Zurich University of Applied Sciences
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Evaluation of 3D hepatic tissue models for bioprinting

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Project goal

The present project aims at evaluating the compatibility of a gelatin-methacryloyl-based bioink with hepatocytes and its characterization. This study is the basis to generate bioprinted liver tissue-like models with co-cultures of human hepatocytes, stellate and endothelial cells

Key findings

- **Printing and characterization of HepG2 3D models as proof-of-concept**
- **Establishment and evaluation of high-density hepatocytes models for bioprinting**

Project data

Printing feasibility study

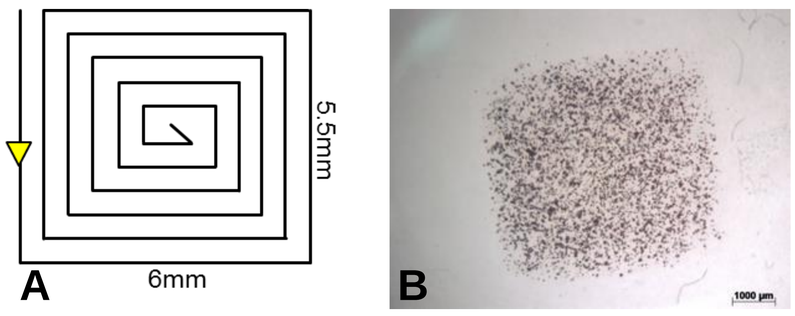


Figure 1
A) Scheme used for layer-by-layer printing, drawn with BioCAD software (regenHU)
B) MTT-staining of a bioprinted 4-layer HepG2-bionk model cultivated for 7 days (blue signal = viable cells)

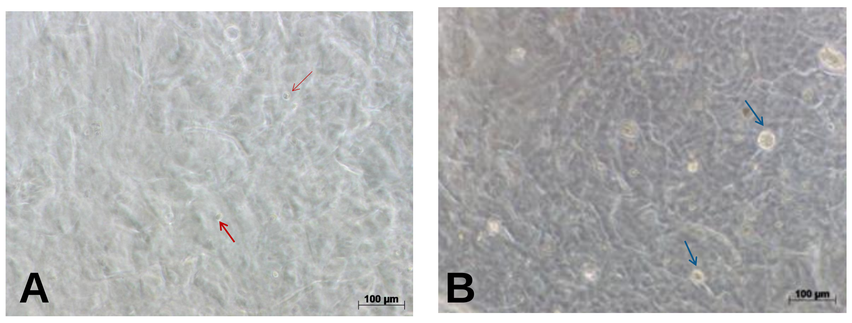


Figure 2
*HepG2 cells / bioink mixture printed in 4-layers and cultivated for 1 (**A**) and 7 days (**B**), respectively.*
Single cells: red arrows; cell agglomerates: blue arrows

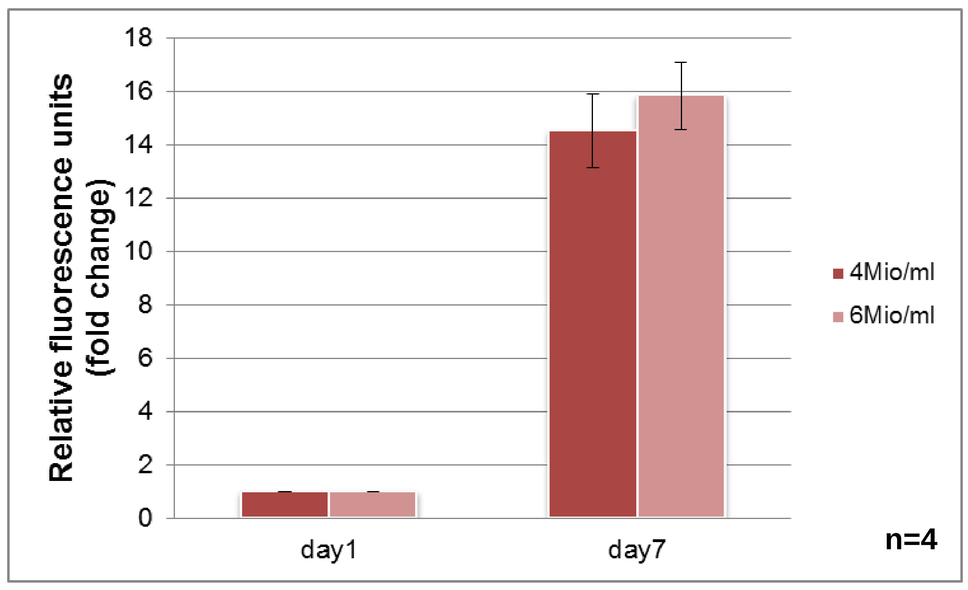


Figure 3
Viability and growth analysis of printed 3D models harbouring HepG2 cells.

Metabolically active cells were analysed by PrestoBlue assay.

The relative fluorescence values of day1 samples were set as reference value at 1

- Bioprinting process as well as bioink is suitable for HepG2 viability and proliferation
- Printed HepG2 cells are homogenously distributed as single cells into the model forming agglomerates increasing in size over time

High-density manually manufactured 3D hepatic models

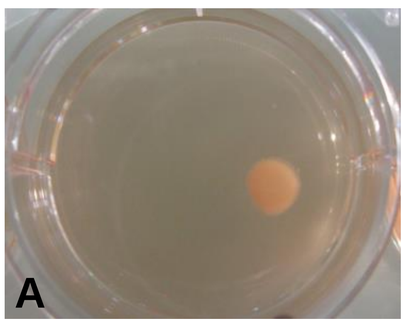
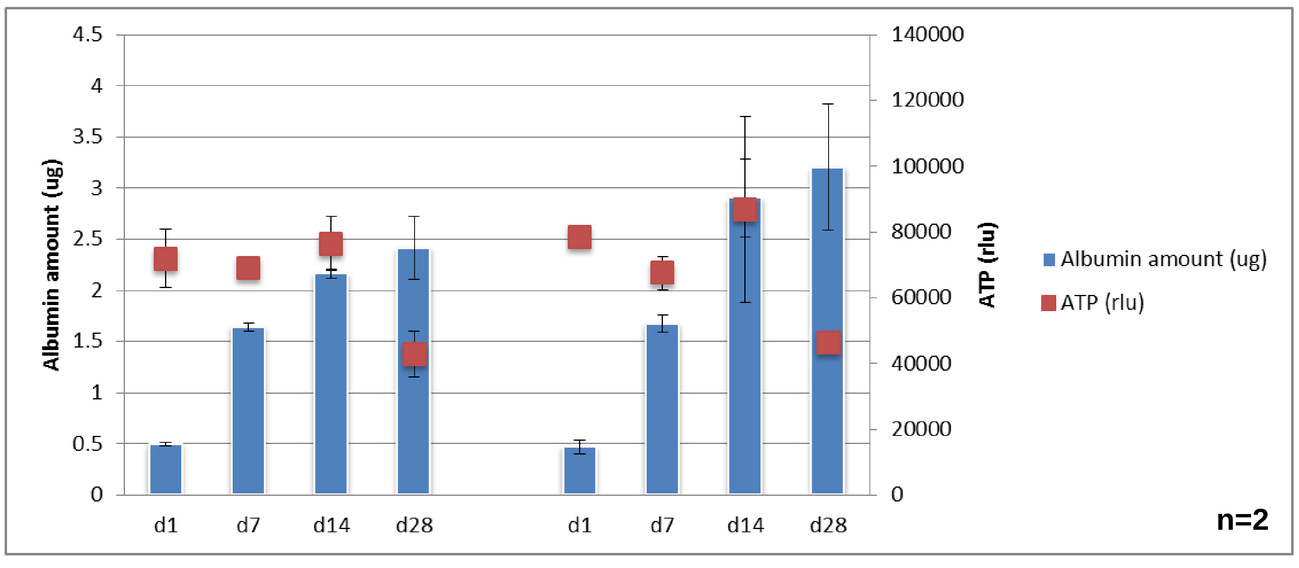


Figure 4
Manually manufactured models made of HepG2 high cell density paste mixed with bioink in a ratio of 2:1.
18 10^6 *cells per disc model (70ul model):*
A) Disc model cultivated 1 day
B) Disc model cultivated up to 28 days and stained with MTT

Figure 5
*HepG2-bioink drop models (7.5 *10^6 *cells per model, 30ul model) cultivated up to 28 days and analysed for cell viability (ATP) and biological activity (albumin secretion) at day 1, 7, 14 and 28*



- High-density HepG2 models can be manually manufactured
- HepG2 3D models show high and constant cell viability up to 14 days with a following decrease (d14 to d28)
- Albumin production increases with time
- Higher albumin amounts are obtained with vitamin C treated HepG2 models (d14 to d28)

Conclusions

Bioprinting set up is established and demonstrates its suitability for the manufacture of well-defined and viable low cell density hepatic models.
In the next step bioprinting will be used to simulate *in vivo* high cellular density conditions (hepatic high-density models) with additional relevant cell types, such as stellate and endothelial cells