## Free paper session 1: Scaffold free biofabrication approaches

**KL1.1**

**Development of scaffold-free 3d tissue & organ fabrication by bio-3D printer**

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Inspired from bone fracture treatments in orthopedic surgery we have established a simple method to fabricate 3D scaffold-free cell construct. This method use spheroids and temporal fixator which enable placement of various types of three-dimensional cells into desired xyz positions without need of hydrogels or biochemical reactive materials. We also developed a robotic system for scaffold-free cell construction named “Bio 3D Printer”.
With this system we already successfully fabricated living cell only construct such as cartilage liver heart blood vessel and so on.
Although we just three-dimensionally placed multi-cellular spheroid roughly to fabricate pre-designed shape the cells in the construct immediately moved to re-organize as normal its original histological alignment. Some projects are now engaging in vivo study like “Bio-3D printed blood vessel” shows good animal experimental results in mini-pig A-V shunt model.
Near future with combination of the robotic technology and the bio technology we may be able to build living organs for autologous transplantation. And this multi-cell construct may be useful research tools for drug development.

**F1.1**

**Optimizing cell viability in droplet-based cell deposition**

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Biofabrication commonly involves the use of liquid droplets to transport cells to the printed structure. High-viability results have been reported but their generality with respect to cell type deposition parameters and surface properties is still poorly understood. Therefore the development of cell spraying inkjet bioprinting and laser-assisted cell transfer could strongly benefit from a clear understanding of the influence of the process parameters on cell survival which is the aim of the current work[1].
Here we present experimental results and a basic theoretical model describing the cell viability as a function of the cell-surrounding droplet characteristics. The model connects (1) the cell survival as a function of the membrane elongation (2) the membrane elongation as a function of the cell-containing droplet size velocity and viscosity [2] and (3) the substrate properties (stiffness). The model is validated by cell-viability measurements in cell spraying which is a method used for both biofabrication and treatment of burns. In these experiments the droplet size impact velocity viscosity and substrate stiffness were varied.
Improved cell survival is observed by reducing the surrounding liquid's viscosity reducing the droplet impact speed or increasing the droplet size. Impact on soft tissues dampens cell deformation during impact also improving cell viability. The analytical cell survival model is validated by these results which supports the herein proposed mechanisms for cell deformation and subsequent cell survival or death. In the application of cell spraying the cell viability can be improved by increasing the nozzle-substrate distance or reducing the spraying pressure.
In conclusion we physically model cell deformation during droplet impact and show how cell deposition parameters can be adapted to increase cell viability [3]. This approach allows for rational optimization of any droplet-based cell deposition technology by controlling the droplet characteristics and surface properties. In particular we expect that spraying devices with a monodisperse droplet size (instead of the usually employed polydisperse droplet size distribution) will result in improved cell viability in (clinical) applications. We are currently working on such solutions and will update our presentation to incorporate the progress made.

The financial support by the MIRA institute for biomedical technology and technical medicine the Foundation for Fundamental Research of Matter (FOM) and the Dutch Arthritis Foundation are gratefylly acknowledged.

1. Malda J. et al. Adv. Mater. 25 5011–28 (2013).
2. Tasoglu S. et.al. Phys. Fluids 22 1–15 (2010).
3. Hendriks J. Visser C.W. et al. Sci. Rep. 5 11304 (2015).

**F1.2**

**High-throughput engineering of single cell microniches with tunable size and elasticity**

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In all tissues individual cells are surrounded by a specialized layer of matrix molecules named pericellular matrix (PCM). The PCM is specifically adapted to support the cell's function and forms the interface between the cell and its macroenvironment  the extracellular matrix (ECM). Conventional tissue engineering lacks the resolution to fabricate constructs comprising the natural multi-level architecture of PCMs within ECM. Here  we report a microfluidic approach for high-throughput engineering of single cell microniches with tunable size and elasticity. This enables the fabrication of tissue-engineered constructs with the spatiostructural complexity that is essential for appropriate tissue function.
Single-cell-laden droplets were produced at a rate of 2kHz by emulsifying in-situ crosslinkable dextran-tyramine (Dex-TA) prepolymer conjugates  horseradish peroxidase and human mesenchymal stromal cells (MSCs) in fluorinated oil using a microfluidic droplet generator. The monodisperse emulsion was subsequently enzymatically crosslinked by controlled supplementation of hydrogen peroxide using a novel diffusion-based microfluidic platform (Fig.1a b). Specifically  we engineered microgels with controlled size (20-30µm) and elasticity (2.5-45kPa) by varying the hydrogen peroxide feed concentration. Encapsulated MSCs remained viable and metabolically active during long-term culture (4w)  as shown by live/dead (Fig.1c) and MTT assays  respectively. Furthermore  differentiation into the adipogenic and osteogenic lineage demonstrated that the cells' multipotency was preserved (Fig.1d).
Here  we report on high-throughput engineering of single cell microniches that can be tuned in a straightforward manner resembling PCMs in terms of size and elasticity. Incorporating PCMs into conventional cell-based tissue-engineered constructs could be the basis of a novel hybrid biomaterials class  mimicking the three-dimensional multi-level tissue architecture of PCMs within ECM as found in natural tissues.
M.K. and J.L. are shared senior author. Funded by Dutch Arthritis Foundation.

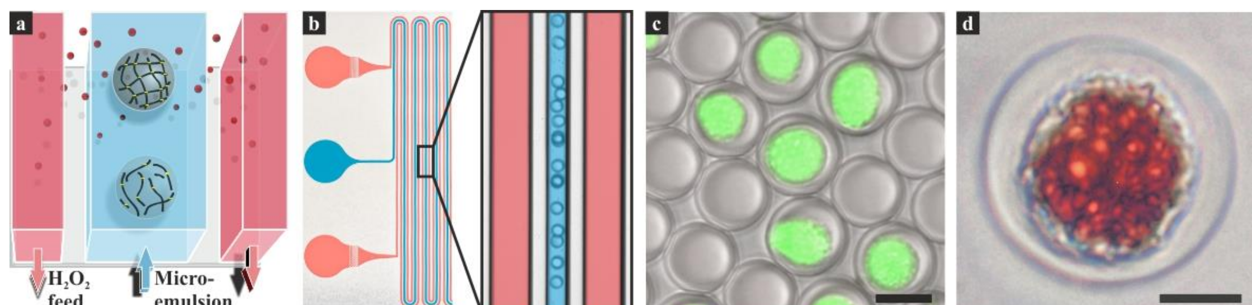

*Figure 1: a,b, Schematic representation (a) and microphotographs (b) of a novel microfluidic platform for diffusion-based enzymatic crosslinking. c, Live/dead staining of single cell Dex-TA microgels (scale bar: 20µm), Oil Red O staining of an encapsulated MSC differentiated into adipogenic lineage (scale bar: 10µm).*

**F1.3**

**3D Direct cell bioprinting of cells for tissue engineering**

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**Aim:** In this research work novel bioprinting methodologies are developed to fabricate 3D artificial biological structures using live cells directly from computer models.
**Method:** Multicellular aggregates made out of at least one cell type are prepared and optimized for direct cell printing. A novel continuous 3D bioprinting is proposed in order to extrude cylindrical multicellular aggregates at predetermined paths. A biocompatible bio-inert thermo-reversible agarose-based hydrogel was used as the support material. Mouse aortic smooth muscle (MOVAS) NIH 3T3 fibroblast cells human umbilical vein endothelial cells (HUVEC) and human dermal fibroblast (HDF) cells were cultured and various combinations of those cells were optimized as bioink. To overcome surface tension-driven droplet formation bioink made out of multicellular aggregates are prepared and optimized.
**Results:** The developed continuous bioprinting methods were used to bioprint 3D tissue constructs with live cells directly from computer models of a targeted tissue. The developed models were used to bioprint cylindrical multicellular aggregates composed of different cell mixture with hydrogel support structures. The results show that the printed constructs show rapid fusion and high cell viability after printing. MOVAS/HUVEC/NIH 3T3 multicellular aggregates fused within 3 days. The cell viability was high (97%) after printing and incubation. The printed constructs were strong enough to be handled and transferred after 4-7 day of incubation.
**Conclusions:** Novel methodologies for totally automated and continuous 3D bioprinting of tissue constructs were developed. 3D artificial biological structures were directly bioprinted from computer models using live multicellular aggregates. The results show high printing accuracy and cell-viability after printing and incubation.

*Acknowledgements:*
This research is supported by The Scientific and Technological Research Council of Turkey (TUBITAK) grant number 112M094 and the Sabanci University Internal Grant.

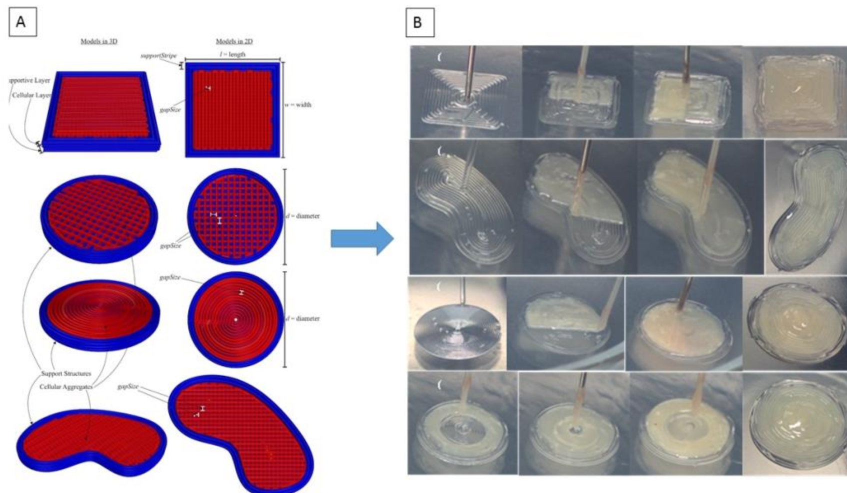


*Figure 1: (A) Computer models and path planning of various tissue constructs (B) 3D Bioprinted constructs with multicellular aggregtes*

**F1.4**

**Biofabrication of hair follicle using microfabricated PDMS spheroids chips**

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**Aim:** Hair loss is a common disorder that affects men  women  and children due to aging  diseases  and medical treatments. Recent researches revealed that hair follicles can be regenerated by merging two aggregates composed of embryonic skin-derived epithelial cells and mesenchymal cells  and transplanting them intracutaneously. In this study  microfabrication approaches were employed for one-step preparation of a large number of epithelial and mesenchymal cell aggregates with a uniform diameter and spacing.
**Methods:** Mouse epithelial and mesenchymal cells were prepared from embryonic skins. The isolated epithelial and mesenchymal cells (1.0×103~1.3×105 cells of each cell type) were suspended in a culture medium and seeded in a poly(dimethylsiloxane) culture chip which had cylindrical wells of 1 mm diameter at a density of 100 wells/cm2. To analyze the follicle formation in vitro  dermal cells were stained by alkaline phosphatase dye. Cell aggregates formed in the wells were intracutaneously transplanted to the back skin of nude mice. Immunostaining was performed to characterize hair follicle stem cells at 18 days after transplantation.
**Results:** In most previous studies  pellets of epithelial and mesenchymal cells were separately prepared and then merged them to fabricate a hair follicle for transplantation. In this study  two cell types were seeded as a mixed cell suspension on the poly(dimethylsiloxane) culture chip. Although these cells formed single aggregates in the wells at 3 days of culture  each cell type was spatially separated in the aggregates. This spontaneous separation of two cell types facilitated the preparation of a large number of cell aggregates (~100 aggregates/cm2). Alkaline phosphatase activity  an indicator of the hair follicle inductivity of dermal papilla cells  was expressed in the cell aggregates at 3 days of culture. Hair follicles were regenerated from transplanted site after 18 days of transplantation. The regenerated hair follicles also showed the hair cycle through the rearrangement of follicular stem cells.
**Conclusions:** A large number of epithelial and mesenchymal cell aggregates could be easily prepared on a poly(dimethylsiloxane) culture chip  which possessed the ability to regenerate hair follicles in vivo.

**F1.5**

**Cell sheet engineering of the glomerular capillary wall**

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**Aim:** There is an increasing need for alternative methods of toxicity screening for pharmaceutical products led by economic ethical and academic factors. A tissue engineered glomerular capillary wall would enable the development of a physiologically relevent toxicty assay which would lead to faster and cheaper toxicity trials a reduction in animal testing and provide a more physiologically relevant model to assess damage and disease mechanisms. Current in vitro nephrotoxicity approaches centre on 2D cell cultures yielding a poor phenotypic comparison and response. The filtration membrane of the glomerular capillary is formed of matrix between glomerular endothelial cells and glomerular epithelial cells known as podocyte. Coculture of the two cells fails to fully reproduce this structure in vitro. A scaffold free bilayer cell sheet can produce the extracellular matrix and cell interactions needed to form the filtration membrane.
**Method:** Human glomerular endothelial cells (hGEnC) and human podocyte (hPods) cells are grown on Nunc™ Dishes with UpCell™ surface. The UpCell surface is thermoresponsive and the cells adhere at physiological temperature. Once a confluent cell sheet is obtained it can be detached from the surface by lowering the temperature. This method ensures that the extracellular matrix and the integrity of the sheet are preserved. The cell sheet bilayer is then characterised for the filtration membrane using EM techniques.
**Results:** Endothelial cell sheets and podocyte have been successfully cultured and differentiated in static and flow conditions and in coculture. A coculture barrier was assembled using lift off surfaces. hGEnCs are viable and form monolayers on cell sheets however the hPods do not grow as well on upcell surface. Bilayers were created by sequential seeding of both cell types. In this arrangement hGEnCs cultured for 7 days on coverslips/aclar followed by seeding of podocytes resulted in good separation of the cells.
**Conclusion:** It is possible to engineer a physiologically relevant cell bilayer with intervening extracellular matrix deposition using a scaffold free cell sheet engineering approach. This is the first step towards creating an in vitro model of the glomerular capillary wall which will have applications for nephrotoxicity testing.

*Acknowledgement:*
The authors would like to acknowledge the following funding bodies - EPSRC and the Wellcome Trust.