Use of Ultrasonic Standing Wave in Biological Studies and Cell Technologies

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In experiments on rat erythrocytes and yeast cells, we studied processes of concentration, separation, and isolation of native cells in suspensions by using standing ultrasonic wave field. Fifteen parameters essential for these processes were determined, and the applications of ultrasonic stratification, concentration, and cell separation methods under experimental conditions were determined.

Key Words: *standing ultrasonic waves; cells; concentration; separation*

Rapid isolation of biological cells in the native state is essential for both biological studies and practical application. Obtaining of different cell types usually involves a series of procedures for cell isolation from tissues. It includes regular replacement of suspension media, separation of native cells from damaged ones, fractionation of cells by different methods, including density gradient ultracentrifugation, use of filters, adsorbents, cell culturing, and preparation of suspensions of a certain concentration. Alternative methods considerably accelerating and simplifying the procedure of sample preparation are ultrasonic (US) methods for cell concentration, isolation, and separation in suspensions.

US field promoting rapid aggregation and precipitation of particles in cell suspensions can be effectively used for concentration of mammalian cells (blood cells, stem cells, cells of various tissues), nanophytoplankton and ultraphytoplankton, bacteria, *etc.*

The use of US standing waves (USSW) will appreciably accelerate isolation of native cells in the needed concentrations. A specific feature of these methods is that the cells after isolation from tissues are placed into a US box, where they can be concentrated, divided into fractions, the culture medium can be replaced, and damaged cells can be eliminated without removal of cells. These methods can be used for the analysis of cell products, pharmacological testing, evaluation of the effects of chemical and physical factors on cells in suspension. The use of FITC-labeled antibodies to different bacteria, for example, salmonella, during cell concentration can lead to intensification of fluorescence and will help to detect and identify these cells. An advantage of the new acoustic separators and concentration devices is the absence of filter contamination, no need in replacement of US boxes, high efficiency of separation, and reliability.

We studied factors essential for fine regulation of separation and concentration of cells used in biological research and biotechnologies in a USSW field.

MATERIALS AND METHODS

The US methods are based on exposure of suspended cells to US fields forces (radiation, Stokes, Bjerknes, Bernulli, gravitation). Summary exposure to these forces leads to migration of cells into USSW pressure nodes [1]. For cells trapping in the suspension volume, ultrasound of 0.88-10 MHz forming standing waves in boxes for cell concentration or

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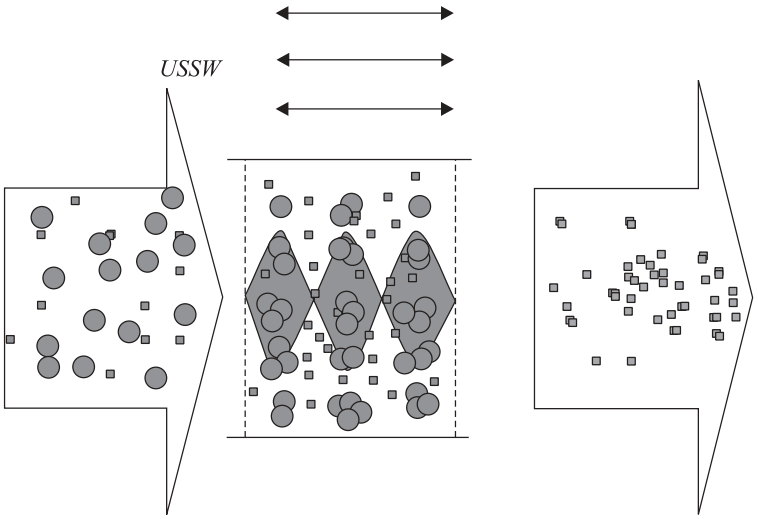

Fig. 1. Stratification and separation of suspended cells in USSW field.

separation is used. In this field, the cells immediately start moving from high pressure to low pressure areas (pressure nodes) and remain there until the field is applied. Due to this, cell concentration in the standing wave nodes increases [3]. The use of US selection flow method leads to separation of cells into fractions. This process depends on some parameters of the test cells, culture media, ultrasound, and medium flow rate through the US box.

The study was carried out on rat erythrocytes and yeast (*Saccharomyces cerevisiae*) cells. Erythrocytes were isolated from the blood of Wistar rats (250-300 g; n=6). The blood was collected from the caudal vein, diluted to a concentration of 10^6 cell/ml with saline containing heparin. Yeast cells were diluted with distilled water, which stopped their division. The initial concentration of yeast cells was 10% (2.5×10^9 cell/ml).

We created laboratory copies of devices and US boxes for evaluation of stratification, concentration, and separation of different types of cells in suspensions. The scheme of cell stratification and separation in USSW field is presented in Fig. 1. The devices worked at 0.88 and 2.64 MHz frequencies at 0.05-1.5 W/cm^2 ultrasound intensity. With a focusing source working at a frequency of 2.64 MHz, the ultrasound intensity in the focal area reached 6 W/cm^2. The time course of cell stratification and rate of this process were evaluated in rectangular cuvettes with flat emitters working at 0.88 and 2.64 MHz and fixed to the bottom. Focusing piezoceramic emitters, working at the frequency of 2.64 MHz (geometrical characteristics of a spherical emitter: focal length 40 mm, focal area length 10 mm, focal area radius 1 mm) were used in a US box for increasing the radiation power. Volume-regulated flow of the medium through the US box was created with a peristaltic pump with a special piece in front of the box ensuring nonpulsatile flow. The US boxes were maintained at 36°C. The medium simultaneously served as a contact medium between the emitter and the box for cell concentration and separation. The parameters of US fields were controlled by the stain-paper method developed at our laboratory [2]. This method presents a visual intensity distribution in different sections of US bundles within less than 1 min; computer processing of these images determines the values of local and mean intensities for various sites of the US bundle. The ultrasound parameters inside the boxes were controlled by a differential thermocouple calibrated by the ultrasound intensity.

The rate of cell stratification was evaluated by the optical method in a box with optical pathway of 3 mm, using a laser and a photodetector. A laser beam passed normally to the cuvette surface and reached the photodetector. Cell stratification rate was recorded using a PC analog-to-digital transfor-

TABLE 1. Parameters Used for Estimation of USSW Forces in Exposure of Yeast Cells

Parameter	Value
Ultrasound velocity in water at 25°C, m/sec	1497
Sound velocity in yeast cells at 25°C, m/sec	1589
Velocity increment (ΔU) for 1 cell, m/sec	$5.35\times10^{-7}\pm2\times10^{-4}$
Percentage of dry weight in *Saccharomyces cerevisiae* cells	25
Water and cell density	1.000-1.085
Cell radius, μ	1.5
Ultrasound pressure on the cells, atm	1.1
Adiabatic compressibility, Bar^{-1}	45

mer. Cell concentration and separation were carried out in a thermostat in a glass box in which a pure standing wave or stable US wave interference was formed.

RESULTS

The main factors essential for cells in the US wave are various forces (radiation, Bjerknes, Bernulli, gravitation), and therefore in order to calculate the forces to which the cells were exposed in a USSW field, the ultrasound velocity in yeast cell suspension, dry weight of cells, cell density and size were measured (Table 1).

Cell stratification in a USSW field under stationary conditions (without culture medium flow) was studied at two frequencies (Fig. 2, 3).

During concentration in the flow, the cells grouped in the zone of the axial intensity of the emitter, because the forces acting on cells were maximum in this zone. As the alternating pressure nodes in the center of the box were filled, its periphery also started to fill. The node regions had finite sizes and after they were completely filled, the cells under the effect of negligible forces acting in the US field outside the pressure nodes migrate into other alternating pressure nodes closer to the box walls (Fig. 4).

Since the maximum forces in the field act on the largest cells or cells maximally differing by the wave characteristics from the culture medium, smaller cells are washed out from US boxes. For further fractionation, the media containing cells not trapped in the box under certain conditions of US exposure and flow rate should pass through a series of boxes with different ultrasound parameters at different medium flow rates.

We determined 15 independent parameters essential for the stratification, condensation, and separation of cells. The parameters characterizing US fields are ultrasound frequency and energy, field shape or spatial distribution of energy (for flat or focusing emitter), type and frequency of ultrasound modulation. The parameters of cells are size and shape, ultrasound velocity in the cell, density of native and damaged cells, and their initial concentration. The ultrasound velocity, medium density and temperature are determined as parameters characterizing the culture medium. The culture medium flow is characterized by the rate of medium pumping through the US box, distribution of velocities in the flow (uniformity of the medium movement in different areas of US box).

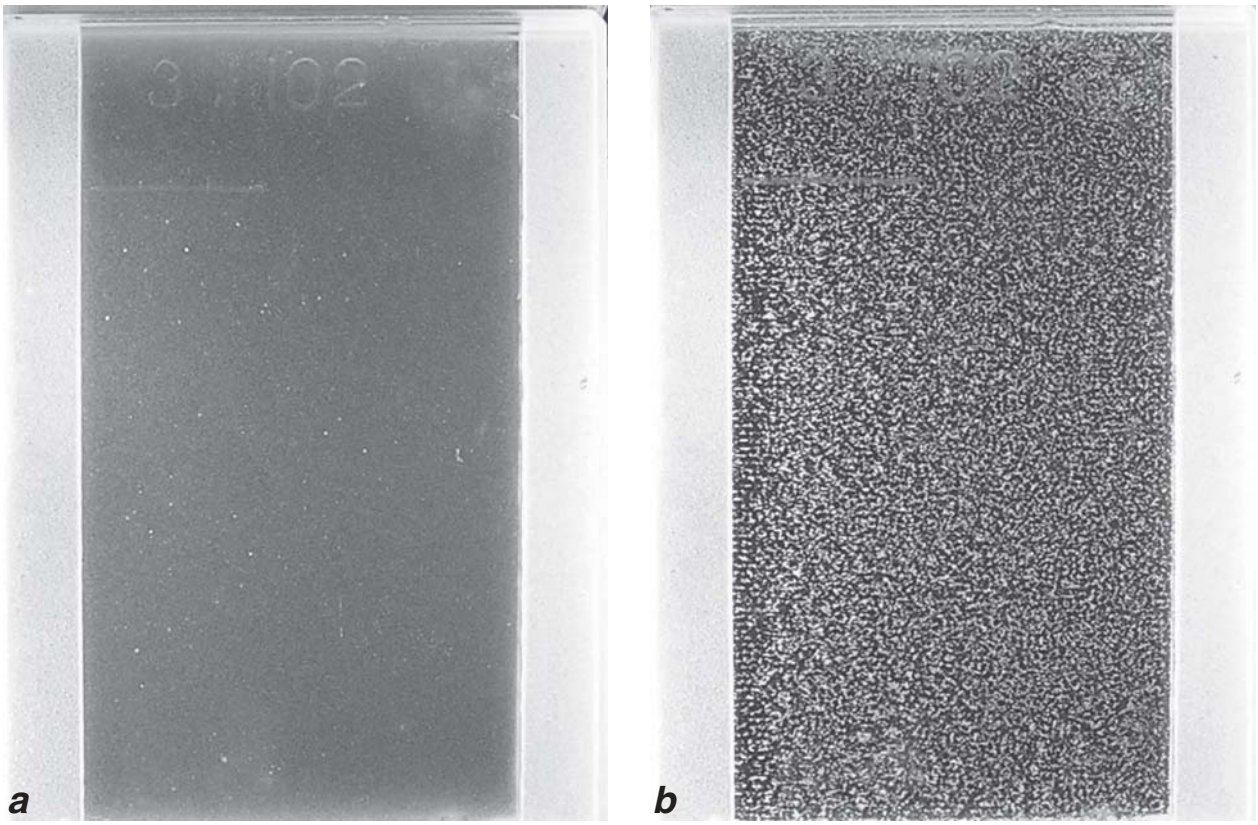

Fig. 2. Stratification and concentration of yeast cells in USSW field at 2.64 MHz. Cell concentration 10^7/ml. *a*) initial distribution of cells in the box; *b*) 10 sec after the ultrasound was switched on.

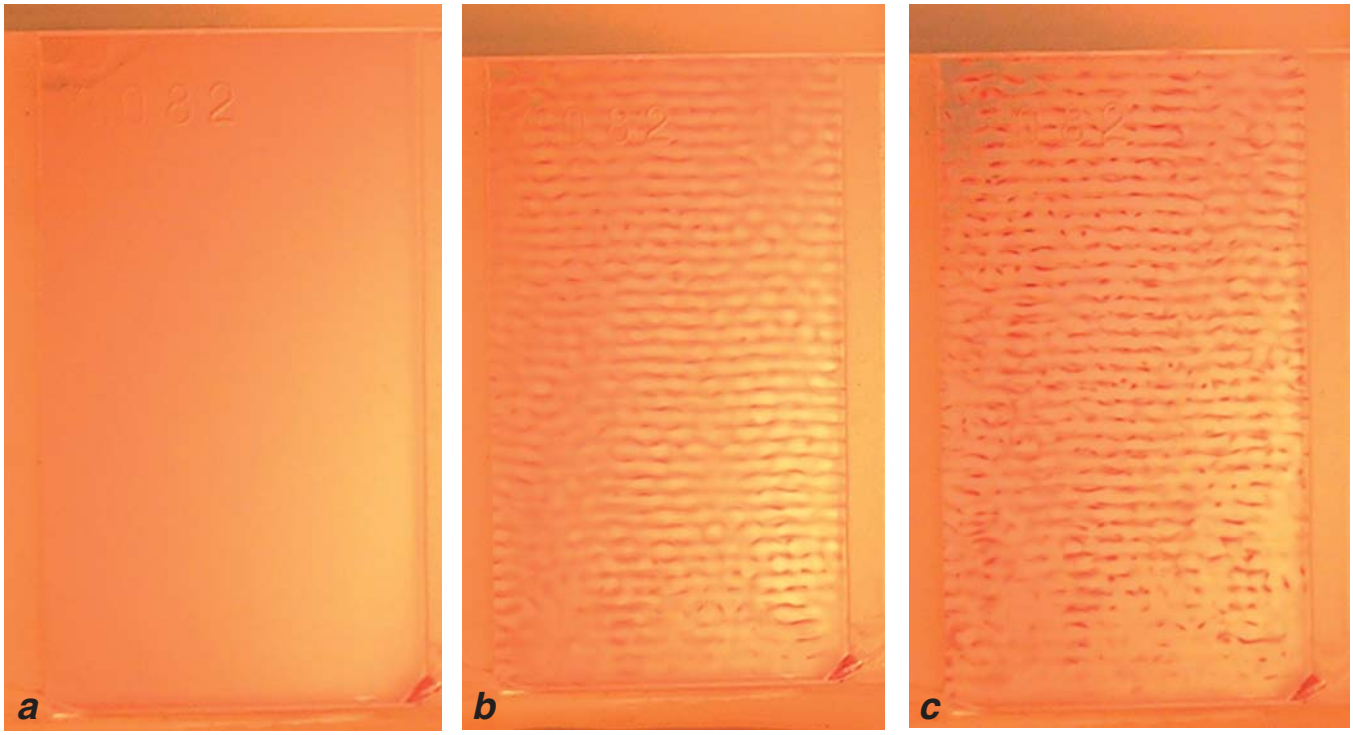

Fig. 3. Stratification and concentration of rat erythrocytes in alternating pressure nodes of USSW field at 0.88 MHz frequency. Initial cell concentration 10^6/ml. *a*) initial distribution of cells in the box; *b*) 5 sec of exposure to ultrasound; *c*) 10 sec of exposure to ultrasound.

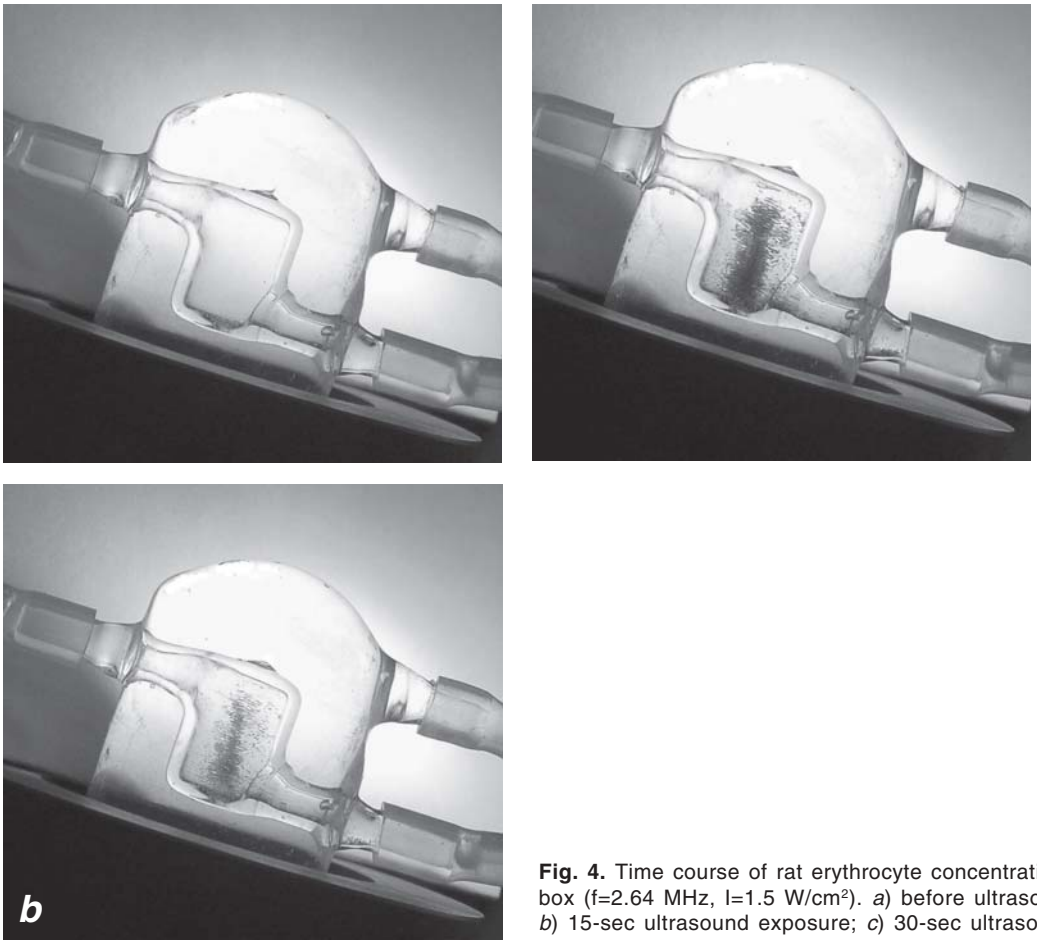

Fig. 4. Time course of rat erythrocyte concentration in a USSW box (f=2.64 MHz, I=1.5 W/cm^2). *a*) before ultrasound exposure; *b*) 15-sec ultrasound exposure; *c*) 30-sec ultrasound exposure.

Hence, the more regulation parameters the operator uses, the greater are the potentialities of modulating cell stratification concentration, and separation in suspension.

The possibility of trapping cells in the US box will presumably allow application of US device for obtaining bioactive compounds in certain volumes and with permanent characteristics.

Analysis of the functional state of cells in the US box with vital stains showed that the percent of viable cells in the suspension initially containing 92-94% native cells increases to 100%.

Only native cells are trapped in the box under certain conditions of US exposure, and therefore this method can be used for rapid isolation of various cells and bacterial strains. In this latter case, the cells damaged during exposure to chemical agents or physical factors are washed away from the box, and only cells resistant to these factors or functioning solely in the culture media of a certain composition remain in the box.

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