Laser Direct Writing of Mouse Embryonic Stem Cells Encapsulated in Alginate Microbeads

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Statement of Purpose: Stem cells can be encapsulated in alginate hydrogel microbeads or microcapsules. Each microbead/capsule serves as a microbioreactor where cells can grow into a three-dimensional (3-D) functional unit. The maintenance and differentiation of stem cells in these microbioreactors can be regulated by the properties of alginate hydrogel microenvironments. Precise placement of these microbioreactors containing stem cells allows the investigation of effects of microenvironments on stem cell fate decisions in 3-D, permitting more effective drug screening and toxicity testing. The aim of this study is to demonstrate the ability of Matrix Assisted Pulsed Laser Evaporated Direct Write (MAPLE DW) to deposit alginate microbioreactors into defined 3-D cellular arrays. This technique grants the ability to precisely position discrete cellular microenvironments for tissue engineering and regenerative medicine studies, and to create pre-bioengineered multi-type micro-tissue constructs for clinically relevant drug screening and toxicity testing.

Methods: Mouse embryonic stem (mES) cells were chosen for this study due to their unique properties; they are unspecialized cells capable of differentiating into many cell types. mES cells were encapsulated in alginate microbeads and microcapsules, respectively. After cultured for 5 days, cell organizations in these microreactors were examined using optical/fluorescence microscopy. The alginate microbeads or microcapsules were dispersed onto an UV transparent quartz ribbon, spin-coated with a layer of 20% w/v gelatin. A pulsed ArF excimer laser (Teosys, Crofton, MD) operating at 193 nm was used to locally vaporize the gelatin matrix (Fig 1). Real-time local ribbon imaging by the built-in CCD camera allowed visual verification of transfer. The interactions of alginate microbeads and microcapsules with gelatin were examined. To verify placement, mES cells were labeled with either Cell Tracker Green CMFDA or Cell Tracker Red CMTPX. The stained mESCs were then encapsulated in alginate microbeads (green) and microcapsules (red), respectively, and deposited onto gelatin/polylysine coated surface.

Results: The difference in hydrogel microenvironments dramatically influences ES cell organization. mES cells cultured in solid alginate hydrogel microbeads formed dispersed small cell aggregates (Fig. 2a). mES cells cultured in semi-liquid alginate microcapsules formed a single embryoid body (EB)-like structure (Fig. 2b), which is an essential step for in vivo-like stem cell differentiation. Alginate microbioreactors were able to stick to the gelatin-coated surface. The addition of polylysine enhanced this adhesion. Alginate microbeads containing green mES cells and microcapsules containing red mES cells were successfully transferred onto gelatin/polylysine-coated surface (Fig. 3), thereby demonstrating the feasibility to form 3-D multifunctional and/or multicellular array using laser direct write.

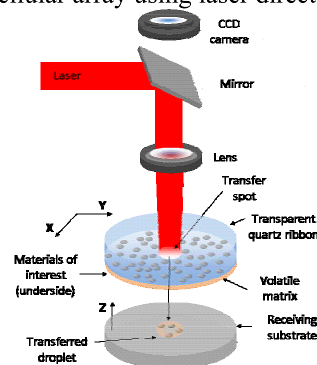

Figure 1. Schematic of matrix assisted pulsed laser evaporation direct write

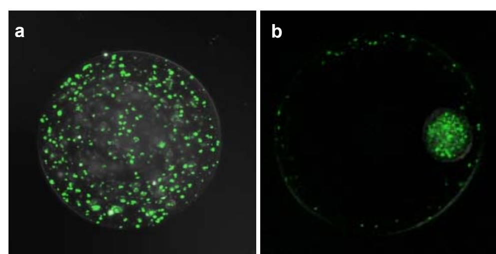

Figure 2. mES cells encapsulated in alginate 3-D hydrogel microbioreactors. (a) solid microbead; (b) microcapsule.

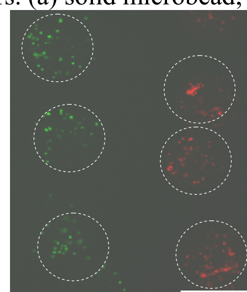

Figure 3. The deposition of 3-D multicellular array of alginate hydrogel beads containing green mES cells and microcapsules containing red cells (Scale bar = 500μm)

Conclusions: This study demonstrates the feasibility of MAPLE DW for the deposition of alginate microbioreactors containing mES cells and the formation of 3-D cellular arrays. These results lay the groundwork for future studies to engineer stem cell micro-environments and understand stem cell differentiation. An engineered 3-D ES-derived micro-tissue array could bridge conventional 2-D culture and animal models for drug screening and toxicity testing, which will significantly reduce the cost and the usage of animal models in regenerative medicine and drug discovery.