Free paper session 10: In vitro models I

F10.1

A 3D bioprinted bone marrow niche to study normal hematopoiesis and bone-residing malignancies

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Introduction: The bone tissue is intricately connected to the bone marrow which constitutes the niche needed for normal haematopoiesis. The niche notably supports and tightly regulates the hematopoietic stem cells (HSC) the cells at the origin of all blood cell types. The bone marrow is also the preferred location of several malignancies among which multiple myeloma (MM). Studying the bone marrow niche is essential to understand the normal hematopoiesis as well as the growth and metastasis process of bone marrow malignancies with their devastating influence on the surrounding bone tissue.
Aim: Both HSCs and incoming MM cells depend on the bone marrow niche to self-renew and to massively proliferate respectively. At present it is not possible to culture these primary cells for a prolonged period of time in vitro. In order to gain better insight in the cellular and molecular interactions taking place in the bone marrow niche it is necessary to have a reliable culture model. In this project we aim to construct this reconstituted bone marrow microenvironment that will be used as a culture model using three-dimensional (3D) bioprinting technology.
Methods: Multipotent mesenchymal stromal cells (MSCs) endothelial progenitor cells (EPCs) hematopoietic progenitor cells (HPCs) or MM cells were cultured in different hydrogels for up to 2 weeks. Cell viability was assessed at different time points. The optimal hydrogel in terms of cell viability and printability was selected for further co-culture experiments. Hydrogels ceramic particles and multiple cell types were combined at varying ratios using 3D bioprinting. To distinguish the different cell types within the co-culture cells were fluorescently labelled. The survival of either HPCs or MM cells was analysed for varying co-culture conditions.
Results: A 3D bone marrow niche was developed supporting the in vitro culture of primary CD34+ HPCs for weeks without using feeder layers. Primary CD138+ MM cells could also be cultured in this model enhancing their in vitro survival from days to weeks displaying not only survival but also proliferation.
Conclusions: A 3D bioprinted bone marrow niche was reconstituted which provides a tool to propagate both hematopoietic progenitor cells as well as multiple myeloma cells. This two sided model is useful for further investigation into hematopoiesis skeletal cancer biology and osteogenesis.

Acknowledgements:
This study was funded by the UU Life Sciences Seed Grant.

F10.2

Open-air Chamber Inkjet head for stable ejection of cell suspensions

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In vitro development of three-dimensional (3D) tissue is in high demand for regenerative medicine and drug screening. Piezoelectric drop-on-demand inkjet heads are attractive devices for patterning living cells with high precision by taking advantage of their features allowing very small droplets to be ejected with high speed [1]. However applying these inkjet heads to tissue construction is not easy because the sedimentation of cells at the flow channel and nozzle often leads to a clogging of the head.
Here we present a novel inkjet head specialized for on-demand ejection of cell suspensions. The head consists of an open-air liquid chamber without a flow channel a membrane with a nozzle for ejecting droplets and a piezoelectric vibrator. Droplets are formed by the membrane vibration which is generated by the pulse actuation of the piezoelectric vibrator. During the non-ejecting period the membrane is vibrated softly to prevent the sedimentation of the cells inside the chamber by the sinusoidal actuation of the vibrator.
We performed an initial feasibility study by ejecting a suspension of normal human dermal fibroblasts (NHDF) using this inkjet head. By combining the ejecting actuation and the stirring actuation the number of cells per droplets remained stable for more than thirty minutes. The cell viability after thirty minutes of actuation was more than 90% both in the chamber and in the jetting droplets.
In order to construct the previous work we reported simple and unique technologies
Next we tried to construct 3D cardiac tissue models by using cardiomyocytes(CM) derived from human induced pluripotent stem cells. In the previous work we reported a unique technology termed “cell accumulation technique” to construct multilayered thick tissue models by cell surface coating with nanometer sized extra cellular matrix (ECM) films [2]. Droplets with ECM coated CM were ejected into culture medium by repeating cycles of fifty seconds ejecting actuation and ten seconds stirring actuation. After four days of incubation the cells in the constructed cardiac tissue showed synchronous beating. From these results this inkjet head has proven to be suitable for the effective ejection of living cells. It would be a promising tool for the biofabrication of living tissues.

Acknowledgements:
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[2] Matsusaki M et. al. Angew. Chem. Int. Ed. 46 46:4689-4692 (2007)

F10.3

Three-dimensional bioprinting of embryonic stem cells

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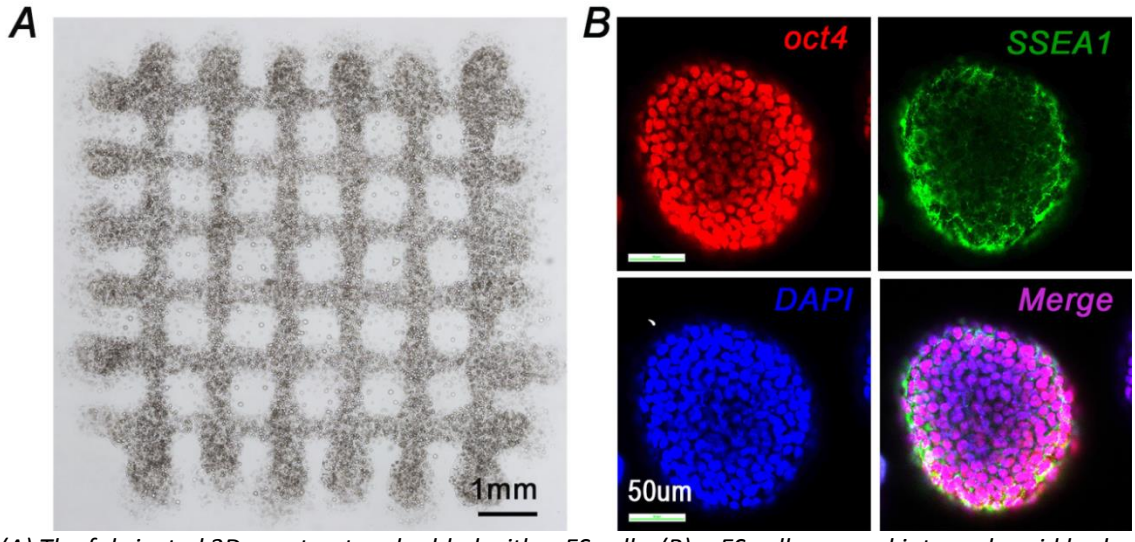
With the ability to manipulate cells temporarily and spatially into three-dimensional (3D) tissue-like construct 3D bioprinitng technology was used in many studies to facilitate the recreation of complex cell niche and/or to better understand the regulation of stem cell proliferation and differentiation by cellular microenvironment factors. Embryonic stem cells (ESCs) have the capacity to differentiate into any specialized cell type of the animal body generally via the formation of embryoid body (EB) which mimics the early stages of embryogenesis. In this study extrusion-based 3D bioprinting technology was utilized for biofabricating ESCs into 3D cell-laden construct. The influence of 3D printing parameters on ESC viability proliferation maintenance of pluripotency and the rule of EB formation was systematically studied in this work. Results demonstrated that ESCs were successfully printed with hydrogel into 3D macroporous construct. Upon process optimization about 90% ESCs remained alive after the process of bioprinting and cell-laden construct formation. ESCs continued proliferating into spheroid EBs in the hydrogel construct while retaining the protein expression and gene expression of pluripotent markers like Oct4 SSEA1 and Nanog. In this novel technology EBs were formed through cell proliferation instead of aggregation and the quantity of EBs was tuned by the initial cell density in the 3D bioprinting process. This study introduces the 3D bioprinting of ESCs into a 3D cell-laden hydrogel construct and showed the production of uniform pluripotent high-throughput and size-controllable EBs which indicated strong potential in ESC large scale expansion stem cell regulation and fabrication of tissue-like structure and drug screening studies.

References:

[1]. Yan Y et al. Biomaterials. 2005; 26(29):5864-71.
[2]. Tasoglu S et al. Trends in biotechnology. 2013; 31(1):10-9.
[3]. Ouyang L et al. Biofabrication. 2015; 7(1):015010.



(A) The fabricated 3D construct embedded with mES cells. (B) mES cells growed into embryoid body with the high maintenance of pluripotency after culturing for one week.

F10.4

Combining bioprinting and microcarrier technology for osteochondral tissue engineering

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Aim: A major challenge in articular cartilage regeneration is to recapitulate the zonal organization. Additional requirements to design functional osteochondral grafts are: i) high cell content which is hard to achieve with standard expansion methods and ii) inclusion of cues to guide cell differentiation in each specific layer.
These demands can be addressed combining bioprinting with microcarrier (MC) technology which allows cell expansion and phenotype control. This work aims to fabricate living constructs via bioprinting of cell-laden microcarriers (MCs) and to assess their potential as components in ostechondral models.
Methods: Mesenchymal Stromal Cells (MSCs) were cultured on collagen-coated polylactide (PLA) MCs (diameter ≈ 120 µm) and encapsulated in a gelatin methacrylamide/gellan gum (GelMA/GG) hydrogel (10% and 1% w/v). The bioink was extruded UV-crosslinked and its mechanical properties were tested. MC-MSCs in gels were assessed for cell morphology viability and osteogenic differentiation (ALP osteocalcin and alizarin red staining). Bioprinting was performed via layer-by-layer deposition of bioink strands with an extrusion-based system. The bioink included MC-MSCs precultured in T-flasks or in a spinner flask bioreactor. Statistical significance was assessed performing Student's t-test (n=3 or 5 $p<0.05$).
Results: GelMA/GG with up to 50 mg/mL MCs was used as a printable bioink. MCs increased the gel compressive modulus up to 2-fold. MSCs in the gel retained a spread morphology with well-defined actin fibers and cell-cell contacts when expanded on the MCs whereas cells encapsulated without MCs were rounded. MCs supported MSC osteogenic commitment inducing matrix mineralization and improving osteocalcin secretion compared to controls. Living constructs were fabricated with high concentrations of MC-MSCs obtained from bioreactor culture. Moreover a bilayered osteochondral model was printed with MC-laden gel as a subchondral bone layer and a gel-only region recapitulating the cartilage zone.
Conclusions: MCs-laden GelMA/GG was proven to be a promising composite material for biofabrication. MCs acted as a mechanical reinforcement for the soft gel. MC-MSCs complexes encapsulation improved osteogenic differentiation and supported bone deposition. MC culture is of great interest for MSC expansion and scaling-up of bone tissue bioprinting and to build advanced constructs for zonal osteochondral repair.

Acknowledgements:
The authors acknowledge the financial support from the Spanish MECD the Dutch NIRM and the Dutch Arthritis Foundation.

F10.5

Cardiac fibroblast laden gelatin methacrylate hydrogels: a mimicry for cardiac fibrosis?

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Purpose: Upon myocardial infarction activation of fibroblasts leads to an increased production of ECM which initially maintains the integrity of the myocardium. However excessive matrix deposition results in perpetuation of pro-fibrotic signaling and eventually in progressive adverse cardiac remodeling during heart failure. Since the underlying processes are poorly defined we developed a three dimensional tunable model of cardiac fibrosis to allow physiological relevant in vitro examination of fibroblast-behavior and to gain insight in cardiac remodeling.
Methods/Results: A photocrosslinkable hydrogel composed of methacrylated gelatin (GelMA) combined with human fetal cardiac fibroblast (hfCF) was used to study fibroblast characteristics. To examine the influence of mechanical differences in a 3D environment cell behavior was studied in both 5%- and 10% GelMA models. Mechanical analysis demonstrated an elastic modulus of 4kPa and 22kPa for 5% GelMA and 10% GelMA respectively mimicking the myocardial changes upon MI. Results showed that hfCF incorporated in 5% and 10% GelMA are both less mechanically activated (3-fold decrease in alpha smooth muscle (a-SMA) expression) compared to hfCF cultured in a 2D environment. Interestingly hfCF appeared to be more active in 5% GelMA based on a higher collagen1a1 (Col1a1) expression and increased matrix metalloproteinase (MMP) activity. Since the mechanical properties of 10% GelMA most closely resembles cardiac tissue hfCF behavior was assessed in these gels in a pro-fibrotic environment. Exogenous stimulation by TGF-β1 of hfCF in 10% GelMA demonstrated activation of hfCF e.g. 2.1-fold increase in a-SMA expression ($p<0.01$) a 10.3-fold increase in COL1a1 expression ($p<0.01$) and an increased collagen deposition in the hydrogels.
Conclusions: Our data demonstrate the influence of environmental cues on fibroblast behavior and thereby underline the importance of using the appropriate 3D cell culture system for translational research. Moreover upon stimulation with the pro-fibrotic protein TGF-β1 our system demonstrates environmental remodeling. These data indicate potential for testing of new therapeutics for adverse cardiac remodeling in heart failure.