

## Late breaking abstracts

### LBA1

#### Changing the fiber diameter “on the fly” with melt electrospinning writing

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**Aim:** Electrospinning of polymer melts produces a defined diameter fiber that is dependent on instrument parameters such as mass flow rate and voltage [1].

**Methods:** A custom-built melt electrospinning device was mounted above an x-y stage where an electrified thread of poly( $\epsilon$ -caprolactone) (PURAC PC-12) melt was direct written onto the metal (titanium) and glass collector. Using a collector gap of 6 mm and a temperature of 85 °C the melt was pushed to the 22G nozzle using between 0.1 and 5 bar of pressure. A standard voltage of 1.5 kV and 5 kV were applied to the nozzle and collector throughout the experiments.

**Results:** The air pressure could be varied between 0.2 and 2 bar to produce fibers with diameters of 5 and 33  $\mu\text{m}$  respectively. Importantly this was performed without changing any other parameter. The maximum time for the diameter to change from one pressure to the other was 2 minutes for the largest pressure differences. The point where the fiber transitions from sinusoidal to straight was coined the “critical translation speed” (CTS) and was measured by writing with a stabilized jet at sequentially lower speeds (Figure 1).

**Conclusions:** The melt flow rate to the nozzle has a significant impact on fiber diameter so the diameters can be altered during the direct writing process using air pressure. The CTS was measured and was found to be inversely proportional to the diameter of the fiber.

#### References:

- 1) Brown TD Dalton PD Hutmacher DW. (2011) Direct Writing by Way of Melt Electrospinning. *Adv Mater* 23 5651-57.

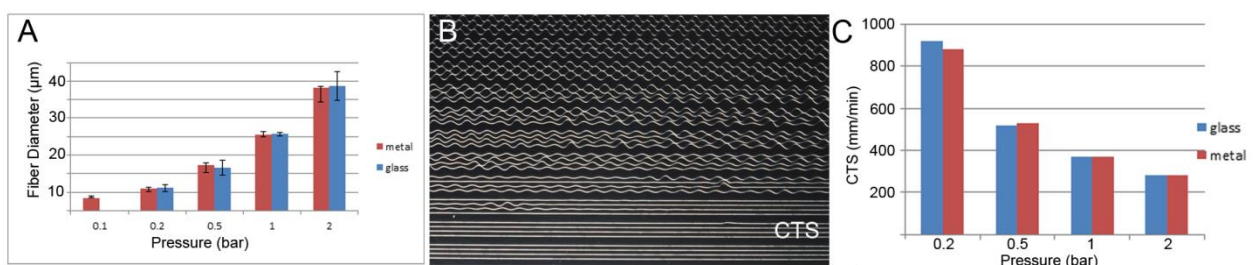


Figure 1: A) Effect of air pressure on fiber diameter; B) example of a CTS measurement with the line where CTS is reached indicated; C) Effect of pressure on the measured CTS.

LBA2

### Cell photoencapsulation 2.0 - characterization and comparison of next generation water-soluble photoinitiators

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**Aim:** Cell encapsulation plays a central role in many biofabrication methods. Photopolymerizable hydrogels provide additional advantage of temporal and spatial control of encapsulation process. Such characteristics of a photoinitiator (PI) as its water-solubility cytocompatibility spectral sensitivity and initiation efficiency are vital to its performance and suitability for a particular application. The aim of this study was to identify characterize and compare a new generation of water-soluble highly reactive and biocompatible visible light photoinitiators (PIs).

**Methods:** The monomer 4-acryloylmorpholine (NAM) and the PI / coinitiator Quantacure BPQ (Q-BPQ) methyldiethanolamine (MDEA) Irgacure 2959 (I2959) and APi-180 are commercially available (TCI Aldrich 3B Scientific Ciba and Shenzhen UV-ChemTech respectively) and were used in the highest purities available. Na-TPO and Li-TPO were synthesized according to literature. Na-BAPO and Li-BAPO were received from BASF where both were synthesized according to published procedures. Absorption properties solubility storage stability cytotoxicity reactivity and efficiency in double bond conversion (DBC) of these compounds were evaluated.

**Results:** The solubility is one of the most important characteristics of a PI for use in water based formulations. While the state of the art PI I2959 and its modified version APi-180 suffer from very low solubility they are substantially outperformed by the new generation MAPO and BAPO PIs. All compounds showed adequate storage stability in neutral basic and acidic solvents. Viability of cells exposed to different concentrations of PIs was shown to be in a similar range as I2959 widely used for cell encapsulation. However absorption characteristics and reactivity of novel PIs around 320-500 nm are superior. This difference in efficiency would potentially allow to use lower PI concentrations for cell encapsulation experiments.

**Conclusions:** In this contribution we have shown that the MAPO and BAPO salts as next generation water-borne photoinitiators not only show storage stability and biocompatibility in the same range as state of the art photoinitiators but outperform them by a large margin in solubility and reactivity. Furthermore it is possible to use the MAPO and BAPO salts as visible light initiators which is a considerable advantage for applications involving living cells. A practical example of encapsulation of MC3T3 preosteoblast cells using I2959 and Li-TPO with 365nm source confirms these conclusions.

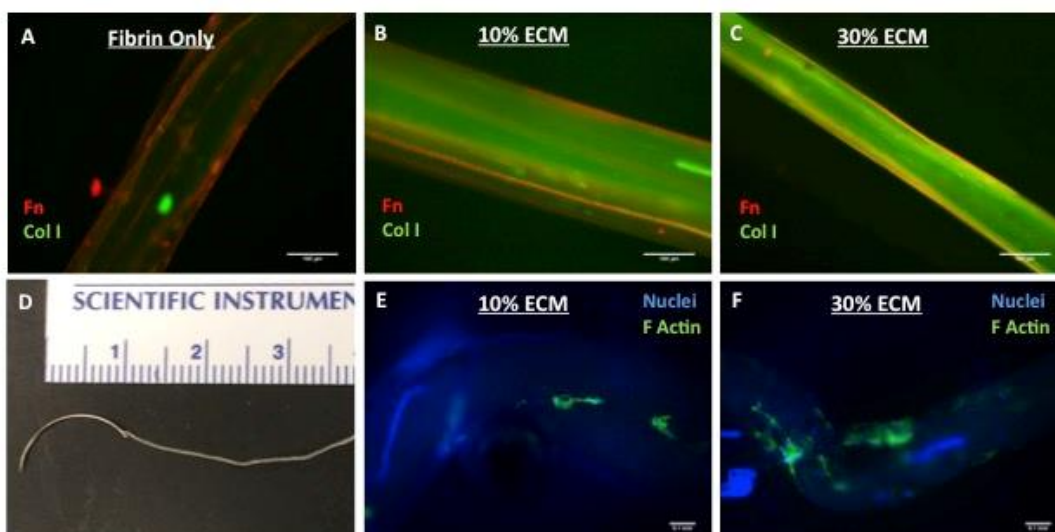


**LBA3**

**Native decellularized cardiac ECM incorporated into fibrin microthreads to provide in vivo-like microenvironment for stem cell adhesion**

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Cardiovascular disease (CVD) is the leading cause of death worldwide accounting for more than 30% of global deaths. One of the most prevalent forms of CVD is myocardial infarction (MI) which results in the formation of non-functional scar within the myocardium. Stem cell therapy has shown promise in regaining the functionality of the scar but there is an issue of low cellular delivery efficiency into the injured myocardium. Previously our group has demonstrated the capability to create fibrin microthread sutures in order to improve upon this engraftment rate. Looking to further improve upon this microthread platform we have begun to investigate the incorporation of native decellularized cardiac extracellular matrix (ECM) into the fibrin microthreads. The native ECM provides complex in vivo-like binding sites for stem cells which has been shown to elicit unique cellular responses. Implantation of complex ECM has also improved vascularization and functionality post-MI. Incorporation of native ECM should allow for more bioactive microthreads which may illicit improved regenerative responses in vivo. ECM was obtained by decellularizing adult rat hearts via treatment with sodium dodecyl sulfate and then solubilization via a pepsin digestion in order to acquire the native ECM. ECM was successfully incorporated into the fibrin microthreads by mixing different concentrations (0 10 30%) of ECM prior to the co-extrusion of fibrinogen and thrombin into fibrin. ECM incorporation was verified through immunohistochemical staining for collagen I and fibronectin (Figure A-C). Within this initial study we found that native ECM can successfully be incorporated into our fibrin microthreads ECM-fibrin microthreads can be made into sutures (Figure D) and that these new ECM-fibrin sutures support cellular adhesion (Figure E F). Future work will be performed to measure the effects of ECM-fibrin microthreads over our fibrin only microthreads. ECM-fibrin microthreads offer a potential bioactive stem cell delivery platform for treatment of infarcted myocardium.



#### LBA4

##### Cell encapsulation by microfabrication with hyaluronic acid based hydrogel precursors

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**Aim:** It was of interest to provide a new type of hydrogel precursors on the basis of hyaluronic acid (HA). The combination of biocompatible macromeres and additive manufacturing technologies (AMT) is a promising approach for the encapsulation of cells. However it is obligatory to have a good understanding of the structure-properties relationships. The aim of the study was to gain this knowledge by a systematic exploration of the material properties.

**Methods:** HA vinyl esters (HA-VE) were synthesized from HA with different m.w. by the transesterification with divinyl adipate. Varied reaction times led to different degrees of substitution (DS). Hydrogels were formulated with varied macromere contents and examined by photorheology. Furthermore the influence of the addition of dithiothreitol (DTT) as chain transfer agent (CTA) to modify macromolecular architecture was investigated. Besides photorheological data the swellability of hydrogels was determined as complementary material data set. Cell compatibility of macromeres was assessed by assays and cells were encapsulated by two-photon polymerization (2PP) based microfabrication. The viability of the encapsulated cells was assessed by LIVE/DEAD staining.

**Results:** The systematic variation of the synthesis/formulation parameter of HA-VE based hydrogels led to conclusive changes of the material properties which can be explained by the modification of the network architecture. An important network parameter is the mesh size ( $L$ ).  $L$  decreased with increasing DS macromere size and -content. Photokinetic parameters (e.g. gel point) and swellability followed the same trend. Formulations with DTT had an optimum at 80 mol%. Macromeres have a moderate to good cytocompatibility. While the influence of DS can be considered as negligible the macromere size was found to have an impact on the cell viability. An exemplary formulation was used for the encapsulation of cells by 2PP microfabrication. The viability of encapsulated cells after 24 and 96 h was confirmed by LIVE/DEAD assay.

**Conclusions:** It was shown that biocompatible HA-VE hydrogels with a wide range of properties are accessible by the careful combination of precursors with suitable macromere size and DS water soluble biocompatible photoinitiators and CTAs. The successful encapsulation of cells proofed the potential of the material system as candidate for the application in regenerative medicine.

**Acknowledgement:** We kindly acknowledge the financial support by the Austrian research funding association (FFG project number 849787) and the European Research Council (Starting Grant-307701 A.O.).



## LBA5

### Cell density determines the amount of stress development in 2D engineered tissues

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Many cell types exert contractile stresses on their surroundings via actin stress-fibers. The magnitude and direction of stress are determined by stress-fiber content and organization which are influenced by the cellular environment e.g. stiffness anisotropy and strain. Understanding and controlling these relationships is important for tissue engineering in order to obtain mechanically functioning tissues with a proper organization. It is unclear however whether the stress development in engineered tissues depends on the cell density of the tissue. Therefore the aim of this study was to determine whether there is a correlation between the cell density and stress development in tissue engineered constructs.

The thin film method [1] was used to determine the stress developed in monolayers of vascular derived human myofibroblasts. Briefly 10  $\mu\text{m}$  wide fibronectin lines were micro-contact printed on thin film constructs. Myofibroblasts were cultured on the constructs for two days after which cell nuclei were stained with Hoechst. After staining films were cut from the constructs in the fibronectin (and thus cell) direction (fig. a). Due to cell contractility the films will curve the curvature is used to calculate the amount of cell stress (fig. b). Afterward cell density was determined by counting the nuclei in tile scans of each individual Hoechst stained film (n=22).

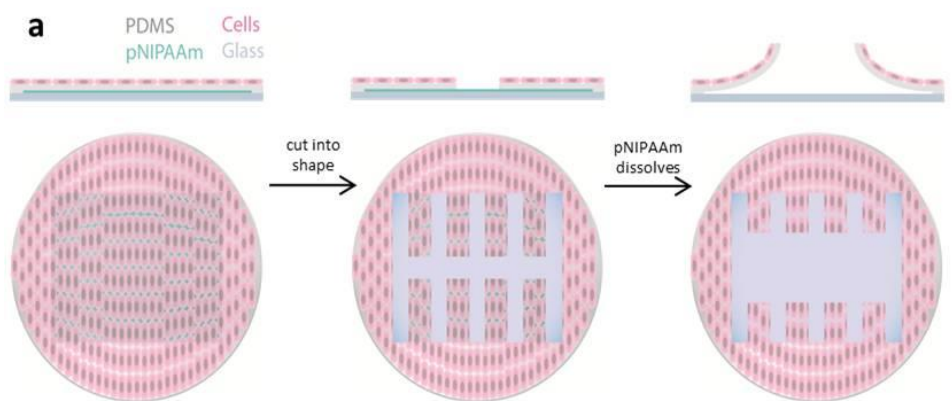
Pearson's correlation coefficient (0.58) showed a clear correlation ( $p=0.004$ ) between cell density and stress development (fig. c) indicating that cells act as individuals and stress developed in engineered constructs therefore depends on the cell density.

As these results are only representative for isotropic tissues like engineered muscle future studies will investigate the same dependency for monolayers with more disperse cell orientations representative for e.g. tissue engineered heart valves.

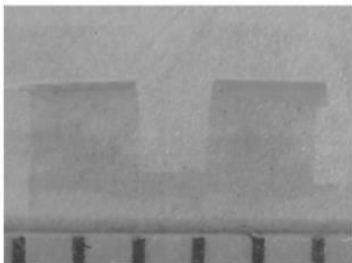
This work was supported by a grant from the Dutch government to the Netherlands Institute for Regenerative Medicine (NIRM grant No. FES0908)

[1] Grosberg 2011 Lab. Chip.

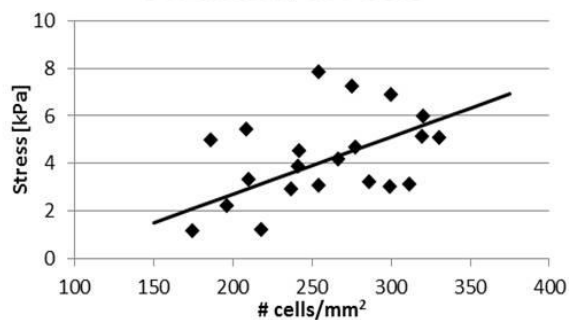




**b**



**c** Cell density vs stress



*a) overview of the thin film method, b) picture of two curved films, and c) correlation between cell density and cell stress.*



## LBA6

### Indirect rapid prototyping as an elegant tool for the production of self-supporting low density scaffolds

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**Aim:** The present work pursues the production of porous low density (<10w/v%) gelatin scaffolds. To this end methacrylated gelatin (gelMOD) was applied as an extracellular matrix mimicking component. Since low concentration gelMOD solutions can't be processed using conventional rapid prototyping (RP) techniques in the absence of additives an indirect RP approach was selected using sacrificial PLLA scaffolds. The scaffolds serve a dual role: hydrogel support while crosslinking and design transfer from scaffold to hydrogel.

**Methods:** Gelatin B was modified with methacrylic anhydride to obtain UV-crosslinkable derivatives (DS 60% & DS 97%). Physical gelation properties were assessed via differential scanning calorimetry (DSC). Crosslinking was performed using 2mol% Irgacure 2959 and UVA light (365nm). Mechanical properties during crosslinking were monitored via rheology. Transparent (365nm) PLLA scaffolds were obtained via FDM (Ultimaker). Post curing the PLLA scaffolds were dissolved using chloroform (15ml 3days) and washed with acetone and water. The obtained gelatin scaffolds were characterized using SEM  $\mu$ -CT optical microscopy and texturometry. Both 5 and 10w/v% scaffolds were successfully produced. Preliminary biocompatibility tests using fibroblasts were performed. (vital staining histology)

**Results:** DSC measurements revealed that physical gelation properties of gelMOD depend on degree of methacrylation and concentration. Previous work indicated that physical gelation of 10w/v% solutions is sufficient to produce 3D scaffolds therefore the resulting mechanical properties were set as a benchmark in the current study. Rheology indicated that cross-linked 5w/v% gelMOD (DS: 97%) exhibits sufficient mechanical properties ( $G' > 1.5\text{kPa}$ ) for transfer to 3D. Furthermore proper transfer of design from scaffold to hydrogel took place as strut sizes of the final construct matched the pore sizes of the PLLA scaffolds. Moreover the approach exhibits the additional benefit that selective scaffold dissolution results in inherently sterile constructs. Preliminary biocompatibility studies revealed cell-interactive and biocompatible behavior of the developed constructs.

**Conclusions:** Physico-chemical testing revealed the scaffold properties (mechanical degradation swelling) to depend on the applied gelatin concentration and the methacrylamide content. Structural and biological analysis indicated the success of the indirect RP approach.

#### *References & Acknowledgement:*

FWO Belgium is acknowledged for financial support.  
1Van Hoorick et al. J Mater Sci: Mater Med (2016)



**LBA7**

**Crosslinked poly( $\epsilon$ -caprolactone) non-woven mat with shape memory properties by a facile combination of electrospinning and sol-gel reaction**

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Shape Memory Polymers are a class of materials able to change their shape in a predefined way from a temporary shape to a permanent one when exposed to an external stimulus such as a temperature change. This peculiar behavior is strictly related to the chemical structure of the polymer that must display both soft chain segments (molecular switchers) and rigid/permanent net-points. The development of shape memory electrospun mats may lead to the realization of smart devices miniaturized structured on a micro/nanoscale of sure interest in the fields of minimally invasive surgery (as easy-to-deliver self-expandable prostheses and drug delivery devices) tissue engineering (as scaffolds capable to orient cells morphology) and sensors/actuators (as tunable porosity membranes). The aim of this work was to develop starting from a previously synthesized  $\alpha$ - $\omega$  triethoxysilane-terminated poly( $\epsilon$ -caprolactone) an electrospun mat characterized by a shape memory behavior. In order to obtain a solution suitable to be processed through the electrospinning technique an increase of the molecular weight of the starting polymer was needed. To this aim a sol-gel reaction that partially crosslinks the macromolecular chains was performed preliminarily to the electrospinning process by exploiting the reactive chain terminals. The crosslinking degree of the polymer was then largely increased after the electrospinning process thanks to an optimized post-crosslinking treatment in acid environment which allowed to increase the mat gel content from 33% to 88%. The shape memory behavior was evaluated by applying a properly designed thermo-mechanical cycle. The results showed that the obtained post-crosslinked electrospun mats had excellent one-way shape memory capabilities being able to fix temporary deformations up to 100% and to recover 100% of it when heated slightly above the melting temperature. Interestingly it was demonstrated (by means of scanning electron microscopy) that mats fibrous morphology was maintained after the application of the thermomechanical cycle.  
Funding: The Italian Ministry of University and Research.





## LBA8

### Ultraporous interweaving electrospun microfibers

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**Aim:** Production of one dimensional nano-materials with secondary morphology exhibiting unique functions is challenging. Here we report that a nanoscale immiscible polymer blend solution electrojet can assemble into ultraporous interweaving microfibers. We further investigated their influence on cell infiltration and colonization.

**Methods:** The polymer solutions were prepared by dissolving PCL (Mw 70 000–90 000 Aldrich) and PEO (Mw 900 000 Aldrich) in DCM/DMF (3:2) at room temperature and the homogeneous solutions were used for electrospinning under the following conditions: applied voltage 18 kV feeding rate 1mL h<sup>-1</sup> and distance between the tip of the needle and collector 12 cm. The morphology crystallinity surface chemistry and wettability of these fibers were studied by scanning electron microscopy (SEM) transmission electron microscopy (TEM) atomic force microscopy (AFM) X-ray diffraction (XRD) X-ray photoelectron spectrometry (XPS) and contact angle (CA) measurements. The NIH3T3 cell viability proliferation and differentiation was measured by LDH MTS. qPCR assay cell attachment and infiltration were assessed by confocal microscopy.

**Results:** Multi-lamellar cylindrical structure was originated from a blend of PCL and PEO in DCM/DMF mixed solution when the ratio between each component reached a threshold and where the electrospinning parameters were delicate controlled. The interplay of the two semi-crystalline polymers and the pair of solvents/non-solvents with the electrospinning processing parameters was found to be critical for the formation of the unique structure.[1] (Fig 1)

The hydrophilic hierarchically porous fibers were applied in culturing fibroblasts and studied the cell infiltration and colonization. Compared to the tight-packed hydrophobic PCL scaffold the hydrophilic micro-porous fibers enhanced the cell infiltration and colonization significantly. Moreover the unique nano-topographical environment that may stimulate cells in a drastically different manner from that of traditional solid smooth electrospun fibers which holds great potential in cardiac tissue engineering.[2]

#### *Acknowledgements:*

This work was supported by the Danish Council for Strategic Research Aarhus University Research Foundation and Carlsberg foundation.

#### *References:*

[1] Li Y; Gregersen H; Nygaard J; Cheng W; Huang Y; Dong M; Besenbacher F; Chen M\*. *Nanoscale* 2015 7 14989 – 14995

[2] Li Y; Rubert M; Yu Y; Dong M; Besenbacher F; Chen M\*. *Nanoscale* 2014 6 3392-402 highlighted in *Global Medical Discovery* 2014 <https://globalmedicaldiscovery.com/key-nanotechnology-articles/ultraporous-interweaving-electrospun-microfibers-from-pcl-peo-binary-blends-and-their-inflammatory-responses/>



**LBA9**

**Novel biodegradable textile for cellular scaffolds based on surface-treated hydrophobized hyaluronic acid**

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The presented work describes a fabrication of a novel biodegradable textile scaffold based on partially hydrophobized hyaluronic acid with a further chemical and physical modifications. Cellular scaffold is based on monofilament fibers prepared within a novel and patented wet spinning technology [1]. The raw fibers based on hydrophobized hyaluronic acid behave rather as a cell-antiadhesive material which is given by two main factors: 1. quasi-non-polar surface character that is incompatible with the polar cellular surface and 2. Fiber-surface smoothness that is generally assumed to be a relevant parameter lowering the cell adhesion efficacy [2]. The cell adhesion is therefore supported by the adsorption and fixation of a cell attractant (fibronectin or fibrinogen). The base material of a partially hydrophobized hyaluronic acid has been chosen due to its special properties comprising mechanical stability within the cultivation medium and on the other hand its biodegradability within 2 months that showed to be potentially variable by the changes of material porosity given by freeze-drying of the textile under various conditions. One of the important goals was the fixation of the cell attractant adsorbed to the fiber surface in order to be stable enough to overcome further cultivation processes. The main role plays the roughness of the fiber surface and its partial porosity that induces a partial swellability of the fiber in the cell-attractant solution. The material swelling supports the incorporation and the entanglement of the cell attractant to the surface layers of the fiber. Pores that are filled by the attractant are further partially closed during the drying that finally stabilizes the complex adhesive layer. The efficacy of the adsorption is evaluated by a specific fluorescence staining of the attractant and further visualized by confocal microscopy. The cell adhesion was further evaluated with use of NHDF cells and by the ATP luminiscence measurements showing a progressive cell proliferation within the 7 days of cultivation

References:

[1] Scudlova J. Betak J. Wolfova L. et. al.: Fibres based on hydrophobized derivatives of hyaluronan method of their preparation and use textiles on base thereof and use thereof. Patent 2014 WO2014082611 A8

[2] Gerberich B. G. Bhatia S. K.: Tissue scaffold surface patterning for clinical applications Biotechnology Journal Special issue: Methods and Advances Vol. 8 Issue 1 73-84 2013.



**LBA10**

**The evaluation of the Poly( $\epsilon$ -CaproLactone) degradation kinetics in an accelerated environment for bone fixation application.**

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Regenerative medicine plays a key role in the development of a healthy age strategy. In the case of bone fracture there is a need for novel strategies. This paper states the major clinical pathways to treat bone fractures the current bone fixation implants and the critical development of the next generation implants in the use of biodegradable polyesters. The degradation process of the biocompatible biodegradable Poly( $\epsilon$ -CaproLactone) (PCL) block units fabricated by an extrusion based technology was investigated in a NaOH aqueous medium in a period of 25 days. The effect of different process parameters for fabrication routes on the degradation kinetics was assessed throughout the observation of the units weight loss percentage. Design of experiments was used to establish control and predict the correlation between different process parameters and its effect on the degradation of the investigated polymer. The study of the process parameters statistical analysis accomplished that the degradation mechanism of PCL non-porous structures fabricated by extrusion additive manufacturing is controllable and predictable. The results show that the increase of temperature deposition velocity and screw rotational velocity will lead to increase the resistance of the materials decreasing the degradation rate and vice versa. In addition results expressed negligible weight loss percentages for the degradation of less than 1% of the total weight. This means that the biodegradable PCL have a proper preservation of its weight loss and its shape morphology during the 25 days hence the biodegradable polyester showed high expectation for surviving in a bone healing environment.



## LBA11

### A novel micro co-extrusion system for biofabrication

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**Aim:** Currently there are already available on the market several devices of different categories which allows the manufacture of three-dimensional structures for biomedical applications (scaffolds). However applying only a single manufacturing technology there are large-scale restrictions within the construction of those structures. Thus the present study aimed to develop a novel micro co-extrusion coupled with an electrospinning system that would enable the development of enhanced hybrid scaffolds.

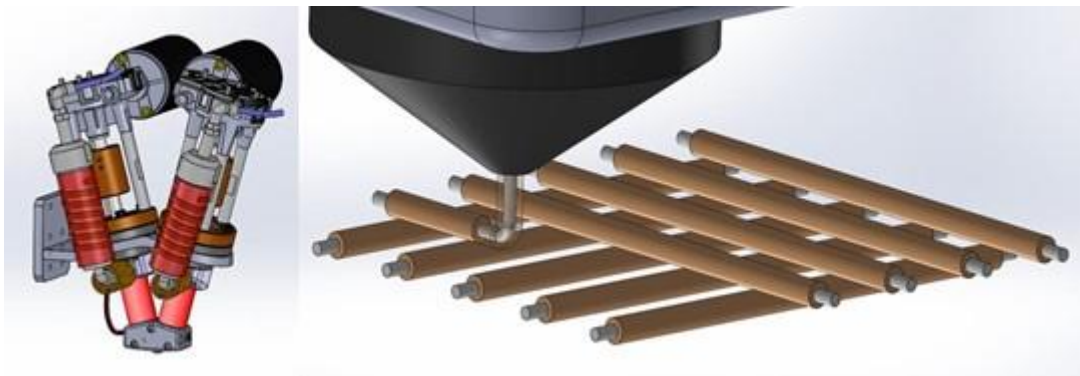
**Methods:** A micro co-extrusion system was developed with 2 reservoirs with a volume of 8.5 cm<sup>3</sup> each a temperature range till 300°C a pressure range of 0-12bar and working with nozzles from 200 till 500µm (cf. Figure 1). An electrospinning system was added through a moving platform. To examine the scaffolds construction different biomaterials were processed.

**Results:** The novel co-extrusion system was able to develop proper structures: the morphological analysis showed high reliability (n=5) between the theoretical and obtained filament and pore size (350µm and 300µm vs. 342±4µm and 302±3µm respectively); extruding two materials/different mixtures another filament was built in a regular and uniform way; adding the electrospinning system hybrid scaffolds with micro and nano fibres were produced.

**Conclusions:** The complexity of human tissue requires the production of differentiated structures both in terms of architecture geometric orientation and material composition as well as scale dimensions. The developed system provided a high controllability in terms of architecture porosity and pore interconnectivity among the structures produced corroborated by a high reliability (ICC ranging from 0.91 to 0.96) among structures. This novel system showed promising results to enhance scaffold properties both creating multi material filaments (e.g. to control the proliferation rate of the inner side material) and/or hybrid scaffolds combining micro and nano fibres.

#### *Acknowledgements:*

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*Micro co-extrusion system*

## LBA12

### Using biobots for standardizing the 3D bioprinting of hydrogel bio-inks

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**Aim:** Bioprinting technologies have emerged as valuable tools for tissue engineering approaches that aim to fabricate small tissue constructs for drug screening applications all the way up to full scale tissues and organs that can replace or repair damaged diseased or lost tissues in the body. Building three dimensional (3D) tissues using bioprinting is essential to move towards these goals because it allows for rapid reproducible and automated fabrication of tissues<sup>1</sup>. Bioprinting is attempting to revolutionize tissue fabrication but it is limited by the lack of commercially available easy to use and affordable bioprinters with standardized bio-inks. We have recently developed a novel and commercially available 3D bioprinter called the BioBot for tissue engineering and biofabrication applications that is easy to use economically friendly and has a small footprint. Here we demonstrate 3D bioprinting capabilities with our first commercially available bio-ink gelatin methacrylate (GelMA).

**Methods:** HepG2 cells (ATCC) were cultured in monolayers at 37 °C and 5% of CO<sub>2</sub> until bioprinting. GelMA was fabricated based on methods of previous studies<sup>2 3</sup>. For bioprinting constructs with and without encapsulated cells a 10% (w/v) aqueous solution of GelMA containing 0.5% (w/v) lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) was used. Printed constructs were crosslinked with blue light (405 nm). To qualitatively and quantitatively assess cell viability of printed constructs tissues were stained using a LIVE/DEAD kit (Life Technologies) or analyzed using a PrestoBlue viability assay (Life Technologies) according to the manufacturer's protocol.

**Results:** Using the BioBot and 10% cell-laden GelMA results demonstrated 100 µm print resolution and the ability to fabricate complex structures with controlled pore size. Quantitative results show that the bioprinting process does not adversely affect cell viability when normalized to non-printed constructs. Further human bioprinted tissues had high cell viabilities at the 24 hr and 1 wk time points as depicted using LIVE/DEAD viability/cytotoxicity stains.

**Conclusions:** The results presented confirm that the commercially available BioBot can achieve the resolutions geometries and post-print tissue viabilities comparable to larger and more expensive commercial and 'in-house' 3D bioprinters. This work is significant because it moves towards the standardization of 3D bioprinting and bio-inks which enables future innovation and progress in 3D bioprinting.

**Funding:** We would like to thank Ben Franklin Technology Partners Dreamit Ventures Microventures FundersClub and Angles for funding and support.

#### *References:*

1. Skardal A. Acta Biomaterialia. 2015. In Press.
2. Nichol J.W. Biomaterials. 31 5536 2010.
3. Kolesky D.B. Advanced Materials. 26 3124 2014.



**LBA 13**

**Fabrication of Poly(2-Oxazoline) Hydrogels with Controlled Porosity by Indirect Printing of Melt Electrospun Templates**

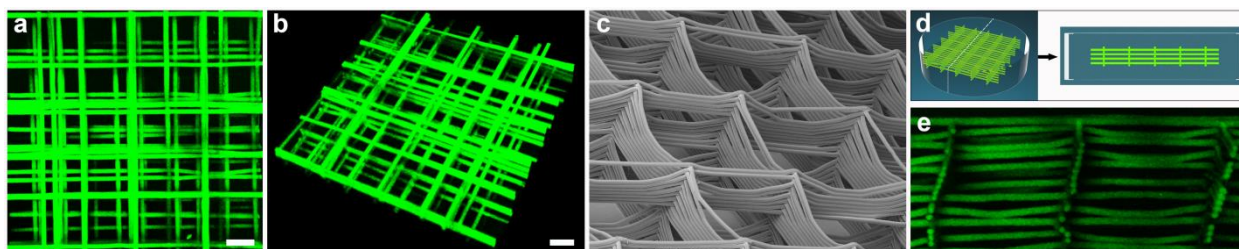
Jodie Haigh, Ya-Mi Chuang, Brooke Farrugia, Richard Hoogenboom, Paul Dalton, Tim Dargaville  
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**Aim:** Fabrication of poly(2-oxazoline) (PAOx) hydrogels with aligned pores from templates produced using melt electrospinning writing (MEW)

**Methods:** Sacrificial polycaprolactone (PCL) templates were produced using MEW and embedded within PAOx hydrogels. Hydrogel crosslinking was completed using UV-initiated photochemistry, using 365 nm light and Irgacure 2959 as photoinitiator and dithiothreitol as crosslinker. Dissolution of PCL templates was completed to obtain porous hydrogels.

**Results:** The MEW PCL sacrificial templates were successfully employed to engineer the microarchitecture of PAOx hydrogels with PCL templates directly influencing the resulting hydrogel pore architecture, as shown in Figure 1, including confocal laser scanning microscopy (CLSM) and scanning electron microscope (SEM) images.

**Conclusions:** The use of MEW sacrificial templates provides a biofabrication technique to produce highly-defined hierarchically-structured within hydrogels. MEW will lead to possibilities of even more detailed porous designs in the near future.



*Figure 1. CLSM images of PAOx hydrogels top-down view (a), and angled view (b). SEM of PCL template (c). Representation of hydrogel cross-section (d) used obtain CLSM image of cross-section (e). Scale bar = 100  $\mu$ m.*

## LBA14

### 3D printing of bioactive glass scaffolds

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Among the several materials used in tissue engineering (TE) bioactive glasses appear as very promising due to its unique characteristic of developing a specific biological and chemical response at their surfaces resulting in a strong bond with the bone tissue [1]. 3D printing is an additive manufacturing technique to produce grafts for TE, either dense or porous, as well as products for drug delivery purposes. [2-3].

The aim of the present work was the fabrication of bioactive glass scaffolds by 3D printing.

Calcium-phosphate glasses were prepared using a melt-quenching method and the obtained glass frit was milled and sieved into different fractions to obtain a morphology and particle size distribution suitable to be applied in 3D printing. Based on a previous characterization of commercial binders, different organic and inorganic binders were prepared and tested.

For the characterization of the formulations, techniques such as differential scanning calorimetry X-ray diffraction, coulter counter technique, scanning electron microscopy, and Fourier transform infrared spectroscopy were used. The printed models were sintered and submitted to mechanical tests and to in vitro mineralization assays. The new powder-binder formulations exhibited characteristics analogous to those of the commercial materials. Besides the morphology the bioactive glass particles presented a bimodal particle size distribution similar to the one of the commercial powders with a largest volume percentage of particles in the range 20-100  $\mu\text{m}$ . The hygroscopic character of the bioactive glass, due the presence of P2O5 contributed for the efficient binding of the particles and the production of resistant green printed models.

In vitro tests carried out by soaking the artefacts in synthetic plasma showed its potential bioactivity confirmed by the precipitation of a Ca-P rich layer on the surface of the printed glass models after 14 days.

The results show that it is possible to produce bioactive glass scaffolds by 3D printing. Further improvement of the mechanical resistance of the sintered models can be achieved by controlled heat treatment of the glass powders to produce glass ceramics.

[1] L. Hench. Journal of Mat. Sci.: Materials in Medicine 17 (2006) 967-978.

[2] S. Bose et al, Materials Today 16 (2013) 496-504.

[3] D.W. Hutmacher et al, Trends in Biotechnology 22 (2004) 354-362.

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