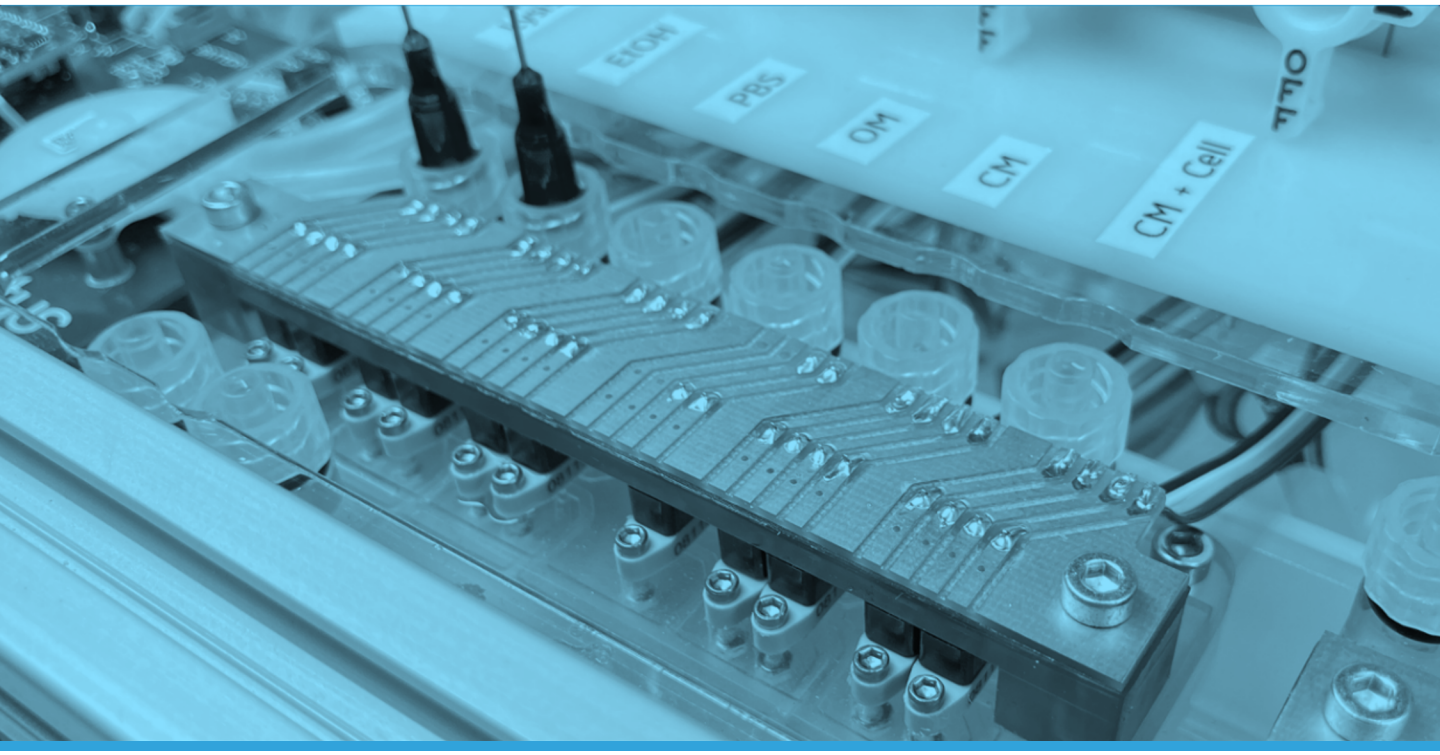
Cell Culture

CASE STUDY:

ULTRA-COMPACT FLUIDICS FOR CELL CULTURE GROWTH AND ANALYSIS



DOLPHIN™ FLUIDICS
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memetis

Cell Culture

In cooperation with:

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PRINTMED-3D :

MICROFLUIDIC CONTROL PLATFORM TO ENABLE THREE-DIMENSIONAL CELL CULTIVATION FOR MEDICAL APPLICATIONS

Even though the world's population is still growing, medical standards are increasing in many regions, and people strive for a long and healthy life up to high age. This is made possible by advanced medical technologies that have experienced exceptional progress over the last decades, which is to a great extend related to a growing understanding of molecular and genetic causes for certain diseases. These technologies enable highly personalized treatments, sometimes even for extremely rare diseases. Since it is not acceptable to conduct experiments or test new drugs directly on real patients for ethical and economic reasons, there is a strong trend for cultivating artificial tissues or even organoids (i.e. functional physical models of an organ, consisting of living cells) in a lab environment and use these as a basis for drug development or surgical training. The great benefit of this approach is that, if necessary, many parallel experiments can be performed in a space-saving, highly controlled and automated environment. By using cell lines (e.g. stem cells) of real patients, experiments can yield strongly personalized results without the need of putting the life and health of a human being at risk. However, the quality of

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the obtained results stands and falls with the conditions under which the artificial cell cultures grow – these should be as realistic as possible. The project PRINTMED-3D (funded by Lombardy Region under POR-FESR 2014-2020) aims to create an infrastructure for the development of solutions for personalized medicine and specialist training, through the combined use of virtual reality and functional additive 3D printing technologies to produce digital and physical organ models with accurate anatomical details, enhanced haptic and functional response. Within this framework, a novel automated cell culture system, designed and developed by Dolphin Fluidics in partnership with memetis, that enables to induce specific differentiation pathways in dynamic cultures of adult stem cells isolated from patients. This approach will foster and accelerate the development of new therapeutic approaches in frequent pathologies related to aging, such as complex bone lesions and degenerative diseases of the joints. A specialized cell culture chip and fluidic platform are developed by the University of Milan, while a control unit for automated fluid handling is provided by Dolphin Fluidics in partnership with memetis.

Designing an automated system for standardized cell culture growth and analysis

Traditional cell culture systems, which require numerous and complex manipulation maneuvers in manual mode, make cell culture difficult to standardize in terms of cell yield and biological activity. The transition to user-friendly automated devices will favor the standardization of the cultivation process, the reduction of production costs and preparation times by reducing the number of interventions by the operator. The aim of the project is to create a platform for advanced cell culture, using of traditional multi-well plates equipped with a dedicated fluidic network and an electronic system for the management and fine control of the liquid flows inside it. The automated fluidic platform will allow to conduct specific cell culture protocols remotely, simplifying the entire process, minimizing the contribution of human operators and allowing the systematic culture of complex cell systems. In addition, the overall system aims for a strong reduction in size to enable mobile use at the Point-of-Need. The system consists of three main units:

1) A **fluidic control unit**, including precision valves and a pump, for defining the transport and perfusion of the fluids of interest for the cell culture within the system, such as culture media (nutrient) or reagents for bioassays.
2) A **fluidic platform** made of polymer, equipped with a network of channels for liquids and integrated sensors for flow and pressure monitoring.

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3) A disposable cell culture unit, consisting of a multi-well transparent plate, each of which will be connected to the fluidic unit and provided with nanostructured biomaterials as growth medium.

Figure 1 gives an overview of the whole system.

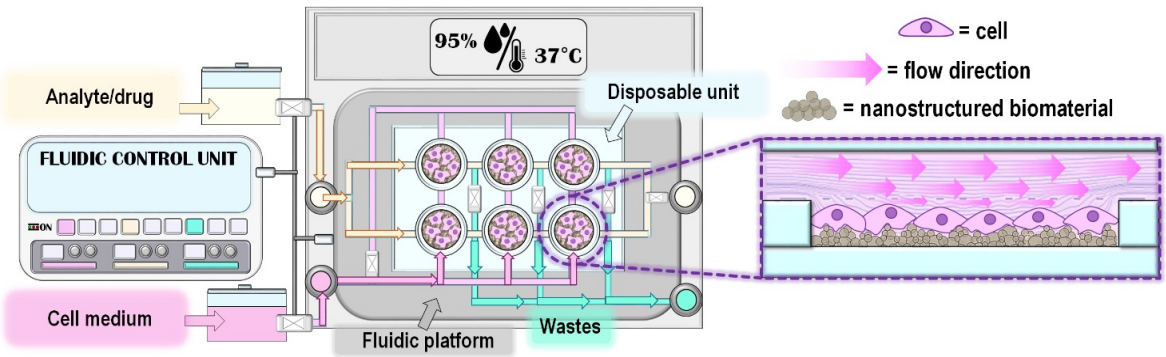

Fig. 1: Schematic of the automated device for cell culture, including the control unit, fluidic platform and disposable unit provided with nanostructured biomaterials in the culture sites

To conduct a successful experiment with meaningful results on stem cells (or any other kind of cell line) in a lab environment, the cells first need to be inserted into the cell culture units, then be kept under optimum growth conditions and nutrient supply, before finally being exposed to the drug under investigation. In the present study, a number of (disposable) cell culture units are inserted into a common fluidic platform, which is kept at body temperature by a heating chamber. This setup allows performing various cell culture experiments in parallel, investigating the effect of the osteogenic medium and nanostructured biomaterials on stem cells.

Fluidic control unit

In order to guarantee stable and controlled experimental conditions, the supply of the cell culture system with fluids shall be managed externally in a fully automated way. For this purpose, a fluidic control unit (see figure 2) is implemented by memetis GmbH and Dolphin Fluidics S.r.l., which makes use of shape-memory-actuated memetis miniature valves. The use of precision miniaturized valves is a fundamental tool on one hand reduce the overall system size to enable mobile use but also to control the shear stresses to which the cells inside the well are continuously subjected during perfusion. Another advantage is the low electrical power consumption of the valves, which makes them ideal for mobile applications and also ensures low heating, so that the media and the cells are

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not thermally affected. These efforts can in fact modify, through mechano-transduction phenomena, the growth and differentiation of cells, thus resulting in a key aspect of the design of the device.

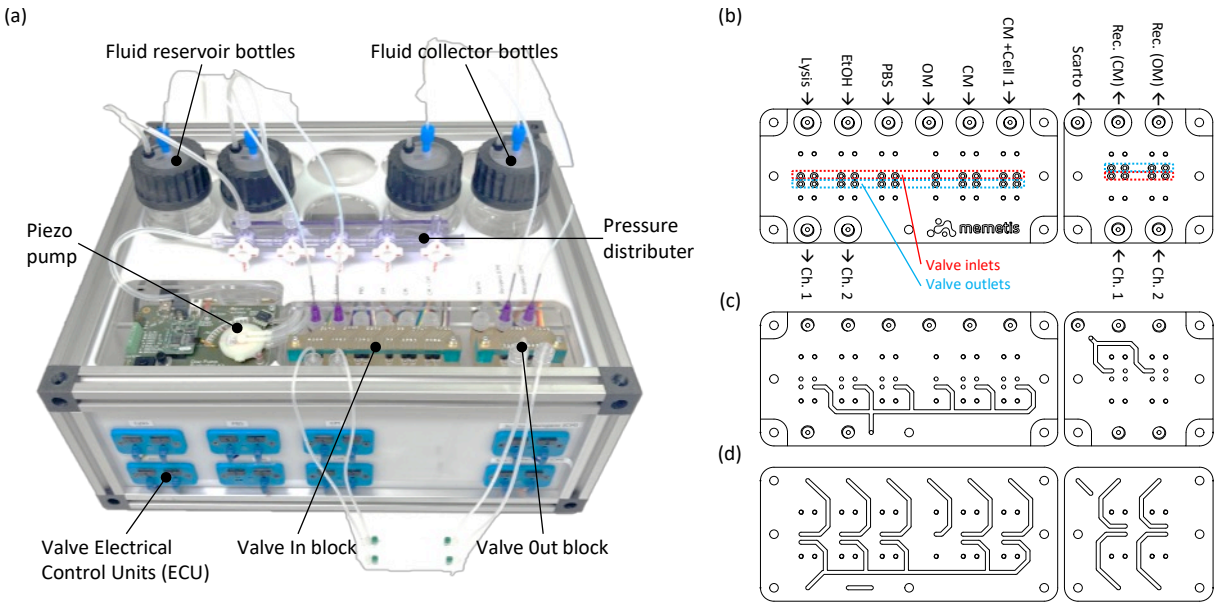

Fig. 2: S(a) Fluidic control unit for cell culture system integrated in a housing; (b) Schematic top view of fluid distribution circuit; (c) cut view of first channel level; (d) cut view of second channel level. The system is designed to distribute different input fluids to the fluidic platform with cell culture units and from there to collector bottles. The fluids are transported by pressure-driven flow.

The on-chip cultivation of stem cells requires sequential flushing of the cell culture units by six different fluids: control medium (CM) without and with cells, osteogenic medium (OM), saline buffer solution (PBS) for washing, lysis buffer as well as ethanol for final cleaning.
After lysis application, the content of the cell culture units is collected in two separate vessels (CM, OM); in other process steps, fluids are directed to a common waste collector.

In order to guarantee a uniform, pulsation-free flow of supply fluids – not disturbing the three-dimensional cell growth within the cell culture units – a pressure-driven flow system is implemented for fluid transport and delivery. The source fluids are stored in six chemically inert glass vessels (fluid reservoir bottles) in the back of the fluidic control unit (figure 2(a)). These vessels can be pressurized by a single piezoelectric air pump from TTP Ventus Ltd., which operates completely silently. A thin PTFE tube (inner diameter 0.8mm) is also reaching through the cap of each vessel, down to its bottom. This way, the internal air pressure will drive fluid from the vessel through the tube when its external end is

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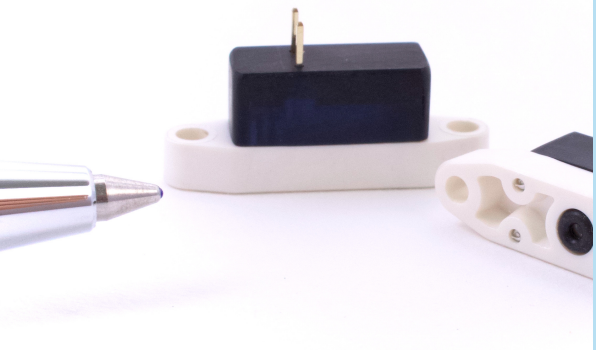
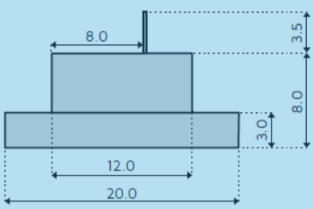
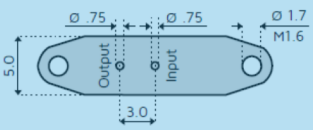
open. Distribution of the six supply fluids to the inlets of the fluidic platform is managed by a custom 3D-printed, biocompatible manifold within the fluidic control unit (figure 2 (b,c,d)), which has two internal levels of flow paths. Eleven normally closed microvalves “Series 09” by memetis are flange-mounted to the manifold to allow automatic control of fluid pathways (“in-block”). Fluids that leave the fluidic platform are handled by four additional valves (“out-block”) on the same manifold and directed to three collector vessels. The control electronics for the silently operating miniature valves, integrated in the front part of the unit, support automated valve control via USB connection and I2C-protocol, as well as manual operation using toggle buttons in the front panel. Due to their functional principle, the valves do not only support fully closed / fully open switching states but can also adopt intermediate states to enable precise flow rate adjustments via closed-loop control. The extreme compactness of the valves with an installation pitch of only 5mm is enabled by a fatigue-free shape memory alloy (SMA) thin film actuator, allowing for more than 20 million switching cycles. A low power consumption of 0.15 W holding power (0.3 W switching power) per valve assures that heat introduction into the controlled media is minimal.



Series 09 normally-closed valve
Specifications

- Internal volume <4 µl
- Max. pressure 2bar
- K_v value ~ 0.007 m^3/h
- Temperature < 50 °C
- Lifetime > 10^7 cycles
- Power < 0.30 W
- Media-separated





Full data sheet **HERE**

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Fluidic platform

The interface between the fluidic control unit and the disposable cell culture units is formed by a dedicated fluidic platform (central part of figure 1). The platform is connected to the control unit via silicone tubes. The platform provides interfaces for insertion of two cell culture units in the initial prototype, allowing to compare two cell cultures, one of which receiving a drug. In the future, a multi-well design will allow the insertion of a higher number of disposable cell culture units, enabling many cell culture experiments at the same time. The multi-well system, connected to the fluidic platform, can then be positioned in an incubator at body temperature, while operations such as the change of the culture medium and the injection of analytes and biomolecules can be timed and controlled externally via the fluidic control unit.

Disposable cell culture unit

In this study, the design of disposable cell culture units is focused on two biological systems: stem cells and organoids. This preclinical approach is in line with the most innovative lines of biomedical research, aimed at understanding the molecular mechanisms underlying diseases and identifying new biomarkers useful for precision medicine.

The disposable unit is designed with cylindrical culture wells with a 15 mm diameter – typical of that of a standard 24 multi-well plate – and is provided with micro-fluidic channels in submillimeter-sized cross section for fluid transportation into the culture sites. The channels are dimensioned to enable low flow rates (10 to 100 µl/min) with associated low values of shear stress affecting the culture cells during chamber perfusion.

The surfaces of the growth medium of the disposable unit will be modified through the deposition of nanostructured thin films, able to favor the proliferation and differentiation of primary and stem cell lines thanks to their particular morphology. This functionalization will be implemented using a state-of-the-art technology originally developed at the University of Milan (supersonic cluster beam deposition, SCBD), which is a fabrication technique compatible with planar fabrication technologies and capable of producing large quantities of devices. By exploiting this nano-fabrication technique, it is possible to control at the nanoscopic level, in a fine and repeatable way, the physical characteristics (such as roughness and porosity) of the surfaces produced, thus reproducing structural

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characteristics of the native environment of the cells. Future applications are expected particularly in the research and medical treatment applications of stem cells and organoids:

Stem cells

Regenerative medicine aims to repair tissues and organs to restore their functional integrity compromised by genetic defects, trauma and aging. It makes use of adult stem cells isolated, if possible, from the same patient and cultured in a microenvironment that hosts them and directs them in the differentiation path. In the field of complex bone lesions, especially where there is osteopenia / osteoporosis with consequent regenerative deficit, osteogenesis aims to repair the damaged structures using artificially grown bone tissues. In a similar way, chondrogenesis is aimed at healing generative diseases of the joints.
Another application field of considerable impact is obtaining in vitro keratinocytes or epidermal flaps to accelerate the repair of wounds in those subjects, including diabetics and the elderly, in which this process is delayed.
An automated cell culture system of this type could also be of interest to space agencies, given that the experiments aboard the International Space Station (ISS) require minimal intervention by the astronaut.

Organoids

The automated culture device will also enable the generation and maintenance of organoids, the last frontier of biomedical research. Organoids are also obtained from individual patient cells, thus reflecting their uniqueness from a genetic and metabolic point of view. In this way it is possible to reproduce various diseases "in test tubes", including hereditary diseases or tumors, useful for precision diagnostic investigations and for developing personalized treatments. On organoids it is possible to conduct diagnostic tests, test experimental drugs, without having an invasive approach on the patient, and evaluate the effects of genome editing using CRISPR / Cas9.

Parallelization of process steps and controlled conditions enabled by automation

A novel cell cultivation system is developed that will enable the cultivation of stem cells and organoids in a parallelized way under highly controlled conditions. Such a system, developed by Dolphin Fluidics in partnership with memetis is expected to be useful for a

wide range of medical and scientific applications. The system design offers several unique advantages:

- Automation and reproducibility of traditional culture and analysis protocols;
- Using a standard disposable culture system (multi-well plate);
- Integrated monitoring and control system
- Possibility of scale-up for a high parallelization of the culture process
- Control on perfusion and culture conditions (static / dynamic)
- Compact device with user friendly interface
- Integration of biomaterials to promote proliferation / differentiation of primary and / or stem cell lines and organoids of particular interest for cell factories and precision medicine.

The combination of these features makes this automated culture system highly innovative compared to the state of the art of this family of devices. In fact, the integration of the technologies developed by the partners allows to cope with typical problems related to the use of devices currently on the market, such as the use of bulky and highly specific equipment and interfaces, the lack of a disposable culture element, the low parallelization of the process and the complexity of assembling the units. The use of precision miniaturized valves and a pulsation-free pump is crucial for avoiding high mechanical stress on the cell cultures which might disturb their growth. The miniaturized fluid handling system developed in this study will thus enable much more gentle and realistic growth conditions than have been possible before.

This system thus represents an important step in combining technologies to produce digital and physical organ models with accurate anatomical details.

A special thank you for contributing to this case study!

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