac{\rho_0 V_c \beta_w}{2\lambda}\right) \times \phi(\beta, \rho) \times \sin(2kx) \quad \text{Eq. 3}$$

where $\rho_0$, $\lambda$, $V_c$, $x$, $k$ are acoustic pressure amplitude, wavelength, particle volume, the distance away from the pressure node, and the wave number respectively. The acoustic contrast factor $\phi$ depends on both, the particle density, $\rho_c$, and its compressibility, $\beta_c$, in relation to the corresponding properties of the surrounding medium $\rho_w$, $\beta_w$.

$$\phi(\beta, \rho) = \frac{5\rho_c - 2\rho_w - \beta_c}{2\rho_c + \rho_w - \beta_w} \quad \text{Eq. 4}$$

where $\rho_c$, $\rho_w$ and $\beta_c$, $\beta_w$ are the density and the compressibility of the particle and the surrounding medium.

The acoustic contrast factor determines the direction of the acoustic force: if $\phi$ is greater than or less than zero, particles will be attracted to the pressure nodes or antinodes, respectively.

III. RESULTS AND DISCUSSION

An experimental system was set up to characterise the performance of the sonotweezers. The device was mounted on the stage of a microscope (BX51, Olympus, UK), and a CMOS camera (ORCA-flash 2.8, Hamamatsu, UK) was connected to the microscope to record the entire process.

In all experiments, particles were trapped by exciting either two or three transducers simultaneously and the position of the particles controlled by shifting the phase of one of the transducers relative to the others.

When two transducers are simultaneously excited, the nodes created are along lines that bisect the angle formed by the two transducers. The distance $d$ between the nodes can be calculated by $d = \lambda/2\sin(\theta/2)$, where $\theta$ is the angle formed by the normal to the planes of the two sides with the active transducers. In the case presented in the paper, $\theta$ is 105° and $\lambda = 375 \mu$m.

A. Patterning and manipulation of functionalized microspheres

In a previous paper, we have demonstrated manipulation of non-functionalised polystyrene microsphere (Bernassau et al. 2011). In this paper, we focussed on 10 µm functionalised microspheres that can be electrically neutral, or negatively or positively charged as a function of the solution pH and of the ionisable chemical groups.
attached to the beads (carboxylate or amino). All experiments were performed using 10 and 6 µm diameter latex carboxylate (CO$_2^-$ group) and amino (NH$_3^+$ group) microspheres (Polysciences Europe, Germany). The transducers were excited via an 8 V peak-to-peak ($V_{pp}$) sinewave at a frequency of 4 MHz. The concentration of the microspheres in the DI water was approximately 4.5 x 10$^6$ beads/ml.

These experiments are a preliminary attempt to develop pH monitored cell-based biosensors, where micro pH sensors (Nemeth et al. 2012) are integrated in the sonotweezers device. In the future, local pH measurements could be used as an indicator of cell metabolic activity.

At first, pH titration of the functionalised microspheres was determined (Fig. 2). The carboxylate microspheres were characterised in a solution of NaOH (pH = 11; 0.1 mol/L) whereas the amino microspheres were characterised in a solution of HCl (pH = 3; 0.001 mol/L). The microspheres can be charged or neutral depending on the pH of the solution. The carboxylate microspheres will be negatively charged (CO$_2^-$) under a solution pH of 5 and higher. The amino microspheres will be charged (NH$_3^+$) under a solution pH of 8 and lower.

**Fig. 2 Titration of carboxylate and amino functionalised microspheres.**

The number of elementary charges on one microsphere can be calculated from the number of beads in the solution and the amount (mL) and concentration (M) of acid or base added to neutralise the microspheres. In our case, the number of carboxylate and amino microspheres in our experimental solution are 4.6 x 10$^6$ and 2.1 x 10$^7$, respectively. 1.5 and 2.5 mL were necessary to shift the titration curve of the carboxylate and amino charged microspheres (Fig. 2). The acid or base added was in a concentration of 10$^{-3}$ M. Using Avogadro number (6.02 x 10$^{23}$ ions/M), we can deduct the total number of charges on the microspheres, 9 x 10$^{17}$ OH$^-$ and 1.5 x 10$^{18}$ H$^+$. So for one carboxylate (respectively amino) microsphere, the number of charged groups is 2 x 10$^{10}$ (respectively 7 x 10$^{10}$). Each group carries an elementary negative or positive charge equals to
1.6 \times 10^{-19} \text{ C}. \text{ So each carboxylate and amino microsphere has a charge of } 3 \times 10^{-9} \text{ C and } 1 \times 10^{-8} \text{ C, respectively.}

Fig. 3a shows the results when two transducers (1-3) are excited simultaneously and the microspheres were negatively charged (carboxylate groups at pH = 11). The microspheres align along lines with a separation distance of 236 \mu m. Fig. 3b shows the effect of a 180° phase shift of transducer 1: the particles move towards transducer 3 by about 118 \mu m. With the same combination, if the phase shift is effected with transducer 3 the particles moved towards transducer 1. The same behaviour is observed with positively charged particles and was also shown to be the behaviour of non-functionalised beads. The net charge carried by the polystyrene particles therefore does not affect their behaviour in the acoustic fields.

With three transducers (1-3-5), the particles cluster around acoustic nodes of minimum energy forming a clear hexagonal pattern (Fig. 3c). When the phase of one of the three transducers is shifted, the nodes and antinodes of acoustic energy are displaced, and the clustered particles follow accordingly.

Therefore, in this heptagonal device, the charged microspheres can be trapped and displaced in a reproducible and predictable manner in two dimensional patterns, as the non-functionalized polystyrene microspheres. The patterns can be displaced while at the same time retaining their relative positions, simply by shifting the phase of one of the transducers.

Fig. 3(a) Photograph of functionalised microspheres aligned along lines with a separation distance of 236 \mu m. Scale bar = 100 \mu m. (b) Photograph of the microspheres patterned with 3 transducers. Scale bar = 100 \mu m. (c) Overlay of two photographs when the phase of one of the transducers is changed to 180° (black = 0°, grey = 180°). For (c) the microscope and sample are kept in a fixed position. ImageJ was used to create a transparent background to generate the overlay.
The electric charge carried by the micro beads nevertheless has an effect on the behaviour of the particles within the lines along which they are aligned. Fig. 4 shows photographs of trapped charged carboxylate microspheres and polystyrene beads at similar concentration. It can be noticed that the polystyrene beads have the tendency to cluster with each other, whereas charged microspheres have not. Charged microspheres tend to repel each other due to their net charge.

![Fig. 4 Photographs of (a) charged carboxylate microspheres, (b) polystyrene microspheres (uncharged) at similar concentration (4.5 x 10^6 beads/ml). Scale bar = 100 µm.](image)

**B. Patterning and manipulation of emulsions**

The emulsions were made by mixing 3 ml of DI water, 1 ml of vegetable oil and 2 ml of isopropanol. Droplets of oil (size of the droplets ranging from 3 to 40 µm) are obtained in suspension between water and isopropanol. The transducers were excited with a 10 V_{pp} 4 MHz sine wave.

Fig. 5a shows a photograph of emulsions trapped by two transducers operating in the excitation combination 1-3. The oil droplets are aligned along the lines and are fixed under the acoustic energy emanating from the transducers. As for the beads experiments, the lines of emulsion have a separation of 236 µm. This picture would however be misleading as it does not indicate whether the droplets are trapped at the minima or maxima of acoustic energy.

As noted in Eq. 3, the contrast factor can be positive or negative depending on the density and compressibility of the entity experiencing the acoustic field. Clearly emulsion micro droplets are of a different nature than polystyrene beads and it is necessary to ascertain their behaviour under acoustic excitation.

This ambiguity is removed by three transducer excitation. Fig. 5b shows a photograph of the emulsions agglomerating when three transducers were active in phase in configuration 1-3-5. As can be seen the pattern of Fig. 5b is completely different than the one of Fig. 3b. According to the simulation of the energy landscape presented in Fig. 1, it can be seen that the droplets concentrate at the energy maxima. The oil droplets cluster around the nodes of maximum energy (antinodes) (Petersson et al. 2004), and form a clear diamond pattern.

Fig. 5c, d shows an overlay of photographs when the particles are trapped with the transducer 1-3 and 1-3-5.
excited, and a change of phase. As in the case of the microspheres, phase changes allow positioning of the droplets at any desired position in the plane.

Fig. 5(a) Photograph of emulsions trapped with two transducers, (b) Photograph of oil droplets clusters at the antinodes with 3 transducers, (c) and (d) Overlay of two photographs when the phase of one of the transducers is changed to 180° (black = 0°, grey = 180°). For (c) and (d) the microscope and sample are kept in a fixed position. ImageJ was again used to create a transparent background for the overlay.

In order to fully demonstrate the different positioning of particles and droplets, an experiment has been conducted to simultaneously subject polystyrene microspheres and emulsion droplets to the acoustic field. Fig. 6a shows beads and emulsions trapped with two transducers in excitation combination 1-3. It can be seen that the line of beads and line of emulsions are in interval of each other as expected from a trapping of beads and droplets to the nodes and antinodes respectively. The effect is even more dramatic with a three transducers excitation as shown in Fig. 6b where the beads and emulsions clustered with three transducers activated in combination 1-3-5. It can be easily seen that the beads are trapped in a hexagonal pattern surrounding the emulsion droplets trapped in a diamond pattern. Fig. 6c shows a comparison between experimental behaviour of beads and the emulsion cluster with three transducers, and the simulation.

Fig. 6 Beads and emulsions trapped at nodes and antinodes, respectively with (a) transducers 1-3, (b) transducers 1-3-5 and (c) simulation showing acoustic nodes (dark grey) and antinodes (light grey).
This dual patterning technique (at nodes and antinodes) could be used to manipulate two carriers possessing different compressibility and carrying two different types of drugs. The different positioning of the two carriers would allow delivering the two drugs at different positions.

C. Patterning and manipulation of ultrasound microbubbles

SonoVue™ (Bracco, UK) ultrasound contrast agent has been used for the following experiments. The mean diameter of the microbubbles is 3 µm, while 95% of the bubbles are smaller than 10 µm (van der Meer et al. 2004). The microbubbles were injected in the ultrasonic cavity at an interface of DI water and mineral oil (Sigma-Aldrich, UK). The mineral oil pushes the bubbles in the acoustic field and allows the patterning and manipulation. The DI water contained a surfactant F-68 pluronic (2% concentration), non-toxic for cells. The surfactant added in the DI water minimised the meniscus formed at the oil-water interface inside the ultrasonic cavity. Without surfactant, the bubbles injected positioned themselves at the edges of the cavity and are out of the ultrasonic field. The data provided by the provider did not allow estimating the amount of bubbles contained in the sample.

The transducers excitation was adjusted not to destroy the fragile microbubbles. As their main purpose in classical experiments is to explode/implode creating cavitation for improved imaging or deliver the enclosed molecules in drug delivery, microbubbles are susceptible to destruction if subjected to too high an excitation power. In the following experiments they were excited with a 0.6 Vpp sine wave at a frequency of 4 MHz, this amplitude being the upper limit before which the patterns are destroyed by microbubble explosions.

The microbubbles collapse slowly with increasing transducer voltage; all the microbubbles have cavitated at 8 Vpp (Fig. 7).
Fig. 7 Microbubbles behaviour under different acoustic voltage excitation. Scale bar = 100 µm.

Fig. 8 shows photographs of the bubbles without ultrasound (a), trapped with two transducers (b), and trapped with three transducers (c). It can be noticed that the microbubbles are trapped in hexagonal shape with three transducers although the pattern is slightly altered.

The microbubbles in a standing waves field would be forced either to the pressure nodes or antinodes, depending on the relation between the resonance frequency of microbubbles and ultrasonic excitation frequency. In our case, it can be observed that the microbubbles are trapped in hexagonal patterns when using three transducers, therefore they are at the minima of the acoustic energy landscape at the pressure nodes. The resonance frequency of the microbubbles (van der Meer et al. 2004) is lower than the transducer frequency of 4 MHz (Wu and Nyborg 2008; Xi et al. 2011). These experiments therefore demonstrate that microbubble manipulation is possible in a non-destructive fashion. Positioning can be achieved by shifting the phase of one of the transducer, in the same way that
functionalised microspheres and emulsion droplets were displaced. The microbubbles represent a very promising drug vector for in vitro applications during trials, since the precise release of their load at the desired site could be triggered by an increased ultrasonic excitation.

IV. CONCLUSION

In this paper we presented a new acoustic device that has been successfully shown to be capable of patterning various micro particles. Functionalised particles, emulsions and microbubbles can be immobilized as well as positioned in a controlled fashion by electronic phase shifts. This precise positioning of drug carriers offers considerable potential in bioengineering applications such as targeted drug delivery. This device offers multiple advantages over previous approaches, including small device size, general biocompatibility and sterile cell culture capability, simplicity in experimental setup and control and the potential for integration with other analytic sensor modules.

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