STREAMLINING CELL BIOLOGY WORKFLOWS: INTEGRATING SUSPENSION CULTURE, CELL LYSIS, PROTEIN EXTRACTION AND NUCLEIC ACID EXTRACTION

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ABSTRACT

We have developed a platform which enables the culture and subsequent analysis of cells in suspension through immunohistology and nucleic acid assays. Implementation of a bucket design and passive pumping described by Young, *et al.* [1] allows for culture of cells in suspension for several days as well as treatment with drugs, cytokines, and protein-based assays. The device has the added functionality of enabling single-step extraction of nucleic acids directly from culture using paramagnetic nucleic acid-binding beads. Extraction of DNA and mRNA from the culture is comparable to that of commercially available kits requiring several wash steps.

KEYWORDS: Nucleic Acid Extraction, Cell Culture, Cancer Biology, Multiple Myeloma.

INTRODUCTION

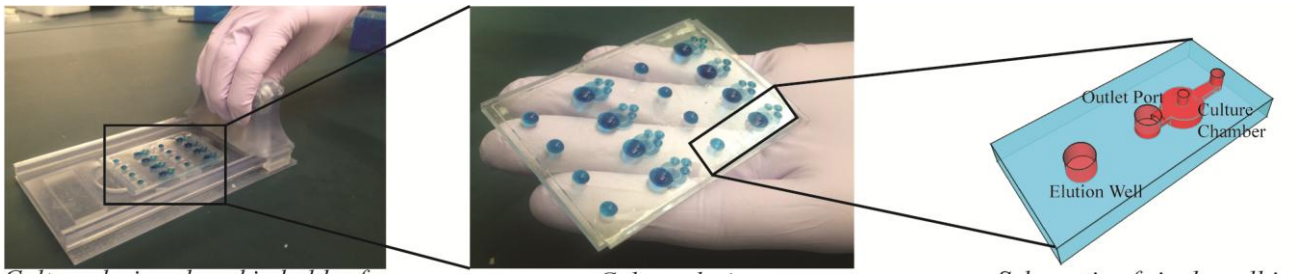
Cancers such as multiple myeloma (MM) have large clonal heterogeneity giving rise to drug resistance, which makes treatment difficult [1]. Improved analysis of MM cells is needed. Current methods allow genetic analysis of the tumor but lack protein and mRNA expression information in response to treatment. More comprehensive analysis of the tumor would enable improved and personalized treatment of the patient. An added challenge is that MM cells are non-adherent, and, thus new methods are needed.

THEORY

Cell culture of non-adherent cell types is difficult due to centrifugation steps required to perform fluid exchange steps for immunohistological staining as well as simple procedures such as feeding cells with fresh media. Integrating culture systems designed suspension cell culture onto a platform which can perform genomic, transcriptional, and protein interrogation of cells *in situ* would be a powerful tool. It allows researchers to increase the variety of experiments performed as well as

EXPERIMENTAL

The system consists of two parts – microchannel suspension culture arrays developed by Young, *et al.* [2] and a reusable platform for performing analyte extraction from the culture channels (Figure 1). Multiple myeloma cells (RPMI-8226) were seeded into the device and cultured for up to 48 hours. After culture the cells can either be analyzed using immunohistological staining directly on the device or DNA or mRNA can be extracted and analyzed. Nucleic acid extraction begins by lysing the cells in a LIDS buffer in the presence of paramagnetic beads which bind either DNA or mRNA. To achieve on-device extraction, we created a novel extraction platform where a magnet is passed over the culture chambers moving the nucleic acid-bound beads to the outlet port where they are collected under the magnet. The hydrophobic surface of the device pins the beads in a droplet on the outlet port. The magnet is then moved across the device to elution well, separating the beads from the outlet droplet. As the beads move across the surface, a magnet of opposite polarity under the elution well repels the transfer magnet upward causing the bottom magnetic field to be dominant and, thus, pulling the beads back onto the device where they can be transferred for amplification or analysis without the use of external tools like a centrifuge (Figure 2).



Culture device placed in holder for nucleic acid extraction | *Culture device* | *Schematic of single well in culture device*

Figure 1: Microfluidic cell culture channel for suspension cells integrated with nucleic acid extraction

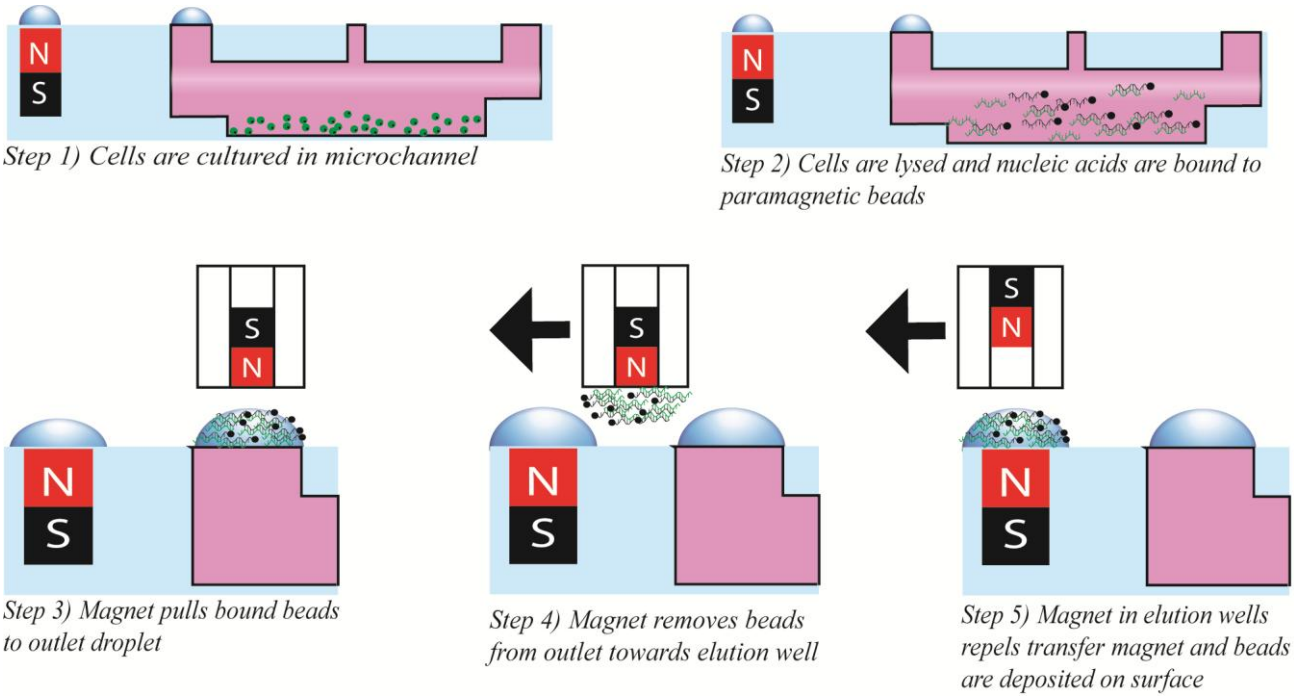


Figure 2: Process flow for cell culture, lysis, and nucleic acid extraction

RESULTS AND DISCUSSION

A comparison between the multiple myeloma integrated culture and nucleic acid isolation device (MM-SLIDE) and commercially available kits were performed to establish the efficacy of extraction and isolation for both cDNA and mRNA was performed. Dynabead paramagnetic bead extraction kits for cDNA and mRNA were purchased from Invitrogen (Grand Island, NY) were used in the experiments.

Two parallel cell culture experiments were started using RPMI-8226 cells. Cells were cultured either in the MM-SLIDE or a 384-well plate. The culture was maintained for 48 hours before extraction. Extraction from the microtiter plate was performed using either the cDNA or mRNA Dynabead kit. Extraction from the MM-SLIDE was performed as described in the experimental section. mRNA recovered from both culture platforms were reverse transcribed to obtain cDNA. The cDNA from mRNA and cDNA obtained directly from culture were run with qPCR and the crossing-point (C_p) value was established to determine relative quantities of nucleic acid recovery between platforms for the target housekeeping gene, GAPDH (Figure 3). The amount of cDNA recovered between both culture and extraction systems were found to comparable with no significant difference across platforms. The amount of mRNA for GAPDH recovered from the macroscale culture with the Dynabead kit was found to be slightly greater than what was recovered from the MM-SLIDE. The amount of mRNA recovered from the MM-SLIDE is still detectable by a large margin and has the potential to be improved to allow for more complete removal of the beads from MM-SLIDE culture surface by increasing the magnet residence time over the bead outlet port and enhancing the hydrophobic patterning on the device surface to optimize pinning thereby decreasing droplet crossover and enhancing purity of the extracted beads.

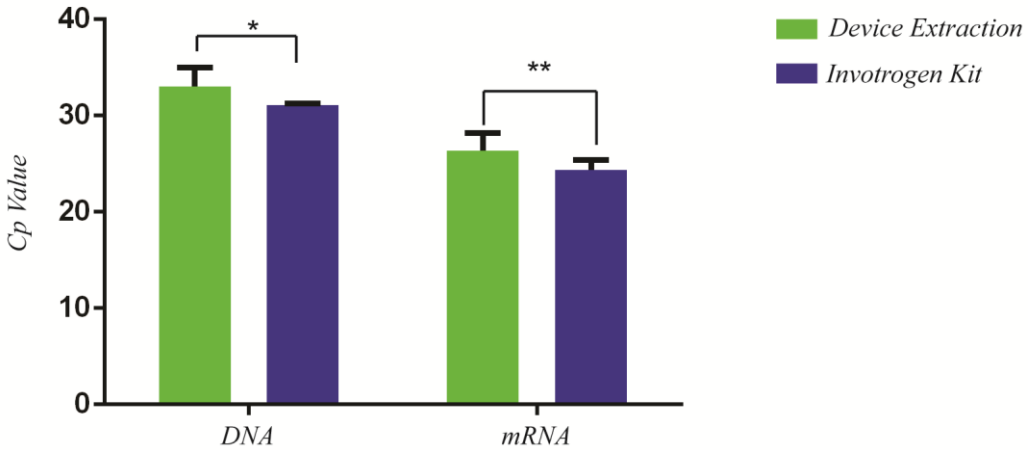


*Figure 3: Comparison between MM-SLIDE and macroscale control for DNA and mRNA of GAPDH in RPMI-8226 cells. *P=.37, **P=.09*

The MM-SLIDE platform was then evaluated its ability to detect signal from both fluorescence and mRNA transcripts. RPMI-8226 cells were prepared and cultured for the MM-SLIDE for a time period of 48-hours and mRNA was extracted, and converted to cDNA as previously indicated. Several culture devices on the MM-SLIDE did not undergo lysis and mRNA extraction and were instead reserved for immunofluoresence staining. Syndecan 1, a protein known to be expressed in RPMI-8226 cells [3], was targeted for detection. qPCR was performed on the extracted cDNA for syndecan 1 and housekeeping gene, GAPDH. Immunohistology for proteins, syndecan 1 and GAPDH was performed on the cell culture devices which were not lysed (Figure 4). The two proteins were detected at both the mRNA transcriptional level through qPCR and at the protein level through immunohistology.

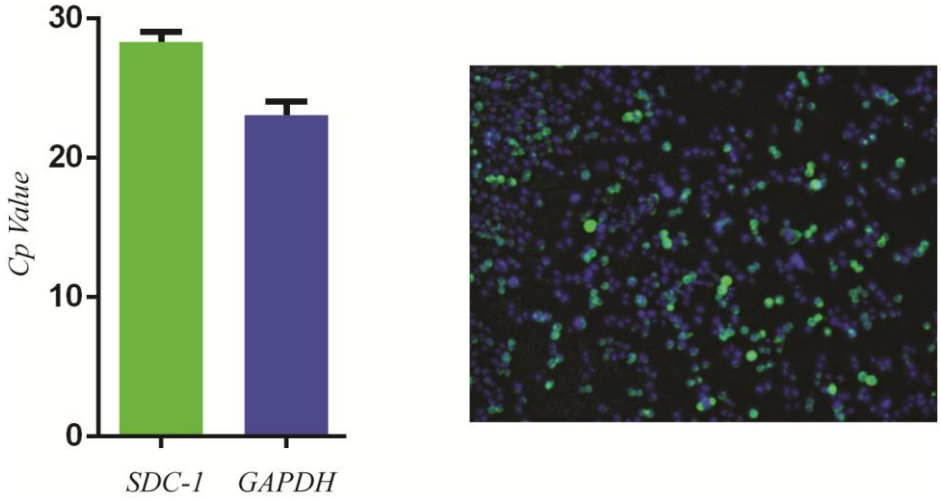


Figure 4: Comparison between mRNA recovery and immnuofluorescence staining of GAPDH (blue) and syndecan 1(green).

CONCLUSION

The platform presented combines both immunohistological and nucleic acid-based analysis of suspension cells in a single culture device. The platform also has the ability to be expanded to encompass even more analytical techniques mediated by paramagnetic beads such as secreted factors through ELISA. Combining platforms enables easier access to accomplish a more complete analysis of a model system to be performed in situ. Streamlining those platforms together allows effective and simple analyte and data collection. The MM-SLIDE provides researchers access to a more complete toolbox to better study and understand diseases like multiple myeloma.

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