3D bioprinting applications for in vitro modeling of cellular interactions and tissues

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Summary

The article considers different strategies for seeding stem cells and their progeny and construction of new tissues and organs, i.e., artificial biocompatible templates, natural decellularized templates, and generating complex tissues directly from stem cells and matrix materials using bioreactors or 3D-printing. Generally, culturing cells under 3D conditions allows to retain fully morphological and functional integrity of specialized cells as shown with hepatocytes. In particular, a promising approach to 3D organ fabrication is to use special bioprinters to prepare tissue scaffolds. Relevant studies have resulted in the production of organ-like cellular complexes, for example tubular/glomerular kidney structures. There are some technical issues which should be considered in any

single case, including type of printing technology, choice of biomatrix type, printing parameters, etc.

Microextrusion technique and laser-induced forward transfer (LIFT) approach are considered as prospective printing technologies. Natural substrates for tissue and organ scaffolds could be obtained by decellularization and subsequent cell seeding, as already shown in animal experiments. Generating 3D tissue models could create promising opportunities for hematopoiesis research in its natural microenvironment.

Keywords

stem cell research, tissue templates, 3D-printing, hematopoiesis, microenvironment, modeling

Introduction

In 2009, over 150 000 people in the US were waiting for an organ transplant, and only 18% of them received one. Nearly 9000 people died while on the waiting list. The lack of available donors is a major driving force for developing the concept of artificial organs. Strategies for construction or reconstruction of new tissues and organs include: 1) Creating biocompatible templates onto which stem cells and their derivative cells can be seeded; 2) Creating natural templates by decellularization, and seeding stem cells or stem cell-derived cells onto these; and 3) Generating complex tissues direct-

ly from stem cells and matrix materials using bioreactors or 3D-printing.

Culturing cells under 3D conditions provides key advantages that make this strategy imperative in approaching the problem of organ replacement. In conventional 2D cultures, primary cells rapidly lose their function, in large part due to perturbed cell-cell contacts. This can lead to rapid loss of polarity and differentiation, as is seen for example in hepatocytes obtained from biopsies. Dissociated hepatocytes in 2D monoculture revert to expression of alpha fetoprotein (AFP) and experience a downregulation of integrins and P450 activity. By contrast, primary hepatocytes cultured under 3D

conditions in a microgravity vessel remain fully functional for at least 6 weeks with respect to albumin secretion, integrin beta 6 expression, P450 responsiveness and downregulation of AFP. In short, a liver-like functional status is retained. Thus, 2D monocultures of animal primary cells or of human immortalized cell lines are not representative of normal human tissue. 3D microenvironments provide more complex and physiological cell-cell and cell-matrix interactions, and provide a more reliable platform for physiologically relevant tissue models, disease models and drug testing.

Figure 1. An example of a 3D-printed tissue scaffold [3].

In designing 3D bioprinting strategies, several important technical issues need to be considered, including:

- 1) Choice of printing technology (microextrusion, laser induced forward transfer – LIFT)
- 2) Choice of biomatrix/hydrogel (alginate, chytosan, etc.)
- 3) Printing parameters (temperature, spatial resolution, cell density, etc.)
- 4) Interactions between multiple cell types
- 5) Use of multiple biomatrices
- 6) The need for associated microfluidics

The choice of printing technology is especially important. Microextrusion technology is essentially the same as used in thermal inkjet printing, and can attain a spatial resolution down to about 100 μm using biogels. Currently available printing platforms provide multiple printing heads and differential temperature control for utilization of diverse materials. Printing parameters must be optimized for each material applied, and material-specific shear forces are relevant when working with labile cells.

Another promising technology is laser-induced forward transfer (LIFT) printing, which uses laser light to force cells from a fluid interface onto a surface, rather than extruding cells through a printing head. This ensures high spatial resolution ($<100\text{ }\mu\text{m}$), corresponding essentially to single-cell seeding, and high-speed cell placement (1000 droplets/sec). LIFT is not dependent on using a biomaterial as vehicle, and thus shear forces are less relevant. However, laser power is a significant factor.

3-D bioprinting

One promising approach to 3D organ fabrication is to use special bioprinters to prepare tissue scaffolds and constructs that reproduce some of the complex interactions occurring among different cell types within a tissue (Figure 1). Relevant studies have resulted in the production of organ-like cellular complexes, for example tubular/glomerular kidney structures (Figure 2).

Figure 2. First 3D printed kidney tissue (<http://ir.organovo.com/news/press-releases/press-releases-details/2015/Organovo-Describes-First-Fully-Cellular-3D-Bioprinted-Kidney-Tissue/>).

Figure 3. An example of a modern bioprinter.

Natural templates

An alternative, and also still experimental, approach to organ manufacture is represented by decellularization and subsequent recellularization of native organs, exemplified by attempts using animal organs, including rat heart [4, 6], rat kidney [1] and monkey lung [2]. As an example of this approach, bone marrow derived mesenchymal stem cells or lung-derived microvascular endothelial cells have been seeded into decellularized lung scaffolds and have generated epithelia that histologically resemble natural respiratory airways [2].

An important challenge when designing tissue scaffolds is the adequate incorporation of vascularization. One potential approach, termed “nano-origami”, involves complex nanostructures that are constructed as topographically patterned 2D substrates that can be seeded with cells and then rolled or folded into a 3D shape [5], see also <http://www.materialsviews.com/advanced-origami-nanostructures-from-flowers-to-boxes/>
<http://nextbigfuture.com/2012/04/logic-gated-nanorobot-for-targeted.html>).

Relevance for hematology

A main area of potential relevance of 3D bioprinting for hematologists is in modeling hematopoiesis in a more natural tissue microenvironment. A variety of differentiating blood cells, accessory cells and immune cells exist within the structural microenvironment of bone marrow, creating intimate functional and regulatory relationships. Thus, generating appropriately patterned 3D tissue models could create promising opportunities for investigations of normal and altered hematopoiesis in a realistic biological niche.

Conclusions

3D bioprinting is a field in rapid development. Increasing numbers of studies are highlighting some of the potential applications, including more physiological and penetrating investigation of tissue and organ development and function, the generation of personalized drug testing and disease models, and scaffold and tissue printing for clinical use. 3D bioprinting is likely to contribute substantially to tissue engineering efforts related to organ replacement.

Conflict of interest

None declared

References

1. Bonandrini B, Figliuzzi M, Papadimou E, Morigi M, Perico N, Casiraghi F, Dipl C, Sangalli F, Conti S, Benigni A, Remuzzi A, Remuzzi G. Recellularization of well-preserved acellular kidney scaffold using embryonic stem cells. *Tissue Eng Part A* 2014;20 (9-10):1486-1498.
2. Bonvillain RW, Scarritt ME, Pashos NC, Mayeaux JP, Meshberger CL, Betancourt AM, Sullivan DE, Bunnell BA. Nonhuman primate lung decellularization and recellularization using a specialized large-organ bioreactor. *J Vis Exp* 2013;82:e50825.
3. Kolesky DB, Truby RL, Gladman AS, Busbee TA, Homan KA, Lewis JA. 3D bioprinting of vascularized, heterogeneous cell-laden tissue constructs. *Adv Mater* 2014;26(19):3124-3130.
4. Ott HC, Matthiesen TS, Goh SK, Black LD, Kren SM, Netoff TI, Taylor DA. Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart. *Nat Med* 2008;14(2):213-221.
5. Sun Y, Weng S, Fu J. Microengineered synthetic cellular microenvironment for stem cells. *Nanomed Nanobiotech* 2012; 4(4):414-427.
6. Vunjak-Novakovic G, Lui KO, Tandon N, Chien KR. Bio-engineering heart muscle: a paradigm for regenerative medicine. *Annu Rev Biomed Eng* 2011;13:245-267.