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Design and implementation of novel multifunctional 3D bioprinter

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Design and implementation of novel multifunctional 3D bioprinter

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Abstract

3D bioprinting is a rapidly emerging biomedical variant of 3D printing technology. 3D bioprinter could be defined as a robotic device for layer by layer biofabrication of 3D human tissues and organs from living cells and hydrogels according to digital model. Development of commercial 3D bioprinters certifiable for clinical use will enable bioprinting 3D human tissues and organs suitable for implantation. The commercial multifunctional clinical 3D bioprinter of extrusion type suitable for robotic biofabrication of 3D human tissues and organs and potentially certifiable for clinical use is presented. The principle constructive feature of 3D bioprinter is a separation of cell printing process from hydrogel spaying which allow to use photo-sensitive hydrogel with ultraviolet-induced polymerization without cell damage. Some of its multiple potential functionalities have been tested and illustrated. Development of clinical 3D bioprinter is an important step toward practical implementation for highly desirable organ printing technology.

Keywords: 3D bioprinter, biofabrication, tissue spheroids, organ printing

Introduction

3D printing could be defined as layer by layer additive manufacturing of 3D constructions according to digital model. 3D bioprinting is a rapidly **emerging** biomedical variant of 3D printing technology. 3D bioprinter could be defined as a robotic device for layer by layer biofabrication of 3D human tissues and organs from living cells and hydrogels according to digital model¹⁻³. The framework of different variants of 3D bioprinting technologies have been outlined in several reviews⁴⁻⁹ and recently published textbooks. It have been demonstrated that there are three main emerging bioprinting technologies: ink-jet bioprinting, extrusion-based bioprinting and laser-based bioprinting⁸. One of the main characteristics of 3D bioprinters is their multifunctionality or capacity to work in different modes for precised deposition of living cells and biomaterials. The production of bioprinted human organs suitable for implantation will require the development of several devices integrated into one organ biofabrication line¹⁰. 3D bioprinter is the most important element of future organ biofabrication lines. The successful translation of organ printing technologies depends on development of commercial 3D bioprinter capable to print human tissue. There is a growing number of companies producing commercial bioprinters. However, according to our knowledge there are no any commercial multifunctional 3D bioprinter officially certified by regulatory agencies for using in clinics.

Thus, the aim of this paper is to present principal constructive features of novel commercial multifunctional 3D bioprinter suitable for bioprinting of human tissues and organs and potentially certifiable for clinical use and illustrate some of its multiple functionalities.

Materials and Methods

Polyurethane

Polyurethane was kindly provided by Dr Xuejun Wen ((EG-85A, Lubrizol, USA).

Human fibroblast cell culture

Normal human dermal fibroblasts were obtained from Lonza (USA). The cells were grown in DMEM (Gibco, USA) containing 10% FBS (Gibco, USA) supplemented with antibiotic/antimycotic mix (Gibco, USA) and 1 mM L-glutamine (Paneco, USA). The cells were cultivated at 37°C in a humidified atmosphere with 5% CO₂ and routinely passaged at 85-95% confluence.

Tissue spheroids biofabrication

The tissue spheroids were formed using the 3D Petri Dishes (Microtissues, USA) according to manufacturing protocol. Briefly, the 3D Petri Dishes were prepared from 2% agarose in PBS. The concentrations of the human fibroblast cells were 6.8×10^6 cells per milliliter. 190 μ l of cell suspension was seeded into the 3D Petri Dishes. In 40 minutes additional culture medium was added to the outside of the 3D Petri Dishes. Human fibroblasts were suspended in cell culture medium and cell suspension was seeded into the 3D Petri Dishes. The 3D Petri Dishes with tissue spheroids were incubated at 37°C in a humidified atmosphere with 5% CO₂.

Electrospinning of polyurethane

Electrospinning of microfibrinous polyurethane matrix have been performed using commercial apparatus Professional Lab Device (Yflow, Spain). Polyurethane have been dissolved to concentration 17% in solvents containing 40% *N,N*-dimethylformamide (DMF) и 60% tetrahydrofuran (THF).

Preparation of collagen hydrogel

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3 For collagen hydrogel 890ul of collagen type I in 0.2M acetic acid, 60ul of 1M NaOH, 250ul of
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5 7.5% NaHCO₃, 300ul of PBS were mixed on ice. The mix is transferred to precooled syringe and
6
7 used for printing. The loaded syringe was inserted into the Fabion's cooling system (4 °C) to keep
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9 the material in low temperature before deposition. **Temperature of collagen inside the syringe**
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11 **before deposition was 4°C. Printing speed was 1 drop each 4 seconds. The dimension of printed**
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13 **colagen hydrogel area in Figure 1 m was 2 cm x 2 cm = 4 cm².** A petri dish was placed on top of
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15 a hot plate (30 °C) where the printing process was conduced. The shape of object was designed in
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17 the proprietary software. A Nordson red tip gauge 25 (0.25mm internal diameter) was used for
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19 the prints.
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23 24 ***Bioprinting of tissue spheroids***

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26 Original multifunctional 3D bioprinter (3D Bioprinting Solutions, Russia) have been used for
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28 dispensation and patterning of biofabricated tissue spheroids on the surface of electrospun matrices
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30 and bioprinted collagen hydrogel according to the digital model. The suspension of tissue
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32 spheroids have been regularly placed according to selected digital model (linear and hexagonal
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34 order) on the surface of electrospun polyurethane matrix using conus-like pipets. **The maximum**
35
36 **printing area is 50 cm². The maximum printing resolution depends on the diameter of the needle**
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38 **and could be 100 µm. The movement axes XY gauranteed a precision of 10µm.**
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43 44 ***Video-recording***

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46 Video recording of tissue spheroids dispensing and patterning have been performed using digital
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48 camera (model dmk41au02) company (Imaging Source, USA) with objective Optem Zoom Kit
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50 70XL (Qioptiq, USA) □
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52 53 ***Scanning electron microscopy***

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55 The samples of tissue spheroids on electrospun polyurethane matrix were fixed with 2.5%
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57 glutaraldehyde/0.1Mcacodylate buffer, dehydrated through ethanol series and then were dried in
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3 a critical point dryer (*HCP-2*, Hitachi Koki Co. Ltd., *Japan*). The samples are mounted on a stub
4 of metal with adhesive, coated with gold using ion coater (*IB-3*, EIKO, *Japan*) and then observed
5 under the microscope *JSM -6510 LV* (*JEOL*, *Japan*).
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10 11 12 13 **Results**

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15 The multifunctional 3D bioprinter (*Fig. 1a*) includes cartesian style X-Y-Z axis robot,
16 automated disposable syringes (*Fishman*, *USA*), sterilizable hydrogel sprayers (*Nordson*, *USA*)
17 and operational control system. Bioprinter has 3 *Fishman* automated syringes on one side and 2
18 *Nordson* style spraying nozzles on another side (*Fig. 1b-f*). Multifunctionality of 3D bioprinter
19 includes: i) capabilities to dispense and precisely place hydrogel filaments with living cells or
20 without cells, cellular rod and separated tissue spheroids according digital design using *Fishman*
21 automated syringes (*Fig. 1b-d, h-j*); ii) capabilities for spraying hydrogel with different principles
22 of polymerization (*Fig. 1 e-f, k-l*); iii) heating system designed to print collagen hydrogel (*Fig.*
23 *1g*). For example, two *Nordson* nozzles allow to spray simultaneously thrombin and fibronogen
24 solution which enables instant polymerization and formation of fibrin hydrogel similar to *Duplojet*
25 system developed by *Baxter* (*USA*) (*Fig. 1k*). Incorporation of UV light source allows rapid
26 polymerization of photo-sensitive bioprintable hydrogels (*Fig. 1e-f,l*). The 3D bioprinter also
27 enables also layer by layer biofabrication of 3D tissue and organ constructs by spraying sequential
28 layers of hydrogel intermitted with precision placing and patterning of tissue spheroids in every
29 sequential layer (*Fig. 1n-o*). Polymerization of bioprinted collagen hydrogel using heating table is
30 demonstrated on *Fig. 1m*. Patterning of tissue spheroids on the surface of electrospun matrix
31 according desirable digital model using 3D bioprinter have been demonstrated (*Fig. 1p*). Finally,
32 the employment of sterile disposable syringes in *Fishman* nozzles (*Fig. 1b,d*) and sterilizable
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3 Nordson nozzles (Fig. 1c,f) is essential for planned clinical certification of 3D bioprinter and
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5 clinical translation of 3D bioprinting technology.
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14 15 **Discussion**

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17 Multifunctional 3D bioprinter have been developed and some of its essential
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19 functionalities have been tested. 3D Bioprinter Fabion includes cartesian type X-Y-Z axis robot,
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21 5 nozzles (3 Fishman type dispensing nozzles and 2 Nordson type spraying nozzles) and
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23 integrated system of operational control. There are three most interesting characteristics of
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25 presented 3D bioprinter: (i) unparallel multifunctionality, (2) unique capability to separate
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27 bioprinting of living cells from **spraying** of hydrogel and (3) realistic potential for certification as
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29 a clinical 3D bioprinter. The multifunctionality of developed 3D bioprinter is manifested by *built*
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31 *in* capabilities to dispense hydrogel filaments, hydrogel filaments containing living cells, cellular
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33 rods and, finally, demonstrated in this report capacity to place tissue spheroids precisely on the
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35 surface of electrospun matrices or in thin layer of hydrogel according to digital model. The
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37 principal novelty of developed 3D bioprinter is a separation of printing of living cells from
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39 spraying of hydrogel, which enables the employment of practically any types of hydrogel
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41 including photo-sensitive printable hydrogel without any UV light induced damage for living
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43 cells. The possibility for sterilization is a most important characteristic for clinical 3D bioprinter
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45 in order to be certified by regulatory agencies for clinical use. It was implemented by using
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47 disposable syringes in Fishman types nozzles and sterilizable Nordson types hydrogel spraying
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49 nozzles.
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3 Taken together three most essential characteristics of bioprinter in combination with high
4 level of printing resolution and large printing area allow to consider presented multifunctional 3D
5 bioprinter Fabion as one of most advanced commercial clinical 3D bioprinter.
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17 **Conclusion**

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19 Multifunctional commercial clinical 3D bioprinter Fabion of extrusion type have been designed
20 and developed and some of its functionalities have been tested. 3D bioprinter enables extrusion
21 and precision placing of hydrogel filaments with or without living cells, continuous cellular rods
22 and separated tissue spheroids as well as independent spraying of biocompatible hydrogels with
23 different principles of induced polymerization. The *built in* multifunctionality of presented 3D
24 bioprinter will allow layer by layer additive biofabrication of complex 3D human organ
25 constructs using tissue spheroids as building blocks. The employing of only disposable and
26 sterilized components in multinozzle system made 3D bioprinter both suitable and certifiable for
27 clinical use. The development of certifiable clinical multifunctional 3D bioprinter is an important
28 step towards practical implementation of desirable organ printing technology
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55 **Author Disclosure Statement**

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57 No competing financial interests exist.
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References

1. Pepper ME, Parzel CA, Burg T, et al. Design and implementation of a two-dimensional inkjet bioprinter. *Conf Proc IEEE Eng Med Biol Soc* 2009; 2009: 6001-6005.
2. Cohen DL, Malone E, Lipson H, et al. Direct freeform fabrication of seeded hydrogels in arbitrary geometries. *Tissue Eng* 2006; 12: 1325-1335.
3. Arai K, Iwanaga S, Toda H, et al. Three-dimensional inkjet biofabrication based on designed images. *Biofabrication* 2011; 3: 034113.
4. Mironov V, Boland T, Trusk T, et al. Organ printing: computer - aided jet-based 3D tissue engineering. *Trends Biotechnol* 2003; 2 :157-161.
5. Mironov V, Kasyanov V, Drake C, et al. Organ printing: promises and challenges. *Regen Med* 2008; 3: 93-103.
6. Mironov V, Visconti RP, Kasyanov V, et al. Organ printing: tissue spheroids as building blocks. *Biomaterials* 2009; 30: 2164-2174.
7. Derby B. Printing and prototyping of tissues and scaffolds. *Science* 2012; 338:b921-b926.
8. Murphy SV, Atala A. 3D bioprinting of tissues and organs. *Nat Biotechnol* 2014; 32: 773-785.
9. Ozbolat IT. Bioprinting scale-up tissue and organ constructs for transplantation. *Trends Biotechnol* 2015; 33: 395-400.
10. Mironov V, Kasyanov V, Markwald RR. Organ printing: from bioprinter to organ biofabrication line. *Curr Opin Biotechnol* 2011; 22: 667-673.

Figures and Legends

Fig. 1. Constructive characteristics and multifunctionality of clinical 3D bioprinter Fabion developed by 3D Bioprinting Solutions, Russia:

- a) Photo of multifunctional 3D bioprinter Fabion;
- b) Graphic design of 3D bioprinter with three Fishman type nozzles (automated syringes) on one side;
- c) Graphic design of 3D Bioprinter with Two Nordson types hydrogel spraying nozzles on another side;
- d) Photo of 3D bioprinter with three Fishman type nozzles (automated syringes) on one side;
- e) Scheme demonstrating UV light-induced polymerization of photo-sensitive polymer during their spraying from Nordson type nozzle;
- f) Photo of 3D Bioprinter with two Nordson types hydrogel spraying nozzles on another side;
- g) Photo of heating table incorporated in 3D bioprinter Fabion;
- h) Dispensing of hydrogel filament containing living cells;
- i) Dispensing of cellular rod;
- j) Dispensing of isolated tissue spheroids;
- k) Spraying of thrombin and fibrinogen with instant formation of fibrin hydrogel;
- l) Spraying of photo-sensitive hydrogel with UV light induced instant hydrogel polymerization;
- m) Polymerized bioprinted collagen hydrogel. **The size of bioprinted collagen hydrogel was 2 cm x 2 cm = 4 cm²;**
- n) Initial step of tissue spheroids dispensing using 3D bioprinter. **Scale Bar = 500 μm;**
- o) Linear patterning of tissue spheroids using 3D bioprinter. **Scale Bar = 500 μm;**
- p) Patterning of tissue spheroids on electrospun polyurethane matrix according to digital model. Scanning electron microscopy.

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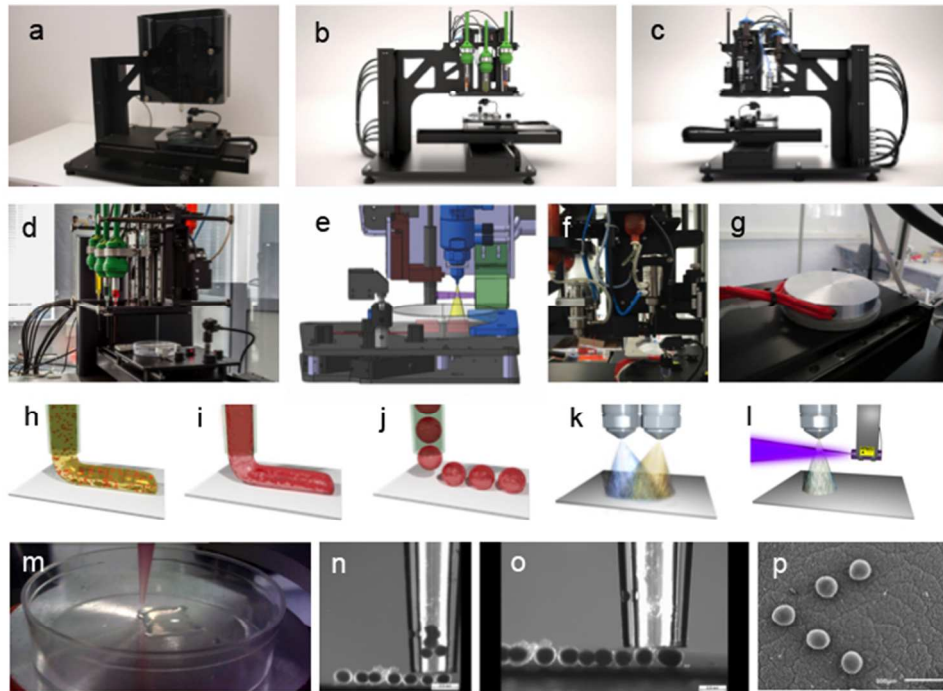


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- n) Initial step of tissue spheroids dispensing using 3D bioprinter. Scale Bar = $500\ \mu\text{m}$;
- o) Linear patterning of tissue spheroids using 3D bioprinter. Scale Bar = $500\ \mu\text{m}$;
- p) Patterning of tissue spheroids on electrospun polyurethane matrix according to digital model. Scanning electron microscopy.

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