Cell-based microarrays: A platform to facilitate patient-specific therapy

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Introduction: Personalized medicine possesses immense promise in improving therapeutic results. Toward that aim, new technologies need to be developed which facilitate the identification of treatment regimens which will be most effective on a per patient basis. Here we report the development of a microarray platform that can perform such analyses using significantly fewer cells than standard *in vitro* assays. As such, screening populations of rare cells isolated from patients is now feasible. In colon cancer for instance, the recent development of ways to identify and isolate colon cancer stem cells (CCSCs), the demonstration that they drive tumorigenesis, and their exceptional rarity presents a unique opportunity to utilize such a strategy[1]. Now it is possible to determine which combinations of chemotherapeutic drugs CCSCs isolated from any given patient are most sensitive to by robust chemosensitivity screening on the cell-based microarray. Additionally, other patient-specific, cell-based therapies become more achievable with this platform technology. For example, a recent strategy to ameliorate type-1 diabetes (T1D), an autoimmune disorder, involves inducing specific immunological tolerance via upregulation of tolerance-promoting dendritic cells (DCs). A substantial barrier to this approach is the ability to screen vaccine formulations intended to increase this tolerogenic phenotype on patient-derived DCs in a robust fashion. We show that utilizing the cell-based microarray described herein to investigate these immunomodulatory effects greatly facilitates such an approach.

Methods: Arrays consisting of amine islands with a PEG-based non-fouling background were manufactured as described previously from our lab (**Fig. 1a, b**)[2]. For CCSC arrays, ethylene vinyl acetate (EVA) was dissolved and mixed with drug-loaded DMSO, homogenized, and printed over the amine islands using the Miniarrayer printer. Primary CCSCs were isolated as previously described[1]. For DC arrays, microparticles were fabricated as previously described[2]. Cells were then cultured on the array and then stained, imaged, and analyzed. Loading and release from thin EVA films was analyzed using spectrophotometry

Results and Conclusions: The tightly controlled specificity of cell attachment allows for co-localization of cells with drug releasing polymer films or particles while eliminating cross-talk between islands (**Fig 1c**). Release kinetics were analyzed and found to have a burst release during the first 24 h followed by steady release for over five days (data not shown). Proliferation and apoptosis of HCT116 cells were characterized on the drug-eluting microarray in a dose-dependent manner (data not shown). Combinatorial drug interactions were investigated on HCT116 cells (data not shown) and CCSC's (**Fig 2-3**). Notably, proliferation of HCT116 cells displayed additive interactions when both drugs were combined while apoptosis revealed an antagonistic relationship. In primary CCSCs, different trends were observed in the two patients when evaluating proliferation. CCSCs from CA1 demonstrated an additive effect whereas cells from CA2 had an antagonistic effect when treatments of nutlin-3a and camptothecin were combined. Ongoing studies are directed toward evaluating a greater number of patient's CCSCs against a library of small molecules to identify chemotherapeutic regimens which display the greatest anticarcinogenic potential.

Additionally, current work is focused on the ability to induce tolerogenic DCs when exposed to a panel of vaccine particle formulations. Specific adhesion of DCs has been achieved. Additionally, a library of particles encapsulated with potential tolerance-inducing small molecules has been manufactured and characterized (data not shown). Microarrays composed of numerous combinations of these microparticles are being used to screen for potential formulations which upregulate tolerogenic DC markers such as production of indoleamine 2,3 deoxygenase and interleukin-10 among others.

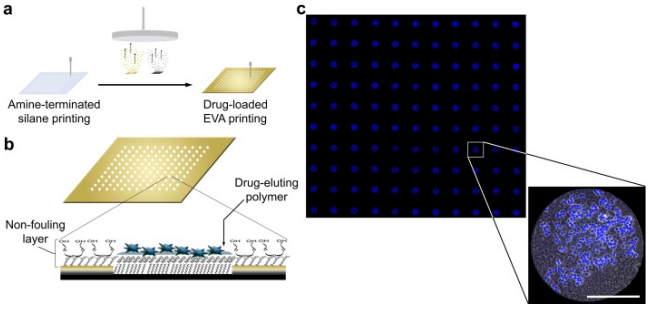

Figure 1. Design of microarray. (a) Production of microarray and, (b) schematic illustrating surface chemistry of final array configuration. (c) Micrograph of a microarray seeded will cells stained for nucleus illustrating specific adhesion to the drug-eluting islands.

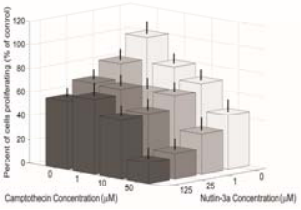

Figure 2. Proliferation of cells from CA1 on camptothecin-nutlin-3a array at 24 hours.

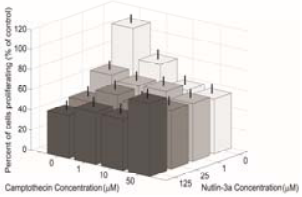

Figure 3. Proliferation of cells from CA2 on camptothecin-nutlin-3a array at 24 hours.

References:

[1] Huang EH et al. Cancer Res. 2009; 69(8): 3382-9
[2] Acharya AP et al. Biomaterials. 2009; 30(25):4168-77