Laser printing of multi-cellular patterns with adjustable cell densities

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Statement of Purpose:
To replicate fully functional natural tissue for regenerative medicine it is necessary to generate complex multi-cellular patterns with an accuracy of a few microns and various cell densities. A promising technique that addresses the issue of combining microscale and unharmed deposition of living cells with free adjustable cell densities is biological laser printing based on laser-induced forward transfer (LIFT). Due to the absence of an orifice, LIFT is capable of printing single to dozens of cells without observable damage in a well defined pattern. By combining cell solutions with materials that can form stable gels it is further possible to establish 3-D tissues layer-by-layer.
These advantages demonstrate LIFT as a promising tool for the generation of *ex vivo* tissue replacements.

Methods:
The biological laser printing setup (Figure 1) based on laser-induced forward transfer consists of two glass slides positioned coplanar in close proximity to each other (~500µm). The upper slide, herein after referred to as "donor slide", is covered with an energy absorbing layer (gold) and a hydrogel layer (2wt% alginate) containing cells to be transferred. The laser pulses (9 ns, 20 Hz, 1064 nm) are focused through the donor slide onto the gold layer generating a local evaporation that propels the subjacent cells compound towards the lower glass slide (collector slide). A thin layer of hydrogel (1wt% alginate) on the collector slide prevents the cells from dehydration by providing a humid environment and cushions the impact occurring during the printing process.

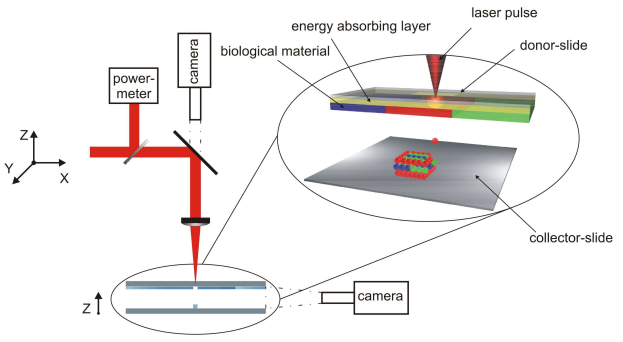

Figure 1. Schematic sketch of the biological laser printing setup and a perspective figure of the principle of laser based cell transfer between two coplanar slides

Results:
In this study it is shown that biological laser printing can be applied for the precise arrangement of living cells. We print porcine mesenchymal stem cells (pMSC) derived from bone marrow together with chondrocytes in a puzzle piece structure with a well defined shape (Figure 2). Furthermore, we investigate the amount of transferred cells in dependence on initial cell density and on the laser pulse energy. Here we are able to show that the cell densities can be varied in a wide range without cell impairment. To exclude cell impairment detailed quantitative viability studies were conducted. Live/dead assay show, that over 95% of the cells survive the transfer. In proliferation studies over six days no significant differences between transferred and non transferred cells were observed. Furthermore, the possibility of DNA-damages was investigated through a single cell gel electrophoresis showing fragmentation of DNA (single and double-fractions) as a comet tail. Here no increased tail was observed.

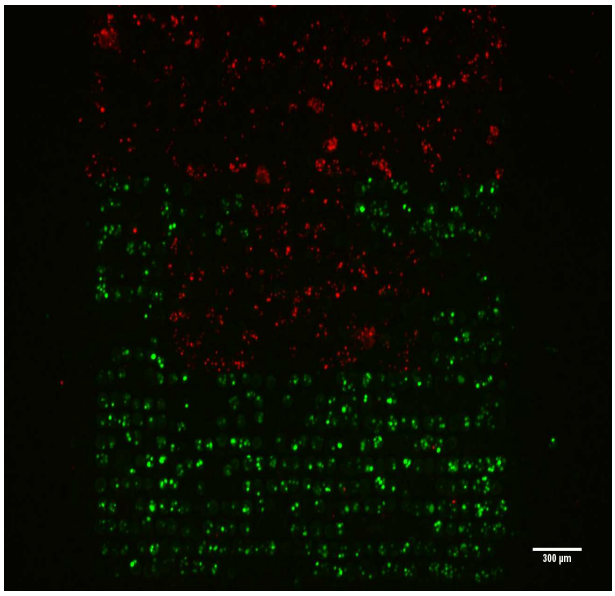

Figure 2. pMSC´s (green) and chondrocytes (red) printed together in a puzzle piece pattern

Conclusions:
The biolocial laser printing technique is suitable for generation of defined complex arrangements using different kinds of living cells. Together with the possibility to choose the cell density arbitrarily, LIFT proves to offer new promising possibilities towards computer-assisted assembly of tissue replacements.