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Comparison of biomaterial-dependent and -independent bioprinting methods for cardiovascular medicine

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Abstract

There is an increasing need and unique opportunities for the development of novel and more powerful tissue engineering methods, among which the 3D bioprinting is one of the most promising. However, after decades of incubation, ingenuous efforts and early success, biomaterial-dependent 3D bioprinting, although showing steady progress, is slow to deliver the expected clinical results. For this reason, alternative 'scaffold-free' 3D bioprinting methods are being developed in parallel at an accelerated pace. In this opinion paper we discuss comparatively the two approaches, with specific examples drawn from the cardiovascular field. Moving the emphasis away from competition, we show that the two platforms have similar goals but evolve in complementary technological niches. We conclude that the biomaterial-dependent bioprinting is better suited for tasks requiring faster, larger, anatomically-true, cell-homogenous and matrix-rich constructs, while the scaffold-free biofabrication is more adequate for cell-heterogeneous, matrix-poor, complex and smaller constructs, but requiring longer preparation time.

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Abbreviations

3DBP, 3D bioprinting; EC, endothelial cells; ECM, extracellular matrix; FB, fibroblasts; GelMA, metacrylate gelatin; HUVEC, human umbilical vein endothelial cells; IC, interstitial cells; MSC, mesenchymal stem cells; SMC, smooth muscle cells.

Introduction

Medicine is facing new challenges in a world with an increasingly aged population. Among them is the massive request of more tissues and organs for transplantation, although fewer than one-third of these patients eventually will receive one [1]. Also, due to their limited efficacy, more robust, possibly radical alternatives to current cell therapy-based methods to treat chronic diseases are needed. Another opportunity for tissue engineering is to replace, or possibly eliminate animal experimentation. This is desirable not only from a bio-ethical standpoint, but also in response to the practical issues derived from species-specific differences in cell function and tissue organization. In addition, more realistic 3D tissue models are increasingly required for toxicological testing and for drug discovery. In all circumstances, tissue engineering is taking a more central position in the emerging bio-medical toolkit [2].

Among the tissue engineering methods, 3D bioprinting (3DBP) holds the promise to become a major revolution in biofabrication of tissues and organs [3•]. This technology might also have an excellent opportunity in the context of deep space exploration: in long-term missions, with very limited resources, the only solution for urgent medical problems could be the on-demand 3D printing of both medical instruments [4] and the required tissues from a patient's own cells [5].

As a form of additive biomanufacturing, 3DBP has been riding so far on the wave of 3D printing. In other words, bioprinting became mainly the biological version of 3D printing [6•]. However, the biomaterials deployed in a layer-wise manner to create the 3D construct, also named 'bioinks' (or 'scaffolds' because of their supporting role), had to coincidently fulfill these often contradictory conditions: i) be printable; ii) protect incorporated cells during bioprinting; iii) sustain their growth and differentiation afterwards; iv) be biocompatible with the recipient organism [7••].

At the interface between scaffold-dependent and scaffold-free bioprinting lies the use of a new generation of 'bioinks' prepared exclusively from natural materials, such as collagen, fibrin or organ-specific extracellular matrices [8•]. Although still experiencing some of the same limitations of their deployment methods as their synthetic correspondents, the latter option is by far more promising in terms of cell support and biocompatibility. But all these difficulties would be absent if the cellular assembling could be performed with cells capable to produce their own extracellular matrix (ECM), i.e., using biomaterial ('scaffold')-free methods.

Terminological issues

One of the consequences of the field's rapid expansion, with contribution of many research groups with expertise blended from different disciplines, is the inhomogeneous (and often confusing) terminology [3•]. For example, *bioprinting* is the name given to: i) layer-by-layer deposition of cells dispersed in a biomaterial; ii) biomaterial-dependent assembling of cellular aggregates; iii) formation of cell aggregates (spheroids or larger constructs) by magnetic pull down, or even by centrifugation; iv) biomaterial-independent 3D assembling of cell cords and spheroids. Correspondingly, as the instrument facilitating the act of 'bioprinting', a *'bioprinter'* may have different meanings. Moreover, for some groups the notion of *'bioink'* represents only the embedding biomaterial used for bioprinting, while for others it includes the living entities used for 3D assembling [9]. Additionally, those procedures where biomaterials are removed shortly after assembling of preformed cellular aggregates as building blocks were also called '*scaffold-free*' [10].

Comparative examples of 3DBP for cardiovascular applications

Commensurate with the exceptional momentum for 3DBP, high-quality reviews of this rapidly evolving field are published almost daily, including many dedicated to cardiovascular applications (e.g. Refs. [7,11•,12]). In what it follows, we will comparatively discuss some recent publications focusing on the cardiovascular field, to help the readers evaluate the strengths and limitations of scaffold-dependent and scaffold-free approaches (see Table 1 for a summary [13•]).

Table 1

Comparative features of biomaterial-dependent and independent bioprinting methods. (reproduced with permission from Moldovan *et al.* 2016 [13]).

	Biomaterial-dependent		Biomaterial-free	
	Attributes	Comments	Attributes	Comments
Object configuration	Direct image input via CAD	Similar to 3D printing	Approximate	Larger 'voxel' size, limited resolution
Structural cohesion ('glue')	Obtained by non-universal, sometimes proprietary and/or expensive bio-inks	New biological bio-inks emerging (e.g. collagen or fibrin based)	Cells produce their own matrix; constructs are dependent on cell type and quality	Matrix deposition can be unpredictable or insufficient
Biomechanics	Hydrogels are essentially soft; hardening can be cell-damaging	'Hybrid' bioprinting as alternative: incorporation of a second (fibrillar) biomaterial	Construct biomechanics less predictable and controllable	Hybrid versions are also likely to be developed
Efficiency	Substantial cell death, for a variety of method-specific reasons	Milder methods are being tested (e.g. laser-assisted bioprinting)	Less or no cell damage Cell-type dependent	By using large spheroids, speed can become comparable or even higher than laser-assisted bioprinting
Cellular cross-talk	Material-limited inter-cellular communication ('encapsulation')	Not a problem for matrix-rich tissues such as bone, cartilage	Direct cellular interactions	Optional addition of hydrogels into or between spheroids still possible
Tissue structure	Simplistic cellular architecture	Biomaterial dissolution allows more spontaneous cell rearrangements	Follows developmental principles	Incorporation of endothelial cells in spheroids may promote micro-vascularization
Bio-compatibility	Cytotoxicity possible, foreign-body reactions likely	Less serious if biological bio-inks are used	Patient-specific cells: MSC, iPSC	Possibly fully autologous constructs
Common technical problems	Nozzle clogging	Limited to ink-jet and micro-extrusion methods	Time of pre-printing preparations	Post-printing maturation time comparable between the two approaches
Scalability	Excellent	***Good for large, cell-homogenous, matrix-rich tissues***	More limited	***Recommended for small, cell-heterogeneous, matrix-poor tissues***