Free paper session 12: In vitro modles II

F12.1

Micro and meso-scale human vascularized organ-specific models to study cancer cell extravasation

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Cancer metastases arise from the dissemination of primary tumors towards specific target organs. Particularly cancer cell extravasation from blood vessels to secondary tissues is a key step of this process and a potential therapeutic target[1]. Hence the development of advanced in vitro models is a key challenge to progress current knowledge on the mechanisms of cancer cell extravasation. The goal of this study is to design micro and meso-scale human vascularized organotypic models and apply them for the study of breast cancer metastases to bone.
Fibrin gels embedding endothelial cells (ECs) and bone marrow mesenchymal stem cells (MSCs) were injected within microfluidic devices or 3D millimeter-size masks and bone- or muscle- specific cells were co-cultured to generate organotypic microenvironments. Confocal imaging allowed to quantify microvascular network (MN) parameters (e.g. total length #branches permeability) and breast cancer cell (BCC) extravasation.
Co-cultures of ECs and MSCs led to the development of perfusable MNs with supporting mural-like cells characterized by superior length (12.86±0.35vs10.13±0.39mm) and reduced vessel diameter (36.2±1.6vs84.2±2.7µm) compared to EC monocultures[2]. BCCs were injected within perfusable MNs showing higher extravasation rate in bone- vs. muscle- mimicking microenvironments (56.5±4.8%vs8.2±2.3%) despite opposite trends in vessel permeability[3]. We demonstrated that muscle-secreted adenosine was surprisingly involved in the regulation of vessel permeability and BCC extravasation. In parallel we developed a meso-scale model to high-throughput screen 35 experimental conditions and quantify the role of selected variables (e.g.cell density culture medium hydrogels) involved in MN formation. We controlled several culture parameters including oxygen gradients and performed genetic analyses following magnetic cell sorting. In this framework we found superior MN total length within normoxic (3 719±734µm) vs. hypoxic (1 179±584µm) regions.
Concluding we have demonstrated that the integration of micro and meso-scale models represents an effective strategy to develop and optimize vascularized organotypic models that can be employed to analyze endothelium/cancer cell interactions through the combination of controlled microenvironments cell-level studies and genetic analyses.

Acknowledgements:
The Italian Ministry of Health is greatly acknowledged.
1. Reymond et al. Nat Rev Cancer 2013 13(12):858-70
2. Jeon Bersini et al. Integr Biol (Camb) 2014 6(5):555-63
3. Jeon Bersini et al. Proc Natl Acad Sci U S A 2015 112(1):214-9

F12.2

3D bioprinting-based in vitro biofabrication and in vivo regeneration of adipose tissue liver and embryonic tissues

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Based on inkjet cell printing and bio-plotting 3D printing technology and stem cell biology tissue-like structures with lobule unit like adipose tissue liver and embryonic tissues were fabricated. Vascularized adipose tissue regeneration with functional blood vessel network and maintained weight and volume were achieved in animal model. An in vitro coculture model composed of human adipocytes and endothelial cells with spatially controlled distribution were fabricated. Detailed studies into the cell-cell interactions between the adipocytes and endothelial cells revealed a mutual-enhanced effect which resembles the in vivo routine. Hepatic stem cells were fabricated into a cell-laden structure and stem cell differentiation was regulated to achieve 3D liver-like microtissues with bile duct network and CYP3A4 expression and metabolic activity. Embryonic stem cells were bioprinted into cell-laden structure and high throughput and highly uniform formation of embryonic bodies were generated. These studies demonstrated powerful methodology for in vitro biological models drug testing and tissue regeneration studies.

F12.3

Multimaterial tandem electrospinning for spatially modulated neural guidance

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The goal of this work is the creation of an in vitro platform to investigate the combined effects of patterned topographical and bioactive cues towards achieving the spatially controlled growth of peripheral sensory neurons. The platform is intended to mimic the nanofibre topographical guidance characteristics of the native extracellular matrix and employ material and biofabrication strategies able to translate results to an implantable scaffold. To achieve a suitable platform the new Tandem Electrospinning technique was developed in order to create spatially defined multimaterial patterns of different populations of oriented nanofibres. Scaffolds were created using two polymer solutions of a PEOT/PBT copolymer dissolved in chloroform/HFIP with both solutions simultaneously electrospun onto a patterned target electrode. The formation of nanofibres was confirmed through scanning electron microscopy and hydrophobic fluorescent dyes were added to each solution to visualize the distribution of each population. To create spatially modulated growth orthogonal copper-click chemistry and maleimide/thiol conjugation additives were added to polymer solutions prior to electrospinning. Confirmation of selective conjugation was performed using ToF-SIMS and functionalized fluorescent dyes. Two laminin peptide sequences GRGDS and p20 were selectively conjugated to the two different fibre populations respectively and ToF-SIMS was used to verify their presence. Dorsal root ganglians (DRGs) from 1 day-old Wistar rat pups were explanted and placed at the overlapping region of the two fibre populations followed by microscopy analysis of fluorescence immunohistochemistry staining for ß(III)-tubulin after 5 days. Through the tuning of electrospinning parameters and target collector design it was possible to achieve a single step deposition of multiple fibre types within a confined space. Each population of fibres exhibited an aligned orientation and there was an observed region of overlap between the fibre types. The presence of selective functional groups on the fibre surface from the conjugation additives was confirmed through the application of functionalized dyes. Successful conjugation of peptide sequences to the fibre surface was also confirmed via ToF-SIMS. Initial in vitro results of neurite outgrowth from explanted DRGs suggest that the oriented nanofibre topography is able to promote directed neurite extension while the regionally defined biofunctionalization is capable of spatially modulating neurite growth. These results validate the newly developed Tandem Electrospinning method to create an in vitro platform that exhibits nanofibre topographical guidance cues and selective functionalization able direct and spatially modulate the growth of neural cells. This work was partly funded by NSERC of Canada.

F12.4

Epicardial application of cardiac progenitor cells in a 3D-printed gelatin/hyaluronic acid patch preserves cardiac function after myocardial infarction

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Aim: Cardiac cell therapy suffers from limitations related to poor engraftment and significant cell death after transplantation. In this regard ex vivo tissue engineering is a tool that has been demonstrated to increase cell retention and survival. The aim of our study was to evaluate the therapeutic potential of a 3D-printed patch composed of human cardiac-derived progenitor cells (hCMPCs) in a hyaluronic acid/gelatin (HA/gel) based matrix.
Methods and Results; hCMPCs were printed in the HA/gel matrix (30 × 106 cells/ml) to form a biocomplex made of six perpendicularly printed layers with a surface of 2 × 2 cm and thickness of 400 µm in which they retained their viability proliferation and differentiation capability. The printed biocomplex was transplanted in a mouse model of myocardial infarction (MI). The application of the patch led to a significant reduction in adverse remodeling and preservation of cardiac performance as was shown by both MRI and histology. Furthermore the matrix supported the long-term in vivo survival and engraftment of hCMPCs which exhibited a temporal increase in cardiac and vascular differentiation markers over the course of the 4 week follow-up period.
Conclusion: Overall the developed 3D printed patch was able to sustain the biological integrity of the cardiac progenitor cells in vitro. Furthermore the fabrication of this patch served as an effective translational approach to enhance hCMPC delivery and action in the injured heart.

F12.5

Developing an in vitro oviduct model: Post-printing treatment of tubular transwell constructs influences oviduct epithelial cell survival

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Oviduct epithelial cells (OEC) provide the microenvironment essential for mammalian fertilization. However in vitro oviduct models lack both morphological and functional characteristics of OECs and the highly folded tubular geometry of the oviduct. Our objective was to combine transwell cell culture and 3D printing technologies to develop an in vivo like OEC culture system to better study the role of the oviduct during fertilization.
Standing transwell inserts were fabricated from a multi-arm acrylate-based resin (PIC100) on an Envisiontec Perfactory P3 stereolithographer. Post-printing treatments of custom-made tubular transwell inserts were compared to determine which best reduced any negative impact of the plastics on cell growth. Inserts were either untreated post-cured with 1000 flashes/side (Otoflash 66 W) post-cured and Soxhlet-extracted overnight in isopropanol or post-cured and Soxhlet-extracted over the weekend in water at 37°C. Equine OECs were isolated from slaughterhouse material (n=3 mares) and cultured for 24 h to allow agglomeration into floating vesicles with outwardly oriented cilia. Vesicles were plated to establish monolayers of de-differentiated OECs which were trypsinized and reseeded in the absence or presence of an insert. In the presence of non-cured inserts monolayers resulting after 1 week were disrupted with detaching cells evident for two of the three donors (5% and 50% of surface area). After 3 weeks detached cell surface area was higher on non-cured inserts (48±38%) than on cured (27±25%) or cured and Soxhlet-extracted inserts (isopropanol 10±10%; water 13±15%). Monolayers in control conditions had the lowest cell loss (7±6%).
Post-cured inserts were mounted with track-etched PET membranes (12 µm thick 0.4 µm pore diameter) to create a 'half-pipe' geometry. OECs were seeded on the membranes grown to confluence and cultured at an air-liquid interface for 21 (bovine) or 40 days (equine). Immunofluorescence with marker proteins for apical and basolateral surfaces indicated that the OECs had re-established polarized morphology.
In conclusion post-curing custom-made inserts and Soxhlet extraction of leachable compounds is crucial to avoid toxic effects on cell growth. This allows the use of custom-designed inserts to create a tube-like surface on which polarized bovine or equine oviduct epithelial cells can be grown. Further studies will examine the ability of the cells to differentiate further and support in vitro fertilization. To this end 3D designs will be tested that allow perfusion of the apical compartment.

F12.6

Epithelial-to-Mesenchymal Transition in the in vitro cervical tumor model established by three-dimensional printing of Hela cells

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An in vitro cervical tumor model has been established by 3D printing to study the epithelial-to-mesenchymal transition (EMT) which is a very important stage of dissemination of carcinoma leading to metastatic tumors. A matrigel/Hela mixture 15% gelatin solution and 4% sodium alginate solution were evenly mixed at a volume ratio of 2:1:1. Finally the mixture was composed of 3.75% gelatin solution 1% sodium alginate solution 20% Matrigel and Hela cells with a density of 106 cells/mL. One milliliter of the cell/biomaterial mixture was drawn into a sterilized commercial syringe with a 25 gauge needle. The mixture was physically crosslinked at 25 ◦C for about 5 min in the syringe and then mounted onto the 3D cell printer. A Hela/hydrogel construct with a grid structure of 8 × 8 × 1 mm3 was fabricated by forced extrusion in a sterile atmosphere of 14 ◦C in a layer-by-layer fashion. CaCl2 (3% w/v) was gently added to chemically crosslinked alginate in the 3D constructs. Each construct was cultured in a 35 mm tissue culture dish with 2 mL culture media per dish. 3D Hela/hydrogel constructs were cultured in H-DMEM supplemented with 1% NEAA 1% penicillin & streptomycin 1% GlutaMAX and 10% FBS at 37 ◦C with 5% CO2 for 7 days (replaced medium every other day) and followed by changing to FBS-free medium for one day. After starving the 3D Hela/hydrogel constructs were changed to the medium with 10 ng/mL TGF-β for another 3 days and then collected for observation and further analysis. Hela cells quickly aggregated in the 3D hydrogel construct within 3 days probably due to the well maintained mass transfer through the hydrogel. Some of the aggregates started to disintegration during the day of starving and most of them collapsed with the induction of TGF-β. Some of the cells were observed to change their shape to fibroblast-like morphology after the 3 days of TGF-β induction showing the EMT phenomenon. Further analysis about protein and gene is going to be confirmed to support the EMT process.

F12.7

Finding the Balance between Oxygen Inhibition and Cell Viability in Biofabrication of Photopolymerised Hydrogels

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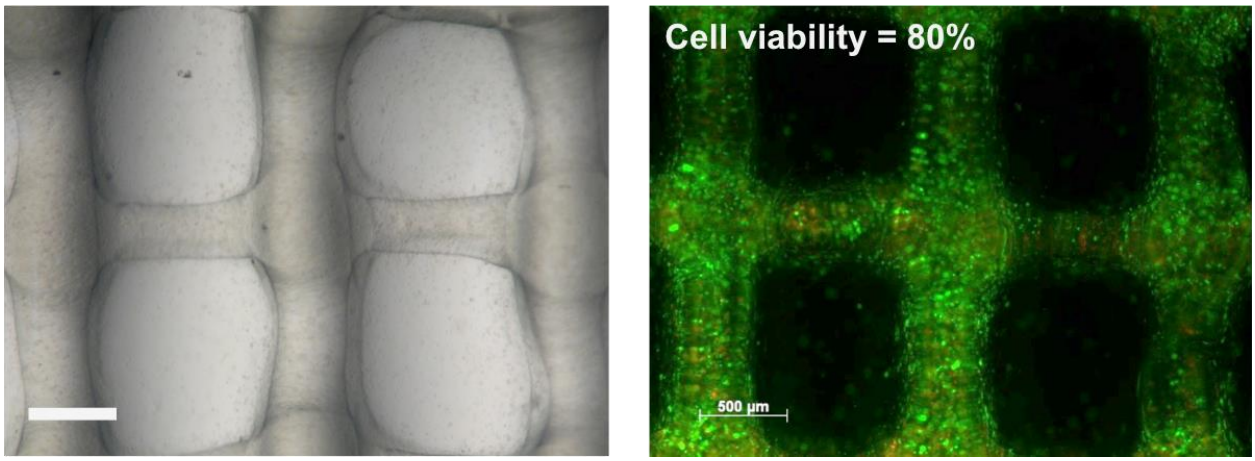
Biofabrication of cell-laden hydrogels (or bioinks) combining 3D bioprinting techniques with photo-polymerisation offers the ability to develop engineered constructs with high shape fidelity as well as supporting cell growth and are hence widely explored [1]. Often this radical based crosslinking process is performed in the presence of oxygen to maintain cell survival and viability. However oxygen is known to quench the radicals causing incomplete crosslinking which in turn leads to physical deformation of the scaffold [1]. Therefore the aim of this study was to minimise the effect of oxygen inhibition in photo-curable hydrogel. 3D printed hydrogel constructs (20x20x3mm) consisting of 10wt% gelatin-methacrylamide + 0.85wt% collagen I + 0.05wt% Irgacure2959 were fabricated using a BioScaffolder (Syseng Germany) then irradiated with UV light (330-450nm) in the presence of oxygen for 15minutes. UV light intensity was varied from 3–100mW/cm2. The reduction in fiber diameter of the photo-crosslinked constructs before and after equilibrium swelling was measured as an indication of oxygen inhibition. For cell encapsulation studies human breast adenocarcinoma cells (MCF-7) were encapsulated at 5 million cells/ml and their viability was assessed at 1d. Constructs photo-cured with commonly adopted UV intensity in literature (3mW/cm2) have poor structural integrity and degraded after 1d in PBS. Increasing light intensity to 30mW/cm2 allowed fabrication of mechanically stable constructs but still had significant reduction in fiber thickness (~70%) after equilibrium swelling. Further increasing the light intensity to 50mW/cm2 successfully reduced the fiber deformation to 25%. Moreover cells encapsulated at this condition were 80% viable (Fig.1) and remained metabolically active after 7d in culture. In conclusion this study shows that oxygen inhibition in photo-polymerised biofabricated hydrogel constructs can be minimised by increasing UV light intensity without detrimentally affecting cell viability. Current work is investigating encapsulation of other cell types such as articular chondrocytes and mesenchymal stromal cells into these constructs.



Microscopy and live/dead images of the 3D printed cell-laden construct; Scale bar = 500µm