Capture and reagent exchange (CARE) wells for cell isolation, labeling, and characterization

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Received: 22 March 2022 / Accepted: 28 June 2022 / Published online: 16 July 2022 © The Author(s) 2022

Abstract

Cell therapy is an emerging field that uses cells as living drugs to treat a broad array of acute and chronic diseases. Most cell therapies in clinical trials are made using standard bench methods, whose open processing require manufacturing in expensive GMP cleanrooms. As cell therapies progress, new methods are needed to enable scalable manufacturing while maintaining process integrity, reducing environmental exposure, and limiting critical cell and reagent use. Here, we introduce capture and reagent exchange (CARE) wells that allow critical processing steps to be integrated into a closed microfluidic device. The unique property of CARE wells is that they allow reagent exchange from an attached channel without cell loss from wells. We show through simulation and experiment that this feature is present in cylindrical wells whose depth is sufficient to generate multiple recirculating vortices and is independent of flow rate in the channel. We demonstrate that CARE wells can be used to perform cell separation, on-chip labeling, and characterization of monocytes as the first steps toward a closed microfluidic system for production of dendritic cell therapies. Immunomagnetic separation of CD14+ monocytes from peripheral blood mononuclear cells (PBMCs) into wells was performed with purity of 97 ± 2% and capture efficiency of 50 ± 17%. On-chip labeling, washing, and characterization were performed using two cell surface markers (CD14 and HLA-DR) on over 3000 cells captured in a 5193-well device. The combination of high purity separation and reagent exchange without cell loss with robust performance over wide range of input and operating conditions makes this technique a promising approach for scalable manufacturing and analysis of cell therapies.

Keywords Cell therapy · Microfluidics · Cell isolation · On-chip labeling · Dendritic cells

1 Introduction

Cell therapy is an emerging paradigm of medical treatment that uses cells to treat a broad array of acute and chronic diseases (Ankrum and Karp 2010; Anguille et al. 2014; Holzinger et al. 2016). Most cell therapy products are made using typical bench cell culture techniques performed inside of a clean room. New methods are needed to enable scalable manufacturing of cell therapies while maintaining protocol integrity and meeting required safety and potency requirements. Closed systems that integrate the manufacturing process into a single instrument are desirable to reduce skilled technician time, reduce reagent use, and reduce or eliminate expensive GMP lab space (Iancu and Kandalaft 2020). Additionally, due to the fragmentation of cell processing and analysis, cell product testing consumes large quantities of cells, increasing both cells harvested from the patient and total costs (Boudousquie et al. 2020). There is a clear need to develop tools for both integrated closed manufacturing of cell therapies and minimally destructive functional test platforms to enable broader access to these life-saving therapies.

Dendritic cells (DCs) are antigen-presenting cells that act as the sentinel of the immune system and can stimulate both naïve and recall responses (Steinman and Banchereau 2007) and have shown promise for use as cancer therapy (Anguille et al. 2014). The most common method for production of therapeutic DCs includes immunomagnetic isolation of CD14+ monocytes from the patient’s blood, culture with cytokines to differentiate them to into immature dendritic cells, and a second culture with cytokines and a target antigen to produce mature, antigen-presenting dendritic cells (Dietz et al. 2006a, b, Laborde et al. 2014). After cell culture is completed, millions of cells are sacrificed for phenotypic
characterization before they are injected back into the patient where they promote an immune response against the target antigen-expressing cells. Depending on the application, the antigen can be tumor lysate (Fadul et al. 2011), autologous tumor lysates (Parney et al. 2020), or peptides that are highly expressed on the target tumor (Bedrosian et al. 2003). These therapies have demonstrated good safety and efficacy in feasibility studies for lymphoma (Lin et al. 2014), glioblastoma (Parney et al. 2020), and ovarian cancer (Knutson 2020). Efforts to develop closed systems for manufacturing DCs have primarily relied on daisy-chaining existing biomanufacturing instruments with closed fluidic connections (Erdmann et al. 2018; Uslu et al. 2019). Their use still requires skilled operators and specialized facilities. A closed, fully integrated system for producing dendritic cells would overcome these limitations and allow much broader deployment of DC-based cell therapies.

Immunomagnetic isolation is an established separation technique that uses magnetic beads conjugated to an antibody against a specific cell surface marker to isolate cells expressing that marker within a mixture of cells (Thiel et al. 1998). When placed in a magnetic field, labeled cells can be drawn away from other cells and collected. A variety of methods have been developed for using this technique in microfluidic devices, including continuous flow isolation (Inglis et al. 2004; Kim et al. 2013; Darabi and Guo 2016), immobilization in a microfluidic channel (Hoshino et al. 2011; Mohamadi et al. 2015), isolation into microwells (Huang et al. 2018; Armbrrecht et al. 2019), and multistage isolations coupled with size-based sorting (Mishra et al. 2020), or in combination with deformability (Chen et al. 2019). There is a large catalog of commercially available magnetic isolation kits based on surface markers, including stem cells, hematopoietic cells, and circulating tumor cells, and many of these have been used in clinical manufacturing of cell therapy products (Van Driessche et al. 2009; Priesner et al. 2016).

Microwells are a versatile tool for performing diverse biological workflows in microscale devices. A single microwell allows cells (Rettig and Folch 2005), organoids (Brandenberg et al. 2020), or other objects to be retained in a fixed location, while the composition of the fluid above the microwell is changed to accomplish a number of analytical tasks. Arrays of microwells allow populations of cells to be quantified, characterized, and monitored with single cell resolution (Wang et al. 2007). Complex biological workflows have been integrated into microfluidic devices using microwells, including digital PCR (Podbiel et al. 2020), single cell RNA-Seq (Yuan and Sims 2016), multiplex secretion profiling (Armbrrecht et al. 2019), nutrient adaptation (Woronoff et al. 2020), immunostaining (Kobayashi et al. 2015), drug discovery (Jorgolli et al. 2019; Ai et al. 2020), organoid culture (Brandenberg et al. 2020), tissue microRNA analysis (Nagarajan et al. 2020), proliferation assays (Park et al. 2010), and lineage analysis (Luro et al. 2020). A feature of microwells that has been used by some groups is to increase the depth of the well to prevent flow from sweeping out cells within the well (Pilat et al. 2017; Avesar et al. 2018; Jorgolli et al. 2019; Luro et al. 2020). Avesar et al. showed with simulations that 200-μm-wide by 100-μm-tall by 400-μm-deep wells had recirculating flow at the well opening and no convective flux between the wells and the channel (Avesar et al. 2018). While this feature of deep microwells has been used by several groups, no one has identified the key parameters that allow microwells to retain cells when there is flow in an attached channel.

In this paper, we present a microfluidic device that allows many key functions of cell therapy manufacturing to be integrated into a single closed system using cell isolation and reagent exchange (CARE) wells. The device is designed to take advantage of the flow properties in deep microwells to allow of integration of immunomagnetic separation and subsequent reagent exchange operations without cell loss in scalable manner. We performed simulations and experiments to understand the well geometries necessary to get these capabilities and then demonstrated the functionality of this technique by performing separation, labeling, and characterization of monocytes from a sample of peripheral blood mononuclear cells (PBMCs) without cell loss during fluidic operations. The combination of high purity separation and reagent exchange without cell loss makes this technique a promising approach for minimally destructive analysis of cell cultures and scalable manufacturing of cell therapies.

2 Results

Capture and reagent exchange (CARE) wells are designed to take advantage of the flow properties of deep microwells to allow for integration of immunomagnetic cell capture and reagent exchange operations, such as immunofluorescent labeling and washing. A schematic of the device operation is shown in Fig. 1. The device is composed of a channel with wells patterned along the upper wall and a single inlet and outlet. Cells labeled with antibody-conjugated magnetic nanoparticles are imported into the channel and pulled into the wells by a magnet, while unlabeled cells remain in the main channel or sediment under the influence of gravity (Fig. 1A). The wells are designed such that flow from the channel does not enter the well, so only cells labeled with magnetic nanoparticles will be captured and unlabeled cells can be flushed from the device (Fig. 1B). After cell capture, the device is flipped so gravity maintains captured cells in the wells (Fig. 1C) and fluid in the main channel can be changed to allow reagent exchange, such as in situ labeling.
with fluorescent markers, washing, and enumeration and characterization (Fig. 1D, E).

To achieve integrated separation and reagent exchange, cells need to be shielded from flow in the channel after capture. Simulations were performed in COMSOL 5.4 (Burlington, MA, USA) to understand the key geometric features necessary to retain cells when there is flow in the channel. Figure 2A shows streamline plots for fluid flow in a geometry composed of a cylindrical well with variable diameter from 10 to 100 microns with 100 micron depth connected to a 200-micron-deep channel with average inlet velocity of 1 mm/s in the channel. The streamline plots show that flow from the channel does not enter the well for any well diameter. Rather, a series of recirculating vortices are generated starting at the entrance of the well with the number of vortices increasing as the well diameter decreases. The number of vortices as a function of well diameter for a fixed well depth of 100 microns is plotted in Fig. 2B (blue squares). Wells with diameters of 50 microns or less have multiple vortices, while those with diameters of 60 microns or more have a single vortex. Cells or objects driven by flow in the main channel will follow streamlines unless acted on by external forces (White 1974). Therefore, the only way for a cell to enter or exit the well is via non-fluidic forces (magnetic, gravitational, thermal, etc.). This ensures that immunomagnetic separation (shown in Fig. 1A) will capture a pure population of cells by surface marker expression.

We tested the effect of vortices in microwells experimentally with beads and found that multiple vortices are needed for it to retain objects during fluidic operations. A microwell device was made that had arrays of wells with variable diameters from 10 to 100 microns patterned inside a channel with a single inlet and outlet (layout shown in Figure S1). 5.8 micron COMPEL magnetic beads (Bangs Laboratories, Fishers, IN, USA) suspended in buffer at 0.01% w/v were pulled into the wells with a magnet, the magnet was removed, and the total number of beads loaded in the array of wells at each diameter was counted. The device was then flushed with 1 mL of liquid at 100 µl/s with a syringe pump and total number of beads remaining in the array of wells at each diameter was counted. The percentage of beads initially loaded into wells that was retained after flushing at each diameter is shown in Fig. 2B (black circles). Wells with diameters between 60 and 100 microns (those shown to only have a single vortex) lost a percentage of beads during the flush, while those with diameters of 50 microns or less (those shown to have multiple vortices) retained all the beads during the flush. This result can be understood through the streamline plots. In a well with a single vortex, an object in the bottom of the well will be driven to the mouth of the well by flow in the channel where it may divert from the streamline due to thermal motion and be lost. In a well with multiple vortices, an object at the bottom of the well will be brought partially up the well and will remain protected from flow in the channel. We do not expect there to be a strong
dependence on cell/bead size and retention in the well so long as the object remains entirely below the first vortex.

Additional simulations established that the recirculating flow pattern in the wells is independent of flow rate. Simulations of 30-micron-diameter wells with 100 micron depth attached to a 200-micron-deep channel were performed with varying average inlet flow rate from 10 cm/s to 1 μm/s. Streamline patterns are plotted as well as the log of velocity magnitude normalized to the inlet velocity (Fig. 2C). The plots show that both the streamlines and the normalized velocity magnitude are nearly identical over five orders of magnitude difference in flow rate and the velocity in the bottom of the well is reduced by a factor greater than $10^8$ from the channel. Cells or beads in the bottom of wells with this geometry will experience essentially no fluid motion (1 nm/s) even when fluid is moving quickly (10 cm/s) in the channel. This is ideal for maintaining a low-shear environment for cell culture and enabling a wide variety of flow rates to be used for various analytical operations, such as perfusion for culture or fast flushing for labeling (Young and Beebe 2010). This result contrasts with inertial vortex capture approaches where the flow pattern in the capture reservoir changes depending on the flow rate (Hur et al. 2011; Chung et al. 2013) and provides a robust performance over a wide range of operating conditions.

Cell retention across a range of flow rates was further verified by exposing beads in wells to a sequence of flushes at varying flow rates and monitoring for bead loss. 207 magnetic beads with 4 micron diameter (Bangs Laboratories, Fishers, IN, USA) were loaded into an array of wells with 30 micron diameter and 100 micron depth. The device was then flushed with 1 mL of buffer at 100 μL/s, imaged, and beads were counted. This was repeated for 10 μL/s, 1.0 μL/s, and 0.1 μL/s in sequence. During this process, which exposed beads to flow rates varying over three orders of magnitude and greater than three hours of flushing, 207 beads were observed after every step and no beads were lost. The process and results are summarized in Table 1. Combined with the simulation results, this verifies that objects captured in
CARE wells will not be lost to flow in the channel during reagent exchange.

We use these simulation results and experiments to define capture and reagent exchange (CARE) wells as those that contain two or more recirculating vortices as shown by streamline plots. Within the geometric space explored in this paper (cylindrical wells with 100 micron depth connected to a 200-micron-deep channel), wells with diameter less than 50 microns exhibited the CARE functionality, while those with larger diameters did not. Because the flow field was independent of flow rate, these criteria will remain consistent if the geometric parameters are scaled up or down by a constant value (Sedov 1982). These criteria may differ for different well shapes and ratios of well depth to channel height and a more in-depth exploration remains for future work.

Using CARE wells, we demonstrated immunomagnetic isolation of CD14 + monocytes from PBMCs with high purity over a wide range of input cell densities. Devices contained 5193 microwells with 30 micron diameter and 100 micron depth patterned over a 2 by 8 mm area. A flow channel with a single inlet and outlet over the well area had 200 micron depth. External connections were made with 23-gauge needle tips mated to EVA plastic tubing to vertical holes punched through the channel layer. One port was connected to a syringe for fluid operations, while the other was open for sample input/output. A schematic of the device layout and cross section is shown in Fig. 3A. PMBCs were isolated from donor blood using Histopaque density gradient separation and labeled with Miltenyi CD14 MicroBeads and fluorescent antibodies against CD14 (monocytes) and CD45 (hematopoietic cells) surface markers. All cells within the PBMC population are expected to express CD45. Cell density, viability, and CD14 abundance were measured in the input cell sample (Fig. 3B). CD14 abundance in the input PBMC samples varied by donor with a range of 13–36% with mean of 24 ± 9%. The input CD14% for each experiment is listed in Table S1. To load cells, 100 μL of cell sample was pulled into the chip at 10 μL/s, flow was stopped and two stacked 32 lb pull force neodymium magnets were placed on top of the device for 10 min to pull bead-labeled cells into wells (Fig. 3C). After 10 min, cells remaining in the channel were flushed with 1–2 ml buffer at 100 μL/s, the magnet was removed, and the chip was flipped to retain captured cells by gravity. This approach yields a uniform density of captured cells across the chip but does not capture any cells outside of the well area. After cell capture, a composite brightfield and fluorescent image was captured of the well area of each device (Fig. 3D) with an EVOS Cell Imaging system (Thermo Fisher, Waltham, MA, USA). For each device, cells were counted using a semi-automated program written in MATLAB to determine the total number of CD45+ cells and CD14+/CD45+ cells. A detailed description of the cell counting program is available in the supplemental material.

Twelve immunomagnetic CD14 isolation experiments, each with a unique donor, were performed with a range of input cell densities from 6.2e5 to 1.6e7 cells/mL, and an average captured CD14+ cell purity of 97 ± 2% was observed with estimated capture efficiency of 50 ± 17%. Purity is defined as number of captured CD14+ cells divided by the total number of captured CD45+ cells. Estimated capture efficiency is defined as the number of captured CD14+ cells divided by the number of captured cells calculated to be in the well area of the chip during separation (live cell density x CD14% in PBMC input x chip volume). Individual experiment results are tabulated in Supplementary Table S1 and purity and capture efficiency as a function of input cell density are plotted in Supplementary Fig. S2. A linear fit shows that purity does not depend strongly on input density, while there is a decrease in capture efficiency with increasing input cell density. Some strategies toward increasing capture efficiency include increasing the magnetic force on cells by increasing capture bead concentration or size or increasing magnetic field strength. Increasing the surface density of wells will increase the capture area the channel area (some cells may be pulled up to the surface of the channel but not into a well). Increasing the channel depth may also allow more cells to be loaded at lower input cell densities if cell clumping at higher densities is limiting capture efficiency.

On-chip labeling with fluorophore-conjugated antibodies, washing, and characterization without cell loss were performed on cells captured in CARE wells. A CD14 isolation with cells only labeled for CD45 was performed and cells were labeled on-chip with antibodies against CD14.

| Step | Flow rate (μL/s) | Mean channel velocity (mm/s) | Time | Beads counted | Beads lost |
|------|------------------|-----------------------------|------|--------------|------------|
| Capture | -- | -- | -- | 207 | -- |
| 1 mL flush 100 | 250 | 10 s | 207 | 0 |
| 1 mL flush 10 | 25 | 1 h 40 s | 207 | 0 |
| 1 mL flush 1.0 | 2.5 | 16 h 40 s | 207 | 0 |
| 1 mL flush 0.1 | 0.25 | 2 h 46 m | 207 | 0 |
and HLA-DR. Low or negative expression of HLA-DR has been associated with an immunosuppressive state that can hinder dendritic cell maturation (Vuk-Pavlovic et al. 2010; Young and Beebe 2010). Labeling was performed by pulling a solution containing fluorescent antibodies into the chip and incubating for 10 min. Washing was performed by flushing the chip with buffer for 10 s at 100 μL/s to remove the labeling solution in the channel and then 30 min at 1 μL/s to allow unbound labeling antibodies to diffuse from the wells. Figure 4A shows a cropped region of the device in each fluorescent channel (CD45/CD14/HLA-DR) before labeling, during labeling, and after washing. Cells that were initially unlabeled for CD14 and HLA are clearly labeled in the wells. A significant fluorescent background can be observed in the labeling step that is removed after the wash, allowing fluorescence intensity of individual cells to be characterized. Figure 4B shows scattergrams (CD45 vs CD14 and CD45 vs HLA-DR) of fluorescence intensity for 3123 CD45 + cells identified after immunomagnetic isolation before and after labeling, with clear separation between unlabeled and labeled populations observed. This capability allows non-disruptive, in situ analysis of surface marker expression of the captured cell population within the device.

The timing of the washing step was informed by a numerical simulation of diffusion of antibodies from the wells during a 1 μL/s flush. Diffusion from the well was simulated in COMSOL 5.4 using the boundary conditions and geometry shown in Fig. 4C. A single 30-micron-diameter, 100-micron-deep well was attached to a 200-micron-deep channel. Average inlet velocity was set to be 2.5 mm/s, which is the equivalent velocity to 1 μL/s on the full device. The concentration was set to 1 mol/m³ in the geometry with a concentration of 0 mol/m³ at the inlet to the channel. The diffusion coefficient was set to 10 μm²/s based on estimates for diffusivity of an antibody at room temperature (Nauman et al. 2007). A time-dependent solution was calculated with 10-s intervals for 30 min. The plot in Fig. 4C shows the average concentration in the well in blue and the concentration at the bottom surface of the well in pink. In 30 min, the
average concentration in the well is $5 \times 10^{-4}$ mol/m$^3$, while the concentration at the bottom of the well is $1 \times 10^{-3}$ mol/m$^3$. This corresponds to a 2000 times reduction in concentration in the well on average and a 1000 times reduction at the bottom of the well, effectively washing the labeled cells from unbound fluorescent antibody. After the first few minutes, both curves show good fit with exponential decay curve with the same decay constant of $-0.24$ min$^{-1}$, so each 9 min and 36 s of additional flushing under these conditions will result in a further 10 times reduction in concentration. Combined with the experimental results, these simulations provide confidence that reagent exchange operations can be performed on cells captured in the wells.

**Fig. 4** On-chip cell surface marker label and wash for phenotypic characterization. A) Fluorescent images of the chip of cells labeled with CD45 prior to chip loading were subsequently labeled with CD14 and HLA-DR on the chip. Scale bar is 50 microns. B) Scatter plots showing fluorescence intensity of CD14 and HLA-DR for each cell detected in the CD45 channel before and after labeling show clear separation between the unlabeled and labeled populations. C) Simulation of washing cells of fluorescent antibody shows that average concentration in well is reduced by 2000 times and concentration at bottom of well is reduced by 1000 times with 30 min of flushing with buffer at 1 μL/s.
3 Conclusion

There is a clear need to develop tools for integrated closed manufacturing of cell therapies and minimally destructive functional test platforms to enable broader access to these life-saving therapies. Here, we have presented a new micowell that allows cell capture and reagent exchange (CARE) to be integrated into a simple, valveless design. We showed through simulation and experiment that fluid motion in CARE wells is characterized by multiple recirculating vortices that shield cells or objects in the wells from flow in an attached channel and allows reagent exchange without cell loss. By exploiting immunomagnetic isolation, gravity, and CARE wells, we can separate cells from a mixture with high purity over a wide range of input densities and perform subsequent reagent exchange without cell loss. With the addition of simple fluorescent imaging and image processing software, the device can be used to count and phenotype cells. These devices could be patterned over a large area to accommodate millions of cells, manufactured in plastic using high volume manufacturing techniques, and operated as a closed system rather than requiring GMP space. In situ analysis of cell surface marker expression without loss could be performed to monitor process steps and ensure the safety and efficacy of the final cell product. The combination of high purity separation and reagent exchange without cell loss, with robust performance over wide range of input and operating conditions, gives this approach broad applicability in cell therapy manufacturing, including diagnostic processes, cell isolation, exposure to differentiating or transformative agents, phenotyping, culture, and expansion.

4 Materials and methods

4.1 Microfluidic device fabrication

Microfluidic devices were designed using CAD software (AutoCAD 2020, AutoDesk, San Rafael, CA, USA) and were fabricated in PDMS using photolithography and soft lithography (Becker and Gartner 2000). The devices consist of a micowell layer with 5193 cylindrical microwells and a channel layer for fluid transport over the microwells. Microwells had 30 micron diameters and 100 micron depth and the channel layer is 200 micron thick. Replica molded PDMS layers were aligned and bonded to each other and a glass slide by oxygen plasma (YES–G1000, Yield Engineering Systems, Livermore, CA, USA). Fluidic connections were made by through holes punched in the channel layer.

4.2 Cell preparation

Cell samples were obtained from blood donated by volunteers at the Division of Transfusion Medicine, Mayo Clinic, Rochester, Minnesota, in accord with current regulations by the AABB and US FDA. Following an apheresis procedure, a leukoreduction system chamber from the apheresis apparatus (Trima Accel, Gambro BCT, Lakewood, CO, USA) was collected, and PBMCs were isolated using density gradient separation in Histopaque (MilliporeSigma, St. Louis, MO, USA) following a previously established protocol (Dietz et al. 2006a, b). Cell density and viability were enumerated using a Countess Cell Counter (Thermo Fisher, Waltham, MA, USA) and trypan blue exclusion. CD14 percentage in PBMC samples was determined by taking a composite fluorescent image of labeled input cells (one channel each for CD14 and CD45 markers) loaded in a Countess Cell Counting Chamber Slide with an EVOS Cell Imaging system (Thermo Fisher, Waltham, MA, USA) and quantifying the number of CD45 and CD14 cells. The CD14 percentage was calculated as the ratio of number CD14 cells detected to the number of CD45 cells detected. Cells were labeled with CD14 MicroBeads (Miltenyi, Bergisch Gladbach, Germany) and stained with antibodies (Biolegend, San Diego, CA) against CD45 and CD14 surface markers following the manufacturers’ protocols before being centrifuged, washed, and resuspended to a desired cell density. In the on-chip labeling experiment, cells were only labeled with CD45 antibodies before on-chip capture.

4.3 Device operation

Devices were wet with a solution of 1% (w/v) Pluronic F-127 in PBS with fluid flow driven by a hand-operated syringe or syringe pump (KD Scientific, Holliston, MA, USA). On-chip magnetic isolation was performed by pulling labeled cell suspensions into the flow channel, stopping flow, and placing a magnet on top of an inverted chip. Two 32 lb. pull force neodymium magnets (K&J Magnetics, Pipersville, PA, USA) were stacked and placed on top of the chip for 10 min, then the flow channel was flushed with greater than 1 mL of buffer at approximately 100 uL/s. The magnets were removed, and the chip was flipped to a wells-down configuration for imaging and other fluidic operations.

4.4 Microscopy and image analysis

Imaging was performed on an EVOS Cell Imaging system. Stitched, composite brightfield, and fluorescent images were captured over the entire well region for each device. Individual channels (brightfield and fluorescent) were split for semi-automated analysis in MATLAB2019a (Mathworks, Natick, MA, USA). Individual wells were identified using
the circular Hough transform and used to mask fluorescent images. After adjusting for brightness/contrast, cell quantification was performed on the masked fluorescent images and detected cells were assigned to a specific well. Any well that contained a fluorescent cell in any channel was then manually inspected to confirm the count. The resulting data included total number of cells detected for each fluorescent channel and the number of cells per well. More detail on this process is available in the supplementary material.

4.5 On-chip labeling

Alexa 488 anti-human HLA-DR (200 μg/mL), PE anti-human CD14 (25 μg/mL), and Alexa 647 anti-human CD45 (150 μg/mL) antibodies were used in the labeling experiment (Biolegend, San Diego, CA, USA). Antibodies were diluted 10:1 in buffer, pulled onto the chip, incubated for 10 min at room temperature, and subsequently flushed from the chip. Washing was performed by flushing the chip with buffer for 10 s at 100 μL/s and then 30 min at 1 μL/s. The initial flush is to remove the label from the main channel, while the secondary flush allows antibodies within the well time to diffuse out. Full chip images were captured in fluorescent channels corresponding to each antibody before staining, during labeling, and after washing. Exposure times were constant in CD45 channel (500 ms) across all steps, while exposure times were reduced during the labeling step in the CD14 (500 ms to 20 ms) and HLA-DR (300 ms to 100 ms) channels before being returned to initial values in the post-wash image.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10404-022-02568-6.

Acknowledgements The authors thank Alexander Revzin for use of his microfabrication facility. This work was supported by the Mayo Clinic Center for Regenerative Medicine and the Department of Lab Medicine and Pathology.

Declarations

Conflict of interest The authors declare no competing interests.

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References

Ai X, Wu Y, Lu W, Zhang X, Zhao L, Tu P, Wang K, Jiang Y (2020) A precise microfluidic assay in single-cell profile for screening of transient receptor potential channel modulators. Adv Sci (Weinh) 7(11):2000111

Anguille S, Smits EL, Lion E, van Tendeloo VF, Berneman ZN (2014) Clinical use of dendritic cells for cancer therapy. Lancet Oncology 15(7):E257–E267

Ankrum J, Karp JM (2010) Mesenchymal stem cell therapy: two steps forward, one step back. Trends Mol Med 16(5):203–209

Armbrecht L, Muller RS, Nikoloff J, Dittrich PS (2019) Single-cell protein profiling in microchambers with barcoded beads. Microsyst Nanoeng 5:55

Avesar J, Blinder Y, Aktin H, Szklanny A, Rosenfeld D, Savir Y, Bercovici M, Levenberg S (2018) Nanoliter cell culture array with tunable chemical gradients. Anal Chem 90(12):7480–7488

Becker H, Gartner C (2000) Polymer microfabrication methods for microfluidic analytical applications. Electrophoresis 21(1):12–26

Bedrosian I, Mick R, Xu SW, Nisenbaum H, Faries M, Zhang P, Cohen PA, Koski G, Czernecki BJ (2003) Intranodal administration of peptide-pulsed mature dendritic cell vaccines results in superior CD8+ T-cell function in melanoma patients. J Clin Oncol 21(20):3826–3835

Boudouquisie C, Boand V, Lingre E, Dutoit L, Balint K, Danilo M, Harari A, Gannon PO, Kandalafte L (2020) Development and optimization of a GMP-compliant manufacturing process for a personalized tumor lysate dendritic cell vaccine. Vaccines 8(1):25

Brandenburg N, Hoehnel S, Kuttlar F, Homiczko K, Ceroni C, Ringel T, Gjorevski N, Schwank G, Coukos G, Turcatti G, Lutolf MP (2020) High-throughput automated organoid culture via stem-cell aggregation in microcavity arrays. Nat Biomed Eng 4(9):863–874

Chen HM, Zhang ZC, Liu HL, Zhang ZF, Lin CM, Wang B (2019) Hybrid magnetic and deformability based isolation of circulating tumor cells using microfluidics. Aip Adv 9(2):025023

Chung AJ, Pulido D, Oka JC, Amini H, Masaeli M, Di Carlo D (2013) Microstructure-induced helical vortices allow single-stream and long-term inertial focusing. Lab Chip 13(15):2942–2949

Darabi J, Guo C (2016) Continuous isolation of monocytes using a magnetophoretic-based microfluidic Chip. Biomed Microdevices 18(5):77

Dietz AB, Bulur PA, Emery RL, Winters JL, Epps DE, Zubair AC, Vuk-Pavlović S (2006a) A novel source of viable peripheral blood mononuclear cells from leukoreduction system chambers. Transfusion 46(12):2083–2089

Dietz AB, Bulur PA, Knuston GJ, Maas ML, Butler GW, Greiner CW, Padley DJ, Gastineau DA, Vuk-Pavlović S (2006b) Central site manufacturing of Th1-polarized dendritic cells for multi-center use. Cancer Res 66(8):681–682

Erdmann M, Uslu U, Wiesinger M, Bruning M, Altmann T, Strasser E, Schulter G, Schulter-Thurner B (2018) Automated closed-system manufacturing of human monocyte-derived dendritic cells for cancer immunotherapy. J Immunol Methods 463:89–96

Erdmann M, Uslu U, Wiesinger M, Bruning M, Altmann T, Strasser E, Schulter G, Schulter-Thurner B (2018) Automated closed-system manufacturing of human monocyte-derived dendritic cells for cancer immunotherapy. J Immunol Methods 463:89–96

Fadul CE, Fisher JL, Hampton TH, Lallana EC, Li ZZ, Gui J, Szczepiorkowski ZM, Tosteson TD, Rhodes CH, Wishart HA, Lewis LD, Ernstoff MS (2011) Immune response in patients with newly diagnosed glioblastoma multiforme treated with intranodal autologous tumor lysate-dendritic cell vaccination after radiation chemotherapy. J Immunother 34(4):382–389

Holzinger A, Barden M, Abken H (2016) The growing world of CAR T cell trials: a systematic review. Cancer Immunol Immunother 65(12):1433–1450
Hoshino K, Huang YY, Lane N, Huebschman M, Uhr JW, Frenkel EP, Zhang X (2011) Microchip-based immunomagnetic detection of circulating tumor cells. Lab Chip 11(20):3449–3457

Huang NT, Hwon YJ, Lai RL (2018) A microfluidic microwell device for immunomagnetic single-cell trapping. Microfluidics Nanofluidics 22(2):16

Hur SC, Mach AJ, Di Carlo D (2011) High-throughput size-based rare cell enrichment using microscale vortices. Biomicrofluidics 5(2):022206

Iancu EM, Kandalafte LE (2020) Challenges and advantages of cell therapy manufacturing under Good Manufacturing Practices within the hospital setting. Curr Opin Biotechnol 65:231–241

Inglis DW, Riehn R, Austin RH, Sturm JC (2004) Continuous microfluidic immunomagnetic cell separation. Appl Phys Lett 85(21):5093–5095

Jorgolli M, Nevill T, Winters A, Chen I, Chong S, Lin FF, Mock M, Chen C, Le K, Tan C, Jess P, Xu H, Hamburger A, Stevens J, Munro T, Wu M, Tagari P, Miranda LP (2019) Nanoscale integration of single cell biology discoveries processes using optofluidic manipulation and monitoring. Biotechnol Bioeng 116(9):2393–2411

Kim S, Han SI, Park MJ, Jeon CW, Joo YD, Choi IH, Han KH (2013) Circulating tumor cell microseparator based on lateral magnetophoresis and immunomagnetic nanobeads. Anal Chem 85(5):2779–2786

Knutson KL, Block MS, Norton N, Erskine CL, Holday TJ, Dietz AB, Padley D, Gustafson MP, Puglisi-Knutson D, Mangskau TK, Chumari S, Dueck AC, Karyampudi L, Wilson G, Degnim AC (2020) Rapid generation of sustainable HER2-specific T-cell immunity in patients with HER2 breast cancer using a degenerate HLA Class II epitope vaccine. Clin Cancer Res 26(5):1045–1053

Kobayashi M, Kim SH, Nakamura H, Kaneda S, Fujii T (2015) Cancer cell analyses at the single cell-level using electroactive microwell array device. PLoS ONE 10(11):e0139980

Laborde RR, Lin Y, Gustafson MP, Burlur P, Dietz AB (2014) Cancer vaccines in the world of immune suppressive monocytes (CD14(+)/HLA-DR1 (lo/neg)) cells: the gateway to improved responses. Front Immunol 5:147

Lin Y, Atwell T, Weisbrod A, Maas M, Armstrong AS, Deeds M, Burlur PA, Gustafson M, Zhang ZJ, Cordes S, Porrata LF, Markovic SN, Johnston PB, Micallef IN, Inwards DJ, Colgan JP, Ansell SM, Gastineau DA, Dietz AB, Witzig TE (2014) Dendritic cell vaccine treatment for B-cell Non-Hodgkin lymphoma: clinical trial in progress. Blood 124(21):4474

Luro S, Potvin-Trottier L, Okumus B, Paulsson J (2020) Isolating live cells after high-throughput, long-term, time-lapse microscopy. Nat Methods 17(1):93–100

Mishra A, Dubash TD, Edd JF, Jewett MK, Garre SG, Karabacak NM, Rabe DC, Mutlu BR, Walsh JR, Kapur R, Stott SL, Maheswaran S, Haber DA, Toner M (2020) Ultra-high-throughput magnetic sorting of large blood volumes for epitope-agnostic isolation of circulating tumor cells. Proc Natl Acad Sci USA 117(29):16839–16847

Mohamadi RM, Besant JD, Mepham A, Green B, Mahmoudian L, Gibbs T, Ivanov I, Malvea A, Stojicic J, Allan AL, Lowes LE, Sargent EH, Nam RK, Kelley SO (2015) Nanoparticle-mediated binning and profiling of heterogeneous circulating tumor cell subpopulations. Angew Chem Int Ed Engl 54(1):139–143

Nagarajan MB, Tentori AM, Zhang WC, Slack FJ, Doyle PS (2020) Spatially resolved and multiplexed MicroRNA quantification from tissue using nanoliter well arrays. Microsyst Nanotech 6:51

Nauman JV, Campbell PG, Lanni F, Anderson JL (2007) Diffusion of insulin-like growth factor-I and ribonuclease through fibrin gels. Biophys J 92(12):4444–4450

Park JY, Morgan M, Sachs AN, Samorezov J, Teller R, Shen Y, Pienta KJ, Takayama S (2010) Single cell trapping in larger microwells capable of supporting cell spreading and proliferation. Microfluid Nanofluidics 8(2):263–268

Parney IF, Gustafson MP, Solseth M, Burlur P, Peterson TE, Smadbeck JB, Johnson SH, Murphy SJ, Vasmatzis G, Dietz AB (2020) Novel strategy for manufacturing autologous dendritic cell/allogeneic tumor lysate vaccines for glioblastoma. Neurooncol Adv 2(1):vdaa105

Pilat Z, Jonas A, Jezek J, Zemanek P (2017) Effects of infrared optical trapping on saccharomyces cerevisiae in a microfluidic system. Sensors (basel) 17(11):2640

Podbiel D, Laermer F, Zengerle R, Hoffmann J (2020) Fusing MEMS technology with lab-on-chip: nanoliter-scale silicon microwav-ity arrays for digital DNA quantification and multiplex testing. Microsyst Nanoeng. https://doi.org/10.1038/s41378-020-00187-1

Priesner C, Aleksandrova K, Esser R, Mockel-Tenbrinck N, Leise J, Drechsel K, Marburger A, Quaiser A, Goudeva L, Arseniev L, Kaiser AD, Gienke W, Koehl U (2016) Automated enrichment, transduction, and expansion of clinical-scale CD62L(+) T cells for manufacturing of gene therapy medicinal products. Hum Gene Ther 27(10):860–869

Rettig JR, Folch A (2005) Large-scale single-cell trapping and imaging using microwell arrays. Anal Chem 77(17):5628–5634

Sedov LI (1982) Similarity and dimensional methods in mechanics. Mir Publishers, Moscow

Steinman RM, Banchereau J (2007) Taking dendritic cells into medicine. Nature 449(7161):419–426

Thiel A, Scheffold A, Radbruch A (1998) Immunomagnetic cell sorting - pushing the limits. Immunotechnology 4(2):89–96

Ulu U, Erdmann M, Wiesinger M, Schuler G, Schuler-Thurner B (2019) Automated Good Manufacturing Practice-compliant generation of human monocyte-derived dendritic cells from a complete apheresis product using a hollow-fiber bioreactor system overcomes a major hurdle in the manufacture of dendritic cells for cancer vaccines. Cytotherapy 21(11):1166–1178

Van Driessche A, Van de Velde ALR, Nijs G, Braeckman T, De Vries JM, Berneman ZN, Van Tendeloo VFI (2009) Clinical-grade manufacturing of autologous mature mRNA-electroporated dendritic cells and safety testing in acute myeloid leukemia patients in a phase I dose-escalation clinical trial. Cytotherapy 11(5):653–668

Vuk-Pavlovic S, Burlur PA, Lin Y, Qin R, Szumlanski CL, Zhao XH, Dietz AB (2010) Immunomuspressive CD14(+)/HLA-DRlow/—pushing the limits. Immunotechnology 4(2):89–96

Wang ZH, Kim MC, Marquez M, Thorsen T (2007) High-density microfluidic arrays for cell cytotoxicity analysis. Lab Chip 7(6):740–745

White FM (1974) Viscous fluid flow. McGraw-Hill, New York

Woronoff G, Nghe P, Baudry J, Boitard L, Braun E, Griffiths AD, Haber DA, Toner M (2020) Large-scale single cell RNA-Seq. Sci Rep 6:33883

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