Efficient femtosecond laser driven SOX 17 delivery into mouse embryonic stem cells: Differentiation study

S.L Thobakgale[1,2], S.L Manoto[1], S. Ombinda-Lemboumba[1], M. Maaza[2], P. Mthunzi-Kufa[1,2*]
[1]Council for Scientific and Industrial Research (CSIR) National Laser Centre, PO Box 395,Pretoria, 0001
[2]University of South Africa (UNISA), Department. of Physics, PO Box 392, Unisa 0003

***Corresponding Author:** PMthunziKufa@csir.co.za

ABSTRACT

Embryonic stem cells (ESC) show great promise in regenerative medicine because of their ability to self-renew and develop into other cell types found in a multicellular organism. The latter occurs via the process of differentiation where a normal stem cell undergoes genetical and physical transformations as it develops into a new cell type. The result of the differentiated cell depends on the environmental conditions surrounding the cell. As such, methods like chemical, viral and physical transfection have been created to influence stem cell differentiation towards a specific lineage. In this presentation, we introduce a laser method of transfection where we porate mouse embryonic stem cells in the presence of plasmid DNA Sox17, to influence differentiation towards endoderm lineage. Secondly, we used fluorescent staining of stage specific embryonic antigen 1 (SSEA-1) to track the extent of differentiation were the intensity of the SSEA-1 decreased as the cells changed from their embryonic state to endoderm. Lastly, we analysed the adenosine tryphosphate (ATP) and Lactate Dehydrogenase (LDH) content in the cells as a measure of cell viability. post irradiation

INTRODUCTION

Embryonic stem cells (ESC) are round, non-specialized cells that are able to self-replicate continuously and differentiate or develop into other cell types of a multicellular organism (1). Differentiation occurs at different levels, as such, ESC are classified according to the various stages of potency, which is the capacity to differentiate to into specialized cell types. Differentiation has afforded ESCs much attention in therapeutic research as a potential aid in regenerative and transplantation medicine (2). ECS can be genetically programmed to treat neural diseases and pancreatic disorders such as diabetes (3-4) . The stages of embryonic development is linked to the potency levels of ESC. Totipotent cells are stem cells that can form all cell types, these cells are found in the zygote and blastomere. Pluripotent cells descend from totipotent and are found in the blastocyst, these cells are capable of producing nearly all cell types because they form the three germ layers: mesoderm, endoderm and ectorderm. The next level is multipotent stem cells and these cells give rise to various types of organ tissue such as neural and hemopoitetic stem cells. Lower levels of potency include oligopotent stem cells which are those that can produce related cell types and unipotent cells, which only produce one type of cell (5).

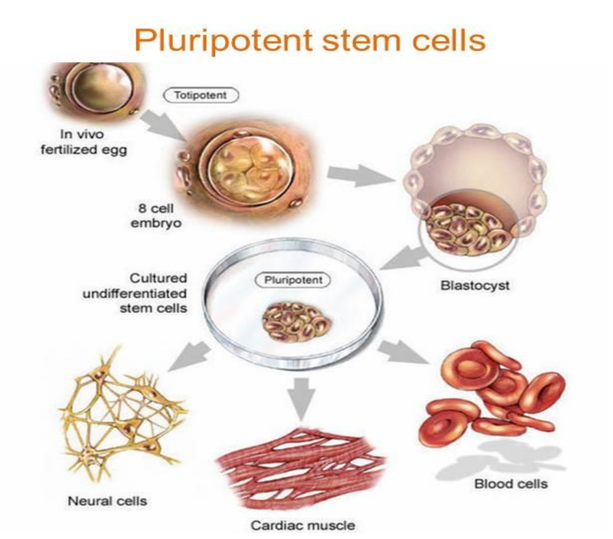

Figure 1: Pluripotent stem cells have the ability to differentiate into different types of specialized cells such as blood, neural, cardiac muscle and liver.

Transfection or insertion of exogenous DNA into ESC is one way of manipulating the differentiation process towards development of specific tissue *in vitro*. The type of DNA used in our study is a transcription factor Sox 17, which is a biomolecule that is associated with the initiation of pluripotent differentiation towards multipotent endoderm tissue (6). Photo-transfection is method that uses photons to porate cell membranes and allow free entry of DNA into the cell. We have built a photo-transfection optical setup connected to a 1kHz femtosecond laser (fig.3) to transfect mouse embryonic stem cells (mES) with Sox 17 to induce endoderm production. Mouse ES cells contain protein markers on their surface such as SSEA-1, which tracks pluripotency in mES that have been genetically modified (7). In our study, we used fluorescence staining of this antigen to visualize the differentiation process post photo-transfection. We also analyse cell viability using molecular assays of ATP and LDH, which signal cell health and early signs of necrosis or cell death.

MATERIALS AND METHODS

mES cell culture, 24 hour incubation at 37^0 5% CO_2 → Photo-transfection, 6µW average laser power at stage, λ=800nm, 24 hour incubation. → Fluorescence imaging, ATP and LDH analysis

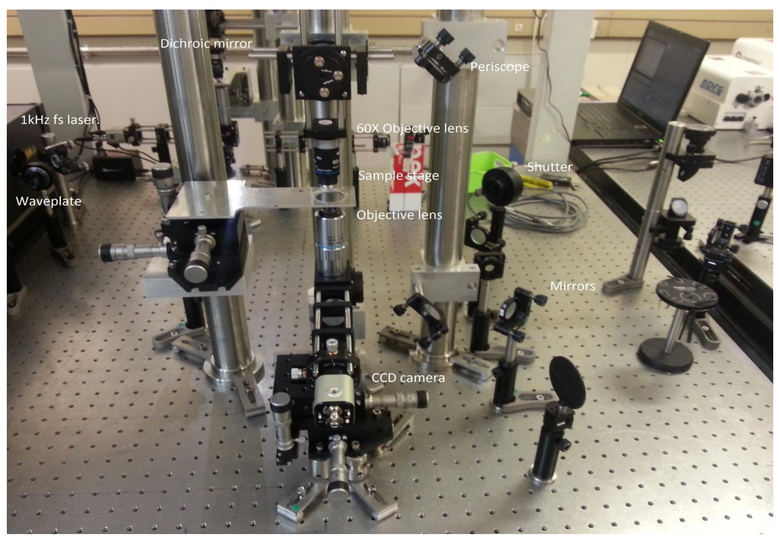

Figure 2: Optical set up for femtosecond photo-transfection. 1 kHz Ti: sapphire laser, Pulse duration <130 femtoseconds, Beam diameter 10-15mm. Average power 1 Watts. 800nm wavelength.

Photo-transfection of mES was done using the optical setup shown in figure 2. The beam propagated from the 1kHz laser was directed using silver mirrors to the waveplate for polarization and power adjustment. Again using silver coated mirrors, the beam was aligned through a microsecond shutter for setting the exposure time. A periscope was used to elevate the beam into the dichroic mirror for wavelength filtering before it reached the 60x objective lens. The beam was then focused onto the sample where laser and matter interaction took place. A second objective lens was attached to collect the light and focus it through a two lens system. A CCD camera was used together with Koehler illumination set up for imaging and background light respectively.

RESULTS

Cell viability and cytotoxicity

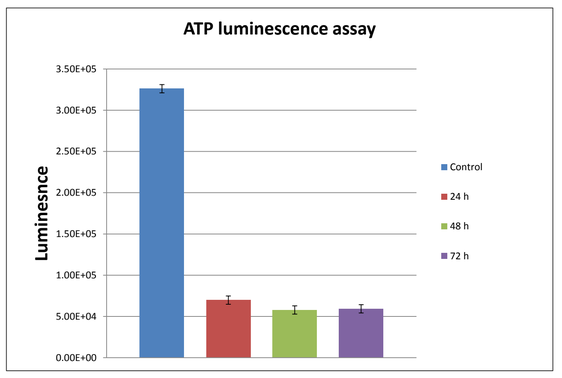

Figure 3: ATP analysis of samples,24,48 and 72 h post irradiation. 60secs integration time with Glomax ™ luminescence instrument

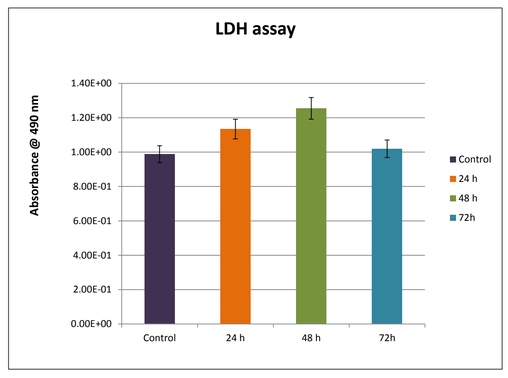

Figure 4: LDH analysis 24,48,72 h post irradiation, 60 secs integration time with Glomax™ absorbance reader

From the above images, ATP content decreased as seen in the experimental samples probably because of the induced differentiation which requires more energy for metabolism (8). Secondly, LDH was released slightly a bit higher in the experiment compared to the control which can be attributed to change in membrane integrity caused by the laser/matter interaction (9). A subsequent decrease in LDH is seen at 72 h, where suspected membrane restoration has taken place.

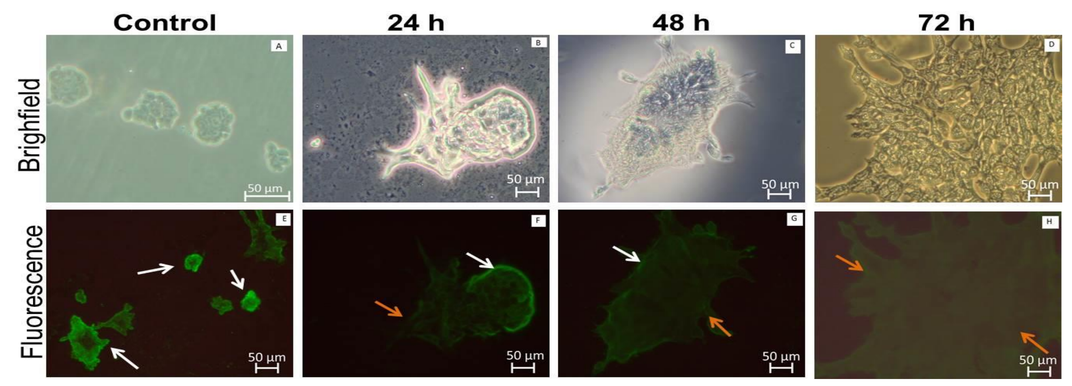

Figure 5: Brightfield (top panel) and fluorescence images of mouse embryonic stem cells. Control cells appeared as round colonies. Expression of the SSEA-1 marker is shown by white arrows and orange arrows show mouse embryonic cells that did not express the marker.

The results in figure 5 show a steady change in morphology of the cells over the course of 72h. This change is related to ongoing differentiation as a new cell type is formed. Fluorescent staining showed a decrease in SSEA-1 expression (24-72h) caused by the induced differentiation.

CONCLUSION

- The study shows that our photo-transfection optical set up was able to deliver Sox17 into mES
- Cell viability were used to give insight on the recovery mechanics of mES post photo-transfection
- From the microscopic analysis on mES, we can conclude that differentiation was successful based on the change in morphology and reduced SSEA-1 content after 72h. Future work will involve confirmation of genetic makers of endoderm tissue via reverse transcriptase PCR.

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