Review paper

Use of 3D bioprinting in biomedical engineering for clinical application

Wykorzystanie biodruku 3D we współczesnej inżynierii biomedycznej i medycynie

Kamil Karzyński, Katarzyna Kosowska, Filip Ambrożkiewicz, Andrzej Berman, Justyna Cichoń, Marta Klak, Marta Serwańska-Świętek, Michał Wszoła

Foundation for Research and Science Development, Warsaw, Poland

Medical Studies/Studia Medyczne 2018; 34 (1): 93-97
DOI: https://doi.org/10.5114/ms.2018.74827

Key words: bionic pancreas, bioprinting, diabetes, regenerative medicine.

Słowa kluczowe: bioniczna trzustka, biodrukowanie, cukrzyca, medycyna regeneratywna.

Abstract

Tissue engineering is a widely developing scientific field, which combines technological solutions with the biology of the living organism. Regenerative medicine that uses tools of tissue engineering offers alternative means of therapy enhancing damaged tissues or organs. One of the development directions of contemporary chemical engineering is the scientific description of novel technologies that will enable production of porous structures – with high utility for biomedical engineering. 3D printing is one of the most popular methods used to produce scaffolds for cell culture. Nowadays a research team, in which authors are currently working, is dealing with the problem of manufacturing 3D constructs that play the role of artificial organ, obtained via 3D bioprinting. In the current article we present the possibilities and limitations of 3D bioprinting method in the context of possible application of manufactured structures as fully functional organs.

Streszczenie

Inżynieria tkankowa stanowi dynamicznie rozwijającą się dziedzinę nauki łączącą rozwiązania techniki z biologią żywego organizmu. Medycyna regeneracyjna, korzystając z narzędzi inżynierii tkankowej, oferuje alternatywne podejście do terapii wspomagających odbudowę zniszczonych tkanek czy narządów. Jednym z kierunków rozwoju współczesnej inżynierii chemicznej jest opracowanie nowoczesnych technologii umożliwiających wywarzanie struktur porowatych o wysokiej użyteczności w inżynierii biomedycznej. Wśród najbardziej popularnych metod wykorzystywanych do wytwarzania rusztowań do hodowli komórek jest technika druku 3D. Obecnie zespół badawczy, w którym pracują autorzy, opracowuje technikę wytwarzania za pomocą biodruku 3D konstruktów spełniających funkcję sztucznych narządów. W artykule przedstawiono zestawienie możliwości i ograniczeń omawianej metody biodruku 3D w kontekście możliwości zastosowania wytworzonych struktur jako funkcjonalnych sztucznych organów.

Introduction

The problem of diabetes is widespread – in Poland alone almost 2.5 million people suffer from diabetes, of whom 200,000 have type 1 diabetes (T1D) [1]. The WHO reports that the number of diabetic patients worldwide could double by 2030 [2]. When diabetes is caused by destruction or dysfunction of the pancreatic beta cells, leading to insulin deficiency, patients should be treated with a protocol of physiologic insulin replacement. Despite optimal glucose level and insulin regimen treatment, many diabetic patients develop further complications due to vascular and nerve damage. They include myocardial infarction, stroke, end-stage renal disease, retinopathy, and foot ulcers, all of which significantly increase diabetic patients' mortality. Another important problem in diabetic patients treated with insulin is severe hypoglycaemia, with mortality estimates ranging from 4 to 10 per cent of deaths. There are two alternative options for achieving near normal glycaemia in patients with T1D: pancreas or islet transplantation. Both methods are able to restore glucose-regulated endogenous insulin secretion, arrest the progression of the complications of diabetes, and improve quality of life. They require difficult surgical procedures and lifelong immunosuppression to prevent rejection. Moreover, there are insufficient available organs for transplantation. Although pancreas islet transplantation is of low invasiveness, simplistic, safe, and has a wide spectrum of potential implementation sites (portal vein, gastric submucosa) [3, 4], the biggest disadvantage is significant loss of islets immediately after the transplantation procedure – as a chronic inflammatory response and absence of extracellular matrix. The solution in the treatment of the T1D disease seems to be a bio-

printed bionic pancreas, which overcomes many of the limitations presented above. This is novel way of dealing with diabetes and is currently under development.

History of bioprinting

A promising method and a solution to organ shortage is the bioprinting of bionic pancreases made of pancreatic islets suspended in an appropriate polymer gel, which corresponds to the natural extracellular matrix – the viability of pancreatic islets grows significantly due to the major reduction of initiation apoptotic processes. The indispensable assets of bioprinting are: innovativeness of process, direct adaptation to the patient's needs by tissue individualisation, independence from a number of donors, and no transplant rejection when pancreatic islets are developed from patients' stem cells using methods of tissue engineering [5]. Nevertheless, the process of bioprinting would not take place if not for the intensified development of 3D printing in the 80s and 90s.

The first bioprinters were developed in 1984 by Charles Hull [6], who patented the stereolithographic method. Four years later the first commercial 3D printer (SLA-250) appeared on the global market called a 'Stereolithography Apparatus' [7, 8]. In the 90s Emmanuel Sachs patented and implemented the term '3D printer'. First plastic, metal, and ceramic elements were 3D printed [9]. In 1996, for the first time in history, biomaterial was used in tissue regeneration (Figure 1). In 2001 direct printing of a scaffold in the shape of a bladder and seeding of a donor's cells took place. Next, in 2002 and 2003 researchers developed a bioprinting method in which cells were characterised by their high viability. That was the moment when Thomas Boland patented inkjet bioprinting [10]. A year later the first tissue was directly printed (without scaffold). This episode led to the invention of a brand new 3D bioprinter in 2009: the Novogen MMX, and resulted in massive commercialisation. The following years saw the introduction of many new bioprinting products, such as: scaffold-free vascular constructs (2009), skin printing and injection of hepatocytes into collagen (2010), articular cartilage (2012), artificial liver (2012), tissue integration with circulatory system (2014), and even heart valves (2016) [5, 11].

Currently, the most fabricated artificial tissues using methods of 3D bioprinting are: (I) blood vessels – focused on geometry optimisation, flow quality prediction, and component diffusion; (II) heart – the most important is bioprinting of valves made of hydrogel and valve interstitial cells (VICs) with high efficiency; (III) bones – scaffolds bioprinted with a focus on precise pore geometry control, cell viability, and the cells' mechanical properties; (IV) liver – bioprinting of liver tissue for biological and medical detection of drugs, toxins, and other chemical compounds; and (V) skin – production of skin substitutes in wound healing and research of skin infection pathophysiology [12]. It is worth noting that the modern study in the field of biomedical engineering is focused only on research and development of the aforementioned methods and not commercially available medical bioproducts [13].

Currently there are three main bioprinting methods: laser-assisted, inkjet, and extrusion [14]. Each of these techniques demand particular requirements for bio-inks in order to achieve high cell viability and desired shape of the 3D bio-artificial organs. Not all types of cells are compatible with a desired bioprinting method. For example, the laser-assisted method is not a cell friendly method due to the heat generated from the laser, and it is not recommended for nondu-

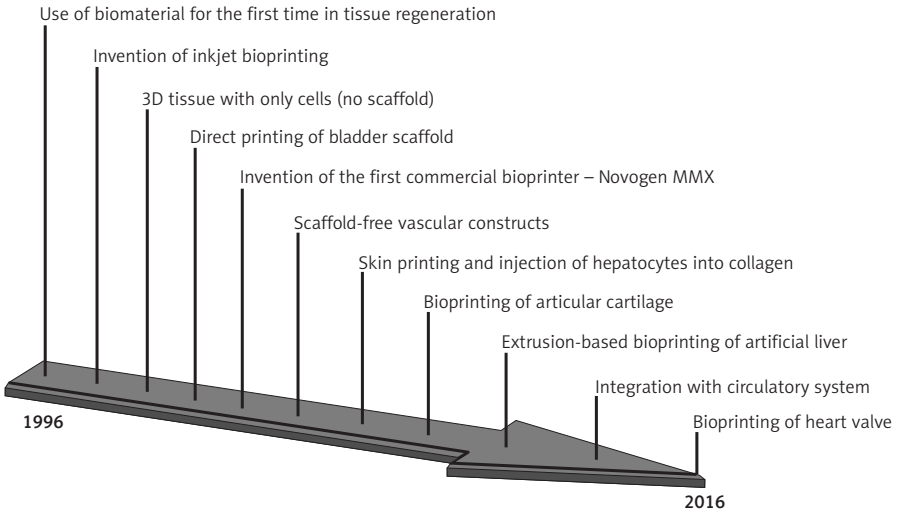

Figure 1. Schematic history of bioprinting

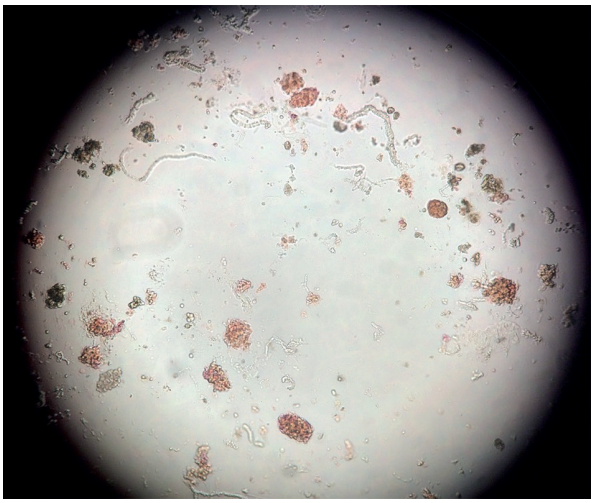

Figure 2. Pancreatic islets after isolation protocol with dithizone staining

rable cells. Inkjet- and extrusion-based methods are one of the best and most widely used in the fabrication of 3D organs because of their high cell viability and lower shear stress [11].

The role of scaffold

Use of the rapid prototyping method in the production of artificial scaffold, which is the foundation for differentiating cells, seems to be promising for contemporary regenerative medicine. As was mentioned before, there are some limitations to pancreas or islets transplantation procedures, mainly due to immunosuppressive drugs and their side effects. Moreover, 'naked' islets lack not only vascularisation but also extracellular matrix, which gives an optimal environment for their development (Figure 2). As a consequence, islet viability decreases, which leads to their functional impairment [15, 16]. An alternative for traditional mode of enhancing patients' quality of life with dysfunction of pancreatic beta cells is fabrication of an artificial organ, fulfilling proper functions, using material taken from a recipient. A bionic pancreas. 3D bioprinted pancreas slices are a subject of research and development by the Foundation for Research and Science Development Team as a part of the Bionic Consortium (Figure 3). This is a promising approach to solve the problem because they are fabricated from cells taken directly from the recipient and placed onto an artificial scaffold made of biocompatible materials. Typical biomaterials used in the production of artificial scaffolds can be divided into two groups: natural and synthetic. Natural-based polymers are: alginate, cellulose, chitosan, hyaluronic acid (HA), collagen, laminin, and fibronectin. Synthetic-based scaffolds include: polyethylene glycol (PEG), polyethylene glycol diacrylate (PEGDA), poly(lactic-co-glycolic acid) (PLGA), and poly(ε-caprolactone) (PCL) [17, 18].

Figure 3. Fragment of 3D bioprinted pancreatic slice

The application of 3D bioprinting in fabrication of artificial organs, especially bionic pancreas, is an innovative solution, but crucial points of procedure need additional research in order to verify the whole process and to minimise potential risk. A major advantage of producing pancreas slices is the possibility of generating a composite construct with a precise deposition of the required number of pancreatic islets at the same time, which would act as a fully functional organ. The impact of the shape and material of the scaffold on the viability and functionality of suspended islets is predictable because there is a hypothesis that states porous structures grant better and efficient transport of nutritional compounds and oxygen inside pancreatic slices and easier interaction between islets [19, 20].

Also, current research is conducted on three different types of polymers, but only to evaluate the viability of seeded human islets (Table 1) [21]. The impact of the shape is unknown.

Method limitations

Undoubtedly the research teams that are focused on the problematics of bioprinting artificial organs face a lot of obstacles. The current milestone in the bioprinting of whole organs is the composition of the bioink. It should be characterised by proper biochemical and physical features – this is crucial and will affect islet viability and cell differentiation. The main problem is fabrication of porous structures layer by layer,

Table 1. Comparison of three polymers after seeding of human islets and seven-day culture

Type of polymer	Morphology of islets	Outgrowth of cells?	GSIS test	FDA approved?
PDLLCL	Spherical with minor aggregation	Yes	Proper response to test	Yes
PEOT/PBT	Large aggregates	Yes	Proper response to test	Yes
Polysulfone	Large aggregates	Yes	Proper response to test	Yes

PDLLCL – poly(D,L-lactide-co-ε-caprolactone), PEOT/PBT – poly(ethylene oxide terephthalate)/polybutylene terephthalate block copolymer

and in the case of 'soft' biological material it can lead to mechanical disruption such as crushing of the lower construct layers. One of the main challenges in the field of tissue engineering is fabrication of vasculature inside the biomaterial scaffold, which would enhance nutritional compounds, oxygen, and metabolite transport – indirectly increasing cell viability. To date, no results have been published about the fabrication of a vascularised organ. This can be linked with current apparatus limitations and the relatively low resolution (circa 100 µm) of bioprinters using bio-ink.

Future perspectives

A fully operational bionic pancreas seems to be a revolutionary solution in dealing with T1D. Nevertheless, it is still a brand new and not fully developed area of bioprinting. For example, the most well known examples of bioprinted organs are connected with heart, liver, and skin but and not the pancreas. The ideal situation will take place when all efforts are be focused on faster development of bioprinted artificial pancreas including the composition of the bio-ink – which is crucial and the most tedious and expensive part of the preparation. This should be resolved by lowering the costs of ECM compounds by direct collection from donors or spare organs and purification in-house. The next perspective includes higher resolution for bioprinters. Future work can be done in lowering the diameter of the printing needle, which should be smaller than 100 µm in order to achieve optimal resolution in vascularised bioprinted organs. We hope that by 2022 a fully operational bionic pancreas will be commercially available.

Acknowledgements

Wszola *et al*., from the Foundation for Research and Science Development, is currently working on research and development of the possibilities of using 3D bioprinting methods to fabricate fully functional artificial organs – especially bionic pancreas – as a STRATEGMED project. Foundation for Research and Science Development is Leader of the Bionic consortium, which consists of teams from: Warsaw University of Technology – team from Materials Science Faculty, under the guidance of Prof. Wojciech Święszkowski; the Nencki Institute – under the guidance of Prof. Agnieszka Dobrzyń; Medical University of Warsaw – Biostructures Centre, under the guidance of Prof. Artur Kamiński; and the Clinical Hospital of the Child Jesus, under the guidance of Prof. Artur Kwiatkowski – Director Institute of Transplantology. Business and part of biotechnology and engineering solutions in consortium is from Medispace Sp. z o.o.

Funding

This study was financially supported by Narodowe Centrum Badań i Rozwoju, STRATEGMED3/305813/2/NCBR/2017.

Conflict of interest

The authors declare no conflict of interest.

References

1. Patterson CC, Dahlquist GG, Gyürüs E, Green A, Soltész G; EURODIAB Study Group. Incidence trends for childhood type 1 diabetes in Europe during 1989-2003 and predicted new cases 2005-20: a multicentre prospective registration study. Lancet 2009; 373: 2027-2033.
2. Imperatore G, Boyle JP, Thompson TJ, Case D, Dabelea D, Hamman RF, Lawrence JM, Liese AD, Liu LL, Mayer-Davis EJ, Rodriguez BL, Standiford D; SEARCH for Diabetes in Youth Study Group. SEARCH for Diabetes in Youth Study Group. Projections of type 1 and type 2 diabetes burden in the U.S. population aged G20 years through 2050. Diabetes Care 2012; 35: 2515-2520.
3. Echeverri GJ, McGrath K, Bottino R, Hara H, Dons EM, van der Windt DJ, Ekser B, Casu A, Houser S, Ezzelarab M, Wagner R, Trucco M, Lakkis FG, Cooper DK. Endoscopic Gastric Submucosal Transplantation of Islets (ENDO-STI): Technique and Initial Results in Diabetic Pigs. Am J Transplant 2009; 9: 2485-2496.
4. Hirshberg B, Rother KI, Digon BJ 3rd, Lee J, Gaglia JL, Hines K, Read EJ, Chang R, Wood BJ, Harlan DM. Benefits and Risks of Solitary Islet Transplantation for Type 1 Diabetes Using Steroid-Sparing Immunosuppression. Diabetes Care 2003; 26: 3288-3295.
5. Duan B. State-Of-The-Art Review of 3D Bioprinting for Cardiovascular Tissue Engineering. Ann Biomed Eng 2017; 45: 195-209.
6. Schubert C, van Langeveld MC, Donoso LA. Innovations in 3D printing: a 3D overview from optics to organs. Br J Ophthalmol 2014; 98: 159-161.

7. Gross BC, Erkal JL, Lockwood SY, Chen C, Spence DM. Evaluation of 3D printing and its potential impact on biotechnology and the chemical sciences. Anal Chem 2014; 86: 3240-3253.
8. Ventola CL. Medical Applications for 3D Printing: Current and Projected Uses. P T 2014; 39: 704-711.
9. Sachs EM, Haggerty JS, Cima MJ, Williams PA. Three-dimensional printing techniques. U.S. Patent 5,204,055, April 20, 1993.
10. Boland T, Wilson WC, Xu T. Ink-jet printing of viable cells. U.S. Patent 7051654, assigned to Clemenson University (Clemenson, SC), May 30, 2006.
11. Dababneh AB, Ozbolat IT. Bioprinting Technology: A Current State-of-the-Art Review. J Manuf Sci Eng 2014; 136.
12. Li J, Chen M, Fan X, Zhou H. Recent advances in bioprinting techniques: approaches, applications and future prospects. J Transl Med 2016; 14: 271.
13. Di Prima M, Coburn J, Hwang D, Kelly J, Khairuzzaman A, Ricles L. Additively manufactured medical products – the FDA perspective. 3D Printing in Medicine 2016; 2: 1.
14. Wei Z, Xuanyi M, Maling G, Deqing M, Kang Z, Shaochen C. 3D printing of functional biomaterials for tissue engineering. Current Opinion in Biotechnology 2016; 40: 103-112.
15. Marchioli G, van Gurp L, van Krieken PP, Stamatialis D, Engelse M, van Blitterswijk CA, Karperien MB, de Koning E, Alblas J, Moroni L, van Apeldoorn AA. Fabrication of three-dimensional bioplotted hydrogel scaffolds for islets of Langerhans transplantation. Biofabrication 2015; 7: 025009.
16. Wszoła M, Constantini M, Berman A, Idaszek J, Górski Ł, Ostaszewska A, Serwańska-Świątek A, Perkowska-Ptasińska A, Chmura A, Swięszkowski W, Kwiatkowski A. Using human pancreatic islets as a bioink for 3D bioprinting for bioengineering an artificial pancreas-preliminary report. IPITA report. Transplantation 2015.
17. Aljohani W, Wajid Ullah M, Zhang X, Yang G. Bioprinting and its Applications in Tissue Engineering and Regenerative Medicine. Int J Biol Macromol 2017; 107 (Pt A): 261-275.
18. Zorlutuna P, Vrana NE, Khademhosseini A. The expanding world of tissue engineering: the building blocks and new applications of tissue engineered constructs. IEEE Rev Biomed Eng 2013; 6: 47-62.
19. Zhu W, Qu X, Zhu J, Ma X, Patel S, Liu J, Wang P, Lai CS, Gou M, Xu Y , Zhang K, Chen S. Direct 3D bioprinting of prevascularized tissue constructs with complex microarchitecture. Biomaterials 2017; 124: 106-115.
20. Ozbolat IT. Bioprinting scale-up tissue and organ constructs for transplantation. Trends Biotechnol 2015; 33: 395-400.
21. Smink A, de Haan BJ, Paredes-Juarez GA, Wolters AH, Kuipers J, Giepmans BN, Schwab L, Engelse MA, van Apeldoorn AA, de Koning E, Faas MM, de Vos P. Selection of polymers for application in scaffolds applicable for human pancreatic islet transplantation. Biomed Mater 2016; 11: 035006.

Address for correspondence:

Michał Wszoła, Dsc
Foundation for Research and Science Development
8 Rydygiera St.
01-793 Warsaw, Poland
Phone: +48 501 506 071
Email: michal.wszola@fundacjabirn.pl