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Multi-Nozzle Industrial Inkjet toward high-throughput Bioprinting for 3D Tissue Engineering

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Introduction

Bioprinting, thanks to the unique flexibility and spatial accuracy it offers, has a promising future for the deposition of cells and extracellular matrices (ECM) toward the fabrication of physiologically relevant 3D tissue models. Most bioprinting platforms offer switching single-nozzle printheads. Such deposition systems are not best suited for the large-scale production of tissue models for pharmaceutical screening or grafting since their throughput is strongly limited. As experts in multi-nozzle inkjet technology, we (iPrint) hypothesized that industrial printheads allow for a reliable, fast, and therefore cost-effective production of 3D tissue models leading bioprinting toward industrialization. Industrial inkjet offers the control of hundreds of nozzles in parallel, allowing the individual tuning of each of them in order to jet drops in physiologically relevant dimensions (20-200pL). To test our hypothesis, we are investigating the reliability of the printing process, its throughput and its impact on cells.

Materials and Methods

A 3D Bioprinting platform, compatible with various inkjet printheads, was internally developed and set up. Multi-nozzle piezoelectric industrial printheads were selected based on their compliance with cell-loaded bioink deposition (nozzle diameter >30um, low dead volume, 128-256 nozzles, aqueous ink compatible). For this first series of tests, a Xaar 128/80L printhead was used. An ink supply to digitally control the purge and the meniscus pressure, while featuring a minimal dead volume, was set up. Dropwatching was implemented to visualize drop formation, characteristics (velocity, volume, shape) and stability of the jetting process. Bioinks based on culture media with viscosity modifiers were prepared and characterized (viscosity, density, dynamic surface tension) to achieve stable cell suspension while remaining liquid enough for inkjet printing (<20mPa*s). Human lung epithelial type II cells (A549) were cultivated and suspended in bioinks ($3x10^6$cells/mL = 100% concentration) and their sedimentation rate was measured. 7mL of the cell-loaded bioinks were fed into the ink system and were kept there for up to 35min of alternating sequences of printing and settling at room temperature. Prior to each printing step, a short purge was implemented to free the nozzles from clogging. Batches of cell-loaded bioinks were produced by printing 75'000 lines (128 nozzles per line) at 1KHz as one big drop in a petri dish that was subsequently divided into n=3 wells for culture. To quantify the reliability of the process, cell concentration was measured after each batch printing. To measure the impact of the process on the cells, a viability test (Trypan Blue exclusion assays) was performed after 4 days in culture.

Results and Discussion

When using DMEM with 10%FBS as a bioink, suspended cell concentration in the printed batches dropped to 31% after 5min settling. Furthermore, large aggregates of cells were observed in the product from the purges. This indicates that the cells sedimented in the printhead during the settling time and were mostly ejected during the purge prior to printing. Increasing the viscosity of the bioink by adding 5% Ficoll PM400 led to an increase of the cell concentration to 81% after 5 minutes settling. This clearly indicates that the rheological properties of the bioink have a direct impact on the overall stability of the printing process. To assess the impact of the printing process on the cells, viability was measured after 4 days in culture. Trypan Blue exclusion assays were performed on the printed batches and compared to the "Gold Standard" of deposition by pipetting (Figure 1). All viabilities were over 85%, suggesting that the time spent in the printing system at room temperature does not have a great impact on the cell. The viability of the printed cells tends to be lower than the ones deposited by pipetting while remaining in an acceptable range. The addition of Ficoll also tends to slightly reduce the viability.

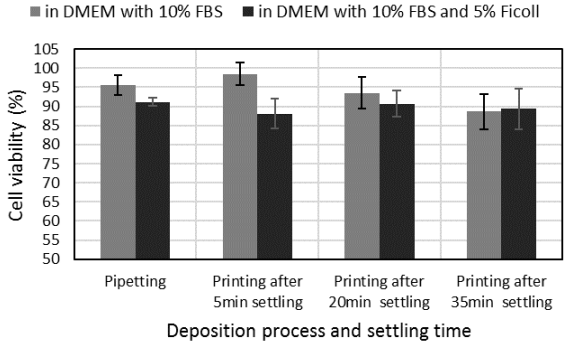

Figure 1: Viability (n=3) of human lung epithelial type II cells (A549) suspended in bioinks after 4 days in culture as a function of the deposition process and time spent in the ink system

Conclusions

We showed that by tuning the rheological properties of the bioinks, the cell concentration over settling time could be increased. The cells printed with the industrial inkjet printhead showed viabilities of over 85% even after prolonged settling times in the ink system. Our study demonstrates that this technology indeed has the potential for a reliable cell deposition and has a low impact on their viability. These encouraging preliminary results support our hypothesis and provide a solid claim for a more thorough investigation of industrial inkjet for bioprinting. Further tests using different cell types, viscosity modifiers, surfactants, hydrogels and printheads will be performed to verify our hypothesis.