Abstract

Living tissues can be constructed in vitro by 3D bioprinting of cells held in hydrogel solutions which are then cross-linked to form 3D geometries analogous to in vivo tissues. Signalling from cell-to-cell and microenvironment-to-cell interaction both effect cell behaviour and life cycle. Artificial cell containing constructs must mimic a sufficient level of the complexity of in vivo tissues to be useful in vitro models for drug testing and fundamental cell biology research. Further demands include maintaining cellular viability and functions during tissue construction while also minimising printing time, cells, cell culture media and reagents required.

Our work involves interdisciplinary collaboration in the fields of bioengineering, stem cell biology, precision engineering and process control. Together we are building a high precision living cell printing system capable of rapidly constructing complex tissues in 3D.

A number of highly sensitive cell types have been successfully printed including human pluripotent stem cell derived cardiomyocytes and hepatocyte-like cells. Cell printing, 2D & 3D constructs, plus a positioning mechanism were all complemented by diagnostics to give the building blocks needed for the manufacture of artificial biological screening models.

1. Introduction

Toxicology testing of substances and efficacy testing of drugs are generally performed on live animals as proxies for humans. Government supported efforts are being made towards replacement, refinement and reduction of animals in research [1, 2]. Current two-dimensional cell-culture methods have limited similarity to in vivo tissues [3, 19] and spheroid models have practical problems [4] as well as geometric constraints. Several technologies are being developed to better represent in vivo tissues including organs-on-chips [5, 6] and bioprinted tissues [7].

Additive manufacturing techniques offer the ability to construct complex geometries with high spatial resolution, a technology expected to have broad economic effects [8]. Since their first demonstration in 2006, induced pluripotent stem cells (iPSC) have become available and may be differentiated into any one of a range of specific cell types found in bodily tissues [9]. Various extracellular matrix (ECM) or ECM-like scaffold materials have been used to construct three dimensional structures [10] into or onto which living cells can be placed to form geometries intended to represent tissues. Structural materials include hydrogels [11, 12], collagen [13, 14], polystyrene [15], and more.

By bringing together specialists in bioengineering, engineering, molecular biology and diagnostics there exists the potential to create a process for manufacturing artificial tissues with sufficiently similar response to a broad range of toxic substances so as to be useful as a more reliable, lower cost, high throughput and repeatable early stage screening tool. This paper presents successful demonstrations of precision cell delivery with high post-printing viability, identification of suitable scaffold material, a mechanism capable of rapidly positioning the cells, and high precision cell viability measurements.

2. The team

This collaborative project includes specialists in bioengineering and mechatronics (Heriot-Watt); biology, toxicology and diagnostics (Clyde Bioscience, Roslin Cellab); as well as engineering and process control (Renishaw). Together we are building a system capable of rapidly constructing complex tissues.

2.1. Cell delivery method and scaffold

Heriot-Watt University have developed a microvalve-based cell printing mechanism [16] capable for delivering cell suspensions with nano-litre resolution. Living cells pass through the delivery system with post-printing viability comparable to cells delivered by pipette tips but at much higher resolution. We demonstrated the delivery process was capable of printing human pluripotent stem cells without affecting their key biological functions including pluripotency (as shown by pluripotency markers) and post-printing differentiation into hepatocyte-like cells with albumin secretion and morphology similar to hepatocytes [17]. Bio-ink droplets containing a liquid suspension of live cells and sodium alginate chains was deposited by one printing valve followed by a droplet of a calcium solution from a second valve. Sodium alginate chains become cross-linked by the calcium ions and form a 3D hydrogel network which contains the cells and forms a self-supporting structure (fig 1).

Refinement of this process has led to self-supporting structures up to centimetre scale while utilizing the properties of biocompatibility, permeability and biodegradability [18]. Sufficient resolution of fluid delivery allows rapid construction on single millimetre scale. Constructs contain sufficient number and density of cells to be statistically useful and for
communications to establish while avoiding the need for excessive use of costly stem cells, associated culture media and reagents.

2.2. Motion platform

In vivo tissues comprise several cell types arranged in specific geometries supported by extracellular matrices. This is where 2D tissues models fail to mimic [19]. Cell development is sensitive to the microenvironment so an artificial tissue must resemble the in vivo environment sufficiently well for a collection of cells within a scaffold to grow into an analogue of the desired tissue. The motion platform used has precision ≤±5 µm to allow the construction of multiple cell types in defined 3D geometries. Platform precision and reliability have been shown over several years while produced and used as a scanning device for dental artefacts [20].

A modified hexapod mechanism is used to avoid the difference in inertia and therefore servo dynamic performance of the motion axes which is inherent to stacked axis designs. Three constraint plates are hinged from a fixed plane, each has two articulating links joining to the moving platform to make parallelograms. All rotational degrees of freedom of the moving platform are thus constrained. Three linear actuators join the fixed plane to the moving platform giving active control of linear motion. Velocity and position feedback loops ensure maximum operational bandwidth in all degree of freedom for fast printing, required to minimise the time cells are outside the incubator. Low hysteresis articulating joints give smooth motion and any errors are continuous, occurring from imperfectly placed joint centres. Error correction is made by a polynomial function which is calibrated by using the mechanism to measure a known artefact.

2.3. High sensitivity cell viability measurement

Commercially available human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) ([Cell2, Cellular Dynamics International, USA] were used to study the baseline behaviour variability using the valve-based cell printing mechanism. To carry out the studies, hiPSC-CMs were plated in glass bottomed 96 well plates using the bioprinter for cell delivery and multichannel pipette as control. The cells were transiently loaded with a voltage sensitive dye (Di-4-Anepps) to ratiometrically measure transmembrane voltage and calcium sensitive dye (Fura 4f-AM) to ratiometrically assess Ca2+, while contractility was measured via cell motion employing the CellOPTIQ® platform. In fig 2 the transient behaviour of printed cells is compared with that of the control. No statistically significant difference was found.

Various hydrogels were tested and a suitable formulation found for 3D structures containing specific cell types.

3. Conclusions

The work has demonstrated each of the building blocks expected to be needed to construct and test in vitro artificial tissues. The most delicate human pluripotent stem cells and their derived cardiomyocytes were delivered using the developed system without measureable damage and a suitable positioning mechanism was modified to carry the printing apparatus. Further work is under way to investigating the manufacture of artificial tissues with statistically repeatable behaviour for toxicology testing.

References

[5] Reardon, Sara 2015 Nature 523 266

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Figure 1. Cells and alginate in suspension are cross-linked by calcium ions to form a structural network [17].

Figure 2. Comparison of three measurements of 2D tissue constructs delivered by manual pipette (control) or bioprinter valve. The three parameters are the electrical activity on the cells (action potential), intracellular Ca2+ (calcium transient) and mechanical activity (contractile kinetic decay). The repolarization parameter refers to the transient return to the equilibrium (resting) after depolarization [21].