Poster session 3: Application for Biofabrication – engineerd tissues 2

P3.1

A modular approach to the biofabrication of a tissue-engineered auricular implant

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Aim: The deformed human auricle is traditionally reconstructed using a carved framework of the patient's own rib cartilage. The disadvantages associated with this procedure lead to increased interest in tissue engineering approaches for the creation of a live implant. However the majority of attempts is plagued by resorption and deformation issues. We hypothesize that these issues are related to nutrient limitation in large constructs such as the human ear hindering growth and development of the immature neocartilage and causing bad quality cartilage or even cell death and tissue degradation. We therefore propose a modular approach in which biofabricated parts of the implant are separately matured until sufficient mechanical strength is attained to withstand the contractive forces of the skin prior to attachment. We aim to demonstrate the feasibility of creating these modular implants using a hybrid bioprinting approach.
Methods: The design of the complete implant is based on the cartilage framework used in auricular reconstruction surgery. For the modules it was ensured that the maximum diffusion distance did not exceed 2 mm. The credibility of the design of the implant was evaluated placing PLA prototypes under a rubber skin. The performance of porcine auricular chondrocytes in GelMA hydrogels was evaluated in vitro (2 weeks) and in vivo (6 and 12 weeks subcutaneous in nude mice). Then hybrid bio-constructs were generated composed of a cell-laden hydrogel reinforced with a fiber scaffold (PCL).
Results: Attached PLA modules form a complete auricular implant with an authentic auricular shape when placed under a tight rubber skin. Auricular chondrocytes produced abundant cartilage matrix in vitro and this was even more prominent after 6-12 weeks of subcutaneous implantation. The reinforcing fiber network appeared to be flexible and hybrid modules with considerable mechanical properties and with convincing structure and shape were successfully created.
Conclusions: The results from this study demonstrate the feasibility of the biofabrication of a hybrid auricular implant using a modular approach. Auricular chondrocytes performed well in the GelMA hydrogel and the PCL reinforcing network provided significant mechanical stability. Further studies are aimed at maintaining size and shape of a tissue engineered auricular construct in vitro and in vivo.

P3.2

Biofabrication of interconnected multi-scale microvessel networks within a gelatin methacrylamide hydrogel system for bone engineering

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For clinical translation many bone tissue engineering (TE) approaches have aimed at mimicking aspects of the natural extracellular matrix (ECM) such as the mechanical strength and porosity. While these strategies show promising results current TE strategies are often limited in size due to a lack of nutrients provided by vascularization[1]. Considering the highly vascularized network existing in natural bone tissue we hypothesized that in-vitro prevascularization is a vital step in TE for the development of bone constructs. Previously the possibility to generate functional vascular networks within a gelatin methacrylamide (gelMA) hydrogel system was demonstrated[2]. Here we present a three dimensional (3D) bioprinting approach for the fabrication of cell-laden gelMA constructs containing endothelial cell-lined channels. Within the gelMA construct itself we aimed to achieve formation of a capillary-like network and to demonstrate interconnectivity of this network with the endothelial cell-lined channels. In addition our goal was to show simultaneous osteogenesis[3] within the gelMA construct.
Constructs were fabricated by co-printing gelMA with Pluronic F-127 wherein the latter was used as sacrificial material to create channeled constructs. Human endothelial colony forming cells (hECFCs) between passages 14 and 18 were seeded into the channels of the gelMA constructs. The gelMA hydrogel included either a monoculture of human multipotent stromal cells (hMSCs) or a co-culture with hECFCs. GelMA hydrogels were cross-linked through UV-initiated radical polymerization.
We are currently investigating the optimal conditions for simultaneous formation of the endothelial lining of the channels and the formation of a capillary-like network and osteogenic differentiation in the gelMA construct. Alkaline phosphatase (ALP) levels are measured as an indicator for osteogenic differentiation. To identify the vascular cells the expression of CD31 and cell morphology are determined in whole mount constructs.
In this study we show long-term cell survival of hECFCs and hMSCs in a 3D bioprinted gelMA construct. Furthermore simultaneous differentiation towards both the osteoblastic lineage and the formation of lined vascular channels were shown within this system. This prevascularized tissue approach could be scaled-up and perfused with bioreactors to overcome current TE size limitations.

References:
1. Nguyen LH et al. Tissue Eng Part B Rev. 2012 18 363.
2. Chen YC et al. Adv Funct Mater. 2012 22 2027.
3. Gawlitta et al. Tissue Eng Part A. 2012 18 208.

P3.3

A new method for fabricating cell-embedded ECM capsules and ECM-loaded spheroids

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Aim: Compared with monolayer cultures three-dimensional culture of hepatocytes is better to imitate the environment of the liver. A typical three-dimensional culture is to embed cells in extracellular matrix (ECM) capsule. In general capsule of ECM gel is formed by an emulsion method. However usage of oil causes cellular damage. In this study we aimed to establish a new method for forming cell-embedded ECM capsules without using oil. We also report "ECM-loaded spheroids" which is a different type of cell-embedded ECM capsules having less amount of ECM.
Methods: To form ECM capsule we utilized the methylcellulose (MC) medium [1]. We injected 1 µl of undiluted or diluted ECM solution (Matrigel or collagen) suspending 2000 Hep G2 cells into the MC medium.
Results: We found that the cellular damage was obviously reduced when we made capsules by the MC medium. However the albumin secretion activity of the Hep G2 cells in ECM capsule was half level compared with normal multicellular spheroids. The reason of functional failure was estimated that too much gels inhibited cell-to-cell interaction. We therefore tried to reduce the concentration of gel to preserve cell-to-cell interaction. When we used 30-times diluted Matrigel the thickness of gel between cells was significantly reduced and this condition was similar to normal spheroids which cell-to-cell interaction was tightly interaction. In this study we called this kind of spheroid with ECM as "ECM-loaded spheroid". When we used undiluted ECM the diameter of ECM capsules was about 1 mm. However the diameter of ECM-loaded spheroids was about 300 µm. This spheroids containing a little amount of ECM were almost the same diameter as normal spheroid. The albumin secretion activity of ECM-loaded spheroids was higher than that of normal spheroids. The effect of Matrigel on the Hep G2 cells was not observed when we used type I collagen gel showing that the induction of hepatic function depends on the type of ECM.
Conclusions: We established a new method to fabricate ECM capsules and ECM-loaded spheroids and ECM-loaded spheroids demonstrated higher hepatic function than conventional spheroids.

[1] Kojima N. Takeuchi S. Sakai Y. "Rapid aggregation of heterogeneous cells and multiple-sized microspheres in methylcellulose medium" Biomaterials 33 2012 4508–4514.

P3.4

Influence of mesoporous silica nanoparticles coated electrospun scaffolds on THP-1 recruitment

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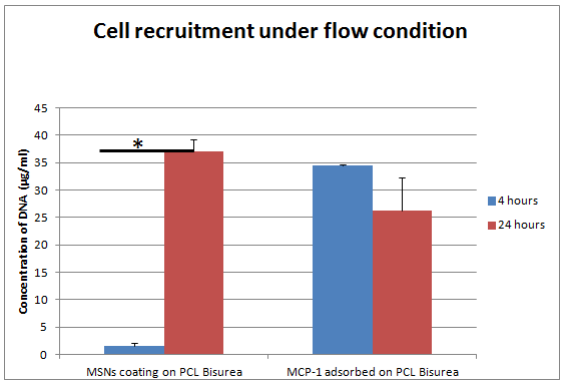
Introduction: In-situ TE is an emerging approach for replacing deteriorating living tissues with a synthetic scaffold that transforms into living tissue.As the nature of infiltrating cells and their differentiation is crucial to initiate immunomodulatory cytokine cascades rapid monocyte infiltration and recruitment have gained a lot of interest.The physiological hemodynamic condition also plays an important role in response of the cells to the 3D scaffold.Mesoporous silica nanoparticles(MSNs) are commonly applied for prolonged release of incorporated drug/bioactives.MSNs of 400nm diameter were synthesised and loaded with MCP-1(Monocyte Chemoattractant Protein-1).An appropriate microenvironment for cell recruitment was provided by coating electrospun PCL bisurea(PCLBU)scaffolds with MSNs.To test the recruitment of human monocytes(THP-1) on MSN coated scaffolds THP-1 cells were cultured in static and hemodynamic conditions.
Method: MSNs were characterized for morphology and degradation for 10 days. Electrospun PCLBUscaffolds were coated with an ethanol-MSN solution by drop casting.As a control PCLBU(from Symochem) scaffolds were coated with MCP-1.Scaffolds were exposed to a THP-1 suspension for up to 24 hours under static and cyclic flow conditions(1).Scaffolds with THP-1 were analysed by staining with phalloidin(actin) and DAPI(nuclei) and MSNs were labelled with TRITC(red).Release of MCP-1(20ng/ml) loaded MSNs was measured with ELISA.
Results: MSN degradation was observed at different time points till day 10 as evident by a decrease in diameter of MSN. In static condition no significant difference in cell infiltration between MSN coated and MCP-1 scaffolds.However MSN-coated scaffolds under flow showed significant increase in cell recruitment over time.As shown in Figure 1 a decrease in cell number was seen for MCP-1-PCLBU scaffolds as determined by DNA content in the scaffolds.Similar results were obtained with fluorescent images. An increased number of cells attached to MSNs coated scaffold compared to control.MSNs may absorb serum proteins thereby increasing cell recruitment(2).Incorporation of MCP-1 in MSNs in the scaffolds is expected to give sustained and comparable release of MCP-1 and enhance cell recruitment over time.A cumulative release of MCP-1 observed from MSNs MCP-1 loaded under both static and dynamic conditions.
Conclusion: The unloaded MSNs coated scaffolds are biocompatible and support THP-1 recruitment on electrospun scaffolds.The next step would be to optimize MCP-1 concentration for efficient recruitment and incorporate additional bioactives in MSN within the scaffolds to promote cell differentiation.
This work is supported by the Dutch government to the Netherlands Institute for Regenerative Medicine
1)Smits et al T.E. part C;Methods 2012 18(6);475-485 2)Monopoli MP et al. J Am Chem Soc 2011 133:2525-2534



Cell recruitment on scaffolds with MSN and with MCP-1 absorbed on PCL bisurea. The cell count was performed at 4 hours and 24 hours. A significant increase in cell recruitment observed on MSN coated PCL bisurea scaffolds.

P3.5

3D multicell simulation during the self-formation of thyroid follicles: a computational approach for the biofabrication of tissues

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Aim: The study of how cells interact and the principles of biological self-assembly during the development of 3D tissue engineered constructs are very important during the biofabrication of organs. Several multicellular spheroids or cellular aggregates recovered by hydrogel (bio-ink particles) have been used as 'building blocks' for tissue and organ printing. However so far their fusion adhesion mitosis growth secretion differentiation phenomena have not been totally elucidated. In silico experiments can help understanding this complex biological system and later help to model the organ blueprint. Thus several researchers have used mathematical models to explore cellular and multicellular behaviors in the context of tissue formation and their subsequent dynamics. Russian Scientists have been developing a bioprinted thyroid gland using stem cells (SC) and endothelial cells (EC) as 'bioink' because this anatomy is a simplistic structure. However there are no studies in silico that comprises the 3D organization and interactions of the aggregates during the thyroid follicular cells differentiation. In order to understand the biological complexity of these events our aim provide a review of the follicular organization of TFC (Thyroid Follicular Cells) and it's in silico replication whereas the TFC are considered as prerequisite for thyroid hormone biosynthesis which occurs under physiological conditions extracellular at the Thyroid Follicular Cells colloid (TFCc) interface using the CompuCell3D.
Methods: The CompuCell3D was used and is an open-source simulation environment for multicell single-cell-based modeling of tissues organs and organisms.
Results: We presented a simplified 3D multicell simulation of angiogenesis during the self-formation of thyroid follicles which can be easily extended and adapted to describe other biological phenomena allowing us to study how the EC and SC interact each other and modulate the growth size differentiation and morphology of the multicellular spheroids currently used in the tissue biofabrication.
Conclusions: The computational approach of thyroid follicles and angiogenesis is important in biofabrication and it's in silico modeling can help the design of bioengineered tissues. This work is motivated by the growing need to understand morphological changes during tissue fabrication in bioengineering in particular in the emergent field of 3D bioprinting.
Figure 1 illustrates the differentiation process of stem and endothelial cells into follicles and blood capillaries of the thyroid.

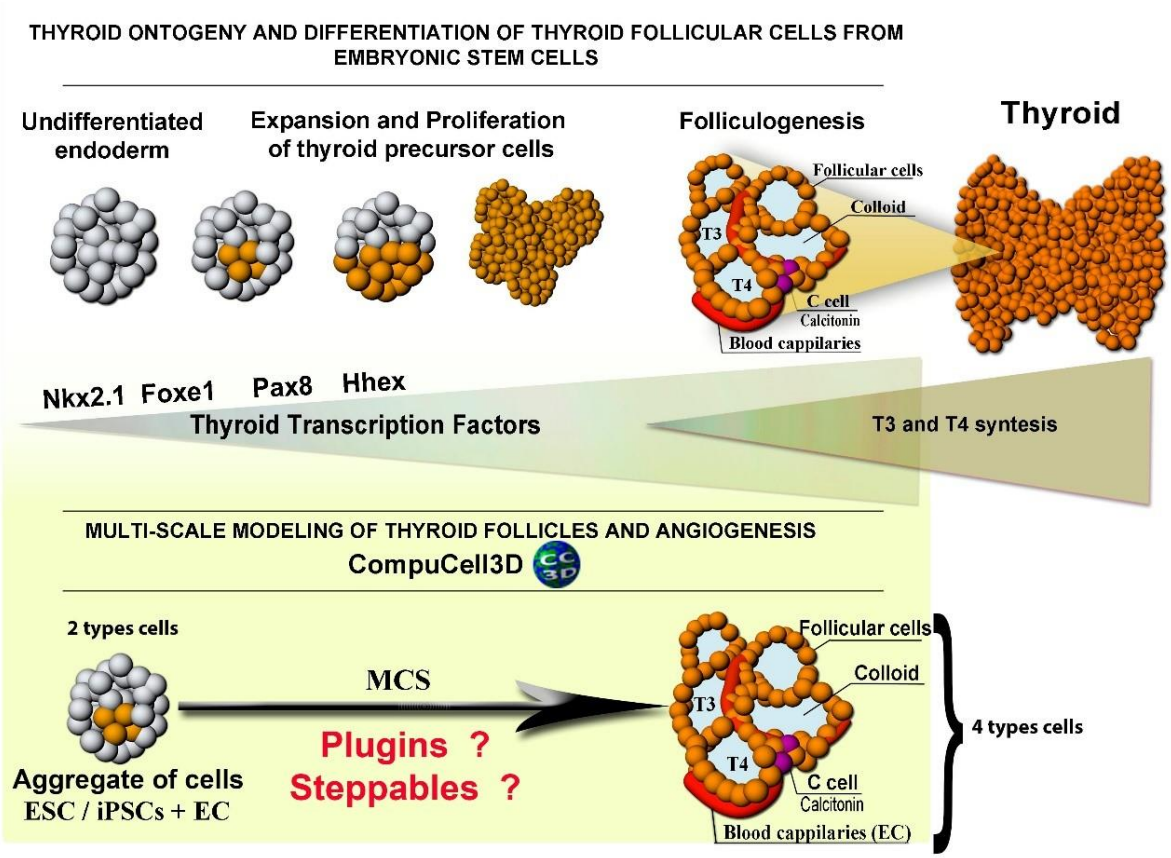

Figure : illustrates the differentiation process of stem and endothelial cells into follicles and blood capillaries of the thyroid.

P3.6

Distribution of follicles in bioprinted organ construct of mouse thyroid gland

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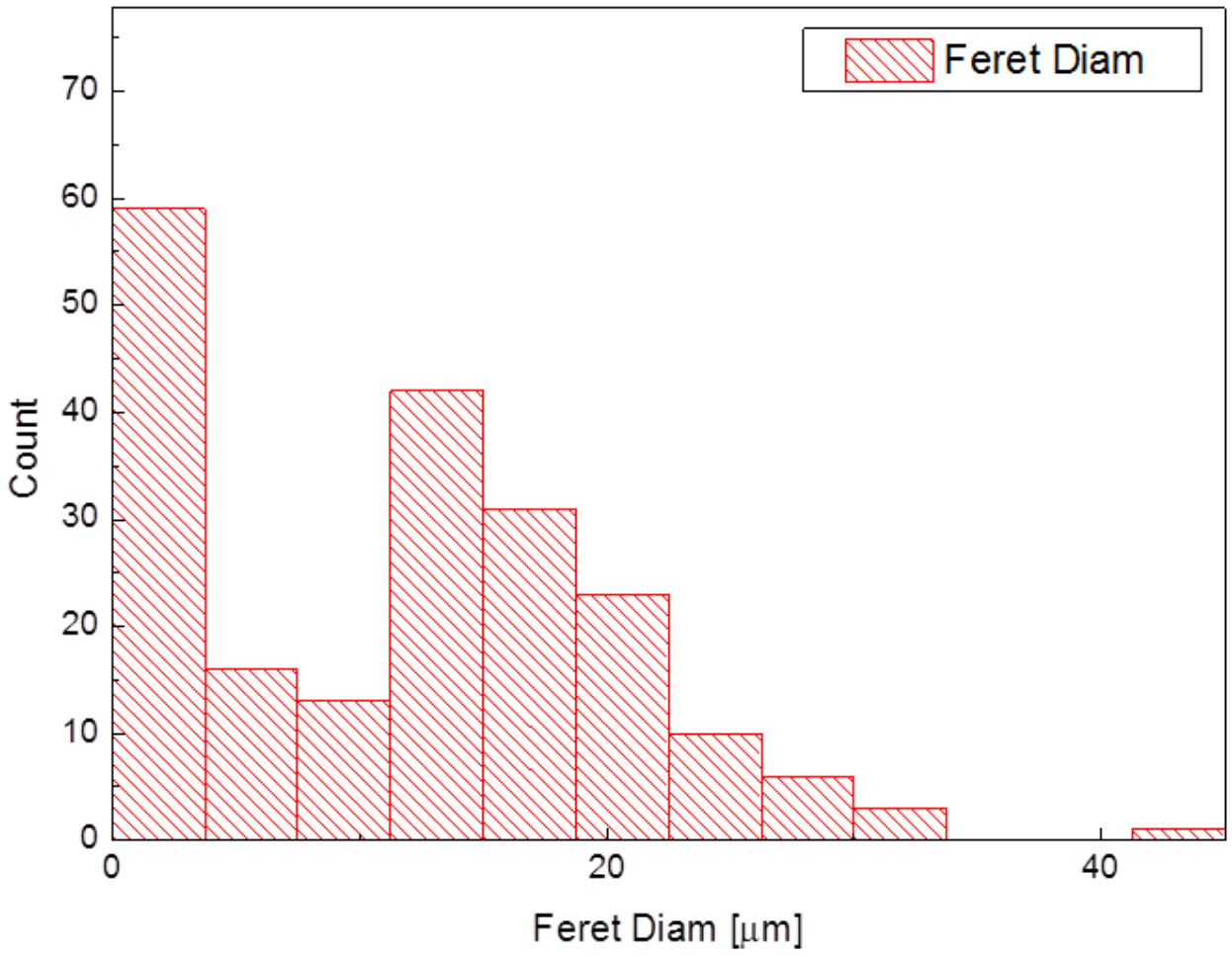
Aim: The main structural-functional components of thyroid gland responsible for realization of its function are angio-follicular units. In order to achieve the desirable optimal functionality on organism level the number of angio-follicular units in bioprinted organ construct must be adequate and be carefully calculated. Such calculations are integral parts of development of rational design and digital model of bioprinted organ construct and they must be based on reliable morphometric data and certain justifiable assumptions. The average diameter & volume density of follicles of thyroid glands have been morphometrically estimated on histological sections of embryonic newborn & adult mice.
Methods: Using specific characterization software average diameter volume density & absolute number of follicles have been estimated. Care was taken to directly differentiate them from mouse embryonic stem cells and implanted under mouse kidney capsule which was capable of compensating lost thyroid gland function (inhibited by treatment with radiactive iode I-131). Using specific calibrated image superimposed on the original one the scaling of images provided with the thyroid gland was extracted (120px=300µm) and calculation of the distribution of the implanted follicles was achieved. However besides the somewhat obvious particles visible on the main area of implanted follicles a plethora of other sites were detected that reflect the effect of other parameters (depth color sorption/adsorption) on the imaging technique which were successfuly filtered out.
Results: Considering this analysis average diameter is 12.035 µm Figure 1. Volume is 477927.0143 µm3. At the initial data on average there is one follicle per 6.3375 micron in the y-direction and 15.13 micron in the x-direction (more statistical uncertainty).
Conclusions: It has been shown that just 5 000 injected thyroid follicles are sufficient to maintain physiological level of thyroxin. Reconstruction based on serial histological sections of rounded 15 days embryonic explants of mouse thyroid gland demonstrated that they contain approximately 1000 follicles with average relatively small diameter (12µm). Thus bioprinting organ construct biofabricated from 5 rounded embryonic mouse explants could gaurantee a desirabe physiological level of thyroxin production. Taken together our data strongly suggest that bioprinted organ construct consisted of 15.000 thyroid follicles will be able to maintain physiological level of hormone thyroxin in mouse blood.



Size distribution of injected follicles in thyroid gland

P3.7

Tissue gun: design fabrication and testing of in situ 3D bioprinter

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Aim: In situ 3D bioprinting is a novel exciting area of research and development on interface of surgical robotics and 3D bioprinting which in case of clinical success will enable rapid in situ biofabrication of 3D human tissue directly in the surgical operation room. Our aim was to design fabricate and test so-called tissue gun or in situ 3D bioprinter which will allow precision robotic hand-aided delivery of tissue spheroids (for example chondrospheres and osteospheres) encaged into iron coated microscaffold using principles of magnetic levitation and fibrin hydrogel spraying.
Methods: Tissue gun has been designed and constructed by combination of three main essential constructive components: i) Duplojet – a fibrin hydrogel spraying device; ii) magnetic solenoid; and iii) commercially available robotic hand or articulated robot . The mechanically enhanced microscaffold has been fabricated using two photon polymerization device from organically modified ceramics or photo-sensitive biomaterials. Microscaffolds have been coated with iron using laser-assisted electroless plating. Tissue spheroids have been biofabricated by cell seeding of recessions of micromolded non-adhesive agarose hydrogel containing microscaffolds already plated inside recessions. Viability morphology and material properties of biofabricated in vitro cartilage and bone tissue constructs have been estimated using standard tests and methods.
Results: Theoretical studies using finite element analysis and experimental testing using parallel plates compression device - microsquisher demonstrated that concentric microscaffolds have dramatically enhanced material properties. Laser-assisted electroless iron plating of microscaffold enabled their rapid translocation in magnetic field generated by magnetic solenoid. Fibrin hydrogel spraying allowed fixing delivered tissue spheroids encaged into microscafolds in desirable space of cartilage and bone defects. The fibrin hydrogel was also permissive for sequential tissue spheroids fusion. In vitro testing demonstrated principal feasibility of rapid in situ biofabrication of cartilage and bone tissue constructs and thus treatment of the damaged areas or defects of cartilage and bone using originally designed and fabricated tissue gun or in situ bioprinter.
Conclusions: Our data demonstrated that tissue spheroids encaged into microscaffold coated with iron by laser-assisted electroless plating could be precisely delivered using magnetic levitation and articulated robotic hand controlled delivery. The sequential fibrin hydrogel spraying enables tissue fusion of closely packed tissue spheroids and rapid in situ biofabrication of cartilage and bone tissue constructs. Our next logical and desirable steps are performing of preclinical testing of tissue gun in animal models and sequential clinical translation of in situ bioprinting technology.