Trypsin-Assisted Cell Depletion Method for Wound Healing Assay

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Abstract: Wound healing assay is a commonly used method in the laboratory to study cell migration ability. Among the methods used to create cell-free zone, the widely used method, called cell depletion, will leave a certain amount of injured cells in the migration regions, which will have an impact on the subsequent healing experiments. To this end, we present a trypsin-assisted cell depletion method in wound healing assay to create cell-free zone without dead cells. This method could rinse the dead cell after applying depleting process without interfering in the attachment of the living cells. All the operation process is accomplished by commonly used equipment and drugs in biological experiments. The effect of the enzyme is controlled by the ambient temperature and processing time. The debris of dead cells are easily detached and removed to avoid the impact on wound healing assay. This method is expected to combine with other 2D and 3D cell patterning methods to form a more reliable cell processing technique.

1 INTRODUCTION

The investigation of wound healing assay would provide more information about cell migration and cell-cell interaction (HE, 2020) for biologist to study cellular mechanisms (Grada, 2017), tumor formation and metastasis (Teleanu, 2019), and inflammation models (Biglari, 2019). The first step in wound healing assay requires creating an artificial cell-free zone which have been well developed by researchers. And most of these methods can be categorized as cell depletion that inevitably causes damage to the cells (Monfared, 2021). Meanwhile, the injured cells may remain on substrate, which seriously affect the wound healing assay of the rest living cells. In this article, we developed a trypsin-assisted method to remove these injured cells by controlling the efficacy of trypsin by temperature and time. After comparison, the most effective processing parameters were obtained. With experimental verification, this method will have negligible side effect on the cells that are prepared for the subsequent wound healing assay. This approach not only addresses the inherent disadvantage of cell depletion, but also makes the process of creating cell-free zone regions more stable and reliable.

2 METHOD

The device with stamping function used to culture cell is shown in Figure 1. The top layer of the chip is the Polydimethylsiloxane (PDMS) with pillars dimensions of 100 μm height and a 400μm diameter fabricated by mold, which is made by photolithography technology. After the PDMS is made, the two ends of the PDMS are punched to fabricate the outlet and inlet of the chip. The middle layer is a spacer with a hollow cavity made of laser-cut Acrylic(PMMA) board. And the bottom layer is the culture dish substrate used for cell attachment.

After the oxygen plasma and ultraviolet treatment, these three parts are aligned and bonded together by double-sided adhesive tapes and use heavy objects to press the device for an hour to obtain a fully sealed channel with 1mm height and 1cm width. The stainless steel needles are inserted at both ends of the channel, and the liquid in the channel can be replaced through the Teflon tube which are connected with syringes. The PDMS layer can be pressed down so that the pillars in the central region can touch the bottom of the channel and realize the stamping function.

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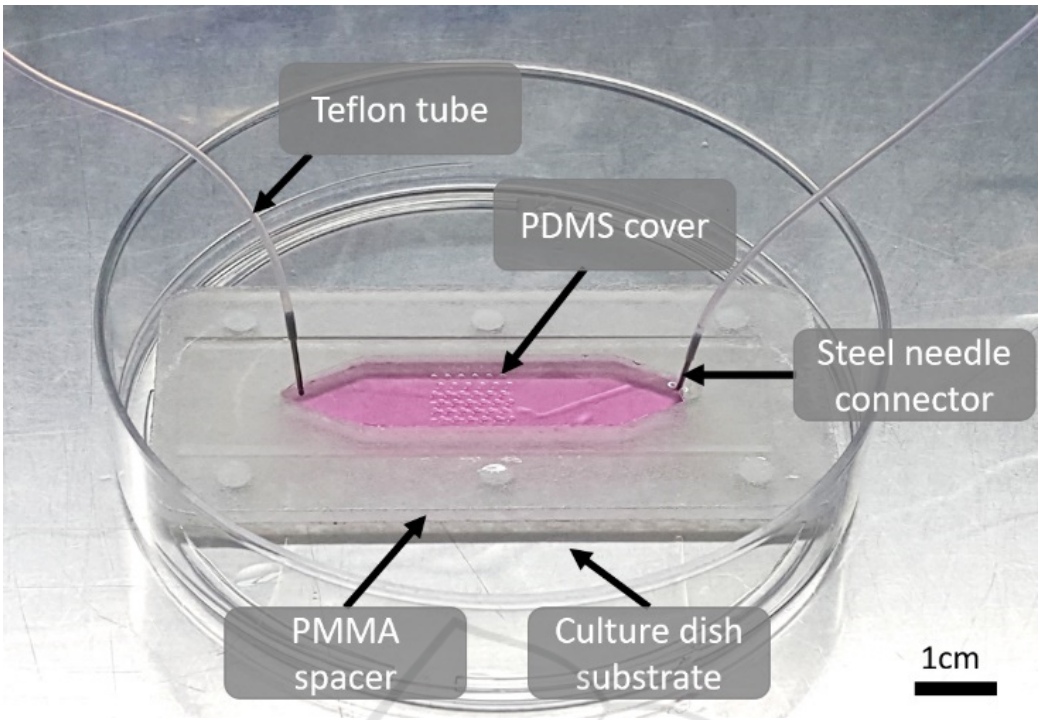

Figure 1: Schematic diagram of cell culturing chip with stamping function. Three layers of the chip are bonded and sealed by two double-sided adhesive tapes. The two ends are connected by tubes to load the nutrients and trypsin that are necessary for the experiment. And both ends of the tubs should be sealed with syringe to prevent leakage of liquid. The device will remain in an incubator except for the trypsin experiment which requires the temperature of the chip to be adjusted.

Before the experiment, the device was filled with liquid 75% ethanol and exposed to ultraviolet light overnight. After disinfection, 5mL phosphate buffer(PBS) was injected into the channel for cleaning. And the PBS was then evacuated from the channel by air, and the device was placed in a sterile vessel to be vacuumed as much gas as possible from the chip. After 30 minutes, the device was moved to a sterile environment, and 75% alcohol was re-injected to drain the air bubbles in the channel. After that, the channel was washed again with PBS, and the chip is ready for use. In the process of the experiment, in addition to trypsin, other liquid injected into the chip, must be put into the incubator in advance, so as not to create bubbles in the chip.

The principle of the trypsin-assisted method is shown in Figure 2 by appending weakened trypsin digestion steps to the traditional cell depletion. In our device, Cells were seeded into the channel as shown in Figure 2(a). After incubation for 24 hours, the cells reached 80% confluent without any liquid flow. The DMEM in the device was replaced by PBS. Then, the pillars were pressed down towards the substrate as shown in Figure 2(c). After 5 minutes, the stamp was reset to the original position. And the trypsin was injected into the channel to replace the PBS and let stand for several minutes as shown in Figure 2(e). Last, DMEM was gently introduced into the channel to rinse the dead cells and resupply the cells with the nutrients that are necessary for the subsequent experiment as shown in Figure 2(f).

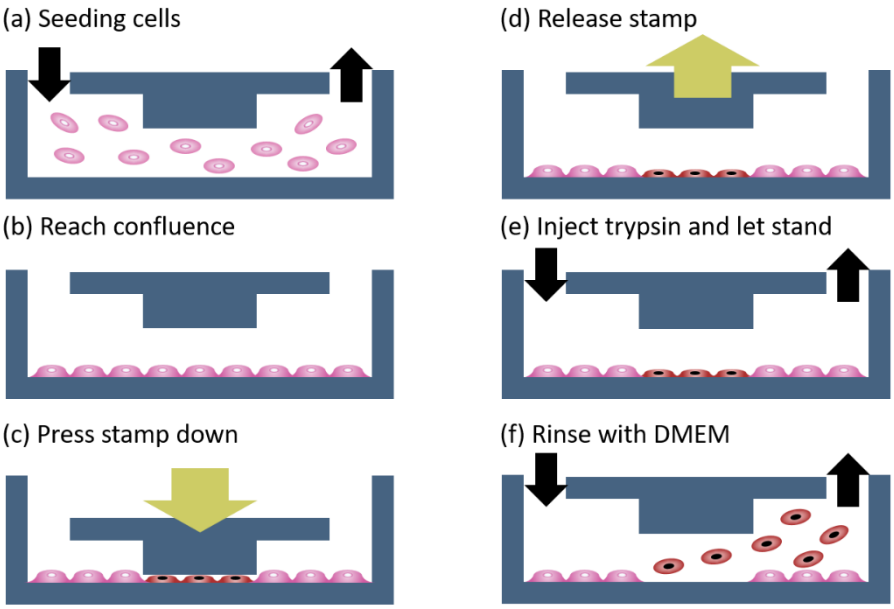

Figure 2: The procedure of the trypsin-assisted method. The dark blue boundary represents the structure around the cell culture chamber, the black arrow represents the flow direction of the different liquid, and the yellow arrow represents the direction of the stamping movement. The stamping process can be performed manually or with other devices. It should be noted that the pressure cannot be too large, otherwise the PDMS will touch the cells out of the target cell-free zone.

3 RESULTS AND DISCUSSIONS

The micrographs of the cells during the procedure was shown in Figure 3. Before stamping, PDMS was washed away with PBS. And the morphology of cells is shown in Figure 3(a). After stamping, it can be observed that the morphology of cells out of cell-free zone was not significantly different from that of the cells before stamping. Compared to the aforementioned cells, the cells below the stamp were heavily stressed, and most of them were dead and thus detached the substrate. But there were still a few injured cells attached to the substrate, which are mainly concentrated in the center and the edge of the cell-free zone, as shown in Figure 3(b). The regions and quantities of these cells are not constant, which is not reliable for studying injured cells. It also can affect other living cells around. Therefore, it needs to be processed immediately. Subsequently, trypsin was slowly injected into the channel and remained at a certain temperature and time. These injured cells would fall off naturally, which were rinsed out of the channel with re-injecting DMEM. And the remaining cells form a well-defined cell-free zone as shown in Figure 3(c).

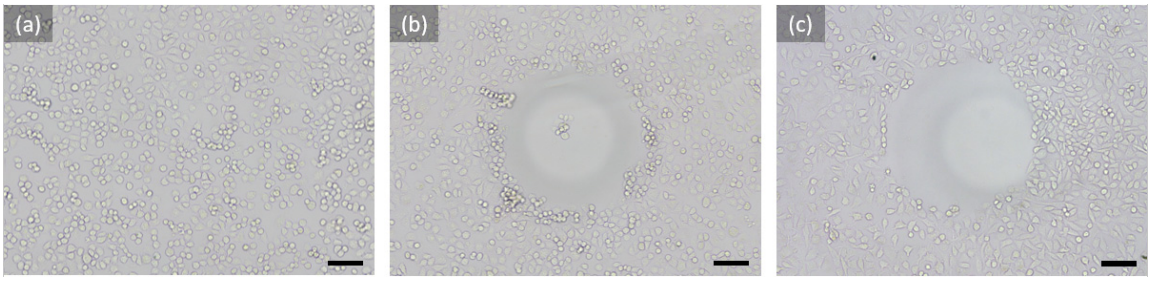

Figure 3: bright-field micrographcs of the cell morphology before and after stamping(a)(b) and after being treated with trypsin(c). The scale bar is 100 μm.

To access the effect of trypsin, which was affected by the ambient temperature and the standing duration, a series of experiments were carried out. Cell activity and cell density at different temperatures are shown

in Figure 4(a). With the increase of temperature, the effect of Trypsin became stronger and a large number of dead cells fell off, leading to the increase of cell viability. But at the same time, the cell density decreased with the increase of temperature. The effect of different standing duration on cells is shown in Figure 4(b). With the increase of standing duration, the shedding of dead cells also leads to the increase of cell viability, while the cell density decreases greatly.

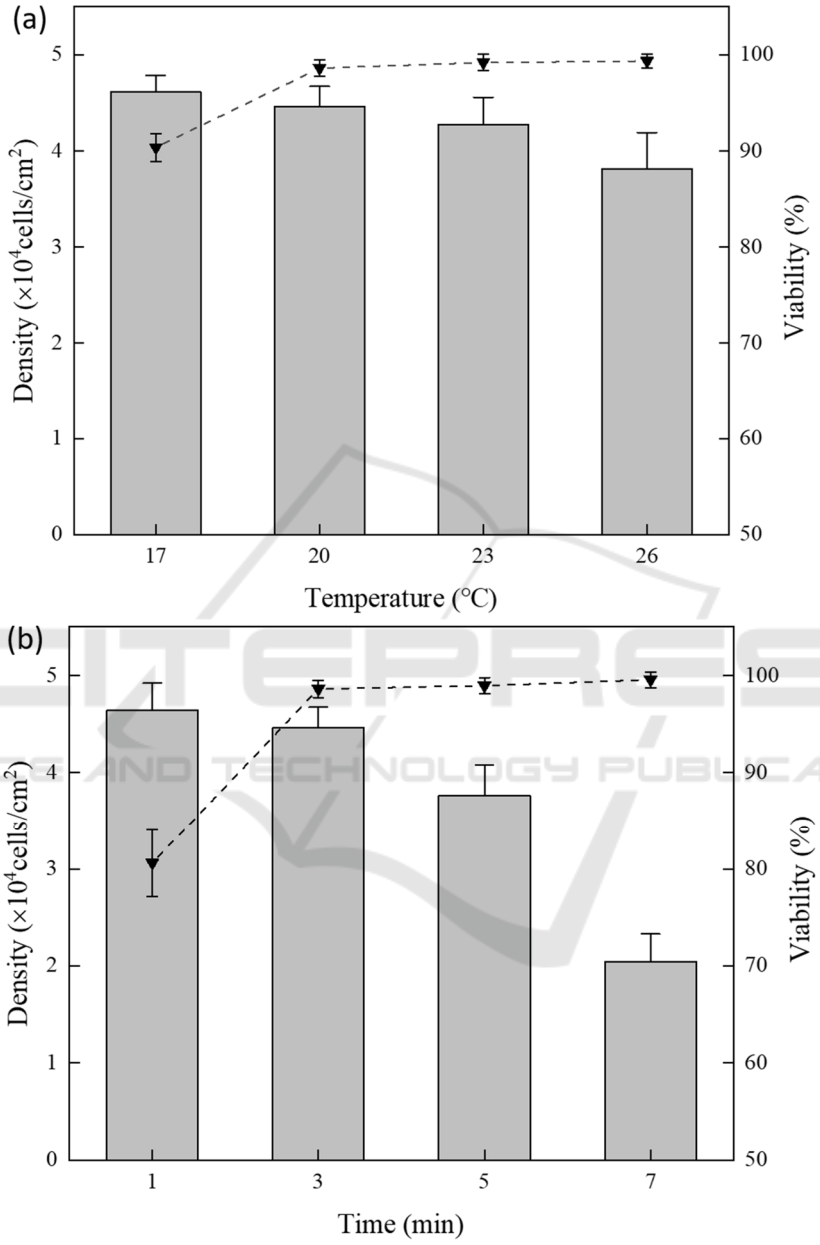

Figure 4: The effect of the trypin in different conditions such like ambient temperature(a) and standing time(b). The cell density is represented by bars and the cell viability is represented by dotted lines.

In order to evaluate the growth of cells treated with or without trypsin, we compared our method with the wound healing of cells in the cell exclusion method within 24 hours. Here, the cells of the experimental group were treated with trypsin at 20 ℃ for three minutes while the control group was treated with DMEM for three minutes at the same temperature. The healing area of cells was recorded every 6 hours. As shown in Figure 5. In each time point, the wound healing area was very close to each

other and all achieved more than 0.95 at 24 hours. Compared with the control group, the effect of trypsin treatment on cells was almost negligible. And the experimental results further verify the feasibility of our method.

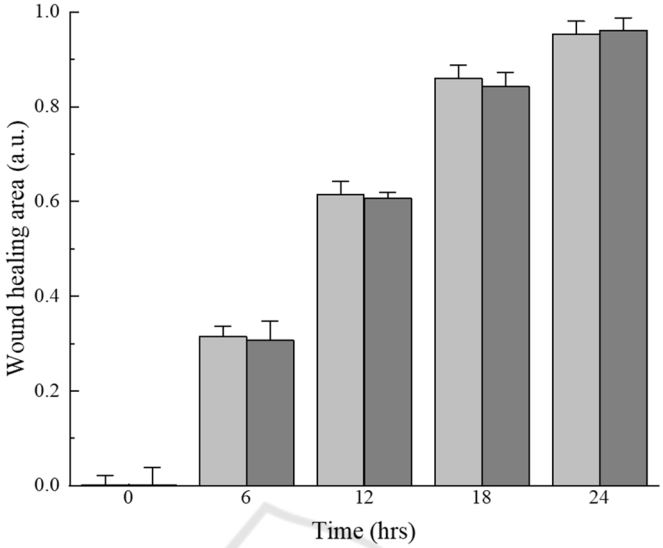

Figure 5: Wound healing area of the cells under treated or non-treated conditions. The light colored columns indicate cells that have not been treated with trypsin, while the dark colored columns indicate cells that have been treated with trypsin. The wound healing area at certain time points was normalized to diagram cell migration.

4 CONCLUSION

The experimental results showed that by adjusting the temperature and time of trypsin, targeted cell clearance could be achieved and well-defined cell-free zone could be obtained. It also proved that the method not only removed the negative effects of the dead cells, but also ensured that the remaining cells treated with the enzyme were no different from the untreated cells in subsequent wound healing experiments. This method overcomes the inevitable defects in the process of cell depletion and it will expand the application prospects of more cell patterning methods.

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REFERENCES

A. Grada, M. Otero-Vinas, F. Prieto-Castrillo, Z. Obagi, V. Falanga.(2017) Research Techniques Made Simple: Analysis of Collective Cell Migration Using the Wound Healing Assay, J Invest Dermatol, 137: e11-e16.

G. Shabestani Monfared, P. Ertl, M. Rothbauer. (2021) Microfluidic and Lab-on-a-Chip Systems for Cutaneous Wound Healing Studies, Pharmaceutics, 13.

H.E. Deal, A.C. Brown, M.A. Daniele. (2020) Microphysiological systems for the modeling of wound healing and evaluation of pro-healing therapies, J Mater Chem B, 8: 7062-7075.

R.I. Teleanu, C. Chircov, A.M. Grumezescu, D.M. Teleanu. (2019) Tumor Angiogenesis and Anti-Angiogenic Strategies for Cancer Treatment, J Clin Med, 9.

S. Biglari, T.Y.L. Le, R.P. Tan, S.G. Wise, A. Zambon, G. Codolo, M. De Bernard, M. Warkiani, A. Schindeler, S. Naficy, P. Valtchev, A. Khademhosseini, F. Dehghani. (2019) Simulating Inflammation in a Wound Microenvironment Using a Dermal Wound-on-a-Chip Model, Adv Healthc Mater, 8 e1801307.