Identify the possibility of cell migration regulation on PLGA coated surface by electrotaxis

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Directed by Professor Jong-Chul Park

The Master's Thesis
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Min Song Kim

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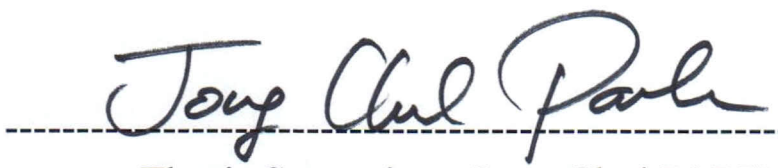
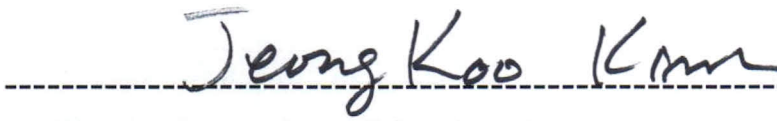
This certifies that the Master's Thesis
of Min Song Kim is approved.



Thesis Supervisor: Jong-Chul PARK



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The Graduate School
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June 2013

Acknowledgement

2008년 무더웠던 여름, 세포제어연구실과 처음으로 인연을 맺고 실험실 생활에 적응하고자 노력하며 지냈던게 엊그제 같은데 어느덧 시간이 흘러 석사학위 졸업을 목전에 두게 되었습니다. 저에게 있어서 지난 2년간의 석사생활은 저의 부족함을 채우고 노력하는 자세와 열정을 배울 수 있었던 뜻깊은 시간이었습니다. 제가 석사과정을 무사히 마칠 수 있도록 많은 도움을 주신 모든 분들께 감사의 말씀을 드리고 싶습니다.

우선 제가 세포제어연구실에서 연구할 수 있는 기회를 주시고 실험을 진행함에 있어 어려움에 부딪힐 때마다 따뜻한 격려와 올바른 방향을 제시해 주셨던 박종철 교수님께 진심으로 감사의 말씀을 드립니다. 교수님의 가르침과 보살핌 덕에 석사학위 논문을 잘 마무리 지을 수 있었습니다. 또한 저의 졸업을 위해 바쁘신 와중에도 시간을 내어주신 이재면 교수님, 먼 곳에서부터 오시느라힘드셨을텐데도 그런 내색없이 자문 및 심사를 해주신 김정구 교수님, 졸업논문 주제와 관련하여 많은 도움을 주신 남기창 박사님께도 감사드립니다.

저의 독특한 성격을 잘 이해해주고 받아준 세포제어연구실 구성원 여러분들께도 감사의 마음을 전합니다. 실험적으로나 정신적으로 큰 도움을 주셔서 너무나도 감사한 미희누나, 언제나 멋지고 유쾌하시며 가슴 속 열정 가득한 혁진형님, 동생인데 누나같은 듬직한 병주, 누나인데 동생같은 여린 마음의 소프라노이자 화가인 경은누나, 얼굴보다 등을 더 많이 보여준 테너톤의 착한 민아, 키가 제일 큰 막내 혜진이, 엠텍의 산 증인이시자 큰 누나이신 현숙누나,

늘 여유와 웃음을 잃지 않으시는 태화누나, 근면성실하시고 꼼꼼하신 능력자 대형이형, 성시경과 다모의 영원한 팬 재경이, 현대중공업서 배가 터지도록 달리고 있을 정현이, 늘 나를 어색해 하시는 연이누나, 결혼준비로 한창 바쁘실 수창형님, 독일서 열심히 공부하시는 동정누나, 푸른 눈 금발머리의 Barbora, 새 직장에서 열심히 일하고 계실 현용형님까지 모두들 고맙습니다. 앞으로도 좋은 인연 쭉 이어가도록 노력하겠습니다.
더운 날씨에도 각자의 목표를 위해 열심히 공부하고 있는 진태와 우상이, 늘 건강하길 바라는 희산이와 상준이, 매일 야근하느라 바쁜 상민이, 유한 성창태, 내가 사랑하는 고등학교 친구들, 지유아빠 규호, 멋진 승제, 미남 기준, 꿀피부 원호, 잘생긴 동석, 이안아빠 병국, 건축가 상우, 갑중의 갑 현덕, 몸짱 태준, 우리나라 금융계를 짊어질 세황, 전약사 사장님, 너무너무 멋지고 본받을 점이 많은 친구 권우까지 모두들 사랑하고 고맙다.
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2013년 7월
김 민 성 드림

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(Directed by Professor Jong-Chul Park)

The area of tissue engineering has been studied in order to satisfy a large number of needs for organs and tissues. The synthetic materials which were developed for tissue engineering often integrate poorly with host tissue and fail over time due to wear and fatigue or adverse body response. Numerous experiments have shown that cells need an appropriate biomatrix or

scaffold as an optimal cell support to develop their typical differentiated phenotype in vitro. In tissue engineering, it is desirable for the cells to be evenly distributed in the scaffold to obtain a uniform mechanical strength of the engineered tissue.

Cell migration is an essential activity of the cells in various biological phenomena such as embryonic development, wound healing of damaged tissue, capillary vascularization in angiogenesis and migration of leukocytes to kill the bacteria around the wound site. Control of cell migration is the one way to spread the cells on the surface of 3D scaffolds and make the cells infiltrate into the scaffolds.

The electrotaxis was used in this study to control the cell migration. We designed a new electrotaxis system because the established system focused on the investigation of electrotaxis mechanism and is not suitable for changing direction of dc EFs. nHDFs showed directional migration towards cathode in dc EFs (1 V/cm), and moved toward opposite direction when the polarity of dc EFs was reversed. The reorganization of actin cytoskelecton and polarization of golgi apparatus were also evaluated by immunostaining. The actin cytoskeleton elongated towards the cathode, and the golgi apparatus were polarized along the EFs. This study identified the possibility of cell migration control on the PLGA scaffold by electrotaxis.

Key words: cell migration , tissue engineering, electrotaxis, fibroblast, PLGA scaffold

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I. Introduction

1. Scaffolds in tissue engineering

The area of tissue engineering has been studied in order to satisfy the tremendous need for organs and tissues[1]. The generation of artificial cartilage and bone is used for treating articular injures or argument defects of ear, nose and neck in plastic surgery. The creation of artificial skin helps burned patients and

artificial kidney is used to hemodialysis [1].

Grafting requires second surgical sites with associated morbidity and is restricted by limited amounts of material, especially for organ replacement. Synthetic materials often integrate poorly with host tissue and fail over time due to wear and fatigue or adverse body response[2]. The concept is to transplant a biofactor (cells, proteins and/or genes) within a porous degradable material known as a scaffold. A successful scaffold should balance mechanical function with biofactor delivery, providing a sequential transition in which the regenerated tissue assumes function as the scaffold degrades[2].

2. Infiltration of cells into scaffolds

Three-dimensional, porous polymer scaffolds are common in many tissue engineering strategies. A wide array of architectural configurations and geometries can be created using scaffold fabrication technologies such as melt extrusion, rapid prototyping, phase separation, emulsion templating, salt leaching, and electrospinning[3].

Synthetic biodegradable polymers are now widely used in tissue engineering due to their generally good strength and adjustable degradation speed. In tissue engineering, it is desirable for the cells to be evenly distributed in the scaffold to obtain a uniform mechanical strength of the engineered tissue. For this reason, cells need to be spread widely and should infiltrate into the 3D scaffolds to give enough mechanical strength and adjustable degradation time to the scaffolds[4].

3. Cell migration control

Control of cell migration is the one way to spread the cells on the surface of 3D scaffolds and make the cells infiltrate into the scaffolds. One important function of the cells is to migrate from the site to the other site, in response to chemical mediators such as chemokines, a process called chemotaxis[5]. Chemotaxis could induce the directional migration of cells, but it is not good for controlling the migration of cell because the chemokines have an extra residue[5]. Fluid shear stress enhanced endothelial cell migration in flow direction and called this ‘‘mechanotaxis’’[6]. Mechanotaxis is an important mechanism controlling endothelial cell migration but changing the direction of flow is difficult. Applied EFs guide migration of many types of cells, including endothelial cells to migrate directionally[7]. We call this “electrotaxis” and in electrotaxis, cells move with a directional tendency toward the anode or cathode under direct-current electric fields (dcEFs), so we use electrotaxis to control the cell migration.

4. Established electrotaxis

Electrotaxis has been established experimentally on a case-by-case basis because the effects of direct current (DC) electric fields on cells are cell-type and species specific. In many cases, cells such as rat osteoblasts, bovine chondrocytes, bovine aortic vascular endothelial cells, and mouse endothelial progenitor cells migrate towards the cathode. However, some cells including rabbit osteoclasts, human osteosarcoma cells, rabbit corneal endothelial cells, and human umbilical vein endothelial cells migrate in the opposite direction, towards the anode[8].

In many cases for electric field application, a DC EF was applied through agar-salt bridges connecting silver/silver chloride electrodes in beakers of Steinberg's solution, to pools of culture medium on either side of the chamber. Using agar-salt bridge and Steinberg's solution is not convenient system for studying electrotaxis.

5. Objectives of this study

The aim of this study is to identify the possibility of controlling the cell migration on the surface of PLGA scaffold using a electrotaxis incubator and chamber system which is a new designed system for more convenient to study electrotaxis. The effect of electrotaxis was evaluated in electrotaxis incubator and chamber system as the replacement of established electrotaxis system. To investigate the possibility of cell migration control, electrotaxis of nHDF was evaluated on PLGA coated slide glass. Also, immunostaining was carried out to confirm the reorganization of cytoskeleton and polarization of golgi apparatus.

II. Materials and methods

1. Cells and cell culture conditions

MDA-MB-231 were purchased from ATCC (Rockville, MD,USA) and maintained in Dullbecco's modification of eagle's minimal essential medium (DMEM) supplemented with 10% fetal bovine serum (FBS, Lonza) and 1% anti-biotics. Neonatal human dermal fibroblasts (nHDFs) were purchased from Lonza Group, Ltd. (Walkersville, MD,USA) and maintained in fibroblast basal medium-2 (FBM-2) supplemented with growth kit (10 ml of fetal bovine serum, 0.5 ml of insulin, 0.5 ml of gentamicin sulfate amphotericin-B (GA-1000) and o.5 ml of r-human fibroblast growth factor-B, Lonza). Cells were incubated at 37 ^{o}C in 5% CO_2 atmosphere. nHDFs were studied between passage 5 and 9 in all experiments.

2. ITO plate electrotaxis system

Electrotaxis system using Indium-Tin-Oxide coated glasses were designed for currents contact cells directly. Indium-Tin-oxide (ITO) coated glasses were kindly provided by Kwangwoon University and were cut with approximately dimensions 2 x 2 cm^2. Cu tape (3M, USA) connected both ends of 2 x 2 cm^2 cut ITO coated glasses (ITO plate) with 5cm long electric wire, then the ITO plate was dipped into 70% ethanol for 30 minutes. The sterilized grease were pasted to one side of the silicon culture insert (Ibidi, Munchen, Germany), then put the silicon insert on the ITO coated glass surface. (Fig. 1)

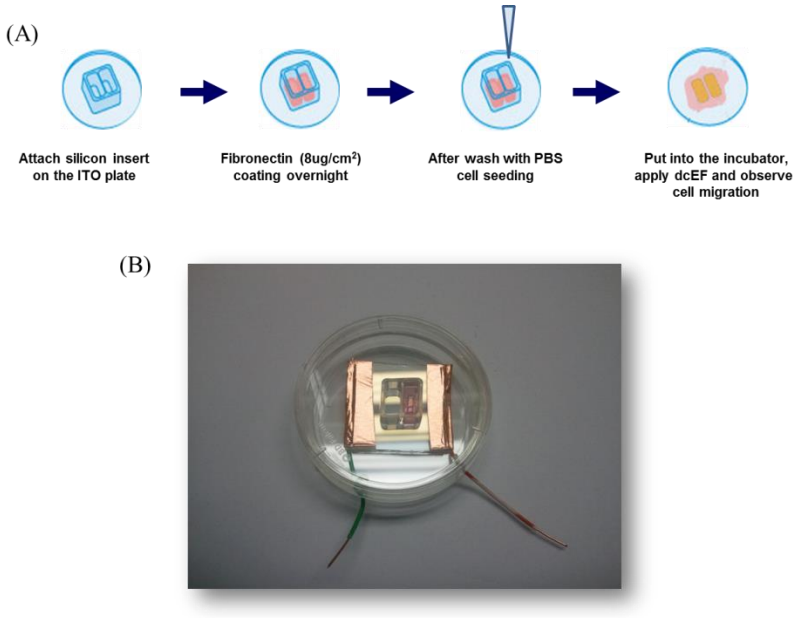

Figure 1. Indium-tin-oxide (ITO) plate electrotaxis system. (A) Schematic diagram of ITO plate electrotaxis system procedure. (B) Picture of finished ITO plate electrotaxis system.

3. Electrotaxis incubator and chamber system

We used electrotaxis incubator and chamber system (Fig. 2A) to apply direct current electric field to nHDFs. The electrotaxis incubator and chamber system consisted of two parts, which are incubator system installed with the microscope to observe live cells and the electrotaxis chamber to apply direct current electric field to the cells. The incubator is regulated by temperature and gas composition controlling program (CCP ver. 3.8) under proper environment for cell (CO_2 5%, 37 ^{o}C). The electrotaxis chamber was made up by chamber top with electric wires for connecting to the gold patterned slide glass, chamber bottom and silicon gasket. Gold patterned slide glass is mounted on the chamber bottom, put the chamber top and silicon gasket on the gold patterned slide glass. The numbered metal poles of chamber top touch the numbered place of gold pattern slide glass. (Fig. 2B) According to Ohm's law, the strength of electric field through a bulk material is where I is the electric current flowing across the material, σ is the conductivity of the material, and A_{eff} is the effective cross-sectional area of the material. (Fig. 2C) $I / \sigma A_{eff}$ is used to calculate the EF inside the electrotaxis chamber.

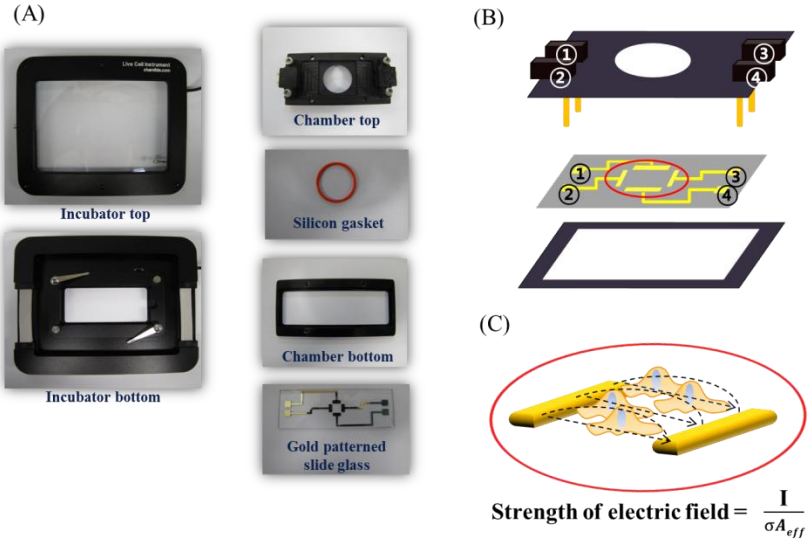

Figure 2. Electrotaxis incubator and chamber system. (A) The composition of electrotaxis incubator and chamber system, (B) Gold pattern slide glass to control the direction of the currents, (C) schematic diagram of current flow in electrotaxis chamber.

4. Time-lapse phase contrast microscopy and analysis of cell migration

The cells were cultured in the electrotaxis incubator placed on the microscope stage, and cell images were recorded every 5 minutes until electric treatment ends by the change-coupled device (CCD) camera (Electric Biomedical Co. Ltd., Osaka, Japan) attached to the inverted microscope (Olympus Optical Co. Ktd., Tokyo, Japan). (Fig. 3) Images were conveyed directly from a frame grabber to computer storage using Tomoro image capture program and saved them as JPEG image files.

For data analysis, captured images were imported into ImageJ (ImageJ 1.37v by W. Rusband, National Institutes of Health, Baltimore, Md). Image analysis was carried out by manual tracking and chemotaxis tool plug-in (v. 1.01, distributed by ibidi GmbH, Munchen, Germany) in ImageJ software. We obtained the datasets of XY coordinates by using manual tracking, then these datasets were imported into chemotaxis plug-in. This tool computed the cell migration speed and directionality of cells and plotted the cell migration pathway. The migration speed was calculated as an accumulated distance of the cell divided by time. The directionality of the cell was defined as the straight-line distance between the start position and the end position divided by accumulated distance. The closer the directionality is to 1, the straighter the cell moved. For each experiment, 20 cells were randomly selected along each edge of the wound. Cells undergoing division, death or migration outside the field of the view were excluded from the analysis.

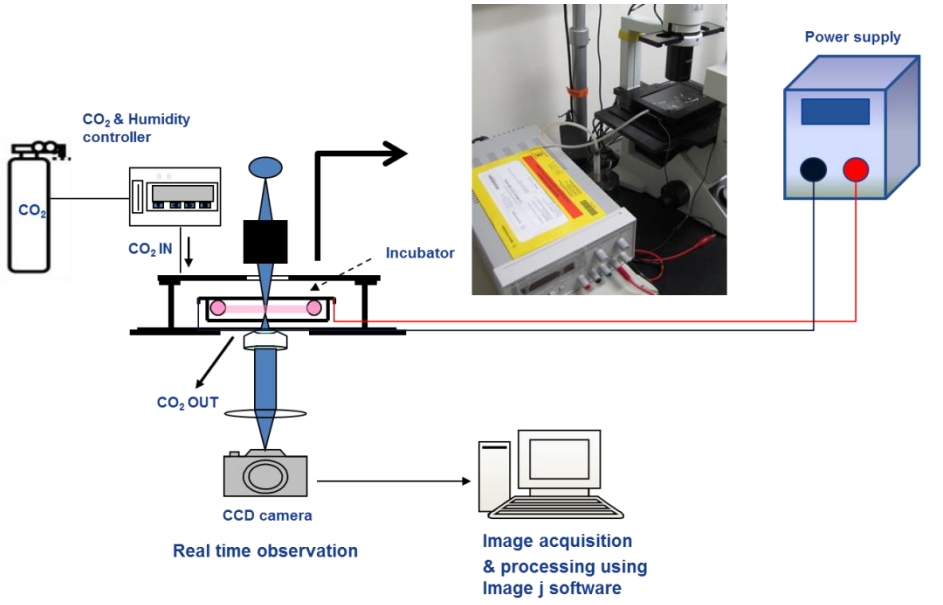

Figure 3. Schematic diagram of electrotaxis incubator and chamber system for real time observation of cell migration.

5. Electrotaxis of MDA-MB-231on ITO plate

Fibronectin needed to be coated on the surface of ITO plate for MDA-MB-231 cell attachment. Add 50 ul of fibronectin sloutions(8 ug/cm^2) to silicon insert and incubate overnight at 37^{o}C. Cells (8 x 10^3 cells/cm^2) were seeded and allowed to grow for at least 24 hr in DMEM supplement with 10% FBS, 1% anti-biotics at 37^{o}C in a 5% CO_2 incubator. Immediately before a test, medium was replaced with DMEM supplemented sth 10% FBS, 1% anti-biotics. Cells were exposed to a direct current electric field for 3hr as indicated at 37^{o}C in a temperature-controlled chamber on an inverted microscope stage.

6. Electrotaxis of nHDF on PLGA coated glass

PLGA polymer (lactide/glycolide = 75:25) was purchased from Lakeshore Biomaterials (Birmingham, USA). PLGA was dissolved in tetrahydrofuran (THF, Duksan pure chemicals co., Ltd.) at a concentration of 0.5% (w/v), then PLGA was coated slightly on the gold patterned slide glass. PLGA coated slide glass was placed in fume hood for 1hr to volatilize the THF, then assembled with electrotaxis chamber tob and bottom. Add 700ul of 70% ethanol to each electrotaxis chamber and PBS washing 3 times after 30 minutes. Cells (1 x 10^4 cells/well) were seeded and allowd to grow for at least 24 hr in FBM-2 supplemented with growth kit (10 ml of fetal bovine serum, 0.5 ml of insulin, 0.5 ml of gentamicin sulfate amphotericin-B (GA-1000) and o.5 ml of r-human fibroblast growth factor-B, Lonza) at 37^{o}C in a 5% CO_2 incubator. Immediately before a test, medium was replaced with FBM-2 supplemented with growth kit. Cells were exposed to a direct current electric field for 8hr as indicated at 37^{o}C in electrotaxis incubator and chamber system.

7. Immunostaining

After electric treatment for 2hr and 3hr, actin cytoskeleton, gogli apparatus and nucleus were visualized by immunostaining. Cells were fixed with 4% paraformaldehyde for 10 min at room temperature and were washed 3 times with PBS. Cells were permeabilized with 0.1% Triton X-100 in PBS for 5min at room temperature and rinsed 3 times with PBS. Nonspecific bindings to cells were blocked with 1% bovine serum albumin (BSA) for 30 minutes at room temperature, followed by and incubated with purified mouse anti-GM130 primary antibody (dilution 1:100, BD Transduction Laboratories™, BD Biosciences, CA, USA) overnight at 4 ^{o}C. Cells were then washed 3 times with PBS and in dark, they were treated with secondary antibody, goat anti-mouse IgG conjugated with Texas Red (dilution 1:100, Santa Cruz, CA, USA) for golgi apparatus staining and Alexa (488)-conjugated phalloidin (5 U/ml, Invitrogen) for actin cytoskeleton staining for 1 hr at room temperature. The slide glass were mounted under a coverslip and were observed by a fluorescence inverted microscope.

8. Statistical analysis

Data are reported as mean ± standard error of the mean (SEM), with n denoting the number of tests except in the migration assay where n denotes the number of cells. Means were compare using one-way analysis of variance (ANOVA) in group comparison. Two-tailed Student's t-test for unpaired data was applied as appropriate. A value of $p < 0.05$ was considered statistically significant.

III. Result

1. Electrotaxis of MDA-MB-231 on ITO plate

We observed the electrotaxis of MDA-MB-231 on ITO plate under direct current electric fields (1 V/cm). MDA-MB-231 moved randomly without electric stimulation (Fig. 4A, Fig. 4B, Fig. 4C) but migrate toward anode under 1 V/cm. (Fig. 4D, Fig. 4E, Fig 4f) Also migration speed was increased under dcEFs (1 V/cm) significantly at 2h. (Fig. 5)

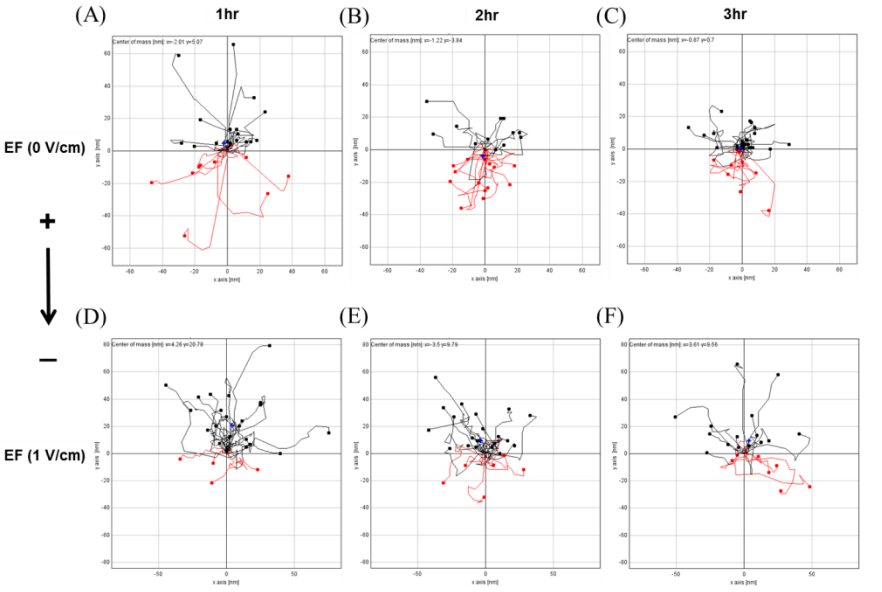

Figure 4. Electrotaxis of MDA-MB-231 on ITO plate. (A-C) Cell tracking data of MDA-MB-231 without electric fields. (D-F) Cell tracking data of MDA-MB-231 under direct current electric fields (1 V/cm). MDA-MB-231 migrates toward anode under 1 V/cm. The starting points (0,0) for cell migration were at the origin.

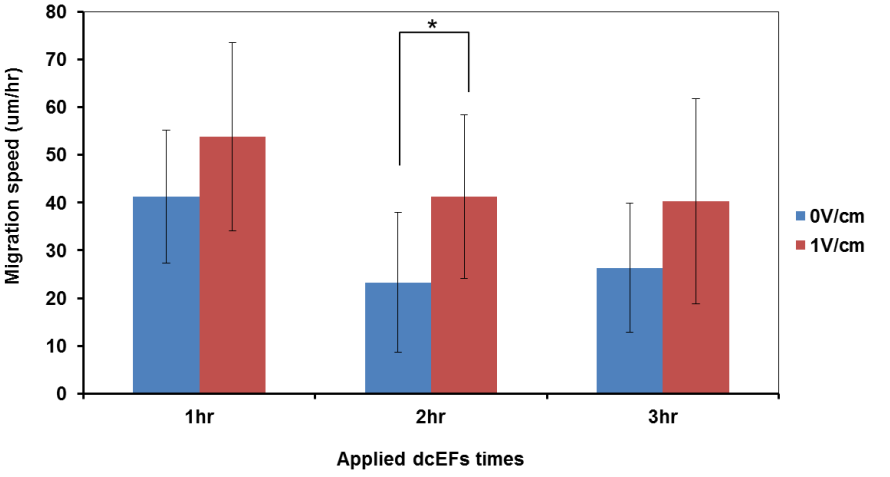

Figure 5. Migration speed of MDA-MB-231. Migration speed of MDA-MB-231 increased significantly at 2hr under direct current electric fields (1 V/cm). * $p < 0.05$ compared with 0 V/cm control group.

2. Electrotaxis of nHDF on PLGA coated gold patterned slide glass

Figure. 6 shows the electrotaxis of nHDF on PLGA coated gold patterned slide glass under 1 V/cm. nHDFs (p7) showed random migration under 0 V/cm (Fig. 6A), but moved toward cathode under 1 V/cm. (fig, 6B) Migration speed under 1 V/cm was slightly decreased. (Fig. 7A) However, directionality under 1 V/cm was increased. (Fig. 7B)

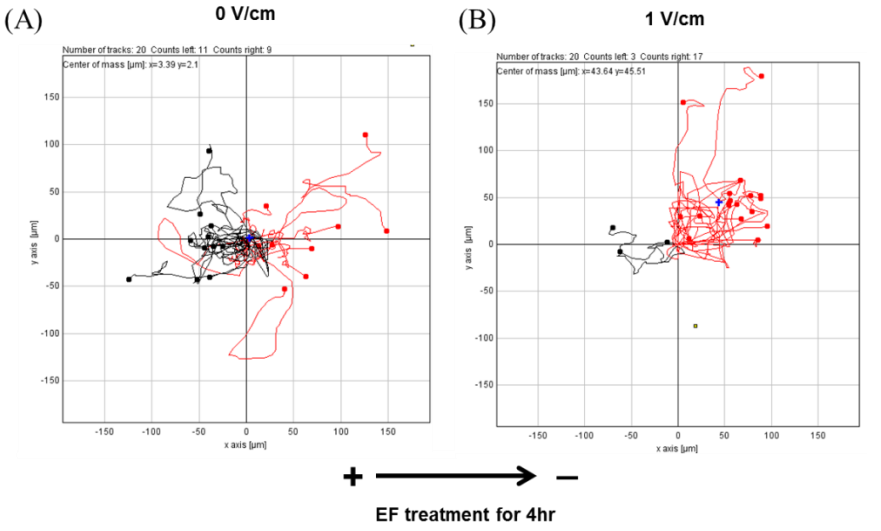

Figure 6. Electrotaxis of nHDFs on PLGA coated gold patterned glass. (A) Cell tracking data of nHDFs without electric fields. (B) Cell tracking data of nHDFs under direct current electric fields (1 V/cm). nHDFs migrate toward cathode under 1 V/cm. The starting points (0,0) for cell migration were at the origin.

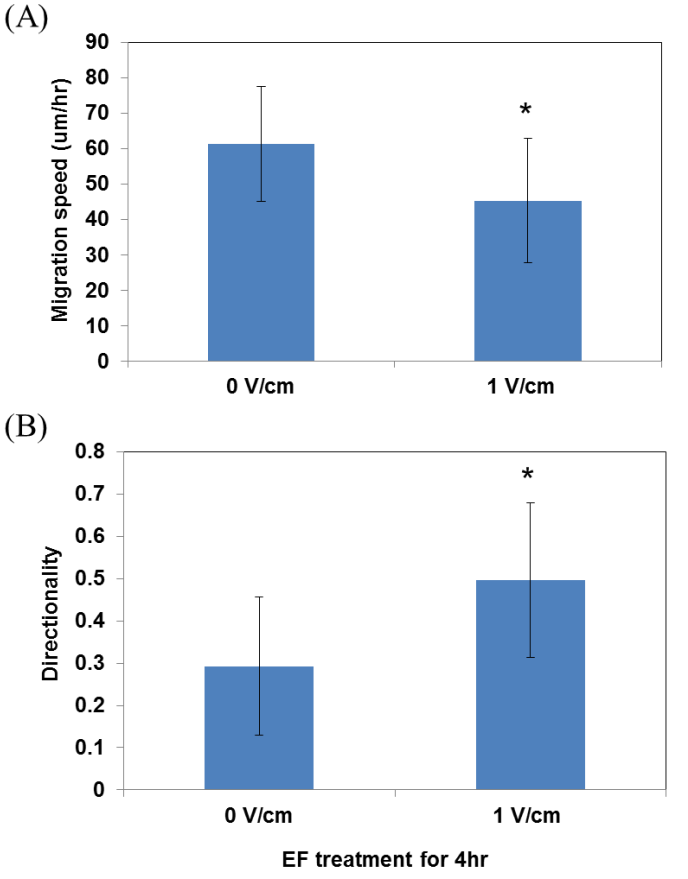

Figure 7. Movement of nHDFs under direct current electric fields. (A) The migration speed of nHDFs . (B) The directionality of nHDFs. * $p < 0.05$ compared with 0 V/cm control group.

3. Migration of nHDF in EF is passage dependent

High passage of nHDFs (p9) showed random migration under 1 V/cm. (Fig. 8) The directional migration was not observed during the electric current flowed from left to right for 2 hours. We switched the direction of current immediately and keep the flow for 2 hours, still nHDFs moved randomly.

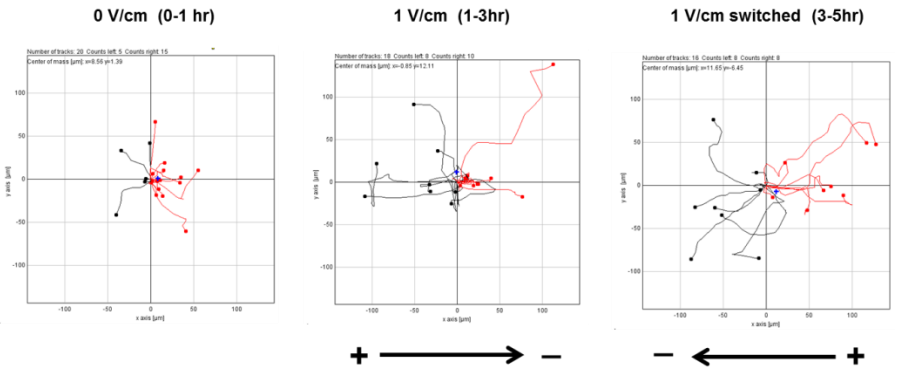

Figure 8. Electrotaxis of high passage nHDFs. During 5 hours all cells were assumed to originate at (0,0) under direct current electric fields (0 V/cm, 1V/cm, 1 V/cm switched). The movement of 20 cells is shown.

4. Migration of nHDF in EF is voltage dependent

nHDFs showed directional migration toward cathode under 1 V/cm. (Fig. 6B) However, there is no electrotaxis effect on nHDFs under 2 V/cm. (Fig. 9) Also, The migration speed of nHDFs under 2 V/cm decreased . (Fig. 10) The changes of nHDFs morphology were observed under 2 V/cm for 10hr. (Fig. 11)

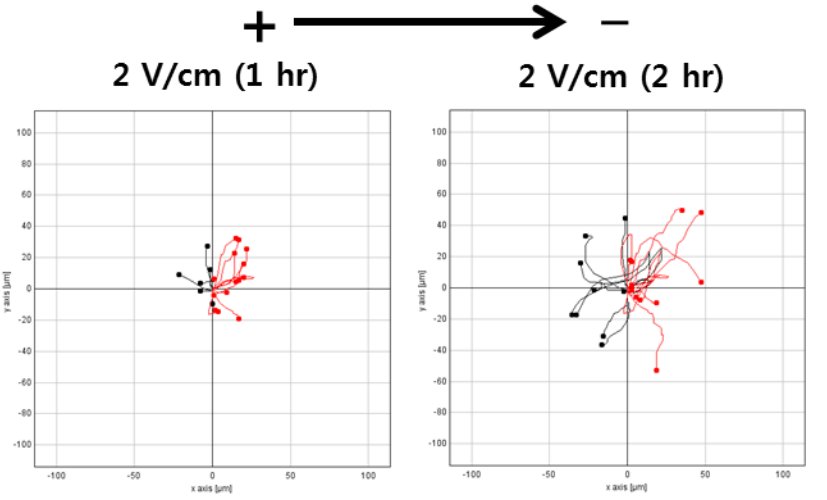

Figure 9. Voltage dependent migration of nHDF in EF. During 2 hours all cells were assumed to originate at (0,0) under direct current electric fields (2 V/cm, 2 V/cm switched). The movement of 20 cells is shown.

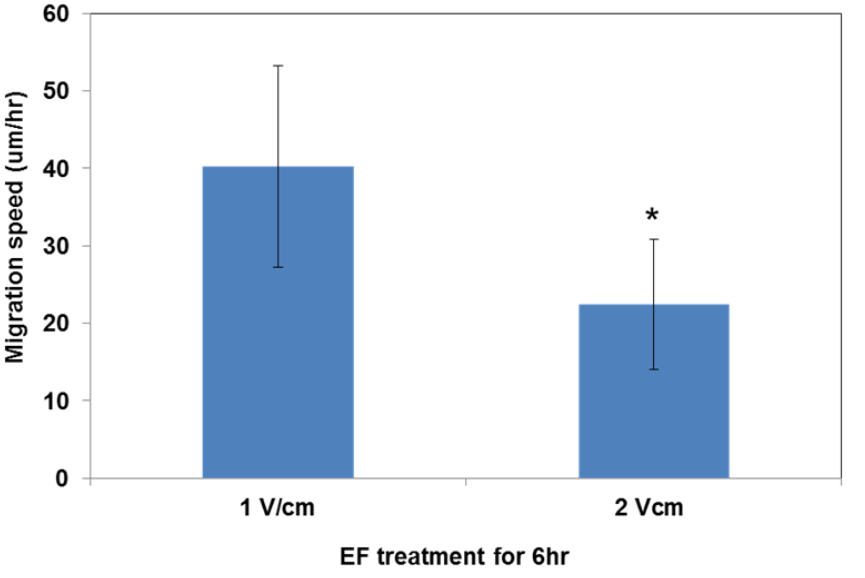

Figure 10. Migration speed of nHDFs under 1 V/cm and 2 V/cm. * $p < 0.05$ compared with migration speed of nHDFs under 1 V/cm.

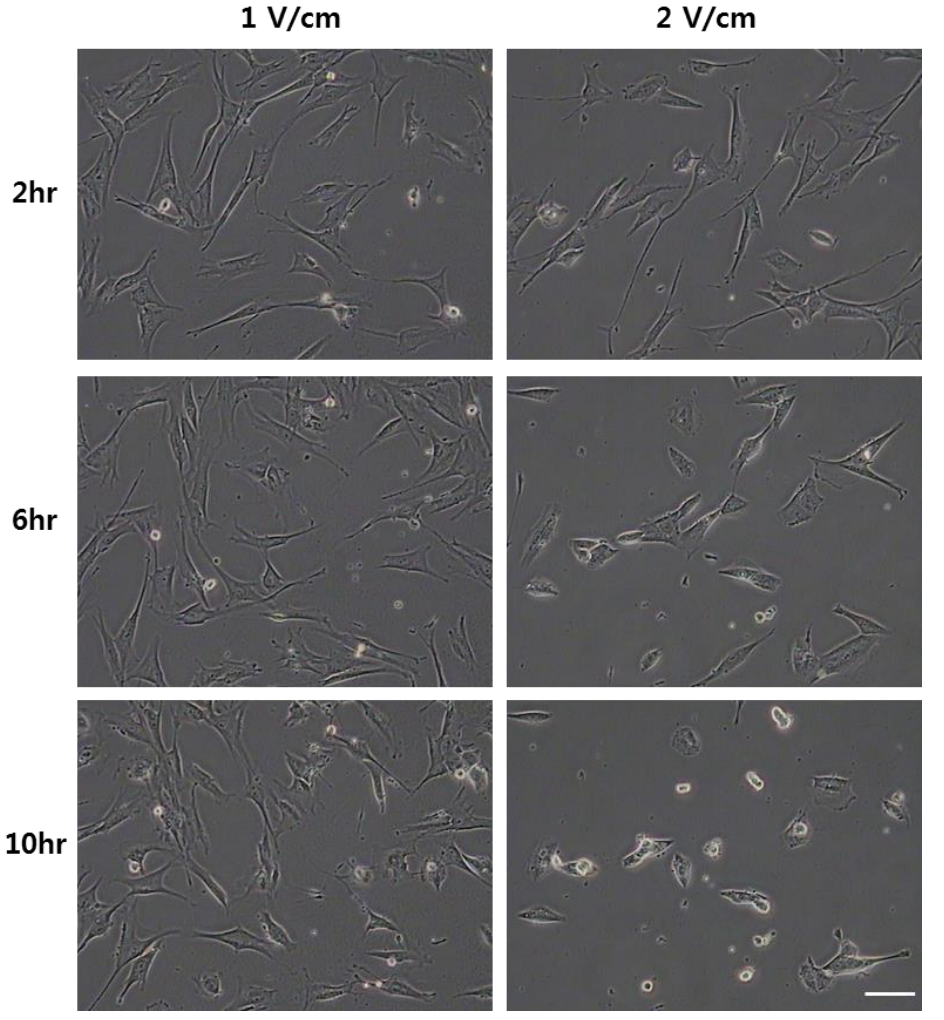

Figure 11. Change of nHDFs morphology under 2 V/cm EF. The cells were exposed to the EF 1 V/cm or 2 V/cm for 10hrs. Scale bar = 100um

5. Switching direction of nHDF migration by Electrotaxis

The electric current flowed from left to right for 2 hours and then current flowed opposite direction for 4 hours. nHDF migrated toward cathode under 1 V/cm (Fig. 12A) and change the direction of migration to new cathode when the current direction was switched. (Fig. 12B) Location of nHDFs were also evaluated. (Fig. 13) The number of nHDFs left side increased gradually when the cathode is left.

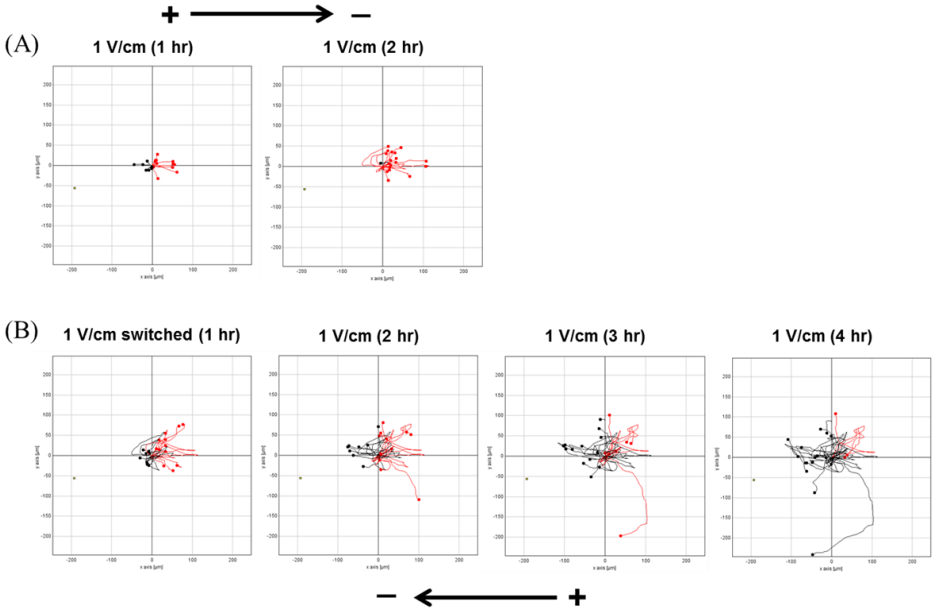

Figure 12. Switching direction of nHDFs migration by electrotaxis. (A) Cell tracking data of nHDFs under 1 V/cm (cathode right). (B) Cell tracking data of nHDFs, under 1 V/cm swithed (cathode left). During 6 hours all cells were assumed to originate at (0,0) under direct current electric fields (1 V/cm, 1 V/cm switched). The movement of 20 cells is shown.

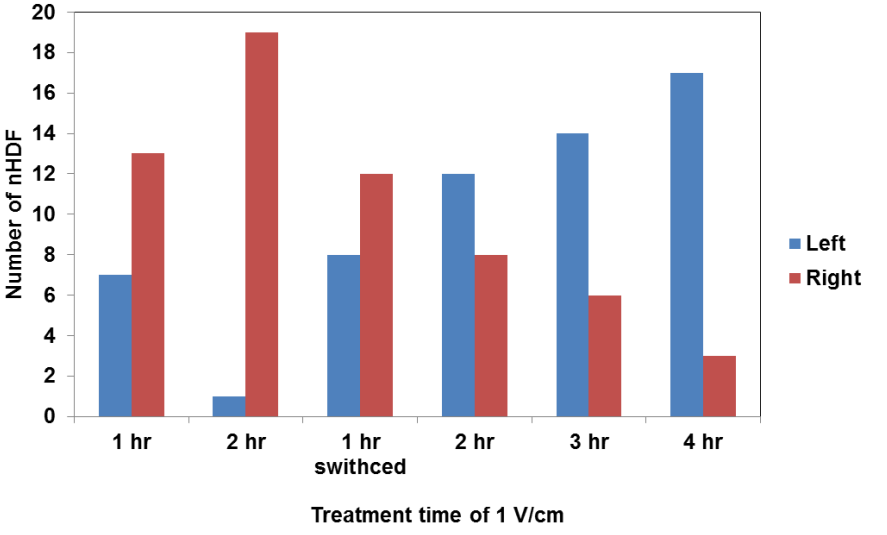

Figure 13. Location of nHDFs under 1 V/cm. The number of cells on the left and right were counted hourly. The datum point of cell location was starting points of each cell.

6. Identifying the possibility of cell migration control

The electric current flowed from left to right for 2 hours and then current flowed from down to up for 3 hours. nHDF migrated toward right under 1 V/cm for 2 hr (Fig. 14, Fig. 15) and change the direction of migration to up for 3 hr when the current direction was changed. (Fig. 14, Fig. 16)

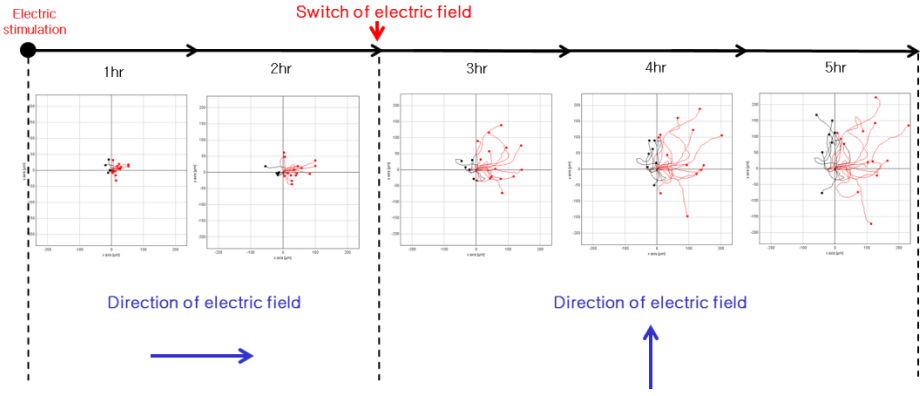

Figure 14. Changing direction of nHDFs migration by electrotaxis. Cell tracking data of nHDFs under 1 V/cm (cathode right) for 2hr then change the EF direction for 3 hr (cathode up). During 5 hours all cells were assumed to originate at (0,0) under direct current electric fields (1 V/cm, 1 V/cm switched). The movement of 20 cells is shown.

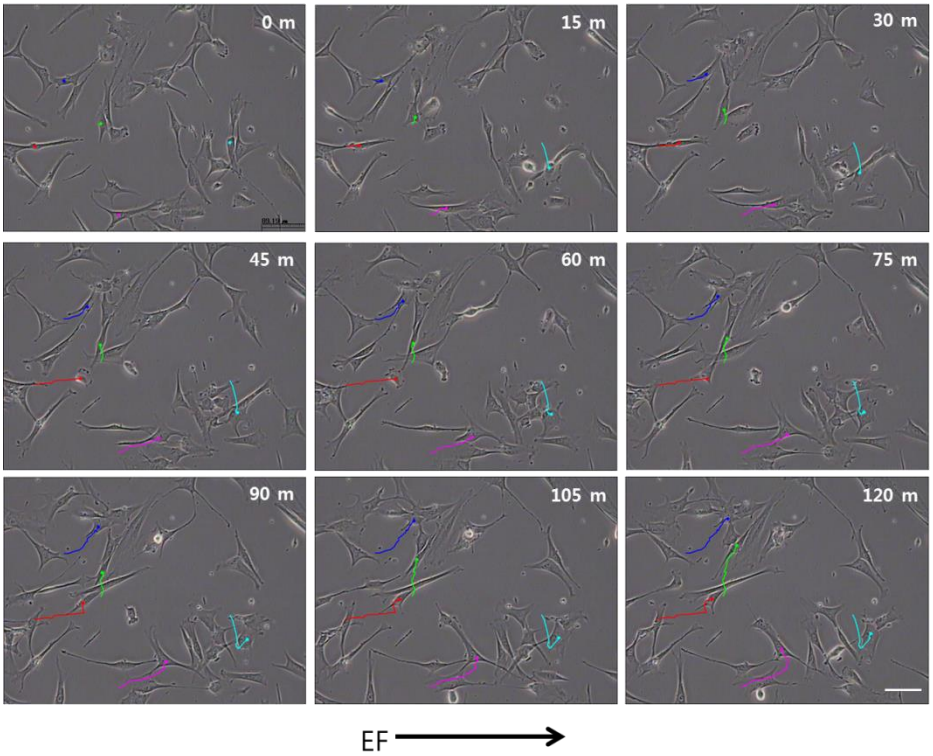

Figure 15. Cell tracking image of nHDFs under 1 V/cm (cathode right). The EFs applied for 2hr from left to right. Each colored point represents the position of each cell nuclei. Scale bar = 100um

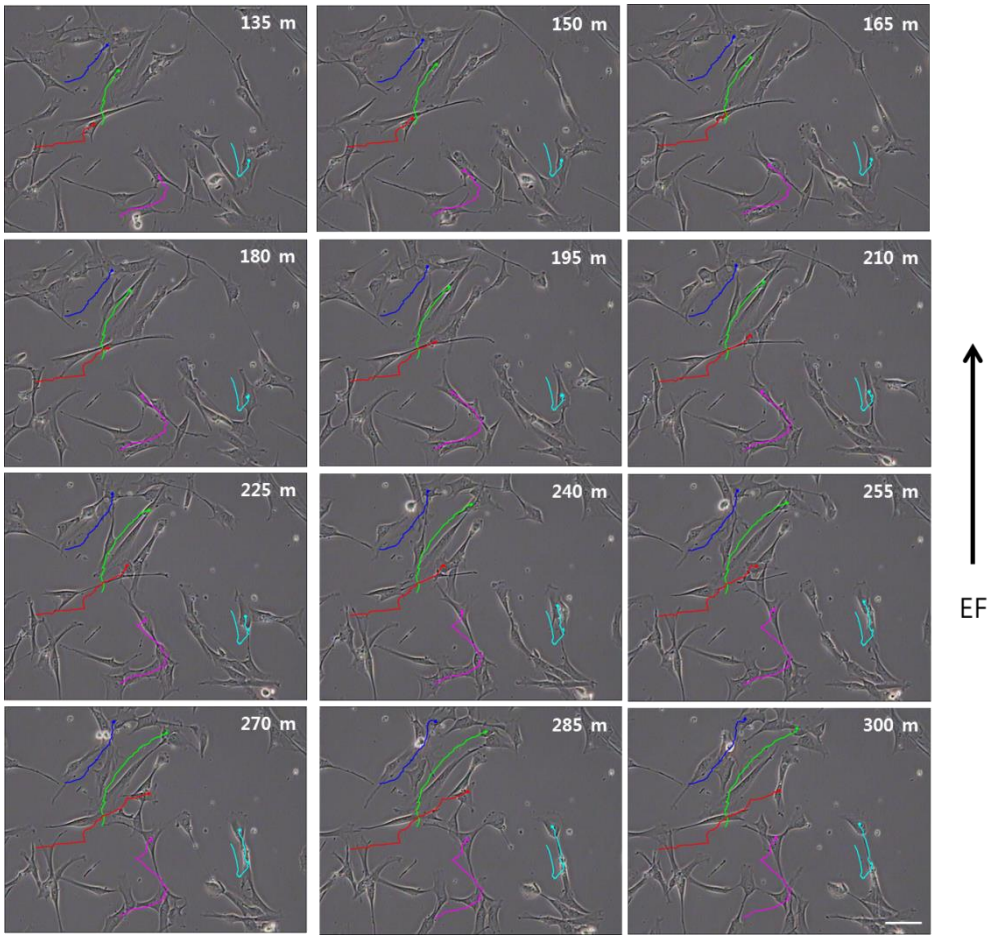

Figure 16. Cell tracking image of nHDFs under 1 V/cm (cathode up). The EFs applied for 3hr from down to up. Each colored point represents the position of each cell nuclei. Scale bar = 100um

7. Observation of actin cytoskeleton reorganization and golgi apparatus polarization

Reorganization of cytoskeleton and polarization of golgi apparatus by electrotaxis were observed.. Figuire 17A shows actin cytoskeletons were not reorganized and golgi apparatus were not polarized under 0 V/cm however, reorganization of cytoskeleton was observed and golgi apparatus polarized toward cathode under 1 V/cm R and 1 V/cm Up. Also, the EF induced significant GA polarization under 1 V/cm R and 1 V/cm Up. (Fig. 18) GA polarization was quantified as the percentage of GA polarized into the quadrant between 45 and 315 degrees in the field direction as shown. (Fig. 17B)

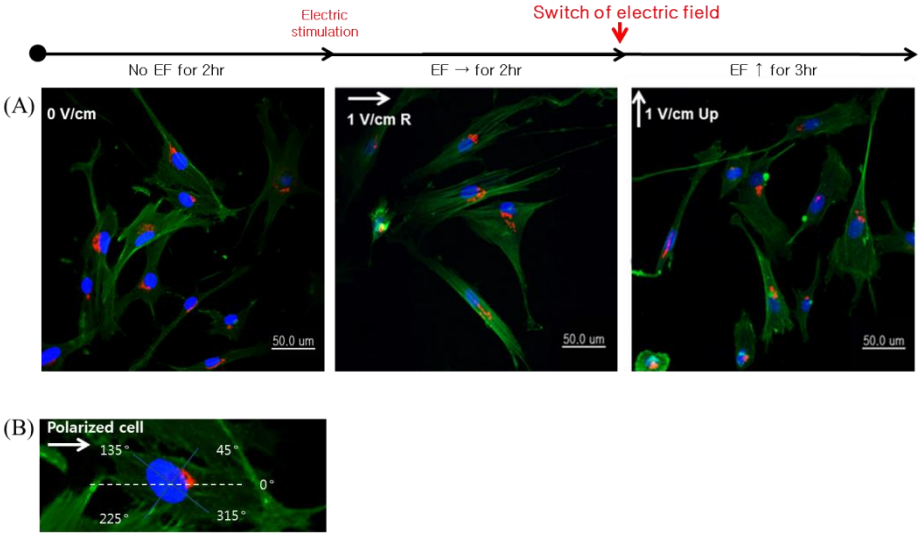

Figure 17. Immunostaining of nucleus, actin cytoskeleton and golgi apparatus under 0 V/cm, 1 V/cm R (cathode right) and 1 V/cm Up (cathode up). The nucleus was stained with Hoechst #33258 and actin cytoskeleton was stained with Alexa (488)-conjugated phalloidin (green) and golgi Apparatus was stained with Texas Red conjugated antibody(red) and (A) merged images and (B) GA polarization quantification were shown. Scale bar = 50um

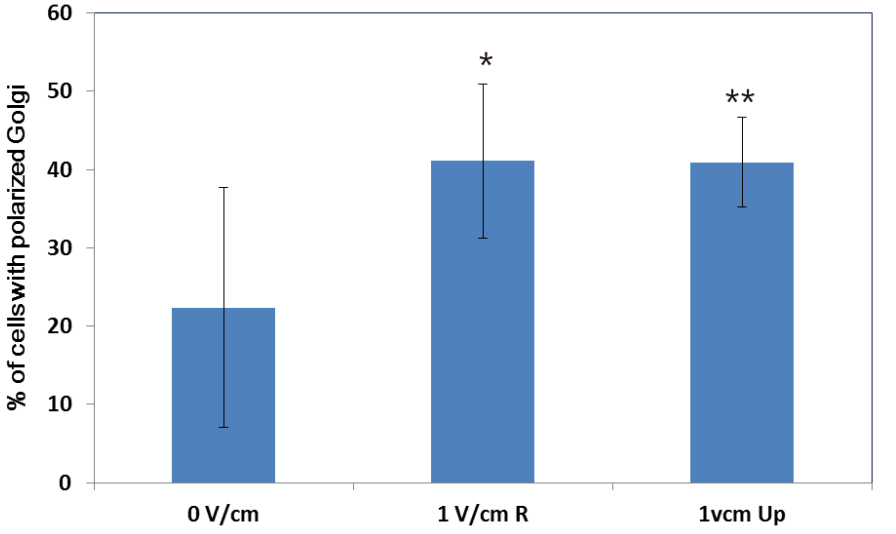

Figure 18. The percentage of cells with polarized GA. * $p < 0.05$, ** $p < 0.05$ compared with percentage of nHDFs with polarized Golgi under 0 V/cm.

IV. Discussion

To make the electrotaxis system more convenient than the established, we considered Indium-tin-oxide coated slide glass[9]. (Fig. 1) The human breast cancer cell line MDA-MB-231 has been studied already that cells responded to applied electric fields with directional migration towards the anode.[10] To identify the function of ITO plate electrotaxis system, MDA-MB-231 were seeded on the ITO plate and electrotaxis was evaluated. We could observe MDA-MB-231 migrated toward anode in this system (Fig.4) and the migration speed was increased in dc EFs. However, the ITO plate had a limit for long-term observing because of the heat generation on the ITO plate surface, so we need to design a new electrotaxis system.

Electrotaxis incubator and chamber system (Fig. 2, Fig. 3) is manufactured to facilitate the long-term observation and changing the direction of current in one of four ways: “up/down”, “down/up”, “left/right”, “right/left”. The incubator maintains the temperature at 37 ^{o}C and the concentration of Co_2 in 5%. The electrotaxis chamber and gold-patterned slide glass could make the switching of current direction possible. nHDF (p7) migrated toward the cathode under 1 V/cm and migration speed decreased (Fig. 6, Fig. 7A). The effect of physiological EFs on the direction of migration and rate are different from those on keratinocytes, which migrate to the cathode with increased speed[11] and human dermal fibroblast, which migrate to the anode with decreased speed[12].

High passage of nHDFs (p9) showed random migration under 1 V/cm. (Fig. 8) Human bone marrow mesenchymal stem cells at early passage (P3-5) or late passage (P7 and P10) clearly migrated towards the anode in an EFs of 200 mV/mm for 2h, but the directedness was decreased at late passage[8]. Cell electrotaxis is passage-dependent with higher passages of nHDFs showing lower

directedness and this appears to be related to cell0intrinsic factors including cell senescence.

Cells need to be spread widely and should infiltrate into the 3D scaffolds to give enough mechanical strength and adjustable degradation time to the scaffolds[4]. To spread the cells on the scaffolds, we need to control the direction of cell migration. The possibility of controlling the cell migration was evaluated by observing the cell migration which were exposed to an EFs (1 V/cm) for 2 hours and for another 3 hours in reversed field polarity (1 V/cm). Cells had moved toward the cathode for 2 hours then changed the direction toward new cathode (opposite direction) for 3 hours. (Fig. 12) The location of nHDFs which means how many cells are located from the starting points, showed right side of cells (first cathode) increased for 2 hours and decreased for 3 hours steadily. (Fig. 13)

It has been already reported that the cytoskeleton of fetal rat calvaria osteoblastic cells and human osteosarcoma cells were redistributed under dc EFs condition[13]. Also, golgi apparatus of chinese hamster ovary cells polarized by dc EFs[14,15]. Electrotaxis induces the reorganization of cytoskeleton and polarization of golgi apparatus, so we observed those in electrotaxis incubator and chamber system. GSK-3β could be a candidate of kinase which mediates EF-directed GA polarization[14]. GSK-3β has been indicated in the regulation of apoptosis, circadian rhythm, transcription, regulation of cell division, stem cell renewal and insulin action[16-23]. Previous study suggested a requirement for PI3 kinase and PKC in phosphorylation of GSK-3β, thus mediating signaling from applied EFs[14, 24-27]. The actin cytoskeleton of nHDFs elongated to the direction of migration (cathode), and the golgi apparatus polarized toward the same direction under 1 V/cm EFs. (Fig. 17, Fig. 18) These data indicates that the function of electrotaxis incubator and chamber system is not different from the established electrotaxis system.

V. Conclusion

In conclusion, we identified the electrotaxis of nHDFs in electrotaxis incubator and chamber system which is designed for more convenient than the established system. Cells moved towards the cathode and after the polarity of EFs reversed, the direction of nHDFs migration followed the EFs polarity. The passage of cells and the strength of the applied EFs were concerned to electrotaxis of nHDFs. Finally, we could identify the possibility of cell migration control on the PLGA film by electrotaxis.

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Abstract (In Korean)

PLGA가 코팅된 표면 위에서의 electrotaxis에 의한
세포 이동 조절의 가능성 확인

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김민성

조직공학은 많은 수의 장기와 조직의 수요를 충족시키기 위해 연구되어져 왔다. 조직공학에 사용되기 위하여 개발된 합성 재료들은 시간이 자남에 따라 마모나 피로도, 혹은 부정적인 생체반응에 의해 숙주 조직과 융합이 잘 안된다는 문제점이 있다. 수많은 연구의 결과들로부터 세포는 주변의 상황에 맞게 잘 분화되기 위하여 적절한 biomatrix이나 지지체를 필요로 한다는 사실을 알 수 있다. 조직공학에서는 지지체의 윤일한 기계적 강도를 얻기위해 세포들이 지지체에 고르게 분포되는 것을 추구한다.

세포 이동은 배아 발달, 조직과 기관의 형태 발생, 손상된 조직의 치료, 혈관 신생 과정에서의 모세혈관 형성, 상처 부위

박테리아를 죽이기 위한 백혈구의 이동 등과 같은 다양한 생물학적 사건에 필수적인 세포의 활동이다. 세포 이동을 조절할 수 있다면 삼차원 입체구조의 지지체 표면에 세포를 고르게 분포시키고, 지지체 안으로 세포를 침투시키는 것이 가능할 것이다.

이번 연구에서는 electrotaxis를 이용하여 세포 이동 조절의 가능성을 연구하였다. 기존의 electrotaxis system은 electrotaxis의 메커니즘 규명에 초점이 맞춰져 있었고 전기장의 방향을 상하좌우로 바꾸기에는 적합하지 않았기에 우리는 이번 연구를 위해 새로운 방식의 electrotaxis system을 고안하였다.인간 피부 섬유아세포는 1 V/cm의 전기장 크기에서 음극 쪽으로 이동하는 것을 관찰할 수 있었고, 전기장의 극성을 반대로 바꾸어 주었을 때 세포의 이동방향 역시 반대로 바뀌는 것을 확인할 수 있었다. 액틴 세포골격과 골치체의 분극현상 역시 면역형광법을 통해 관찰하였다. 액틴 세포골격은 음극 방향으로 길게 늘어나는 모습을 보였고, 골지체 역시 음극 쪽으로 몰려서 분극현상이 일어남을 관찰하였다. 이번 연구를 통하여 electrotaxis를 이용한 PLGA 지지체에서의 세포 이동 조절의 가능성을 확인할 수 있었다.

핵심되는 말: 세포이동, 조직공학, 전기주성, 표피섬유세포, PLGA 지지체