Engineering Challenges in Biofabrication

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Abstract

Biofabrication describes the inkjet application of bioink which may include active compounds such as drugs and living cells as well as non active, scaffolding materials to build two- and three-dimensional constructs for medical treatment. Many of the challenges in tissue engineering generally and biofabrication specifically are biological in nature; however, many appear to fall within the realm of imaging and science and technology. One challenge is to arrange the donor cells into the exact patterns that will promote growth towards the desired tissue form and function. Of the many approaches that have been suggested to accurately place cells the inkjet printing approach is one of the more interesting. In these devices researchers have tailored their bioinks by two approaches, namely using new biomaterials that fit the processing window of commercial printers or developing new systems that use the biomaterials as bioink directly. Tailoring the physical properties of these inks, and developing printheads optimized for these properties will improve cell density, and the tissue fabrication speed. Biofabricated tissues can be used to build models of the effects of local environment on different cell types. The models can be incorporated into computer design and simulation environment in order to predict tissue function.

Introduction

There is an acute shortage of human organs, such as heart, lungs, liver, kidney, pancreas for transplantation which prompted several approaches to solve the problem. Biological based approaches such as gene therapy, stem cells or, xenotransplantation are still decades away from success. In addition, there is still great concern about potential spreading of animal viruses[1] and the long-term psychological effects of immunosuppressants that were revealed in the hand allograft studies[2], in which patients preferred to be taken off the medication and suffer the loss of the limb .

Tissue Engineering is a promising approach that is partly rooted in materials science and engineering on engineering to solving the need for replacement of failed organs. This tissue approach is based on seeding isolated and expanded cells onto pre-formed solid rigid scaffolds[3]. While this approach has yielded in some unprecedented success for hollow organs[4], there are, however, at least three concerns with applying this approach to non hollow organs:

a) Cell penetration and seeding is not very effective. Tissue maturation proceeds on the time scale of months and is not uniform throughout the scaffold. Although there is significant progress in designing scaffold allowing effective seeding and cell migration[5], the approach is still far from optimal.

b) Organs usually consist of many cell types and placing different cell types in specific positions represents a challenge that is still far from being resolved.

c) The pre-formed rigid scaffolds made from PLGA are not optimal for engineering contractile tissue such as heart, vascular tubes, or capillaries.

It is a generally accepted hypothesis that effective vascularization of tissue engineered constructs is a key to build larger tissues or organs[6]. One approach to solve the vascularization issue is to engineer small diameter vessels and capillaries within the scaffold through a combined solid freeform fabrication and cell placement approach[7].

Several groups have designed and built scaffolds with controlled architecture. Complex hierarchical scaffold designs can only be built using layer-by-layer fabrication processes known collectively as solid free-form fabrication (SFF). A number of recent articles have reviewed and contrasted SFF scaffold fabrication techniques.[8,9,10,11] All SFF systems build a 3D structure by layering a 2D material onto a moving platform. Commercially available systems either photopolymerize liquid monomer, sinter powdered materials, process material either thermally or chemically as it passes through a nozzle, or print material, such as chemical binder onto powders. Moreover, SFF techniques can be easily automated and integrated with imaging techniques to produce constructs that are customized in size and shape allowing tissue-engineering grafts to be tailored for specific applications or individuals.[9]

Ink Jet Printing of Biomaterials

Recently, the inkjet technology has been successfully adapted to medicine and biomedical engineering applications, such as drug screening, genomics, and biosensors.[12,13,14] Although biological molecules and structures are often viewed as fragile, molecules such as DNA have been directed onto glass by commercial inkjet printers to fabricate high-density DNA micro-arrays without molecular degradation.[15] In addition, proteins such as horseradish peroxidase have been deposited onto cellulose paper to create active enzyme arrays for bioanalytical assays.[16] We have shown that active biosensors based on biotin-streptavidin linkages can be deposited onto glass.[17] Commercially available desktop printers were modified to perform diverse tasks, such as printing self-assembled monolayers, proteins, and other molecules.

More recently, a novel concept of inkjet printing cells and biomaterials by using the off-the-self printers to generate 3D scaffolds and cellular structures has been proposed.[18] Organ printing, defined as computer-aided jet based tissue engineering, is an advance in SFF as it allows constructing a 3D object with living biological material, such as a specific cell type, tissue or organism. A fundament requirement of this process is its capability of simultaneously delivery scaffolding materials, living cells, nutrients, therapeutic drugs, growth factors, and or other important chemical components at the right time, right position, right amount and within the right environment to form living cells/ECM (or scaffold) for in vitro or in vivo growth. Here, we

will present an overview of our work demonstrating that a single device can perform these tasks.

Bio ink

One of the significant challenges in tissue engineering is to arrange the donor cells into 2-D and 3-D configurations that will promote growth towards the desired tissue form and function. Many approaches have been suggested to accurately place cells. The processes are typically highly specific in that the type and form of material that can be processed. In the past, researchers have tailored their bioinks to be tissue-engineering specific by two approaches, namely using new biomaterials that fit the processing window of commercial inkjet printers[19] or developing new systems that use the biomaterials as bioink directly [20,21]. There is a need to improve the biomaterials that can be used as bioinks, which currently include natural hydrogels,[19] living cells[22] and collagen solutions[23]. Tailoring the rheological and surface properties of the inks, and develop printheads optimized for these properties will improve cell density, and the speed at which tissues may be manufactured. Incorporating controlled release particles loaded with growth factors or signaling molecules into bioinks opens interesting avenues for combining cell printing with other potential therapeutic modalities.

3D printing of biomaterials using thermal inkjet printers

In order to build a 3-dimensional structure, z-axis control through a moving platform are typically implemented using an electronically controlled chamber with an elevator stage. The chamber is typically filled with a polymeric solution that is known to crosslink chemically or physically by pH or temperature change. By applying the crosslinker or physical stimulus layer-by-layer, deep to superficial onto the platform, 3D structures will be generated. A number of acellular structures have been published that were printed with this setup, including tubes, branched tubes, and hollow cones (Figure 1). The diffusion of the crosslinker throughout the sample during and after biofabrication is a key element to be understood. Typically, the reaction rates are concentration dependent; thus, by changing the concentrations of hydrogel and crosslinkers, a variety of structures can be obtained, ranging from amorphous to dots to channels.

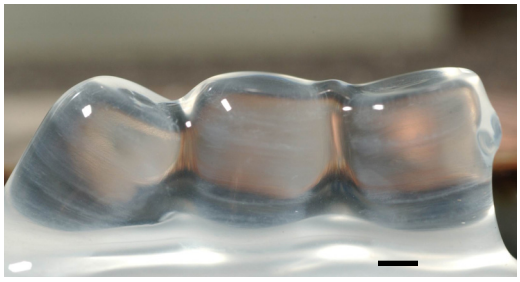

Figure 1. *Photographs of printed tubes. (A) Parallel tubes are shown immediately after printing with the stage raised above the liquid level. (C) A branched chambered structure.*

Detailed studies on how different concentrations of alginate and crosslinker affect the biofabricated structrures have been published elsewhere (ref). As diffusion of the crosslinker and its reaction with the polymeric materials are typically proceeding in axisymetric fashion, but the printing rate is much different for x, y, z directions, being slowest in z, the resulting structures are highly anisotropic. Furthermore, the fabrication of hollow structures is typically limited to the x/y plane as it relies on rapid crosslinker diffusion. Thus optimizing print speed with respect to reactivities of the various bioinks will be most critical in achieving desired microstructures of biofabricated materials.

Simultaneous printing of materials and cells

Recently, we examined endothelial cell attachment to biofabricated hydrogels. We have evidence of good cell proliferation inside microchannels, proving that the cells are able to attach and migrate in these gels after being exposed to the crosslinker, and that the biofabricated structures may serve as a printable tissue engineering matrix.

Conclusions

Much of the early work, during the past six years, has focused on the used on the use of off-the-shelf technology from commercial printers (such as the HP DeskJet). This has proven to be a low-cost means of obtaining high tech micro-, electromechanical systems at a very low cost. The current approach has been to design the bioink, e.g. viscosity and surface tension, to match the ink printing process. It appears there is now an opportunity to re-examine the control of the bubble jet mechanism and tailor the printing parameters to new bioinks. Furthermore, with the basic process of cell printing being established it may be possible to redesign the basic ink ejection mechanism to better suit bioink. MEMS devices have the advantage over single nozzle devices as allowing for parallel deposition of many bioinks. In thermal inkjet printers the drop size is controlled by the volume of the firing chamber and the orifice diameter. The heat is usually controlled by the amperage reaching the chamber, which needs to be tuned to allow for evaporation of a small volume of liquid, while preventing the chamber to heat up significantly during prolonged firing. In piezo driven devices, the drop size is controlled by the waveform sent to printheads. While each of these types of heads has their advantages, the cost savings and flexibility in nozzle sizes above 50 microns of thermal printers are viewed favorably over the piezo printers.

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