3D BIOPRINTING: CAN WE CREATE THE STRUCTURES IN LIVING CELLS?

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The assembly of structures to interact with living systems requires the ability to distribute multiple materials and different cell types in three dimensions. This assembly places a number of demands on the hardware required, and on the bioinks (liquid cell suspensions housed in printing cartridges).

In this article, we will review our progress to date in creating 3D structures for use in:

- cartilage regeneration (in collaboration with St Vincent's Hospital, Melbourne)
- carriers for islet cell transplantation (in collaboration with the Royal Adelaide Hospital)
- wound healing (in collaboration with St Vincent's Hospital, Melbourne).

These applications demand a customised approach for each clinical challenge.

Customised bioinks

Ink formulation should consider the requirements of a printing modality. For instance, for extrusion printing, a bioink should possess the desired viscoelastic characteristics for high-fidelity cell printing, including both shear-thinning and fast shear-recovery properties. Furthermore, the chemical structure of a bioink should be amenable to a rapid and cytocompatible crosslinking process to stabilise the printed 3D structure. Ideally, the 3D printed structure should be cell/tissue-specific, with the biochemical and biomechanical cues that are critical to elicit the desired cellular phenotype and functions.

Cartilage regeneration

We have developed a chondrogenic bioink based on modified gelatin and hyaluronic acid. In tandem with a customised, handheld extrusion device called the Biopen, we have devised a unique printing platform, namely, co-axial Biopen printing, for achieving both mechanically and biologically enhanced constructs. This offers the potential for regeneration of articular cartilage. The building blocks of the constructs consist of bioactive cell-laden cores that are supported by acellular polymer sheaths.

Islet transplantation

Bioprinting of islets of Langerhans cells has recently been demonstrated with good viability and morphology, using alginate/gelatin as the bioink. To enhance islet survival, it is crucial to re-establish the native environment, including vascular support, while minimising the immune rejection. 3D printing of islets with other islet pro-survival cells, including endothelial progenitor cells and immunomodulatory cells, offers a promising strategy to improve islet survival and engraftment.

Wound healing

Though still at an early stage, bioprinting offers the potential to create complex skin substitutes. Research in skin printing benefits from a rich heritage of knowledge of skin-tissue engineering and skin grafts for clinical use, and is further accelerated by the involvement of three leading firms, including L'Oréal, P&G and BASF. Current skin-printing activities often employ a collagen-based bioink to produce multi-layered cellular structures of skin fibroblast and

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keratinocytes. Studies have shown that the printed skin cells survived well and formed keratinised skin-like tissue with ingrowth of some blood vessels from the wound bed.

Customised hardware

Each of the identified applications presents its own challenges in terms of hardware, each influenced by a range of factors. These factors, including the biomaterial's physical properties, the working environment and required feature resolution, are typically interdependent, and result in the need for fabrication tools to be tailored to the specific application.

The physical properties of the biomaterial dictate the form of printing to be employed. The most common deposition method relies on extrusion of fluid through a nozzle at a controlled rate or applied pressure, with commercial examples including EnvisionTec, regenHu and INKREDIBLE. Discrete deposition of finer droplet volumes of fluid (pL–nL range) is achieved through micro-valve jetting or ink jetting. It is possible to have inkjet capability included in the most recent of commercial biofabrication systems; however, inkjettable materials have very constrained physical limits for viscosity and surface tension, limiting where higher resolution can be achieved. Extrusion printing works over a much wider operating window through adjustment of parameters such as temperature (to modulate viscosity), applied pressure, and nozzle diameter to control extrusion rate and ensure a continuous, uniform extrusion pattern.

In the case of the working environment, we have to consider how the hardware is intended to be used. Automated fabrication systems are not always the most effective approach. The Biopen is an excellent example of this, as the dexterity of the clinician's hand enables the controlled and structured delivery, and the 'sculpting' of patient cells directly to the defect site to facilitate effective repair and cartilage regeneration. In this case, there is typically no way of predicating the exact geometry of the defect prior to the operation. For this reason, a handheld, free-form fabrication method is essential. In a case where a level of repeatability and accuracy has to be ensured—such as in the production of a porous lattice structure for the entrapment of islet cells—automated positioning of the biomaterial delivery device, seen in the commercial examples mentioned above, is critical. High-resolution, three-axis stages allow fine positioning within computer-controlled coordinate systems, but present systems have limitations in terms of hardware and control.

Biofabrication holds great potential, but is still in its infancy. We are not yet at the point where a clinician can place an automated defect data capture and printing system in their surgical theatre for on-demand production of patient-specific implants. When this might occur will depend on continuing advances in inks, materials, stem cell biology and printing hardware. Such advances depend critically on our ability to build collaborative research teams capable of interdisciplinary research and training.

Gordon Wallace will be speaking at the 17th International Biotechnology Symposium (IBS 2016).