A step towards on-chip biochemical energy cascade of microorganisms: carbon dioxide generation induced by ethanol fermentation in 3D printed modular lab-on-a-chip

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Abstract. The concept of biochemical energy cascade of microorganisms towards oxygen generation in 3D printed lab-on-a-chip has been presented. In this work, carbon dioxide – a product of ethanol fermentation of yeasts has been utilized to enable light-initialized photosynthesis of euglenas and as a result of their metabolic transitions produce pure oxygen.

1. Introduction
Culturing of microscopic creatures in lab-on-a-chip environment is one of the most investigated subjects standing on the borderline of microfluidics and microbiology [1-4]. For instance, several works concerning the growth and study of euglena and yeast biological potential have been demonstrated recently [5-8]. Euglenas are commonly known as distinctive microorganisms that easily generate oxygen – a bypass product of their metabolism, based on CO₂ photosynthesis process. On the other hand, generation of CO₂ is an obvious effect of organic compounds’ fermentation, especially ethanol fermentation of yeasts.

In this paper, a lab-on-a-chip, in which both of the aforementioned processes are used to obtain an energy cascade, has been presented. In the cascade, a colony of yeasts generates gaseous carbon dioxide (CO₂) which next, diffuses through a semi-permeable membrane and nourishes a colony of euglenas. Specified metabolism of euglenas (photosynthesis) results in production of gaseous oxygen (O₂) that penetrates through the next diaphragm and reaches the final microchamber. Eventually, gaseous oxygen can be used to supply a new generation of bio-based fuel cells.

Among different fabrication methods of the lab-on-a-chip devices, 3D print technique has been selected as the most appropriate in this solution. The lab-chip construction and principle of working, as well as results of preliminary experiments are shown in the next section.

2. Experiment
As mentioned above, two colonies of microorganisms have been cultured in the lab-on-a-chip device (Fig. 1).
Figure 1. Scheme of the microorganisms’ biochemical energy cascade.

In the module 1, solution of baker’s yeasts (*Saccharomyces cerevisiae*), glucose and H$_2$O mixed in the relation of 1:4:10 (w/w) is used to start the ethanol fermentation. The photosynthesizing microorganism, *Euglena gracilis* (Blades Biological Ltd) is cultured in a buffer prepared according to the guidelines (Blades Biological Ltd, Protozoa and algae culture instructions) in the module 2. As a result of euglenas’ photosynthesis (process initialized by CO$_2$ and light) gaseous oxygen is generated and transferred through the membrane to the module 3.

2.1. Lab-on-a-chip construction

All of the processes leading to the microorganisms’ energy conversion have been conducted inside the 3D printed modular lab-on-a-chip. The 3D printer (model: Projet 3510, 3D Systems, USA) utilizing ink-jet UV photo curable materials (Visijet M3 Crystal and support – Visijet S300, 3D Systems, USA) has been exploited for fabrication of the device. The use of 3D printing method and modularity of the chip structure enables for a reconfigurable and rapid extension of the system. As mentioned, the chip contains three modules. The modules are arranged horizontally, creating a sandwich structure of the chip (Fig. 2). Every module has a cavity for o-ring sealing, which provides tightness and protects samples from the external environment. Volume of each module chamber is 132 µl. Semi-permeable polydimethylsiloxane (PDMS) membranes are indispensable elements of the chip, since they separate both the second and the third modules, enabling the diffusion of gases from one chamber to another. The membranes have been fabricated utilizing moulding technique. Glass slide (Borofloat 3.3, Schott) have been covered with polymer mask (Avery Graph) with laser-cut pattern of the membrane and isotropically etched in the solution of 50% HF:69% HNO$_3$. Next, polydimethylsiloxane (Sylgard 184, Dow Corning) mixed with its curing agent at the ratio of 10:1 (w/w) and degassed in ultrasonic washer, has been casted onto master mould. Ultimately, after 24 hours of polymerization, circle-shaped membranes with a diameter of 12 mm and thickness of 120 µm have been obtained. Every diaphragm is combined with the module by a pressing ring, which prevents it from damage while screwing. On a top of the chip, a cap with a glass window is attached for microscopic observations and illumination of euglenas’ colony.

3. Results and discussion

Yeasts have been cultured in the module 1, according to the conditions mentioned earlier. After 24 hours, generation of carbon dioxide has been observed in the module 2 lasting for 5 days. The detection of CO$_2$ has been obtained by the hydrogencarbonate indicator [9], solution that changes its colour from red to orange, if pH value in the environment increases. The indicator has been introduced to the module 2 and full colour pictures have been taken by CMOS minicamera to visualize the solution colour before and after the start of ethanol fermentation. Acquired pictures have been then
Figure 2. Construction and functioning of the 3D printed lab-chip: a) exploded schematic view, b) lab-chip at a glance, c) euglenas’ colony in the module 2, scale bar – 100 µm.

digitized to obtain basic RGB colour histograms (Fig. 3). In the next experimental step, colonies of yeasts and euglenas have been cultured in the module 1 and 2, respectively. CO₂ generated by yeasts in the module 1 permeates through the first membrane and supplies euglenas’ colony, cultured in the module 2 and lightened by daylight. The colony of euglenas absorbs CO₂ and generates O₂. Production of O₂ is in turn detected by solution of reduced methylene blue [10], introduced into the module 3. On the contrary to the long-lasting colony of yeasts (5 days easily), in our first experiment the culture of euglenas has survived solely 3 hours. It seems that concentration of generated in the system CO₂ (>0.04%, according to the colour transition of hydrogen carbonate indicator) might be too high and causes evaporation of the buffer with euglena colony. The culturing conditions of this microorganism have to be optimized in the future works to enable the implementation of the energy cascade.

Figure 3. The change of colour of hydrogencarbonate (pH) indicator in the lab-on-a-chip chamber after 24 hours of yeasts’ culturing: a) on the left: initial red colour of solution in the chamber, on the right: colour histogram of the representative area, b) on the left: change of the colour to orange hue in the chamber, on the right: colour histogram of the representative area.
4. Conclusions

3D printed lab-on-a-chip consisting of the three modules has been presented. The chip has provided diffusion of gases between the modules and enabled for simultaneous yeasts’ and euglenas’ culturing. In the first module, generation of CO$_2$ has been obtained and next utilized as nourishment for euglenas. Euglenas cultured in the second module, has absorbed CO$_2$ in the photosynthesis process and produced O$_2$ that has been then gathered in the third module of the chip. The culture of yeasts has lasted for 5 days, while euglenas’ stimulated by based on ethanol fermentation CO$_2$ – 3 hours.

It may be contended that the construction of the chip and application of 3D printing technique is suitable to fit the requirements of the microorganisms’ energy cascade, however further works have to be conducted to improve the functioning of the biological system and allow for its future application in fuel cells.

Acknowledgments

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