Bulgarian Chemical Communications, Volume 52, Special Issue F (pp. 68-73) 2020 DOI: 10.34049/bcc.52.F.0012

Approaches to mass transfer modeling in micro-channels inside gel

B. G. Pokusaev[1], A. V. Vyazmin[2*], D. A. Nekrasov[1], N. S. Zakharov[1], D. P. Khramtsov[2], N. V. Shumova[2]

[1]*Department of Chemistry and Biotechnology, Moscow Polytechnic University, 107023, Moscow, Russia;*
[2]*Department of Chemical Engineering, MIREA – Russian Technological University, 119454, Moscow, Russia*

Received: July 31, 2020; Accepted: August 10, 2020

The aim of the work is to find ways of effective cultivation of living microorganisms in the gel volume by designing a specific structure of internal channels, when the delivery of nutrients to the gel volume is carried out by the convective-diffusion mechanism. The nutrients penetration depth into the gel under microorganisms stable living conditions is estimated. The possibility of forming time-stable linear channels in the gel volume has been experimentally established. The possibility of their usage for convective nutrient supply to the volume was tested. The regularities of the nutrients diffusion from the channels into the gel volume with immobilized cells are established.

Keywords: gel, immobilized cells, microchannels, diffusion-convection mass transfer

INTRODUCTION

The current state of society can be characterized as the period of the fourth technological revolution. One of its features is the use of additive 3D technologies [1]. In regenerative medicine this technology is commonly referred to as 3D bioprinting. Its goal is to create artificial human organs from stem cells using additive methods [2]. Like any new technology, bioprinting requires new materials that have a whole set of specific biochemical and technological properties. One of the promising materials for these purposes is gel [3, 4]. The network of capillaries in gels can be used to supply nutrients and oxygen to the cells and remove the products of their metabolism. In additive 3D printing the suitable peculiarity of these media is the specific rheological characteristics, which permits, when applied under high stress conditions, these materials to have features similar to liquids [5].

The future potential of gels applications puts them among some of the most promising materials for 3D printing technologies, particularly for forming objects with complex geometric shapes where gel can be used as a bioink [6]. Gels can be used for tissue building and serve as a framework for cells growth [7]. For example, gels can be applied for vascular tissue formation [8]. Such vascular structure can be initially formed with a bioresorbable gel with immobilized cells and after solidification serve as a framework for cells growth which will be subsequently resorbed after vascular tissue is formed in the gel [9]. In particular, it is possible to apply to this 3D bioprinting technology the possibility of predicting and controlling the thermophysical characteristics that affect the mass transfer characteristics of the gel-based matrix [10].

The task of creating a tissue structure is complicated due to presence of living cells in the forming gel sample. In this regard, it is necessary to provide conditions suitable for the vital activity of cells. For this reason, it is absolutely essential to supply nutrients and oxygen to the cells, which can be implemented by forming artificial channels inside the gel, which are similar to the structure of blood capillaries in the organs [11-13]. To study this process, it is convenient that there is a formal analogy between the transfer of nutrients in gels and filtration with the formation of deposits in the pores [14].

Since gels can be produced from different chemical components, the question of choosing the right gel for tissue growth is of high importance. Moreover, it is important to know the thermophysical properties of gels [15, 16]. As minimum, such gels must solve two purposes: be a medium for cells immobilization and be able to be extruded *via* a 3D printer [17]. Hence, gels must melt and solidify in normal conditions. Pure agarose gels [18] and complicated gels with bioresorbable additives [19, 20] are considered as promising materials for 3D bioprinting.

Previously was shown the possibility of forming stable microchannel structures inside the gel, capable of transferring liquid to gel volume [21, 22] Similar channels were produced in order to form a pattern for a vascular tissue [23]. The complexity of the problem requires the development of a model of the movement of a liquid through the capillaries in

* To whom all correspondence should be sent:
E-mail: *av1958@list.ru*

the gel structure, which will significantly reduce the time of research. This approach will allow to test the effectiveness of different configurations of the channels in the gel. Due to the irregularity, the problem of modeling the gel structure is nontrivial. There is also an open question about the optimal structure of the channels inside the gel, providing the effective mode of supply of nutrients to living organisms immobilized in the gel.

EXPERIMENTAL

Materials, Methods and Approaches

The mass transfer study in gels, including those with internal channels and living microorganisms, was carried out on model systems. For primary studies of the mass transfer the individual properties inherent to various living cells and gels are not of fundamental importance. At the present research stage, it is important to understand the fundamental mass transfer laws during bulk microorganism's cultivation in gels with artificial microchannels.

Pure gels based on agarose "Chemapol" and gels with the addition of yeast culture with a nutritious bouillon were used as model systems. The weight concentration of agarose in gels varied in the range of 0.6-1.5 %. Such gels are optically transparent, which makes it possible to conduct mass transfer studies using non-contact optical methods without disturbing the metabolism conditions.

The yeast culture *Pichia polymorpha Y-314* was selected as a model microorganism, since its cells size is close to that of human somatic cells. The culture is viable if glucose is used as the only source of carbon. The yeast concentration was controlled by measuring the optical permittivity at a wavelength of 540 nm.

To visualize mass transfer processes and measure the diffusion rate, a 1.0 % aqueous solution of fuchsin was used, which is sometimes added to nutrient media. Aqueous solutions of fuchsin have a purple-red color and high-contrast against the gel background.

Optical methods of spectrometry and visualization with computer image processing were used to measure the mass transfer rate. Fig. 1 shows the experimental setup scheme used for the experiments [21]. The equipment consists of two working sections. Section 1 is designed to study mass transfer by scanning the test gel samples along the cuvette height. Section 2 is designed to study mass transfer in layered gel samples with flow microchannels.

A two-beam spectrophotometer UV-1280 manufactured by Shimadzu (7) was used for spectrometric measurements. The technical characteristics of the equipment provide measurements of light transmission and absorption spectra at several wavelengths in the range of 190 - 1100 nm in automatic mode. A special system was installed in the working area to measure the gel sample position in space (3, 5, 6).

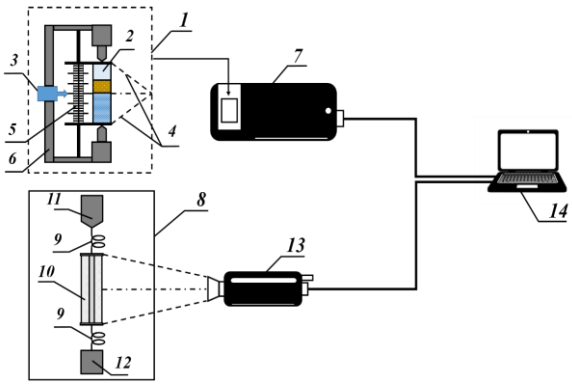

Figure 1. Scheme of the experimental setup: 1 - working area 1: 2 - optical cell with gel, 3 - level indicator, 4 - working area of scanning, 5 - scale of height measurement, 6 - cell holder, 7 - Shimadzu spectrophotometer, 8 - working area 2: 9 – pipeline system with control valve, 10 - optical cell with gel with channels, 11 - tank with nutrient medium, 12 - collection tank, 13 - camera, 14 - computer

To visualize mass transfer in gels with artificial microchannels, an automatic high-resolution photo recorder was used. A cuvette with a gel having channels (10) is placed in the working area, connected to a pipeline, through which the colored liquid from the container (11) enters the channel. The flow rate of the colored liquid is regulated by a valve on the pipeline (9). The colored liquid flows after the channel into a measuring container (12) with a graduated scale. Using a stopwatch, the average flow liquid rate that has passed through the channel is monitored. A photo-recording device (13) writes the diffusion front propagation in the gel, then the photos are processed on a computer (14) using the method [22] to calculate the diffusion coefficient value for mass transfer from the channel to the gel.

The method of forming artificial channels in the gel is the following: a metal wire is placed in an experimental cuvette which is filled with a gel forming agarose solution at a temperature not lower than 40°C (above the gel formation temperature). After lowering the temperature below the gel formation temperature and stabilizing the gel, the wire is removed. However, a time-stable channel of the required diameter and shape remains in the gel.

Using the described method, straight-flowing and branched channels with a thickness of 1.5 mm

to 0.3 mm were formed in agarose gels with different agarose concentrations. After removing the wire, the channels are filled with dispersion moisture, which is replaced by an aqueous solution of fuchsin. In the experimental setup described above, a flow of an aqueous solution of fuchsin with a flow rate of up to 2×10^{-10} m^3/s takes place through direct channels.

RESULTS AND DISCUSSION

In traditional deep cells cultivation in the gel volume, the transfer of nutrients and oxygen to the cells is carried out through the external boundary in a diffusive regime. Diffusion processes in gels are slow; their rate decreases as the dispersed phase concentration increases and may be by an order of magnitude lower than for a pure dispersion medium. As a result, the microorganisms' growth rate in the gel volume is limited by the supply rate of substances necessary for their immobilization. Internal artificial channels allow to partially take off these restrictions by reducing the path necessary for the nutrients penetration into the volume and the medium convective movement inside the channel.

Comparable experiments were performed to measure nutrients mass flow rate in the gel volume through the external boundary and internal artificial microchannels. For this purpose, temporal dynamics measurements of filling with fuchsin (transported substance) of the experimental cuvette test volume with the gel for both methods of mass transfer were performed. Further, it is assumed, that the fuchsin concentration in the gel is proportional to the transmitted light absorption intensity.

The experiment was carried out on the equipment described above and consisted in determining the absorption intensity of light at a 540 nm wavelength in two agarose gel samples with a 0.6% weight agarose concentration. In one sample, fuchsin was poured onto the upper gel boundary and transferred by diffusion into the bulk. In the other sample, fuchsin was fed into a single central channel 0.8 mm in diameter. The integral light absorption intensity was measured over the entire width of the experimental cell equal to 10 mm to a 10 mm depth from the upper surface with a 1 mm step. The measurements were carried out at different time intervals after the fuchsin was poured.

Data on the fuchsin diffusion temporal dynamics into the gel bulk from the upper surface as the relative light absorption intensity D depending on the penetration depth l at different times are shown in Fig. 2. The experimental results fully correspond to the mass transfer theoretical concepts described by the exact solution of the non-stationary diffusion equation for a semi-infinite space under the third boundary value problem conditions [24]. Analysis of the experimental dependences form shows that there is diffusion resistance to the nutrients mass transfer in the liquid layer above the gel surface. In particular, at the 220 min time from the start of the diffusion process the fuchsin penetration depth does not exceed 4 mm. Estimations based on Fig. 2 show that for this time the average relative light absorption for a test gel volume of $10 \times 10 \times 10$ mm has a value lower than 0.4.

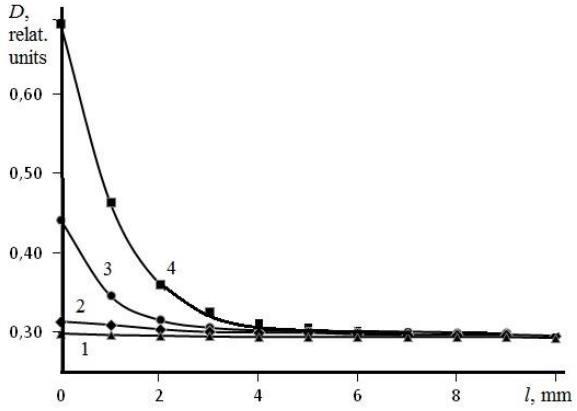

Figure 2. Dependence of the light absorption relative intensity *D* (relative units) of the gel sample on the depth *l* (mm) when diffusing fuchsin into its volume from the upper surface in different time moments from the start of the process, min: 1 – 0, 2 – 40, 3 – 160, 4 – 220.

When the fuchsin solution is flowing through the central rectilinear channel, its transverse diffusion into the gel volume takes place. As an example, Fig. 3 shows a photo visualizing this process for a channel with a diameter of 0.8 mm in a gel with a 0.6% agarose weight concentration. Since fuchsin absorbs light more strongly than pure gel, its diffusion appears in the photo as an increase in the channel diameter with time. It can be seen that under this supplying fuchsin method, the cuvette volume with the gel is filled much faster than when the fuchsin diffuses only from the upper boundary.

Interpretation of the data shown in Fig. 3 can be made in two ways. One can follow the approach proposed in [22] and determine the dependence of the fuchsin penetration depth from the channel into the gel volume under different time framesth. Alternatively, using computer image processing, it is possible to determine the light absorption average intensity dependence in the test gel sample volume (which will characterize the average concentration of fuchsin in its volume) on the diffusion time. The influence on the internal channel result, which

reduces less than 1.0% of the testing volume, is neglected.

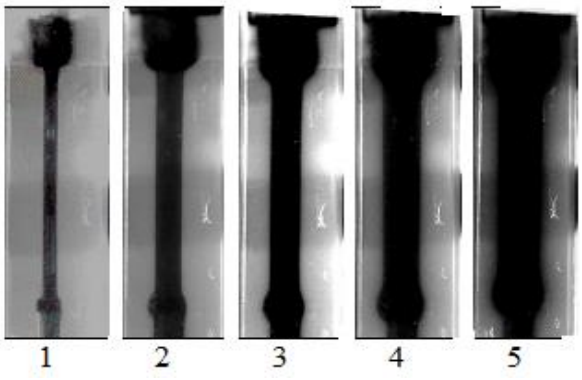

Figure 3. Photos visualizing the fuchsin transverse diffusion from a flow channel with a diameter of 0.8 mm into the gel volume with a weight agarose concentration of 0.6% at times from the experiment beginning, min: 1 – 0, 2 – 30, 3 – 60, 4 – 120, 5 – 200.

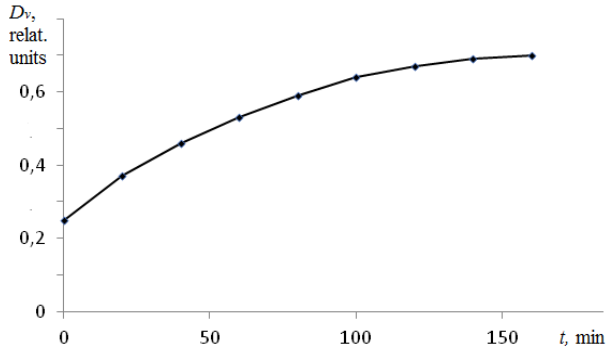

Figure 4. Dependence of the average volume relative light absorption intensity *Dv* (relative units) in the gel on the time *t* (min) when fuchsin diffuses into the volume from the flow channel.

Data on the temporal dynamics of the fuchsin average volumetric concentration growth in the gel volume during diffusion from the channel in the form of the volume average relative light absorption intensity D_v dependence on time *t* are shown in Fig. 4. In the absence of diffusion resistance to fuchsin mass transfer inside the channel due to its flow, its average volume concentration in the test volume increases. For example, the average relative light absorption for the test gel volume at 160 minutes is 0.7.

The abovementioned results confirm the simple assumption that filling the gel volume with a substance through artificial channels is more efficient than through its outer boundary. More interesting are the spectral study results of the gel sample with cells during diffusion from the channel into the gel volume of a nutrient broth that ensures cell growth and division instead of a model medium. In this case, it is possible to determine not only the temporal filling dynamics for the gel with the nutrient medium, but also the cell concentration growth during bulk cultivation in the gel.

Further, the possibility of cultivating microorganisms (using yeast, as example) in the gel volume under feeding nutrient conditions through an artificial internal microchannel was experimentally investigated. Based on the data obtained, the increase in the yeast cells concentration as their division result was evaluated by measuring the light absorption intensity for two adjacent identical gels: with living yeast cells and without cells.

The research was carried out on the experimental equipment described above. The light absorption intensity was measured at a 540 nm light wavelength. The wavelength choice is due to the fact that it is used in microbiology to measure the yeast cells concentration by a spectrophotometric method.

An investigated gel sample with a 0.6% agarose weight concentration consists of four alternating layers: a pure gel layer, then a gel with cells layer, and so on. The studied gel system photos are shown in Fig. 5. Since the cells presence leads to high light absorption at the selected wavelength, the gel layers with cells in the photo appear darker than those without cells. An artificial channel with a 0.8 mm diameter was formed in the sample, which was filled with nutrient bouillon. It should be noted that, unlike fuchsin, the nutrient bouillon is not a high-contrast substance in relation to the gel, therefore, the presence of a channel with bouillon in the photo in Fig. 5 is almost invisible. However, the optical transmission of broth is weaker than that of water (absorption is greater). For this reason, the bouillon spread in the gel sample can be monitored by the light absorption intensity.

The measurements were carried out along the 10 mm height of the cuvette with a 0.5 mm step at time moments: 0 (immediately), 2, 15 hours after the bouillon was injected into the channel. Fig. 6 shows the absolute absorption intensity for passing through the gel sample light, depending on the depth at different microorganism's cultivation times.

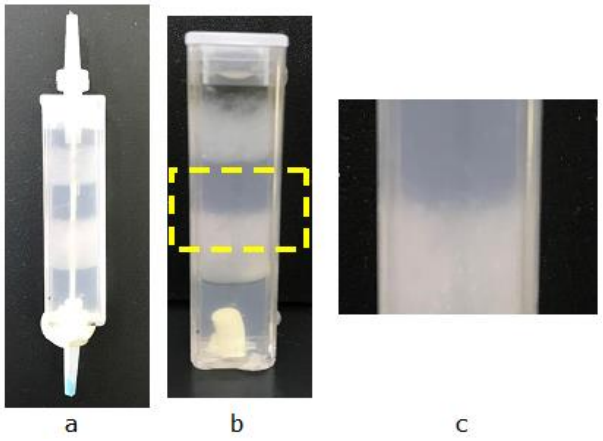

Figure 5. Photo of a four-layer gel sample with a 0.6% agarose weight concentration and an internal central channel of 0.8 mm diameter. Gel layers with cells and without cells alternate (darker in the photo - layers with cells). Designations under the photos: a - original sample with an empty channel; b - sample with a channel filled with nutrient broth; c - the investigated part of the layered gel sample (highlighted by a dotted line in photo 5b).

In the studied area, there was a layer of pure gel (4 mm) - on the right in the graph, the section meniscus between the layers (2 mm) - in the center and a gel layer with cells (4 mm) - on the left in the graph. Thus, as an experimental result, it is possible to see the difference in each gel layer optical density at the start of the experiment and after 15 hours with constant nutrition diffusion from the channel into the gel, which allows maintaining the cells inside the gel in an immobilized state.

As follows from Fig. 6, at the initial moment when feeding bouillon into the channel, the light absorption intensity in the layer with cells is much higher than in pure gel (curve 1). This is because the overall light absorption is due to both inside the gel and the cells. In 2 hours after the start of the experiment, the absorption intensity in both gel sample regions insignificantly increases (curve 2). This is probably due to the bouillon diffusion transfer into the gel. Moreover, in the meniscus section filled with the dispersion liquid, this difference becomes noticeable, since the bouillon mass transfer along the meniscus occurs much faster than in the gel volume.

After 15 hours of the experiment, the light absorption intensity in both sample parts significantly increases (curve 3 in Fig. 6). Moreover, in the gel area with cells, such growth is noticeably greater than in a pure gel. This is due to two reasons. Firstly, both gel samples volumes are filled with nutrient from the channel, which leads to an increase in absorption in both samples.

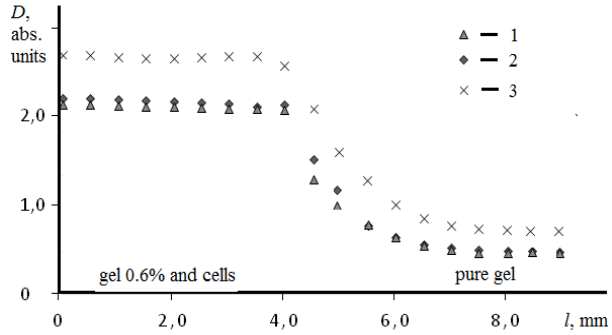

Figure. 6. Absolute absorption intensity for light with a 540 nm wavelength passing through a gel with a 0.6% agarose weight concentration and having a central channel of 0.8 mm diameter, filled with nutrient bouillon, depending on the depth at the gel layers' junction with seeded yeast cells and without cells at different cultivation microorganisms times. To a depth of 4 mm - gel with cells, from 4 to 6 mm - section meniscus with boundary layers, under 6 mm - pure gel. The curves designations correspond to the times from the experiment beginning, h: 1 - 0; 2 - 2; 3 - 15.

Secondly, an increase in the yeast cells concentration is possible due to division, which causes an additional increase in the light absorption intensity in the area with cells in comparison with pure gel.

Suppose that the light absorption intensity is additively dependent on the magnitude of three different factors that determine the light absorption: gel, bouillon and cells. Therefore, from the measurement data in Fig. 6 it can be estimated that the increase in the microorganism's concentration during 15 hours is about 15% in relation to the initial concentration. This proves the possibility of effective bulk cells culturing in the gel volume in the microchannels presence for supplying microorganisms with nutrients. It is important to note that due to the microchannels stability in the gel, such a supply can be carried out over a long time period.

The estimates made regarding the increase in the yeast cells concentration in the gel with an artificial channel seem to be justified. Cell growth with subsequent division is possible only under conditions of sufficient supply of nutrients and oxygen. According to the abovementioned measurements this is achieved within 3-4 hours using the channel. Under optimal cultivation conditions, the selected yeast culture doubles the cell concentration within 4-6 hours. The optimum temperature for yeast cultivation is 36°C. The experiment was carried out at non-optimal temperature in the range of 23-25°C. As a result, the time for doubling the microorganisms number during growth and division increased several times.

Accordingly, the yeast concentration growth was much slower.

CONCLUSIONS

It is shown that the mass transfer of substances into the gel volume can be carried out through an artificial microchannel system which is stable in time. In this case, the average volumetric concentration growth of the transferred substances in the gel volume occurs several times faster than in the case when it is transferred through outer boundary.

An optical method based on the light absorption intensity measurement established the cultivating microorganism's possibility (using the example of an increase in the yeast concentration) throughout the entire gel volume when nutrients and oxygen are supplied through the flow channel. With this cultivation method, the possibility of a uniform increase in the microorganism's concentration by volume, and not only near the gel sample outer boundary, was shown.

As a result, for further research and subsequent practical application, the problem of finding the optimal diameter and structure of channels for cultivation of microorganisms in a gel in a volumetric manner becomes of high importance. At the same time, it is important to understand how to create and use a branched flowing microchannels system in a gel in order to increase the efficiency and uniformity of nutrient transfer into its volume.

Acknowledgements: *The paper was prepared in full within the state assignment of Ministry of Education and Science of the Russian Federation for 2020-2022 (project No. FZRR-2020-0027).*

REFERENCES

1. K. Schwab, N. Davis, Shaping the Fourth Industrial Revolution, Cologny, World Economic Forum, 2018.
2. V. Lee, G. Dai, *Adv. Health Care Technol*., **1**, 23 (2015).
3. K. Kajiwara, Yo. Osada (eds.), Gels Handbook, Elsevier, 2000.
4. M. R Placzek, I.-M. Chung, H. M. Macedo, S. Ismail, T. M. Blanco, M. Lim, J. M. Cha, I. Fauzi, Yu. Kang, D. C. L. Yeo, C. Y. J. Ma, Ju. M. Polak, N. Panoskaltsis, A. Mantalaris, *J. R. Soc. Interface*, **6**, 209 (2009).
5. D. Chimene, K. K. Lennox, R. R. Kaunas, A.K. Gaharwar, *Ann. Biomed. Eng*., **44**, 2090 (2016).
6. H. Gudapati, M. Dey, I. Ozbolat, *Biomaterials*, **102**, 20 (2016).
7. H. Jian, M. Wang, S. Wang, A. Wang, S. Bai, *Bio-Des. Manuf.*, **1**, 45 (2018).
8. D. Richards, J. Jia, M. Yost, R. Markwald, Y. Mei, *Ann. Biomed. Eng*., **45**, 132 (2017).
9. P. Sasmal, P. Datta, Y. Wu, I. T. Ozbolat, *Microphysiol. Syst.*, **2**, 9 (2018).
10. S. Wang, J. M. Lee, W. Y. Yeong, *Int. J. Bioprinting*, **1**, 3 (2015).
11. K. Jakab, C. Norotte, F. Marga, K. Murphy, G. Vunjak-Novakovic, G. Forgacs, *Biofabrication*, **2**, 20 (2010).
12. B. G. Pokusaev, S. P. Karlov, A. V. Vyazmin, D. A. Nekrasov, *Theor. Found. Chem. Eng.*, **49**(6), 617 (2015),
13. B. G. Pokusaev, D. A. Nekrasov, N. S. Zakharov, D. P. Khramtsov, S. P. Karlov, A. V. Vyazmin, *Theor. Found. Chem. Eng.*, **54**(1), 91 (2020).
14. Yu. A. Taran, A. V. Kozlov, A. L. Taran, *Fine Chem. Technol.*, **14**(2), 15 (2019).
15. B. Gao, Q. Yang, X. Zhao, G. Jin, Y. Ma, F. Xu, *Trends Biotechnol*., **34**, 746 (2016).
16. B. Pokusaev, A. Vyazmin, N. Zakharov, S. Karlov, D. Nekrasov, V. Reznik, D. Kramtsov, *Thermal Sci.*, **24**, 347 (2020).
17. C. Norotte, F. S. Margaa, L. E. Niklason, G. Forgacs, *Biomaterials*, **30**, 5910 (2009).
18. G. R. López-Marcial, A. Y. Zeng, C. Osuna, J. Dennis, J. M. García, G. D. O'Connell, *ACS Biomater. Sci. Eng.*, **4**, 3610 (2018).
19. R. Suntornnond, J. An, C. K. Chua, Macromol. Mater. Eng., **302**, 1 (2017).
20. B. G. Pokusaev, S. P. Karlov, D. A. Nekrasov, N.S. Zakharov, D. P. Khramtsov, V. V. Reznik, A.V. Vyazmin, N. V. Shumova, *Chem. Eng. Trans*., **74**, 1171 (2019).
21. B. G. Pokusaev, A. V. Vyazmin, D. A. Nekrasov, N. S. Zakharov, D. P. Khramtsov, N. V. Shumova, D. A. Belova, *Chem. Eng. Trans*., **79**, 13 (2020).
22. B. G. Pokusaev, A. V. Vyazmin, N. S. Zakharov, D. P. Khramtsov, D. A. Nekrasov, *Theor. Found. Chem. Eng.*, **54**(2), 277 (2020).
23. L. E. Bertassoni, M. Cecconi, V. Manoharan, M. Nikkhah, J. Hjortnaes, A. L. Cristino, G. Barabaschi, D. Demarchi, M. R. Dokmeci, Y. Yang, A. Khademhosseini, *Lap Chip*, **13**, 2202 (2014).
24. A. D. Polyanin, A. M. Kutepov, A. V. Vyazmin, D. A. Kazenin, Hydrodynamics, Mass and Heat Transfer in Chemical Engineering, London, Taylor & Francis, 2002.