Controlling directional migration
Of neural stem cells electrically

Dr. Amira Aumari
Department of animal biology – faculty of Sciences – Damascus University – Syria

Abstract

Embryonic stem cells from mouse strain (OCT4-GIP) was cultured in medium (DMEM/F12) supplemented with leukaemia inhibiting factor (LIF) in Petri dishes treated with gelatin 0.1% and the cultures incubated in an incubator CO2-temperature 36.9–36.7 °C, and CO2 partial pressure of 9.4 to 2.4%. Cultured cells responded to factors of culture and proliferated well, and dispersed new cultures new daily. Quoted cultures cellular processing of a special called electrotactic chamber contains the medium of culture and provided of circuit electrical particular, and provided chamber to control the PH, and also provided by installation of electrical control ensures the strong from 5.5V to 12V for the cells cultured. For these cultured cells the experiment revalated this cell (OCT4-GIP) move effectively and activity apparent when they exposed of the electric field in the electrotactic chamber, and that this movement much larger than the movement of the same cells cultured in the same conditions without electric field (control). This phenomenon is useful stimulating the migration of stem cells in vivo tissue towards the target of a terrorist (like wounds) to expedite and facilitate the healing, as well as directs the context of these cells to repair nerve tissue battered.

Key words:

Embryonic stem cells, Electric field, wound healing, Migration of embryonic stem cells.
Introduction

Stem cells have been known long time ago. They were called different names in biology and histology, for example, Hematopoietic stem cells. The features of these cells have been studied again in order to identify it and understand their growing and contrasting features, as well as, their restoration ability. (Kelly, 2007). (Marshak, et al, 2001). Researchers described it as pluripotents– have the ability to take different forms – able to give many series of cells, behavior and contrasting controlled, and able to restore and renew their selves. (Institute of Health, 2004). National (Nagano, et al, 2008). After thoroughly studies, these cells have been divided into two categories: Embrionic stem cells (ESCs) and adult stem cells (ASCs). The first category works during the embryonic life, however, the second works in adults bodies after childbirth. Different studies have been studying these cells, in order to use the formation and plasticity abilities of these cells in generating contrasting cells different from those given in normal place. (Hongan, et al, 2009) (Reubionoff, et al, 2009) Thus, Hemtopoietic stem cells could generate non-blood cells, neuronal, muscle, and bone cells, for example. Therefore, there is a tendency to use stem cells in what is called “cell therapy” to treat some diseases that cannot be treated by traditional means. For example, they could be cultured to recover resected neuronal tissues, to replace cancer blood cells with healthy cells or to treat Barkion disease and other diseases. (Wobus, et al, 2008) (Motton and others, 2010). The difficulties in studying these cells are: how to separate these cells from their normal environment, how to identify them precisely, how to transport them, how to control their contrasting forms as desired, and finally how to transport them into the body.

Different modern means have been applied for this purpose. In this research, the researcher used the ability of these cells to move and emigrate within electrical field to separate these cells in glass – In Vitro – and examined the possibility of applying the same procedure for cuts restoration, for example.

Not long time ago, it was known that some cells emigrate if put under weak pressure – from electrical field produced by direct current. Many scientists studied this phenomena like Bing Song, Min Zhao and others. They found that electric field motivate the cells to move and emigrate. Nowadays, the effects of electric signs of physiological strengths on the controlled cells during the emigration cuts recovering. This study opens the door for observing the cells responses to electric signs as shown, to some extent, in electric fields during increasing and cuts recovering.

The direct current in electric field is generated naturally inside the living body (in vivo). Duboi Reymond was the first to discover this fact, in details, 150 years ago. He did that after having a cut in his finger, and using various modern techniques to measure the strength of the direct current which was approximately self generated 1 µA. (Min zhao, et al, 2006) (Bing Song et al, 2007). Other researchers like Bing Song and Min zhao reached similar results regarding the strength of the direct current inside cuts in all types of skin and cornea, including the human skin. The results of these studies show that cuts generate an electric field soon after hurting the epithelium, and then continue until other epithelium formed. The second epithelium regains its job as electric resistance barrier. The strength of these electric fields are about more than 40 – 50 mVmm -1 for corneous (fabaceous) cuts and 100-150 mVmm – 1 for skin cuts. (Bing Song et al, 2007).

Many experiment indicate the essential role of these electric signs which control and direct the cells emigration, directly into the cuts until it recovered. The strength of the direct current in electric field
was measured during its endogenous, its development, its increasing, and after the damage in non-epithelium cells. It was found that electric fields generate due to place and time diversity in culturing the epithelium. It - K+, Na+ and the place electric resistance of the epithelial sheets. (Bing Song et al., 2007).

This research leads to study the response of the embryo stem cells towards electric field by studying the emigration of these cells after applying different strengths of direct current for several hours. The result of these experiments could be used in the future treatment applications. The stem cells inside the organ have the ability to emigrate towards the damaged areas, because of the self electric current which motivate them to emigrate. This could be applied in clinical therapies for patient with neural diseases like those who suffer from Parkinson. (Malin Parmar and Menyli, 2007). In this study electric fields were applied into cultured cells, where they were put in an electrical chamber designed directly before the experiment. In this chamber a medium was put together with the inhibiting factor to stop the increasing ability.

**Material and Methods**

**Cells lines used in the experiment:**

The experiments were applied on mice embryonic stem cells (mEs), which were got by Dr. Smith, Research Institute of Stem Cells, Andhra.

Cells line: OCT4 – GFPmE. (OCT4- Gip cell line). The embryonic stem cells are OCT4 Gip, taken from mouse race 129 Ola which carry OCT4 – GF pirespace transgene.

- **Cells culture:**

In stem cells laboratory (Medicine, Science School, Ebriden, Scotland), the embryonic stem cells taken from a mouse embryo – blastocyst – were cultured in special transplantation containers that contain medium DMEM/F12 and the Leukemia inhibiting factor (LIF). Before using these culturing containers, they should be painted with % 0.1 bovid gelatin (sigma cat G9391).

- **Embryonic Stem Cells Feeding:**

The normal medium was changed in embryonic stem cells daily. The culturing containers were kept inside incubator at 36.9-36.7 c and 4.9-4.2 % co2.

- **Embryonic Stem Cells Splitting:**

The content of each transplantation (culturing) container were distributing after splitting into four containers (1:4) within the medium and LIF factor. This was done by connected processes specially designed for embryonic stem cells splitting.

These containers were kept inside the incubater again. The cells were examined by the microscope (Leica DMIL) with camera Lecia DF C42c, connected to a computer (DELL), through all the mentioned practical processes.
- Centrifuge PK110 Centrifuge Model. THREO ELECTRON CORPORATION – was used for stem cells gathered, taking ball forms inside special tubes in the device, as well as, inside the culture tent – LAMINAR Hood, TRIMAT2 CIASS II.


- **Direct current electric field application on embryonic stem cells:**

Embryonic stem cells were put in electrotactic chamber, made from a sterilized container specially designed for tissues culture, painted with 0.001% gelatin and sometime with polyornithin-laminin. This was done by practical connected processes specially designed for this purpose. These chambers require also glass slips, Agar bridges %2 (Agar, sigma, cat. no A7002), high vacuum grease, steinbergs solution liquid, as well as, HEPES to control the medium PH about 7.4. Figure(1)
A- A photo taken from the protocol for the researchers (Bing Song et al., 2007).

B- A photo of metamorph microscope and other devices used for the research purposes.

C- A photo of electrotactic chamber during the preparation phase and when put under metamorph microscope connected to a computer and a device specially designed for generating electric energy.

D- The electrotactic chamber, prepared by the researcher according to the protocol (Bing et al., 2007).

"Note: photos 2, 3, 4 were taken by the researcher" metamorph system was also.

**Devices used in this system:**

- Energy device model (CONSERTE 143). Direct current power supply with cables.
- Another device connected with the first one LAMBAD 10-2 sutter instrument co.
- Florescence device model: Florescentes model eb q100ISOLATED.
- Voltage meter device.
- Silver wire electrode (A dvent cat, no. AG549109).
- Metamorph microscope model ZEISS AX10Vert s 100 tv., connected to DELL computer 36.9 c.

**Results and Discussion**

After directing the direct electric current with different strengths - starting from 5.5 v, ending with 12 v, in the electrotactic chamber which contains monolayer embryonic stem cells OCT4, for 4-7 hours. The instance was used under the same conditions without applying electric field. The movement of the cells and its emigration was filmed by Time – Lapse system, programmed together with other computer programmes which give all the coordinates related to cells emigration within the three dimension X, Y, Z, with multiple wavelength recording, green fluorescence protein (GFP) to express the same embryonic stem cells used in bright field as they are healthy, good, fit, indistinct, and finally bright light field.

Also, a photo of cells emigration was taken by the metamorph imaging system (molecular devices UK).

To get data and results, image J-mtrackJ system was used, and then the digital photos were analysed by Time – lapse system. The same conditions were applied on the instance embryonic stem cells without exposing them to electric field.

It is worth mentioned to say that before executing the experiment with the electric field, metamorph systems must be prepared at least two hours before, however the electrotactic chamber must be prepared one to two days earlier.
The following forms, show the embryonic stem cells OCT4 before splitting process and after it. This process is very important, difficult and complicated, aimed to culture embryonic stem cells continuously and connectedly without being effected by pollution. This technique requires patience, hard work, and careful attention to details during the execution phase, taking into consideration that getting these embryonic stem cells is not an easy task before culturing and splitting these cells.
Before splitting x400 Oct4 p42

After splitting x200 Oct4 p43

After splitting by 2.5 h x200 Oct4 p43
The following forms show the results of the digital photos analysis by image J-mtrack J programme. The forms were chosen from many other forms. Different current strengths were also chosen 10.8 v for 4.5 hours on OCT4 P43 cells, 11.5 v for 4.5 hours on the OCT4 P39 cells, and 11.5 for 7 hours on other OCT4 cells, because the research pages should be restricted into a particular number according to the publication terms.

These forms show the movement and emigration of embryonic stem cells using Time lapse system. The movement and emigration of all embryonic stem cells could be clearly noticed, not only the ones circled as an example. These photos were taken at the starting point of the experiment, another photos after 2-2.5 hours of the experiment and a third group after 4-5 hours, in bright light field condition and in FIIC fluorescents model after exposing the cells in the experiment to different strengths of electric current mentioned before.
0h Oct4 P43 pol-lam. E.F 10.8 V x100 Bright Field

2.5h Oct4 P43 pol-lam. E.F 10.8 V x100 Bright Field

4-5h Oct4 P43 pol-lam. E.F 10.8 V x100 Bright Field
0h Oct4 P43 pol-lam. E.F 10.8 V x100 FITC

2h Oct4 P43 pol-lam. E.F 10.8 V x100 FITC

4-5h Oct4 P43 pol-lam. E.F 10.8 V x100 FITC
0h Oct4 P46 pol-lam. E.F 11.5 V x100 Bright Field

3.5h Oct4 P46 pol-lam. E.F 11.5 V x100 Bright Field

7h Oct4 P46 pol-lam. E.F 11.5 V x100 Bright Field
0h Control Oct4 P44 pol-lam. x100 Bright Field

2h control Oct4 P44 pol-lam. x100 Bright Field

4-5h control Oct4 P44 pol-lam. x100 Bright Field
It is clear from the precious photos that the instant pluripotents embryonic stem cells movement or their emigration is so limited, due to the distance they passed towards other direction, compared with pluripotents embryonic stem cells exposed to electric field produced by direct current, as far as the duration extend and the electric strength increase, the emigration distance would be longer. This important result means that embryonic stem cells have responded well to electric field so they emigrated. In fact, It was not an easy task to reach such practical result.

Min Zhao, Bing Song, and others improve by their researches on the treatment of cuts in mouse skin and cornea, that the direction of the direct current in cornea and skin cuts P110Y in experimental mice was similar to that in wild mice.

The researchers noticed a movement in the surrounding of epithelium tissues of the cornea cuts in wild mice. That movement was significant (P=0.027). The movement accelerate when applying electric field (E.F) towards the negative pole inside the cut (Min Zhoa et al., 2006).

Bing Song and his colleagues say that emigration levels of different types of cells, are significantly different. Zhao agreed with him and stressed that some cells are quick in their movement like the neutrophils with emigration levels 20-30 µm min-1.

Applying the electrotactic experiment on such types of cells requires a stability in PH, heat and other particular condition during the experiments. It was noticed when applying 30-60 m of experiment on other types of cells, a slow emigration of these cells, so they need longer time to pass the distance required for a cell to emigrate. The same was noticed regarding their speed limits and direction. Therefore, such experiment need stability in its condition like heat and PH.

Other scientists used chemotaxis chamber to analyze the controlled emigration of nerve progenitor cells. They noticed that the movement of these cells was quick especially after the first hour of preparation. After 4-8 hours, the cells moved and emigrated as a three – dimensioned radial connected series starting in culturing centre towards its peripheral. (weiner, ..2008).

When Lei Li and his colleagues used electric fields on neuronal/progenitor embryonic cells in 17-18 – day –old mice embryo. They found that these electric fields motivated the movement of neuronal/progenitor stem cells (NSPCs) towards the cathode – negative pole. The activation of N- methyl – D – a separate receptors was proved to lead to increasing in physical tie between one of the enzymes the Rho GTPase Rae 1 (which is GTPase connected with the receptors NMDARs) and Intracellular actin cytoskeleton (Lei Li et al., 2008). These experiment led to using the electric field as a controller specifies the emigration of neuronal stem cells. They also set a role for the RACI / NMDAR actine in transporting the motivation signs through the electric field.

The results of this study are similar to those got by Lei Li and his colleagues, when he measured the movement of the cells. They found that every cell move directly in X level is moving towards the negative pole has -1 value, however every cell move directly towards positive pole has +1 value. (Lei Li et al.…..2008).

This study stressed the results of other studies used the electric current application on the brains of Parcnion disease patients, and depression patients. These clinical studies show that electric current application on brain by alerting particular deep areas inside the brain, ease the Parcnion disease and other depression diseases. (Kringlby M, others, 2010).
To sum up, results reached firstly stressed that culturing processes of embryonic stem cells OCT4 requires hard work, patience and careful attention in order to success and get healthy, good, and fit culturing. Secondly, these embryonic stem cells OCT4 respond significantly to electric field. This leads to using these experiment in cells response to electric fields produced by direct current. These results lead also to using safe physical mean in medical field by applying the electric fields to treat the damaged areas in the brain. This could be done by directing the neurotic stem cells emigration towards these damaged areas to correct them.
REFERENCES


