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FABRICATION OF COMPLEX HYDROGEL STRUCTURE VIA FREE-FORM BIOPRINTING: A REVIEW

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ABSTRACT: Different bioprinting techniques has been used in recent years to produce complex structures with hydrogels, however these approaches have been limited to simple 3 or 2.5 dimensional structures. This paper reviews the existing methods that are able to fabricate free-form complex shapes via bioprinting for tissue engineering purpose. The principle behind these free-form bioprinting techniques can be broadly categorized into 1) Providing a physical scaffolding or support structure that lifts the build material 2) Organizing complex cellular structures (termed tissue spheroids) in a controlled manner. The techniques reviewed are F.R.E.S.H. bioprinting, stereolithography, magnetic bioprinting and organovo extrusion based bioprinting. We discussed the processes critically in term of materials and resolution achieved by these techniques. The advantages and disadvantages of these techniques are presented as well.

KEYWORDS: Additive Manufacturing, Bioprinting, Rapid Prototyping, 3D Printing.

Introduction

3-Dimensional (3D) cell culture has been the standard for cell culturing for recent years because it has the ability to mimic the natural state of the cells in the in-vivo study compared to the conventional 2D culturing technique(Cukierman, Pankov, Stevens, & Yamada, 2001). Significant improvement in cellular response has been identified in areas such as differentiation, gene expression, proliferation and morphology. Thus, various methods have been developed to 3D culture cells. The methods ranges from the use of rapid prototyped scaffolds(Yeong, Chua, Leong, Chandrasekaran, & Lee, 2005), electrospinning(Sun et al., 2007), cell coated hydrogel beads(Tsuda, Morimoto, & Takeuchi, 2009) and recently, the use of 3D printing of cells seeded hydrogels(Lee & Yeong, 2015; Mironov, Boland, Trusk, Forgacs, & Markwald, 2003).

Bioprinting applies the principles of additive manufacturing by using layer-by-layer deposition of cells and matrix (Mironov, Reis, & Derby, 2006; Tan & Yeong, 2014). Such use of a technology enables the advancement of porous construct and multi-material printing. The ability to determine the shape and size of printable hydrogels enable researchers to develop complex hydrogel designs that mimic the natural state of cellular environment and provide effective nutrient transfer across a 3D material. However, there are current challenges that limit the choice of materials in bioprinting. Materials that are used for bioprinting are usually limited by their viscosity and gelling speed, limiting the number of potential hydrogel formulations that matches the bioprinting process window (Malda et al., 2013).

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This paper reviews the existing methods that are able to fabricate free-form complex shapes via bioprinting for tissue engineering purpose. The principle behind these free-form bioprinting techniques can be broadly categorized into 2 distinct groups, i.e. 1) Providing a physical scaffolding or support structure that lifts the build material 2) Organizing complex cellular structures (termed tissue spheroids) in a controlled manner. This paper describes the processes critically in term of materials and resolution achieved by these techniques. The advantages and disadvantages of these techniques are presented as well.

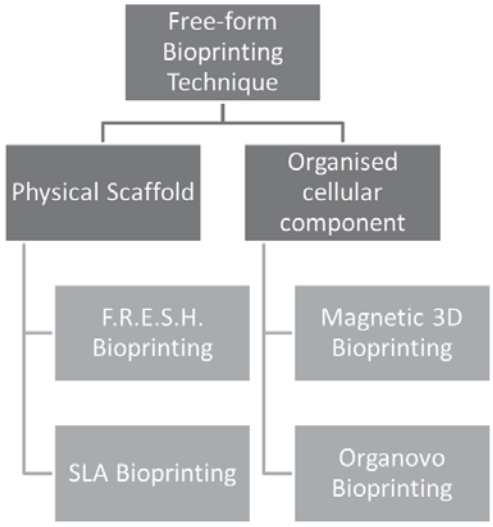

Figure 1: Free-form Bioprinting Techniques

Physical Scaffolding Approach

Physical scaffolds represents the use of hydrogels or other forms of supporting scaffold to align and manipulate individual cells to conform into their structural shape. Such forms of physical scaffolding usually can present itself to be able to create relatively accurate representation of organs in the macroscale. However cell density within the scaffold are usually lower as compared to cellular printing. Cell death generally occurs due to inhibition of nutrient diffusion across the support material

FRESH Bioprinting(Hinton et al., 2015)

Modified from a commercial 3D printer, this printing method uses a gelatin slurry base to extrude ink into the solution. The slurry acts as a Bingham Plastic, not yielding until a specific shear force has been applied. It allows the use of multiple types of material ranging from calcium alginate hydrogels to and has the ability to print free hanging structures in 3-Dimensional space. However, solution has to be printed slowly so as not to disrupt the printing. The resulting resolution (200-1000um) of the print is also limited by the nozzle size, the cross linking kinetics, gelatin microparticle size, extruder translation speed, flow rate and the material's elastic

modulus(<500kPa). The material has to be calibrated to account for osmosis and density during printing. The process has to be printed in a controlled temperature environment (22 ± 1 ^{o}C).

Stereolithography Bioprinting

This form of bioprinting uses the SLA technology to cure photocurable hydrogels using a beam from a laser or a projector. One such process of bioprinting was developed by the University of British Columbia(Wang et al., 2015). In this process, Poly(ethylene glycol) diacrylate (PEGDA) and gelatin methyl acrylate (GELMA) with a white light photo-initiator (Eosin Y) was used to cure the hydrogel . The use of a white light photo initiator as opposed to the commonly used UV initiator such as Irgacure 2959 helps to improves the cytocompatibility of the process by reducing photo damage on the DNA which could eventually induce cancer on the cells. Although the process was able to produce a resolution of up to 50um, it could be ascertain that this process of printing can only produce a 2.5 Dimensional structure as over-hangs cannot be printed. White light stereolithography uses white light for curing, the printing time is about 2-4minutes, compared to the use of UV light which has a printing speed of about 4 seconds

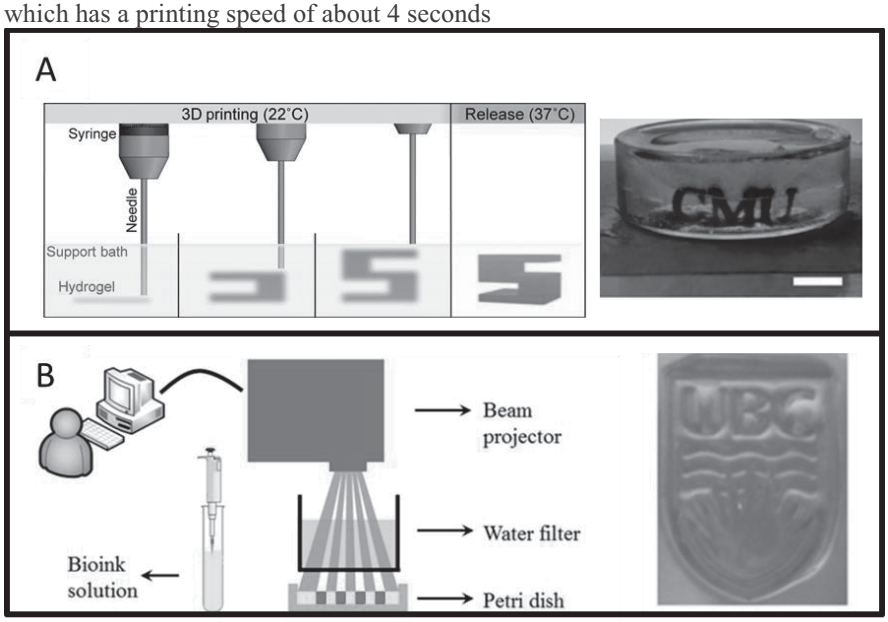

Figure 2: Examples of Bioprinting (A) FRESH Bioprinting (B) SLA Bioprinting ,adapted from (Hinton et al., 2015; Norotte, Marga, Niklason, & Forgacs, 2009; Wang et al., 2015))

Organizing Complex Cellular Structures

Another approach to produce complex cellular structure is through organizing complex cellular structures. This method of fabrication uses a cluster of cells (cell spheroids) as a unit of fabrication. Due to the 3-Dimensional interactions between the cells. Noticeable improvements in the cellular functions are usually identified in these tissue models. However, processes to control the fusion and placement are relatively complex and require simulations to predict the eventual shape of the printed construct. Cell death may occur when the cell spheroids have over-developed above their critical size, leading to low nutrient exchange.

Organovo's Extrusion Bioprinting

This form of extrusion printing can create small tissue models via cell printing. One example is Organovo's NovoGen MMX Bioprinter™. The printing platform consists of two deposition heads for dispensing different materials, enabling materials to be constructed with spatial control in the *X*, *Y*, and *Z* axes. The cellular bio-ink units serves as build material and agarose material serves as support material. Thus, complex cellular patterns or structures can be produced, which mimics key aspects of *in vivo* native tissues. The method is able to extrude spherical or cylindrical cellular aggregates with diameters of 500 or 260 μm, through a preloaded micropipette that serves as cartridges(Chua & Yeong, 2014). The Organovo's bioprinting process utilises the principles of spheroid cell behaviours such as self-organisation, self-assembly and tissue fusion(Mironov et al., 2016; Mironov et al., 2009).

Magnetic Bioprinting

Another method of controlling and placement of cell spheroids is through the use of magnetic particles. Bioprinted magnetically labelled cells and spheroids can be manipulated and moved towards a magnetic field(Tseng et al., 2015). One such process of using magnetic bioprinting to organize a tissue structure is through the combinatory use of bioprinting and magnetic nanoparticle(Kivilcim et al., 2009). By mixing hydrogel and magnetic nanoparticle, the magnetically labelled cells and bioactive factors becomes magnetically active. Using a static magnetic field, it was noted that the nanoparticles can be controlled within the hydrogel. However these form of nanoparticle manipulation can only be done on soft hydrogels such as on a 1% Calcium Alginate solution. At stiffer hydrogels, the nanoparticles are not able to reposition itself. Moreover, it was noted that the magnetic nanoparticles did indeed cause internal stress on the cells leading to eventual cell death.

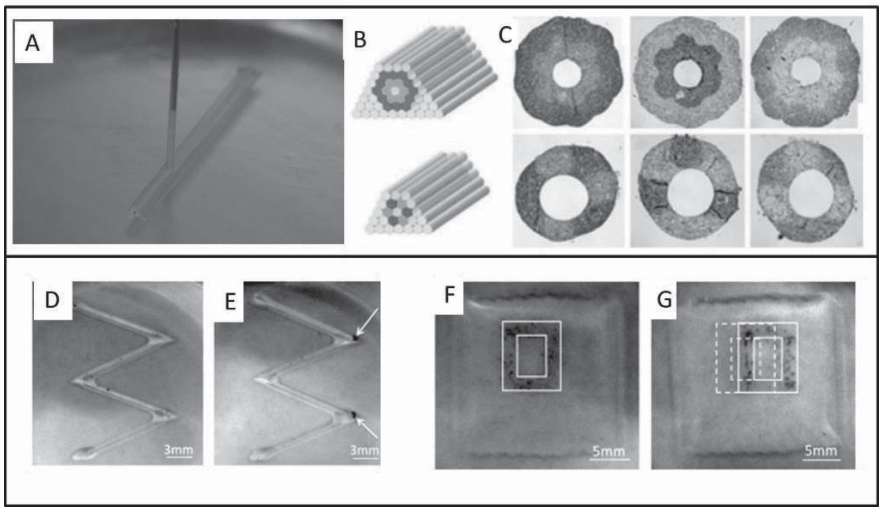

Figure 3: Examples of organized cellular components (A-C) Process of Extrusion based Bioprinting and tissue spheroid fusion (D-G) Effects of magnetic nanoparticles manipulation (black areas are magnetic nanoparticles), adapted from (Kivilcim, Wonjin, Wei, & Alisa Morss, 2009; Norotte et al., 2009)

Resolution and Materials

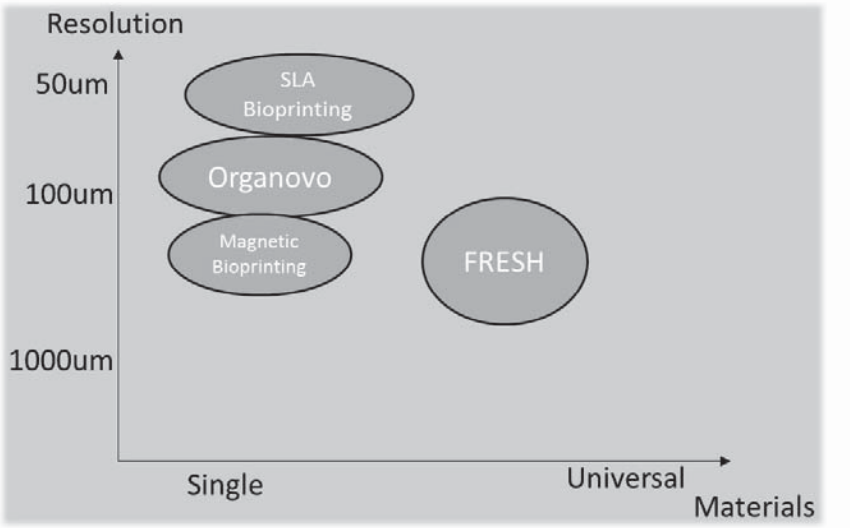

Figure 4: Comparison between the resolution of printing with the number of bioinks usable on the process

A major concern in bioprinting is the resolution and the adaptability of the process on the types of material to be used. The resolution depicts how accurately cells and the scaffolds can be positioned on the 3-Dimensional plane, while the types and number of materials shows how flexible the process can be tune to the different characteristics of materials to be used. From the comparison, it is noted that stereolithography bioprinting is able to produce hydrogels that have extremely high resolution of up to 50μm however, this process is only applicable to be used for materials that cross-links through photopolymerisation. Also notable is the use of F.R.E.S.H. process, where a large variety of hydrogel can be printed, but due to the use of an extrusion process within a slurry, the resolution of the printed scaffold is limited.

Conclusion

A review on the methods for bioprinting hydrogel with defined strut thickness and complex 3D configuration has been presented. For each of the process, their critical parameters affecting the cell viability and resolution has been discussed in details. Although many methods have been developed, each has their own specific strengths and weaknesses. The process of free-form bioprinting brings humanity a step closer to develop an organotypic culture that mimics the human organ. However, more research are needed to improve both the resolutions and materials printable for free-form complex cellular structures.

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