ON-CHIP CULTURE OF OSTEOCYTES

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ABSTRACT

This paper presents a microfluidic device (PDMS micro channels bonded with glass slides) enabling culture of osteocytes (murine long bone osteocyte Y4, MLO-Y4). In this study, collagen coating, cell seeding and culture as well as immunostaining of E11/gp38 (bench mark of osteocytes) were demonstrated where gravity was used as the driving force for liquid transportation. To investigate the effects of surface modification on osteocyte proliferation and phenotype expression, MLO-Y4 cells were cultured in the microfluidic channels with and without collagen coating. Furthermore, the effects of channel dimensions (variations in width and height) on the viability of MLO-Y4 cells were explored. As a platform technology, this microfluidic device may function as a new cell culture model enabling further studies of osteocytes.

KEYWORDS: Osteocytes, Microfluidics, Cell Culture, Collagen Coating, Channel Geometry

INTRODUCTION

Osteocytes (bone cells embedded in bone matrix) have long been regarded as coordinating cell types in bone metabolism and their dysfunctions are responsible for osteoporosis[1]. However, current studies of osteocytes are based on conventional cell culture, which are not capable of regulating the spatial/temporal distribution of cells and biomolecules and thus cannot recapitulate local in vivo microenvironments[2].

Microfluidics is the science and technology of manipulating and detecting fluids in the microscale. Due to its dimensional comparison with biological cells and capabilities of defining local biophysical, biochemical and physiological cues, microfluidics has been used for more in vivo like cell culture[3]. However, in the field of microfluidics based bone studies, preliminary demonstrations were conducted to study osteoblasts[4] while no systematic study of on-chip culture of osteocytes has been demonstrated.

To address this issue, we proposed a microfluidic platform (Figure 1) for on-chip culture of osteocytes (MLO-Y4) where collagen coating, cell seeding and culture, as well as immunostaining were conducted in a tubing-free manner. More specifically, the effects of surface modification (with or without collagen coating) on the proliferation and phenotype expression of osteocytes cultured in microfluidic channels were investigated. Furthermore, the influence of channel dimensions (variations in width and height) on cellular viability was studied. Meanwhile, as gravity was used as the driving force for cell loading and immunostaining, this platform doesn’t need external pumps and tubes, which can be operated in biological labs with low access requirement.

MATERIALS AND METHODS

Figure 1: Schematic of the microfluidic platform for culture of MLO-Y4 cells where gravity was used for collagen coating ((a)-(b)), MLO-Y4 cell seeding, culture and medium replacement ((c)-(e)), as well as immunostaining ((f)-(h)).

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MLO-Y4 cells (gift from Dr. Linda BoneWald, University of Missouri-Kansas City) were cultured in a PDMS microfluidic platform where gravity was used for collagen coating, osteocyte seeding and culture as well as immunostaining of E11. Cell passage generations used in this study were between P5 and P14.

Prior to cell-culture experiment, the glass surface in microfluidic channels were coated with collagen (Figure 1(a) and (b)). Briefly, 1 mg/ml collagen solutions were flushed into the channels through the inlet using micro pipets. Then, microfluidic channels were soaked with collagen solutions for 1 hour, followed by three-time rinse of supplemented culture medium. The microfluidic devices without collagen coating were used as negative control by flushing supplemented culture medium into the channels before cell loading.

The cells were loaded into microfluidic channels prefilled with supplemented culture medium based on gravity (Figure 1(c)-(e)). Solutions in two ports were removed and replaced with cell suspension solutions at 20 vs. 15 μL (0.5 million cells per mL). Due to the volume difference, cells were pushed into the microfluidic channels due to gravity for 5 minutes. Then, each device was placed in a petri dish containing 2 ml of distilled water to limit culture medium evaporation and then transferred to an incubator. Culture medium in each port was replaced with 20 μl of fresh supplemented culture medium every 12 hours.

In the process of on-chip immunostaining (Figure 1(f)-(h)), cells within the microfluidic channels were rinsed three times with PBS solutions, fixed by 4% paraformaldehyde in PBS for 10 minutes at 4°C, blocked with 10% goat serum/PBS for 45 minutes. Then cells were incubated with primary antibody of 4 μg/ml overnight at 4°C, rinsed three times with PBS, incubated with secondary antibody of 10 μg/ml for 45 minutes at 25°C. Note that in all the steps, solutions of 20 vs. 10 μl were applied into two ports for medium replacement.

To evaluate the osteocyte proliferation and viability inside microfluidic channels, cell numbers and cellular process numbers were counted based on manual processing of phase contrast images at 12 hour, 24 hour, 36 hour, 48 hour, 60 hour and 72 hour. All results were expressed as means ± standard deviations. In the statistical analysis the Student’s t-test was used for two-group comparisons. P<0.01 was considered statistically significant.

RESULTS AND DISCUSSION

Figure 2 shows the effects of collagen coating on cellular morphology and proliferation of osteocytes over time. Within the first 12 hours of cell seeding, MLO-Y4 cells on collagen-coated surfaces were shown to spread more (Figure 2(a)) than cells on glass surfaces without collagen coating (Figure 2(b)). As time goes on, elongation in the cellular processes with decrease in the area of cell bodies was noticed for both types of surfaces and at 72 hours of cell seeding, more processes were located for MLO-Y4 cells on collagen-coated surfaces than cells cultured on glass surfaces only.

Figure 2: The effects of extracellular matrix on MLO-Y4 phenotype. MLO-Y4 cells cultured in microfluidic channels with collagen coating spread more and developed more processes (a) compared to MLO-Y4 cells on glass surfaces without collagen coating (b). Quantified process numbers where a significant difference with and without collagen coating was located (c). Compared to MLO-Y4 cells on glass surface, MLO-Y4 cells on the collagen-coated surface showed lower proliferation rates (d).
Based on the manual counting, at 72 hours following cell seeding, the process number per cell for MLO-Y4 cells cultured on collagen-coated surfaces was quantified as 2.57±0.11 (12 Hour), 2.60±0.17 (24 Hour), 2.63±0.19 (36 Hour), 2.65±0.22 (48 Hour), 2.70±0.31 (60 Hour) and 2.72±0.42 (72 Hour) while the process number per cell for MLO-Y4 cells seeded on glass surfaces was quantified as 2.44±0.20 (12 Hour), 2.51±0.23 (24 Hour), 2.52±0.24 (36 Hour), 2.52±0.20 (48 Hour), 2.53±0.20 (60 Hour) and 2.59±0.15 (72 Hour). In summary, a significant difference on cell process number per cell was located as 2.68±0.27 vs. 2.53±0.20 for MLO-Y4 cells seeded on collagen-coated and glass surfaces, respectively (Figure 2(c)), consistent with previous studies.

As to cellular proliferation, a similar trend was located for cells seeded on glass surfaces coated with and without collagen in microfluidic channels. Cell densities on collagen-coated surfaces were quantified as 47.5±10.8/mm² (12 Hour), 43.01±11.53/mm² (24 Hour), 39.13±11.49/mm² (36 Hour), 39.13±10.05/mm² (48 Hour), 40.97±8.66/mm² (60 Hour), and 40.41±14.31/mm² (72 Hour) while 32.96±8.81/mm² (12 Hour), 25.58±5.63/mm² (24 Hour), 25.83±5.25/mm² (36 Hour), 33.71±7.15/mm² (48 Hour), 40.54±8.28/mm² (60 Hour), and 48.42±8.91/mm² (72 Hour) were quantified values for cells seeded on glass surfaces without coating (see Figure 2(d)). For cells seeded in microfluidic channels with and without collagen coating, initial cell density decreases followed by graduate increases in cell numbers were observed. Compared to cells seeded in collagen-coated microfluidic channels, cells seeded on bare glass surfaces were noticed to have a higher proliferation rate, consistent with previous publications[5].

Figure 3 shows the microscopic images of immunostaining of E11/gp38 as the benchmark of osteocytes, confirming the osteocyte source of MLO-Y4 cells and the feasibility of on-chip osteocyte immunostaining.

As the channel width was decreased from 800 μm to 200 μm (channel dimensions of 10.00 L × 0.80 W × 0.10 H (mm)), a significant decrease in cell viability was noticed (Figure 4). At 12 hours after cell seeding, osteocytes seeded in the middle of the micro channels didn’t adhere to the substrate as a sign of cell death while osteocytes near the channel inlets were noticed to adhere to the substrate and survive well. However, most osteocytes near the channel inlets showed elongated cell bodies at 72 hours after cell seeding, indicating poor cell status. The same trend was observed for MLO-Y4 cells seeded in microfluidic channels with a decrease of channel height from 100 μm to 20 μm (channel dimensions of 10.00 L × 0.80 W × 0.02 H (mm)) (Figure 5).

Figure 3: Microscopic pictures of on-chip immunostaining of E11/gp38 as the benchmark of osteocytes (channel dimensions of 10.00 L × 0.80 W × 0.10 H (mm)).

Figure 4: Microscopic pictures of MLO-Y4 cells cultured in microfluidic channels with channel width of 800 μm (a) and 200 μm (b), indicating a higher cellular viability for MLO-Y4 cells seeded with a larger channel width.
These differences may result from nutrition level distribution, which decreases from the locations near the channel inlets to the middle of micro channels. In addition, the decreasing dimensions may cause the insufficiency of oxygen which can result in the cells apoptosis. This study indicates that geometrical parameters can affect MLO-Y4 cell viability significantly, which is consistent with previous studies on other cell types inside microfluidic channels.

CONCLUSION

In this paper, a tubing-free microfluidic device was fabricated for MLO-Y4 culture where collagen coating, cell seeding, culture and immunostaining were demonstrated. Compared to glass surface, collagen-coated substrates lead to significant differences in MLO-Y4 proliferation and morphology in a time sequence. Microfluidic channels with decreased dimensions may lead to lower viability of MLO-Y4 cells due to limited nutrition and oxygen transportation. As a platform technology, this microfluidic device may function as a new cell culture model enabling further studies of osteocytes.

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