Drug-Eluting Microarrays

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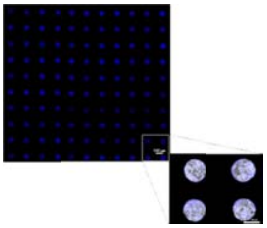
Introduction: The ability to fabricate an ex vivo device capable of accurately mimicking the in vivo microenvironment responsible for signaling which modulates cellular function would possess enormous potential in the study of cell biology. Microarrays can be utilized to delineate the various interactions of molecular signaling that affect cellular responses in vivo as well as embody a device that mimics a particular class of cell's microenvironment. 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 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. Patient-derived 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. Toward this aim, here we report a method for performing such analyses on HCT116 cells, a well characterized epithelial colon cancer cell line, with future studies directed at colon cancer stem cells isolated from human patients. Development of such a method alongside a clinical collaboration allows for a personalized medicine approach to colon cancer.

Methods: Arrays consisting of amine islands with a PEG-based non-fouling background were manufactured as described previously from our lab [3]. Biocompatible ethylene vinyl acetate (EVA) (Sigma**)** was dissolved in cyclohexanol (Acros, Geel, Belgium. Small, hydrophilic molecules were loaded into water while hydrophobic molecules were loaded in cyclohexanol. Drug-loaded polymer was then printed over the amine islands. HCT116 cells were seeded over the array and allowed to incubate until cell attachment on the EVA islands occurred. The arrays were then gently washed and placed in an incubator for 24 or 72 hrs. Arrays were then stained for Annexin V and BrdU, fixed in paraformaldehyde with Hoechst, and mounted with Fluoro-Gel (Electron Microscope Sciences, Hatfield, PA). The arrays were then imaged using an Axiovert 200M microscope and analyzed. Analysis was performed by quantifying the area of fluorescence and reported as RFI.

Results and Conclusions: Cellular arrays can be manufactured with tightly controlled specificity of cell attachment allowing for co-localization of cells with drug releasing polymer while eliminating cross-talk between islands as shown in Figure 1. Proliferation of HCT116 cells was characterized via cell populations on the drug-eluting islands in a dose-dependent manner as shown in Figures 2 and 5. Apoptosis was quantified via Annexin V [Fig 3]. Proliferation was quantified via BrdU [Fig 4]. Ongoing studies are testing the efficacy of drug release and cellular uptake of a library of small molecules on drug-loaded cellular arrays by quantifying apoptosis and proliferation. Additionally, 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.



	24 Hours
Misprints	1.24% +/- 0.61
% Islands with cell coverage	95.89% +/- 4.20
Under-populated islands	0.42+/- 3.01
% Cells on islands	94.72% +/- 3.48

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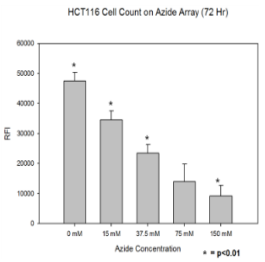

Figure 2. Cell density on azide array.

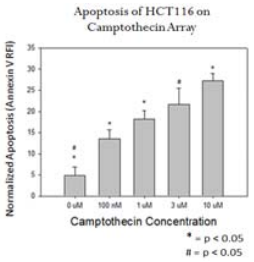

Figure 3. Apoptosis on camptothecin array

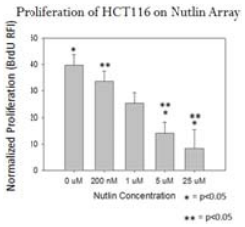

Figure 4. Proliferation on nutlin-3a array at 24 hours.

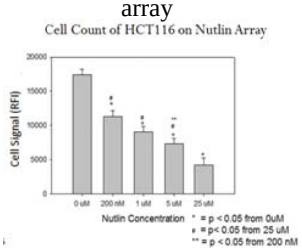

Figure 5. Cell density on nutlin-3a array at 72 hours.

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
[3] Acharya AP et al. Biomaterials. 2009; 30(25):4168-77