Development of a microfluidic approach to study autocrine and paracrine signaling in co-culture systems

M. Vetralla[1,2], L. Vitiello[3], N. Elvassore[2,4], C. Bolego[1]

[1]Dept. of Pharmaceutical and Pharmacological Sciences, University of Padua, Italy
[2]Venetian Institute of Molecular Medicine , Padua, Italy
[3]Dept. of Industrial Engineering, University of Padua, Italy
[4]Dept. of Biology, University of Padua, Italy

BACKGROUND AND AIMS: Autocrine and paracrine signaling contribute to many cellular processes, such as cell migration, angiogenesis and cell-cell interaction [1]. Co-culture systems and conditioned media assays are commonly used when studying cell-cell interaction *in vitro;* they allow to identify autocrine factors, which are involved in processes like embryonic stem cell (ESC) pluripotency and lineage commitment, as well as paracrine signals. The most commonly used methods involve use of transwells [2,3], but this type of assay can be affected by factors like cell seeding density, growth time and preparation of conditioned media. Besides, they require relatively large amounts of cells and media. On the other hand, culturing cells in enclosed microfluidic environments gives the possibility to maximize the concentration of secreted soluble ligands using small scale culture systems, and/or to minimize it by continuously exchanging the medium in the culture chambers [5]. Importantly, microfluidic approaches are also easily adapted to provide real-time readouts of cellular pathways. The long-term objective of this study is to create a robust co-culture system with endothelial and immune cells, to study cell-cell interaction under physiological and pathological conditions (e.g., aging, diabetes). To this aim, we are setting up a cell-culture micro-scale model that allows to precisely measure the activation of a specific pathway by soluble factors, either released from cell cultures or exogenously added.

METHODS. Experiments were carried out using a transparent microfluidic platform –chemically inert, impermeable to water, permeable to gases, non-toxic to cells and optically transparent– into which we cultivated a reporter cell line consisting of immortalized human keratinocytes transfected with the TGF-ß1 inducible $(CAGA)_{12}$ luciferase-reporter construct. Cells were seeded to a density of 10^3 cell/mm^2. Conditioned media containing TGF-ß1 were added to induce luciferase expression and luminescence signal was detected by Vivo Vision IVIS 100 Series (XENOGEN) instrument equipped with Living Image Software version 3.0. Cell viability was evaluated by the LIVE/DEAD® system (Molecular Probes, Invitrogen), a two-colour fluorescence assay based on membrane integrity and esterase activity assay.

RESULTS: Here we report the design of a microfluidic system comprising two cell-culture multiple chambers, fabricated from polydimethylsiloxane through soft-lithography and replica molding, connected with polytetrafluoroethylene micro capillaries. Such a device allowed us to transfer by capillarity specific amounts of media (in the microliter range) between the micro-chambers. We then performed a time-dependent activation of TGF-ß1 inducible luciferase-reporter construct, demonstrating the possibility to study and manipulate diffusible signals by controlling the media transfer in the microfluidic cell-culture system. In addition, we carried out quantitative analyses of the luciferase activity by measuring the luminescence signal. Finally, we also set up methods to detect proteins and gene expression, with western blot and real-time PCR respectively, in a micro-scaled system.

CONCLUSIONS: Activation of TGF-ß1 specific pathway mediated by soluble factors validated our microfluidic cell-culture system and represents a proof of concept for studying cell activation programs. This model could be useful for studying large numbers of soluble factors, including drugs, involved in cell-cell interaction.

REFERENCES:

1. Freeman M Nature, 2000; 408: 313-319
2. Toutovskaia A et al. Lab on a Chip, 2005; 5:401-406
3. Lecault V et al. Nat Meth, 2011; 8:581-586
4. Greber B et al. Stem Cells, 2007; 25(2):455-464
5. XU R-H et al. Cell Stem Cells, 2008; 3(2):196-206