Biofabrication

Trends in Biotechnology

CellPress REVIEWS

Special Issue: Tissue Engineering

Feature Review

Biofabrication: A Guide to Technology and Terminology

Lorenzo Moroni,[1,*,@,@] Thomas Boland,[2] Jason A. Burdick,[3] Carmelo De Maria,[4] Brian Derby,[5] Gabor Forgacs,[6,7] Jürgen Groll,[8] Qing Li,[9] Jos Malda,[10,11] Vladimir A. Mironov,[12,13] Carlos Mota,[1] Makoto Nakamura,[14] Wenmiao Shu,[15] Shoji Takeuchi,[16] Tim B.F. Woodfield,[17] Tao Xu,[18] James J. Yoo,[19] and Giovanni Vozzi[4]

Biofabrication holds the potential to generate constructs that more closely recapitulate the complexity and heterogeneity of tissues and organs than do currently available regenerative medicine therapies. Such constructs can be applied for tissue regeneration or as *in vitro* 3D models. Biofabrication is maturing and growing, and scientists with different backgrounds are joining this field, underscoring the need for unity regarding the use of terminology. We therefore believe that there is a compelling need to clarify the relationship between the different concepts, technologies, and descriptions of biofabrication that are often used interchangeably or inconsistently in the current literature. Our objective is to provide a guide to the terminology for different technologies in the field which may serve as a reference for the biofabrication community.

Trends

Biofabrication holds great potential in the fields of regenerative medicine and physiological 3D *in vitro* models by allowing the manufacture of complex tissue constructs with a higher degree of biomimicry to native tissues than do current biomedical solutions.

As the number of biofabrication technologies being developed continues to expand, it is of paramount importance to adopt a concerted terminology framework and avoid generalizations.

The ratio between the spatial resolution and the timescale of manufacture could be considered as a reliable measure to aid in the selection of an appropriate biofabrication technology for a desired application.

What Do We Mean by Biofabrication?

By combining the principles of engineering, biology, and material science, biofabrication holds great promise to change the toolbox for many biotechnological disciplines. Recently, in the context of tissue engineering and regenerative medicine applications, the definition of biofabrication as a research field was updated as 'the automated generation of biologically functional products with structural organization from living cells, bioactive molecules, **biomaterials** (see Glossary), cell aggregates such as micro-tissues, or hybrid cell-material constructs, through **bioprinting** or **bioassembly** and subsequent tissue maturation processes' [1]. This definition includes the fabrication of scaffolds with hierarchical structural properties or smart-surface properties within the realm of bioprinting. It was reasoned that the design of such features would be indispensable to obtain structurally functional biological substitutes. This work provided an overview of the historical evolution and broader meaning of the term, and also specified the research field with a focus on applications in tissue engineering and regenerative medicine, and proposed bioprinting and bioassembly as the two major approaches to biofabrication. Despite this definition and positioning of the field, as well as recent reviews that nicely provide a common framework to the additive manufacturing field at large [2–5], the terminology commonly used, especially in recent literature, is not clearly defined and lacks consensus. This absence of an agreed and accepted terminology can, and partially already does, lead to uncertainty or confusion in the description of new approaches and possible misunderstanding as to where a new report fits in relation to previous reports. This could impede the development of the field by making it difficult to correctly map progress in the science and technology of biofabrication.

We intend here to follow-up from our previous review updating the definition of biofabrication for tissue engineering and regenerative medicine applications [1]. We aim to set an overarching

[1]MERLN Institute for Technology-Inspired Regenerative Medicine, Department of Complex Tissue Regeneration, Maastricht University, Maastricht, The Netherlands
[2]Department of Metallurgical, Materials and Biomedical Engineering, The University of Texas at El Paso, El Paso, TX, USA
[3]Department of Bioengineering, University of Pennsylvania, Philadelphia, PA, USA
[4]Research Center 'E. Piaggio' and Dipartimento di Ingegneria dell'Informazione, University of Pisa, Pisa, Italy
[5]School of Materials, University of Manchester, Manchester, M13 9PL, UK
[6]Department of Physics, Biology and Biomedical Engineering, University of

 https://doi.org/10.1016/j.tibtech.2017.10.015

terminology framework by clarifying the technologies used within biofabrication strategies, as well as rationalizing appropriate terminologies, as an integral basis for communication in all the different application fields of biofabrication. We therefore feel that a brief review of classical and novel biofabrication approaches, with the aim to point out the differences among them and the limitations that must still be overcome, is timely. A Glossary of the main different terminologies clarified in this article is also provided in this article. We also point out potential future research directions where we believe biofabrication may have a major impact, with the objective to collaborate with industry to bring biofabrication strategies to the clinic in a more efficient and consensual manner, and to collectively overcome future regulatory and ethical challenges.

Technologies Used for Biofabrication

As previously indicated, biofabrication strategies employed for tissue engineering and regenerative medicine can be identified as either bioprinting or bioassembly. To further classify the various technologies used for these strategies in terms of efficiency of fabrication, we introduce the spatial **resolution/time for manufacturing (RTM) ratio** as a quantitative characterization of the process underlying a specific technology, considering the ability to produce scaffolds with fine details in a short time as a measure of merit. The RTM ratio is defined as:

$$RTM = \frac{Spatial\,resolution}{Time\,for\,manufacturing} \cong R \cdot P = \frac{1}{d} \cdot \frac{V}{t} \quad (1)$$

Here, R is the best spatial resolution that can be achieved within the technology, and P is the delivery rate of the material being printed or assembled. R is expressed as the inverse of the **minimum feature dimension**, d: d is measured in m, thus R is measured in 1/m; P is expressed as the volume, V, of material (measured in m^3) delivered per unit of time, t (in minutes). As a consequence, the physical dimensions of the RTM ratio are square-length/time. In the biofabrication field, the order of magnitude of R and P are 1/μm and mm^3/minute respectively: hence, the RTM ratio must be expressed in 10^{-3} m^2/minute for an easier comparison between different technologies. Note that, for each specific technology, R and P may vary, depending on the material delivered, as well as on the geometry of the scaffold and on its placement in the building chamber.

In the following sections, standard operating procedures are considered (such as commonly used materials, average printing parameters, single or multiple material deposition head) for building a 1 cm^3 cube of material ($V = 10^{-6}$ m^3), lying on one of its faces. Table 1 lists the average RTM ratios for the biofabrication technologies that will be considered in the present work. Figure 1 (Key Figure) gives a graphical representation of the distribution of the various biofabrication technologies in the parameter space with axes of minimum feature dimension d and delivery rate P: as explained above R and P may vary for each technology (represented by circles). In Figure 1 the contour lines of the RTM function are also plotted: technologies along the same contour line have the same RTM ratio. Generally speaking, the higher the value of the RTM ratio, the more efficient the process. Most technologies are placed along the diagonal of the '*d*-*P*' parameter space, indicating that higher delivery rates result in lower resolution, and fabricating fine details is in contrast with fast manufacturing. We briefly here outline the most common technologies to illustrate and illuminate their differences, advantages, and limitations.

The most commonly used technologies in biofabrication with a major and active role of biomaterials in the printing process comprise (i) **3D printing**; (ii) light-based technologies such as **selective laser sintering** (SLS), **selective laser ablation**, **stereolithography** (SLA), and **two-photon polymerization** (2PP); (iii) **fused deposition modeling (FDM)/3D fiber deposition (3DF)/bioextrusion**; (iv) **wet-spun automated extrusion systems**; (v) **3D plotting/bioplotting/robotic dispensing/extrusion bioprinting**; (vi) **ink-jet and**

Missouri, Columbia, MO, USA
[7]Modern Meadow Inc., Brooklyn, NY, USA
[8]Department of Functional Materials in Medicine and Dentistry, University of Würzburg, Würzburg, Germany
[9]School of Aerospace, Mechanical and Mechatronic Engineering, University of Sydney, Sydney, Australia
[10]Department of Orthopedics, University Medical Center Utrecht, Utrecht, The Netherlands
[11]Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands
[12]3D Bioprinting Solutions, Moscow, Russian Federation
[13]Sechenov Medical University, Moscow, Russia
[14]Graduate School of Science and Engineering for Research, University of Toyama, Toyama, Japan
[15]Department of Biomedical Engineering, University of Strathclyde, Glasgow, UK
[16]Institute of Industrial Science, University of Tokyo, Tokyo, Japan
[17]Department of Orthopedic Surgery and Centre for Bioengineering and Nanomedicine, University of Otago, Christchurch, New Zealand
[18]Department of Mechanical Engineering, Tsinghua University, Beijing, China
[19]Wake Forest Institute for Regenerative Medicine, Wake Forest School of Medicine, Winston-Salem, NC, USA
@Twitters: @MoroniGroup, @BioFabUnipi

*Correspondence: l.moroni@maastrichtuniveristy.nl (L. Moroni).

Table 1. Main features and Limitations of Biofabrication Techniques

Technique	RTM ratio (10^{-3} m^2/minute)	Minimum feature width ($\mu m = 10^{-6}$ m)	Limitations	Refs
3D™ Printing	Medium (~0.1)	~300	Presence of polymeric grains and of excess solvent	[6,7]
Selective laser sintering	Medium to high (~1)	<400	Presence of polymeric grains; limited to non-thermolabile materials	[8–10]
Laser ablation	Medium to high (~1)	<400	Thermolabile materials (cells and proteins) can be damaged during scaffold fabrication	[12,15]
Stereolithography	Medium (~0.5)	~30–70	Use of photosensitive polymers and initiators, which may be toxic	[18,20]
Two-photo polymerization	Medium (~0.05)	<1	Use of photosensitive polymers and initiators, which may be toxic	[21,22]
Digital light processing	Medium to high (~2)	~70–100	Use of photosensitive polymers and initiators, which may be toxic	[19]
Fused deposition Modeling	Medium to high (~1)	~200	Limited use with thermolabile materials. Evident layered structure	[23–25]
PAM and wet-spun technologies	Medium (~0.5)	~20	Limited range of material available, and low vertical dimension processing time increases with the number of material heads used	[35,36]
Indirect additive manufacturing	Low (~0.03)	~200	Limited mould materials	[40–42]
Bioplotting	Medium (~0.5)	~100	Need a self-sustaining gel (bioink) with a high degree of shear thinning	[46–48]
Ink-jet bioprinting	Medium (~0.1)	~100	Limited range of gels (bioinks) available; inks must be of low viscosity	[52,117]
Valve-jet bioprinting	Medium (~0.3)	~200	Delivery rate not sufficient for building clinically relevant constructs	[56,57]
Electrospinning	Medium (~0.1)	<1	Difficult to realize thick scaffolds	[58,59]
Laser-assisted bioassembly	Low (~0.04)	~30	Difficult to realize thick scaffolds; limited range of gels available	[78,79]
Spheroid-based approaches	Low to very low (<0.001)	~300	Long time for fabrication owing to cell spheroid production and construct maturation	[71–74]

valve-jet bioprinting; and (vii) **electrospinning** (Figure 2). Most of these technologies were originally developed as additive manufacturing technologies for rapid prototyping, but are included as biofabrication strategies when used for biomedical applications.

3D Printing

With 3D printing, a jet of binder is directed at a powder bed to define a pattern controlled by computer-aided design/computer-aided manufacturing (CAD/CAM) software. The solvent binds the powder, thus forming a slice of solid material; subsequently a new layer of powder is laid down and the process is repeated to build the scaffold layer-by-layer [6,7]. The unbound powder acts as a support for the object during building, allowing the easy fabrication of re-entrant and hollow objects. It can be difficult to remove excess unbound grains and remnants of used solvents/binder. In this respect, it is important to highlight that the term 3D printing should only refer to this specific additive manufacturing technology. This technology allows the

Glossary

Bioassembly: the fabrication of hierarchical constructs with a prescribed 2D or 3D organization through automated assembly of preformed cell-containing fabrication units generated via cell-driven self-organization or through preparation of hybrid cell–material building blocks, typically by applying enabling technologies, including microfabricated molds or microfluidics.

Bio-engineered structures: biological constructs engineered by using in a predefined manner cells, biomaterials, and/or biological factors alone or in combination with each other.

Bioink: formulation of material(s) and biological molecules or cells processed using bioprinting technologies.

Biomaterial: a material that is used as (part of) a medical device or an advanced therapy medicinal product to replace, restore, or regenerate a tissue or organ and its function.

Bioprinting: the use of computer-aided transfer processes for patterning and assembly of living and non-living materials with a prescribed 2D or 3D organization to produce bio-engineered structures serving in regenerative medicine, pharmacokinetics, and basic cell biology studies. In this context, additive manufacturing of 3D scaffolds able to instruct or induce the cells to develop into a tissue mimetic or tissue analog structure, for example, through distinctive cell interaction, hierarchical induction of differentiation, or functional evolution of the manufactured scaffolds falls within bioprinting.

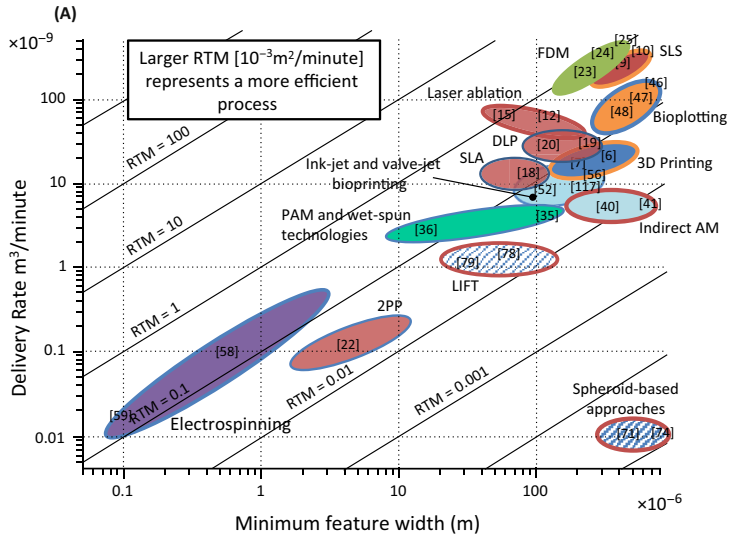
Cell spheroid: a cluster of cells with a spherical shape, typically formed by allowing a cell suspension to settle into a droplet of media.

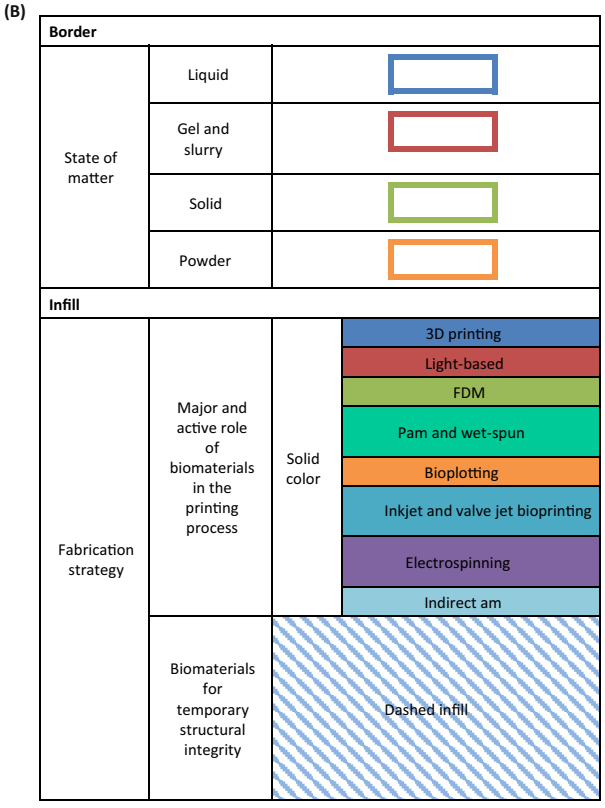
Electrospinning: a material processing technology that uses high electrical voltage to fabricate fine fibers from polymer solution or molten polymer. Fibers are deposited onto a collector, with a random or defined alignment.

Fabrication rate: the rate of fabrication of a scaffold or of a bioprinted construct using biofabrication technology. In the RTM ratio it can be calculated as the time

Key Figure

Distribution of Various Biofabrication Technologies According to Their Minimum Feature Width and Printing Rate





Trends in Biotechnology

to fabricate a 10^{-6} m^3 (1 cm^3) cube, lying on one of its faces.

Fused deposition modeling/3D fiber deposition (3DF)/ bioextrusion: additive manufacturing technologies that can be used for bioprinting in which a thermoplastic material, in shape of filament or pellet, is hot-extruded and deposited to form a layer of solid material. Using a layer-by-layer approach a 3D scaffold or a 3D construct is built.

Indirect additive manufacturing: a biofabrication approach that uses an additive manufactured mold in which a bioink is cast, injected, or compressed.

Ink-jet and valve-jet bioprinting: printing systems able to bioprint constructs in a layer-by-layer manner by ejecting bioinks in the form of droplets via the nozzle head. Droplet ejection is controlled either by piezo- or thermal actuators (ink-jet), or by solenoid microvalves (valve-jet).

Laser-assisted bioprinting (LAB): also known as laser-induced forward transfer (LIFT), a bioprinting technique that uses laser pulses to deposit a bioink from a donor slide onto a substrate.

Microfluidic technology: a technology based on geometrically constrained minute volume transport in micro-channels. This technology can also be used to fabricate strands of hydrogels that are suitable as building blocks for successive assembling processes.

Micro-masonry concept: biofabrication approach in which microunits of cell-laden hydrogels are used as regenerative building blocks.

Minimum feature dimension: the smallest detail that can be fabricated using a biofabrication technology.

3D plotting/bioplotting/robotic dispensing/extrusion bioprinting: bioprinting technologies that dispense continuous filaments of hydrogel materials that are extruded through nozzles using a piston, a screwing system, or pneumatic pressure as the driving force.

Pressure-assisted microsyringe deposition (PAM)/wet-spun automated extrusion systems: additive manufacturing technologies that can be used for bioprinting based on the extrusion of polymers dissolved in volatile solvents. The quick evaporation of the solvent

(See figure legend on the bottom of the next page.)

fabrication of structures with a lateral resolution controlled by a combination of the powder particle size and the volume that the solvent penetrates by capillary action. Current commercial systems have a feature resolution around 300 μm, which is suboptimal if precise control of cell positioning is required after scaffold fabrication. Its RTM ratio is about 0.1×10^{-3} m^2/minute, and is thus at the medium level of manufacturing efficiency.

Light-Based Technologies

SLS uses a laser where the beam of light is selectively directed to a powder bed, generating local heat and forming patterns of fused material; after its solidification, a new layer of powder is laid down and the process is repeated to build the scaffold layer-by-layer. A variety of thermoplastic polymers [8], metals (in this case SLS is called 'selective laser melting'), ceramics, or mixtures of polymer-ceramic [9] and polymer-encapsulated ceramic [10] can be used, but the required high temperatures limit the utility of SLS for biofabrication processes. The source materials that are normally used require extra processing to obtain these in powder form with precise and narrow size granulometry. Selective laser ablation works in the opposite way by ablating a solid material using a very short duration laser pulse [11,12]. If the ablation process is conducted in all of the three directions, or if laminated porous films are stacked and bonded on top of each other, a 3D structure can be created. These techniques have proved useful for the fabrication of improved tissue constructs upon seeding the scaffold with cells [13–15]. These technologies do not allow the direct incorporation of pharmaceuticals, biomacromolecules (proteins, growth factors), or cells into the scaffold. Thus, they could be considered as biofabrication technologies only when the fabricated scaffolds are designed following hierarchical or smart principles to influence cell activity and achieve functional biological constructs, as previously discussed [1]. In these methods the resolution of the printed pattern depends on the laser spot size, the thermal conductivity, the surface tension, the absorption of the raw materials, and grain size. Owing to heat conduction, resolution is inevitably larger than the spot size. The RTM ratio is $\sim 1 \times 10^{-3}$ m^2/minute for both technologies, which means that these techniques are fast even if the resolution is comparable to that of other methods.

A promising modification of SLS that can release active compounds (e.g., ribonuclease, an exceptionally stable enzyme) is surface selective laser sintering (SSLS) [16], which uses an infrared laser to sinter powder substrates. In this case the radiation is not absorbed by the polymer particles but by carbon microparticles spread on the surface of the polymer particles. However, owing to several challenges, such as the use of carbon microparticles without a proven track record of biocompatibility, the processing of polymers into powder, and the lack of extensive studies with unstable biologically active compounds, this technology is not yet ready for the clinic.

SLA, in which light is used to solidify a photosensitive resin, has been typically used to produce a negative replica that is filled with ceramic or metallic slurries, and is subsequently removed by sintering at high temperatures [17]. Biocompatible and biodegradable photosensitive polymers that can be used in SLA to directly fabricate 3D scaffolds are also being developed and investigated. A few newly developed photosensitive resins are starting to appear in the biomaterials field for this purpose, and these have also opened the possibility to use a light

Figure 1. (A) The contour lines represent the resolution/time for manufacturing (RTM) ratio, a figure that ranks the biofabrication technologies according to their efficiency, taking into account resolution (*x* axis) and fabrication throughput (*y* axis). A larger ratio represents a more efficient process. (B) An illustration defining the colors used in panel (A).

allows shape retention of the 2D pattern deposited by the 3D micropositioner. With a layer-by-layer approach a 3D scaffold can be fabricated.

3D printing: an additive manufacturing technology that can be used for bioprinting in which a jet of binder is directed at a powder-bed to define a pattern. The solvent binds to the powder, forming a slice of solid material; subsequently a new layer of powder is laid down, and the process is repeated to build the scaffold layer-by-layer.

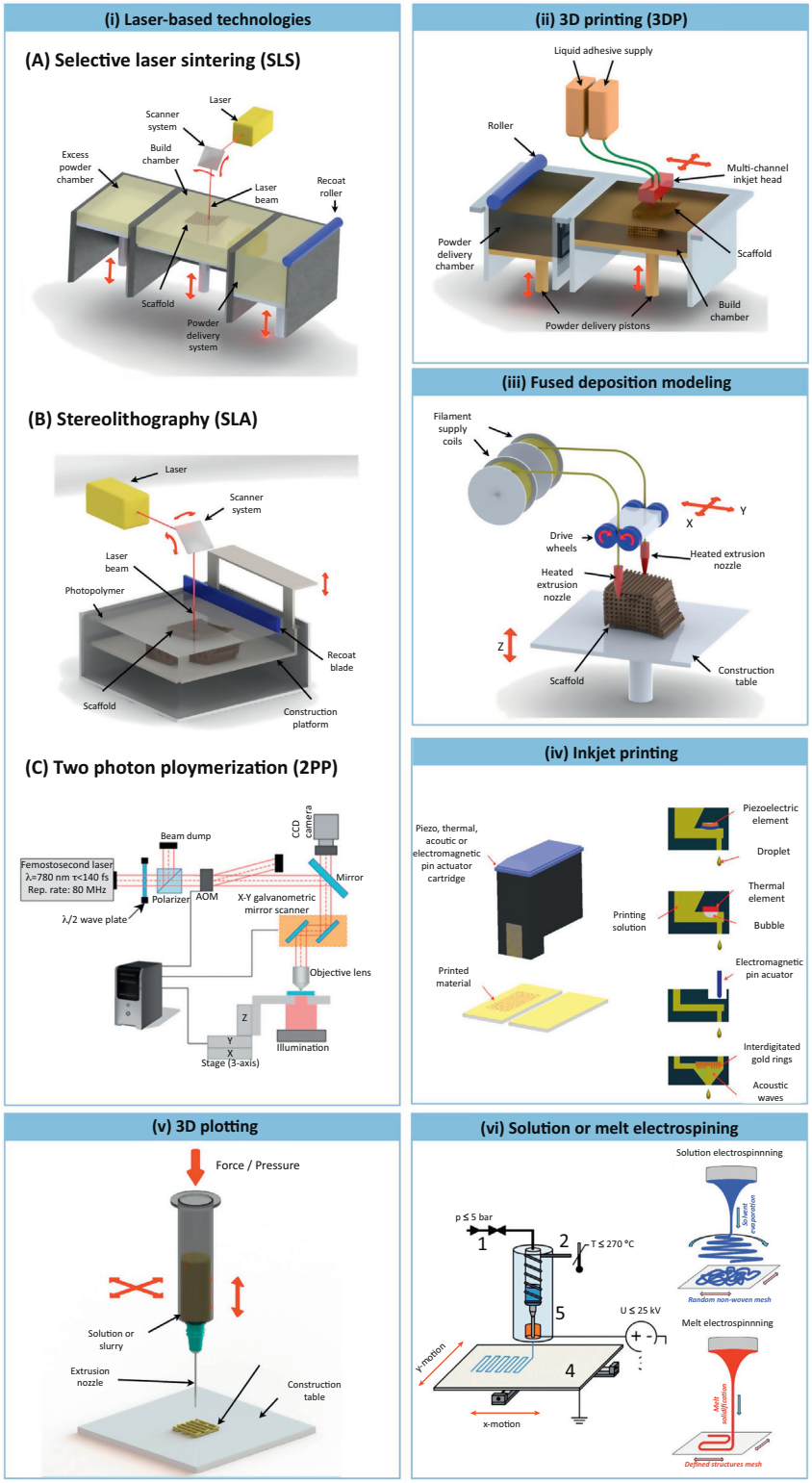
Resolution/time of manufacturing (RTM) ratio: a measure used to rank the performance of biofabrication technologies. The RTM ratio is defined as the ratio of the spatial resolution to the time required for biofabricating a bio-engineered structure; a larger RTM ratio represents a more efficient process.

Selective laser ablation: an additive manufacturing technology that can be used for bioprinting in which a solid material is ablated using a very short time-duration laser pulse. If the ablation process is conducted in all the three directions, or if laminated porous films are stacked and bonded on top of each other, a 3D structure can be created.

Selective laser sintering (SLS): an additive manufacturing technology that can be used for bioprinting in which a beam of laser light is selectively directed to a powder-bed, generating local heat and forming patterns of fused material; after its solidification, a new layer of powder is laid down and the process is repeated to build the scaffold layer-by-layer.

Stereolithography (SLA): an additive manufacturing technology that can be used for bioprinting in which light is used to cure a photosensitive resin. Through different irradiation approaches the various stereolithographic systems use a layer-by-layer approach: the energy delivered by the light is sufficient to solidify a particular thickness of the exposed resin and join this layer with the previous one.

Tissue liquidity: the notion, introduced by Malcolm Steinberg, that tissues or multicellular aggregates composed of motile and adhesive cells have properties



Trends in Biotechnology

analogous to liquids, evidenced by the fact that irregular tissue fragments spontaneously round up into spheroids, and two fragments composed of different cell types mutually envelope each other. Such tissues can be quantified in terms of apparent tissue surface tension.

Two-photon polymerization (2PP): a laser-based technique that uses a near-infrared ultrashort-pulsed laser to excite in a precise way and to confine space molecules (photoinitiators) to a two-photon state, triggering the polymerization of monomers in solution. This was the first technique that allowed the manufacturing of true 3D nano/micro-structures without supports.

(See figure legend on the bottom of the next page.)

projection system instead of a laser source (known as digital light processing, DLP™) [18,19]. Some of these also comprise hydrogel formulations, which would then allow the incorporation of cells into the biofabrication process. Despite enhanced resolution (~20–40 μm) with SLA, the biocompatibility of these photopolymers and of the photoinitiators used for their cross-linking remains to be fully validated. Incorporation of any biological material, including cells, depends on their sensitivity to the light source used [20]. In addition, this method is limited to a cell type that could be incorporated into a hydrogel. Using data in the literature, an RTM ratio of about 0.5×10^{-3} m^2/minute can be estimated for SLA, which places this method among the more efficient techniques. In case of DLP, the RTM ratio can be increased to 2×10^{-3} m^2/minute by parallelization of the light beam. An improvement of SLA with respect to spatial resolution is two-photon polymerization (2PP), where a spatially and temporally confined laser beam polymerizes a photosensitive resin [21,22]. The resolution of this technology can reach the sub-micron scale. Although new photocurable biomaterials with satisfactory biocompatibility are being developed, and the speed of this fabrication process has improved, further progress in both materials development and **fabrication rate** is needed. The RTM ratio is 0.05×10^{-3} m^2/minute, which means that 2PP has a very high resolution despite taking a long time to manufacture large structures.

Fused Deposition Modeling

FDM has been extensively used to fabricate custom-made scaffolds and to modulate their mechanical properties for tissue engineering applications, with encouraging results [23–25]. In FDM, molten thermoplastic polymers are extruded into filaments. These filaments are deposited to form a layer, and a 3D scaffold is built layer-by-layer. The entire process is controlled by a CAD design. Several biocompatible thermoplastics have been developed and processed with this technique. However, the majority of published work to date has used polycaprolactone as the polymer of choice because of its relatively low melting temperature and its commercial availability in medical grades. Many other extrusion-based tools inspired by FDM have been developed to fabricate 3D scaffolds. These also comprise multi-dispensing systems, such as 3D fiber deposition (3DF) and bioextrusion, that allow the deposition of different materials and at the same time produce constructs with locally differing physicochemical properties [4,23,26]. The main difference between FDM on the one hand, and 3DF and bioextrusion on the other, is that in 3DF and bioextrusion the biomaterials are loaded into a cartridge as pellets or particles instead of being extruded in a filament form. This approach has the advantage of expanding the palette of biomaterials that can be used compared to FDM. An associated disadvantage of 3DF and bioextrusion is higher susceptibility to thermal degradation owing to the long residence times of the raw material at high temperatures. Although FDM, 3DF, and bioextrusion techniques have considerably improved the quality of tissue engineered constructs with respect to their functional performance [25,27–29], the high temperatures involved during the fabrication of molten polymers may limit the direct incorporation of biological factors by this technique. A solution could be envisaged if not only metallic [30] or ceramic pastes [31] are employed, but also polymeric pastes that can be processed at room temperature. Alternatively, surface modification techniques could be used to functionalize the fibers and allow grafting of bioactive agents at specific sites [32]. In addition, hydrogels that encapsulate biological components could be deposited together with thermoplastic materials, thereby circumventing the limitations [33,34] imposed by the high temperatures. FDM, 3DF, and bioextrusion have the highest RTM

Figure 2. Commonly Used Technologies in Biofabrication with a Major and Active Role of Biomaterials in the Printing Process. (i) Light-based technologies (selective laser sintering, stereolithography, two-photon polymerization), (ii) 3D printing, (iii) fused deposition modeling, (iv) ink-jet printing, (v) 3D plotting, and (vi) solution and melt electrospinning. Adapted, with permission, from [118–120].

ratio of most of all methods, around 1×10^{-3} m^2/minute. Because the post-processing phase is practically nonexistent, there is a limited need for intervening layers or binders and solvents to remove excess material, as with most other techniques.

Pressure-assisted microsyringe deposition (PAM)/wet-spun automated extrusion systems have been developed to solve the disadvantages associated with high temperatures in FDM, at the same time achieving scaffolds with a higher fiber resolution (~80 μm for FDM vs 10 μm for PAM) [35,36]. PAM is part of the PAM2 system in which several working modules can be mounted in parallel on a robotic micropositioner to allow simultaneous processing of synthetic and natural polymer solutions as well as living cell suspensions [36,37]. The main drawback of wet-spun extrusion-based technologies is the low vertical dimension (when high resolution is sought), resulting in a medium RTM ratio of 0.5×10^{-3} m^2/minute. This implies that thick constructs take more time to be fabricated than with other extrusion technologies. Recent progress by Lewis and colleagues might, however, solve such limitations by fabricating arrays of nozzles that can deposit multiple filaments at the same time [38].

The major limitation of most scaffold fabrication processes reported in the literature is that each technique is applicable only in particular conditions (e.g., rheology, pressure, temperature, voltage) that restrict the choice of materials. Hydrogels composed of natural polymers (e.g., collagen, gelatin, hyaluronic acid), either in combination with additional biological factors or used alone, are intrinsically biocompatible and biodegradable, and possess biological cues [39]. However, these natural-polymer hydrogels are difficult to process with the techniques discussed so far. As an alternative, scaffolds made of natural biomaterials can be produced by indirect fabrication techniques (e.g., casting a biomaterial into a sacrificial mould formed by additive manufacturing processes). Indirect methods to produce additive manufactured scaffolds have emerged in several different approaches, with promising results [40]. The development of an alkali-soluble photopolymer allowed the use of **indirect additive manufacturing** with hydrogels [41]. In particular, gelatin and collagen scaffolds could be produced by applying indirect SLA manufactured moulds with high resolution and a minimum pore or strut size on the scale of several tens of micrometers [42,43]. Indirect methods could be also combined with conventional techniques, such as salt leaching and phase inversion, to fabricate dual-pore scaffolds [44,45]. Some drawbacks still exist with indirect approaches, including the poor resolution of the additive manufacturing techniques, the casting procedure, and the extraction methods. For these reasons the RTM ratio is $\sim 0.03 \times 10^{-3}$ m^2/minute.

Bioplotting and Ink-Jet Bioprinting

Whereas all of the above-mentioned methods have demonstrated different degrees of success in fabricating 3D scaffolds to accommodate cells that can develop into tissues or proto-tissue structures, most are incapable of simultaneously depositing biomaterials and cells. Therefore, cells need to be separately seeded into the scaffolds produced by these techniques. This limits the flexibility to mimic cell distributions in native tissues, particularly when strategies for the regeneration of multiple tissue interfaces or organs are to be developed. Three main sets of technologies have demonstrated the ability to incorporate cells during the process of additive manufacturing into a biomaterial carrier: 3D plotting (also known as bioplotting and extrusion bioprinting), ink-jet bioprinting, and valve-jet bioprinting. In bioplotting, the cells are typically encapsulated into a hydrogel carrier biomaterial and extruded by the application of pressure, similarly to wet-spun extrusion based technologies [46–48]. This technique allows the deposition of different cell types in different hydrogel formulations, but is still limited in terms of speed of production and fabrication of constructs with a complex shape, mostly because of the lack of optimal hydrogel carriers (also called **bioinks**). The technique also has some limitations if a

stable structure is to be formed. For example, it may be necessary to use a plotting bath containing a fluid of matching density and viscosity to the extruded material to prevent sagging or deformation of the construct immediately after extrusion, or alternatively to use a hydrogel with sufficient viscosity to self-sustain its own weight after processing. An additional approach that has emerged is the extrusion of material into 3D space of another material, in contrast to building a structure from a surface [49–51]. This has been most commonly performed with hydrogels (e.g., continuous or colloidal suspensions) where the material displaces as another material is extruded into it, and has been used to print suspended objects or open-channel structures with the use of sacrificial materials.

In ink-jet bioprinting, cells encapsulated into hydrogel carriers are dispensed in droplet fashion. By ink-jet bioprinting it is possible to exquisitely control the number of cells per deposited droplet, thus resulting in a finer control over cell distribution in the fabricated constructs [52,53]. The development of optimal hydrogels as bioinks for both bioplotting and ink-jet bioprinting remains a challenge, and the fluid requirements for both methods are very different in terms of fluid viscosity and surface tension [54]. The RTM ratio is $\sim 0.5 \times 10^{-3}$ m^2/minute for bioplotting as a consequence of rapid scaffold production but low resolution, and $\sim 0.1 \times 10^{-3}$ m^2/minute for ink-jet bioprinting due to high resolution but also high fabrication time per unit volume. In both technologies the presence of living cells in the bioink limits the RTM ratio: higher extrusion flow and smaller nozzles can induce damage due to shear stress on cell membranes [46–53].

Similar to the ink-jet technique, valve-jet bioprinting is a non-contact, droplet-based method where cells are printed with or without hydrogel carriers [55]. The actuation mechanism for the valve-jet is based on pneumatic pressure, and the ejection of droplets is controlled by solenoid microvalves instead of by piezo- or thermal actuators as in ink-jet printing [56]. Currently, the printing resolution (e.g., nanoliter droplet) and throughput (e.g., 1–1000 Hz) of valve-jet bioprinting lie between bioplotting and ink-jet bioprinting, as does the printable fluid viscosity range (up to 100 Pa.s). Because the technology is not limited by the nozzle size (as with ink-jet printing), shear stress can be minimized and therefore the technology is amenable to print delicate human pluripotent stem cells [57]. The RTM ratio is therefore $\sim 0.3 \times 10^{-3}$ m^2/minute for valve-jet bioprinting.

Electrospinning

A further promising technique for scaffold fabrication is electrospinning. This technique produces fiber meshes with physical features mimicking those of the native extracellular matrix (ECM). The fiber meshes are created by passing a biomaterial solution through a high-voltage electric field near the deposition nozzle. At a defined voltage threshold, which is specific for a defined biomaterial solution, the surface tension of the biomaterial solution is overcome by the applied electric field, resulting in the formation of an electrohydrodynamic Taylor cone from which fibers are spun and collected on a grounded target plate [58,59]. Despite being a relatively old technology, originally developed for textile fiber production, this technique is now widely used by the tissue engineering and regenerative medicine community because of the wide range of materials available for the technique and the methods of fiber collection that allow an expansive spectrum of structures and shapes to be fabricated [60,61]. An important recent development in the field of electrospinning is the possibility to control the deposition of fibers at the scale of a single fiber. This has been achieved by the so-called near-field electrospinning technique for biomaterials in solution and by melt electrospinning writing for molten polymers [62]. Together they constitute a method of electrohydrodynamic writing in which predictable fiber paths are used to direct-write small-diameter fibers onto a translating collector. This new electrospinning modality can potentially be used to create scaffold structures that can better

mimic the native ECM not only by the physical dimensions of the constituent fibers but also in terms of structural organization. Although cells have been shown to maintain their viability after electrospinning [63,64], reports describing the possibility of electrospinning cell-laden hydrogels are still limited [65,66]. The RTM ratio of this technique is currently around 0.1×10^{-3} m^2/minute.

In addition to the techniques listed so far, several alternative biofabrication strategies that use biomaterials only to provide structural integrity have been developed (Figure 3). The first examples of these strategies were provided by the work of Forgacs and coworkers who dispensed **cell spheroids** and cylinders into a hydrogel bed using special-purpose extrusion bioprinters [67–69]. The hydrogel is used as a support, while a tissue-like structure forms by exploiting the biophysical principles of **tissue liquidity** that governs the fusion of adjacent cell aggregates. In this manner, branched vascular networks [67,70], nerve grafts [68,71], and other tissue modules [69] have been successfully fabricated. Additional applications of this technology resulted in commercial products in the form of architecturally and functionally correct human tissue constructs for drug toxicology essays [72]. The principal limitations of this approach are (i) the relatively slow fusion of the cell aggregates, which takes typically at least 24–48 h depending on the cells used, and may lead to a somewhat inhomogeneous construct; (ii) low spatial resolution owing to the use of micropipettes of relatively large diameter (300 or 500 μm) for the preparation and deposition of gels and cells; and (iii) the limited diffusion of nutrients when large constructs are fabricated. Owing to these limitations and to the low resolution of this method, its RTM ratio is less than 0.001×10^{-3} m^2/minute.

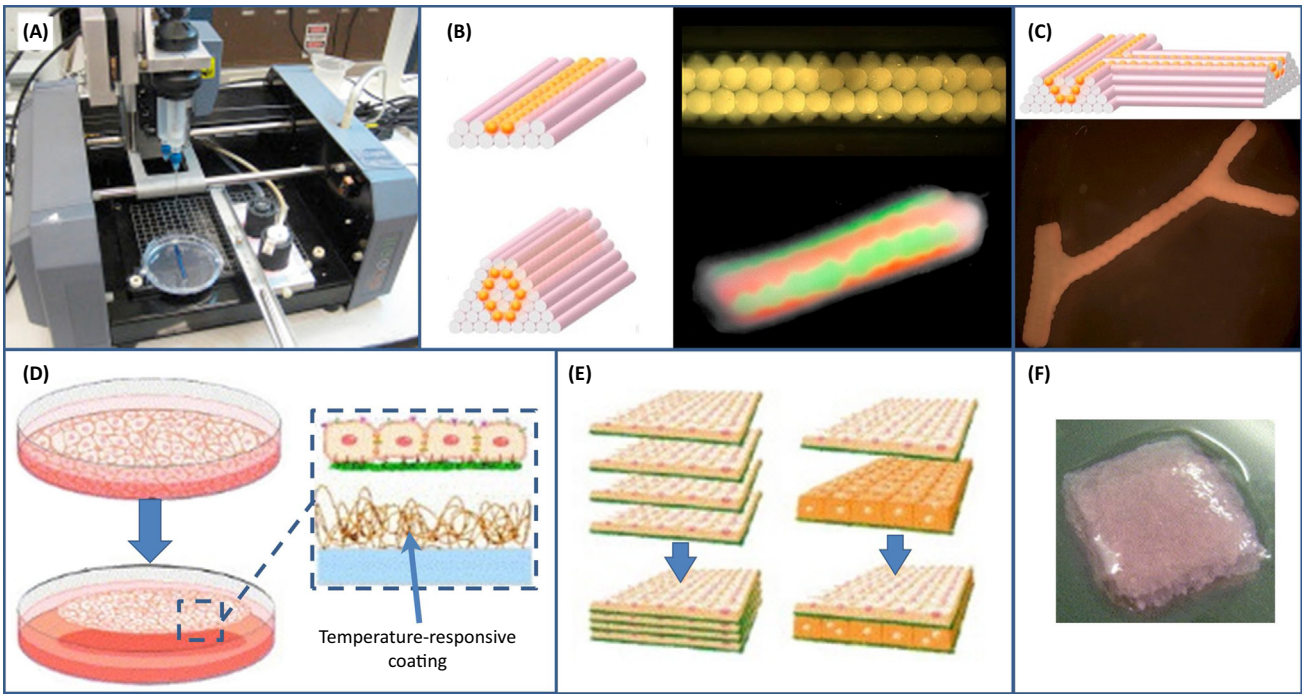
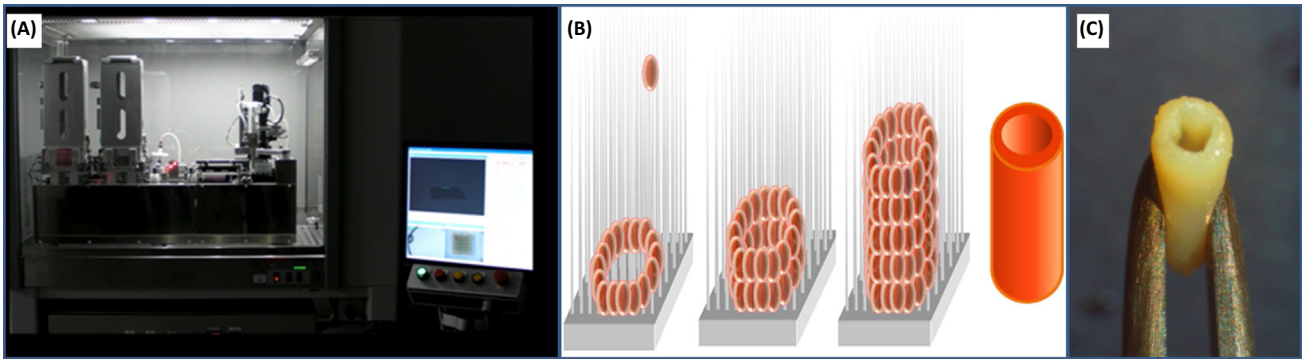


Figure 3. Example of Biofabrication Strategies that use Biomaterials To Provide Only Structural Integrity. (A) An extrusion bioprinter is used to deposit cell aggregates as spheroids and/or cylinders. (B) Deposition strategy for fabricating a straight or (C) branched hollow shape. (D) Representation of cells culture on thermoresponsive culture plates, and (E) cell-sheet stacking as steps for (F) obtaining a complex structure in a cell-sheet biofabrication method. Adapted, with permission, from [121] and [81].

A similar technology was developed by Nakayama and coworkers who used an ingenious skewering system [73,74] wherein the cell spheroids are placed on fine metallic needles (i.e., skewers), geometrically positioned as to be consistent with the shape of the desired organ structure (e.g., tubular construct; Figure 4). The novelty in this technology is that the needles prevent the otherwise unavoidable shrinking of the construct upon fusion of the spheroids (at least in one direction). Others have used a tangram-based concept in which different cellular shapes were left to fuse with each other and self-assemble into a macroscopic tissue construct [75–77]. Achieving fully vascularized large constructs with these tissue liquidity-based strategies is still an open challenge. Owing to the long aggregation time of the cell aggregates, the RTM ratio is slow, less than 0.001×10^{-3} m^2/minute.

Another approach developed originally by Chrisey and colleagues [78], later further adopted by Guillemot and colleagues [79], is based on **laser-assisted bioprinting** (LAB). This technique has also been known as laser-induced forward transfer (LIFT) and matrix-assisted pulsed laser evaporation (MAPLE). The deposition system is composed of three components: (i) a pulsed laser source, (ii) a target from which a biological material is printed, and (iii) a receiving substrate that collects the printed material. The target is made of a thin absorbing layer of metal (such as gold or titanium) coated onto a laser-transparent support (e.g., glass or a transparent polymer film). Organic materials (molecules or cells) are prepared in a liquid solution (e.g., culture media or a hydrogel precursor) and are deposited at the surface of the metal film. The laser pulse induces vaporization of the metal-absorbing layer, resulting in the production of a jet of liquid solution which is deposited onto the substrate [80]. The resolution of this system depends on parameters such as the thickness of the bioink layer coated onto the target, the surface tension and viscosity of the bioink, the wettability of the substrate, the laser fluence, and the air gap between the target and the substrate. LAB has a fairly low RTM ratio of around 0.04×10^{-3} m^2/minute owing high resolution and long fabrication time, which implies that thick constructs need more time for manufacture compared to the other techniques described before, and sometimes the production time of large size structures is not compatible with cell processing times.

A different approach for the biofabrication of tissues and organs consists of bottom-up approaches (Figure 3D–F) in which micro and nano modules are first engineered and used as building blocks to fabricate the targeted tissues. One of the most successful bottom-up approaches is represented by the cell-sheet engineering method developed by Okano and colleagues, where cells are cultured until they reach confluence on thermoresponsive culture



Trends in Biotechnology

Figure 4. Biofabrication Strategy Based on Skewering of Tissue Spheroids. (A) Custom-made device for automatic skewering. (B) Stacking strategy for obtaining a hollow shape. (C) Vascular graft generated from mesenchymal stem cells using the skewering approach. Adapted, with permission, from [74].

plates, which can easily release the formed cell layers by switching the temperature from 37°C to room temperature [81]. Larger constructs comprising multiple layers placed together in a conventional layer-by-layer method have been fabricated and successfully brought to the clinic [82]. With a similar approach, L'Hereux and coworkers developed and brought to the clinic a layer-by-layer approach for fabricating vascular biological grafts from cell sheets [83].

Another classic example is the **micro-masonry concept** pioneered by the laboratories of Khademhosseini and Demirci, among others, where micro-units of cell laden hydrogels are used as regenerative building blocks [84–86]. The modularity of this approach is limitless. Hydrogels of different compositions that embed different cells can be mixed. Recently, functionalization with DNA segments was also demonstrated, and this resulted in a more biological dynamic recognition of different building blocks during *in vitro* assembly [87]. Furthermore, such blocks can be also precisely positioned using microrobots [88].

Despite the great flexibility promised by these methods, further studies will be necessary to effectively demonstrate the fabrication of clinically relevant large vascularized constructs to compensate for the known nutrient-diffusion limitations of most hydrogel systems. Similar approaches have been also developed by combining solid micro- and nanoparticles with cells, thus offering the advantage of engineering the shape and size of such objects, which can possibly offer further stimuli to direct cell differentiation, particularly when stem cells are used [89,90]. Their use in combination with cells offers the possibility to impinge on cellular condensation, which results in tissue shrinking, thus offering the opportunity to maintain the dimensionality of large tissue constructs. For bottom-up approaches the RTM ratio is lower than 0.001×10^{-3} m^2/minute; these low values are principally due to the long fabrication time for the production of cell sheets or microunits and the time required for the fusion of the different cell sheets or microunits.

Microfluidic technology has also boomed in the recent years to create tissue-on-chip platforms that can recapitulate key functions of targeted tissues and organs. These platforms are typically used in association with a biomaterial formulation, namely hydrogel networks, to culture cells in 3D and study mechanisms behind pathological events and possible treatments. Examples comprise studies on cancer metastasis, lung, liver, intestine, and vessels, among others [91–95]. Further development of these platforms will comprise the integration of sensors to monitor in real-time cell and tissue functionality, as well as other biomaterial formulations, to better replicate native ECM of the targeted tissues to be studied. A further advancement in these bio-assembly biofabrication strategies has been reported by Takeuchi and coworkers, where meter-long cellular fibers have been created through microfluidic technology and proved to be efficacious in the regeneration of several tissues in preclinical animal models [96]. Cellular fibers are created by encapsulating cell-containing ECM proteins in a pre-gel state in mechanically stable Ca alginate hydrogel carrier in a coaxial manner, and by dissolving the carrier hydrogel upon the gelation of the cell-containing ECM. These fibers have also been weaved, thus creating cellular fiber scaffolds [97].

Implications for an Integral Terminology and Future Perspectives

In this review we have made a first attempt to define a metric that can compare the fabrication efficiency of the main current biofabrication technologies and that is usable with new methods as they are being developed. If used in the common range of biofabrication applications (i.e., minimum feature size d less than 500 μm), although dimensionally complex, the RTM ratio will objectively classify the continuous optimization and advances in current biofabrication technologies (Box 1). A practical example is the recent development of a new SLA technology by the

Box 1. Factors Influencing the RTM Ratio

The RTM ratio and the '*d-P*' parameter space can quantitatively and visually classify the various biofabrication technologies, respectively, and they can also indicate possible research directions to improve the efficiency of a specific technology. The RTM ratio is given by the printing rate and the minimum feature size achievable with a specific technology. This minimum feature size is determined by the physics beyond the fabrication process, strictly depending on the involved physical principles (e.g., photopolymerization, heat diffusion, and so on) and on the materials (polymers, ceramics, metals) and their physical state (solid, powder, liquid, gel, slurry). The spatial resolution can be improved by working on the development of new materials and processes rather than on technological optimization.

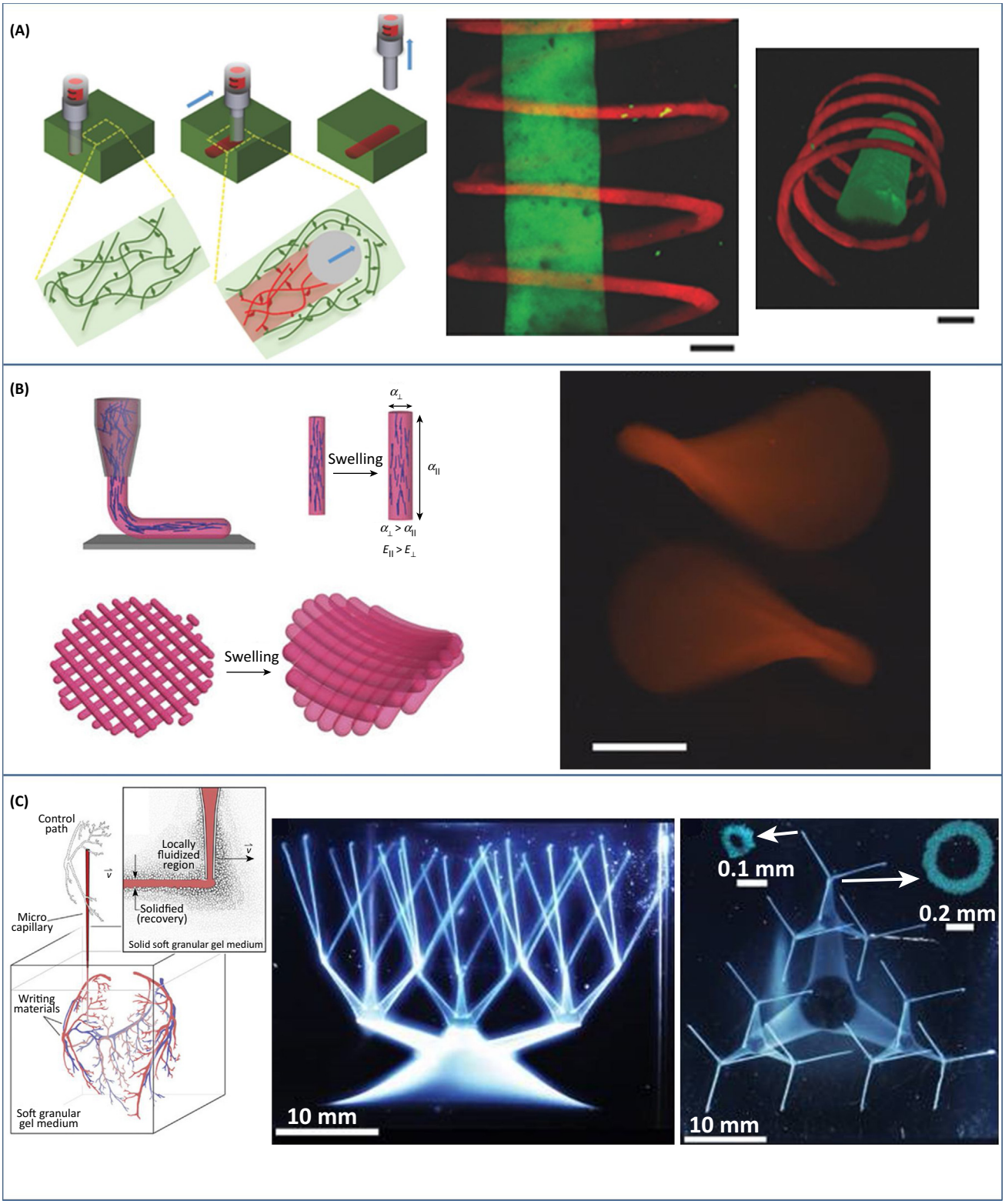
The printing rate can depend on the complexity of the printed shape, both in terms of geometry and multi-material composition (Table I). Biofabrication technologies based on a 'vector scanning' strategy for tracing the trajectories that lead to construct fabrication result in longer printing time in case of complex geometries, compared to those based on 'raster scanning' or 'projection' strategies. Multimaterial composition can also increase the fabrication time if different materials cannot be processed at the same time.

Table I. Factors Influencing the Printing Time

Technique	Geometrical complexity	Multimaterial fabrication
Vector		
Selective laser sintering	↑	Not allowed
Laser ablation	↑	Not allowed
Stereolithography	↑	Not allowed
Two-photon polymerization	↑	Not allowed
Fused deposition modeling	↑	↑
PAM^2	↑	↑
Bioplotting	↑	↑
LAB	↑	↑
Raster		
3D printing	=	Not allowed
Ink-jet bioprinting	=	=
Valve-jet bioprinting	↑	↑
Projection		
Digital light processing	=	Not allowed

Symbols: ↑, increase; =, no substantial change.

group of DeSimone, called continuous liquid interface production (CLIP), which allows creating 3D objects 100-fold faster than conventional SLA [98]. Whether this technology could be translated to biomaterials or cell-laden hydrogels remains to be demonstrated, but an estimate of its RTM ratio is 5×10^{-3} m^2/minute. In addition to constraints associated with the physical principles behind each technology and the chemistry of currently available biomaterials, the RTM ratio can be increased by parallelization of printing heads: light-based technologies better than others can exploit this route, and projection SLA (known as digital light processing, DLP^{TM}) is a clear and extreme example in this direction [19]. Another technological challenge is the fabrication of complex anatomically shaped constructs. Whereas current bioprinting technologies can already achieve non-intricate structures, the recent development of new colloidal inks and optimized bathing stages in which the bioprinting process takes place could offer new solutions to further increase the degree of complexity in mimicking native organs [50,51]. In particular, the use of high buoyant density liquids [99], sacrificial fugitive materials [100], or granular gels [50,101] as media into which bioinks are deposited is becoming a new exciting



Trends in Biotechnology

(See figure legend on the bottom of the next page.)

avenue to fabricate larger and more complex constructs. For a more in-depth review on supporting temporary sacrificial materials we refer elsewhere [102].

With the development of new bioprinting processes, it is nowadays possible to manufacture tissues with different levels of complexity (Figure 5A–C). This is achieved by heterogeneous combinations of different cells and bioinks. The degree of complexity needed to mimic and eventually replace a tissue and ultimately an organ has started to be considered for constructs tested *in vitro* [103,104] and *in vivo* [105]. With initial minor functional outcomes, further understanding of the level of mimicry and complexity necessary to achieve optimal functional tissue or organ replacement is expected as the field matures.

In the perspective of commercial-scale production, other standards of quality should be taken into consideration, such as: (i) accuracy, or how closely the output of a manufacturing output conforms to a tolerance within a specified dimensional range, and (ii) repeatability, which captures the ability of the equipment to produce consistent output, time after time. These parameters are necessary to reduce the extrinsic variability of the advanced tissue models owing to microenvironmental properties, limiting the intrinsic variability related to the cells themselves [40]. Quality control requires a consensus on metrology: limiting the discussion to geometrical consideration, a least-squares fit of a point cloud representing the scaffold (e.g., from a μCT scan) to its CAD model, can give a measure of the fabrication error [106,107]. Interestingly, additive manufacturing processes allow for in-process inspection of the internal structure of a component. Furthermore, the accuracy over time (long-term stability) is directly related to the off-the-shelf availability and, in a more prosaic way, to shipping and storing methods [108].

Finally, new hydrogels need to be developed that are able to maintain at the same time cell viability and activity, as well as the physical shape of the final printed construct [109–111]. The dynamic behavior of native ECM is an appealing feature that could be incorporated into new bioinks in future biofabrication strategies. In this respect, what is nowadays called 4D printing could allow such integration where the use of stimulus-responsive materials allows a spatio-temporal change in a 3D object [112,113]. Whether we are really witnessing 4D printing, a process that should be defined as a programmed temporal shape change occurring during the 3D manufacturing itself, or not, is still to be clarified in the field. We advocate for cautious use of the term 4D printing because all reports so far published in literature show 3D objects that can change shape after the 3D manufacturing process. Nonetheless, these time-morphing 3D objects are certainly an exciting new development of conventional additive manufacturing, which would be thrilling to see to be translated into novel biofabrication strategies.

Concluding Remarks and Recommended Guidelines

As the biofabrication community expands and the applications of this technology grow, it is important to establish a set of definitions and terminology that will help to normalize discussion and reports of developments in the field (see Outstanding Questions). Some attempts have been already made in the case of 3D scaffolds; for example, the National Institute for Standards and Technology of the United States of America is adopting 3D scaffolds fabricated by additive manufacturing technologies as standards for 3D cell culture [114]. Further standards could be

Figure 5. Examples of Complex Heterogeneous Bioprinted Structures. (A) Bioprinting of shear-thinning supramolecular hydrogels into self-healing support gels allowing continuous manufacturing in 3D space while patterning multiple inks and cells. (B) Example of 4D printing for stimulus-responsive materials that allow spatiotemporal changes (e.g., by swelling). (C) Example of hierarchically branched tubular networks generated by granular gel printing. Adapted, with permission, from [49,50,121]. Scale bars in A and B are 200 μm and 2,5 mm, respectively.

sought into the realm of advanced manufacturing, as recently pointed out by Hutmacher and coworkers, who coined the term 'additive biomanufacturing' when standard norms such as ASTM or ISO are applied to the biofabrication field [115]. In this context it is important to note that biomanufacturing means the use of living organisms to manufacture a product. In a recent review the term has been defined more precisely as 'a type of manufacturing that utilizes biological systems (e.g., living microorganisms, resting cells, plants, animals, tissues, enzymes, or *in vitro* synthetic (enzymatic) systems) to produce commercially important value-added biomolecules for use in the agricultural, food, energy, material, and pharmaceutical industries' [116]. Therefore, it is not clear what the combined term 'additive biomanufacturing' actually means. This should be defined before taking into account its use as part of an industrial norm. In the case that the biological systems considered by Zhang and colleagues are used as part of a fabrication approach, we wish to stress that, according to the most recent definition of biofabrication [1], 'additive biomanufacturing' is a subfield of biofabrication.

We propose here a clarification and classification of the different biofabrication terminologies in current use. In this respect, we recommend that the term 3D printing no longer be used as a general term for all additive manufacturing technologies applied to biofabrication strategies because 3D printing represents only one such technology, as previously described. Instead, we recommend using the name of the specific technology used to create a biofabricated construct, as outlined in this article. When referring to more general biofabrication strategies in tissue engineering and regenerative medicine, we recommend using the two general terms bioprinting or bioassembly. As we previously defined [1], bioprinting refers to the use of computer-aided transfer processes for patterning and assembling living and non-living materials with a prescribed 2D or 3D organization to produce **bio-engineered structures**; bioassembly refers to the fabrication of hierarchical constructs with a prescribed 2D or 3D organization through automated assembly of pre-formed cell-containing fabrication units generated via cell-driven self-organization or through preparation of hybrid cell–material building blocks. We advise members of the community to adopt this terminology approach in their new studies and that the media will report advances in the field with the correct language, instead of broadly using only the term 3D printing.

Outstanding Questions

What is the level of biomimicry needed to obtain a fully functional tissue, organ, or a bio-inspired product?

Which cells, cell organizations, and biomaterials are necessary to develop self-sustaining tissues, tissue models, organs, or bio-inspired products?

Which (combination of) biofabrication technologies should be used?

References

1. Groll, J. *et al.* (2016) Biofabrication: reappraising the definition of an evolving field. *Biofabrication* 8, 013001
2. Gao, W. *et al.* (2015) The status, challenges, and future of additive manufacturing in engineering. *Comput. Aided Des.* 69, 65–89
3. Melchels, F.P.W. *et al.* (2012) Additive manufacturing of tissues and organs. *Prog. Polym. Sci.* 37, 1079–1104
4. Mota, C. *et al.* (2015) Additive manufacturing techniques for the production of tissue engineering constructs. *J. Tissue Eng. Regen. Med.* 9, 174–190
5. Williams, C.B. *et al.* (2011) A functional classification framework for the conceptual design of additive manufacturing technologies. *J. Mech. Des.* 133, 121002
6. Giordano, R.A. *et al.* (1996) Mechanical properties of dense polylactic acid structures fabricated by three dimensional printing. *J. Biomater. Sci. Polym. Ed.* 8, 63–75
7. Pfister, A. *et al.* (2004) Biofunctional rapid prototyping for tissue-engineering applications: 3D bioplotting versus 3D printing. *J. Polym. Sci. A Polym. Chem.* 42, 624–638
8. Tan, K.H. *et al.* (2005) Selective laser sintering of biocompatible polymers for applications in tissue engineering. *Biomed. Mater. Eng.* 15, 113–124
9. Simpson, R.L. *et al.* (2008) Development of a 95/5 poly(L-lactide-co-glycolide)/hydroxylapatite and beta-tricalcium phosphate scaffold as bone replacement material via selective laser sintering. *J. Biomed. Mater. Res. B Appl. Biomater.* 84, 17–25
10. Zhou, W.Y. *et al.* (2008) Selective laser sintering of porous tissue engineering scaffolds from poly(L-lactide)/carbonated hydroxyapatite nanocomposite microspheres. *J. Mater. Sci. Mater. Med.* 19, 2535–2540
11. Takesada, M. *et al.* (2003) Micro-character printing on a diamond plate by femtosecond infrared optical pulses. *Jpn. J. Appl. Phys.* 42, 4613–4616
12. De Maria, C. *et al.* (2014) Development of a novel micro-ablation system to realise micrometric and well-defined hydrogel structures for tissue engineering applications. *Rapid Prototyp. J.* 20, 490–498
13. Sherwood, J.K. *et al.* (2002) A three-dimensional osteochondral composite scaffold for articular cartilage repair. *Biomaterials* 23, 4739–4751
14. Williams, J.M. *et al.* (2005) Bone tissue engineering using polycaprolactone scaffolds fabricated via selective laser sintering. *Biomaterials* 26, 4817–4827
15. Sarig-Nadir, O. *et al.* (2009) Laser photoablation of guidance microchannels into hydrogels directs cell growth in three dimensions. *Biophys. J.* 96, 4743–4752
16. Antonov, E.N. *et al.* (2005) Three-dimensional bioactive and biodegradable scaffolds fabricated by surface-selective laser sintering. *Adv. Mater.* 17, 327–330

17. Chu, T.M. *et al.* (2002) Mechanical and in vivo performance of hydroxyapatite implants with controlled architectures. *Biomaterials* 23, 1283–1293
18. Melchels, F.P. *et al.* (2009) A poly(D,L-lactide) resin for the preparation of tissue engineering scaffolds by stereolithography. *Biomaterials* 30, 3801–3809
19. Gauvin, R. *et al.* (2012) Microfabrication of complex porous tissue engineering scaffolds using 3D projection stereolithography. *Biomaterials* 33, 3824–3834
20. Wang, Z. *et al.* (2015) A simple and high-resolution stereolithography-based 3D bioprinting system using visible light crosslinkable bioinks. *Biofabrication* 7, 045009
21. Ovsianikov, A. and Chichkov, B.N. (2012) Three-dimensional microfabrication by two-photon polymerization technique. *Methods Mol. Biol.* 868, 311–325
22. Ovsianikov, A. *et al.* (2007) Two-photon polymerization technique for microfabrication of CAD-designed 3D scaffolds from commercially available photosensitive materials. *J. Tissue Eng. Regen. Med.* 1, 443–449
23. Woodfield, T.B. *et al.* (2004) Design of porous scaffolds for cartilage tissue engineering using a three-dimensional fiber-deposition technique. *Biomaterials* 25, 4149–4161
24. Moroni, L. *et al.* (2006) 3D fiber-deposited scaffolds for tissue engineering: Influence of pores geometry and architecture on dynamic mechanical properties. *Biomaterials* 27, 974–985
25. Woodfield, T.B. *et al.* (2005) Polymer scaffolds fabricated with pore-size gradients as a model for studying the zonal organization within tissue-engineered cartilage constructs. *Tissue Eng.* 11, 1297–1311
26. Yan, Y. *et al.* (2003) Layered manufacturing of tissue engineering scaffolds via multi-nozzle deposition. *Mater. Lett.* 57, 2623–2628
27. Hutmacher, D.W. *et al.* (2003) Elastic cartilage engineering using novel scaffold architectures in combination with a biomimetic cell carrier. *Biomaterials* 24, 4445–4458
28. Schantz, J.T. *et al.* (2005) Osteogenic differentiation of mesenchymal progenitor cells in computer designed fibrin-polymer-ceramic scaffolds manufactured by fused deposition modeling. *J. Mater. Sci. Mater. Med.* 16, 807–819
29. Hoque, M.E. *et al.* (2005) Fabrication using a rapid prototyping system and in vitro characterization of PEG-PCL-PLA scaffolds for tissue engineering. *J. Biomater. Sci. Polym. Ed.* 16, 1595–1610
30. Li, J.P. *et al.* (2005) Porous Ti(6)Al(4)V scaffold directly fabricating by rapid prototyping: preparation and in vitro experiment. *Biomaterials*
31. Michna, S. *et al.* (2005) Concentrated hydroxyapatite inks for direct-write assembly of 3-D periodic scaffolds. *Biomaterials* 26, 5632–5639
32. Hubbell, J.A. (1996) Hydrogel systems for barriers and local drug delivery in the control of wound healing. *J. Control. Release* 39, 305–313
33. Schuurman, W. *et al.* (2011) Bioprinting of hybrid tissue constructs with tailorable mechanical properties. *Biofabrication* 3, 021001
34. Pati, F. *et al.* (2014) Printing three-dimensional tissue analogues with decellularized extracellular matrix bioink. *Nat. Commun.* 5, 3935
35. Puppi, D. *et al.* (2012) Additive manufacturing of wet-spun polymeric scaffolds for bone tissue engineering. *Biomed. Microdevices* 14, 1115–1127
36. Vozzi, G. *et al.* (2002) Microsyringe-based deposition of two-dimensional and three-dimensional polymer scaffolds with a well-defined geometry for application to tissue engineering. *Tissue Eng.* 8, 1089–1098
37. Tirella, A. *et al.* (2011) PAM2 (piston assisted microsyringe): a new rapid prototyping technique for biofabrication of cell incorporated scaffolds. *Tissue Eng. C Methods* 17, 229–237
38. Hansen, C.J. *et al.* (2013) High-throughput printing via microvascular multinozzle arrays. *Adv. Mater.* 25, 96–102
39. Zhu, J. and Marchant, R.E. (2011) Design properties of hydrogel tissue-engineering scaffolds. *Expert Rev. Med. Devices* 8, 607–626
40. De Maria, C. *et al.* (2015) *Indirect Rapid Prototyping for Tissue Engineering, Essentials of 3D Biofabrication and Translation,* Elsevier
41. Liska, R. *et al.* (2005) Water-soluble photopolymers for rapid prototyping of cellular materials. *J. Appl. Polym. Sci.* 97, 2286–2298
42. Tan, J.Y. *et al.* (2010) Indirect fabrication of tissue engineering scaffolds using rapid prototyping and a foaming process. Innovative Developments in Design and Manufacturing 51–57
43. Sachlos, E. *et al.* (2003) Novel collagen scaffolds with predefined internal morphology made by solid freeform fabrication. *Biomaterials* 24, 1487–1497
44. Bose, S. *et al.* (2003) Pore size and pore volume effects on alumina and TCP ceramic scaffolds. *Mat. Sci. Eng. C* 23, 479–486
45. Miller, J.S. *et al.* (2012) Rapid casting of patterned vascular networks for perfusable engineered three-dimensional tissues. *Nat. Mater.* 11, 768–774
46. Landers, R. *et al.* (2002) Rapid prototyping of scaffolds derived from thermoreversible hydrogels and tailored for applications in tissue engineering. *Biomaterials* 23, 4437–4447
47. Schuurman, W. *et al.* (2013) Gelatin-methacrylamide hydrogels as potential biomaterials for fabrication of tissue-engineered cartilage constructs. *Macromol. Biosci.* 13, 551–561
48. Fedorovich, N.E. *et al.* (2012) Biofabrication of osteochondral tissue equivalents by printing topologically defined, cell-laden hydrogel scaffolds. *Tissue Eng. C Methods* 18, 33–44
49. Highley, C.B. *et al.* (2015) Direct 3D printing of shear-thinning hydrogels into self-healing hydrogels. *Adv. Mater.* 27, 5075–5079
50. Bhattacharjee, T. *et al.* (2015) Writing in the granular gel medium. *Sci. Adv.* 1, e1500655
51. Hinton, T.J. *et al.* (2015) Three-dimensional printing of complex biological structures by freeform reversible embedding of suspended hydrogels. *Sci. Adv.* 1, e1500758
52. Villar, G. *et al.* (2013) A tissue-like printed material. *Science* 340, 48–52
53. Yanez, M. *et al.* (2015) In vivo assessment of printed microvasculature in a bilayer skin graft to treat full-thickness wounds. *Tissue Eng. A* 21, 224–233
54. Malda, J. *et al.* (2013) 25th anniversary article. Engineering hydrogels for biofabrication. *Adv. Mater.* 25, 5011–5028
55. Xu, F. *et al.* (2010) A droplet-based building block approach for bladder smooth muscle cell (SMC) proliferation. *Biofabrication* 2, 014105
56. Faulkner-Jones, A. *et al.* (2015) Bioprinting of human pluripotent stem cells and their directed differentiation into hepatocyte-like cells for the generation of mini-livers in 3D. *Biofabrication* 7, 044102
57. Faulkner-Jones, A. *et al.* (2013) Development of a valve-based cell printer for the formation of human embryonic stem cell spheroid aggregates. *Biofabrication* 5, 015013
58. Deitzel, J. *et al.* (2001) The effect of processing variables on the morphology of electrospun nanofibers and textiles. *Polymer* 42, 261–272
59. Bashur, C.A. *et al.* (2006) Effect of fiber diameter and orientation on fibroblast morphology and proliferation on electrospun poly(D,L-lactic-co-glycolic acid) meshes. *Biomaterials* 27, 5681–5688
60. Carlberg, B. *et al.* (2010) Direct photolithographic patterning of electrospun films for defined nanofibrillar microarchitectures. *Langmuir* 26, 2235–2239

61. Wang, Y. *et al.* (2009) Electrospun nanofiber meshes with tailored architectures and patterns as potential tissue-engineering scaffolds. *Biofabrication* 1, 015001
62. Brown, T.D. *et al.* (2011) Direct writing by way of melt electrospinning. *Adv. Mater.* 23, 5651–5657
63. Jayasinghe, S.N. *et al.* (2006) Electrohydrodynamic jet processing: an advanced electric-field-driven jetting phenomenon for processing living cells. *Small* 2, 216–219
64. Sampson, S.L. *et al.* (2014) Cell electrospinning: an in vitro and in vivo study. *Small* 10, 78–82
65. Ji, Y. *et al.* (2006) Dual-syringe reactive electrospinning of cross-linked hyaluronic acid hydrogel nanofibers for tissue engineering applications. *Macromol. Biosci.* 6, 811–817
66. Jin, G. *et al.* (2014) Bicomponent electrospinning to fabricate three-dimensional hydrogel-hybrid nanofibrous scaffolds with spatial fiber tortuosity. *Biomed. Microdevices* 16, 793–804
67. Jakab, K. *et al.* (2004) Engineering biological structures of prescribed shape using self-assembling multicellular systems. *Proc. Natl. Acad. Sci. U. S. A.* 101, 2864–2869
68. Jakab, K. *et al.* (2006) Three-dimensional tissue constructs built by bioprinting. *Biorheology* 43, 509–513
69. Jakab, K. *et al.* (2008) Tissue engineering by self-assembly of cells printed into topologically defined structures. *Tissue Eng. A* 14, 413–421
70. Kelm, J.M. *et al.* (2007) In vitro vascularization of human connective microtissues. *Methods Mol. Med.* 140, 153–166
71. Marga, F. *et al.* (2012) Toward engineering functional organ modules by additive manufacturing. *Biofabrication* 4, 022001
72. Nguyen, D.G. *et al.* (2016) Bioprinted 3D primary liver tissues allow assessment of organ-level response to clinical drug induced toxicity in vitro. *PLoS One* 11, e0158674
73. Murata, D. *et al.* (2015) A preliminary study of osteochondral regeneration using a scaffold-free three-dimensional construct of porcine adipose tissue-derived mesenchymal stem cells. *J. Orthop. Surg. Res.* 10, 35
74. Itoh, M. *et al.* (2015) Scaffold-free tubular tissues created by a Bio-3D printer undergo remodeling and endothelialization when implanted in rat aortae. *PLoS One* 10, e0136681
75. Frey, O. *et al.* (2014) Reconfigurable microfluidic hanging drop network for multi-tissue interaction and analysis. *Nat. Commun.* 5, 4250
76. Rivron, N.C. *et al.* (2012) Tissue deformation spatially modulates VEGF signaling and angiogenesis. *Proc. Natl. Acad. Sci. U. S. A.* 109, 6886–6891
77. Rago, A.P. *et al.* (2009) Controlling cell position in complex heterotypic 3D microtissues by tissue fusion. *Biotechnol. Bioeng.* 102, 1231–1241
78. Ringeisen, B.R. *et al.* (2004) Laser printing of pluripotent embryonal carcinoma cells. *Tissue Eng.* 10, 483–491
79. Guillemot, F. *et al.* (2010) Laser-assisted cell printing: principle, physical parameters versus cell fate and perspectives in tissue engineering. *Nanomedicine* 5, 507–515
80. Devillard, R. *et al.* (2014) Cell patterning by laser-assisted bioprinting. *Methods Cell Biol.* 119, 159–174
81. Yang, Y. *et al.* (2005) Cell sheet engineering: recreating tissues without biodegradable scaffolds. *Biomaterials* 26, 6415–6422
82. Egami, M. *et al.* (2014) Latest status of the clinical and industrial applications of cell sheet engineering and regenerative medicine. *Arch. Pharm. Res.* 37, 96–106
83. L'Heureux, N. *et al.* (1998) A completely biological tissue-engineered human blood vessel. *FASEB J.* 12, 47–56
84. Fernandez, J.G. and Khademhosseini, A. (2010) Micromasonry: construction of 3D structures by microscale self-assembly. *Adv. Mater.* 22, 2538–2541
85. Hancock, M.J. *et al.* (2012) Designer hydrophilic regions regulate droplet shape for controlled surface patterning and 3D microgel synthesis. *Small* 8, 393–403
86. Ling, Y. *et al.* (2007) A cell-laden microfluidic hydrogel. *Lab Chip* 7, 756–762
87. Qi, H. *et al.* (2013) DNA-directed self-assembly of shape-controlled hydrogels. *Nat. Commun.* 4, 2275
88. Tasoglu, S. *et al.* (2014) Untethered micro-robotic coding of three-dimensional material composition. *Nat. Commun.* 5, 3124
89. Leferink, A. *et al.* (2014) Engineered micro-objects as scaffolding elements in cellular building blocks for bottom-up tissue engineering approaches. *Adv. Mater.* 26, 2592–2599
90. Zhao, S. *et al.* (2011) Stereomask lithography (SML): a universal multi-object micro-patterning technique for biological applications. *Lab Chip* 11, 224–230
91. Huh, D. *et al.* (2010) Reconstituting organ-level lung functions on a chip. *Science* 328, 1662–1668
92. Ho, C.T. *et al.* (2013) Liver-cell patterning lab chip: mimicking the morphology of liver lobule tissue. *Lab Chip* 13, 3578–3587
93. Imura, Y. *et al.* (2009) A microfluidic system to evaluate intestinal absorption. *Anal. Sci.* 25, 1403–1437
94. Franco, C. and Gerhardt, H. (2012) Tissue engineering: blood vessels on a chip. *Nature* 488, 465–466
95. Shin, M.K. *et al.* (2011) Integration of intra- and extravasation in one cell-based microfluidic chip for the study of cancer metastasis. *Lab Chip* 11, 3880–3887
96. Onoe, H. *et al.* (2013) Metre-long cell-laden microfibres exhibit tissue morphologies and functions. *Nat. Mater.* 12, 584–590
97. Morimoto, Y. *et al.* (2015) Point-, line-, and plane-shaped cellular constructs for 3D tissue assembly. *Adv. Drug Deliv. Rev.* 95, 29–39
98. Tumbleston, J.R. *et al.* (2015) Continuous liquid interface production of 3D objects. *Science* 347, 1349–1352
99. Blaeser, A. *et al.* (2013) Biofabrication under fluorocarbon: a novel freeform fabrication technique to generate high aspect ratio tissue-engineered constructs. *Biores. Open Access* 2, 374–384
100. Blaeser, A. *et al.* (2016) Controlling shear stress in 3D bioprinting is a key factor to balance printing resolution and stem cell integrity. *Adv. Healthc. Mater.* 5, 326–333
101. O'Bryan, C.S. *et al.* (2017) Self-assembled micro-organogels for 3D printing silicone structures. *Sci. Adv.* 3, e1602800
102. O'Bryan, C.S. *et al.* (2017) Three-dimensional printing with sacrificial materials for soft matter manufacturing. *MRS Bull.* 42, 571–577
103. Kolesky, D.B. *et al.* (2014) 3D bioprinting of vascularized, heterogeneous cell-laden tissue constructs. *Adv. Mater.* 26, 31243130
104. Lee, V.K. *et al.* (2014) Creating perfused functional vascular channels using 3D bio-printing technology. *Biomaterials* 35, 8092–8102
105. Gaebel, R. *et al.* (2011) Patterning human stem cells and endothelial cells with laser printing for cardiac regeneration. *Biomaterials* 32, 9218–9230
106. Hockaday, L.A. *et al.* (2012) Rapid 3D printing of anatomically accurate and mechanically heterogeneous aortic valve hydrogel scaffolds. *Biofabrication* 4, 035005
107. Besl, P.J. and Mckay, N.D. (1992) A method for registration of 3-D shapes. *IEEE Trans. Pattern Anal.* 14, 239–256
108. Freytes, D.O. *et al.* (2008) Hydrated versus lyophilized forms of porcine extracellular matrix derived from the urinary bladder. *J. Biomed. Mater. Res. A* 87A, 862–872
109. Chimene, D. *et al.* (2016) Advanced bioinks for 3D printing: a materials science perspective. *Ann. Biomed. Eng.* 44, 2090–2102
110. Jungst, T. *et al.* (2016) Strategies and molecular design criteria for 3D printable hydrogels. *Chem. Rev.* 116, 1496–1539
111. Nakamura, M. *et al.* (2010) Biomatrices and biomaterials for future developments of bioprinting and biofabrication. *Biofabrication* 2, 014110
112. Gladman, A.S. *et al.* (2016) Biomimetic 4D printing. *Nat. Mater.* 15, 413–418

113. Tibbits, S. (2014) 4D printing: multi-material shape change. *Archit. Des.* 84, 116–121

114. Simon, C.G., Jr *et al.* (2015) ASTM international workshop on standards and measurements for tissue engineering scaffolds. *J. Biomed. Mater. Res. B Appl. Biomater.* 103, 949–959

115. Chhaya, M.P. *et al.* (2015) Additive manufacturing in biomedical sciences and the need for definitions and norms. *Expert Rev. Med. Devices* 12, 537–543

116. Zhang, Y.P. *et al.* (2017) Biomanufacturing: history and perspective. *J. Ind. Microbiol. Biotechnol.* 44, 773–784

117. Boland, T. *et al.* (2003) Cell and organ printing 2: fusion of cell aggregates in three-dimensional gels. *J. Biomed. Mater. Res. B Appl. Biomater.* 272, 497–502

118. Hochleitner, G. *et al.* (2015) Additive manufacturing of scaffolds with sub-micron filaments via melt electrospinning writing. *Biofabrication* 7, 035002

119. Obata, K. *et al.* (2013) High-aspect 3D two-photon polymerization structuring with widened objective working range (WOW-2PP). *Light Sci. Appl.* 2, e116

120. Mota, C. and Moroni, L. (2015) High throughput screening with biofabrication platforms. In *Essentials of 3D Biofabrication and Translation* (Atala, A. and Yoo, J.J., eds), pp. 187–213, Academic Press

121. Norotte, C. *et al.* (2009) Scaffold-free vascular tissue engineering using bioprinting. *Biomaterials* 30, 5910–5917