Microfluidic chip for long-term cell co-culture

Craig Watson[1] · Chao Liu[1] · Ali Ansari, PhD[2] · Helen Miranda[1], PhD · Samuel Senyo, PhD[1]

[1] Case Western Reserve University, Cleveland OH USA; [2] Bucknell University, Lewisburg PA USA

CASE WESTERN RESERVE UNIVERSITY EST. 1826

Introduction

Paracrine interactions are challenging to study in vitro, as conventional in vitro cell culture techniques provide poor models of cell-cell interaction. To address this, we have developed a microfluidic chip designed specifically to co-culture cells for signaling studies.

Results

Chip design (fig 1)

- 32 individually-addressable units
- 16 inputs, 2 outputs
- 4 chambers/unit, separated by short valved channels
- Plastic substrate (Ibidi polymer coverslip)

Cell culture

- Cells can be allowed to exchange secreted factors by opening/closing valve between chambers
- Media delivery by perfusion through chambers, or by diffusion from adjacent chambers
- Small volumes ensure that autocrine/paracrine signals are not diluted in media
- Validated with broad range of cells, including cell lines (3T3, HeLa, RAW 264.7, Hep3B, HepG2) and primary cells (neonatal rat cardiac fibroblasts and cardiomyocytes, human skeletal muscle and motor neurons)
- Cells have been routinely cultured for up to 2 weeks, enabling differentiation studies and other experiments

Read-outs

- Compatible with optical assays (immunostaining, fluorescent reporters)
- Cells/lysate/media can be recovered for off-chip analysis (e.g. qPCR)

Conclusion

The co-culture chip has been validated as a platform for long-term cell co-culture, with support for up to 128 different conditions. Complex experiments and assays such as immunostaining can be run autonomously using our microfluidic control system.

Current and future directions include applying to chip to stuying stem cell differentiation, immune-cancer cell interactions, and cardiac regeneration.

References

C. Watson and S. Senyo, "All-in-one automated microfluidics control system," HardwareX, vol. 5, p. e00063, Apr. 2019

Acknowledgments

R01 NS121374-01 Miranda (PI)

R25 HL145817 Senyo (PI)

Chip design

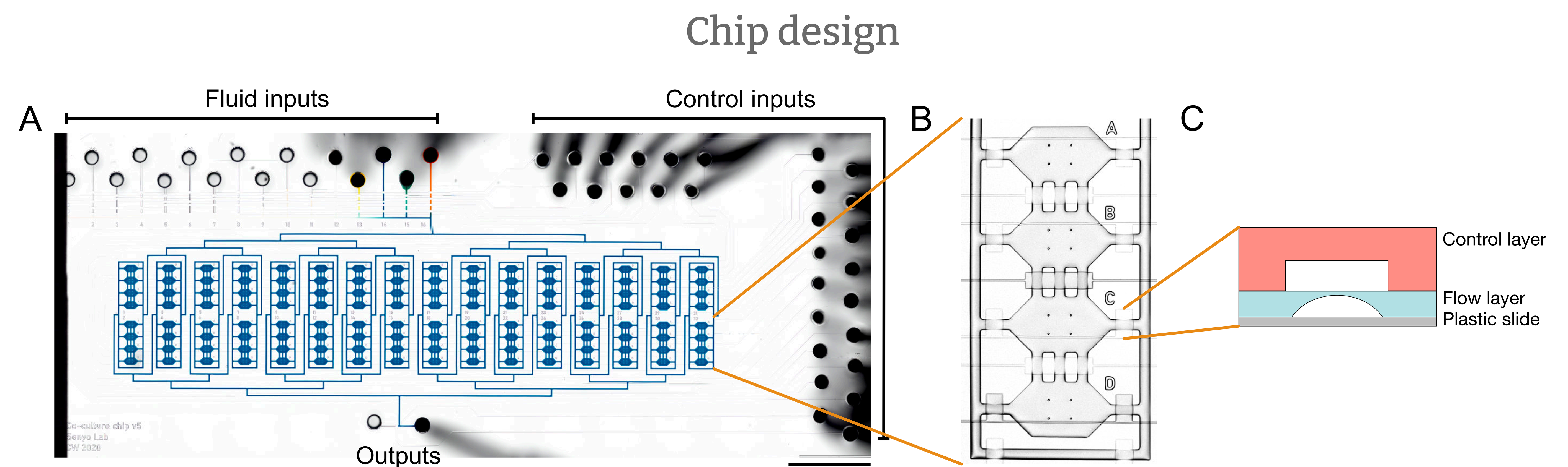

Figure 1. A) Overview of the co-culture chip, featuring 16 fluidic inputs, two outputs, and 32 individually-addressable units. Multiplexers are used on the inputs and channels to each unit, to minimize number of control lines needed. When flowing reagents to multiple units simultaneously, the branched channel design guarantees equal flow rate everywhere. Scale bar: 5 mm **B)** Detail of one unit, comprising 4 chambers and a rinse channel. Valves between chambers allow control over diffusion of media or cell-secreted signals. Scale bar: 500 µm **C)** Cross-section schematic. A standard 2-layer design is used, with a plastic slide providing a gas-permeable, 180µm thick substrate enabling high quality, high resolution imaging

Metabolic modeling

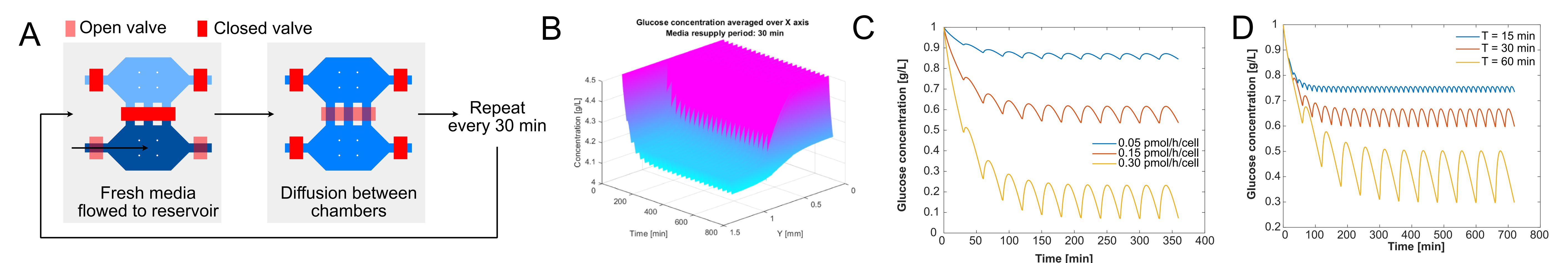

Figure 2. A glucose consumption and diffusion model is used to ensure that media delivery frequency is sufficient to allow cell survival while minimizing dilution of cell-secreted signals. The same model can also be applied to tracking paracrine signal secretion, diffusion and uptake. **A)** Media delivery by diffusion scheme. **B)** Simulated glucose concentration over time and distance from the media reservoir, with fresh medium (4.5 g/L glucose) delivered every 30 minutes. **C)** Effect of consumption rate on glucose concentration profile at the end of the chamber farthest from the reservoir, with 30 minute supply period. **D)** Effect of media supply period on glucose concentration profile, with 0.13 pmol/h/cell consumption rate.

Long-duration experiments

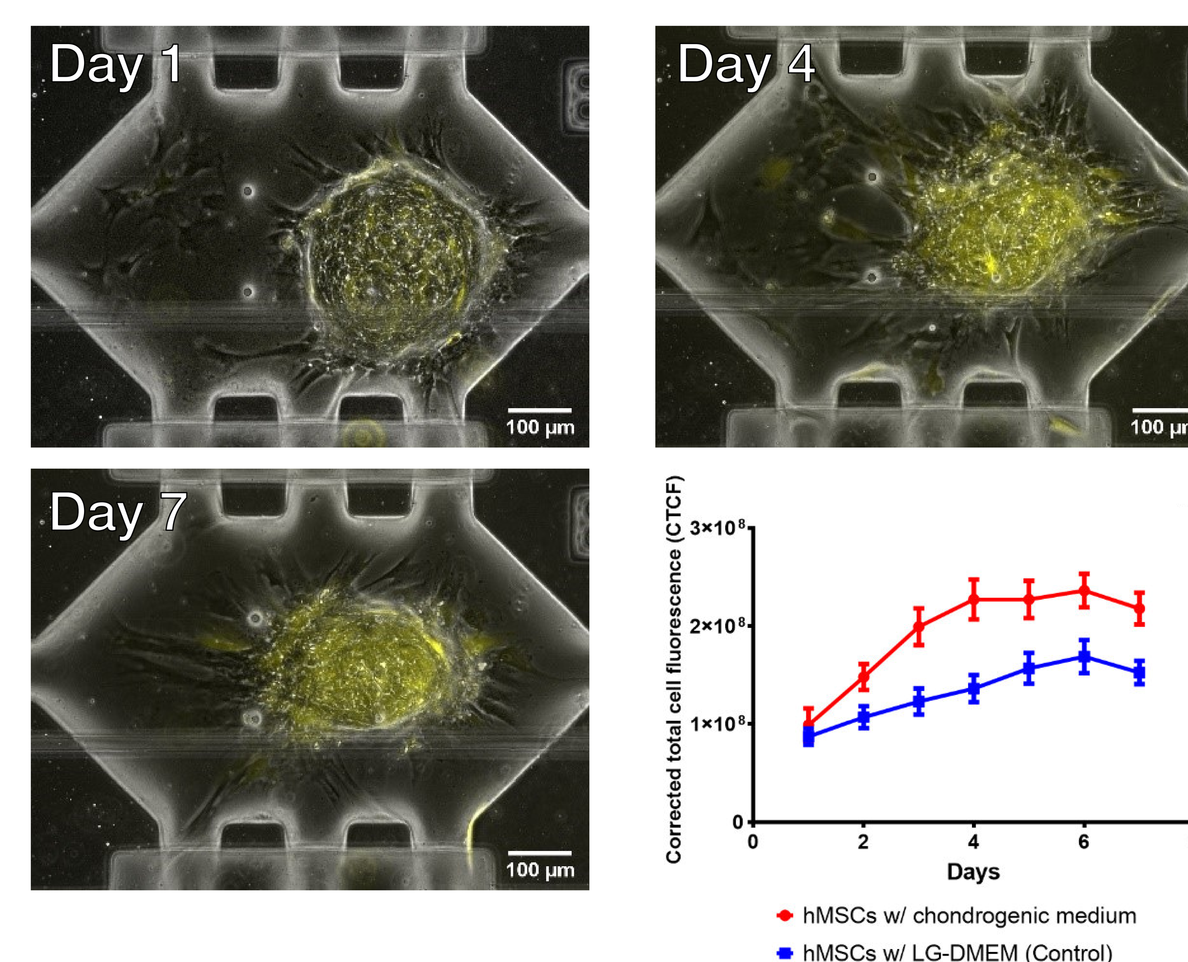

Figure 3. Primary mesenchymal stem cells (MSCs) expressing aggrecan reporter and treated with differentiation medium show stronger progression towards chondrogenic state compared to control. Phase contrast/fluorescence overlay

Cell co-culture

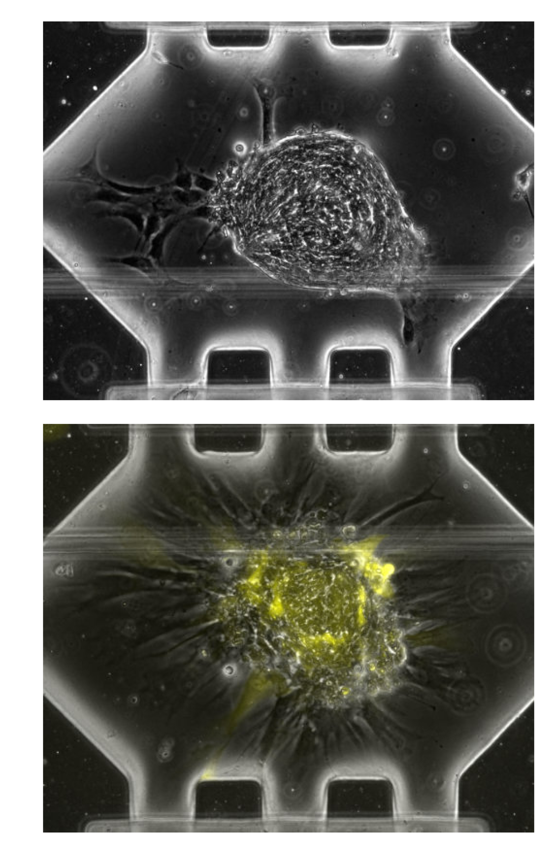

Figure 4. Chondrocytes (top) and MSCs (bottom) in adjacent chambers at day 7 of culture in a study on the effect of co-culture on differentiation. MSCs express aggrecan dTomato reporter. Phase contrast/fluorescence overlay. Scale bar: 500 µm