BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, March 2019. Vol. **16**(1), p. 15-17

4D Bioprinting as New Tissue Engineering Perspective

Anna Lapomarda[1,2] and Giovanni Vozzi[1,2]

[1]Research Centre 'E. Piaggio' of University of Pisa, Pisa, Italy.
[2]Department of Ingegneria dell'Informazione of University of Pisa, Pisa, Italy.

http://dx.doi.org/10.13005/bbra/2715

(Received: 04 March 2019; accepted: 15 March 2019)

Three-dimensional (3D) biological substitutes (scaffolds) able to restore the functions and anatomical properties of living tissues play a key role in Tissue Engineering (TE). Scaffolds can be used to develop patient specific tissue constructs, which once seeded and colonized by the patient cells, can be implanted to induce tissue regeneration avoiding rejection effects. Moreover, they could be used as template for drug screening or to define models of physiological and pathological states.

The identification of the chemical and physical properties that should characterize an ideal scaffoldis still one of the main challenges of TE.Nonetheless, it is well known that an ideal scaffold has to mimic the natural tissue on the macro- and micro- scale. As the natural extracellular matrix (ECM) surrounding the cells, the scaffold represents the framework for dissociated cells to reform an appropriate tissue structure. Thescaffold should reproduce the topological properties (e.g. three-dimensionality, macro- and micro-porosity, pore interconnectivity, surface roughness), mechanical properties (e.g. elastic modulus) and biochemical signalling (e.g. ECM composition) of the living tissue. The more the scaffold is similar to the natural tissue, the higher is the chance that cells recognize it as their natural environment and start to adhere, migrate, proliferate, differentiate and vascularize it.

The scaffold features are crucially determined by materials and their bio fabrication techniques. Biocompatible and biodegradable polymers (natural or synthetic) and ceramics, with or without inclusion of bioactive molecules, are the main materials used to produce 3D scaffolds. Different fabrication techniques are utilised to obtain these scaffolds, such as solvent casting, freeze drying or bioprinting which include a series of computer aided fabrication techniques[1-2]. Theselast techniquesallowto obtain tailored and reproducible scaffolds with a high control over the scaffold architecture, pore geometry and pore interconnectivity by layer-by-layer deposition of biomaterials and living cells. Despite all the advantages, 3D bioprinted scaffolds are not able to reproduce the dynamic activities and functions of living tissues and organs (e.g. heart contractility, gut peristaltic activity and bone remodelling)[3], which are very important for the maintenance of the homeostasis. 3D bioprinting considers, in fact,the scaffold as an inanimate and static

*Corresponding author E-mail:

support neglecting the bidirectional cell-scaffold interactions.

Four-dimensional (4D) bioprinting is currently emerging as a promising and innovative biofabrication approach which may have a positive impact and mayrevolutionize the TE paradigm. Particularly, 4D bioprinting is so called since it introduces the variable 'time' as fourth dimension in the 3D bioprinting process. It enables to obtain scaffolds that replicate not only the complex geometry of natural organs but also the ability of living tissues to react to external stimuli at the macro- and micro-scale. This is possible mainly by two different approaches. The first is based on the 3D printing of 'smart' materials, namely responsive materials, which are able to reshape or transform themselves in response to external stimuli (e.g. variations of temperature, pH, humidity, electric fields, magnetic fields, light)[3]. Biocompatible responsive hydrogels are promising material candidates for 4D bioprinting approaches. These materials, in fact, change their shape by swelling or deswelling in response to external stimuli. Furthermore, hydrogels mimic the physical properties of the natural ECM and many of these are printable at physiological temperature (37°C) supporting viable cells during 3D printingprocess[3-4].One of the most studied hydrogels for 4D bioprinting applications are the thermoresponsivepoly(N-isopropylacrylamide)-based polymers that undergo a reversible volume transition at a critical solution temperature close to physiological temperature[5]. Natural polymers with cell laden have also been used in 4D bioprinting approach. In this regard, engineered blood vessels were obtained by 3D bioprinting cells encapsulated in alginate and hyaluronic acid on a flat surface. The tube shape was subsequently obtained upon immersion the 3D bioprinted structure in cell culture media. Blood vessels with 20 μm diameter were obtained (which are not yet achievable by other existing biofabrication approaches) and a high cellular alignment on the vessel walls was observed[3-5]. The second approach refers to the production of active forces produced upon the maturation of engineered tissue constructs after printing[3]. Cell traction forces, originated from intracellular actin polymerization and actomyosin interactions, are used in the method called 'cell origami' to produce 3D microstructures by culturing cells on two-dimensional (2D) microplates. The active cellular force causes the folding of the 2D surface in predefined shapes. By changing the geometry of the patterned 2D microplates, various cell-laden structures can be obtained after folding[5].

Both these approaches allow to obtain programmable ECM-mimicking scaffolds, namely dynamic constructs whose external stimuli responses are programmed *a priori*. Particularly, mathematical models provide an important support to simulate and optimize both the scaffold architecture and its composition to obtain predefined functional scaffolds. Compared to 3D scaffolds, 4D scaffolds provides extra stimuli to cell adhesion, proliferation and differentiation to a specific cell phenotype obtaining more realistic engineered tissues. This may have a positive contribution to solve unaddressed worldwide medical needs, such as organ transplantation, by inducing the regeneration of the intended tissue. Finally, thanks to 4D bioprinting it is possible to obtain scaffolds that mimic the complex geometry of organs which can not be obtained by traditional 3D biofabrication techniques to date.

Being a new biofabrication approach, 4D bioprinting methods and applications are still currently investigating and several challenges need to be addressed. One of the main challenges of 4D bioprinting is to reproduce the cyclic activity of living organs, such as cardiac contraction, at the same extent and frequency as naturally occurring. Controlling the spatiotemporal response of 4D scaffolds in terms of variation of shape, orientation and/or functionality is in fact arduous. Moreover, the number of printable responsive biomaterials with high sensitivity to specific stimuli at physiological temperatures is limited.Further studies need to be carried out both to synthesize new processable responsive biomaterials and to identify new actuation forms in order to better mimic the functionalities of living tissues. Finally, the effect of the fourth dimension on *in vivo* tissue regeneration has also to be investigated.

In conclusion, 4D bioprinting opens up new perspectives to find interesting and innovative solutions to overcome TE issues.

REFERENCES

1. Moroni, Lorenzo, *et al*. "Biofabrication: a guide to technology and terminology." *Trends in biotechnology,* 2018; **36**(4): 384-402.
2. Groll, Jürgen, *et al*. "Biofabrication: reappraising the definition of an evolving field." *Biofabrication*, 2016; **8**(1): 013001.
3. Gao, Bin, *et al*. "4D bioprinting for biomedical applications." *Trends in biotechnology,* 2016; **34**(9): 746-756.
4. Morouço, Pedro, and João Gil. "Four-Dimensional Bioprinting for Regenerative Medicine: Mechanisms to Induce Shape Variation and Potential Applications." Innovations (2019).
5. Ashammakhi, Nureddin, *et al*. "Advances and future perspectives in 4D bioprinting." *Biotechnology journal,* 2018; **13**(12): 1800148.