Agnieszka Krakos
Department of Microsystems
Wroclaw University of Science and Technology
agnieszka.krakos@pwr.edu.pl

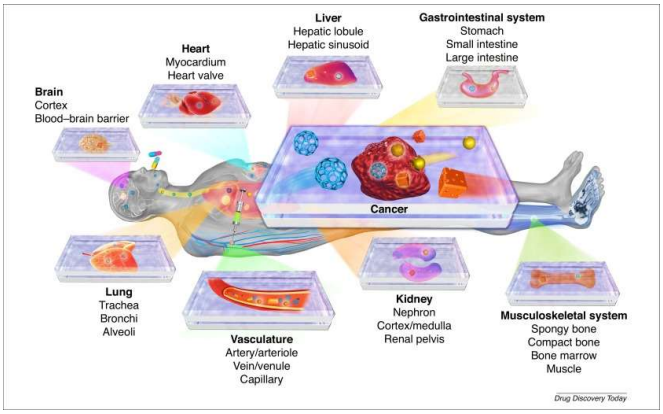
DOI: 10.35117/A_ENG_25_07_08_05

Miniature lab-on-chip instruments – role and strategies in development of microgravity biomedicine

Abstract: In the paper, the analysis of the current interest in the miniaturization of the space instruments, especially for the biomedical research has been shown. The review shows lab-on-a-chip devices which have been applied in the studies performed both on ISS, as well as on nanosatellites boards. Special attention has been paid to NASA and ESA programs (e.g. Tissue chips), nevertheless other scientific and commercial initiatives have also been presented. As the ground-based research utilizing simulated microgravity instruments constitutes a significant step prior to the missions, this issue has also been described. The paper ends with the conclusion regarding NASA and ESA roadmaps and further trends on miniaturization of space analytical instruments.

Keywords: Lab-on-a-chip; Microfluidics; Space biology; Microgravitional medicine; Biological nanosatellite

Labs-on-a-chip (LOC) are miniaturized analytical devices used widely in biochemical and biomedical research. Although initially, the microscale laboratories discussed herein were mainly used in protein and DNA analysis, they have recently become the first choice in broadly understood medical diagnostics, including, among others, cell culture studies [1-2]. The processes of biological objects growth "on chip" include both the analysis of single cells, as well as the larger fragments of tissues or organs. In the literature, this type of research already has its own nomenclature and terms, such as cell/tissue/organ on-chip, has been used for almost a decade [3]. The application possibilities of LOC in cancer cell culture are also becoming increasingly popular (Fig. **1**). LOC devices are designed as full-value, automated and autonomous instruments enabling diagnostics and selection of appropriate oncological therapy [4-5]. Before it is proposed to the patient, biological samples are subjected to tests determining cell survival in response to stimulation with drugs of different composition and concentration. This is possible thanks to the use of microfluidic phenomena, which allow for effective management of samples, with a volume of several to several dozen microliters [6].



1. The concept of research conducted "on-chip" concerning multi-organ cancer cell culture [7]

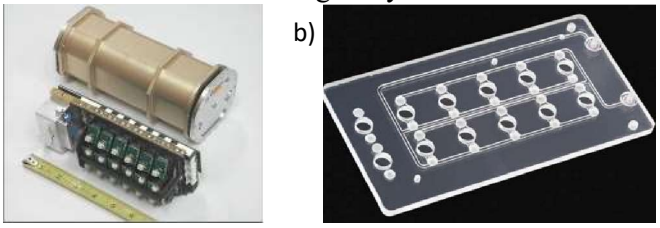
Chemotherapy is not the only form of oncological research currently conducted "on chip". The development of microengineering techniques enables miniaturization of complex analytical devices, in which a biological sample can be subjected to multi-stage stimulation. In addition to testing the response to drugs, within a single experiment, the sample can also be irradiated and/or treated with an electrical pulse. For years, scientists have been describing promising results of works on the use of LOC instruments in the development of new chemotherapeutic and radiotherapeutic strategies, as well as photodynamic, sono- and electroporation therapies [8-10].

The boldness of LOC applications seems to be unlimited, especially if we take the considerations contained in this article beyond the framework of the earth laboratories. In recent years, interest in space and the possibility of conducting research in microgravity and radiation conditions has been significant. Extreme technologies using microelectronics and photonics, precise mechatronic microcomponents, MEMS (Micro-Electro-Mechanical System) microsystems, MOEMS (Micro-Opto-Electro-Mechanical System), as well as multiparametric data acquisition and intelligent processing systems, are increasingly used to build a new class of miniature instruments for, so-called, space biomedicine. The small dimensions of such devices significantly reduce the costs of launch into orbit, and their autonomous nature ensures to use them not only on the International Space Station (ISS, Fig. **2**), but also in unmanned missions, for example utilizing nanosatellites.

2. Examples of lab-chips used in research on the ISS. Images courtesy of NASA and the University of Pennsylvania School of Engineering and Applied Science, and Children, Äôs Hospital of Philadelphia

Although there are few projects implemented so far involving, so-called biological nanosatellites (8), it has been shown that microgravity has a significant impact on the development of living organisms. Examples include missions proposed mainly by NASA, e.g. GeneSat-1 (2006) and EcAMSat (2017), Fig. **3**. In both cases, a payload designed as an integrated analytical microlaboratory was used. Equipped with a LOC structure and microfluidic and optical components, it enabled precise dosing, management and analysis of the sample. In both missions, it was decided to study the development of model cultures of *E. coli* bacteria, with simultaneous assessment of changes in their metabolism and proliferation using excited fluorescence techniques. As a result of the conducted experiments, for instance, inhibited growth of *E. coli* colonies in a microgravity environment was observed [11-13].

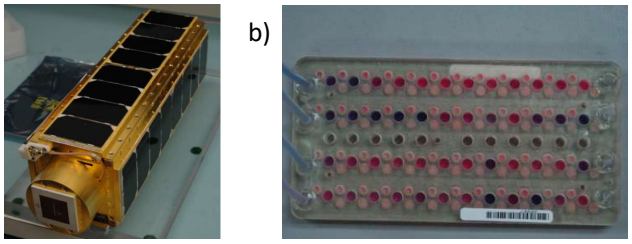
a) b)



3. Payload of the GeneSat-1 biological nanosatellite (a), lab-chip used to study the development of *E. coli* bacteria cultures in microgravity conditions (b). Images courtesy of NASA

The PharmaSat mission (2009, NASA), as well as the currently conducted Biosentinel mission (2022, NASA), proposed experiments on the yeast strain *S. cerevisiae* [14-16]. The development and resistance of *S. cerevisiae* to fungicides were studied on board the PharmaSat nanosatellite (Fig. **4**). Among other things, significant drug resistance of these objects was demonstrated, significantly exceeding terrestrial cultures, indicating greater "virulence" of *S. cerevisiae* in microgravity, as well as the ability to develop new immune mechanisms. Biosentinel is the first nanosatellite mission carried out in cis-lunar space, beyond Low Earth Orbit (LEO). This is a mission aimed mainly at assessing the potential degradation of DNA of *S. cerevisiae* under conditions of ionizing radiation and microgravity. We are still waiting for the publication of the first results of this project.

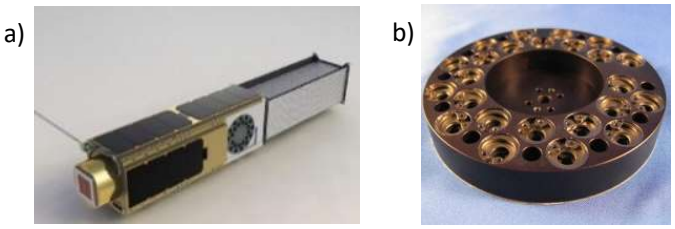
a) b)



4. Payload of the PharmaSat biological nanosatellite (a), lab-chip used to study the development of *S. cerevisiae* yeast culture and its drug resistance in microgravity conditions (b). Images courtesy of NASA

A slightly different approach to the mission implementation was proposed within the O/OREOS (2010, NASA) and LabSat projects (2022, Wrocław University of Science and Technology, SatRev, Poland). The common feature of the O/OREOS and LabSat missions was the simultaneous conduction of two different experiments using the LOC microfluidic laboratory. In O/OREOS, the LOC platform and an optical spectrometer were used to determine changes in organic compounds, i.e. polycyclic aromatic hydrocarbons, amino acids and quinones in space (Fig. **5**). Among other things, the processes of decomposition of selected samples were observed, but these results were not unambiguous. In parallel, the O/OREOS mission studied the development of *B. subtilis* and *H. chaoviatoris* bacteria "on-chip" and for both strains, inhibited colony growth was noted compared to terrestrial conditions [17-18].

a) b)

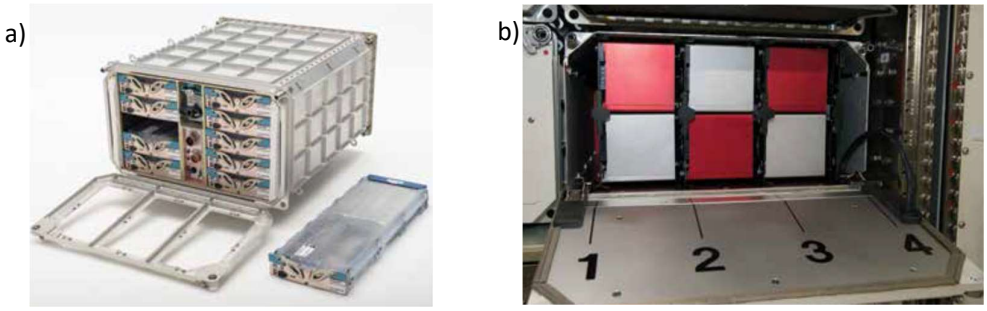


5. Payload of the O/OREOS biological nanosatellite (a), SEVO microfluidic device for studying the effect of microgravity on selected organic compounds (b), [17]

LabSat mission in turn was the first European nanosatellite research aimed at investigating the effect of microgravity on the cultivation of cosmopolitan microscopic fungi *F. culmorum* and plant seeds (*L. sativum*). A complex microlaboratory was constructed,

equipped with a number of microfluidic and optical components, enabling the examination of biological samples directly in orbit. Both fungal spores and cress seeds were placed in lab-chips in a "dry" form. The beginning of the cultivation process, conditioned by the supply of necessary nutrients, took place after the nanosatellite reached the appropriate altitude and established radio communication with the Earth laboratory. The vision system placed within the payload was programmed to acquire the images of the cultivation several times a day. As a result of the LabSat mission, it was possible to observe the beginning of the cress seed germination process in LEO, which is interesting due to the possibility of disturbed geotropism in plants in microgravity conditions [19-20].

As reported in this paper, none of the so-called biological nanosatellite missions carried out so far have conducted experiments on more demanding biosamples, e.g. those originating from humans. This is due to the difficulty of protecting cells for the rocket launch (often the waiting time for the flight is even several months), especially considering the wide range of temperature changes, vibrations and overloads. The leaders in the field of stem and cancer cell research in microgravity conditions remain projects carried out on the ISS. The ISS infrastructure has a number of laboratories enabling various types of biomedical tests (e.g. KUBIK, ABRS, EMCS, Bioculture System Facility, TangoLab-1, Fig. **6**), but the problem is the availability of equipment, as well as the need to involve astronauts. The lack of autonomy may cause conflicts in the field of intellectual property and, ultimately, affect the success of the experiments. In this respect, nanosatellites are still a better choice, which may be a kind of development engine for their further use in biomedical research in space.

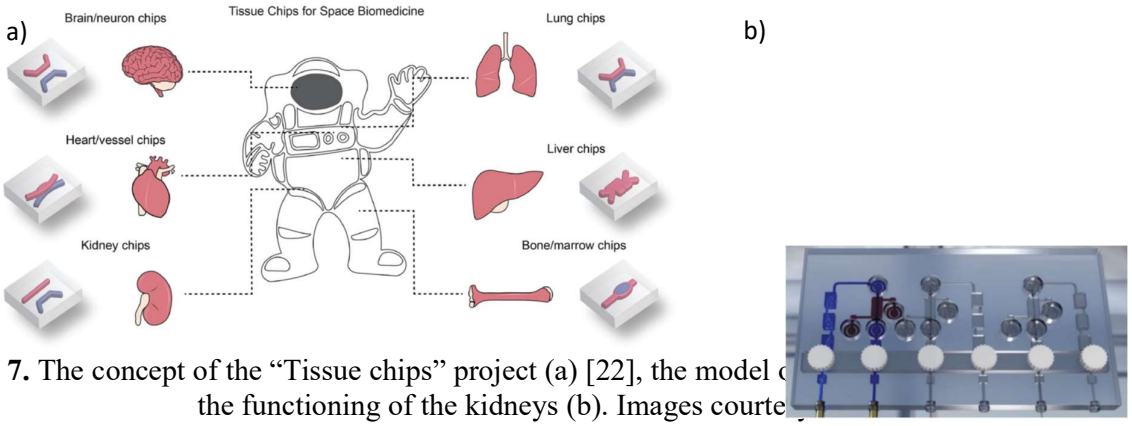


6. The examples of ISS instruments enabling biomedical testing: a) Bioculture System Facility, b) Tango-Lab-1. Images courtesy of NASA

As mentioned, there are currently several projects run on the ISS to determine the effect of microgravity on the development of human cells. Of particular interest to NASA and ESA are experiments related to the three-dimensional growth of cancer cells *in vitro*. It has been shown that in a microgravity environment, cell cultures take the form of spheroids, which is what we naturally observe in the human body. This is currently the subject of ESA research, among others, as part of the ESA-SPHEROIDS campaign [21] and M4PM (Microgravity for Personalized Medicine). The aim of these initiatives is to create new oncological models using high-quality three-dimensional biological structures and to determine the effectiveness and toxicity of chemotherapeutics. Cancer cells will also be examined for aging and the possibility of developing regenerative mechanisms. So far, reduced proliferation, differentiation and changes in cell morphology have been noted, as well as the ability to form aggregations, which can reduce the effect of shear forces and stresses in the extracorporeal environment.

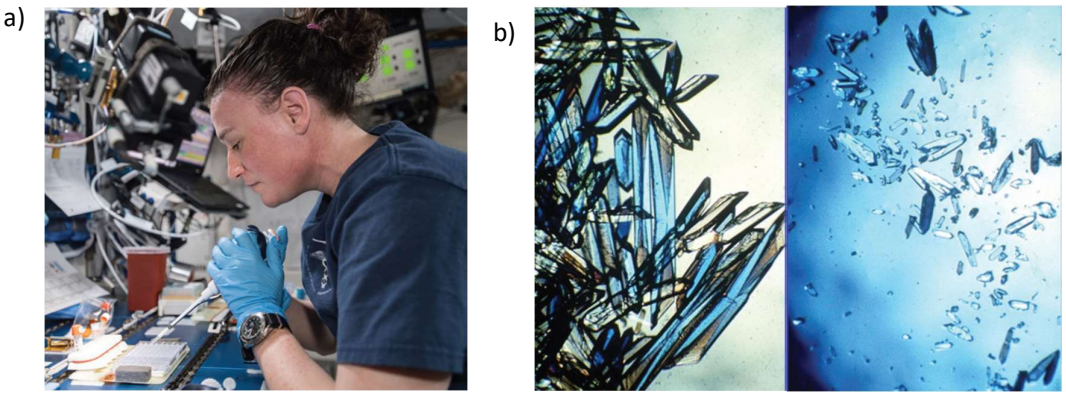
For several years, NASA, together with a scientific consortium (National Center for Advancing Translational Sciences, NCATS, National Institute for Biomedical Imaging, NIBIB), has been implementing the "Tissue chips" project [22-23]. For this purpose, ISS facilities are used, afor instance, to study cell cultures, including cancer cells and drug resistance mechanisms, especially in the context of kidney and heart tissue dysfunction, as well as

immunological changes or deconditioning of the entire musculoskeletal system (Fig. **7**). In 2023, the last of the "Tissue chips" campaign was carried out on the ISS, led by a consortium of Stanford University and Johns Hopkins Medicine. The so-called Engineered Heart Tissues Chip (EHTs chip) was proposed, in which the functioning of heart cells was studied while modeling diseases and determining the drug resistance of cardiomyocytes. Further plans of NCATS, NIBIB and NASA institutions are related to the continuation of the "Tissue chips" project, especially in the field of studying kidney dysfunction. The microfluidic structure (Fig. 7) would be filled with a hydrogel enabling spatial growth of kidney cells, and the culture medium containing cells would be introduced into microchannels created in the hydrogel, imitating blood vessels. The cells, adhering to the walls of these microchannels, would form a proximal renal tubule. Such a microenvironment could then be used to study drug resistance and absorption processes.



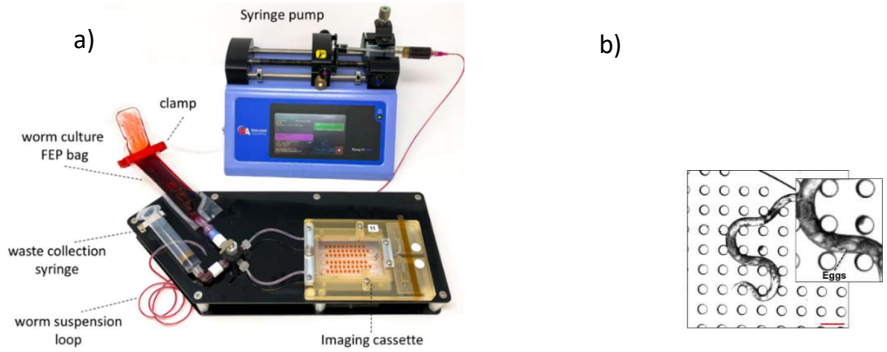
7. The concept of the “Tissue chips” project (a) [22], the model c
the functioning of the kidneys (b). Images courte

In parallel to research on the behavior of human cells in m g conducted on the development of new generation drugs. Wo f biotherapeutic compounds is being conducted by, e.g., NASA, Ja . According to the current reports, the reduction of gravitational forces, such as sedimentation and convection, allows protein molecules (e.g. lysozyme and thaumatin) to incorporate into the crystal lattice more slowly and in a more orderly manner, which significantly improves crystal growth [24]. Research in this area is also being conducted by the University of Toledo and the companies Dover Lifesciences and MicroQuin. Protein crystals of appropriate size and quality (Fig. **8**) would be used in the development of new drugs for obesity, cancer and rare genetic disorders [25].

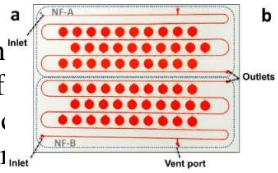


8. Protein crystallization experiments conducted on the ISS: a) NASA astronaut Serena Auñón-Chancellor preparing the samples, b) comparison of protein crystals grown in space (left) and on Earth (right) [25]. Images courtesy of NASA

Genetic studies are also conducted at the ISS. Using lab-on-chip platforms, such as Nemaflex (Fig. **9**), changes in gene expression are determined based on the behavior of model organisms, including *C. elegans*. In these experiments, reduced sensitivity of *C. elegans* to mechanical stimuli has been observed so far [26]. The MinION DNA sequencer, proposed by Oxford Nanopore Technologies (Oxford, UK), is also being tested at the ISS. The MinION DNA has been used, among others, for DNA sequencing and genome composition studies on lambda bacteriophages, *E. coli* and *M. musculus* [27].

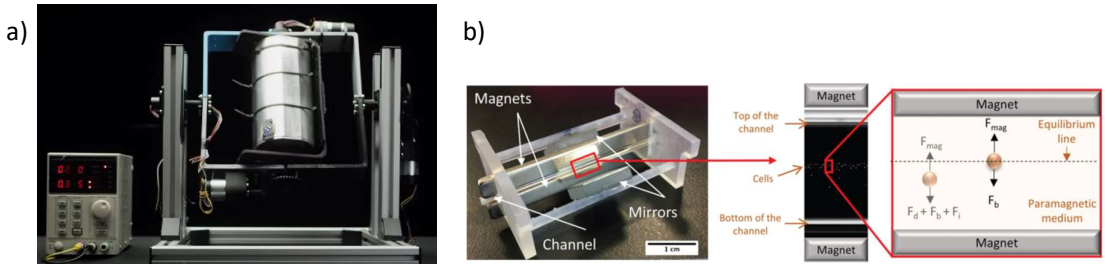


9. Nemaflex microfluidic platform (a), lab-chip scheme with magnified image of *C. elegans* nematode passing through micromechanical obstacles (b), [26]



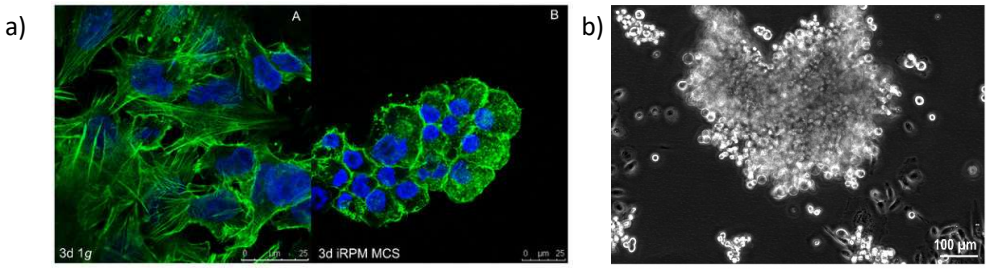
The paths of development of new methodologies in : and genetic research implemented on the ISS, combined with the f th increased penetration, may in the near future lead to the creation of pies". If it is possible to achieve positive therapeutic effects in microgr shorter time, while reducing the drug dose and thus, the side effects of treatment, a real biopharmaceutical revolution may occur. An increase in financial outlays on research in the field of microgravity biomedicine by global giants may be only a matter of time here, and representatives of the cosmetics or food industry may follow the trend.

The current efforts undertaken mainly by academic communities in microgravity biomedical research also have to be mentioned. Due to the limited implementation capabilities of the ISS and the construction of own research nanosatellites, microgravity simulators are becoming the first choice for identifying phenomena caused by a quasi-microgravity environment. Instruments such as RPM (Random Position Machine), RWV (Rotating Wall Vessel) and RCCS (Rotating Cell Culture System) are widely used in the study of biological samples. An interesting idea provided recently is also the use of the, so-called diamagnetic levitation (Fig. **10**). Limited graviperception is realized herein by magnetic force, not rotation as in the case of the aforementioned simulators [28].



10. Examples of microgravity simulators: a) RPM (own work), b) diamagnetic levitation instrument [29]

The leading center in biomedical research conducted on microgravity simulators is the team of Dr. Marcus Kruger from the Otto-von-Guericke University in Magdeburg. As part of the work conducted to date using RPM, scientists have demonstrated, e.g., the possibility of lung, pancreatic, prostate and breast cancer cells to be grown in the form of three-dimensional spheroids (Fig. **11**) [30-32]. By obtaining an imitation of *in vivo* conditions, they also conducted research on drug resistance, genetic and proteomic changes, defining new development paths for personalized medicine.



11. Microscopic images of cancer cells cultured in normal conditions and on microgravity simulators: a) visible changes in the cytoskeleton of lung cancer cells induced by simulated microgravity [30], b) formation of spheroids of prostate cancer cells under simulated microgravity conditions [31]

Microgravity biomedicine is a new, extremely interesting research direction, the development of which requires the cooperation of many other scientific branches. The use of extreme technologies including microelectronics, innovative materials and intelligent, fully integrated autonomous computer systems is becoming necessary to produce instruments capable of working in difficult space conditions. Further miniaturization of analytical devices, although it is a challenge in terms of construction, as well as the occurrence of phenomena characteristic only for the microscale, seems to be an appropriate approach to increase the availability of research in the field of microgravity biomedicine. The use of microfluidic lab-on-a-chip devices is increasingly proposed within the framework of ISS projects, biological nanosatellite missions and in further research paths of NASA and ESA, including those going beyond the LEO framework.

Source materials

[1] Azizipour, N., Avazpour, R., Rosenzweig, D.H., Sawan, M., Ajji, A., Evolution of Biochip Technology: A Review from Lab-on-a-Chip to Organ-on-a-Chip, Micromachines, vol. 11, 599, 2020
[2] Castillo-Leon, J., Svendsen, W.E., Lab-on-a-Chip Devices and Micro-Total Analysis Systems: A Practical Guide, London, 2015, Springer
[3] Young, R.E., Dongeun Huh, D., Organ-on-a-chip technology for the study of the female reproductive system, Advanced Drug Delivery Reviews, vol. 173, pp. 461-478, 2021, https://doi.org/10.1016/j.addr.2021.03.010
[4] Moghimi, N., Hosseini, S.A., Poudineh, M., Kohandel, M., Recent advances on cancer-on-chip models: Development of 3D tumors and tumor microenvironment, Bioprinting, vol. 28, e00238, 2022
[5] Chakrabarty, S., et al., A Microfluidic Cancer-on-Chip Platform Predicts Drug Response Using Organotypic Tumor Slice Culture, *Cancer Res*, vol. 82, pp. 510–520, 2022

[6] Iannone, E., Labs on Chip: Principles, Design and Technology, Boca Raton, 2015, CRC Press Taylor & Francis Group
[7] Zhang, Y.S., Zhang, Y.-N., Zhang, W., Cancer-on-a-chip systems at the frontier of nanomedicine, Drug Discovery Today, vol. 22, pp. 1392-1399, 2017
[8] Sung, H.W., Choi, S.-E., Chu, C.H., Ouyang, M., Kalyan, S., Scott, N., et al., Sensitizing drug-resistant cancer cells from blood using microfluidic electroporator, PLoS ONE 17(3): e0264907, 2022
[9] Chermat, R., et al., Radiotherapy on-chip: microfluidics for translational radiation oncology, Lab Chip, vol. 22, pp. 2065-2079, 2022
[10] Cieslak, A., Krakos, A., Kulbacka, J., Detyna, J., Overview of research on additive manufacturing of hydrogel-assisted lab-on-chip platforms for cell engineering applications in photodynamic therapy research, *Microchim Acta,* vol. 191, 608, 2024
[11] Kitts, C., Flight Results from the GeneSat-1 Biological Microsatellite Mission, The Proceedings of the 21st Annual AIAA/USU Conference on Small Satellites, pp. 1-11, 2007
[12] Padgen, M.R., et al., The EcAMSat fluidic system to study antibiotic resistance in low earth orbit: Development and lessons learned from space flight, Acta Astronautica, vol. 173, pp. 449-459, 2020
[13] Padgen, M.R., et al., EcAMSat spaceflight measurements of the role of σs in antibiotic resistance of stationary phase Escherichia coli in microgravity, Life Sciences in Space Research, vol. 24, pp. 18-24, 2020
[14] Diaz-Aguado, M.F., Ghassemieh, S., Van Outryve, C., Beasley, C., Schooley, A., Small Class-D spacecraft thermal design, test and analysis - PharmaSat biological experiment, 2009 IEEE Aerospace conference, Big Sky, MT, USA, 2009, pp. 1-9
[15] Tieze, S.M., et al., BioSentinel: A Biological CubeSat for Deep Space Exploration, Astrobiology, 2020
[16] Padgen, M.R., et al., BioSentinel: A Biofluidic Nanosatellite Monitoring Microbial Growth and Activity in Deep Space, Astrobiology, 2021
[17] Ehrenfreund, P., et al., The O/OREOS mission—Astrobiology in low Earth orbit, Acta Astronautica, vol. 93, pp. 501-508, 2014
[18] Wayne L., et al., The O/OREOS Mission: First Science Data from the Space Environment Survivability of Living Organisms (SESLO) Payload, Astrobiology, vol. 11, pp. 951-958, 2011
[19] Krakos, A., Lab-on-chip technologies for space research — current trends and prospects. *Microchim Acta,* vol. 191, 31, 2024
[20] Krakos, A., When Earth is not enough: lab-on-a-chip accelerating space research, Lab-on-a-chip devices for advanced biomedicines: laboratory scale engineering to clinical ecosystem / eds. Arpana Parihar, Piyush Pradeep Mehta. Cambridge: The Royal Society of Chemistry, cop. 2024. s. 402-429, (Detection Science, ISSN 2052-3068; 25)
[21] Kruger, M., et al., Growth of Endothelial Cells in Space and in Simulated Microgravity – a Comparison on the Secretory Level, Cell Physiol Biochem, vol. 52 pp. 1039-1060, 2019
[22] Mu, X, e al., Small tissue chips with big opportunities for space medicine, Life Sciences in Space Research, vol. 35, pp. 150-157, 2022
[23] Low, L.A.,Giulianotti, M.A., Tissue Chips in Space: Modeling Human Diseases in Microgravity, Pharm Res, vol. 17, 2019
[24] Woerd, M.J., Brasseur, M.M., Spearing, S.F., Whitaker, A.F., Lab-on-a-chip based protein crystallization, SmallTalk 2001 Conference, San Diego, January, 2001, https://ntrs.nasa.gov/citations/20010075161 (available in July 2023)
[25] Williamson Smith, A., Probing Proteins: Leveraging Microgravity for Medically Important Molecular Crystallization, ISS National Laboratory Center for the

Advancement of Science in Space, 2019, https://www.issnationallab.org/probing-proteins-leveraging-microgravity-formedically-important-molecular-crystallization/ (available in July 2023)
[26] Soni, P., Anupom, T., Lesanpezeshki, L., et al., Microfluidics-integrated spaceflight hardware for measuring muscle strength of Caenorhabditis elegans on the International Space Station, npj Microgravity, vol. 8, 50, 2022
[27] Castro-Wallace, S.L., Chiu, C.Y., John, K.K., et al., Nanopore DNA Sequencing and Genome Assembly on the International Space Station, Sci Rep, vol. 7, 18022, 2017
[28] Vashi, A., Sreejith, K.R., Nguyen, N.-T., Lab-on-a-Chip Technologies for Microgravity Simulation and Space Applications, Micromachines, vol. 14, 116, 2023
[29] Anil-Inevi, M., Yaman, S., Yildiz, A.A., et al., Biofabrication of in situ Self Assembled 3D Cell Cultures in a Weightlessness Environment Generated using Magnetic Levitation, Sci Rep, vol. 8, 7239, 2018
[30] Corydon, T.J., et al., Current Knowledge about the Impact of Microgravity on Gene Regulation, *Cells, vol. 12*, 1043, 2023
[31] Grimm, D., et al., The Fight against Cancer by Microgravity: The Multicellular Spheroid as a Metastasis Model, Int. J. Mol. Sci, vol. 23, 3073, 2022
[32] Grimm, D., Recent Advances in Thyroid Cancer Research, *Int. J. Mol. Sci.*, vol. *23*, 4631, 2022