High throughput assembly of spatially controlled microscale tissue subunits

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Introduction: Guided assembly of microscale tissue subunits has found applications in tissue engineering, cell and developmental biology, and drug discovery. Different cell types (e.g. hepatocytes, pancreatic cells, embryonic stem cells, and cardiomyocytes) have been reported to exhibit a more in vivo-like behavior when cultured in 3D aggregates in comparison to traditional 2D dispersion cultures. Aggregate size and geometry have been known to influence cell responses. The ability to control aggregate formation in a high throughput manner could be advantageous for biomedical applications. Using micro and nanofabrication techniques, we developed a platform that allows controlled formation of microscale tissue subunits.

Methods: The platform consisted of a microfabricated array of through-thickness wells bound to a sheet of electrospun fibers. Different well geometries (square, circular, triangular) and dimensions (100-500 µm) have been produced. Soft lithography based techniques were used to fabricate the microwell array from various polymers (e.g. polycaprolactone -PCL-, polystyrene –PS-, polymethylmethacrylate –PMMA-). A non-woven network of fibers was obtained via electrospinning. Fibers with different sizes and chemistries (e.g. PCL, gelatin, and polyethersulfone) were synthesized. The fibers were attached to the microwell array via solvent-assisted binding. The ability of the platform to allow controlled formation of microscale tissue subunits was studied using different cell lines (C3A hepatocytes, HL1 cardiomyocytes, PANC-1 ductal epithelial cells, and murine embryonic stem cells). The specimens were characterized via fluorescence, confocal and scanning electron microscopy. Finally, the ability of the device to support studies on heterotypic interactions was demonstrated by co-culturing C3A cells (on the microwell side) and NIH 3T3 fibroblasts (on the opposite side of the fibers). Viability and functionality studies of the microtissues were conducted with C3A cells over a period of 8 days. The proliferative/metabolic activity of the cells was quantified with alamar blue. The functionality was analyzed in terms of urea production.

Results and Discussion: Fig. 1 A and B show electron micrographs of the platform. The materials used to fabricate the device can be specifically selected to meet certain requirements in different applications. Fiber morphology and chemistry, as well as microwell geometry and dimensions can be altered to elicit desired cell responses. Cell culture experiments revealed preferential cell adhesion to the exposed fibrous surfaces. This allowed for spatially controlled cell distribution throughout the platform (Fig. 1 C-E). Vacuum could also be applied through the fiber mat to achieve localized cell settling. The proliferative/metabolic activity of the C3A cells showed a tendency to increase over time (Fig. 1 F). Urea production by the hepatocyte microtissues on the platform was enhanced by ~2-fold in comparison to cells cultured on tissue culture polystyrene controls. C3A – 3T3 co-cultures were successfully conducted (Fig. 1 G, H). C3A cells showed enhanced urea production when co-cultured with the 3T3 cells.

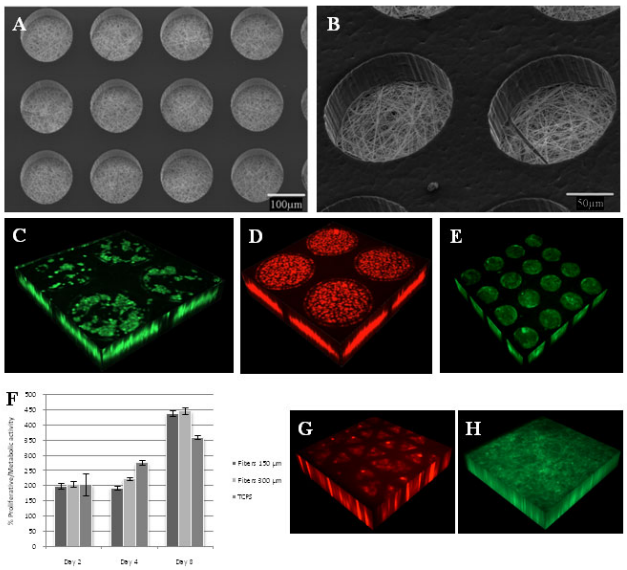

Fig. 1. A. PS microwells (150 µm)/PCL fibers, **B.** PCL microwells (300 µm)/PCL fibers, **C.** HL1 cells cultured for 2 days (cells stained with Calcein, 300 µm wells), **D.** PANC-1 cells cultured for 4 days (cell nuclei stained with PI/RNase, 300 µm wells), **E.** C3A cells cultured for 8 days (cells stained with Calcein, 150 µm wells). **F.** Proliferative/metabolic activity, **G.** C3A cells on the microwell side, **H.** 3T3 cells on the opposite side.

Conclusions: A platform that supports high throughput assembly of spatially controlled microtissue subunits was successfully developed by combining cost-effective micro and nanofabrication techniques. The potential applications of this device were demonstrated with different cell types. Our platform possesses several advantages over previously developed microwell arrays intended for the same application. These are: (1) The fibrous substrate could provide topographical stimulation to the cells in addition to resembling a more in-vivo like environment in certain cases; (2) The underlying porous fiber sheet could facilitate more suitable nutrient/waste exchange conditions to the cell clusters which can be a critical factor for achieving adequate cell viability and functionality; (3) The platform can easily be integrated with transwell plate-like systems to conduct studies on the delivery of biochemical compounds of interest (e.g., growth factors, hormones, drugs, etc) to the developing tissues through the fiber mat; fiber chemistry and morphology can be easily modified to create different delivery conditions.