제 2 회 학사학위 졸업논문
지도교수 이 동 현

Effect of Electric Stimuli to MC3T3-E1 Cell on Graphene Substrate

천 혜 정

School of Integrative Engineering
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이 논문을 학사학위 졸업논문으로 제출합니다.

중앙대학교 공과대학
융합공학부
천 혜 정

2014년 12월

중앙대학교 공과대학 융합공학과 천 혜 정 의 공학사 학위 취득을 위한 요구 사항 중 그 일부인 본 졸업논문을 인정함.

2014년 12월

지도교수 : 이 동 현 (인)

약 력

천혜정은 1992년 서울특별시 송파구 오금동에서 천경필씨와 이연우씨의 장녀로 태어났다. 2011년 수지고등학교를 졸업하고, 2011년도에 중앙대학교 공과대학 융합공학부로 입학하여 현재 4학년에 재학 중이며 2014년 2월에 졸업할 예정이다.

감사의 글

참 시간이 빠르게 흘렀습니다. 약 4년 전 입학하여 교정에 발을 딛었던 기억이 선명한데, 입학 할 때 공사 중이던 정문의 광장은 어느덧 노란 잔디로 덮였습니다. 그 위에 눈이 소복이 쌓이는 계절은 네 번이나 지났고, 이는 제가 학교를 떠날 시기가 다가왔음을 실감하게 하였습니다.

중앙대학교 공과대학에 신설된 학부인 융합공학부에서 처음으로 무언가를 했던 일이 많았습니다. 저를 비롯한 신입생들은 첫 입학생으로서 융합공학부의 체계를 꾸려나가야 했고, 이는 학부 내, 외적으로 물심양면 도움을 주신 교수님들이 아니었다면 완성될 수 없었을 것입니다. 이 자리를 통해 다시 한 번 제게 가르침을 주시고 융합공학부를 위해 노력하신 교수님들에게 감사의 말씀을 전합니다. 가장 먼저 저를 오랫동안 지켜봐주시고, 아낌없는 지원으로 책상위의 학문이 아닌 실험적 경험을 하게 해 주신 지도교수님, 이동현 교수님께 감사드립니다. 교수님의 지도하에서 실험을 했던 제 학부시절의 기억은 졸업 후에도 잊지 못할 것 같습니다. 뿐만 아니라 융합공학부를 위해 고군분투하시고 항상 밝은 얼굴로 맞아주시는 표성규 교수님, 열정적인 강의로 수업의 흥미를 이끌어주신 김수길 교수님, 강의와 강의 외의 질문에도 성심껏 답해주시는 윤성훈 교수님, 흥미롭고 유용한 주제의 프로젝트 과제를 경험하게 해 주신 손형빈 교수님, 오랜 시간 뵌 것은 아니었지만 마지막 학기에 조언과 격려를 아끼지 않으셨던 왕동환 교수님, 진로에 대해 고민할 때 직업에 대한 의미를 되새겨보게 해주신 민준홍 교수님, 잊지 못할 프레젠테이션을 경험하게 해 주신 박한수 교수님, 프로그래밍 언어의 실습에 재미를 알게 해 주신 박경주 교수님, 그리고 학생들의 입장에서 공감하고 경청해주셨던 이상근 교수님께 감사드리며 제가 수업을 듣진 못했지만 융합공학부를 위해 노력해주신 모든 교수님께 제 감사의 인사를 전해드리고 싶습니다.

그리고 졸업논문을 쓰는데 있어서 많은 분들의 도움과 조언이 있었지만, 가장 큰 힘을 주셨던 생체복합소재 및 조직공학 실험실의 연구원, 석사, 박사, 학부생인 유나언니, 지연언니, 근선오빠, 덕일오빠, 압둘라, 학림오빠, 군 오빠, 수 오빠, 문호오빠 그리고 같이 동고동락했던 재연언니, 상원이, 윤정이 외에도 실험실을 거쳤던 모든 사람들과 함께 실험했던 다른 실험실의 학생, 연구원분들에게도 감사하고 덕분에 즐겁게 실험을 할 수 있었다는 말을 전해주고 싶습니다.

끝으로 나와 입학하여 함께 해준 11학번 동기들과 항상 사랑하는 가족들, 4년 동안 만난 여러 인연들 모두에게 감사합니다.

목 차

Effect of Electric Stimuli to MC3T3-E1 Cell on Graphene Substrate

1. Introduction

Numerous studies have demonstrated that electric stimuli is important in directing stem cell differentiation, and endogenous electromagnetic fields are effective for developing and regenerating tissues, either in the extracellular space or in the cell cytoplasm. To study the effects of electric stimuli and electromagnetic field in cell behavior, various kinds of biomaterial are used and developed, such as carbon-based materials like carbon nanotube, graphene, nanowire and conducting polymer. [1, 2, 9]

Graphene, a single layer of sp2 bonded carbon atoms in a two-dimensional hexagonal lattice, has attracted considerable biological attention as a potential biomaterial because of its exotic properties such as electric, physical and mechanical properties, including a large surface area and high electric conductivity, so it is expected to be applied for cell behavior. Because of its biocompatibility at low concentration and 2D nature with ultra-large surface area, graphene has recently captured interests as cell culture substrates. Substrates coated with graphene have enabled the culture of several mammalian cells. [7, 8] High electric conductivity property of graphene is applied in neural prosthetics field as strategies for the diagnosis, therapy, and treatment of neural disorders increasingly relying on electrical stimulation techniques. Electricity-conduction property of graphene affects cells in proliferation and communication of electric signals. [10, 11]

In this study, to figure out the relationship between electric stimuli and neuro cells differentiation and proliferation, we use graphene as cell culture substrate. Original purpose of experiment is demonstration of relationship in neuro cell, but prior to that, we conducted preliminary experiment in MC3T3-E1, osteoblast which could be differentiated into bone cell, because it is already proved that pulsed electromagnetic field stimulation could also increase bone formation by enhancing the recruitment, proliferation, and differentiation of osteochondroprogenitors, osteogenesis, and chondrogeneisis resulting in a larger cartilage mass and a corresponding greater amount of new bone. [4, 5, 6] Before using graphene substrate for experiment, we designed Teflon template to prevent electrical agglomeration of additives in cell culture medium.

2. Materials and Methods

2-1. Design of Teflon template

To prevent electrolysis and agglomeration of proteins and additives in culture media, we designed a Teflon template which has size of 46.4㎜×34.4㎜×40㎜. Cell culture area is 30㎜×30㎜. Considering cell culture media is state of liquid, top cover and bottom is packed with silicon ring (60028, MG-SEAL). The design of Teflon template is shown in figure 1 (a) and the Teflon template containing graphene substrate is also shown in figure 1 (b).

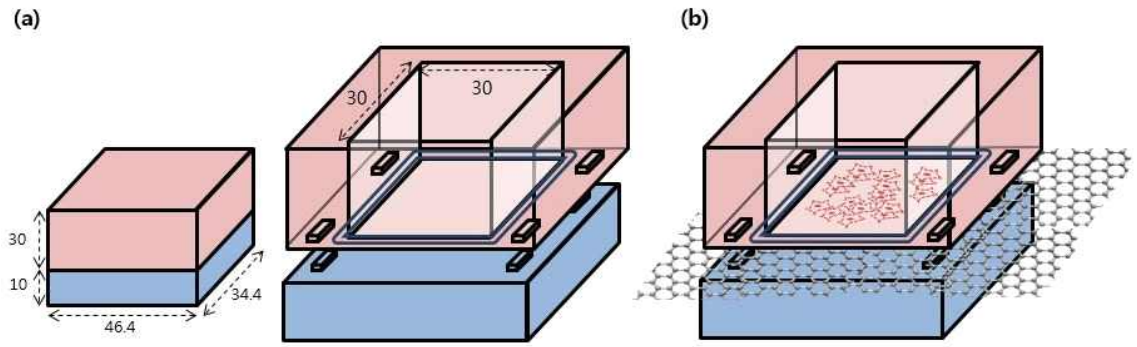


Figure 1 (a) Designed Teflon template (b) Scheme of cultured MC3T3-E1 cells on graphene substrate in Teflon template

2-2. Cell Culture and Seeding

The preosteoblastic MC3T3-E1 cell line was used for the cell culture assays. The cells were cultured in α-MEM supplemented with 10% FBS and 1% penicillin-streptomycin at 37℃ in a humidified atmosphere with 5% CO_2. The medium was changed every 2-3 days. The MC3T3-E1 cells were detached using 0.25% trypsin-EDTA and then suspended in α-MEM supplemented with 10% FBS and 1% penicillin-streptomycin. Prior to cell seeding, graphene substrate and Teflon template were sterilized by exposure to ultraviolet (UV) light for overnight followed by immersion in 70% ethanol for 3 hours. After sterilization, the Teflon template containing graphene substrate was rinsed thrice with PBS and pre-wetted in

serum-free α-MEM for 2 h at 37 ℃, followed by seeding 100μL of MC3T3-E1 cell suspension containing 1×10^5cells onto the graphene substrate. Same process is conducted on 12-well plate (EW-01930-29, NUNC) for control group and it is chosen for the most similar culture area compared to 30×30㎟ graphene substrate. [3] Cells on 12-well plate is not applied by electric stimuli as control group, so the number of cells on 12-well plate is compared to that on graphene where electric stimuli is applied.

2-3 Pulsed electric stimuli on graphene substrate

MC3T3-E1 cells are attached graphene substrate and culture medium is sealed with silicon ring by strong force of steel bolt and nut as figure 2 (a) and (b).

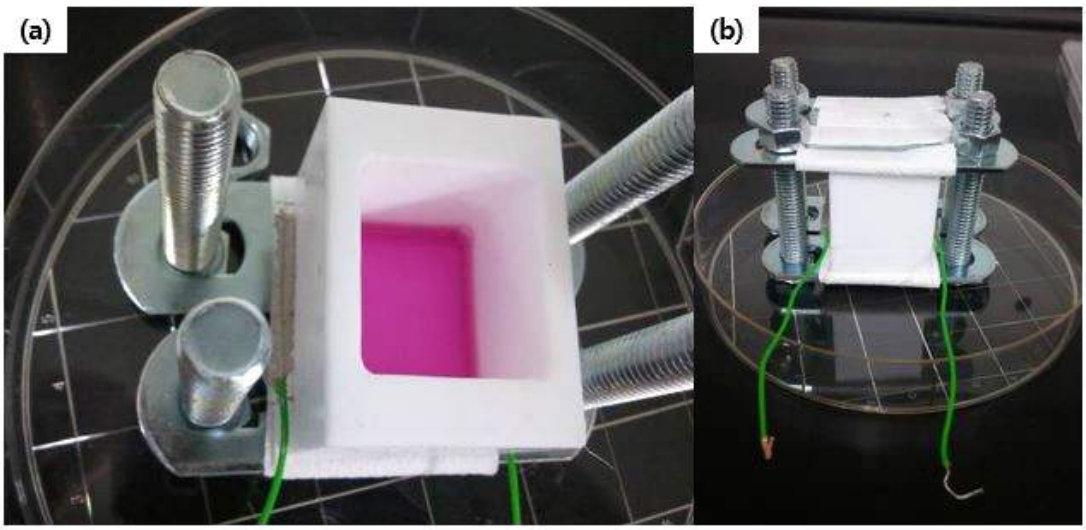
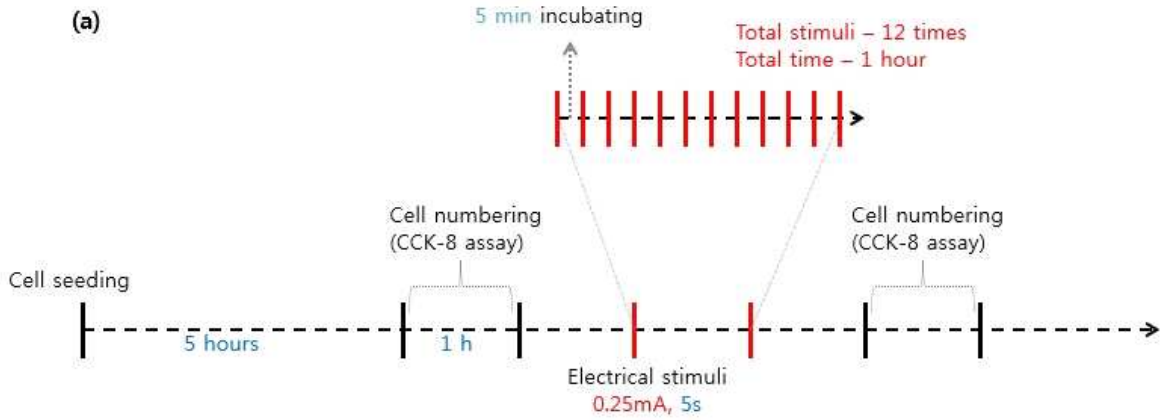


Figure 2 (a), (b) Preparation before electric stimuli. Two electric wires are attached on graphene substrate by silver paste.

For electric pulse, electric wires are attached on graphene film by silver paste, and electric current is supplied by Keithley 2401 Sourcemeter (Keithley). The electric currents applied to cells are 0.1mA and 0.25mA each, for 5 sec 12 times with 5 min interval, therefore total applied time is 1 h. The time schedule of cell culture and electric stimuli in each

experimental group is shown as figure 3 (a) and (b).



(b)

	Exp. 1	Exp. 2
Applied current	0.25mA	0.1mA
Cell seeding number	$1x10^5$	$1x10^5$
Time	5s	5s
Time interval	5min	5min
Total stimuli times	12	12

Figure 3 (a) Total time schedule of cell culture, electric stimuli and cell counting (b) Experimental group 1 and 2

2-4. Cell Adhesion and Proliferation

To assess cell adhesion and proliferation, after incubating the seeded MC3T3-E1 cells on the graphene substrate and 12-well plate for 5 h, the number of adherent cells was determined using an optical microscope to get optical image by camera (CKX41, Olympus) and a CCK-8 cell counting kit (Dojindo Laboratories, Kumamoto, Japan). Briefly, the CCK-8 solution and serum-free α-MEM were combined at a ratio of 1:10 and added to each well. After incubating for 1 h at 37 ℃ in a humidified atmosphere with 5% CO_2, the optical density (O.D.) values at 450nm were measured using a Synergy H1 microplatereader (BioTek,Winooski,VT,USA). Using the O.D.

values, the number of cells was calculated based on a standard curve generated using MC3T3-E1 cells. After cell adhesion analysis, the Teflon template containing graphene substrate was added to α-MEM supplemented with 10% FBS and 1% penicillin-streptomycin and applied electric stimuli for further analysis. After electric stimuli, same cell counting method is used again for comparing cell numbers before/after electric stimuli.

3. Result and Discussion

3-1. Cell adhesion by optical image

Optical images of MC3T3-E1 cells on graphene substrate are viewed by magnification of 40, 100, 200 and these are shown in figure 4 (a), (b), (c), respectively.

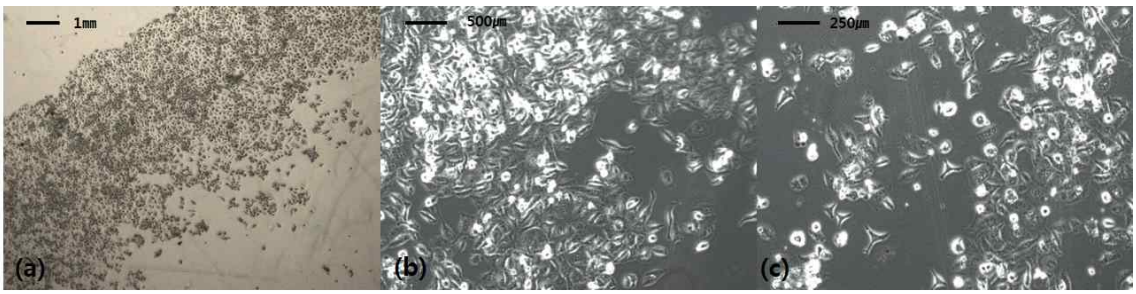


Figure 4 Microscopic view of MC3T3-E1 cells attached on graphene substrate after 5 h seeding (a: X40, b: X100, c: X200)

Cells are attached well on graphene substrate with shapes of cells in radial form, so cells are not floated in culture media. This implies graphene substate is effectively biocompatible substrate for cell culture, especially MC3T3-E1 cells, and electric stimuli which is applied to graphene substrate is also transferred to cells on substrate directly.

3-2. Proliferation after electric stimuli compared to control group

Cell number counted by cell counting method is shown in figure 5.

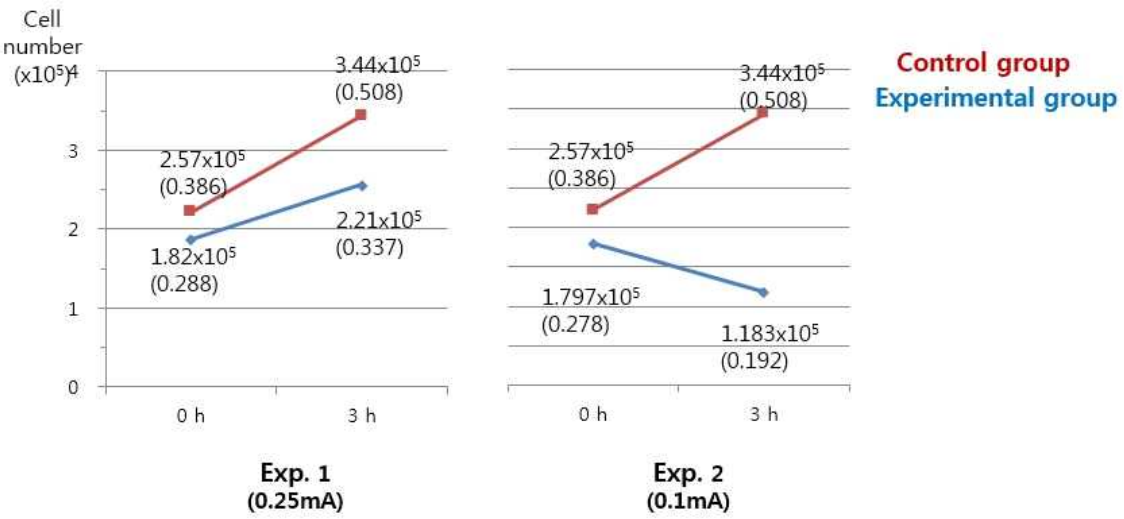


Figure 5 The number of cells before/after electric stimuli compared to control group. Exp. 1 depicts experiment conducted with 0.25mA and Exp. 2 depicts experiment conducted with 0.1mA

Comparing adhesion rate with cell culture dish, cell numbers of control groups before electric stimuli are $2.57x10^5$ each in experiment 1 and 2, cell number of experimental group, where cells attached on the graphene substrate is $1.82x10^5$ in experiment 1 and $1.797x10^5$ in experiment 2. This difference is resulted that 12-well plate is treated by additives which enhance cell adhesion artificially, so the average value of 72.3% adhesion rate of graphene substrate compared with 12-well plate is quite large in value. Inexperiment 1, where 0.25mA of electric stimuli are given, cell number increases to $2.21x10^5$ and cell number of control group increases to $3.44x10^5$. Each growth rate is +21% in experimental group and +33% in control group. In experiment 2, where 0.1mA of electric stimuli are given, cell number decreases to $1.183x10^5$ and cell number of control group increases to $3.44x10^5$. Each growth rate is -34% on graphene substrate and +33% on 12-well dish. Theses result regards that difference in electrical stimuli makes different result in cell growth and there is one specific electric current supply to increase cell growth. Although in these experiments, result of cell growth rate on graphene substrate is less than that on 12-well cell culture dish, and even in experiment 2 cell number decreases as electric stimuli 0.1mA, it shows further study should be done

to find effective electric stimuli range for cell growth and differentiation. The possible reason of decreasing cell number in experiment 2 is due to cell apoptosis caused by exotic electric stimuli which results blocking communication of electrolytes upon cell membrane.

4. Conclusion

In this study, we cultured MC3T3-E1 cells on graphene substrate with Teflon template. Electric stimuli 0.25mA, 0.1mA are applied to cells on graphene and the number of cells is estimated by cell counting method before and after stimuli. The result shows adhesion on graphene substrate is quite good compared to cell culture dish, and different electric stimuli, 0.25mA and 0.1mA, affects differently in cell growth. Therefore, for exact relationship with electric stimuli and cell growth, further studies should be conducted to find effective electric current value for proliferation and differentiation. Especially, more experiments with osteogenic media for preosteoblast differentiation with electric stimuli is needed.

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