Sensors & Actuators: B. Chemical 280 (2019) 86–93

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Wound-on-a-chip: High-throughput 3D wound healing assay with a novel SU-8 mesh chip

Yachao Wang[1], Jinchi Zhu[1], Peng Chen, Yiwei Li, Shuangqian Yan, Jie Wang, Wei Du, Bi-Feng Liu*

The Key Laboratory for Biomedical Photonics of MOE at Wuhan National Laboratory for Optoelectronics-Hubei Bioinformatics & Molecular Imaging Key Laboratory, Systems Biology Theme, Department of Biomedical Engineering, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan 430074, China

ARTICLE INFO

Keywords:
SU-8 mesh chip
3D cell culture
Wound-on-a-chip
Wound healing

ABSTRACT

The dynamic process of cells is the basis of many biological problems in living organisms and has been a major research subject over several decades. In this paper, we present a novel SU-8 mesh chip (SMC) with thousands of chamber arrays for high-throughput 3D wound healing assay, which could directly and efficiently evaluate 3D cell growth process and cellular drug responses *in vitro*. Four kinds of adherent cells (HeLa, HepG2, HUVEC, NIH-3T3) were successfully seeded into the SMC to form three-dimensional hollow cell aggregates models with precisely pre-defined sizes and shapes, followed by monitored their spontaneous growth process in real time. Compared with traditional 2D wound healing assay, this 3D model provided more complex cellular processes and some tissue-specific properties, which conferred a high degree of clinical and biological relevance to *in vitro* models. NIH-3T3 and HUVEC cell aggregates in this chip that termed as "wound on a chip" model had been developed and further investigated the drug response in these 3D wound healing processes by applying different concentrations of candidate drugs.

1. Introduction

The proliferation, migration and self-organization of cells play an important role in the process of growth, development, and diseases of living organisms. Understanding the underlying mechanisms is thus critical for exploring the occurrence and treatment of some health issues [1–3]. For example, the processes of embryo development, cancer metastasis and wound healing *etc. in vivo* are complex and the cell dynamic processes among them are very important [4,5] In order to investigate these dynamic processes, researchers are committed to establish *in vitro* models to monitor the occurrence and development of these cell processes in real time.

Traditional approaches for *in vitro* cell progress study are mainly based on scratch assay [6–8] and some more high-throughput and controllable assays based on micropatterning cell culture methods [9,10], which are straightforward and economical method to probe cell migration and proliferation in two-dimensional. However, since cells in any multicellular organism are organized into three-dimensional (3D) structures, and reside within 3D environments, all above mentioned methods suffer from the limitations of monolayer cell culture models: lacking of tissue-specific properties and increasing uncertainty about their validity as a model system [11,12].

Currently, to overcome some of limitations of 2D cell culture methods, a variety of *in vitro* 3D cell culture models have been developed, such as 3D cell co-culture models [13,14], cell spheroid culture [15–17], "organs on a chip" system [18–20] and even "organisms on a chip" system [21,22], which were widely applied in the field of cell therapy [23,24], the *in vitro* model for drug discovery [25,26] and tissue engineering [27–29]. With the construction of these systems, lots of microfabrication technologies including microwell arrays [30–32], microdroplets [33–35] and microfluidic chips [15,19,36–39], have been developed correspondingly. Although these technologies are good replacements for the traditional approaches *in vitro*, to monitor and quantitative analysis the 3D dynamic cell progress just like the cell monolayers cultured on flat substrates remains a great challenge [3,40,41]. The recent advent methods focusing on tumor progression [42] and embryoid bodies (EBs) development [21,31,43] offer some strategies for these issues. For example, Nassoy and colleagues applied microfluidic devices to make tumor cellular capsules and investigate the mechanics of tumor progression *in vitro* [44]; Rivron and colleagues

* Corresponding author.
E-mail address: bfliu@mail.hust.edu.cn (B.-F. Liu).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.snb.2018.10.050
Received 19 March 2018; Received in revised form 29 September 2018; Accepted 8 October 2018
Available online 10 October 2018
0925-4005/

introduced a microwell platform, which was integrated in standard culture plates, for 3D high throughput screening and profiling of embryoid bodies [31]. Despite the improvements have been made in the relevant areas, it still need complex operation and quantitative analysis. Thus a 3D model that could straightforward and economical study this cell growth processes, and further investigate the effect of candidate drugs about these health issues remain to be fully realized.

Here, a novel SU-8 mesh chip (SMC) with thousands of chamber arrays was developed for investigating and monitoring the 3D growth process of cells in real time, and a new 3D hollow cell aggregates culture method based on mesh culture method [45–47] had been established. Specifically, the cells were first seeded on the SU-8 mesh walls of chip at the beginning, and most of them were adhered to the lateral face of the mesh structure to form 3D hollow cell aggregates with a "3D wound" in the middle, then new cells would spontaneously grow to the wound area based on the original cells and extracellular matrix until they completely filled with the 3D space, which was similar to the process of wound healing in the body. As a proof of concept, four kinds of cells (HeLa, HepG2, HUVEC, NIH-3T3) were successfully formed the hollow cell spheroid, and among them, NIH-3T3 and HUVEC 3D hollow cells spheroids were regarded as "wound on a chip" model. By applying different concentrations of arginine vasopressin and rh-VEGF on these wound models, and comparing the results of our experiments with the known effects of these drugs and the results of previous studies, the validity of related biological applications for such a novel three-dimensional cell culture model had been proved. Finally, we determined that the novel SMC 3D cell culture model opens up a new avenue for the *in vitro* study on the related problems of cell process.

2. Experimental

2.1. Chip fabrication

The proposed hybrid chip consisted of two parts, a SU-8 net and a cover slip. The SU-8 mesh structure was fabricated through standard photolithography techniques (Fig.1A), which included the following a series of steps: silicon wafer cleaning, SU-8 spin coating, UV exposure, and developing in PGMEA. First, a silicon substrate was cleaned using Piranha (3:1, H2SO4:H2O2), then rinsed with deionized water and dried at 150 °C for 1 h. SU-8 1075 (Gersteltec Sarl, Switzerland) was spun on the wafer at 1700 rpm using a spin coater (KW-4 A, Institute of microelectronics, Chinese Academy of Sciences) to a final thickness of approximately 100 μm and baked at 40 °C for 30 min., 120 °C for 5 min., and was cooled to room temperature. The SU-8 was then exposed to 365 nm light using a lithography machine (JKG-2 A, Shanghai Xueze, China) through predesigned mask. Lastly, the substrate was baked at 95 °C for 30 min., cooled to room temperature and placed in the PGMEA developer (Microchem) for about 10 min. The SU-8 mesh was developed and released from the substrate by immersion in PGMEA, and then sterilized by soaking it in isopropanol for 20 min. and washing 3 times with sterile phosphate buffered saline (PBS). After obtaining the SU-8 mesh, we sandwiched it between two pieces of cover slip, and incubated them into water bath (above 80 °C) for a few seconds clamped by tweezers, then one of the cover slips was removed immediately and the mesh was leaved on the other one forming microchambers.

2.2. Cell culture and seeding

Four cell lines were used for the experiments: human cervical cancer cells (HeLa), human liver carcinoma cells (HepG2), human umbilical vein endothelial cells (HUVEC) and mouse embryonic fibroblast cells (NIH-3T3). All the cell lines were cultured in high-glucose (25 mM) Dulbecco's Modified Eagle's Medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco) and 1% penicillin/streptomycin. All the cell lines were incubated (5% CO_2, 90% humidified) at 37 °C in an incubator (Innova-Co 170; New Brunswick Scientific, U.K.). Prior to use, cells were detached from the flask with the treatment of 0.25% (w/v) trypsin-EDTA solution (Gibco) for several mins.

Before the cell suspension was seeded to the chip, a kind of non-adhesive tape was used to stick the edge of the SU-8 net to the cover slip in case all the cells falling off from the SU-8 net. Then put them to a twelve well plate, and each well added 3 mL cell suspension with about 3×10^5 cells. After several hours of culture, a part of the cells next to the mesh walls were adhered to the surface of the mesh, then the SU-8 net with cells was removed from the cover slip and suspended in fresh DMEM cell culture medium for further experiments.

2.3. Drug screening

NIH-3T3 cells and HUVECs were selected to construct the wound model [48,49]. Arginine vasopressin (Dalian Meilun Biotech Co., Ltd., China) and rh-VEGF 165(Bersee, Beijing) that could respectively promote NIH-3T3 and HUVEC cell growth were used as the candidate drugs. The experiments were performed according to the following steps: firstly, 3D hollow cell aggregates were fabricated as 3D wound models according to the method mentioned above; secondly, different concentrations candidate drugs were respectively applied to the model's culture medium, and the medium with drugs was exchanged every day; thirdly, observing and recording the location of the cells in the chip every day; at last, analyzing the results.

2.4. Cell viability tests

The viability of cells cultured in the meshes was analyzed using Live/Dead assay. Briefly, cells in the chip were washed twice with PBS and incubated for 10 min. with 2 μg mL^{-1} calcein-AM (AnaSpec) and 2 μg mL^{-1} propidium iodide (PI) (Sigma). The stained cells were analyzed using a confocal fluorescence microscopy (FV1000). Calcein-AM on behalf of living cells with green fluorescence, whereas the PI indicate dead cells with fluoresces in red.

2.5. Scanning electron microscopy

Cells in the meshes should be pretreated before SEM imaging. They were fixed by 4% Polyformaldehyde in phosphate-buffered saline (PBS) buffer for 30 min. at room temperature and then washed 3–5 times with deionized water. The fixed cells were subsequently dehydrated with a series of graded ethanol (20%, 40%, 60%, 80%, and 100%) for 15 min at each concentration. After dehydration, the cells were frozen at -20 °C, and followed by freeze-dried for several hours. The freeze-dried cells in the mesh were coated with a layer of gold before observed under a scanning electron microscope.

2.6. Monitor cell growth process

The decreasing areas and the migration distance of hollow cell aggregates edge position in the SU-8 mesh were monitored and used to calculate cell growth rate. Facilities for imaging the 3D wound areas in the SMC were an ordinary inverted microscope (IX71, Olympus, Japan) with a charge-coupled device (CCD) camera (Evolve 512, photometrics, USA). As the cells began to grow stably in the SMC after about a day of culture, data began to be collected. The position with cells in the images was darker than the empty position, so the change of the hollow area could be easily calculated by the image analysis software Image-Pro Plus 6.0.

2.7. Two-dimensional control experiment

The two-dimensional (2D) control experiments were carried out by a microfluidic chip, which was consisted of a PDMS column array and a glass slide. The middle blank areas were formed by the PDMS column that could reversible bonded to the slide surface. Briefly, cells were

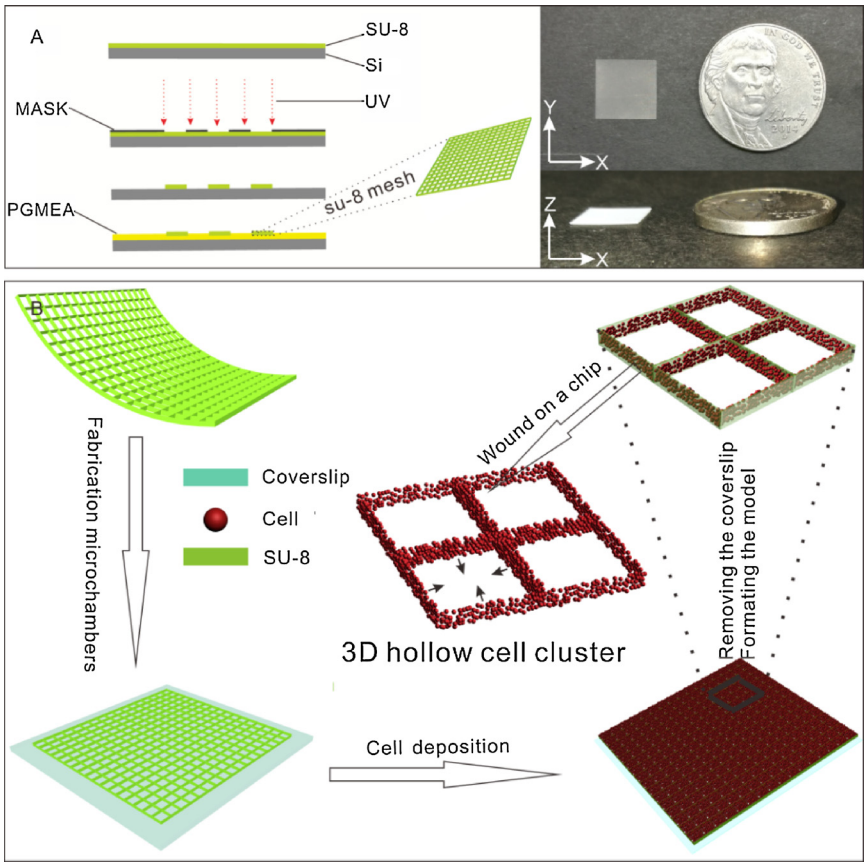

Fig. 1. Fabrication processes of the 3D cell culture chip. (A) Schematic of the SMC fabrication process, and its comparison with a coin. (B) The work-flow of the assay. 3D hollow cell aggregates that applied for wound on a chip model in flow-up study were successfully constructed by the SMC, and the growth process of cells that used to simulate the process of wound healing was shown by the arrows.

firstly injected into the chip, followed by removing the PDMS after cells had attached to the slide, so the places covered by a PDMS column on a slide formed the blank areas of cells. Through subsequent culture, the cells gradually grow into the wound area of the slide in a two-dimensional way compare to this three dimensional experiments.

2.8. Statistical analysis

Each experiment was applied in more than 9 meshes and had been repeated at least three times, in which the wound areas counted in Fig. 4 were the average number of 9 meshes. The mean and standard deviation (SD) for each measured parameter were calculated for reflecting the change of the 3D wound areas under investigation. Comparisons of cell growth rates under different drug concentrations were performed using the *t*-test (IBM SPSS 22), and statistical significance of differences were determined at $P < 0.05$ and $P < 0.01$.

3. Results and discussion

3.1. Characterization of the controllable chip structure

In this paper 'wound on a chip' model had been successfully constructed by the SMC with controllable shape and size meshes. Thousands of meshes constituted the SMC in square centimeter size (Fig. 1A), and each of the mesh could be used as an independent experimental unit (Fig. 1B). A variety of chip structures (rectangular (Fig. 2A and Fig. S1), triangular (Fig. 2B), hexagon (Fig. 2C), circular (Fig. 2D) and cross (Fig. 2E)) were fabricated in our study to construct various wound shapes. The widths of the rectangular meshes were changed from 50 μm to 150 μm (Fig. S1), and the thickness of their high aspect ratio structure walls was 20 μm and the height was 150 μm, which was referred by the white arrow in Fig. 2A. Three different depths of the meshes were also characterized by PDMS columns got from a SU-8 mesh mold (Fig. S2B), and we could make any height between the three by controlling the speed of the spin coater. In a word, the meshes in the chip were with high aspect ratio walls and various structures, compared with the traditional two dimensional wound healing assay, they had offered enough 3D space for cell growth.

3.2. Cell seeding of the chip

The diagrammatic sketch was a sectional view of the whole process of an experimental unit (Fig. 3A). For details, cells in suspension were firstly aggregated into each mesh (Fig. 3A (a)), then most cells slid from the mesh as the cover slip was removed after several hours, and the cells attached to the SU-8 scaffold were left behind, in which the cells attached to the lateral faces of the mesh walls had formed the 3D wounds on the SMC (Fig. 3A (b)). The key point here was that the SU-8 net and cover slip should be compact, and the cell number should be sufficient to fill the chamber. The cells would also adhere or move to the top or bottom surface of the mesh because the same chemical properties of the SU-8 scaffold surfaces, but they would not affect the 3D wound healing process of the cells in the lateral faces of the mesh. The images obtained by SEM (Fig. 3B) and confocal microscopy (Fig. 3C) were the 3D wound models, just like the scratch test, the 3D wound areas in this model had been successfully scratched without damaging the cells, and in the related subsequent experiments, the three-dimensional wound space in the middle of the mesh would be occupied by the cells through a cell process defined as 3D wound healing (Fig. 3A (c–d)).

3.3. Monitor high-throughput 3D wound healing process

In this paper, thousands of duplicate mesh units for cell culture could be assembled into a one cm^2 chip. Due to range of the vision was about 3 mm^2 for 10X microscope, there were only 100 meshes of the net were imaged and monitored in our actual experimental operation. As

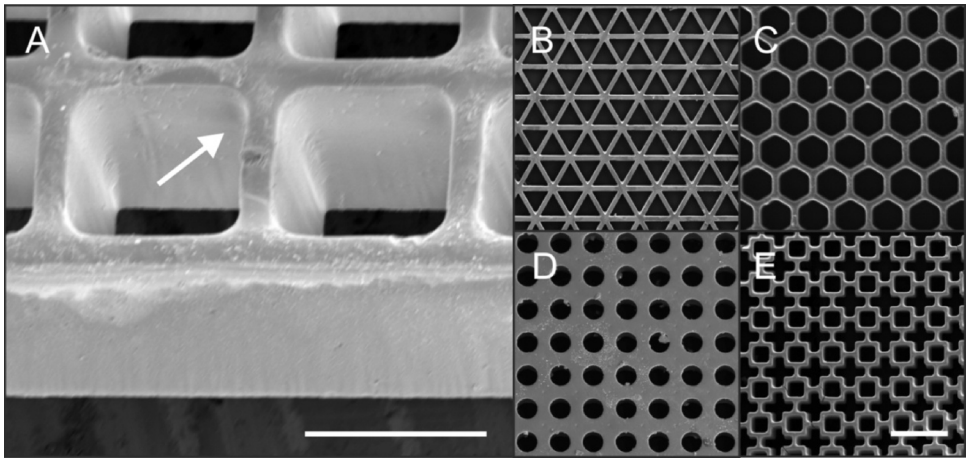

Fig. 2. SEM images of the SMC structure. (A) Ultra-high aspect ratios SMC structure as the white arrow shows; (B–E) SEM images for different shapes of the SMC, (B) Triangle, (C) Regular hexagon, (D) Circular, (E) Cross. Scale bars, 200 μm.

shown in Fig. S3, HUVEC cells were evenly distributed into mesh to form three-dimensional wound models at first, then the wound areas gradually reduced with the passage of time, and the statistical results indicated the growth curve of the cells. Through analyzing the growth curve, we could further investigate the 3D wound healing process and provide a high-throughput platform for subsequent drug discovery research.

3.4. Cells generation in the chip

After cells were seeded into the chip, they began to grow spontaneously into the wound areas, and the edge position of the hollow cell aggregates began to be recorded daily, then the size of the wound areas and the migration distance of the hollow cell aggregates edge position were calculated to accurately demonstrate the growth process of cells by data analysis software image pro plus. The SEM images with red arrows in Fig. 3B showed that the cells were firstly attached to the inner surface of the SU-8 and formed three-dimensional wound areas, then

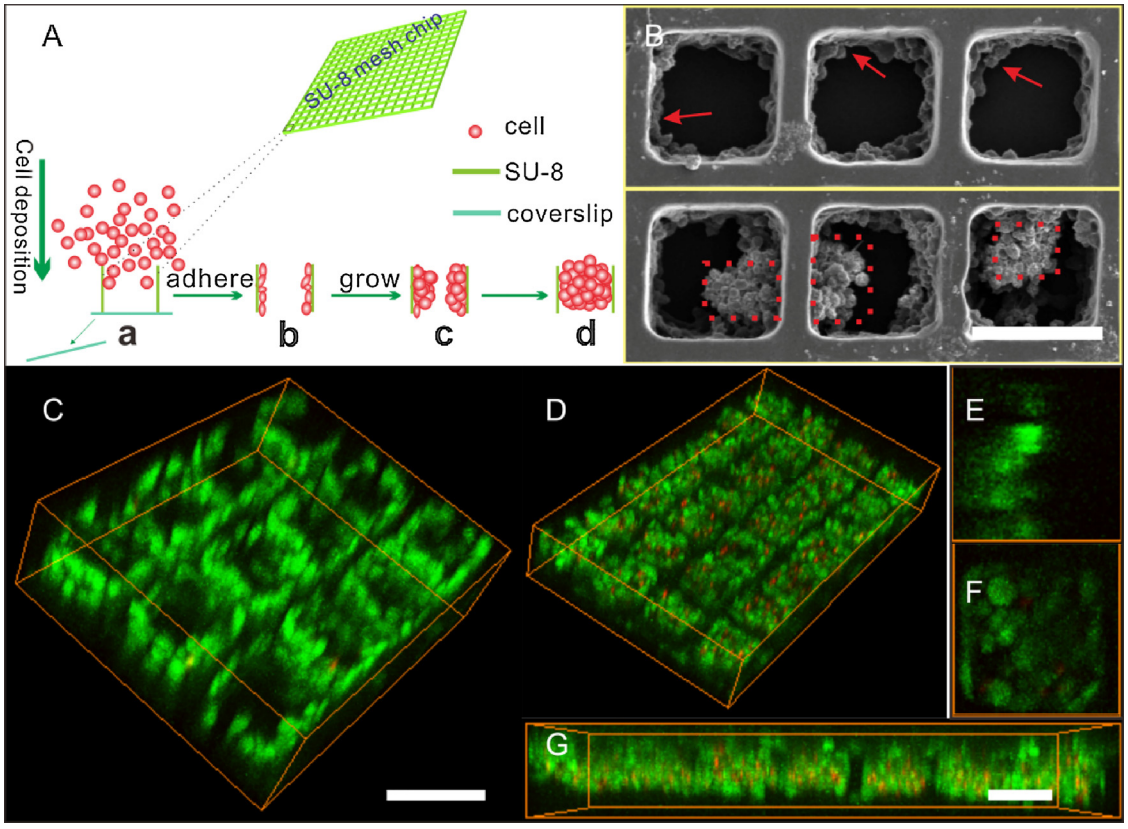

Fig. 3. Construction of 3D wound healing models on SMC. (A) Schematic of the 3D wound healing process on chip, (B) SEM images of the cell position during the experiments. The red arrows indicated that cells had adhered to the 3D SU-8 wall surface and red dashed boxes indicated that cells had grown into the wound area. (C) 3D confocal fluorescence images showed the stereoscopic distribution of cells that had just been adhered to the walls, (D) 3D confocal fluorescence images of cell spheroids that had already filled the SMC chambers, (E) Longitudinal section of the cell spheroids, (F) Transverse section of the cell spheroids, (G) YZ section of the cell spheroids. Scale bar 100 μm (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

after a period of time culture, they had grown into the wound areas. The SEM images with red dashed box in Fig. 3B and Fig. S4 had displayed this dynamic process. Thus, by comparing these two sets of images, a kind of *in vitro* 3D wound healing process had been successfully monitored in this proposed novel SMC.

In this 3D wound healing process, cell activity had been tested, and the 3D confocal images (Fig. 3C and D) were the results of cell live/dead staining at different experimental stages respectively. There were only a few dead cells after they were seeded into SU-8 mesh for 24 h (Fig. 3C), and most of the cells were still alive until they had filled the meshes after about 3 days of culture (Fig. 3D), which suggesting that there are no toxic effects from the SU-8 net.

Although the scanning electron microscope and confocal microscopy had proved that the cells indeed grew into the mesh in a three-dimensional way, were there hollow structures inside the 3D cell cluster was still not sure. Due to we used mesh area reduction to react the cell growth rate, so it is critical to make sure that no internal hollow structures in the cell aggregates. The images shown in Fig. 3E–G were the transverse, longitudinal section and the Z axial field of Fig. 3D, from which we determined that the final cell aggregates were solid and the method to monitor cells proliferation rate was credible.

3.5. Tracking multiple kinds of cell growth processes in multiple kinds shape meshes

Multiple kinds of cells including cancer cells (HeLa, HepG2) and normal cells (HUVEC, NIH-3T3) were applied to the SMC, and their growth processes were recorded by a microscope. All the four kinds of cell lines began growing spontaneously to the middle of the chip until they fully filled with the meshes within a few days (Fig. 4A), which demonstrated that all these four kinds of cells could be 3D cultured by our method. In other words, all these cells could spontaneously grow into the 3D hollow regions just based with the original cell masses and the extracellular matrix without any other supports.

Besides the basic square chip structure, several other chip structures (triangular, hexagon, circular and cross) had also been applied in this model. HUVECs were first formed cell aggregates of these structures successfully based on pre-designed chips (Fig. S5), then the cells in these chip structures began spontaneously heal the wounds, and their dynamic processes were recorded for several days before they completely filled with the 3D space of meshes in these SMCs (Fig. 5). The experimental results showed that this model was not restricted by cell types and chip structures, which had provided more possibilities for biological applications.

The wound areas in the meshes of various SMCs had been quantified by the image processing software Image-Pro Plus, and the pixels of the areas had been counted and analyzed by the software Origin 9.0. The results showed that the reduction of the blank area caused by the increased number of cells decreased linearly with time (Figs. 4B–E and 5 E–H). We inferred the change of cell growth rate by calculating the slope change of fitting line, such as the effects of candidate drugs on these cell processes in the follow-up experiments. It is well known that the number of cells increase exponentially with time for an ideal exponential growth curve of cell proliferation [50]. The cells and the culture conditions in each chip were the same in our experiments, which resulted in the size of the newborn cells were similar in each mesh (Fig. 3B). Thus the reduction of the wound area caused by the increase of cell number should decrease exponentially as well. Therefore, the limitation of cell proliferation possibly occurred unlike ideal logarithmic proliferation in our model, and the final statistical results of our model were linear. Moreover, we could also analyze the wound healing process by calculating the migration distance of hollow cell aggregates edge position, which had provided another idea for some biological application (Fig. S6).

As a control experiment of this paper, the 2D wound healing assay had been carried out on a glass slide surface. The size of the "two-dimensional wound" on the slide was 400 square microns. When the cells had covered with the glass substrate around the wound areas, their wound healing processes began to be monitored, and after only about 9 h, the cells had completely occupied the wound area (Fig. 6). Compared with the time of the two-dimensional experiment, we believed that the three-dimensional wound healing assay introduced in this article showed more complex cell growth processes with some tissue-specific properties. What's more, the substrate used in two-dimensional experiments would also impact the wound healing process. However, we needn't to consider these in the three dimensional experiments here.

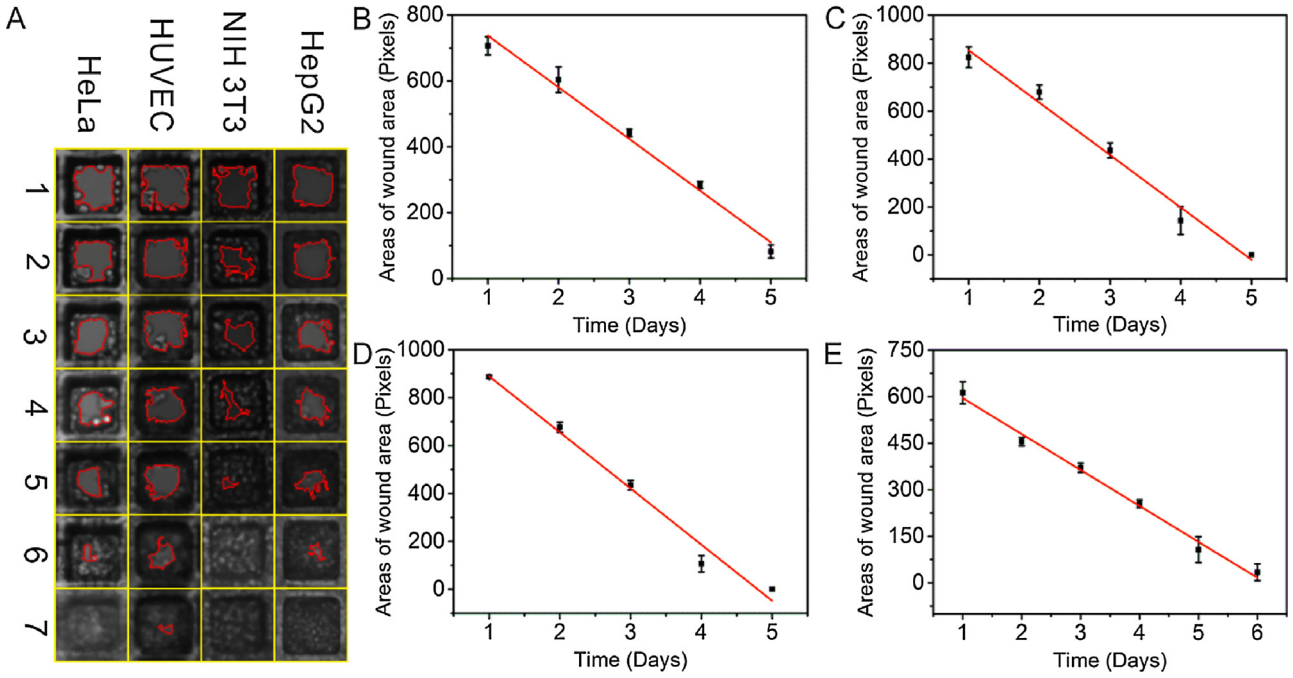

Fig. 4. The growth processes of different kinds of cells. (A) Images of the four kinds of cells growth processes (HeLa, HUVEC, NIH-3T3, HepG2) that were tracked for several days, (B–E) Growth curves of these cells that were 3D cultured in SMC, (B) HeLa, (C) HUVEC, (D) NIH-3T3, (E) HepG2.

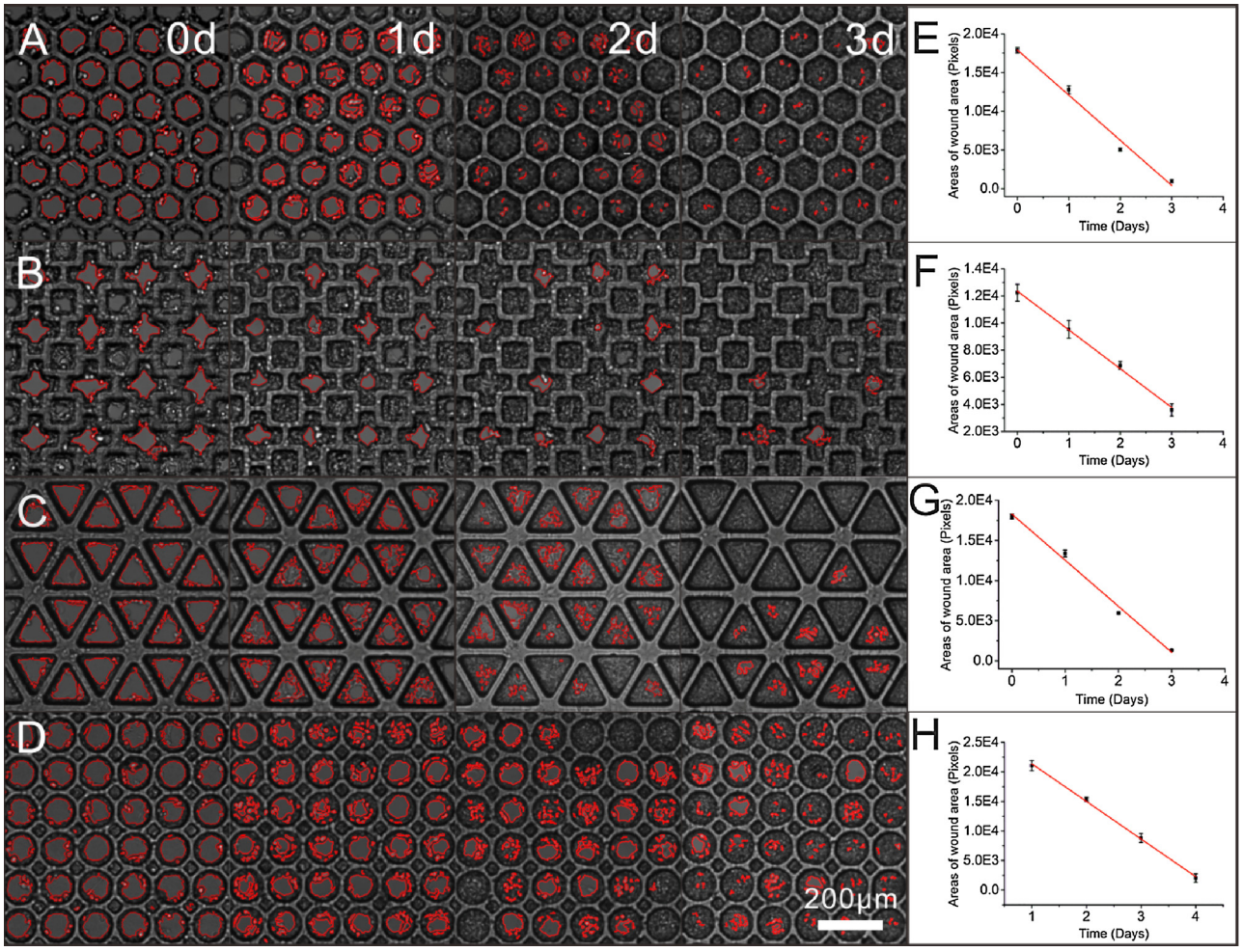

Fig. 5. Cell growth processes in multiple kinds of meshes. (A–D) The monitoring results of cell growth in different chip structures (From top to bottom: (A) Hexagons, (B) Crosses, (C) Triangles, (D) Circles). (E–H) Cell growth curves of cells in these chips. (E) Hexagons, (F) Crosses, (G) Triangles, (H) Circles.

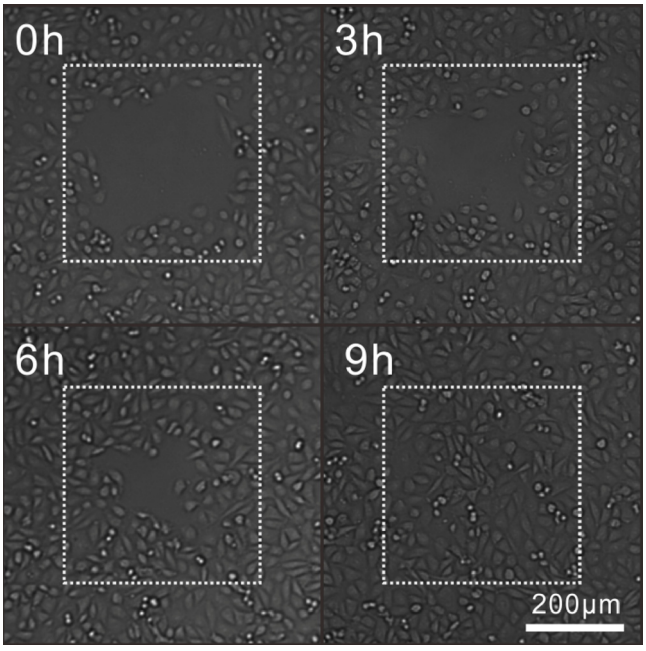

Fig. 6. Two dimensional wound healing experiment on glass slide substrate. The areas in white dotted line frames were the pre-designed blank area, and the cells had filled with about 400 μm^2 blank areas in 9 h.

3.6. Wound on a chip study

In this paper, our model was used as a wound on a chip model to investigate the effect of candidate drugs on cell growth process. NIH-3T3 cells and HUVECs were selected to construct 3D wound model, and different concentrations of candidate drugs were applied to stimulate the cells proliferation. According to the previous researches, vasopressin could significantly promote 3T3 cells proliferation at 1 or 10 ng/mL and VEGF could significantly promote HUVECs proliferation at 50–100 ng/mL, and these drugs were also applied in our 3D wound healing models at these concentrations. The growth curve of these 3D cell aggregates at different concentrations of candidate drugs were shown in Fig. 7A and C and the calculation results indicated that our method had accurately reflected the utility of candidate drugs (Fig. 7B and D). In summary, a three-dimensional cell culture model for studying cell dynamic process was successfully constructed, and we applied this method for wound on a chip study which had successfully captured the response of the cell dynamics process to the changes of the microenvironment around the cells consistent with previous studies.

4. Conclusions

We have developed a novel microchip based on SU-8 net with high-throughput mesh arrays, and a three-dimensional model for studying cellular dynamic processes *in vitro* had been constructed based on the SMC. In this study, adherent cells cultured on the lateral faces of the mesh arrays were used to form 3D hollow cell aggregates, then the cells

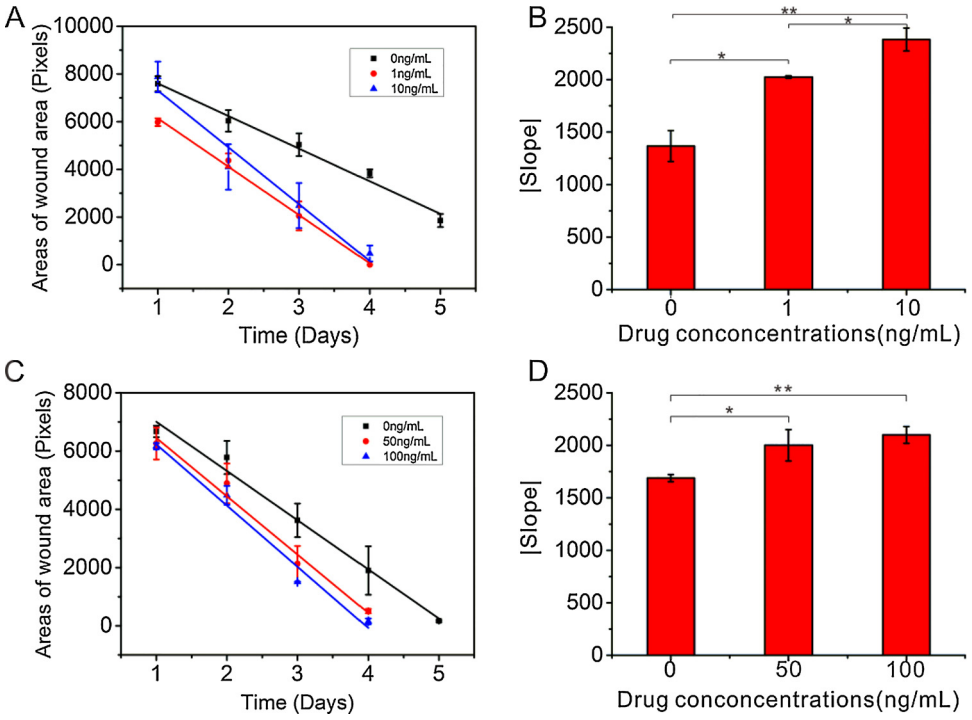

Fig. 7. Wound on a chip analysis. (A and C) The growth curves of 3T3 and HUVEC cells under different drug concentrations. (B and D) The absolute value of the slopes of cell growth curves with different concentrations of drugs. n = 3, *P < 0.05; **P < 0.01.

would grow spontaneously into the hollow spaces until they filled it, by monitoring the reducing space of the mesh every day the growth law of cells was quantified. Through this simple and cost-effective approach, we had not only successfully constructed a 3D wound healing model, but also achieved agreement results in drug screening applications compared with the existing researches.

Moreover, to prove the practicability of proposed method, four kinds of cells had been successfully applied to form the hollow cell spheroid, which included two kinds of cancer cells (HeLa, HepG2) and two kinds of normal cells (HUVEC, NIH-3T3). In the process of building the model, a variety of chip structures were constructed, and each mesh chamber in the chip with a high aspect ratio structure wall, which ensured the 3D structure of the cell aggregates cultured based on the wall surface. In summary, different kind of cells had been seeded into the chips with various structures, and then their subsequent spontaneous growth process was recorded and analyzed to obtain the growth curves of cells cultured in this model. The novel SMC 3D cell culture model opens up a new avenue for the *in vitro* study on the related problems of cell process compared with two-dimensional wound healing experiments.

Acknowledgements

We gratefully acknowledge the financial support from the National Natural Science Foundation of China (21775049, 21475049, 31471257 and 31700746), the National Key R&D Program of China (2016YFF0100801) and China Postdoctoral Science Foundation funded project (2018M630847 and 2018T110753).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.snb.2018.10.050.

References

[1] P. Friedl, D. Gilmour, Collective cell migration in morphogenesis, regeneration and cancer, Nat. Rev. Mol. Cell Biol. 10 (2009) 445–457.
[2] K.O. Okeyo, T. Adachi, J. Sunaga, M. Hojo, Actomyosin contractility spatio-temporally regulates actin network dynamics in migrating cells, J. Biomech. 42 (2009) 2540–2548.
[3] Z. Guan, S. Jia, Z. Zhu, M. Zhang, C.J. Yang, Facile and rapid generation of large-scale microcollagen gel array for long-term single-cell 3D culture and cell proliferation heterogeneity analysis, Anal. Chem. 86 (2014) 2789–2797.
[4] M. Eiraku, N. Takata, H. Ishibashi, M. Kawada, E. Sakakura, S. Okuda, et al., Self-organizing optic-cup morphogenesis in three-dimensional culture, Nature 472 (2011) 51–56.
[5] Y. Cui, Y. An, T. Jin, F. Zhang, P. He, Real-time monitoring of skin wound healing on nano-grooves topography using electric cell-substrate impedance sensing (ECIS), Sens. Actuators B Chem. 250 (2017) 461–468.
[6] C.C. Liang, A.Y. Park, J.L. Guan, In vitro scratch assay: a convenient and inexpensive method for analysis of cell migration in vitro, Nat. Protoc. 2 (2007) 329–333.
[7] K.P. Goetsch, C.U. Niesler, Optimization of the scratch assay for in vitro skeletal muscle wound healing analysis, Anal. Biochem. 411 (2011) 158–160.
[8] M. Fronza, B. Heinzmann, M. Hamburger, S. Laufer, I. Merfort, Determination of the wound healing effect of calendula extracts using the scratch assay with 3T3 fibroblasts, J. Ethnopharmacol. 126 (2009) 463–467.
[9] S. Zhang, L. Yan, M. Altman, M. Lassle, H. Nugent, F. Frankel, et al., Biological surface engineering: a simple system for cell pattern formation, Biomaterials 20 (1999) 1213–1220.
[10] C. Zheng, Z. Yu, Y. Zhou, L. Tao, Y. Pang, T. Chen, et al., Live cell imaging analysis of the epigenetic regulation of the human endothelial cell migration at single-cell resolution, Lab Chip 12 (2012) 3063–3072.
[11] A. Ranga, S. Gobaa, Y. Okawa, K. Mosiewicz, A. Negro, M.P. Lutolf, 3D niche microarrays for systems-level analyses of cell fate, Nat. Commun. 5 (2014) 4324.
[12] K.M. Yamada, E. Cukierman, Modeling tissue morphogenesis and cancer in 3D, Cell 130 (2007) 601–610.
[13] G. Adriani, D. Ma, A. Pavesi, R.D. Kamm, E.L. Goh, A 3D neurovascular microfluidic model consisting of neurons, astrocytes and cerebral endothelial cells as a blood-brain barrier, Lab Chip 17 (2017) 448–459.
[14] C.J. Kirkpatrick, S. Fuchs, R.E. Unger, Co-culture systems for vascularization–learning from nature, Adv. Drug Deliv. Rev. 63 (2011) 291–299.
[15] K. Kwapiszewska, A. Michalczuk, M. Rybka, R. Kwapiszewski, Z. Brzozka, A microfluidic-based platform for tumour spheroid culture, monitoring and drug screening, Lab Chip 14 (2014) 2096–2104.
[16] E. Fennema, N. Rivron, J. Rouwkema, C. van Blitterswijk, J. de Boer, Spheroid culture as a tool for creating 3D complex tissues, Trends. Biotechnol. 31 (2013) 108–115.
[17] R.Z. Lin, H.Y. Chang, Recent advances in three-dimensional multicellular spheroid culture for biomedical research, Biotechnol. J. 3 (2008) 1172–1184.

[18] D. Huh, G.A. Hamilton, D.E. Ingber, From 3D cell culture to organs-on-chips, Trends. Cell Biol. 21 (2011) 745–754.
[19] S.N. Bhatia, D.E. Ingber, Microfluidic organs-on-chips, Nat. Biotechnol. 32 (2014) 760–772.
[20] K.-W. Chang, P.-Y. Chang, H.-Y. Huang, C.-J. Li, C.-H. Tien, D.-J. Yao, et al., Womb-on-a-chip biomimetic system for improved embryo culture and development, Sens. Actuators B Chem. 226 (2016) 218–226.
[21] J. Akagi, K. Khoshmanesh, C.J. Hall, J.M. Cooper, K.E. Crosier, P.S. Crosier, et al., Fish on chips: microfluidic living embryo array for accelerated in vivo angiogenesis assays, Sens. Actuators B Chem. 189 (2013) 11–20.
[22] A. Ge, L. Hu, X. Wang, J. Zhu, X. Feng, W. Du, et al., Logarithmic bacterial gradient chip for analyzing the effects of dietary restriction on *C. elegans* growth, Sens. Actuators B Chem. 255 (2018) 735–744.
[23] T.J. Bartosh, J.H. Ylostalo, A. Mohammadipoor, N. Bazhanov, K. Coble, K. Claypool, et al., Aggregation of human mesenchymal stromal cells (MSCs) into 3D spheroids enhances their antiinflammatory properties, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 13724–13729.
[24] S. Uchida, K. Itaka, T. Nomoto, T. Endo, Y. Matsumoto, T. Ishii, et al., An injectable spheroid system with genetic modification for cell transplantation therapy, Biomaterials 35 (2014) 2499–2506.
[25] E.W. Esch, A. Bahinski, D. Huh, Organs-on-chips at the frontiers of drug discovery, Nat. Rev. Drug Discov. 14 (2015) 248–260.
[26] A. van de Stolpe, J. den Toonder, Workshop meeting report organs-on-chips: human disease models, Lab Chip 13 (2013) 3449–3470.
[27] L.G. Griffith, M.A. Swartz, Capturing complex 3D tissue physiology in vitro, Nat. Rev. Mol. Cell Biol. 7 (2006) 211–224.
[28] Y.T. Matsunaga, Y. Morimoto, S. Takeuchi, Molding cell beads for rapid construction of macroscopic 3D tissue architecture, Adv. Mater. 23 (2011) H90–94.
[29] Y. Li, X. Feng, Y. Wang, W. Du, P. Chen, C. Liu, et al., Assembly of multiple cell gradients directed by three-dimensional microfluidic channels, Lab Chip 15 (2015) 3203–3210.
[30] T.G. Fernandes, M.M. Diogo, D.S. Clark, J.S. Dordick, J.M. Cabral, High-throughput cellular microarray platforms: applications in drug discovery, toxicology and stem cell research, Trends. Biotechnol. 27 (2009) 342–349.
[31] E.J. Vrij, S. Espinoza, M. Heilig, A. Kolew, M. Schneider, C.A. van Blitterswijk, et al., 3D high throughput screening and profiling of embryoid bodies in thermoformed microwell plates, Lab Chip. 16 (2016) 734–742.
[32] Y.Y. Choi, B.G. Chung, D.H. Lee, A. Khademhosseini, J.H. Kim, S.H. Lee, Controlled-size embryoid body formation in concave microwell arrays, Biomaterials 31 (2010) 4296–4303.
[33] A.Y. Hsiao, Y.C. Tung, C.H. Kuo, B. Mosadegh, R. Bedenis, K.J. Pienta, et al., Micro-ring structures stabilize microdroplets to enable long term spheroid culture in 384 hanging drop array plates, Biomed. Microdevices 14 (2012) 313–323.
[34] Y. Li, P. Chen, Y. Wang, S. Yan, X. Feng, W. Du, et al., Rapid assembly of heterogeneous 3D cell microenvironments in a microgel array, Adv. Mater. 28 (2016) 3543–3548.
[35] O. Frey, P.M. Misun, D.A. Fluri, J.G. Hengstler, A. Hierlemann, Reconfigurable microfluidic hanging drop network for multi-tissue interaction and analysis, Nat. Commun. 5 (2014) 4250.
[36] Y. Jun, M.J. Kim, Y.H. Hwang, E.A. Jeon, A.R. Kang, S.H. Lee, et al., Microfluidics-generated pancreatic islet microfibers for enhanced immunoprotection, Biomaterials 34 (2013) 8122–8130.
[37] M.-H. Wu, Y.-H. Chang, Y.-T. Liu, Y.-M. Chen, S.-S. Wang, H.-Y. Wang, et al., Development of high throughput microfluidic cell culture chip for perfusion 3-dimensional cell culture-based chemosensitivity assay, Sens. Actuators B Chem. 155 (2011) 397–407.
[38] P. Chen, X. Feng, D. Chen, C. Liu, W. Du, B.-F. Liu, Investigating intercellular calcium waves by microfluidic gated pinched-flow, Sens. Actuators B Chem. 234 (2016) 583–592.
[39] P. Chen, Y. Guo, J. Wang, W. Du, X. Feng, B.-F. Liu, A localized chemical pulse generator for the development of a microfluidic cell-based biosensor, Sens. Actuators B Chem. 251 (2017) 112–119.
[40] C. Kim, J.H. Bang, Y.E. Kim, S.H. Lee, J.Y. Kang, On-chip anticancer drug test of regular tumor spheroids formed in microwells by a distributive microchannel network, Lab Chip 12 (2012) 4135–4142.
[41] R. Limame, A. Wouters, B. Pauwels, E. Fransen, M. Peeters, F. Lardon, et al., Comparative analysis of dynamic cell viability, migration and invasion assessments by novel real-time technology and classic endpoint assays, PLoS One 7 (2012) e46536.
[42] M.S. Gajeski, E.L. Beckman, J.A. Fronczak, G.B. Krishnan, Development of a 3-dimensional cell invasion assay compatible with high content screening, Mol. Biol. Cell 24 (2013).
[43] V. Du, N. Luciani, S. Richard, G. Mary, C. Gay, F. Mazuel, et al., A 3D magnetic tissue stretcher for remote mechanical control of embryonic stem cell differentiation, Nat. Commun. 8 (2017) 400.
[44] K. Alessandri, B.R. Sarangi, V.V. Gurchenkov, B. Sinha, T.R. Kiessling, L. Fetler, et al., Cellular capsules as a tool for multicellular spheroid production and for investigating the mechanics of tumor progression in vitro, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 14843–14848.
[45] B. Tian, J. Liu, T. Dvir, L. Jin, J.H. Tsui, Q. Qing, et al., Macroporous nanowire nanoelectronic scaffolds for synthetic tissues, Nat. Mater. 11 (2012) 986–994.
[46] K.O. Okeyo, O. Kurosawa, S. Yamazaki, H. Oana, H. Kotera, H. Nakauchi, et al., Cell adhesion minimization by a novel mesh culture method mechanically directs trophoblast differentiation and self-assembly organization of human pluripotent stem cells, tissue eng, Part C Methods 21 (2015) 1105–1115.
[47] K.O. Okeyo, M. Tanabe, O. Kurosawa, H. Oana, M. Washizu, Self-organization of human iPS cells into trophectoderm mimicking cysts induced by adhesion restriction using microstructured mesh scaffolds, Dev. Growth Differ. 60 (2018) 183–194.
[48] F.Q. Nie, M. Yamada, J. Kobayashi, M. Yamato, A. Kikuchi, T. Okano, On-chip cell migration assay using microfluidic channels, Biomaterials 28 (2007) 4017–4022.
[49] Y. Luo, H. Diao, S. Xia, L. Dong, J. Chen, J. Zhang, A physiologically active polysaccharide hydrogel promotes wound healing, J. Biomed. Mater. Res. A 94 (2010) 193–204.
[50] J.L. Sherley, P.B. Stadler, J. Scott Stadler, A quantitative method for the analysis of mammalian cell proliferation in culture in terms of dividing and non-dividingcells, Cell prolif. 28 (1995) 137–144.

Yachao Wang is a Ph.D. candidate in Huazhong University of Science and Technology (HUST), China. His main research interest focuses on microfluidic-based methods for biological applications and bioengineering.

Jinchi Zhu is a Ph.D. candidate in HUST, China. His main research interest focuses on microfluidic-based methods for biological applications and bioengineering.

Peng Chen received his Ph.D. degree in HUST, China. His main research interest focuses on microfluidic-based methods for cell analysis

Yiwei Li received his Ph.D. degree in HUST, China. His main research interest focuses on microfluidic-based methods for biological applications and bioengineering.

Shuangqian Yan received his Ph.D. degree in HUST, China. His main research interest focuses on microfluidic-based methods for tumor diagnosis and therapy.

Jie Wang is a Ph.D. candidate in HUST. Her main research interest focuses on microfluidic-based methods for POCT and exosomes analysis.

Wei Du received his Ph.D. degree in Biology from Wuhan University, China in 2005. He is currently an associate professor at HUST. His main research interest focuses on the development of microfluidic analysis of *C. elegans*.

Bi-Feng Liu received his Ph.D. degree in Analytical Chemistry from Wuhan University (China) in 1999 and continued his postdoctoral research in the College of Life Science. From 2001, he moved to Japan and worked in Prof. Terabe's group (Japan) as a JSPS postdoctoral fellow. He joined HUST as a professor in 2003. He is now serving as deputy Dean of the College of Life Science & Technology. His research focuses on systems biology-oriented analytical science in the areas of mass spectrometry coupled micro-separation, microfluidics and molecular imaging.