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REVIEWS

Opinion

Multitechnology Biofabrication: A New Approach for the Manufacturing of Functional Tissue Structures?

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Most available 3D biofabrication technologies rely on single-component deposition methods, such as inkjet, extrusion, or light-assisted printing. It is unlikely that any of these technologies used individually would be able to replicate the complexity and functionality of living tissues. Recently, new biofabrication approaches have emerged that integrate multiple manufacturing technologies into a single biofabrication platform. This has led to fabricated structures with improved functionality. In this review, we provide a comprehensive overview of recent advances in the integration of different manufacturing technologies with the aim to fabricate more functional tissue structures. We provide our vision on the future of additive manufacturing (AM) technology, digital design, and the use of artificial intelligence (AI) in the field of biofabrication.

From Cell Therapy to the Biofabrication of Tissues and Organs

Every day, ~18 people die in Europe alone due to the shortage of human donor organs. In 2017, it was estimated that only 19% of 34 000 patients on waiting lists for organs would receive an organ transplant [1]. To overcome this, great efforts have been devoted to regenerative medicine (RM) strategies that could restore damaged tissues and organs. Since first appearing during the early 1960s, regenerative strategies have come a long way from the first stem-cell transplantation [2] to the 3D **biofabrication** (see Glossary) of artificial tissue-like structures of today [3].

Stem-cell therapies have proved successful when applied to diseased or injured tissues in small animal models, such as rodents [4]. Unfortunately, clinical trials in large animal models and humans have rendered conflicting results, with the best scenarios supporting only minor benefits mostly regarded as nonregenerative and limited to paracrine effects [4]. This situation has not been improved significantly by the use of 3D cellular aggregates with improved cell–cell interactions and a protective self-secreted extracellular matrix (ECM) layer [5]. More recently, developmental biologists have shown that cultured pluripotent stem cells can differentiate into organ-specific cells and further self-organize into small 3D organ-like structures, such as intestinal or kidney organoids [6,7]. However, none of these strategies or *in vitro* organ developmental approaches have yet shown the ability to recreate biological structures with the functional richness, multiscale structure, and size of a living tissue [5–7].

It is known that living tissues comprise some of the most complex and hierarchically functional materials and are composed of different cell types and ECM components, including bioactive molecules and structural elements. The complex interplay between the components of native tissue suggests that, to recreate tissue equivalents that result in a functional *in vivo* outcome, the tissue equivalent should be as similar to the native tissue organization and composition as

Highlights

Single-deposition biofabrication methods mimic form but have only limited ability to replicate function of biological tissues.

Multitechnology biofabrication brings new perspectives towards functional tissue manufacturing.

Integration of digital design and AI-powered real-time monitoring tools with multitechnology bioprinting will allow for high-throughput biofabrication.

Although simple purpose-built bioprinting systems may find use in clinical environments, laboratory environments will strongly benefit from AI-driven multitechnology bioprinting systems.

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possible. Bioprinting, one of the main emergent biofabrication approaches, allows the *in vitro* fabrication of biological constructs with precise combinations of cells and biomaterials, while complimentary digital manufacturing processes enable biological structures to be shaped into the geometry of the target tissue or organ [3]. Biofabrication comprises a growing toolbox of a range of fabrication strategies, of which the most established are droplet, extrusion, light- and laser-assisted bioprinting. Each of these strategies has a different working principle, which has its own associated advantages and drawbacks with regards to cell processing, resolution and material selection (Box 1 and Figure 1A). So far, researchers have predominantly adopted a single fabrication strategy based on the target tissue composition and/or size. However, similar challenges as for cell-based therapies and *in vitro* organ developmental approaches have been observed (i.e., limited ECM deposition and organization, and absence of required functionality) [8]. These challenges are mainly due to insufficient synergy between material composition and organization, because the self-organization capacity of cells was not sufficient enough to recreate tissue functionality and single fabrication approaches are not mature enough to recreate the tissue mimicking environment to guide those cells.

We believe that an important current development in the evolution of manufacturing functional tissue and organ structures is the potential to combine different manufacturing processes into a single biofabrication platform (Figure 1B). Recent evidence suggests that the simultaneous use of complementary fabrication processes allows for the strategic arrangement of multiple cells and ECM components at different length scales, taking us closer to the heterogenous composition and complex multiscale organization of living tissues [3]. Additionally, recent advances in information technologies enable user-friendly access to **AI** systems that provide help for optimal design and decision making, and with that accelerate progress in manufacturing of tissue mimicking equivalents.

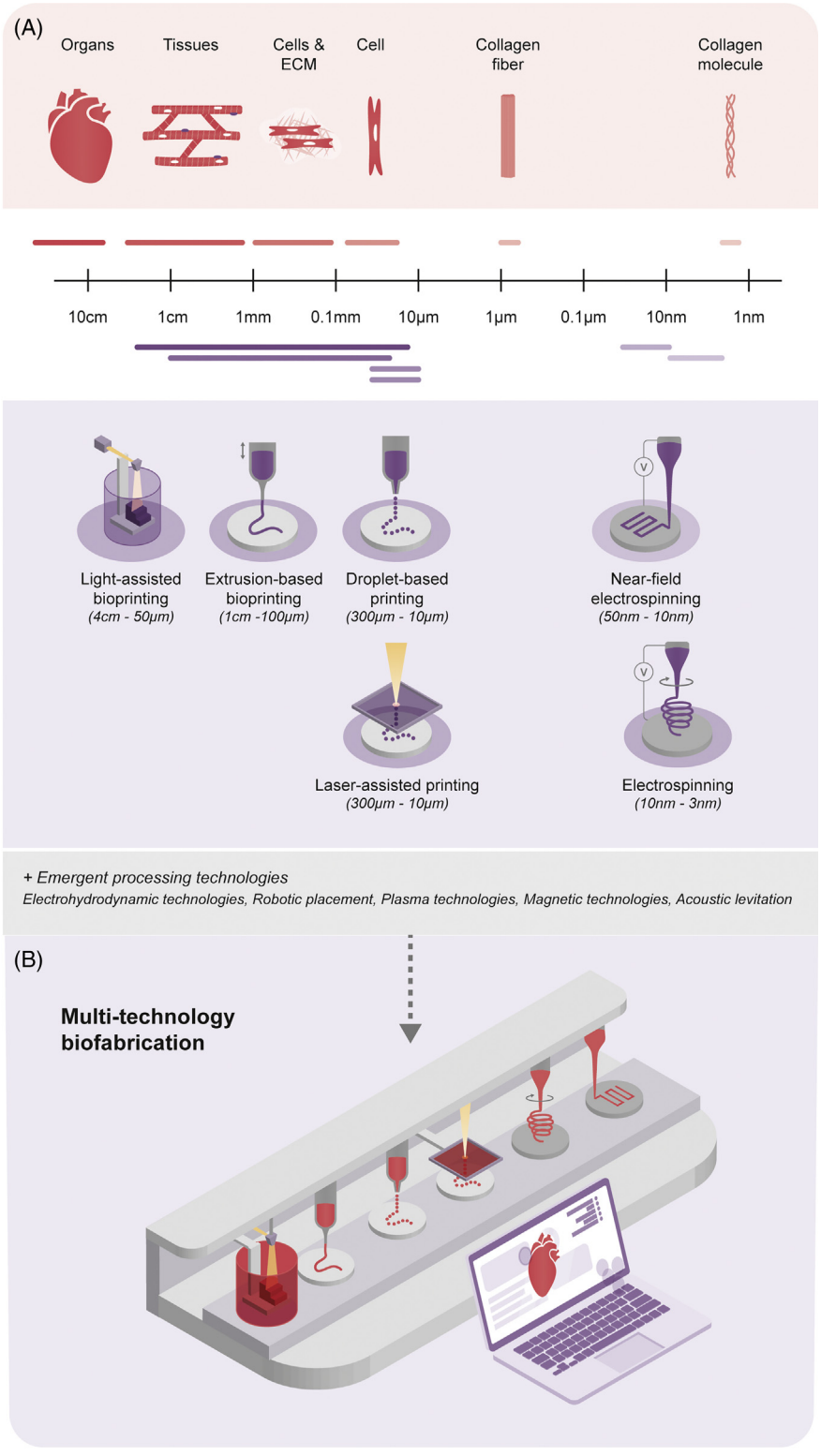
Here, we provide an overview of the current advances on combining biofabrication technologies and discuss opportunities and challenges for converging existent and emerging processing

Box 1. Single Deposition Biofabrication Methods Mimic Shape but Compromise Function

The cornerstone of bioprinting lies in sequential layer-by-layer material deposition, which allows the manufacturing of anatomically inspired tissue equivalents potentially from patients' own cells. Since their first description during the early 21st century, the focus has been on four bioprinting technologies: droplet [49], extrusion [50], light [51,52] and laser [53] based bioprinting. Until now, the majority of these technologies have relied on a single deposition method, which cannot fully replicate the complexity and composition of living tissues. **Droplet-based bioprinting**, which involves selective deposition of cell-containing material droplets, can produce 3D structures from more than one cell suspension with micron resolution, but cannot achieve biologically relevant cell densities (achieved cell density $<10^6$ cells/ml) [49] or large tissue sizes (achieved size <3 cm thickness) [54,55]. **Extrusion-based printing**, which encompasses the selective dispensing of a material through an extrusion nozzle, allows for the deposition of more physiologically relevant cell densities, yet compromises on printing resolution (achieved resolution >100 μm), and prevention of cell damage during extrusion remains challenging [56,57]. Further research showed that **electrohydrodynamic biofabrication** technologies, which combine extrusion-assisted printing within an electrical field, can increase resolution down to the submicron range and, therefore, potentially resemble the complex ECM microenvironments of biological tissues; however, cell compatibility and low reproducibility are still of concern [58]. **Light-based bioprinting**, which comprises the selective solidification of a cell-containing hydrogel layer by applying a light energy source (e.g., UV or visible light), is not limited by shear stresses and typically allows for the manufacture of volumetric constructs (cm scale) [52] with considerably higher resolutions (>50 μm) [51]. Important disadvantages are related to the limited flexibility regarding the use of multicell types and material combinations. By contrast, **laser-based bioprinting** involves the selective application of a pulsed laser to an absorbing layer, containing a cell-laden hydrogel ink, which induces the transfer of a cell-hydrogel droplet to a receiver substrate [53]. The innovative contactless material deposition involved in this bioprinting strategy allows for higher cell densities than the previously discussed bioprinting technologies. However, its potential to print large, volumetric structures and incorporate multiple biological components requires further attention. One limitation that is common to all the described manufacturing technologies is the low mechanical resistance of the bioprinted constructs. To date, most bioprinting technologies use intrinsically weak hydrogels that can provide the right conditions for cell survival, yet fail to withstand the harsh mechanical environment observed *in vivo* [59,60].

Glossary

Acoustophoresis (AP) and magnetophoresis (MP): arranging microparticles and/or cells by applying a controlled acoustic or magnetic field, respectively, to a material.
Artificial intelligence (AI): set of numerical algorithms able to make decisions without being explicitly programed.
Biofabrication: automated generation of biologically functional products with structural organization from living cells, bioactive molecules, biomaterials, and cell aggregates through bioprinting or bioassembly and subsequent tissue maturation processes.
Digital design: process of generating 3D models with a computer-based software followed by evaluation of their performance (e.g., structural, mechanical or biological) using numerical simulation tools.
Digital light processing (DLP): process of generating a 3D structure by light- or laser-assisted resin curing.
Droplet-based: process of accurate droplet deposition by generating pulses in the nozzle with acoustics (piezoelectric or ultrasound) or fluctuations in air pressure (microfluidic systems).
Electrohydrodynamic processing: generation of nanometer to micrometer-scale fibers by establishing an electrical field between the deposition material and collecting surface; includes solution electrospinning, melt electro-spinning, and writing
Extrusion-based printing: (micro)-extrusion of a material through a nozzle to allow fiber deposition in a layer-by-layer fashion. Extrusion can be regulated pneumatically, or by use of a mechanical piston or screw system.
Inline printing process monitoring: access fidelity of cell/biomaterial deposition during the printing process using machine vision and inspection sensor systems of key printing parameters and printing environment conditions.
Multitechnology biofabrication: automated process that integrates complementary manufacturing technologies into a single biofabrication platform to produce biological structures. Integrated technologies operate in a collaborative way and allow in-process variation of printing length scale and simultaneous processing of different materials.



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technologies towards functional tissue manufacturing. Furthermore, we identify future directions, in particular how **digital design** can be synergized with multitechnology biofabrication platforms. In a decade of exponential growth of information technologies, particularly machine learning and AI, we believe that numerical technologies when coupled to advanced bioprinting systems will have a key role in realizing high-throughput fabrication of **functional living tissues**.

Convergence of Biofabrication Technologies

What Is Multitechnology Biofabrication?

Multitechnology bioprinting can be defined as the integration of complementary fabrication technologies into a single biofabrication platform, wherein they operate in a synergistic manner to deliver living, functional constructs. Complementary processes can include different, but compatible, component deposition methods, such as the primary bioprinting classes reviewed in Box 1, but also other emergent manufacturing technologies, such as fiber deposition methods, magnetic technologies, acoustic levitation, or plasma technologies.

The foreseen integration of complementary technologies in a single-printing platform is not a trivial challenge, but would allow in-process variation of printing length scales, materials, and deposition methods, which is a clear deviation from trends in the conventional 3D (bio-)printing space. True convergence can only occur where the interchange between different manufacturing techniques occurs automatically without the need for operator intervention, thus moving away from existing multistep assembly methods. Furthermore, while most bioprinting techniques have inherent commonality in the use of three-axis positioning systems, there are distinct differences in the resolution and positional accuracy of these systems, as well as the software used to manage them. Convergence will lead to hardware design compromises, where higher resolution (and more expensive) positioning systems necessary for high-resolution deposition techniques have to be used for low-resolution systems. Additionally, in process characterization technologies (e.g., optical or ultrasound) and appropriate software to enable the detection of cell damage and print errors, and subsequent adaptation, will be required. These adaptive software tools will potentially need to determine whether a printing process has 'failed' or if it can be 'recovered' by adapting printing parameters or trajectories, so that machine efficiency as well as efficient use of cell-laden bioinks is maximized. It is here that the use of AI will become fundamental for accounting for all printing scenarios and parameters selection. In addition, these digital tools will help in determining the optimal shaping of bioprinted constructs to guide matrix deposition for functional tissue formation.

In our opinion, multitechnology biofabrication represents a paradigm shift in tissue manufacturing because it allows the combination and spatial organization of different cell types and biological or artificial components, which is not possible with single-process printing methods or conventional manufacturing processes. This current trend in the fabrication of living tissues is observed in the most recent scientific literature, as reviewed later.

Converged Biofabrication Technologies and Increased Functionality of Manufactured Tissues

While the first report on leveraging functionality of biomedical devices by combining 3D printing technologies with traditional manufacturing methods dates back to the 1990s [9], only very recently have researchers focused on integrating the working principles of different biofabrication

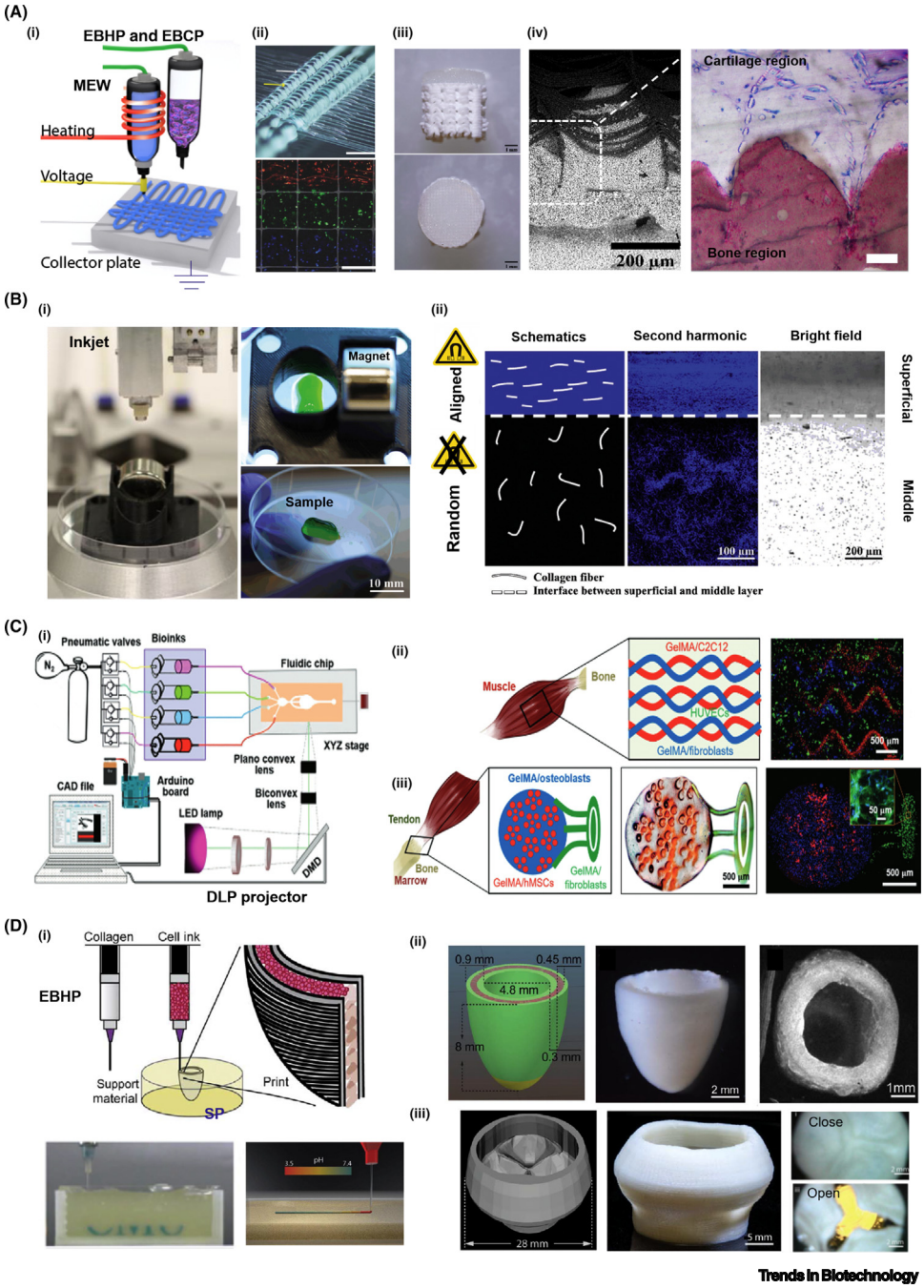
Figure 1. Schematic Illustration of Multitechnology Biofabrication. (A) Comparison of the typical operation length of single deposition biofabrication technologies with the size and hierarchical structure of tissues and organs. (B) In-process variation of printing length scales and simultaneous material processing potential when complementary biofabrication technologies are combined in a single-printing platform. Abbreviation: ECM, extracellular matrix.

technologies to enhance the functionality of artificially generated tissues. One of the earliest reports presented the combination of electro-driven fabrication technologies with bioprinting strategies, in particular, the combination of melt spinning technologies with extrusion- [10,11] or light-assisted bioprinting [12]. For example, melt electrowriting (MEW) and extrusion-based bioprinting have been successfully integrated in a single biofabrication platform, allowing for the fabrication of constructs with a spatial distribution of different cell types and improved mechanical functionality without compromising cell viability and differentiation (e.g., cartilage [10] and osteochondral tissue repair [13]) (Figure 2A). This combination of technologies provided the groundwork to solve one of the current biofabrication conundrums, namely the lack of biomechanical properties of the bioprinted constructs. From a scale-up perspective, the lengthy fabrication time of the fiber technologies (e.g., >1 h for MEW constructs with 600 mm^3 [10]) remains a challenge and negatively impacts cell viability due to hydrogel drying. We anticipate that future hybrid fiber-cell printing apparatus will move from the conventional multinozzle approach towards gradually implementing needleless printheads. A similar strategy is already used for industrial-scale production of fiber yarns [14]. Alongside this strategy, we foresee that the collector platform could be implemented on a climatized, fluid nebulizer system that could prevent hydrogel drying and maintain cell survival during extended manufacturing times. This will require further decoupling of the high-voltage components from the main components of the climatization platform, and the design of low-conductivity fluids that experience minimal effects within electrical fields.

An alternate combinatorial approach that can precisely control the local material composition and orientation on printed structures is the combination of magnetic fields with droplet- [15] or light-based printing technologies [16]. For example, Betsch and coworkers incorporated a magnetic field into a droplet-based bioprinter to align chondrocyte-loaded collagen fibers during bioprinting (Figure 2B), while Martin and colleagues proposed a hybrid system that integrates magnetic control with digital light processing (DLP) to fabricate graded composite structures, including an 'osteon-like' microstructure [16]. A major limitation of present set-ups is the low intensity and the bidirectionality of the magnetic field generated (in the milli-tesla range, and along collector plate plane). To overcome this, we anticipate that the next generation of droplet- or light-based bioprinter could be placed inside large magnetic coils, preferably covering the three cartesian coordinate axes (X, Y, and Z), which could generate higher intensity magnetic fields (in the tesla range) and control the direction of the field throughout a spherical volume. Special attention will have to be paid to ensure that the magnetic forces do not interfere with the operation of the dispensing systems being used.

Other emerging approaches comprise extrusion-based printing with light-based bioprinting [17] or sacrificial support materials [18]. Such strategies have the potential to process biologically relevant materials that were previously marked as 'unprintable', while maintaining high cell viability and allowing for the fabrication of tissue-like constructs with biologically relevant structures and sizes. For example, Miri and coworkers integrated an automated extrusion-based microfluidic chamber with DLP in a single bioprinting device (Figure 2C). By combining multiple cell-laden hydrogels at high cell densities and with refined spatial resolution, sophisticated biological structures, such as a skeletal muscle strip and a tendon-to-bone insertion on a millimeter size scale, were achieved [17].

The groups of Feinberg, Miller, and Grover developed a suspended layer bioprinting process that has been shown to allow the fabrication of complex biological systems on a centimeter scale [18–20]. A microgel suspension (fluid gel) was used to structure a secondary extrusion-based printed cell-laden solution, which was cured post printing and subsequently removed as a separate construct. This allowed a range of different living tissue structures to be printed, from an osteochondral unit of 2 mm in height [20] to a trileaflet heart valve of ~3 cm in diameter



Trends in Biotechnology

Figure 2. Examples of Multitechnology Biofabricated Tissues with Improved Material Combinations and Hierarchical Structures. (A) (i) Osteochondral implant obtained by extrusion-based thermoplastic printing (EBHP) and melt electrowriting (MEW); (ii) MEW fibers of polycaprolactone (PCL) guided over a pluronics hydrogel strand; (iii) osteochondral unit comprising a GelMA hydrogel reinforced with a MEW printed fiber scaffold at the chondral region and a printed calcium phosphate (CaP) at the bone layer. (iv) SEM image and histology section of cross-section of the osteochondral unit revealing embedded MEW fibers within the (CaP) region and new cartilage and bone tissue being formed at the chondral and osteo regions, respectively. Scale bars: 500 μm (ii) and 100 μm (iv). (B) (i) Chondrocyte-laden constructs with a zonal collagen

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(Figure 2D) [21]. Although this approach deviates from the combination of two or more technologies into a single device, it is, in our opinion, also a form of multitechnology biofabrication because it merges the principles of in-liquid printing, initially introduced by the stereolithographic systems, with conventional extrusion-based printing. Moreover, this approach shows great promise towards tackling organ fabrication upscaling and vascularization challenges, and might become one of the most impactful technologies in the biofabrication field if converged with other emerging fabrication technologies, such as acoustophoresis [22,23]. We foresee that, by manipulating acoustic waves in bulk fluid gels, or even directly in cell-suspended media, it will be feasible to improve cellular organization and further introduce an extra level of control over the cellular nano and microenvironment. The first hybrid apparatus of this configuration will potentially implement low-frequency sound generators positioned around the suspended bath container. Focus should lie on preventing interference of resonant frequencies with the bioprinter hardware. Additionally, acoustic field propagations and forces in cell-laden biological fluid gels should be controlled.

Furthermore, promising results on improving cell–material interactions and in directing stem cell behavior have been reported by combining extrusion-based bioprinting with plasma technologies [24] or droplet-based printing [25]. The combination of atmospheric plasma and extrusion-based printing allowed the selective introduction of biological cues (e.g., different growth factors) on extruded polymer filaments, which can potentially guide and accelerate tissue renewal [24]. Alternatively, Liu and coworkers combined extrusion-based printing with inertial force jetting, a derivative of droplet-based bioprinting that uses alternated viscous and inertial force jetting. This integrated method allowed the precise deposition of cells on designated locations, thereby inducing cell interactions at a distance of <100 μm [25]. In this process, fine control of cell distribution was achieved through imaging of an extrusion-based printed cell-laden material to manually determine the ideal locations for single cell depositions. We envision that the next progressive steps will be incorporating the co-extrusion of materials to achieve a gradient in cell densities in the bulk substrate and automation of the required secondary cell location, in short, adaptive gradient control. An overview of current efforts towards multitechnology biofabrication and their main characteristics is summarized in Table 1.

From Digital Design to 'Semiautonomous' High-Throughput Devices

The idea of engineering living tissues by digitally controlling the organization of tissue equivalents based on model predictions is both intriguing and potent and, in our opinion, will have a key role in the development of high-throughput biofabrication of functional tissues. The boost in computational power, big data collection, and AI techniques have the potential to enable systematization, automation, and control of design and biofabrication. Together with rational digital design technologies grounded on formalized scientific experience and mechanistic understanding, supervised and unsupervised machine learning should be harnessed to support semiautonomous biofabrication solutions to complex design problems.

Digital Design Technologies for Instructed Bioprinting

Typically, the flexibility of bioprinting draws from numerous design parameters, including multiscale architecture, material composition, and dynamic cell–cell and cell–material interactions

organization obtained by combining inkjet bioprinting with a magnetic field; (ii) collagen bioinks with aligned collagen fibers in a superficial layer increase the compressive properties of printed structures. (C) (i) Skeletal muscle strip and tendon to bone interphase obtained by combining a extrusion-assisted microfluidic chamber with digital light processing. (ii) Muscle strip comprising interwoven GelMA containing C2C12 cells (red) and gelMA containing fibroblast (blue) filaments; (iii) tendon-to bone insertion containing patterned gelMA with osteoblasts (blue), human mesenchymal stem cell (hMSC; red), and fibroblasts (green) filaments. (D) (i) Human-scale ventricle model and trileaflet heart valve printed by extrusion-based hydrogel printing inside a suspended bath; (ii) ventricle model comprising cardiac cells (pink) and supporting collagen shells (green); (iii) trileaflet heart valve supporting pulsatile flow. Reproduced under a Creative Commons Attribution Non-Commercial License CC BY-NC from [10] (A2); reproduced with permission from [15] (B), [17] (C), and [21] (D).

Table 1. Representative Examples of Converged 3D (Bio) Fabrication Technologies[a]

Converged technologies		Cell printing	Main characteristics	Refs
AVIF	EBHP	Yes	Structuring cell/biomaterials with 100-μm precision	[25]
DLP	EBHP	Yes	Control over cell and biological gradients; flexibility over multimaterial deposition	[17]
EBCP	MEW	Yes	Improved soft–hard interface tissue regeneration and interfacial strength	[13]
EBHP	MEW	Yes	Improved mechanical properties of soft cell-laden hydrogels; control over reinforcing architectures	[10]
	A	Yes	Control over macro and microarchitectural characteristics of living tissues; contact-less cell organization	[23]
	SP	Yes	Tissue upscaling; incorporation of large cell densities and viable vasculature networks	[61]
EBTP	MES	No	Fabrication at larger length scale (macro and micro)	[11]
	AP	No	Guided new cell and new tissue formation by biomaterials functionalization with biologics	[24]
	EBHP	Yes	Anatomically shaped constructs; improved mechanical properties of soft microtissues	[62]
IJ	M	Yes	Optimal for anisotropic living tissue fabrication; contact-less control of micro and nano-sized bioinks	[15]
	SE	Yes	Tissue upscaling; improved mechanical properties of soft microtissues	[63]
SL	SE	Yes	Guided new tissue formation and improved mechanical properties of soft microtissues	[12]

[a]Abbreviations: A, acoustophoresis; AP, atmospheric plasma; AVIF, alternating viscous and force jetting; DLP, digital light processing; EBCP, extrusion-based cement printing; EBHP, extrusion-based hydrogel printing; EBTP, extrusion-based thermoplastic printing; IJ, inkjet; M, magnetophoresis; MEW, melt electrowriting; MES, melt electrospinning; SE, solution electrospinning; SL, stereolithography; SP, suspended printing.

[26,27]. Understanding and predicting the effect of multiple interacting design features involves different disciplines and creates a challenge in developing design principles and strategies for functional 3D bioprinting. Various tissue-engineering (TE) design parameters are interdependent, and conflicting objectives need to be addressed in the design, such as the necessity for both significant scaffold stiffness and high porosity, in the case of bone TE [26]. Interdependent and conflicting design parameters and objectives require comprehensive methodical optimization techniques [28,29], which can only realistically be solved using computing.

Optimization techniques involve building numerical models of the multiphysics processes at play in a bioprinted construct to predict the influence of design parameter changes on the construct properties. 'Parametric design' is well suited to these aims, and refers to a design methodology that is built on algorithmic thinking and relies on the definition of a family of initial parameters and the relationships they keep with each other and with the final design. Parametric design naturally enables systematic parameter space exploration and subsequent selection of optimal design parameter set [30]. Another powerful digital design approach that is well established in structural/mechanical engineering is topology optimization, where the best distribution of material within a selected design space is numerically derived to comply with a set of constraints [31]. 3D fabrication appears to synergize with topology optimization because it allows for practical production of the resulting organic shaped structures that are often incompatible with conventional fabrication techniques. Promising attempts have been made at implementing topology optimization for TE construct designs, in particular to jointly meet both stiffness and permeability criteria [28,29,32].

Despite these computational advances, bioprinting designs still rely on arbitrary parameter selection and decisions are made based on a trial and error approach. This suboptimal design methodology incurs substantial costs in both time and expenses related to *in vitro* or *in vivo* experiments [26]. Computational efficiency is one of the greatest current obstacles to the large-scale use of digital design optimization for bioprinting constructs. Microscale continuum models are used to predict the mechanical behavior of the scaffold with a resolution relevant to cellular processes. In addition, inclusion of multiscale, multiphysics, time-dependent phenomena, such as fluid–solid interactions and mechanobiology processes, dramatically increases the number of variables to solve [33,34]. A common solution to overcome computational complexity is to reduce the scope of the simulation to a smaller number of structural elements [33,35] although the direct implication is the necessity to use homogeneous cellular structures. We envision that implementing 'soft computing' techniques, such as metamodels, to integrate empirical evidence and human-like 'vagueness' in computational modeling will significantly increase the design flexibility and leverage design-centered biofabrication for better biomimicry [36–38]. AI techniques, including regression models and neural networks [39], are ideal candidates for such fast-running metamodels, with only limited reduction in accuracy compared with complex multiphysics mechanistic models. Empirical knowledge that is not directly or homogeneously interpretable by humans can be harnessed via unsupervised machine learning [40].

Flexible and 'Semiautonomous' Bioprinting Platforms for Functional Tissue Fabrication

We envision that the next generation of bioprinters will become more practical, user-independent, 'semiautonomous' systems. Advances such as process parameter selection and real-time monitoring of cell function and material properties during bioprinting will become commonplace. Given that functional tissue fabrication also includes complexity in anatomical design, it is conceivable that collaborative robotic systems (robotic arms) will work in unison to create functionally heterogenous structures; however, this will not be viable if the positional accuracy and software control of these robotic arms cannot at least match the accuracy of more established three-axis platforms. Up to now, conventional approaches to biofabrication have relied on deposition technologies integrated with three-axis positioning systems. In most cases, this means that structures are deposited onto planar substrates. This inherent requirement is a limitation that is not representative of the natural anatomical relevant structures that the biofabrication field aims to recreate. Several groups have taken inspiration from established computer numerical control (CNC) machining techniques to include additional axes, such as a fourth rotary axis beneath the deposition head to produce structures of increasing complexity on cylindrical mandrels, including radial stents [41] and valve structures [42].

Moreover, as a means to work outside of the restrictions of automated positioning systems, there are a growing number of examples of handheld, freeform biofabrication [43,44] devices with the intent to rapidly translate advances in biomaterials research into the clinical environment. Operation of these devices is reliant on the surgeon's expertise and fine motor control to directly deposit biomaterials to the *in vivo* point of need. To mimic the surgeon's motion in an automated platform, it is necessary to begin using six-axis robotic arms. Multiaxis robotic arms will not only facilitate handheld biofabrication, but also significantly improve the process flexibility of multitechnology bioprinting platforms, allowing cell and/or biomaterial deposition onto clinically relevant geometries and facilitating the interchange between printing technologies. However, adopting these systems will present challenges to the biofabrication research community due to the need for significantly more sophisticated control programming and user interface development. Current digital design tools, slicing algorithms, and tool path generation software, typically outputting G-code instructions, are not compatible with robotic system language. Therefore, we envision that the already interdisciplinary biofabrication field will need an influx of advanced robotics engineers and

computer engineers as the use of systems with increased degrees of freedom becomes more common. In addition, we foresee that AI will be fundamental for linking digital design tools with control instructions for positioning systems. Due to the fabrication constraints of some bioprinting technologies, specific AI algorithms will have to be developed to automatically remove unnecessary geometrical features from digitally design models and adequately position objects within the building substrate. For example, bioprinting technologies, such as EHD, operate in the base of a single, continuous filament deposition, while light-based technologies require homogeneous light penetration, which will significantly limit tool path direction and cell distribution on a converged set-up.

The next generation of multitechnology bioprinters will also encompass real-time inline monitoring of the printing process through combinations of machine vision, inspection sensors, and feedback control systems so that deviation from preplanned designed structures can be detected and the printing process automatically adjusted to compensate for the error. We believe that a profound impact will be seen from the area of machine vision, extending the application of AI to monitor and control the bioprinting process. To make this feasible, three main challenges must be solved. First, optical- and laser-profiling technologies will have to be integrated into the multitechnology bioprinting platforms to allow for screening of biofabricated constructs within different length scales (i.e., at both the cellular and tissue/organ scale). Here, the use of lens-free microscopes should be attractive due to their small form factor, only a few cm^3, and their potential to monitor dynamic biological processes without the need for cell labels [45]. An alternative 'machine vision' strategy was shown by Ruland *et al.* with a quantitative ultrasound imaging system that allows for cell growth and new tissue formation monitoring within bioprinted constructs [46]. Second, different inspection sensors, such as speed, material reservoir volume, temperature, CO_2 and O_2, humidity, and pH, will have to be integrated in the printing platform to monitor key instrument parameters and environmental conditions. The first imperative steps to integrating sensors for motion/vibration, temperature, and humidity within standard 3D printers have already been taken [47]. Third, specific AI software for bioprinting will have to be developed to analyze the large data sets that are collected from machine vision and inspection sensors. Based on the information gathered, AI will have to produce real-time predictions on how printing parameters (e.g., dispensing rate, light intensity, fabrication temperature, collection speed, and/or printing chamber environment conditions) should be adapted to correct identified flaws on bioprinted constructs. AI will also be essential to identify crossover points between multiple fabrication technologies and incompatible printing parameters. However, reliable AI algorithms will have to trained based on test cases and first be proven with simple technology and material combinations before full technology convergency. Here, deep neural networks is perhaps the most promising AI method due to the large data set that can be processed and recent experience with the specificities of each technology [48].

Concluding Remarks and Future Perspectives

We are now at the crossroads where biofabrication technologies have opened exciting perspectives to restore or replace damaged tissues and organs, although each technology individually has not yet been able to deliver functional tissue structures of biologically relevant size. The integration of complementary fabrication technologies in a single-printing platform has recently given rise to what we believe is a new biofabrication era (see Outstanding Questions). The in-process variation of printing length scale and different material processing capabilities of multitechnology biofabrication platforms is a clear deviation from conventional 3D (bio-)printing, which is opening new perspectives for the fabrication of hierarchical structures with relevant sizes and combinations of different cell types and ECM components. Examples of functional multitechnology bioprinted constructs are diverse, ranging from mechanical robust articular cartilage constructs that are manufactured by melt-spinning technologies and extrusion-based bioprinting, to skeletal muscle strips obtained by extrusion-assisted microfluidic chamber combined with digital light

Outstanding Questions

Can we further recapitulate the functionality of native tissue by combining complementary manufacturing technologies into a single biofabrication platform?

Which biofabrication technologies can successfully be combined and which combinatorial approaches can achieve the required resolution and material combinations to further mimic tissue structure, composition, and function?

How can we achieve high-throughput biofabrication?

processing, and human-sized trileaflet heart valves manufactured by merging extrusion-based printing with suspended manufacturing. Further evidence of the impact and potential of multitechnology bioprinting in the RM field are the different commercially available multitechnology bioprinters recently introduced by bioprinting companies.

However, one of the current challenges is that the complexity of the equipment is increasing exponentially, making multitechnology printers more viable in a laboratory environment than for

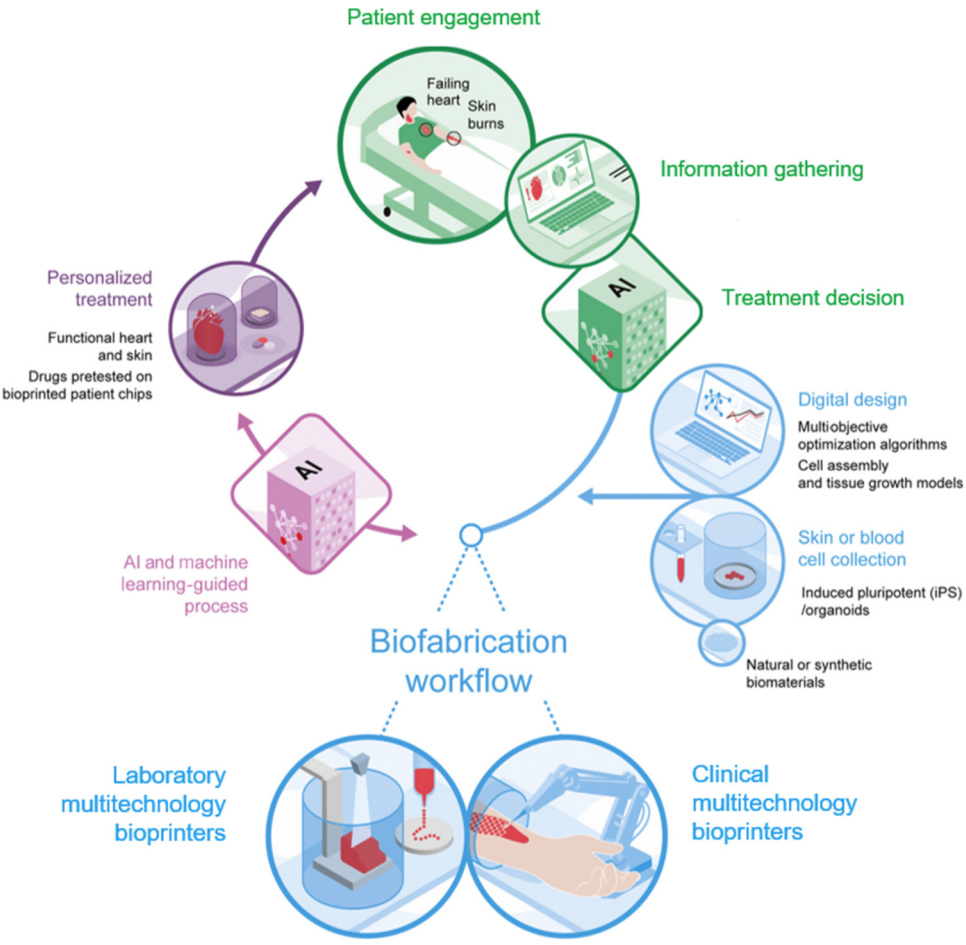

Figure 3. How Will Multitechnology Bioprinters Improve Patient Care? Example of a future patient treatment process chain where multitechnology bioprinters will support artificial tissue and organ fabrication. The process chain starts with a patient engaging with the healthcare system after experienced health problems, such as a failing heart and skin burns (Step 1. Patient engagement). Then, the gathering of patient clinical information begins based on physical examinations and diagnostic tests (Step 2. Information gathering). Subsequently, a treatment decision is taken by clinicians with the support of artificial intelligence (AI) algorithms to facilitate the integration and classification of lesions or affected organs (Step 3. Treatment decision). Depending on the affected tissue, two biofabrication routes will be followed: *in situ* tissue bioprinting for outer tissue fabrication using simple multitechnology bioprinters (clinical multitechnology bioprinters) and laboratory tissue bioprinting for solid organs and personalized organ-on-a-chip fabrication using a more complex combination of bioprinting technologies (laboratory multitechnology bioprinters) (Step 4. Biofabrication workflow). Biofabricated tissues will be developed from the patient's own cells [induced pluripotent stem cells (iPSCs) or organoids] and combined with natural or synthetic materials. Tissue organ design will be conducted by using a digital design tool and the biofabrication process will be assisted by AI algorithms. The personalized heart, skin, and drug (tested on patient chips) are then delivered to the patient (Step 5. Personalized treatment).

clinical use. Alternatively, the realization of less complex systems customized for particular applications is beginning. For example, handheld devices are gaining more attention for *in situ* fabrication of 'outer tissues', such as skin, cornea, and cartilage. In addition, we believe the recent advancements in multiaxis robotic systems will allow printing onto surfaces that more closely resemble the contours of natural structures in our body, while inspection sensors and real-time monitoring of the printing process will improve process reproducibility. In parallel, we believe that AI will have an important role in this new biofabrication era. Given that humans can only analyze limited information simultaneously, new multitechnology biofabrication hardware will be powered by AI tools to aid simultaneous monitoring of printing parameters and printed parts. To help in organizing our view, we have developed a conceptual model that illustrates how multitechnology bioprinters could be used in a future patient treatment process chain (Figure 3).

Finally, another important challenge that remains is the synergistic potential of the combination of digital design and biofabrication. Integrated computer models of biosystems, as well as numerical optimization techniques, are key for the fast and reliable design and manufacture of advanced functional biological and biomedical constructs. Smart and flexible computing will be pivotal to enable on-demand tailored and cost-effective biofabrication solutions to complex design problems. In our opinion, the next-generation biofabrication systems should transcend native tissue structural replication and actively direct promote the development of functional tissue structures.

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