Development and Characterization of Drug Delivering Cellular Microarrays

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Introduction: Microarray technology has emerged as a valuable tool in biological sciences, particularly for high-throughput applications. While small molecule microarrays have demonstrated their capacity to screen a large variety of drugs on a small cell population [1], a microarray consisting of discreet islands of cells, thereby mitigating potential cross talk and diffusion concerns, has yet to be shown. An application for such technology would be to screen drug efficacy on rare cell populations. Colon cancer stem cells are one such population, having only recently been recognized as a potential cause of colon cancer with several cell markers identified [2]. As such, this cell population has been targeted for future therapeutics. One approach to therapy lies in manipulating signaling pathways, which govern self-renewal, using factors such as KAAD-Cyclopamine, a sonic hedgehog antagonist, DKK-1, a WNT inhibitor, Compound E, a notch pathway antagonist, and rapamycin, an mTOR inhibitor. Here we report a method for performing such analyses using a limited number of cells.

Methods: Glass coverslips were cleaned in an oxygen plasma etcher (Terra Universal, Fullerton, CA). Arrays of (3-Aminopropyl) trimethoxysilane (NH2-terminated silane) (Sigma-Aldrich, St. Louis, MO) were then printed on clean coverslips using a Calligrapher Miniarrayer printer (Bio-Rad, Hercules, CA) with 1500 µm center to center distance and a pin diameter of 360 µm. The silane printed coverslips were then coated with 175 Å of titanium (Ti-99.995% pure) and 225 Å of gold (Au-99.999% pure) (Williams Advanced Materials, Buffalo, NY). Following coating, gold-coated arrays were sonicated to remove gold from the amine spots, exposing NH_2-terminated silane islands. The coverslips were then incubated with 0.1 M, methyl-terminated alkanethiol ($CH_3(CH_2)_{11}SH$, Sigma–Aldrich). Substrates were then incubated in 10% Pluronic® F-127 (BASF Corporation, USA) for 3 h to create a non-fouling surface around the amine islands.

Biodegradable poly(D,L lactide-co-glycolide) (PLGA) (Lactel, Pelham, AL) was dissolved in propylene carbonate (Sigma-Aldrich, St. Louis, MO) at a 10% concentration and printed over the amine islands using the Miniarrayer printer. Immediately following, collagen (29 µg/ml) and fibronectin (20 µg/ml) were over-spotted onto the PLGA films and the array was then placed in a 1% heat-denatured BSA solution for 30 min. HCE-T cells were then seeded over the array in 2 ml serum-free media and allowed to incubate until cell attachment on the PLGA occurred. The arrays were then gently washed in PBS, placed in a 35 mm petri dish with complete media and placed in an incubator for 24 hr.

Arrays were then fixed in 4% paraformaldehyde and mounted with DAPI mounting media and imaged using an Axiovert 200M microscope.

For release studies, 10% PLGA was loaded with 20% (w/w) 7-Diethylamino-4-methycoumarin (Acros Organics, Morris Plains, NJ) and printed onto glass coverslips in an arrayed fashion as described above. Following printing, arrays were placed in 35 mm petri dishes with 2 ml PBS and 20 µl samples were taken at defined intervals. Samples were then analyzed on a Wallac 1420 Multilabel Counter (PerkinElmer, Waltham, MA).

Results and Conclusions: Cellular arrays can be manufactured in a robust fashion. The tightly controlled specificity of cell attachment allows for co-localization of cells with drug releasing polymer while eliminating cross-talk between islands as shown in Figure 1. Small molecules have been shown to exhibit release profiles consisting of a burst release for 18 hrs. Ongoing studies are being directed toward two initial applications. First, efficacy of drug release and cellular uptake is being tested on colon cell lines with known signaling pathway mutations by seeding on drug-loaded cellular arrays and quantifying proliferation using BrdU. Second, colon cancer stem cells are being seeded on arrays loaded with multiple signaling pathway inhibitors in a randomized fashion. Proliferation and differentiation will be quantified providing critical information about these rare cell populations and providing a platform for future treatment.

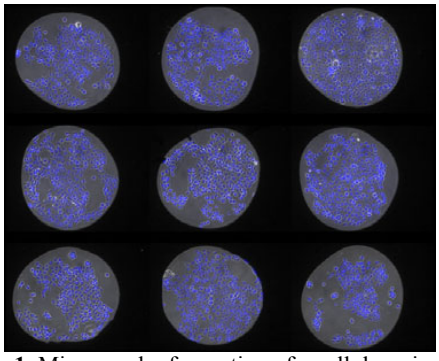

Figure 1. Micrograph of a portion of a cellular microarray seeded with HCE-T cells.

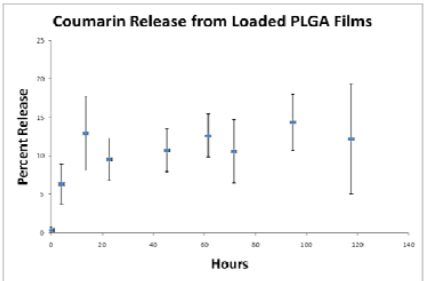

Figure 2. Release profile of arrayed PLGA films loaded with coumarin dye.

References:

[1] Bailey SN et al. Proc. Natl. Acad. Sci. 2004; 101: 16144-16149
[2] Huang EH et al. Cancer Res. 2009; 69(8): 3382-9