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Organ printing: from bioprinter to organ biofabrication line

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Organ printing, or the layer by layer additive robotic biofabrication of functional three-dimensional tissue and organ constructs using self-assembling tissue spheroid building blocks, is a rapidly emerging technology that promises to transform tissue engineering into a commercially successful biomedical industry. It is increasingly obvious that similar well-established industries implement automated robotic systems on the path to commercial translation and economic success. The use of robotic bioprinters alone however is not sufficient for the development of large industrial scale organ biofabrication. The design and development of a fully integrated organ biofabrication line is imperative for the commercial translation of organ printing technology. This paper presents recent progress and challenges in the development of the essential components of an organ biofabrication line.

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Introduction

Since its inception [1••] the concept of organ printing using robotic bioprinters for the layer-by-layer additive biofabrication of functional 3D tissues and organ constructs using self-assembling tissue spheroids has undergone progressive development [2,3,4••,5,6] and gradually gained recognition as a reasonable bottom-up solid scaffold-free alternative to the classic top-down or solid scaffold-based approach to tissue engineering [7]. As Dr. David Williams stated in recent influential review: ''*There is obviously some way to go before such a paradigm [. . .directed tissue self-assembly. . .] could be translated into a practical reality, but many steps have been taken*'' [8]. The report on the 4th International Bioprinting and Biofabrication Conference (2009) that took place in Bordeaux, France, stated that '*bioprinting is coming of age*' [9]. An increasing number of papers and reviews, publication of the first books [10], the rapid development of new bioprinting research centers around the world, creation of the new Biofabrication journal and International Society for Biofabrication (2010) and, most importantly, the development of commercially available bioprinters are all important progress milestones.

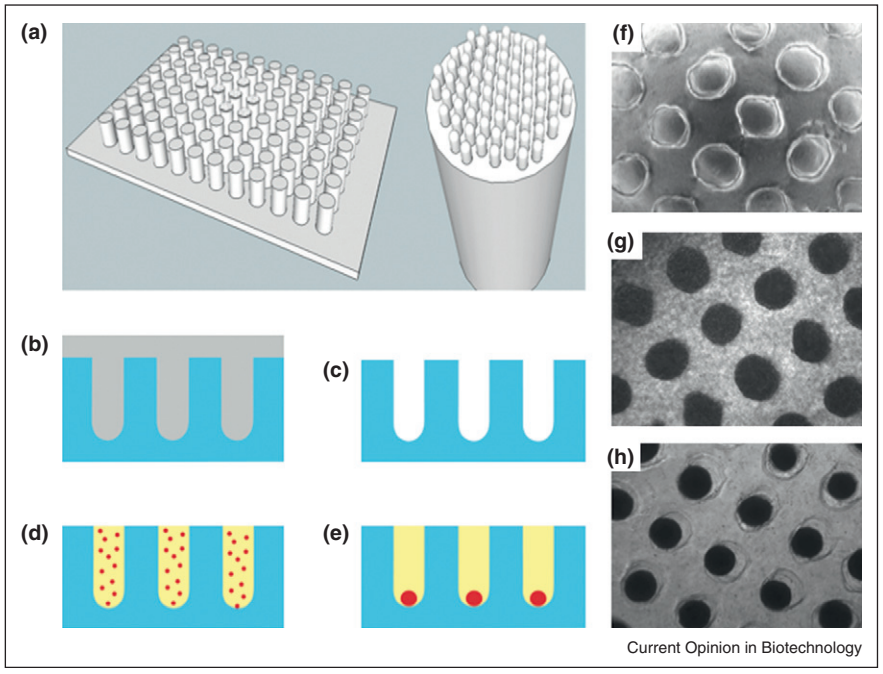
The potential competitive advantage with the use of self-assembling tissue spheroids for organ printing has been recently reviewed [3,4••,6]. It has been suggested that the bottom-up solid scaffold-free approach can enhance the development of tissue engineering technology by enabling the automated and robotic industrial scale organ biofabrication [4••]. History of the automobile industry and the emergence of microelectronic industry have taught us that an automated robotic approach is required for the successful development of new commercially profitable industries. The combination of computer-aided robotics and tissue engineering will not only enable tissue and organ bioassembly at large industrial scale, but will also provide the necessary level of flexibility for patient specific, customized organ biofabrication [2,4••,5,11–14].

It is become increasingly obvious that, from a systems engineering point of view, it will take more then just bioprinters to biofabricate complex human tissues and organs. Indications suggest that the development of series of integrated automated robotic tools, or an organ biofabrication line (OBL) is required. Components of the OBL must include a clinical cell sorter, stem cell propagation bioreactor, cell differentiator, tissue spheroid biofabricator (Figure 1), tissue spheroids encapsulator, robotic bioprinter, and perfusion bioreactor. Certain components of the OBL, such as clinical cell sorters (for example, Celution, Cytori Therapeutics, USA), stem cells propagation bioreactors (for example, Aastrom Biosciences, USA), cell and tissue encapsulators (for example, Nisco Engineering Inc., Switzerland), and robotic bioprinters (for example, Envisiontech, Germany and BioAssembly Tool, Sciperio/nScript, USA) (Figure 2) are already commercially available whereas other components are still under development. Ideally, all components of an OBL must be compatible and able to integrate. In this review we will present progress and discuss the related challenges in the development of the essential components of OBL, such as tissue spheroid biofabricators, robotic bioprinters and bioreactors.

Design principles of organ biofabrication line

The OBL must be developed using carefully formulated engineering principles. For example, the engineering

Figure 1



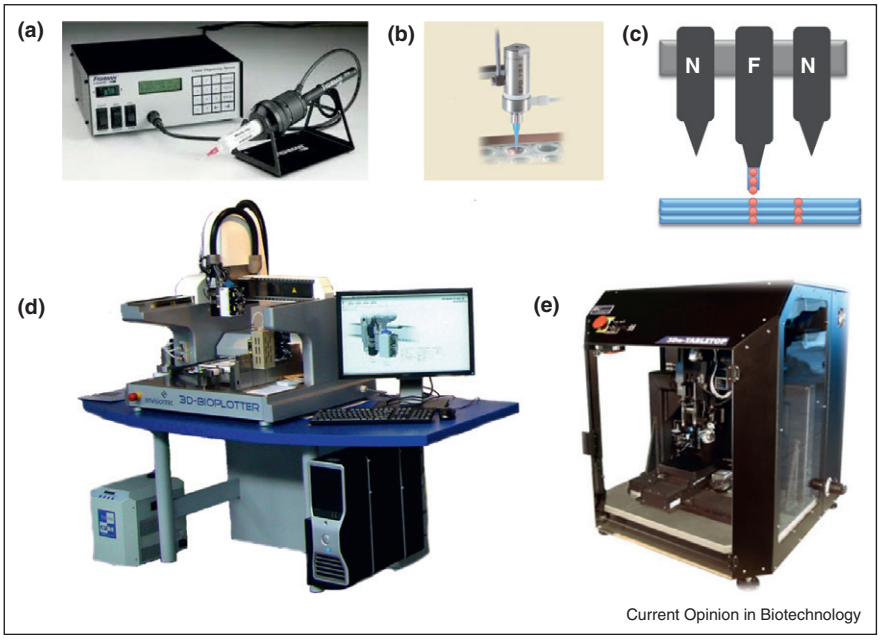
A scalable biofabricator for the production of tissue spheroids.
(a) Computer-aided design of the 96 multiwell micromolding device.
(b) Scheme of the process for fabricating micromolded recessions in agarose hydrogel.
(c) Scheme showing micromolded recessions in agarose hydrogel.
(d) Scheme demonstrating a cell suspension in micromolded recessions in agarose hydrogel.
(e) Scheme demonstrating tissue spheroid fabrication in micromolded recessions in agarose hydrogel.
(f) Empty micromolded recessions in non-adhesive agarose hydrogel.
(g) Gravity forced sedimentation of cells suspended in micromolded recessions in non-adhesive agarose hydrogel.
(h) Tissue spheroid formation in micromolded recessions in non-adhesive agarose hydrogel.

principles in synthetic biology include standardization, decoupling and abstraction [15,16]. The standardization of parts, or building blocks, is a necessary engineering requirement for any large-scale assembly process. The decoupling of design from fabrication is a standard approach because building without design is engineering nonsense. Finally, abstraction allows for reduction of the entire project into series of small tasks without the loss of an integrated comprehensive vision of entire project. In the case of engineering an organ biofabrication line, we believe it is logical to introduce the following basic design principles. The first principle – do not re-invent wheel. It is important to optimize the potential of existing technologies and integrate them before trying to develop something entirely new. The second principle is to never forget that you are dealing with living tissue – this is probably the most important engineering restraint. The third principle is to maximize the automation of all OBL processes and operations because scalability cannot be achieved without automation. The fourth principle is that all components must be compatible and able to seamlessly integrate together. Finally, the fifth principle is that the whole OBL must be automatically controlled and every step be non-destructively biomonitored in real time, using sophisticated 'built in' sensors and an automated system of quality control. The implementation of an automated system of quality control is key for success and regulatory agency approval.

Tissue spheroids biofabricator

The development of scalable methods to biofabricate uniformly sized tissue spheroids is essential for enabling the bioprinting of large tissue and organ constructs because each require millions of tissue spheroids [4••]. The majority of existing methods of tissue spheroid biofabrication are not scalable [17]. For example, it would take 100 Petri dishes to generate 5 thousands tissue spheroids using the hanging drop method of tissue spheroid fabrication [18–20,21•,22–25]. Recently, a novel elegant method was introduced whereby tissue spheroids were biofabricated using micromolded non-adhesive hydrogel (agarose) [26–28,29•,30]. These densely placed micromolded recessions with rounded bottoms in hydrogel allowed for the biofabrication of uniformly sized tissue spheroids [31]. The use of this approach in combination with a robotic dispenser (for example, 'EpMotion-5070',

Figure 2



Design of robotic bioprinters.
(a) Fishman automated dispenser.
(b) Nordson aseptic valve sprayer.
(c) Design of a robotic bioprinter (N – two Nordson aseptic valve sprayers for spraying thrombin and fibrinogen, and the fabrication sequential layers of fibrin hydrogel; F – Fishman robotic dispenser for punching tissue spheroids into sequentially sprayed layers of fibrin hydrogel. Both Nordson sprayers and the Fishman automated dispenser are fixed on an *XYZ*-axis robot).
(d) Envisiontech commercial bioprinter.
(e) Sciperio/nScript commercial bioprinter.

Eppendorf) increases the productivity of tissue spheroids biofabrication for production of up to five thousand tissue spheroids of standard size on one 96 well multiwall plate (Figure 1). Similar approaches have also been developed using increasingly sophisticated microfabrication techniques such as lithography [32–36]. However, the flat bottom of the microfabricated microwells is an obvious disadvantage because a round shaped bottom is essential for the rapid fabrication of tissue spheroid into the desired ball-like shape. The use of novel stimuli-sensitive biomaterials unlocks a unique opportunity for the automatically controlled retrieval of tissue spheroids by providing the appropriate stimulus for the specially designed hydrogel [37•]. Recent advances in digital (droplet-based) microfluidics offered a new exciting perspective to biofabricate thousand tissue spheroids with complex internal structure and composition in seconds using a relatively cheap and elegantly designed cascade droplet generator [38]. Thus, the development of scalable robotic tissue spheroid biofabricators for the automated biofabrication of uniform sized tissue spheroids is a feasible and achievable goal. The storage of biofabricated tissue spheroids and the prevention of undesirable fusion are other challenges. One possible engineering approach is to integrate tissue spheroid biofabricators with robotic bioprinters using microfluidic devices. However, from a systems engineering viewpoint, the increasing complexity of bioprinters and the combination of two or more functionalities in a single device can increase the chance of failure and associated downtime.

Robotic bioprinters

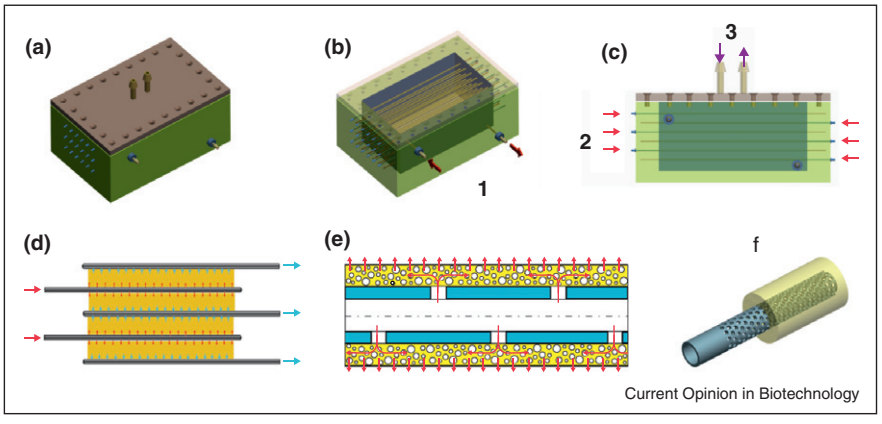
Bioprinters are a key element of organ printing technology [2,4••]. The emergence of commercially available bioprinters (Figure 2) is probably the most remarkable development of the past decade. The explosive growth of different variants of bioprinting technology resembles the early development phase of rapid prototyping technology 10–15 years ago, when many completing technologies were developed but not all of them successfully commercialized. Robotic bioprinters for the precise dispensation of tissue spheroids include three essential elements: *X*–*Y*–*Z* axis robotic precision position system, automated biomaterial dispensers and computer-based software enabled operational control. Tissue spheroid printing of satisfactory resolution is already achievable by robotic deposition [2,4••,5,6,39]. Achieving the desired level of cell density, effective vascularization and accelerated tissue maturation are remaining challenges [3,5,6]. It has been

shown that adequate cell density can be achieved by dielectrophoresis in combination with stereolithography [40]. This investigation revealed that cell density increased progressively, even in an initially low-density printed cell-hydrogel construct, as the result of either post-printed cell proliferation or the elimination of a sacrificial biodegradable hydrogel [41–43]. The initial high cell density can be achieved immediately after bioprinting using self-assembling tissue spheroids [4••,5,6,39,44••] or continuous self-assembling tissue rods or longitudinal pellets [45]. There has also been certain progress in solving the problem of vascularization of thick 3D bioprinted tissue and organ constructs [6]. Branched vascular tissue constructs have been bioprinted using continuous rod dispensation [45••]. Vascular tubes have also been bioassembled from vascular tissue spheroids [2,4••,5,6,21•,39,46]. In other developments, new *in vitro* assays have been developed for the systematic screening of potential chemical maturogenic factors necessary for accelerated tissue maturation [47]. It has also been demonstrated that mechanical conditioning can improve vascular tissue maturation [21•]. Further progress in bioprinter development must focus on improving nozzle and cartridge design, the development of more flexible functionality and the design of collectors or bioreactor for bioprinted constructs. In this context, recently reports have found nozzle-free bioprinting to be an innovative engineering concept [48•]. Another interesting development is magnetic force driven tissue engineering with tissue spheroids labeled with magnetic nanoparticles, which could lead to the development of a magnetic bioprinter [17,49,50]. Thus, bioprinting technology has matured enough for the bioprinting of large thick vascularized tissue and organ constructs.

Bioreactors for organ printing

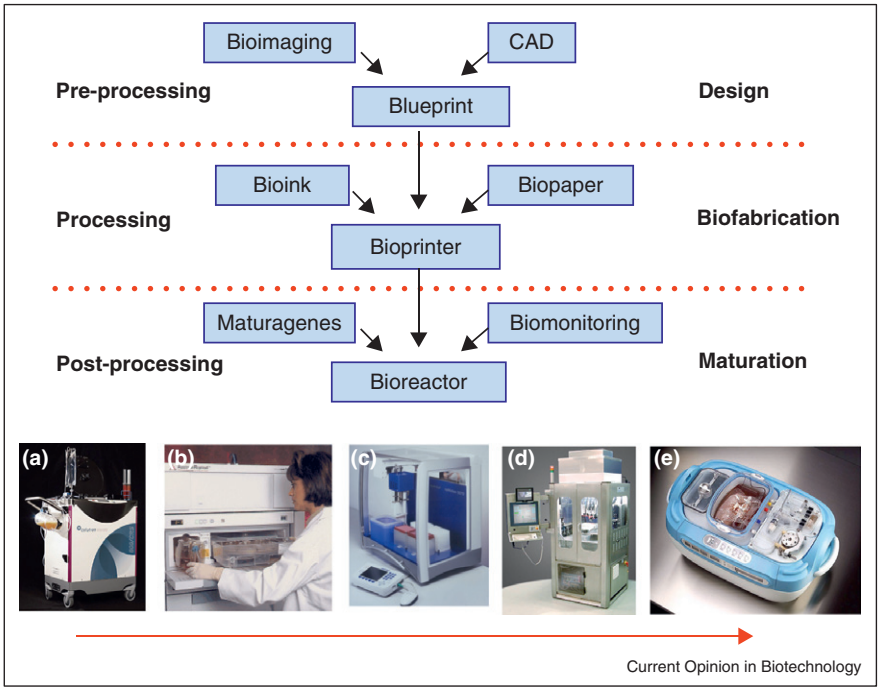
Bioprinting *per se* is not sufficient for the creation functional tissue or organ constructs immediately suitable for implantation. It takes time for bioprinted tissue spheroids to fuse and bioprinted tissue to assemble, compact, remodel and mature into functional tissue constructs. Post-processing is probably the most essentially crucial step in organ printing technology, and effective post-processing or accelerated tissue maturation will require the development of new types of bioreactors, more efficient accelerated tissue maturation technologies as well as methods of non-invasive and non-destructive biomonitoring. The conceptual design of novel irrigation dripping tripled perfusion bioreactors with temporally removable porous minitubes suitable for bioprinting has already been developed [3,4••] (Figure 3). The design is a triple perfusion bioreactor because it has three circuits: one for maintaining a wet environment around the bioprinted construct, the second for media perfusion through an intraorgan branched vascular tree, and the third and most essential circuit, for temporal perfusion. The last type of perfusion is undertaken using extremely strong, thin, porous, non-biodegradable, removable minitubes that serve as temporal supports and artificial microchannels. The main goal of the proposed dripping irrigation circuit system is to 'buy' time until the 'built in' intra-organ branched vascular system will mature sufficiently for the initiation of intravascular perfusion. Moreover, this

Figure 3



Design of an irrigating triple perfusion bioreactor.
(a) Design of the triple perfusion bioreactor chamber.
(b) Design for the placement of temporally removable porous tubes into the triple perfusion bioreactor chamber. The inlet and outlet for the extra-construct perfusion circuit (1).
(c) Design of the second circuit (2) based on the use of temporally removable porous tubes for interstitial intra-construct perfusion, and the inlet and outlet of third circuit (3) for intravascular perfusion.
(d) Scheme demonstrating the fluid flow pattern between temporally removable porous tubes in the triple perfusion bioreactor.
(e) Scheme demonstrating a longitudinal section through the wall of temporally removable porous tube and the flow of fluid through its porous wall.
(f) Computer-aided design of a temporally removable porous tube for the triple perfusion bioreactor.

Figure 4



Essential steps in organ printing technology and an organ biofabrication line.
(a) Celution – clinical sell sorter for the scalable isolation of autologous adipose tissue derived adult stem cells (Cytori Therapeutics, USA).
(b) Bioreactor for the scalable propagation of stem cells (Aastrom Bioscience, USA).
(c) EpMotion-5070 – robot for the automated scalable biofabrication of uniformly sized tissue spheroids (Eppendorf, Germany).
(d) Bioasembly Tool – robotic bioprinter (Sciperio/nScript, USA).
(e) LifePort – Perfusion apparatus (Organ Recovery System, USA).

temporal perfusion system can be used for the delivery cells, soluble extracellular matrix molecules and maturogens. The rational design behind such a bioreactor, especially the level of porosity and distance between minitubes, must be based on systematic mathematical modeling and computer simulation of interstitial flow. The identification of proper materials and coatings of these minitubes, and the optimal way to retrieve the inert minitubes without severe tissue injury are other important engineering challenges.

Conclusion

The only economic and reasonable way to commercialize organ-printing technology is to systematically employ scalable automated robotic technology and to build an integrated organ biofabrication line. It is not sufficient to develop just one robotic device – a bioprinter. The biofabrication of a human organ will require the development of series of integrated automated robotic devices, or an organ biofabrication line (Figure 4). The development of such a production line is an important technological imperative and an exciting engineering challenge. Certain components of the organ biofabrication line are already commercially available, whereas others need adaptation to organ printing technology, and some must be entirely developed from new. The clinical cell sorter (Celution, Cytori Therapeutics, USA) and bioreactor for stem cell propagation (Aastrom Biosciences, USA) are examples of commercially available components of the future organ biofabrication line.

The progress in tissue spheroid biofabrication clearly indicates that the development of a scalable automated robotic tissue spheroid biofabricator is a feasible and achievable goal. The recent explosion of interest to build different types of laboratory bioprinters and the emergence of commercial bioprinters also strongly suggests that the design and fabrication of a robotic bioprinter suitable for the automated and precise dispensation of tissue spheroids is just a matter of time, proper investment and optimization. Finally, the design and fabrication of a perfusion bioreactor suitable for organ printing is still a great challenge.

To guarantee the bioprinting of consistently high quality products (failure is not an option in case of human organ biofabrication), the whole OBL must be automatically controlled and every bioassembly step must be non-destructively biomonitored in real time by sophisticated 'built in' sensors and automated systems of quality

control. It is logical to assume that close collaboration between biologists and engineers, and use of mathematical modeling and computer simulation to predict tissue biofabrication processes, will significantly enhance, optimize and accelerate the OBL design process.

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