Microfabrication for multi-organ platforms

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Investigating cross-talks between different organs is of paramount importance to have a deeper understanding of human patho-physiology [1]. Owing to microfabrication techniques, cell patterning and compartmentalization have been widely shown to play a major role in modeling physiological environments and in recapitulating tissue interactions *in vitro* [2]. Along with micropatterning, multi-organs-on-chip devices equipped with microchannels or user-controlled valves are appealing to finely tailor biological interactions among different cell compartments [3]. Photo- and soft-lithographic techniques are here adopted to fabricate PDMS-based microfluidic devices to be subsequently employed as multi-organ platforms for liver-tumor and liver-heart models, and neuro-muscular circuits.

Liver-Tumor on-a-chip for drug efficacy testing

- **AIM:** Developing techniques that allow the culture of liver and tumor cells on specifically designed micropatterns within microfluidic devices
- **Devices Layout:** the chip is composed by two 2D culture chambers, one for liver patterned cultures (pink) and one for tumor cultures (grey). The chip is characterized by two different set of valves, one for chamber communication and one for fluid confinement
- µPCCs (µ-patterned co-cultures) of HepG2 and fibroblasts were generated inside the liver chamber of the chip that was then validated for pharmacokinetic-based drug screenings involving diffusion of the anticancer drug Tegafur (UFT) after liver metabolism (UFTL) (Fig.1A)
- µLOBULE: Recapitulating the architecture of hepatic lobule by means of a plasma-enhanced micropatterning technique (Fig.1B)

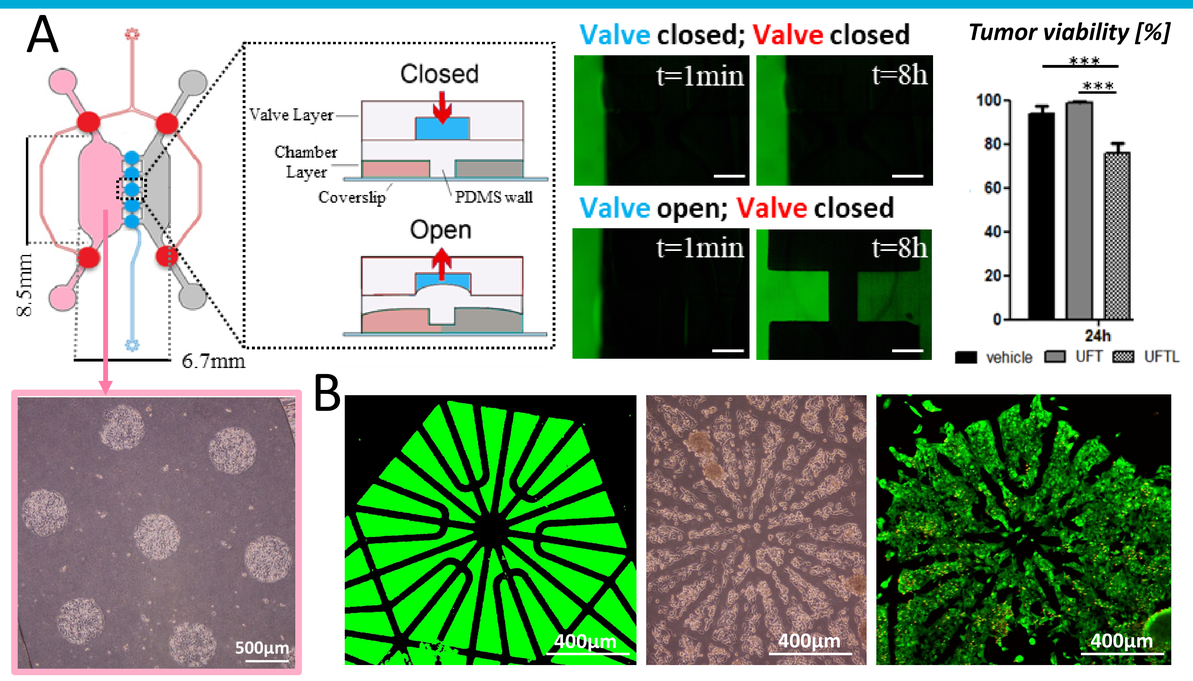

Fig. 1 – A) Design and working principle of Liver-Tumor device, containing µPCCs as liver model. Exemplification of diffusion phenomenon within the platform, scale bars are 250 µm. Viability of HCT-116 cells 24h after liver metabolism of Tegafur (UFTL); **B)** µLOBULE micropatterned inside a microfluidic device. Patterned PLL, HepG2 cells and LIVE/DEAD after 7 days of culture.

Liver-Heart on-a-chip for drug safety testing

- **AIM:** Developing microfluidic platforms that allow the simultaneous culture of both liver and heart organ models to study the cardiac toxicity of compounds upon liver metabolism (Fig.2)
- **Devices Layout:** the chip is composed by two culture chambers, one for 2D liver µPCCs (pink) and one for 3D neonatal rat cardiac microtissues (blue) cultures. The chambers are separated either by an array of µchannels (5µm high, 3µm wide, 1mm long, Fig.2A) or by a valve system (Fig.2B) to impose different and controlled diffusion-based exchange of compounds between compartments
- Ongoing experiments are aimed at validating the platform for pharmacokinetic-based drug screenings (e.g. Terfenadine and Cyclophosphamide) with the purpose to demonstrate the ability of the chip to detect the predicted effects (i.e. cardiac structural or functional toxicity) of commercially available compounds

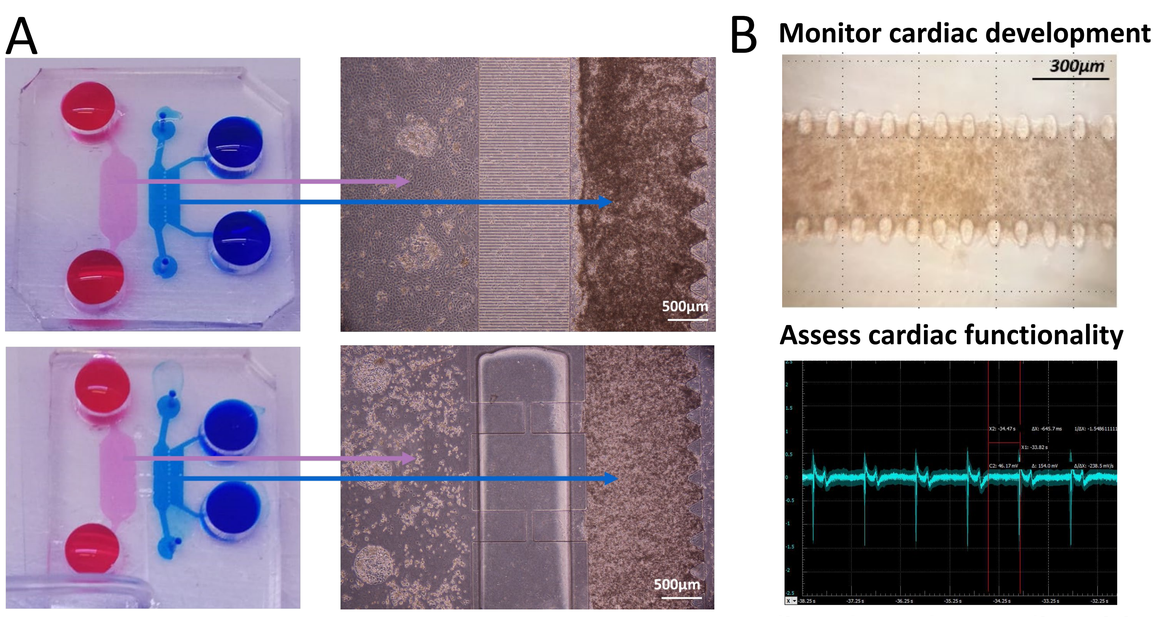

Fig. 2 – A) Design and layout of the devices consisting of 2 culture chambers separated by µchannels or by a set of valves. Phase contrast images of HepG2 micropatterned islands in the liver compartment and cardiac microtissues in the heart compartment; **B)** What we can do with our platforms? Monitor cardiac development/functionality and assess the effect of liver metabolism on cardiac cells in a controlled way.

Neuro-muscular circuit on-a-chip

- **AIM:** Development of a compartmentalized microfluidic platform (Fig. 3A) for the co-culture of neuronal organoids that recapitulate the dorsal root ganglia (DRGOs) together with human intrafusal myofibers mimicking the proprioception sensory pathway
- **Device layout**: The culture chamber layer (Fig.3B) has two compartments, i.e. one for neuronal organoids (h=43µm) and one for intrafusal myofibers (h=143µm), separated by an array of microchannels (h=5µm). The muscle chamber is formed by a central channel for 3D cell laden gel injection and a medium channel, separated by an array of hanging pillars. An additional layer, the actuation compartment, allows the myofibers to be mechanically stimulated (Fig. 3C)
- DRGOs successfully extended their axons through the microchannels, contacting myoblasts in the muscle compartment (Fig. 3D). Ongoing studies focus on evaluating the functionality of the neuro-muscular connection

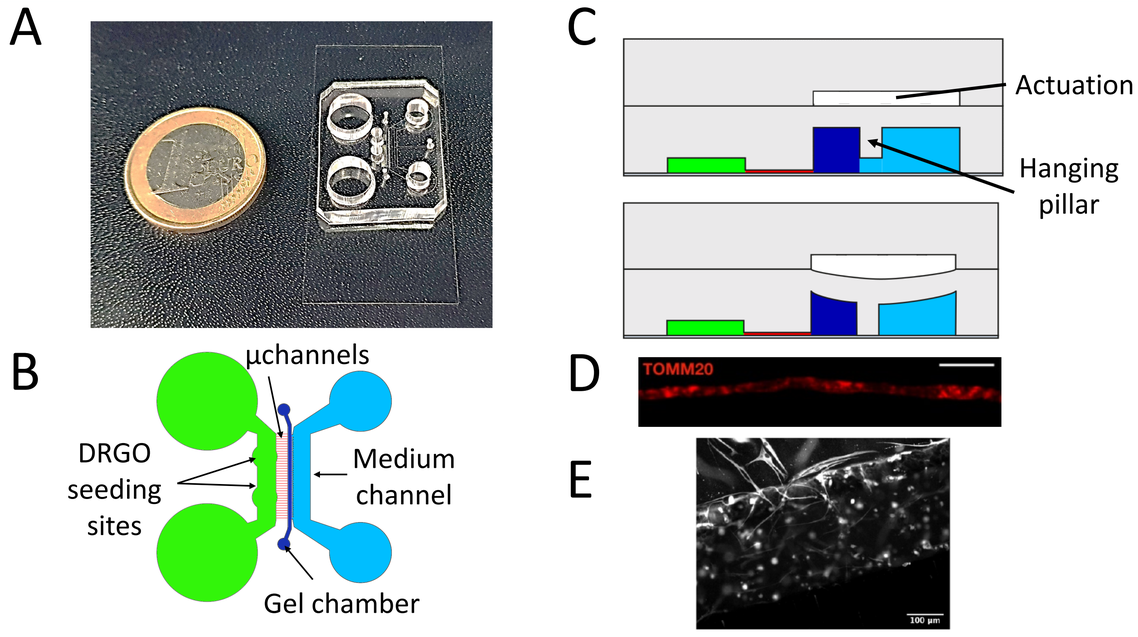

Fig. 3 – **A)** Assembled microdevice. **B)** Layout of the device (green: DRGO compartment, blue: myofibers compartment, red: microchannels). **C)** Side view of the device showing the culture chamber layer (bottom) and the actuation layer (top). **D)** Representative images of DRGO axons within the microgrooves stained for TOMM20 (red) to visualize the number and morphology of mitochondria. **E)** Representative images of DRGO axons entering the muscle compartment where myoblasts are seeded.

References

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Acknowledgements

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