Platform for in Vitro Angiogenesis Research

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Abstract
A microfluidic chip-based platform for in vitro angiogenesis research has been developed that allows reproducing the phenomenon of functioning self-developing endothelial capillary networks in endothelial cell culture and conducting a wide range of tissue engineering and biotechnological research on them.

Keywords:
endothelium, endothelial capillary networks, in vitro angiogenesis, tissue engineering, biotechnologies, regenerative medicine.

1. Introduction

Setting up the experimental studies of angiogenesis in vitro is a mostly complicated scientific and technical task that can only be done by technologically advanced, well-funded research groups in cell technology laboratories with the appropriate equipment, staff and competencies. Laboratory equipment, glassware, reagents, nutrient substrates, primary cell cultures of endothelial cells, microfluidic chips (MFC) and others, as a rule, are not produced on the territory of the Russian Federation and are purchased through domestic intermediary companies that are dealers of large foreign companies Cell Applications, Inc .; Dolomite Microfluidics and others [1, 2, 3], financing their further development. The Russian Federation is in complete technological dependence on the developed biotechnological powers and this disadvantage is growing rapidly, the RAS does not control the situation in this area. In Russia, such research can, unfortunately, be afforded only by a few large, high-budget research centers and laboratories with a modern scientific infrastructure. At the same time, they are almost always in the position of catch-up, repeating, complementary, led, but not leading. At the same time, in highly developed biotechnological countries such as South Korea, USA, Israel, China, Japan, experimental studies of in vitro angiogenesis are developing at an ever-increasing rates. This is starting to resemble the nuclear race that began after Lisa Meitner and Otto Hahn discovered a new type of nuclear fission reaction of uranium nucleus by using neutrons in the late 1930s.

2. The main idea of research and technology

Researchers and engineers under the guidance of professor Noo Lee Chong from Seoul national university (South Korea) managed to develop and create an experimental platform in which the phenomenon of development in endothelial cells culture in vitro, combined with microflows of a nutrient medium, 3-D functioning self-developing endothelial capillary networks (abbreviated SEKS) capable of mass transfer was reproduced. The key problem of experimental angiogenesis has been solved (Fig. 1) [4-8].

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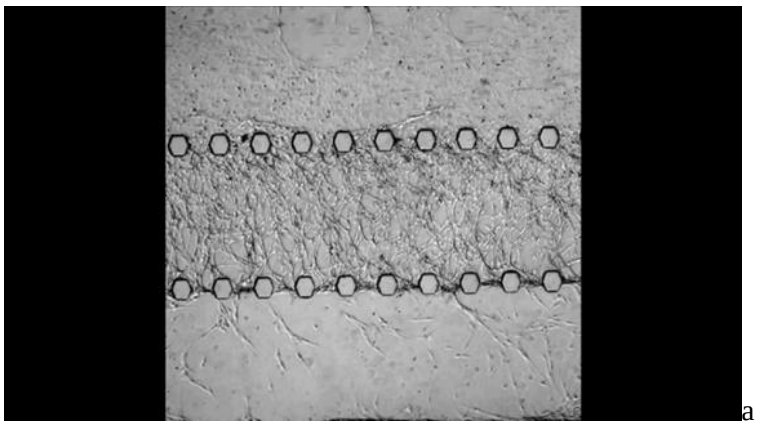
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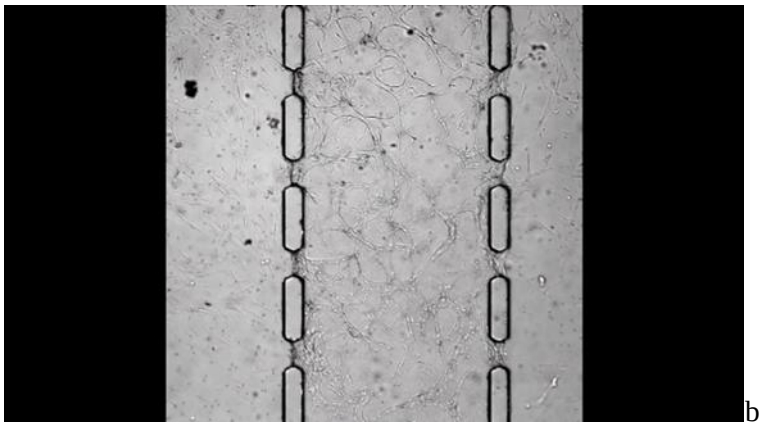
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Figure 1a, b. This is how artificial functioning SEKS, capable of mass transfer in vitro, look like [6]. Full video recordings of the reproduction of the phenomenon of functioning SEKS, capable of mass transfer in vitro, are presented by the links:

1a – https://yadi.sk/i/bP2wtwvU6Lyjzw [6], 1b – https://yadi.sk/i/-ruxrxZv76J4xQ [6]

A colossal biotechnological breakthrough has been made in cellular and tissue engineering of the endothelium field. A real perspective has appeared to start the experiments of in vitro construction of artificial tissue- and organ-like formations with given projected biological properties on the basis of the technology of functioning SEKS. Such experiments are based on the cooperative cultivation of SECS and primary cultures of various cells' types (SMC, muscle cells, nerve cells, β-cells of the Langerhans islets, etc.).

The technologies of functioning SEKS open up fantastic prospects for mankind: the creation of a meat-like biomass with given biological properties, as a semi-finished product for the food industry and thus, the preconditions, in the future, for the transition of mankind to a pseudoautotrophic type of nutrition; artificial muscles - movers for robotic complexes of natural-like type and cyborgs; artificial placenta and uterus - capable to achieve the embryonic and fetal periods of human development in vitro from a zygote to a newborn child - a step towards controlled demography, the possibility of rapid population growth in a single country or in extraterrestrial colonies; creation in vitro of artificial organs of a natural-like type suitable for transplantation without immunological problems, one of the first such organs more likely will be artificial islets of Langerhans; research laboratory complexes for studying the action and testing of medicinal drugs on nature-like models and much, much more (Fig. 2a, b).

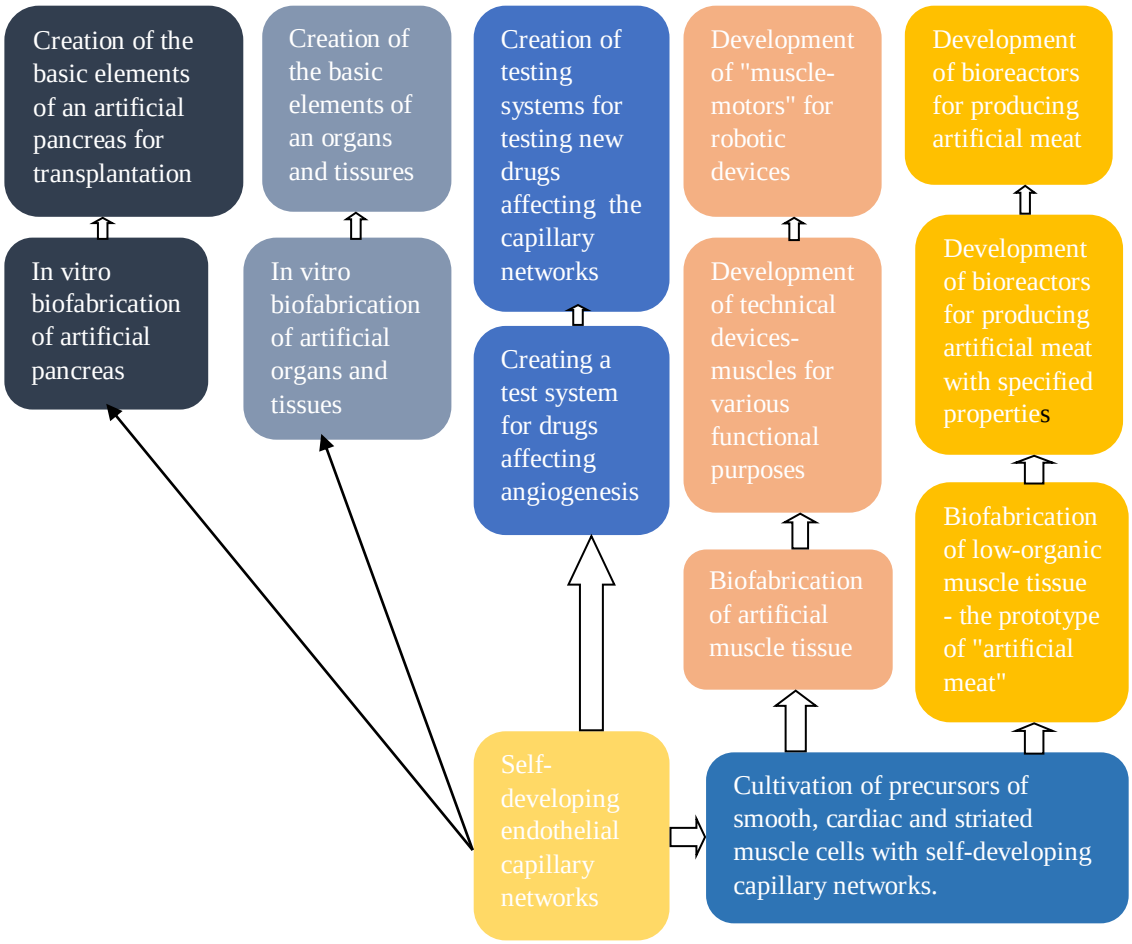
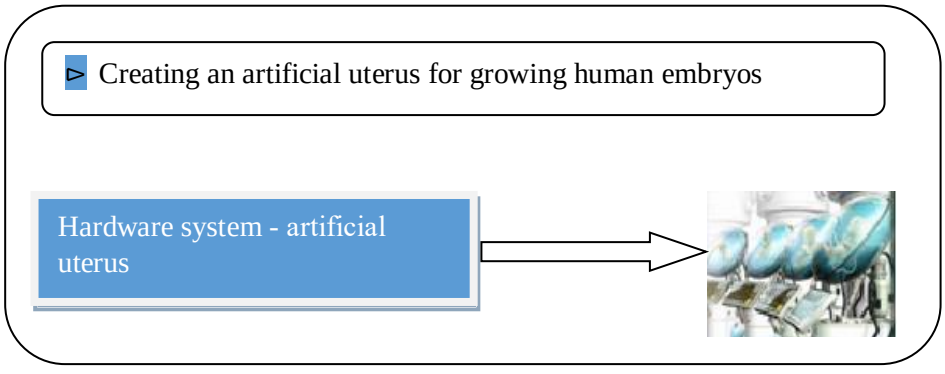

Creation of the basic elements of an artificial pancreas for transplantation
Creation of the basic elements of an organs and tissures
Creation of testing systems for testing new drugs affecting the capillary networks
Development of "muscle-motors" for robotic devices
Development of bioreactors for producing artificial meat
In vitro biofabrication of artificial pancreas
In vitro biofabrication of artificial organs and tissues
Creating a test system for drugs affecting angiogenesis
Development of technical devices-muscles for various functional purposes
Development of bioreactors for producing artificial meat with specified properties
Biofabrication of artificial muscle tissue
Biofabrication of low-organic muscle tissue - the prototype of "artificial meat"
Self-developing endothelial capillary networks
Cultivation of precursors of smooth, cardiac and striated muscle cells with self-developing capillary networks.

Figure 2a


Creating an artificial uterus for growing human embryos
Hardware system - artificial uterus

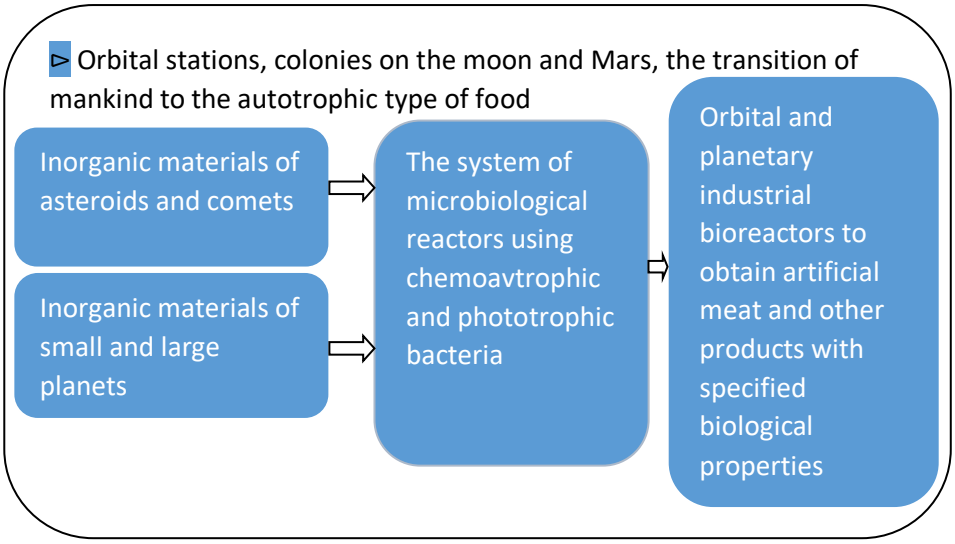


Figure 2b

Figure 2a, b. Nearest (a) and remote (b) perspective of the technology of functioning SEKS. (Project of the Skolkovo Foundation "Universal platform" Frankenstein "for biofabrication of artificial tissues and organs" (Scientific supervisor - V. A. Glotov).

3. Platform

Such kind of studies require the creation of special experimental platforms with the highest level of engineering support. Design enjineering and creation of such platforms has moved from the scientific sphere to the engineering and scientific sphere now.

The platform being developed is a complex structure with many parameters; it is a new area of research. Finding the optimal parameters of pressure, temperature, humidity, flow rate of the nutrient fluid, at which a capillary network is forming, - all of that is an independent complex mathematical and engineering task, since the values of the parameters are strongly depending on the platform construction. The solution of the problem could be the creation of self-regulating, adaptive systems with mathematician-programmer possible interactive intervention. The platform is generally described by nonsmooth Lipschitz functions, therefore, to find the optimal ones, it is required to use new nonsmooth optimization methods developed by mathematicians and engineers of the Smolensk state medical university.

In 2020, at the Department of human anatomy of Smolensk state medical university, a totally original R&D was completed with the support of the Small Enterprise Assistance Fund in the scientific and technical sphere: Project NTI-50323 "Development of a platform on a microfluidic chip base for ex vivo angiogenesis research" (Scientific supervisor - MD, prof. V.A. Glotov, chief designer - V.V. Zhilkin) (Fig. 3-7) [9].

In the course of the project, the development of the platform based on a microfluidic chip for ex vivo angiogenesis research was performed. The platform will allow to study the combined effect of inducers and inhibitors of angiogenesis on the functioning SEKS formation.

The developed microfluidic chip-based platform for ex vivo angiogenesis research has the following parameters and provides the following functionality:

- the possibility of cell lines cultivating (both single and compositions from some of them), including the reconstruction of a tissue-like cell structure on a collagen matrix of biological origin;

- the ability of cell line maintaining for at least 20 days period;

- system for maintaining the vital functions of cell lines cultivated in the MFC, to provide the specified parameters of the environment for at least 24 hours without operator intervention;

- operating temperature regime of the platform + 25 ° C to + 60 ° C, mode of cells vital activity maintaining - + 25 ° C- + 45 ° C;

- the hydraulic system of the platform equipment (system of pipes, branch connectors, locking valves) is made of biocompatible materials resistant to weak acids and alkalis;

- the possibility of dosed supply of chemical factors in concentrations specified by the requirements of the experiment, in a volume from 1 μl to 100 μl;

- the possibility of optical registration with resolution of at least 600x600 pixels, at a rate of at least 10 shots per second;

- the ability to describe the process of vascular bed formation and make quantitative assessment of the following parameters: the number of processes, total length, sections and intensity of branching, the size of the lumen in different parts of the channel, and also to fix the time parameters of the ongoing processes.

The developed platform (Fig. 3-7) corresponds to the technical enquiry for the project. The planned results of the project have been achieved.

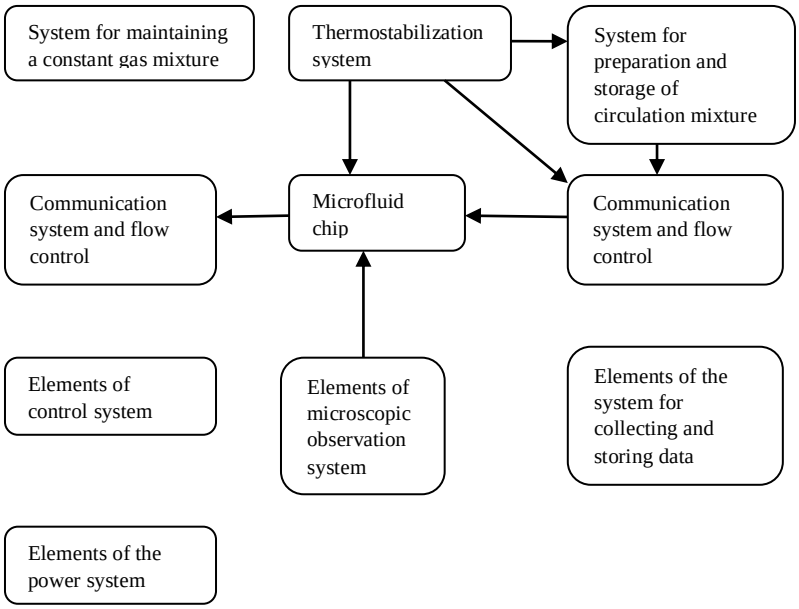


Figure 3. Block diagram of the platform for in vitro angiogenesis research.

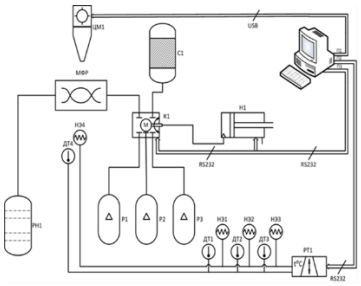


Figure 4. Functional diagram of the platform for in vitro angiogenesis research.

Figure 5. External view of the platform for in vitro angiogenesis research. OBJ

Figure 6. Platform assembly without case. 1. Control computer. 2. Drivers of the devices. 3. GSM modem. 4. Microcontroller of the control. 4.5.6 Valves - selectors. 7.8. Piston pumps - microdosers. 9.10. Gas mixture recirculators. 11. USB microscope. 12. Battery of containers with nutrient fluid, inductors and inhibitors of angiogenesis, etc. 13. Electronic thermostat with temperature sensor. 14. Microfluidic chip in focus of the microscope objective. 15. Mounting panel. 16 Heating mat. 17. CO2 bottle with reducer and electromagnetic valve. Peristatic pump with mixture circulation pipes.

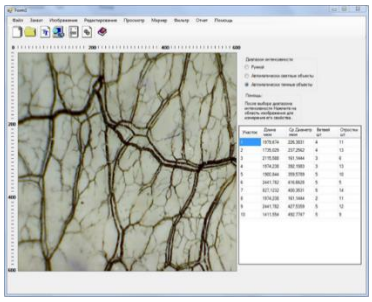


Figure 7. Graphical interface of the program of technical vision of the platform.

The developed platform is a mobile, autonomous, cybernetic biotechnical system that allows, subject to the appropriate research protocols, to reproduce the phenomenon of functioning SEKS in the culture of endothelial cells and to carry out a wide range of tissue engineering and biotechnological studies on them. The development of this area of research and R&D in the Russian Federation will allow us to quickly (in 2-3 years) overcome our biotechnological retardation behind the leading biotechnological powers, and with proper political, organizational and financial support, become leaders in this strategically important area.

4. Acknowledgements

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