NETRI's APP NOTE

THE DUALINK CHIPS: HOW TO IMPROVE REPRODUCIBILITY IN COMPARTMENTALIZED CO-CULTURES

- Easy access to compartmentalized co-culture with different medium, even for a long culture duration.
- Capacity to expose a compound on either axonal or soma compartment with minimal leakage.
- Better reproducibility allows increased statistical discrimination between test conditions.
- Enhanced reproducible readouts whatever the operator's experience in microfluidics.

OVERVIEW

The development of advanced *in vitro* models to mimic the physiological micro-environment of cells is a challenge, especially for the co-culture of different cell types within the same chip, which requires specific culture media for proper differentiation and maintenance. Moreover, the access to only somas or axons/dendrites in conventional culture systems prevent specific exposure of molecules when studying neural networks under healthy or pathological states. NETRI's microfluidic devices are designed with several compartments connected by microchannels (either tunnels or grooves), allowing the neuronal cell bodies (somas) to be seeded in one channel and their axons to growth in another channel1. However, manual handling of microfluidic devices can lead to leakage of medium from one compartment to the other through microchannels, a phenomenon which can also greatly differ between operators, leading to lower reproducibility of results.

In this application note, we introduce a device in which a narrow channel has been inserted between the two main compartment. This channel acts as a "garbage collector" for fluids, thus enhancing the fluid isolation between cell compartments.

To reproduce classical handling of *in vitro* cultured microfluidic devices and analyze the effects on fluids leakage, the devices were filled under the laminar flow hood and moved to the microscope (1st move), then moved to the incubator (2nd move) and finally moved back to the microscope (3rd move). To be representative of the end-users' profile heterogeneity, the experiments were done by a panel of operators composed of trained and non-trained technicians for the use of microfluidic devices. Moreover, to avoid biasing the study, the different chips were fixed on the same microplate for each operator, ensuring the same handling and moves between the different chip's architectures, whether with or without hydrostatic pressure difference between channels.

As presented in the first part of this application note, the commonly used technique of volume difference between compartments (creating a hydrostatic pressure difference) can only partially thwart the fluid leakage. Using the "garbage collector" channel, the DuaLink chip (with microchannels tunnels) and DuaLink Ultra chip (with microchannels grooves), where developed. By comparing the performance of such microfluidic devices to the former device, the second part of the document shows the increase in fluidic isolation and the reduced intra- and inter- operators' variability.

Altogether, this demonstrates the ability of this thin channel to maintain the respective cellular micro- environments, leading to optimal cell phenotype and results reproducibility.

RESULTS

Performance of the conventional two- channel chip under hydrostatic pressure

Fluids leakage between channels was evaluated thanks to relative fluorescent measurement. Hydrostatic pressure was created by adding only 80 µL in channel 1 (stained with Red-dextran 3-5kDa) while adding 110 µL in channel 2 (stained with FITC- dextran 3-5kDa) (Figure 1A). Since the channels are symmetric, the differences of volumes induce a gravity driven hydrostatic pressure from channel 2 to channel 1, preventing the liquid from channel 1 to passe through channel 2.

After 1 and 3 motions of the device (Figure 1B), the fluorescence levels were quantified and normalized to express the percentage of dextran in both channel (Figure 1C).

Results showed that, under hydrostatic pressure, red- dextran compound present in channel 1has a limited leakage to the channel 2 (red-dextran present in channel 2 at 0,5 % ± 0,5 after 1 move and 2,7 % ± 4,5 after 3 moves). However, under hydrostatic pressure, the FITC-dextran compound leaked from channel 2 to channel 1 with up to 48 % leakage after 3 moves (FITC-dextran present in channel 1 at 10,8 % ± 8,6 after 1 move and 27,7 % ± 16,2 after 3 moves), furthermore leading to a large and highly variable dilution of red-dextran compound present in channel 1 (Figure 1C). Although leakage was expected because of the hydrostatic pressure, our results show that this technique cannot be properly used to performed pharmaceutical compounds screening that precise application of compound concentration, which cannot be achieved if leakage happens between compartments.

With this experiment, we highlighted that using hydrostatic pressure cannot be used when culturing different cell types or testing a compound on one compartment, because it leads to mixed culture media and compounds and/or dilution of them. That is why NETRI developed a new architecture to rise fluidic isolation without the use of any hydrostatic pressure.

NETRI's DuaLink architectures rise fluidic isolation and intra- and inter-operators' reproducibility

NETRIs DuaLink architectures overcome the fluid leakage and low reproducibility of the two-channel microfluidic device. The DuaLink and DuaLink Ultra chips consist of two main channels separated by a thin one (making them three-channel devices) connected with microchannels tunnels or grooves, respectively. The performances of those two DuaLink formats have been compared to the two-channel device. The fluid isolation was analyzed with red-dextran added in the channel 1 of each device (the other channels were filled with PBS), after 1 and 3 moves (Figure 2), without hydrostatic pressure difference between channels. The operators performed each 2 independent experiments.

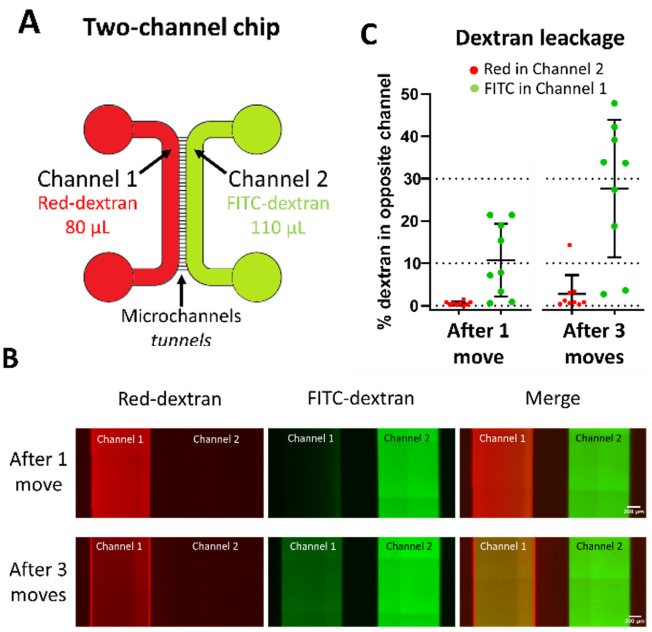

Figure 1. Analysis of fluidic leakage between channels under hydrostatic pressure in the two-channel architecture.
(A) Schematic representation of the two-channel microfluidic device. At T0, channel 1 was filled with 80 µL of Red- dextran and channel 2 with 110 µL of FITC-dextran, thus the difference of volume creating a hydrostatic pressure between channels.
(B) Representative fluorescent pictures of the devices after 1 move or 3 moves.
(C) Representation of the fluidic leakage between channels by the quantification of the percentage of each compound (dextran) in the opposite channel after 1 and 3 moves. Average ± SD, n=9 (3 operators x 3 replicates).

Results showed a significant decreased leakage of fluids between opposite channels (channel 1 to 2 in the two-channel device, channel 1 to 3 in the DuaLink chips) with the new architecture after 1 or 3 moves (Figure 2C), with a maximal leakage value divided by 2 to 3.

Moreover, the F test showed a significant decreased variance of the results with the DuaLink chips compared to the two-channel one ($p<0,001$ for 1 move and $p<0,01$ for 3 moves, whether for tunnels or grooves), with reduced intra- and inter-operators' variance.

We demonstrated that the addition of the thin "garbage collector" channel improved fluidic isolation between the two main channels. Moreover, the fall in variance between operators and between replicates enables a better reproducibility in the results.

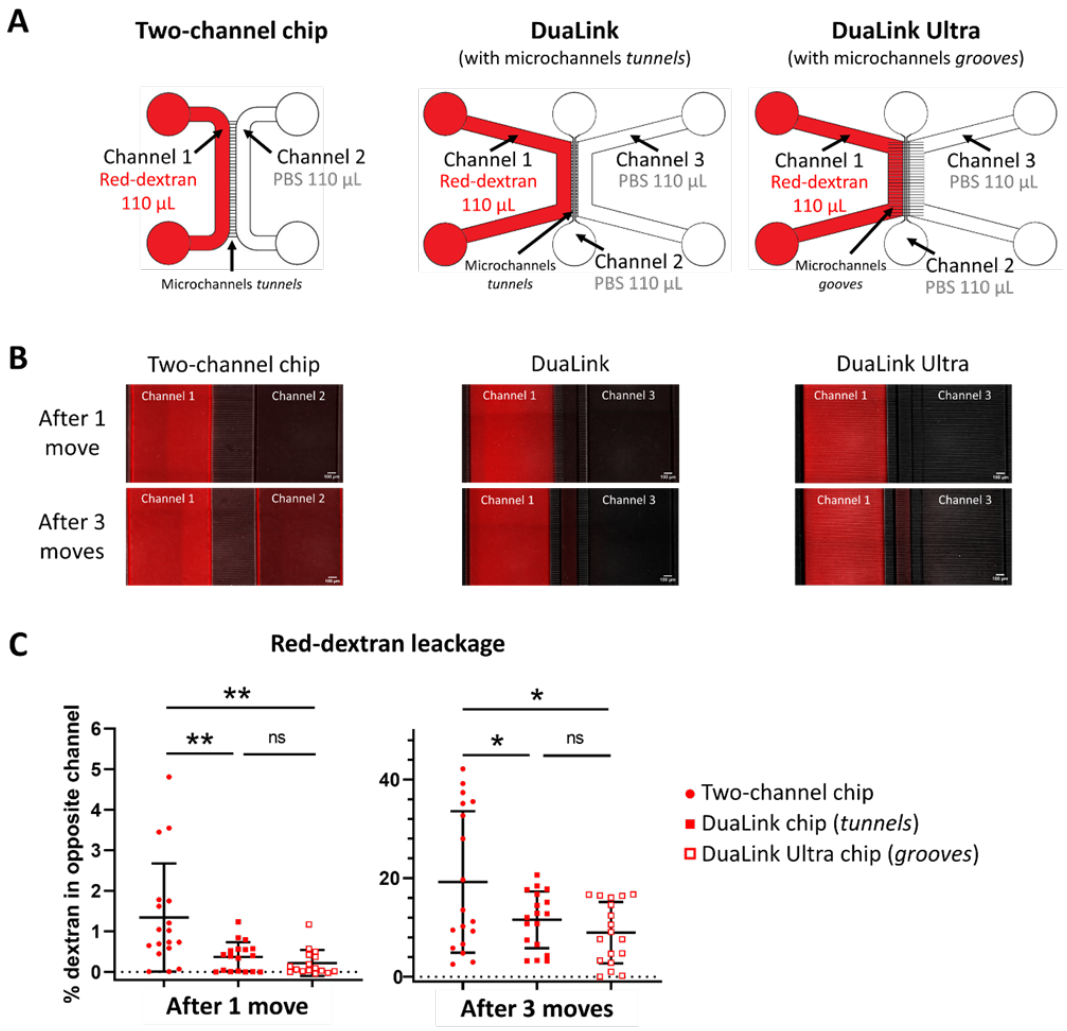

Figure 2. Improved fluidic isolation and reproducibility with the NETRI's DuaLink and DuaLink Ultra chips, compared to the two-channel device.
(A) Schematic representation of the compared devices (left, the two-channel chip; middle, the DuaLink chip, right, the DuaLink Ultra chip). At T0, channels 1 were filled with 110 µL of Red-dextran and channels 2 and 3 with 110 µL of PBS, thus not creating hydrostatic pressure difference between channels.
(B) Representative fluorescent pictures of the devices after 1 move or 3 moves.
*(C) Representation of the fluidic leakage between channels by the quantification of the percentage of red-dextran in the opposite channel after 1 and 3 moves. Average ± SD, n=16-18 (3 operators x 4-6 replicates) for 1 move, n=18 for 3 moves (3 operators x 6 replicates). Welch's t-test, ns: non-significant, * p<0,05, ** p<0,01.*

CONCLUSION

This Application Note reports the analysis of fluidic isolation between the main channels of the DuaLink microfluidic chips, compared to the former two- channel device. We demonstrated that:

- The fluid isolation between channels permits to create compartmentalized micro-environments, allowing to optimally culture different cell types in each channel and specifically test compound in one compartment, i.e. axonal/soma compartment.
- Handling of microfluidic devices leads to high and variable fluidic leakage between channels, which is worsen by number of microplates motions by operator even under hydrostatic pressure.
- NETRI's DuaLink and DuaLink Ultra microfluidic devices are designed with an additional thin central channel which acts as a "garbage collector".
- In the DuaLink devices, either with microchannels tunnels or grooves, the fluid leakage generated by the handling is significantly reduced, compared to the two-channel device, with a maximal leakage value divided by 2 to 3.
- There is less than 1 % of leakage between opposite channels of the DuaLink chips with a typical move like from the laminar flow hood to the incubator
- The thin central channel in the DuaLink chip allows to improve intra- and inter-operator reproducibility

RESOURCES

Available upon request

- DuaLink – Technical specifications
- DuaLink Ultra – Technical specifications

1. Taylor AM, Blurton-Jones M, Rhee SW, Cribbs DH, Cotman CW, Jeon NL. A microfluidic culture platform for CNS axonal injury, regeneration and transport. Nature Methods. 2005;2(8):599-605.

Based on 10 years of scientific research, NETRI has developed a unique know-how in designing organs/organoids-on-Chip by integrating disruptive building blocks into the same microfluidic devices, while maintaining industrial production standards compatible with pharma industry equipments & requirements.

Thanks to our patented technologies, we are capable of manufacturing prototypes and validating their biological function using primary animal or human induced pluripotent stem cells differentiated in our chip. Our unique infrastructure allows us also to scale up chip production for mass production.

Need more information about NETRI's products and services or how Organs-on-Chip revolutionize pre-clinical trials and human *in-vitro* models? Contact us!

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