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PERSONAL VIEW

Generation of microfluidic gradients and their effects on cells behaviours

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Introduction

The concept of "gradients" has been widely demonstrated and applicated in biology. For example, concentration gradients and potential gradients in the body can regulate the homeostasis as well as the balance of physiological environment; oxygen gradients play a vital role in cellular gene expression and migration. Moreover, biomolecular gradient guidance is also very important in biological processes such as embryonic development, cancer spread, immune response, wound healing and osteogenesis [1]. These biological processes have been studied in vitro to explore various cellular behaviours under molecular gradients, such as cell proliferation, migration, differentiation and adsorption [2]. The importance of biomolecular gradients in regulating cells behaviours leads to the development of multiple methods for the generation of chemical gradients in vitro by performing them in vivo studies. It has been revealed that gradient signal transmission is a complex and highly regulated process since the cellular response depends on the gradient concentration and its temporal and spatial characteristics [3].

As early as 1977, Sally Zigmond proposed the "Zigmond chamber". In this traditional chemotaxis assay system, it can be observed that cells tend to migrate towards a source chemoattractant at a narrow constriction (3–10 micrometres). Based on this research, biologists subsequently developed the "Dunn chamber" and "Insall chamber". These advances had greatly improved the stable gradient, high resolution and long-term imaging capabilities of the traditional visual chemotaxis assays [4]. However, compared with microfluidic technology, the traditional methods of gradient-generating in vitro are laborious and limited. Besides, it is difficult to accurately control the diffusion of molecules and maintain it in a relatively stable state in time and space by traditional methods.

Microfluidic technology has offered great potential for next generation of chemotaxis assays, it can generate highly dynamic, controllable and micron-level gradients in a small integrated chip which exposes cells into one or more custom-made spatial–temporal biomolecular gradients. This new method shows a great advance in many fields: (1) the microfluidic multi-channel environments are relatively independent of each other, and they can match the cell in sizes; (2) the transfer of mass and heat in microfluidic channels is very fast; (3) the multiple operating units in the microfluidic platform can be flexibly combined and processed in a large number of tests in parallel, which can meet the requirements of high-throughput analysis [5]. Therefore, studying the behaviours and chemotaxis of the cells by microfluidic platforms will bring a revolution to biological gradients research. In this context, the relationship between the generation of microfluidic gradients and their effects on cells behaviours should be revealed.

Generation of microfluidic gradients

The most common method is the use of microfluidic gradient generators, which can generate various gradients in solutions or on surfaces. Researchers designed a variety of microfluidic chips that can generate a series of gradients in a solution, such as the "Christmas Tree" microfluidic chips and the multi-channel microfluidic device. The "Christmas

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Tree" microfluidic chip is named after its shape. As shown in Fig. 1a, the single unit has two inlets, a gradient generator and six screening chambers. Six identical tree-like units constitute an integrated microfluidic screening chip, which can perform multi-unit screening of target molecules by integrated injection pathways without complicated control accessories and pipelines [6]. The multi-channel microfluidic device is relatively simple, as shown in Fig. 1b, and the outer channels contain chemical attractant solution and buffer solution, thereby establishing a chemoattractant gradient in the central channel to achieve nanoparticle sorting [7]. Because of the effects caused by physicochemical properties of the substrate on cell behaviour, researchers also studied the generation of physicochemical gradients based on the surface of substrates [2, 8]. The combination of microfluidics and photopolymerization can effectively and controllably produce gradient compliance distribution substrates in microscale [9].

Microfluidic gradient generators mainly based on convection mixing [10], laminar flow diffusion [11], static diffusion [12] and geometric metering mixing [13]. The microfluidic generator can precisely control the formation, direction and cell microenvironment of the gradient and show less effect by shear stress, which has been demonstrated to be a good platform for biological research.

Effects of microfluidic gradients on cells behaviours

Chemotaxis is the basic process of cell and microbial biochemical signalling and migration [14]. To our knowledge, cells are stimulated by their biochemical microenvironment all the time. These biochemical signals affect cells behaviours and functions during the organismic development and tissue regeneration [15]. In general, the expression of these signals that control organismic development and tissue regeneration is complex and usually involves biological gradients. Research shows that these signal gradients can regulate cells behaviours and functions. Therefore, precisely controlling gradients in temporal and spatial is important during the research of cells behaviours. Complex gradient signal regulation is used to control cells attachment, growth, differentiation and migration during the formation of tissue (such as the formation of cartilage and osteogenesis, neurogenesis, angiogenesis and wound healing), the occurrence and spread of cancer and bacterial chemotaxis. Gradient area control of cells behaviours and functions based on the microfluidic platform is of great significance for understanding the process of biological tissue regeneration and the design of cell-based therapy in the area of regenerative medicine.

Effects of gradients on cells behaviours in bone tissue engineering

Tissue engineering is a promising technology for the repair of damaged tissues/organs and the reconstruction of physiological functions. Hydrogel, a three-dimensional insoluble hydrophilic polymer network formed by the crosslink of hydrophilic homopolymers, copolymers or macromers, shows unique biocompatibility and ideal physical properties [16]. Therefore, incorporating signal gradients into hydrogel scaffolds is widely used for studying the effect of the

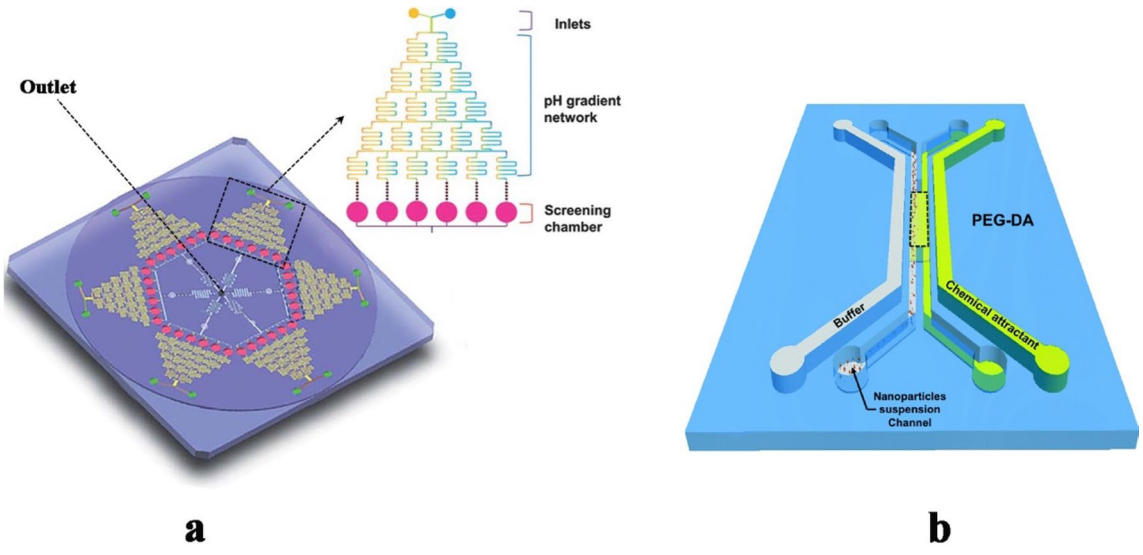

Fig. 1 Examples of microfluidic gradient generators. **a** "Christmas Tree" microfluidic chips. Reproduced with permission from [6]. **b** Multi-channel microfluidic device. Reproduced with permission from [7]

gradients on cells behaviours and promoting the engineering of biomimetically functional tissues. A potential application for studying the spatiotemporal gradients of biological materials is the regeneration of the interface between bone and cartilage in the area of bone tissue engineering [15]. An advantageous method is to use a microfluidic technology platform to achieve the formation of a growth-factor gradient in the hydrogel. Also, by using microfluidic technology we can observe the behaviour and proliferation of the cells, such as a gradual biomimetic interface between bone and cartilage.

Movilla et al. diffused growth factors in a hydrogel to form a chemical gradient of growth factors by a microfluidic gradient generator based on static diffusion. The results showed that the migration of osteoblasts was affected by the characteristics of the hydrogels and the gradients of growth factors [17]. Shi et al. reported a microfluidic gradient generator formed by a polydimethylsiloxane (PDMS) pool containing a hydrogel cultured with adipose-derived mesenchymal stem cells (ADMSCs) and a microchannel PDMS sheet covered with a polycarbonate microporous membrane. This generator formed a gradient at the transition interface of bone and cartilage in the hydrogel plate. Finally, the cells gradually changed from osteoblasts to mineralized chondrocytes at first and to chondrocytes, finally, reflected the gradient of cell differentiation (Fig. 2a) [18].

Effects of microfluidic gradients on tumour cells behaviours

Tumour metastasis is one of the most important biological behaviours of malignant tumours, influencing the success of the treatment of tumour patients. Invasion of the extracellular matrix by tumour cells is a key step in the process of metastasis. During this process, cells are regulated by a variety of factors. One of the most important factors is the specific chemokine gradients that can direct the movement of cells [19]. How to better quantify the migratory capability of cancer cells had attracted a lot of attention. Chaw et al. separated the parallel microchannels in a microfluidic chip by a series of microcolumns that were filled with matrigel with a 15 micron-wide micro-gap. This chip could generate a biophysical barrier for migrating cells and guide the movement of the migrated cancer cells through a serum concentration gradient because of the serum diffusion in matrigel [20]. Zou et al. detected the chemotactic migration behaviour of a single cancer cell in real time under multiple serum gradients [21].

Oxygen is a key substance for human metabolism, and it involves the proliferation and migration of cells, the angiogenesis and tissue repairing. The tumour grows rapidly, leading to the lack of enough vessels that transport nutrients and oxygen in the tumour location and then causing the cancer cells to adapt to the hypoxic environment and

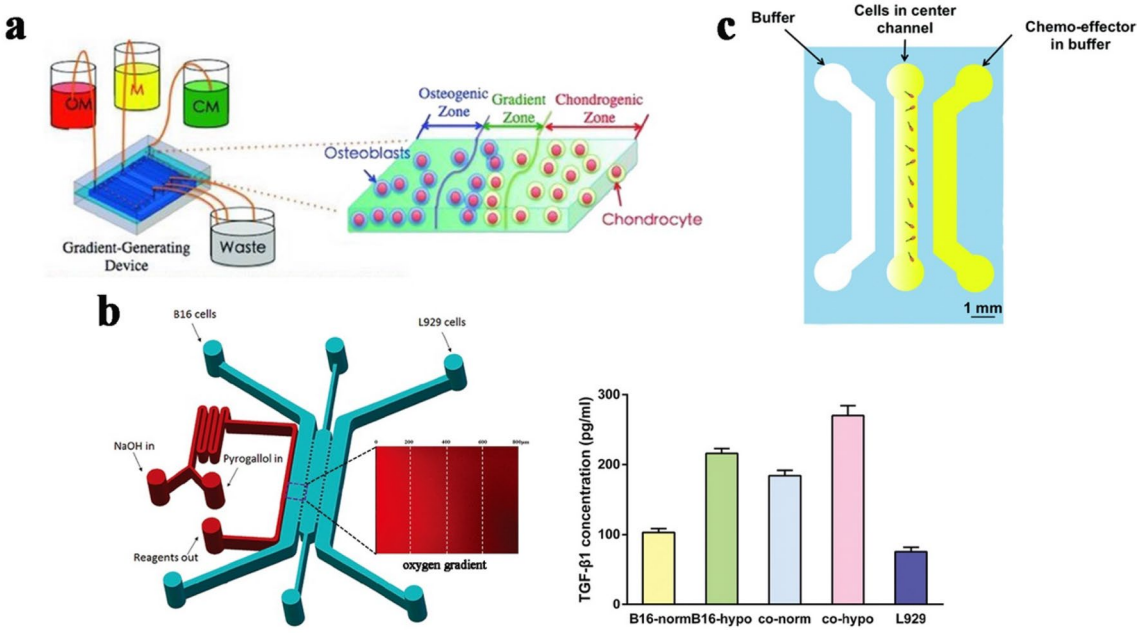

Fig. 2 Schematic illustration of the effects of microfluidic gradients on cell behaviours. **a** Formation of osteogenesis/chondrogenesis gradient zone on the hydrogel slab by a microfluidic device. Reproduced with permission from [18]. **b** Interaction study of cancer cells and fibroblasts on a microfluidic oxygen gradient. Reproduced with permission from [22]. **c** High-throughput sorting of nanoparticles in microfluidic gradient. Reproduced with permission from [7]

proliferate under hypoxic conditions [22]. The interaction of light and cells induces the generation of reactive oxygen species (ROS) in the cells, which will affect the migration behaviour of the cells. Therefore, it is essential to study the response of tumour cells under hypoxic conditions and their phototaxis. Sun et al. built a model of the interaction between cancer cells and fibroblasts in an oxygen gradient environment. They found that the co-culture of cancer cells and fibroblasts in hypoxia could accelerate the migration of cancer cells and the hypoxic cancer cells are more active and aggressive than normoxic fibroblasts (Fig. 2b) [22]. Lin et al. developed a microfluidic chip used for culturing cells and then illuminated them with blue light-emitting diodes to generate a blue light gradient for guiding the cell migration. Their research proposed a new strategy to reveal the phototaxis behaviours of cancer cells [23].

Chemotaxis of bacteria in microfluidic gradients

Bacteria are usually able to sense to chemical gradients and move towards the area with higher concentrations of favourable chemistry or lower concentrations of unfavourable chemistry [24]. Comparing with the conventional chemotaxis of adherent cells, the chemotaxis of free bacteria is more widely studied by using microfluidic technology because of its spatial and temporal space controllability and high throughput (for example, drug screening). In 2005, DiLuzio et al. produced PDMS sheets with microchannels based on soft lithography and seeded *E. coli* in the microchannels to limit their motion in a range of two-dimensional space. The movement of *E. coli* showed clear chemotaxis; moreover, the material gradient has a guiding effect on the movement of bacteria [25]. Suh et al. used a diffusion-based microfluidic platform to assemble particles on the surfaces of motile *E. coli* and achieved high-throughput sorting of nanoparticles relied on their chemotaxis. After 45 min, the bacteria migrated along the concentration gradient of chemical attractant and separated from the freely diffusing particles (Fig. 2c) [7].

Other applications of microfluidic gradients

In addition to bone tissue engineering, microfluidic gradients have very important applications in neurogenesis, vascular tissue and other cell biology fields. In research on Parkinson's disease, biologists created an in vitro model of Parkinson's disease based on the high-throughput cytotoxicity assay of microfluidic technology. Studies have found that in the concentration gradient channel of the neurotoxic agent 6-hydroxydopamine, neurons show graded death [26]. We have discussed chemical and biological gradients a lot in the previous section. However, the physical gradient of biological materials is another important factor affecting cell behaviour, especially cell migration and proliferation, such as the stiffness of the material. Generally, neurons are more suitable for soft materials with Young's modulus of 50 Pa; smooth muscle cells prefer stiff materials with Young's modulus of 8–10 kPa [9]. Combining photopolymerization and patterning technology, Wong et al. found that vascular smooth muscle cells migrated from the soft area to stiff area on the radial gradient compliant substrate [27].

Expectations for chemotaxis of microfluidic technology

Owing to the advantages of microfluidic technology on integrated miniaturization, high throughput, low cost and precise control in conditions, this technology was used to handle many challenges of chemotaxis in biomaterials. This technology provides a quick and precise change on the conditions around the moving cells, helping us to better understand the system mechanism of cells behaviours in response to chemical gradients. By the combining of the mathematical model and a simple T-shaped microfluidic device that can generate a stable chemical gradient, this system can be used for accurately observing the behaviours of a single cell and its chemotactic sensitivity coefficient in response to chemical gradients [28]. In the future, the challenge of more complex cell chemotaxis requires the skilled usage of mathematical and physical models. Hence, we should combine the new microfluidic technologies and traditional molecular tools, develop spatiotemporal control of cells environment and integrate new molecular biological technologies. We believe that by the revealing of the relationship between gradients and cells behaviours, we will obtain an important scientific basis for chemotaxis research.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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

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