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Combination of two laser processes for the creation of relevant bio-models for therapeutical applications

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Printing techniques applied to biology have begun to develop in the 2000s and have since been greatly improved and perfected. Based on interdisciplinary approaches they make use of cell biology, chemistry, engineering and a combination of sophisticated protocols to create organized 2D or 3D patterns of biomaterials or living cells. Their applications range from tissue engineering or organ creation to regenerative medicine and new drug discovery. In this context, at LP3 and in close collaboration with MMG, we took advantage of our experience on the Laser-Induced Forward Transfer (LIFT) technique (previously developed for electronic purpose) to print bio-inks containing living cells for the creation of *in vitro* bio-models. This work will be focused on the printing of muscular stem cells and the process optimisation for stem cell differentiation in the prospect of creating a versatile tool aimed at improving differentiation toward the skeletal muscle lineage and formation of neuromuscular junctions (NMJ).

LIFT is a two-part printing method using laser-matter interaction to transfer tiny amounts of material from a thin donor film to a receptor substrate, both separated by a few hundreds of micrometres. A short laser pulse induces the formation of a controlled jet propagating perpendicularly to the donor substrate. The bio-ink previously spread as a thin film (few tens of microns) on this donor substrate is thus collected as a droplet on the receiver.

This method allows us to precisely control the amount and the location of the targeted material deposited and, by extension, the number of living cells printed. In contrary to conventional bio-printing methods using extrusion or ink-jet systems, LIFT is a nozzle-free process that allows the use of a large range of bio-inks viscosity and a high cell concentration without clogging.

Here, we will present the LIFT process and its optimisation allowing us to achieve a controlled, reliable, precise printing of muscle progenitor cells, ensuring a high post-printing cell survival rate and proliferation. The cell survival rate will be controlled by fluorescence staining of printed cells and the monitoring of the cell development over several days.

In parallel with the optimisation of the LIFT process, a laser surface structuring technique is currently being developed to topographically modify the surface of the receiver substrate to improve the survival rate, the proliferation and even the differentiation of the printed cells. Two different kinds of structuration have been investigated: (i) the creation of micro-channels to guide the growth of muscle progenitors to force them to stretch and should thus promote their differentiation into myotubes and (ii) the creation of nano-porosity at the surface of the receiver to make them better suited for cell development.