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RESEARCH ARTICLE

A high-throughput three-dimensional cell culture platform for drug screening

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Abstract

Traditional two-dimensional (2D) cell cultures lack the extracellular matrix (ECM)-like structure or dynamic fluidic microenvironment for cells to maintain in vivo functionality. Three-dimensional (3D) tissue scaffolds, on the other hand, could provide the ECM-like microenvironment for cells to reformulate into tissue or organoids that are highly useful for in vitro drug screening. In this study, a high-throughput two-chamber 3D microscale tissue model platform is developed. Porous scaffolds are selectively foamed on a commercially available compact disk using laser. Perfusion of cell culture medium is achieved with centrifugal force-driven diffusion by disk rotation. Experimental studies were conducted on the fabrication process under various gas saturation and laser power conditions. Cell cultures were performed with two types of human cell lines: M059K and C3A-sub28. It is shown that the structure of microscale porous scaffolds can be controlled with laser foaming parameters and that coating with polydopamine these scaffolds are inducive for cell attachment and aggregation, forming a 3D network. With many such two-chamber models fabricated on a single CD and perfusion driven by the centrifugal force from rotation, the proposed platform provides a simple solution to the high-cost and lengthy drug development process with a high-throughput and physiologically more relevant tissue model system.

Keywords High-throughput screening · Three-dimensional tissue model · Drug screening · Centrifugal force-driven diffusion · Laser foaming

Introduction

Screening based on toxicity and efficacy is a critical step in drug discovery and development. A series of in vitro and in vivo studies need to be conducted once a lead compound is identified. Drug screening is currently performed using two-dimensional (2D) cell cultures in Petri dishes and microtiter plates. These traditional 2D cell culture platforms lack extracellular matrix (ECM)-like structure or dynamic fluidic microenvironment. Cells are forced to form an arbitrary monolayer that could lose in vivo functionality [1]. About 50% experimental drugs fail in clinical trials due to initial screening inaccuracy [2].

Three-dimensional (3D) cell cultures, on the other hand, could provide ECM-like microstructure for cells to reformulate into tissue and organoids. They can be integrated with microfluidic channels to mimic key physiological and pathological processes [3]. Thus, 3D cell cultures provide a more realistic model for many biomedical studies, including drug discovery and development. For example, 3D tissue model systems have been used to study functional behavior of liver cells in vitro [4, 5]. A two-chamber perfusion-based 3D tissue model system has been developed to improve the testing result of chemotherapy drugs by mimicking in vivo functionality of human liver metabolism [3]. However, for high-throughput screening applications, the size of the two-chamber tissue model system needs to be further reduced such that a large number of these systems can be packed on a single platform to achieve parallel studies.

In this research, a high-throughput 3D cell culture platform is proposed based on selective laser foaming of polymeric material [6]. Localized porous scaffolds together with reservoirs for cell culture medium are fabricated radially in line on a commercially available compact disk (CD) [7]. These scaffolds and reservoirs are connected with microchannels to provide nutrient flow, which is driven by the centrifugal force via CD rotation. We report the fabrication

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of such a high-throughput two-chamber cell culture platform and the cell culture result with human liver and brain tumor cell lines, C3A-sub28 and M059K. With many such two-chamber models fabricated on a single CD and perfusion driven by the centrifugal force from rotation, the proposed platform provides a simple solution to the high-cost and lengthy drug development process with a physiologically more relevant 3D tissue model system.

Experimental

Platform fabrication

Figure 1 shows the design of the high-throughput multi-chamber cell culture platform on a CD. A pair of foamed scaffolds are fabricated with a culture medium reservoir on one side and a waste collecting well on the other. All the chambers are radially arranged in line. Cell seeding and nutrient diffusion are assisted with the centrifugal force generated by disk rotation. A key step to create the proposed high-throughput tissue model platform is to fabricate the microscale tissue scaffolds on the CD, for which a previously developed selective laser foaming process is employed [6, 8]. Commercially available CDs made of polycarbonate (PC) are used in this study. The glass transition temperature of PC is 150 °C, allowing for the fabricated platform to be sterilized using an autoclave, a benefit over other polymeric materials such as polylactic acid (PLA) and polystyrene (PS).

Polycarbonate CDs were first foamed via a two-stage selective laser foaming process, as shown in Fig. 2 [8, 9]. In the gas saturation stage, the CDs were saturated with CO_2 in a pressure vessel at 2 MPa for 11, 22, 33, and 44 h at room temperature. Gas-saturated samples were taken out of the pressure vessel and their weights measured to determine the CO_2 concentration. CO_2-saturated CDs were mounted on a computer-controlled positioner (MAXNC 10 from MAXNC, Inc.) for laser foaming. A continuous CO_2 laser was used (Synrad Firestar v30 from Synrad, Inc., WA). The wavelength of the laser was 10.6 μm, and the beam diameter was 2.5 ± 0.5 mm. A LabVIEW program was developed to control the laser exposure time and pulse rate. Both a continuous pulse and a pulse train at 50% duty cycle were used. The fabrication conditions used in this study are shown in Table 1.

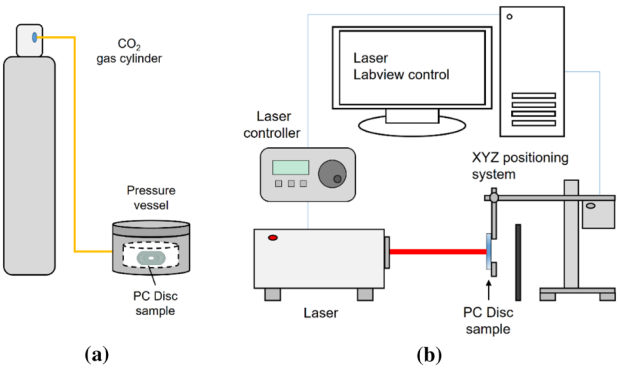

Fig. 2 Schematics of the selective laser foaming process [6, 8]

Foamed CDs were exposed to the ambient condition for a week to desorb the remaining CO_2, after which the samples were placed in liquid nitrogen for 1 min before the skin formed in the foaming process removed with a razor blade. Micro-channels and reservoirs were machined using a PCB prototyping machine (cirQoid, Latvia). After machining,

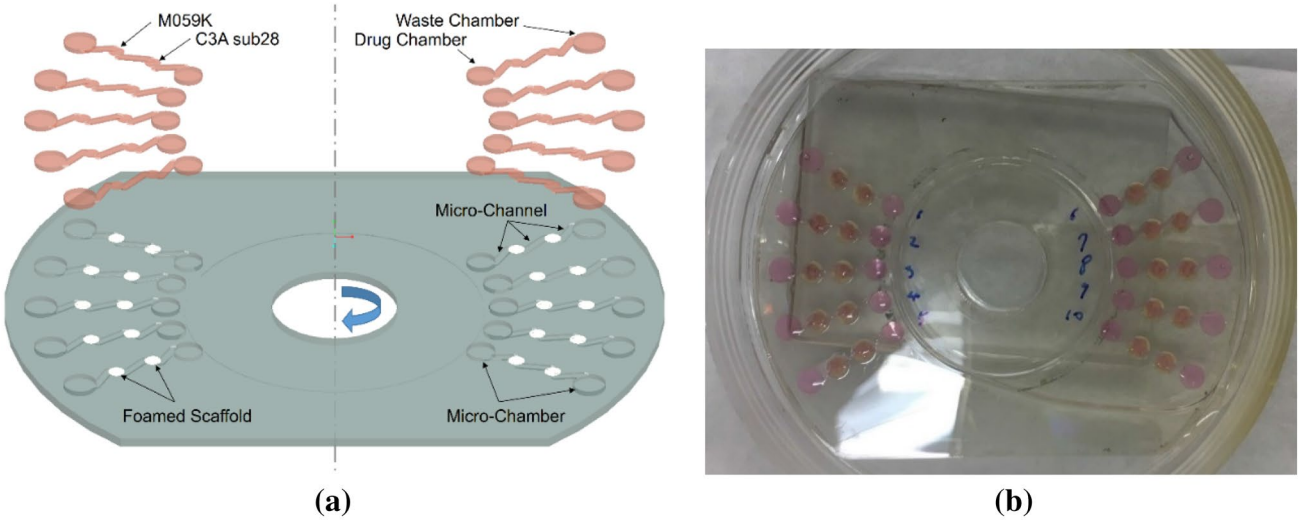

Fig. 1 **a** Design of the proposed high-throughput multi-chamber tissue model platform and **b** the fabricated platform in a Petri dish for cell culture

Table 1 Parameters for laser foaming of polycarbonate CD

Parameter	Value
Saturation pressure (MPa)	2
Saturation temperature (°C)	21
Saturation time (h)	11, 22, 33, 44
Laser power (W)	0.54–33.7
Laser exposure time (s)	0.016–1
Laser pulses	1, 10

the CD samples were cleaned with 100% ethanol and sonicated for 1 h using a Branson 3510 ultrasonic cleaner. The CD samples were then rinsed with distilled water for 1 min and dried in a vacuum oven at 35 °C overnight before polydopamine (PDA) coating. For polydopamine coating, 1.8 g of dopamine HCl was dissolved in 900 ml of (tris(hydroxymethyl)-aminomethane)-hydrochloride (Tris–HCl) (10 mM, pH 8.5). The solution was stirred for 1 min at 850 rpm (mass concentration 2 mg/mL) following a procedure described in [10]. The CD samples were then immersed in the solution for 4 h. The solution was stirred at 400 rpm while immersion. The polydopamine-coated CDs were rinsed with deionized water thoroughly and dried in a vacuum oven at 25 °C for 1 day before sterilization and cell culturing.

Cell culture study

Polydopamine-coated samples were sterilized with 70% ethanol for 30 min. The samples were placed in a vacuum oven at 25 °C overnight and then exposed to ultraviolet light for 30 min. Cell culture medium (1.5 µl) was pipetted in the foamed scaffolds. The samples with cell culture medium were placed in an incubator at 37 °C and 5% CO_2 for more than 2 days. The cell culture medium was removed before cell seeding. Human brain tumor cells (M059K) and human liver cells (C3A-sub28) were seeded in the two foamed scaffolds. The samples were then placed in the incubator for 2 h for cell attachment. The micro-channel and reservoirs were then filled with cell culture medium for continuous culturing.

The human brain tumor cells glioblastoma multiforme (GBM) M09K and hepatoblastoma cells C3A-sub28 were purchased from American Type Culture Collection (ATCC, Manassas, VA). The cell culture medium was made of Dulbecco's Modified Eagle Medium (DMEM) mixed with 10% of fetal bovine serum (FBS) and 1% of penicillin/streptomycin (PS). The blended solution was filtered using Stericup®/Steritop® and a vacuum pump. Dulbecco's Modified Eagle Medium (DMEM) with 4500 mg/L glucose, L-glutamine, sodium pyruvate, and sodium bicarbonate was acquired from Sigma–Aldrich (St Louis, Missouri). FBS with endotoxin ($\leq$ 5EU/ml) and hemoglobin ($\leq$ 10 mg/dl) was purchased from Thermo Fisher Scientific (Waltham, MA). PS and Dulbecco's phosphate-buffered saline (DPBS) were also purchased from Sigma–Aldrich (St Louis, Missouri).

Microstructure characterization and cell viability assessment

The fabricated samples were freeze-fractured in liquid nitrogen for microstructure examination. The fractured foamed wells were sputter coated with gold and palladium using an EMS 500 × sputter coater (Electron Microscopy Science, Hatfield, PA). The microstructure of the laser foamed samples was characterized using a JEOL NeoScope JCM-5000 (Nikon®, UK) scanning electron microscope (SEM) under 5–10 kV accelerating voltage and 0.1 nA beam current. The image processing software, ImageJ, from National Institutes of Health was used to analyze the SEM image [11]. Static water contact angles of the bare surface and the PDA coating were measured using FTÅ 200 contact angle analyzer (First Ten Ångstroms, Inc., Portsmouth, Virginia). The static sessile drop method was used to perform the contact angle measurement. Deionized water was delivered on the surfaces using a syringe controlled by the FTA program, and images of the droplets were taken within 3 s of placing the droplets.

Cell viability was qualitatively evaluated using the live/dead staining solution. The live/dead stain assay was obtained from Invitrogen Inc. (Carlsbad, CA). Cell concentration was measured using a microscope and a hemocytometer. The live/dead dyes in the viability/cytotoxicity kit (calcein AM and ethidium homodimer-1) were diluted with DPBS before use for cell staining. The culture medium in the system, including the scaffolds, micro-channel, and reservoirs, was removed, and the staining solution was added in the system. The samples were incubated for 20 min and then examined with an upright fluorescent stereomicroscope (LEICA M205 FA) and an inverted fluorescence confocal microscope (LEICA SP8).

Diffusion enhancement test

The high-throughput device in this study relies on centrifugal force to assist the nutrient or drug diffusion through the scaffolds on which cells are cultured. This centrifugal force effect was tested with food coloring diluted in distilled water (4% by weight). 2 µL of the solution was dropped in the nutrient reservoir that is closer to the disk center. In order to minimize evaporation, the sample was placed on a spindle platter in a humidity chamber. Static diffusion and diffusion assisted with rotation are assessed visually with the color change in the radial direction of the multi-chamber system.

Results and discussion

Laser foamed micro-wells

Table 2 shows the CO_2 absorption behavior of the polycarbonate CD sample under 2 MPa. Four different gas sorption times were chosen to determine the CO_2 gas concentration. The results were in agreement with previous experiments using other materials such as polymethyl methacrylate (PMMA) and polylactic acid (PLA) [6, 12, 13]. The gas concentration increases with the saturation time until an equilibrium is achieved. Typical microstructure of laser foamed scaffolds is shown in Fig. 3. All the samples were saturated for 44 h under 2 MPa. The resulting CO_2 concentration was 3.78 wt%. The laser foaming parameters are shown on the SEM images. The difference between laser foaming and bulk foaming is that a solid skin layer will form in the bulk foaming process, whereas in laser foaming, the top surface of the foamed region has open cell structure. The pore size in the open cell structure tends to be bigger than that underneath, indicating that the surface of the laser processed region may be molten during the foaming process. This open cell structure is needed to allow cells to enter the porous structure underneath the surface. The pore size of the open cell structure ranges from 100 to 300 μm, which is suitable for cell culture [14–19]. As can be seen from the SEM images, the porous structure of scaffolds can be controlled by both the laser power and time. In general, the higher the energy input, the larger the porous scaffolds. However, comparing Fig. 3c and d, even the total energy input is the same, the resulted microstructure could be different. This is due to the nonlinear heating and heat dissipation effects in the laser foaming process. A higher power and shorter time will produce a smaller scaffold with better defined microstructure.

When pulsed laser is used, the foamed scaffolds will be generally smaller than the ones with a single pulse, because the time in between the pulses allows for heat dissipation instead of accumulation for generating the porous structure.

Table 2 Gas concentration of polycarbonate CDs

Parameter	Value			
Absorption time (h)	11	22	33	44
Weight of CD before gas sorption (g)	5.2571	5.2612	5.6009	5.8245
Weight of CD after gas sorption (g)	5.3661	5.4198	5.7905	6.0447
Gas concentration (wt%)	2.07	3.01	3.39	3.78

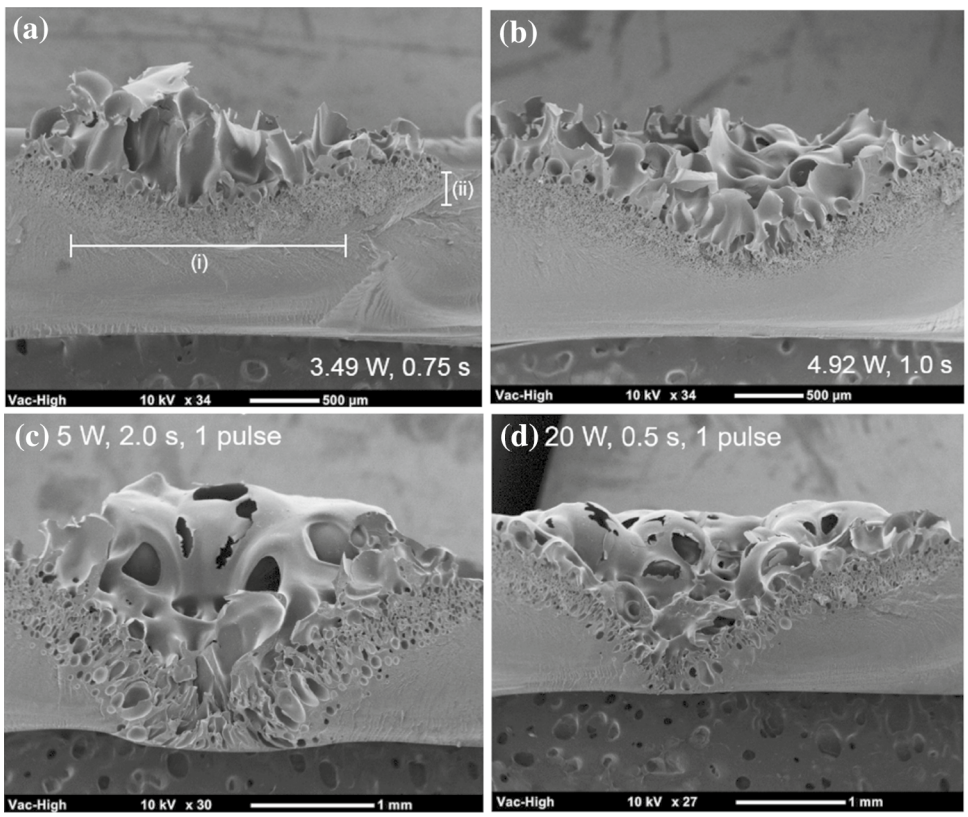

Fig. 3 Microstructure of laser foamed scaffolds

Cell culture results

C3A-sub28 and M059K cells were cultured in foamed scaffolds after PDA coating. Figure 4 shows the images after 13 h of culturing. The porous scaffold is also stained with the staining kit, so that the depth of open pores can be estimated by focusing the upright microscope. The pore depth is determined to be from 150 ± 0.5 μm to 250 ± 0.5 μm. Cells are found to attached to the side walls of the pores, although more cells are found at the bottom due to gravity. Nonetheless, the cells followed the porous structure to form a 3D organization. The 3D network would be easier for the cells to connect spatially rather than forming a monolayer as in the 2D cell culture case. In addition, the small pores underneath the big pores are beneficial for cell adhesion and storing cell culture medium [20]. Figure 5 shows a comparison between 2D and 3D cell culture results using breast cancer cells. In 2D cell culture, cells formed a monolayer, while in 3D cells aggregated to form a spheroidal structure. Other examples of the differences between 2D and 3D cell cultures can be found in [21].

Figure 6 shows confocal images of C3A-sub28 and M059K cells after 24 h and 72 h of culturing, respectively. It is observed that both cell lines maintained high viability, and the cells began to aggregate to form organoid structures. Cell aggregation occurs inside the open porous structure, which is conducive for cell–cell interaction. Such 3D cell culture structure mimics the in vivo microenvironment of cells better than a 2D cell culture system [22]; therefore, it could provide more realistic results for anticancer drug studies [3, 23, 24]. For a long-term cell culture study, the two types of cells were seeded on the laser foamed scaffolds with a much lower cell seeding concentration, about 10 times lower. The cell culture medium was changed every 24 h during the first 14 days and after every 12 h. Cells remain viable in aggregations for at least 28 days.

Effect of centrifugal force on diffusion

The effect of centrifugal force on diffusion is shown in Fig. 7. Without disk rotation, it took 120 min for food color to diffuse from the inner reservoir to the outer one, while with the disk

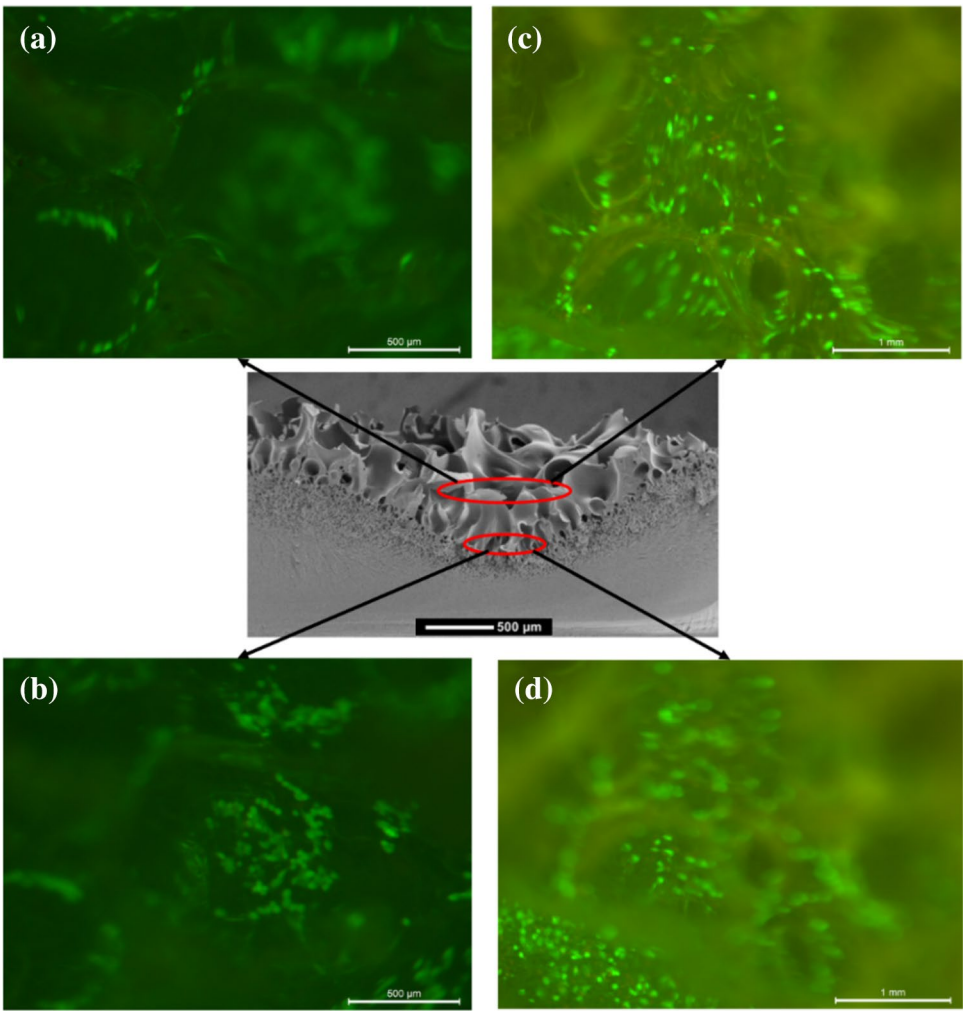

Fig. 4 Cell attachment after 13 h of culturing: **a**, **b** C3A-sub28 cells with seeding density 982,500 cells/ml and **c**, **d** M059K cells for C3A-sub28, seeding density 1,170,000 cells/ml

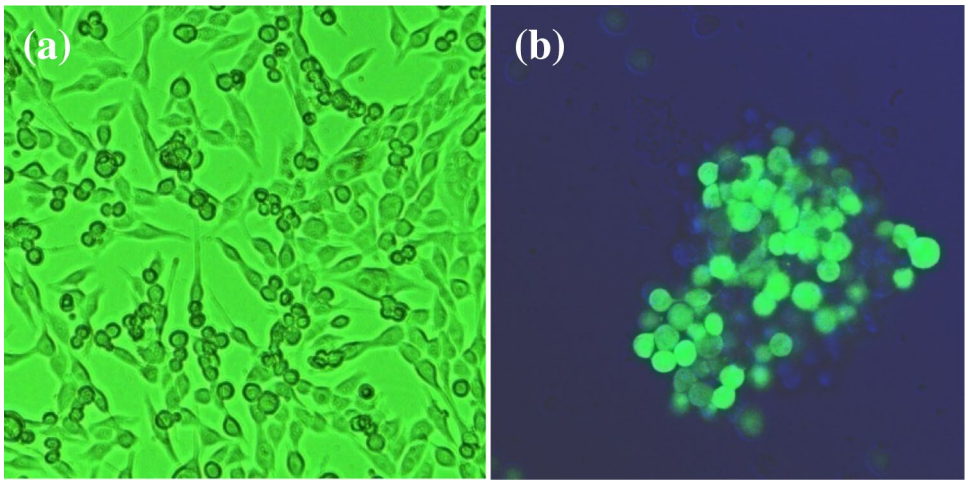

Fig. 5 Comparison of 2D and 3D cell culture results: **a** breast cancer cells cultured in 2D Petri dish and **b** breast cancer cells cultured in 3D scaffold for 21 days, pore size 300μm

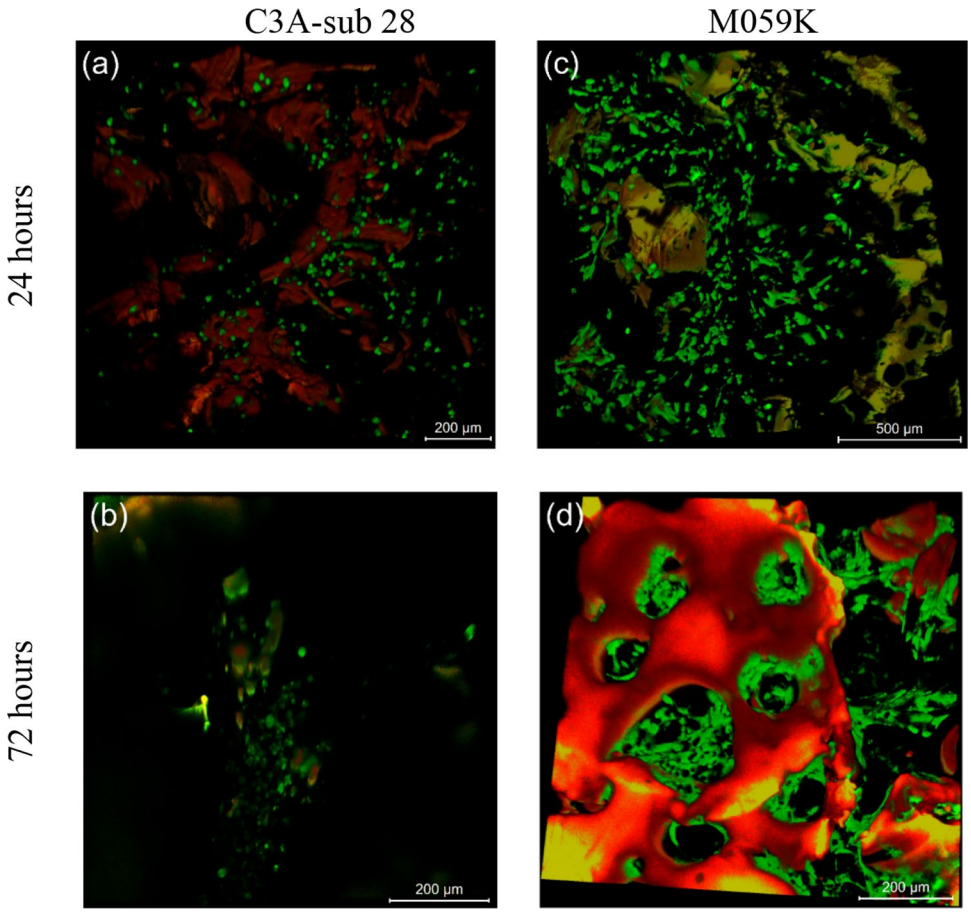

Fig. 6 Cell morphology of C3A-sub28 cells along with time: **a** 24 h and **b** 72 h from a confocal microscope (17,635,000 cells/ml). Cell morphology of M059K cells along with time: **c** 24 h and **d** 72 h from a confocal microscope (7,022,500 cells/ml)

rotation at 60 rpm the diffusion process only took 10 min. With the normal disk size, it has been shown that cells will remain undamaged until a rotation speed of 1200 rpm [25]. Therefore, it is reasonable to assume that the centrifugal force generated with 60 rpm would have minimum effect on cell viability. On the other hand, the diffusion driven by the concentration difference between the inner and outer chambers is dramatically accelerated by the centrifugal force. This result agrees with prior studies where gravitational and centrifugal forces were found to increase the diffusion rate in liquid solutions [26–28].

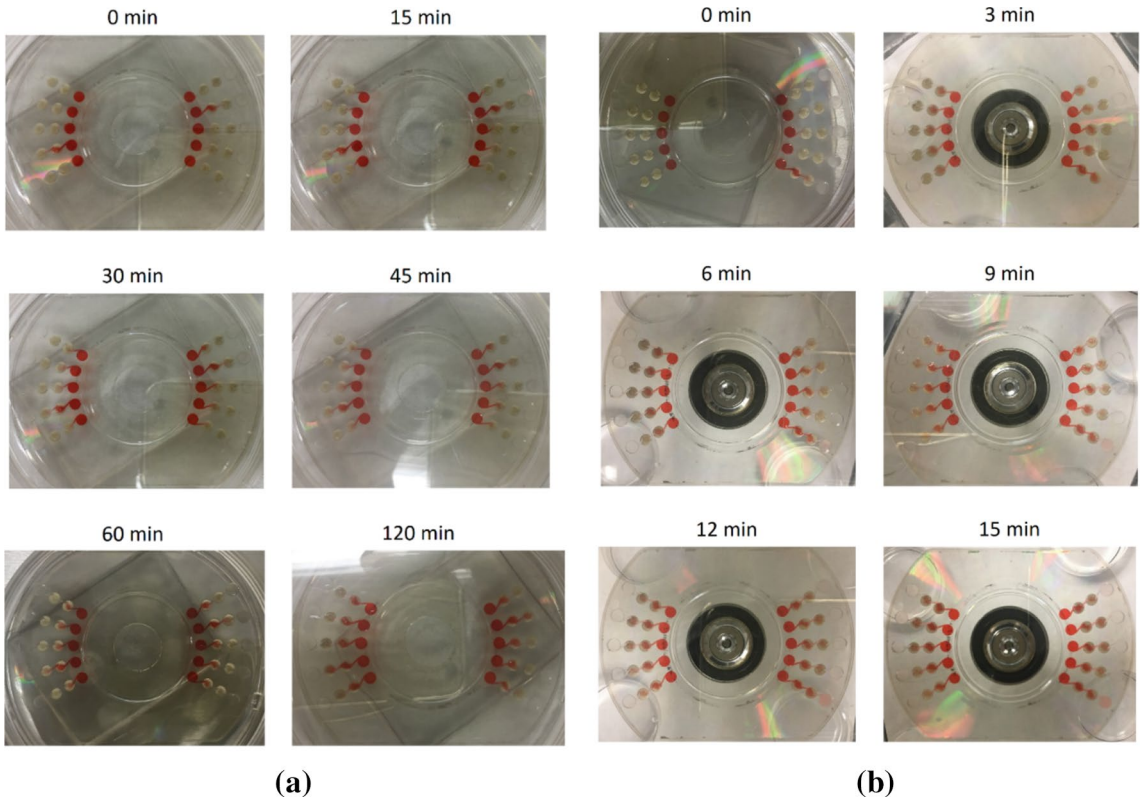

Fig. 7 **a** Diffusion test without rotation and **b** diffusion test with 60 rpm rotation

Conclusion

A high-throughput two-chamber 3D microscale tissue model platform is developed for drug screening studies. Porous scaffolds are selectively foamed using laser on a commercially available compact disk. Perfusion of cell culture medium is achieved with centrifugal force-driven diffusion by disk rotation. Experimental studies were conducted on the fabrication process under various gas saturation and laser power conditions. Cell cultures were performed with two types of human cell lines M059K and C3A-sub28. It is shown that the laser foaming process created microscale scaffolds with perforated surface covering the porous structure underneath. The size of the foamed region is dependent on laser power and time through a nonlinear heating and heat dissipation effect. A higher laser power and shorter laser time will produce a smaller scaffold with better defined microstructure. Cell culture study results demonstrate that polydopamine-coated micro-porous scaffolds are inducive for cell attachment and aggregation. With the potential of fabricating many such two-chamber systems on a single CD and the centrifugal force-driven diffusion, the proposed platform provides a simple solution to achieving high-throughput drug screening with more realistic 3D tissue model systems.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

Ethical approval This study does not contain any studies with human or animal subjects performed by any of the authors.

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