Statistical analysis of the integration of additive and information technologies for the artificial cultivation of plant cells

Ekaterina Bogodukhova[1*], *Valentina* Britvina[1], *Vladimir* Konyukhov[2], *Alexander* Semenov[3], *Shakizada* Niyazbekova[3], and *Asiiat* Mottaeva[3]

[1] Moscow Polytechnic University, 107023 Moscow, Russia
[2] Russian University Sport (SCOLIPE), 105122 Moscow, Russia
[3] Moscow Witte University, 115432 Moscow, Russia

Abstract. This article discusses the prospects for the development of biopreparations as a leading direction for tissue regeneration by integrating several advanced technologies. A statistical analysis of data on the current state of plant production in the world was made, the change in the rate of destruction of organic matter with a decrease in species diversity was demonstrated. A study aimed at identifying the main tissues of isolated cells in bioprinting was also carried out, which was taken as a basis for developing an extruder for layer-by-layer cultivation of biotissues, the main technical characteristics of which are also presented in the materials of this work. Presented data confirming the practical relevance of additive technologies for the development of crop production and bioengineering are aimed at stabilizing climate change. The article is supplied with graphical materials and tables, as well as a detailed description of each stage of the research.

1 Introduction

Currently, there is an increased interest in the field of bioengineering based on artificial cultivation and regeneration of living plant cells. Extinct plant species in the world make up 40 % of the total diversity of flora, in 7 years the number of endangered plant species has increased by 20%. The rate at which the planet is losing its green cover threatens the existence of many living organisms, including humans.

According to the World Wildlife Fund, climate change, including frequent and prolonged droughts, may destroy about 50% of vegetation by 2080. Agriculture will also suffer: Alexei Eliseyev, a leading researcher at the Institute of Atmospheric Physics of the Russian Academy of Sciences, noted that due to global warming Russia may not have any territories suitable for growing crops [1].

Experts from the UN Food and Agriculture Organization (FAO) predict that 16 percent to 22 percent of potato, bean and nut species will disappear by 2055 because of global warming. These are wild varieties, but their genetic material is important for breeding new varieties that can withstand drought, soil salinity and other adverse conditions. Natural

* Corresponding author: bogodukhova_katerina@mail.ru

ecosystems have declined by 47% on average relative to the earliest parameters recorded by science, and approximately 25% of species are threatened in most of the plant groups studied [2].

That is why artificial cultivation of plant cells is an urgent direction for the development of synthesis of multicellular three-dimensional microenvironment with intercellular communication, which is the key to understanding the symbiosis of additive and informational cellular reprogramming.

2 Materials and methods of research

In the current environment, it is particularly important to increase the efficiency of the regeneration potential of living cells through digital technologies, since the further development of not only bioengineering, but also biosynthesis in general, depends on it. At the moment, however, the development potential in this field has a low coefficient, in contrast to the ever-increasing proportion of irreversible destruction of ecosystems, which cannot be restored by traditional methods (Table 1).

Table 1. Number of species on the Red List of the International Union for Conservation of Nature

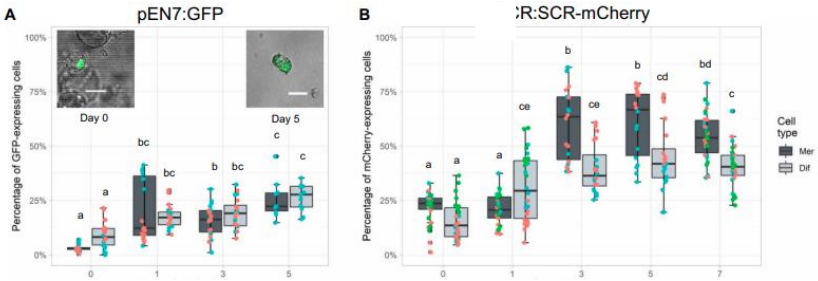
Protected Status	Plants	Fungi and protozoa
1	2	3
Disappeared	122	0
Extinct in the wild	42	0
Endangered	4674	27
Disappearing	8593	86
Vulnerable	8459	133
Near Vulnerable	3181	53
Dependent on conservation efforts	157	0
Least Concern	24809	87
Insufficient data for threat assessment	4090	39
Note – Compiled from source data [3]		

Numerous studies have established that traditional methods of growing plant cells do not allow to fully simulate the volumetric structure of the microenvironment observed in natural conditions [4, 5], so to better simulate natural plant conditions, additive construction technology is used, which allows to create specially designed spatial cell structures [6].

3D printed cell biomaterial is a mobile and controllable systematic framework for fabrication and implementation into complex systems that not only capture cellular dynamics, but also interact in a physiologically precise way to restore plant tissues [7-9]. For example, human induced pluripotent stem cells have been bioprinted to study cell fate, phenotypic variability and tissue regeneration. The study of plant tissues with further application of the symbiosis of bioprinting and digitalization promotes the development of a regenerative cell repair system, and the economic feasibility of these studies will allow the production of supplies for laboratory research in other countries [10].

Turning to an in-depth study of the identity of plant cells from the original tissue, it was revealed that bioprinted meristematic and differentiated root cells were visualized on days 1, 3, 5 and 7 (Figure 1). Representative high-resolution confocal images of 3D bioprinted cells are given: scale bars, 20 microns. Heatmap of the transcriptional changes for endodermal identity genes on days 0, 1, and 3 upon bioprinting of differentiated root cells. Stars indicate

significant DEGs. Percentage of viable cells isolated from meristematic (left) and differentiated (right) root cells 0, 1, and 3 days upon bioprinting [11]. High-salinity treatment was performed on day 2, representative high-resolution confocal images of 3D bioprinted cells are given: scale bars, 20 microns. Arrows indicate nucleus-located SCR expression. Colored dots represent the independent experiments, number of bioprinted constructs 20 and 30, letters indicate significant differences ($P < 0.05$).



a) b)

Fig. 1. Ground tissue identity of isolated root cells bioprinting: a – pEN7:GFP; b – pSCR:SCR-mCherry

The study showed that isolated single cells taken as a basis, which represent an excellent system for studying aspects of cellular physiology, cellular identity, intercellular communication and re-entry into the cell cycle [11], can be preserved with high viability for several days using a reproducible 3D bioprinting extruder. The percentage of isolated cells expressing endodermal markers increases over time, which suggests that bioprinted cells may either partially change identity or switch to a previously unknown identity.

The logarithm of the ratio of experimental productivity to product control is a measure for evaluating the positive and negative effects under given conditions [12]. According to the data presented in Table 1, the negative effect arising from various globally acting factors with a decrease in plant species diversity will be 95%, which will lead to an increase in CO2 concentration and the intensity of ultraviolet radiation, as well as to climate warming. With a further depletion of the composition of destructors, the rate of decomposition of plant residues decreases by about 20% (Figure 2) [13]. Accordingly, there is a depletion of soil ecosystems that perform ecological functions and are regulators of the content of CO2, N2, O2 in the air, as well as absorbers of harmful gas impurities [13, 14], the soil formers of which slow down the destruction process and have a significant impact on the full existence of ecosystems (Figure 2).

To prevent this plant effect, it is necessary to use technologies capable of growing living cells in artificial nutrient media [14]. 3D-bioprinting help cultivate plants without a natural environment with the use of additive technologies, creating the required conditions. The structure of the market for additive technologies as a percentage by areas of energy use, aviation and space, industry, medicine and other products, including energy, food, construction and bioprinting, the percentage of which is less than one (Figure 3), therefore, the cultivation of natural fabrics is still underdeveloped and has enormous potential for further development.

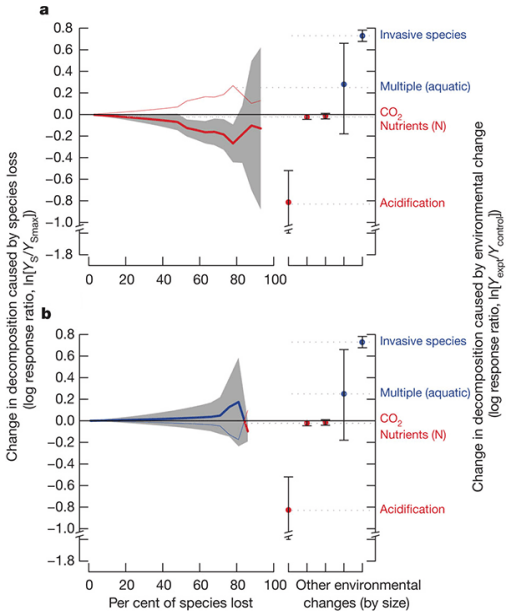


Fig. 2. Change in the rate of destruction of organic matter with a decrease in species diversity: a – destructors; b – producens

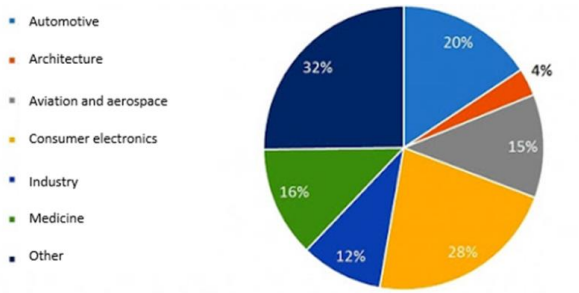


Fig. 3. The use of additive technologies in various industries

3 Results

Based on the data obtained in the course of the study, it can be confirmed that this technology of layered cultivation of crops is relevant for many countries; it combines the development of three-dimensional tissue modeling and special additive equipment based on FDM-printing. The first stage is the construction of the plant cell and the construction of a three-dimensional drawing, on the basis of which a file in STL format is created. The digital model contains information about the relative position of the points, after which the model is processed in a special program. The computerized process of layer-by-layer bioprinting takes place directly with the selection of a suitable medium in the bioreactor for conducting experiments and monitoring the process of cell maturation. Cells of future plants are placed in an artificial nutrient medium for growth and/or proliferation. The main factors required for various cell growth are: elevated temperature, cell attachment substrate (for cell attachment to cells), suitable culture medium, CO2 incubator to control the pH level, use of osmotic. the most important point to ensure the overall growth/proliferation of cells is the choice of a suitable culture medium. The culture medium is a gel observed for cell uptake/proliferation

growth. The cell culture medium consists of certain amino acids, vitamins, salts, glucose, hormones, nutritional supplements, growth carbohydrates; Supporting buffer system and osmotic pressure [15-20].

Since the basis of plant growth is cell division, therefore, in the process of growing a plant using 3D printing , specific additives are provided that can accelerate the process of cell division, stimulating their development. They contain phytohormones - organic substances, on the action of which the processes of growth and development of plants directly depend. Such substances differ in their chemical structure, the degree of influence on biological organisms, and, accordingly, the results [16]. Depending on whether these substances are manifested, the functions of the regulators are activated. It is important that such dressings also include not only hormones, but also other components, essential plants, in natural proportions. This is their proper nutrition, rapid growth and a high probability of stress, stimulation of development. The most relevant application of the stimulant formula with a visible balance is for growing for this work a complex of phytohormones, humic and fulvic acids, monosaccharides, macro- and mesoelements, as well as amino acids and fatty acids.

Within the framework of this work, a 3 D-printing biological head was designed for layer-by-layer growing of living cells, which is an optimized design of a classic 3D printing head for FDM- printing with a micro-screw. A special working body allows not only moving living cells along, but also the function of a nozzle that forms each layer. After that, the calculations of the geometric parameters of the 3 D -printing head were called. All calculations were carried out according to the methodological manual of Y I. Litvinets "Technological and energy calculations in the processing of polymers by extrusion" [17] using the formulas specified in it (Table 2).

Table 2. Calculation of the geometric parameters of the extruder

Parameter	Designation	Meaning	Units	Formula	Note	Variable	Meaning
1	2	3	4	5	6	7	8
volumetric productivity	Q	0.6	mm^3/s	$Q = 0.6D^{2.5}$			
screw diameter	D	1	mm				
screw pitch	t	0.8	mm	$t = k_1 \cdot D$	k_1 varies from 0.8 to 1.2. usually equal to 1	k_1	0.8
screw length	L	10.3	mm	$L = k_2 \cdot D$	k_2 varies from 8 to 40	k_2	10.3
screw length to compression zone	L_0	4.12	mm	$L_0 = 0.4L$			
screw head length	L_H	6.18	mm	$L_H = 0.6L$			
channel depth in the feed zone	h_1	0.12	mm	$h_1 = k_3 \cdot D$	k_3 varies from 0.12 to 0.16	k_3	0.12
in the plasticization zone	h2	0.1	mm	$h2 = h1 - \frac{(h1-h3)}{L} \cdot L_0$			

in the dosing area	h3	0.05	mm	$h3 = 0.5\left[D - \sqrt{D^2 - \frac{4h1}{i}(D - h1)}\right]$	material compression ratioifrom table 2	i	2
coil crest width	e	0.1	mm	$e = k_4 \cdot D$	k_4 varies from 0.06 to 0.1	k_4	0.1
radial clearance	δ	0.010	mm	$\delta = k_5 \cdot D$	k_5 varies from 0.002 to 0005	k_5	0.01
Performance Q = 44.7 cm^3/hour							

The operation of the extruder presented in the article for the synthesis of plant cells is carried out as follows: a distribution coupling transmits rotation from a stepper motor to a micro-screw installed inside the structure, then plant cells move [18] into a special nozzle that looks like a thin needle with a hole of no more than 0.1 microns, with which plant cells of the plant are synthesized and grown layer by layer. The whole process is carried out in such an environment for a certain type of plant, which improves the adhesion of plant cells during their 3d printing. The proposed cultivation technology is relevant not only for botany, but also for those areas where living cells are used as the main material that needs to be grown quickly and efficiently, without forgetting about the medium for adhesion of cellular material, for example, in bioengineering [19-41].

4 Discussion

Based on the results of the study, the following conclusion can be made: the proposed technology for synthesizing plant cells will regenerate tissues, which will prevent a decrease in species diversity and lead to a biobalance of the primary ecosystem, thereby reducing the acidity and concentration of carbon dioxide. The negative effect (Table 1) will be reduced to 50%, thereby not having a strong impact on climate change (Figure 1), performing environmental functions to the full and absorbing harmful gases of impurities.

Summing up the work done, it can be noted that the artificial plant cultivation project is a symbiosis of advanced technologies: additive and informational, in which the positive effect is achieved much faster and better with minimal investment and loss of time, reducing the cycle of growing and regenerating plant cells for rapid prototyping, proves its economic feasibility.

The lack of foreign and domestic developments in the manufacture of an extruder for bioprinting confirmed the practical importance of the project for further study of the possibilities of growing various plant species, not only those that are on the verge of extinction, but also the hybridization of new unique properties that will help stabilize the climatic situation.

5 Conclusion

In conclusion, I would like to add that the market for bioprinting with plant cells is far from oversaturated and is innovative in many areas where the integration of additive and information technologies can have a huge potential for active development not only in the field of bioengineering, but also medical research when crossing technologies.

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