Design Artificial Stem Cell Nests for Stem Cell Niche in a Microfluidic Petri Dish Programmed by a Cell Phone

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This article describes a technology to transform a petri dish into a microfluidic chip with a stem cell nest for stem cell niche engineering. A permanent magnet sphere is put in a 30 mm petri dish as an oscillator pump (O-pump). An earphone-sized actuator outside the petri dish receives the programmed audio signal from an MP3 player or a mobile phone to drive the in-dish O-pump for a microflow. There are guiding walls to form a wake area for the cell nest at the dish’s center. The cell nest uses the wake to create a stable, ultraslow internal microcirculation flow. The retention half-life of nanoparticles in the cell nest is 1419.8 ± 1.3 s. By setting the microfluidic program and supplementing five drops of the culture medium outside the cell nest every 2 d, the growth rate of embryonic stem cells in the cell nest during the 8 d automatic culture appears significantly optimized. The single clone area of embryonic stem cells increases from (3.57 ± 0.52) × 10^5 to (11.67 ± 1.33) × 10^5 µm^2 after 2 d of the automatic culture and advances to (53.34 ± 8.37) × 10^5 µm^2 after 4 d.

1. Introduction

The chemical and life processes on the earth are all carried out based on fluids. The human body is a 3D fluid system with interactions among 50 trillion cells. It concludes fluid transport, molecular diffusion, and nanoparticle transport, such as the nanoflow of the lymphatic system[1] or exosomes’ release.[2] We need to simulate the human body’s life process on a full scale (body-on-a-chip)[3] before fully understanding the life system. However, the ideal body-on-a-chip does not exist because the current microfluidic technology has many disadvantages. For example, the price of microfluidic chips and related equipment is exceptionally high and challenging to use. As a result, biologists still use petri dishes without microfluidic control. The petri dish is just a container with a lid, which is easy to operate and has a low cost. Building a microfluidic chip in a petri dish will solve cost and ease of use issues.[4] Besides that, microfluidic technology faces a core problem: niche biological question.[5] To solve the question, we must consider the following technical details: the local circulation; the speed control; the transportation of molecular, granular, and nanomaterials; microfluidic programming technology; the chemical and physical interference. As fluid driving technology is indispensable, the key to the question is how to get an ideal micropump. The current micropump technology either fails to meet the microfluidic control requirements or interferes with the system. For example, the latest technology of constructing microfluidic structures in petri dishes[5] still uses a syringe drive. The same is true for other applications (white adipose tissue-on-a-chip,[6] metastasis-on-a-chip,[7] tumor cell separation,[8] blood-vessel-on-a-chip,[9] bone marrow structure analysis,[10] and double syringe perfusion for circulating tumor cells[11]). The syringe perfusion takes away the molecules around the cell. The large volume of the syringe dilutes the signal molecule, modifying the biological niche.

Although there are many kinds of driving technologies for microfluidics, if these technologies are adopted, the niche biological questions will likely not be solved while losing the cost and convenience. For example, gravity[12] or vacuum pump[13–15] lose precise flow control (liver-on-chip). Diaphragm pumps rely on deformable materials, which are imperfect optically and in biocompatibility (liver spheroids-on-a-chip for the insulin-regulated glucose model[16] or drug retention studies[17]). Ferrofluid,[18] electroosmotic,[19] or electrochemical[20] pumps do not avoid the interference of ferrofluids, voltage, and current on the system. The acoustic wave-based pump[21,22] has high-energy sound waves that may interfere with the niche. These technologies cannot solve niche biological questions.

However, biologists cannot wait; they need to pay close attention to niche biological questions. The hematopoietic stem cell niche promotes metastasis.[23] Stem cells accelerate tumor progression[24] while microenvironmental changes attract immune cells and stem cells.[25] There are studies about hematopoietic stem cell niche for transplantation or therapies[26] cancer stem cell niche for cancer treatment,[27,28] bone marrow
niche for leukemia;[29] mesenchymal stem cell niche for periodontal ligament remodeling;[30] vascular niche for tumor growth and metastasis;[31] hematopoietic stem cell niche for hematopoiesis;[32] adult neural stem cell niche for adult neurogenesis;[33] adult mammalian retinal stem cell niche for its activation in vivo;[34] bone marrow niches for hematological disease;[35] and stem cell niche factors for congenital enteropathy with ocular dysgenesis.[36] The niche brings intractable complexity. We must also consider the electrical niche[37] for generating stem cell-derived neurons, macrophages[38] for the muscle stem cell niche and the pathogen’s interaction with the stem cell niche for potential inflammation.[39] Biologists even consider the niche ecology of cancer differentiation therapy.[40] These studies are exceedingly tricky due to the lack of experimental simulation niche technology. Stem cell niche engineering is expected to be the key to modeling tissue development and disease and personalized medicine, drug screening, and cell therapy.[41]

We need to build microfluidic technology in biologists’ petri dishes[4] and solve niche biological questions.[5] Among all niche biological questions, the stem cell niche is the most representative. The stem cell niche is an area where stem cells are present in an undifferentiated and self-renewable state. The stem cell niche requires niche architecture cells and the extracellular matrix (e.g., collagen, fibronectin, and basement membrane), provides chemical support by molecular or nanoparticle diffusion (e.g., exosomes, chemokines, and hormones),[42] and avoids strong fluid shear forces. Therefore, niche engineering can use a semi-enclosed cell nest, which provides a tiny space for physical contact between stem cells and other structural cells. The niche also needs an opening to exchange substances with the outside world and transmit molecular information within the nest through microcirculation. Microfluidic stem cell–culture reports are rare because stem cell clones quickly collapse under the flow field. While cells build their microenvironment in a semi-closed cell nest, they also need to exchange materials with the outside world. If done too quickly, this exchange will destroy the microenvironment created by cells; however, it will lose the nutrient support and condition control if done too slowly. There should be internal self-circulation and interaction with the outside simultaneously in the cell nest’s semi-enclosed space, such as stem cell nests,[43] tumor cell nests,[44] and solid cell nests.[45]

We have recently developed a microfluidic drive technology to help solve this common problem. This drive technology only requires a magnetic 1.8 mm diameter microsphere for the fluid movement in a petri dish. If the stem cell nest is constructed in a petri dish, it is possible to achieve experimental control of the stem cell niche. Our goal is to design a semi-closed artificial cell nest in a petri dish and test its primary function using embryonic stem cells’ automatic microflow culture. We aim to provide new, ultralow-cost microfluidic petri dish technology for stem cell niche programming.

2. Results and Discussion

2.1. The Operation of a Microfluidic Petri Dish and False-Color Images of Flow Fields

We developed an MP3 music playlist control microfluidic petri dish technology for the experiment (Figure 1). After being placed in an O-pump (oscillator pump, 1.88 ± 0.07 mm, sphericity < 1.003), the mobile phone plays audio (1–25 Hz square waves) to drive the earphone-sized actuator, and the

Figure 1. The microfluidic petri dish experimental system and image processing. a) The audio of the mobile phone directly drives the O-pump in the petri dish. We add a drop of carbon nanoparticle solution to indicate b) the fluid’s movement and c) use a color scale to create d) the false-color image for each pixel’s absorbance value. e) We build guiding walls (black and red) in PMMA (polymethyl methacrylate) or PC (polycarbonate) petri dishes. f) Each 30 mm petri dish has a 10 mm cell nest (red) at the center with its opening direction to E, NE, N, NW, W, SW, S, and SE (see Movie S1, Supporting Information).
actuator causes the O-pump in a petri dish (Figure 1a) to form a flow field. We add carbon nanoparticles (100 nm) or red ink (formula weight 692) to indicate fluid flow and diffusion. The nanoparticles’ diameter is relatively close to the exosomes’ diameter (≈100 nm, see Figure S3, Supporting Information). We write software to calculate each pixel’s absorbance in the picture and use the color space to convert the absorbance data into a false-color image for a petri dish's detailed flow field (Figure 1b–d; see Movie S1, Supporting Information). Our experiment mainly uses a Petri dish with an inner diameter of 30 mm (Figure 1e). However, the size of the Petri dish is not limited. We design some structures (in black or red, Figure 1f) in the Petri dish to guide the microflow and the central cell nest has eight different orientations. When testing, we use 12 Hz to get a faster speed. When culturing cells, we use 8 Hz to get a 1–2 mm s$^{-1}$ flow velocity around the cell nest (see Movie S1, Supporting Information).

2.2. The Primary Flow Field of a Microfluidic Petri Dish

In a petri dish filled with water, the right O-pump vibration will drive the water to form a flow field (Figure 2a). We add two drops of carbon nanoparticles to indicate the flow field (Figure 2a,t1). Subsequently, the carbon nanoparticles flow to all parts of the petri dish, clearly showing three pairs of vortices (Figure 2a,t2–t4). We designed a ring-shaped partition wall to guide the flow (Figure 2b). The O-pump sucks water from the central region into the outer wall channel (Figure 2b,t1). The outer wall channel guides the water to the west, returns the water to the central area (Figure 2b,t2), and completes the water circulation at the east gate (Figure 2b,t3–t4). We then design a circular barrier wall (Figure 2c, red) in the central area. The water flow bypasses the right exit’s circular barrier (c,t1–t4). We can see a unique steady flow wake by zooming in the right barrier wall (Figure 2d). Figure 2c’s design transforms the multivortex flow field into a standard flow field from west to east, which is essential for artificial cell nests (see Movie S2, Supporting Information).

2.3. Determination of the Orientation of the Central Cell Nest

We plan to design cell nests in the central area because the flow here has a definite direction (from west to east) and velocity (1–2 mm s$^{-1}$) (Figure 3a). The cell nest’s internal diameter is
10 mm, which is equivalent to the early diagnosed tumor. The cell nest has a door for fluid exchange. There are eight choices for the direction of the entry (Figure 3b). We need to confirm the flow field and stability of each orientation through experiments. We add carbon nanoparticles to the external channel, start audio playback to drive the circulation outside the cell nest, and use a charge-coupled device (CCD) camera to record how these black nanoparticles enter the cell nest (Figure 3c). As the external carbon nanoparticles enter through the doorway, the absorbance distribution in the cell nest shows a microfluidic field. Figure 3c is the absorbance field in the cell nest with different orientations at 30 min. Seven cell nests showed prominent absorbance gradients (NE, N, NW, W, SW, S, SE) and one showed a more uniform absorbance field (E). The cell nest facing E has a unique flow field with four whirlpools and the outer material can quickly reach the depth of the cell nest through these whirlpools (see Movie S3, Supporting Information). We have repeatedly conducted anti-interference experiments and found that the E-oriented cell nest is very stable and is not affected by levelness. Cell nests in other directions sometimes form such a four-ring microcirculation, sometimes not. The cell nest E benefits from a stable wake (Figure 2d). In terms of stability in use, the performance of E meets the control requirements. Moreover, the cell nest E’s flow field has a clear and easily identifiable pattern, which can send the material to the depth so that the entire cell nest area is under programmed control. Therefore, in the following research, we focus on the cell nest E (see Movie S3, Supporting Information).

2.4. The Velocity Measurements of the Microcirculation

With the 3D false-color image, we can see that this is the four-circular flow field of the cell nest E (Figure 4a). It has a very thin cross-shaped circulation running through the entire cell nest. The four circulations are clockwise and counterclockwise in pair. The grid in the figure is 500 µm. The thickness of the circulation is about 100 µm. As the nanoparticles do not show the flow’s velocity, we add 100 µm plastic particles for velocity calculation (Figure 4b,c). By tracking the particle movement in each part, we obtain the velocity field in the entire cell nest (Figure 4d) and the average and standard deviation of the local velocity per square millimeter (Figure 4e). The velocity distribution percentage of the entire cell nest is obtained (Figure 4f). The maximum speed at the entrance is 35.5 ± 9.0 µm s⁻¹. This top speed inside the cell nest is very weak, only 2% compared to 1–2 mm s⁻¹ laminar flow outside the cell nest. The velocity at the center of the vortex or near the edge is around 15 µm s⁻¹ (see Movie S4, Supporting Information). In this open petri dish system, the flow velocity is proportional to the driving frequency. If the driving frequency is 1 Hz, the maximum speed is under four µm s⁻¹. In cell culture, we use 8 Hz to control the velocity at less than 26 µm s⁻¹. Our purpose is to control the ultraslow microflow pass over the stem cells precisely. Stem cells are susceptible to flow rate. Even at such a slow speed, it cannot continue to be applied to stem cells. Through the song playlist as a program, a microfluidic flow is triggered by

Figure 4. The velocity and scale of the microcirculation inside the cell nest. a) The carbon nanoparticles enter the cell nest and indicate the circulation of ≈100 µm in width. b,c) We add plastic 100 µm particles (red arrows) to show the flow rate. a) The fastest flow rate appears on the path from the east entrance to the center. b,c) Two particles move in 60 s. We measure five particles for each square mm to get their speed values (d, red arrows) and average values with standard error (e, error bars). The percentage of arrow counts represents the velocity distribution in the velocity interval of 2 µm s⁻¹ (f, see Movie S4, Supporting Information).
the control system every hour for 1 min or every day for 1 min (1 min D−1, Figure 6).

2.5. The Exchange Rate of the Cell Nests

The cell nest E has a stable internal circulation because of the steady wake. If we zoom in on the gate (Figure 5a,b), we will find a 500 μm vortex pair at the inner side of the doorway (see Movie S5, Supporting Information). This vortex pair prevents the water at the door from entering the cell nest and uses the wire-drawing effect to squeeze the water to 100 μm. If there is no such vortex pair at the door (Figure 5c,d), the water slowly passes through the door and fans out as in other cell nests (Figure 5d). The wake gives this vortex pair stability and this stability allows us to control the experimental conditions precisely. We measure influx and outflux by integrating the absorbance of all pixels (Figure 5e,f). Data show that the flux of cell nest E is significantly higher than that of the other seven cell nests. Although the two vortices make the incoming flow thinner, they do not block the flux increase. They stabilize the flow field and make it easy to control. We calculate the half-life of each cell nest (Figure 5g, N = 1200). The half-life without flow is 11 120.6 ± 10.4 s (=3.09 h), and E is 1419.8 ± 1.3 s (=23.7 min). Other orientations are 3486.6 to 7614.3 s (standard error of mean < 16.3 s).

2.6. The Embryonic Stem Cell (H9)–Cancer Cell (A549) Auto Coculture via a Programable Microfluidic Petri Dish

Stem cells are susceptible to culture conditions, requiring Matrigel and a particular medium, which needs refreshing after 1 or 2 d. We must operate gently. After forming the circular stem cell clone, we start the microfluidics. Although the microfluidic fluid circulates in the petri dish, the stem cell clones collapsed after about a day, including the stem cell clone at the weakest flow position. We designed an artificial cell nest to protect the stem cells and chose the E-oriented cell nest to culture embryonic stem cells because of its stable microcirculation. However, a constant flow will make the cell nest lose half its material in 23.7 min and the embryonic stem cells cannot form their niche for survival. Therefore, we looked for the best conditions for stem cell survival by further reducing the flow rate and setting the programmed flow control. The flow starts every hour for 1 min (that is, 24 times a day) and the clones of the stem cells still show no apparent signs of growth. However, when we reduce the flow to 1 min per day (1 min D−1, 8 Hz), the stem cell clone becomes healthy and proliferates (Figure 6a–d). Therefore, an ultralow flow rate and flowing time are essential conditions. In the human body, the structure of tumor or stem cell nests protects the cells. Nevertheless, when we use artificial cell nests to cultivate stem cells, we know that the flow’s intensity and time control are delicate.
In the stem cell–cancer cell coculture experiment, all artificial cell nests are eastward (Figure 6e). All chip materials are transparent PC (polycarbonate). The chip has no edges and is sterilized with O-pump and placed directly in a traditional 30 mm petri dish. The cell nest door has a 1 mm slit to retain the Matrigel. We plant the cells mixed in Matrigel in the center of the artificial cell nest, with the cancer cell mimic tumor on the bottom and stem cells on top. By the playlist, we drive the flow at 8 Hz for the 60 s every day (1 min D⁻¹). We do not refresh the cell nest culture medium. We add five drops of the new medium to the external channels every 2 d for an automatic medium refresh. Results show that the automation of medium refresh is effective (Figure 6f,g), and the single clone area of embryonic stem cells increases from $(3.57 \pm 0.52) \times 10^5 \mu m^2$ to $(11.67 \pm 1.33) \times 10^5 \mu m^2$ (about three times) after 2 d of automatic culture and advanced to $(53.34 \pm 8.37) \times 10^5 \mu m^2$ (about 15 times) after 4 d. Both the stem cells and cancer cells survive the automation and the stem cell clones (green fluorescent) of

Figure 6. The culture of cancer cells and stem cells (H9) in the artificial cell nest. a) We culture embryonic stem cells (ES cells) in cell nests and take bright field pictures every 2 d. We measure the area of stem cell clones (b, N = 4, error bars are SEM) and take confocal fluorescence images (GF: green fluorescence) at the end of the experiment. a,b) The initial stem cell clones in the first experiment are small and c,d) the stem cells in the second experiment are large. c) The 5 min D⁻¹ procedure was too much, and the stem cell clones collapsed and disappeared entirely on the sixth day (red cross in (d) contrast with a green arrow in (b), showing fast-growing connected clones unmeasurable as singles after 5 d). e) We top the mimic tumor (A549, red fluorescent) with a stem cell (human embryonic stem cell H9, green fluorescent) Matrigel drop and cover them with 20x Matrigel. The flow starts once every day for 60 s. After 14 d of coculture, we take the confocal red fluorescent images for cancer cells (red fluorescent protein) and stem cells (green fluorescent protein) and the bright-field images for both f) experiment and g) control (see high-resolution image Figures S1 and S2, Supporting Information).
the 1 min D⁻¹ over the mimic tumor (red fluorescent) show higher activity than that of 0 min D⁻¹ (no flow, Figure 6f). More importantly, the scattered stem cells accumulate to a dozen big stem cell clones. After the 14 d of culture, these stem cell clones already go deep down into the mimic tumor and touch the bottom (see Movie S7, Supporting Information).

3. Conclusions
The key to eliminating the difference between in vitro and in vivo is simulating the in vivo microenvironment. As the high-cost microfluidic chips are challenging to use and have niche biological problems, the traditional petri dishes without microfluidic functions are still the first choice. Here, we show a novel microfluidic petri dish with microfluidic functions. Its operation and cost are nearly the same as those for a traditional petri dish. To get a microfluidic control, we need only an earplug-sized actuator driven by cell phone audio and programmed by song playlists. We design artificial cell nests in microfluidic petri dishes. The ultraslow circulation and the material exchange rate are adjustable to find the optimum conditions for both embryonic stem cell culture and coculture with tumor cells in an artificial cell nest. The microfluidic petri dish is a new and convenient cell or tissue microenvironment simulation technology. It realizes the function of a lab-on-a-chip with the operation and cost of a traditional petri dish. Besides that, this microfluidic petri dish with an artificial cell nest is designed to solve the niche biological question. It has ultraslow (<40 µm s⁻¹) microcirculation (5 mm) precisely controlled by a 1.8 mm pump. Its flow field determines the transportation of molecular, granular, and nanomaterials and the programming technology regulates the rhythm of the stream supply. Its pure motion drive method excludes chemical and physical interference factors. It does not have the problem of clogging or bubbles, nor does it have the problem of service life. It is very suitable for simulating life processes.

4. Experimental Section

**Microfluidic Petri Dish:** A computer numerical control (CNC) milling machine was used to process polycarbonate microfluidic petri dish. The bottom and wall of the microfluidic petri dish were processed separately. After processing, trimming, cleaning, and assembly, the chip and O-pump would be sterilized at 120 °C. The chip could be sandwiched between two glass pieces during high-temperature sterilization to prevent deformation. The sterilized chip and its O-pump were placed in a petri dish in a sterile room. After adding the cells and culture medium, the microfluidic culture dish was put on the controller that generated the scanning magnetic field, connected the MP3 player, and put them into the incubator. When the MP3 playlist was played, the petri dish would be automatically played the playlist program to control the microflow (see Movie S8, Supporting Information).

**O-Pump Manufacturing:** O-pumps were manufactured from magnet beads (<2 mm). If the magnet bead's sphericity and size were not good enough, their shape was needed to be polished. One piece of sandpaper and one magnet bead were put in a petri dish; the petri dish was fixed on a shaker to vibrate and shine the bead until its diameter and sphericity met the requirements. Generally, it can roll freely on the glass (see Movie S8, Supporting Information).

**Microparticle Solution:** The plastic particles were created by scratching plastic plates of similar specific gravity to the fluid. A CNC milling machine was used to produce particles. The smaller the tool, the smaller the particles were. In the experiment, 100 µm particles are used to measure ultraslow flow (see Movie S8, Supporting Information).

**Nanoparticle Solution:** Carbon ink could be used directly as nanoparticles to indicate microcirculation and calculate the flux in and out of cell nests. However, the nanoparticles' size should be confirmed with an electron microscope before use, which should generally be about 100 nm (see Movie S8, Supporting Information).

**Cell Culture:** The cancer cell (A549-red fluorescent protein) culture medium was Dulbecco's Modified Eagle Medium-F12K and the stem cell culture (embryonic stem cell hESC-H9-eGFP) medium was mTeSR1. Approximately 80 µL of the cell suspension was mixed with 20 µL Matrigel and then dropped into the cell nest to form 5x Matrigel tumor cells or stem cell drops, 1–2 mm in diameter. After the shape was stable (usually 5–10 min in the incubator), the cell drops were covered with 20x Matrigel, stem cell culture medium was added, and they are put on the controller in the incubator. The MP3 player played the program audio for auto culture (see Movie S8, Supporting Information). To avoid disturbing its environment, two to five drops of stem cell culture medium were supplemented outside the cell nest every 2 d and a bright-field inverted microscope was used to record cell growth images (finished in 15 min). Fluorescence images were taken later 7–14 d later.

**Flow Field Velocity Measurement:** Plastic particles with a specific gravity similar to water were added into the water for fluid movement tracing. The acquired pictures were played back frame by frame to track the particles, measure the distance, and get the particle movement's direction and speed (see Movie S4, Supporting Information).

**Image Processing:** The nanoparticles were mixed into the water to indicate the flow field. The microfluidic petri dish was placed on a light-emitting diode backlight plate. The light generated by the backlight passed through the cell nest from bottom to top. The ink containing nanoparticles in the cell nest absorbed light and was recorded by a CCD camera above. Self-editing software was used to obtain each pixel's Red Green Blue (RGB) value; the RGB value was converted into the absorbance value, and it was converted into a false color. The bright-field picture of the cells was taken with a 4X objective lens and then stitched together to obtain a complete picture of the cell nest. The edge-finding function of Photoshop then enhanced the stitched image. The fluorescence image's stitching was automatically completed by the fluorescence microscope software's built-in function (see Figures S1 and S2, Supporting Information).

**Free Sample:** Some free sample products are provided to help readers build their microfluidic petri dish system, including O-pumps, actuators, plastic particles, MP3 audio files, microfluidic Petri dishes, etc. Readers can send requests via email (contact@qiyuexm.com).

Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest
The authors declare no conflict of interest.
Author Contribution
Y.G. and L.P. contributed equally to this work. Author contribution is as follows: X.Y. (L.) P. (experiments and manuscript); L.P. (music technology); Y.G. (diffusion experiments); J.L. (flow field velocity).

Data Availability Statement
The data that supports the findings of this study are available in the supplementary material of this article.

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