

Estimation of cancer cell migration in biomimetic random/oriented collagen fiber microenvironments

Jingru Yao(姚静如), Guoqiang Li(李国强), Xiyao Yao(姚喜耀), Lianjie Zhou(周连杰), Zhikai Ye(叶志凯), Yanping Liu(刘艳平), Dongtian Zheng(郑栋天), Ting Tang(唐婷), Kena Song(宋克纳), Guo Chen(陈果), and Liyu Liu(刘雳宇)

Citation: Chin. Phys. B, 2024, 33 (**5**): 058706. DOI: 10.1088/1674-1056/ad334c

What follows is a list of articles you may be interested in

Molecular dynamics simulations of membrane deformation induced by amphiphilic helices of Epsin, Sar1p, and Arf1

Zhen-Lu Li(李振鲁)

Chin. Phys. B, 2018, 27 (**3**): 038703. DOI: 10.1088/1674-1056/27/3/038703

Anisotropic formation mechanism and nanomechanics for the self-assembly process of cross-*β* peptides

Li Deng(邓礼), Yurong Zhao(赵玉荣), Peng Zhou(周鹏), Hai Xu(徐海), Yanting Wang(王延颋)

Chin. Phys. B, 2017, 26 (**12**): 128701. DOI: 10.1088/1674-1056/26/12/128701

Modulation of intra- and inter-sheet interactions in short peptide self-assembly by acetonitrile in aqueous solution

Li Deng(邓礼), Yurong Zhao(赵玉荣), Peng Zhou(周鹏), Hai Xu(徐海), Yanting Wang(王延颋)

Chin. Phys. B, 2016, 25 (**12**): 128704. DOI: 10.1088/1674-1056/25/12/128704

In vitro three-dimensional cancer metastasis modeling: Past, present, and future

Wei-jing Han(韩伟静), Wei Yuan(袁伟), Jiang-rui Zhu(朱江瑞), Qihui Fan(樊琪慧), Junle Qu(屈军乐), Li-yu Liu(刘雳宇), on behalf of the U.S.--China Physical Sciences-Oncology Alliance

Chin. Phys. B, 2016, 25 (**1**): 018709. DOI: 10.1088/1674-1056/25/1/018709

Hierarchical processes in *β*-sheet peptide self-assembly from the microscopic to the mesoscopic level

Li Deng(邓礼) and Hai Xu(徐海)

Chin. Phys. B, 2016, 25 (**1**): 018701. DOI: 10.1088/1674-1056/25/1/018701

Estimation of cancer cell migration in biomimetic random/oriented collagen fiber microenvironments

Jingru Yao(姚静如)[1,†], Guoqiang Li(李国强)[2,†,‡], Xiyao Yao(姚喜耀)[1], Lianjie Zhou(周连杰)[1], Zhikai Ye(叶志凯)[3], Yanping Liu(刘艳平)[4], Dongtian Zheng(郑栋天)[1], Ting Tang(唐婷)[2], Kena Song(宋克纳)[5], Guo Chen(陈果)[1], and Liyu Liu(刘雳宇)[1,§]

[1] *Chongqing Key Laboratory of Soft Condensed Matter Physics and Smart Materials, College of Physics, Chongqing University, Chongqing* 401331, *China*
[2] *Chongqing Key Laboratory for Resource Utilization of Heavy Metal Wastewater, Aquatic Ecosystems in the Three Gorges Reservoir Region of Chongqing Observation and Research Station, Chongqing University of Arts and Sciences, Yongchuan* 402160, *China*
[3] *Wenzhou Institute, University of Chinese Academy of Sciences, Wenzhou* 325000, *China*
[4] *Department of Biomedical Engineering, Chongqing University of Posts and Telecommunications, Chongqing* 400065, *China*
[5] *College of Medical Technology and Engineering, Henan University of Science and Technology, Henan* 471023, *China*

(Received 2 February 2024; revised manuscript received 2 March 2024; accepted manuscript online 13 March 2024)

Increasing data indicate that cancer cell migration is regulated by extracellular matrixes and their surrounding biochemical microenvironment, playing a crucial role in pathological processes such as tumor invasion and metastasis. However, conventional two-dimensional cell culture and animal models have limitations in studying the influence of tumor microenvironment on cancer cell migration. Fortunately, the further development of microfluidic technology has provided solutions for the study of such questions. We utilize microfluidic chip to build a random collagen fiber microenvironment (RFM) model and an oriented collagen fiber microenvironment (OFM) model that resemble early stage and late stage breast cancer microenvironments, respectively. By combining cell culture, biochemical concentration gradient construction, and microscopic imaging techniques, we investigate the impact of different collagen fiber biochemical microenvironments on the migration of breast cancer MDA-MB-231-RFP cells. The results show that MDA-MB-231-RFP cells migrate further in the OFM model compared to the RFM model, with significant differences observed. Furthermore, we establish concentration gradients of the anticancer drug paclitaxel in both the RFM and OFM models and find that paclitaxel significantly inhibits the migration of MDA-MB-231-RFP cells in the RFM model, with stronger inhibition on the high concentration side compared to the low concentration side. However, the inhibitory effect of paclitaxel on the migration of MDA-MB-231-RFP cells in the OFM model is weak. These findings suggest that the oriented collagen fiber microenvironment resembling the late-stage tumor microenvironment is more favorable for cancer cell migration and that the effectiveness of anticancer drugs is diminished. The RFM and OFM models constructed in this study not only provide a platform for studying the mechanism of cancer development, but also serve as a tool for the initial measurement of drug screening.

Keywords: microfluidic chip, random collagen fiber microenvironment (RFM), oriented collagen fiber microenvironment (OFM), cancer cell migration

PACS: 87.85.dh, 87.80.–y, 87.18.Gh, 87.50.cf **DOI:** 10.1088/1674-1056/ad334c

1. Introduction

With the rapid development of disciplines such as medicine, biology, and engineering, composition and physiological structures of tumor microenvironments have been gradually discovered, and their impact on cancer cell proliferation, invasion, and metastasis has also been gradually revealed.[1–3] Among them, extracellular matrixes (ECMs) play a pivotal role as a key constituent of tumor microenvironments, providing a supportive medium for cell growth and migration while exerting significant regulatory functions in cancer initiation and progression.[4–6] Collagen fibers, as integral elements of an ECM, interact with collagen receptors possessing tyrosine kinase activity in cancer cells, facilitating collagen fiber alignment, and promoting tumor growth and invasion.[7,8] Clinical studies have corroborated that collagen fibers in early stage breast cancer tissue exhibit random and non-directional pattern, whereas in late-stage breast cancer tissue, they exhibit oriented and highly linear patterns.[9,10] Notably, the presence of oriented collagen fibers is associated with shorter survival periods in breast cancer patients.[11] In addition to physical structures of collagen fibers in tumor microenvironments, biochemical gradients also exert a profound influence on cancer cell physiology, regulating crucial processes such as proliferation, differentiation, and migration.[12–14] Over the past decade, various biochemical gradient models have been developed for studying cell chemotaxis and conducting high-throughput drug screening.[15–17] However, there is a scarcity of biophysical models that combine the biochemical gradients with the physical characteristics of collagen fibers within microenvironments to investigate migration of cancer cells at dif-

[†]These authors contributed equally to this work.
[‡]Corresponding author. E-mail: guoqiangli1989@126.com
[§]Corresponding author. E-mail: lyliu@cqu.edu.cn

© 2024 Chinese Physical Society and IOP Publishing Ltd

ferent stages of tumor progression.

Due to their integrated, miniaturized, and highly controllable nature, microfluidic chips have become valuable tools for constructing biomimetic biophysical models to study physiological and pathological mechanisms, as well as to assess the effects of drugs on cellular kinetic behavior.[18–20] Bionic modeling in microfluidic chips has been a popular research area in recent years. Researchers have conducted related studies in various aspects, such as material selection, chip design, and biological function bionics,[21] and have made progresses in several directions of cancer research modeling. For instance, Nagaraju *et al.* developed a model consisting of tumor, matrix, and spontaneously formed vascular layers and found that the number of MDA-MB-231 cells invading the three-dimensional matrix was increased in the presence of the vascular network.[22] Jeon *et al.* constructed a breast cancer extravasation model and found that the introduction of the tumor cells into channels fused to the endothelial monolayer, endothelial cell permeability increased by 3.8-fold compared to the permeability before introduction.[23] Additionally, a co-culture model of breast cancer and patient-derived fibroblasts was used to study tumor-stroma interactions.[24] However, it is worth noting that few of these studies constructed early stage and late-stage cancer models for comparative analysis.

In this work, a random collagen fiber microenvironment (RFM) model and an oriented collagen fiber microenvironment (OFM) model resembling the migration of cancer cells in a tumor microenvironment of early stage/late-stage breast cancer patients (Fig. 1, upper) are first constructed by employing microfluidic chip technology, and the effect of the collagen fiber structure on the migration behavior of invasive breast cancer cells MDA-MB-231-RFP is studied. Subsequently, based on the biochemical gradient microenvironment observed in early stage/late-stage breast cancer chemotherapy patients (Fig. 1, lower), we utilize the unique design of the chip and the permeability of collagen proteins to establish the concentration gradient of paclitaxel, a commonly used anticancer drug, to investigate the migration behavior of cancer cells in the drug concentration gradient and explore the combined effects of collagen fiber structure and drug gradient on cancer cell migration within the tumor microenvironment. The similarity between our study and other research lies in the construction of tumor models with microfluidic chips. The difference is that the present study focuses on the construction of early stage and late-stage extracellular matrix microenvironments of breast cancer with random/oriented collagen fibers, and the construction of biomimetic biochemical concentration gradients by diffusion. Finally, our RFM and OFM models not only provide insights into cellular dynamics, but also offer guidance for clinical drug treatment strategies in cancer patients.

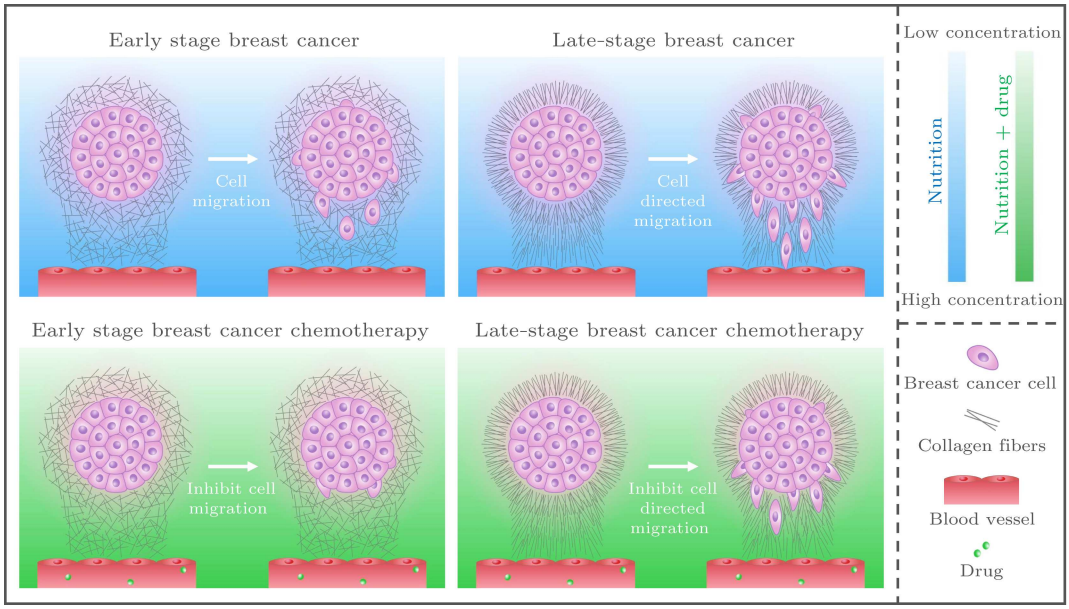


Fig. 1. Cancer cell migration in different stages of breast cancer. The migration process of cancer cells in the random/oriented collagen microenvironment of early stage/late-stage breast cancer patients (upper). The migration process of cancer cells in the biochemically gradient collagen microenvironment of early stage/late-stage breast cancer chemotherapy patients (lower).

2. Methods

2.1. Preparation of the RFM chip and the OFM chip

The chips of RFM and OFM were both fabricated using SU-8 photolithography and poly-dimethylsiloxane (PDMS) (Sylgard, 184 Dow Corning, USA) soft etching technology. Firstly, the structural diagrams of the RFM chip and OFM chip were separately designed using Auto CAD software (2016, Autodesk) and printed them to generate high-precision masks. Next, a smooth layer of SU-8 photoresist (Zhong xin qi heng, China) was spin-coated onto a 4-inch silicon wafer, with the spin coater set at 1000 rpm/min for 5 s. Subsequently, the

photoresist-coated silicon wafers were heated on a constant-temperature hotplate at 95 °C for 3 h, after completion, the mask and silicon wafer were aligned and subjected to UV lithography. Excess photoresist was removed by developing solution (Zhong xin qi heng, China), resulting in silicon molds for the RFM chip and OFM chip. Following that, PDMS was mixed with its curing agent at a ratio of 10 : 1 and poured onto the silicon molds, after vacuum deaeration, they were heated in a constant-temperature oven at 80 °C for 2 h. Once the PDMS chips cooled down to room temperature, they were peeled off from the silicon molds, cut, punched, and bonded to glass slides to make the RFM chip and OFM chip, which were cleaned with 75% ethanol and treated with UV sterilization before being prepared for use.

2.2. Preparation of collagen

The collagen used to mimic the ECM in this study was selected from mouse tail type-I collagen (354236, Corning, NY, USA) with a concentration of 10.21 mg/ml.[12,25] The collagen was adjusted to the concentration of 5.0 mg/ml and the pH of 7.2, using 10×PBS (Corning, NY, USA), sterile water, and 1 mol/L NaOH. The prepared collagen solution was kept in an ice bath for later use.

2.3. Culture and preparation of breast cells

The cell used in this study was invasive breast cancer cell line MDA-MB-231 labeled with red fluorescent protein (RFP), was obtained from China Infrastructure of Cell Line Resources (Beijing, China). MDA-MB-231-RFP cells were cultured in RPMI-1640 medium (Corning, NY, USA) supplemented with 10.0% fetal bovine serum (Gibco, NY, USA) and 1.0% penicillin/streptomycin (Corning, NY, USA), the cells were cultured in an incubator with 5.0% CO_2 at 37.0 °C. The medium was replaced every two days, and when the cell density reached 80% to 90%, the cell suspension with a density of 1.0×10^6 cells/ml was prepared using 0.25% trypsin/EDTA (Corning, NY, USA). To prepare the collagen solution with a concentration of 10.0 mg/ml according to the above-mentioned method of collagen blending, the collagen solution was mixed with the cell suspension in a 1 : 1 ratio (the final concentrations of cells and collagen were 5.0×10^5 cells/ml and 5.0 mg/ml, respectively).

3. Construction of RFM and OFM models

To investigate the impact of random collagen fiber structure in early stage breast cancer and oriented collagen fiber structure in late-stage breast cancer on the migration behavior of breast cancer cells, we designed and fabricated the RFM model and the OFM model, as depicted in Figs. 2(a1) and 2(a2). Both the models consisted of three parts: coverslip, PDMS chip, and medium pool. Among them, PDMS chips were designed with microfluidic channels and trapezoidal micropillars. Through the microfluidic channels, nutrients or drugs could be delivered to the breast cancer cells. The function of trapezoidal micropillars was to form a collagen microenvironment region in the middle, separating collagen microenvironment region and microfluidic channels. Specifically, the difference between the RFM model and the OFM model was that the OFM model contained the leaf-shaped micropillars for the formation of oriented collagen fibers, whereas the RFM model did not, creating the random collagen fiber microenvironment. Figure 2(b) illustrates the production process of the RFM and OFM models: (I) the RFM and OFM chips were prepared following the steps outlined in Subsection 2.1. (II) collagen solution was prepared using the method described in Subsection 2.2, and it was injected into the respective collagen microenvironment regions of the RFM and OFM chips at a rate of 250 ul/min by a syringe pump, then the RFM and OFM chips were placed in thermostat set at 37 °C and 20 °C, respectively, allowing the collagen to solidify after 1 h. (III) A mixture of collagen solution and cell suspension was prepared in the proportions specified in Subsection 2.3. This mixture was then injected into the RFM and OFM chips, followed by incubation at 37 °C and 20 °C, respectively, for 1 h to allow solidification. (IV) The medium or the medium with paclitaxel was introduced into the microfluidic channels and medium pool of both RFM and OFM chips, and was changed every 12 h. The migration behavior of the breast cells was observed or tracked using an inverted fluorescence microscope (Nikon, Ti-E) from 0 h to 72.0 h every 12 h. Shooting conditions: red fluorescence excitation channel, constant temperature 37.0 °C, carbon dioxide concentration 5.0%, exposure time 60.0 ms, contrast 180-1200. Each experiment was repeated three times, and the shooting conditions were consistent.

4. Results and discussion

4.1. Characterization of random and oriented collagen fibers

Researchers have shown that the transformation of collagen fibers from disordered to ordered morphology in breast cancer is regarded as an important indicator for assessing the stage and prognosis of breast cancer.[26–28] However, there are very few models available for studying the effects of disordered and ordered collagen fibers on the dynamical behavior of cancer cells. In order to investigate the influence of different collagen fiber structures on cancer cell migration behavior, we designed and fabricated the RFM and OFM models based on microfluidic chip. Both the models were used to create microenvironments with random collagen fibers and oriented collagen fibers similar to the clinical features, respectively. The detailed process is shown in Fig. 2.

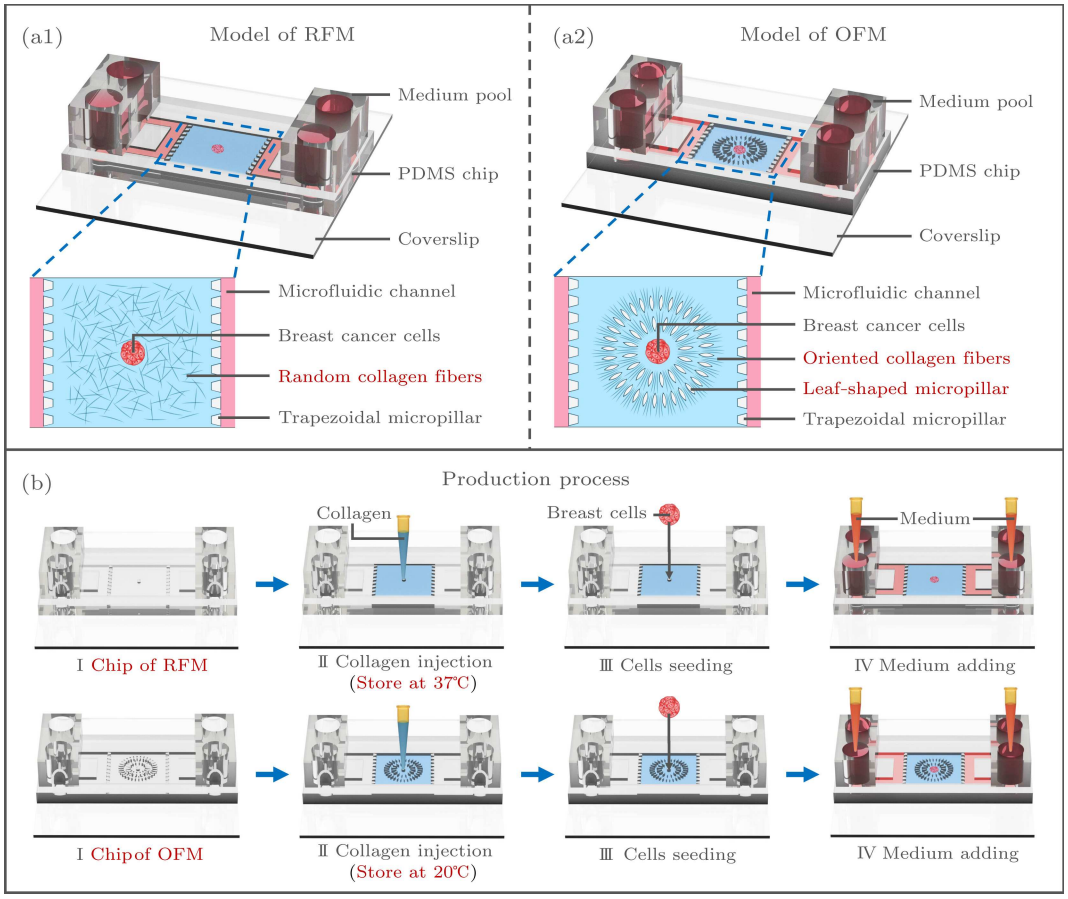


Fig. 2. Construction of the RFM and OFM models. [(a1), (a2)] Three-dimensional (3D) structural diagram and two-dimensional (2D) localized enlargement of the RFM and OFM models, respectively. (b) The production processes of the RFM and OFM models.

In the experiment, 5.0 mg/ml collagen solution was injected into both the RFM and OFM models, respectively. The reason for choosing 5.0 mg/ml collagen solution is that the collagen fiber density and orientation characteristics formed by this concentration of collagen solution are more similar to those in tissue sections, which can better simulate the *in vivo* microenvironment in which the cancer cells are located.[29] In the RFM model, there is no restriction of leaf-shaped micropillars within the collagen microenvironment region of the chip, and the collagen solution is able to diffuse and fill the entire collagen microenvironment region in an approximately steady-state manner, as shown in Fig. 3(a1). The collagen is not affected by stress and easily forms a random collagen fiber structure. Among them, Fig. 3(a1)a shows the second harmonic generation (SHG) imaging of random collagen fibers obtained through two-photon laser scanning microscopy, and the enlarged image is shown in Fig. 3(a1)b. In contrast, in the OFM model, the collagen microenvironment region of the chip had a circular array of leaf-shaped micropillars. After the collagen solution was injected, it was hindered by the leaf-shaped micropillar array. Due to the effects of the flow field and stress field in the gap between the leaf-shaped micropillars, an oriented arrangement of collagen fibers was formed, as shown in Fig. 3(a2)a, and Fig. 3(a2)b is an enlarged view of it. To explain the formation of random and oriented collagen fibers in RFM and OFM chips, we simulated the flow and stress fields inside the two chips using COMSOL (Multiphysics 5.3a, COMSOL, Sweden), respectively, as shown in Fig. S1 in the supplementary information, and the results are consistent with the above description.

In order to comparatively analyze the degree of collagen fiber alignment in the RFM and OFM models, we counted the angular distribution of collagen fibers at three locations of the chips. It is worth noting that the oriented collagen fibers in the OFM chip are emission-shaped, and the oriented collagen fiber images at three selected locations will be adjusted to the state shown in Fig. 3(a2)b. Following the analysis method reported in the literature, the alignment direction of collagen fibers was analyzed using curvelet-based alignment analysis software (University of Wisconsin at Madison, USA),[30,31] and the results are shown in Figs. 3(b1) and 3(b2). The comparative analysis shows that the angle distribution of collagen fibers in the RFM model is more uniform from 0° to 180°, while in the OFM model, the angle distribution of collagen fibers is more concentrated around 90°. To better illustrate that the RFM and OFM models can be used to simulate randomly and orientedly arranged collagen fibers in the early and late matrix, we statistically analyzed the angular distribution of collagen fibers in the acquired tissue sections and in the models, as shown in Fig. S2, which showed that the collagen fibers

in the early/late tissue sections and in the RFM/OFM models remained highly consistent. This indicates that the RFM and OFM models can be respectively used to construct microenvironments with random collagen fibers resembling early stage breast cancer and microenvironments with oriented collagen fibers resembling late-stage breast cancer, and to study the effects of different collagen fiber microenvironments on cancer cell dynamics.

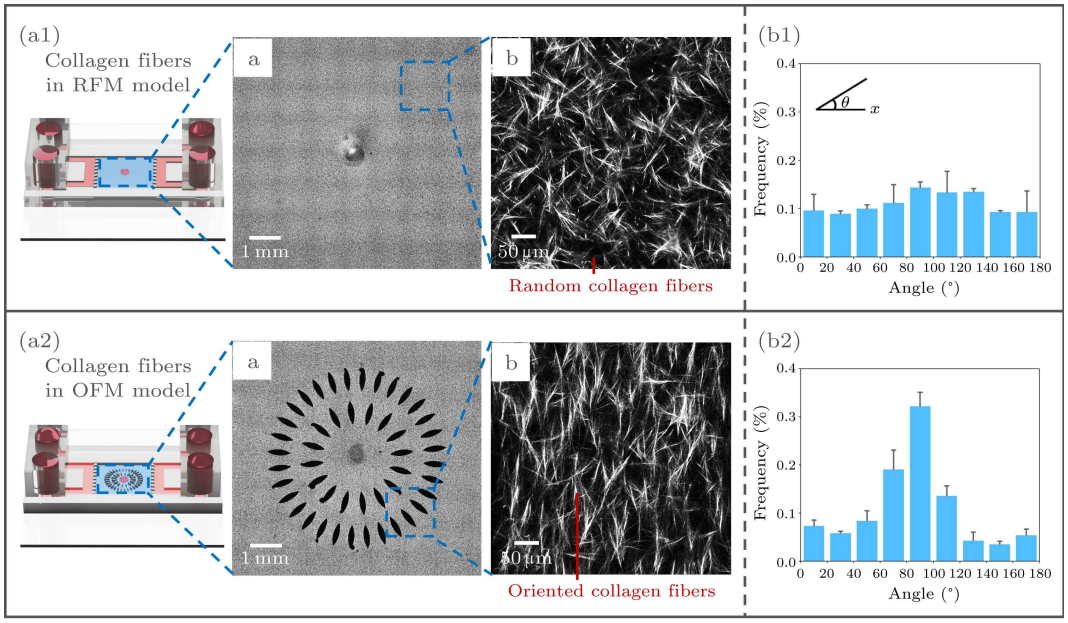


Fig. 3. Characterization of collagen fibers in the RFM and OFM models. (a1) Illustration of random collagen fibers in the RFM model (a) and local magnification (b). (a2) Illustration of oriented collagen fibers in the OFM model (a) and local magnification (b). [(b1), (b2)] Statistical analysis of the angle distribution of random and oriented collagen fibers, respectively.

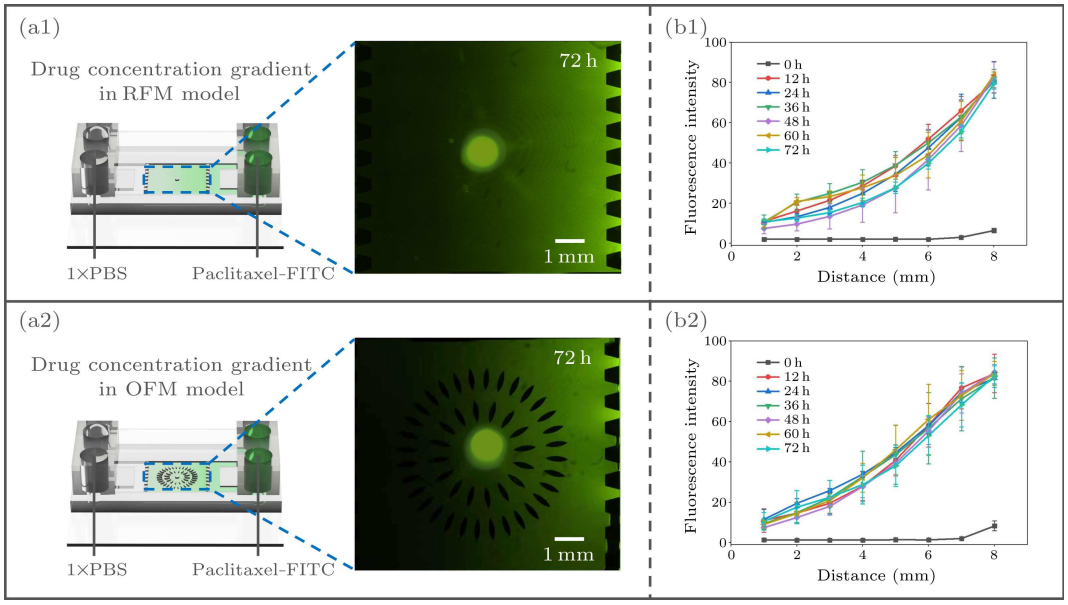


Fig. 4. Construction and characterization of the paclitaxel-FITC gradient. [(a1), (b1)] The construction of the paclitaxel-FITC gradient in the RFM model and statistical analysis of their variations over time and space. [(a2), (b2)] The construction of the paclitaxel-FITC gradient in the OFM model and statistical analysis of their variations over time and space.

4.2. Construction and validation of biochemical gradients

The RFM and OFM models can not only mimic the collagen fiber microenvironment in breast cancer, but also construct the biomimetic biochemical gradient microenvironment similarly *in vivo*, which is used to study the combined effects of collagen fiber structure and biochemical gradient on cancer cell dynamics. In this study, collagen was used to construct the matrix microenvironment for cell growth. Due to its porous structure, collagen protein facilitates the stable diffusion of small molecule compounds.[32,33] Therefore, we expect to construct biomimetic biochemical gradient microenvironments in the RFM and OFM models, which can also serve as platforms for drug testing and screening.

In order to verify that the stable biochemical concentra-

tion gradient can be constructed in the RFM and OFM models, we chose paclitaxel-FITC (green) for diffusion experiments to visualize the biochemical concentration gradient. Paclitaxel is known to significantly inhibit cancer cell proliferation, migration, and invasion, making it a commonly used drug in clinical breast cancer treatment.[34–36] In this study, paclitaxel will be used to test the feasibility of RFM and OFM models as a drug detection platform. In the experiments, we labeled paclitaxel as paclitaxel-FITC, and the solution of paclitaxel-FITC with a concentration of 80.00 nM were loaded into the microfluidic channels on the right side of the chip in the RFM model and the OFM model, the left side of the chip were loaded with 1×PBS (Corning, NY, USA), and observed under an inverted fluorescence microscope (Ti-E, Nikon, Japan). It was found that paclitaxel-FITC would gradually diffuse to the left side to form a stable concentration gradient, i.e., the concentration of paclitaxel-FITC was higher closer to the right channel, as shown in Figs. 4(a1) and 4(a2). It is noteworthy that the concentration gradients generated in the RFM and OFM models were very similar, and unaffected by collagen fiber alignment.

Furthermore, we utilize MATLAB software (MathWorks, USA) to perform quantitative analysis of the fluorescence intensity changes of paclitaxel-FITC over time and space, as shown in Figs. 4(b1) and 4(b2). The results show that the fluorescence intensity of paclitaxel-FITC at a specific position is proportional to the distance from that position to the right channel. As time progresses, the biochemical gradient will approach a stable state. From the analysis plots, it can be observed that the concentration gradients of paclitaxel-FITC in both the RFM and OFM models have reached a stable state within 12.0 h. Therefore, the model constructed in this study is not only able to simulate the cancer bionic microenvironment, but also can be used as a tool for preliminary drug detection or screening.

4.3. Combined effect of collagen fiber structure and biochemical gradient on the migration of breast cancer cells

After confirming that the RFM and OFM models can construct random collagen fiber structures and oriented collagen fiber structures, and the corresponding biochemical gradients, respectively, we further explored the migration behaviors of breast cancer cells in these bionic microenvironments. Firstly, the RFM and OFM models were fabricated according to the procedure in Fig. 2(b), respectively. Subsequently, the RFM or OFM chips loaded with culture medium were placed in an inverted fluorescence microscope containing a live cell device, and the migration of breast cancer MDA-MB-231-RFP cells was tracked. The results are shown in Fig. 5(a1), indicating that after 72.0 h of incubation, MDA-MB-231-RFP cells migrated in both the RFM and OFM chips. During all experiments, there were no differences in breast cancer cell lines, collagen solution, and the transport of oxygen and nutrients. In our chips, the oxygen and nutrients both gradually diffuse with lower oxygen and nutrient concentrations in the center, and similar to the tumor environment. However, notably the migration behavior of MDA-MB-231-RFP cells in the OFM chip was stronger compared to that in the RFM chip. This observation indicates that the oriented collagen fiber structure influences cancer cell migration and acts as a stronger facilitator in this process.

Furthermore, to quantitatively analyze the impact of collagen fiber structure on cancer cell migration, we performed fluorescence intensity analysis on the acquired cell fluorescence images using MATLAB software. According to the existing studies, fluorescence intensity serves as a reasonable indicator of cell quantity,[37,38] which shows that the statistical analysis based on fluorescence intensity is reasonable in this study. For the fluorescence intensity analysis of MDA-MB-231-RFP cells, first the cellular fluorescence images were divided into six subregions, as depicted in Fig. 5(a1) for the RFM model. These sub-regions represented angles of 60°, 120°, 180°, 240°, 300°, and 360°, respectively. Next, the boundary positions of the cell clusters at 0 h were identified. Finally, we measured the distance from each cell outside the boundary to the boundary position at 72 h, which represents the migration distance of MDA-MB-231-RFP cells, as shown in Fig. S3. According to this method, we statistically analyzed the migration of MDA-MB-231-RFP cells in both the RFM and OFM models, and three experiments were performed for each model. The results are shown in Fig. 5(b1), the migration behavior of cells in each subregion in the OFM model was stronger than that in the RFM model, and the differences were statistically significant. These findings align with the observations in Fig. 5(a1), indicating that the presence of oriented collagen fiber structures in the OFM model promotes cancer cell migration, which is consistent with the notion that oriented collagen fibers in late-stage breast cancer patients contribute to cell migration. The biological mechanisms of the differential cell migration between the random and oriented collagen fiber are complex, and the reason may be the mechanical information transmission caused by the contact of collagen fibers,[30] or the different arrangements of collagen fibers led to differences in the energy consumption of breast cancer cells. In future studies, we will further investigate the deep-seated reasons for the different migration of cells in the two types of microenvironments.

Early stage and late-stage cancer patients take some anticancer drugs during chemotherapy, the efficacy of these drugs can vary among patients at different stages, and this difference may be related to collagen fiber structures. In light of these questions, we designed the following experiments to investigate the migration of breast cancer cells under a con-

centration gradient of paclitaxel in random collagen fiber microenvironment (early stage breast cancer) and oriented collagen fiber microenvironment (advanced stage breast cancer), respectively. Specifically, RFM chips and OFM chips were prepared according to Fig. 2(b), and medium was loaded in both of their left channels, and medium + paclitaxel (PTX) with a concentration of 80.00 nM was loaded in both of their right channels, as shown in Fig. 5(a2). In comparison with Fig. 5(a1), it can be observed that paclitaxel concentration gradient had an inhibitory effect on the migration of MDA-MB-231-RFP cells in both the RFM and OFM models, and the inhibitory effect on cell migration was significantly stronger on the closer to the right channel than on the left side. Further, the impact of paclitaxel concentration gradient on the migration of breast cancer MDA-MB-231-RFP cells in both the RFM and OFM models were quantitatively analyzed by the same method shown in Fig. S1, and each model was tested three times. The results shown in Fig. 5(b2) indicate that the migration of MDA-MB-231-RFP cells was inhibited in each subregion in the RFM model, with no significant inhibition of cell migration in the subregion 180°, significant inhibition of cell migration in the subregion 240°, and extremely significant inhibition of cell migration in subregions 60°, 120°, 300°, and 360°. This suggests that the higher concentrations of paclitaxel in the RFM model lead to more pronounced inhibition of MDA-MB-231-RFP cell migration. In the OFM model, the migration of MDA-MB-231-RFP cells was also inhibited in each sub-region, but the inhibition is not significant in any sub-region. In summary, the inhibitory effect of paclitaxel concentration gradient on breast cancer cell migration in the RFM model was significantly greater than that in the OFM model, i.e., paclitaxel had a weaker inhibitory effect on breast cancer cell migration in the oriented collagen fiber microenvironment.

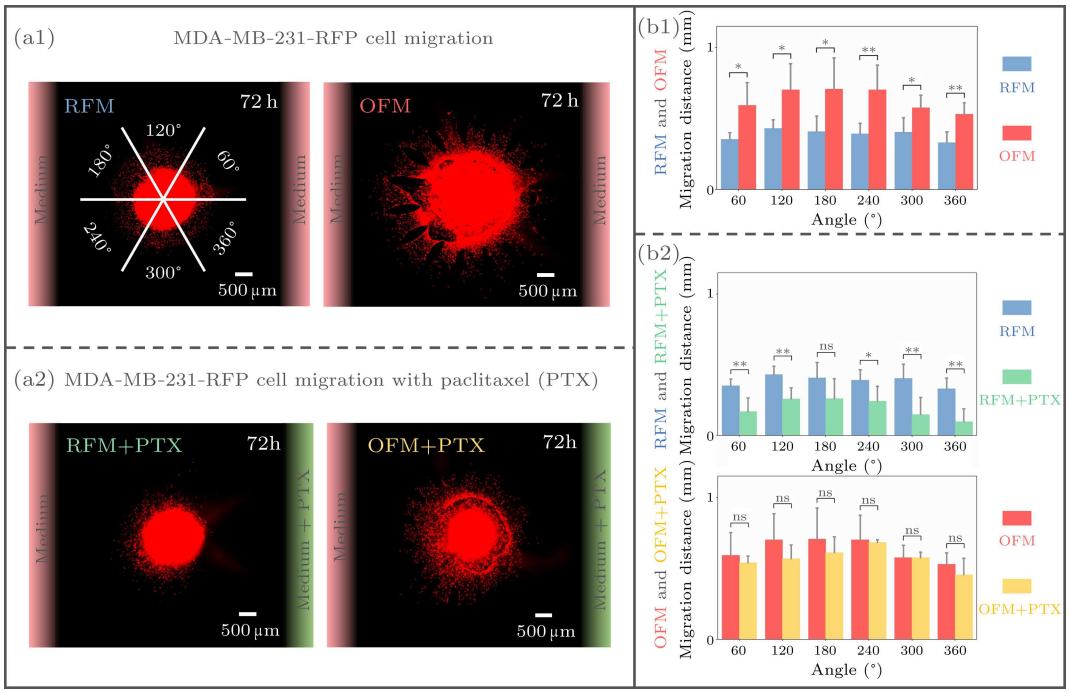


Fig. 5. Breast cancer MDA-MB-231-RFP cells (red) migration in complex biomimetic microenvironments. [(a1), (b1)] Comparative analysis of MDA-MB-231-RFP cells migration in the RFM and OFM models. (a2) The migration of MDA-MB-231-RFP cells under paclitaxel concentration gradient in the RFM and OFM models, respectively. (b2) Statistical and comparative analysis of MDA-MB-231-RFP cells migration in RFM and OFM models before and after the addition of paclitaxel gradient.

5. Conclusions

Based on the random and oriented collagen fiber microenvironments within the bodies of early stage and late-stage cancer patients, we have employed microfluidic chip technology to construct RFM and OFM models. Both the models facilitate the controlled formation of random/oriented collagen fiber structures *in vitro*, allowing for the investigation of the impact of such structures on the migration behavior of breast cancer MDA-MB-231-RFP cells. The results indicate that the oriented collagen fiber structure is more conducive to cancer cell migration. Additionally, taking advantage of collagen protein's ability to permeate small molecules, biomimetic biochemical gradient microenvironments are created in both the RFM and OFM models. The migration behavior of MDA-MB-231-RFP cells under a concentration gradient of the anticancer drug paclitaxel is studied. The results demonstrate that the concentration gradient of paclitaxel inhibited cancer cell migration in both the models but the inhibitory effect is significantly stronger in the RFM model compared to the OFM model. These findings suggest that the poor efficacy of chemotherapy in late-stage cancer patients may be closely

associated with the oriented collagen fiber microenvironment within the body. In the future, it may be possible to enhance chemotherapy effectiveness by modifying the microenvironment within the patient's body. Furthermore, by optimizing parameters of the RFM and OFM models and combining them with organoid technology, the models can be utilized for drug testing, personalized diagnosis, and treatment.

Acknowledgments

We gratefully acknowledge support from the National Natural Science Foundation of China (Grant Nos. 11974066, 12174041, 12104134, T2350007, and 12347178) and the Fundamental and Advanced Research Program of Chongqing (Grant No. cstc2019jcyj-msxmX0477), and the Natural Science Foundation of Chongqing (Grant No. CSTB2022NSCQ-MSX1260), the Science and Technology Research Program of Chongqing Municipal Education Commission (Grant No. KJQN202301333), the Scientific Research Fund of Chongqing University of Arts and Sciences (Grant Nos. R2023HH03 and P2022HH05), College Students' Innovation and Entrepreneurship Training Program of Chongqing Municipal (Grant No. S202310642002). We would like to thank Miss Qin Deng at the Analytical and Testing Center of Chongqing University for her assistance with immunofluorescence imaging and analysis.

References

[1] Spill F, Reynolds D S, Kamm R D and Zaman M H 2016 *Curr. Opin. Biotech.* **40** 41
[2] Klein-Goldberg A, Maman S and Witz I P 2014 *Cancer Lett.* **352** 54
[3] Anderson N M and Simon M C 2020 *Curr. Biol.* **30** R905
[4] Gordon-Weeks A and Yuzhalin A E 2020 *Cancers* **12** 3331
[5] Patwardhan S, Mahadik P, Shetty O and Sen S 2021 *Biomaterials* **279** 121185
[6] Vasudevan J, Jiang K, Fernandez J G and PhD C T L 2023 *Acta Biomater.* **163** 351
[7] Sun X, Wu B, Chiang H C, Deng H, Zhang X, Xiong W, Liu J, Rozeboom A M, Harris B T, Blommaert E, Gomez A, Garcia R E, Zhou Y, Mitra P, Prevost M, Zhang D, Banik D, Isaacs C, Berry D, Lai C, Chaldekas K, Latham P S, Brantner C A, Popratiloff A, Jin V X, Zhang N, Hu Y, Pujana M A, Curiel T J, An Z and Li R 2021 *Nature* **599** 673
[8] Riching K M, Cox B L, Salick M R, Pehlke C, Riching A S, Ponik S M, Bass B R, Crone W C, Jiang Y, Weaver A M, Eliceiri K W and Keely P J 2014 *Biophys. J* **107** 2546
[9] Tomko L A, Hill R C, Barrett A, Szulczewski J M, Conklin M W, Eliceiri K W, Keely P J, Hansen K C and Ponik S M 2018 *Sci. Rep.* **8** 12941
[10] Conklin M W, Eickhoff J C, Riching K M, Pehlke C A, Eliceiri K W, Provenzano P P, Friedl A and Keely P J 2011 *Am. J. Pathol.* **178** 1221
[11] Locker J and Segall J E 2011 *Am. J Pathol.* **178** 966
[12] Li G, Liu Y, Yao J, Song K, Wang G, Zhou L, Chen G and Liu L 2022 *Chin. Phys. B* **31** 028703
[13] Uzel S G M, Amadi O C, Pearl T M, Lee R T, So P T C and Kamm R D 2016 *Small* **12** 612
[14] Shimizu A, Goh W H, Itai S, Karyappa R, Hashimoto M and Onoe H 2020 *Biomicrofluidics* **14** 044106
[15] Morrow C M, Mukherjee A, Traore M A, Leaman E J, Kim A, Smith E M, Nain A S and Behkam B 2019 *Lab Chip* **19** 3641
[16] Tong X, Jiang J, Zhu D and Yang F 2016 *ACS Biomater. Sci. Eng.* **2** 845
[17] Shen S, Zhang F, Gao M and Niu Y 2020 *Micromachines* **11** 493
[18] Tian C, Tu Q, Liu W and Wang J 2019 *Trend. Anal. Chem.* **117** 146
[19] Mao M, He J, Lu Y, Li X, Li T, Zhou W and Li D 2018 *Biofabrication* **10** 025008
[20] Zhang X, Karim M, Hasan M M, Hooper J, Wahab R, Roy S and Al-Hilal T A 2022 *Cancers* **14** 648
[21] Liu Y, Liu R, Liu H, Lyu T, Chen K, Jin K and Tian Y 2023 *Analyst* **148** 5822
[22] Nagaraju S, Truong D, Mouneimne G and Nikkhah M 2018 *Adv. Health. Mater.* **7** 1701257
[23] Jeon J S, Zervantonakis I K, Chung S, Kamm R D and Charest J L 2013 *PLoS One* **8** e56910
[24] Truong D D, Kratz A, Park J G, Barrientos E S, Saini H, Nguyen T, Pockaj B, Mouneimne G, LaBaer J and Nikkhah M 2019 *Cancer Res.* **79** 3139
[25] Graf F, Horn P, Ho A D, Boutros M and Maercker C 2021 *FASEB J.* **35** e21692
[26] Gurrala R, Byrne C E, Brown L M, Tiongco R F P, Matossian M D, Savoie J J, Collins-Burow B M, Burow M E, Martin E C and Lau F H 2021 *Front. Bioeng. Biotech.* **9** 618448
[27] Liu C, Chiang B, Mejia D L, Luker K E, Luker G D and Lee A 2019 *Acta Biomater.* **83** 221
[28] Li H, Bera K, Toro P, Fu P, Zhang Z, Lu C, Feldman M, Ganesan S, Goldstein L J, Davidson N E, Glasgow A, Harbhajanka A, Gilmore H and Madabhushi A 2021 *npj Breast Cancer* **7** 104
[29] Yao J, Li G, Jiao Y, Zheng Y, Liu Y, Wang G, Zhou L, Zhang H, Zhang X, Shuai J, Fan Q, Ye F, Lou S, Chen G, Song K, Liao Y and Liu L 2021 *Lab Chip* **21** 3004
[30] Han W, Chen S, Yuan W, Fan Q, Tian J, Wang X, Chen L, Zhang X, Wei W, Liu R, Qu J, Jiao Y, Austin R H and Liu L 2016 *Proc. Natl. Acad. Sci. USA* **113** 11208
[31] Hai M, Liu Y, Xiong L, Li G, Wang G, Zhang H, Shuai J, Chen G and Liu L 2020 *Chin. Phys. B* **29** 098702
[32] Aizel K, Clark A G, Simon A, Geraldo S, Funfak A, Vargas P, Bibette J, Vignjevic D M and Bremond N 2017 *Lab Chip* **17** 3851
[33] Liu H, Wang Y, Cui K, Guo Y, Zhang X and Qin J 2019 *Adv. Mater.* **31** 1902042
[34] Patt D, Gauthier M and Giordano S 2006 *Womens Health* **2** 11
[35] Samaan T M A, Samec M, Liskova A, Kubatka P and Büsselberg D 2019 *Biomolecules* **9** 789
[36] Murray S, Briasoulis E, Linardou H, Bafaloukos D and Papadimitriou C 2012 *Cancer Treat. Rev.* **38** 890
[37] Krtolica A, Solorzano C O, Lockett S and Campisi J 2002 *Cytometry* **49** 73
[38] Caffrey T A M, Agarwal L A and Weksler B B 1988 *In Vitro. Cell. Dev.* **24** 247