DOI: 10.15825/1995-1191-2023-2-63-81

# PROSPECTS FOR FABRICATION OF ARTIFICIAL HUMAN TISSUES AND ORGANS BASED ON 3D BIOPRINTING

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Three-dimensional (3D) printing is a method of creating a material object layer-by-layer in space from a virtual, mathematical model. 3D printing is based on additive technologies – a step-by-step formation of a structure by adding material to the base. 3D bioprinting is the fabrication of functional biological structures that mimic human organs and tissues. Analysis of scientific publications showed that in the near future, viable and fully functional artificial copies of individual human organs and tissues can be obtained.

*Keywords: 3D bioprinting, additive technologies, biofabrication, tissue-engineered constructs, artificial organs, transplantology.*

## INTRODUCTION

Organ and tissue transplantation is a widely used method of treating severe organ pathology, extensive, irreparable damage to internal organs and tissues [1, 2]. Unfortunately, this method has major drawbacks – graft rejection, graft dysfunction, internal bleeding, postoperative infection, risk of malignant tumors, and complications associated with the use of nonspecific immunosuppressants [3, 4].

Another unresolved problem in transplantology is the global shortage of donor material. A working group of the Russian Transplant Society and the Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation annually collect, process and analyze data on organ donation and transplantation in Russia. According to estimates by Russian experts, only one tenth of those in need of organ transplantation in Russia have their annual need met (the need for organ transplantation in Russia is at least 11,000 kidney transplants per year; 2,000 for liver; 1,100 for heart, including heart-lung; 800 for lungs; 300 for pancreas) [5–8].

The development of additive technologies, research in regenerative medicine, tissue engineering, immunology (search for solutions to the biocompatibility problem), cryobiology (technologies for long-term storage of organs and tissues), materials science (biomaterials, synthetic materials, composite/hybrid materials), are essential for the development of modern methods of compensating the functions of damaged or lost organs and tissues [9–19].

The promise of 3D bioprinting was first demonstrated in 1988. Using ordinary office equipment (an inkjet printer) and software (standard graphic editor), it was shown that cells and cell adhesion proteins can be accurately positioned in space according to predetermined coordinates [20]. Currently, functional biological systems for *in vitro* studies, anatomical bioequivalents of various human tissues and organs with a complex, multicomponent structure are created using 3D bioprinting [21]. In the technological process, highly specialized (organ-specific) cells, growth factors, and various biocompatible materials are used [22], which provides adequate conditions for long-term functioning of the created tissue-engineered construct [23, 24]. In the global 3D bioprinting industry, consumer trends have been formed, the main research groups of developers and manufacturers have been identified. Based on existing basic additive technologies and the 3D bioprinting technique, methods for obtaining artificial organs and tissues, biocompatible matrices are being actively developed. The global 3D bioprinting market is valued at $1.4 billion and is projected to reach $4.4 billion by 2028 [25].

The main leading companies in the field of 3D bioprinting are presented in Table 1 [26, 27].

Chinese company Sichuan Revotek and American company Organovo are the two leading companies by the number of received patents for inventions related to 3D bioprinting [28].

The leading country in this field is the USA, where a kind of "roadmap" – a scenario for the commercializa-

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tion of regenerative medicine technologies in the field of tissue engineering and organ regeneration from 2000 to 2060 – has been created (Fig. 1) [29].

This scenario consists of the following stages [10]:

**2000–2015**, using the results of research in the field of tissue engineering and regenerative medicine to form a new global market of technologies, equipment and consumables;

**2015**, creation of new kinds of biopolymers to completely replace synthetic biodegradable matrices;

**2025**, creation of industrial biotechnological complexes for cultivation of autologous cells and development of tissue engineering technologies based on these cells;

**2050**, development of technologies for converting allogeneic cell genotype into autologous cell genotype;

**2060**, opening of a network of commercial repositories (tissue banks) for obtaining and long-term storage of personalized artificial bioequivalents of organs for a particular recipient.

## BASICS OF 3D BIOPRINTING

The main component of any 3D bioprinter is a three-axis (X-Y-Z) positioning manipulator (Fig. 2).

The software controls the trajectory of automated system movement along the X, Y, Z axes and dosed supply of cellular elements, growth factors and other biomaterials into the created 3D structure. Thus, this technology turns virtual computer models (prototypes) of various organs into real artificial organs [30].

Currently, manufacturing companies offer a wide range of bioprinters for printing with live cells, which have different design and technical solutions. However, these devices retain the same operating principle for all models – layer-by-layer application of cell populations placed in a biocompatible support base (soluble hydro-

Table 1

**Major companies leading the global 3D bioprinting market**

| America | Europe | Asia |
|---|---|---|
| Countries:<br>– USA;<br>– Canada.<br>Companies:<br>– Aspect, Aether, SE3D, Organovo, Tevido, BIOLIFE 4D, Seraph Robotics, BioRobots, ASLS, nScrypt | Countries:<br>– Germany;<br>– France;<br>– Switzerland;<br>– Sweden.<br>Companies:<br>– Ourobotics, Poietis, 3Dynamic, EnvisionTEC, regenHU, REGEMAT 3D, GeSiM, CELLINK, and 3D Bio | Countries:<br>– China;<br>– Japan;<br>– South Korea;<br>– Singapore.<br>Companies:<br>– Sichuan Revotek, Regenovo Biotech, ROKIT, Cyfuse, Pensees and Bio3D Tech |

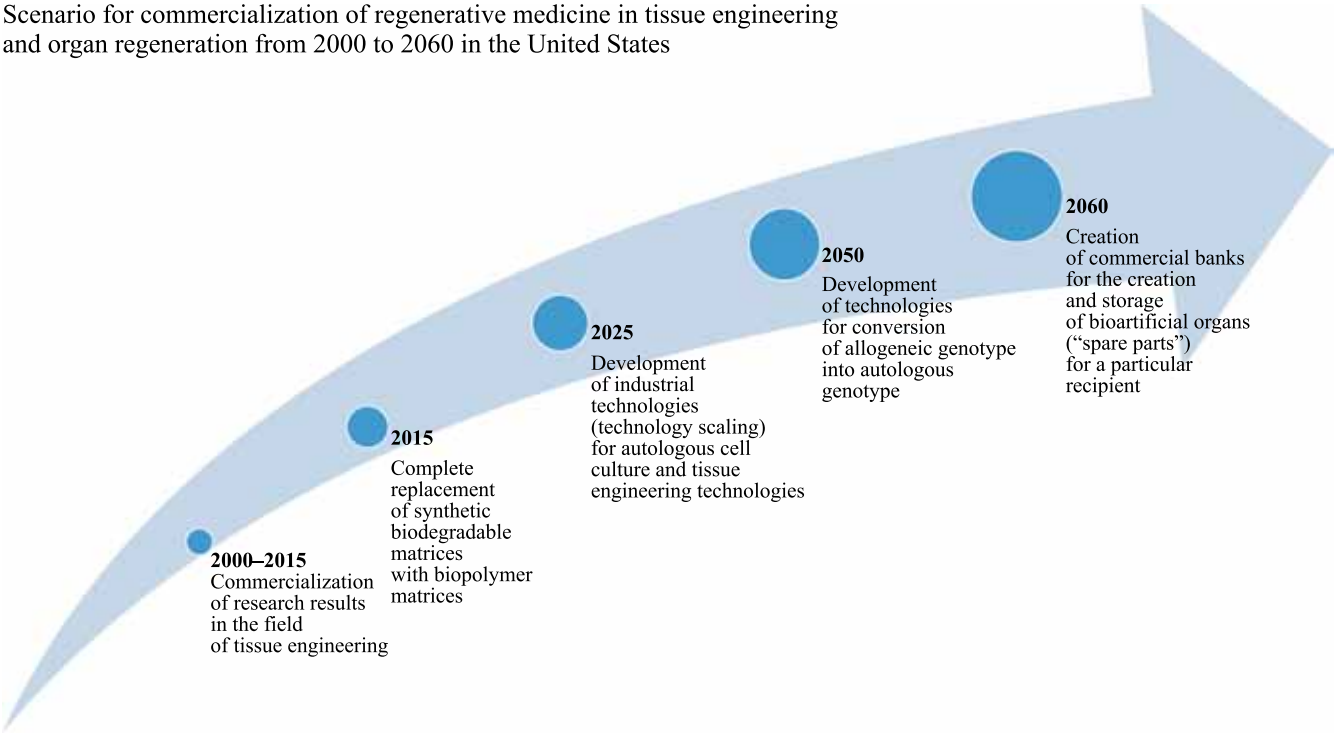


Fig. 1. Roadmap for the commercialization of regenerative medicine technologies in the United States [29]

gels) from the print head to the cell population building surface [31].

## POSSIBLE OPTIONS FOR 3D BIOPRINTING

3D bioprinting is mainly based on three central approaches [32].

1. Continuous (extrusion) method: a constant stream comes from a syringe or special dispenser. Extrusion-based bioprinters use a mechanically or pneumatically driven system that places cells in the form of a filament.
2. Intermittent (droplet) method: inflow of microdroplets. Droplet-based bioprinters use heat-, piezo-, or acoustic-driven mechanisms to deposit droplets of cell suspension at high throughput.
3. Laser bioprinters use a non-contact method of applying a biomaterial, where high-frequency pulsed energy of the laser beam transfers a hydrogel drop containing cells to the receiving surface. This bioprinting method is referred to as "laser direct writing". This technology makes it possible to create structures with a density of $10^8$ cells per 1 $cm^3$ and a resolution of 1 cell at high speed [33].

## BIOMATERIALS FOR 3D BIOPRINTING

To obtain a functioning tissue-engineered construct, it is necessary to use carriers made of biomaterials with predetermined characteristics – natural, synthetic or composite materials. When choosing the most suitable materials and their production methods, it is necessary to simultaneously take into account many biological, physical and chemical parameters which determine internal architectonics, resorption time, biocompatibility (*immunological reactivity*), controlled release of bioactive substances (*specific extracellular matrix proteins, growth factors, cytokines*) in the matrix, which are responsible for proliferation and growth of cells regulating parenchymal-stromal and intercellular interactions [34–36]. Pore size and overall matrix porosity (Fig. 3 and 4) influence the rate of diffusion, drainage and delivery of oxygen, nutrients, various regulatory factors, removal of metabolic products due to formation of microvasculature, other homeostasis processes that are necessary to prevent ischemic injury and long-term preservation of full-fledged biological properties and physiological functions of the created tissue-engineered construct [36, 37].

It has been experimentally proven that with pore diameters exceeding 500 μm, cell migration is impossible because cells do not recognize the surface. Matrices with multiple, homogeneous and communicating pores (up to 70% porosity), having diameters from 50 μm to 500 μm, are ideal for the creation of tissue-engineered constructs [38, 39].

In recent years, biopolymers have been increasingly used as materials for creating biodegradable 3D matrices (Table 2). Unlike biodegradable synthetic polymers, biopolymers or their composites containing bioactive substances meet, to the greatest extent, the main requirements for matrices in tissue-engineered constructs, such as [40]:

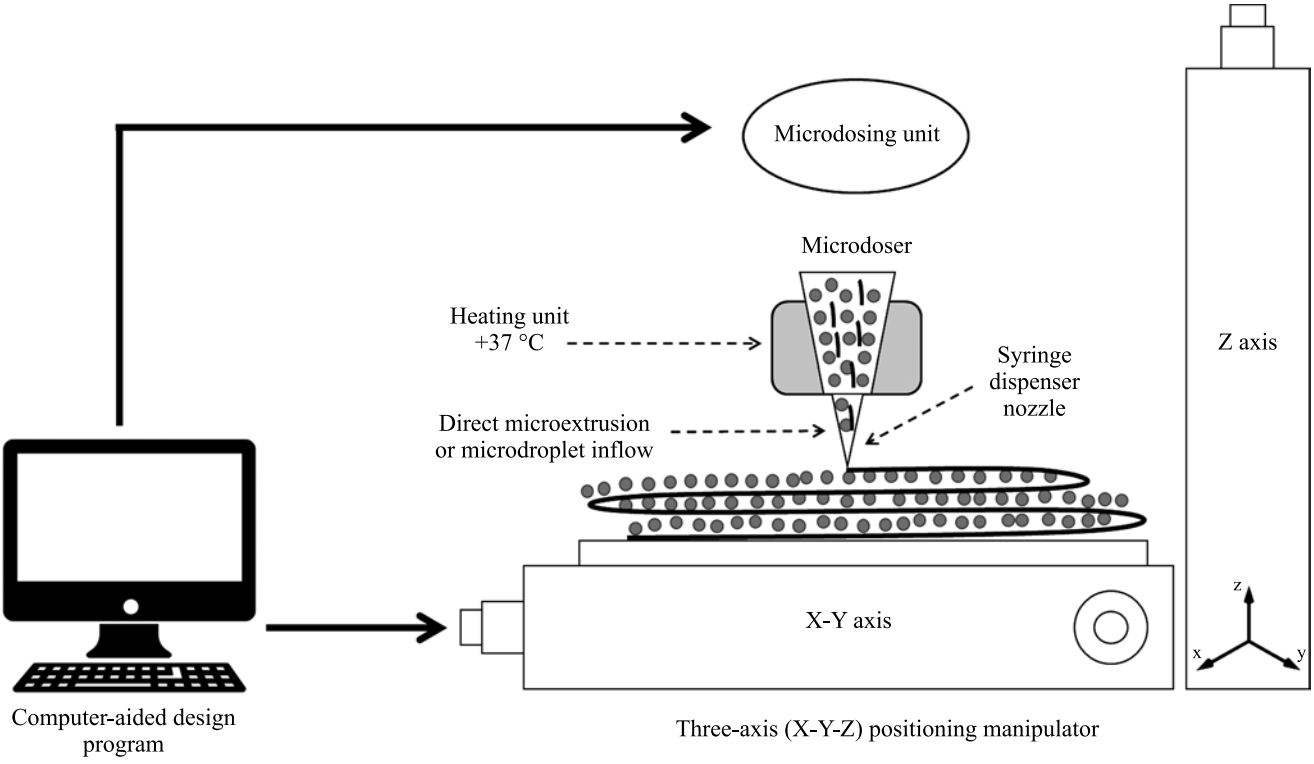


Fig. 2. Schematic representation of 3D bioprinting device

– biocompatibility of the product and its decomposition products;
– presence of biostimulating properties;
– ability to regulate biodegradation time;
– ability to neovascularize and neoinervate;
– withstand loads, provide strength and stability of tissue-engineered constructs, maintain viability of cellular elements;
– full connection to cell populations, stimulation and control of their growth;
– sterilization with preservation of biological and medical-technical characteristics of the obtained structure.

Encapsulation of cells within a semi-permeable biopolymer hydrogel is an attractive procedure that allows preserving the viability of cell populations during bioprinting [41]. Swedish researchers suggested using cellulose nanofibers in combination with cells. Chondrocytes bioprinted in nanocellulose exhibited a cell viability of 86% in the printed structure after 7 days of 3D culture [42]. Biodegradable matrices with up to 70% volumetric porosity were created based on aliphatic polyethers containing bioactive components such as hydroxyapatite, enzymes, growth factors and drugs [43]. It is important to consider the effect of various bioactive substances produced by the body in the course of responding to the implantation of a tissue-engineered construct – development of oxidative stress characterized by a high content of compounds that react by a free-radical mechanism [44]. Free radicals are capable of destroying cell membranes, damaging DNA molecules, and causing oxidative destruction of mitochondria. The method of creating tissue-engineered constructs based on microstructured biopolymer hydrogel matrices with antioxidant and antiradical activity seems promising [10, 45]. Advantages, disadvantages, as well as prospects of using some of the materials studied so far are presented in Table 3 [35, 46].

It should be noted that matrix elasticity has an influence on cell growth and differentiation. This should

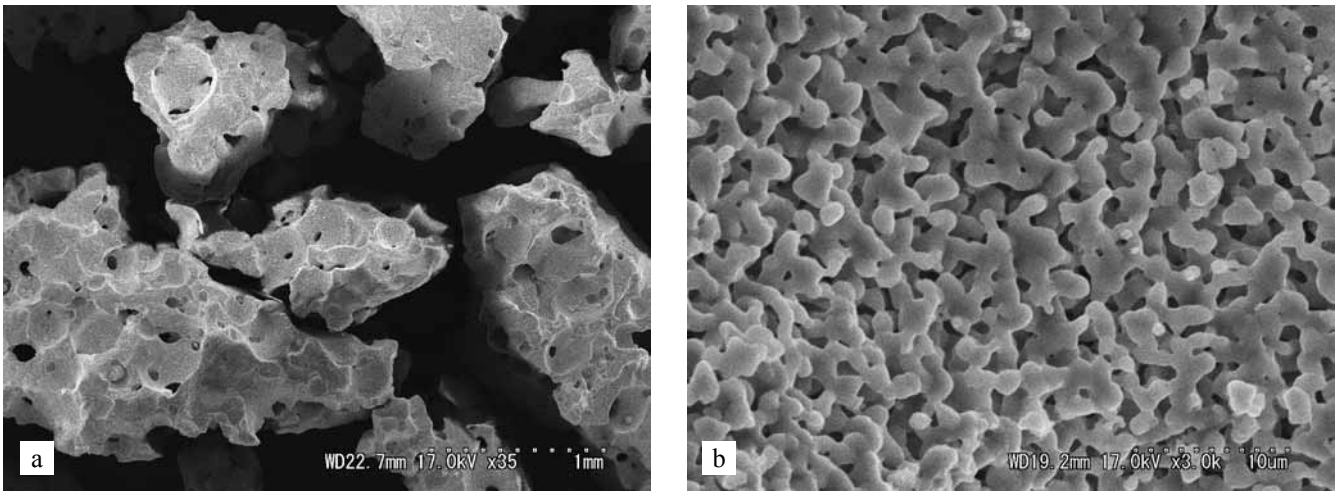


Fig. 3. Micrograph of a matrix based on beta-tricalcium phosphate (β-TCP) obtained by scanning electron microscopy. β-TCP granules contain multiple micropores ranging in size from 100 μm to 400 μm; total matrix porosity 75%. (a), macrostructure; (b), microstructure

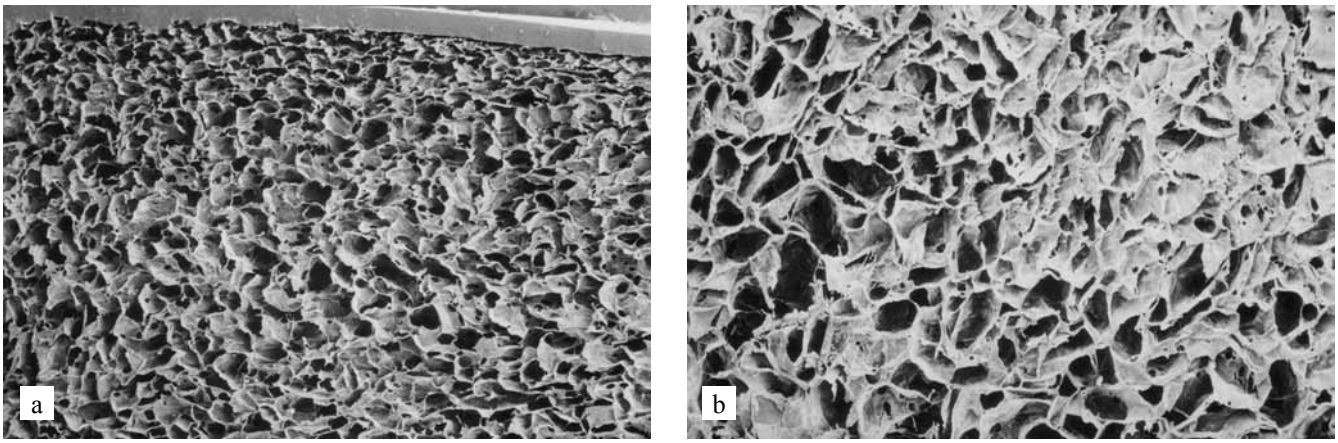


Fig. 4. Micrograph of collagen-based matrix obtained by scanning electron microscopy. (a), macrostructure; (b), microstructure, numerous micropores ranging in size from 50 μm to 500 μm

Table 2

**Biopolymer materials most commonly used in tissue engineering and regenerative medicine [10]**

| Biopolymer | Source |
|---|---|
| Alginates | Polysaccharide from brown seaweed |
| Collagen, elastin | Extracellular matrix protein |
| Gelatin | Thermally denatured collagen |
| Chitosan | Chitin derivative (source: crayfish, crabs, shrimp) |
| Silk fibroin | Cocoon protein (silkworm) |
| Spidroin | Cobweb protein |
| Hyaluronic acid | Extracellular matrix component |

be considered when choosing a carrier. However, soft polymers do not allow recreating an organ structure at micro- and nano-levels (Fig. 5).

## THE CURRENT STATE OF RESEARCH IN THE FIELD OF 3D ORGAN BIOPRINTING

Numerous scientific publications confirm the promising use of 3D bioprinting both for research purposes and in clinical practice [47]. Constructs mimicking the myocardium [48], bone and cartilage tissue [49], blood vessels with multiple branches [50], skin [51], and peripheral nerves [52] were created. A liver model was presented to study pharmacokinetic processes (absorption, excretion, distribution and metabolism) *in vitro* [53]. For 3D bioprinting of spheroids, Japanese researchers used spherical cell aggregates consisting of chondrocytes, fibroblasts, bone marrow mesenchymal stem cells to create a miniature model of the trachea [54]. Scientists from Switzerland have created a functioning model of the alveolar-capillary membrane consisting of endothelial cells, basal membrane and alveolar epithelial cells [55]. Successful experiments were performed on models of laboratory primates to implant individual structural and functional components of the bronchopulmonary complex [56]. A technology has been developed for creating single-layer models of alveolar, bronchial and intestinal epithelium cells as a basis for complex structures of the airways and gastrointestinal tract, which can be used to assess the toxicity of pharmacological drugs [57]. A method for printing blood vessels using tissue spheroids with lumen, which form a complete vascular network when fused with each other, has been proposed [58]. It has been shown that vessels made only of cells, without any dense supporting scaffolds, can rapidly mature in a bioreactor and acquire properties comparable to those of natural blood vessels [59]. Multicellular spheroids composed of human umbilical vein endothelial cells (40% of all cell populations), human aortic smooth muscle cells (10%) and normal human dermal fibroblasts (50%) were used for 3D bioprinting of the blood vessel model. After culturing in a perfusion bioreactor, the resulting model in the form of a tubular structure (inner diameter of 1.5 mm) was successfully implanted into the abdominal aorta in a rat [60]. Researchers from Carnegie Mellon University (Pittsburgh, USA) developed a method for bioprinting heart and blood vessels using collagen, alginate, and fibrin as supporting materials. Since the structures made of the materials chosen by the researchers collapsed under their own weight during 3D printing, it was decided

Table 3

**Main groups of materials for 3D bioprinting (advantages, disadvantages and prospects for use)**

| Material | Advantages | Disadvantages | Challenges | Prospects |
|---|---|---|---|---|
| Biomaterials | Natural origin, biocompatible, properties of natural tissues are preserved | Limitations in fabrication of materials with specified parameters | Risk of immune response, biodegradation, difficulties in fabricating multicomponent matrices with addition of synthetic materials | Development of bioactive matrices with predetermined characteristics, obtaining new composite materials |
| Synthetic materials | Polymeric materials with reproducible properties | Risk of developing an immune response, chemical instability, disruption of homeostasis in surrounding tissues | Fabrication of materials (biomimetics) based on the principles realized in living nature | Fabrication of composite biomaterials with predetermined characteristics, development of bioactive matrixes |
| Hybrid materials | Ideal combination of natural and synthetic polymer properties | None | Obtaining non-immunogenic matrixes with natural tissue properties and possibility of biodegradation | Development of bioactive matrixes with predetermined properties |
| Materials derived from decellularized tissues and organs | Natural origin, preservation of the tissue structural architectonics that existed before decellularization | Donor material is required | Risk of rejection reaction as a consequence of possible failures in the organ decellularization technology; obtaining a carrier while preserving all the characteristics of natural tissue | Obtaining organoids and functional models of bioengineered organs |

to use a special gelatin scaffold to create organs. Then the temperature of the finished model was raised to a cell-friendly 37 °C, causing the gelatin support bath to melt in a nondestructive manner. This method was named FRESH (Freeform Reversible Embedding of Suspended Hydrogels) [61]. Using single cell-derived spheroids from human mesenchymal stem cells, a model of the urethra was created. The resulting structure was placed in a bioreactor for subsequent differentiation of stem cells into uroepithelial cells. After 10 days of maturation in the bioreactor, the tissue-engineered construct was successfully transplanted into a rat [62]. Recent preclinical studies indicate the possibility of transplanting 3D constructs from allogeneic human pancreatic beta cells in the treatment of type 1 diabetes [63]. Preclinical studies on animal models of acute liver failure are being conducted on the possibility of using allogeneic 3D constructs consisting of a combination of primary hepatocytes and human mesenchymal stem cells in the treatment of patients suffering from acquired or genetic liver diseases [64]. A technology of neural tissue creation using human-induced pluripotent stem cells (hiPSCs) derived from neural progenitor cells (NPCs) has been developed [65].

We obtained ring models of smooth muscle tissue of the human respiratory tract and intestine that responded to chemical stimulation in the form of contraction and relaxation of smooth muscle fibers. The fibers contracted when exposed to physiological histamine levels (0.01–100 μM) and relaxed when exposed to salbutamol, a drug used to relieve asthma attacks. Addition of transforming growth factor beta (TGF-β) to the airway muscle rings caused an increase in unstimulated muscle contraction and a decrease in response to salbutamol, a phenomenon also seen in chronic lung disease. The results show that 3D bioprinted smooth muscle is a physiologically relevant model *in vitro*, which can be used to study disease pathways and the effect of novel therapeutic agents on acute contraction and chronic tissue stenosis [66]. Researchers from Cornell University, USA, have developed a method of individual 3D bioprinting of intervertebral discs, which is ideal for a particular patient [67]. Note-

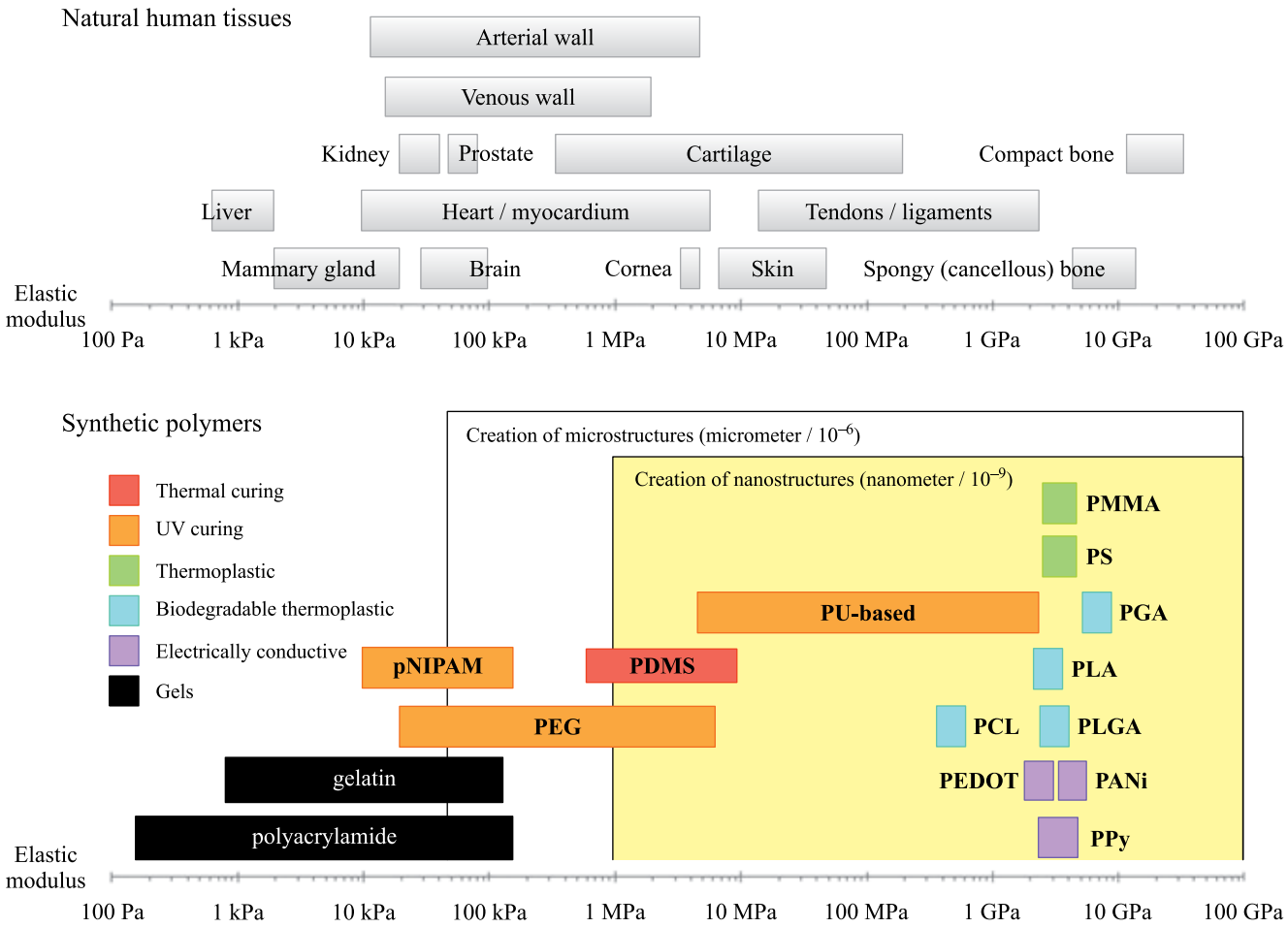


Fig. 5. Mechanical properties of natural human tissues and synthetic polymers. PDMS, polydimethylsiloxane; PU, polyurethane; PEG, polyethylene glycol; pNIPAM, poly-N-isopropylacrylamide; PMMA, polymethylmethacrylate; PS, polystyrene; PLGA, polylactic-co-glycolic acid; PGA, polyglycolic acid; PLA, polylactide; PCL, polycaprolactone; PANi, polyaniline; PPy, polypyrrole; PEDOT, poly-3,4-ehtylenedioxythiophene. Source: Annals of Biomedical Engineering, 2012; 40 (6), 1339–1355

worthy is the report on the creation of a bionic ear based on calf chondrocytes, hydrogel (alginate) and silver nanoparticles. The created construct repeats the anatomical shape of the human ear, has a built-in inductive antenna for capturing electromagnetic vibrations in the Hz and GHz ranges [68].

## EXAMPLES OF SUCCESSFUL COMMERCIALIZATION OF 3D BIOPRINTING METHODS

**Organovo** (*San Diego, CA, USA*). Organovo was the first company to develop and market NovoGen Bioprinter® Platform, a 3D bioprinting equipment. The technological parameters of the platform make it possible to create functional models of bone tissue, tissue of the liver, kidney, intestine, skin, blood vessels, skeletal muscle, eye tissue, malignant tumors of the breast and pancreas [69, 70]. Multicellular tissue-engineered constructs with predetermined functions are created for pharmaceutical companies [71–73]. A significant success achieved by the company was the creation of an *in vitro* functioning 3D model of liver tissue (ExVive™ Human Liver Tissue). Primary human hepatocytes, Kupffer cells, stellate cells (Ito cells) and endotheliocytes were used in the creation of the model [74]. The resulting model functioned stably for 40 days [75, 76]. The company's specialists presented a three-layer model of the human vessel wall. All cell populations within the created construct were functionally active [77, 78]. The company is actively developing the technology to create a bioequivalent of the kidney [79].

**TeVido Biodevices** (*Austin, Texas, USA*). The company specializes in the production of a personalized artificial nipple-areola complex used in the final stage of breast reconstruction after radical mastectomy [80]. Another area of activity is the development of vascularized skin substitutes for the treatment of vitiligo, chronic wounds and burns. Autologous stem cells isolated from the patient's adipose tissue and dermis are used in the process of creating bioconstructs [81].

**Nano 3D Biosciences** (*Houston, Texas, USA*). The company develops a technology for creating tissue spheroids in a magnetic field (magnetic 3D bioprinting) for subsequent use in bioprinting. This technology makes it possible to obtain tissue models for *in vitro* studies in the shortest possible time [82–84].

**Tissue Regeneration Systems** (*Plymouth, Michigan, USA*). The company develops and manufactures polymeric implants for replacement of bone tissue defects. The company's products are certified by the U.S. Food and Drug Administration and are widely used in dentistry, maxillofacial surgery, traumatology and orthopedics, and neurosurgery [85].

**nScrypt** (*Orlando, Florida, USA*). Software development, production of biocompatible materials and equipment for 3D bioprinting – BFF (BioFabrication Facility) complex. In the process of printing, it is possible to simultaneously use up to 4 different types of biocompatible materials, including live cell populations. The capabilities of the complex allow creating defined structures up to 10 μm in diameter (the diameter of a human red blood cell is 7 to 10 μm), with a minimum working volume of material in the dispenser of 100 picoliters. In 2019, 3D bioprinting of human myocardial tissue was performed onboard the International Space Station, in zero gravity, together with the research biotechnology (space biotechnology) company Techshot (USA). Earth's gravity does not allow printing biological objects of large size – hydrogel bases do not hold their shape, spreading out under their own weight. The experiment proved the efficiency of a specially designed additive system under weightlessness [86].

The same companies (nScrypt and Techshot), with financial support from The Geneva Foundation (a nonprofit organization that funds research in military medicine), together with the United States Military Academy West Point, the Uniformed Services University, within the framework of research program 4D Bio3 (4-Dimensional Bioprinting, Biofabrication and Biomanufacturing – an interdisciplinary program of biomedical research and practical implementation of advanced biotechnologies for the US Army needs) [87], tested a shockproof version of the BFF – nRugged bioprinter. The equipment was deployed at the base of a U.S. Army medical unit in the desert terrain of North Africa, in the immediate vicinity of the active combat zone [88, 89].

During BFF field trials, a variety of tools and medical consumables were produced for both the military medical service and large multidisciplinary military hospitals, such as:

– disposable blade holder pens;
– hemostatic supplies;
– dressing material using antibacterial hydrogel;
– A functional meniscus model based on human mesenchymal stem cells and hydrogel as a matrix;
– acsurgical model of the 9th thoracic vertebra (Th 9) [90].

The choice of the meniscus as the object of the experiment was due to the high frequency of knee joint injuries among military personnel (meniscus injuries in military personnel occur 10 times more frequently than in civilians) [91]. The digital model used to print the meniscus was sent as an electronic file from the United States – this was the first demonstration of cyberfabrication, in which information about complex structures is transmitted via satellite communication to a remote location to produce a functional model [90].

**Advanced Solutions Life Sciences,** (*Louisville, Kentucky, USA*). The company develops 3D bioprinting software. These programs are used to create 3D computer models for subsequent fabrication of complex tissue-engineered constructs [92]. The in-house bioprinting

equipment BioAssemblyBot is a certified, fully robotic multifunctional device with a 6-axis EPSON robotic arm [93] for printing functional models of different tissues and organs, and implants with complex geometric forms [94]. The design features of the equipment allow printing vascularized tissue-engineered constructs for clinical application directly in the operating room – *in situ* bioprinting under aseptic conditions [95].

**MicroFab Technologies Inc** (*Plaino, Texas, USA*). The company is a pioneer in the field of liquid bioprinting (ink-jet dispensing). Currently, together with the US Armed Forces Institute of Regenerative Medicine and one of the leading medical research centers, Wake Forest Institute for Regenerative Medicine, the company is developing the technology of accelerated regeneration of skin burn wounds. The main goal of this project is to develop a method of bioprinting the skin directly onto the damaged area [96]. Another promising area of the company's activity is the creation of special sheath-conductors (bioabsorbable nerve guidance conduits) used for the growth of peripheral nerves. This construct is placed between the damaged sections of the nerve. The distal and proximal ends of the injured nerve are connected to the guidance conduit, and the nerve grows and regenerates within the conduit. Later the guidance conduit is completely resorbed [97].

**ETEC** (*Dearborn, Michigan, USA)*. The company produces 3D Bioplotter system using technologies developed at Freiburg Materials Research Center. They produce complex tissue-engineered constructs from various biocompatible materials [98–100]. 3D Bioplotter can simultaneously print using five different materials and their mixtures (living cell populations, polymer hydrogels, ceramics, metals) of different consistency (from paste-like to liquid), it is possible to use material of any origin, different concentration and with any additives (Table 4). Each user can use their own printing parameters [101].

The technology is based on extrusion from a syringe. The advantage of using a syringe-based material delivery system is the ability to 3D print at room temperature, which allows for inclusion of live cellular material in your printed designs. 3D Bioplotter comes with four types of print heads:

– Low temperature (2 °C to 70 °C);
– High temperature (30 °C to 250 °C);
– Ultra-high temperature (30 °C to 500 °C);
– UV-emitting (when used for printing photopolymer materials).

**Cyfuse Biomedical** (*Tokyo, Japan*). Tissue-engineered constructs are created on in-house equipment Regenova Bio 3D Printer using the scaffold-free biofabrication method. In the process of creation, spheroids are used – spherical cell aggregates formed from autologous or allogeneic cell populations of various origin. The method is based on the ability of living cells to form spherical aggregates when cultured on non-adhesive surfaces. Tissue spheroid is a group of 15 to 20 thousand cells interconnected to form a spatial three-dimensional structure in the shape of a sphere. Spheroids ranging in size from 400 to 600 μm can be single-cell, consisting of one type of cells, or multi-cellular, formed from different types of cells and biomaterials. During printing, fabric spheroids are "threaded" on a metal base formed from the thinnest needles (reminiscent of the kenzan, a base for attaching flowers when making Ikebana flower). Each needle is 1 cm long and 170 μm in diameter; the needles are arranged in a strictly defined sequence (9 × 9 or 26 × 26) at intervals of 400 μm from each other) [102]. The capabilities of Micro Needle Array Technology (MNAT) make it possible to make tissue constructs from different types of cell populations. Then, the resulting construct is incubated until the spheroids join together to form large cellular associates capable of independently synthesizing extracellular matrix components and forming a given structure. This technology opens up great opportunities for tissue and organ bioengineering [103]. In the future, it is possible to print pancreatic islets, myocardium, and skin [104, 105].

**Regenovo Biotechnology** (*Hangzhou, China*). The company designs and manufactures 3D bioprinting equipment – Regenovo 3D bioprinter, BIO-ARCHITECT X. A distinctive feature of the device is the high speed of model making. Special nozzles allow you to simultaneously create different types of fabrics with a high level of resolution. The presence of a high-precision infrared laser makes it possible to check the quality of the internal structure of the fabric during production. The 3D bioprinter uses an innovative microcomputed tomography system to print a wide range of tissues and organs (including skin, muscle, cartilage, bone, tendons, liver tissue). According to forecasts by the company, it

Table 4

**Materials used when working with 3D Bioplotter**

| Bone tissue regeneration | Targeted drug transport (drug release) | Soft tissue biofabrication, organ bioprinting | Prototyping 3D models |
|---|---|---|---|
| Hydroxyapatite (HA) | Polycaprolactone (PCL) | Suspensions of living cell populations | Polyurethane (PU) |
| Tricalcium phosphate (TCP) | Poly-D,L-lactide-co-glycolide (PLGA) | Agar, chitosan, alginates, hyaluronic acid | Silicone |
| Titanium (paste) | Poly-L-lactide (PLLA) | Gelatin, fibrin, agarose, collagen | Acrylates |

will be possible to carry out mass production of artificial tissues and organs for transplantation in 15–20 years. In addition to the equipment production, the company produces 3D printing biomaterials. Currently, the company offers more than 20 types of biomaterials from organic and inorganic polymers. Cell survival in Regenovo materials is 90%; they function for up to four months [106].

**RegenHU** (*Switzerland).* A software developer and manufacturer of equipment (bioprinters) and consumables based on collagen hydrogels. In the process of 3D bioprinting of functionally active bioequivalents of human skin, bone and cartilage tissues, up to 9 different components (cells, tissue spheroids, various biomaterials) are used simultaneously [107, 108]. A personalized 3D model of the human medial meniscus based on collagen hydrogel and autologous mesenchymal stem cells isolated from the patient's bone marrow was created. The obtained prototype was the starting point for subsequent development of technologies for manufacturing individual implants designed to replace damaged menisci [109]. The technology for creating a skin bioequivalent that is morphologically and functionally comparable with the native human skin has been developed [110]. A new concept of creating personalized myocardial tissue has been proposed. Cell populations and extracellular matrices were isolated from patients' adipose tissue (omentum). The cells were reprogrammed into pluripotent stem cells, and the extracellular matrix was transformed into a personalized collagen hydrogel. After mixing the cells with the hydrogel, the cells were differentiated into cardiomyocytes to create immunocompatible and vascularized patient-specific myocardial tissue [111].

**Osteopore International,** *Singapore.* Production of personalized implants for neurosurgery, traumatology, maxillofacial surgery and dentistry made of biodegradable polycaprolactone (PLC). PLC is a biodegradable polymer that can be completely disintegrated and reabsorbed *in vivo* through hydrolysis. The porous microstructure of the material, which mimics the structure of natural human cancellous bone, ensures colonization of bone marrow by cell populations, development of a network of vessels of the microvasculature. Complete replacement (bioresorption) of a PLC-based implant by the patient's own bone tissue occurs within 18–24 months [112–114].

**OxSyBio**, *United Kingdom*. 3D bioprinting technologies are based on the use of hydrogel microdroplets (polymersomes) covered with a lipid layer. Living cells are placed in the polymersomes, which protects the cellular material from damage during the printing process. Each droplet is the same size as a cell and can be positioned to within 1 μm. With this printing method, structures of various geometric shapes can be formed. The created constructs conduct electrical impulses, like nerve cells, in a certain direction. Significant advances have been made in the development of biomaterials for the treatment of wound surfaces. There are plans to create complex organs by combining synthetic materials with live cell cultures to create organs and tissues for transplantation [115, 116].

## FUTURE PROSPECTS AND DUAL-USE TECHNOLOGIES

Analysis of domestic and foreign research publications on this topic has indicated that it is possible to come up with technologies for creating fully functioning artificial organs using 3D bioprinting by the end of the next decade [117]. However, at present, the use of bioprinted tissues and organs in preclinical studies and in clinical practice is very limited [118, 119]. A number of significant technological problems need to be solved for this purpose. The resulting 3D printed constructs are static, they are not capable of reproducing the natural dynamic nature of tissue – processes of natural regeneration and repair, which include conformational changes in the structure [120]. It is necessary to improve the characteristics of biomaterials capable of supporting cell proliferation and differentiation [121–123]. A promising direction is the creation of biocompatible matrices made from biomaterials and cellular elements that respond to stimuli, such as temperature, pH, humidity, electricity, magnetic field, light, sound waves or to a combination of these stimuli [124]. The development of models that change their morphology over time, according to the given stimuli from the environment, has already begun [125]. Creation of vascularized models is an extremely difficult task [126, 127]. For human tissues and organs of normal anatomic shape and size, it is necessary to develop technologies that allow integrating blood vessels into the created model. The existing 3D bioprinting methods do not allow for simultaneous formation of blood vessels and other elements forming the parenchyma and stroma of the organ [128]. Full-fledged vascularization ensures long-term, adequate functioning of the bioprinted construct [129]. More advanced bioprinters are needed to create the vascular component in the printed model; the resolution and speed of current equipment are insufficient [130, 131]. Below are the optimal technical characteristics of the equipment for 3D bioprinting of the future [132]:

- high degree of freedom and speed of movement in space, allowing to apply biomaterials to uneven surfaces of the damaged organ and to restore lost tissue ex tempore;
- high resolution and accuracy of printing, allowing to apply biomaterials an accuracy that corresponds to the structure of native tissue;
- possibility of simultaneous use of various types of biomaterials for making heterocellular tissues similar by structure and functions to those of native tissue;
- compactness for work in sterile conditions (laminar flow box);

– possibility to sterilize biomaterials in the process of bioprinting;
– full automation that facilitates bioprinting without user intervention;
– versatility, which allows users to modify and expand the technical capabilities of the equipment for multipurpose use;
– ease of use, allowing users with minimal skills and experience to operate the equipment.

It should be noted that any revolutionary technology always has dual-use potential [133, 134]. The possibilities of using the 3D bioprinting method in the creation of new classes of weapons, means of combat support, special and dual-use products are presented in Table 5 [135, 136].

## CONCLUSION

Further improvement of 3D bioprinting technologies will solve the problem of donor material shortage and significantly expand the possibilities of practical transplantology [137–140]. Broad prospects are opening up for the development of new medical devices and pharmacological preparations, *in vitro* studies of the effects of various bacteriological, chemical and physical factors on the human body: bacteriology, immunology (*ex vivo* creation of an artificial immune system), toxicology, radiation biology, and radiation medicine [141–143]. The use of 3D printing for preoperative planning and production of phantom organs for educational purposes will improve the professional skills of surgeons and enable them to repeatedly refine the surgical technique, thus requiring less time to perform the operation. Organ models can completely replace experiments on laboratory animals, significantly reduce the cost of drug development and reduce the time required for laboratory trials [144–146].

We hope that the information presented in this review will be informative for creating fully functional anatomical bioequivalents of human organs using additive

Table 5

**Potential for 3D bioprinting**

| Application | Description |
|---|---|
| Camouflage | The use of hybrid biomaterials with stealth characteristics to create clothing and coatings that are hardly visible in radar, infrared and other spectrums |
| Combat identification | Biomarkers for identifying one's own and allied soldiers (the biological analogue of the friend-or-foe identification system) |
| Computers, databases | DNA-based computers, biological models for computer algorithms. Associative memory, computing devices using biomaterials. Artificial intelligence – proteins as a means of working with information and energy |
| Foodstuff | Nutritional supplements to protect the digestive system from adverse environmental factors |
| Remote monitoring of soldier's health | Creation of implantable biosensors allowing real-time remote monitoring of body vital functions in combat conditions, environmental control for timely warning of enemy use of weapons of mass destruction |
| Lightweight armor | Protection of soldiers and combat systems, protective coatings with living tissue characteristics, creation of self-healing armor for body protection |
| Protection of combat electronic systems from ionizing radiation and electromagnetic radiation | Incorporation of hybrid biomolecules into components of electronic systems, biomolecular-based diodes and transistors |
| Combat robotics | Biological prototype constructs for creating self-propelled bionic platforms, creation of anthropomorphic robot |
| Reducing equipment size and weight | Molecular electronics, biochips, nanotechnology |
| Environmental monitoring systems in a battle zone | Creation of miniature diagnostic systems (mini lab on a chip) to detect and recognize chemical, biological and radioactive substances |
| Military field therapy, military field surgery | Acceleration of wound regeneration, creation of artificial tissues and organs |
| Artificial immune system (creation of 3D human immune system) | Vaccines with a shortened period of immunity, creation of protection (on the basis of gene and cell technologies) against weapons of mass destruction, new methods of treatment of wounded servicemen.<br>Biological approach to maintaining combat capability in extreme conditions:<br>The possibility of designing a fundamentally new complex protein (protein machine) that can neutralize a pathogenic organism within 24 hours;<br>Studying the mechanisms of regulation and expression of new genes and substances created by the body as it enters and exits extreme conditions;<br>DNA editing in a living organism;<br>Biomolecules that can neutralize the effects of prolonged lack of sleep |

technologies based on 3D bioprinting. The near future will confirm or refute our expectations and predictions.

*The authors declare no conflict of interest.*

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*The article was submitted to the journal on 05.03.2023*