Cover Page

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A Scientific Rationale for 3D Bio-Printing of Soft Matter (Human Tissues And Organs) in Microgravity & under Reduced Gravity During Deep-Space Exploration Using Electric-Field-Assisted Direct Ink Writing (e-DIW)

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Background of 3D Bio-printing and Its Use in Space

Astronauts experience higher radiation and altered gravity levels in space, which increase the risk of health threatening incidents, such as bone demineralisation, bones mass reduction, diminished cardiovascular activity, injuries, or even cancer. Severe health incidents such as extensive burns, bone fractures or even organ failure, can lead to serious emergency situations, and potentially to the death of the astronaut if not treated correctly in time. The probability of these severe health risks increases in longer and more distant deep-space exploration missions, while the crew cannot access medical support from Earth anymore [1-3]. An on-site medical treatment is, therefore, crucial to supporting manned space exploration missions, and especially future deep-space exploration and colonization. New technologies such as additive manufacturing (AM) and 3D bio-printing offer promising perspectives for on-site medical treatment applications [4-12].

Additive Manufacturing (AM) technologies, also known as 3D Printing, which fabricate three-dimensional objects directly from a digital model by accumulating materials, have been widely investigated in the past few years for on-site manufacturing applications in space [10-14]. Conventional AM can be used to maintain the clinical infrastructure in space by manufacturing medical tools, splints for medical orthoses, tailored casts and dental equipment, such as implants or fillings [15].

3D bio-printing of soft matter (e.g. human tissues and organs) is an additive manufacturing methodology which employs simultaneous layer by layer deposition of cell types and cyto-compatible biomaterials such as hydrogels which, together provide a supporting structure capable of generating organoids or potentially entire functional organs [16-22]. It has been successfully used on Earth to produce ear-shaped cartilage for auricular reconstruction [16], and print tumorous tissues for biomedical research [23].

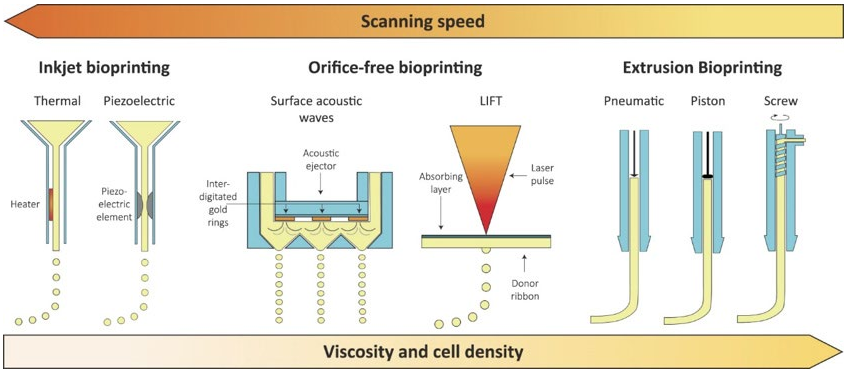

Fig.1 Mostly used AM techniques for 3D bioprinting and the associated performance in terms of scanning speed and viscosity and cell density obtained: inkjet, orifice-free and extrusion bioprinting [18]

3D bio-printing is considered as a promising solution for direct medical support in space, which can offer a broad range of potential future applications ranging from simple tissue constructs for treating skin lesions and bone defects, to the potential fabrication of complex, vascularized tissue constructs including organs such as kidney and liver (and potentially, even heart and brain tissues). [9-14, 24] Notably, DIW based 3D bioprinting can produce in situ artificially-grown organs from cells of a particular patient (an austronaut). Figure 1 summarizes the mostly used AM technologies for 3D bio-printing, including inkjet bioprinting, orifice-free bioprinting, and extrusion bioprinting which is also known as Direct Ink Writing (DIW) [25].

Among these 3D bioprinting techniques, the extrusion bioprinting, also known as DIW, is the most popular and broadly used bioprinting technique on Earth and also in space, due to its ease of use in microgravity conditions and versatile platform. It is rapidly developing worldwide in the last couple of years [11]. In July 2019, the so-called 'Biofabrication Facility' developed by the

U.S. companies TechShot and nSrypt, containing a DIW bioprinter, was launched and has been installed at the International Space Station (ISS). Media reported that it has successfully printed a large volume of human heart muscle cells aboard the ISS [26]. The European Space Agency ESA also has started to develop a 3D bioprinting system which shall be integrated in the so-called Biolab at ISS [27]. Several USA companies are actively working on the development of certified space 3D bioprinters of the extrusion type for performing bioprinting experiments at ISS [28].

Challenges

Despite the tremendous potential and recent progress in microgravity bio-printing, to accomplish the aim of supporting medical treatment in space, many scientific challenges remain to address. The major limitation is how to create volumetric structures with required properties that provide good conditions for cell survival and development and contain perfusable, vasculature-like support systems [17-21]. From a manufacturing technology standpoint, faster printing processes, higher printing resolution for vasculature production, and complex bioreactors for tissue maturation are needed to better recreate the natural structures of the final tissue equivalents and to increase long-term cell viability [17, 19].

However, the current DIW bioprinting technique cannot fully accomplish these manufacturing aims. Its printing speed is very low, i.e., only a few millimeters per second. The minimum feature size that it can fabricate is usually around a few hundred micrometers, which is not adequate for fabricating complex vascular systems. Another major challenge for current DIW bioprinting is finding the optimal conditions between the printability and fidelity of bioprinted constructs and their biocompatibility [29-36]. In order to be printable, the ink has to have appropriate rheological properties, so that it can be extruded and then, retain its shape after the extrusion. To achieve the desired printability, additives are usually added into hydrogels to increase their viscosity, while the effect of the most important physical property- viscoelasticity- is currently unexplored at all [37]. The viscous bioinks have bioprintability with a high level of fidelity, but their biocompatibility and permissiveness for post-printed sprouting angiogenesis are usually compromised [38].

To address those challenges of the current DIW bioprinting technique, we propose to deploy our patented e-DIW technique [1, 39-44] to bioprinting and investigate its use in space. The preliminary results revealed that compared to the current commercial DIW systems, our e-DIW prototype machines at UIC print at a speed of several meters per second (e.g., 13.2 m/s), which is orders of magnitudes faster. In addition, the e-DIW process can print features with sizes as small as 5 μm. Due to the effect of the electric field, a wide range of inks which are difficult or even impossible to be printed in current DIW systems, can be printed successfully in the novel e-DIW system. Figure 5 depicts some test cases printed by the e-DIW system [45]. The preliminary study of e-DIW on Earth indicates that it has the potential to replace the conventional DIW technology to be used for on-site bioprinting in space and advance the on-site tissue engineering and even organ printing in space. To test this hypothesis, this project will investigate e-DIW for printing of additive-free renewable biopolymers like alginate and methylcellulose, and potentially human-cell cultures, and the influence of reduced gravity on e-DIW bioprinting.

Electric-field-assisted Direct Ink Writing

This white paper focuses on the scientific rationale for deploying an electric-field-assisted Direct Ink Writing (e-DIW) technology under different gravity conditions ranging from partial (lunar, martian) to zero- or microgravity environment to print patient-specific tissue constructs in manned space exploration missions. The PIs recently patented a novel extrusion-based additive manufacturing technology, electric-field-assisted Direct Ink Writing (e-DIW), which can extrude almost any material (from aqueous liquids to viscous pastes and slurries, from polymer solutions to cell-loaded hydrogels, etc.) onto a substrate to build a 3D construct at a super-high speed (at least, several meters per second, 30 times faster than the current-of-the-art) at a very high resolution (~ 10 μm).

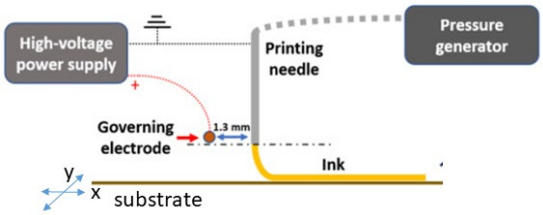

Fig.2. Schematic of e-DIW setup [45]

As illustrated in Fig. 2, in e-DIW, the printing needle is grounded and an external electric field is applied by applying voltage to the governing electrode near the grounded nozzle. By manipulating the air pressure and the electric field strength, the ink extrusion and ink-substrate wetting can be dynamically and locally controlled, thus allowing for printing at a much higher speed and use of inks and substrates that are impossible in other existing DIW systems, in particular on substrates with complicated surface landscape and high roughness. As shown in Fig. 3, compared to the state-of-the-art DIW process, our patented e-DIW process successfully addressed the ink instability and the speed limitation issues, and thus achieved a higher print reliability and orders of magnitude faster print speed [45]. Compared to the millimeters per second printing speed typically found in commercial DIW systems, the e-DIW prototype machines developed by our group at UIC print at a speed of several meters per second and a resolution of a few micrometers.

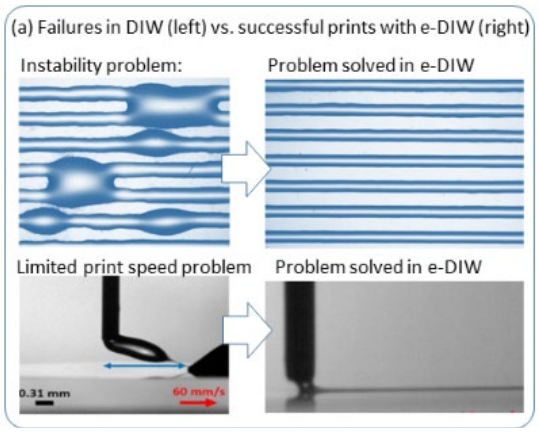

Fig.3. Comparisons of conventional DIW process and the novel e-DIW process. [1]

To accelerate the adoption of this new 3D printing process for bioprinting in space and achieve faster and more accurate manufacturing of patient-specific tissue constructs and even organs which would be required in space exploration in the future, this project will investigate the following aspects: 1) study the liquid ink-gas interfaces, liquid ink-cell interaction, and cell activity in the ink in ground, lower-gravity and microgravity environments; 2) experiments will be conducted on Earth under normal gravity, using drop towers, sound rockets, unmanned aerial vehicle (UAV), parabolic flights, and potentially on Space stations, on the Moon and Mars surfaces; 3) study the shape of gas-liquid ink- solidified ink interface affected by liquid ink flow, cell flow, and reduction in gravity or microgravity, 4) study the effect of electric field and gravity vector magnitude on printing performance including accuracy, resolution, and speed. In particular, the effect of the Coulomb force on ink-substrate wetting, ink solidification, manufacturing resolution, and printing of overhanging features in microgravity environment will be characterized and modeled; and 5) especially, bio-hydrogels will be used as inks for e-DIW. As shown in Fig.3 and

Fig.4, on Earth under normal gravity, the electric field effect could change the gas-liquid ink-substrate interface effectively to enhance the wetting of liquid ink on substrate by pulling the ink downward to the substrate and counteract the surface tension-driven instabilities. We assume that under microgravity conditions, the liquid ink-substrate wetting problem in an extrusion-based 3D printing process (i.e., DIW) will be more severe due to the reduced gravity, as reported in literature [46-48], and our e-DIW technology could effectively solve this problem by inducing the pulling downward effect using an electric field.

If successful, the process will provide a facile, low-cost, high throughput and high resolution method to fabricate customized 3D tissue constructs for on-site medical treatments in manned space exploration missions.

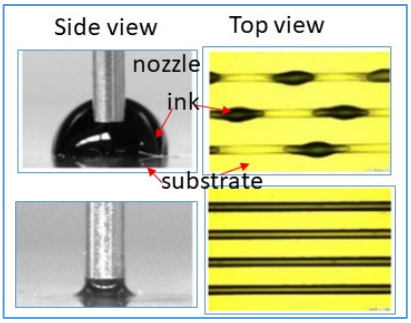

Fig.4. Comparisons of gas- liquid ink-substrate interfaces (side view) and corresponding printed results (top view): using an extrusion 3D printing process without an electric field (i.e., DIW) and with an electric field (i.e., e-DIW).

Future Tasks & Conclusion

Accordingly, the following research tasks are required for fundamental understanding and scalability to facilitate research as identified in NASA's "Grand Challenges in Soft Matter Science: Prospects for Microgravity Research" [49]:

Task 1. Characterization of e-DIW bio-printing First, biopolymers including alginate and methylcellulose-loaded with cells will be tested in e-DIW process. The printed geometry fidelity and resolution will be characterized and modeled as functions of the electric field strength and bio-ink composition. The printed trace morphology will also be investigated and its correlation with the e-DIW process parameter settings will be analyzed. Furthermore, the cell viability and proliferation rate in the printed structure will be measured. Comparisons between the conventional extrusion-based bioprinting and our novel e-DIW bioprinting will be made, in terms of the printable bioink viscosity range, viscoelastic relaxation time, scaffold porosity, and biocompatibility. A systematic and deep understanding of the influence of electric field on bio-ink printability, tissue construct integrity, and cell viability will be established.

Task 2. Multiphysics-based modeling and experimental investigation of microgravity e-DIW printing The printing process include ink extrusion from the nozzle, ink trace deposition, and ink trace solidification. The micro-gravity deposition of bio-ink will be mimicked on Earth by manipulating it into perpendicularly depositing on a vertical substrate, using the method described in [50], as well as tested using drop tower experiments, and tests in sound rockets, parabolic flights, unmanned aerial vehicles (UAV), and parabolic flights, to prepare this technology to deployment on Space stations and on the celestial body surfaces. The prevailing physical mechanisms of gravity and electric field on bio-ink deposition and solidification can be modeled using the approach developed in our previous work

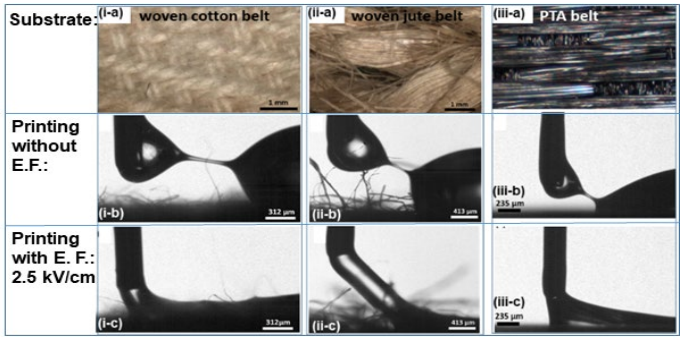

Fig. 5 A comparison of extrusion-based 3D printing of bioinks without electric field (i.e., conventional DIW) and with electric field (i.e., the e-DIW process) on 3 different substrates.

[45]. As shown in Fig.5, the ink jets deposited on different substrates exhibit significantly different configurations in a conventional DIW system, which has no electric field (E.F.), and in the e-DIW system which has an external E.F. applied. Under the normal gravity, when the substrate is too rough, the liquid ink tends to break into large drops and cannot wet the substrate in the conventional DIW process if the substrate is too rough. In the e-DIW process, the applied electric field effectively changes the interactions of liquid ink-nozzle-substrate and pulls the ink towards the substrate surface, working similarly as the gravity force, which holds great promise to applications of e-DIW in space exploration. The configurations of ink jet without E.F. and with E.F. have been analytically modeled and experimentally validated for on Earth in our previous work [45]. In this task, the effect of Coulomb force on bio-ink extrusion, the jet configuration, and ink-substrate wetting interface, under microgravity conditions will be modeled and verified by experiments under different gravity conditions, including microgravity. Furthermore, numerical simulations should be conducted to simulate the bio-ink extrusion and deposition in microgravity and reduced zero-gravity conditions. The liquid ink-gas interfaces, liquid ink-cell interaction in microgravity environment will be explored and elucidated through the multi-physics modeling and numerical simulations. Numerical simulations will also need to be used to study the shape of gas-liquid ink-solidified ink interface affected by liquid ink flow, cell flow, and reduced gravity.

Task 3. Study of bio-ink solidification and bi-oink-cell interaction in space via drop tower experiments, tests in sound rockets, parabolic flights, and UAV-assisted microgravity simulation.
The study of bio-ink solidification requires utilization of different gravity fields for different durations. Different platforms (e.g.- drop tower, sound rocket, UAV) will be required. As an example, a hex-rotor unmanned aerial vehicle (UAV) system can be utilized as a microgravity-enabling platform. A constant acceleration of the UAV can be made equal to the freefall acceleration and thus, any payload on-board will experience microgravity. In particular, a feedback linearization-based acceleration control law and a parameter estimation scheme, as developed in [51] will be employed to ensure the convergence of acceleration to the desired value under a certain condition and maintain it to achieve the desired reduced- or microgravity. With the UAV-based microgravity enabling platform, the liquid biopolymer ink trace or layer samples, cell-loaded bio-ink trace samples, and 3D bio-ink tissue construct samples printed on a small-size substrate will be placed on the platform to experience reduced or microgravity. Accordingly, the liquid ink- gas interfaces, liquid ink-cell interaction, and cell activity in the ink in microgravity environment will be recorded right after the UAV microgravity simulation flights. In addition, the time needed for ink solidification and the solidified ink trace geometry in microgravity conditions will be characterized, to understand the effect of microgravity. Similar experiments will also be conducted using all other available experimental techniques: drop tower experiments, tests in sound rockets, parabolic flights to prepare e-DIW of human tissues and organs to deployment in space exploration.

With the knowledge and manufacturing technology developed in this project, it is expected that e-DIW platform will be a key component of NASA's focus of deep space exploration. The fundamental questions answered in this important area of soft matter research will expand the present commercially available DIW-based bio-printing technique by >100 times faster printing and >5 times higher resolution under Earth and the reduced-gravity and microgravity conditions. It is also expected that the e-DIW holds great potential of an order-of-magnitude higher cell viability resulting from the combined effect of the improved printing conditions and use of biomaterials that are more biocompatible but not printable by conventional DIW systems.

References

1. Hellweg, C. E., & Baumstark-Khan, C. (2007). Getting ready for the manned mission to Mars: the astronauts' risk from space radiation. *Naturwissenschaften*, *94*(7), 517-526. DOI 10.1007/s00114-006-0204-0
2. Barcellos-Hoff, M.H., Blakely, E.A., Burma, S., Fornace Jr, A.J., Gerson, S., Hlatky, L., Kirsch, D.G., Luderer, U., Shay, J., Wang, Y. and Weil, M.M. (2015). Concepts and challenges in cancer risk prediction for the space radiation environment. *Life Sciences in Space Research*, *6*, 92-103. https://doi.org/10.1016/j.lssr.2015.07.006
3. Horneck, G., Facius, R., Reichert, M., Rettberg, P., Seboldt, W., Manzey, D., Comet, B., Maillet, A., Preiss, H., Schauer, L. and Dussap, C.G. (2006). HUMEX, a study on the survivability and adaptation of humans to long-duration exploratory missions, part II: missions to Mars. *Advances in Space Research*, *38*(4), 752-759. https://doi.org/10.1016/j.asr.2005.06.072
4. Ghidini, T. (2018). Regenerative medicine and 3D bioprinting for human space exploration and planet colonisation. *Journal of Thoracic Disease*, *10*(Suppl 20), S2363. doi: 10.21037/jtd.2018.03.19
5. Kang, H. W., Lee, S. J., Ko, I. K., Kengla, C., Yoo, J. J., & Atala, A. (2016). A 3D bioprinting system to produce human-scale tissue constructs with structural integrity. *Nature Biotechnology*, *34*(3), 312-319. https://doi.org/10.1038/nbt.3413
6. Zhou, X., Nowicki, M., Sun, H., Hann, S.Y., Cui, H., Esworthy, T., Lee, J.D., Plesniak, M. and Zhang, L.G. (2020). 3D bioprinting-tunable small-diameter blood vessels with biomimetic biphasic cell layers. *ACS Applied Materials & Interfaces*, *12*(41), 45904-45915. https://doi.org/10.1021/acsami.0c14871
7. Dordlofva, C., Lindwall, A., & Törlind, P. (2016). Opportunities and challenges for additive manufacturing in space applications. *DS 85-1: Proceedings of NordDesign 2016, Volume 1, Trondheim, Norway, 10th-12th August 2016*, 401-410.
8. Heinrich, M.A., Liu, W., Jimenez, A., Yang, J., Akpek, A., Liu, X., Pi, Q., Mu, X., Hu, N., Schiffelers, R.M. and Prakash, J. (2019). 3D bioprinting: from benches to translational applications. *Small*, *15*(23), 1805510. DOI: 10.1002/smll.201805510
9. Sacco, E., & Moon, S. K. (2019). Additive manufacturing for space: status and promises. *The International Journal of Advanced Manufacturing Technology*, *105*(10), 4123-4146. https://doi.org/10.1007/s00170-019-03786-z
10. Najmon, J. C., Raeisi, S., & Tovar, A. (2019). Review of additive manufacturing technologies and applications in the aerospace industry. *Additive Manufacturing for the Aerospace Industry*, 7-31. https://doi.org/10.1016/B978-0-12-814062-8.00002-9
11. Cubo-Mateo, N., Podhajsky, S., Knickmann, D., Slenzka, K., Ghidini, T., & Gelinsky, M. (2020). Can 3D bioprinting be a key for exploratory missions and human settlements on the Moon and Mars?. *Biofabrication*, *12*(4), 043001. https://doi.org/10.1088/1758-5090/abb53a
12. Braddock, M. (2019). Tissue engineering and human regenerative therapies in space: benefits for Earth and opportunities for long term extra-terrestrial exploration. *Innovations in Tissue Engineering and Regenerative Medicine*, *1*(3), 1-5. ITERM.000512.2019
13. Sobel, A. (2020). Update on bioprinting and biofabrication in support of aerospace missions and the human condition. *Aerospace Medicine and Human Performance*, *91*(5), 457-458. https://doi.org/10.3357/AMHP.5570.2020

14. Grimm, D., Egli, M., Krüger, M., Riwaldt, S., Corydon, T.J., Kopp, S., Wehland, M., Wise, P., Infanger, M., Mann, V. and Sundaresan, A. (2018). Tissue engineering under microgravity conditions–use of stem cells and specialized cells. *Stem Cells and Development*, *27*(12), 787-804. https://doi.org/10.1089/scd.2017.0242
15. Dordlofva, C., Lindwall, A., & Törlind, P. (2016). Opportunities and challenges for additive manufacturing in space applications. *DS 85-1: Proceedings of NordDesign 2016, Volume 1, Trondheim, Norway, 10th-12th August 2016*, 401-410.
16. Zhou, G., Jiang, H., Yin, Z., Liu, Y., Zhang, Q., Zhang, C., Pan, B., Zhou, J., Zhou, X., Sun, H. and Li, D.(2018). In vitro regeneration of patient-specific ear-shaped cartilage and its first clinical application for auricular reconstruction. *EBioMedicine*, *28*, 287-302. https://doi.org/10.1016/j.ebiom.2018.01.011
17. Ballard, D. H., Mills, P., Duszak Jr, R., Weisman, J. A., Rybicki, F. J., & Woodard, P. K. (2020). Medical 3D printing cost-savings in orthopedic and maxillofacial surgery: cost analysis of operating room time saved with 3D printed anatomic models and surgical guides. *Academic Radiology*, *27*(8), 1103-1113. https://doi.org/10.1016/j.acra.2019.08.011
18. Tappa, K., Jammalamadaka, U., Ballard, D. H., Bruno, T., Israel, M. R., Vemula, H., Meacham, J.M., Mills, D.K., Woodard, P.K., & Weisman, J. A. (2017). Medication eluting devices for the field of OBGYN (MEDOBGYN): 3D printed biodegradable hormone eluting constructs, a proof of concept study. *PLoS One*, *12*(8), e0182929. https://doi.org/10.1371/journal.pone.0182929
19. Ballard, D. H., Weisman, J. A., Jammalamadaka, U., Tappa, K., Alexander, J. S., & Griffen, F. D. (2017). Three-dimensional printing of bioactive hernia meshes: In vitro proof of principle. *Surgery*, *161*(6), 1479-1481. https://doi.org/10.1016/j.surg.2016.08.033
20. Tappa, K., Jammalamadaka, U., Weisman, J. A., Ballard, D. H., Wolford, D. D., Pascual-Garrido, C., ... & Mills, D. K. (2019). 3D printing custom bioactive and absorbable surgical screws, pins, and bone plates for localized drug delivery. *Journal of Functional Biomaterials*, *10*(2), 17. https://doi.org/10.3390/jfb10020017
21. Boyer, C. J., Ballard, D. H., Weisman, J. A., Hurst, S., McGee, D. J., Mills, D. K., ... & Alexander, J. S. (2018). Three-dimensional printing antimicrobial and radiopaque constructs. *3D Printing and Additive Manufacturing*, *5*(1), 29-36. https://doi.org/10.1089/3dp.2017.0099
22. Mills, D., Tappa, K., Jammalamadaka, U., Weisman, J., & Woerner, J. (2018). The use of 3D printing in the fabrication of nasal stents. *Inventions*, *3*(1), 1. https://doi.org/10.3390/inventions3010001
23. Neufeld, L, Yeini, E, Reisman, N, & Satchi-Fainaro, R. (2021). Microengineered perfusable 3D-bioprinted glioblastoma model for in vivo mimicry of tumor microenvironment, *Science Advances*, *7*(34) 2.
24. Three dimensional bioprinting in space. https://bioprinting.ru/en/ press-center/publications/nasa-organaut/. Retrieved on Sep. 23, 2021.
25. Hölzl, K., Lin, S., Tytgat, L., Van Vlierberghe, S., Gu, L., & Ovsianikov, A. (2016). Bioink properties before, during and after 3D bioprinting. *Biofabrication*, *8*(3), 032002. doi:10.1088/1758-5090/8/3/032002
26. Braddock, M. (2019). Tissue engineering and human regenerative therapies in space: benefits for Earth and opportunities for long term extra-terrestrial

exploration. *Innovations in Tissue Engineering and Regenerative Medicine*, *1*(3), 1-5. ITERM.000512. 1(3).2019
27. ESA (2019) Upside-down 3D-printed skin and bone, for humans to Mars. *European Space Agency* (www.esa.int/Enabling_Support/Space_Engineering_Technology/Upside-down_3Dprinted_skin_and_bone_for_hufmans_to_Mars)
28. Allevi 2020 Bioprinting in Space Allevi Inc. (http://allevi3d.com/allevi-bioprinting-in-space)
29. Wang, X., Wang, Q., & Xu, C. (2020). Nanocellulose-based inks for 3D bioprinting: Key Aspects in research development and challenging perspectives in applications—a mini review. *Bioengineering*, *7*(2), 40. https://doi.org/10.3390/bioengineering7020040
30. Tan, J. J. Y., Lee, C. P., & Hashimoto, M. (2020). Preheating of gelatin improves its printability with transglutaminase in direct ink writing 3D printing. *International Journal of Bioprinting*, *6*(4). doi: 10.18063/ijb.v6i4.296
31. Gao, T., Gillispie, G. J., Copus, J. S., Pr, A. K., Seol, Y. J., Atala, A., ... & Lee, S. J. (2018). Optimization of gelatin–alginate composite bioink printability using rheological parameters: a systematic approach. *Biofabrication*, *10*(3), 034106. https://doi.org/10.1088/1758-5090/aacdc7
32. Mazzocchi, A., Devarasetty, M., Huntwork, R., Soker, S., & Skardal, A. (2018). Optimization of collagen type I-hyaluronan hybrid bioink for 3D bioprinted liver microenvironments. *Biofabrication*, *11*(1), 015003. https://doi.org/10.1088/1758-5090/aae543
33. Rutz, A. L., Gargus, E. S., Hyland, K. E., Lewis, P. L., Setty, A., Burghardt, W. R., & Shah, R. N. (2019). Employing PEG crosslinkers to optimize cell viability in gel phase bioinks and tailor post printing mechanical properties. *Acta Biomaterialia*, *99*, 121-132. https://doi.org/10.1016/j.actbio.2019.09.007
34. Zhang, Z., Jin, Y., Yin, J., Xu, C., Xiong, R., Christensen, K., Ringeisen, B.R., Chrisey, D.B., & Huang, Y. (2018). Evaluation of bioink printability for bioprinting applications. *Applied Physics Reviews*, *5*(4), 041304. https://doi.org/10.1063/1.5053979
35. Chung, J. H., Naficy, S., Yue, Z., Kapsa, R., Quigley, A., Moulton, S. E., & Wallace, G. G. (2013). Bio-ink properties and printability for extrusion printing living cells. *Biomaterials Science*, *1*(7), 763-773. DOI: 10.1039/C3BM00012E
36. Ouyang, L., Yao, R., Zhao, Y., & Sun, W. (2016). Effect of bioink properties on printability and cell viability for 3D bioplotting of embryonic stem cells. *Biofabrication*, *8*(3), 035020. https://doi.org/10.1088/1758-5090/8/3/035020
37. Jammalamadaka, U., Tappa, K., Weisman, J. A., Nicholson, J. C., & Mills, D. K. (2017). Effect of barium-coated halloysite nanotube addition on the cytocompatibility, mechanical and contrast properties of poly (methyl methacrylate) cement. *Nanotechnology, Science and Applications*, *10*, 105. doi: 10.2147/NSA.S131412
38. Sun, W., Starly, B., Daly, A.C., Burdick, J.A., Groll, J., Skeldon, G., Shu, W., Sakai, Y., Shinohara, M., Nishikawa, M. and Jang, J. (2020). The bioprinting roadmap. *Biofabrication*, *12*(2), 022002. https://doi.org/10.1088/1758-5090/ab5158
39. Jiang, Y., Wang, X., Plog, J., Yarin, A. L., & Pan, Y. (2021). Electrowetting-assisted direct ink writing for low-viscosity liquids. *Journal of Manufacturing Processes*, *69*, 173-180. https://doi.org/10.1016/j.jmapro.2021.07.028

40. Plog, J., Jiang, Y., Pan, Y., & Yarin, A. L. (2021). Coalescence of sessile droplets driven by electric field in the jetting-based 3D printing framework. *Experiments in Fluids*, *62*(3), 1-9. https://doi.org/10.1007/s00348-021-03153-3
41. Plog, J., Jiang, Y., Pan, Y., & Yarin, A. L. (2020). Electrostatic charging and deflection of droplets for drop-on-demand 3D printing within confinements. *Additive Manufacturing*, *36*, 101400. https://doi.org/10.1016/j.addma.2020.101400
42. Plog, J., Lowe, J., Jiang, Y., Pan, Y., & Yarin, A. (2019, November). Drop Manipulation by Electrowetting for 3D Printing. In *APS Division of Fluid Dynamics Meeting Abstracts* (pp. B23-007).
43. Plog, J., Löwe, J. M., Jiang, Y., Pan, Y., & Yarin, A. L. (2019). Control of direct written ink droplets using electrowetting. *Langmuir*, *35*(34), 11023-11036. https://doi.org/10.1021/acs.langmuir.9b01061
44. Löwe, J. M., Plog, J., Jiang, Y., Pan, Y., & Yarin, A. L. (2019). Drop deposition affected by electrowetting in direct ink writing process. *Journal of Applied Physics*, *126*(3), 035302. https://doi.org/10.1063/1.5109385
45. Plog, J., Jiang, Y., Pan, Y., & Yarin, A. L. (2021). Electrostatically-assisted direct ink writing for additive manufacturing. *Additive Manufacturing*, *39*, 101644. https://doi.org/10.1016/j.addma.2020.101644
46. Huang, J., Qi, L., Luo, J., & Hou, X. (2021). Insights into the impact and solidification of metal droplets in ground-based investigation of droplet deposition 3D printing under microgravity. *Applied Thermal Engineering*, *183*, 116176. https://doi.org/10.1016/j.applthermaleng.2020.116176
47. Snyder, M., Dunn, J., & Gonzalez, E. (2013). The effects of microgravity on extrusion based additive manufacturing. In *AIAA SPACE 2013 Conference and Exposition* (p. 5439). https://doi.org/10.2514/6.2013-5439
48. Cowley, A., Perrin, J., Meurisse, A., Micallef, A., Fateri, M., Rinaldo, L., ... & Sperl, M. (2019). Effects of variable gravity conditions on additive manufacture by fused filament fabrication using polylactic acid thermoplastic filament. *Additive Manufacturing*, *28*, 814-820. https://doi.org/10.1016/j.addma.2019.06.018
49. Chaikin, P., Clark, N., Nagel, S. Grand Challenges in Soft Matter Science: Prospects for Microgravity Research. https://ntrs.nasa.gov/citations/20205010493.
50. Huang, J., Qi, L., Luo, J., & Hou, X. (2021). Insights into the impact and solidification of metal droplets in ground-based investigation of droplet deposition 3D printing under microgravity. *Applied Thermal Engineering*, *183*, 116176. https://doi.org/10.1016/j.applthermaleng.2020.116176
51. Kedarisetty, S., & Manathara, J. G. (2019). Acceleration control of a multi-rotor UAV towards achieving microgravity. *Aerospace Systems*, *2*(2), 175-188. https://doi.org/10.1007/s42401-019-00031-z