Design, construction and testing of a COC 3D flow-over flow-through bioreactor for hepatic cell culture

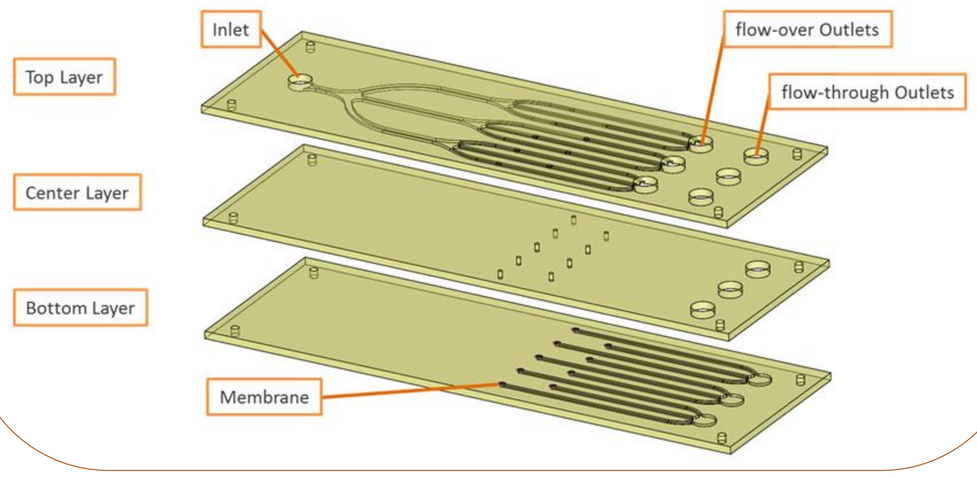
Jindrich Windels*, Rik Verplancke*, Amir Jahanshahi*, Marcus Heimann°, Sofia B.Leite#, Tiffany Roosens#, Leo A. van Grunsven#, Laurent Barbe[1], Sebastian Prill§, Magnus Jaeger§, Claus Duschl§, Jan Vanfleteren*

Goal

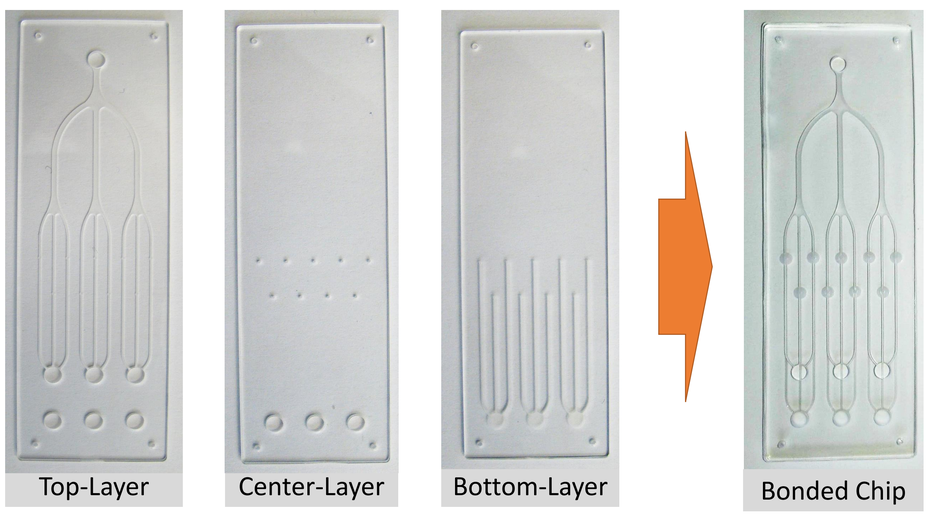
- Liver is 3D structure: closer mimic of in-vivo than available bioreactors
- Bioreactor environment for maintaining pre-formed hepatic co-culture spheroids cells and exposing to toxicants for > 1week
- Spheroids rest on a membrane allowing the medium to flow-through
- Flow-over is maintained to avoid an excess pressure on the cells

Material properties & design

- Topas COC (Cyclic Olefin Copolymer)
- Good properties for cell toxicolology: low adsorption, low auto-fluorescence, excellent optical & characteristics, sterilizable
- More challenging to structure than traditional microfluidics materials
- Structuring of 3 slides, afterwards thermal bonding with membrane

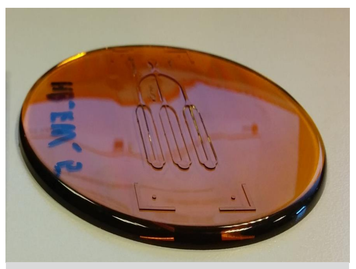


Fast prototyping
Mechanical milling & bonding

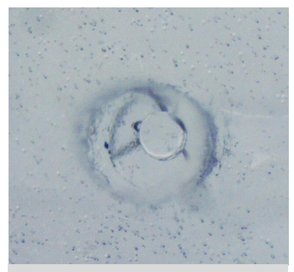


- Fast prototyping, rapid testing of design variations
- Using Topas 6013 and Polycarbonate membranes
- Milling take 1 hours and thermal bonding 30 minutes per reactor
- Relatively difficult to scale to mass production (serial process)

Medium scale production
Hot embossing with epoxy tools

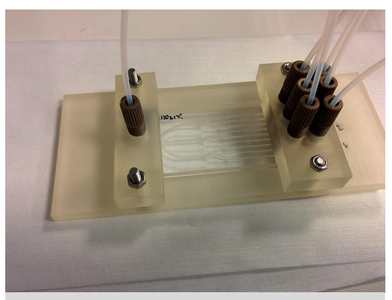

Epoxy mold for embossing


Embossed slides


Glass bead in well

- Longer time needed to adapt to different design (+-1 week)
- After initial set-up: fast process (embossing of multiple slides in parallel)
- Embossing cycle with epoxy tool(s) is 45 minutes
- Theoretical limit of our equipment: A4 size mold & substrate (32 slides)
- Cheaper and faster to prototype than traditional metal molds
- Temperature limits of epoxy limit prototyping to Topas 9506 and 8007
- Allows small to medium scale production of design very close to bioreactor as they would be produced with metal molds
- Visual inspection inside well with top side view and embossed reactors is possible due to optical quality surface finish

Cell seeding and culturing


Bioreactor in 3D printed holder


Spheroid in channel during seeding


Spheroid in well after seeding

- 3D printed holder for bioreactor to provide connections to pump and medium collection recipient or sensors
- Seeding of spheroids in milled reactors is successful, but time consuming
- Issues with spontaneous generation of bubbles in the culturing medium that can block the microchannels
- Viability and presence of cells after 1 week could not be confirmed with bottom side inspection (surface roughness and membrane opacity)

Conclusions

- Most of the technological hurdles for producing the COC flow-over flow-through bioreactor have been overcome
- Design can still be optimized to improve flow and avoid blocked channels
- Further work still is needed to (1) facilitate seeding, (2) ensure adequate flow to all wells, (3) verify toxicology testing

Author affiliations: *CMST, imec & Ghent University, Technologiepark 914A, 9052 Ghent, Belgium,
°Fraunhofer Institute for Reliability and Microintegration IZM, Gustav-Meyer-Allee 25, 13355 Berlin, Germany
#Liver Cell Biology Laboratory, Vrije Universiteit Brussel, Laarbeeklaan 103, 1090 Brussel, Belgium,
[1]CSEM (Centre Suisse d'Electronique et Microtechnique), Bahnhofstrasse 1, 7302 Landquart, Switzerland
§Fraunhofer Institute for Cell Therapy and Immunology, Branch Bioanalytics and Bioprocesses (IZI-BB), Am Muehlenberg 13, 14476 Potsdam-Golm, Germany

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