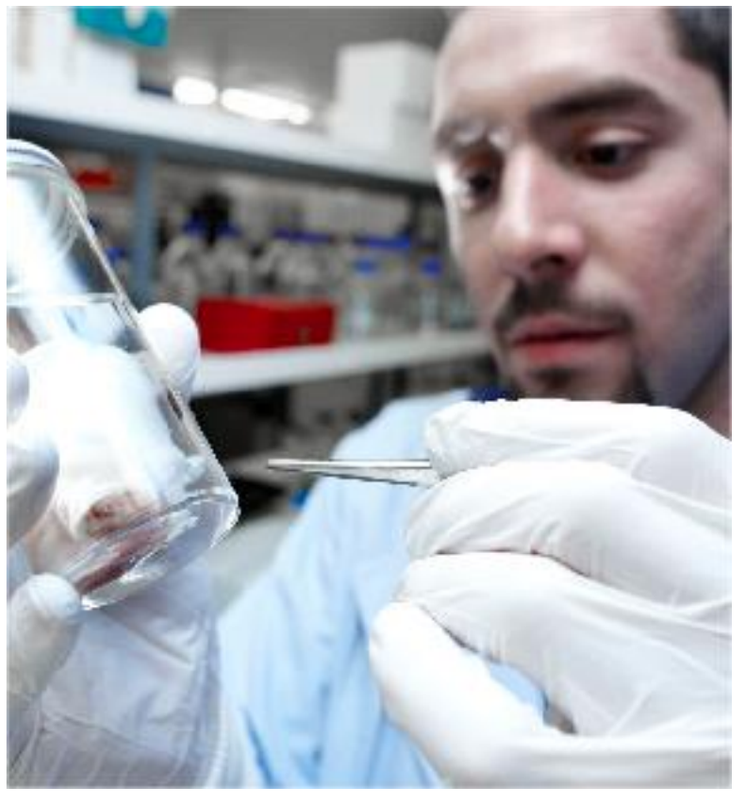
The Design of Droplet Based Bioprinting Systems for Reliable Bioink Delivery

Matthew Benning
School of Mechanical in Systems Engineering
Newcastle University

MeDe Innovation
EPSRC Centre for Innovative Manufacturing in Medical Devices

Introduction

- Our Research Aim and Objectives
- Printing systems at Newcastle University
- Bioprinter Technologies
- Issues associated with Bioink Jetting
- Results
- Conclusions



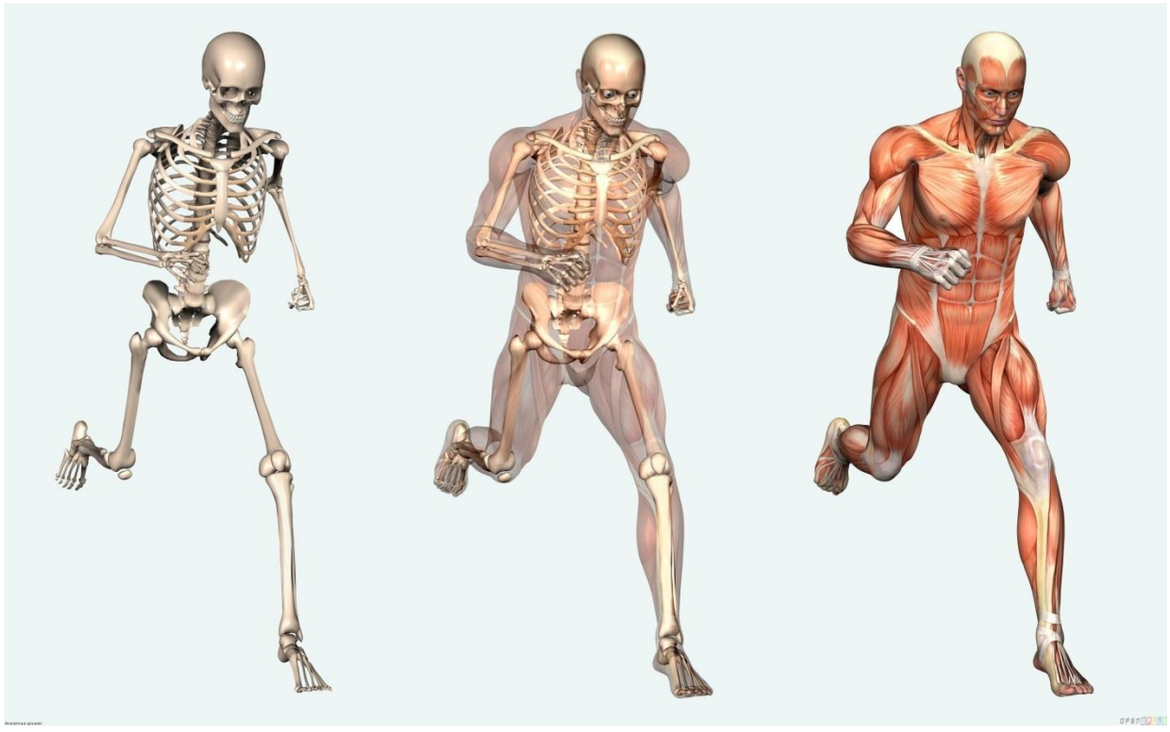
The University Of Sheffield. | The University of Nottingham | Newcastle University | UNIVERSITY OF BRADFORD | UNIVERSITY OF LEEDS

EPSRC Pioneering research and skills

MeDe Innovation
EPSRC Centre for Innovative Manufacturing in Medical Devices

Project Background

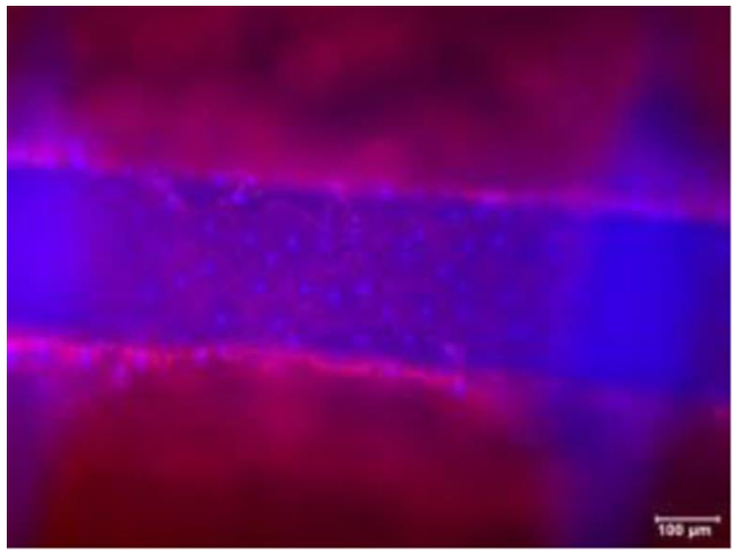
- There are many different tissues with many different mechanical properties
- Soft tissue scaffolds can often be produced pre-seeded with cells.
- Rigid scaffolds have to be seeded with cells after production which can be time consuming.
- In clinic production of pre-seeded scaffolds requires rapid turnaround if autologous cells are to be used.
- This poses a significant problem for the in clinic production of rigid scaffolds.



MeDe Innovation
EPSRC Centre for Innovative Manufacturing in Medical Devices

Research Aim and Objectives

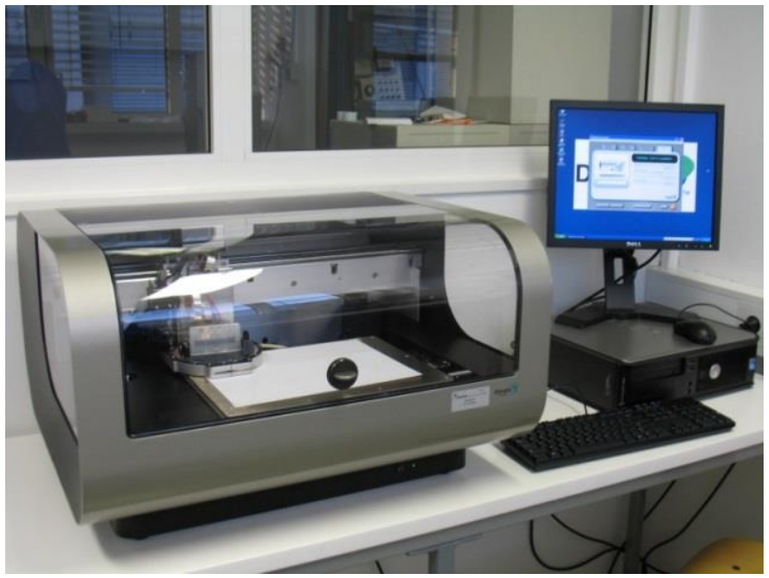
- **Aim –** to produce tissue scaffolds pre seeded with cell material using an additive layer manufacturing approach



- **Objective 1 –** Print viable cells, in a robust and repeatable manner
- **Objective 2 –** Print monomers and polymerise in situ
- **Objective 3 –** Print both together while maintaining cell viability

MeDe Innovation
EPSRC Centre for Innovative Manufacturing in Medical Devices

Our Commercial Printing Systems



Fujifilm Dimatix Printer
(multi orifice piezo DOD)

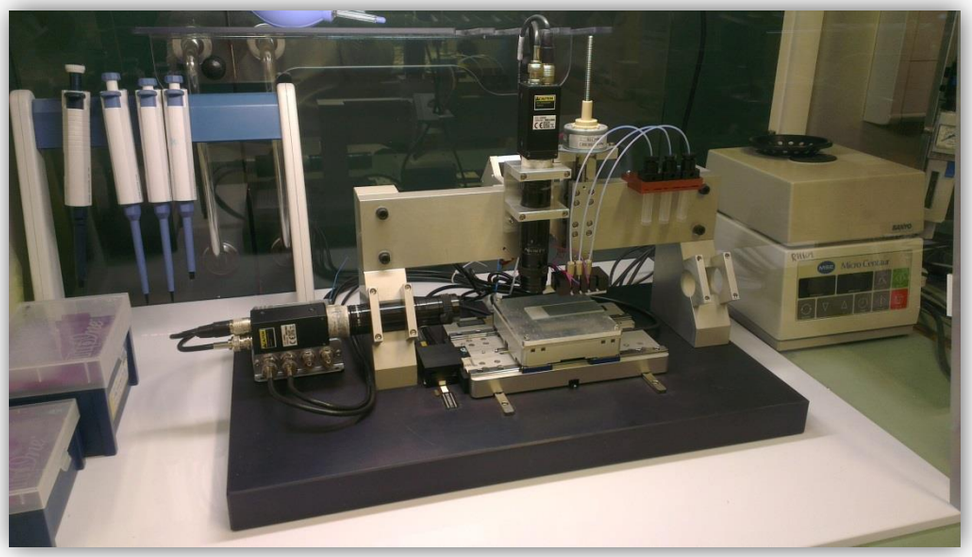
nScrypt Micro Dispensing
(Hot on cold extrusion)

Microfab Inc. Jetlab
(single orifice piezo DOD)

MeDe Innovation
EPSRC Centre for Innovative Manufacturing in Medical Devices

Our Experimental Apparatus

- We have JetLab 4 printer
  - Drop on Demand (DOD) Piezoelectric System with up to four independent reservoirs.
  - Retrofitted micro valve technology
- A bespoke, in house designed, dispensing system.
  - Capable of both inkjet and micro valve deposition.
  - High accuracy stages (nm/µm)
  - Multiple microscope video imaging sensors



MeDe Innovation
EPSRC Centre for Innovative Manufacturing in Medical Devices

Bio-Printing Technologies - Bioplotters

- The Systems are extrusion based, processing cells/biomaterials in gels
- Needle and syringe type architecture
- Used by Orgonovo Inc.
- Resolution limited

Wake Forest Institute For Regenerative Medicine

Organovo's Bio Plotter

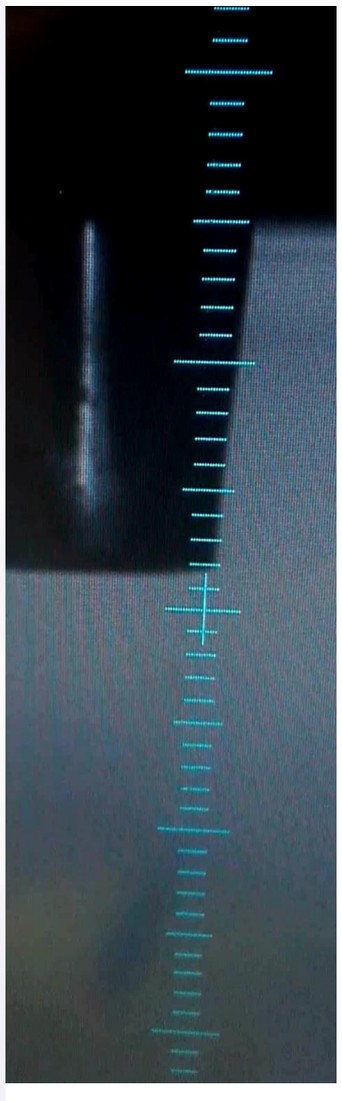
MeDe Innovation
EPSRC Centre for Innovative Manufacturing in Medical Devices

Bio-Printing Technologies – Droplet Based

- Inkjets produc
  - Inkjets
    - ✓ High res
    - ✓ High acc
    - X Low drop
    - X Low relia
- Microvalves p
  - Microvalve
    - ✓ High volu
    - ✓ Good rel
    - X Low reso
    - X Low accu

Micro-valve

Inkjet



Inkjet Printing - Material Properties

A materials rheological properties dictate weather it's printable.

Important factors included:

•Density

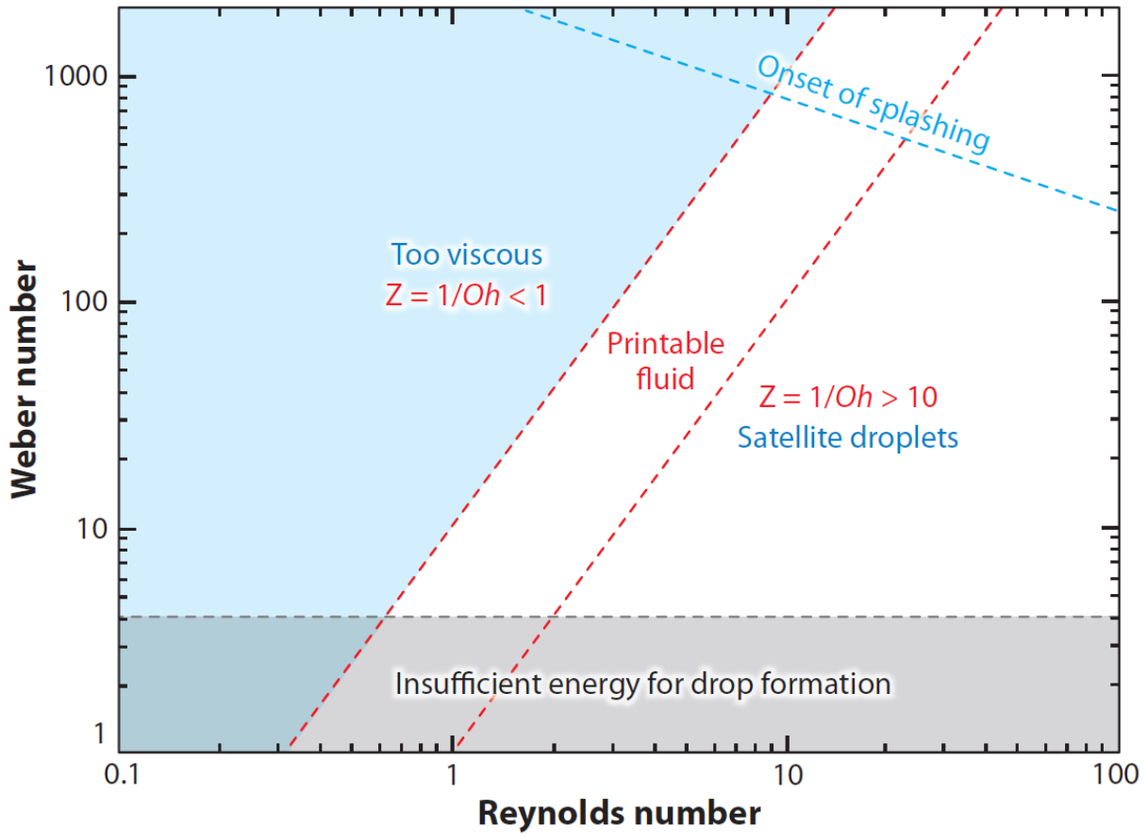
•Dynamic viscosity

•Surface tension

The relationship of these material properties can be characterised by a number of dimensionless physical constants:

•Reynolds, Weber and Ohnesorge numbers

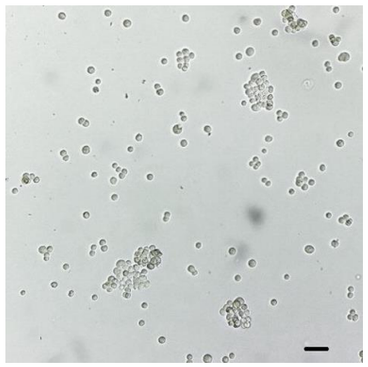
•**Problems arise when printing bioinks through inkjets**



(Derby 2010)

Inkjet Printing of Cells

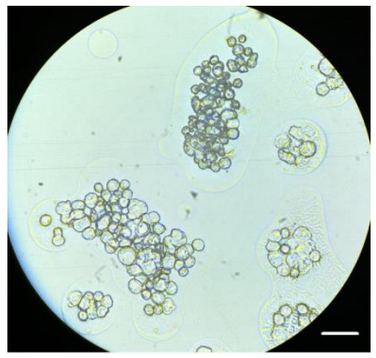
- A bio-ink is a suspension of biological material in a carrier fluid.
  - As the rheological properties dictate weather a material is printable, large particles (micro scale) can reduce printing efficacy due to local changes in rheology at the print orifice.
  - Often, cells agglomerate within a bioink.



Bioink - Osteosarcoma cells in media (1m/mL)

Printing

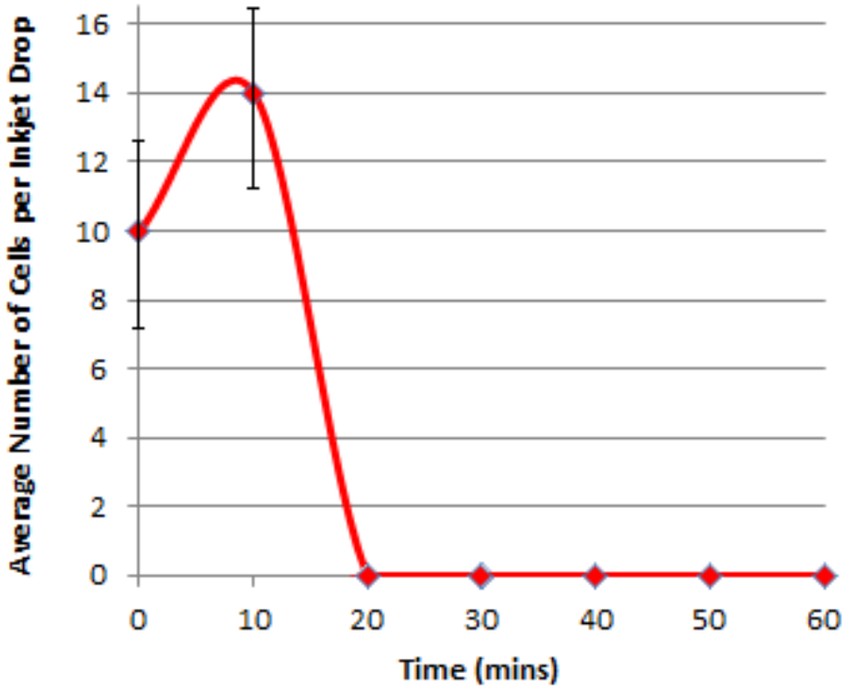
Process



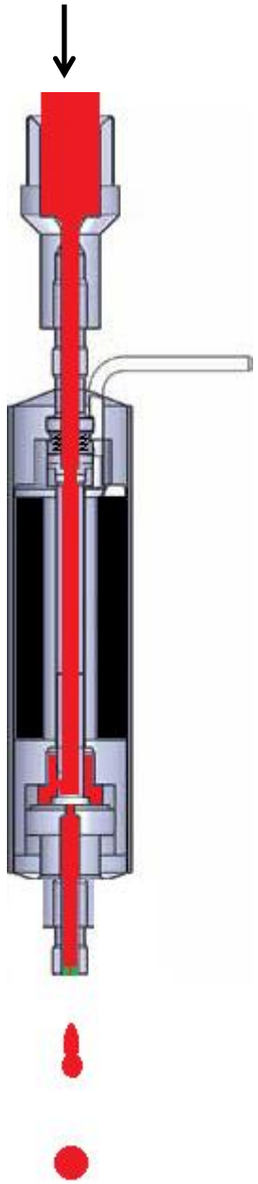
50 drops

Orifice Obstruction

Bio-ink Sedimentation

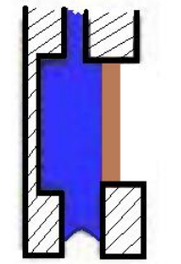


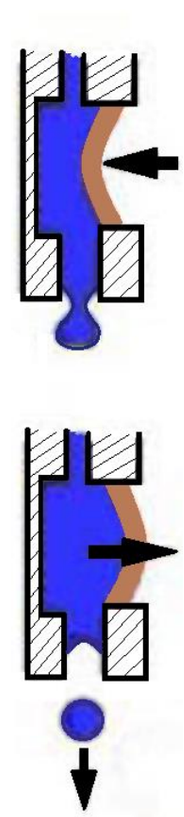
Inkjet vs Microvalve



Inkjets rely on the material properties to create a droplet -

- Microvalves rely on a mechanical valve allowing fluid to pass from a hyperbaric chamber.

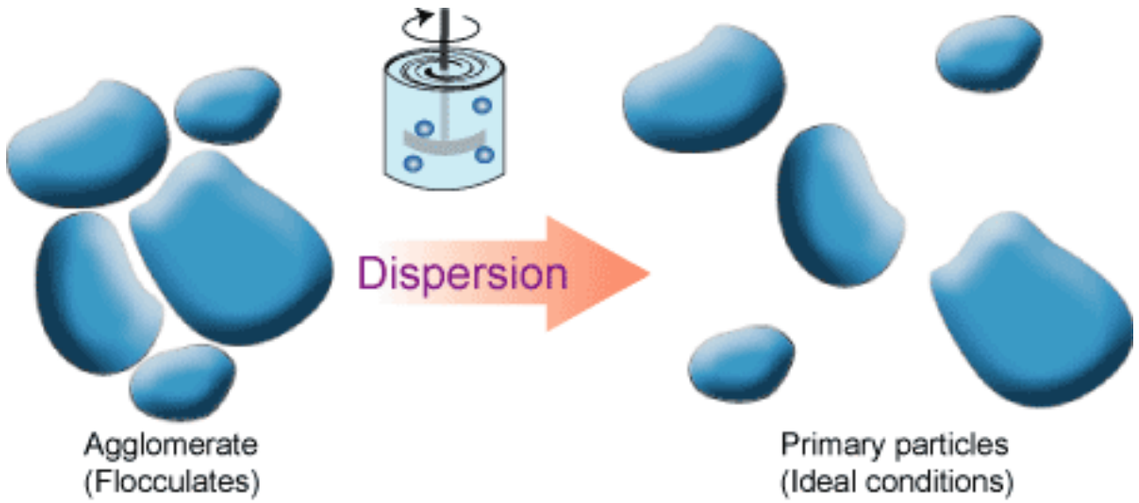




MeDe Innovation
EPSRC Centre for Innovative Manufacturing in Medical Devices

Stabilisation of Bio-inks

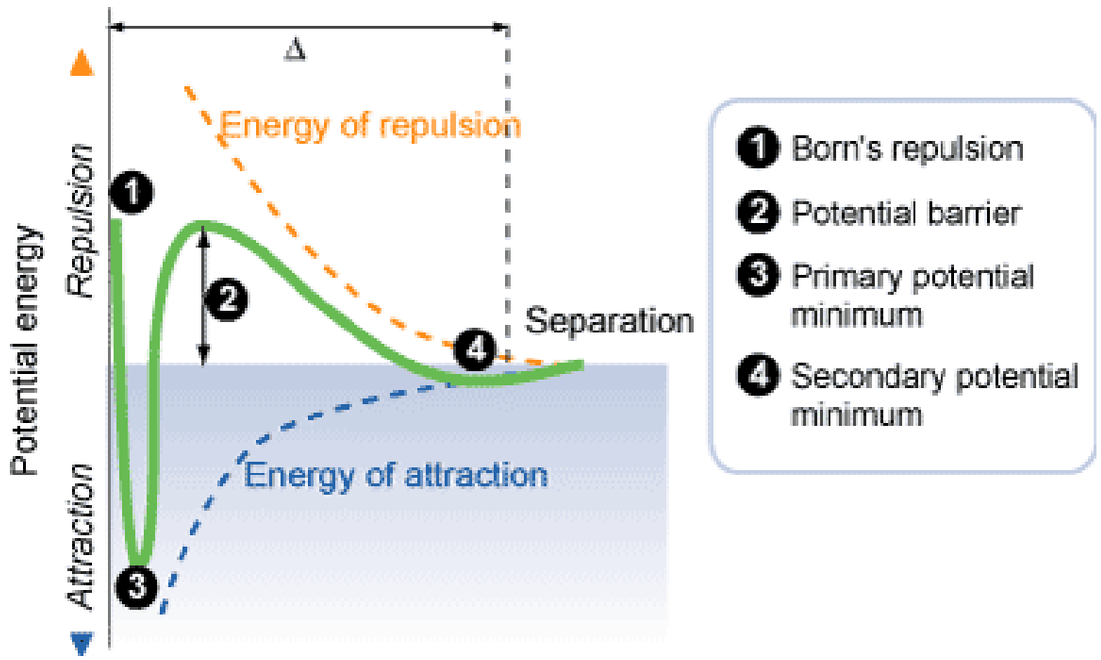
- Printer manufacturers have been formulating suspensions of pigments in carrier fluids for many years and have come up with a number of solutions to stabilise them.
- What makes an ink unstable?
  - Within standard inks, small forces such as Van der Waals force can hold particles together, leading to flocculation.
- Density differences cause flocculants to settle out of suspension
- Mechanical agitation will temporarily de-aggregate, but the particles will soon re-aggregate as they come into contact with each other

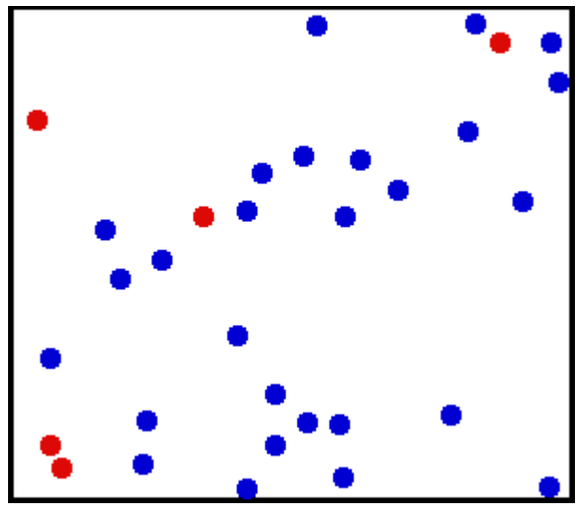


MeDe Innovation
EPSRC Centre for Innovative Manufacturing in Medical Devices

Brownian Motion and The Potential Barrier

> Brownian motion, or particle theory, describes the random interaction of a small number of large particles in a large number of small particles, i.e cells and water molecules.





> In a system where the potential barrier is low, this constant collision between ‘pigment’ particles results in agglomeration.

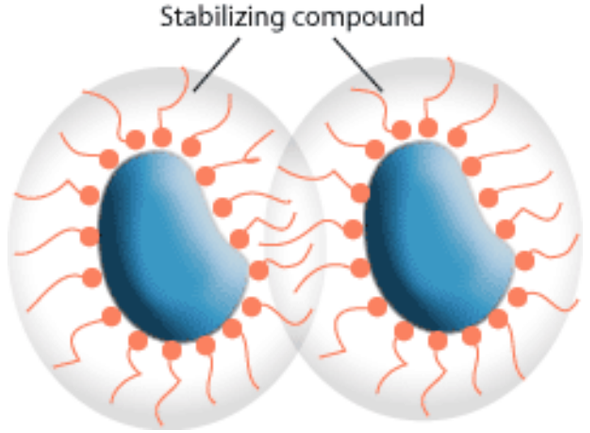
MeDe Innovation
EPSRC Centre for Innovative Manufacturing in Medical Devices

Increasing The Potential Barrier

- There are two recognised ways:

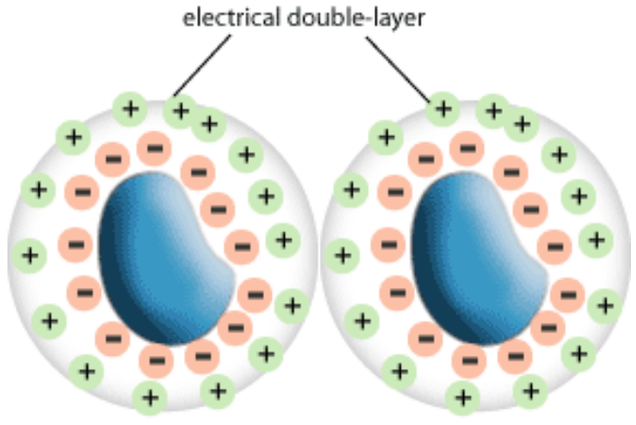
Steric Stabilisation

- Amplify the osmotic repulsion effect by maintaining a thinker fluid barrier layer on the particle surface.



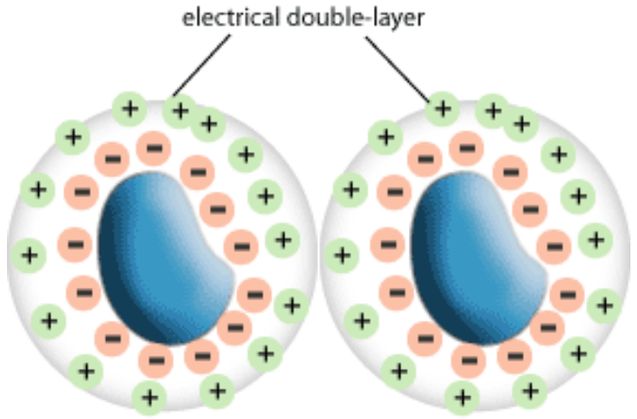
Electrostatic Stabilisation

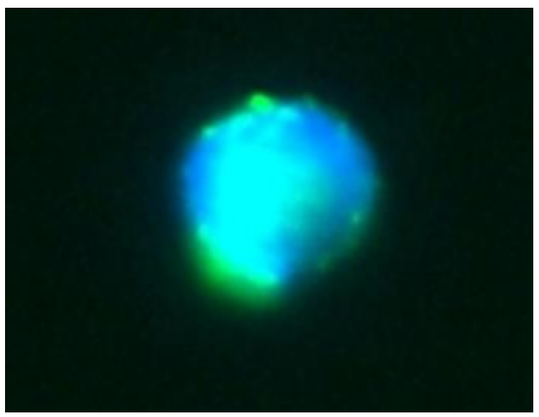
- Introduce an additional repulsion force by coating the particle with a charged material
  - The higher the uniform potential between particle and carrier fluid, the more stable the suspension.



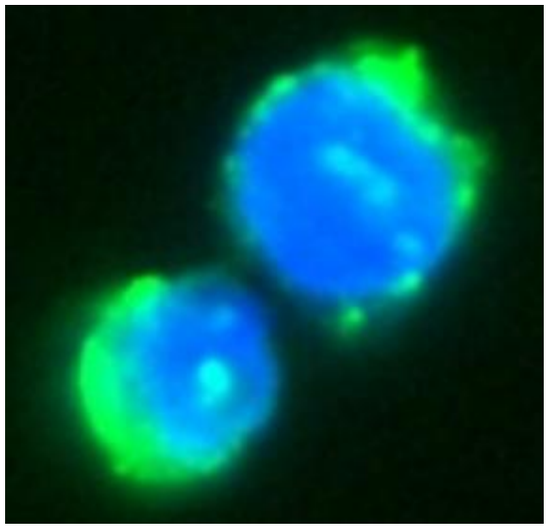
Electrostatic Bio ink Stabilisation

- Introduce an additional repulsion force by coating the particle with a charged material, in this case a cationic polymer Poly-L-Lysine
- Osteosarcoma cells were coated in a number of different concentrations of PLL and their viability assesed.

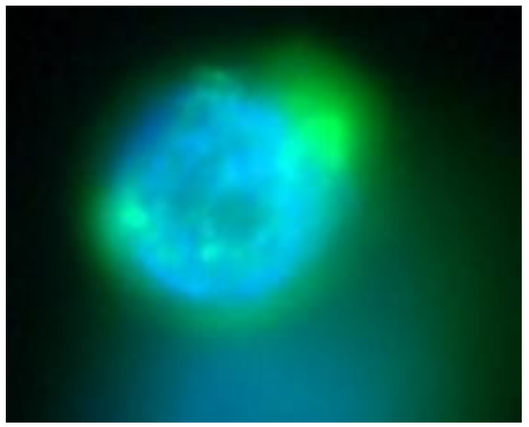




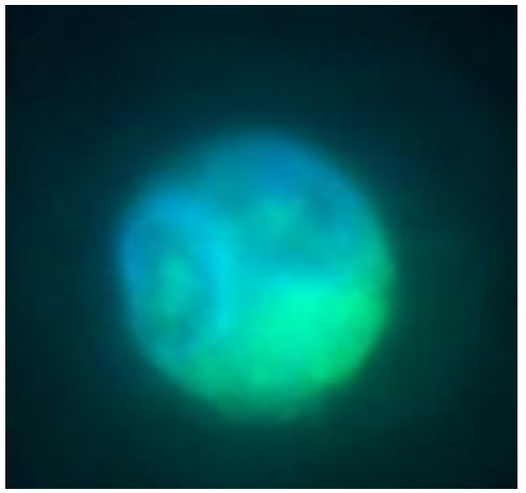
➢ 50 µg/mL



➢ 100 µg/mL



➢ 200 µg/mL



➢ 400 µg/mL

Day 0

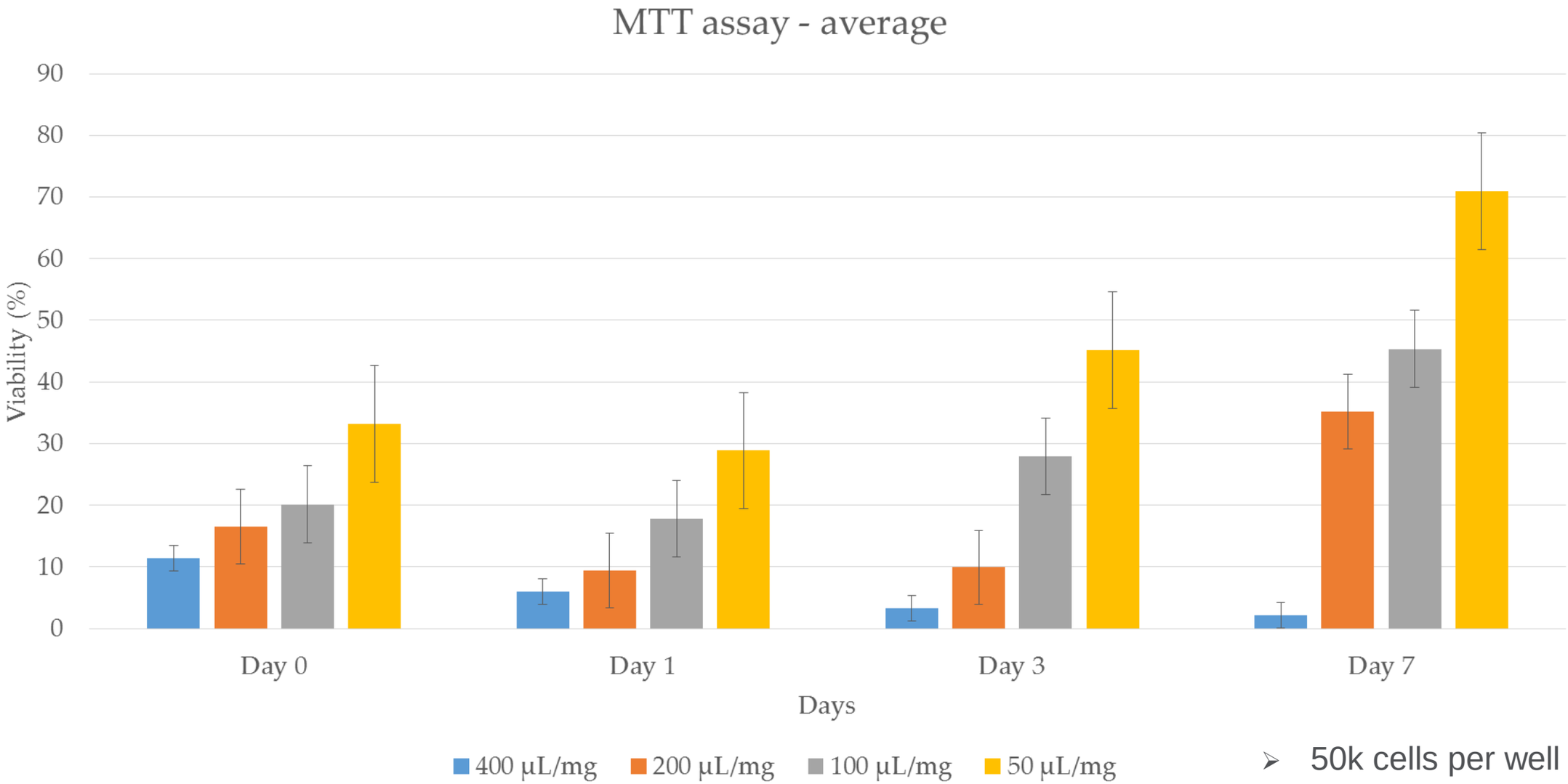
Blue: DAPI

Green: PLL-FTIC

10 µm

MeDe Innovation
EPSRC Centre for Innovative Manufacturing in Medical Devices

Encapsulated Cell Viability



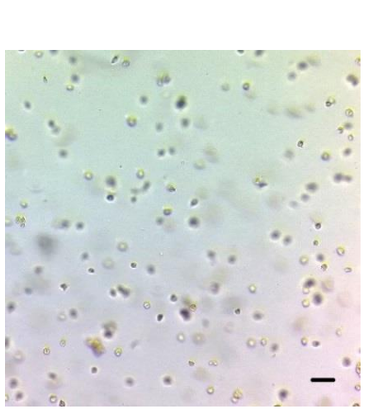
- 50k cells per well

The University Of Sheffield. | The University of Nottingham | Newcastle University | UNIVERSITY OF BRADFORD | UNIVERSITY OF LEEDS

EPSRC Pioneering research and skills

MeDe Innovation
EPSRC Centre for Innovative Manufacturing in Medical Devices

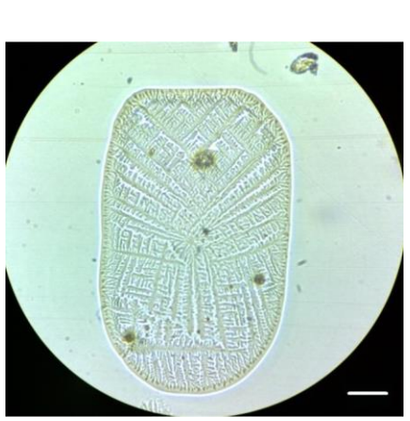
Printing with a Stabilizsed Bioink



Bioink - Osteosarcoma cells, coated with 50µg/mL of PLL, in media (1m/mL)

Printing

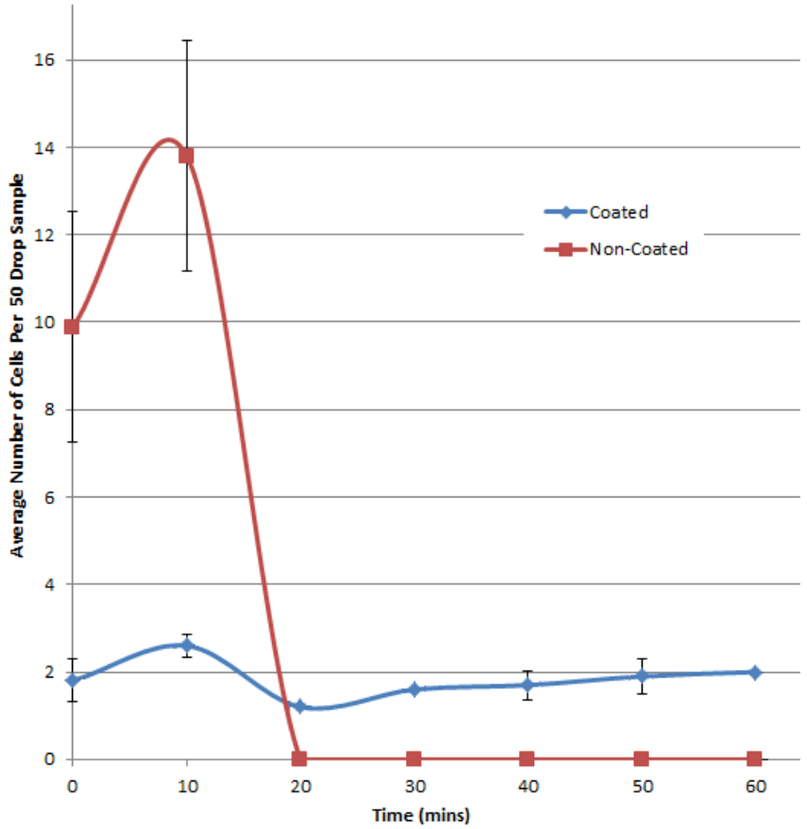
Process



50 drops

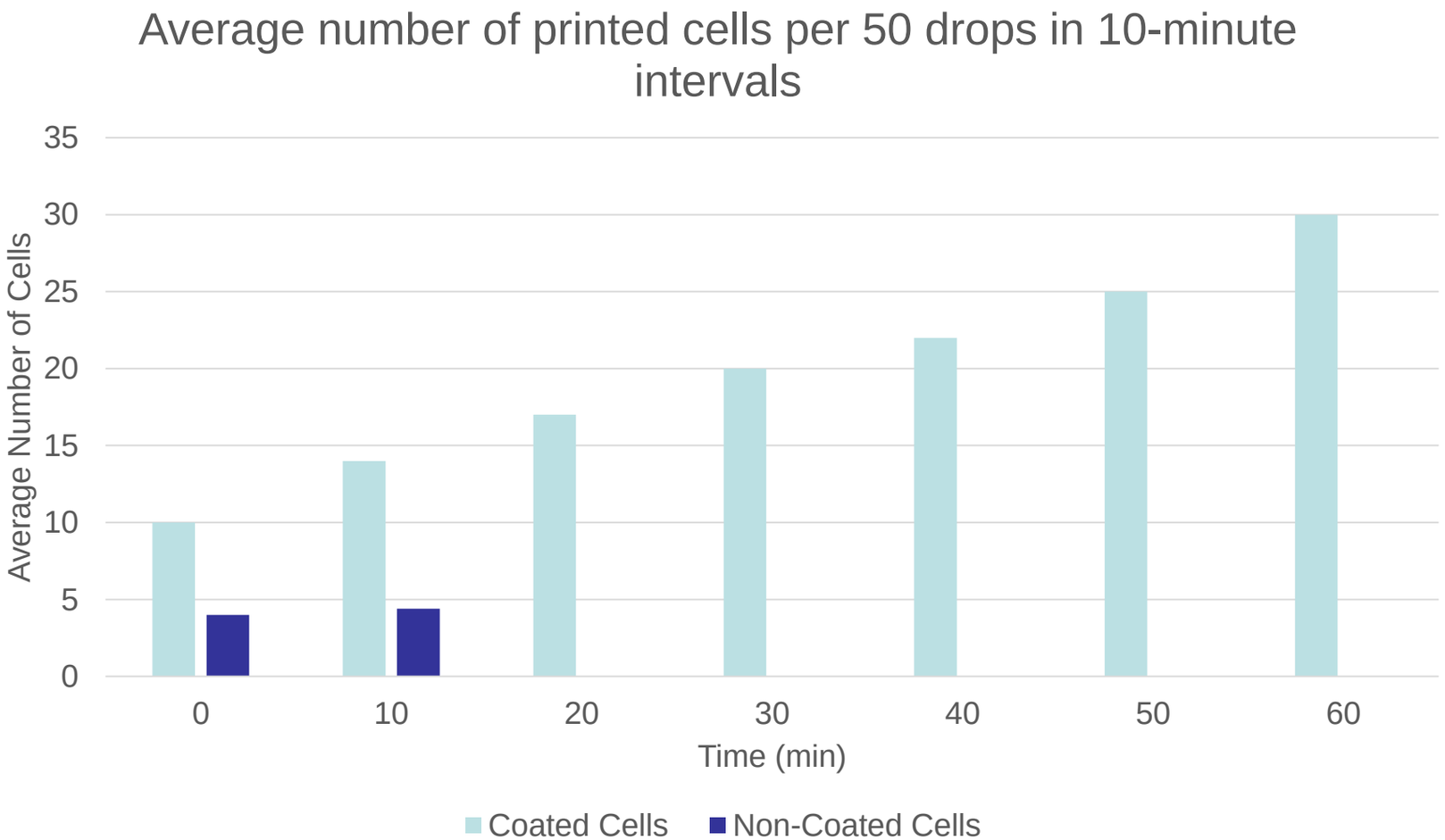
Predictable Printing

Stable Bio-ink



MeDe Innovation
EPSRC Centre for Innovative Manufacturing in Medical Devices

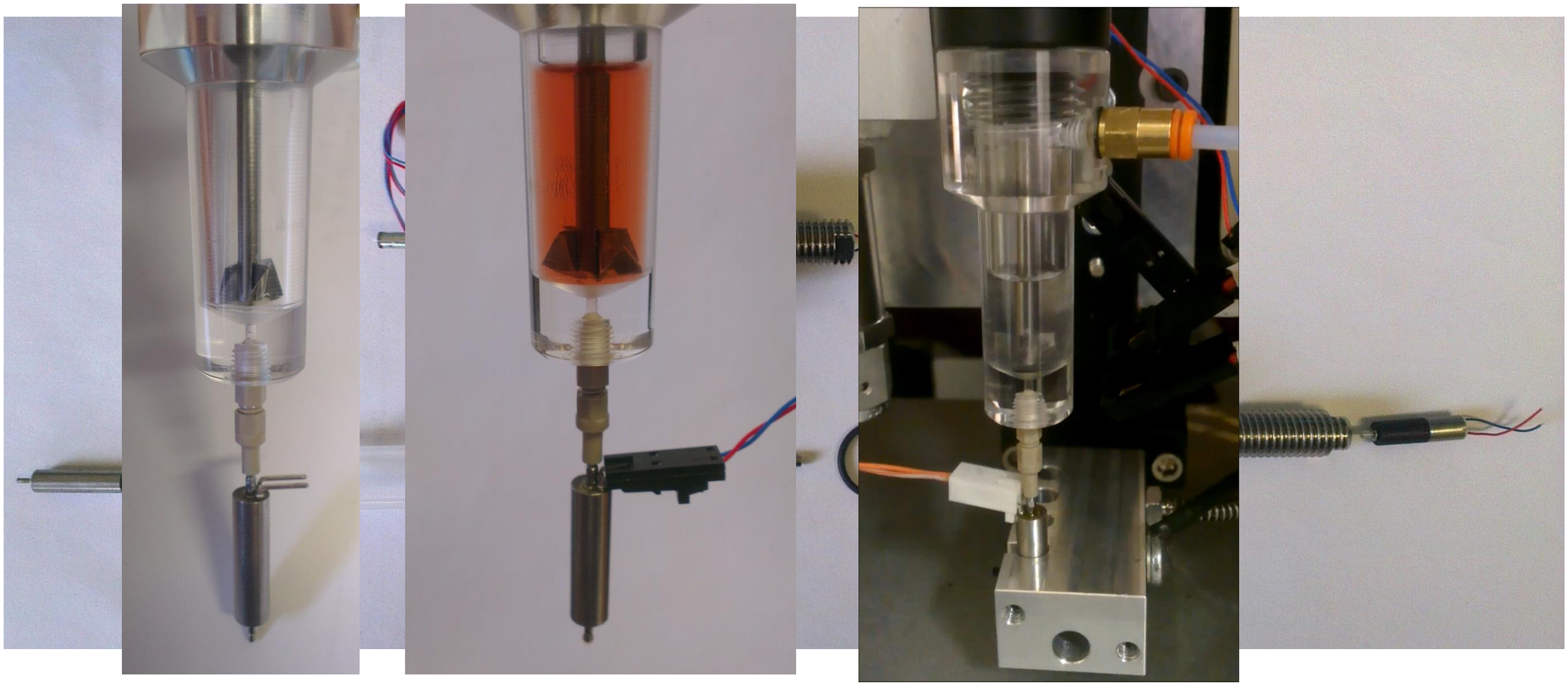
Results from Further Experiments



- The uncoated cells still lead to blockage
- An increase in coated cell numbers over time was noted
  - Cells settling due to density difference

MeDe Innovation
EPSRC Centre for Innovative Manufacturing in Medical Devices

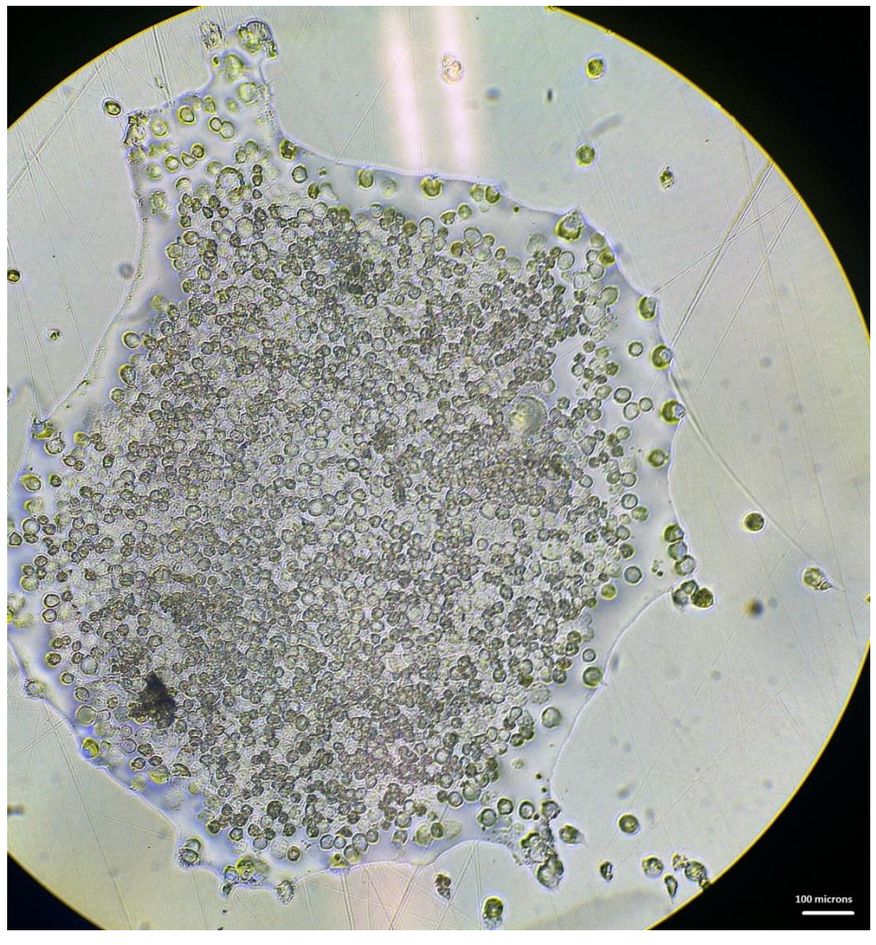
Cartridge Re-design



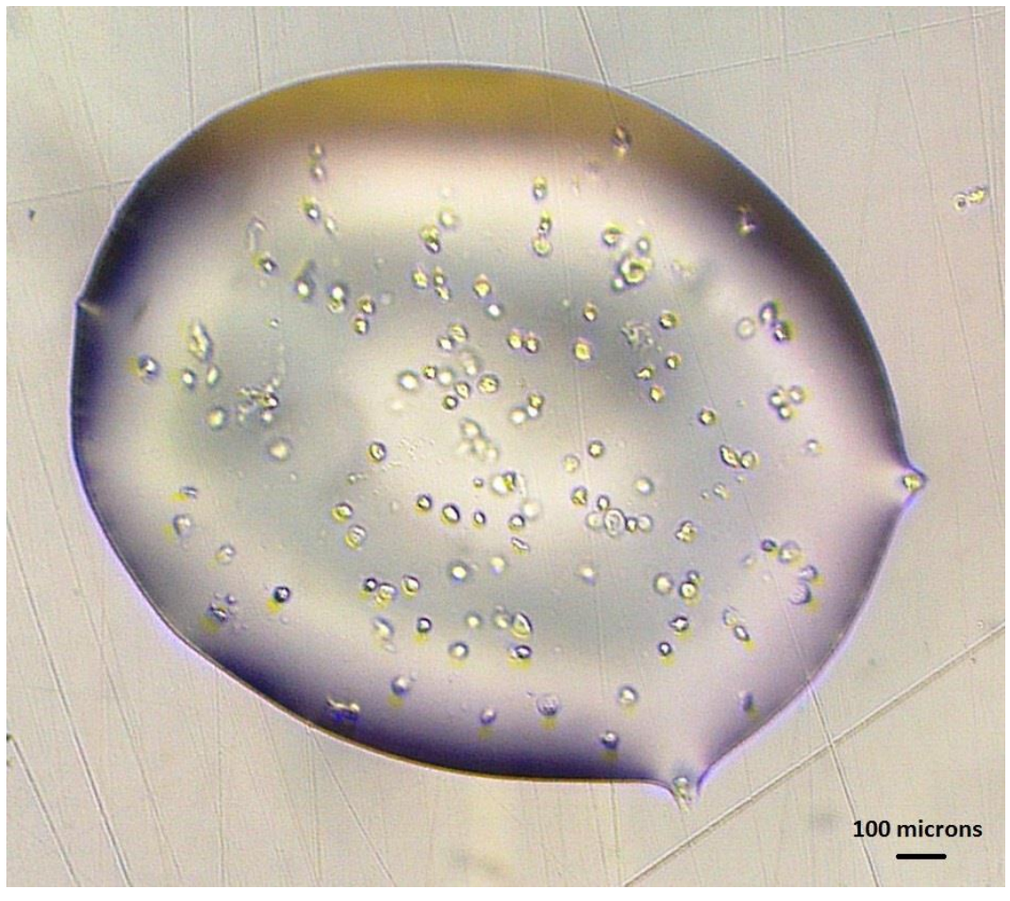
MeDe Innovation
EPSRC Centre for Innovative Manufacturing in Medical Devices

Printing with the Agitator

Left for 30 minuets without agitation



Left for 30 minuets with agitation

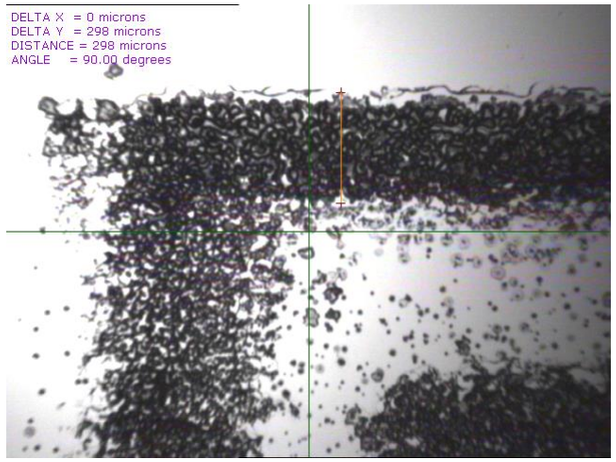


Bioink - Osteosarcoma cells, coated with 50µg/mL of PLL, in media (1m/mL)

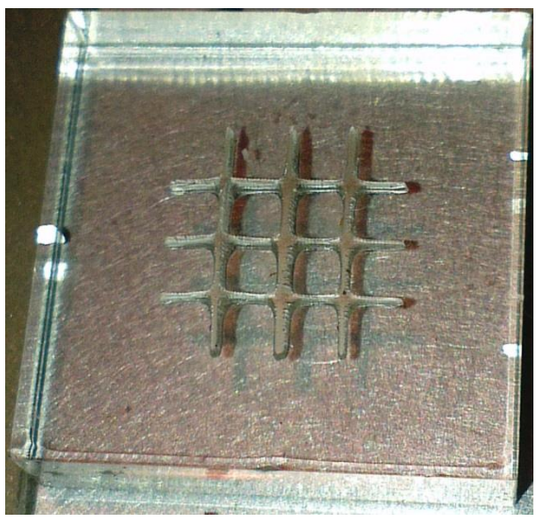
MeDe Innovation
EPSRC Centre for Innovative Manufacturing in Medical Devices

Rigid Scaffold Printing

- Evaporative polymer printing
  - Coffee staining effect
  - Low deposition rates
- Two component polymerization
  - Poor mixing on substrate
  - Monomer and initiator cytotoxic
- Photoinitiated Monomers
  - Acrylates + camphorquinone initiator (470nm)
  - Polymerisation inhibited by oxygen
    - Nitrogen sheilding gas allowed for a higher cure



Polymer from solvent
~300µm line width

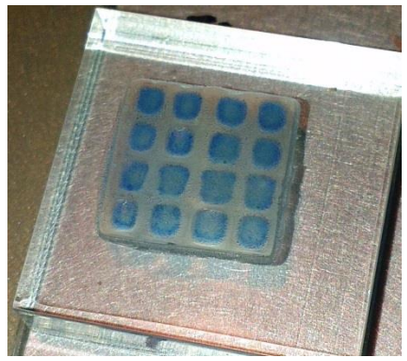


10x10x0.7mm blue light resin printed grid

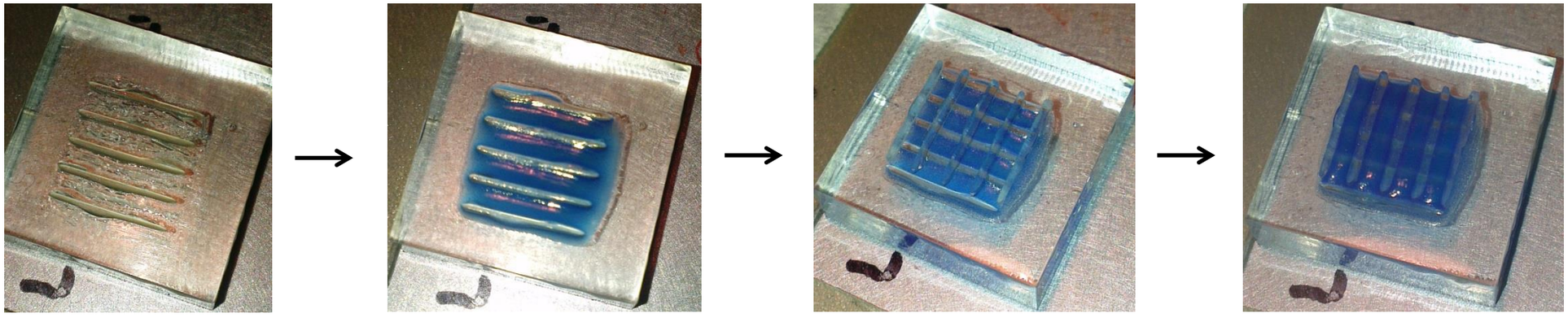
MeDe Innovation
EPSRC Centre for Innovative Manufacturing in Medical Devices

Co-processing Cell and Polymer

- Creating single layer gratings back filled with a second material proved to be possible.
- The scaffolds were formed using inkjet deposition of the rigid acrylate scaffold material (transparent material) and microvalve deposition of the bioink (blue material).



10x10x0.7mm filled grating



Summary

- Seeding of ‘in clinic produced’ rigid scaffold with autologous cells poses a significant tissue engineering problem.
- The stability of traditional bioinks for reliable and predictable inkjet printing is not good enough for printing over extended periods.
- The polyelectrolyte coating of cells within bioinks reduces the propensity for agglomeration and a slow mechanical agitator may reduce the effects of differing densities between bioink components.
- A combination of inkjet and micro valve technology may allow the rapid production of cell seed rigid scaffolds..

Acknowledgments

- This work has been supported by:
  - Ria Toumpaniari
  - Simon Partridge
  - Marina Ferreira-Duarte
  - Kenny Dalgarno