Identification of putative mesenchymal stem cells in benign prostate hyperplasia using immuno-microfluidics

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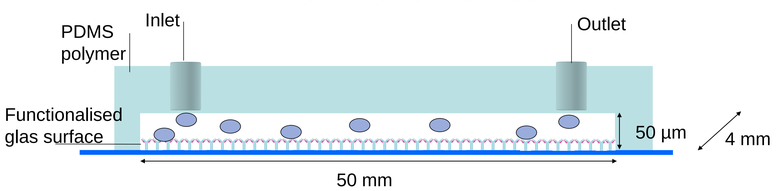
" Aims: To develop microfluidic methods for sorting stem cells, to design a controlled environment for monitoring cell-cell interactions, and to screen for stem cell markers."

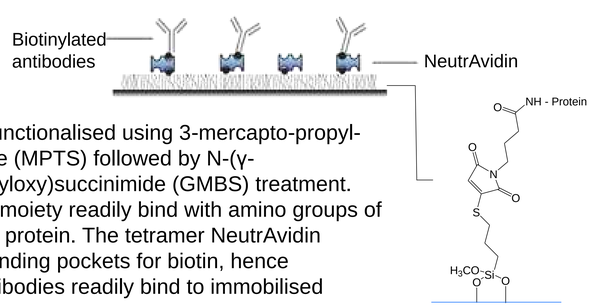
Introduction

The isolation and collection of adult stem cells from tissues or organs plays a crucial role in stem cell research. Current methods of cell separation such as fluorescent or magnetic activated cell sorting (FACS and MACS) are widely used; however, these techniques suffer from issues relating to compromised cell viability after sorting due to high mechanical stresses experienced by the cells during the separation process. Moreover, stem cells are rare in tissues and organs, hence it is crucial to obtain a high yield of viable cells. Microfluidics allows a more gentle approach to sorting and collecting cells. We propose a stem cell sorting method using a functionalised microfulidic device which is based on antibody selection.

Microfluidic device

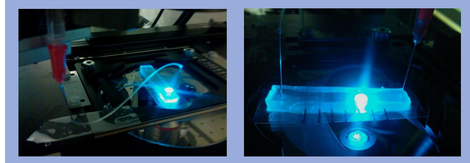


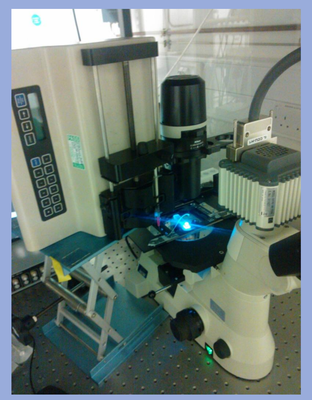
Surface functionalisation



The device is functionalised using 3-mercapto-propyl-trimethoxysilane (MPTS) followed by N-(γ-maleimidobutyryloxy)succinimide (GMBS) treatment. The maleimide moiety readily bind with amino groups of the NeutrAvidin protein. The tetramer NeutrAvidin contains four binding pockets for biotin, hence biotinylated antibodies readily bind to immobilised NeutrAvidin.

Experimental setup



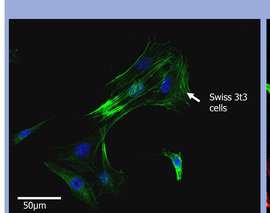


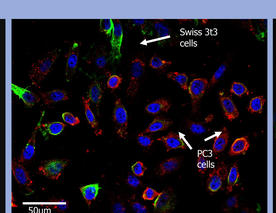
The PDMS polymer is transparent and thus suitable for imaging.
The sample is injected using a high precision infusion pump into the inlet of the microfluidic device. The microfluidic device can be placed on a confocal microscope for time/lapsed live imaging.

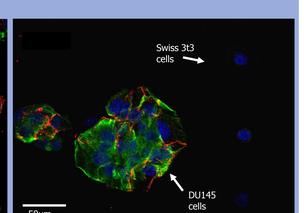
Cell capturing process:

1. Cells are trypsinised into a single cell suspension.
2. The sample is then injected into the microfluidic device at a rate of 30 µm/min.
3. A series of images is obtained and the captured cells are counted.
4. The captured cells can be released for further processing or cultured and characterised *in situ* (ex. Immunostaining)

Antibody selection for device optimisation





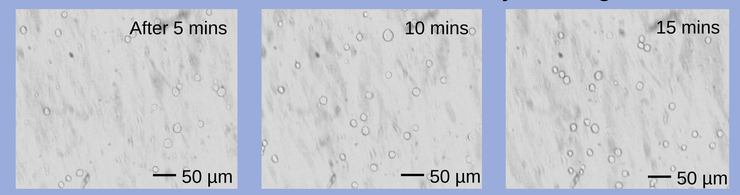


Swiss 3t3 cells | **Co-culture of PC3 and Swiss 3t3 cells** | **Co-culture of DU145 and Swiss 3t3 cells**

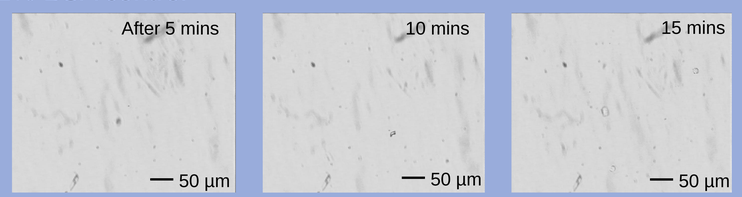
We have used anti-Prostate Cell Surface Antigen (PCSA) to capture prostate cells (DU145 and PC3 cancer cell lines) from a co-culture with non-prostatic cells (3T3 Swiss fibroblasts). Immunohistochemical staining of PCSA antibody (red), phalloidin (green) and DAPI (blue) showed that PC3 and DU145 cells are PCSA -positive and that Swiss 3t3 cells are negative.

Immunocapture of cells in microfluidic channels

Functionalised device with PCSA antibody coating



1% BSA control



Captured cells PC3 cells in functionalised device and non-specific capture of PC3 cells in control.

Summary

- The microfluidic channel has been functionalised with MPTS, GMBS, NeutrAvidin and to graft a biotinylated PCSA antibody.
- The antibody affinity to the PCSA protein has been optimised using a two cell line (one prostate and one non-prostate) co-culture system.
- Cells have been selectively captured using the antibody coated device.
- The findings from this study have illustrated the potential of designing a microfluidic device for selective enrichment of specific cell type by functionalising the surface chemistry of the channels.
- The study provides a conceptual framework for the fabrication of a microfluidic system that can efficiently isolate and phenotypically identify mesenchymal stem cells in prostate tissue.