Research talks

The Development of an OOAC Automated Platform: Breast-on-a-Chip (BOAC)

A. Aly[1], L. Osorio[2], E. Silva[2], R. Mackay[1]

[1]Department of Mechanical, Aerospace and Civil Engineering, Brunel University London, Uxbridge, UB8 3PH, Greater London
[2]Depatment of Life Sciences, Brunel University London, Uxbridge, UB8 3PH, Greater London

Corresponding Author Email: aya.aly@brunel.ac.uk

INTRODUCTION

Breast cancer is the most common cancer in women in both the developed and less developed world. Due to its complex aetiology and pathophysiology, breast cancer requires substantial research to increase understanding of the risk factors associated with the disease and to develop novel therapies. As such, we require the development of a reliable experimental model, which recapitulates breast cancer in humans. Based on the increasing ethical and financial issues concerning animal testing and the fact that animals often lack the same physiology as humans, there is significant demands for the development of OOAC technologies, which replicate the mammary gland and the process of carcinogenesis, allowing the robust study of the disease. This project aims to develop a BOAC system and an automated integrated OOAC platform for long-term monitoring of breast cell culture.

METHODS

This project incorporates two elements; the BOAC and the design of the platform. The design process includes iterations of CAD embodiments, 3D printing, soft lithography, and an electronic control framework. The electronic framework incorporates miniature piezoelectric pumps and feedback using a set of sensors. Poly (dimethylsiloxane) (PDMS) devices were developed with a trapezoidal chamber for BOAC experiments. Breast epithelial cells (MCF-12A) were embedded into Matrigel® and placed in the well of the PDMS chip prior to sealing. Piezoelectric pumps were used to flow cell media through the device at a flow rate of 15.32µL/min. Due to the high accumulation of bubbles in the first sets of tests, a bubble trap was designed, 3D printed using the Ultimaker 2+ and added to the fluidic circuit.

RESULTS

With Matrigel® as the scaffolding, the BOAC produced organised 3D acini structures over 12 days. The structures formed were comparable, in morphology and size, to those in human tissues, as well as those reported in the literature[1]. The experimentations were conducted in a 5% CO_2, 37°C incubator and at a flow rate of 15.32µL/min – which will be replaced by the platform.

DISCUSSION AND CONCLUSIONS

The microfluidic chips used were capable of emulating the morphology of breast tissue *in vitro*. The BOAC is a great alternative to animal and traditional cell culture testing in the future, as it is versatile, less time consuming and a lower monetary investment. The automated platform possesses many advantages, such as experimental reproducibility and continuous monitoring of the cell culture. The platform and the OOAC have the potential to become a technology that can be used to accommodate growth and analysis of other human organ cells.

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A human 3D neural culture model utilising iPSCs from an Alzheimer's patient to study neurodegenerative diseases

Elodie J Siney[1], Preeti Prasannan[1], Shreyasi Chatterjee[2], Amrit Mudher[2] & Sandrine Willaime-Morawek[1]

[1]Clinical Neurosciences, Faculty of Medicine, University of Southampton.UK
[2]Biological Sciences, Institute for Life Sciences, University of Southampton.UK

Corresponding Author Email: E.Siney@soton.ac.uk

INTRODUCTION

Organ specific research has advanced greatly using biopsy tissues in creating *ex vivo* and *in vitro* models, however the collection of live human brain tissue from an Alzheimer's disease (AD) patient is almost unheard of. In the last decade, human stem cells have been an invaluable source of research material for neuroscience and we can now obtain iPSCs from the fibroblasts of AD patients containing the exact genetic mutations that give rise to the disease. Our aim was to create a physiologically relevant neural tissue-like 3D model to model some pathological and biochemical aspects of AD.

METHODS

Using IPSCs from an early onset AD patient containing the Presenilin 1 mutation (L286V) we have created 3D human neural cultures for the study of early onset AD, we are currently validating this model using immunofluorescence, western blotting and electrophysiology.

RESULTS

IPSC-derived neural progenitors were plated in Matrigel, self-organised into 3D structures by week 3 and were maintained *in vitro* for 18+ weeks. These 3D cultures contained a heterogeneous cell population expressing differentiation markers for neurons and astrocytes as well as the mature 4R tau isoform by week 12. It also features AD-associated proteins, phospho-Tau and Abeta oligomers.

DISCUSSION AND CONCLUSIONS

3D neural cultures, derived from the fibroblasts of an AD patient, can now be utilised to study the disease pathology in a fully human model. Here we present a viable and physiologically relevant *in vitro* neural model for the study of neurodegeneration. We believe this model can be further developed into a medium throughput assay for small molecule screening by utilising current OOAC technology or by developing new technology through interdisciplinary collaborations.

Conceptual Design and Development of an Organ on a Chip Research tool: Understand and investigate the functionality of Epithelial Tissue of the Lower Female Reproductive Organ and the Prevalence of Bacterial Vaginosis.

A.Naveenathayalan. [1], P. Kaur[2,] R. Mackay. [1] E. Silva[2,] A.Houlden[2]

[1]Department of Mechanical, Aerospace and Civil Engineering, Brunel University London, Uxbridge, UB8 3PH, Greater London
[2]Department of Life Sciences, Brunel University London, Uxbridge, UB8 3PH, Greater London
Corresponding Author Email: Angel.Naveenathayalan2@brunel.ac.uk, 1604134@brunel.ac.uk

INTRODUCTION

Bacterial Vaginosis (BV) is one of the most common vaginal disorders suffered by women, yet its aetiology is unknown. This condition desperately needs a new approach to developing effective treatment, as it is known to affect ~ 50% of the female population in the developing world and ~ 33% of women in the developed world [1,2]. The disease is thought to be a result of ecosystem disruption with overgrowth of opportunistic pathogens rather than a single pathogen infection. Multi-species microbial biofilms and decrease in *Lactobacillus crispatus* are known to be the major aspects in BV; however, the initial process which causes this is unclear. Even though *Gardenella vaginalis* is the most prevalent BV-associated anaerobe, it is also found in the vaginal microbiome of healthy women. The factors of host and bacteria such as genetic differences may be the reason behind the different pathological features and virulence factors of *G. vaginalis* [3]. Antibiotics or vaginal cream/gel are usually prescribed to treat the infection, but reoccurrence is common. At present, there is no effective treatment for this condition. New studies are now focusing on the new paradigm Organ-on-Chip (OOC). This project involves creating a powerful 3D microfluidic tool the Vagina-on-a-Chip (VOC) which involves multidisciplinary research that will mimic the mechanical, biochemical and physical aspects of the vaginal tissue. The main aim of our work is to use VOC platform to study these factors that cause an interruption in the vaginal microbiome and the interaction between the vaginal epithelial cells and bacteria strains, to improve female healthcare.

METHODS

The Vagina-on-Chip will be developed using multiple techniques that combines micro-engineering, 3D printing, and cell culture. The layers of the chip are created using 3D printed moulds and soft lithography method using silicone polymer (PDMS) to fabricate each layer to form the platform. *L. crispatus* has been cultured *in vitro* to monitor its growth characteristics and measure the impact that different culture conditions have on growth such as aerobic and anaerobic conditions. To mimic the normal vaginal environment, the bacteria will be inoculated onto the top layer of the platform in order to seed the epithelial layer to create a biofilm. Electrospinning will be used to create a biocompatible membrane which forms part of the middle layer. This is where 3D cell culture will take place to form the epithelial layer of the vagina. The shear force will be used to stimulate the tissue creating 3D tissues and the device will incorporate a nutrient channel to allow growth media to be continuously distributed to the cells. The model represents the function of the organ which will act as the platform for experimental use, to understand enigmatic conditions such as Bacterial Vaginosis (BV).

RESULTS

The VOC is a novel approach to study BV and the interaction between vaginal epithelial cells and *L. crispatus*. Biocompatibility tests have been done on electrospun membranes and have shown viable results. Optimal design of the platform has been completed. The next stage is to combine all layers with the membrane and create functional, physiological vaginal microtissues within the device. To explore this condition, we will add *G. vaginalis* and *P. bivia* bacteria to see the biofilm formation which is an important aspect of bacterial vaginosis. To find the specific aetiology, we will further investigate the genetics of different bacterial strains and how they affect the biofilm formation.

DISCUSSION AND CONCLUSIONS

The VOC platform can be used to realistically model and obtain a better understanding of the BV. The platform can be used by researchers to find better treatment for BV by challenging the bacterial ecosystem and examine the bacteria with different treatments such as antibiotics, prebiotics and probiotics. Our goal is to have a 3D model that could potentially replace animal testing for the new development of drugs and toxins and can be used to study pathogens that affect the epithelial layer of the lower reproductive system in females.

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HIGH THROUGHPUT MICROFLUIDIC GUT-ON-A-CHIP MODEL FOR DRUG DISCOVERY AND TARGET VALIDATION IN INFLAMMATORY BOWEL DISEASE

Claudia Beaurivage[1,2], Yee Xiang Chang[1], Kai Erdmann[2], Jan Stallen[1], Richard Janssen[1]
[1]Galapagos BV, the Netherlands
[2]University of Sheffield, United-Kingdom

Corresponding author: Claudia.beaurivage@glpg.com

Inflammatory bowel disease (IBD) is a group of chronic relapsing inflammatory diseases of the gastrointestinal tract. Patients suffering from IBD have presently limited options in terms of treatment due to the lack of physiologically-relevant models to study IBD. With the advent of Organ-on-a-chip technology, a few gut-on-a-chip models have been recently developed and show great promise; however most of them use cancerous cell lines which do not reflect the physiology of IBD patients.

This study reports a novel high throughput microfluidic model of the gut composed exclusively of human primary material. Our first experiments show that primary intestinal epithelial cells (IEC) can be grown in a leak-tight polarized epithelial tubule in the OrganoPlate® platform developed by Mimetas. These cells exhibit intact tight junctions and retain the capacity of differentiating into the different cell types of the intestinal epithelium. An inflammatory state can also be created by exposing IEC to a cytokine trigger; IEC will respond by secreting key inflammatory cytokines such as CCL20, IP-10 and IL-8. Finally, we were able to establish a co-culture model between IEC from healthy donors but also from IBD patients together with primary human immune cells to further investigate IBD mechanisms.

Overall, we show a novel 3D gut-on-chip model entirely composed of human primary material that is suitable for high throughput experiments and screenings. We are presently investigating whether CRISPR-engineered epithelial cells can be grown in this device and therefore be a great tool for target validation. We hope that this model offers an increased relevance compared to existing *in vitro* models and that it will be suitable to validate potential IBD drug targets and implement it in Galapagos' drug discovery process on a daily basis.

AN IN VITRO 3D MODEL TO EVALUATE BEHAVIOUR OF BREAST CANCER CELLS AND RESPONSE TO TREATMENT

Tayebeh Azimi[1], Miriam Dwek[1], Marilena Loizidou[2]
[1]Department of biomedical Science, University of Westminster, London, UK
[2]Department of Surgical Biotechnology, University College London, London, UK

Corresponding Author Email: T.azimi@my.westminster.ac.uk

INTRODUCTION

The field of 3D culture models of disease has started to move towards systems that aim to recapitulate the complexity of human tissues. However, despite recent improvements, current 3D systems remain overly simplistic, lacking the biophysical characteristics and diverse structures found in most organs. In this project, the cellular behaviour of breast cancer and their responsiveness to chemotherapeutic agents were evaluated under different 3D cell culture conditions.

METHODS

MDA-MB231 and SKBR3 cells were prepared as spheroids using ultra-low attachment plates and as 'artificial cancer masses' (ACM) by embedding cells in a dense collagen type-I. The ACMs were maintained under flow (150 µL/min) and flow/pressure (550 µL/min, ~19 mmHg) conditions.

RESULTS

A significant reduction in cell viability was observed when cancer cells were grown as ACM compared to 2D culture. Cell viability also declined significantly when ACMs were maintained in flow/pressure condition compared to static condition. Similarly, an increase in the expression levels of markers of EMT was observed when cells were cultured as ACM. However, compared to static 3D incorporation of flow and pressure was associated with decreased expression levels of vimentin, HIF1-α, whilst MMP14 expression increased and Snail remained unchanged. HER2 levels were increased in SKBR3 when the cells were cultured as static ACM (1.34 fold) and under flow/pressure (1.5 fold) compared to 2D. Overall, cells cultured as ACMs exhibited reduced responsiveness to doxorubicin compared to those grown in the conventional 2D culture. A decrease sensitivity was also observed in 3D/flow/pressure and 3D/flow compared to 3D/static condition.

DISCUSSION AND CONCLUSIONS

The results obtained in this study show that cancer cell behaviour and their response to therapeutic agents are affected by different microenvironments. Therefore, a new-generation of 3D in vitro models need to be developed as pre-clinical drug testing platforms.

TOWARDS REALISTIC BRAIN MODELS: TRANSITION FROM STATIC TO MICROFLUIDIC MULTI-ELECTRODE ARRAYS

D. Moschou[1], P. R. F. Rocha[1]

[1] Centre for Biosensors, Bioelectronics and Biodevices (C3Bio) and Department of Electronic and Electrical Engineering, University of Bath, Claverton Down, Bath, BA2 7AY, United Kingdom

Corresponding Author Email: P.Rocha@bath.ac.uk, D.Moschou@bath.ac.uk

INTRODUCTION: Cells or tissues are currently cultured on microchips to replicate organ-level functions, with measurements of cell electrical activity routinely taken via static multi-electrode arrays (MEA). Nonetheless, they are not able to precisely renovate cell medium as occurs in the human body. Microfluidics can be integrated to replicate more accurately the in vivo microenvironment and micro physiological behaviour, resulting in a higher predictive power in terms of disease understanding, compound testing and late adverse drug effects. In this work we demonstrate a microfluidic MEA device with ultra-low background noise, capable of realistically monitoring and modulating brain cells in-vitro. We accomplish this by transitioning from a static and ultra-low noise MEA to a dynamic one, assuring a continuous and homogeneous transfer of nutrients across the growth/measuring chamber.

METHODS: COMSOL Multiphysics® was used to simulate the concentration profiles of the designed microfluidic structures under steady flow. The optimum microfluidic design was rapid prototyped via Poly (methyl methacrylate) (PMMA) laser patterning and tested for fluidic tightness. The MEA device was implemented in a standard gold-plated Printed Circuit Board (PCB[1]) for the first time.

RESULTS: Different microfluidic designs were simulated, aiming for a uniform growth medium distribution across the custom-MEA[2,3]. The optimum design comprised a 3-inlet, 3-outlet made rounded corner chamber following the electrode periphery (Fig.1a). The microfluidic was integrated on a PCB fabricated MEA and hydrophilized, so as to avoid bubble accumulation over the electrode surface (Fig1b).

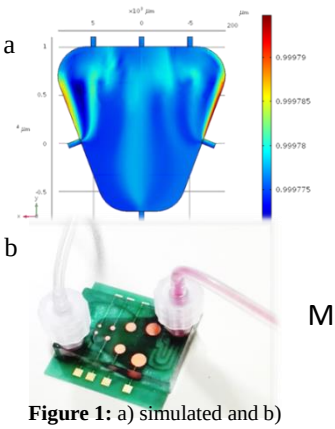

Figure 1: a) simulated and b) prototyped microfluidic MEA

DISCUSSION AND CONCLUSIONS: To transition from no-flow structures to continuous flow ones, the design of the microfluidic structure needs to be carefully selected, assuring uniform flow across the complete chip surface; non-uniformities could result in cell death and poor cell adhesion in specific parts of the array, thus biasing the electrical recordings. It is also very critical to assure a bubble free operation under flow, since bubbles over the electrodes will compromise the signal transduction. In this work, we have demonstrated a systematic study in achieving efficiently all of the above, exploiting a PCB fabricated MEA. Future studies include the characterization of signal-to-noise ratios of our chip using cell growth medium, their dependence on alternative flow rates and brain cell electrical signal recordings under different flow rates

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SOL-GEL DERIVED INKS FOR 3D BIOFABRICATION

G. Poologasundarampillai[1], S. Jayash[1], G.C. Machado[2], R.M. Shelton[1], P.R. Cooper[1]

[1]School of Dentistry, University of Birmingham, Birmingham, B5 7EG, UK
[2]School of Materials, University of Manchester, Manchester, UK

Corresponding Author Email: g.poologasundarampillai@bham.ac.uk

INTRODUCTION

Combination of bottom-up 'soft-chemistry' sol-gel processing and top-down manufacturing processes have allowed the synthesis of complex structures for biomedical applications. One of the potential barriers to future developments in biofabrication is the limited choice of suitable materials. At present, hydrogels from natural biopolymers are mainly used for 3D biofabrication. These have several limitations, such as batch-to-batch variation, low shape fidelity, limited post-printing strength and uncontrolled degradation. Promising synthetic alternatives are advanced organic-inorganic hybrid materials developed using the sol-gel process. This work presents the development of novel sol-gel derived bioinks for the 3D biofabrication of tissue constructs.

METHODS

Polyol-modified silanes (PMS) and biopolymers (chitosan and gelatine) were reacted to produce organic-inorganic hybrid hydrogels. Briefly, biopolymer dissolved in aqueous media was functionalised with an organosilane coupling agent. To this PMS was added and allows to react. PMS undergoes hydrolysis and condensation reactions *in vitro* to form organic-inorganic hybrid gels with strong covalent bonding between silica and biopolymer. The structure, chemistry, physical properties as well as the biological properties of the gels were investigated.

RESULTS

Homogeneous gels were formed starting from solutions within few minutes to several days depending on factors such as the pH, type of buffer solution used, volume ratio of buffer:PMS and proportions of organic/inorganic. Gel formation was observed to follow the formation of stable colloids which under critical concentration assemble to form stable gels, Fig. 1a shows SEM image of dried gel. Gels could be produced in 3D structures adopting a top-down biofabrication approach (Fig. 1b).

DISCUSSION AND CONCLUSIONS

This study presents a bottom-up approach to produce bioinks that could be used in biofabrication of tissues and constructs for drug discovery and delivery. The materials produced promises to be a better alternative to biopolymers which have several drawbacks.

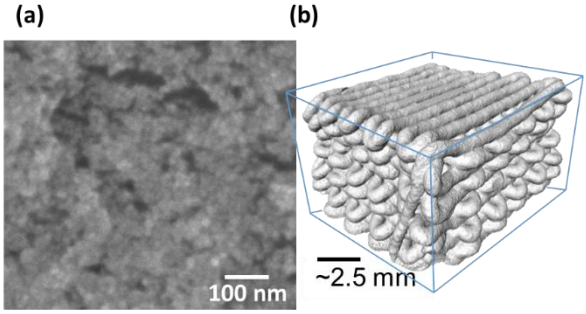

Fig. 1: (a) SEM image showing the colloidal silica nanoparticles (light grey) that has assembled together to form a gel with interpenetraing organic (dark) and (b) μCT image of a 3D printed gel scaffold with 0/90 layer by layer structure.

Studying bacteria propagation in a foetal membranes-on-a-chip

V. Mancini[1], V. Pensabene[1,2]

[1]School of Electronic and Electrical Engineering, University of Leeds, Leeds, LS2 9JT, United Kingdom
[2] School of Medicine, University of Leeds, Leeds, LS2 9JT, United Kingdom

Corresponding Author Email: V.Pensabene@leeds.ac.uk

INTRODUCTION

Preterm birth (PTB) is a leading cause of premature death in new-borns and children under the age of five. While the causes and the process triggering PTB are still not understood, 70% of the PTB cases have been correlated with bacterial infection of the foetal membrane, so called "chorioamnionitis" (CAM). CAM per se' does not lead to premature rupture of the foetal membranes (PPROM) and this is possibly due to the innate maternal and foetal response. In order to clarify the origin of PPROM and the spatio-temporal progress of CAM we aim to define a new human model of the foetal membranes were bacterial infection and propagation through the foetal membrane can be observed and studied.

METHODS

The planar Organ-on-a-chip (OoC) includes two microfluidic channels separated by a central gel-filled channel, which aims to mimic the extracellular matrix in the chorionamnion structure. We tested collagen type I, Matrigel and Jellyfish-derived collagen (Jellagen, UK) to identify their compatibility with human cells and potential effects on macrophages activation. A transwell model has been used as control to optimize combined culture of decidualized stroma, cytotrophoblasts and macrophages (MΦ). Primary stroma, placental macrophages isolated from human, un-identified placenta (collected from programmed C-sections at Saint James's University Hospital at Leeds, after ethical approval). As control, human immortalized THESC (ATCC® CRL-4003) and phorbol 12-myristate 13-acetate (PMA)-stimulated THP.1 (ATCC® TIB-202) were used. Lipopolysaccharides (LPS) exposure was used as model for inducing CAM in the transwell and in the microfluidic system. Cytokines secretion (IL-8) after 24 hours LPS -exposure using enzyme-linked immunosorbent assay (ELISA). Cell morphology was visualized by ActinGreen™ and Hoechst-nuclei staining.

RESULTS

The OoC allowed to culture THESCs in two individually perfused, communicating channels. Diffusion of dextran (70, 140 kDa) modelled by FEM COMSOL Multiphysics was observed through collagen I, Matrigel and Jellagen. Gel type and culture surfaces (ie polycarbonate membranes, polystyrene) affected mΦ NFkB activation magnitude. We measured increased IL-8 secretion by THESCs at 24 h-LPS exposure.

DISCUSSION AND CONCLUSIONS

This OoC foetal membrane model is a promising platform to study complex maternal foetal interactions in presence of toxins or pathogenic microbes and to link those to their potential impacts on PTB.

Posters

ESTABLISHMENT AND VALIDATION OF *IN-VITRO* MODEL FOR LOWE SYNDROME AND DENT II DISEASE.

Sindhu Naik[1], H. Lanz[2], J. Stallen[3], E.Smythe[1], K.S.Erdmann[1]

[1]Department of Biomedical Science, University of Sheffield, Sheffield, S10 2TN, UK.
[2]Mimetas B V, Leiden, 2333 CH, The Netherlands.
[3]Galapagos B V, Leiden, 2800 A3, The Netherlands

Corresponding Author Email: k.erdmann@sheffield.ac.uk

INTRODUCTION

Lowe syndrome and Dent II disease are rare X-linked recessive genetic disorders affecting eye, kidney and brain. The PtdIns(4,5)P2 5-phosphatase, encoded by the OCRL gene, dephosphorylates (PI(4,5)P2 & PI(4,5)P3 present on cell membranes and other vesicles into PI4P. Mutations in OCRL lead to the accumulation of PI(4,5)P2 and PI(4,5)P3 causing cataracts, mental disabilities, and kidney dysfunction in Lowe syndrome patients. Dent II disease displays less severe phenotypes of kidney dysfunction, also due to mutations in OCRL gene. Research has shown that OCRL is involved in multiple cellular processes such as endocytosis, membrane trafficking and actin skeleton dynamics[4]. The underlying molecular mechanism due to which the disease manifests is still largely unknown.

METHODS

We have created a unique model to study OCRL1-related disease phenotypes by combining an *in-vitro* genetically modified adult human proximal tubule cell line (HK-2), generated through CRISPR-Cas9 gene editing by knock-out of OCRL gene, with the MIMETAS OrganoPlate® technology enabling culturing of 3D proximal tubules with fluid flow applying shear stress and mimicking the *in-vivo* environment.

RESULTS

We have successfully characterized our disease model to show phenotypes recorded in literature such as defects in primary cilium maintenance[5], transport defects (early endosomes to Golgi network[6], actin cytoskeleton abnormalities[7], and megalin mediated endocytosis defect.

DISCUSSION AND CONCLUSIONS

The OrganoPlate® 3D diseased kidney model offers a novel way of studying Lowe syndrome and its underlying disease mechanisms and a unique opportunity to screen for drugs in a high throughput manner, which could ameliorate the disease condition. Furthermore, the ongoing functional characterizations might give us an insight into yet unknown disease mechanism.

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ESTABLISHMENT AND VALIDATION OF AN IN VITRO MODEL FOR CROHN´S DISEASE

K. Kosim[1], D. Kurek[2], K. Erdmann[1]

[1]Department of Biomedical Science, The University of Sheffield, Sheffield, S10 2TN, UK
[2]Mimetas - The Organ on a Chip Company, Leiden, 2333 CH, The Netherlands

Corresponding Author Email: k.kosim@sheffield.ac.uk

This project aims at providing a tool to investigate Crohn's disease in vitro. Crohn's disease is one of the two major variations of inflammatory bowel disease (IBD) – it is characterized by inflammation and ulceration of the gut mucosa. IBD is a very complex disease with multiple etiological factors associated (genetic, environmental and microbial). The precise mechanism causing the disease is still not uncovered but some genes have been identified to predispose patients for this disease. NOD2 (nucleotide-binding and oligomerization domain 2) has been reported as one of the potential players in Crohn's disease due to its genomic localization and its role in recognition of bacterial cell wall components[8]. Several mutations in CARD15 gene coding for NOD2 protein have been found in Crohn's patients[9] [10]. This protein is part of the innate immune system of intestinal epithelia and its signalling pathway leads to regulation of NFκB activation, which is disrupted in Crohn´s disease.

To model Crohn's on a chip I am using an OrganoPlate® developed by Mimetas. The platform based on a microtiter plate harbours up to 96 chips and enables the culturing of perfused 3D tube-like structures in a membrane-free manner[11]. In our model we are using both, the gut epithelial cell lines as well as primary material. As the project focuses on NOD2 involvement in the disease, we induce the inflammation state with MDP (muramyl dipeptide) that is a minimal bioactive peptidoglycan motif common to all bacteria and a NOD2-specific ligand.

We established human gut on a chip model that mimics an intestinal barrier inside the microfluidic channel of the OrganoPlate®. Cell polarization, formation of tight junctions and expression of intestinal markers have been confirmed. Importantly, we are able to show the pro-inflammatory reaction of the epithelium in response to the trigger. The final aim of the project is to demonstrate the suitability of the model for high-throughput drug screening.

There is a great need for more reliable *in vitro* disease models that are suitable for testing drug toxicity and efficiency at early stages of drug development. Having such models in place would greatly impact on drug development costs and increase safety of newly developed medicines.

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STUDY OF BONE CELL POPULATION MODELS OF S-SYSTEM TYPE

J. Simonović[1], T. E. Woolley[2]

[1] Biomedical Engineering Department School of Engineering, Cardiff University, Cardiff, CF24 3AA, UK

[2] Cardiff School of Mathematics, Cardiff University, Senghennydd Road, Cardiff, CF24 4AG, UK

Corresponding Author Email: simonovicj@cardiff.ac.uk

INTRODUCTION

This study represents a deterministic and stochastic analysis of the S-System of bone remodelling. We explore this system through its homogeneous coupled ordinary nonlinear differential equations,

$$\frac{du_i}{dx} = \alpha_i \prod_{j=1}^{2} u_j^{g_{ij}} - \beta_i u_i, \quad i = 1, 2, \tag{1}$$

as well as through its probabilistic analogue,

$$\emptyset \xrightarrow{\alpha_i u_i u_j} u_i, \quad u_i \xrightarrow{\beta_i} \emptyset, \quad i = 1, 2, \text{ and } \quad j \neq i, \tag{2}$$

to investigate whether the model can capture the essential autocrine, paracrine and synergistic characteristics of bone cell communication processes, both in targeted and random remodeling processes.

METHODS

Eq. (1) is used to analyse steady state stability, bifurcations and system sensitivity to parameters and initial conditions changes. The analogous probabilistic model (2) cannot be analysed so easily, thus, we turn to simulation. Namely, stochastically simulating the system 1000 times allows us to extract probabilistic distributions of the outcomes, which capture the noisy features of cell division and death, especially in the transition regimes on the beginning and ending of the processes.

RESULTS

Population dynamics are illustrated using time series plots, phase portraits, histograms and bifurcation diagrams. These motivate discussions regarding the model viability and parameter's value range and importance.

DISCUSSION AND CONCLUSIONS

Mathematical models are a great way of cementing biological verbal models. Specifically, they can provide causative mechanisms linking inputs and outputs and they can illuminate underlying assumptions that determine a biological system's dynamics. Finally, they offer a means of predicting new outcomes, as well as highlighting the most sensitive modelled components, resulting in the construction of new experimental hypotheses and reducing experimental waste.

Continuum models (such as (1)) assume that the simulated populations are large enough that a continuum approximation is valid. In such cases the stochastic and deterministic descriptions are equivalent as noise reduces relative to population size. However, in the bone creation-degradation application, which these equations describe, cell population numbers often fall below 10 cells. Thus, the stochastic description is more apt. Critically, we see that dynamics that are often present in the deterministic equation, which used to explain a variety of observed experimental dynamics, do not occur in the stochastic model. Thus, we must question biological reality that these equations present.

Acoustic Microfluidic Chips for Separating Microparticles and Cells

Xin Yang[1]

[1]Department of Electrical and Electronic Engineering, School of Engineering, Cardiff University, Cardiff, UK CF24 3AA
Corresponding Author Email: yangx26@cf.ac.uk

INTRODUCTION

Acoustofluidic techniques are increasingly used to manipulate nano- and micro-particles in microfluidics. A wide range of acoustofluidic devices integrating microchannel and acoustic source have been developed for applications in biochemistry and biomedicine.

METHODS

We have developed surface acoustic wave (SAW) based acoustofluidic devices as the schematic shown in Fig. 1 to separate microparticles and cells. Two interdigital transducers (IDTs) are made on piezoelectric substrate with a microfluidic channel bonded to host the sample. Standing SAW (SSAW) are produced with pressure nodes positioned near the microchannel walls. Microparticles such as polystyrene microspheres and cells experiencing acoustic radiation force and streaming drag force can migrate towards the pressure nodes. The migration velocity is dependent on difference particle's volume, density and compressibility. Microparticles with different mechanical properties will be separated and collected in different outlets.

RESULTS

The separation experiment
results shown in Fig. 2(a) is the
relationship between the
microparticle separation
rate and the acoustic input power.
The microparticle sizes are 5, 6, 8,
10, 15 µm, which can be driven in
the microchannel by applying
different input powers. Fig.
2 (b) shows the separation
20 µm microspheres from a
mixture with 10 µm denoting a
separation of 86%. Fig. 2(c) shows
the microscopic image of the
microchannel of the
acoustofluidic device, in which
the separation of large-size (>10 µm) mouse fibroblast cells from small-size population is recorded.

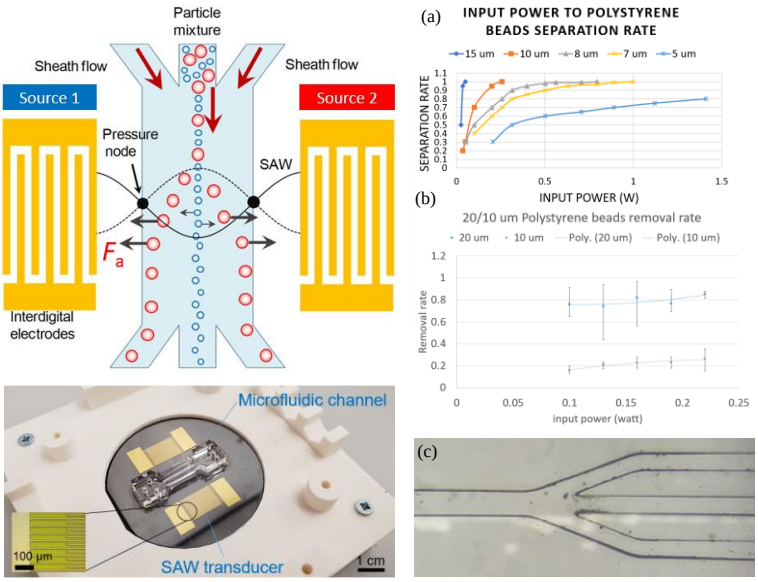

Fig. 1 Schematic of the SAW device for separation. Fig. 2 Particle trajectories in simulation and experiment.

DISCUSSION AND CONCLUSIONS

The acoustofluidic device using SAW to actuate microparticles and biological cells has been demonstrated. The device can be part of the microfluidic or organ-on-a-chip-system to function as cell sorting and characterisation unit.

TISSUE SPECIFIC INVASION OF BREAST CANCER CELLS IN A LAB-ON-A-CHIP SYSTEM: THE INVASION AND CHEMOTAXIS (IC) CHIP

B. Firatligil[1], G. Bati-Ayaz[1], D. Pesen-Okvur[1,2], O. Yalcin-Ozuysal[1]

[1]Izmir Institute of Technology, Department of Molecular Biology and Genetics, Izmir, 35430, Turkey
[2]Initio Cell Ltd., Cheshire, SK10 4TG, United Kingdom

Corresponding Authors Emails: dpesenokvur@gmail.com, ozdenyalcinozuysal@gmail.com

INTRODUCTION

Classical drug discovery uses 2D cell culture coupled with animal testing for preclinical studies. Neither 2D cell culture nor animal testing truly recapitulate the in vivo microenvironments of cells in a human body. Using 3D cell culture in lab-on-a- chip devices that can faithfully mimic the in vivo conditions, costs can be reduced ten-fold, results can be achieved ten times faster, animal testing can be significantly reduced and personalized medicine can be realized. Being the leading cause of cancer related deaths, it is essential to understand the complex metastasis phenomenon of breast cancer to develop new diagnostic and therapeutic strategies. In this study, a lab-on-a-chip (LOC) system was used to detect the metastatic
capacities of breast cancer cells to different target tissues. For this purpose, lung, liver, breast and bone microenvironments were generated and homing choices of metastatic breast cancer cells to each microenvironment were determined.

METHODS

The triple negative breast cancer cell line MDA-MB-231 was selected based on its high metastatic capacity, while non-metastatic breast cancer cell line MCF7 was chosen for control. Normal lung, liver, breast and bone cells, WI-38, BRL3A, MCF10A and hFOB were used to generate target tissue microenvironments. A three-channel LOC system, the IC-chip (Invasion and Chemotaxis chip), one for addition of cancer cells, one for mimicking target tissue microenvironment and one for media supply, was used. The seeding cell densities, common medium composition and ECM components for microenvironment were optimized for the LOC system. Following addition of cancer cell lines to the IC-chip, invasion capacities to different target sites were determined by tracking 3D distribution of fluorescently labelled cancer cells up to four days using a Leica SP8 confocal microscope.

RESULTS

The obvious difference in the invasion of MDA-MB-231 cells towards empty microenvironment generated only by matrigel having either serum-containing or serum-free medium revealed that LOC system is working. A differential invasion of MDA-MB-231 cells was observed towards in vitro generated microenvironments. Breast and lung microenvironments were the most preferred sites for MDA-MB-231 cells to invade, while the invasion towards bone microenvironment was limited. The non-metastatic MCF7 cells showed no invasion towards neither conditions.

DISCUSSION AND CONCLUSIONS

The results here revealed that tissue-specific cell lines in a LOC system can be used to mimic target tissue microenvironments and evaluate different invasion capacities of breast cancer cell lines to different target sites.

A novel autologous vascular cell bioassay with the potential for patient phenotyping and tissue engineering

B. Ahmetaj-Shala[1], R. Kawai[1,2], I Marei[1,3], C. Shih[4], B. Konain[1], R. Mongey[1], N. S Kirkby[1] and J. A. Mitchell[1]

[1]Cardiovascular Division, National Heart and Lung Institute, Imperial College London, SW36LY, UK.
[2]Daiichi-Sankyo Co.Ltd., Medicinal Safety Research Laboratories, Tokyo, Japan.
[3]Qatar Foundation Research and Development Division, Doha, Qatar.
[4]National Defense Medical Center, Department of Pharmacology, Taipei, R.O.C., Taiwan.
Corresponding Author Email: j.a.mitchell@ic.ac.uk

INTRODUCTION

Blood vessels are comprised of endothelial and smooth muscle cells, however growing these cells directly from human blood vessels is difficult. Vascular cells grown out from blood progenitors offer a noninvasive solution. Although 'blood outgrowth endothelial cells' (BOECs) have been studied extensively there has been very little research on 'blood outgrowth smooth muscle cells' (BO-SMCs). We suggest that combining autologous cultures of BOECs and BO-SMCs from healthy donors and patients with cardiovascular (or other) disease could revolutionize how we study cardiovascular function, stratify patient treatment or screen drugs. Here we describe, for the first time, pilot data where combinations of autologous BOEC, BO-SMCs and peripheral blood monocytes (PBMCs) from donors have been studied in co-culture with the ultimate future aim of developing a 3D co-culture system.

METHODS

Autologous BOECs and BO-SMCs were isolated and expanded as described previously from PBMCs of healthy donors (n=3). Both cell types were characterised by morphology and FACS analysis for CD31 and α-smooth muscle actin (α-SMA), markers for ECs and SMCs respectively. A co-culture of BO-SMCs, BOECs and PBMCs was created and the vascular mediators endothelin-1 (ET-1) and prostacyclin (measured as 6-ketoPGF$_{1\alpha}$) released into the media after 24 hours measured using ELISA. Data are given as mean±SEM (n=3) and analysis was performed using an unpaired Students t-test (*$p<0.05$).

RESULTS

BOECs were obtained from ≈80-90% of donors and BO-SMCs from ≈20% of donors. Each cell type showed typical morphology and expressed requisite phenotypic markers (BOECs, CD31 positive; BO-SMCs α-SMA positive). Autologous co-culture studies showed that BOECs, but not SMCs and PBMCs, are the predominant source of ET-1 release (109.1±26.0 pg/ml) which was not affected by the addition of SMCs or PBMCs. PBMCs, SMCs and BOECs all produced prostacyclin (105.0±24.4pg/ml, 176.8±56.3 pg/ml and 193.5±40pg/ml respectively). PBMCs significantly exacerbated prostacyclin release by BOECs in combination with SMCs.

DISCUSSION AND CONCLUSIONS

Whilst still exploratory, our data provides an important platform for clinical translation in vascular biology research areas such as drug testing, vascular cell therapy, organ regeneration and other stem cell applications.

Can Articular Cartilage Degeneration Be Attributed to Underlying Subchondral Bone Health?

T. Hopkins[1,2], N.J. Kuiper[1,2], K. Wright[1,2], S. Roberts[1,2], J-H. Kuiper[1,2].

[1]The Robert Jones and Agnes Hunt Orthopaedic Hospital, Oswestry, SY10 7AG, UK.
[2] The Institute for Science and Technology in Medicine, Keele University, Stoke-on-Trent, ST5 5BG.

Corresponding Author Email: t.hopkins@keele.ac.uk

INTRODUCTION

Osteoarthritis is now regarded as a disease of the whole articular joint, with articular cartilage (AC) degeneration being seen as one symptom of an 'organ-level failure', rather than the defining feature. This has led to an increased interest in the other joint tissues. Of particular interest is the subchondral bone (SB), which has a close physical association and often demonstrates degenerative changes that parallel or even precede those seen in the AC. This includes bone-marrow lesions, which are often found distal to areas of chondropathy on MRI. Here we report a comparison of cells from regions of the SB with and without pathological changes from a single patient and the development of an in vitro model system to assess the interactions between chondrocytes and SB cells isolated from healthy and diseased regions.

METHODS

Pre-operative MRIs of patients scheduled for total knee replacement (TKR) were assessed to locate SB lesions distal to the tibial plateau. Bone chips were collected separately from macroscopically 'normal' and lesioned SB sites on the excised plateau and used for outgrowth cultures of SB osteoblasts. Half of each lesion and 'normal' site was left intact to allow for histological analysis. mRNA was extracted from each of the confluent cell populations and reverse-transcribed to cDNA. Subsequently, qRT-PCR was carried out to interrogate several genes assessing the chondrogenic and osteogenic characteristics of each cell population. A trans-well co-culture system was developed to assess the interactions between chondrocytes in agarose 3D culture above monolayer cultures of SB cells.

RESULTS

A bone lesion site was found distal to the area of greatest cartilage erosion (ICRS grade 4). The different bone regions identified on MRI showed distinct histological characteristics, including the presence of bone marrow fibrosis and cartilaginous regions in the subchondral bone of the lesioned site, but not in the 'normal' site. However, no apparent differences were found between the two populations of SB osteoblasts that grew from the distinct regions, including cell growth characteristics, gene expression and alkaline phosphatase staining intensity. Results from co-culture systems will be available for presentation at the Organ on a Chip event.

DISCUSSION

We plan to build on this work with further patients and subsequently to use cells isolated from regions with and without subchondral bone pathologies in our trans-well co-culture model to assess their effect on the quantity and quality of extracellular matrix components produced by donor-matched chondrocytes.

Path selection of a spherical capsule in a microfluidic branched channel

Z. Wang[1], A. V. Salsac[2], D. Barthes-Biesel[2], W. Wang[1], Y. Sui[1]

[1]School of Engineering and Materials Science, Queen Mary University of London, London, E1 4NS, UK
[2]Université de Technologie de Compiègne, CS 60319, 60203 Compiègne, France

y.sui@qmul.ac.uk

INTRODUCTION

Path selection of a deformable capsule suspended in an external fluid flowing through a branched microchannel is an important problem that is relevant to understand blood-related flows in the microcirculation, aerosol particle deposition in respiratory airways, or to develop microfluidic technologies to separate or enrich suspensions. In the present study we consider an initially spherical capsule flowing through a straight channel with an orthogonal lateral branch, focusing on the influence of the geometry of the side branch on the capsule path selection.

METHODS

We use computer simulation, with a well-tested three-dimensional immersed-boundary lattice-Boltzmann method. The capsule is enclosed by a strain-hardening membrane and contains an internal fluid of the same viscosity as the fluid in which it is suspended.

RESULTS

A major finding is that, at equal flow rate split between the two downstream branches, the capsule will enter a branch which is narrow in the spanwise direction, but will not enter a branch which is narrow in the flow direction. For $Re < 5$, this novel intriguing phenomenon primarily results from the background flow, which is strongly influenced by the side branch geometry. For higher values of Re, the capsule relative size and deformability also play specific roles in the path selection. The capsule trajectory does not always obey the classical Fung's bifurcation law[12], which stipulates that a particle (in Fung's case, a red blood cell) enters the bifurcation branch with the highest flow rate. We also consider the same branched channels operating under constant pressure drop conditions and show that such systems are difficult to control due to the transient additional pressure drop caused by the capsule.

DISCUSSION AND CONCLUSIONS

The present results obtained for dilute systems open new perspectives on the design of microfluidic systems, with optimal channel geometries and flow conditions to enrich cell and particle suspensions.

[12] Y. C. FUNG (1973) Stochastic flow in capillary blood vessels. Microvasc. Res. 5, 34–48.