A New Aspiration-Assisted Bioprinting Method for Tissue Fabrication

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Statement of Purpose: In contrast to traditional two-dimensional cultures, tissue spheroids offer many advantages, such as the ability of cells to secrete their extracellular matrix with an effective communication between them in a native-like microenvironment [1]. Bioprinting of tissue spheroids has gained noteworthy attention in tissue engineering [2,3]. We present an aspiration-assisted bioprinting (AAB) system that provide the required back-pressure for picking and placing spheroids. AAB system is composed of micro-valves, customized glass pipette, mounted on the arm, the aspiration pressure for picking the spheroids, and placing them onto fibrin one by one. Afterward, fibrinogen and thrombin droplets are generated via micro-valves that can enable layer-by-layer deposition of fibrin hydrogel. Using the 3D bioprinter with fully automated control system, spheroids can be bioprinted on desired location precisely.

Methods: Mouse fibroblast cell line (3T3), mouse breast cancer cell line (4T1) and human umbilical vein endothelial cell (HUVEC) spheroids were fabricated using U-bottom 96-well microplate (Greiner Bio-one, Monroe, NC). Cell nuclei were then stained with NucBlue® ReadyProbes™ reagent (Life Technologies, Grand Island, NY); actin cytoskeletal fibers were stained with ActinGreen™ 488 ReadyProbes™ reagent according to manufacturer’s instructions. CellTracker™ Orange CMTMR (Thermo Fisher Scientific, USA) and CellTracker™ Green CMFDA (Thermo Fisher Scientific) were used according to manufacturer’s instruction. For constructing fibrin scaffolds, 5 mg/ml fibrinogen (Sigma-Aldrich, United Kingdom) and 1.5 μ/ml thrombin (Sigma-Aldrich) were mixed at ratio of 1:1 and were incubated at 37°C in 20 mins.

Results: In Figure 1A-C, we have demonstrated the ability of AAB in placing spheroids precisely, through the fabrication of various complex patterns such as: PSU and matrix patterns. The viability of the control group was determined to be 94.19 ± 2.55%. The cell viability was 88.07 ± 2.02% when spheroids were processed with picking and placing into cell media. When printing onto fibrin, the cell viability was measured as 82.53 ± 7.03%.

Conclusions: During AAB process, tissue spheroids can be inspected with charged couple device (CCD) cameras to allow bioprinting with different size and type of spheroids. Developed bioprinting system showed high cell viability and without any fusion to other spheroids before bioprinting. This bioprinting approach can be used large-scale tissue fabrication and organ-on-a-chip applications.

References: