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Quantitative evaluation of in-air and droplet microfluidics techniques in the determination of hydrogel particle size, velocity and quantity

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Comparative analysis of air and droplet microfluidics methods performed with respect to experimental data obtained at close flow rates of a two-phase liquid on initial diameters, velocities of movement and the number of hydrogel particles formed. The expediency of using these methods in bioengineering is substantiated.

Keywords: bioprinting, in-air microfluidics, droplet microfluidics, tissue engineering.

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Microfluidics is a popular technology for droplet and particle production in the biomedical industry, in particular for 3D bioprinting and targeted drug delivery. There is a distinction between droplet microfluidics (DM) and in-air microfluidics (IAM). DM is based on the controlled mixing of liquids in microfluidic chips using a network of microchannels [1], while AM is based on the interaction of microjets of liquids directly in air [2]. The application of microfluidic chips has limitations, one of which is the presence of a continuous phase. Separation of the continuous phase is, in most cases, challenging and involves an additional manufacturing step. There is also a risk of clogging the microchannels of the chip. Forming liquid particles in air prevents this problem and makes the production a one-step process. The formed particles can be immediately deposited into three-dimensional structures, for example in bioprinting tasks. The AM method also has limitations. An external perturbing influence (e.g., through a reverse piezoelectric effect or acoustic influence) must be applied to form the microdroplet flow of the polymer solution in a controlled manner. AM is designed to form a soft gel, i.e. the formed particles are not solid capsules, but microspheres with a thin, fragile shell, vulnerable to various external influences. Therefore, AM is mainly used in tissue engineering [3]. DM has an advantage in this case. In configuration of the microfluidic chip a mini-reactor [4] is provided. As the particles move through it, the shell becomes controllably denser and the output is a fully cross-linked particle with a solid shell. Such particles can be used as microcapsules for drug and live cell transport with their controlled extraction. The aim of the present work is to quantitatively compare in-air and droplet microfluidics methods in terms of varying the size and velocity of the formed particles as well as the process performance.

A microchannel device with a coaxial arrangement of the dispersed phase channel ($d = 0.22$ mm) inside the continuous phase channel ($D = 0.90$ mm) is used for particle formation by the DM method. The flow rate range of the dispersed phase is $Q_d = 0.01-0.06$ ml/min, and of the continuous phase — $Q_c = 0.8-3.2$ ml/min. Aqueous solution of sodium alginate (ALG) with calcium carbonate ($CaCO_3$) used as the dispersed phase, and refined sunflower oil, acetic acid and emulsifier Polysorbate 80 were used as the continuous phase (Table 1). The formation of particles in air is carried out by the interaction of two liquid microflows involving the implementation of ionic crosslinking of the biopolymer. To crosslink the two microflows in air, a framework for attaching needles with an inner diameter of 0.21 mm was designed and printed on a 3D printer. Working fluids are fed into the needles through tubes using syringe pumps. Aqueous solution of ALG as a polymer (polymer flow rate $Q_d = 12$ ml/min) and aqueous solution of calcium chloride ($CaCl_2$) with ethyl alcohol (C_2H_5OH) as a crosslinking agent (crosslinker flow rate varied in the range $Q_c = 8-18$ ml/min) were used to form hydrogel particles by the AM method (Table 2). A more detailed description of the experimental setups is given in [4] (for DM) and [5] (for AM).

One of the main advantages of microfluidics is the control of the size of the formed hydrogel particles: D_0/D_{nozzle} (D_0 — diameter of the formed particles, D_{nozzle} — nozzle

Table 1. Component composition of hydrogel particles formed by the DM method

Phase	Name component	Concentration, wt.%
Dispersed	Distilled water	98.9
	Sodium alginate	0.6
	Calcium carbonate	0.5
Continuous	Sunflower Oil	98.7
	Polysorbate 80	1.0
	Glacial acetic acid	0.3

Table 2. Component composition of hydrogel particles produced by AM method

Sample of hydrogel	Polymer	Crosslinker	
	ALG concentration, mg/ml	Concentration $CaCl_2$, mg/ml	Concentration C_2H_5OH, wt.%
*G*1	2	15	10
*G*2	4	15	10
*G*3	6	15	10
*G*4	8	15	10

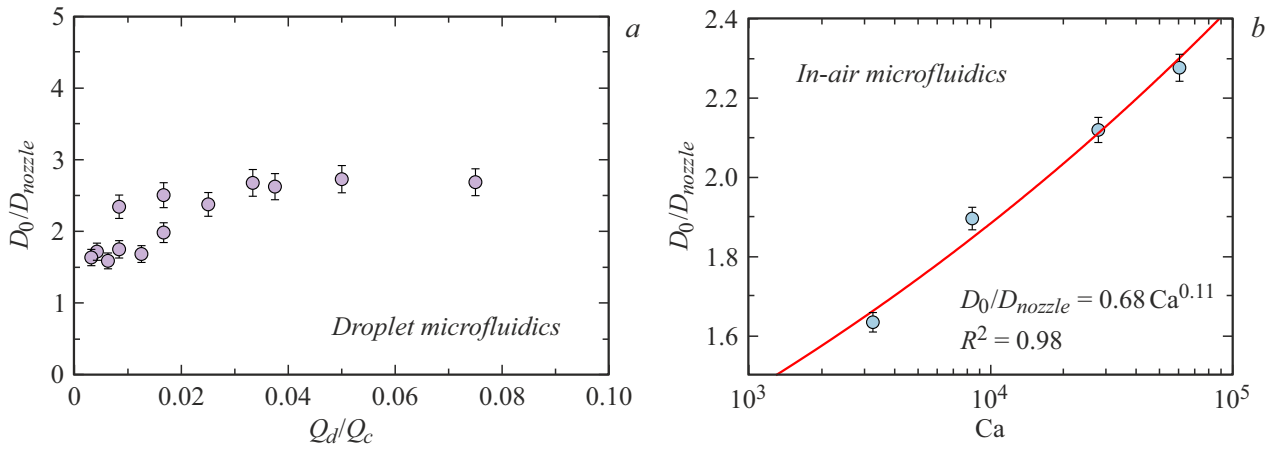

Figure 1. Dimensionless hydrogel particle diameter D_0/D_{nozzle} when varying the ratio of dispersed and continuous phase flow rates Q_d/Q_c (droplet microfluidics) (a) and capillarity number Ca for hydrogel particles (in-air microfluidics) (b).

diameter). This study showed that for the DM method, the changing of D_0/D_{nozzle} is accomplished mainly by varying the ratio of the dispersed and continuous phase flow rates Q_d/Q_c (Fig. 1, a). Increasing Q_d/Q_c leads to an almost linear increase in the D_0/D_{nozzle} values. In case of AM, changing Q_d/Q_c does not affect the hydrogel particle size. It was found that D_0/D_{nozzle} increases with increasing capillarity number Ca (Fig. 1, b). The Ca number accounts for the properties of hydrogel particles (viscosity and surface tension), which in turn depend on the polymer concentration. The size of particles formed by the two mentioned methods is comparable. DM is more promising in this respect and allows to obtain particles of several tens of micrometers in size [1]. In microchannels monodisperse particles are formed with complete control of their size and sphericity of shape. In air it is more difficult to control these parameters. Therefore, in this case, the size spread is larger and the particle shape is an ellipsoid elongated at both ends. Fig. 1, b shows the empirical dependence that allows predicting the size of formed hydrogel particles for AM. The data are approximated by a stepped allometric function with the coefficient of determination $R^2 = 0.98$ (Fig. 1, b).

The rate of hydrogel particle formation determines the performance of the process in the conjugate tasks of tissue engineering and targeted drug delivery to tissues. The initial velocity of hydrogel particles in the microchannel device U_0, expressed through the Reynolds number Re, is changed by varying Q_d/Q_c. From Fig. 2, a we can see a linear decrease in Re values as Q_d/Q_c ratio is increased. Increasing Q_d/Q_c leads to an increase in Re according to a linear law in the case of particle formation in air (Fig. 2, b). The initial velocity of hydrogel particles formed by the AM method is higher than U_0 in the case of DM. This is also reflected in the performance of the hydrogel particle formation processes (Fig. 3). The productivity of the AM method is two orders of magnitude higher than that of the DM method. The number of hydrogel particles (N) formed in the microfluidic chip during 1 s decreases when the ratio of the dispersed and continuous phase flow rates Q_d/Q_c increases (Fig. 3, a). In its turn, the number of N particles formed during 1 s in air decreases with increasing Ca number (Fig. 3, b). The data in Figs. 2, a and 3, a are for $Q_d = 0.04$ ml/min. At other flow rates, the appearance of the function remains the same. Figs. 2 and 3 show empirical functions for predicting the Re number (as a way to estimate hydrogel particle velocities) and the number of hydrogel particles to be formed N with a high coefficient of determination ($R^2 > 0.97$).

Thus, for applications and technologies in which high throughput and particle formation rate are important, the AM method is more suitable. However, as stated above, the AM method forms particles with a shell that is vulnerable to external factors. Therefore, a promising challenge is to select the component composition in such a way that acceptable

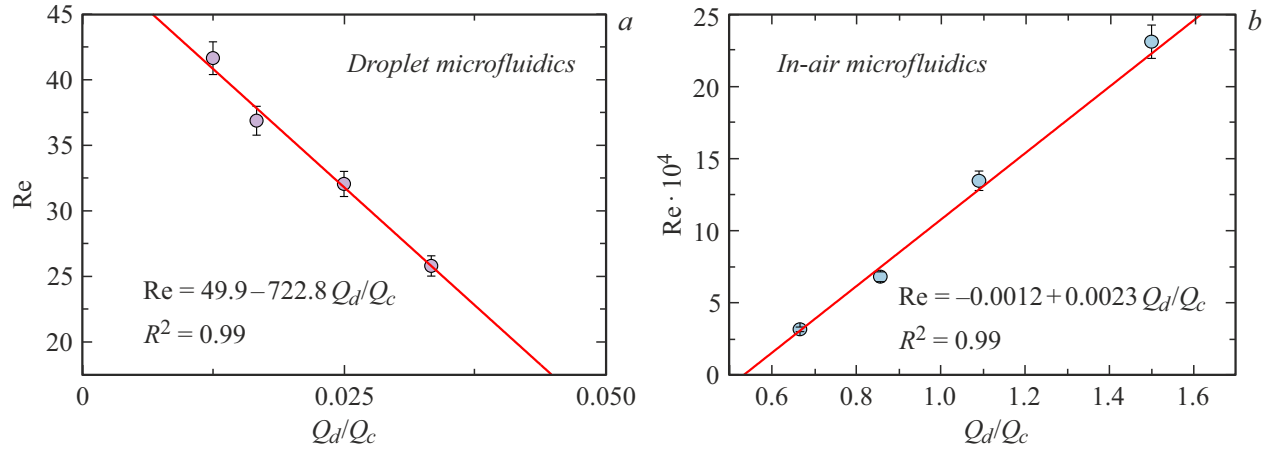

Figure 2. The Reynolds number Re for hydrogel particles when varying the ratio of the dispersed and continuous phase flow rates Q_d/Q_c. *a* — droplet microfluidics, *b* — air microfluidics.

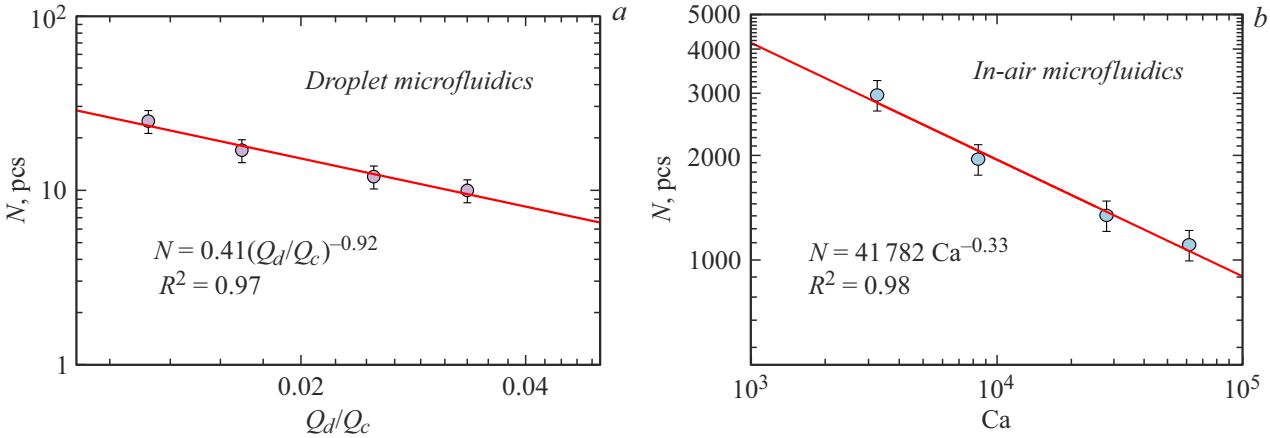

Figure 3. The number of hydrogel particles N formed in 1 s by varying the dispersed and continuous phase flow rate ratio Q_d/Q_c (droplet microfluidics) (*a*) and the capillarity number Ca for hydrogel particles (air microfluidics) (*b*).

crosslinking is achieved. DM remains an efficient technique for the production of droplets and particles with different sizes and shapes.

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Conflict of interest

The authors declare that they have no conflict of interest.

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