ABSTRACT

We report the development of a digital microfluidic (DμF) platform for automated spheroid-based migration and invasion assays. Digital microfluidic devices were fabricated with through-holes in the bottom plate that permit the formation of hanging drops for culturing multicellular spheroids. For invasion assays, spheroids were encapsulated in a collagen solution that readily gels at the ambient cell culture temperature. Migration modulating agents were delivered to gel-encapsulated spheroids to either stimulate or inhibit invasion. By automating the liquid handling protocols for in-situ spheroid formation and gel encapsulation, digital microfluidics could help reduce the barriers to adoption for three-dimensional cell invasion assays.

KEYWORDS: Cell Spheroid, Hanging Drop, Invasion, Migration Assay, Digital Microfluidics, EWOD

INTRODUCTION

Cell invasion and migration assays probe cell motility in response to particular micro-environmental conditions. Cell migration is an important factor in many physiological phenomena such as wound healing, tissue growth, and cancer metastasis [1]. The majority of cell migration and invasion assays measure cell migration in two-dimensional monolayers, which cannot recapitulate many of the complexities associated with three-dimensional physiological tissues. Three-dimensional, spheroid-based invasion assays provide a more physiologically relevant tissue and disease model, but they are typically labor intensive, requiring numerous manual pipetting steps. This can limit the throughput and the reproducibility of the assays.

With automated and flexible liquid handling capabilities, digital (droplet) microfluidics (DμF) can address many of the limitations associated with manual spheroid invasion assays. Digital microfluidics enables the manipulation (dispensing, translation, splitting, and mixing) of discrete, pL–to– L droplets of liquid through the spatially and temporally controlled application of electric fields [2]. A wide variety of bioanalytical techniques and cell culture protocols have been developed for the DμF platform, making it a useful tool for automated cell-based assays. Here we present a novel DμF device and protocol for performing automated, spheroid-based cell invasion assays.

EXPERIMENTAL

Digital microfluidic devices were fabricated according to previously described protocols [3], with the addition of through-holes, or “wells,” in the bottom plate as shown in Figure 1. The hydrophobic coating was removed from the inside surface of the wells to expose the glass substrate and render the wells hydrophilic. Consequently, drops that made contact with a well were spontaneously drawn into the well via capillary forces, resulting in the formation of a hanging drop. Droplet actuation was achieved by applying a voltage of ~110V at 18.5 kHz.

Suspensions of human foreskin fibroblasts at ~1e6 cells/mL were used to form spheroids in-situ. Droplets were dispensed from on-chip reservoirs and delivered to the wells to form hanging drops with volumes of ~8 µL and cell densities of ~8000 cells/drop. After the hanging drops were formed, the devices were placed in an incubator and maintained at 37 °C and 95% relative humidity for 24 h to allow the cells to aggregate into compact spheroids. The medium in the hanging drops was then replaced with 8-10 µL of medium containing 1 mg/mL bovine collagen-I, which was allowed to gel overnight in the incubator. To demonstrate the ability to assay the encapsulated spheroids, the spheroids were exposed to either standard cell culture medium, or medium solution containing either 100 ng/mL bone morphogenic
protein-2 (BMP-2) or 5 µg/mL prostaglandin-E2 (PGE2). All liquid handling steps were performed using DμF droplet actuation.

After 24 and 48 h of collagen encapsulation, spheroids were stained with calcein-am and ethidium homodimer-1 to identify living and dead cells, respectively, via confocal microscopy. Acti-stain™ 488 phalloidin stain was used to stain actin filaments within the migrating cells to observe their invasion into the collagen. ImageJ software was used to analyze and quantify the degree of cell migration using the radial profile tool. The collagen structure was visualized by scanning electron microscopy (SEM) after a step-wise dehydration process. The elastic moduli of the collagen gels were determined by atomic force microscopy (AFM).

RESULTS and DISCUSSION

Collagen solutions of up to 4 mg/mL could be dispensed and translated on the DμF device, allowing for the formation of gels with elastic moduli up to ~50 kPa. This modulus range spans the elastic moduli of a variety of tissue types, including lung, breast tumor, kidney, liver, brain, cardiac muscle, skeletal muscle, spinal cord and lymph node [4]. Collagen solutions of 1 mg/mL were used for spheroid encapsulation (Figure 2). Cell attachment and elongation within the gel could also be seen by SEM, indicating cell invasion into the matrix.

Spheroids cultured in-situ exhibited >95% cell viability following 24 h of hanging drop culture and 48 h of encapsulation within a collagen hanging drop. Within the collagen gels, cells migrated up to ~300 µm from the spheroid body after 24 h and up to ~500 µm after 48 h. Figure 3 shows representative images of cell migration from spheroids after 24 h of encapsulation within collagen. The addition of BMP-2 to the medium resulted in an 81% increase in cell invasion compared to standard medium over a 24 h period, whereas treatment with PGE2 caused a 59% decrease in cell invasion after 24 h.

Figure 2: (a) SEM image showing the collagen scaffold peeled away to reveal the cell spheroid encapsulated inside. (b) Magnification of the box in (a) showing fibroblast adhesion and invasion into the collagen matrix.
Figure 3: (a) Representative images of cell migration from spheroids after 24 h in a collagen gel and exposure to either standard medium, BMP-2, or PGE2. Green corresponds to fluorescently labeled actin filaments. (b) Migration profiles for standard media, BMP-2 treated, and PGE2 treated spheroids after 24 h. The plotted lines correspond to the average fluorescence intensity of the stained spheroids at increasing radial distances from the perimeter of the spheroids. The dashed lines are measurements from individual spheroids; the solid lines are averages for the various conditions. As expected, the addition of BMP-2 increased cellular invasion while PGE2 inhibited invasion.

CONCLUSIONS

We have developed a D µF platform that automates spheroid-based migration assays. The incorporation of through-holes into the bottom plate of the device enables the formation of hanging drops that can be addressed and assayed individually, in-situ, allowing for the culture of cell spheroids. Replacing the growth medium with a medium solution containing collagen allows spheroids to be encapsulated within a collagen gel, providing a substrate for cell migration and invasion. The ability to stimulate or inhibit cell migration into through the addition of exogenous agents has been demonstrated. By automating the liquid handing steps necessary for spheroid culture, a DµF platform like the one described here may facilitate greater adoption of more sophisticated, three-dimensional cell-based assays, which could ultimately yield deeper insights into important physiological processes such wound healing, organogenesis, or tumor metastasis.

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