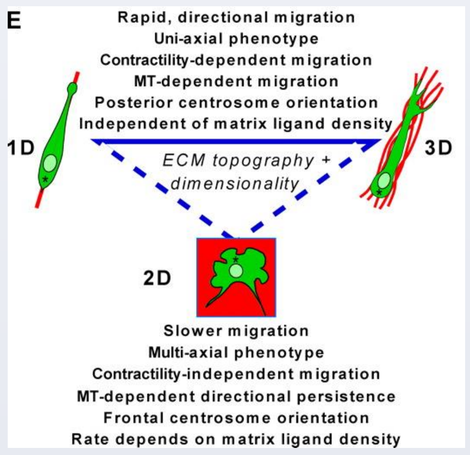
A combined cell placement and migration assay device for cancer cell anti-migration drug screening

Colin L. Hisey[1,2], Oihane Mitxelena-Iribarren[2,3], Miguel Martinez-Calderon[2], Sergio Arana[2,3], Maite Mujika[2,3], S.M. Olaizola[2], Derek J. Hansford[1]

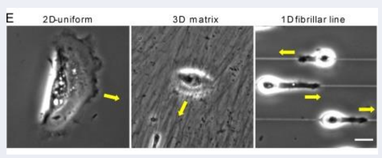
1) The Ohio State University, Biomedical Engineering, Columbus, Ohio, USA
2) CEIT and Tecnun (University of Navarra), Manuel de Lardizábal 15, 20018 San Sebastián, Spain
3) Centro de Ingeniería Biomédica (University of Navarra), Campus Universitario, 31009 Pamplona, Spain

Introduction

Highly migratory cancer cells often lead to recurrence and secondary tumors and are responsible for high mortality rates in cancers such as glioblastoma multiforme. Current treatments focus on resection and destruction of the primary tumor with radiation as well as chemical targeting of proliferative cells. Recently, drugs which specifically target highly migratory cells have been developed, but robust *in vitro* platforms for quantifying the efficacy of these drugs are still lacking. We have developed a microfluidic device capable of hydrodynamically trapping cancer cells from solution onto 10 and 15 micron polystyrene lines which encourage 1D migratory behavior based on the cells' tendency to follow topographical cues.



Andrew D. Doyle et al. J Cell Biol 2009;184:481-490

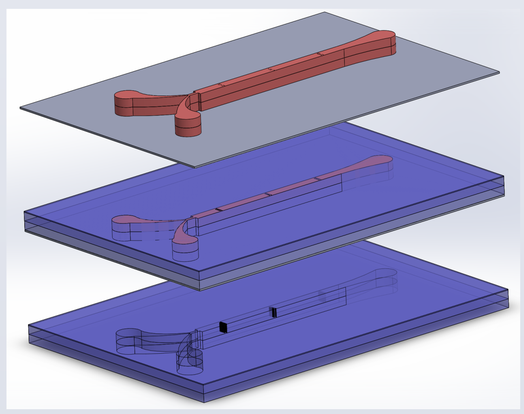


Andrew D. Doyle et al. J Cell Biol 2009;184:481-490

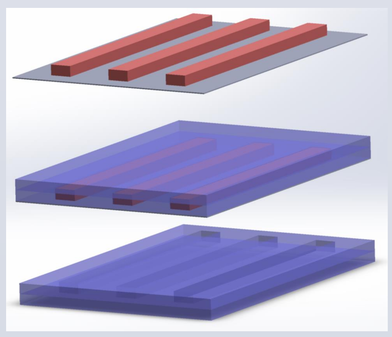
This device design will allow precise microfluidic assessment of potential drug targets to limit cancer cell migration due to its high cell seeding predictability and fluidic control.

Methods

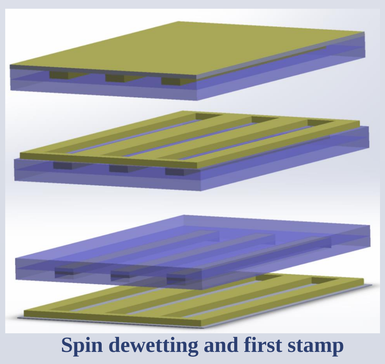
Microchannels are fabricated using a multilayer aligned photolithography process, with the upper SU-8 layer containing traps and bottom layer containing no features in order to leave room for the migration lines. Microchannels are then molded by pouring PDMS over the patterned SU-8.



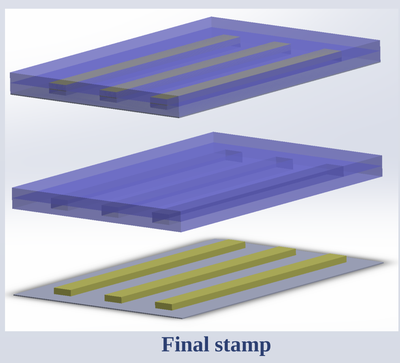
Lines and rectangular bases for femtosecond laser ablation were created using PDMS molds of SU-8 line structures. 10 wt% polystyrene in anisole is spin-coated at 4,500 RPM to achieve partial dewetting. The dewetted layer is stamped off at 170°C and low pressure (manual) and the inner layer is stamped at 170°C and high pressure (manual).



PDMS mold of SU-8 pattern

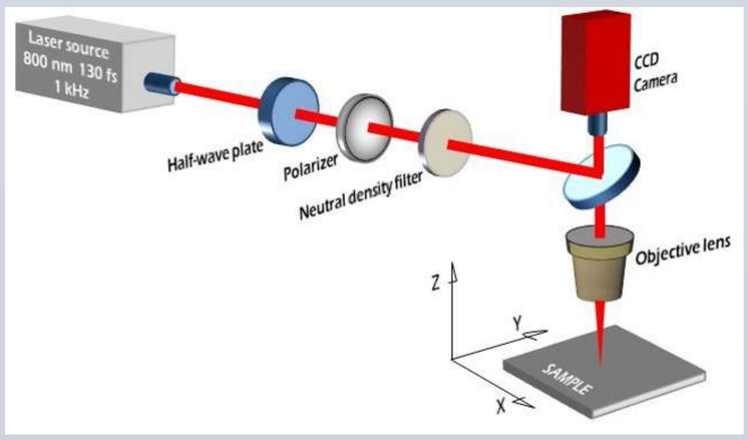


Spin dewetting and first stamp

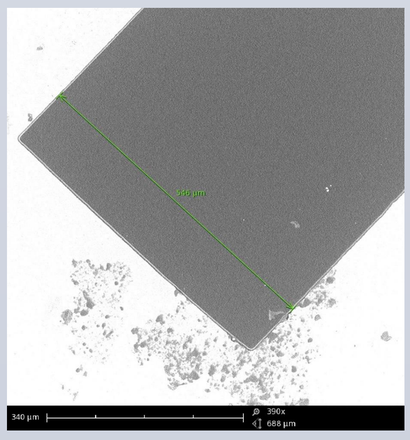


Final stamp

Samples were also machined in open air atmosphere with a Ti:Sapphire laser system that generates 130 fs pulses at a central wavelength of 800 nm, with a 1 kHz repetition rate. The 8 mm diameter laser beam was focused on the samples using a 10x microscope objective with a NA of 0.16 to aω_0 of 17 µm. A three dimensional translational stage moved the sample under the beam with a velocity of 1.5 mm/s which results in 12 pulses per spot. Pulse energies of 1 and 3 µJ which correspond to laser fluencies of 0.9 and 2.7 J/cm^2. Several scans were performed in each groove with different spacing in order to obtain the desired line width.



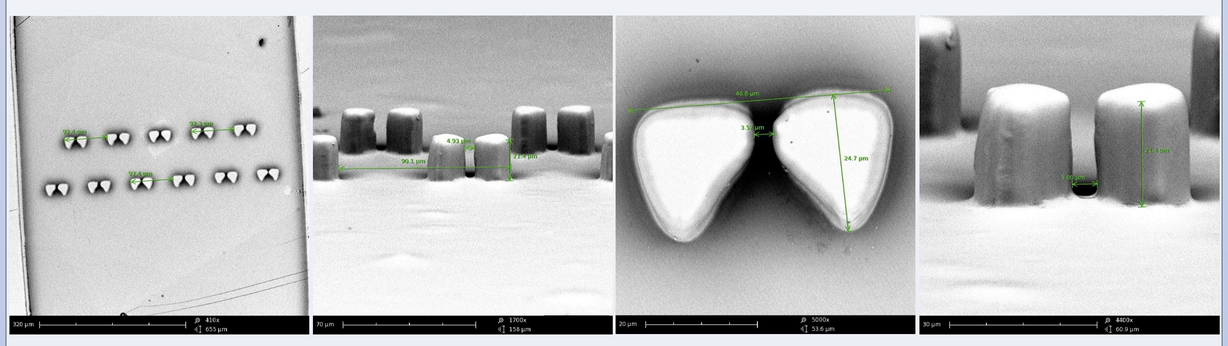
Femtosecond laser schematic



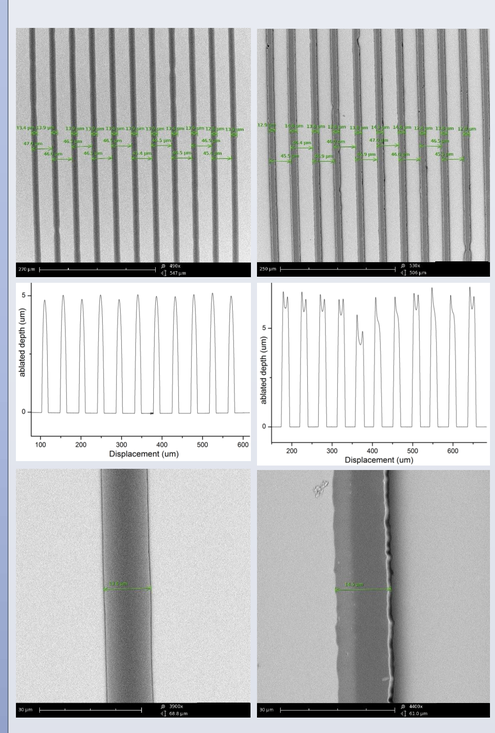
Polystyrene before ablation

Results & Discussion

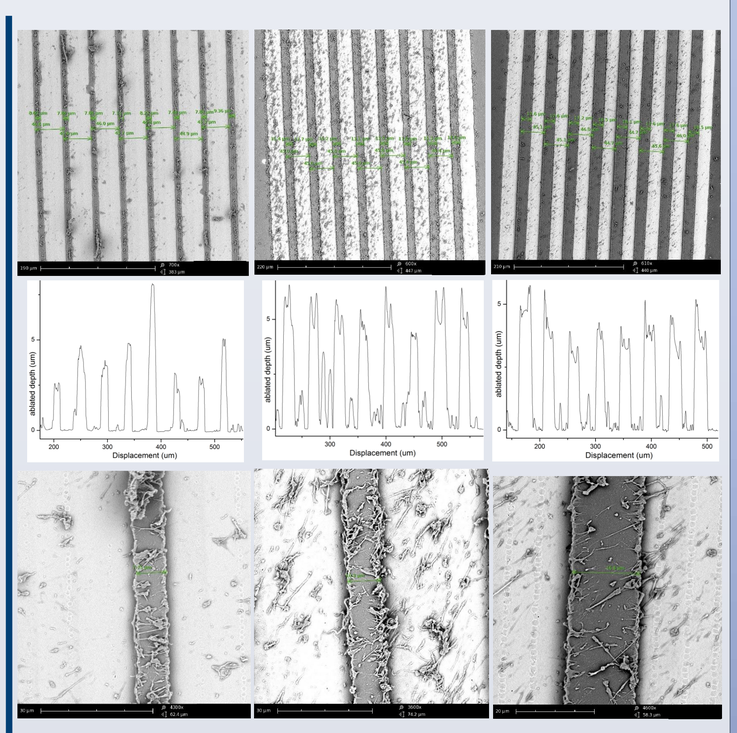
Microfluidic channels were successfully fabricated from an SU-8 mold using a 10:1 ratio of PDMS base to curing agent. Hydrodynamic traps with ~5µm gaps are located in the upper portion of the channel with 45 µm spacing between traps, while the lower portion remains empty until bonded with the desired polymer substrate.



Polystyrene lines for guided 1D migration were fabricated using both spin-dewetting/stamping and femtosecond laser ablation. Stamping produced more consistent line heights and edges and did not produce debris as in ablation, but the pressure from stamping caused some lateral displacement which lead to slight misalignment with the traps. The debris from ablation may play a significant factor as cells typically prefer areas of higher surface energy for focal adhesion formation.

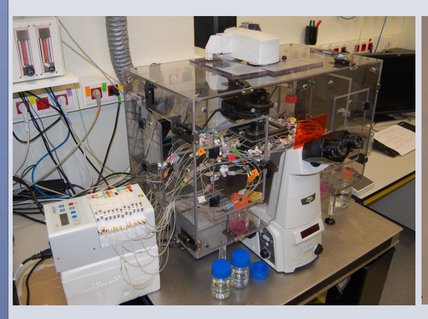


Dewetting and Stamping

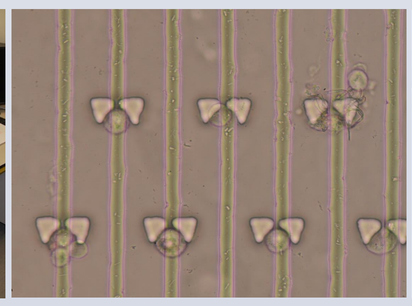


Femtosecond Laser Ablation

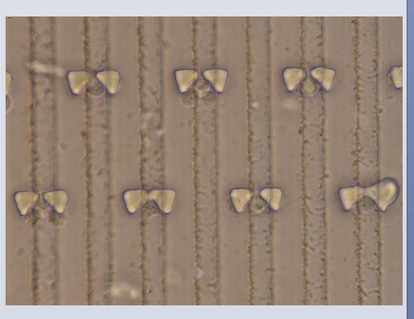
Polystyrene migration substrates have been manually aligned and bonded with hydrodynamic microfluidic traps using oxygen plasma. U2 osteosarcoma and U87 glioblastoma cells are seeded at low flow rates (<3µl/min) to minimize damage from shear forces and encourage adhesion to the substrate. Cells are allowed to adhere for ~12 hours, then the flow is reversed and cells migrate out and away from the traps for 48-72 hours with images every 10 minutes.



Incubating microscope



Cells trapped over stamped lines



Cells trapped over ablated lines

Future Work

Currently, cell migration experiments are underway to quantify the effects of line width, surface chemistry treatment and fabrication method on migration velocity, distance and directional persistence. Future studies will include implementation of nanofiber substrates, other polymers of interest to mimic the stiffness and surface chemistry of various *in vivo* fiber-like structures and a concentration gradient generator to test a range of anti-migratory therapy concentration levels.

References

Doyle AD, Wang FW, Matsumoto K, Yamada KM. One-dimensional topography underlies three-dimensional fibrillar cell migration. The Journal of Cell Biology. 2009;184(4):481-490. doi:10.1083/jcb.200810041.

Acknowledgements

Funding for this project was provided by the Whitaker International Program, operated by the International Institute of Education. Also, a special thanks to CEIT and Tecnun for hosting me during my year abroad.