RaftNote

Applications and protocols for use with CellRaft™ Technology

Imaging and Isolation of Non-adherent Single Cells on the CellRaft AIR™ System

May 2018

Jacquelyn DuVall, PhD, Nick Trotta, PhD, M. Arwen LaDine, Nick Dobes, PhD, Rob McClellan, and Steven Gebhart, PhD

Cell Microsystems, Inc.; Research Triangle Park, NC

Abstract

Research involving non-adherent cells such as lymphocytes and other primary blood cells is immensely important for applications including drug discovery, cell therapy, and immunology [1]. Non-adherent cells are also of interest in understanding the activity of the Cas9 nuclease, as transfection of these cells is highly variable and inefficient [2]. The CellRaft™ technology developed by Cell Microsystems allows for single cell imaging and isolation using the CellRaft AIR™ System, with applications including single nucleus sequencing, RNA-seq, and colony propagation for CRISPR/Cas9 genome editing. To render this technology amenable for use with non-adherent cell lines, we investigated seven common surface coatings and developed recommended protocols. Each coating was tested for the percentage of cells that remained adherent after vigorous rinsing of the array and release and transfer operations into a 96 well plate on the AIR™ System. The methods detailed here are intended to serve as general guidance but use of specific non-adherent cells and other workflow steps should be independently validated on the CellRaft Technology.

The CellRaft™ Technology

The single cell isolation and recovery platform developed by Cell Microsystems allows for imaging, sorting, and isolation of living adherent cells in a cell culture environment which closely replicates standard in vitro conditions. In its current form the CellRaft™ technology is preferentially suited for use with adherent cells without the requirement for surface modification of the CytoSort™ Array; however, the surface coatings evaluated here can be employed to render the surface amenable for use with non-adherent cell lines such as lymphocytes and various other primary blood cells.

The CellRaft AIR™ System

The AIR™ System is a bench-top instrument comprising an internal microscope with both brightfield and three-channel fluorescent imaging capabilities (**Figure 1**). The system employs the CytoSort™ Array as a substrate for single cell imaging and culture, as well as the proprietary CellRaft Technology for the isolation and collection of single cells. The AIR™ System software allows users to automatically scan a CytoSort Array for microwells containing individual cells. The system then allows either user-specified selection of cells to be collected as individuals, or cytometric analysis to automatically sort cells based on desirable characteristics according to fluorescent markers. While any type of fluorescent marker can be imaged on the AIR™ System, including transgenic proteins and antibody conjugates, live-cell nuclear stains are used to identify cells to determine which CellRafts on the CytoSort Array contain individual cells. To

Figure 1: The CellRaft AIR™ System, an automated, bench-top system for single cell imaging, sorting and isolation.

Coating	Manufacturer	Recommended concentration	Incubation	Wash steps
Poly-L-lysine	Sigma	1.5 mL of 0.01% solution	1 hour, 37°C	Sterile water (x1)
Cell-Tak	Corning	31.5 ug/mL (in PBS)	1 hour, 37°C	Sterile water (x3)
Fibronectin	Corning	10 ug/mL (in PBS)	1 hour, 37°C	PBS (x2)
Gelatin	Sigma	1 mL of 2% solution	1 hour, 37°C	None
RGD peptides	Selleckchem	10 ug/mL (in PBS)	1 hour, 37°C	Sterile water (x1)
Matrigel	Corning	1 mL of 1% solution	1 hour, 37°C	PBS (x2)
Collagen type IV	Sigma	10 ug/mL (in PBS)	1 hour, 37°C	PBS (x2)

Table 1: Coating information and application conditions for use with CytoSort™ Arrays.

expand the utility of this powerful technology, we describe here methods for imaging and isolation of non-adherent cells. We provide protocols and efficiency data regarding the use of various cell culture coatings to enable the isolation of non-adherent single cells.

Surface Coatings on the CytoSort™ Array

The CytoSort™ Array comprises thousands of individual CellRafts, fabricated from a polymeric material loaded with magnetic nanoparticles. This allows for the isolation and retrieval of a single CellRaft and the attached cell. To increase the value of this technology for applications requiring non-adherent cells, we tested several common surface coatings and developed recommended protocols for each (**Table 1**). Following an initial array preparation (rinse three times with 1X PBS for three minutes) each surface coating is prepared at the recommended concentration, applied directly to the CytoSort™ Array, and incubated at 37˚C for 1 hour. Following incubation, the coating is aspirated and the array is rinsed according to the manufacturer's protocol with either PBS or sterile water (with the exception of gelatin which requires no rinse step). Non-adherent cells are then seeded at the desired density and allowed to adhere and recover for several hours, up to overnight.

Efficiency of surface coatings on CytoSort™ Array

All surface coatings were applied to the CytoSort™ Array and tested with two different non-adherent cell lines, B lymphocytes (2PK-3, ATCC® TIB-203™) and T lymphocytes (Jurkat clone E6-1, ATCC® TIB-152™). The cells were seeded on the coated arrays and allowed to adhere overnight for 2PK-3 and 2-3 hours for E6-1. The cells were stained with SYTO 13 green fluorescent dye (ThermoFisher) and imaged on the AIR™ System (**Figure 2**). The "Full Array Scan" mode was used, which scans the full array and generates and stores images in the fluorescence and brightfield channels for each of the array's 340 fields of view. To test for adherence of each cell line with various coatings, the CytoSort™ Arrays were rinsed twice with 1X PBS and imaged a second time on the AIR™ System. Offline software was used to interrogate ten randomly selected fields of view for each array to determine the total number of cells present before and after the PBS rinses (**Figure 3**). The percentage of adherent cells ranged from 30-85% depending on the coating and cell line. This

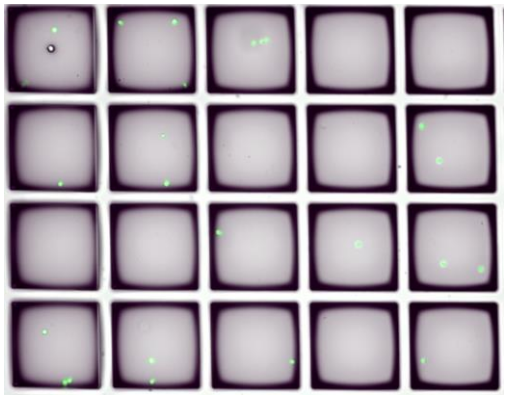

Figure 2: Representative field of view showing Jurkat E6-1 cells on poly-L-lysine coated array. Cells were stained with SYTO 13 and imaged on the AIR™ system.

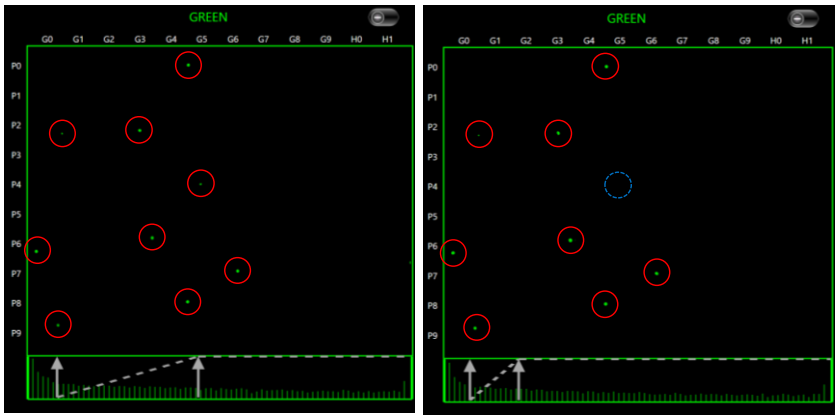

Figure 3: Poly-L-lysine (left) before wash and (right) after two PBS washes. 8 out of 9 cells remained attached to CytoSort™ Array after two vigorous PBS washes. Blue circle indicates missing cell.

data is intended only as a basic guideline, with results expected to vary depending on the type of non-adherent cells used and specific cell culture conditions. These data are summarized in **Figure 4**.

Release and transfer on the AIR™ System

To validate the use of surface coatings on the AIR™ System, each coated array was tested for successful collection by the magnetic wand. 32 releases and transfers were performed with each of the surface coated CytoSort™ Arrays seeded with 2PK-3 cells. **Figure 5** (an 'X' for each coating's release and transfer efficiency is plotted) shows the results from these experiments. Magnetic wand-based retrieval was 100% efficicent with every surface coating tested with the exception of Matrigel (30 out of 32 successful collection), demonstrating that the surface coatings have no significant effect on collection efficiency.

To further investigate the surface coatings, we tested the efficiency of each coating in maintaining the attachment of a non-adherent cell to the CellRaft™ surface through the single cell isolation workflow on the AIR™ System. This process includes needle-based release of the CellRaft™ from the elastomeric floor of the CytoSort™ Array, magnetic wand collection, and deposition of the raft into a 96 well plate. For each of the 32 release and transfer operations described above, the surface coated CellRafts were visually inspected with a fluorescence microscope following deposit into a 96 well plate to confirm the presence of the cell. **Figure 5** shows the results from these experiments, with the percentage of rafts with an attached cell ranging from 40-83% (bars plotted on the histogram). Control experiments were performed using adherent cells, with 100% still present on the CellRaft™ after release and transfer operations. Representative images of 2PK-3 cells on CellRafts coated with Fibronectin, Poly-L-lysine, and Cell-Tak are shown in **Figure 6.**

These recommended protocols are intended only as a starting point for further optimization of coating protocols for varying cell lines and culture conditions. Contact Cell Microsystems for more information.

Literature Cited

[1] Deutsh, M., et al., *A novel miniature cell retainer for correlative high-content analysis of individual untethered non-adherent cells.* Lab Chip, 2006. **6**: p. 995-1000

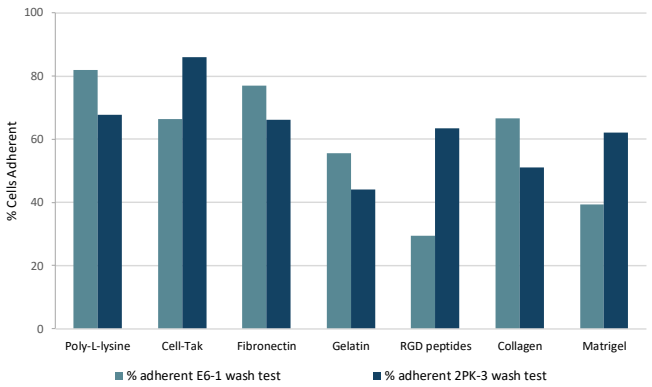

Figure 4: Arrays for each coating and cell type were scanned before and after two vigorous washes with PBS and the percentage of adherent cells was calculated.

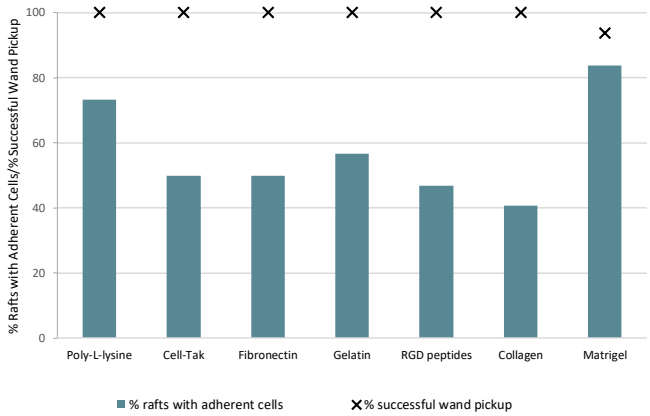

Figure 5: Isolation efficiency on the AIR™ System. 32 releases were attempted for each coating and the number of rafts successfully picked up by the magnetic wand was recorded (percent efficiency plotted as 'X' marks). For 2PK-3 cells after release and transfer of the coated rafts into a 96 well plate, the number of rafts with cells remaining adherent was visually confirmed using a fluorescence microscope (efficiency plotted via bars).

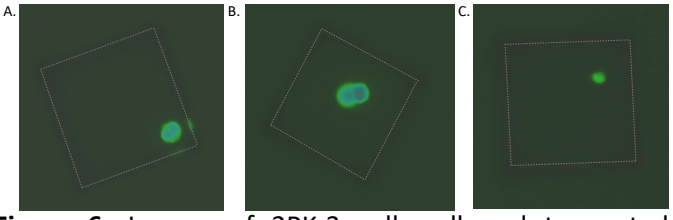

Figure 6: Images of 2PK-3 cells adhered to coated CellRafts following release and transfer into a 96 well plate. Images were obtained using a fluorescence microscope. Fibronectin (A), Poly-L-lysine (B), and Cell-Tak (C) are shown above. Grey lines were added to indicate the edges of the raft.

[2] Attayek, P.J., et al., *Automated microraft platform to identify and collect non-adherent cells successfully gene-edited with CRISPR-Cas9.* Biosens. Bioelectrons., 2017. **91**: p. 175-182

For more information:

info@cellmicrosystems.com