

Experimental Investigation of Ultrasonic Trapping of Bioparticles Using Parallel Plane Cavity

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ABSTRACT

This paper explores the use of acoustics for manipulation of bioparticles. A series of experimental studies were conducted using acoustic standing waves in resonant cavities as means of trapping bioparticles and enhancing bioparticles separation. Parallel Plane Cavity was explored. The effect of cell size and host media concentration on trapping efficiency, trapping time and velocity were investigated. Monitoring of the cells behavior during ultrasonic manipulation by using three simple techniques (light microscopy, dielectric measurement and optical absorption technique) was studied. Study indicates that increasing ultrasound intensity increases the trapping efficiency of cells and decreases the time consumed to trap cells. Also force increases with increasing cell diameter. Increasing of cell media concentration tends to increase the trapping time and decrease the cell velocity. Osmotic fragility studies of the trapped cells indicate that there is no effect on cell membrane integrity of the biological cell exposed to ultrasonic standing wave.

KEY WORDS:

Bio particles trapping, Cell manipulation, Ultrasound standing waves, Parallel plane cavity, RBC,s Yeast, E-Coli.

1. INTRODUCTION

The sorting and trapping of target cells and suspended particles from a medium is of great importance to cell biology, drug delivery and related fields in biomedicine. Furthermore; the ability to separate and trap micro-particles is of profound interest to the biomedical community to obviate current clinical limitations^[1]. The conventional regimes for cell separation, manipulation and trapping need not only expensive equipment but also the skillful management of equipment's. Sorting technology using acoustic waves has several benefits over the other methods such as mechanical^[2], biochemical^[3], magnetic^[4], and dielectrophoresis separation^[5]. Ultrasonic manipulation technology has recently emerged as a powerful tool for handling micrometer-sized biomaterials (such as cells and bio-functionalized beads) in microfluidic chips^[6-10], with application examples such as separation and fractionation^[11-13] washing^[14,15], positioning^[16] and aggregation and retention^[17-19]. Ultrasonic- standing –wave technology shows promise for both efficient, as well as gentle manipulation of cells. It provides an accurate, low-power method of trapping that cause's minimal damage to cells^[20]. Also, acoustic standing wave manipulation is characterized by its long-range force field, determined by the pressure node spacing in the axial direction^[21]. A number of studies indicated that the acoustic fields used caused no variations in particle integrity and cell viability in yeast, mammalian cells or erythrocytes. Various resonator

systems are designed to exploit acoustic radiation forces originating from geometrically well-defined resonant ultrasonic fields to move particles or cells in suspension^[22-24].

In the present work three cell trapping monitoring techniques were investigated. Different experimental trapping conditions were studied.

Materials and Methods

In the present work, three biological cell samples are used, red blood cells (R.B.C's), yeast cells and Escherichia coli (E. coli).

2. RED BLOOD CELLS (R.B.C'S).

Ten blood samples were taken from healthy volunteers following a protocol approved by Medical Research Institute ethics committee guidelines. Ten ml of blood was drawn and anti-coagulated by (EDTA) and then plasma and buffy coat were removed by centrifugation (800 rpm for 10 min.), the remaining R.B.C's were washed in phosphate buffered solution (PBS) pH 7.4. The obtained R.B.C's were divided into small parts and diluted by different media e.g. phosphate buffered solution (PBS), agarose, and polyacrylamide gel to yield a final cell suspension containing approximately 1.5×10^5 cell/ml. The cell concentration was counted using Neubauer haemocytometer.

Preparation of yeast samples

Dried yeast cells (*Saccharomyces cerevisiae*) were suspended in PBS pH 7.4 and diluted to yield a final suspension containing approximately 1.5×10^5 cell/ml.

Preparation of E. coli

E. coli obtained from the Department of Microbiology, Medical Research Institute, was suspended in PBS to prepare a final suspension containing approximately 1×10^6 cells/ml.

Ultrasonic trapping system

To investigate biological cells manipulation and separation, a simple homemade ultrasonic cell trapping system was designed and constructed. This system consists of three main parts: ultrasonic generator, trapping cavity, and monitoring system.

1-Ultrasonic Generator

Ultrasonic Generator (Model CSL Shanghai, No822 Factory, China) operates at 0.8 MHz continuous wave mode with output intensity from 0.5 to 3 W/cm² was used as a source for ultrasonic waves. The ultrasonic wave emitted from the transducer is coupled to the trapping cavity using an aluminum sheet extended inside the cavity.

2-Parallel plane trapping cavity

The parallel trapping cavity consists of two glass slides (75x25 mm) separated from each other by two glass spacer of thickness 0.5 mm at lateral sides and by a rectangular aluminium coupler (30 mm length, 20 mm width and 0.5 mm thickness) and stainless steel reflector at the other sides. The aluminium coupler transfers the ultrasonic waves from the transducer to separation cavity. The ultrasonic waves pass through the cell suspension and reflected back by stainless steel

reflector to form standing waves pattern Figure 1. The distance between two successive nodes or antinodes within the standing waves was calculated as half of the ultrasonic wavelength ($\lambda/2$). In the present work 0.8 MHz ultrasonic waves were used, the distance between the aluminium coupler and stainless steel reflector was chosen to be 27.75 mm which is multiple of ultrasonic wavelength.

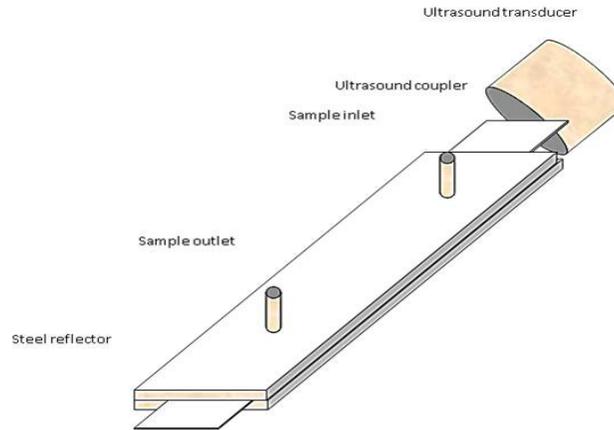


Fig. 1. Basic constituents of home-made parallel trapping cavity.

3-MONITORING SYSTEM

The cells trapping behavior under the effect of US application was monitored by using three experimental methods: Light microscopy inspection, dielectric measurement, and optical absorption measurement techniques.

3.1 -Light microscopic inspection monitoring

To study the behaviour of the biological cells inside the trapping cavity under the effect of the ultrasonic waves, a light microscope system (Type: BX41; Olympus America Inc.) was used Figure 2. The trapping cavity was fixed over a microscopic stage, and nearly 1.5 ml of the cells suspension was injected inside the trapping cavity, then left for a minute to be get stable and was subjected to ultrasonic waves. The imaging capture system was consisted of CCD camera mounted on the light microscope column. The microscopic image was projected onto the CCD camera. The camera was connected to a personal computer (PC) across an interface Fly TV Prime3 XTV tuner and video capture card 3.22. Images were recorded on the PC in order to perform imaging processing procedures. Recording of cell behaviour film under US effect was performed using Life View TVR program (version 3.21.400). At the beginning the of US application, the life view program was opened in the same time and by mouse cursor on the PC screen, the film recording was started. Also images were captured by the snapshot button.

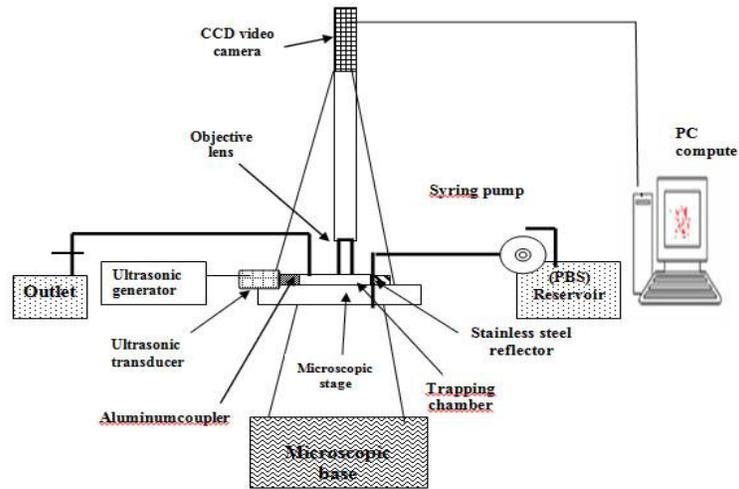


Fig. 2. Schematic diagram for light microscopy monitoring system

3.2-Dielectric measurement monitoring

In dielectric method, resistance and capacitance of R.B.C's suspended in 8.5 % (w/v) non-electrolytic sucrose suspension was monitored using 50 kHz alternative current passing through the suspended medium via two silver electrodes connected to LCR meter (Instek-LCR meter 819). Silver electrodes are of interspaced distance (d) 1 mm, and the immersed electrode cross-sectional area $1 \times 1 \text{ mm}^2$ at estimated node position. The permittivity (ϵ) and conductivity (σ) of the manipulating cells were calculated from Equations (1 and 2).

$$C = \epsilon \cdot \frac{A}{d} \quad (1)$$

Where C is capacitance, ϵ is permittivity; d is interring spaced distance of electrode.

$$G = \sigma \cdot \frac{A}{d} \quad (2)$$

Where G is conductance, σ is conductivity.

3.3-Optical absorption monitoring

Optical method provides an inexpensive and very sensitive technique for monitoring cell movement. In this method, incident light from red laser diode of nearly 640 nm wavelength is passing through trapping cavity. The transmitted light was received by a pin-photodiode detector (model GCPD, china) fixed at the other side of the cavity. The output voltage from the detector was amplified and recorded using a Chart Recorder. The whole experiment was carried out at a dark room to avoid any background light overlapping.

4. EXPERIMENTAL PARAMETERS AFFECTING THE TRAPPING EFFICIENCY

Effect of ultrasound intensity

In the present study, the effect of different ultrasound intensities ranged from 0.5 to 3 W/cm² on cell trapping fraction and osmotic fragility of the cell were done.

4.1-Cell trapping fraction

To study R.B.Cs trapping efficiency for applying US intensity from 0.5 to 3 W/cm², trapping fraction of different intensities was calculated. Trapping fraction is defined as the percentage of the trapped cells after constant time. Captured images (at 400x) were processed by using Scion image program version 4.0.3.2 to calculate cell trapping fraction. Cell trapping fraction was calculated from the relation:

$$\text{Cell trapping fraction (\%)} = \frac{\text{mean no. of trapped cells/image}}{\text{mean total no. of cells/image}} \times 100$$

4.2-R.B.Cs osmotic fragility

To determine the cell integrity and viability after different US intensities application, R.B.Cs osmotic fragility test was performed according to (Arthur *et al.* 1947) ^[25]. 20 μL of outlet R.B.Cs obtained was added to two ml of saline concentrations from 0 to 0.9 % NaCl. shake well and leave for 30 min in dark at room temperature then centrifuge at 8000 r.p.m for 15 min. Read the absorbance (A) of the supernatant for each saline concentration against 100 % lysis (Tube marked 0 concentrations) at a wavelength of 540 nm using Jenway UV-visible spectrophotometer. The osmotic fragility curve (NaCl concentration on X-axis and % Hemolysis on Y-axis) was plotted. The concentrations of NaCl at which 50% hemolysis occur for control and test samples (median corpuscular fragility MCF) were determined from the curve.

$$\% \text{ Hemolysis} = \left(\frac{\text{Optical density of sample}}{\text{Optical density of 100\% lysis}} \right) \times 100$$

Effect of suspending media concentration

To study the effect of suspended medium concentration on trapping behaviour (cell trapping velocity and time) of R.B.C's suspensions were prepared at phosphate buffer solution (PBS) and different concentration of agarose and polyacrylamide gel were added to form a gel media concentration ranged from 0.063 to 1 %. Film was recorded for the cell movement using a video camera fixed on the light microscope. From the recorded film of the cell during trapping, the instant cell velocity was calculated by measuring the time required for the cell travel 10 mm on the screen along the trapping path after 15 seconds from ultrasound application. The cell movement distance was calibrated using standard latex sphere particles of diameter equal to 1.151 μm. The velocity of the cell was estimated from the relation:

$$\text{Cell velocity} = \frac{\text{Distance(mm)}}{\text{Time consumed(seconds)}}$$

5. RESULTS AND DISCUSSION

Monitoring of biological cells in the trapping cavity

1.5 ml of each cell suspension was injected inside the trapping cavity, and left for a minute to get stable. The movement of the cells inside the trapping cavity was monitored using three techniques:-

Ultrasonic Cell manipulation was tested by light microscope under different ultrasonic intensities ranged from 0.5 to 3 W/cm² after a trapping time of 30 s. As shown in Figure 3, ultrasonic intensity of 3 W/cm² gives a maximum trapping efficiency for RBC's suspension. So, all measurements in the following experiments were performed under the effect of this intensity at room temperature (25 C⁰).

Figure 4 shows light microscopic image of R.B.C's (a&b) and Yeast (c&d) patterns before and after sonocation at intensity of 3 W/cm². Before sonocation the cells were distributed randomly Figure (a&c) and then after sonication, the cells begin to stratify in organized bands after nearly thirty seconds Figure (b&d). Studying of the trapping pattern indicate that the interspaces distances between the center of the bands is nearly 0.9 mm which is nearly equal to half wavelength of the ultrasonic waves used.

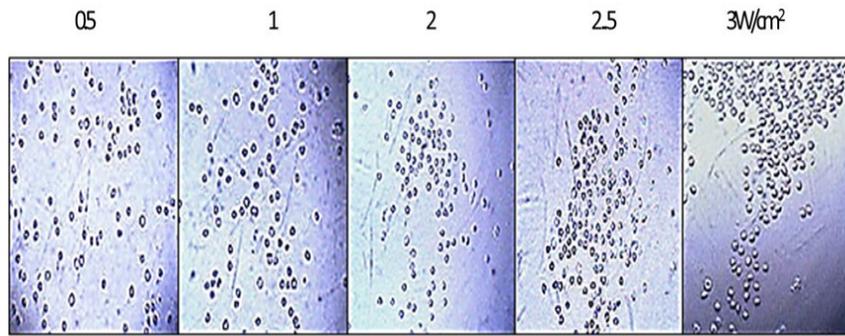


Fig. 3. Effect of different ultrasonic intensities on trapping R.B.C's.(100X)

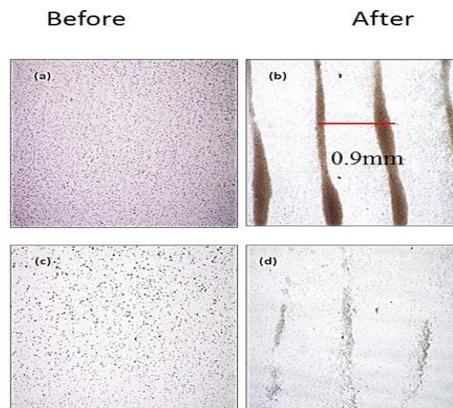


Fig. 4. Light microscopic image of R.B.Cs (a and b) and yeast cells(c and d) before and after sonication in parallel cavity.

To determine the cell separation ability of the parallel plane resonator, a mixture of R.B.Cs. and *E.coli* cells 1:1 Volume/Volume was made and injected into the trapping chamber Figure 5. It is clear that before sonication the cells were heterogeneously distributed. After sonication, the R.B.Cs begin stratify in organized bands at the left of the image, while *E.coli* of relatively smaller in diameter ($0.5 \mu\text{m}$) than R.B.C's ($7\mu\text{m}$) are still distributed within the image field. These bacterial cells can be separated and eluted to outlet from stratified R.B.Cs bands by the aid of Phosphate buffer saline (PBS) passing through the separation cell.

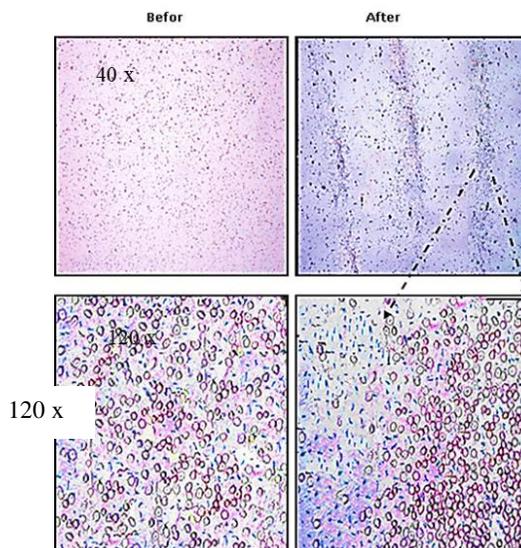


Fig. 5 .Light microscope images (x40) and (x120) of R.B.Cs (round shape) and *E. Coli* (blue rods) before and after sonication at 3 W/cm^2 ultrasound waves.

The force induced on particles in an acoustic standing wave field is the result of both the primary and secondary radiation forces, where the primary force originates from the standing wave and the secondary forces are due to sound waves scattered by the particles [8, 20]. At the beginning of US application suspended cells were driven by primary acoustic radiation force and moved to the pressure nodal planes and started to form cell bands perpendicular to the direction of acoustic waves. Cell bands were formed in the interval of every half-wavelength in the standing wave fields Figure 4. US longitudinal waves are often called pressure waves, then standing waves can be described in terms of the pressure variations in the trapping cavity. Thus when the standing waves are created within the cavity, variation in pressure points will occur including maxima and minima pressure points (nodes and antinodes) corresponding to velocity antinodes and nodes respectively (Lilliehorn and Simu (2005) [26]).

In case of R.B.C's and yeast (Figure 5) cells that have positive contrast factors (ϕ) the cells suffer the acoustic radiation force between the nodal and antinodal position till these cells are trapped at node positions. As a result waves pressure diminishes as cells are driven towards the pressure node (velocity anti-node), thus these cells produce definite pattern of standing waves pressure nodes of cells inter-spaced by anti-nodes of space ($\lambda/2$) [27].

6. CONDUCTIMETRIC AND DIELECTRIC MONITORING

In this experiment continuous measurement of resistance and capacitance were recorded at several time intervals following the application of 0.8 MHz, ultrasonic waves of intensity 3

W/cm^2 at frequency 50 kHz. The corresponding conductivity and permittivity were calculated using equations 1 and 2. At a high frequency of 50 kHz the injected current penetrates the cells membrane of RBC's and the RBC's aggregation during sonocation causes changes in the conductivity and permittivity of the suspended medium. Results shown in Figure 6, 7 indicate that the permittivity and conductivity of the suspended cells increases with increasing of the sonication time and reach saturation after a trapping time in the range after nearly 50 seconds. Obtained plot can be correlated to the aggregation of the RBC's.

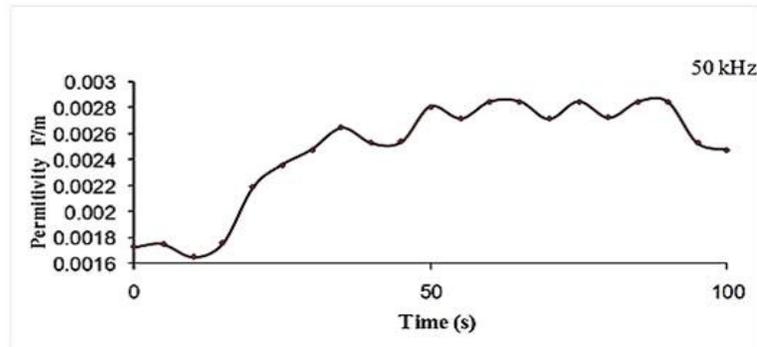


Fig. 6. Shows monitoring of the trapped R.B.Cs. by variation in the permittivity in relative to time (seconds) at 50 kHz frequency.

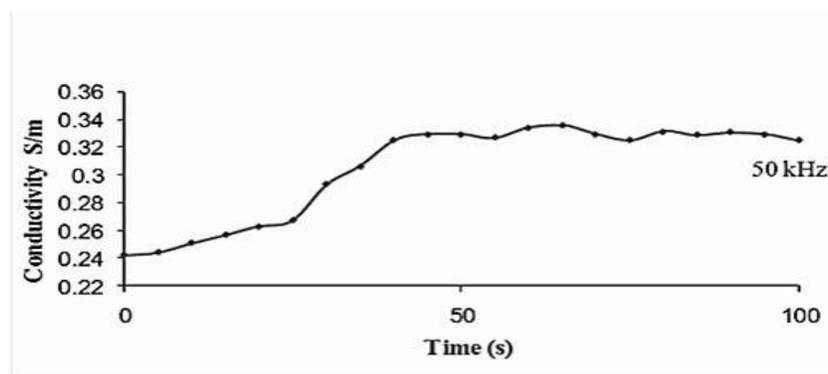


Fig. 7. Shows variation in the conductivity of R.B.Cs.in relative to time (seconds) at frequency of 50 kHz

Conductimetric detection has been investigated for chip-based separation and is a good technique in particular for small ions which are otherwise not readily detected. Permittivity is a physical quantity that describes how electric field affects, and is affected by a dielectric media. It is determined by the ability of a material to polarize in response to the field and thereby reduce the total electric field inside the material. Thus permittivity relates to a material's ability to transmit an electric field ^[28]. Dielectric monitoring gives a non-destructive, accurate and error-free kinetic manner. This method exploits alternating current (AC) technique that can provide valuable mechanistic information as it provides data on both electrode capacitance and charge-transfer kinetics (Bruce and Frazier 1999) ^[29].

7. OPTICAL ABSORPTION MONITORING

In the present work, the intensity of transmitted light through stained yeast cells is measured using a photodiode detector. Figure 8 illustrates x-t chart of the electrical signal of transmitted light passes through the trapping cavity with stained yeast cells and measured by photodiode detector during the trapping process. The recorded signal decreases with increasing of time. Nearly most cells are trapped after nearly 32 seconds and almost remain stable. Although these detection techniques provide high sensitivity and are well characterized and developed, they have several limitations.

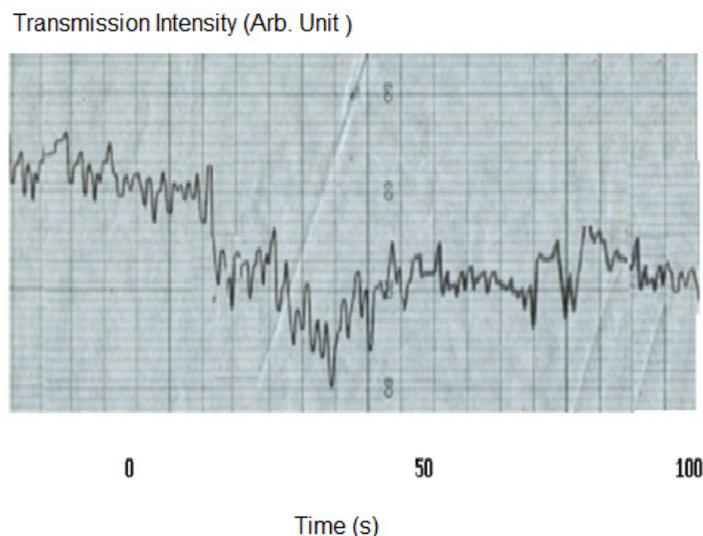


Fig. 8. x-t diagram of the transmitted light signal after starting of ultrasonic trapping process of yeast cells.

8. OPTIMAL CONDITIONS FOR THE CELL TRAPPING

Understanding the characteristics of the primary radiation forces is sufficient to interpret the acoustic cell manipulating concept. The magnitude of the ultrasound radiation force is proportional to the ultrasonic wave intensity, cells size, and the difference in the mechanical properties between the cells and the host medium which are density and compressibility.

Effect of US intensity on trapping efficiency and trapping time

Figure 9 shows the effect of increasing of applied US intensity on trapping efficiency. It was found that the increasing in the applied US intensity greatly increases the percentage of trapping fraction per image studied. That is an indication of increasing in trapping efficiency. Cell movement was investigated using light microscope and the time required to trap all cells and form a trapping band for both gel media (agarose & polyacrylamide) concentration was recorded using a stop watch. Figure 10 shows the changes in the number of cells trapped with time per seconds. With increasing of the sonication time the number of trapped cells increases. At the beginning the cell are randomly distributed (a) as the time proceeds the cells behave to trap (b) till formation of visible bands (c) then at prolonged time the cells become to adhere more to each

other (d). As shown in Figure 11 increasing of the ultrasonic wave intensity tends to decrease in the trapping time. That is an indication of increasing in trapping efficiency.

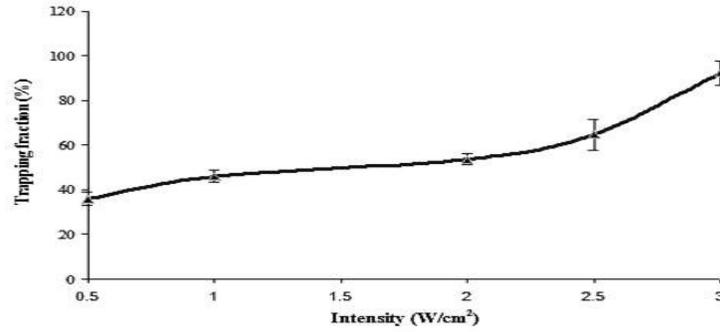


Fig. 9 . Shows relation between trapping fraction and ultrasound intensity.

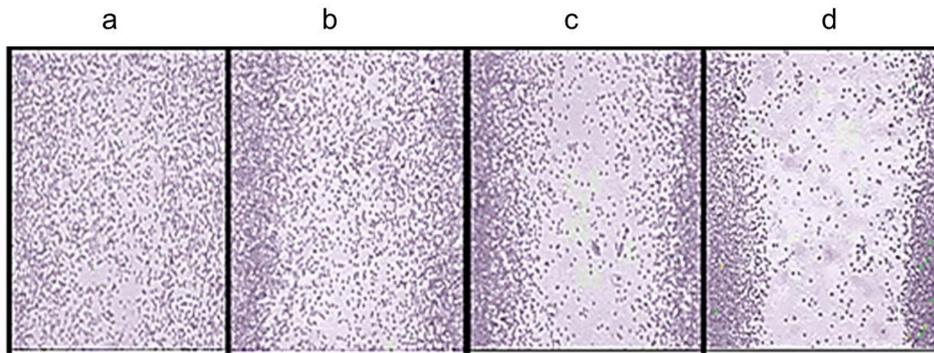


Fig. 10. The effect of sonocation time on cell trapping band formation.

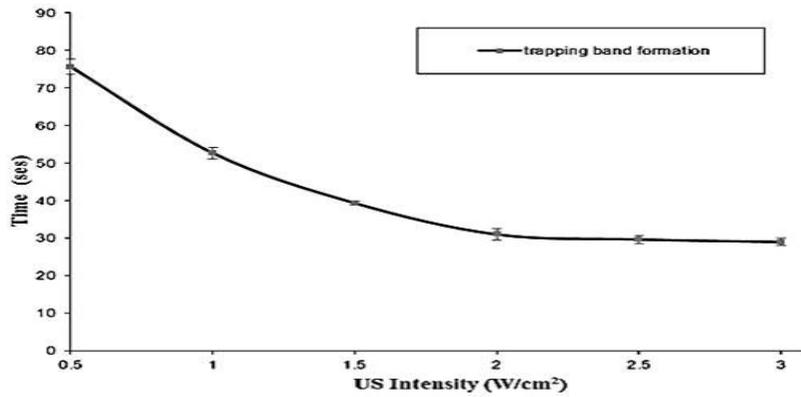


Fig. 11. Relation between ultrasound intensity and trapping time

8.1-R.B.Cs osmotic fragility

Figure 12 shows the osmotic fragility curve of R.B.Cs exposed to different ultrasound intensity, there is no effect on cells membrane integrity. In comparison with untreated cells the mean corpuscle fragilities (MCF) for exposed cells still remain within the normal R.B.Cs. range.

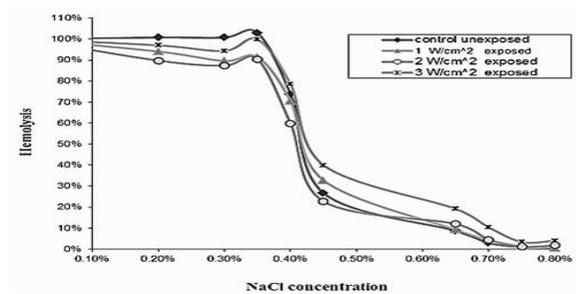


Fig. 12. Relation between osmotic fragility (hemolysis) of R.B.Cs and NaCL concentration at different ultrasound intensities.

When the ultrasonic waves of intensity is applied to the cell suspension e.g R.B.C's, the cells suffer acoustic force which can be calculated for parallel trapping cavity^[30]. At low intensity some of cells (R.B.C's) were trapped and move more freely in the field across the ultrasonic standing wave nodes. When the ultrasound intensity increases the cells begin to trap and the trapping efficiency (expressed as cells trapping fraction) increases as the ultrasound intensity increases, and the most of cells were trapped at intensity 3 W/cm² (Figure 3, 9).

With increasing of sonication time the number of trapped cells increases. Also increasing of the ultrasonic wave intensity tends to decrease the trapping time (Figure 10,11). As time proceeded, more and more cells arrived at potential wells and striated cell columns thickened and were clearer to observe (Figure 10). Then, probably under the influence of secondary radiation force generated from inter-particle attraction, cell clusters were concentrated and compressed toward the central axis of the cavity^[35].

Applying mixture of different cells that have variation in their diameter will appear different trapping behaviors: According to our mathematical estimation Table 1, for R.B.Cs and *E .coli* mixture, the R.B.Cs was trapped in pressure node relatively faster than *E .coli* cells. However, it is difficult for *E.coli* to stay on pressure nodal planes because acoustic microstreaming force imposed on bacterial cells is comparable to the primary radiation force and could make *E. coli* circulated between nodal planes. Hence applying the separation liquid flow rate, the cells will spend more time in the acoustic field and thereby experience more force. Even so, it was demonstrated that very low concentrations of submicron-sized bacteria could be harvested from suspension with larger cells such as R.B.C's^[26, 31,32].

9. The effect of suspending media concentration

From the recorded film stripe on the PC computer screen, the time required for the focused single red blood cell to move a fixed distance along the trapping path were recorded (Figure 13) and used to calculate the cell trapping velocity.

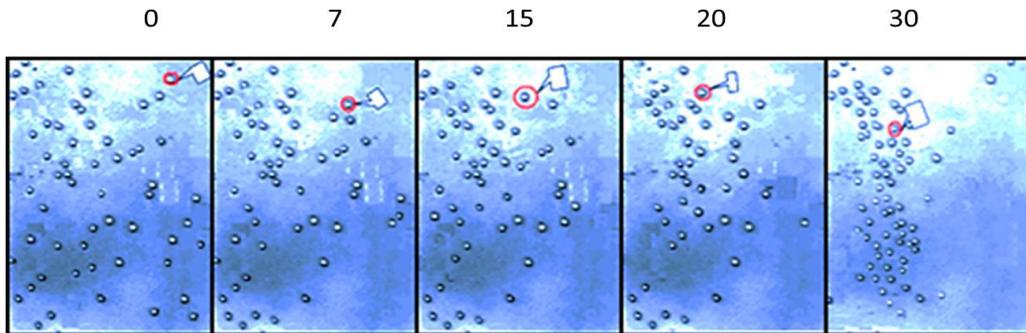


Fig. 13. Film stripes represent the behavior of a red blood cell in 1% agarose media sonicated with 3 W/cm² continuous ultrasound waves.

9.1-The effect of medium concentration on cell trapping Velocity

Figure 14 shows the effect of agarose and polyacrylamide at different concentration on cell velocity. It is clear that, increasing of the medium concentration decrease cell velocity.

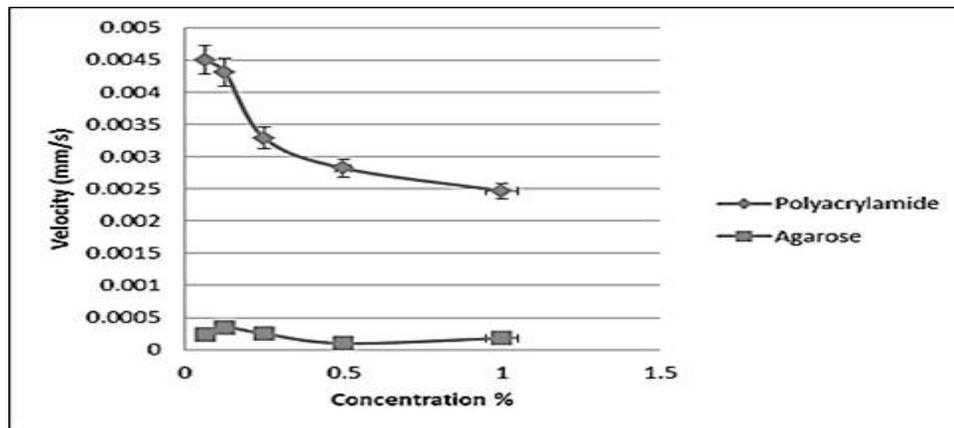


Fig. 14. Change in cell velocity relative to the concentration of agarose and polyacrylamide .

9.2-The effect of medium concentration on the average trapping time

The effect of medium concentration on the average trapping time is shown in Figure 15. Increasing of gel concentration tends to increase the trapping time. The higher relative viscosity of polyacrylamide (nearly 4 times the density of the saline solution) is tending to increase the trapping time level than that of lower agarose one (nearly 2 times the density of the saline solution).

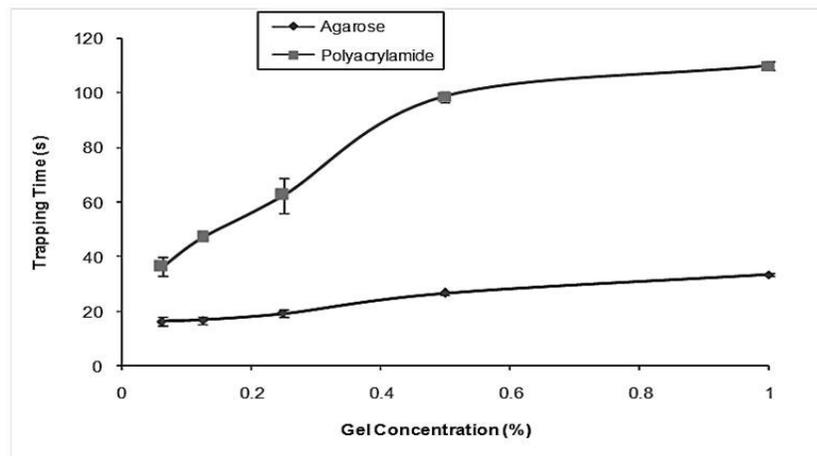


Fig. 15. Effect of medium concentration on the mean trapping time.

10. CONCLUSIONS

This work presented here deals with experimental investigation of ultrasonic trapping of bioparticles using parallel plane cavity and monitoring of the cells behavior during ultrasonic manipulation by using three simple techniques (light microscopy, dielectric measurement and optical absorption technique). Dielectric monitoring and optical absorption using photodiode detector to measure transmitted light through stained cells are deemed likely to become strong complementary methods to detect optimal time for manipulation. Study indicates that increasing ultrasound intensity increases the trapping efficiency of bioparticles and decreases the time consumed to trap bioparticles. Increasing of trapping media concentration tends to increase the trapping time and decrease the cell velocity. No effects on cell membrane of the biological cell exposed to ultrasonic standing wave were observed.

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