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A novel Roll Porous Scaffold 3D bioprinting technology

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ABSTRACT

Biomanufacturing is a novel promising technology and an important field of research, offering hope for bridging the gap between organs shortage and transplantation needs. The paper describes the ways to overcome the main technological barriers and to accelerate biofabrication of 3D cellular tissue constructs greatly, to build multi-cell implantations of high density and accuracy for vascular system. It should be noted that though the Roll Porous Scaffold (RPS) 3D is a novel technology and never being used but derived from the time-proved bioprinting methods and components. The potential of the RPS are micron and submicron precision, performance of up to 5 *l* per hour with 20 μm layer thickness, which makes it more applicable than traditional methods. Features of the RPS are based on employing the Archimedean spiral and manufacturing 3D objects layer-by-layer with their transformation into a support ribbon. RPS may be one of the possible ways to solve the serious problem of "3D bioprinting" for tissue engineering and regenerative medicine in the nearest future.

1. Introduction

For the past three decades [1,14], tissue engineering technology (TET) has emerged as a multidisciplinary research field connecting biologists, engineers, and physicians, for the purpose of creating biological substitutes mimicking native tissue to replace damaged tissues and to restore malfunctioning organs [2]. TET brought the hope of fabricating tissue substitutes with biological functions [3,4] and of reducing the need for organ replacements in the very near future. It could greatly accelerate the development of new drugs that may cure patients and thus eliminate the need for organ transplantations altogether. For example, bone tissue engineering scaffolds can readily be designed as 3D data model and realized with titanium known for its excellent osseointegration behavior. Such scaffolds allow a high degree of microarchitectural freedom to generate rod lattice structures and to determine the optimal distance between rods and the optimal diameter of rods for osteoconduction (bone ingrowth into scaffolds) and bone regeneration after 4 weeks of healing [50].

Although TET conjures up visions of living organs built from the scratch in the laboratory from a person's own cells, ready to be transplanted into desperately ill patients, there is still a long way to go to practically realize this ambitious vision. To develop seamless automated technology from stem cell isolation to transplantation, one needs to beat the current impediments in TET for *in-vivo* integration.

The usual TET strategy is to seed cells onto porous scaffolds [9–12], which can then cause cell proliferation and differentiation into volumetric functioning structures, and then these cells are guided to form various desired tissues [5,6].

Nevertheless, though significant success has been achieved in the past decades both in research and clinical applications [13], it is obvious that complex 3D organs require more precise multi-cellular structures with vascular network integration, which cannot be fulfilled by the traditional methods [1].

As stated in Ref. [7], nowadays varieties of tissue engineering technologies based on scaffold structures are rapidly developing, but there still remain some defects to be solved: it takes too much time to produce a tissue organ which delays the treatment; (2) they are unable to carry out a multi-cell implantation, and the cells cannot achieve distribution with high density and precise spatial position; (3) it is hard to achieve the required growth of blood vessels and there are still has problems with the nutrition supply for large organs [3,8]. A new fabrication method to achieve fast organ production with precise spatial position of multi-cells/materials and blood vessel orientation is urgently needed.

Many countries currently regard 3D bioprinting as key development project [14], and a large number of universities and companies are carrying out in-depth study in this area [49], due to the tremendous potential of 3D biofabrication.

The purpose of RPS is to offer new techniques for solving difficulties in 3D bioprinting mentioned above.

2. Methods and materials

2.1. Spiral coordinate system feature

One of basic features of RPS is slicing the 3D object into a 2D stripe by

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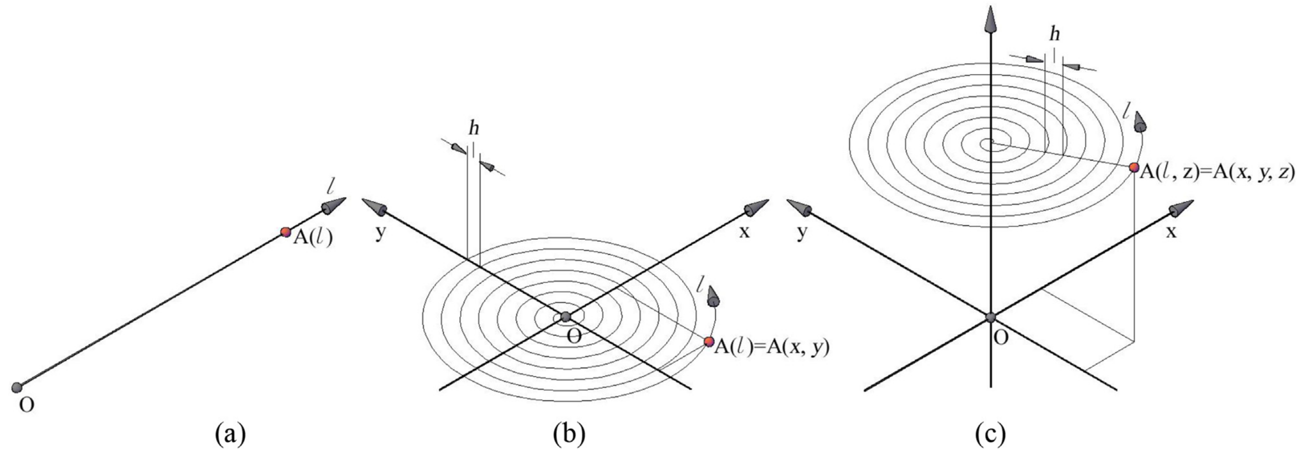

Fig. 1. Definition of "A" point's location in 1D space (a) and features of spiral coordinates systems for 2D (b) and 3D (c) spaces.

the spiral coordinate system [15] before printing. This coordinate system makes it possible to define a point's location in the volume with only two coordinates (Fig. 1) and even one (Fig. 2) [16] instead of three, the third dimension appears if the plane is transformed into a roll, like a carpet.

The spiral coordinate system is derived from the spiral of Archimedes. It is different from the Cartesian coordinate systems requiring two coordinates (*x, y*) to determine the point's location in a plane (Fig. 1(b)) and three coordinates (*x, y, z*) in 3D case (Fig. 1(c)) because (*l*) and (*l, z*) coordinates accordingly are sufficient enough for the spiral one. The point's position is defined by the spiral length within the accuracy, which depends on '*h*' constant (the distance between successive turnings), in RPS, with '*h*' being the height of a scaffold tape.

To make things clear, Fig. 3 shows a simplified sequence of the flat tape coordinates conversion into volume. In other words, it is easy to convert any 3D object into a stripe, similarly to veneer sheet manufacturing. The transformation of a three-dimensional space to a two-dimensional one can be easily performed by the equations of equivalence between these spaces [15]. Algorithms with its analyses [17] and software for the coordinate's transformation are described in Refs. [18–23] accordingly.

Fig. 4(b) illustrates creating the impeller inside a roll with the ribbon. The impeller is converted into a series of multitude frames without a gap, Fig. 4(a). The support stripe is marked with length labels (L_i) on a wavy edge through its manufacturing. There are other labels on the opposite edge (F_n) in the shape of blue triangles. These labels are made for the period of support stripe reeling and are necessary for precise rolling control. This feature is explained in more detail and described further.

2.2. Filling the support ribbon

Fundamental RPS workflow algorithm is demonstrated in Fig. 5(a). At the beginning, an empty roll of a support ribbon (porous scaffold) is reeling in an object roll. When being rewound, the scaffold is perforated and filled with bioinks droplets, Fig. 5(b). This process continues until the necessary object is completely shaped. In the cross section, the rewound object roll is similar to the spiral of Archimedes.

Scaffolds structure and its material has a great significance for 3D bioprinting [24]. It must be stable enough to form a large tissue, avoiding any collapse and destruction in the printing process, it should have certain porosity for cell growth [25], and it should support the whole structure [26,27].

A scaffold should have excellent biocompatibility, good mechanical strength, outstanding shaping performance and high solubility in water, which means that the material can degrade in the required time, and the by-product of the degradation will not have any impact on the cells and other parts of human body, this feature known as cytotoxicity free [28]. Materials should have sufficient supporting ability and provide space for cells survival with the ability to switch between solid and liquid conditions.

In the process of RPS, it is suggested to use a spongy support ribbon (produced by salt leaching, electrospinning or other foaming techniques to form various desired tissues [5,6]) with reinforcement structure shown in Fig. 6. This configuration provides mechanical strength and at the same time appropriate degradation ability.

Fig. 7 depicts simplified general view of proposed RPS 3D bioprinter.

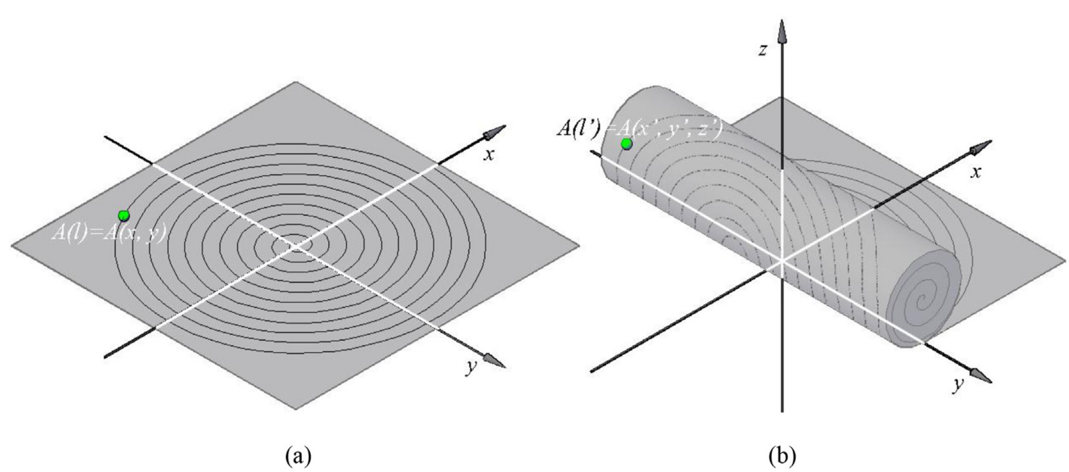

Fig. 2. Simplified diagram of conformal coordinate's transformation 1D spiral space (a) into 3D one (b).

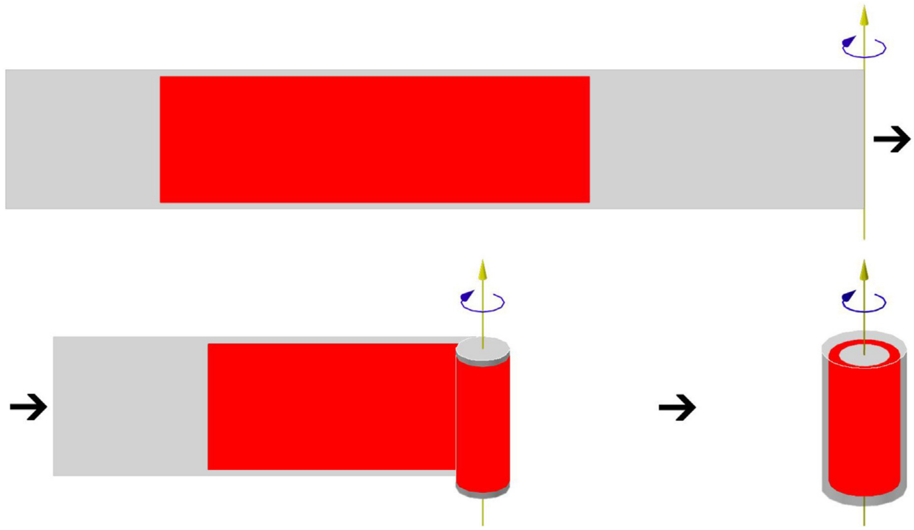

Fig. 3. Sequence of conversion of the stripe's red area into a tube inside the roll. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

It is similar to described [16,29–32] and patented [33–35] devices for production plastic, ceramic and metal details with micron and submicron precision, smoother surfaces and hundredfold increased performance over dominant additive manufacturing technological processes currently available the market. Additive technology, mentioned above, is a kind of Selective Laser Melting (SLM) suitable for useful valuable, such as creating 3D bioprinted bones [50,52] which are under human clinical trials and have potential in future medical applications [53].

According to RPS algorithm of manufacturing objects, at the beginning, while the scaffold roll (1) is reeling in the object roll (2) by extending rollers (3), (4) and transfer belt (5), the droplets filling systems (12), (13) drip different bioinks, from tanks (14) and (15): a type of bioink is determined by the required tissue. Moreover, these systems make labels on the support ribbon at the beginning of each frame edge for "Frame position on the scaffold roll control system" (8). The winding process is executing under the control of "Take-up roll reeling layer merging control systems" (7). Both systems are using photo detectors and recognition modules. As calculated 3D CAD model of the manufacturing object is sliced into frames, the position of each frame on the scaffold tape is known beforehand by the length. There is possibility to shift the next frame (by software) forward or backwards, if it is necessary to fix a distortion, according to the locations of labels (Fig. 7, left down corner): of the top layer (L_j), the previous bottom layer (L_i), and the frame start ("F_n" on Fig. 4, blue triangles).

RPS strongly depends on the winding processes: high-precision winding is mandatory. The component ribbon's reeling precision is

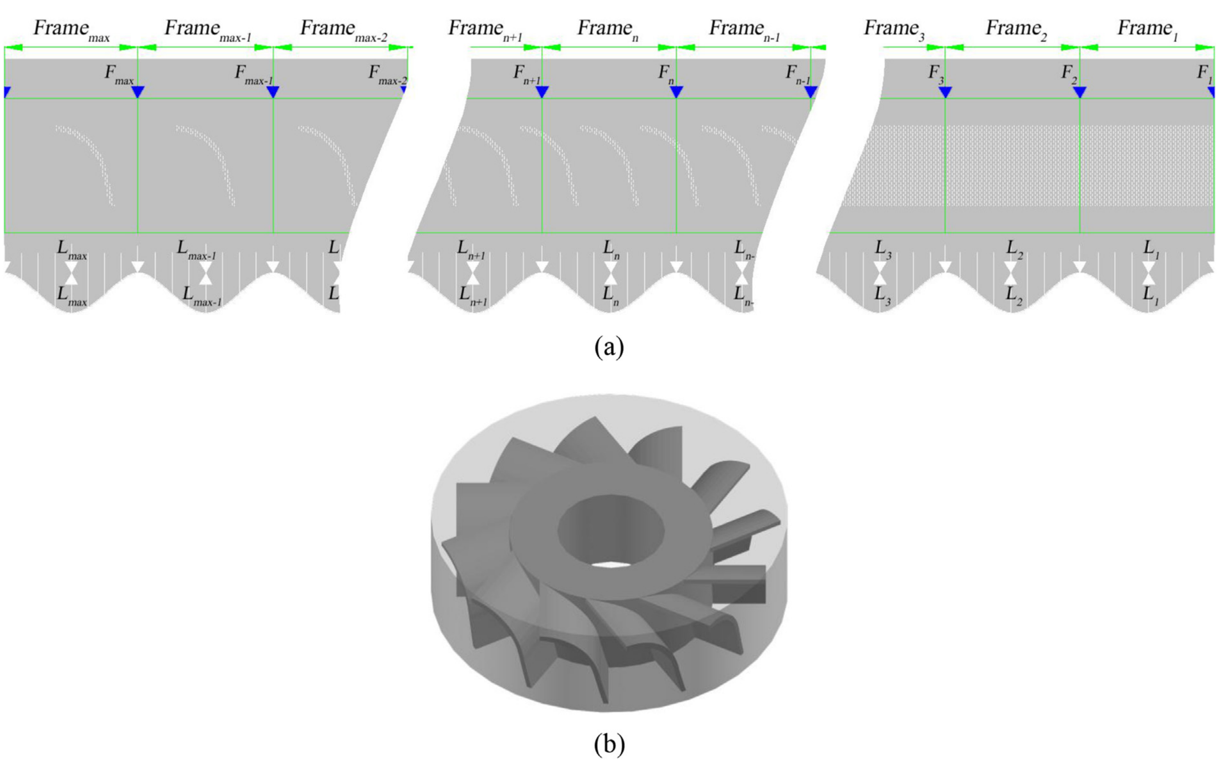

Fig. 4. Linear sequence of frames on the support ribbon (a) for impeller's manufacturing inside the roll (b).

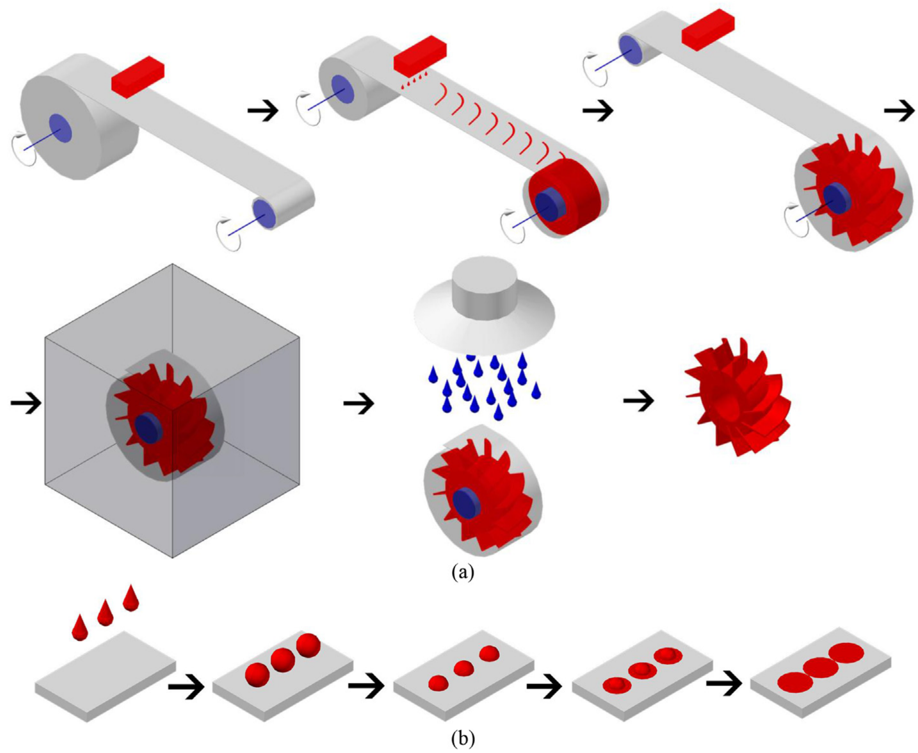

Fig. 5. Fundamental RPS workflow algorithm (a) and filling support ribbon with droplets (b).

provided by its inextensible structure and perforations along the sides similar to a cinema film. Such solution makes ribbon's move without slipping.

Obviously, the smaller is frame the higher is the precision. Usual home inkjet printer provides droplets with the volume of ~1 *pl*, 9600 *dpi* accuracy and prints A4 page per second (~300 mm/s), i.e. if the layer thickness is ~20 μm then the creating performance will be ~5 *l* per hour. This velocity is enough for the centrifugal force to splash tissue liquid from a rotating component roll when its radius is small. The solution to this is either to significantly increase a starting radius of a take up roll to save the performance or to reduce the reeling speed for a small radius. There is another variant for cells transference in RPS with higher resolution and higher cell density – laser assisted printing [36–38]. In short, when the laser beam acts on cells or droplets which contain cells, it will produce an effect of two components located in the different directions from the droplets, so that the cells will move in the direction of parallel and vertical beam. Therefore, the laser beam will accurately control the whole the process, beginning from separation, passing on to the movement and to the arrival at the base plate. This technology is called laser-guided direct writing (LGDW) [14].

Consequently, RPS machine based on inkjet or LGDW droplets filling system makes it possible for 3D bioprinting to have not only with high precision but also high performance with different tissue.

The propose 3D bioprinting technology shows many technical features for building functional biological tissue constructs by dispensing the individual or group of cells into specific locations along with various types of bio-scaffold materials and extra cellular matrices. RPS describes many technical methods and elements for bioprinting implicate new opportunities for on-demand individualized construction of biological organs. Obviously, a concept is not hardly feasible due to founded on tested techniques and components.

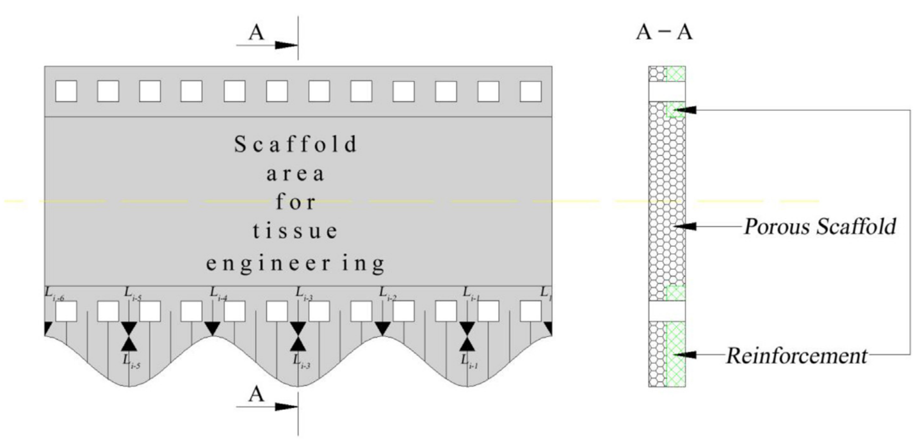

Fig. 6. Simplified scheme of RPS support ribbon and its cross section with length labels (L_i and the black triangle), reinforcement and perforation along the sides similar to a cinema film.

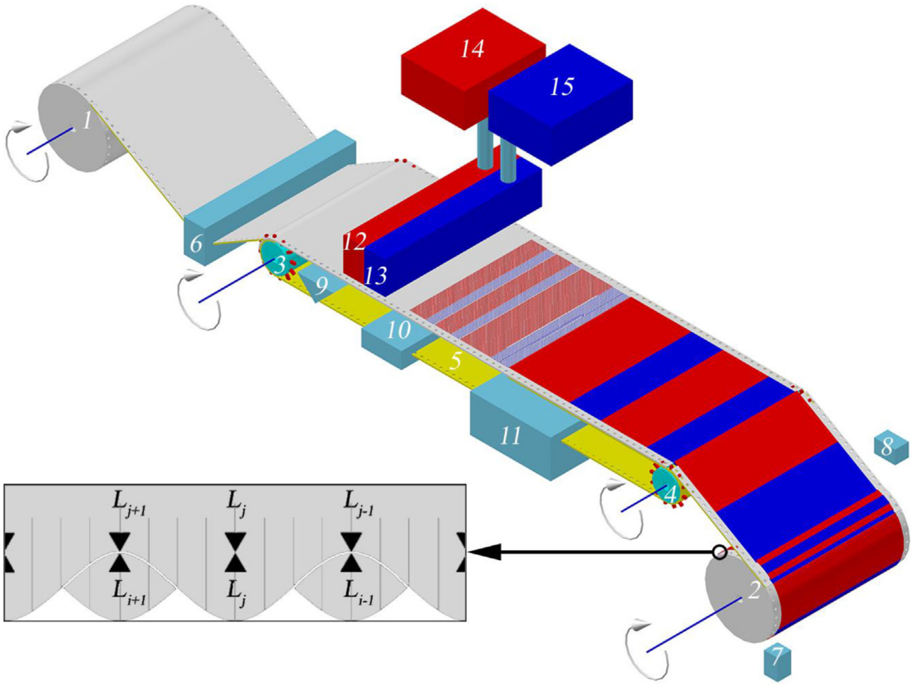

Fig. 7. Simplified general view of the RPS 3D bioprinter. (1) Scaffold roll, (2) Object roll, (3) and (4) Extending rollers, (5) Transfer belt, (6) Supply roll unreeling tension control system, (7) Layer merging control system, (8) Frame position on the scaffold roll control system, (9) Safety reeling protector, (10) Transfer belt tension control system, (11) Transfer belt refinement system, (12) Droplets filling system for fist bioink, (13) Droplets filling system for second bioink, (14) Tank for first bioink, (15) Tank for second bioink.

3. Results and discussion

Nowadays, bioprinting technologies generally use the following three methods: laser printing, inkjet and extrusion printing. There are many commercial and non-commercial models. For example, at Technical University Munich, The Scripps Research Institute, Tokyo University of Science and Wuhan University of Technology produce bioprinters for proteins and mammalian cells based on a modified Canon Bubble Jet printer (BJC-2100) and modified Hewlett-Packard Deskjet printers (HP 550C, HP 500, and HP 340). Ulsan National Institute of Science and Technology (UNIST) with Osaka University and Japan Science and Technology Agency (JST) manufacture "Fujifilm Dimatrix (DMP-2800) printer" and "Cluster Technology DeskViewer™" accordingly. All of them are easy to accommodate for RPS [65].

RPS 3D bioprinting technology is developed for wide use in various application areas of regenerative medicine, transplantation, tissue and organ fabrication and pharmaceutics, drug screening. This new technology is emerging to advancing the tissue fabrication toward physiologically relevant tissue constructs, tissue models, tissues and organs and organs-on-a-chip models for medicine and pharmaceutics. RPS shows the methods to overcome the main technological barriers for fabrication of native-like tissues with a heterocellular microenvironment with great improvement in velocity and precise placement of multiple cell types, including living cells, nucleic acids, drug particles, proteins and growth factors.

Table 1 shows the potential of RPS 3D Bioprinting Technology for the future industry use with same resolution, a faster performance and more versatility than the previous one.

The new proposed methods for RPS 3D bioprinting significantly decrease the costs of producing tissue, make it possible to carry out multi-cell/materials structure with high cell density at high precision spatial position including tissue of blood vessels for the nutrition supply in large

Table 1
Features of different bioprinting methods.

Print methods	Bioinks	Resolution, μm	Cell viability	Cell density, *cells/ml*	Print speed	Target tissue
Laser-assisted printing[a]	Fibrinogen, collagen, GelMA	1–50	>97%	10^8	100–1600, mm/s	Skin, vessel
Inkjet printing[b]	Collagen, poly (ethylene glycol) dimethacrylate (PEGDMA), fibrinogen, alginate, GelMA	50–500	85–98%	$<5 \times 10^6$	1000–5000, droplets/s	Skin, cartilage, bone, tumor, liver
Extrusion printing[c]	Gelatin, polycaprolactone (PCL), polyethylene glycol (PEG), alginate, hyaluronic acid (HA), polyamide (PA), polydimethylsiloxane (PDMS) dECM, nanocellulose	>50	80–96%	Cell spheroid	5–20, mm/s	Skin, cartilage, vessel, bone, muscle, tumor, heart
The potential of a Novel Roll Porous Scaffold 3D Bioprinting Technology for the future industry use[d]						
RPS	Fibrinogen, collagen, GelMA, Collagen, poly (ethylene glycol) dimethacrylate (PEGDMA), alginate, Gelatin, polycaprolactone (PCL), alginate, hyaluronic acid (HA), polyamide (PA), polydimethylsiloxane (PDMS) dECM, nanocellulose	1–9	85–98%	$<5 \times 10^6$–10^8 Cell spheroid	100–1600, mm/s 1000–5000, droplets/s ~5 *l* per hour	Skin, vessel, cartilage, bone, muscle, tumor, liver, heart

[a] [39–41].
[b] [42–53].
[c] [54–64].
[d] RPS bioprinting specification is theoretical but based on the time-proved bioprinting methods at a large number of universities, companies and widespread, commercially available inexpensive components.

volumes. RPS provides a new fabrication method for tissue engineering technologies.

The RPS workflow described here, in contrast to the other technologies, does not require an expensive equipment for multimaterial 3D biofabrication and thus opens the door for the application of this technology to new fields of medical research. This a layer-by-layer manner of producing a three-dimensional object can readily be designed as 3D CAD model and realized in a short time with excellent precision.

In addition, the RPS ability to create different precise structures inside easily dissolved scaffold, while winding it into a roll, with the volume of liters within an hour, is one of the key factors for technology development in medicine and bioengineering to achieve better results. The use of foamed scaffolds with the reinforcement structure in a tape with length labels on its sides together with the winding control systems and laser-assisted printing provides micron and submicron accuracy of three-dimensional layer lattice structure and determines more suitable distance between cells than the existing volume biomanufacturing technologies.

The simplified general view of RPS 3D bioprinter and its fundamental workflow sequence is shown in comparison to the dominating technologies.

4. Conclusion

The goal of RPS is to resolve the current difficulties of 3D biofabricating by fast, precise and inexpensive methods. RPS is offering to rise above the main technological barriers of bioprinting and to build a multi-cell tissue with high density, accuracy and performance in 3D. It suggests that easily solved biocompatible and biodegradable porous scaffolds should be used (salt leaching, electrospinning or other foaming techniques with the mechanical strength to support cell attachment), based on reinforcement synthetic and natural polymers for tissue engineering.

Successful implementation of the proposed techniques with micron and submicron precision, performance of up to 5 *l* per hour with precision of 9600 *dpi* and layer thickness of 20 μm may greatly improve the properties of manufactured tissue substitutes with biological functions and advance regenerative medicine in the nearest future.

Therefore, RPS proposes methods for overcome the main technological barriers and, at the same time, to greatly accelerate the biofabrication of 3D cellular tissue structures with high density and accuracy for multi-cell implantation with integrated vascular system. This paper is an example of using special creativity and technical imagination, as it illustrates the creation of a new process for biomanufacturing that could change the way of industrial bioproduction

Disclosure statement

No potential conflict of interest was reported by the authors.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.bprint.2019.e00042.

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