Free paper session 4: New Technologies

F4.1

Additive manufacturing of scaffolds with sub-micron filaments via melt electrospinning writing

Gernot Hochleitner, Tomasz Jüngst, Toby David Brown, Kathrin Hahn, Claus Moseke, Franz Jakob, Paul Donald Dalton, Jürgen Groll
Department for Functional Materials in Medicine and Dentistry University of Würzburg, Würzburg, Germany

Aim: We optimized the process parameters of Melt Electrospinning Writing (MEW) to produce highly ordered structures composed of sub-micron filaments for Tissue Engineering (TE) purposes. [1]
Methods: MEW has recently been introduced as a novel electrohydrodynamically assisted additive manufacturing (AM) process that allows a controlled deposition of electrically non-conductive fibers of particular biocompatible and biodegradable thermoplastic polymers [2 3]. To direct write thin and homogeneous filaments from poly(epsilon-caprolactone) (PCL) with our custom-made device we investigated and adjusted the process parameters including: spinning temperature ≤ 120 °C feeding pressure ≤ 4 bar nozzle diameter ≤ 22 G acceleration voltage ≤ 7 kV and nozzle/collector distance ≤ 7 mm. During processing a grounded collector plate was moved in planar directions under the nozzle by a computer-aided system in order to collect straight filaments. [1 3]
Results: While PCL is often used for AM in TE direct writing molten filaments with sub-micron dimensions has not yet been demonstrated. We found that MEW could break through this micron diameter barrier and allow the deposition of accurately stacked sub-micron filaments (817 ± 165 nm) to highly regular structures (100.6 ± 5.1 µm). Thus PCL fibers can be deposited with a diameter range of 800 nm to 40 µm. This adjustment of the specific surface allows a tailoring of the degradation time in combination with a highly porous and well defined structure which provides cell migration into the scaffold. First experiments for cell adhesion have been conducted and initial data will be included in the presentation. [1]
Conclusion: While other 3D printing methods such as fused deposition modeling allow a fabrication of fibers in a range of 100 µm microns and more MEW can be used to print even sub-micron filaments. In contrast to solution electrospinning drawing fibers from melts enables the processing more amenable to direct writing and stacking without often toxic solvents for TE approaches.

Acknowledgements:
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[1] G. Hochleitner Biofabrication 7 (2015) 035002
[2] G. Hochleitner Polymer 55 (2014) 5017-5023
[3] T. D. Brown Advanced Materials 23 (2011) 5651–5657

F4.2

Femtosecond versus picosecond pulses for laser-induced forward transfer of biomaterials

Raphaël Devillard, Stéphane Petit, Jean-Christophe Delagnes, Olivia Kérourédan, Florent Deloison, Eric Cormier
INSERM, Bordeaux, France

Aim: Laser induced forward transfer has been modified to transfer living cells or biomolecules. Laser assisted bioprinting (LAB) has emerged as an alternative to ink-jet bioprinting technique due to its resolution [1]. In parallel needle free (NF) injection systems have been developed to increase vaccination rate and to overcome conventional needles issues. LAB and NF injection are both based on the creation of bubbles near a free liquid-air interface by laser pulse illumination. The accurate control of the jet's characteristics is required in term of maximal height velocity and diameter. The benefit of picosecond and femtosecond laser sources compare to nanosecond sources is to induce optical breakdown and the creation of bubbles without absorbing sacrificial layer. We investigated the influence of the pulse duration on the jet creation dynamics.
Methods: A fiber chirp pulse amplification laser system emitting 400 fs pulses at 1030 nm has been used. The pulse duration can be continuously tuned from 400 fs to 20 ps by translating the imaging system of a lens-based near to zero-dispersion stretcher unit. A polarizer-based attenuator controled energy up to 14 µJ. The liquid sample was placed on a 2mm-thick transparent glass plate while the laser beam was focused through the glass plate close to the liquid surface by a vertical X20 microscope objective placed on a micrometer translation stage. We used time-resolved Schlieren imaging technics to characterize the jets. Images were recorded for given energies pulse durations and observation times and all data were then processed by a homemade image processing software extracting jet characteristics (speed height and full width half maximum).
Results: As initial speeds varied jet morphologies were different. 410 fs pulses produced medium height and fine jets with low initial speed. The 8 ps pulses induced higher and larger jet with increased velocity. The 14 ps pulses revealed even higher velocities with a constant jet width and an energy sensitive length.
Conclusions: The selection of specific pulse duration is critical for each biomedical application. For LAB application femtosecond sources inducing lower speed and lower acceleration could minimize cell damages during droplet landing with high resolution optimized by the production of fine jet. At the opposite needle free injection would benefit of high velocity and acceleration at high distance to optimize penetration and minimize dispersion with 14ps pulse duration.
[1] Devillard R et al. Methods Cell Biol. 2014;119: 159–74 (2014).

F4.3

Bioprinting of encapsulated pancreatic islets

Dirk Jan Cornelissen, John Casey, Aidan Courtney, Jason King, Wenmiao Shu
Heriot Watt University, Edinburgh, United Kingdom

Type 1 diabetes affects an estimated 320.000 persons in the United Kingdom costing the National Health Service roughly £1 billion (or €1.4 billion) per year. In this disease the patients' beta-cells in the pancreatic islets are incapable of producing enough insulin to maintain a normal blood glucose level. Pancreatic islet transplantation is an established treatment. However there are two major problems associated with the current method of transplantation: a majority of the islets are lost during transplantation due to immune reactions; and the transplantation is currently limited by the availability of donors.
Aim: By encapsulating islets in a hydrogel to shield them from the immune system islet survival post transplantation could be enhanced.[1] In this ongoing research a novel way of encapsulating islets is investigated. By using a bioprinting technique we are investigating the method to create encapsulated islets with a higher rate of reproducibility than standard air-aided encapsulation methods.
Methods: Islets (whole or dissociated) are encapsulated in an alginate hydrogel through electrospraying. Alginate has been chosen as the encapsulation hydrogel of choice since it is biocompatible easy to handle widely used and cheap. However most commercially available alginate is contaminated with foreign bodies which could set of the immune-system when transplanted. We are developing a novel purification process based on the work of Klöck et al.[2] to get rid of the impurities making the natural occurring materials suited for transplantation.
Results and Conclusion: Using electrospraying we can encapsulate islets in a fast and safe manner. With our shortened purification protocol we can lower protein levels in the alginate to 3mg/gram of alginate and endotoxin levels to .8 EU/gram of alginate. The cellular viability and function of encapsulated islets will be reported.

1. de Vos P. et al;.Advanced Drug Delivery Reviews 2014. 67–68(0): p. 15-34
2. Klöck G. et al.. Applied Microbiology and Biotechnology 1994. 40(5): p. 638-643

F4.4

Innovative biofabrication of 3D conductive scaffolds for cardiac tissue modelling

Carmelo De Maria, Francesca Montemurro, Michele Ibrahimi, Ilenia Guerrazzi, Giuseppe Criscenti, Federico Vozzi, Giovanni Vozzi
Research Center E. Piaggio - University of Pisa, Pisa, Italy

Aim: Cardiovascular disease is the most frequent cause of death in industrialized countries and heart failure is steadily increasing in the aging population with a 5-year mortality up to 75%. New cost-efficient treatments are urgently needed. The present work aims at fabricating using a new biofabrication approach a 3D scaffold which mimics the mechanical and electrical properties of the healthy and pathological heart tissue to be used as a benchmark for improving therapies.
Methods: The fabrication process consists into an innovative multistep and cyclic procedure combining chemical crosslinker deposition and UV curing. In particular a physical crosslinked gelatin type A matrix is stabilized by the patterning of genipin (chemical crosslinker) and decorated with single-walled carbon nanotubes (SWCNTs) to create conductive pathways through the an inkjet printer. In order to accelerate the crosslinking procedure the polymeric system is exposed to UV light contextually to the printing procedure. A layer-by-layer approach allows to build 3D scaffolds removing the gelatin not crosslinked with genipin by immersing the structure into a 40°C water bath at the end of the process. Microfabrication procedures are tuned to guarantee repeatability of the entire process.
The stability of the structures is analysed with swelling test while the electromechanical properties were modulated varying genipin and carbon nanotubes concentration. Results are explained by a mathematical analysis using the homogenization approach the percolation theory and lumped parameters models. Once the scaffolds are fabricated the focus is shifted on how micro and macro-structural properties influence survival and growth of H9c2 cardiomyocytes evaluating cell density (DAPI assay) and viability (Alamar blue assay). Morphological analysis on the cell alignment over the conductive patterns is also investigated by electrical stimulation of scaffold-cells construct.
Results: The proposed fabrication procedure allows to modulate the mechanical properties over a range of 700 – 1300 kPa while the impedance can vary (at low frequencies) from 6 MOhm to 500 kOhm. Cell alignment onto conductive patterns is verified while cell vitality is slightly influenced by gelatin and genipin concentration.
Conclusion: A new fabrication procedure combining the 3D inkjet printing of crosslinker molecules and UV photocuring is proposed and tested to build a cardiac tissue model. The process allows to create conductive pathways by printing SWCNTs that are able to guide cell alignment

F4.5

Assessment of electromagnetic device for label-free magnetic cell assembly

Yoshitake Akiyama
Shinshu University, Matsumoto, Japan

We are developing a 3D tissue formation system by means of label-free cell assembly based on the magneto-Archimedes effect. In this study we assembled microbeads and cells around a cylinder-shaped mandrel and assessed the uniformity on the mandrel surface. We have already proposed the label-free magnetic assembly method based on the magneto-Archimedes effect [1]. The principle of the method is described briefly as follows. The magnetic force acting on the cell in the medium is proportional to the difference between magnetic susceptibilities of the cell and the medium. Therefore cells in the paramagnetic medium are acting as a diamagnetic object since The electromagnetic device was composed of 2 pairs of mutually-perpendicular electromagnets and z-axis automatic stage. When applying an equal current to all the electromagnets the magnetic fields generated by the electromagnet was repelled each other and the spot with lower magnetic flux density would appeared on the center of the electromagnets. So as to apply the magnetic field to the whole chamber the z-axis stage was moving upward and downward repeatedly during experiments at the constant velocity. We confirmed that almost all microbeads or cells attached on the mandrel surface after a few hours later as shown in Figure and observed the mandrel from all directions by rotating the chamber. The obtained projection showed that the microbeads or the cells are assembled almost uniformly on the mandrel. The result indicates that our system has a high possibility as a suitable candidate to assemble cells on a scaffold.

References:
[1] Y. Akiyama K. Morishima Appl. Phys. Lett. 98 163702 (2011)

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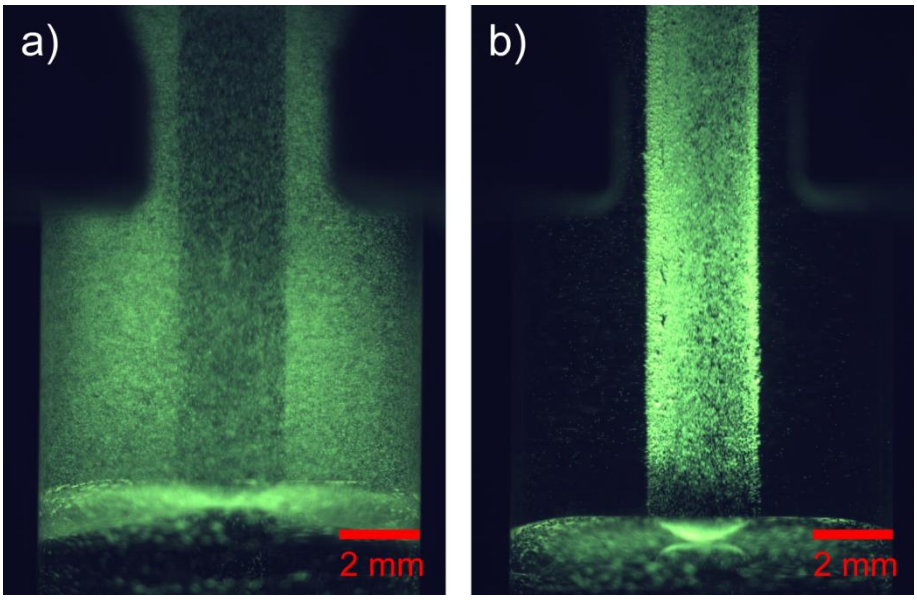

Microscopic images of fluorescent microbeads in the chamber from the side immediately after the beginning (a) and over 3 h later (b).