Ultrasonic Concentration of Tissue Culture Cells

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A new principle of ultrasonic fractionation of suspensions is proposed, namely flow ultrasonic selection. This method allows for selective retention of particles by the field of a standing ultrasonic wave. Conditions are defined for its use to perform a number of tasks in biomedical studies. The principle and conditions of ultrasonic concentration of tissue culture cells are discussed.

Key Words: *ultrasonic fractionation of suspensions; concentration of tissue culture cells*

Biomedical studies often require preparation of material on the basis of suspension fractionation, namely concentration of suspensions, replacement of suspending medium, separation of suspensions into fractions, purification of suspensions and solutions, separation of substances failing to bind in immunological analysis, and so on.

The importance of these tasks dictates the need to constantly develop new methods of fractionation. One such approach is the use of ultrasound (US).

Several known methods of using US for the concentration of suspensions differ fundamentally from one another [2]. One of them involves sonication of the suspension, followed by gravity sedimentation of the coagulated particles [2]. Another consists in stratification of the suspension flow by the field of a standing US wave directed perpendicular to the flow and layer-by-layer selection of fractions [2]. Yet another method compriseses stratification of the suspension with shifting of the strata via a periodic stepwise alteration of the US frequency and a subsequent selection of the concentrate.

This paper describes a new method of selecting suspensions which opens up new horizons in US fractionation.

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MATERIALS AND METHODS

The essence of the method is as follows. Let us superimpose the field of a standing US wave onto a suspension moving in a laminar flow. Radiation pressure forces directed toward the zones corresponding to the minimal potential energies of the particles in the US field will then act upon the suspension particles. If the fluid flow forces (F_F) do not exceed the forces of the radiation pressure (F_{US}) on the particles, the latter will be retained in these zones:

$$F_{US} > F_F \qquad (1)$$

For particles of a spherical shape and a radius short in comparison with the US wavelength, force F_{US} can be estimated as described previously [1], while force F_F acting on a particle due to the flow is determined according to Stokes' formula. In this case the velocity of laminar flow of the suspension, with condition (1) fulfilled, can be described as:

$$V \leq 2/3 \times 1/2 \times k \times \bar{E} \times a^2/\Phi/, \qquad (2)$$

where $\Phi=[\rho_o+2/3(\rho_o-\rho)]/[2\rho_o+\rho]-\rho U^2/3\rho_o U_o^2$; $k=2\pi/\lambda$, η is the dynamic viscosity of the fluid, $\bar{E}$ the mean density of the US field energy, λ is the US wavelength, a is the radius of a particle, Φ is the compressibility density factor, ρ and ρ_o are the density of the fluid and of the particles, respectively, and U and U_o are the velocity of US in the fluid and of the particle material, respectively.

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Hence, a laminar mode of suspension delivery makes it possible to concentrate the suspension particles in a volume limited by the superimposed US field.

Relation (2) determines the conditions for the potential application of the new method.

RESULTS

For the experimental studies we developed a fractionation device which creates in the flow unit a field of a standing US wave with an energy density of up to 7 J/m^3 in the 0.5 to 3.2 MHz band, can alter the velocity of the suspension flow from 0.2 to 20 ml/min, and can also thermostatically control the working volume of the flow unit.

In the first stage we defined the permissible conditions of exposure to the US field allowing the cells in the suspension to remain native. Despite the fact that there have been many studies of the biological effects of US, the physical mechanisms of this effect are still not fully understood. The leading role of temperature and cavitation phenomena in the injurious effect of US on cells in suspension has been noted. Unfortunately, however, it is practically impossible to process all the material on this subject, because some data on the effects of US are contradictory, and the majority of researchers have presented just partial information about the conditions of sonication.

With this in mind, we carried out experiments directly in the flow unit of the fractionation device. The flow unit was filled with the suspension to be studied and sonicated for 30 min, a period which is longer than that needed for actual concentrations of suspensions. In the course of sonication the suspension was thermostatically controlled at 25-30°C. The viability of cells after sonication was assessed using the vital stains trypan blue, eosin, etc.

Experiments were carried out on suspensions of BHK-21 cells, Chinese hamster fibroblasts (an aleuploid clone 431 of the B-II-d-ii-TAF-28 strain), and rat hepatocytes and red cells. Normal saline, medium 199, and medium 199 with 10% calf serum were used as suspending media.

The data indicate that the injurious effect of US declines as its frequency increases and demonstrate the possibility of using the field of a standing US wave at 3.2 MHz and an energy density of up to 7 J/m^3 for intravitam concentration of cell suspensions.

The next stage of the investigations involved the concentration of cell suspensions. Our aim was to find the maximum velocity of movement of the suspension at which the cells are effectively retained by the US field, to compare the experimental data with the estimates calculated using relation (2), and to assess the effect of US on the concentrated cells. The concentration was carried out on the cell suspension mentioned above. After each concentration its efficiency was assessed σ as the percent ratio of the content of particles in the concentrate to their content in the initial suspension. The concentration was repeated while decreasing or increasing the rate of the suspension flow so as to attain the established efficiency (95% in our experiments).

As an example, we will present the experimental data of concentrating fibroblasts suspended in 50 ml of medium 199 with 10% calf serum: volume of concentrate (volume of flow unit) 5 ml, velocity of suspension flow 7 ml/min, efficiency of concentration σ=95%, cell viability 97%.

Besides the experiments described above, we concentrated cells with replacement of the suspending medium. In this case the new medium was passed through the unit after the suspension, and this new medium displaced the previous one, while the cells were retained by the US field.

The results indicate that the principle of flow US selection may be used for effective intravital concentration of cell suspensions. The experimental value of the maximum velocity of the suspension flow (at σ=95%) is 9-12 times higher than its estimated value obtained from the relation (2), a fact which should be taken into account when using the above relation for estimation purposes.

The proposed principle of flow US selection opens new doors for the use of US for fractionation of suspensions. Studies of the biological effects of US on cells in suspension and experiments on their concentration have demonstrated the possibility of using this method for effective intravitam concentration of large easily deformed cells, specifically tissue cell cultures. The chief advantage of this approach is the ease of automating the fractionation operations, consisting in organization of the suspension flow through the flow unit. This means that the method can be used to design both laboratory devices intended to replace centrifuges and biotechnological lines, for example, controlled cultivators.

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