Micro-pattern to discriminate differentiated cell depending on migratory behavior through electrotaxis analysis

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Engineering and Management
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Directed by Professor Jong-Chul Park

The Master's Thesis
Submitted to the Department of Medical DeviceEngineering and Management,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree of
Master of Medical Science

Eun Jeong Go

December 2022

This certifies that the Masters's Thesis
of Eun Jeong Go is approved.

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The Graduate School
Yonsei University
December 2022

ACKNOWLEDGEMENTS

기대로 가득 찬 상태로 처음 연구실에 들어왔을 때가 생생하게 기억나는데 어느덧 2년의 시간이 지나 졸업을 맞이하게 되었습니다. 짧고도 긴 시간 동안 좋은 인연의 사람들을 만나 행복한 시간이었습니다. 제 인생에서 소중히 여길 수 있는 많은 것들을 깨닫고 배우고 떠나며, 이 자리를 빌려 저에게 관심과 조언을 해주셨던 분들께 진심을 담아 감사의 뜻을 전합니다.

우선 아낌없는 조언과 지도로 부족한 저를 무사히 졸업할 수 있도록 이끌어 주신 박종철 교수님께 감사의 인사를 드립니다. 연구 설계 초반부터 연구의 방향을 설정해주시고 석사 학위 과정 동안 꼼꼼한 피드백을 통해 큰 그림을 볼 수 있도록 깨달음을 주셔서 학문적인 배움을 더욱 넓혀나갈 수 있게 되었습니다. 진심으로 감사드립니다. 또한, 바쁘신 와중에도 자문 심사 때부터 많은 관심과 지도로 저의 학위 논문의 완성도를 높여 주신 성학준 교수님, 논문의 부족한 점을 잘 채워 주신 김자영 교수님. 심사를 맡아 주셨던 세 분께 진심으로 존경과 감사를 표합니다.

함께 연구실 생활을 함께 한 셀 바이오 컨트롤 연구실의 모든 분께 감사드립니다. 늘 격려해주시고 다독여 주시면서 전폭적인 지원을 아끼지 않으셨던 미희 박사님, 힘든 상황에서도 따뜻한 격려와 웃음을 주셨던 현숙 선생님, 늘 웃는 얼굴로 인사해주시고, 춘식이를 보면 늘 저한테 알려주시는 금손 혜진 선생님, 연구실에 처음 온 날부터 지금까지 연구실 생활에서 힘들고 어려운 일이 있을 때마다 항상 마음 써주시고 의지할 수 있었던 러블리 승희 언니 이 모든 분이 함께 있었기에 즐거운 대학원 생활을 할 수 있었습니다.

마지막으로 언제나 곁에서 지지해주시고 믿고 기다려주시는 부모님과 가깝지만 먼 우리 오빠에게도 사랑과 감사의 말을 전합니다. 아들 같은 딸로서 하고 싶은 일이라면 늘 응원해주시고 지원 해주셔서 이제까지 아무 탈 없이 잘 성장할 수 있었습니다. 아직은 어리게만 보인다며 다 큰 딸 곁에 두고 싶어 하셨던 엄마, 고민을 편하게 털어놓을 수 있고 진지하게 들어 주셨던 아빠, 매일 티격태격하지만, 나의 상황을 누구보다 더

공감해주고 조언해준 우리 오빠. 가족의 무한한 사랑에 보답할 수 있는 딸이 될 수 있도록 노력하겠습니다.

앞으로 이곳에서의 배움을 바탕으로 더 넓은 곳으로 나아가 큰 사람이 되겠습니다. 저를 응원해주신 모든 분께 고개 숙여 감사의 말씀 올립니다. 진심으로 감사합니다.

2022년 12월
고은정 드림.

Table of Contents

LIST OF FIGURES

Abstract

Micro-pattern to discriminate differentiated cell

depending on migratory behavior through electrotaxis analysis

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(Directed by Professor Jong-Chul Park)

Human tonsil-derived mesenchymal stem cells (TMSCs) isolated from human palate tonsil tissue have been suggested an important candidate for tissue regeneration due to their relatively high proliferation rate and versatility. Adipose derived stem cell(ADSCs) have potential for cell-based treatment because osteogenesis differentiation is not affected by the environment.

This study aims to analyze the migratory behavior of differentiated cells and stem cells through electrical stimulation (ES) and to suggest the possibility of cell discrimination and quality control using characteristics related to speed and direction. ES may induce

directional movement of cells to the anode or cathode. Depending on the cell type, organism, and species, cell migration may show different patterns for the cathode or anode during current flow. Discrimination of stem cells and differentiated cells depends on different migratory behaviors.

Electrotaxis experiments are conducted in customized agar-salt electrotaxis chamber wherein TMSCs are exposure to a 1200 μA electric current for 3 hr. When ADSCs were under 1200 μA ES, cell migration speed decreased as osteogenic differentiation progressed during electrical stimulation. For TMSCs, the directionality was completely changed to a cathode during myogenic differentiation period. In addition, to maximize the difference in the direction of cell movement due to electrical stimulation, it is intended to be confirmed using a micro-patterned surface. When investigating the migration direction of both types of stem cells (differentiated cells and stem cells) in relation to the ES, we found significant changes that the direction of cell migration increased on the micro-pattern surface. And then, we identified the possibility of controlling cells using microchannel. Analyzing cell migration on the same line can suggest an efficient method for observation compared to real-time analysis methods.

Key words : adipose derived stem cell, tonsil-derived mesenchymal stem cell, electrotaxis, migration, micro-patterned printing

Micro-pattern to discriminate differentiated cell depending on migratory behavior through electrotaxis anaysis

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I. INTRODUCTION

1. Stem cell-based therapy

Cell therapy using the regenerative potential of stem cells present in tissues, blood, and bone marrow has been used clinically over the last decade. These stem cells have offered potential regeneration of tissue and organs in clinical trials.[1,2] Adult stem cells, the stem cells often used in stem cell therapy, are found in the blood, fat, bone marrow, nerves, muscle, skin, cord blood, and placenta of the adult. Adult stem cells can differentiate into cells of specific tissues. They are exploited to grow and proliferate as tissue substitutes for

defective tissues. Mesenchymal stem cells are currently developing cell-based therapies for various diseases.[3]

Mesenchymal stem cells (MSCs) are clinical paradigm because of their properties that are tissue repair, regeneration and signaling repertoire such as immunomodulatory and anti-inflammatory effects. Therefore, MSCs commonly use in autologous transplantation. Several *in vitro* and *in vivo* studies are aimed at differentiation potential of mesenchymal stem cells into specific cell types and their ability to regenerate various tissue types.[4]

Many studies have been conducted on stem cell therapy to treat muscle-related diseases. Previous studies have demonstrated that human tonsil-derived MSCs (TMSCs) can differentiate from *in vitro* to muscle cells and that transplantation of muscle cells promotes recovery of muscle function after damage.[2, 5, 6] Adipose-derived stem cells (ADSCs) are known to play an important role in the treatment of osteoporosis, a common age-related disease in the world. Previous studies have demonstrated that treatment with ADSC prevents bone loss and can be used to treat osteoporosis caused by aging.[7-9]

2. Discrimination between differentiated cells and stem cells

Although MSCs have been an important candidate of treatment, heterogeneity of MSC properties hinders the comparing study conditions and becomes a source of confusion in cell therapy development.[4, 8, 10] Therefore, the heterogeneity of MSCs is potentially at risk when observed for a long time after treatment.[3] In recent studies, there is an immunological response to the risk caused by heterogeneity of MSCs. Clinical examples include injection of MSC into the joint to treat osteoarthritis and accompanied by pain and joint exudation or side effects. MSC suggests that low immunogenicity has been known as an advantage, but in many studies, it can induce a strong immune response *in vivo*.[12-15] For specific indications, the need for research and development of stem cell discrimination technology through detailed characterization of cells is increasing.[16-18] However, stem cell isolation methods are widely used, their efficiency and specificity are still lacking.[13]

3. Electrotaxis migration

Cell migration plays an important role in various physiological development, and disease-related processes, The basic principles of this process have been revealed over the years.[19] Cell migration occurs during the development and maintenance of multicellular organisms for wound healing, tissue regeneration, immunosuppressive and pathophysiological responses.[20] In addition, cell migration contributes to the pathology of injury and recovery, vascular disease, osteoporosis, infection, cancer, and deformity.[21, 20] Electrotaxis refers to the phenomenon of cells movement according to or opposite to the direction of electrical stimulation.[23] Electrotaxis can promote wound treatment and can activate regeneration for non-treatable wounds. The effect of external electrical stimulation has been studied through *in vitro* cells. Various types of cells exhibit directional movement under external electrical stimulation.

4. Controlling the cell movement through micro-patterning and microchannel chip

The mechanical properties of extracellular matrix (ECM) are well known to have a great influence on cell behavior. Cells form bonds with the ECM and move along topography. The ECM used here is composed of basic elements that make up complex tissue structures such as collagen, membrane, nerves, muscle bundles, and fat cells. ECM contacts with the surface to form a structural chemical complex. Cells bind to specific receptors and attach to ECM. This is the most important factor in signaling. The cells move along the guidance in the direction of current flow.[24] Because these characteristics, cell movement is not irregular and can induce high-direction movement.

The environment of microchannel has a unique characteristics of micro-scale flow phenomena, and has recently gained prominence as efficient tools for the control of cells.[25, 26] Therefore, controlling cells using microchannel can suggest an efficient method for cell migration observation.

5. Objective of this study

Various types of cells demonstrate different directional movement under electrical stimulation. We intend to observe the movement of stem cells by electrical stimulation to efficiently discriminate cells. By identifying the specific properties of cells movement to ES, we want to determine the possibility of cell discrimination and quality control.

In previous studies, when distinguishing differentiated cells and undifferentiated cells using electrotaxis analysis, it was difficult to define the direction of cells due to irregular movement. Therefore, we would like to identify that techniques for discriminating cells using micro-patterning can improve cell directionality. Furthermore, cell migration had to be observed in real time. Evaluation of cell movement needed many process. To solve these problems, we want to study the method that can efficiently separate cell movement through microchannel chip without continuous observation.

II. MATERIALS AND METHODS

1. Cell culture

ADSCs (Lonza, Basel, Switzerland) were cultured in ADSC growth medium (ADSC-GM, Lonza). TMSCs were provided by Dr. Jo in Ewha woman's university (Seoul, Korea) and cultured in Dulbecco's Modified Eagle's Medium low glucose (DMEM low glucose, Welgene, Seoul, Korea). Cells were incubated at 37˚C in a 5% CO_2 environment.

2. Osteogenic differentiation of ADSCs

Osteogenic differentiation (OsD) of stem cells was performed at defined passages 4 ~ 8. To induce OsD, the cells were seeded at a density of 1.0×10^5 cells per cm^2 into 75 T flask and cultured in Mesenchymal Stem Cell Osteogenic Differentiation (MODM, Sciencell, Carlsbad, CA, USA) until they reached 70 ~ 80% confluence. The cells should not reach 100% confluence before inducing OsD. The OsD medium was replaced twice a week for 14 days.

3. Calcium deposits analysis via Alizarin Red S assay

OsD cells were stained with Alizarin Red S Staining kit. (ARed, Sciencell) On 7 and 14 days, the cells were washed twice with Dulbeco's Phosphate-Buffered Saline (DPBS, Welgene), fixed with 4% paraformaldehyde (PFA) in PBS for 15 min at room temperature (RT). The fixative removed and rinsed the cells were rinsed 3 times with deionized water (diH_2O). After removing the diH_2O, the cells were cultured 2% Alizarin Red S Stain Solution for 20 ~ 30 min at RT. The cells were washed 3-5 times with diH_2O to remove non-stain solution. To imaging the cells morphology, the cells were put into diH_2O.

4. Myogenic differentiation of TMSCs

TMSCs were cultured in DMEM low glucose (Welgene) at 37˚C in a 5% CO_2 environment. After 70 ~ 80% confluent, the cells were seeded at a density of 3 ~ 4 × 10^6 cells in a 10 mm Petri dish with DMEM low-glucose containing 10% fetal bovine serum (Welgene) and 1% antibiotic-antimycotic solution 100 x (ABS 100 x, Welgene). After 1 ~ 2 days, a sphere about 50-100 μm in size formed and identified through a microscope (Fig. 1A). To induce the myogenic differentiation, the medium was changed from growth media to differentiation medium, DMEM/nutrient mixture F-12 (DMEM/F-12, Gibco, Thermo Fisher Scientific, Inc, USA) containing 1ng/ml transforming growth factor-β (TGF-β, R&D Systems Minneapolis, MN, USA), non-essential amino acids (NEAA, Invitrogen, Carlbad, CA, USA), insulin-transferrin-selenium (ITS, Gibco, Thermo Fisher Scientific, Inc) and ABS in collagen coated dish for 12 days.[2] After one day of incubation, cells were attached to the collagen-coated surface (Fig. 1B), and morphology of spheres spreading along the surface was confirmed after 3 days (Fig. 1C).

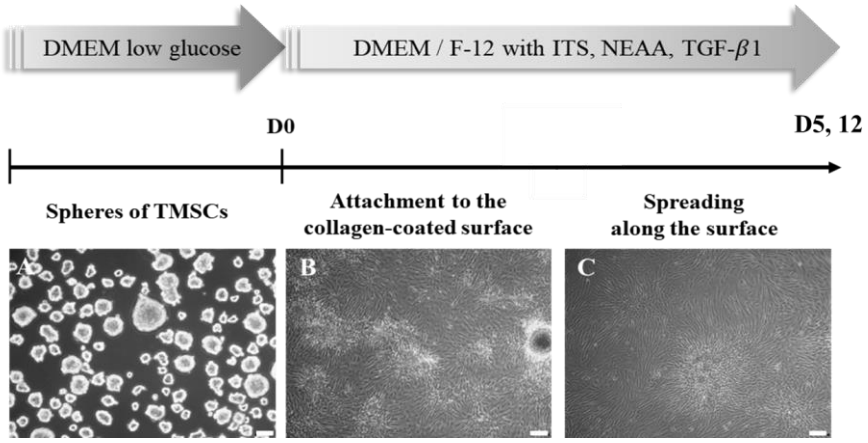

Fig. 1. Schematic representation of protocol of differentiation into muscle cell with corresponding cell morphology changes. (A) about 50-100 μm - sized sphere formed on a petri-dish. (B) TMSCs were attached to the collagen-coated surface. (C) morphology of spheres spreading along the surface. Scale bar = 100 μm.

5. Immunofluorescence of myogenic differentiation cells

The morphology of myogenic difference cells ware observed at each time using immunofluorescence. For immunofluorescence, cultured myogenic differentiation cells were seeded into a collagen coated 24-well culture plate with DMEM/F-12 containing 1 ng/ml TGF-β, NEAA, ITS and ABS. The cells were fixed with 4% PFA (Biosesang, Korea) for 15 min at RT and rinsed twice with DPBS. The cells were permeabilized with 0.25% Triton X-100 (Sigma-Aldrich, St. Louis, MO, USA) in DPBS for 10 min at RT and rinsed three times with PBS, then blocked with 1% bovine serum albumin (Bovogen Biologicals, East Keilor, Australia) for 30 min at RT. The cells were incubated with CD34, Pax7 (1:100; abcam, Cambridge, UK, ab187339), Myo D(1:200; novusbio, UK, NB100-56511), Dystrophin (1:1000; abcam,ab7164), α-SMA (1:200; cell signaling , Beverly, MA , #19245) and Calponin (1:100; cell signaling, #17819) for overnight at 4˚C. Subsequently, Alexa Fluor® goat anti-Rabbit IgG H&L (1:1000; Invitrogen) and Alexa Fluor® goat anti-Mouse IgG H+L (1:1000; Invitrogen) was used as secondary antibody for 1 hr at RT. Cell nuclei were stained with Hoechst#33258 (B2883, Sigma-Aldrich) for 1min at RT in the dark. The cell images were obtained using fluorescence-inverted microscope (LSM700, Carl Zeiss, New York, USA).

6. Electrotaxis migration

To stimulate differentiated cells and stem cells, Customized agar-salt electrotaxis incubator and chamber system were used. The system has a charge-coupled device (CCD) camera equipped to an inverted microscope (Olympus Optical Co. Kid. Tokyo. Japan) for observing cells and an incubator that creates an environment (5% CO_2, 37˚C) that cells are not damaged when the cells were observed. The cells were seeded in slide glass at density of 3 ~ 5×10^3 cells and incubated overnight at 5% CO_2, 37˚C. Then, slide glass was put on the electrotaxis migration chamber and used incubator and chamber system. The cells are affected electrical stimulation at 1200 μA for 3 hr or pulsed.

Cell movement against electrical stimulation was carried out using a CCD camera to obtain an image each 5 min, and it was observed for a total of 3 hr. chemotaxis tool plug-in of Image J software (v. 1.01, distributed by ibidi GmbH, Munchen, Germany) was used for cell migration analysis. Tracking was performed along the nucleus of cell, and the value of cell movement speed and direction was calculated. The speed indicates how fast the movement of cells against electrical stimulation, which was calculated by dividing the total movement distance by the measurement time. The directedness indicated that the + value moves toward anode and the – value moves toward cathode. Therefore, increasing of directedness value indicates that cells move more horizontal. That is, +1 is defined to move horizontally in the anode direction, and -1 is defined to move horizontally in the cathode direction. Directedness is the division of the X-axis distance in cell migration by the shortest travel distance and is the equal as the $\cos\theta$ value for the angle between X-axis and

shortest travel distance (Fig. 2).

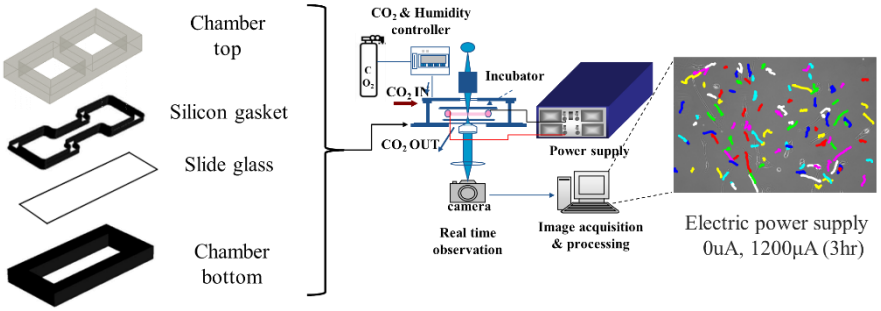

Fig. 2. Schematic representation of system electrotaxis chamber and incubator for the evaluation of cell migration.

7. Micro-patterned printing

For producing the Poly dimethyl siloxane (PDMS) mold, SiO_2 wafer was designed. The SiO_2 wafer was consisted of an adhesive region of 80μm and antiadhesive region of 150 μm. PDMS (SYLGARD™ 184 Silicone Elastomer, dow corning, USA) was used as mold for micro-patterned printing. PDMS mold was mixed at 10:1 (w:w) base : curing agent and heated at 90˚C for 6 hr. After the solution hardens, the mold was cut size of 1 x 1cm Fluorescent fibronectin conjugated Alexa 488 was placed on the PDMS mold for 30 min to coat the surface. Then, the surface was rinsed twice with sterile water and dried with a stream of compressed nitrogen gas. Nitrogen gas is an inert gas that prevents other substances from binding on the mold.

Before micro-patterned printing, the surface of slide glass was activated through an ultraviolet ozone cleaner for 10 min. The fibronectin-coated mold was then placed in conformal contact with the surface for 10 min (Fig. 3). Surfaces patterned with fluorescently conjugated proteins images were acquired through fluorescence-inverted microscope (LSM700).

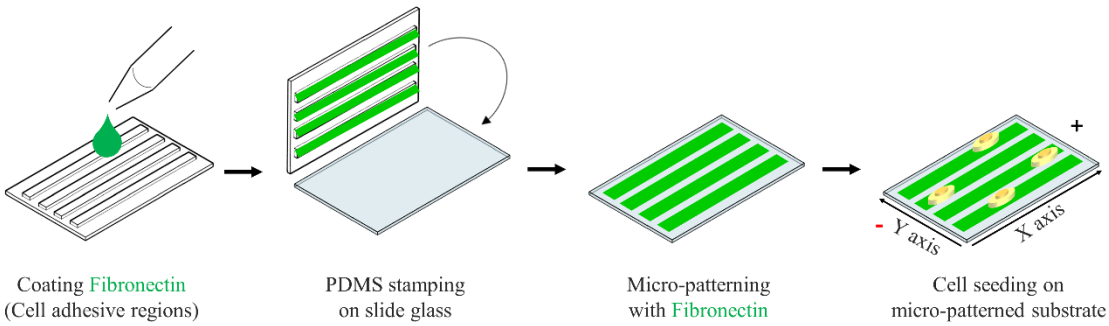

Fig. 3. Schematic representation of protocol of micro-patterned printing of fluorescent fibronectin (green) for the evaluation of the cell migration. Scale bar = 100 μm

8. Start line using microchannel chip

PDMS chip of microchannel was produced SiO_2 with designed wafer. The SiO_2 wafer was consisted of an adhesive region of 100μm (Fig. 4A). PDMS chip was mixed at 10:1 (w:w) base curing agent and heated at 90˚C for 6 hr. After applying the microchannel chip on micro-pattered surface to block the cell antiadhesive regional attachment, the cells were seeded on the same line (Fig. 4B). After incubation for 2 hr, microchannel chip was removed. The cells seeded on same line were identified through a microscope.

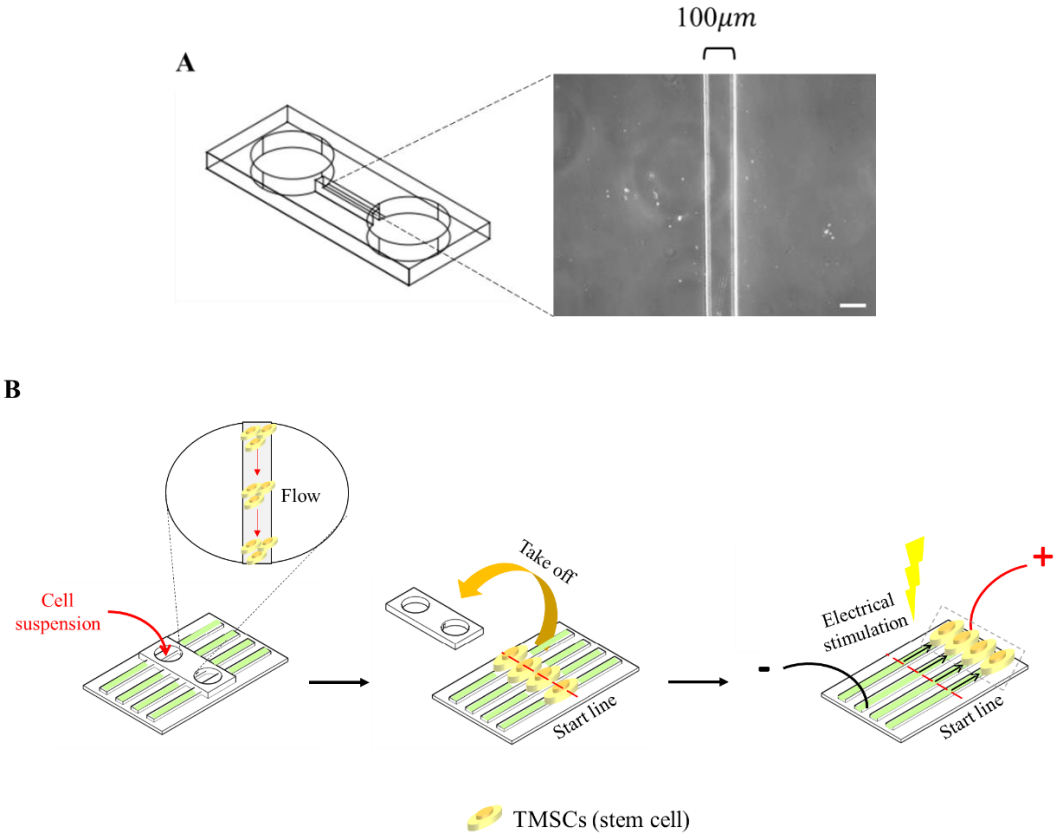

Fig. 4. Schematic representation of protocol of start line using microchannel.

(A) topographies of microchannel chip of PDMS. Scale bar = 100 μm, (B) start line with microchannel.

9. Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM). All statistical analyses were performed using SPSS 23.0. Means were compared using one-way analyses of variance (ANOVA). A value of $p < 0.05$ was considered statistically significant.

III. RESULT

1. Osteogenic differentiation of ADSCs and Alizarin Red S assay

ADSCs have potential to differentiate into osteoblast cells.[18, 19] So, ADSCs were induced to osteogenic differentiation and investigated whether cells are differentiated well through Alizarin Red S staining. These assays can observe the calcium deposition in osteogenic differentiation cells. Calcification deposits are observed as bright red or orange dots[17] (Fig. 5A) and demonstrates microscopy images of ADSCs and osteogenic differentiated cells. After 7 days, orange dots were found to have increased gradually. As the differentiation period increased, calcium deposition was strongly expressed in red (Fig. 5B). After 14 days, osteogenic differentiation was complete (Fig. 5C, D).

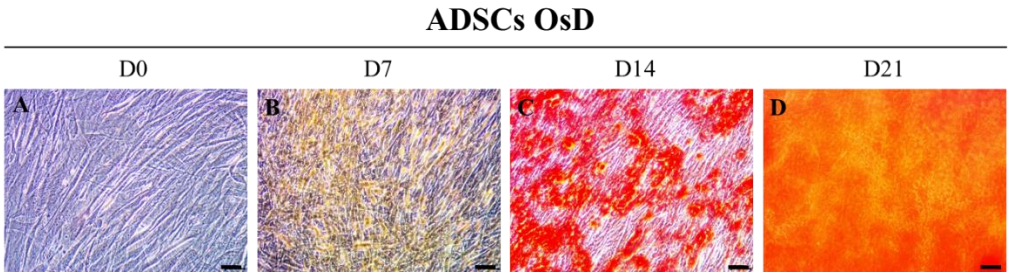

Fig. 5. The Alizarin Red S staining analyses for the ADSCs during osteogenic differentiation. The microscopy images of ADSCs osteogenic differentiation on (A) day 0, (B) day 7, (C) day 14, (D) day 21. Scale bar = 50 μm.

2. Immunofluorescence of myogenic differentiation of TMSCs

In order to confirm the presence of muscle cell differentiation of TMSC, the expression of major markers was observed through immunofluorescence staining. The markers used stem cells (CD34), satellite cells (Pax7), Myoblast cells and skeleton muscle cells (MyoD), Myoblast cells and smooth muscle cells (a-SMAs), and smooth muscle cells (calponin). CD34 and pax7 were expressed in undifferentiated TMSCs, but other antibodies were not. Specifically, we found that the expression patterns of MyoD and calponin were different between myogenic differentiation cells and smooth muscle cells. In the case of MyoD, it was strongly expressed in day 3, which is an early myogenic differentiation stage. These results can be seen as differentiation into muscle cells over differentiation periods. However, during differentiation and later in life, MyoD was not expressed, and the expression of muscle cells and smooth muscle cell markers was increased. These results indicate that during and after differentiation, they are derived as skeleton muscle cells in the early stages of differentiation, but they are differentiated into smooth muscle cells (Fig. 6).

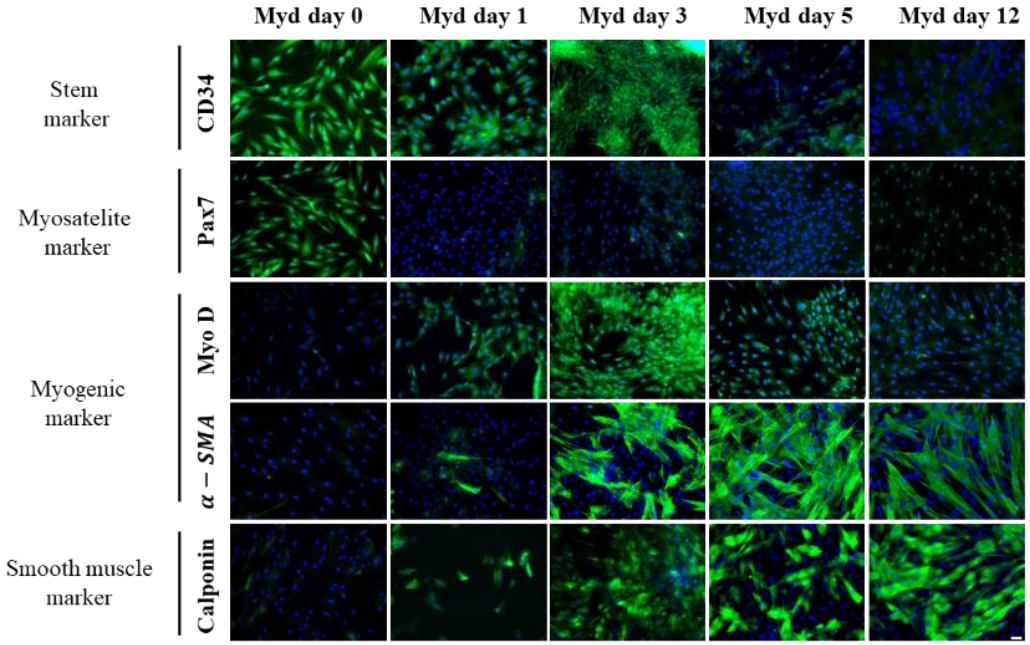

Fig. 6. Immunofluorescence of myogenic differentiation of TMSCs. Scale bar = 50 μm.

3. Discrimination of differentiated cells and stem cells using electrotaxis migration

Cell migration in response to electrical stimulation helped analyze characteristics of differentiated and undifferentiated cells. Both ADSCs and TMSCs were observed one day after seeding. For ADSCs, electrical stimulation was applied for 3 hr using the customized electrotaxis chamber and incubator system on osteogenic differentiation days 7 and 14. As a result, there was no specific change in cell migration directionality during electrical stimulation. However, cell migration speed of ADSCs decreased as osteogenic differentiation progressed (Fig. 7A). The orientation of both undifferentiated and osteogenic differentiated cells migrated toward to the anode direction (Fig. 7B, C).

TMSCs migration were observed on day 1, 3, 5, and 12 of myogenic differentiation. TMSCs migration speed did not appear to change during electrical stimulation (Fig. 8A). However, when electric stimulation was given, it was confirmed that the directionality changed to anode on day 0 in the state of undifferentiated cells, but the directionality started to decrease at day 1 and changed toward the cathode on day 3. During the subsequent differentiation period, the direction was completely changed to a cathode (Fig. 8B, C).

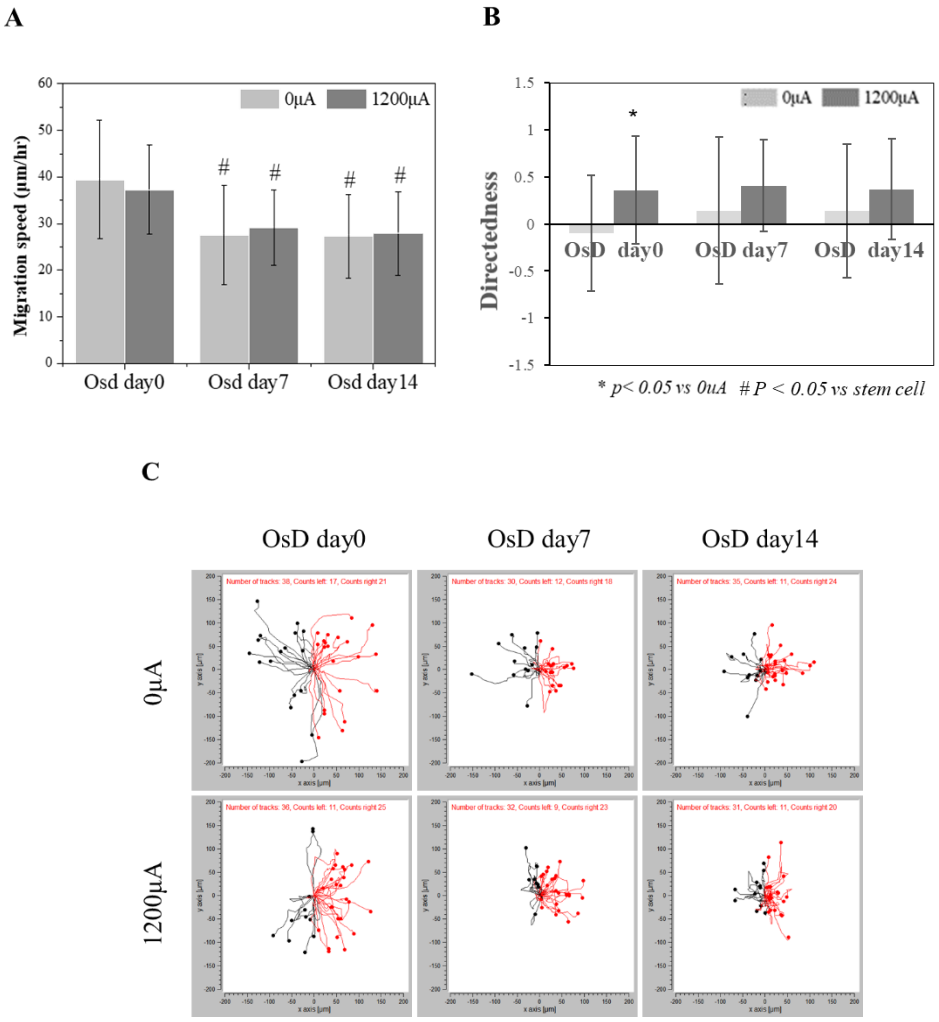

Fig. 7. Cell migration of ADSCs on 1200 μA electrical stimulation for 3 hr. (A) migration speed of TMSCs, (B) directedness of ADSCs and osteogenic differentiation cells, (C) cell tracking images Scale bar = 50 μm.

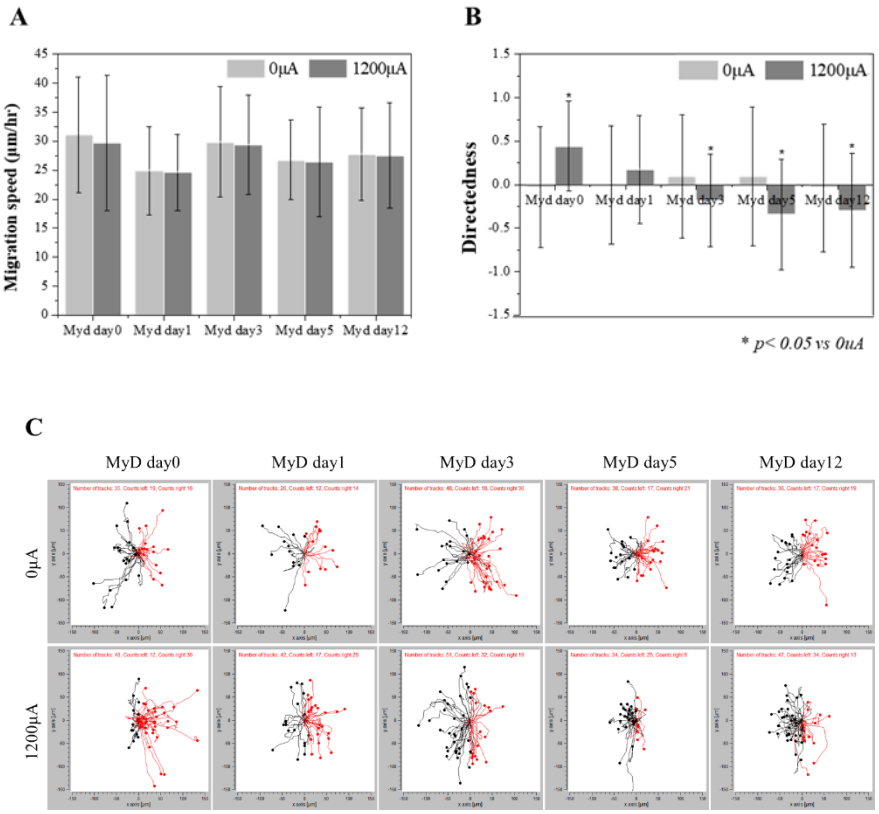

Fig. 8. Cell migration of TMSCs on 1200 μA electrical stimulation for 3hr. (A) migration speed of TMSCs, (B) directedness of TMSCs and myogenic differentiation cells, (C) cell tracking images. Scale bar = 50 μm.

4. Enhancement of directionality on Micro-patterned surface

Cells have the property of moving randomly. Therefore, it was difficult to objectively analyze the direction of cell movement in previous experiments. To solve this problem, a guide was manufactured on a surface with fibronectin, one of the types of ECM. The width of the guide to which the cell was attached was 80 μm, and the width of the part to which the cell was not attached is 150 μm. The fibronectin coated with Fluor 488 was identified through a fluorescence microscope using ground fibronectin (Fig. 9). Before seeding the cells, PLL-g-PEG was treated to prevent cells from adhering to any part of the surface except on the guide. The subsequent change in cell migration on the reformed surface was analyzed. As a result, there was no specific change in cell migration speed compared to randomly seeded cells (Fig. 10, 11A). However, there was a marked increase in direction (Fig. 10, 11B). These results were able to obviously discriminate the directionality because the cell migrates along a guide consisting of fibronectin (Fig. 10, 11C). In the case of muscle cell differentiation cells of TMSC, it was observed that their direction was opposite to that of undifferentiated cells. Therefore, using micro-patterned printing increased objective identification of cell characteristics.

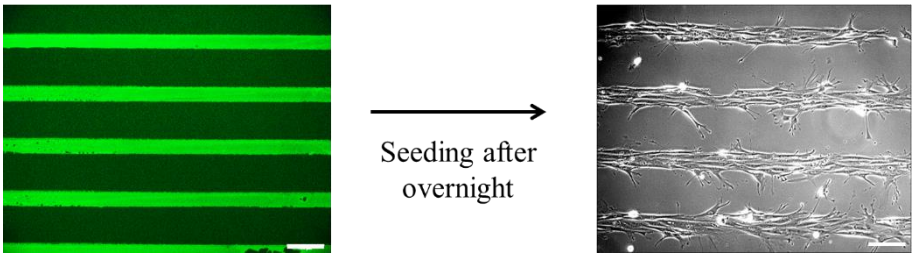

Fig. 9. Morphology of TMSCs on micro-patterned surface. Scale bar = 100 μm.

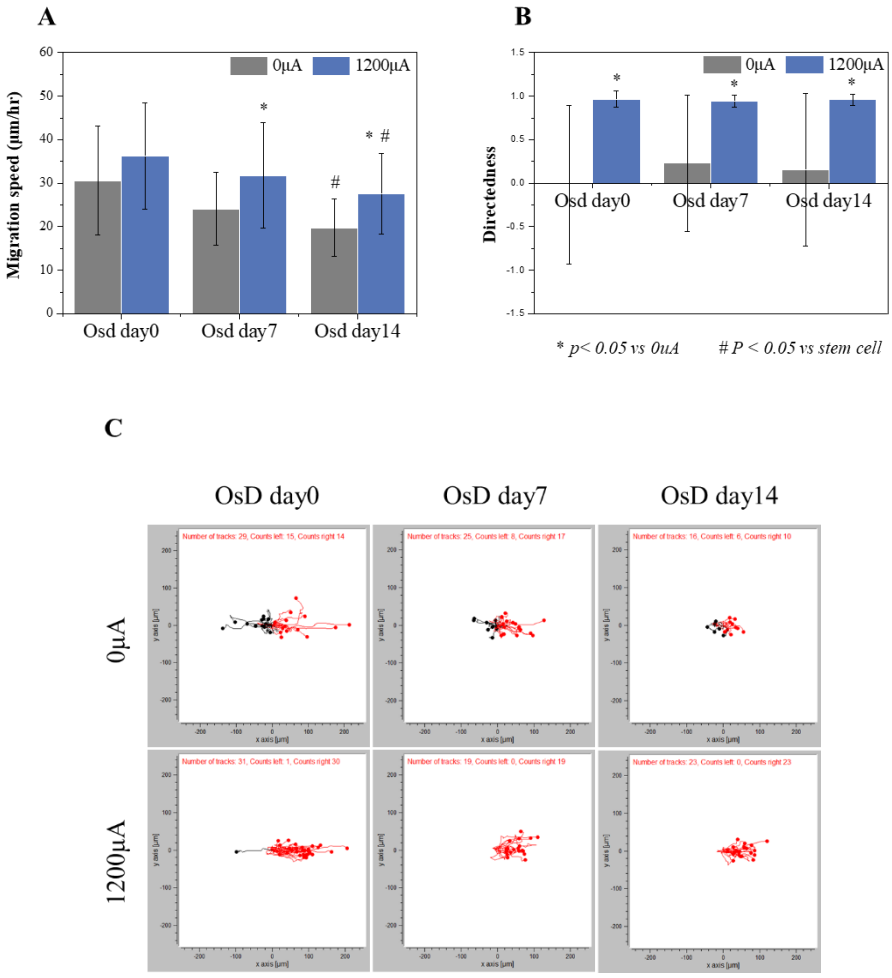

Fig. 10. Cell migration of ADSCs on 1200 μA electrical stimulation for 3 hr on micro-patterned surface. (A) migration speed of TMSCs, (B) directedness of TMSCs and myogenic differentiation cells, (C) cell tracking images. Scale bar = 50 μm.

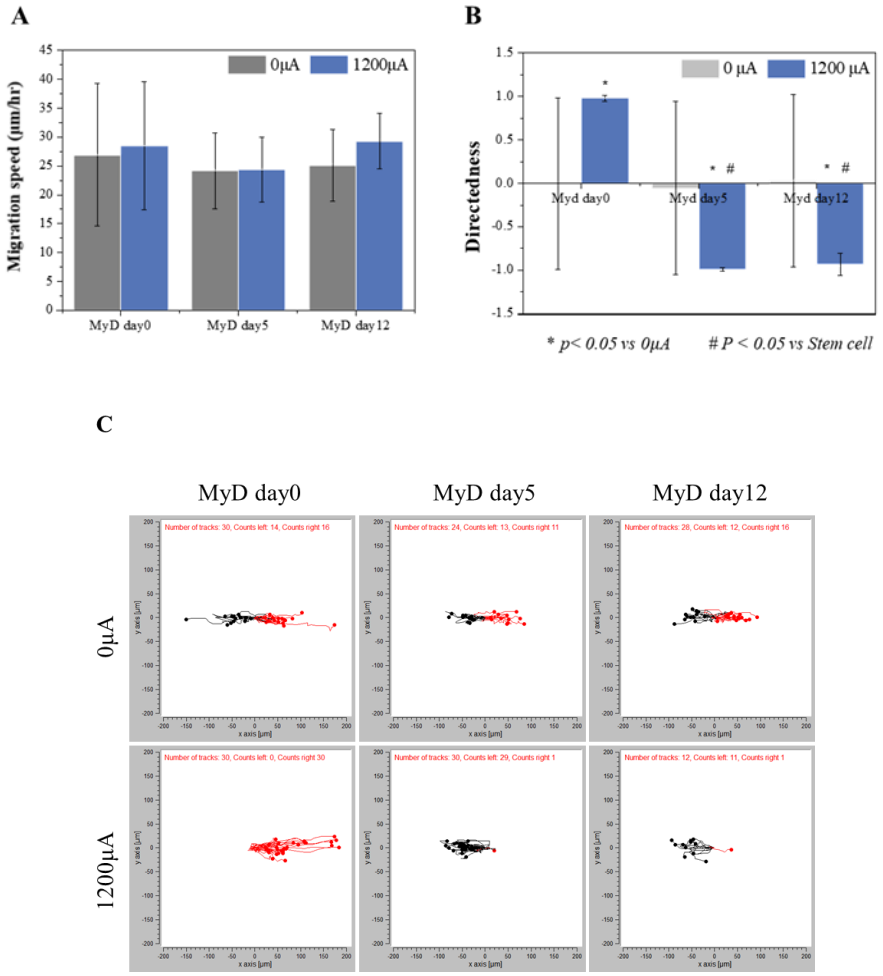

Fig. 11. Cell migration of TMSCs on 1200 μA electrical stimulation for 3 hr on micro-patterned surface. (A) migration speed of TMSCs, (B) directedness of TMSCs and myogenic differentiation cells, (C) cell tracking images, Scale bar = 50 μm.

5. Start line using microchannel chip

We observed cells mobility on start line surface. It was confirmed that the cells flow by the microchannel, when the stem cell was seeded after the microchannel chip was placed on the micro-patterning surface. After incubation for 2 hr, cells were attached to the inside of the microchannel (Fig. 12). And then, when the microchannel chip was removed, it was confirmed that the cells were attached on the same line and electrotaxis experiment was performed. The results of the experiment demonstrated that the cell started at the same location, but at 0uA, it moved randomly in the anode and cathode (Fig. 13A). However, when 1200μA of electrical stimulation was applied, it was confirmed that cells were located in a specific region when electrical stimulation was applied with pulsed while moving in the anode (Fig. 13B).

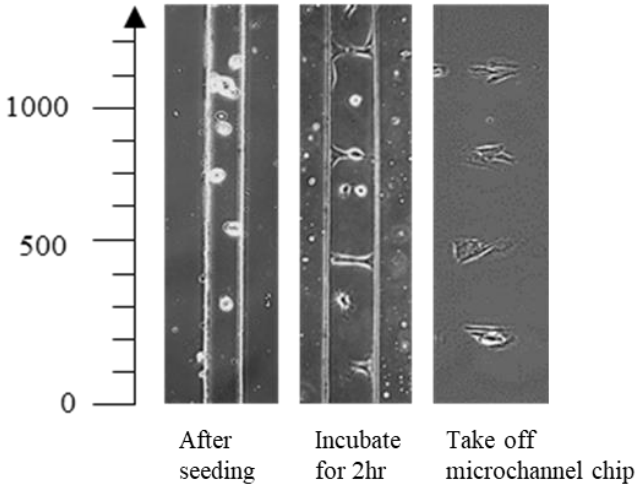

Fig. 12. Morphology of TMSCs on start line surface.

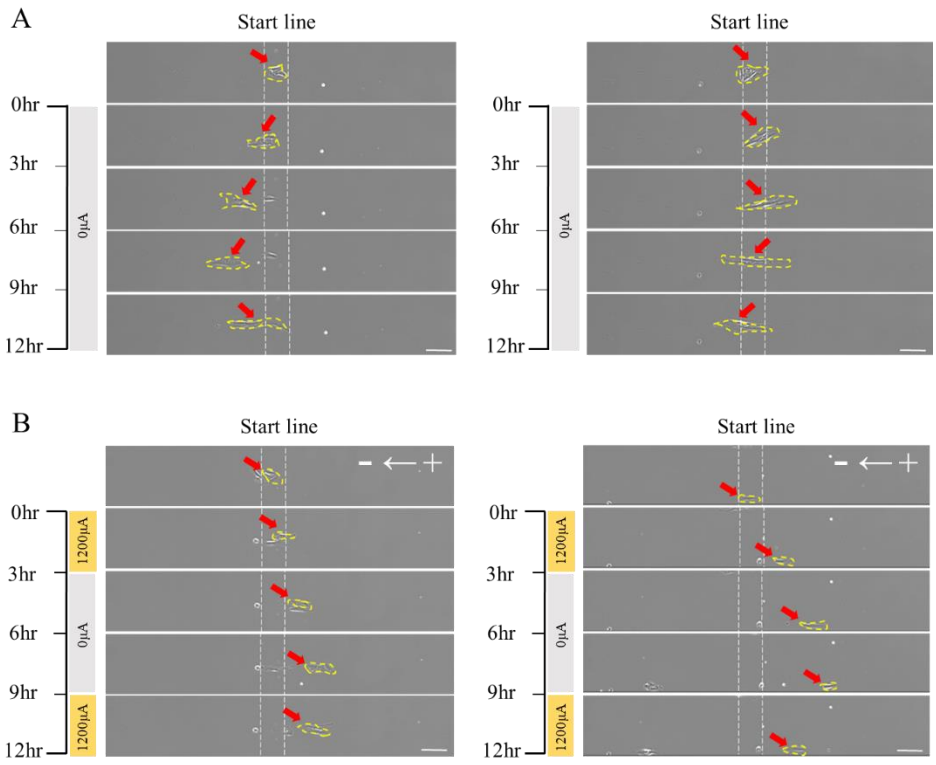

Fig. 13. Cell tracking images of TMSCs. (A) on 0 μA electrical stimulation for 12 hr (B) on 1200 μA electrical stimulation for 3 hr, followed by rest re-stimulation for 3 hr on start line surface, Scale bar = 100 μm.

IV. DISCUSSION

In the stem cell therapy, high-purity for therapeutic purposes is critical, because there are serious side effects to cell therapy, such as immune responses. Electrotaxis is characterized by movement according to cell type by electrical simulations. Through this property, when electrical stimulation was applied in *in vitro,* it was intended to check the change in movement speed and movement direction between undifferentiated and differentiated cells.[27] The mechanism of cell movement is known to be promoted by physical signals such as electrical stimulation. Recent studies have supported the hypothesis that electrical signals result in movement by inducing cell surface or signal molecule reorientation.[28] Using these properties, we observe movement to electrical stimulation of differentiated and undifferentiated cells, and based on the results, we suggest the possibility of cell discrimination.

In order to observe the movement of differentiated cells to electrical stimulation between differentiated cells and undifferentiated cells, ADSC was induced to differentiate osteoblasts. Osteogenic differentiation medium induced differentiation of osteoblasts. Since calcium deposition is produced on the cell surface over the differentiation period, the presence or absence of differentiation was confirmed through Alizarin Red Stain. As a result, a pale orange color was formed on the 7 days of differentiation, and on the 14 days, bone differentiation was confirmed through the generation of noticeable red dots. TMSCs were differentiated into muscle cells. In the early stages of differentiation, the skeleton muscle cell differentiation marker was strongly expressed during and after differentiation.

This study analyzed cell migration to electrical stimulation with ADSCs and TMSCs. ADSCs induced bone differentiation and TMSCs were differentiated into muscle cells. The movement characteristics of each differentiated cell were compared against the undifferentiated stem cells.

ADSCs decreased in speed as they differentiated into osteoblasts both with and without electrical stimulation of 1200μA. Regarding the directional aspect of the cell, both cells moved to anodes. There was no specific change in directionality. TMSCs had no specific change in speed due to the presence or absence of electric stimulation. However, the direction changed from the anode to cathode as differentiation progressed with electric stimulation of 1200 μA. These results are thought to change directionality by specific cell signals as TMSCs differentiate into muscle cells.

Further research is needed on the principle. A study related to the movement of Myoblast's electrical stimulation suggested the possibility that external mechanical stretching results in satellite cell activation, which can affect intracellular signal transmission to cathodes.[9]

In previous studies, cell migration was random, making it difficult to objectively analyze. Therefore, a way to clearly analyze the direction was sought. And a guide was prepared by micro-patterned surface with fibronectin, one of the types of ECM. ECM consists of basic elements that make up complex tissue structures such as collagen, membrane, nerves, muscle bundles, and fat cells. ECM encounters the surface to form a structural chemical complex. It binds to a particular receptor and attaches to the ECM.[29] Therefore, cells move

along the guide printed with the ECM, which is horizontal, so the directionality is increased. As a result, the direction could be analyzed more clearly.[29, 30]

V. CONCLUSION

In conclusion, applied electrical stimulation can discriminate between differentiated and undifferentiated ADSCs using the characteristics of speed change and for TMSCs using the characteristics of directional change. As the differentiation progresses, the speed of ADSCs decreases, and the directionality of TMSCs is reversed. It was confirmed that micro-patterned printing can be used to further maximize cell orientation and objectively analyze it. Compared to when there was no electrical stimulation, the directionality was clearly distinguished. In addition, when TMSCs are differentiated into muscle cells using myogenic differentiation medium, they are differentiated into myogenic differentiation cells at the early stages of differentiation, but they are induced to smooth muscle cells as the differentiation progressed. These results suggest the possibility of quality control and regenerative medicine of stem cell therapy.

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ABSTRACT (IN KOREA)

전기 주성 분석을 이용한 이동 행동에 따른 분화 세포를 분별하는 마이크로 패턴

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고은정

인간의 편도 조직으로부터 분리된 인간 편도 유도 줄기세포(TMSC)는 상대적으로 높은 증식 속도와 다능성으로 인해 조직 재생의 중요한 후보로 제시되어 왔다. 그리고 지방 유래 기질 세포(ADSC)는 골형성 분화가 환경에 많은 영향을 받지 않기 때문에 세포 기반 치료의 잠재적인 가능성이 있다.

본 연구의 목적은 전기자극을 이용한 분화세포와 줄기세포의 이동 패턴을 분석하고 속도와 방향성에 관한 특성을 이용하여, 세포 분별 및 품질관리의 가능성을 제시하고자 한다. 직류 전기자극(ES)는 세포의 양극 또는 음극으로 이동 방향을 유도할 수 있다. 세포의 종류, 유기체 및 종에 따라, 세포 이동은 양극 또는 음극에 대해 다른 패턴을 보일 수 있다. 따라서 줄기세포와 분화 세포를 분별하는 것은 전류에 의한 이동 행동에 따른다.

전기 주성은 1200μA의 전류를 3시간 동안 노출시키 위해 맞춤형 한천염 전기 주성 챔버에서 수행되었다. 1200μA의 전류에 의해 ADSC는 골세포분화가 진행됨에 따라 이동 속도가 감소하였다. TMSC의 경우, 근세포분화가 진행됨에 따라 방향성이 양극으로 완전히 변화하였다. 또한, 전기 자극에 대한 세포의 이동 방향의 차이를 극대화하기 위하여 마이크로 패턴화된 표면을 이용하여 확인하였다.

두 종류의 줄기세포(분화 세포와 줄기세포)의 이동 방향을 분석하였을 때, 마이크로 패턴 표면에서 세포 이동 방향이 증가하는 유의한 변화를 확인하였다. 또한, 마이크로 채널을 사용하여 세포를 제어할 수 있는 가능성을 확인하였다. 동일선상에 위치한 세포 이동을 분석하는 것은 실시간 분석법과 비교하여 효율적인 방법을 제안할 수 있다.

핵심되는 말 : 지방유래 성체줄기세포, 편도 유래 중간엽 줄기세포, 전기주성, 세포 이동, 마이크로 패턴 프린팅