Precision Assembly of Cellular Microarchitectures for Investigation of Cell to Cell Interactions in Early Tissue Development

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Introduction

Stem cells are defined by their ability to self renew and produce terminally differentiated daughter cells. There are many different stem cell types within the body with varying potencies, or cell types that they can differentiate into. The 3D arrangement of cells within an organ, tissue or stem cell niche can have a profound effect on the differentiation and function of cells within the tissue. However, the accurate study of cellular microenvironments and complex cell-cell signalling is limited by the lack of technologies that are able to manipulate cells in 3D at a sufficiently high enough resolution. The ability to build and manipulate multicellular microscopic structures will facilitate a more detailed understanding of cellular function in fields such as developmental and stem cell biology. In this poster I present a holographic optical tweezers based technology to accurately generate specific cellular micro-architectures. Using embryonic stem cells, 3D structures of varying geometries were created and stabilized using hydrogels and cell-cell adhesion methods. The application of holographic optical tweezers-based micromanipulation will enable novel insights into biological microenvironments by allowing researchers to form complex architectures with sub-micron precision of cells, matrices and molecules.

Chemical Treatment of Cell Surfaces

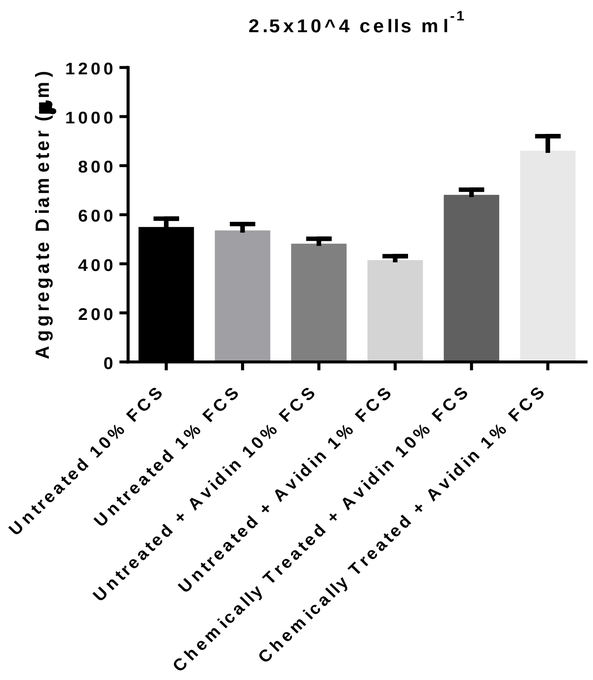

Figure 1. Graph showing the effect of chemical treatment on aggregate size. The effect of chemical treatment of the cell surface on the "stickiness" of the cells was assessed by looking at the diameter of aggregates formed by agitation at a seeding densities of 2.5x10^4 cells ml^{-1}. Cells were agitated for 6 hours on a rocker, following treatment, before the FCS concentration was made up to 10% and were incubated at 36°C for 72 hours.

To investigate cell-cell interactions and their affect on stem cell differentiation I needed to create stable structures that persist over prolonged periods. One possible way to do this was to adapt an existing technique to increase cell-cell adhesion or "stickiness" pioneered by De Bank and later Gothard. Their research showed that it was possible to increase cellular aggregation by specific oxidation of sialic acid residues on the cell surfaces, using sodium periodate. Biotin hydrazide could then be conjugated to the aldehyde groups. Once the cells surfaces were coated in biotin they could be cross-linked in medium containing avidin.

Small Scale Patterning Using Cell Surface Chemical Modification

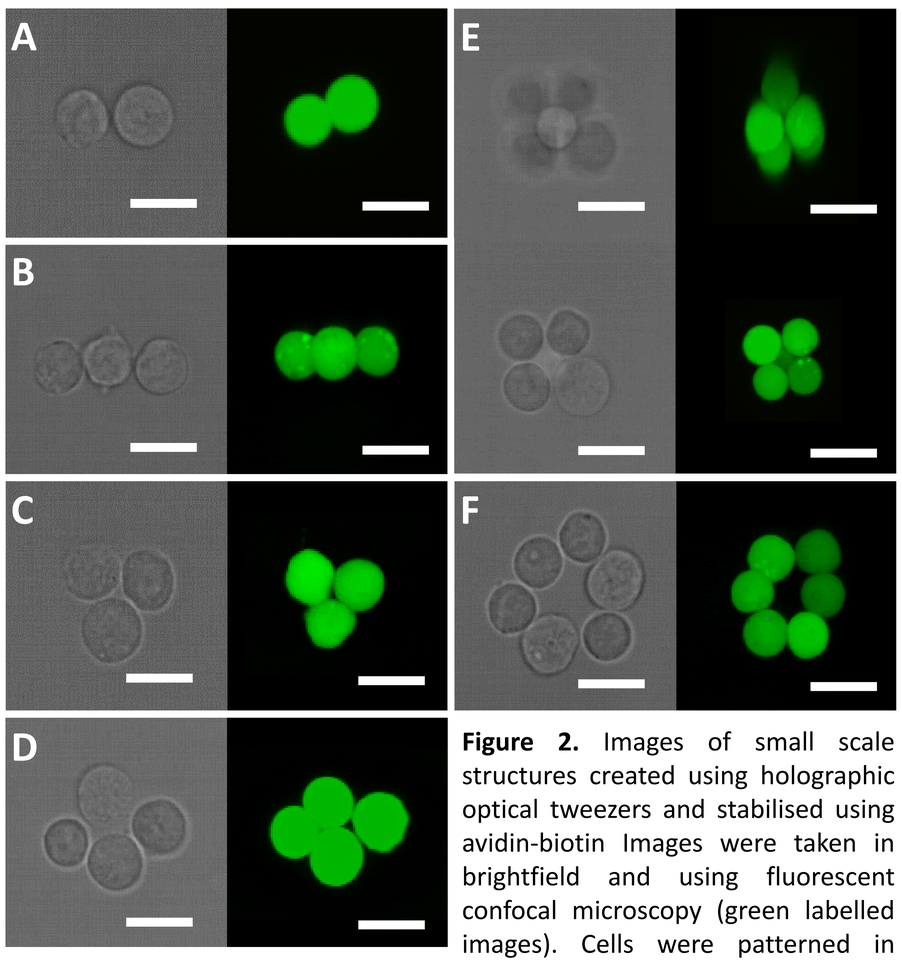

Figure 2. Images of small scale structures created using holographic optical tweezers and stabilised using avidin-biotin Images were taken in brightfield and using fluorescent confocal microscopy (green labelled images). Cells were patterned in Calcein AM containing medium. Scale bars = 12 mm

Modifying the same chemical modification of cell surfaces method as used to aggregate cells in figure 1 it was possible to stick cells together in defined patterns. These patterns were stable enough to be moved from one building to another for imaging and maintain their structures.

Larger Structures Using PEG Based Hydrogel

Many of the patterns created using the biotin-avidin stabilisation technique were lost as they were created in a liquid medium containing other cells, not used for patterning. One way to combat this is to use a hydrogel to stop cells from moving around and drifting into the patterned structures.

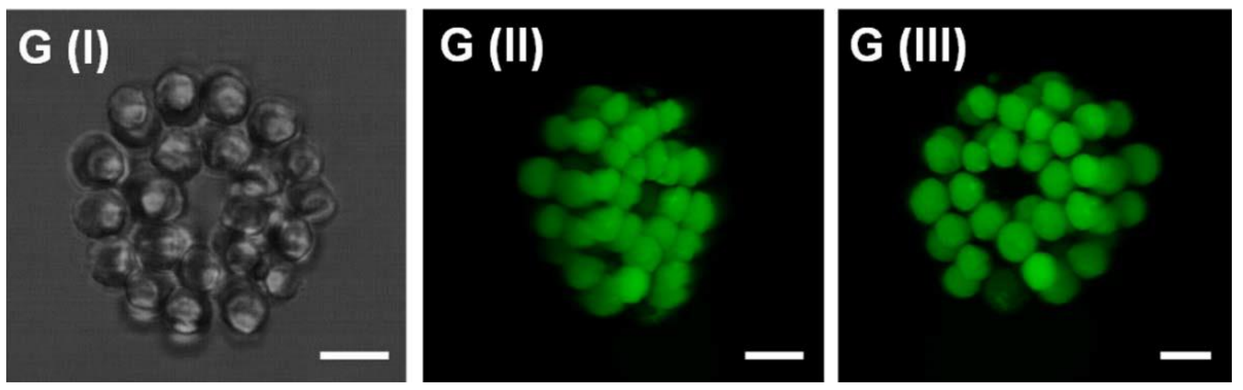

Figure 3. Images of larger structures created using holographic optical tweezers and stabilised using a PEG based hydrogel Images were taken in brightfield and using fluorescent confocal microscopy (green labelled images). Cells were patterned in Calcein AM containing medium. Scale bars = 12 mm

By using a hydrogel I was able to create large patterns of cells in 3D and incubate them for a prolonged period without fear of having these structures destroyed by other cells drifting into them.

Proliferation of Aggregates

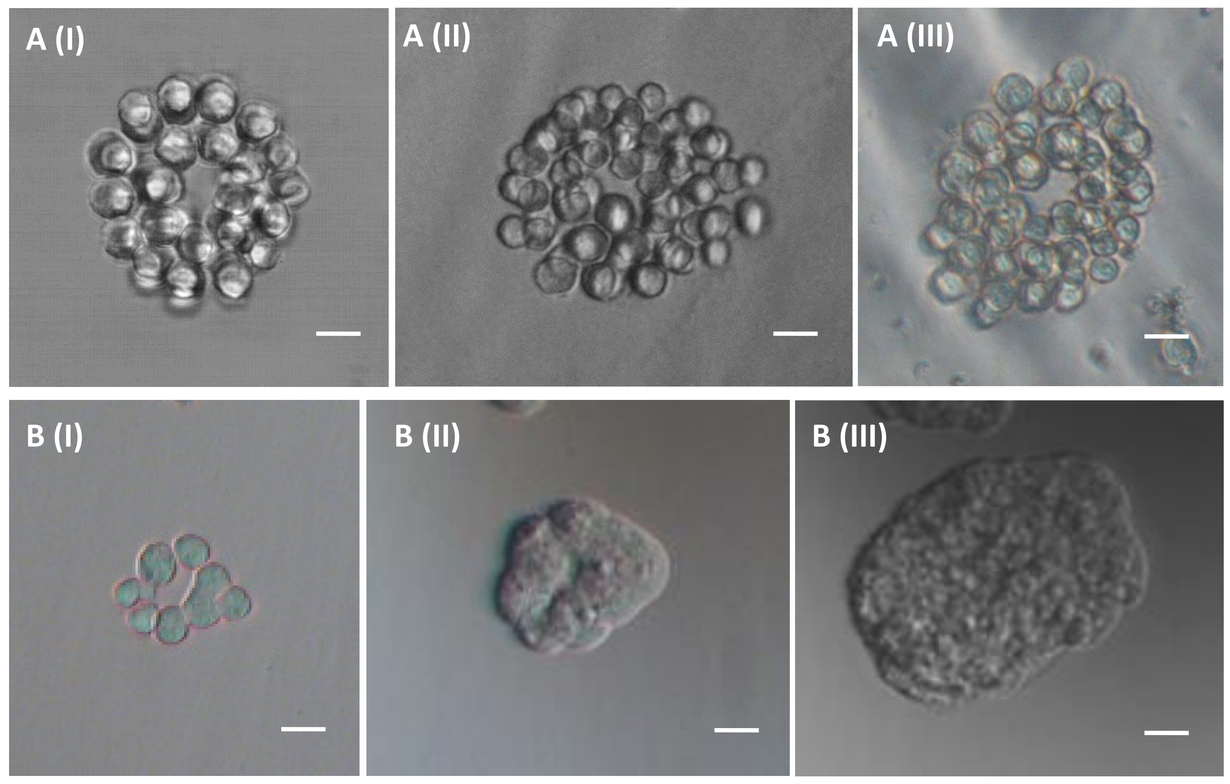

Figure 4. Images of larger structures created using holographic optical tweezers and stabilised using a PEG based hydrogel. (A) Shows a 66 cell structure at patterned in the presence of the old LED (I) 0 hours, (II) 48 hours and (III) 120 hours after patterning, (B) shows an 8 cell structure patterned in the presence of the red LED shown in (C) at (I) 0 hours, (II) 48 hours and (III) 120 hours after patterning. Cells were incubated at 37°C and 5% CO_2 in a humidified environment.
Scale bars = 12 mm

When the 66 cell pattern (fig 4A(I)) was incubated for 120 hours (fig 4A(III)) there was no visible proliferation of cells within the aggregate. After further investigation it was discovered that the LED light source used to illuminate the cells during pattering was inhibiting the proliferation of cells. The LED light source was switched to a red light source (right) with the hope that with the reduction of the higher energy wavelengths the inhibition of proliferation would be eliminated. Figure 4B(III) shows that the 8 cell structure patterned in fig 4B(I) had proliferated over the period of 120 hours.

The complex patterning of cells in 3D at such a high resolution offers a powerful tool to investigate complex cell-cell patterning in more physiologically representative environment.

Acknowledgements

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