Migration of Osteoblast-like Cells on Extracellular Matrices-coated Culture Plate Using Cell Tracking System

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In this study, the migration of MC3T3-E1 osteoblast-like cells was examined on the culture plate coated with ECM, such as type I collagen, laminin or fibronectin. The cells were incubated in a self-made mini-incubator with the same conditions as commercial CO₂ incubator, placed on an inverted microscope and observed as a real-time mode. After 24 hr of incubation, the edge and center of single cell were detected by an automated cell tracking system. From the cell movement path, it was shown that the cells had a tendency to move randomly without any certain direction. Furthermore, it could be found that the cell movement speed was divided into three phases, attachment preparation phase, from 0 to T1 hr, accelerated movement phase, from T1 to T2 hr and speed maintenance phase, till 24 hr. At first phase, a cell moved slightly as like searching a local attachment site. Afterwards, the cell accelerated its speed and reached the maximum speed, \( V_{\text{max}} \). To estimate the cell migration speed, the migration index was defined as the value of \( V_{\text{max}} \) divided by \( T2-T1 \). The migration index of the MC3T3-E1 cells on fibronectin was 8.9, while those indexes on the type I collagen and laminin were respectively 5.3 and 5.1. These results suggested that all cells might have their own migration index according to their species or phenotypes and ECM type, and the migration speed could be inferred from those values without further study.

Key words: Cell migration, Extracellular matrix, Osteoblast-like cell, Cell tracking system

INTRODUCTION

Cell migration is central to normal and pathological processes, such as angiogenesis, embryogenesis, neuron development, inflammation, tumor invasion and metastasis, repair of wounded vascular wall and cell colonization of a biomaterial.⁵⁻⁶ Especially on occasion of bone regeneration process, the osteoblasts from the surrounding intact bone are recruited to the resorption site.⁷ Many recent studies have focused on cell seeding on porous biomaterials used as the scaffold at an injured site.⁸ In order to extend the life span of the applied biomaterials, the cells should be directed to migrate and cover the pores of the scaffold surface homogeneously. Several methods such as Boyden-chamber assay and wound healing assays were used in the previous studies for cell migration.¹⁻³ However, these methods might not be effective to evaluate the cell migration attributed to some problems.¹⁴

Cells cannot function properly without the appropriate extracellular matrix (ECM) in the formation of new tissue. It is due to that cell-ECM signals give especially important influences to the cell signal, except for the multiple factors such as soluble survival growth factors and signals from cell-cell interaction.¹⁷ Fibronectin (FN) is a general cell adhesion molecule by interacting specifically with different cell surface receptors, namely the integrins and transmembrane proteoglycans. FN also can act to organize cellular interaction with the ECM by binding to different components of the ECM and to membrane-bound FN receptors on cell surface, thus can affect cellular signaling mechanisms.¹⁸

In this study, a new computer-aided time-lapse video-microscope system equipped with an image processing software, programmed for automated image analysis, were developed for the rapid and precise cell tracking. The migration of MC3T3-E1 osteoblast-like cells on the culture plate coated with different ECMs such as type I collagen (CN), laminin (LN) and FN was investigated using this cell tracking system, and the migration index (MI) was determined from the cell movement path and speed in order to estimate the time-lapse cell migration speed without further study at each time.

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MATERIALS AND METHODS

Materials
An inverted microscope (model IX70, Olympus Optical Co. Ltd., Tokyo, Japan) with a color charge-coupled device (CCD) camera (model VCC-3974, SANYO Electric Biomedical Co. Ltd., Osaka, Japan) was employed. The computer system with Intel Pentium III processor (CPU clock speed: 933MHz), 512MB main memory, 40GB hard disk drive and color line monitor was manufactured by SAMSUNG Electronics Co. Ltd. (Seoul, Korea). A temperature controller (model DX-4, Hanyoung Co., Seoul, Korea) with two temperature sensors and heating-tape was used to detect the mini-incubator’s temperature. All reagents were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) unless otherwise. Cell culture flask and 4-well cell culture plate for cell migration observation were obtained from Nalge Nunc International Corp. (Naperville, IL, USA).

Cell Culture and Conditions
The mouse pre-osteoblasts (MC3T3-E1) were obtained from ATCC (Manassas, VA, USA) and routinely maintained in α-modified minimum essential medium Eagle (αMEM, GIBCO BRL, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS) and a 1% antibiotic antimycotic solution (including 10,000 units penicillin, 10 mg streptomycin and 25 mg amphotericin B per ml) at 37°C in a humidified atmosphere of 5% CO₂ in air.

CO₂ Mini-Incubator
In order to incubate the cells on the stage of an inverted microscope, a CO₂ mini-incubator with 150 mm in length, 130 mm in width and 40 mm in height was self-made, and a double-layered acryl plate with 5 mm in thickness was covered on it. Two temperature sensors and heating-tape were used for monitoring and maintaining the temperature of the mini-incubator to 37°C. The mini-incubator was connected with a CO₂ incubator (model MCO-15AC, SANYO Electric Biomedical Co. Ltd.) and a mini-pump (model MP-603T) for supplying CO₂, and placed on the stage of an inverted microscope (Figure 1). After the inverted microscope with a color CCD camera conveyed the images from the mini-incubator as a real time-mode, a frame grabber card in computer captured these images at regular intervals and memorized them as bmp image files (Figure 1).

Cell Migration Assay
For analyzing the cell migration on the various ECM-coated culture plates, each surface of the culture plate was coated with 100 μg/ml CN, LN and FN, respectively and stabilized overnight at 4°C. The excess ECMs were completely removed and the ECM-coated culture plates were blocked with 2%

heat-treated bovine serum albumin for 2 hr at room temperature, followed by washing the surface of the plate twice with phosphate-buffered saline. On the culture plates prepared as above, MC3T3-E1 cell suspension was seeded at the density of 2x10⁵ cells/well and incubated for 24 hr in the mini-incubator. The mini-incubator was made as a closed system by sealing its edge with vacuum grease and covering it with a double-layered acryl plate. To test the quality of this system, a dot with micrometer diameter was marked on the surface of the same culture plate as that used in cell culture.

Image Processing for Single Cell Tracking
The captured 8-bit color images were incorporated into the image analysis software programmed by MATLAB V5.3 (Math-Work Inc., USA) and Visual Basic V6.0 language (Microsoft, USA) and converted into gray scale images. The edge point of each cell was detected and then the center of the detected edge was determined by the same method as previously described. The migration of the center was recorded and expressed as cell movement path and speed.

As shown in Figure 2, the cell movement speed was divided into three phases, attachment preparation phase (from 0 to T1...
hr), accelerated movement phase (from T1 to T2 hr) and speed maintenance phase (from T2 to 24 hr). In order to estimate the cell migration speed, the MI was determined by the following equation:

\[ MI = \frac{V_{max}}{(T2 - T1)} \]

where \( V_{max} \) is the maximum speed where the cell accelerated its speed and reached, T1 as the time where the accelerated movement phase began and T2 is the time where the speed maintenance phase began before reaching at the \( V_{max} \).

**Statistical Analysis**

All the variables were tested in an independent incubation for each experiment, and each experiment was repeated quadruplicate (n = 4). The results were reported as a mean ± standard deviation and analyzed by Student t-tests. Statistical significance was considered at \( p < 0.05 \).

**RESULTS AND DISCUSSION**

**Test of Self-Made System Quality**

The movement speed of a dot marked on the culture plate surface might be originally zero because the dot was a lifeless object. However, it was found that under an actual condition, the dot was regarded as an object moving at 16 μm/hr of an average speed by this cell tracking system (Figure 3). This phenomenon was shown to be attributed to a fine vibration or subtle temperature change.

**Cell Movement Path**

Figure 4 showed the morphologies of the migrated MC3T3-E1 cells on the FN-coated culture plate, which were obtained from the images captured at a 6 hr interval. As shown in Figure 5, the X and Y positions of the cells at each image were linked to generate the motility. The cell marked with 1 changed its position to right down via right up and the cell marked with 2 moved to right up, whereas the cell marked with 3 drew an irregular circle clockwise. Those three individual cells showed the different movement path and distance one another. The cell movement path on the CN- or LN-coated culture plate was similar to that on the FN-coated culture plate (data not shown here). From these results, it was revealed that the cell movement path had no common feature, suggesting that the cells might migrate without specific direction unless given signals to influence the direction. Furthermore, the cell movement was shown to be influenced by not the cell characteristics, but the ECM type.

**Cell Movement Speed**

Figure 6 showed that the cell moved slightly at the attachment preparation phase, from 0 to about 4 hr. It was well
known that most cells fully attached to the culture plate at 4 to 6 hr after seeding. In this study, 0 hr was at approximately 30 min after cell seeding, due to setting the culture plate into the mini-incubator. Considering this situation, T1 might be considered as the time when the cells prepared initial attachment. Then over the time T1, cell moved fast suddenly. From T1 to T2, the cell movement speed accelerated, resulting in an appreciable slope as steep as an angle of almost 45°. After the T2 time, the cell maintained its maximum speed at the $V_{\text{max}}$. Although the cell migration pattern after 24 hr could not be observed due to limitations of the cell culture period to 24 hr, it seemed that the $V_{\text{max}}$ would be maintained. The pattern of cell movement speed on the CN- or LN-coated culture plate showed similar results to that on the FN-coated culture plate (data not shown here).

The MI of the MC3T3-E1 cells on the FN-coated culture plate was 8.9, while those indexes on the CN- and LN-coated culture plates were respectively 5.3 and 5.1 (Table 1). The MI of the cells on the FN-coated plate was significantly ($p < 0.05$) than that on the CN- or LN-coated plate, but there were no significant difference between the plates coated with the CN and the LN. Greater value of MI was associated with a faster migration speed of the cell. These results suggested that all cells might have their own MI according to their species or phenotypes and ECM type, and the migration speed could be inferred from those values without further experimental study. In relation to this result, it was found that cultured fetal fibroblasts moved faster than neonatal fibroblast on CN, FN and hyaluronic acid.

A large number of studies have reported the cell migration through the change of cell population area. However, the precise movement path and speed of single cell could not be analyzed efficiently. To overcome these problems, an automated cell tracking system was developed and applied in this study. This system showed that the cells moved without any certain direction and the cell movement speed was divided into three phases, attachment preparation, accelerated movement and speed maintenance, though the concept of vector would not be contained in the speed. If the MI would be applied, it might be determined whether the stimuli, such as the electrical signal, cytokines and growth factor, might affect on the cell migration or not. Moreover, the accumulated cell movement distance from the cell movement speed could be obtained, which demonstrated the all distance of the cell movement for 24 hr regardless of direction. Further studies aimed at examining the entire mechanism of the cell migration on the different ECM are recommended.

**CONCLUSIONS**

This study was intended to examine the migration of MC3T3-E1 osteoblast-like cells on the culture plate coated with different ECMs such as CN, LN and FN using a newly developed cell tracking system, that was computer-aided time-lapse video-microscope system equipped with an image processing software programmed for automated image analysis. From the cell movement path and speed, the MI was determined in order to estimate the time-lapse cell migration speed. The MI of the MC3T3-E1 cells on the FN-coated culture plate was much greater than on the CN- or LN-coated culture plate. These results suggested that all cells might have their own MI according to their species or phenotypes and ECM type, and the migration speed could be inferred from those values without further study. Also, the cell tracking sys-

![Figure 5. Traced paths of the MC3T3-E1 cells on the FN-coated culture plate. Arrows indicated the starting points. The numbers represented the individual cells to be interested.](image1)

![Figure 6. MC3T3-E1 cell movement speed on the FN-coated plate.](image2)

| Table 1. Migration index of the MC3T3-E1 cells on the different ECM-coated culture plates |
|-----------------|----------------|
| ECM-coated      | MI$^a$        |
| on FN-coated    | 8.9±1.7*      |
| on CN-coated    | 5.3±0.4       |
| on LN-coated    | 5.1±2.9       |

$^a$MI = $V_{\text{max}}$ / ($T_2 - T_1$) from Figure 2.  
$^*$significantly different from the others, $p < 0.05$ ($n = 4$).
term would be exploited to craft strategies for the preparation of scaffolds for tissue engineering.

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