Chapter 3
Development of optically sensitive cells

3.1. Introduction

Over the past few years, the field of optogenetics has gained widespread attention, particularly in developing optically sensitive cells. Although this emerging research opportunity was exploited first in the field of neuroscience, a combination of optics and genetic engineering has spread its wings into other domains as well, mainly because it enables understanding and studying of the underlying biological functionality. The technique is considered non-invasive and non-contact and importantly facilitates wireless control with high spatial resolution and temporal precision. Optogenetics involves steps including (1) development of gene constructs responsible for the expression of light sensitive proteins, (2) insertion and expression of such genes in the host cells, (3) stimulation of the optically sensitive cells and finally, (4) observation of the biological responses to derive the underlying mechanism (Deisseroth 2011; Pastrana 2011).

Ion channels are located in the cell membrane, comprising of a diverse group of proteins that can be stimulated by a variety of stimuli such as voltage, temperature, ligands and even light (Alberts et al. 2002). Ion channels have been long known for their roles in the excitability of neurons and muscles, whereas it is now well recognized for its participation in the physiology of non-excitable cells (epithelial cells) as well (Ambrosi et al. 2014; Pinto et al. 2009). It helps in maintaining the optimum concentration of salt and water in the cytoplasm; and also modulate cellular volume and pH of cells (Subramanyam and Colecraft 2015). Such ion channels can prove their impact in gaining an insight into the structure-function mechanisms of some potentially fatal degenerative diseases. Because of their extensive distribution over cell membrane and their association in plethora of biological processes, ion channels can be exploited as potential therapeutic targets (Bagal et al. 2013). Research findings have indicated a crucial involvement of ion channels in generation of reactive oxygen species (ROS) (Simon, Varela, and Cabello-Verrugio

2013), inducing apoptosis, cell proliferation and cancer (Bortner and Cidlowski 2014; Lang et al. 2005). Ion channels have been of vital importance in human physiology and toxicology (Restrepo-Angulo, Vizcaya-Ruiz, and Camacho 2010) as they have been critically involved in several human diseases such as neurological disorders (Kumar et al. 2016), skeletal muscle disorders (Barchi 1993), lung diseases (Salomon et al. 2016), liver diseases (Ramírez et al. 2016) and many more (Niemeyer et al. 2001). Application of engineered light-gated ion channels as biosensors further supports their use in reducing the harmful effects and identifying the symptoms of a disease at an early stage (Banghart, Volgraf, and Trauner 2006). Light-gated ion channels such as channelrhodopsin 2 (ChR2) are amongst the most exploited ion channels since their discovery in the last decade. Channelrhodopsin-2 (ChR2) is a light activated channel derived from the algae *C. Reinhardtii* (Nagel et al. 2005).

ChR2 as an optogenetic tool provides high spatial and temporal control of the cells, which has led to the development of optically sensitive in vitro tissue models. Researchers from the University of Bonn have combined optogenetic tools with induced pluripotent stem cells (iPSCs) derived cardiomyocytes for the development of a high-throughput drug screening platform that has tremendous potential especially in the field of cardiology. In their study they have demonstrated the transfection of commercially available iPSC derived cardiomyocytes, infected with adeno-associated virus for stable expression of ChR2. Thereby, characterization of the unfavourable effects of the known ion channel blockers and pro-arrhythmogenic drugs on $Na^{2+,}$ $Ca^{2+,}$ and K^+ channels, was performed. The results from such experiments provide insights into developing strategies for ion channel related drug discoveries (Rizzetto et al. 2018). Another recent work by Uzel et al. reports development of a microfluidic device for the formation of physiologically relevant, optically excitable, three-dimensional neuromuscular junction. The microfluidic

device used in the study facilitates the co-culture of optically excitable ChR2 transfected motor neurons along with muscle strips derived from myoblasts in a 3D gel. An essential component of the system is the muscle strip formed in between PDMS pillars. Such an arrangement enables measurement of contraction forces produced by the muscle strip on optical stimulation of ChR2 transfected motor neurons. Thus, the device has tremendous potential for studying normal neuromuscular junction physiology, pathology and drug discovery (Uzel et al. 2016). Various studies have been carried out to create optically sensitive skeletal muscle cells. These optically sensitive skeletal muscle cells have been used to understand the physiological and pathological aspects of skeletal muscle myogenesis (Asano, Ishizua, and Yawo 2012; Sebille et al. 2017; Cvetkovic et al. 2014; Raman et al. 2016).

The chapter demonstrates, the transfection of C2C12 skeletal myoblasts with a plasmid construct AAV-CAG-ChR2-GFP to render C2C12 skeletal myoblast cells light sensitive. Along with C2C12 cells, cell lines like NIH 3T3 and HepG2 are used as model cells for the establishment of transfection procedures. For the purpose of transfection, a liposomal-based transfection method was utilized. The plasmid DNA contains ubiquitous mammalian promoter CAG, ChR2 gene, and a green fluorescence protein (GFP) gene within it. The expression of the plasmid enables incorporation of a ChR2 membrane protein along with identification of transfected cells with the help of emission of green light. When illuminated with ~470 nm wavelength, the retinal chromophore inside the channel isomerizes from *cis* to *trans* configuration and vice versa and therefore opens the channel (Hegemann and Möglich 2011). This opening and closing of the ChR2 has mostly been associated with non-specific influx of cations like H^+, Na^+, K^+, and Ca^{2+} which may have positive implications in governing cell functionality (Barritt, Chen, and Rychkov 2008; J. Y. Lin et al. 2009). The transfection of ChR2 channels has been previously

investigated in excitable cells, and to the best of our knowledge, its first-time integration of ChR2 in HepG2, which is a non-excitable cell.

3.2. Materials and method

3.2.1. Materials

The *E. Coli* XL 10 gold bacteria with the plasmid DNA AAV-CAG-ChR2-GFP (Addgene plasmid #26929) (a gift from Edward Boyden) was procured from [Addgene, Massachusetts, USA]. C2C12 mouse myoblast cells, Human liver hepatocellular carcinoma cells (HepG2) and mouse embryonic fibroblast cells (NIH-3T3) cells were procured from National Center for Cell Science (NCCS) Lab, Pune, India. NZY broth [Himedia, Mumbai, India], Luria Bertani (LB) agar [Himedia, Mumbai, India], Dulbecco's Modified Eagle Medium (DMEM) [Himedia, Mumbai, India], Fetal Bovine Serum (FBS) [Himedia, Mumbai, India, Penicillin-streptomycin [Himedia, Mumbai, India, Trypsin-EDTA [Himedia, Mumbai, India and Phosphate buffer saline (PBS) [Himedia, Mumbai, India], Lipofectamine™ 3000 [Thermo Fisher Scientific, Bengaluru, India] and Opti-MEM™ [Thermo Fisher Scientific, Bengaluru, India].

3.2.2. Bacterial culture and isolation of plasmid AAV-CAG-ChR2-GFP using alkaline lysis method

The *E. Coli* XL 10 gold bacteria with the plasmid AAV-CAG-ChR2-GFP was a gift from Edward Boyden. The bacteria were grown in NZY broth with ampicillin antibiotic at 37°C and 160 rpm for 20 hours in a bacteriological incubator shaker. Further, streak method was performed on a standard Luria bertani (LB) agar plate to obtain isolated bacterial colonies. Handpicked bacterial colony was inoculated in NZY broth (at a temperature of 37°C and 160 rpm for 20 hours) and observed for turbidity in the broth. The bacterial culture was pelleted, and alkaline lysis was performed to isolate the plasmid DNA. Phenol chloroform extraction method followed by ethanol precipitation was applied to purify the plasmid DNA. After isolation of plasmid DNA, preliminary agarose

gel electrophoresis was also performed to determine the presence of single intact plasmid DNA. The obtained plasmid DNA was quantified with respect to concentration and purity by performing spectrophotometry [BioSpectrometer, Eppendorf] (Sambrook, Fritsch, and Maniatis 1989).

3.2.3. Culture of C2C12, HepG2, and NIH 3T3 cell lines

The cells were cultured in polystyrene culture plates with a culture medium containing 89% Dulbecco's Modified Eagle Medium (DMEM), 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (100 µg/mL Penicillin, 100 µg/mL Streptomycin). The cells were grown under standard conditions of 37 ºC temperature, and 5% CO_2 in a CO_2 incubator [Galaxy 170S, Eppendorf]. The cells were subcultured by trypsin-EDTA (0.25%) dissociation after they reached 80% confluency. The cells were counted using standard hemocytometer and seeded.

3.2.4. Transfection of C2C12, HepG2, and NIH 3T3 cell line with the isolated plasmid DNA

Transfection was performed by using Lipofectamine™ 3000 [Thermo Fisher Scientific] reagent kit. The kit contained Lipofectamine™ 3000 [Thermo Fisher Scientific] reagent and P3000™ [Thermo Fisher Scientific] reagent. Transfection was performed on C2C12, HepG2, and NIH 3T3 cell lines according to manufacturer's protocol. The initial cell density of 10,000 cells was used for plating. Cells were plated a day before the experiment in 96 well plate and transfection was performed after the cells reached a confluency of 70-80%. 0.3 µL Lipofectamine™ 3000 reagent was diluted in 5 µL of Opti-MEM™ medium. A master mix of DNA (0.2 µg) was prepared by diluting it into 10 µL of Opti-MEM™ medium, and then 0.4 µL of P3000™ reagent was added to the solution. The two mixtures; diluted Lipofectamine™ 3000 and diluted DNA was added in 1: 1 ratio (5 µL each) and was incubated for 15 minutes at room temperature. The complex was then added to the cells in a drop wise manner. The cells with transfection complex medium

were top upped with Opti-MEM™ medium to make the final volume 100 µL. These cells were then incubated for 2-4 days at 37°C in a CO_2 incubator. The cells were observed 3 days post-transfection. Transfection media was changed to cell culture media without penicillin-streptomycin. The media was changed every alternate day. Equal numbers of cells were seeded for the control experiments. Only Opti-MEM™ medium was added to the control wells.

3.2.5. Microscopic observations of the cells

An inverted fluorescence microscope [Nikon TiU, Nikon, Japan] was used to observe the morphology of the cells under bright field, and fluorescence images were collected using the appropriate filter. The presence of ChR2 was confirmed using the same blue band pass filter. The expression of GFP suggests successful transfection of the cell lines with the plasmid AAV-CAG-ChR2-GFP.

3.2.6. Methodology for fluorescence intensity analysis

The images were converted to grey scale using ImageJ software and background was subtracted by 50 pixels. In order to visualize the transfected cells, the subtracted image was added to itself by using the image calculator module. Using multi point tool in the software the cells were marked, and the mean intensity values of the fluorescence were measured using analyze module (Rueden et al. 2017).

3.2.7. Statistical analysis

A set of three experiments were performed. Statistical analysis was performed by using one way ANOVA post hoc Tukey means of comparison. The data were represented in the form of mean±standard deviation.

3.3. Results and discussion

3.3.1. Culture of *E. coli* XL 10 gold bacteria containing plasmid DNA (AAV-CAG-ChR2-GFP)

Standard streak plate method was followed to obtain isolated colony of bacteria on a

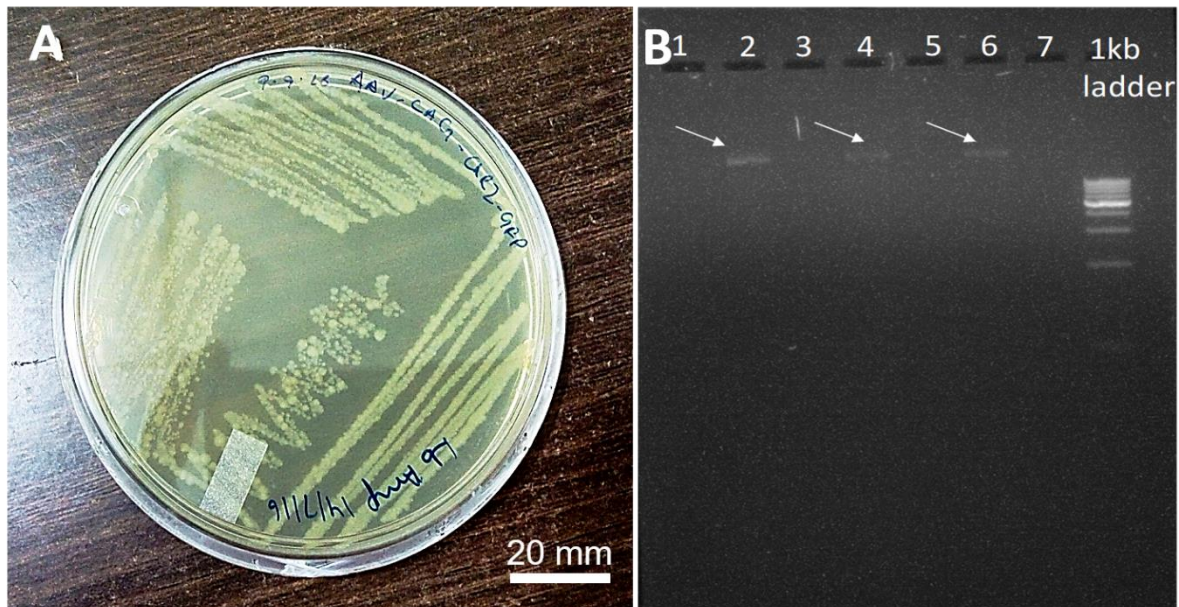standard LB agar plate. The results of streak plate method are as shown in Figure 3.1A.

Figure 3.1 (A) Streak plate culture of *E. Coli* XL 10 gold bacteria on Luria bertani (LB) agar plate, showing isolated colonies of bacteria after incubation at 37°C for 20 hours in bacteriological incubator. (B) Agarose gel electrophoresis of plasmid DNA AAV-CAG-ChR2-GFP. Numbers 1-7 indicate the well numbers. White arrows indicating the AAV-CAG-ChR2-GFP plasmid bands isolated by alkaline lysis method from *E. Coli* XL-10 gold bacteria.

The single isolated colony was picked from a trail of isolated bacterial colonies present

at the centre of the LB agar plate. The picked bacterial colony was inoculated into NZY

broth and incubated at 37°C. Furthermore, after 20 h, the NZY broth was observed for

turbidity, and the alkaline lysis method was carried out to isolate the plasmid DNA.

3.3.2. Isolation and estimation of concentration and purity of the plasmid DNA (AAV-CAG-ChR2-GFP)

The plasmid DNA obtained after alkaline lysis was further subjected to phenol

chloroform precipitation to obtain a purified form of the plasmid DNA. A preliminary

agarose gel electrophoresis of the isolated plasmid DNA was carried out to ensure the

presence of single intact plasmid DNA. Figure 3.1 (B) shows the results of the electrophoresis experiment. In the representative image, observation of three light bands corresponding to wells 2, 4, and 6 respectively can be seen (moving from right to left). A 1 kb DNA ladder was added for reference; as seen in the figure, all the bands are intact with no extra bands or tails. The results indicate that the sample contains pure plasmid DNA without any impurities of RNA or proteins.

Further, the characterization of isolated plasmid DNA was performed using spectrophotometry for estimating the concentration of DNA. The results of spectrophotometric analysis are presented in Table 3.1.

Table 3.1 Showing the results of spectrophotometry of plasmid DNA isolated by alkaline lysis method.

Concentration of DNA obtained	1.150 µg/µl
Absorbance at 260 nm/Absorbance at 280 nm	1.79 (~1.8)
Absorbance at 260nm /Absorbance at 230 nm	1.98
Absorbance at 230 nm	0.140
Absorbance at 280 nm	0.150
Background absorbance	0.047

The concentration of plasmid DNA was found to be 1.150 µg/µL. The concentration of plasmid DNA obtained is considered good enough for proceeding with the transfection process (Cardarelli et al. 2016). Absorbance values at wavelengths 230 and 280 were found to be 0.140 and 0.150, respectively. Ratios of absorbance at 260/280 nm, 260/230 nm were found to be 1.79 and 1.98, respectively. The 260/280 nm absorbance is generally used for assessing the purity of the plasmid DNA. The ratio obtained for isolated plasmid

DNA was found to be very close to the standard value of reported 1.8 (Ehrt and Schnappinger 2003), indicating the highly pure nature of the plasmid DNA. Thus, the plasmid DNA isolated by the alkaline lysis method was found to be intact, pure with the desired concentration. These parameters of plasmid DNA met the requirements of the Lipofectamine™ 3000 (Thermo Fisher Scientific) based transfection process.

3.3.3. Microscopic observation of transfected cells

The fluorescence-based observation is a convenient and standard method for evaluation of transfected cells. The plasmid DNA used for transfection contains genes encoding for ChR2 coupled with green fluorescent protein (GFP). Following successful transfection, the green fluorescence of GFP along the periphery of cells ensures the incorporation of ChR2 proteins into the cell membrane.

The significant level of fluorescence intensity was observed in case of NIH 3T3 cells after 3 days of transfection as shown in Figure 3.2 (A, B, C). Figure 3.2A shows the bright field image of the transfected cells. Figure 3.2B shows the fluorescence image corresponding to the bright field image with expression of GFP on the cell membranes indicative of successful transfection of Chr2 plasmid within the NIH 3T3 cells. Figure 3.2C is merged image of the bright field and fluorescent image, which allows a clear visualization of GFP expression on the cell membranes (inset). Figure 3.2 C and D represent the corresponding bright field and fluorescence images for NIH 3T3 cells respectively.
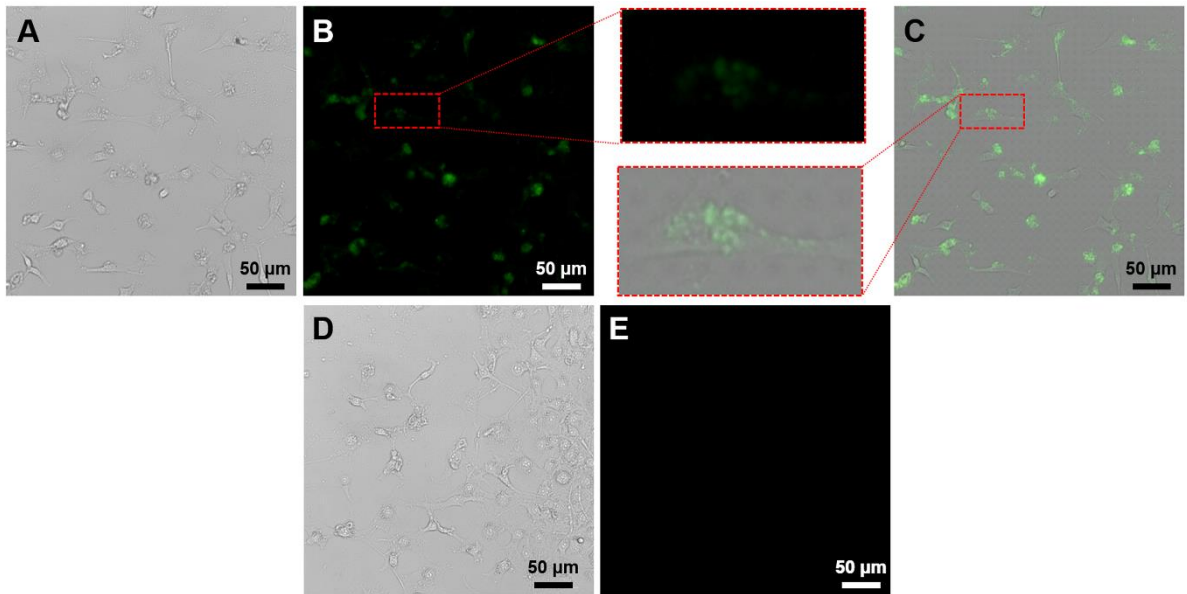
Figure 3.2 Shows transfected NIH 3T3 cells observed after 3 days of transfection. **(A)** Bright field image showing NIH 3T3 cells after 3 days of transfection, (B) Fluorescent image of A showing the NIH 3T3 cells which are transfected (visible in the inset). (C) Merged image of (A) and (B) showing localized maximum intensity regions of fluorescence indicating the cell surfaces with expression of membrane bound channelrhodopsin proteins on NIH 3T3 cells (visible in the inset).
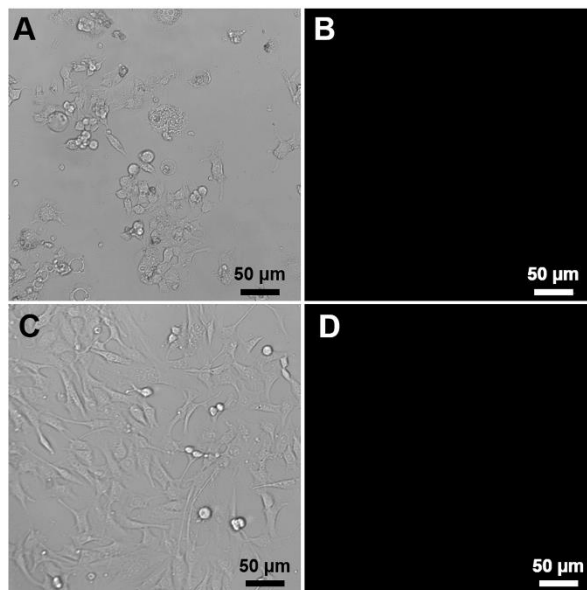

Figure 3.3 Shows no transfection in HepG2 and C2C12 cells after 3 days of transfection. **(A)** Bright field image showing HepG2 cells after 3 days of transfection, (B) Fluorescent image of A showing the HepG2 cells which are not transfected. **(C)** Bright field image showing C2C12 cells after 3 days of transfection, (D) Fluorescent image of C showing the C2C12 cells which are not transfected.

During the same period, HepG2 cells and C2C12 cells after 3 days of transfection did not

exhibit any notable intensity level of fluorescence as shown in Figure 3.3 (A, B, C & D).

Interestingly, 6 days post-transfection, HepG2 cells displayed remarkable intensity levels
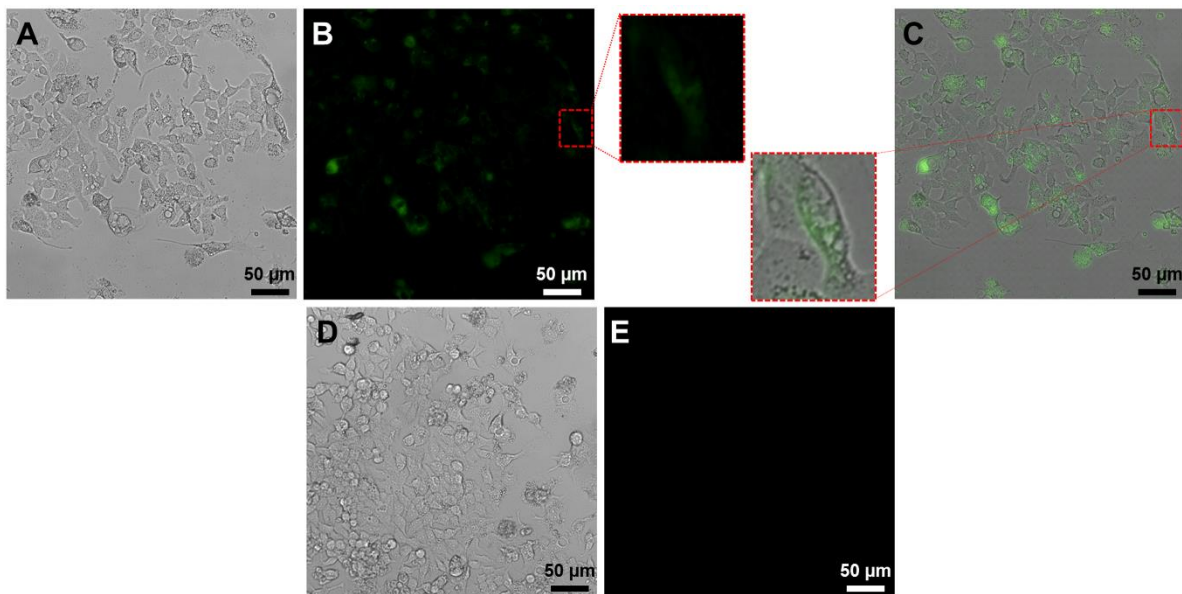
of fluorescence as shown in Figure 3.4 (A, B, C & D).

Figure 3.4 Shows transfected HepG2 cells observed after 6 days of transfection. (A) Bright field image showing HepG2 cells after 6 days of transfection, (B) Fluorescent image of (A) showing the HepG2 cells which are transfected (visible in the inset), (C) Merged image of (A) and (B) showing localized maximum intensity regions of fluorescence indicating the cell surfaces with expression of membrane bound channel rhodopsin proteins on HepG2 cells (visible in the inset), (D) A bright field image showing HepG2 control cells after 6 days of transfection, (E) Fluorescent image showing HepG2 control cells after 6 days of transfection.

Figure 3.4A shows the bright field image of the transfected HepG2 cells. Figure 3.4B

shows the fluorescence image of the cells corresponding to the bright field image with

expression of GFP on the cell membranes indicative of successful transfection of Chr2

plasmid within the HepG2 cells. Figure 3.4C is merged image of the bright field and

fluorescent image, which allows a clear visualization of GFP expression on the cell

membranes (inset). Figure 3.4D and E represent the corresponding bright field and

fluorescence images for HepG2 cells respectively. By this time, there was no notable

intensity level of fluorescence in transfected C2C12 as shown in Figure 3.5A, B.
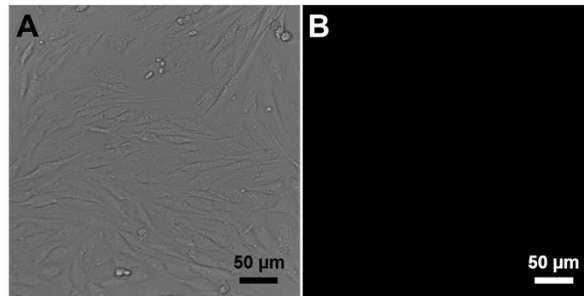
Figure 3.5 Shows no transfection in C2C12 cells after 6 days of transfection. **(A)** Bright field image showing C2C12 cells after 6 days of transfection, (B) Fluorescent image of A showing the C2C12 cells which are not transfected.
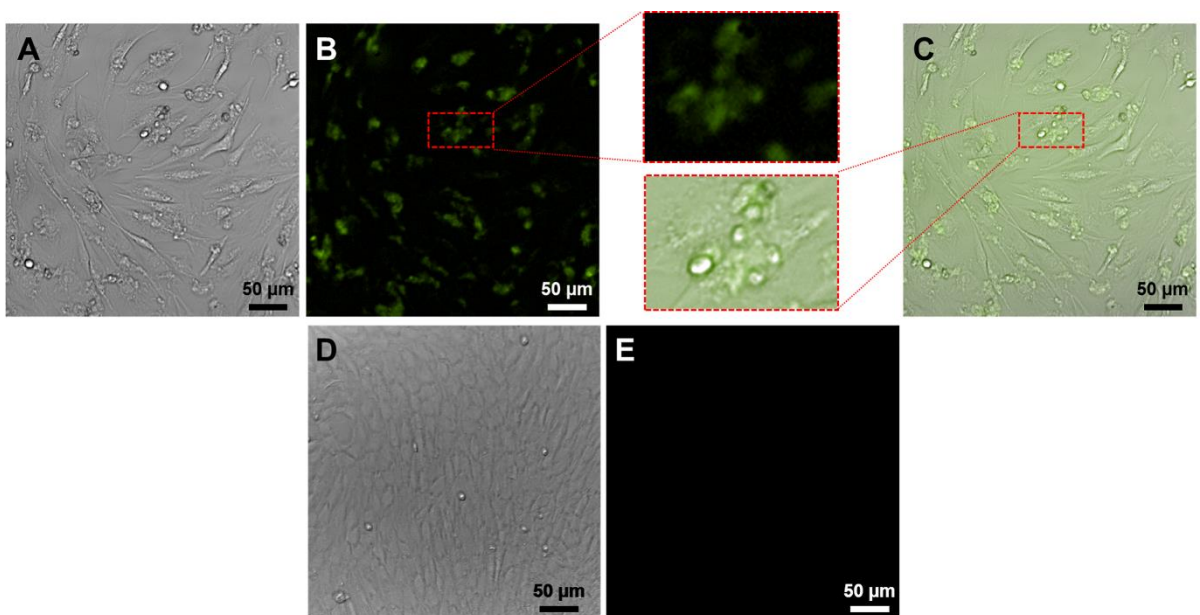

Figure 3.6 Shows transfected C2C12 cells observed after 9 days of transfection. (A) Bright field image showing C2C12 cells after 9 days of transfection, (B) Fluorescent image of (A) showing the C2C12 cells which are transfected (visible in the inset), (C) Merged image of (A) and (B) showing localized maximum intensity regions of fluorescence indicating the cell surfaces with expression of membrane bound channel rhodopsin proteins on C2C12 cells (visible in the inset), (D) A bright field image showing C2C12 control cells after 9 days of transfection, (E) Fluorescent image showing C2C12 control cells after 9 days of transfection.

It can be observed that, the C2C12 myoblast cells showed the expression of GFP after 9 days of transfection as shown in Figure 3.6. Figure 3.6A shows the bright field image of the transfected C2C12 cells. Figure 3.6B shows the fluorescence image of the cells corresponding to the bright field image with expression of GFP on the cell membranes indicative of successful transfection of Chr2 plasmid within the C2C12 cells. Figure 3.6C

is merged image of the bright field and fluorescent image, which allows a clear visualization of GFP expression on the cell membranes (inset). Figure 3.6D and E represent the corresponding bright field and fluorescence images for C2C12 cells respectively.

3.3.4. Fluorescence intensity analysis of transfected cells

Further, the fluorescence intensity analysis of the cells present in transfected wells in comparison to the fluorescence intensity from control wells for each of the transfected cells was done. The results of the analysis are represented in Figure 3.7A, Figure 3.7B and Figure 3.7C for NIH 3T3, HepG2 and C2C12 cells respectively. These results suggest that, in comparison to NIH 3T3 cells, HepG2 and C2C12 cells require relatively a larger amount of time to express the outcome of successful transfection. In addition, the results also indicate that the incorporation of ChR2 within the membranes of HepG2 and C2C12 through the transfection process is notably a "slow" event most likely due to the basic differences in their inherent biological behaviors (Romano et al. 2009). The plot represented in Figure 3.8 illustrates the percentage of the transfected cells in all the three cell types. The plot thus indicates the efficiency of liposomal agent-based transfection process in each of the transfected cells. The percentage of transfected NIH 3T3, HepG2 and C2C12 cells were found to be around 46.042%, 41.26% and 58.16% respectively.
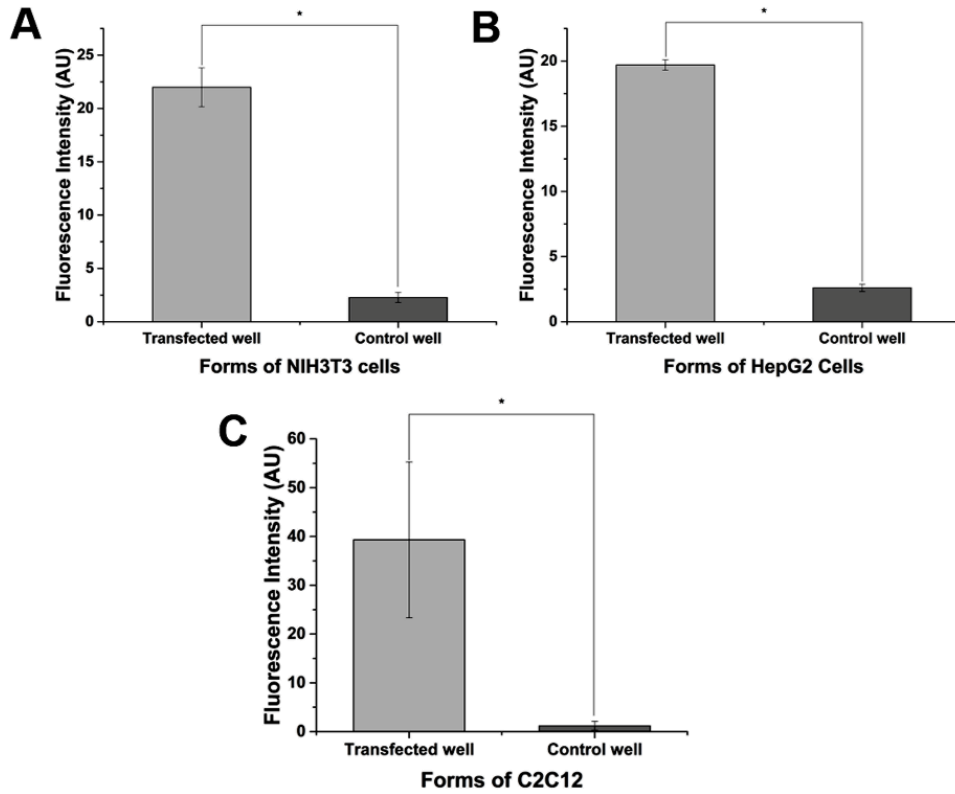
Figure 3.7 (A) Graph depicting the difference in the fluorescence intensity of the NIH 3T3 transfected cells and the control. Asterisk indicates the level of significant difference ($p < 0.05$). (B) Graph depicting the difference in the fluorescence intensity of the transfected HepG2 cells and their control. Asterisk indicates the level of significant difference ($p < 0.05$). (C) Graph depicting the difference in fluorescent intensity of transfected C2C12 cells and their control. Asterisk indicates the level of significant difference ($p < 0.05$)
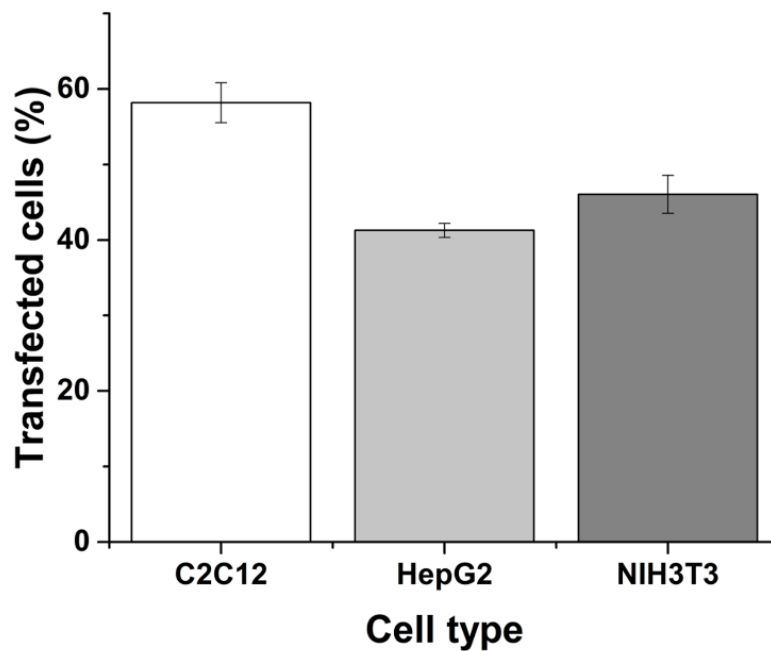

Figure 3.8 Graph depicting the various cell types used for liposomal transfection and their respective percentage of transfected cells.

Light-gated ion channels such as channelrhodopsin (ChR) are amongst the most exploited ion channels since its discovery in the last decade. These channels show changes in response to exposure with ultraviolet or visible light. Engineered light-gated ion channels propose a superlative form of spatial and temporal control. Channelrhodopsin is a cationic channel which allows non-selective conductance of the cations in the order $H^+>Na^+>K^+>Ca^{2+}$ (J. Y. Lin et al. 2009).

NIH3T3 cells have been previously reported to be transfected with ChR2 plasmid. The study involved co-culture of ChR2 transfected NIH3T3 cells with neonatal cardiac muscle cells. The light excited NIH 3T3 cells were responsible for gap junction mediated pacing of neonatal cardiac muscle cells. This indicates that there was direct transmission of signals from NIH3T3 cells to cardiomyocytes leading to their enhanced functionality (Nussinovitch, Shinnawi, and Gepstein 2014). A study involving co-culture of C2C12 cells by plating on fibroblast feeder layer showed the formation of myotubes with enhanced functionality with respect to their adherence and contractility (S. T. Cooper et al. 2004). However, another study involving the co-culture of C2C12 cells along with 3T3 fibroblast cells showed that, the fibroblast cells inhibited differentiation of C2C12 cells independent of contact between the two cells. Under contact condition, the myoblasts showed better alignment of myotubes upon differentiation (N. Rao et al. 2013). These studies suggest that co-culture of light sensitive NIH3T3 cell along with C2C12 cells may be prospect of research. In case of positive implications, the C2C12 cells may show enhanced functionality as in the first study by cooper et.al. However, if there is enhanced inhibition of differentiation, such a co-culture may lead to development of skeletal muscle disease model.

Furthermore, co-culture of ChR2 transfected NIH3T3 cells with HepG2 cells would enhance the functional properties of hepatocytes. This idea further gains strength from

the previous studies involving co-culture of hepatocytes with NIH 3T3. The co-culture environment not only favoured maintenance of normal hepatocyte phenotype but also allowed for deposition of extracellular matrix (Goulet, Normand, and Morin 1988; Shimaoka, Nakamura, and Ichihara 1987). In addition, enhancement in functional properties of hepatocytes like albumin secretion, cytochrome p450 expression, gap junctional communication was reported (Bhandari et al. 2001; Bhatia, Yarmush, and Toner 1997; He et al. 2018; Ito et al. 2007; Seo et al. 2006). Thus, co-culture with the transfected cells could lead to an enhanced tissue model with optimal functionality.

Generation of light sensitive C2C12 cells by incorporation of ChR2 was first reported by Asano et al. (Asano, Ishizua, and Yawo 2012). Same group conducted another study wherein they showed that rhythmic optical stimulation training of immature light sensitive C2C12 cells enhanced the morphological and functional development of myotubes (Asano et al. 2015). Thus, these studies show the potential of the transfected C2C12 cells for studying myogenesis. Furthermore, various studies have shown that these optically sensitive C2C12 cells could be used to create tissue models and bio robots, which can be used in potential applications including non-invasive drug delivery, multifunctional implants and high throughput drug screening (Asano et al. 2015; Sakar et al. 2012; Raman et al. 2016; Cvetkovic et al. 2014; Sebille et al. 2017). Raman et al. explored the application optically sensitive C2C12 to fabricate biological robots and studied the ability of a skeletal muscle to regenerate under conditions of mechanical damages like lacerations (Raman et al. 2016). ChR2 incorporation into the cellular membrane of HepG2 cells may have some possible implications. Opening of the channel on light stimulation may lead to influx of cations H^+, Na^+, K^+ and Ca^{2+}. The increase in the concentrations of these ions in the cytoplasm may have various effects on the cell. Na^+ ions have been found to be having an important role in the proliferation of rat

hepatocytes (Koch and Leffert 1979). Calcium plays a significant role in the metabolism of glucose as it modulates the phosphorylation and dephosphorylation of intracellular enzymes- glycogen synthase and glycogen phosphorylase involved in glycogen synthesis and breakdown, respectively (Blackmore et al. 1986; Exton 1987; E. G. Krebs 1989). Cell proliferation and cell cycle are also dependent upon spatiotemporal variations in Ca^{2+} concentrations within the subcellular components of the hepatocytes (Poenie et al. 1986; Steinhardt and Alderton 1988; Twigg, Patel, and Whitaker 1988; Rasmussen and Means 1989; Groigno and Whitaker 1998; R. M. Roman et al. 1998; Kahl and Means 2003). It has been found that nuclear calcium concentrations are essential for cell proliferation of SkHep1 and HepG2 cell lines. All the above studies suggest that the cations have a major role to play in governing cell functionality.

Thus, the chapter reports, liposomal based transfection methodology to transfect channelrhodopsin based AAV-CAG-ChR2-GFP plasmid to develop optically sensitive C2C12, HepG2 and NIH 3T3 cells. The novelty of the study is given by the comparative analysis of transfection efficiency of AAV-CAG-ChR2-GFP plasmid among the three cell lines. In addition to it the study reported first-time transfection of HepG2 cells with channelrhodopsin rendering them light sensitive. Various studies have been reported for transfection of C2C12 cells (Asano, Ishizua, and Yawo 2012; Asano et al. 2015; Sakar et al. 2012; Raman et al. 2016; Cvetkovic et al. 2014; Sebille et al. 2017) and NIH3T3 cells (Nussinovitch, Shinnawi, and Gepstein 2014). However, there is a paucity of studies relating to application of optical sensitive cells in view of myoblast regeneration (Asano et al. 2015; Raman et al. 2016). On similar lines, coculture cell systems involving optically sensitive C2C12 cells and NIH3T3 cells can potentially lead to skeletal muscle models with physiological and pathological significance (S. T. Cooper et al. 2004; N. Rao

et al. 2013). Thus, further exploration of optically sensitive cells in coculture systems may lead to better understanding of skeletal muscle regeneration.

In the current study, after transfection of cells confirmative studies could not be carried out due to non availability of facilities like Fluroscent activated cell sorting (FACS) and Optical stimulation setup. FACS setup is required for characterization and separation of transfected optically sensitive cells. The stimulation of the optically sensitive cells requires a specialized stimulation and control setup consisting of blue light laser and focusing system.

3.4. Summary

Here, the chapter demonstrates the development of optically controllable C2C12, HepG2 and NIH 3T3 cells through incorporation of a membrane-bound ChR2 protein by the transfection process. Such a strategy of developing light sensitive forms of cells can further be used under single and co-cultured environments to form promising tissue models for toxicological and pharmacological studies. Channelrhodopsin protein is known to act as a photo switch that perceives the presence of light or change in its intensity and offer a completely new mechanism for the exploration and alteration of physiological and cellular processes. Such ion channels can prove very impactful in gaining an insight into the structure-function mechanism of some potentially fatal degenerative diseases. Engineered light-gated ion channels can be utilized as biosensors that further supports its application in reducing the harmful effects and identifying the symptoms of a disease at an early stage (Banghart, Volgraf, and Trauner 2006).