Importance of cell-substrate impact during drop-based deposition

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Abstract

The development of new technology for the realisation of biocompatible and bioactive structures is an emerging field in the field of tissue engineering, regenerative medicine and biosensors. These techniques are generally based on the computer-aided design approach. Recently the use of ink-jet printers has been extensively studied both using bio-molecules and cells. Ink-jet systems were noted to cause cell damage in the microfluidic system and in the drop generation system (e.g. heating or piezo-electric), and different thermal inkjet printer heads have been used to address these points. In this work we underline that the impact of the drop on the substrate is more relevant, and show how the properties of the deposition surface can influence cell viability. Firstly the printing process and the drop impact were modelled using fluid-dynamics and fluid-structure interaction equations. To validate the model, the Olivetti BioJet system was used to print cells onto different substrates with different elastic and viscous properties and cell viability was monitored in time.

Introduction

Rapid Prototyping (RP) techniques have been receiving a great deal of attention in recent years in the area of tissue engineering, regenerative medicine and biosensors. Of particular interest is the concept of cell printing. Rather than post-process seeding of biodegradable RP scaffolds with cells, they are printed directly into a matrix made with natural polymers (e.g. alginate, collagen, matrigel). Boland [1] and Mironov [2] pioneered this field realising the first printing processes using living cells as ink. Nowadays different techniques are used to fabricate these kinds of structures. These techniques can be divided in non-impact printing and in direct-writing. In the first group ink-jet and laser [3] based technologies have been used to precisely position viable (cells) and non-viable (biological molecules) patterns. Using these systems patterns with resolution of a few micrometers can be easily obtained. However one of the main limitations of these methods is that only thin structures (few micrometers) can be realised. This is sufficient for basic studies but not for most tissue engineering applications. On the other hand, with direct writing techniques it is possible to process more viscous materials, so that the final structures are somewhat thicker. Examples of direct writing techniques are the PAM2 system [4] and Multi-nozzle deposition system [5].

In this study we demonstrate that the main factor which influences cell viability in all cell-incorporated drop-based printing methods is the impact of a drop on the deposition surface. A finite element model was used to evaluate the forces acting on a droplet during their impact on the deposition surface. Different materials were investigated, and the deformation of elastic bodies was taken into account during impact. The model was validated using the Olivetti BioJet system. Fibroblasts were printed onto different surfaces, and their viability was monitored in time. The results highlight the influence of the viscoelastic properties of the deposition surface on cell viability after printing and shows that cells will not survive if drop printed on a rigid surface such as glass or plastic. A significant and often overlooked consequence is that using a soft or viscous surface implies that the resultant spatial resolution of printed cells is lost in time.

Materials and Methods

Deposition substrate

The printing process was performed in a laminar flow hood under sterile conditions. Cells were printed directly into polystyrene (PS) multiwell plates. During the experiment different deposition substrates were used (i.e. solid, liquid, visco-elastic substrates) in order to determine the influence of viability on impact. The multiwell plates used in the experiments were previously prepared using with different solutions: medium with serum (liquid substrate), medium with 1% w/v of gelatin (viscous substrate), and 3 mg/ml collagen cross-linked with M199 10X culture medium (visco-elastic substrate), polystyrene (solid substrate).

Cell culture

Fibroblasts 3T3 (mouse embryonic fibroblast cell line, ATCC-LGC, UK) were cultured in DMEM medium supplemented with 10% v/v Fetal Bovine Serum (FBS), 5% v/v L-glutamine 200 mM, 5% v/v pennicilline-streptomycine mixture (containing 100 U of pennicilline and 100 µg of streptomycine). All the experiments were performed using cells at the same passage. After trypsinisation and cell counting with a hemocytometer, a suspension of $5 \cdot 10^6$ cells/ml was prepared. The suspension was added to the print head reservoir, and the printing process started. Printed cells were then cultured in 1 ml of medium at 37°C in incubator for 48 hours. In parallel a cell control was performed using approximately the same amount of cells pipetted into a multiwell plate.

BioJet Inkjet system

BioJet [figure 1] is a flexible non-contact nano-dispensing system that enables to produce high quality, precise and dense arrays in any format for genomic and proteomic applications. Using patented thermal ink-jet technology, BioJet is able to

precisely and accurately dispense picoliter to nanoliter volumes of liquid: just the involved material quantity is needed, so it is the ideal solution for micro-depositing active and expensive fluids. The robot X-Y-Z stage enables extremely precise positioning of the sample spots. The latest BioJet release hosts up to 12 different printheads working simultaneously to print on 18 substrates (1 x 3 inches). The software can perform electrical and functional tests on each printhead separately, and can align the printheads between themselves with a resolution of 10µm. BioJet is interfaced by a user-friendly software that allows you to manage several kinds of printheads loaded with different liquids. Depending upon chosen printhead and biological fluid, optimal printing parameters can be set: driving energy, printing frequency, pulse length. It is possible to use different printheads with different drop volumes as well and "multipass deposition" is allowed with Olivetti BioJet system, too; driving software can reside on a remote PC interfaced by a LAN connection. In described tests a previous BioJet release was used, hosting up to 6 different printheads simultaneously.

Figure 1. *Olivetti BioJet system*

Printing process

The first step was to fill the cartridge reservoir with 300 µL of cell suspension (as described in the Cell culture section), such that 35,000 cells were printed in each well. This approximation was performed counting the printed cells with a hemocytometer.

Modelling the impact

To evaluate the forces acting on cells during the printing process the ejection phase was evaluated with a finite element model. Attention was focused on the generation of the droplet, its motion and finally the impact on a specific substrate. All the models in this work were implemented using Comsol Multiphysics. The drop generation and motion were simulated using a two-phase fluid-dynamics simulation: this model was used as the starting condition for evaluating substrate impact. In the latter model a fluid domain was inserted to represent a liquid or viscous deposition substrate (i.e. medium or gelatin 1% w/v), and used to investigate the forces acting on the drop during the impact. The fluid with the cell suspension was represented as a liquid phase (ρ= 1.2 Kg/m^3; η=1 mPa·s), while the other fluid domain had different viscous properties according to the substrate used.
The boundary condition was set as a wetted wall with hydrophilic contact angle. In the case of PS and collagen which represent rigid or elastic deposition substrates, a fluid-structure interaction module was used and the deformation and forces acting on the droplet during impact were evaluated. The domain properties were set to 5 MPa and 5kPa, respectively for PS and collagen. The boundary was set as a moving wetted wall, with less hydrophilic interaction. This evaluation is particularly important to predict cell viability after the printing process.

Viability test

Cells viability was measured using the Cell Titer-Blue-Cell Viability Assay (Promega, Madison, WI, USA), based on a resazurin-based compound metabolized by mitochondrial cytosolic enzymes to resorufin, which can be detected with a fluorimeter [6]

Results

Modelling the impact with the surface

Assuming that the impact can control cell fate, we decided to investigate how the substrate properties change cell viability. Different substrates were used in the experiments and the same materials used in the experiments were modelled and analysed. Results show how substrate properties can change the forces acting on the droplet [figure 2].

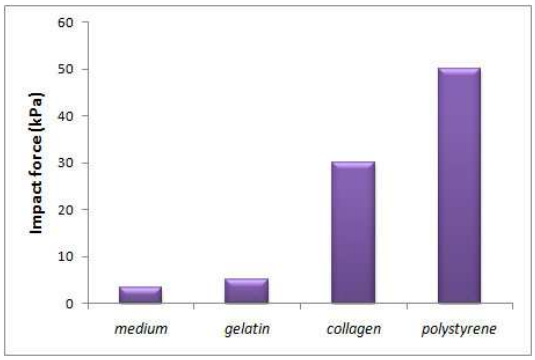

Figure 2*. Forces acting on the droplet with different substrates*

In particular it is interesting to see how the droplet shape changes as a function of the substrate [figure 3].

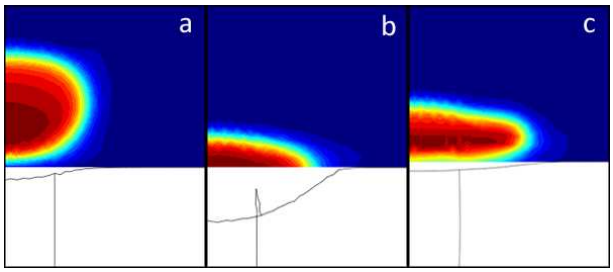

Figure 3*. Droplet shape before the impact (a). Droplet shape and substrate deformation after the impact with different surfaces: a) collagen, b) polystyrene*

Cell viability as function of deposition surface

To validate the model computed with the Comsol Multiphysics solver, printed cells were cultured in an incubator after the printing phase and their viability measured at 4, 24 and 48 hours. The most interesting result is observed at 4 hours after the printing process (figure 4f): in fact viability decreases with increasing stiffness of the deposition surface. As shown in figure 4a-e (cells at 4 hours after printing, 10X magnification) the distribution of cells is quite homogenous over all the substrates, apart from the PS. Droplet shape and impact forces influence the viability and the recovery of cultured cells. It is possible to correlate the influence of the substrate properties after 4 hours from the printing phase: the viability is linearly proportional with the impact force [figure 2].

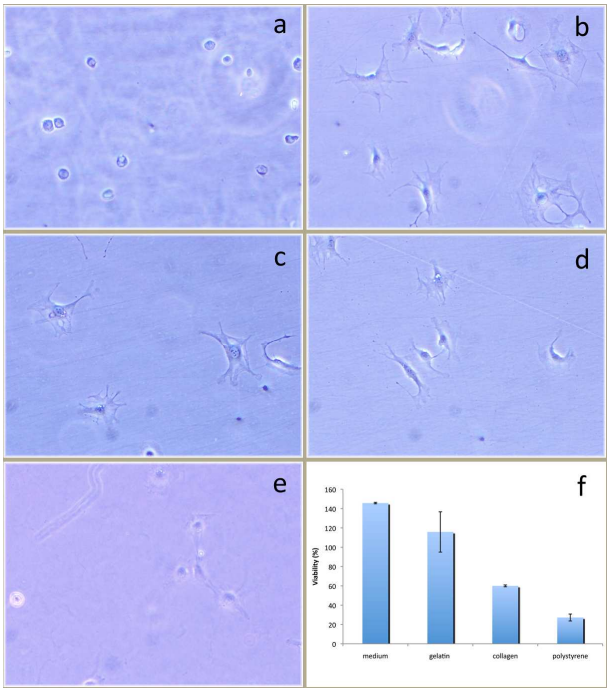

***Figure 4**. Cells 4 hours after the printing process on different substrates: a) PS; b) control; c) medium; d) gelatin; e) collagen; f) viability (n=6) for each substrate*

Cells printed directly into medium seem to be more viable with respect to all the other deposition materials, while cells printed on relatively soft visco-elastic materials (i.e. gelatin 1% w/v solution and 3 mg/ml cross-linked collagen) show a similar viability. Cells printed on PS surfaces are not viable: cell damage occurs due to the high stiffness of the deposition surface which causes high impact forces. After 48 hours the few living cells show a very low recovery in proliferation rate. On the other surfaces, during the first 24 hours surface adhesion processes occur and cell numbers begin to increase. Between 24 and 48 hours there is a high proliferative activity similar to controls as shown in figure 5.

Conclusions

Olivetti BioJet is suitable for the ejection of cells. Cell viability is not significantly influenced by the micro-fluidics system of the print head. On the other hand, the mechanical properties of the deposition surface are critical to cell survival because they define the force with which cells impact on the substrate. In all drop based cell printing methods the substrate must either be soft or viscous to avoid cell damage and this inevitably compromises spatial resolution during printing.

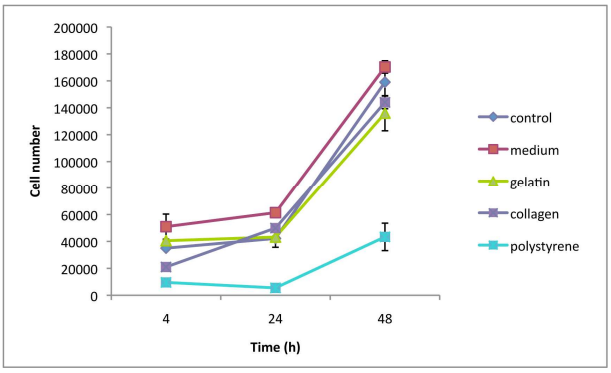

***Figure 5.** Cell number in time*

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Author Biography

Annalisa Tirella is a PhD student in Materials for Environment and Energy at the University of Rome "Tor Vergata". She is working on the design of a micro-fabrication system able to reproduce the complexity of biological environments. She is also interested in material properties (i.e. natural and synthetic polymers), and in materials able to form a well shaped hydrogel with controlled mechanical properties, suitable for tissue engineering applications.