Bioink as a type of biologically active substances: issues of technology and legal regulation

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Abstract. 3D bioprinting as a digital innovation gives hope to patients in need of organs and awaiting transplantation, as well as other patients. The prospects for bioprinting are significant; many types of bioink are being developed, without which this digital technology may not exist. However, the legal regulation of thing developing sphere of medical science is in its initial stage. Given the complexity and duration of creating legal norms for new directions in medical science, we consider it important to start developing legislative acts that will regulate 3D bioprinting and establish requirements for bioink so that they are ready by the time the first organ (tissue) constructs suitable for clinical use appear. It is emphasized that the study of technological and legal aspects of bioinks as biologically active substances is an extremely relevant and promising scientific task.
Keywords: biologically active substances, 3D bioprinting, 3D bioprinter, digital health, ethics, law, digital medicine

1 Introduction

Bioprinting is the newest field of research related to the creation of tissue structures consisting of living cells, cellular aggregates, and bioactive molecules. Bioactive materials include micro-tissues, structures of hybrid cellular materials or biomaterials as a component in production. This innovative technology can be used to create structures that mimic the composition and hierarchical architecture of organ (tissue) constructs. The main component of 3D bioprinting is bioink, which is crucial for the formation of functional organs or tissue structures. However, despite their actual diversity, modern bioinks have not yet fully implemented attempts to create a natural tissue. Bioinks used in 3D printing technology must have many vital properties that must be taken into account when choosing one. Given the importance of bioink, we believe that the requirements for their development and application should be determined at the level of legislation.

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2 Materials and Methods

The materials for the work were the provisions of the Russian legislation, as well as normative-legal acts in force in the field of health care, and theoretical views of authors who have researched a similar topic. The reliability of the obtained results is provided by the study of legislative norms, as well as the use of modern methods of research method: logical, formal, legal, comparative-legal, system-structural and other methods of scientific cognition.

3 Results

3D bioprinting is a new type of high-tech medical care designed to revolutionize medicine and bring it to a new level. It certainly offers a number of significant advantages, including a possibility to replace diseased or damaged organs with healthy ones, to address the shortage of organs and tissues for transplantation, and to replace bones, cartilage, blood vessels and internal organs such as the heart, kidneys, liver and others in humans. The production of organs using bioprinters can reduce the time required for surgeries and thus contribute to saving the lives of those in need of organ and/or tissue transplants. When bioprinted organs are created from "own" deoxyribonucleic acid (further – DNA), the organ is not rejected by the human body. According to scientists, most organizations now consider this technology morally justified if it has a therapeutic effect [1].

One may note that today various bioprinting technologies have overcome medical and political restrictions on their implementation. It should be stated that commercialization of relations in the sphere of bioprinting technology has already emerged.

The need to study the problem of legal regulation of bioprinting in all possible aspects creates difficulties in conducting the research. The development of bioprinting technology will inevitably require changes in legislation, including norms regulating transplantation.

One of the key stages in the bioprinting process is the bioprinting (processing) [2] stage per se, which involves printing organ (tissue) constructs using a digital model (digital template), living cells (individual cells or densely packed cell aggregates called "tissue spheroids"), and biomaterials (e.g., hydrogels).

3D bioprinting is done with so-called bio-ink, which is made up of living cells and used in place of ink in conventional printing. The bioprinting process uses a solution of biomaterial or a mixture of several biomaterials in hydrogel form to create organ (tissue) constructs; it usually encapsulates the desired cell types and is called bio-ink [3].

Bioink for bioprinting can be created from natural or synthetic biomaterials, or a mixture of both called hybrid materials. In some cases, aggregates of cells without adding other biomaterials can be used for bioprinting. However, to be used in bioprinting, bioink must have certain properties such as mechanical strength, rheological characteristics, and biocompatibility with target tissues to ensure proper functioning of the printed tissues and organs [3].

To date, various natural and synthetic biomaterials with specific characteristics have been used as bioinks [4]. Currently, biotechnologists have many questions regarding the composition of bioinks [5]. Specialists are making efforts to find effective and safe technologies to produce this important component of bioprinting.

We believe that the turnover of bioinks should be standardized. That is, it should comply with medical care standards, treatment protocols or clinical recommendations, as well as with ethical and legal principles. The latter, in our opinion, must be taken into account in the development of norms aimed at regulating the relevant legal relations.

This said, the procedure of applying bioinks in bioprinting should be carried out in accordance with the standards of medical care (medical service).

Undoubtedly, great importance should be given to the quality of bioinks.

It is important to pay special attention to the quality and safety of the tissue material, as well as compliance with the requirements for cleanliness of the premises and storage conditions. During the bioprinting process, no high temperatures and no chemicals are used to protect cells from destruction. Afterwards, the human cells are embedded in the selected biomaterial, such as hydrogels consisting of water and various polymers. Therefore, we believe it necessary that quality studies on the bioinks viability are performed, as well as bioinks testing for viral, bacterial, protozoal, fungal, and prion infection. Hydrogels are commonly used as bioink material in scaffold-based bioprinting and are characterized by several important properties: biodegradability, biocompatibility and the presence of cell binding points for cell attachment, growth or differentiation [6].

Safe storage and transportation of bioinks in a biobank should be ensured, since printing of the organ (tissue) construct takes place in another medical institution. Undoubtedly, the clinical efficacy of bioinks application must be proved. For example, scientists at the Korea Institute of Science and Technology have recently developed bioink based on multisystem phosphazene hydrogel. It has no toxic photocuring agent and requires no photocuring. After application it is heated to body temperature and induces tissue regeneration, degrading in the body after a certain period of time. It contains no cytotoxic linking agents, so the living tissues created with them will cause minimal side effects during implantation [7]. The biological functionality of bioprinted constructs depends on the properties of the bioink used [8]. The lack of effective bioinks limits progress in tissue engineering and hinders the application of research results in clinical practice [9].

The choice of bioink depends on the specific application and cell type, as well as the bioprinter used. Although there has been a significant advance in bioprinting techniques in this field, the use of bioprinting depends on the bioinks that must both meet the requirements of bioprinting and have proper bioactivity of different cells [10].

One should agree that it is necessary to assess all possible risks at any stage of bioprinting and digital technology development: from the creation of bioinks to the transplantation of organ (tissue) constructs into the human body [11].

In addition, questions will inevitably arise concerning the bioprinters safety, related to the labor protection of workers directly involved in bioprinting and creating bioinks, as well as other persons in their operation. This is due to the fact that bioprinting uses biocompatible plastics, polymers, bioinks, biofibers, powders and various substrates, as well as high temperatures. A bioprinter should be located in a sufficiently safe (sterile) environment or in a special cabinet (box).

4 Discussion

Undoubtedly, it is necessary to determine what the developed bioinks will be and what legal regime will apply to them. Will they be subject to the legal regime of civil rights objects? In this regard, justified are the researchers' concerns related to defining special requirements for "bioinks", as well as for specialists in the field of additive technologies used in biomedicine [12].

The question will arise whether bioink is a medical device, biomedical cell product or drug [13]. In Russia there have already been cases of bringing to administrative responsibility customers who included goods in the auction documentation that, as it turned out, were not registered as medical devices (bioink for bioprinter BIO X containing lamin 111; bioink for bioprinter BIO X; set for preparation of bioink for bioprinter 6BIO X) [14]. This conclusion also confirms the fact of legal uncertainty in the legal nature of human biomaterials, of which, inter alia, bioinks are made.

In addition, a rather serious problem is the availability of a sufficient amount of cellular material for bioprinting and its origin. Therefore, one should agree with the researchers that

modern technologies do not always allow creating "bioink" from the patient's own cells: to create some tissues, stem cells or materials of animal or organic origin are used [15]. According to medical professionals, the sources of cellular material and substrate can be different. For cells, autologous cells, including induced pluripotent, allogeneic, embryonic, or xenogeneic cells can be used. The substrate may consist of allogeneic or xenogeneic materials, as well as of biological or synthetic materials [16].

Social and religious factors should be considered when using xenogeneic (animal) cells. In addition, patients with religious beliefs may disapprove of the use of cells from certain animal species, such as pigs. When they are used, testing is required because of the risk of carrying zoonotic infections and viruses [16]. Therefore, there is a question about the ethics of using such designs.

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However, according to researchers, modern genetic engineering technologies allow creating genetically modified animals that can become donors of connective tissue scaffolds (substrates) to be subsequently used by human cells to develop transplants [17].

The problem of increasing the number of cells needed to create organs remains very important. However, it should be taken into account that a significant amount of cellular material is needed to print an entire organ. On the contrary, if just the functioning of an organ (liver, thyroid gland) is disturbed, it should be assumed that less cellular material is needed.

5 Conclusions

Thus, the study of technological and legal aspects of bioinks as biologically active substances is extremely relevant for science [18-21] and practice [22-24].

At the moment, depending on the type of 3D printing, there are several printing technologies, each requiring independent clinical studies (trials) and standardization. At that, a significant role should be given to legal regulation in order to avoid risks and threats in the future. There are many issues that require regulation and are related to the cells origin, the nature of the substrate, stability, potential side effects and a patient's reaction after the bioprinting procedure, especially when using allogeneic cell lines, which may cause a reverse reaction. All these issues must be regulated by medical care standards.

Until an effective regulatory system for bioprinting is developed, we consider it possible that the subjects involved in the process, including medical professionals, are guided by ethical principles. In our opinion, compliance with them may prevent potential negative consequences as a result of their application [25].

Speaking of the nature of bioink origin, one cannot but consider the need for legislative stipulation of its definition in the legislation regulating the citizens' health protection. We propose the following definition of the concept of bioink – a mixture of donor cells and synthetic substances that mimic the extracellular matrix used for the production of tissue (organ) constructs.

References

1. K.M. Belikova. Bioprinting and growing of natural tissues and organs in BRICS countries (by the example of Brazil, India, China and South Africa): approaches of intellectual property legislation. Pravo i politika, **5**, 35-57 (2020).

2. M.T. Sultan, O.J. Lee, J.S. Lee, C.H. Park. Three-Dimensional Digital Light-Processing Bioprinting Using Silk Fibroin-Based Bio-Ink: Recent Advancements in Biomedical Applications. Biomedicines, **10**, 3224 (2022). doi: 10.3390/biomedicines10123224
3. P.S. Gungor-Ozkerim, I. Inci, Y.S. Zhang, A. Khademhosseini, M.R. Dokmeci. Bioinks for 3D bioprinting: an overview. Biomater Sci, **6(5),** 915-946 (2018). doi: 10.1039/c7bm00765e.
4. D-Y. Lee, H. Lee, Y. Kim, SY. Yoo, W-J. Chung, G. Kim. Phage as versatile nanoink for printing 3-D cell-laden scaffolds. Acta biomaterialia, **29**, 112-124 (2016).
5. M.I. Galyukova. Prerequisites for criminal liability for bioprinting. Lex Russica, **76(1),** 85-97 (2023).
6. R.K. Bregg. *Current Topics in Polymer Research* (Nova Publishers, 2005) 128-154.
7. URL: https://www.gazeta.ru/science/news/2023/04/14/20215063.shtml?updated
8. S.V. Murphy, P. De Coppi, A Atala. Opportunities and challenges of translational 3D bioprinting. Nat Biomed Eng, **4(4),** 370-380 (2020) doi: 10.1038/s41551-019-0471-7.
9. A. De Mori, M. Peña Fernández, G. Blunn, G. Tozzi, M. Roldo. 3D Printing and Electrospinning of Composite Hydrogels for Cartilage and Bone Tissue Engineering. Polymers, **10(3),** 285 (2018). doi: 10.3390/polym10030285
10. P.S. Gungor-Ozkerim, I. Inci, Y.S. Zhang, A. Khademhosseini, M.R. Dokmeci. Bioinks for 3D bioprinting: an overview. Biomater Sci, **6(5),** 915-946 (2018). doi: 10.1039/c7bm00765e.
11. A. Kirillova, S. Bushev, A. Abubakirov, G. Sukikh. Bioethical and Legal Issues in 3D Bioprinting. Int J Bioprint, **6(3),** 272 (2020). doi: 10.18063/ijb.v6i3.272.
12. O.V. Romanovskaya, G.B. Romanovskiy. Legal regulation of additive technologies in modern biomedicine. RUDN Journal of Law, **27(1),** 21-40 (2023).
13. I.R. Begishev, A.A. Shutova, D.V. Kirpichnikov [et al.]. Bioprinting Medical Devices: Criminal Evaluation Issues. AIP Conference Proceedings, **2701**, 020032 (2023). doi: 10.1063/5.0121700.
14. Decision of the Administration of the Federal Antimonopoly Service in Rostov oblast of June 23, 2021 No. 061/06/64-1078/2021 (key topics: medical devices – procurement participants – state and municipal needs – goods originating from foreign countries – contract system in procurement). The document is published at http://www.zakupki.gov.ru/, cited as of 28.06.2020.
15. I.Z. Ayusheyeva. Exercise of personal non-property rights in the creation of bioprinted human organs. Lex Russica, **7(164),** 24-33 (2020).
16. A. Melerzanov, A. Mishra, N. Manturova. Three-dimensional organ bioprinting: ethical and legislative aspects. Vrach, **5**, 11-13 (2016).
17. N.A. Zinovieva, A.V. Melerzanov, E.V. Petersen [et al.]. Use of transgenic GAL-KO pigs in xenotransplantation: problems and prospects. Selskokhozyaystvennaya biologiya, **2**, 42-49 (2014).
18. E.A. Kirillova, O.E. Blinkov, N.I. Ogneva, A.S. Vrazhnov, N.V. Sergeeva. Artificial intelligence as a new category of civil law. Journal of Advanced Research in Law and Economics, **11(1),** 91-98(2020).
19. P.S. Gulyaeva. Medical nanorobots in the focus of law. Journal of Digital Technologies and Law, **1(1),** 89-122 (2023). doi:10.21202/jdtl.2023.4
20. E.A. Kirillova, V.V. Bogdan, A.N. Surkov, A.B. Baumshteyn, V.E. Novichkov. Digital rights and digital objects: Features of control and regulation. Journal of Advanced Research in Dynamical and Control Systems, **11**(10 Special Issue), 567-573(2019).

21. Y.S. Berdova, G.E. Emaletdinova. International legal format for trans regionalisation of trade and economic partnership within BRICS in global development. Space and Culture, India, **7(3),** 76-85 (2019). doi: 10.20896/saci.v7i3.508
22. T.V. Luzina, E.A. Dudareva, E.M. Akhmetshin. The international and legal framework for transregionalization of trade and economic cooperation of the BRICS countries. European Research Studies Journal, **21**(Special Issue 3), 166-176 (2018). doi: 10.35808/ersj/1370
23. A.L. Zolkin, V.D. Munister, O.Y. Bogaevskaya, A.V. Yumashev, A.N. Kornetov. Promising directions of information technologies applications for improving of control and measuring devices in medical industry. Journal of Physics: Conference Series. **2094(3),** 032001 (2021).
24. Yu Bogaevskaya, O., Yumashev, A.V., Zolkin, A.L., Smirnova, O.A., Chistyakov, M.S. Application of progressive information technologies in medicine: Computer diagnostics and 3D technologies. Journal of Physics: Conference Series, **1889(5),** 052001 (2021).
25. A.A. Shutova, I.R. Begishev. Pilot project of an Ethical code of subjects implementing activity of creating, applying and utilizing medical products based on bioprinting technologies, interacting with patients and cell donors, turnover of donor cells, bioink and bioprint tissue (organ) constructs: Preprint **3**, 1-23, (2023). doi: 10.21202/978-5-8399-0803-1_2023_3_23