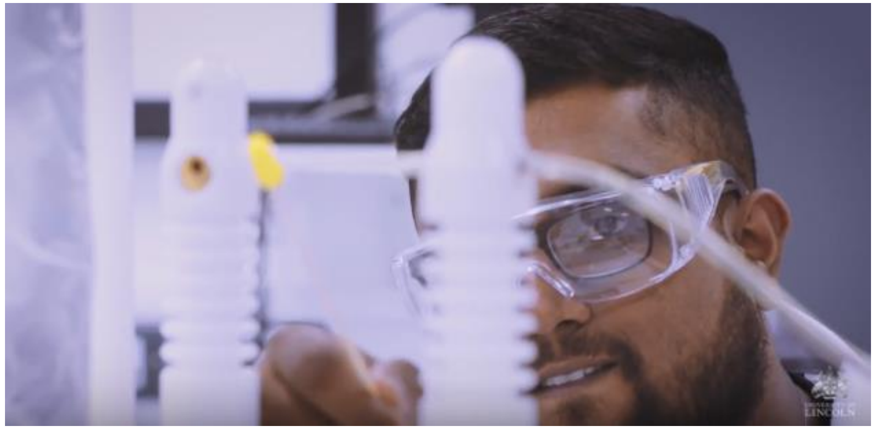
INCORPORATING NANOFIBRES INTO SYNTHETIC BONE MATERIAL

MEng Project

School of Engineering, University of Lincoln

University of Lincoln, School of Engineering

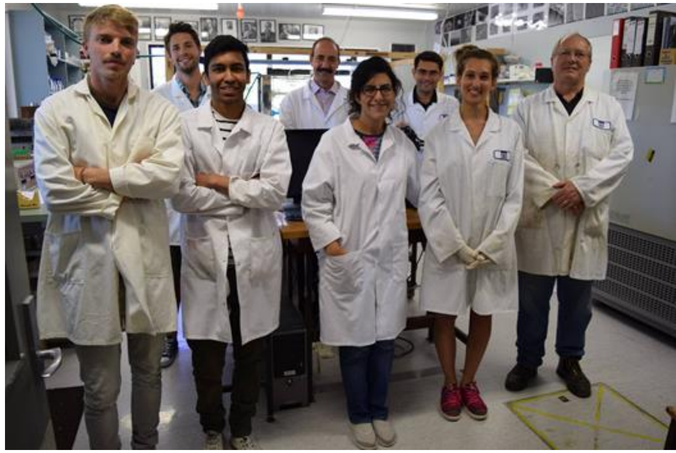
- The School of Engineering combines state-of-the-art R&D and teaching facilities with research informed teaching and industrial links.
- Listed as a principal partner of Siemens Industrial Turbomachinery Limited
- Key research areas in Power and Energy, and Intelligent Systems

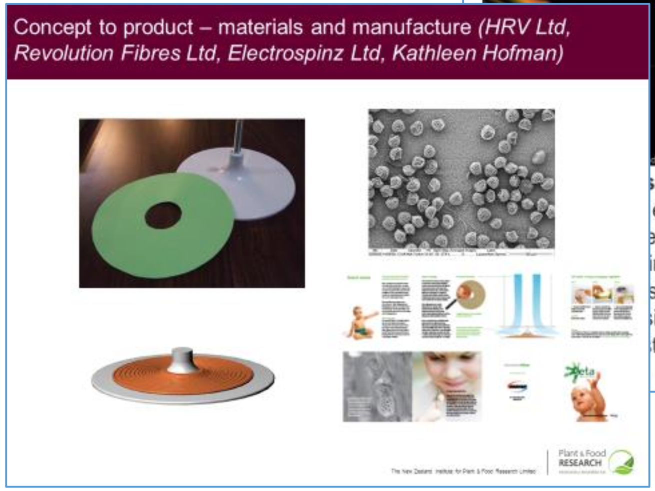


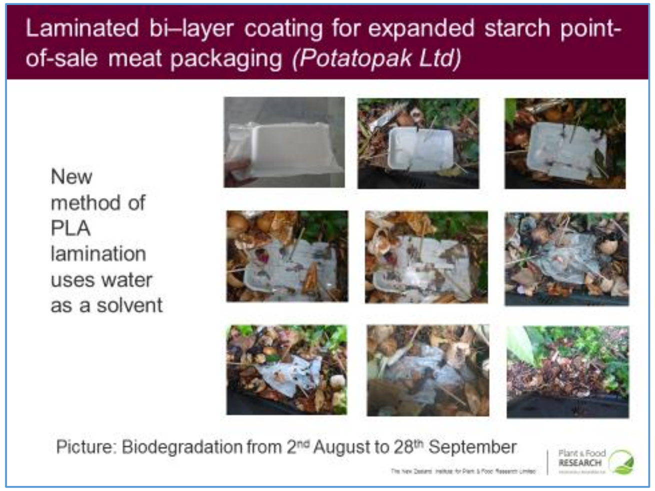
Meet the team

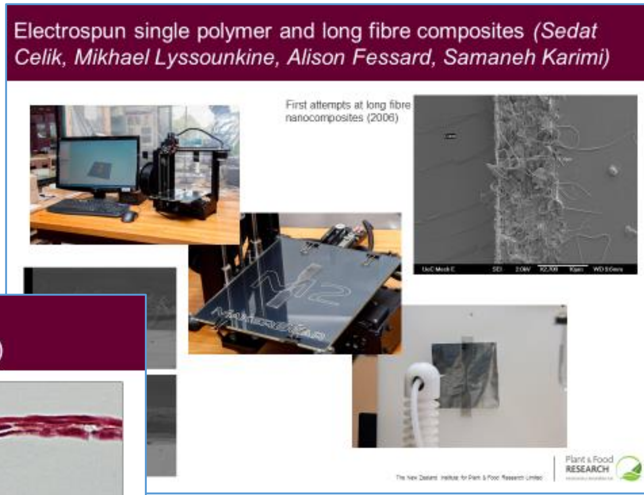
Meet the team – Dr Nick Tucker

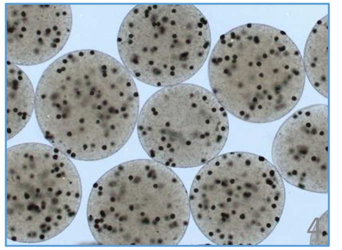
- Senior Lecturer – Materials and Manufacturing
- PhD in mechanical and manufacturing systems engineering
- Peer and professional review approved assessor for the Institute of Materials, Minerals and Mining





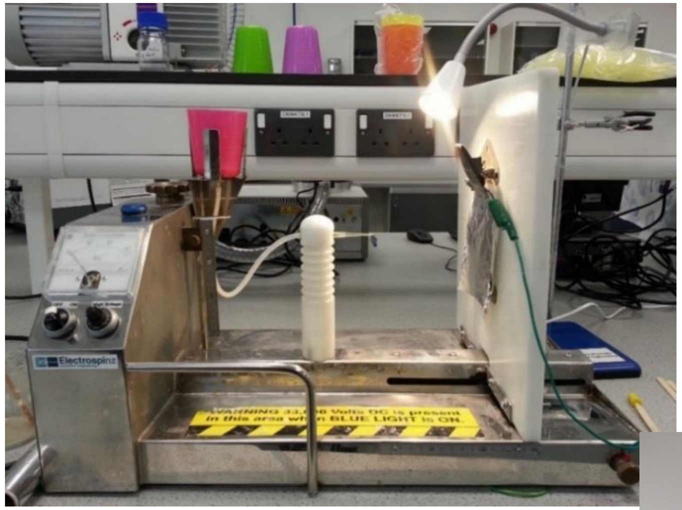


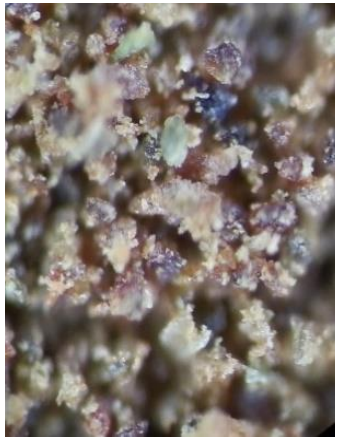




Meet the team – Natalie Czarnocki

- **Background** in electrospraying and electrospinning
- **Role within Project** – Electrospinning and SEM
- **Career aspiration** is to work within testing and within the research and development department of materials





Dissertation Title:

Electro-Encapsulation of a Bioactive Material - 2017

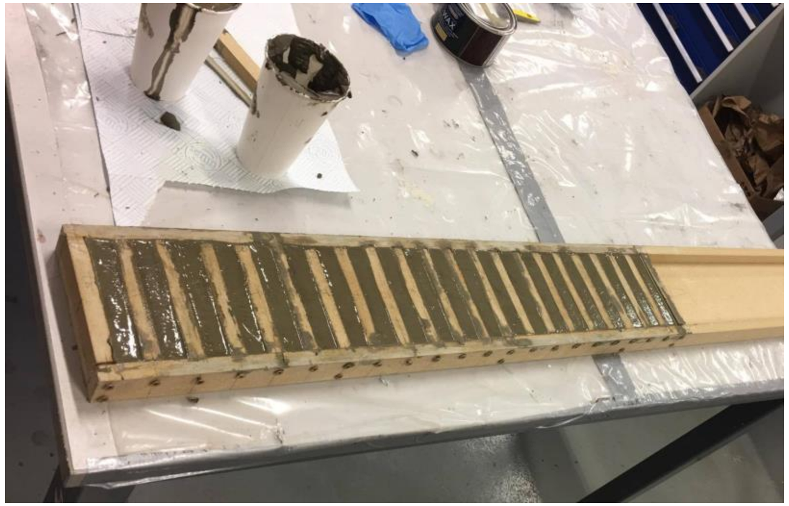
Meet the team – Divarshan Sivakumar

- **Background** in electrospinning and composite materials

- **Role within Project** – manufacturing scaffolds and castings

- **Career aspiration** is to work towards achieving chartership and work in a managerial environment

Dissertation Title: *Nanofibre Reinforced Concrete - 2017*



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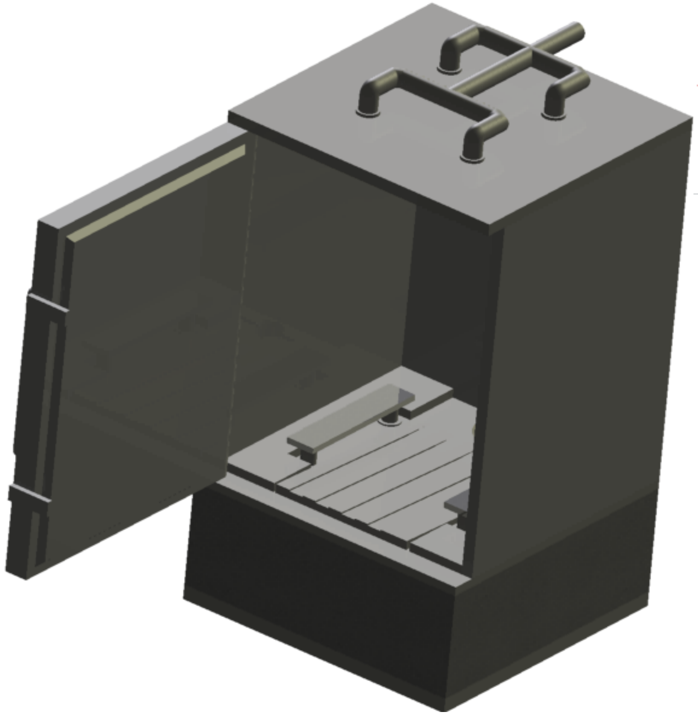
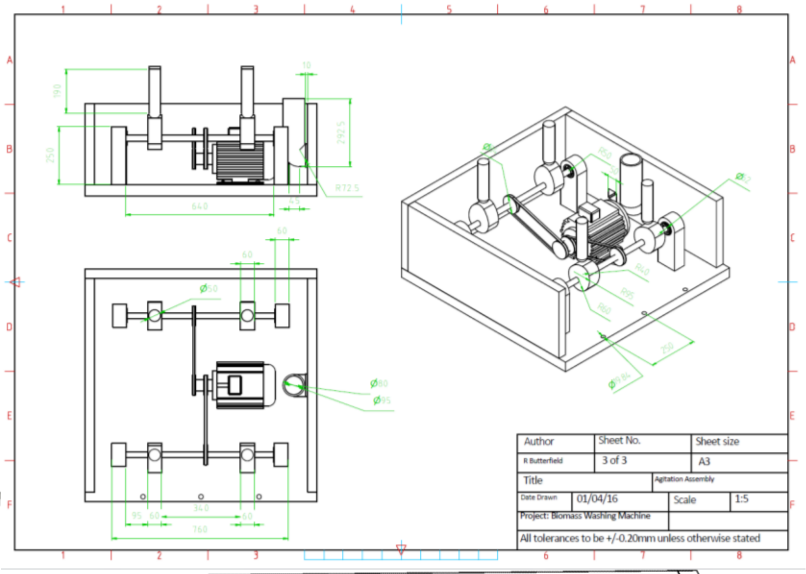
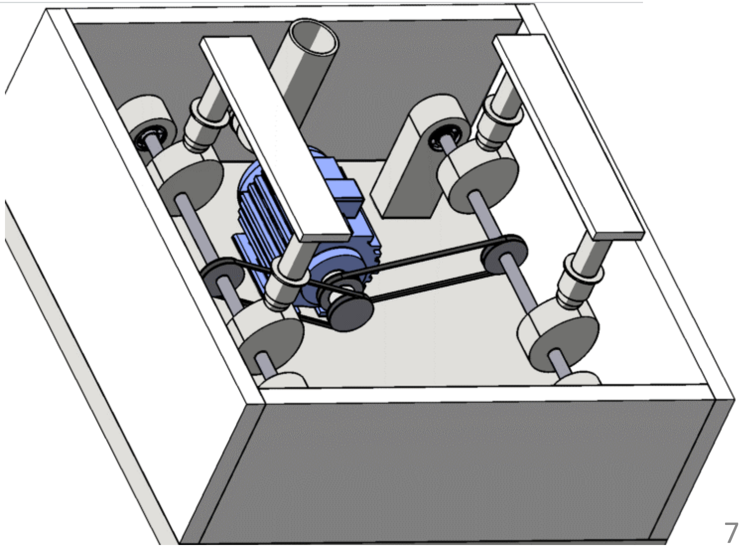
Meet the team – Rebecca Butterfield

- **Background** in CAD and Power & Energy
- **Role within Project –** Key researcher and Initial design
- **Career aspiration** is to work in the Industrial Gas sector in a Technical Role

Dissertation Title:
Novel Design of a Biomass Pre-treatment Washing Machine - 2016



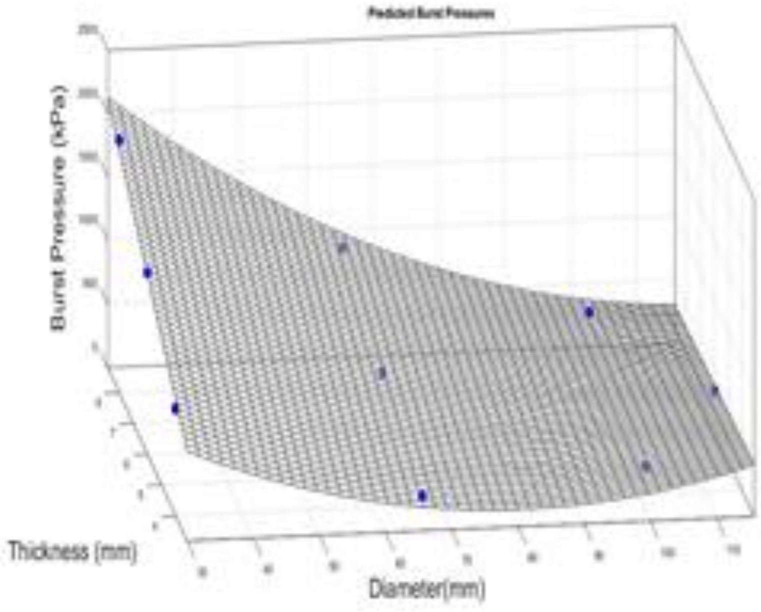


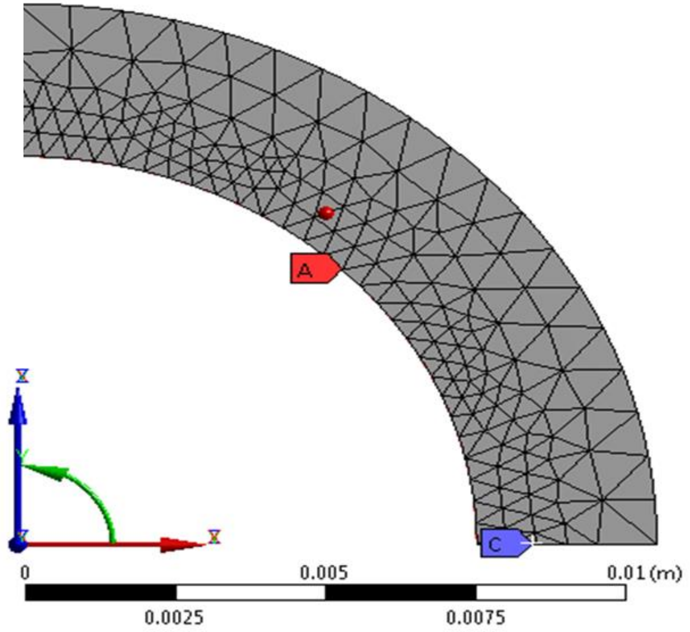


Meet the team – Matthew Warnes

- **Background** in 3D modelling and Finite Element Analysis
- **Role within Project** – Modelling the moulds via CNC Machine and Casting Plaster
- **Career aspiration** is to use FEA modelling within structural analysis and work towards chartership

Dissertation Title: *Hyper elastic model of Neoprene hose - 2017*





Incorporating Nanofibres into Synthetic Bone Material

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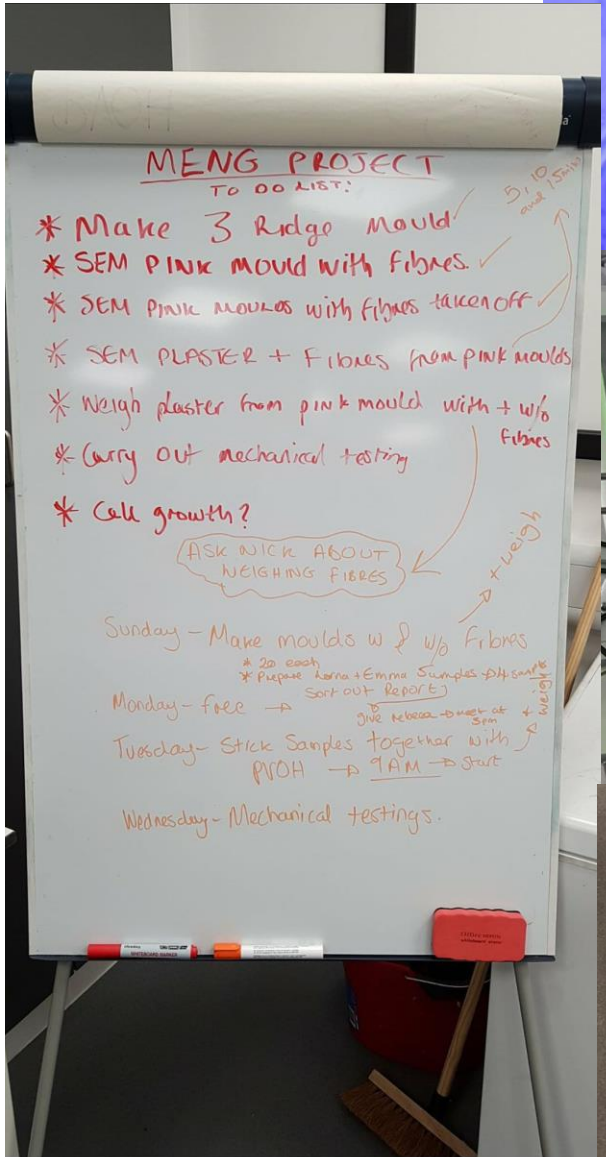
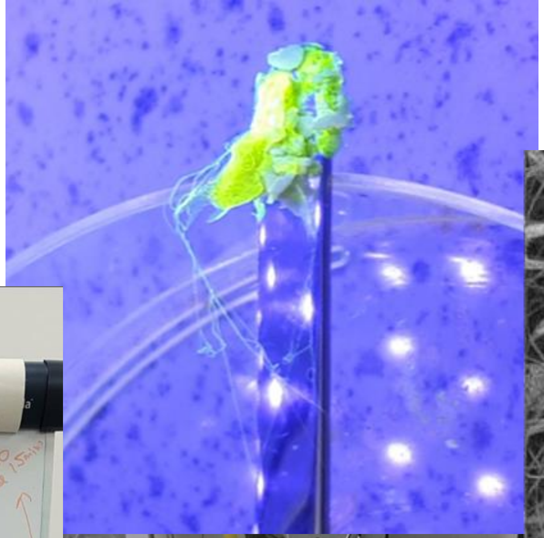
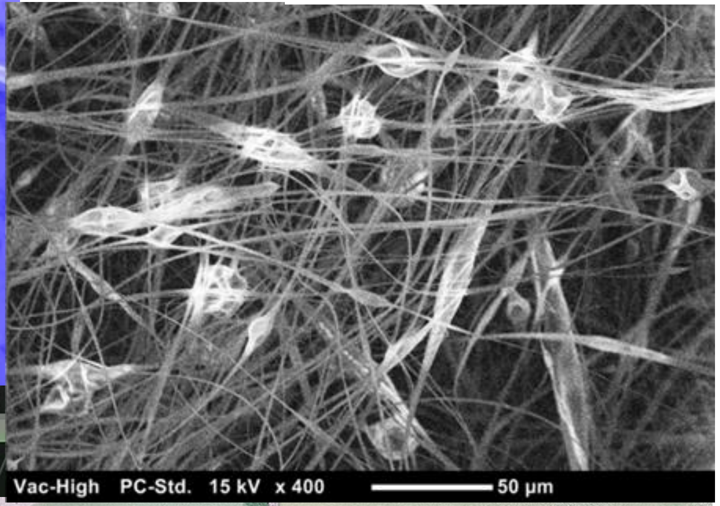
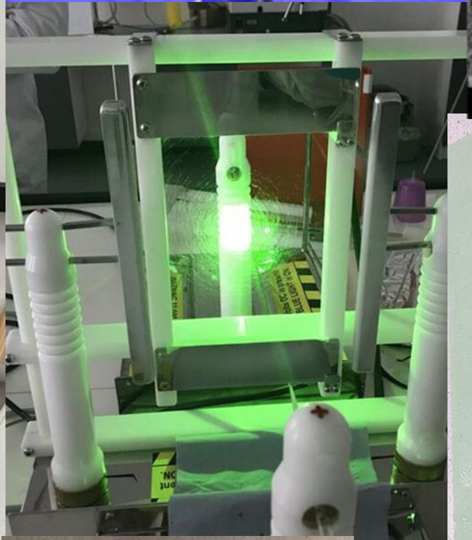
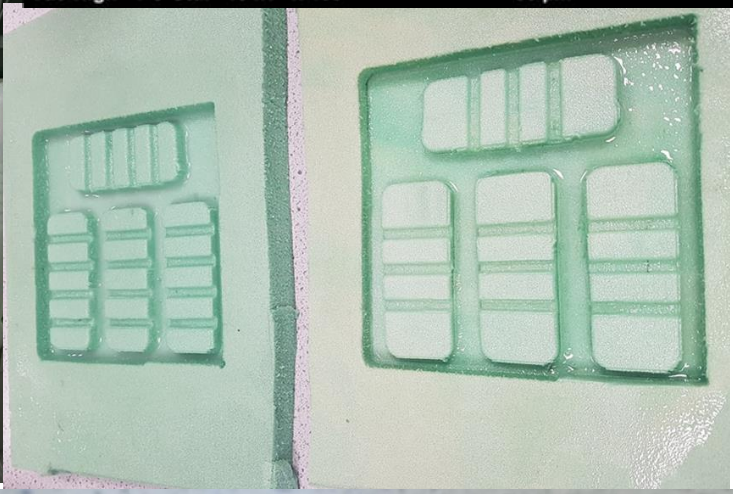
- Electrospinning fibres for to use as reinforcement
- Creating an effective Scaffold for testing
- Establishing an effective incorporation method
- Mechanically test Scaffold to establish effect of fibre reinforcement





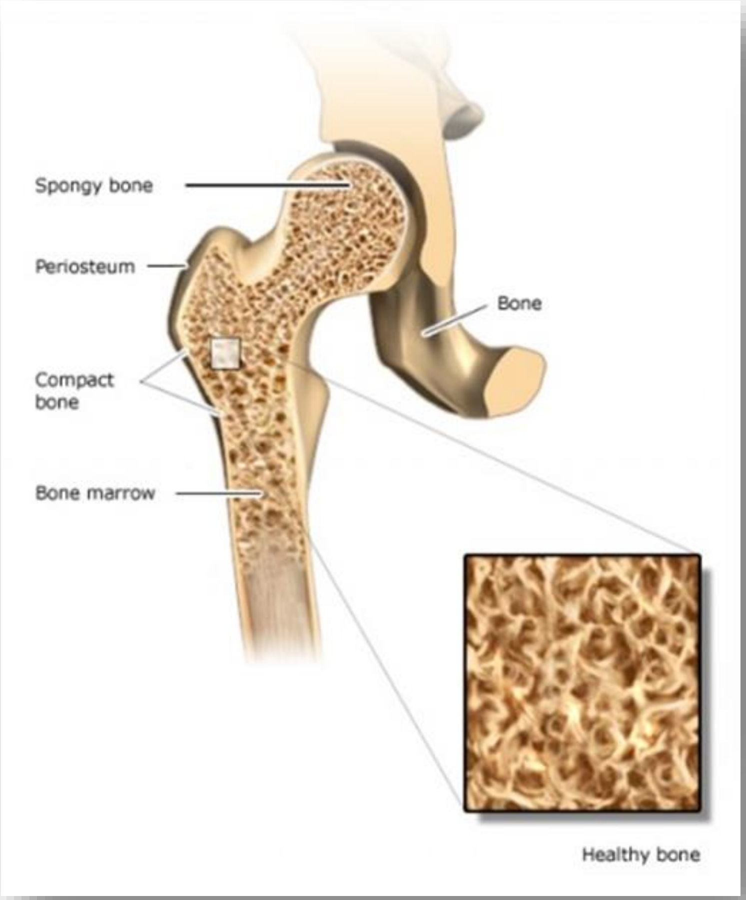




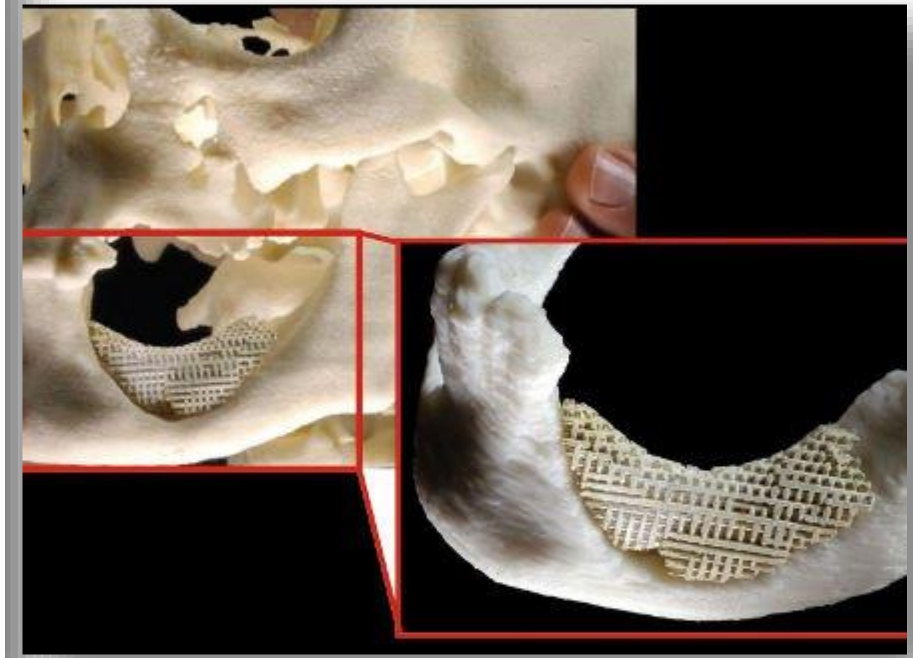


Initial Research

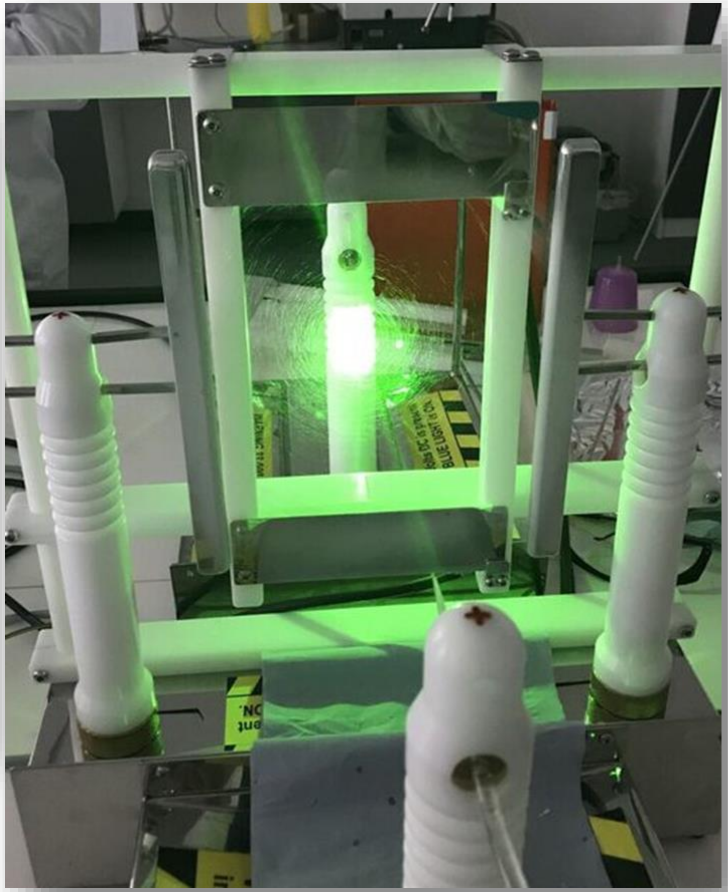
Bones



Synthetic Bones



Nanofibres



Electrospinning Fibres – Equipment set up

Benefits of using electrospinning:

Large surface area – allows for a greater and better area for cells to grow on.

3D assembly – allows for intercellular growth, for the bone analogue it will add mechanical properties such as strength and flexibility.

Porosity – a porous structure will allow for cellular impalement which should be between 60-90%.

Initial Fibre Exploration - parameters

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Acetic Acid and Zein
Acetic Acid, Zein and Fluorescence
PVOH
Cellulose and PVOH
Cellulose Nitrate
PVOH
Polyethylene Glycol and Collagen
Acetic Acid and Collagen
Acetic Acid and Gelatin
Acetic Acid, Citric Acid and Gelatin

Electrospinning Parameters:

- Voltage
- Duration
- Spineret distance
- Electrode distances
- Min/Max alternating voltage

Conditions:

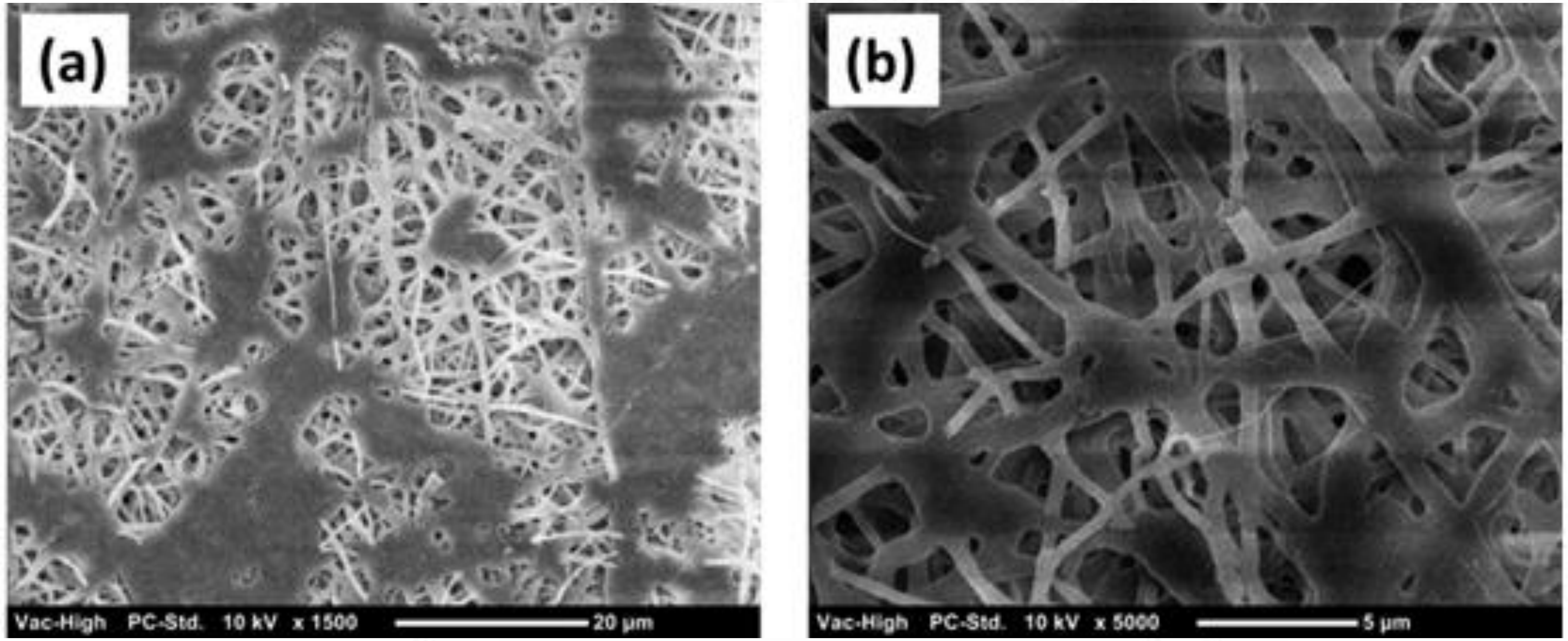
- Humidity
- Temperature

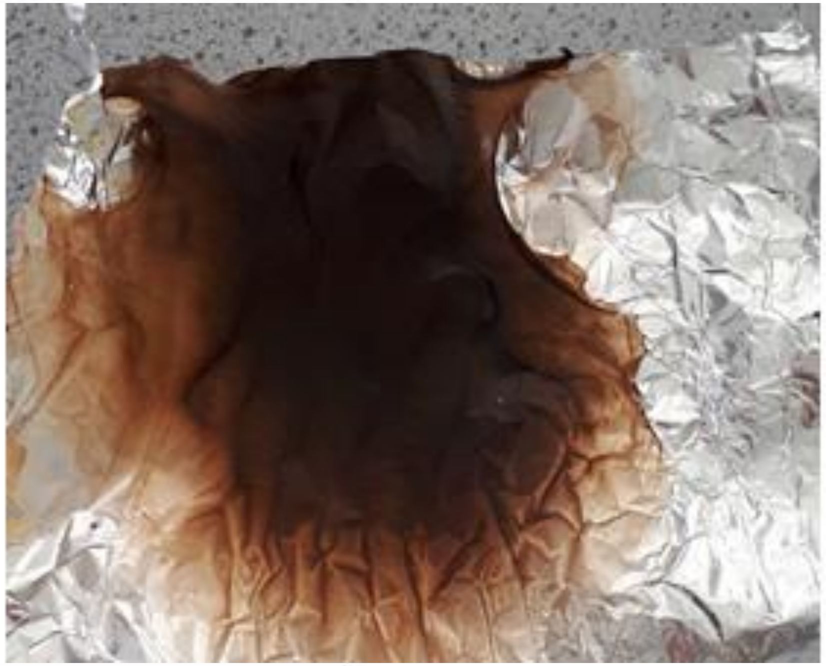
Preparation Conditions:

- Solution type
- Stirring times
- Temperature

Initial Fibre Exploration – SEM Images

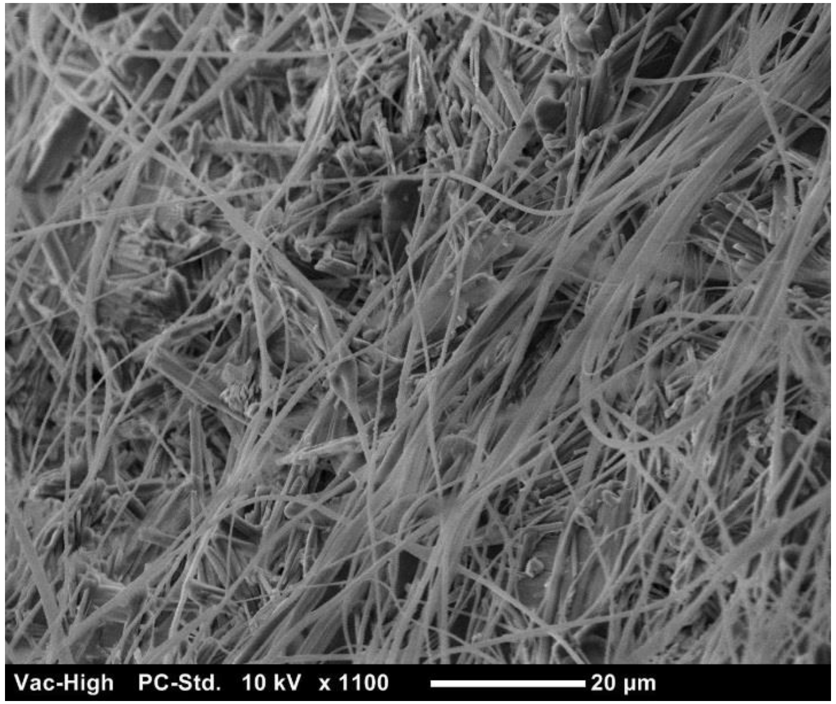
Experiment Order	Substance Used and Amounts	Voltage (kV)	Duration (min)
J(2)	• Acetic Acid – 17.2g (66%) • Citric Acid – 1.2g (8%) • Gelatin – 1.6g (6%) **(Oven-Cured, 140°C, 2 hrs)**	12	20

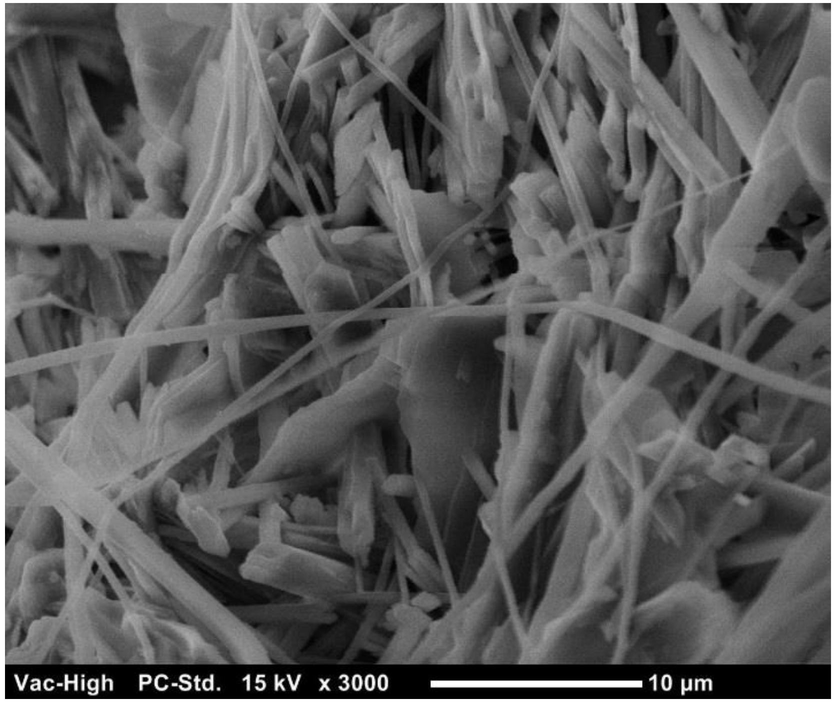




Fibre Integration analysis – Cellulose Nitrate

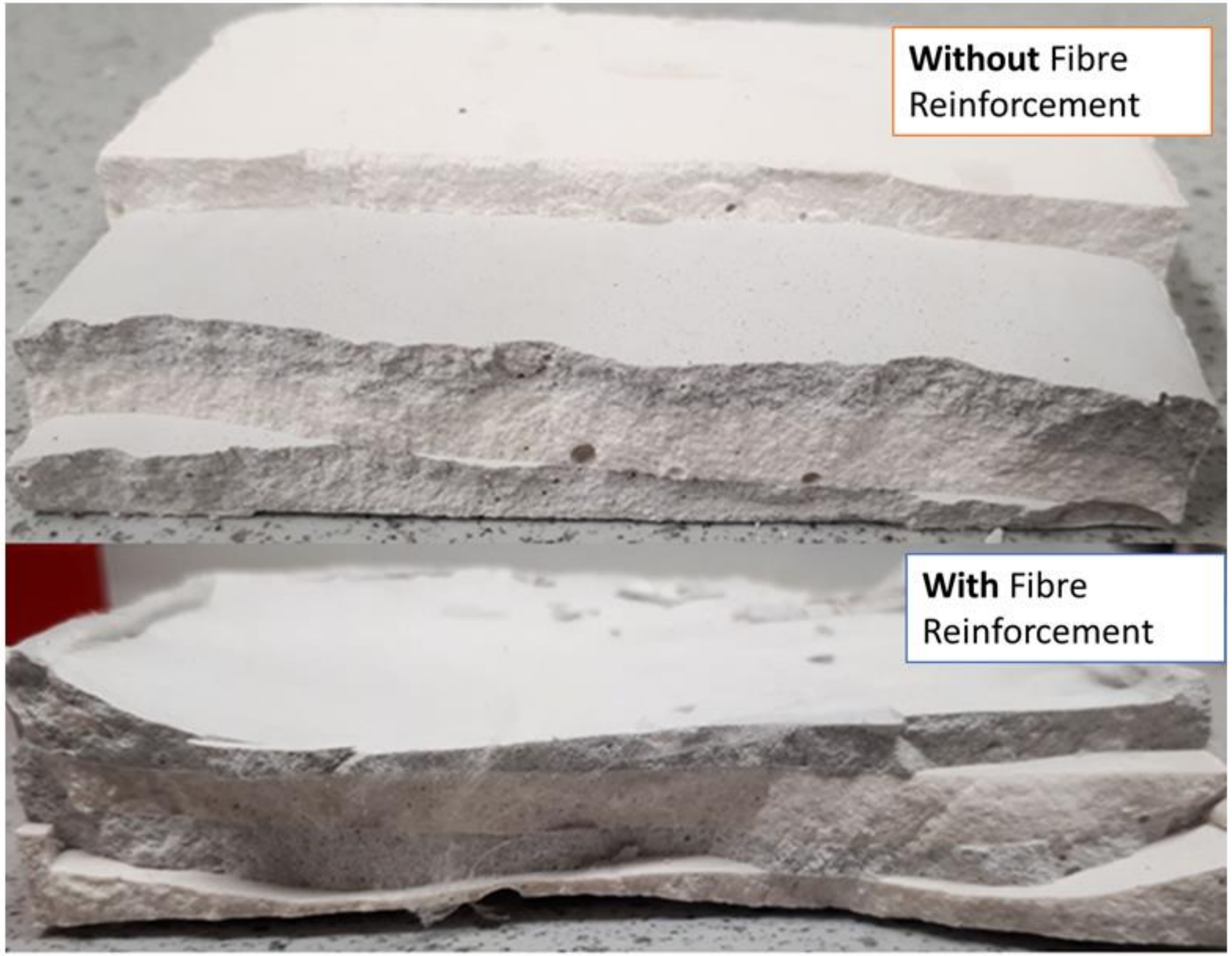
- Cellulose Nitrate chosen as fibre reinforcement – Hydrophobic
- Average Fibre Diameter:
  - Freshly Electrospun – 1.714μm
  - After Plaster is applied – 0.891μm
- Average Weight of fibres in one scaffold: 9.24mg
- Fibres are Electrospun for 10 minutes – shows high level of integration with Plaster of Paris





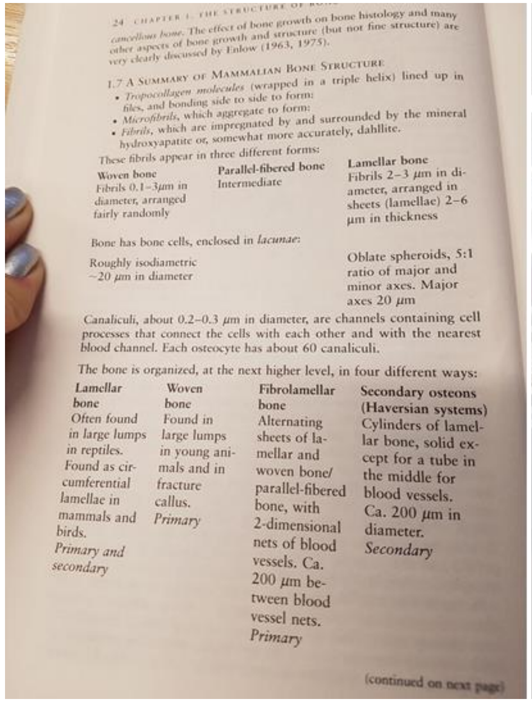
Initial mechanical testing – Plaster of Paris, with and without Reinforcement

	Breaking Loads for Plaster of Paris Specimens	
Specimen	Plaster of Paris Composite with Fibres	Plaster of Paris with Fibres
1	225.27156	154.34
2	290.42374	158.77
3	316.5459	166.73
4	326.27103	188.60
Average Breaking Loads (N)	289.62806	167.11
Standard Deviation (N)	45.496042	15.22



Conclusions drawn from Initial Testing

- Plaster of Paris to be used as the analogue material (due to cost, availability and mechanical properties)
- Cellulose Nitrate to be used as fibre reinforcement (due to spin capability and insolubility)
- Scaffold to be a layered composite with fibre reinforcement


24 CHAPTER 1. THE STRUCTURE OF BONE

cancellous bone. The effect of bone growth on bone histology and many other aspects of bone growth and structure (but not fine structure) are very clearly discussed by Enlow (1963, 1975).

1.7 A SUMMARY OF MAMMALIAN BONE STRUCTURE

- *Tropocollagen molecules* (wrapped in a triple helix) lined up in files, and bonding side to side to form:
- *Microfibrils*, which aggregate to form:
- *Fibrils*, which are impregnated by and surrounded by the mineral hydroxyapatite or, somewhat more accurately, dahllite.

These fibrils appear in three different forms:

Woven bone	Parallel-fibered bone	Lamellar bone
Fibrils 0.1–3μm in diameter, arranged fairly randomly	Intermediate	Fibrils 2–3 μm in diameter, arranged in sheets (lamellae) 2–6 μm in thickness

Bone has bone cells, enclosed in *lacunae*:

Roughly isodiametric ~20 μm in diameter	Oblate spheroids, 5:1 ratio of major and minor axes. Major axes 20 μm

Canaliculi, about 0.2–0.3 μm in diameter, are channels containing cell processes that connect the cells with each other and with the nearest blood channel. Each osteocyte has about 60 canaliculi.

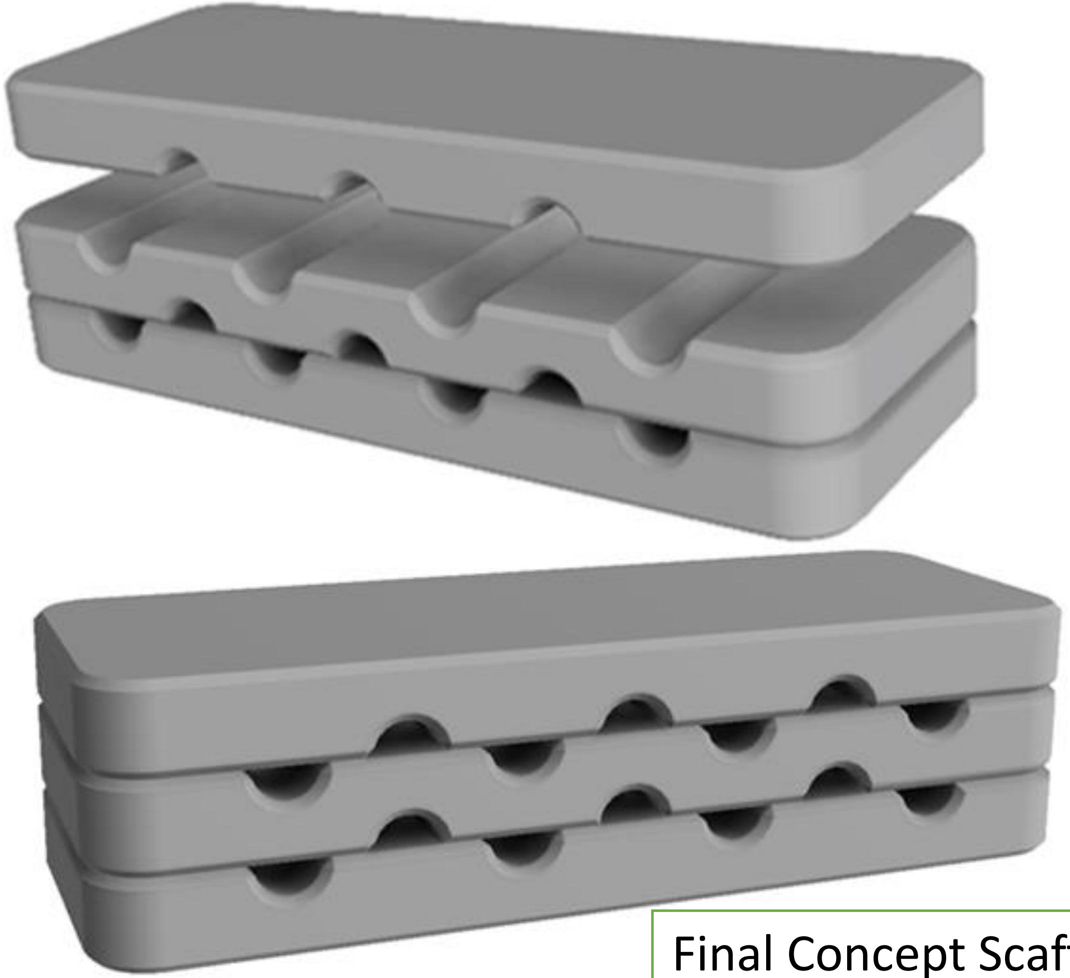
The bone is organized, at the next higher level, in four different ways:

Lamellar bone	Woven bone	Fibrolamellar bone	Secondary osteons (Haversian systems)
Often found in large lumps in reptiles. Found as circumferential lamellae in mammals and birds. *Primary and secondary*	Found in large lumps in young animals and in fracture callus. *Primary*	Alternating sheets of lamellar and woven bone/parallel-fibered bone, with 2-dimensional nets of blood vessels. Ca. 200 μm between blood vessel nets. *Primary*	Cylinders of lamellar bone, solid except for a tube in the middle for blood vessels. Ca. 200 μm in diameter. *Secondary*

(continued on next page)

Scaffold design – Requirements and Concepts

Scaffold Design Requirements	Composite structure	To replicate natural bone structure
	Simplistic Geometry	To aid layering to achieve composite structure and for aid of manufacture and repeatability
	Pores of approximately 5mm radius	Aid cell growth and migration, whilst retaining adhesion
	Biologically sound material	To prevent rejection from the body, and to biodegrade



Final Concept Scaffold – *Arrangement 2*

Tray Design – Concept Development

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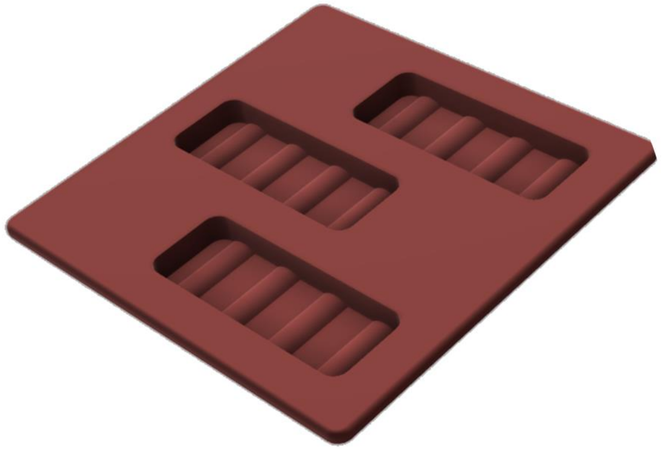


Tray Concept 1

- Large number of analogues to be produced at once
- Increase production efficiency
- Testing found that adding fibres by directly spraying onto tray was better than manually applying (using silicone backing paper)

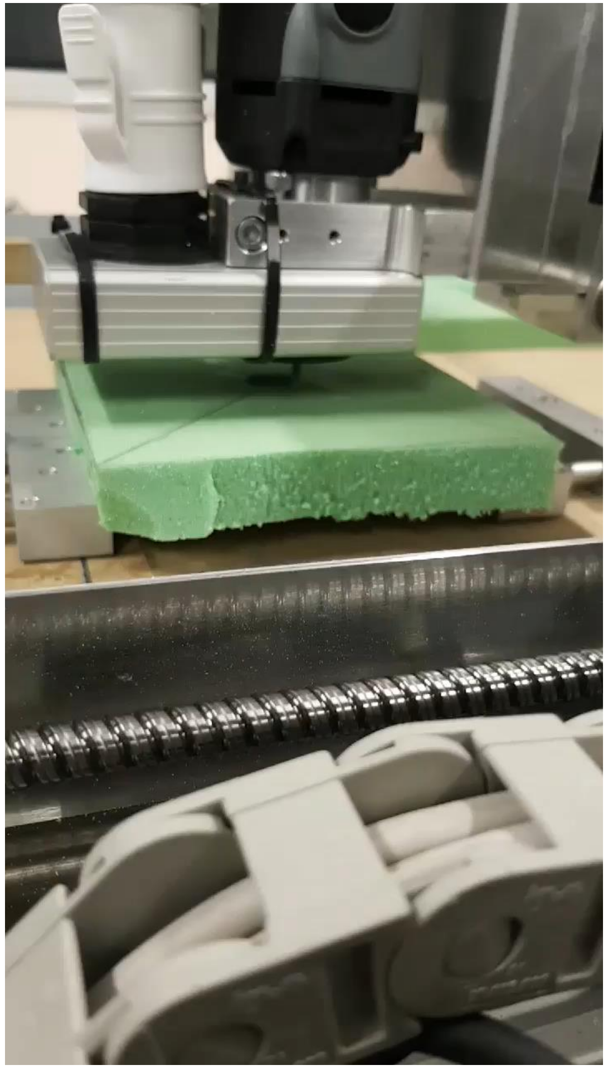
Tray Concept 2

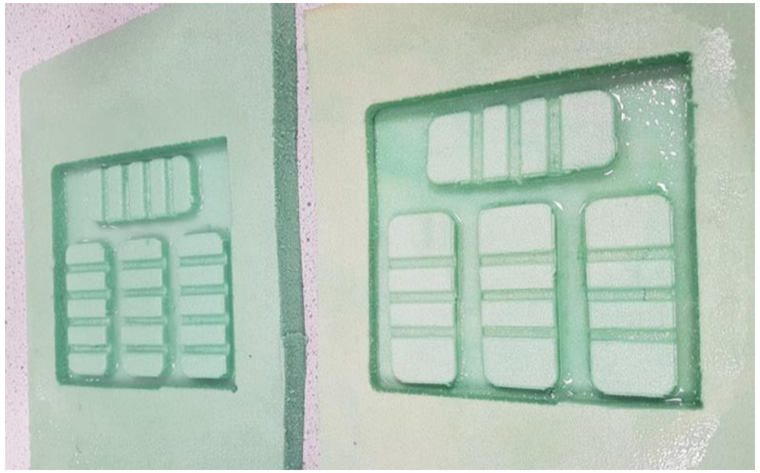
- Less analogues can be produced
- Creates analogues *30mm x 80mm x 10mm*
- Size allows for tray to be attached to Electrospinner – fibres sprayed directly

Tray Overlay

- Negative of Tray 3 (Refined)
- Acts as a press to create the centre layer of the Scaffold

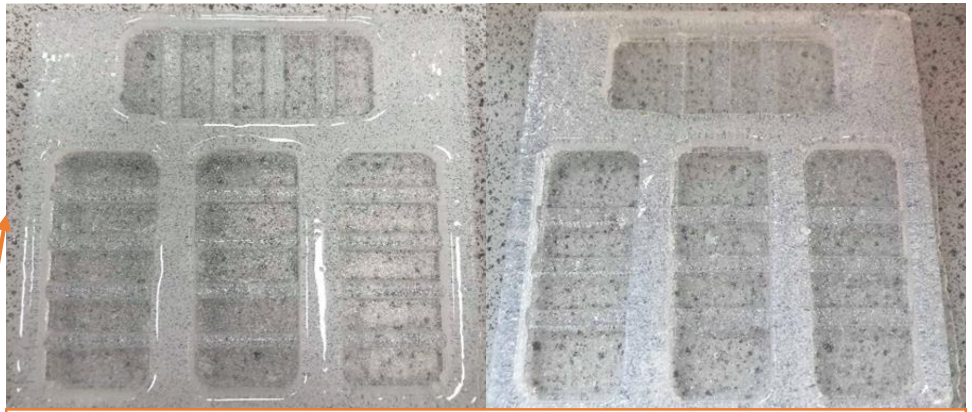
Tray Production – Manufacturing and Material refinement





Production Method

- Positive mould cut out of foam, edges and grooves tidied using manual tooling
- Positive moulds cleaned, then coated with epoxy resin
- After resin dried, silicone rubber was poured in and allowed to set for 24 hours



Silicone Rubber 1: easycomposites™ , clear, **non-matte finish**

Silicone Rubber 1: Polycraft™ ,pink, **matte finish**

Fibre testing on Prototype tray - Parameters

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PARAMETERS

Voltage: 12kV

Height of Cup: 250mm

Spinneret Tip to Target Distance: 200mm

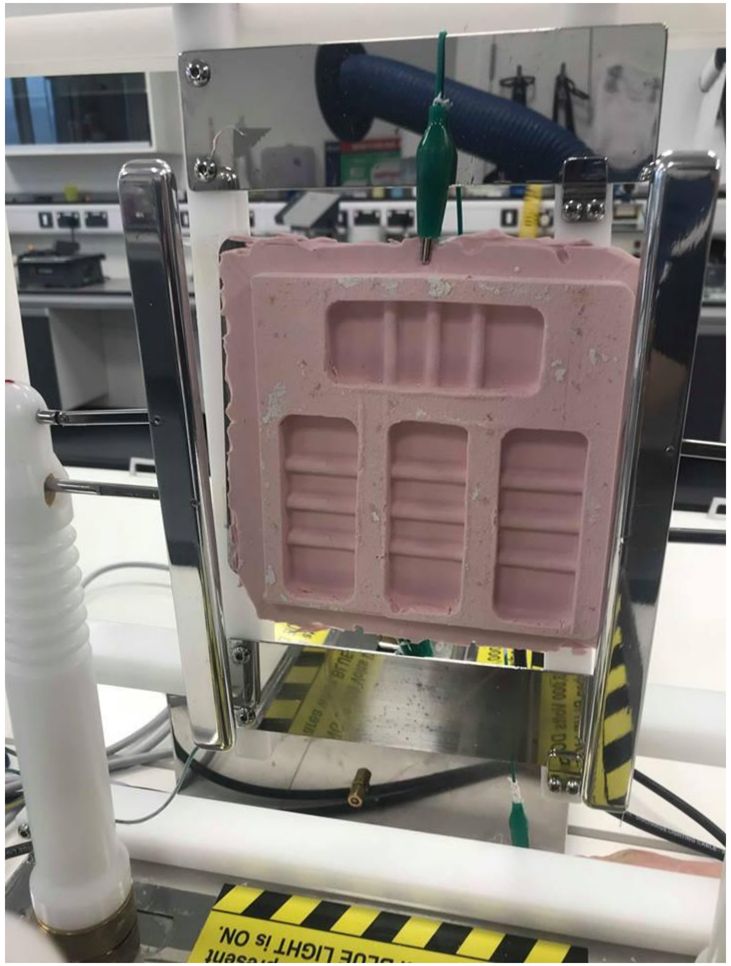
Spinneret Tip Diameter: 1mm

Electrode Gap: 180mm

Alternating Voltage between Electrodes: 2 and 5kV

Optimum Room Temperature: 18 – 20^{o}C

Optimum Humidity: 1.4 – 1.6g/m^3



Prototype tray – Analogue Comparisons

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easycomposites™ clear tray – analogues

- Fairly rouch surface texture
- Well defined grooves
- Some irregularities on outer edges

Polycraft™ pink tray – analogues

- Sligtly smoother surface texture
- Grooves and rigdges well defined
- Successful creation of ‘middle’ analogue

Weight testing – Fibre Spinning and Scaffold Drying

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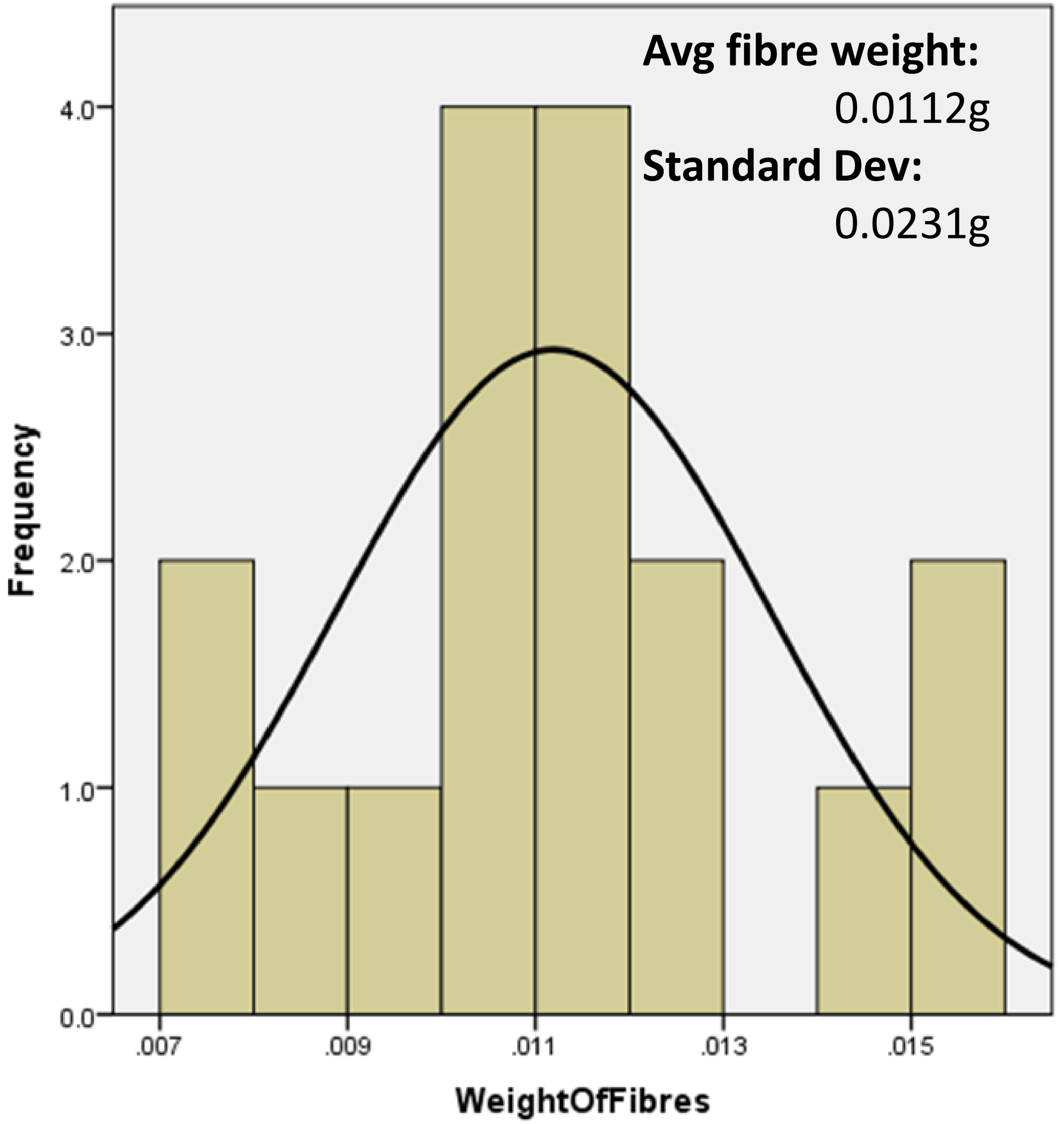
Fibre weight after 5 min Spin Duration



Analogue Mass after 4 hours curing

Average weight of each analogue (Hour 0)	40.02g
Average weight of each analogue (Hour 4)	38.92g
Average Weight loss of specimen	1.10g

Fibre testing on Prototype tray - *easycomposites™* clear tray

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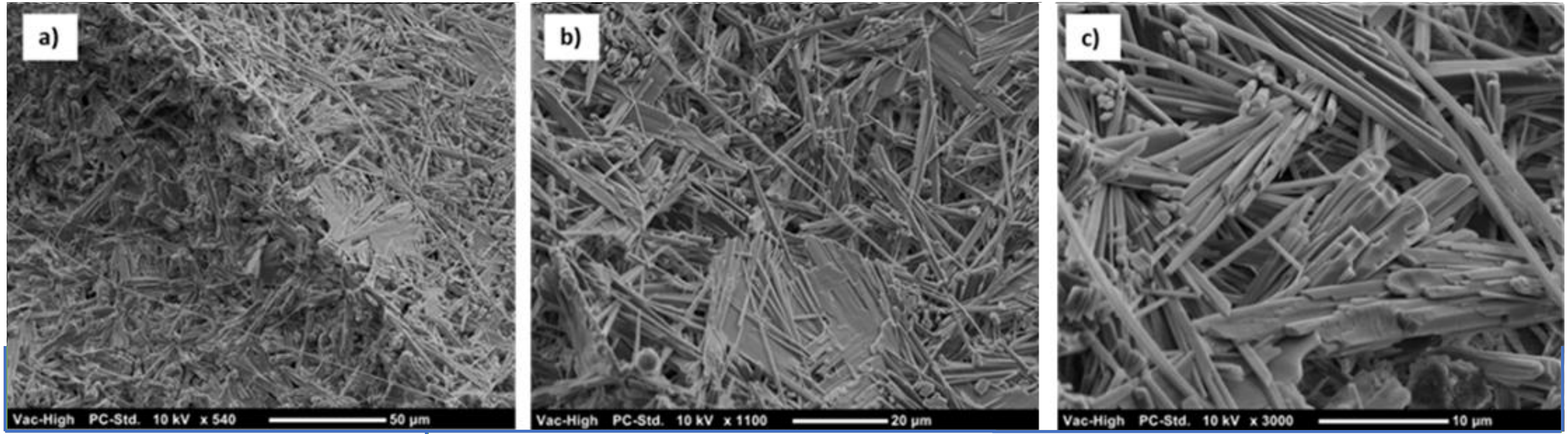
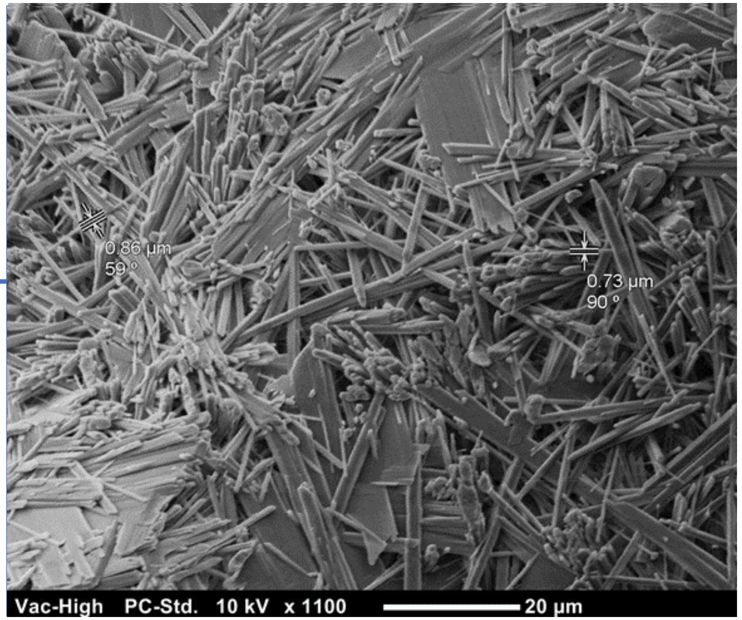


Images of Cellulose Nitrate electrospun onto Clear Silicon ***for 15 Minutes*** with Addition of Plaster

Electrospun Cellulose Nitrate Fibres (15 minutes) with Plaster of Paris from Clear Silicon - Diameter and Orientation



Fibre testing on Prototype tray - *Polycraft™* pink tray

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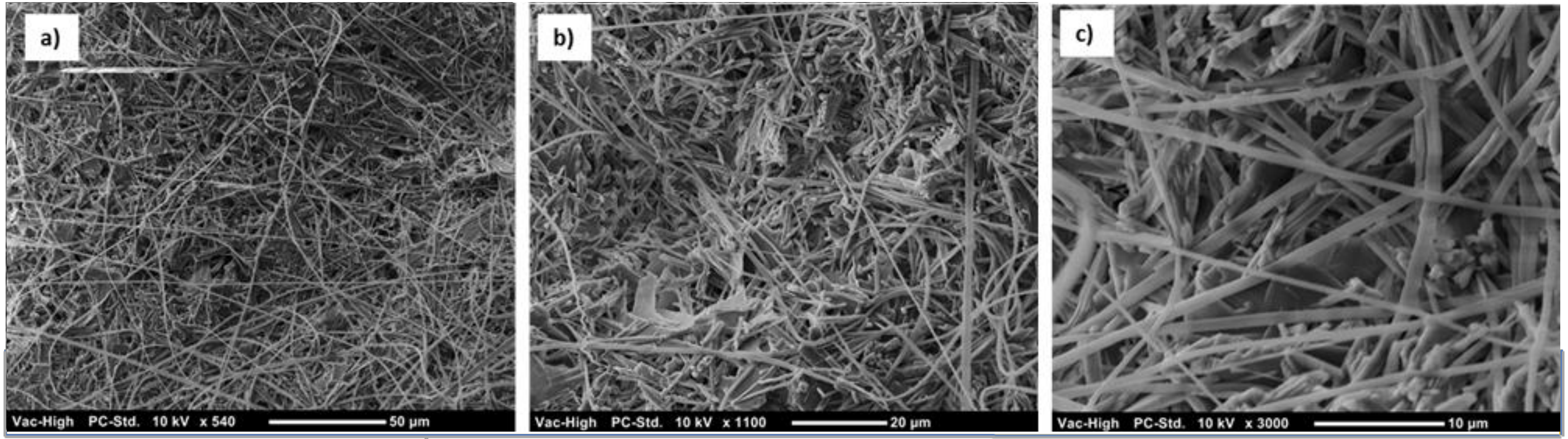
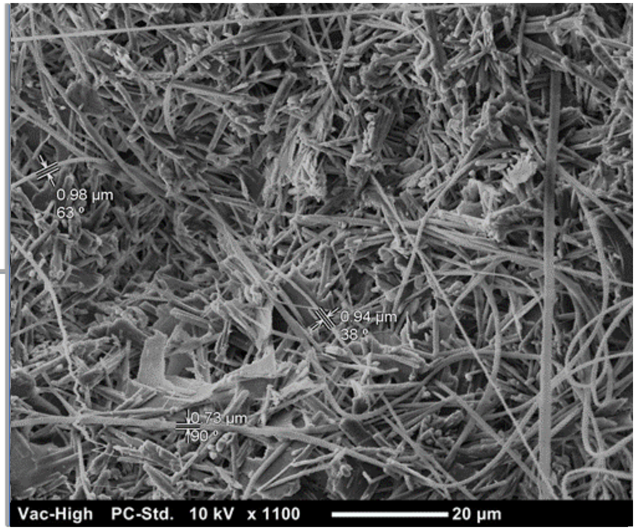


Images of Cellulose Nitrate electrospun onto Pink Silicon ***for 15 Minutes*** with Addition of Plaster

Electrospun Cellulose Nitrate Fibres (15 minutes) with Plaster of Paris from Pink Silicon - Diameter and Orientation



Fibre testing on Prototype tray – Residue Comparison

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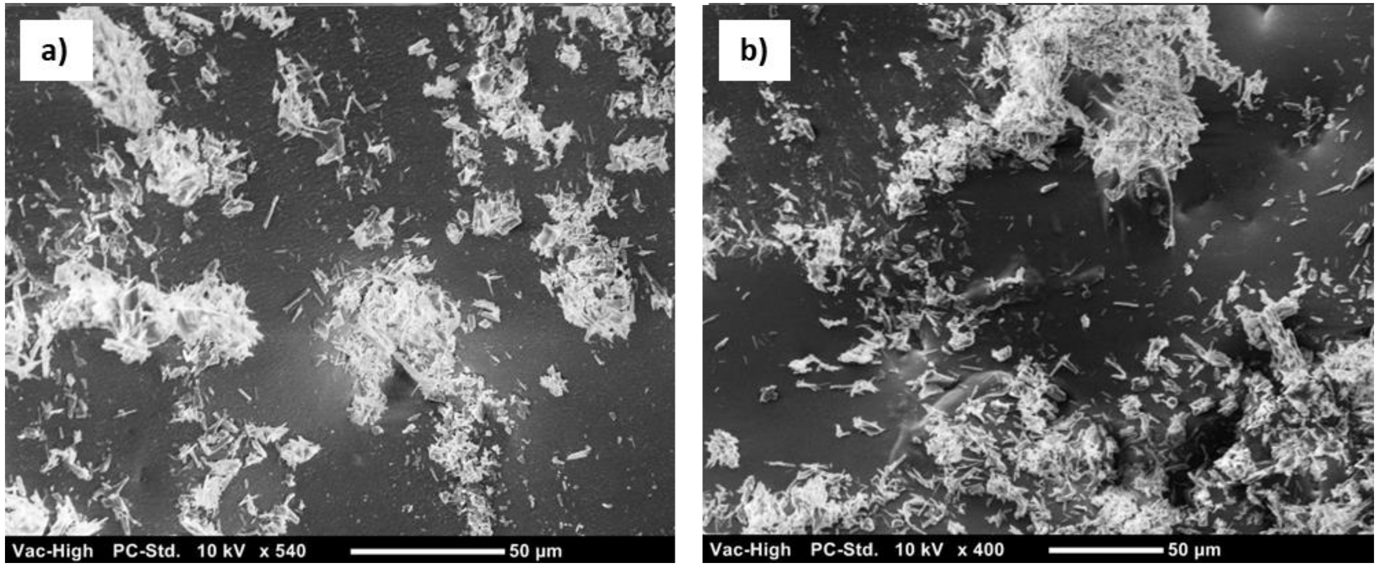
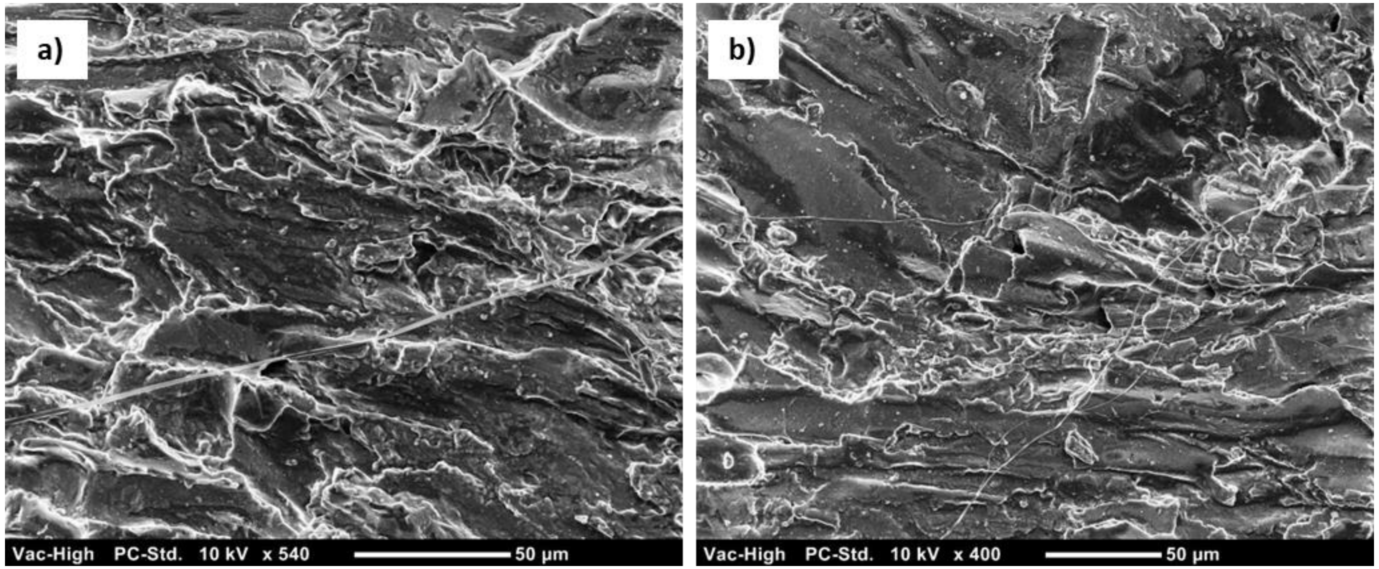
Images of Plaster residue after analogues are removed

easycomposites™ clear tray

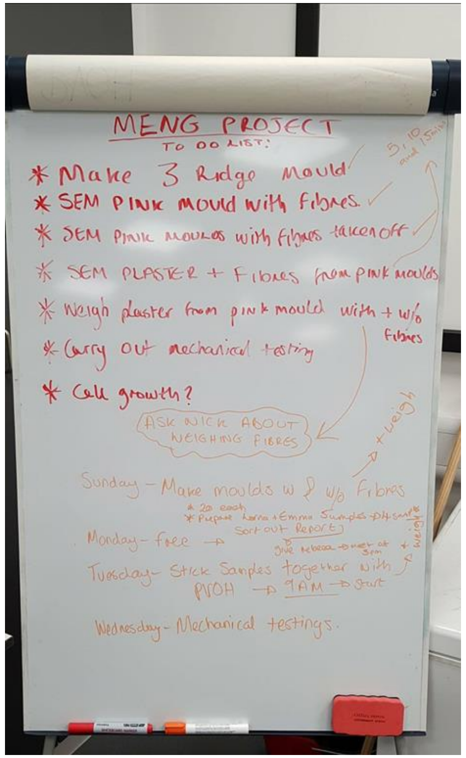


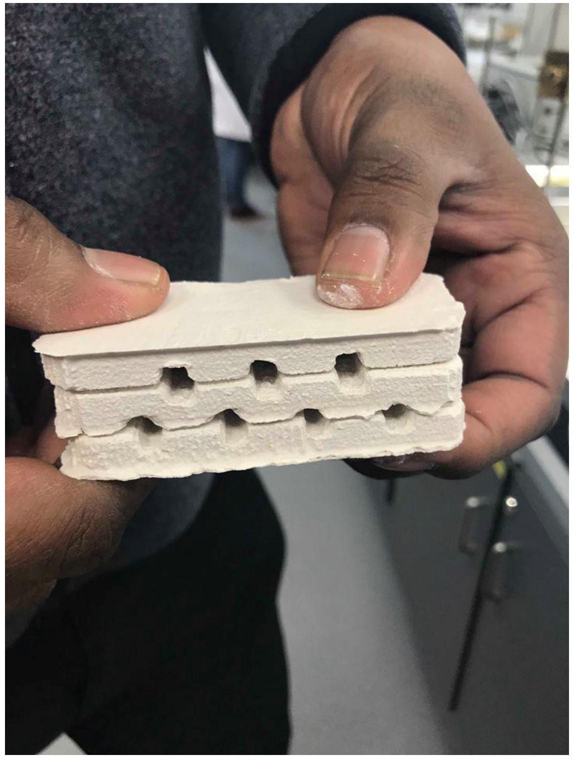
Polycraft™ pink tray

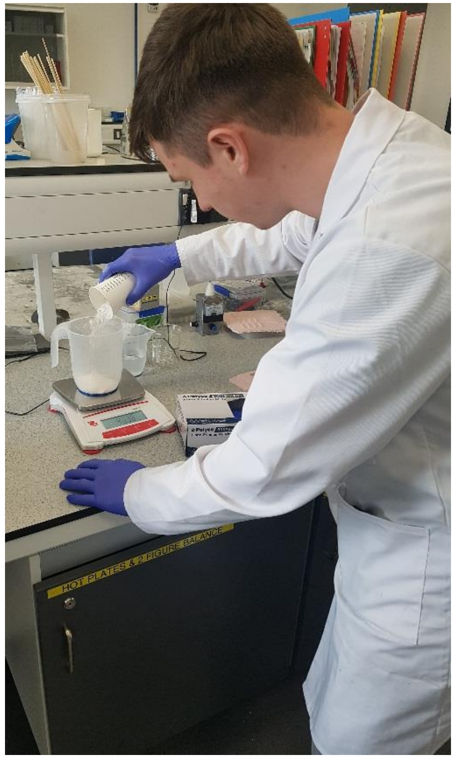


Conclusions drawn from Refined Testing

- Cellulose Nitrate to be spun directly onto tray for a duration of 10 minutes
- Pink matte tray to be used for experimentation, as it produced well defined analogues, and greater fibre incorporation
- Adhesive between each layer to be PVOH (approved by School of Pharmacy consultation)







Scaffold production – Method of fibre incorporation

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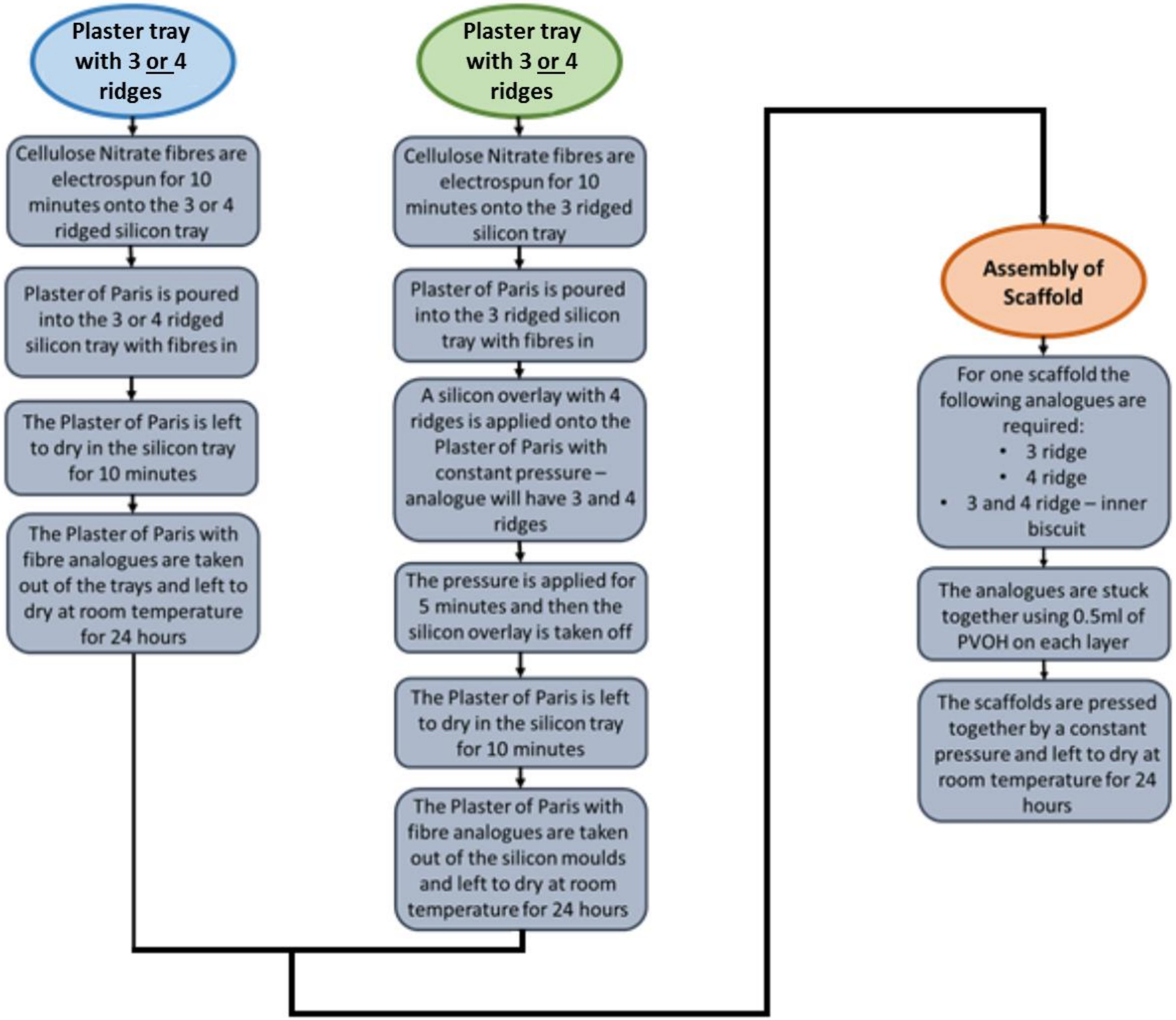
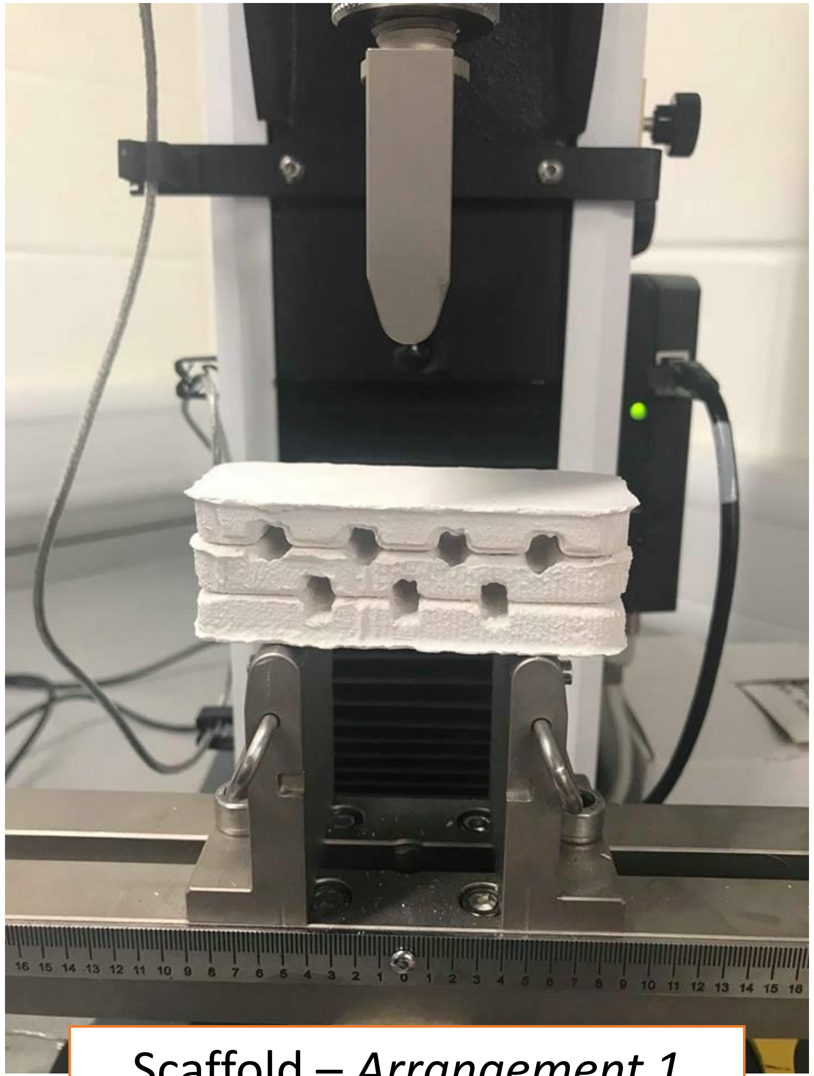


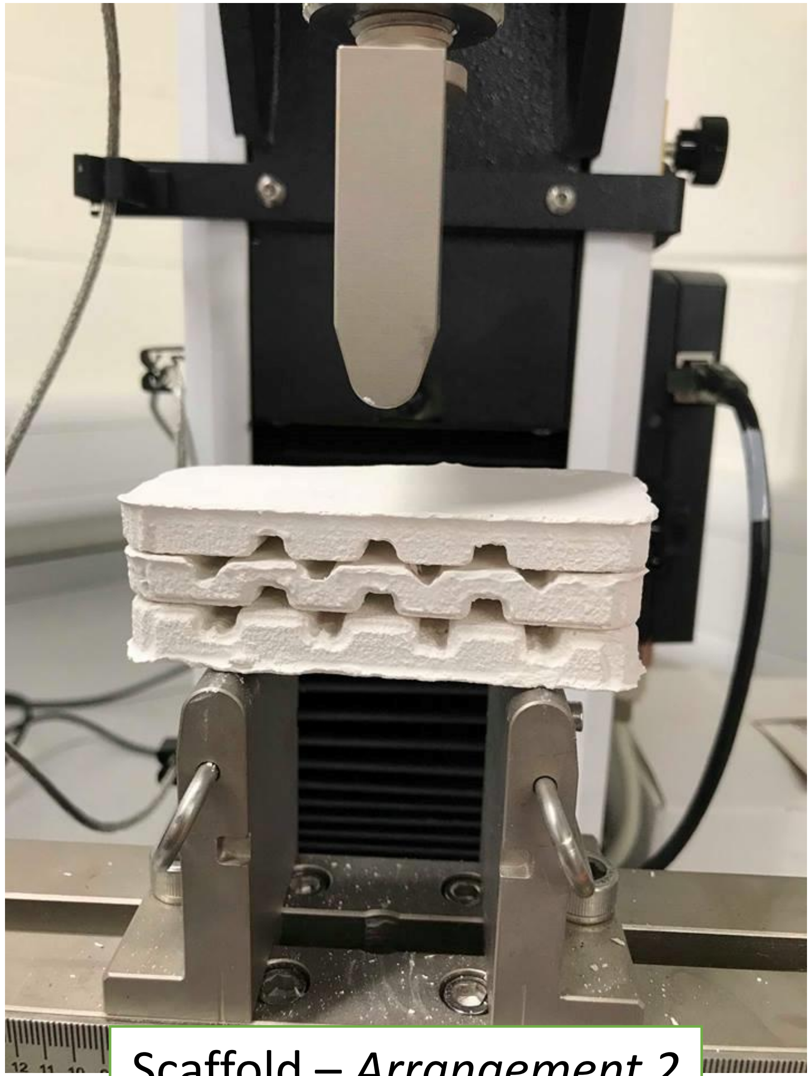
Scaffolds assembled, pressed & dried

Scaffold testing – Analogue layer arrangement



Scaffold – *Arrangement 1*

Average Breaking Load: 110.13*N*



Scaffold – *Arrangement 2*

Average Breaking Load: 97.55*N*

Scaffold testing – With Fibres & Without Fibres

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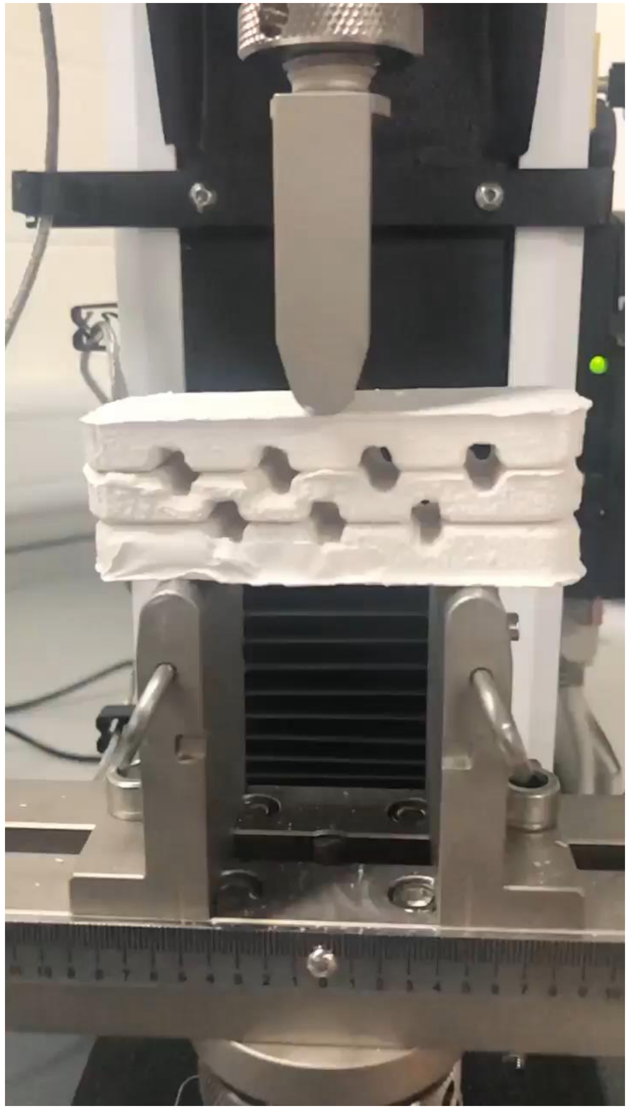
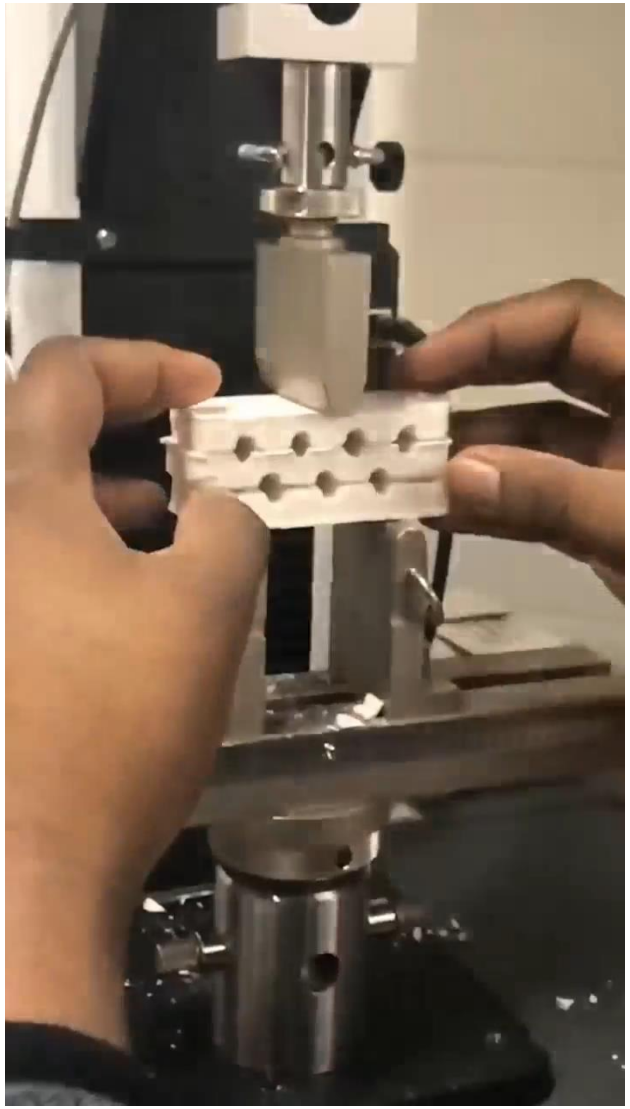
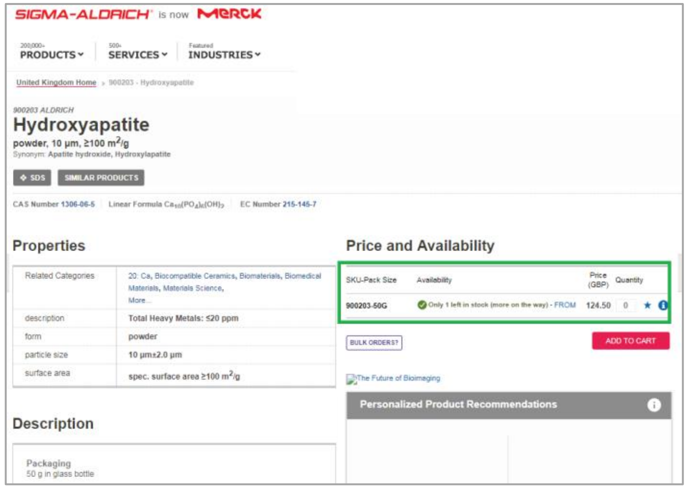




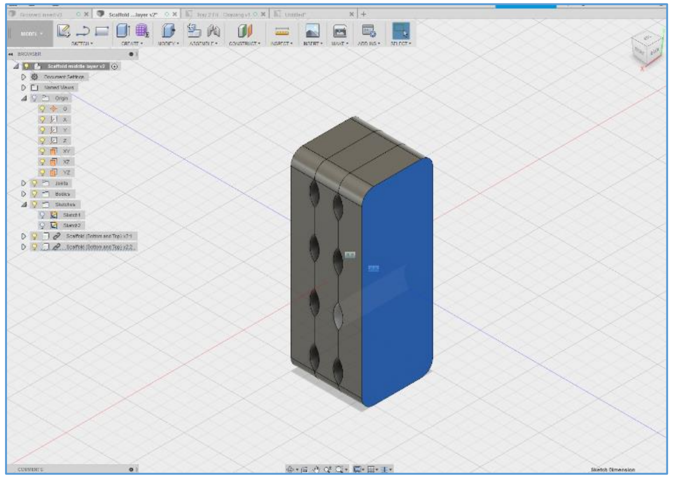
Average Breaking Load (N)	
With Fibres	Without Fibres
439.90N	298.60N

32% increase in breaking load required for Fibre reinforced Scaffolds

Recommendations and Next Steps



Expand range of 'bone material'



Bespoke geometries for particular purposes

Test wider sample size to eliminate further anomalous results

Utilise PVOH as a reinforcement – alignment and biodegradability

Examination of cell growth properties along Scaffold grooves

Thank-you for your time

We are happy to take any questions

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