Video Article

Robotic Production of Cancer Cell Spheroids with an Aqueous Two-phase System for Drug Testing

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Abstract

Cancer cell spheroids present a relevant *in vitro* model of avascular tumors for anti-cancer drug testing applications. A detailed protocol for producing both mono-culture and co-culture spheroids in a high throughput 96-well plate format is described in this work. This approach utilizes an aqueous two-phase system to confine cells into a drop of the denser aqueous phase immersed within the second aqueous phase. The drop rests on the well surface and keeps cells in close proximity to form a single spheroid. This technology has been adapted to a robotic liquid handler to produce size-controlled spheroids and expedite the process of spheroid production for compound screening applications. Spheroids treated with a clinically-used drug show reduced cell viability with increase in the drug dose. The use of a standard micro-well plate for spheroid generation makes it straightforward to analyze viability of cancer cells of drug-treated spheroids with a micro-plate reader. This technology is straightforward to implement both robotically and with other liquid handling tools such as manual pipettes.

Video Link

The video component of this article can be found at https://www.jove.com/video/52754/

Introduction

Cell-based assays provide an important tool for the development and discovery of new anti-cancer drugs.[1,2] Historically, monolayer cultures of cancer cells have been employed to investigate the efficacy of candidate compounds against particular types of cancer cells. The ease of maintenance of monolayer cultures in standard culture plates, the compatibility of standard plates with commercial robotic tools for addition of reagents, and with screening equipment for downstream analysis of cellular responses to chemical compounds are the major benefits that render 2D cultures an attractive tool for drug testing.[3] Unfortunately, monolayer cell assays often fail to predict the efficacy of compounds *in vivo,* making drug development and discovery an extremely costly process.[4,5] Despite significant investment and effort by pharmaceutical companies and academic units, only ~1% of anti-cancer drugs in clinical trials were approved by the FDA over the past two decades.[6] Disparity between 2D cultures and the complex 3D environment of cancer cells *in vivo* is a major shortcoming of monolayer culture systems.[7] Therefore, screening of candidate compounds against tumor cells in a setting that more closely resembles the 3D tumor environment may expedite development of novel chemotherapy drugs.[8]

Cancer cell spheroids present a relevant 3D tumor model *in vitro*.[9,10] Spheroids are compact clusters that form through spontaneous or induced assembly of cancer cells on non-adherent surfaces or in suspension using techniques such as spinner flask, liquid overlay, microfabricated micro-well arrays, microfluidics, and hanging drops.[11-16] Spheroids mimic key features of solid tumors including geometry and limited transport of oxygen, nutrients, and drug compounds into the central zone; hence, they more closely regenerate drug response of solid tumors compared to monolayer cultures.[17-19] Despite this marked benefit, spheroids are not routinely used for screening of chemical compounds against cancer cells. Difficulty of producing uniform sized spheroids in a standard high throughput setting that is compatible with commercially available robotics and screening/imaging tools impedes incorporation of spheroid culture into drug development pipeline. Although custom materials and plates have recently become commercially available to address this need, cost considerations deter their widespread use.

Two major techniques with the capability of producing consistent sized spheroids in high throughput use a new hanging drop platform and microfabricated micro-wells.[13,16,20] However, both approaches require special plates and devices that are expensive to fabricate and inconvenient for endpoint users in core research centers and pharmaceutical industries where the most major efforts for the discovery of new anti-cancer drugs are made. Despite some improvements in the stability of cell-containing drops with a recent design of hanging drop plates, only every other hole of the plate is still used during culture to avoid spreading/merging of drops.[16] This significantly decreases experimental throughput. Drug addition and renewal is difficult with manual or robotic pipetting and spheroids need to be transferred into a standard plate for biochemical analysis because this plate configuration is not readily compatible with conventional screening equipment such as plate readers.[21] Micro-wells fabricated using soft lithography also allow controlled size spheroid production.[13,20] However, incompatibility of this platform with standard pipetting tools prevents treating of individual spheroids with different drug compounds/concentrations, exposing all spheroids to a single

treatment condition. Thus, this method is not appropriate for high throughput compound screening that requires simultaneous testing of multiple compounds/concentrations.

To overcome these obstacles, a new technique for high throughput production of consistently sized cancer cell spheroids in standard 96-well plates has been developed.[22,23] The approach is based on a polymeric aqueous two-phase system (ATPS) with polyethylene glycol (PEG) and dextran (DEX) as phase-forming polymers.[24] ATPSs have recently been utilized in a variety of novel cell biological applications to enable cell micropatterning and localized delivery of biological reagents to cells in highly aqueous media.[25-32] To form a spheroid, cancer cells are mixed with the aqueous DEX phase and a sub-microliter drop of the resulting suspension is pipetted into a well containing the immersion aqueous PEG phase solution. The drop remains immiscible from the immersion phase and confines cells to facilitate formation of a spheroid. Importantly, the highly aqueous immersion phase provides nutrients to cells of the spheroid and minimizes the well-known problem of media evaporation common to some other assays that causes changes in media osmolality and fluctuations of drug concentrations. This technique enables spheroid production and drug treatment only using commercially-available reagents and pipetting tools in standard 96-well plates. Importantly, analysis of cellular responses of spheroids is performed in the same plate using standard biochemical assays and plate readers. The ease of working with ATPS and adaptability of the approach to robotic liquid handling makes high throughput generation of both mono-culture and co-culture spheroids a straightforward laboratory technique. This new approach will be a major step forward toward integration of cancer cell spheroids into drug development and discovery processes with improved testing throughput and cost-effectiveness (increasing numbers of tested compounds and reduced reagent consumption) and efficiency (reducing hands-on time).

A detailed protocol for robotic production of cancer cell spheroids in 96-well plates using the ATPS approach is described below. In addition, details of drug treatment of resulting spheroids and downstream analysis of cellular responses using a commercial biochemical assay are presented.

Protocol

1. Preparation of Polymeric Aqueous Two Phase System (ATPS)

1. Weigh 0.5 g of polyethylene glycol (PEG) (MW: 35,000) and add it to 9.5 ml of complete growth medium in a sterile 15 ml conical to prepare 10 ml of 5% (w/v) aqueous PEG phase.
   Note: Adding half of the medium to the conical first followed by adding the polymer and then the remaining amount of medium minimizes adhesion of the polymer to the conical walls and helps the polymer dissolve faster.
2. Weigh 0.128 g of dextran (DEX) (MW: 500,000) and add it to 0.872 ml of complete growth medium in a sterile 1.5 ml microcentrifuge tube to prepare 1 ml of 12.8% (w/v) aqueous DEX phase.
3. Vortex both solutions for about 1 min to dissolve the polymers in the medium.
4. Keep both solutions in a water bath at 37 °C for 1 hr to ensure dissolution and homogenous mixtures. Keep the caps above the water level to avoid the possibility of contamination of solutions from the bath water.
5. Load the PEG phase solution into a sterile, plastic syringe and pass it through a syringe filter of 0.2 µm pore size to remove impurities.
   Note: Fill the syringe with the PEG solution, place it in the insert of the filter on top of a conical tube and using force, slowly push the solution through the filter. It is normal to experience resistance against movement of the syringe plunger. Minor loss of the polymer solution occurs as some of the solution will remain in the filter, but the polymer concentration will not change.
6. Pipette 10 µl of the DEX phase solution and dilute it in 10 µl of medium in a separate tube to result in 6.4% (w/v) DEX phase solution. Aspirate 100 µl of the PEG phase solution and dispense it into a Petri dish.
   1. Dispense 0.5 µl of the 6.4% (w/v) DEX phase solution into the PEG phase solution. Visually confirm successful ATPS formation by observing a DEX drop under a microscope. The drop boundary should remain visible, indicating presence of two stable, separate aqueous phases.
7. Store the stock aqueous PEG and DEX phase solutions in 4 °C until use.
   Note: It is recommended that polymer solutions are prepared fresh prior to each experiment and used within 24 hr of storage.

2. Preparation for Printing of Cancer Cell Spheroids

1. Grow cancer cells of interest (*e.g.,* MDA-MB-157 breast cancer cells) to a 90%-100% confluent monolayer. For MDA-MB-157 cells, use a medium composed of DMEM containing 10% FBS, 1% glutamine, and 1% antibiotic.
   1. Harvest cells using a cell dissociation buffer (according to manufacturer's protocol) and load the suspension into a 15 ml conical. Centrifuge it for 5 min at 173.3 x g, remove supernatant, and resuspend cells in 1 ml of complete growth medium.
2. Load cells onto a hemocytometer and count them to calculate the required number of cells for a desired spheroid cell density. A confluent monolayer of MDA-MB-157 cells grown in a T75 flask usually gives ~7 x 10^6 cells.
   Note: Required cell density for a drop volume to generate a single spheroid will depend on the cell type.[22] For example, a density of 1.5 x 10^4 or larger for MDA-MB-157 cells is recommended per 0.3 µl DEX phase drop to ensure formation of a single spheroid.
3. Centrifuge cells for a second time for 5 min at 173.3 x g and resuspend them in an appropriate volume of growth medium to concentrate the suspension to a desired cell density.
   Note: For example if 7 x 10^6 cancer cells were harvested, the total volume of cell suspension required to form a spheroid of 1.5 x 10^4 cell density in a 0.3 µl DEX phase drop will be 140 µl. However due to dilution with the DEX phase solution in the next step, only use 70 µl of cell culture medium to resuspend cells.
4. Add to the resulting cell suspension an equal volume of the 12.8% (w/v) aqueous DEX phase solution prepared in 1.2. For the example of 1.5 x 10^4 cell density spheroid, 70 µl of the DEX phase solution is added to 70 µl of cell suspension.

5. Thoroughly mix the cell suspension to ensure uniform distribution of cells and mixing of DEX solution. Pipetting up and down should be done gently to prevent bubble formation.

3. Preparation for Printing of Co-cultured Spheroids

1. Grow cancer cells (*e.g.,* MDA-MB-157 human breast cancer cells) and support cells (*e.g.,* human fibroblasts) to a 90%-100% confluent monolayer. Harvest each cell type using a cell dissociation buffer (according to manufacturer's protocol).
    1. Load each suspension into a 15 ml conical, centrifuge them for 5 min at 173.3 x g, and aspirate supernatant from each conical. Resuspend cells of each conical in 1 ml of complete growth medium.
    Note: Fluorescent dyes such as Calcein AM (live cell stain) and nuclear dyes (Hoechst) can be used to distinguish the two cell types.
2. Load each cell type separately onto a hemocytometer and count them to calculate the required number of each cell type for a desired ratio of cancer cells to supporting cells in co-cultured spheroids. Confluent monolayers of MDA-MB-157 cells and fibroblasts grown in T75 flasks usually give ~7 x 10^6 and ~6 x 10^6 cells, respectively.
3. Add the correct volume from the suspension of supporting cells to cancer cells suspension to give a desired ratio of the number of two cell types. For example, use a ratio of 50 cancer cells to 1 fibroblast cell.
4. Centrifuge the conical containing the mixed cell suspension for 5 min at 173.3 x g and resuspend cells in an appropriate volume to result in final density comprising of equal volumes of growth medium and the 12.8% (w/v) aqueous DEX phase solution (prepared in 1.2).
5. Gently pipette the resulting cell suspension up and down to ensure homogeneity of the suspension.

4. Printing of Tumor Spheroids into a 96-well Plate

1. One day prior to experiments, coat non-treated, round-bottom 96-well plates with 1% (w/v) Pluronic at 37 °C for 24 hr. This coating prevents cell attachment over the culture period.
2. Dispense 50 µl of the filtered 5% (w/v) aqueous PEG phase into each well of a 96-well plate (destination plate).
3. Using a pipette, mix the cell suspension (prepared in step 2 or step 3 for mono- and co-culture, respectively) and add 20 µl of the suspension to every other well from one column of a 384-well plate (source plate).
4. Turn on the liquid handler and "home" the pipetting head to register coordinates. Then compile a previously defined protocol (below in 4.6) to ensure labware and parameters are defined correctly. This "homing" step may be different for different liquid handlers.
Note: The flow of the protocol, shown step-by-step below in 4.6, will be similar regardless of the robotic liquid handler used; however, the programming interface may look different due to the use of different software.
5. Place the source plate, the destination plate, and pipette tip boxes at defined positions on the workstation of the liquid handler as shown in **Figure 1**.
6. Execute the protocol that includes the following steps:
    1. Load one column of barrels from the pipetting head of the liquid handler with mixing pipette tips (8 tips) from the workstation (**Figure 1**).
    2. Mix the cell suspension in wells of the source plate (**Figure 1**). Select a mixing volume smaller than the cell suspension volume in wells to avoid bubble formation.
    3. Eject tips into an empty waste tips box (**Figure 1**).
    4. Load one column of barrels from the pipetting head with 10 µl dispensing pipette tips (**Figure 1**).
    5. Aspirate 0.3 µl of the cell suspension from the source plate (**Figure 1**) into each pipette tip.
    6. Dispense the cell suspension into wells of one column of the destination plate (**Figure 1**). Use a dispense height of 0.5 mm and a dispense flow rate of smaller than 1 µl/sec.
    7. Repeat steps 4.6.1 through 4.6.6 until the column-by-column printing into the entire destination plate is complete.
7. Carefully remove the destination plate from the workstation surface and place it in a humid incubator at 37 °C and 5% CO_2. Maintain the plate horizontal when carrying it into the incubator to avoid disrupting of DEX phase drops containing cells.
8. Incubate the plate for 24 hr to allow aggregation of cells into a spheroid within the DEX phase drop.

5. Drug Treatment of Cancer Cell Spheroids

Note that the following protocol is for a 4-day drug treatment and includes a renewal with fresh drug after day 2. It can be modified for other treatment periods.

1. Visually confirm spheroid formation in 96-well plates after 24 hr. If needed, image wells for measurement of size of spheroids by averaging the smallest and largest diameters of each spheroid.
2. Prepare the stock solution of a desired drug by dissolving it in a solvent recommended by the manufacturer at a predetermined solubility concentration. For example, dissolve cisplatin in water at 2 mg/ml.
Note: Protect the stock solution of the drug from light if the drug is light-sensitive.
3. Using the serial dilution technique, prepare diluted drug concentrations with cell culture medium from the stock solution prepared in the previous step. Each dilution should be made twice the desired concentration. Dilution ratios will vary depending on the starting stock concentration and desired working concentrations.
4. Add 50 µl of drug solution prepared in the previous step to each well. This can be done robotically or by manual pipetting.
Note: Each drug concentration will be diluted in half to the desired concentration by the 50 µl aqueous PEG phase already in each well.
    1. Use spheroids from two columns of a 96-well plate (n=16) for each concentration.
    2. In control wells, add only 50 µl of fresh culture medium to the existing 50 µl of the aqueous PEG phase.
5. Incubate spheroids with the drug for 48 hr at 37 °C and 5% CO_2, protected from light.

6. After 48 hr of incubation, prepare fresh dilutions of the drug at desired concentrations and renew drug by adding 50 µl of fresh drug solution to each well.
   Note: The wells already contain the desired drug concentration from the first drug treatment. Therefore in this renewal phase, each concentration is prepared at the desired concentration since no dilution will occur.
7. Incubate spheroids with the drug for another 48 hr at 37 °C and 5% CO_2, protected from light.

6. Analysis of Cellular Viability in Spheroids

1. After 48 hr of incubation with renewed drug (*i.e.*, total time of treatment of spheroids with a drug is 4 days), measure the total volume of medium in one well by manual pipetting.
   Note: Typical volume in one well is ~140 µl (a small decrease occurs due to evaporation).
2. Calculate the volume of cell viability reagent (*e.g.,* PrestoBlue) as 10% concentration of the total well volume. Add this volume of cell viability reagent to each well.
   Note: With an existing media volume of 140 µl in a well, a volume of 15.6 µl is required to give a 10% concentration of the total well. This is calculated by dividing the existing media volume by 9 (140 µl / 9 = 15.6 µl).
3. Incubate the plate with the cell viability reagent added to each well for 6 hr at 37 °C, protected from light.
4. Place the plate in a micro-plate reader and read the fluorescence signal at 560 nm and 590 nm excitation and emission wavelengths, respectively.
5. Calculate the percent viability of cells in spheroids by normalizing the fluorescent signal from treated wells to that of control wells after averaging the values from the same treatment conditions.

Representative Results

The workstation of the robotic liquid handler is shown in **Figure 1**. The pipetting head and all stations used in the robotic printing of spheroids in section 4.6 are labeled. The image shows the use of two different stations for tip boxes (one set of tips for mixing and the second set for aspirating/dispensing of cell suspension–aqueous DEX phase mixture). The entire setup is housed within a standard biological safety cabinet to maintain sterility. **Figure 2** depicts a schematic of the “printing” process with the aqueous two-phase system. A pipette tip loaded with the cell suspension in the aqueous DEX phase (blue, patterning phase) is lowered into a round-bottom well of a 96-well plate containing the aqueous PEG phase (pink, immersion phase) to dispense its content close to the well bottom. The resulting DEX phase drop restricts the cancer cells from dispersing and encourages cell-cell interactions that result in aggregation of cells and spheroid formation. Spheroid formation is spontaneous and happens within 24 hr of incubation as shown in the experimental image of **Figure 2**. In practice, this spheroid printing is performed column-by-column in 96-well plates. After spheroids form, culture medium is renewed by direct addition of fresh medium, converting the two-phase system to a single aqueous phase. This transition is due to reduction in concentrations of PEG and DEX below a threshold concentration required for separation of aqueous PEG and DEX phases.

Dispensing from a conventional air displacement pipetting mechanism of the liquid handler was parametrically optimized and it was found that a dispense height of 0.5 mm, a dispense flow rate of smaller than 1 µl/sec followed by dispensing 0.2 µl of pre-aspirated air volume produces the most consistent size of aqueous DEX drops, and hence spheroids.[23] **Figure 3a** displays the distribution of diameter of 96 spheroids from one plate with an average diameter of 332 ± 31 µm. Previous experience shows that this approach typically generates spheroids with 8%-10% standard error around the mean diameter. **Figure 3b** shows the frequency distribution of diameter of spheroids of the same plate as in **Figure 3a**, demonstrating that diameters are normally distributed.

Next, the potential of this approach for compound screening in standard 96-well plates and analysis of cellular responses in the same plates, without a need to transfer spheroids to new plates was demonstrated. **Figure 4** shows a dose-dependent study of viability of MDA-MB-157 breast cancer cell spheroids treated with a clinical chemotherapeutic drug, cisplatin. Drug-treated and control spheroids were incubated with a commercial PrestoBlue reagent (cell viability reagent). Enzymatically-active cells reduced the reagent and resulted in a change in the media color and a shift in the fluorescent signal. The shift in signal was greatest with live cells (*e.g.,* in control spheroids). The viability of cancer cells in spheroids decreased with increasing drug concentration above 1 µM. This test resulted in a 50% lethal dose drug concentration of LD50 = 4.67 µM.

This aqueous two-phase system technology for 3D culture allows straightforward production of more realistic tumor models by including other components of cellular microenvironments such as supporting stromal cells. It has been shown previously that including stromal cells in 3D cultures modulates growth, proliferation, and invasion of cancer cells.[33-35] As a proof-of-concept experiment, co-culture spheroids were generated by combining MDA-MB-157 human breast cancer cells and human fibroblasts at a ratio of 50:1.[36] Prior to experiments, MDA-MB-157 and fibroblast cells were stained with a nuclear dye (blue) and live cell dye (green), respectively, to allow detection of cells in fluorescent images. **Figure 5a** shows fluorescent and phase images of co-culture spheroids with a density of 1.5×10^4 cells and a 50:1 ratio of cancer cells and fibroblasts. **Figure 5b** shows control spheroids of MDA-MB-157 cells for comparison. This approach will enable evaluating the effect of stromal cells on various phenotypes of cancer cells.

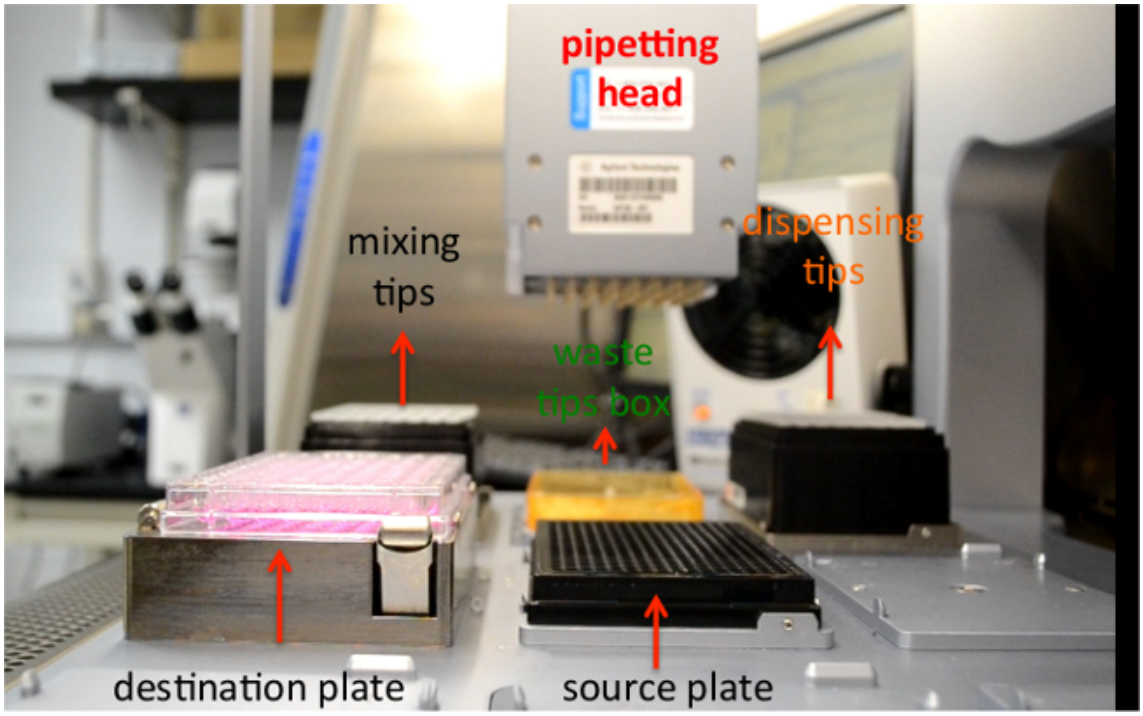

Figure 1. Liquid Handling Platform. The liquid handler consists of stations where mixing tips, dispensing tips, waste tips box, source plate, and destination plate locations are placed. The location of each station is defined in the protocol. Mixing tips are loaded onto one column of the pipetting head and used to mix the solution of aqueous DEX phase containing cells in the source plate. These tips are then disposed of in the waste tips box and the dispensing tips are inserted onto the same column of the pipetting head. These tips aspirate 0.3 µl of the aqueous DEX phase containing cells from the source plate and dispense it into the destination plate to form cell-containing drops of the DEX phase within the immersion PEG phase already in the destination plate wells. Finally these tips are also disposed of in the waste tips box to complete one cycle of printing.

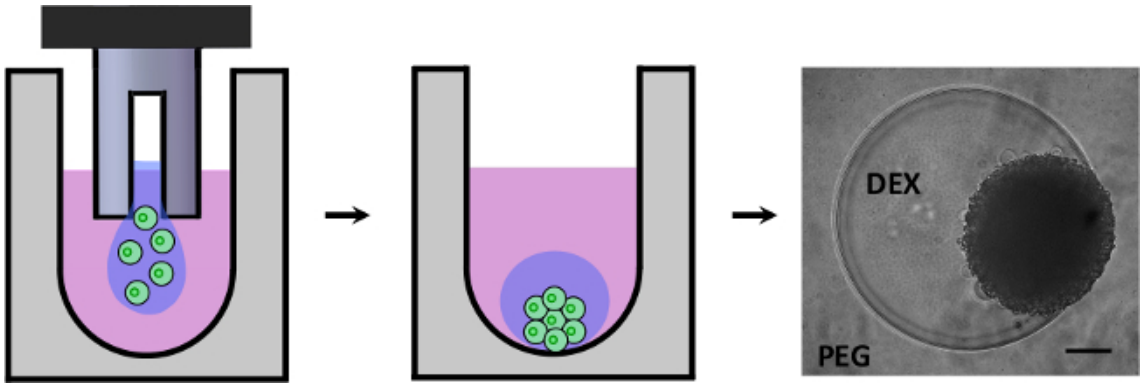

Figure 2. Spheroid Formation. The liquid handler dispenses 0.3 µl of the aqueous DEX phase (blue) containing cells (green) into a well containing the aqueous PEG solution (pink). This results in formation of a spheroid after 24 hr of incubation as shown in the image on right. Scale bar 200 µm. Please click here to view a larger version of this figure.

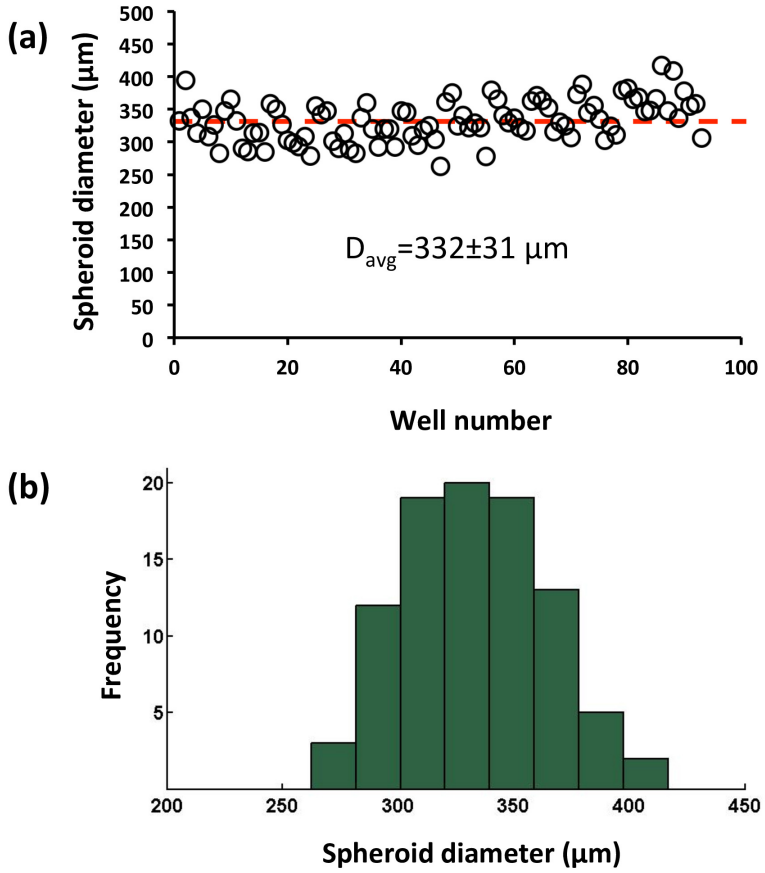

Figure 3. Spheroid Consistency Data. (**a**) Distribution of diameter of spheroids measured from a 96-well plate, each point represents one spheroid. (**b**) Frequency distribution of diameters shows normal distribution of the data.

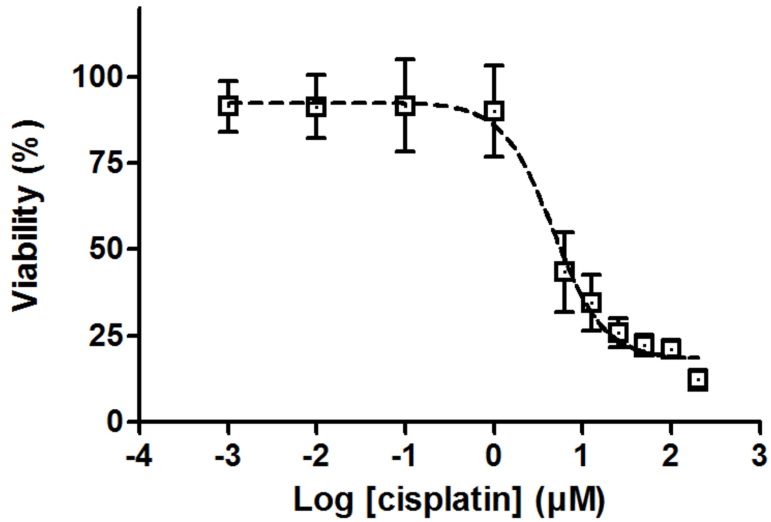

Figure 4. Drug Response of Spheroids. The viability of cells in MDA-MB-157 breast cancer cell spheroids reduces with increase in the concentration of cisplatin over 1 nM-200 µM range. The dashed line is a sigmoidal fit to the viability data.

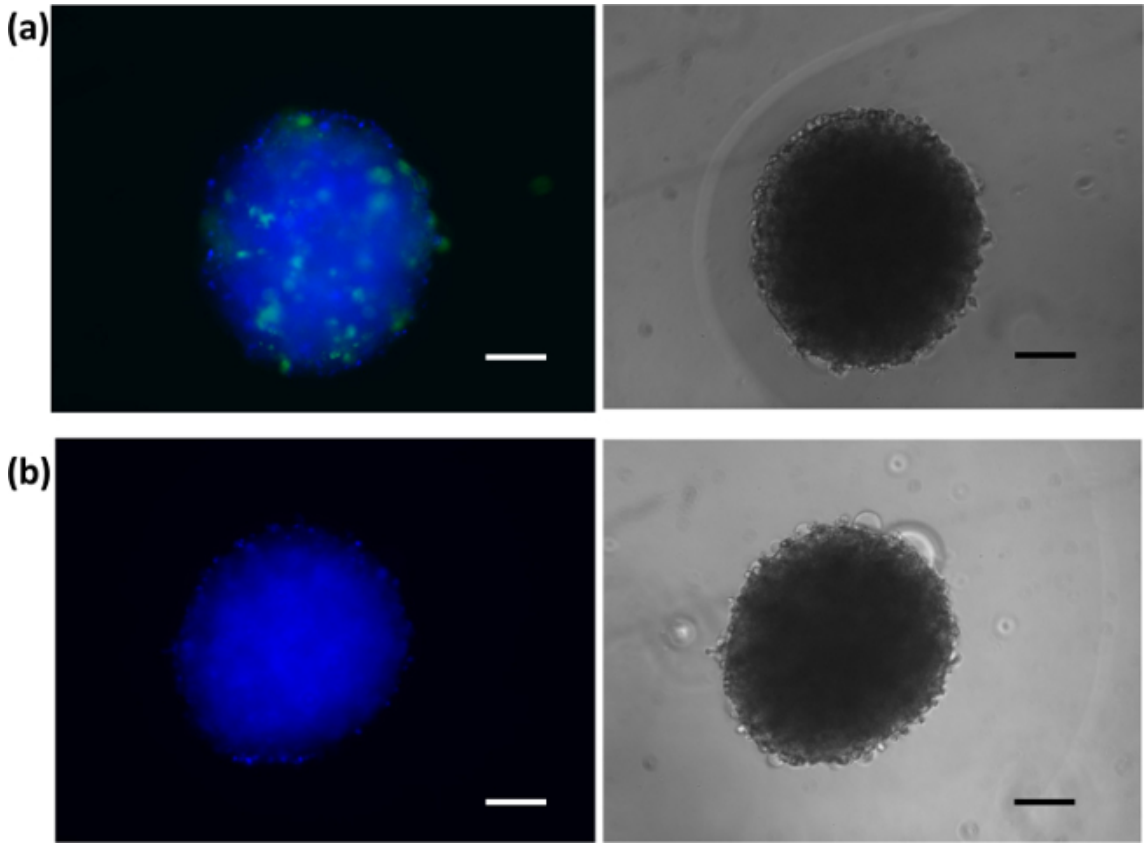

Figure 5. Co-Culture Spheroids. (**a**) Fluorescent (left) and phase-contrast (right) images of spheroids of MDA-MB-157 breast cancer cells (blue) co-cultured with fibroblasts (green) at a 50:1 ratio. (**b**) Spheroids of MDA-MB-157 cells are shown for comparison. Scale bar 100 µm.
Please click here to view a larger version of this figure.

Discussion

Spheroids present a realistic model to better understand tumor physiology and drug efficacy and provide a useful tool for anti-cancer drug discovery. Such applications would greatly benefit from simple spheroid generation and maintenance techniques that only require standard labware, liquid handling tools and screening equipment. The use of an aqueous two-phase system to spontaneously aggregate cancer cells within the drop phase allows efficient production and maintenance of spheroids with robotic liquid handlers, and *in situ* drug treatment and endpoint analysis of cellular responses with commercial reagents and tools. Thus, this technology is a major advantage over existing approaches of 3D cancer cell cultures and presents a screening platform to expedite drug discovery.

Generating uniform sized spheroids within a micro-well plate is crucial for high throughput screening applications to ensure a similar baseline cellular activity level in all spheroids. Using liquid handlers with an air displacement pipetting mechanism, it is important to optimize the dispensing process through quantification of the effect of key liquid handling parameters (dispense height, dispense flow rate, and pre-aspirated air volume) on dispensing of the aqueous DEX phase. This optimization allows the pipetting head to sweep out the viscous DEX phase solution and cancer cells into wells and produce homogenous spheroids. Although specific quantities for these three parameters have been determined in the protocol for the SRT Bravo liquid handler, a similar approach should be employed with other liquid handlers to determine optimum dispensing conditions that result in consistent DEX phase drop size dispensed into the immersion PEG phase. In addition, it is possible to use 96 tips and to simultaneously print spheroids into all 96 wells. This however significantly increases the number of cells required to fill the source plate with the aqueous DEX phase containing cells. Regardless of selecting column-wise or whole-plate printing approaches, it is crucial to mix the content of the source plate prior to the aspiration step. This ensures that the dense cell suspension in the DEX phase is homogeneous and reduces variations in the size of spheroids.

Several studies show that cancer cell spheroids mimic several key properties of avascular tumors such as morphology and diffusion limitations of reagents; hence they present a physiologically relevant model for evaluating the efficacy of novel and conventional compounds against cancer cells compared to traditional monolayer cell cultures.[8,17,18,21,23,37] It is shown that the aqueous two-phase system approach to producing spheroids conveniently allows drug screening in the same plate, simply by addition and renewal of a drug compound at desired time points and automated screening of cell viability using a standard micro-plate reader. This is a major advantage over techniques such as hanging drops that would require transferring spheroids to a standard plate for analysis of cellular responses to drugs.[21] In addition, it is known that supporting cells in the tumor microenvironment and their interactions with cancer cells are implicated in various malignant phenotypes of cancer cells.[33-35,38,39] Therefore the ability to generate co-culture spheroids of cancer and stromal cells using the aqueous two-phase technology will help evaluate the influence of supporting cells of tumor microenvironment on responses of cancer cells to various treatments. The co-culture experiment performed in this study serves as a proof-of-concept that this technology can successfully generate spheroids containing cancer cells and stromal cells. Future studies will harness this capability and investigate differential characteristics of mono- and co-cultured spheroids such as proliferation

and drug responses. In addition, this technology offers a platform to more closely mimic the *in vivo* tumor microenvironment by including other stromal components in the tumor model.[40]

In summary, this protocol enables spontaneous formation of spheroids in 96-well plates without using any external forces. Adapting the technique to a robotic liquid handler enhances the speed and efficiency of spheroid production in a standard high throughput format. The compatibility of the technology with commercial imaging and assay analyses platforms allows using different instruments available in academic and industrial laboratories. For future applications, this protocol will streamline drug screening with 3D cancer cell cultures a routine technique. In addition, this approach can be easily modified to form spheroids of different sizes, different cell types, different combinations of cancer and stromal cells in co-culture spheroids, and be scaled up to higher throughput micro-well plates to further expedite anti-cancer drug screening.

Disclosures

The authors have nothing to disclose.

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