Gene Expression of Diabetic Fibroblast and Normal Fibroblast after Irradiating with Light Emitting Diode by Using Microarray

Pongsathorn Chotikasemsri

A Thesis Submitted in Fulfillment of the Requirements for the Degree of
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I hereby certify that this work has not been accepted in substance for any degree, and is not being currently submitted in candidature for any degree.

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Pongsathorn Chotikasemsri

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CHAPTER 1

INTRODUCTION

Background and Rationale

Diabetic wounds can become chronic and difficult to be treated. One of the treatment techniques is Phototherapy. Phototherapy is a process that photon energy from one particular wavelength can control the behavior of a cell. The mechanism behind this idea is that a number of important cellular molecules with multi-ring structure can be triggered by photons when it is bombarded. This also influences many other downstream pathways. Researchers found that any cellular molecules whose structures consist of ring structure with free electron can effectively receive photon energy and then transfer or activate the activity of any relating molecules with no ligand required. Current applications of phototherapy are mood and sleep related disorder, seasonal affective disorder, circadian rhythm sleep disorder, laser acupuncture, and skin disease treatments, such as psoriasis, vitiligo, and acne vulgaris. Therefore, the researchers want to investigate whether phototherapy can be used to treat a chronic wound disease like diabetic wounds. In addition, the researchers want to know what pathways can be affected from phototherapy.

Review of Literature

Diabetes and its wound

Diabetes mellitus is a main cause of tissue-repair impairment. Ones with this disease tend to have difficulty to heal these wounds. A simple wound could turn to be chronic and get infectious wound. Moreover, the amputation rate for such diabetics is much higher than non-diabetic patients. Many factors are involved in the development of the classic "diabetic" ulcer that develops on the sole of the foot beneath the head of the first or second metatarsal. Because healing problems are so common and devastating, several models of tissue repair have been developed in both animals and in vitro studies (Greenhalgh DG, 2003). However, one must have an understanding of how wound healing is altered in human diabetes mellitus. First of all, diabetic patients frequently have peripheral vascular disease that interferes with blood supply to the feet. In addition to the macrovascular disease, the microvasculature is also affected so that there is altered blood flow to the foot. Another key factor is the neuropathy of diabetes mellitus. The lack of sensation can lead to deeper wounds and loss of the normal arch positioning causes increased pressure over the head of the metatarsals. The loss of autonomic nerves leads to decrease sweating, and dry, cracking skin that causes the skin to have an

increased risk of breakdown. Finally, diabetes mellitus leads to impaired immune function so that a small wound has a higher propensity for infection. Typically, the process of wound healing consists of six steps as shown in figure 1. First step is “injury phase”. When a wound occurs, red blood cells, platelets, and white blood cells come to the wounded area. Secondly, fibrin proteins will come to the area to close the wound and to stop bleeding. This step is called “coagulation phase”. Third is “early inflammation phase”. Polymorphonuclear leukocytes will come to the site where they can clean up the wound. Fourth is late inflammation phase. In this phase, macrophage will come to eliminate all left over germs again. Fifth is “proliferation phase”. New fibroblast cells and collagen will come to fill up the wound. And the last step is remodeling phase which all fibroblast cells and collagen will try to smoothen and completely close the wound’s surface (Shaw TJ, Martin P, 2009).

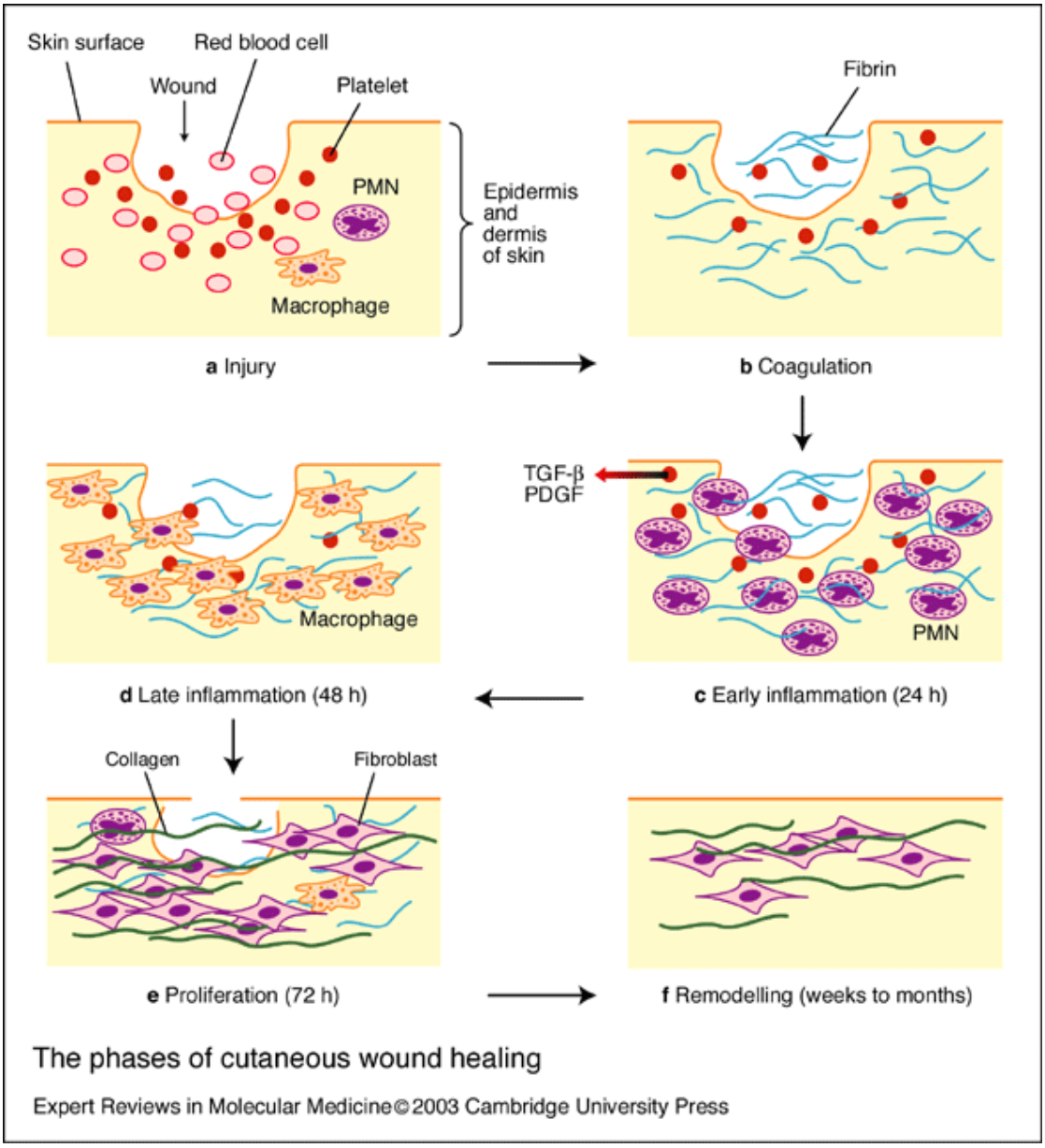


Figure 1. Six steps of wound healing process.

Genes relating to wound healing process

According to literature reviews, 4 major groups of genes relating to wound healing process are categorized:

1. Extracellular Matrix & Cell Adhesion function:

    a. ECM Components are groups of extracellular molecules that are secreted by cells to provide structural and biochemical support to the surrounding cells. These are collagen genes, which are relating to wound healing process: COL5A2, COL5A3, and VTN.

    b. Remodeling Enzymes are proteins that facilitate cells to form correctly. Here are genes which are relating to wound healing process: FGA (Fibrinogen), F3 (Tissue Factor), SERPINE1 (PAI-1), and TIMP1.

    c. Cellular Adhesion is the binding mechanism between cells or to a surface or substrate. Cellular adhesion can be referred to the linkage within the cytoplasm of cells and signal transduction. Cell adhesion is also essential for the pathogenesis of infectious organisms. Here are

cellular adhesion genes, relating to wound healing process: CDH1 (E-cadherin), ITGA1, ITGA2, ITGA3, ITGA4, and ITGA5.

d. Cytoskeleton is an intracellular protein that supports cell shape and function. Its function is capable of rapid assembly or disassembly dependent on the cell's requirements. Here are cytoskeleton genes, relating to wound healing process: ACTB, ACTA2 (a-SMA), and ACTC1.

2. Inflammatory Cytokines & Chemokines function is a protective response that may involves immune cells, blood vessels, and molecular mediators. In this case, at molecular levels, cellular molecules, such as cytokine & chemokine proteins, are responsible for inflammatory response. Here are genes, relating to wound healing process: IL8, IL6, IL4, CXCL1, and CXCL11 (ITACIP-9).

3. Growth Factors function consists of proteins that are capable of stimulating cell cycle, cellular growth, proliferation, healing, and cellular differentiation. Growth factors are important for regulating a variety of cellular processes. Here

are genes, relating to wound healing process: VEGFA, FGA, HGF, FGF2, ANGPT1, FGF7, IGF1, and TNF.

4. Signal Transduction function:

    a. Transforming growth factor beta (TGFβ) are proteins that controls proliferation and cellular differentiation, such as TGFB1, TGFBR3, and STAT3.

    b. Wint signaling pathway (WNT) is a communicative protein group of signal transduction molecules that pass signals from outside of a cell through cell surface receptors to the inside of the cell. Once this signaling pathway is activated, other downstream pathways, such as cell proliferation and cell migration, will also be induced: CTNNB1, WISP1, WNT5A.

    c. Phosphorylation is a protein group that work as a switch to turn on and off other molecules by reducing or adding phosphate to the target molecules: MAPK1 (ERK2), MAPK3 (ERK1), PTEN.

Properties of light from different light sources

Theoretically, light is a kind of electromagnetic waves that consist of electric field and magnetic field with perpendicular orientation. The visible wavelength starts from 400 nanometers to 700 nanometers as shown in figure 2. In contrast, below 400 nanometers and beyond 700 nanometers are invisible lights. Currently, we can utilize benefits from them. For example, most of beyond 700 nanometers lights can be used in communication field, such as radio wave, Radar, and Broadcasting. On the other hand, most of below 400 nanometers lights are popularly used in medical and science field. For example, we used UV and Gamma rays to sterilize objects and X-rays to inspect the bones in a patient (Reddy GK, Stehno-Bittel L, Enwemeka CS, 2001).

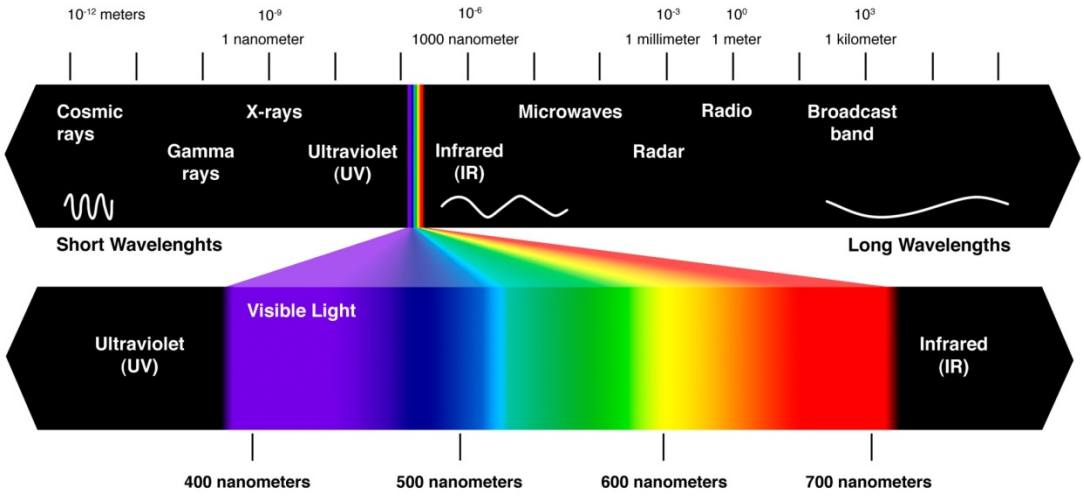


Figure 2. Spectrum of visible and invisible light.

In addition, another interesting property of light is that different light source can present different unique properties. There are 3 major types of light sources. As shown in figure 3, Sunlight provides full spectrum of wavelengths with variety of colors. Therefore, the wave pattern of sunlight is not in the same phase (non-coherent) and not in the same color (different wavelengths). However, a light emitting diode (LED) can provide specific wavelength if it is built from a combination of certain elements. So, we can control the color that we only desire, but they are still non-coherent. Lastly, LASER is an expensive scientific instrument that can generate a true color of one specific wavelength and coherent pattern. Thus, LASER is a perfect tool for a highly accurate experiment (Whelan HT, Smits RL Jr, Buchman EV et al., 2001)

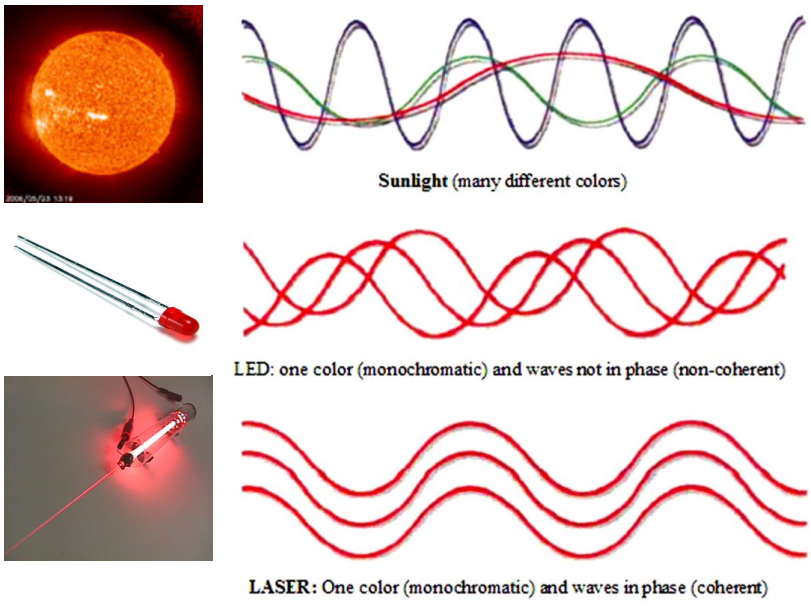


Figure 3. Different properties and patterns from different light sources.

In addition to the wave pattern from different light sources, the ability of each color (different wavelength) is also significantly different. As shown in Figure 4, at the same energy output it seems that yellow, orange, and red color can penetrate into the deepest skin into the dermis level which is around 80 percent or 5 millimeters. So, one has to consider this property in order to suit the purpose of each treatment and target cells (Lee SY, You CE, Park MY, 2007).

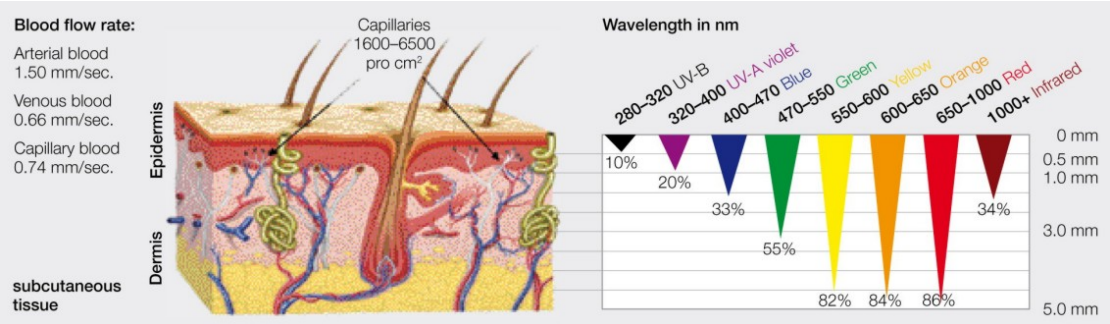


Figure 4. Penetrating levels of different wavelengths into human skin.

The reason behind this property is that our skin consists of both solid and liquid phase, such as blood vessels, hemoglobin, melanin, and water. These molecules can only allow some certain wavelengths to pass through. According to figure 5, it shows an absorbance value at particular wavelengths from Karu's experiment in 2005.

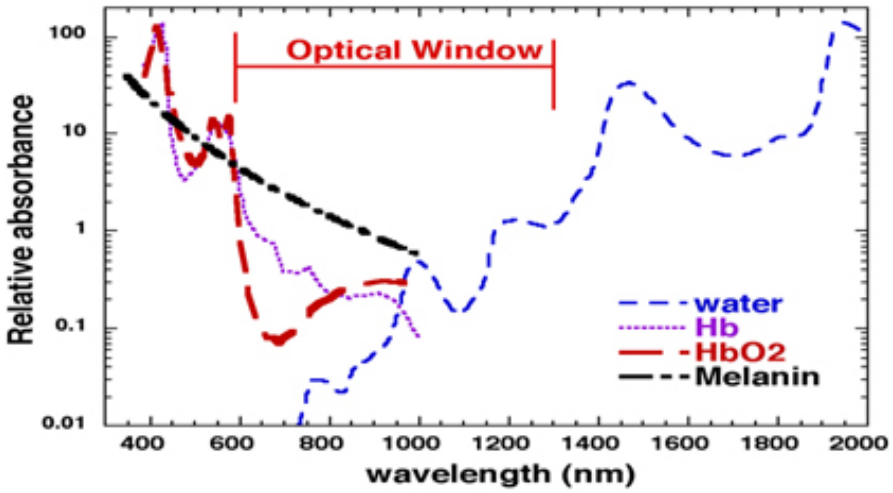


Figure 5. Absorbance values of different wavelength into human skin.

LED and Phototherapy as Photobiomodulation

In terms of the benefits of each wavelength to human body, physician currently used a technique, called "Phototherapy", to treat many kinds of skin diseases, such as Vitiligo, Psoriasis, and Acne Vulgaris. Here are previous reviews in the past 15 years that has been mentioned about therapy. In 2001, Whelan HT and his team mentioned that NASA has used 670-nm LED light to study its effects on injury repair and found that it grows at 40 -100 % more in mouse-derived fibroblasts, and increases in growth of 155–171% of normal human epithelial cells. In 2005, Kuru mentioned that 613.5 - 623.5 nm, 677.5 – 683.7 nm, 750.7 – 772.3 nm, and 812.5 – 846 nm stimulate DNA and RNA synthesis rate and cell adhesion to glass matrix. In 2006, Hawkins DH and Abrahamse H. demonstrated that using helium-neon laser at

632.8 nm with higher doses (10 and 16 J/cm^2) were characterized by a decrease in cell viability and cell proliferation with a significant amount of damage to the cell membrane and DNA. In 2009, Bossini PS et al. mentioned about using 670 nm can reduce necrosis when use higher density power. In 2011, Kaviani A, et al. provide evidence that Low Level LASER Therapy (LLLT) at 685 nm with energy density 10 J/cm^2 can accelerate the healing process of chronic diabetic foot ulcers, and it can be presumed that LLLT may shorten the time period needed to achieve complete healing; however, the average time of healing comparing with placebo group was not statistically significant. In summary, they have clearly identified that using LED light between 600 -700 nm is suited for wound treatment. Therefore, one purpose of this experiment is to explore the in-depth changes occurring after 630 nm LED irradiation, such as cell proliferation rate and gene expressions in order to make sure that this approach can be used in both normal and diabetic wound patients.

Mechanisms of wound healing in molecular level after LED treatment

Wound healing in diabetic is a common problem in primary care, intermediate care, and emergency medical facilities. In any healthy cells, the metabolism rate should be stable and have to be recovered as soon as the wound presents. These cellular chemical reactions will trigger the

amount of ATP inside mitochondria. Absorption of photons from LED by molecules leads to electronically excited states and consequently can lead to acceleration of electron transfer reactions at cytochrome c oxidase – an intermediate molecule in cellular respiration. The more electron transport reaction increases, the more ATP production increases. And, the more ATP is produced, the higher rate of energy for a cell to gain recovery from wound (Hong WX, Hu MS, Esquivel M et al., 2014).

In addition, another evidence about cellular structures that we have already known can be brought to explain how phototherapy works. There are a number of cellular structures that are porphyrin, heterocyclic, or polycyclic aromatic compounds. These kinds of structures usually have free electrons and are abundant in living cells. Shown in figure 6, one of porphyrin compounds in plant cells is chlorophyll which has a magnesium ion in the center of the molecule. Additionally, there is a molecule in mitochondria, called “Cytochrome C” which serves as cellular respiration process. This molecule is a porphyrin structure that has iron ion in the center. Theoretically, photons from light can be trapped with porphyrin structure and transfer this photon energy via free electrons along its structure. Thus, this means that photons can activate or trigger the activity of such molecules without any direct contact from proper ligands (Houreld NN, Abrahamse H, 2008).

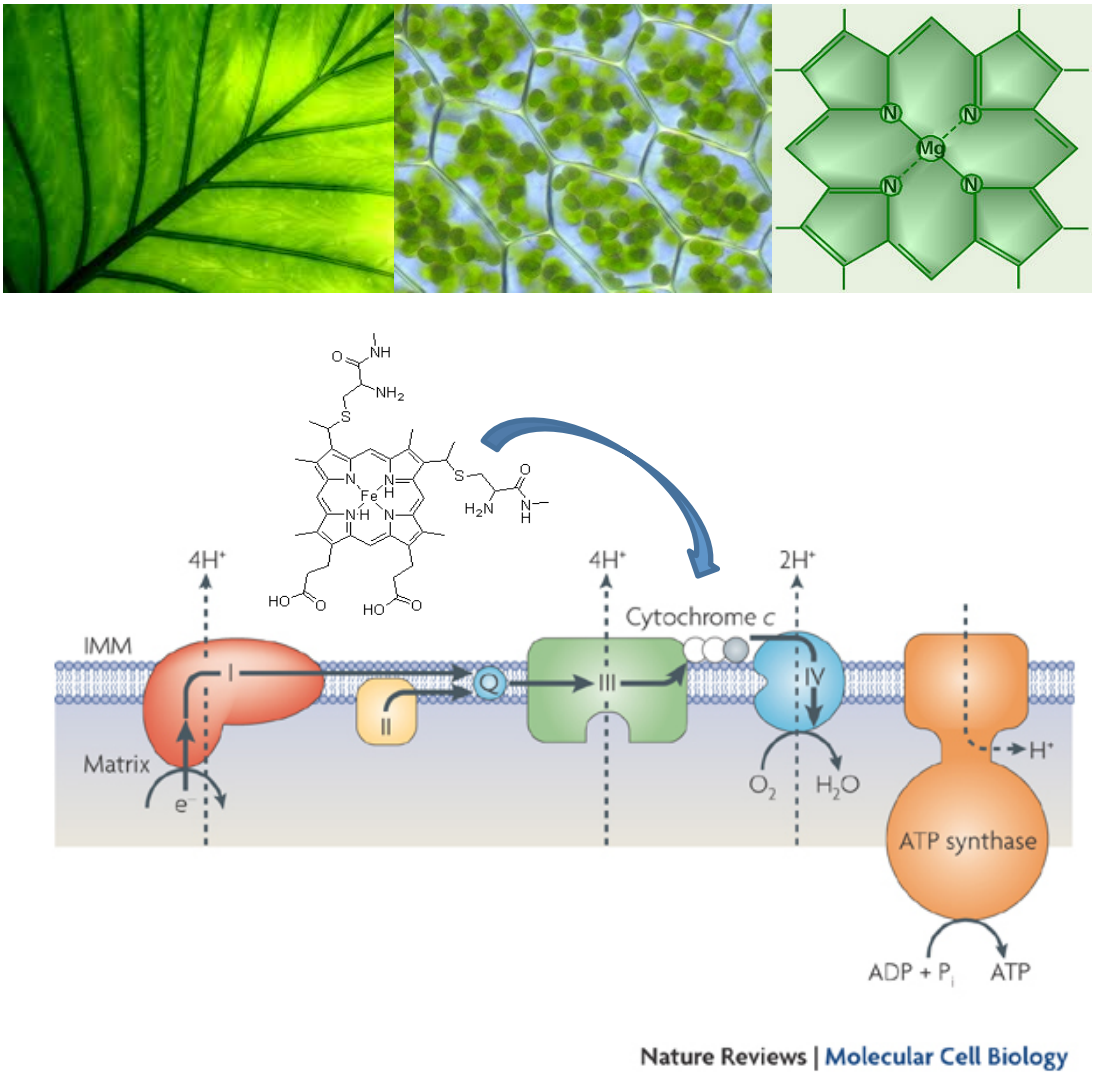


Figure 6. Chloroplast in plant and cytochrome C in mitochondria are porphyrin structures, which can receive photon energy from light.

Microarray

A microarray is a lab-on-a-chip technique, which is used to simultaneously detect the present of a large number of target DNA or RNA from a sample. Technically, microarray is a 2D array of oligonucleotides on a solid substrate, usually a glass slide. This is a high-throughput screening miniaturized, multiplexed and parallel processing and hybridized detection methods (Lei zhang, Shen qu, Aibin liang et al., 2013). Theoretically, as shown in Figure 7, 8, and 9, a large number of small micro-size spots of short single-stranded oligonucleotides, which can be either DNA or RNA, drop onto a glass slide as templates or fixed probes. When a sample, which is a mixture of either DNA or RNA, is extracted from the treated group, it will be hybridized or bound to the templates of the slide. If the hybridization occurs, it means the detection of such oligonucleotides or genes is observed. However, the way to process these binding molecules is via chemical illumination or light detecting system by a high-resolution camera as an intensity ratio. The color of such chemical illumination system could be one (as green color) or two colors (as red and green) depending on the purpose of each experiment. Then, those intensity ratios will be compared and analyzed to

observe the expression levels of each gene (Zhang Y, Song S, Fong CC et al., 2003).

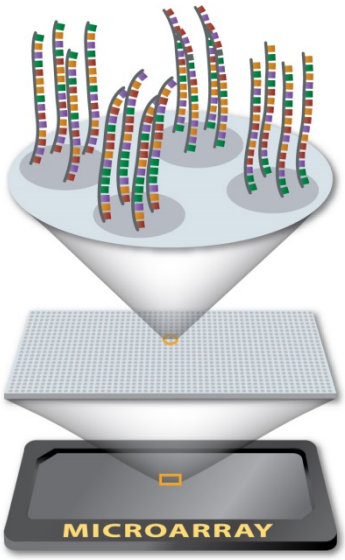


Figure 7. A microarray chip consists of thousands of micrometer-size spots with 15 to 25 oligonucleotide strands attaching onto the surface of glass slide which serve as template or probes.

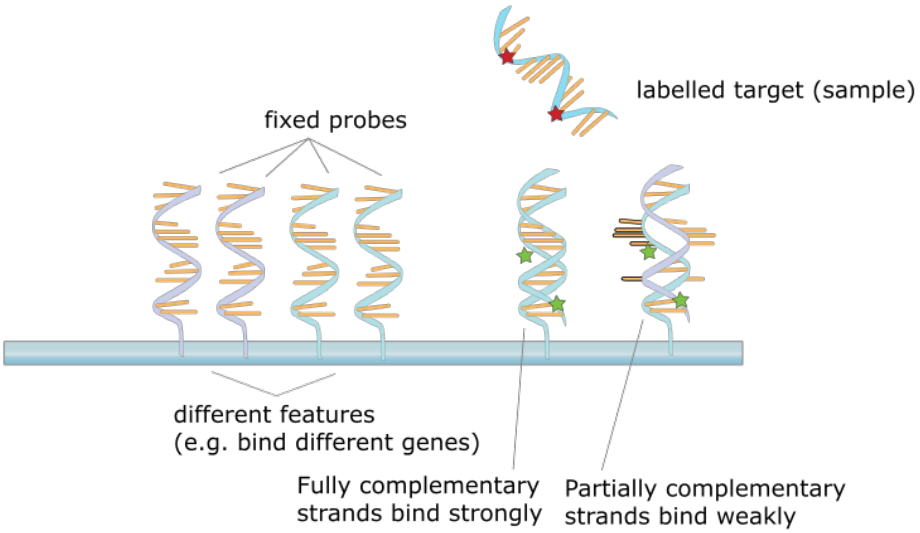


Figure 8. A 2-color hybridization method between probes and target oligonucleotides. It is also required chemical dyes (as green color on probes and red color on target molecules) to confirm the binding affinity between samples and probes and present as light intensity ratios.

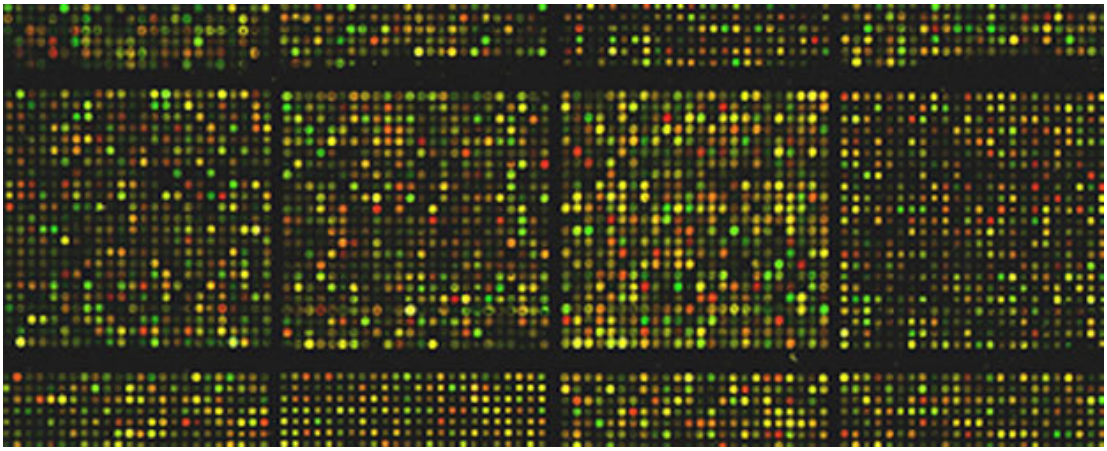


Figure 9. An example of pictures taken from high-resolution detecting camera shows a different intensity of each spots. Then, the intensity values of each spot will be analyzed and compared.

Gap knowledge

There are 2 gap knowledges that have not been solved; therefore, this study needs to answer.

1. No whole gene expression study has been done in human diabetic fibroblast cell line with red, blue, and green LED light.

2. Proper energy doses have not been identify

Objectives

1. To investigate whether LED exposure can promote wound healing in fibroblast cells.

2. To investigate the mRNA expression response of both diabetic and normal fibroblast cells after LED irradiation.

3. To investigate advantages and disadvantages of LED phototherapy relating to wound healing process after LED irradiation.

CHAPTER 2

RESEARCH METHODOLOGY

An overview of this research is shown in Figure 10 as a conceptual framework diagram. It started investigating by finding the optimum dose with red light because a number of previous studies from other researchers have been claimed that red light tends to promote wound healing than other lights do. Another reason is that red light provides the best penetrating level which means that it may provide better performance at the lower energy. Then, the corrected power will be used for next step, which are an 8 important genes step by real-time PCR and whole gene expression analysis with mRNA microarray.

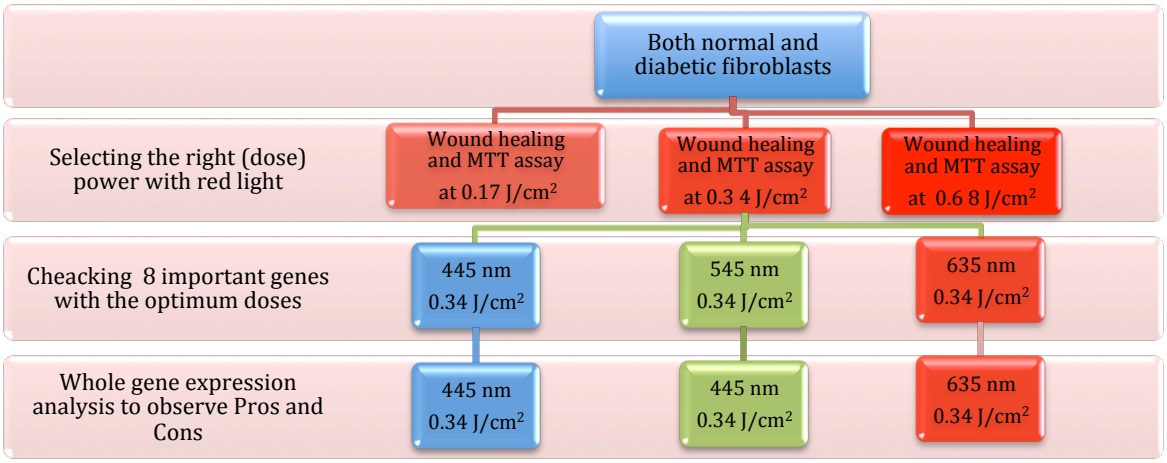


Figure 10. Conceptual framework of this research

Materials

1. Fibroblast cell lines

1.1. Both type 2 diabetic fibroblast cell line and healthy normal fibroblast cell line (AG06083 and GM03440) were taken from Coriell Institute for Medical Research.

2. Primers

2.1. All primers were designed in exon-exon overlap orientation and checked the binding performance with Primer-Blast from NCBI database. They were ordered as liquid primers from Sigma-aldrich. Based on literature reviews, only some of 8 important genes relating to wound healing pathway were selected as follows:

ACTB For- CTGGGACGACATGGAGAAAA;

Rev- AAGGAAGGCTGGAAGAGTGC,

CDH1 For- TCATGAGTGTCCCCCGGTAT;

Rev- TCTTGAAGCGATTGCCCCAT,

FGA For- AGAGCCTACCCGGAAGTGAT;

Rev- TGAGCCGTGCCTATCTTTGT,

GAPDH For- GAA GGT GAA GGT CGG AGTC;

Rev- GAA GAT GGT GAT GGG ATT TC,

HGF For- CTGGTTCCCCTTCAATAGCA;

Rev- GTCGGGATATCTTTCAGGCA,

IL8 For-GGTGCAGTTTTGCCAAGGAG;

Rev- TTCCTTGGGGTCCAGACAGA,

LEPTIN For –CTGTGCCCATCCAAAAAGTCC;

Rev- TCTGTGGAGTAGCCTGAAGC,

VEGFA For- AAGGAGGAGGGCAGAATCAT;

Rev- GAGGCTCCAGGGCATTAGAC).

3. Reagents

3.1. Phosphate-buffered saline (PBS)

3.2. Sodium Bicarbonate

3.3. Typsin-EDTA

3.4. Dulbecco's Modified Eagle's medium (DMEM)

3.5. Dimethyl sulfoxide (DMSO)

3.6. Pen-strep is penicillin and streptomycin, which are anti-micro organism drugs. 1% per volume of penicillin and streptomycin were added into fresh cell culture media to prevent contamination during the experiments.

3.7. Fetal Bovine Serum (FBS) was bought from GIBCO as regular nutrient for cells to grow.

4. Commercial provided kits

4.1. mRNA extraction kit: GeneJet purification kits were bought from ThermoScientific

4.2. MTT solution: was bought from Sigma-Aldrich. This MTT was used to evaluate the cell viability after treatment.

5. Scientific instruments

5.1. LED array set

5.1.1. An apparatus equipped with red, blue, and green light–emitting diodes (LED) was used in this experiment. The diode array assembled by Department of Electrical Engineering, Prince of Songkhla University consists of LED array panel that perfectly cover culturing plates. Blue LED, green LED, and

red LED were bought from electronic source Co., Ltd. 144 LEDs of each color were all assembled into 6 by 6 inches. A monochrome meter and a power meter from Department of Physics, Prince of Songkhla University, provided analysis of the light's emission spectra and power. The analysis of peak wavelength of each color is shown in Figure 11-13.The peak wavelength of blue LED is 470 nm. The peak wavelength of green LED is 520 nm. The peak wavelength of red LED is 630 nm.

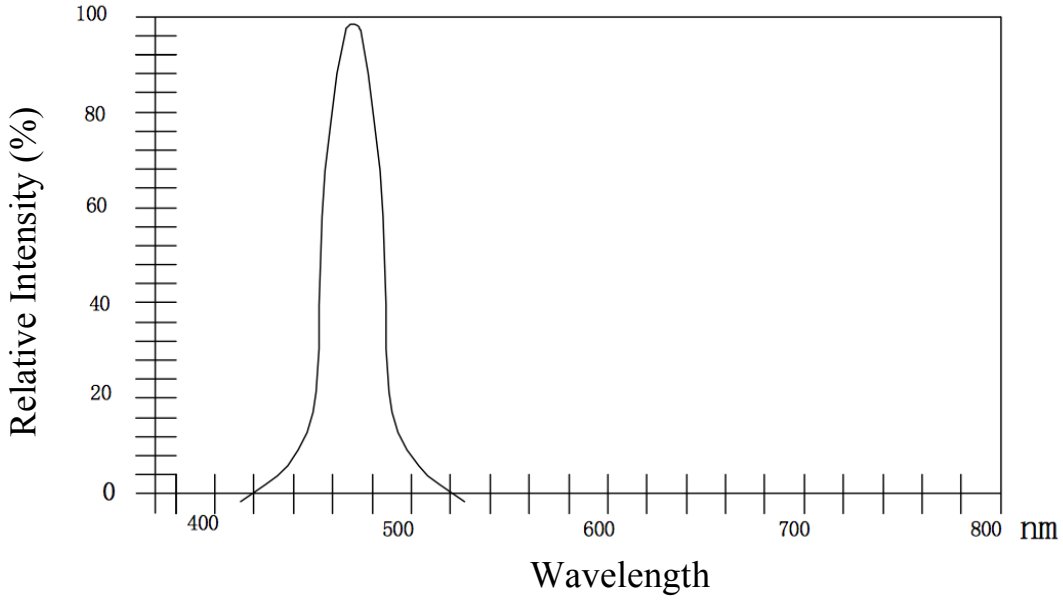


Figure 11. The highest peak wavelength of blue LED is at 470 nm.

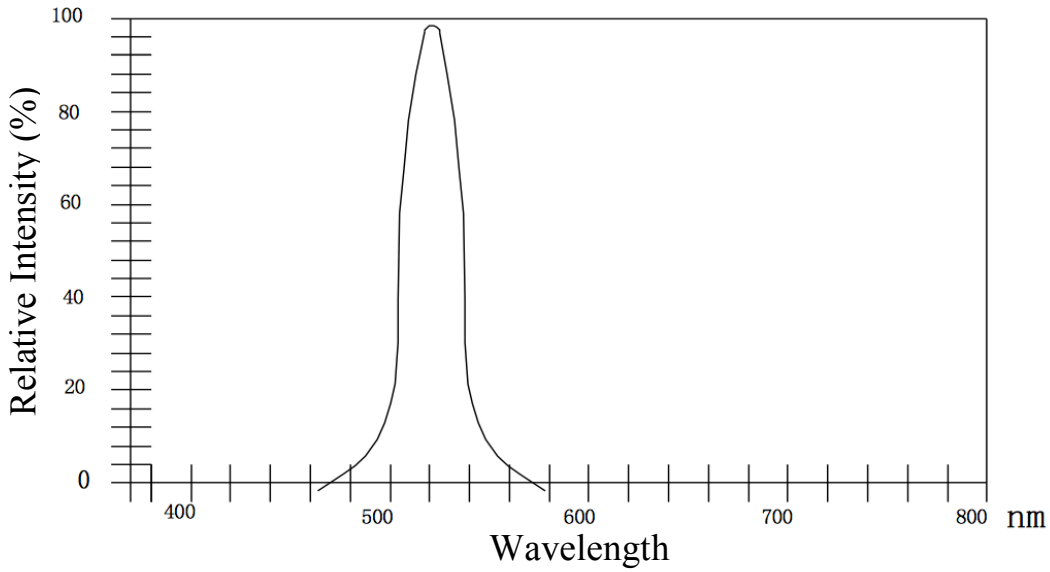


Figure 12. The highest peak wavelength of green LED is at 520 nm.

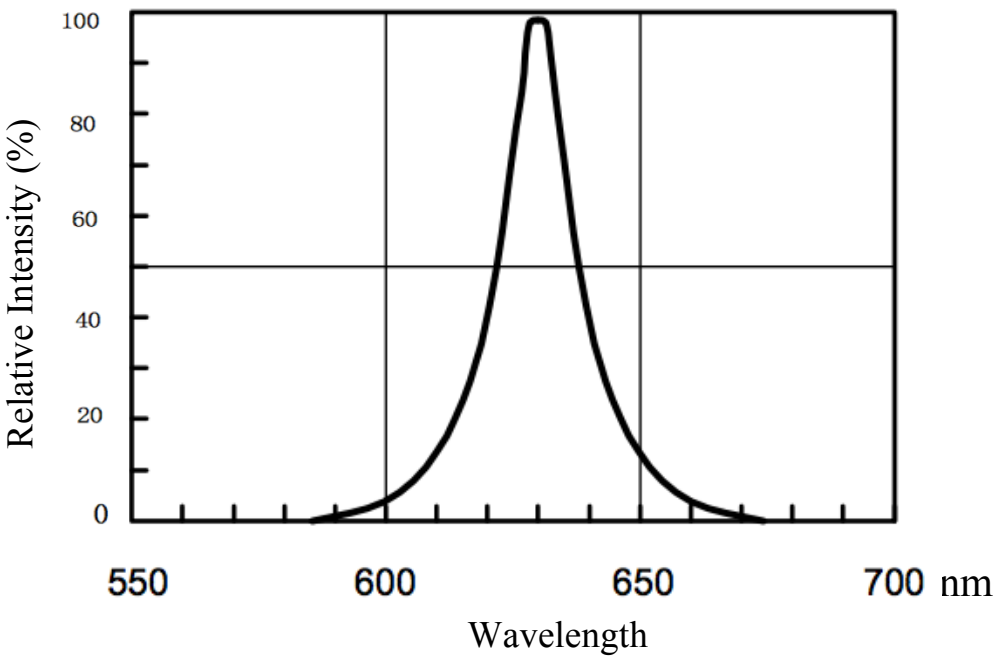


Figure 13. The highest peak wavelength of green LED is at 630 nm

5.1.2. Adapter for LED was assembled by a staff from electrical engineering department with maximum output is 1 A. The power of this adapter can be adjust to suite the optimal energy doses for cell viability.

5.2. Scientific instruments for cell culture

5.2.1. Lamina flow from ESCO class II was used for cell culture

5.2.2. Pipetters and Pipette tips

5.2.3. 96-well plates and 10-centrimeter culture plates were bought from Axygen for cell culture.

5.2.4. A carbon dioxide incubator was used to culture the cells at 37 degree Celsius with 5% carbon dioxide.

5.2.5. An autoclave was used to sterilize all instruments before experiments start with 121 degree Celsius and for 20 minutes.

5.2.6. 0.2 micro meters bottletop filter for medium cell culture was used to filter out leftover particle and to sterile medium

5.2.7. pH meter is used to adjust the pH condition of cell culturing medium.

5.3. Scientific instruments for RNA extraction

5.3.1. Pipeters and Pipet tips

5.3.2. Centrifugal machine

5.4. Scientific instruments for real-time PCR

5.4.1. NanoDrop spectophotometer

5.4.2. Bio-Rad 96FCX Connect

5.4.3. PCR plate and plastic film cover

5.4.4. Pipeters and Pipet tips

5.5. Scientific instruments for MTT assay

5.5.1. Pipetters and Pipette tips

5.5.2. 96 well culture plate

5.5.3. Microtiter plate reader

Methods

1. Media preparation

A packet of DMEM from GIBCO was mixed with sterilized water and then added 1.6 gram of sodium bicarbonate. Next, it was taken to

pH meter to adjust to 7.4. After that, it was filtered by air suction flask with 0.2 micrometer filter.

2. Cell culture

Both type 2 diabetic fibroblast cell line and healthy normal fibroblast cell line (AG06083 and GM03440) were cultured in DMEM media with 1% penicillin, 1% streptomycin, and 10% Fetal Bovine Serum in a 10 cm. plate with 2,000,000 cells seeding density and in 96 wells plate with 10,000 cells seeding density.

3. LED irradiation assay

After being cultured for 24 hours, both diabetic and normal fibroblasts were received either no light (as a control group) or being exposed to the light (as treated groups). The irradiation distance in the apparatus was 1 cm from media surface. This apparatus had been used in lamina flow (ESCO class II); therefore, cooling airflow fan constantly maintained a constant temperature during light exposure.

4. Cell proliferation assay with MTT

MTT ((3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was used to measure a number of living cells after treatment. Both normal and diabetic fibroblast cell lines were

separately cultured in 96 wells plate with DMEM media with 1% penicillin-streptomycin, and 10% Fetal Bovine Serum. The seeding density for 96 well-plate is 10,000 cells. Then, samples were daily treated with LED light at the power of 0.17 J/cm^2, 0.34 J/cm^2, 0.67 J/cm^2. On the day 3 after treatment, all samples were treated with MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide for 10 micro liter. Next, they were incubated at 37 degree Celsius for 4 hours. Before measuring their absorbance with a Microtitor plate reader as a spectrophotometer, they were aspirated and added with DMSO for 100 microliter.

5. Wound healing assay

Once the cells were cultured until it reached 95% confluence in 10 centimeter culture plates, the wounds were created with a 1 ml plastic tip as a wide line, a cross line, a triangle line, and a square line. Next day, all cells were treated with LED light daily with the power at 0.17 J/cm^2, 0.34 J/cm^2, and 0.67 J/cm^2. Light was administered for 4 consecutive days. Cells were taken pictures to inspect the rate of healing until wounds completely close. Then, these pictures were analyzed with ImageJ software.

6. Eight important genes expression assay with Real-time PCR

6.1. RNA extraction for gene expression assay

Both normal fibroblast and diabetic fibroblast cell line were received either no light (as a control group) or exposed to one of three different fluxes of red, blue and green LED light. For treatment groups, they were daily received at 0.34 J/cm^2. Then, right after the irradiation finished, all cells were trypsinized and extracted their mRNA. All samples were extracted their mRNA by GeneJet RNA Purification kits from ThermoScienctific. These doses are in the range that has been shown in cell proliferation assay.

6.2. cDNA synthesis

All extracted mRNA samples were brought to synthesize for cDNA with Maxima H minus First strand cDNA sysnthesis kit from ThermoScientific. This process also requires a thermal cycling machine. For this research, Bio-Rad CFX96connect was used to perfectly control the temperature.

6.3. Eight important gene expression with Real-time PCR

All cDNA were mixed with SYBR green Premix Ex Taq II from TAKARA. Then, they were loaded into 96 well PCR plates and each of 8 genes was added into each well with 3 repeats. All samples were run in Bio-Rad CFX96connect. Temperature was set for this assay was 93 degree Celsius for denaturing phase, 50 degree Celsius for annealing phase, and 70 for elongating phase. ACTB and GAPDH served as housekeeping gene to normalize the other 6 gene expression results. The PCR efficiency of all genes was in the range between 98.5% and 99.5%.

7. Whole gene expression assay with Microarray

7.1. LED irradiation

Both normal fibroblast and Diabetic fibroblast cell line were received either no light (as a control group) or exposed to one of three different fluxes of red, blue, and green LED light. For treatment groups, they were daily received at 0.34 J/cm^2.

7.2. RNA extraction for gene expression assay

Then, right after the irradiation finished, all cells were trypsinized and extracted their mRNA. All samples were extracted

their mRNA by GeneJet RNA Purification kits from ThermoScienctific. The extraction procedure was clearly stated in its manual.

7.3. Microarray analysis

All mRNA samples were submitted to a certified Agilent microarray service lab in India and were tested the purity of mRNA by Bioanalyzer with RIN higher than 8.0 before carrying on to Microarray assay. The SurePrint G3 Human gene expression 8x60k V2 chips were used.

7.4. Statistical analysis

GeneSpring 13 was used to analyze the raw image data. All raw data were analyzed by Gene Spring 13. All expression with two fold differences was selected and followed by Up and Down expression analysis, Gene Ontology analysis, and Pathway analysis. Cut-off level for all analysis was P less than 0.05. The raw data will be also submitted to NCBI database for other researchers.

CHAPTER 3

RESULTS

1. Cell proliferation assay with MTT results

After the cells were seeded and irradiated with red (630nm) lights at 5 different conditions (control, 0.17 J/cm^2, 0.34 J/cm^2, 0.67 J/cm^2, and 1.34 J/cm^2), MTT reagent was added into all wells and further incubated for 6 more hours. Then, all cells were taken to measure the absorbance values under Microtiterplate reader at 595nm, and the results are shown in Table 1.

According to the MTT results from Table 1., the number of cell proliferation was starting to be flattened out at 1.34J/cm^2. Therefore, this condition of light was not further used for the next step of experiment, which is wound healing assay.

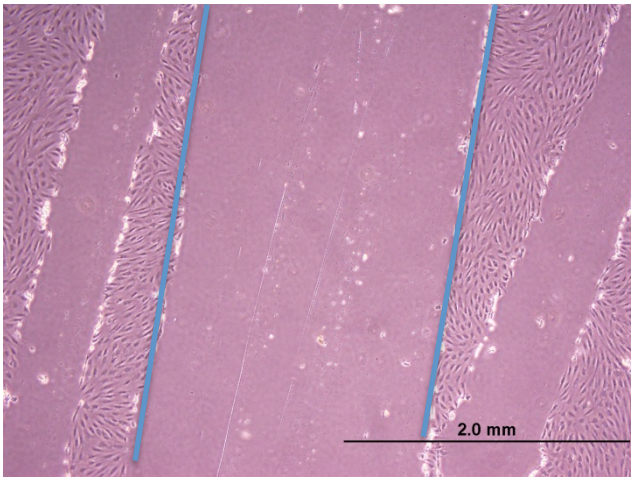
Table 1.The absorbance values of both normal and diabetic fibroblast cells that were exposed to different power of red LED light from MTT assay.

Power	Control	0.17 J/cm^2	0.34 J/cm^2	0.67 J/cm^2	1.34J/cm^2
Normal Fibroblast	0.080833333 0.078923213 0.081736489 0.081029384	0.107184759 0.110714286 0.112373649 0.110239849	0.137441611 0.141168373 0.131382849 0.132384784	0.123249953 0.142952381 0.121253445 0.127323830	0.08852381 0.08891663 0.08327281 0.09013838
Average ±SD	0.080630605 ± 0.0012	0.110128136 ± 0.0021	0.135594404 ± 0.0045	0.128694902 ± 0.0098	0.08771290 ± 0.0030
Diabetic Fibroblast	0.065523810 0.065309524 0.064428274 0.065983928	0.086102935 0.085186537 0.081829129 0.089459839	0.137416618 0.121738095 0.120657143 0.111397774	0.094837392 0.098292153 0.101392893 0.096823928	0.07218991 0.07517237 0.07028327 0.07128238
Average±SD	0.065311384 ± 0.0006	0.08564461 ± 0.0031	0.122802408 ± 0.0107	0.097836592 ± 0.0027	0.07223198 ± 0.0021

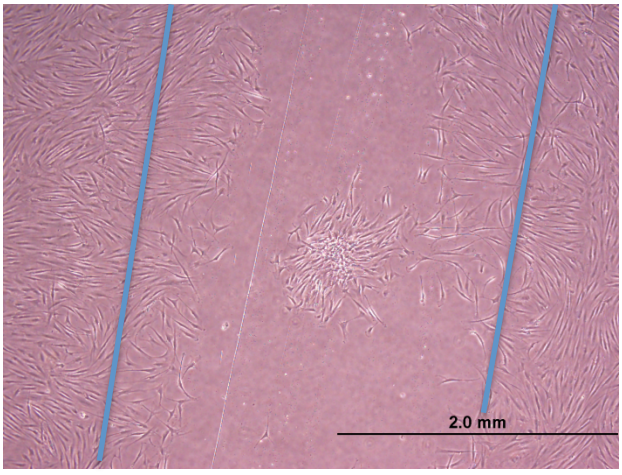
Therefore, for this cell proliferation assay, there are 30% more cells number than control group at 0.17 J/cm^2. At 0.34 J/cm^2, there are 70% more numbers of cells. At 0.67 J/cm^2 there are 50% more numbers of cells; and 10% more numbers of cells at 1.34 J/cm^2.

2. Wound healing assay results

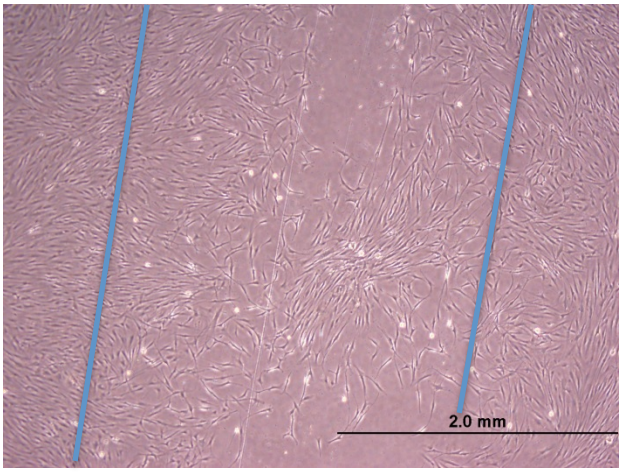
Normal fibroblast cells were sub-cultured to DMEM with 10% FBS, 1 % penicillin and 1% streptomycin. After culturing until fully grow in 10 centimeter plate, the wounds were created with a 1ml plastic tip as a wide line, and a cross line. Next day, all cells were treated with red LED light daily with a condition stated above. On the following day, pictures were taken to inspect the rate of healing until wounds completely close and here are the results. According to Figure 14 to Figure 20, it shows the changes of how fibroblast cells migrate to cover the wounded area. Within 8 days, most wounds were completely healed.



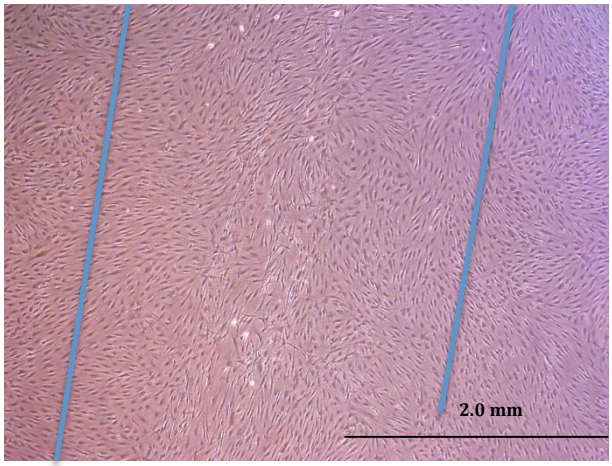
WIDE LINE WOUND 2 DAY 1 CONTROL CONDITION



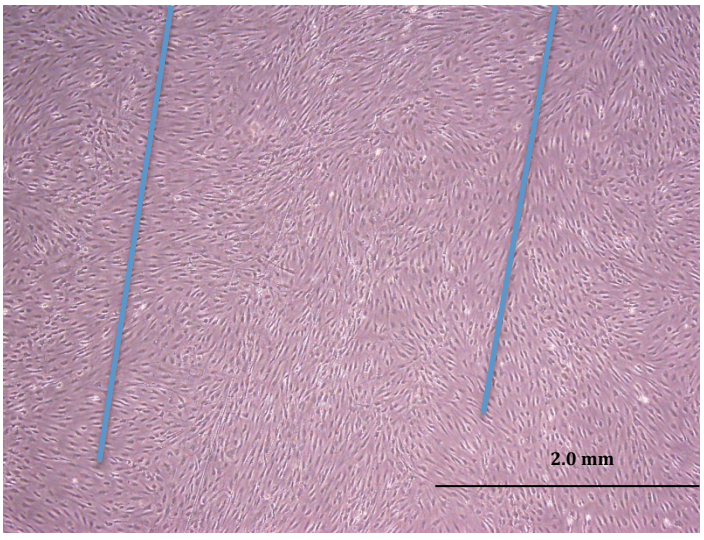
WIDE LINE WOUND 2 DAY 2 CONTROL CONDITION



WIDE LINE WOUND 2 DAY 3 CONTROL CONDITION

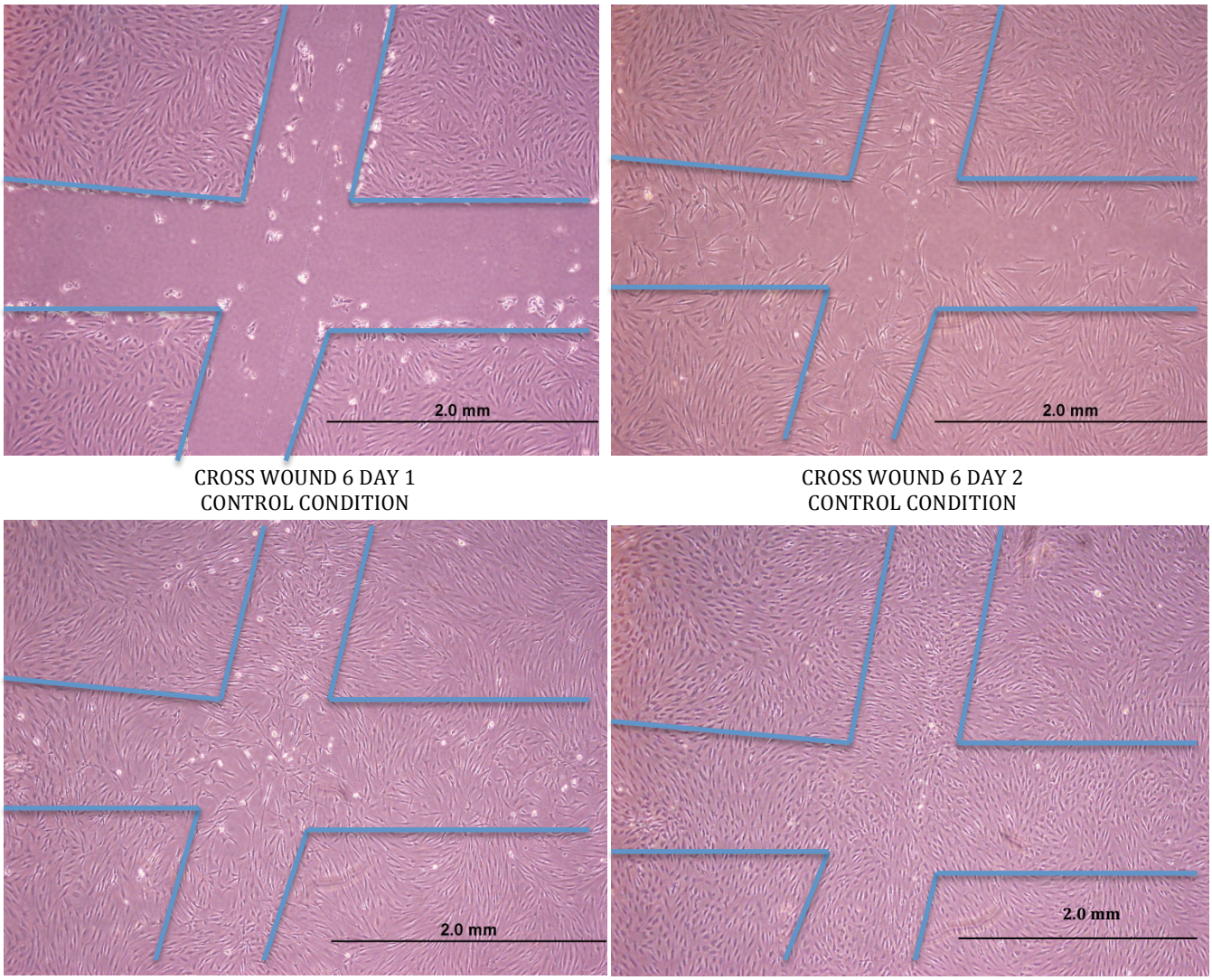


WIDE LINE WOUND 2 DAY 6 CONTROL CONDITION



WIDE LINE WOUND 2 DAY 8 CONTROL CONDITION

Figure 14. Wound healing assay as wide line wound of control fibroblast group. Blue lines represent the edge of the wound on day 1.



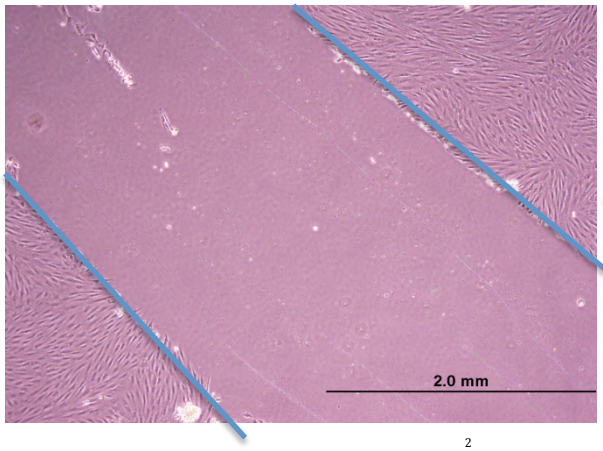
CROSS WOUND 6 DAY 1
CONTROL CONDITION

CROSS WOUND 6 DAY 2
CONTROL CONDITION

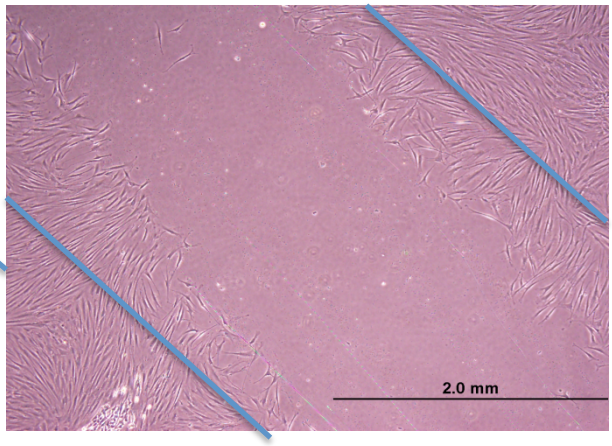
CROSS WOUND 6 DAY 3
CONTROL CONDITION

CROSS WOUND 6 DAY 6
CONTROL CONDITION

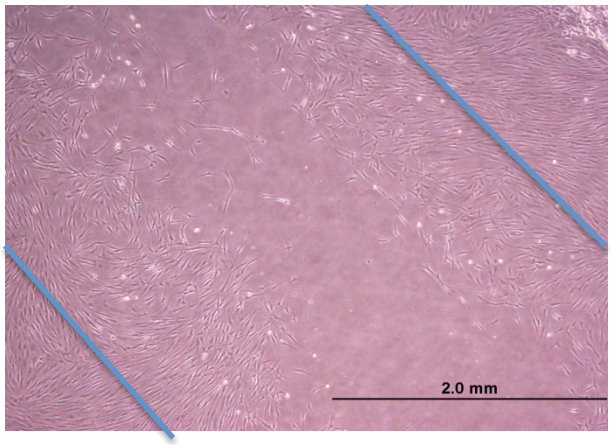
Figure 15. Wound healing assay as cross wound of control fibroblast group. Blue lines represent the edge of the wound on day 1.



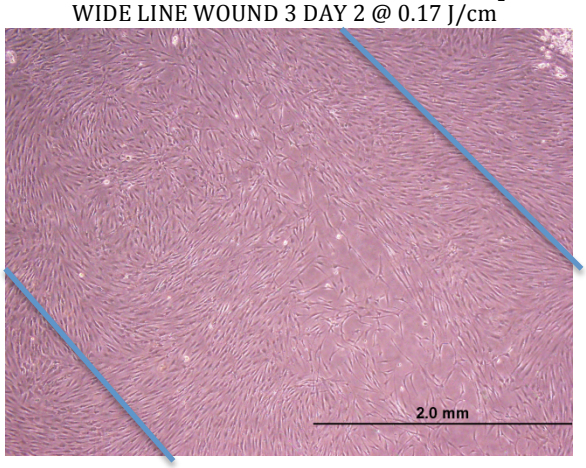
WIDE LINE WOUND 3 DAY 1 @ 0.17 J/cm^2



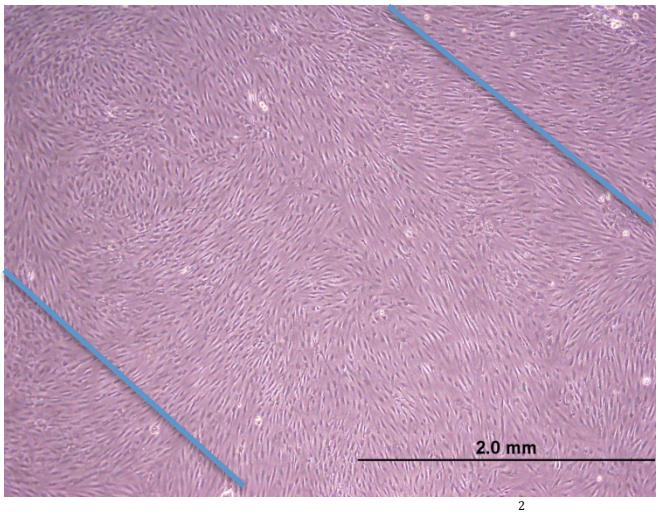
WIDE LINE WOUND 3 DAY 2 @ 0.17 J/cm^2



WIDE LINE WOUND 3 DAY 3 @ 0.17 J/cm^2

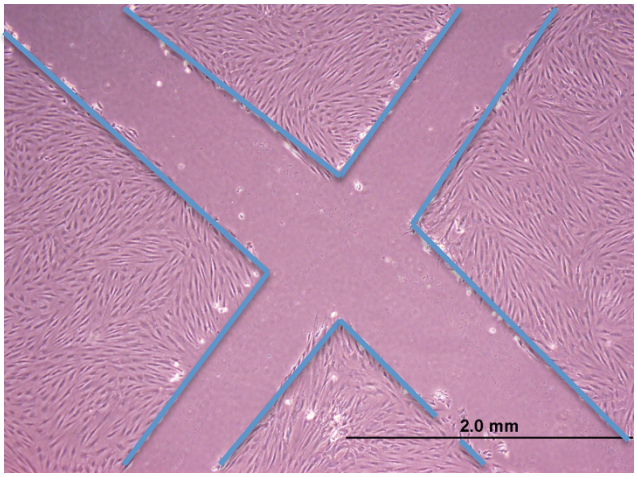


WIDE LINE WOUND 3 DAY 6 @ 0.17 J/cm^2

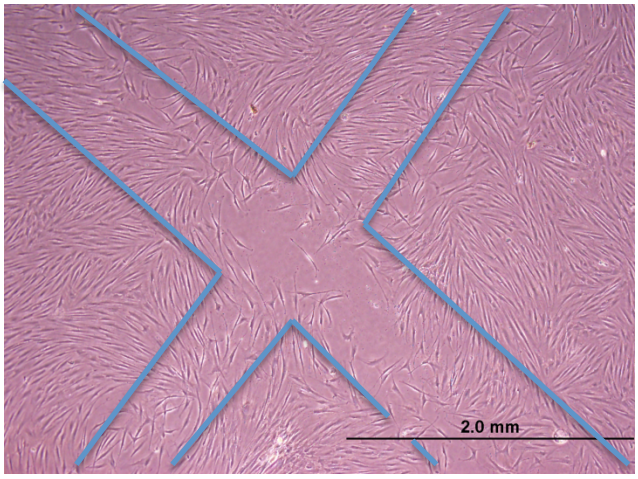


WIDE LINE WOUND 3 DAY 8 @ 0.17 J/cm^2

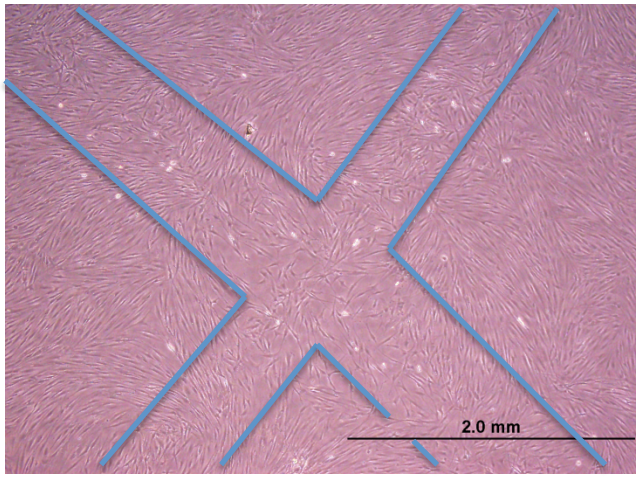
Figure 16. Wound healing assay as wide line wound of treated fibroblast group with 0.17 J/cm^2. Blue lines represent the edge of the wound on day 1.



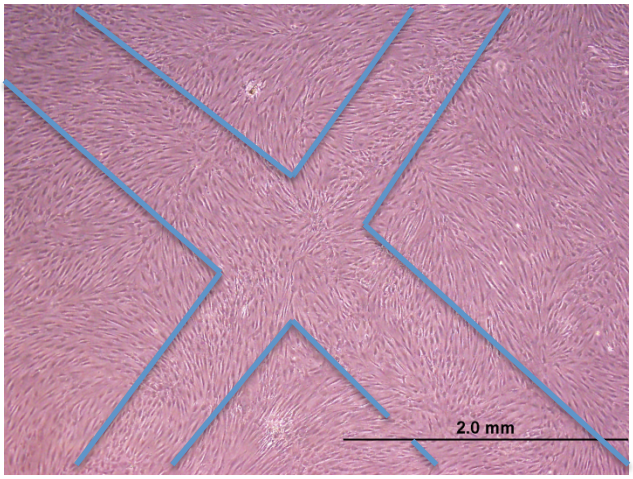
CROSS WOUND 4 DAY 1 @ 0.17 J/cm^2



CROSS WOUND 4 DAY 2 @ 0.17 J/cm^2

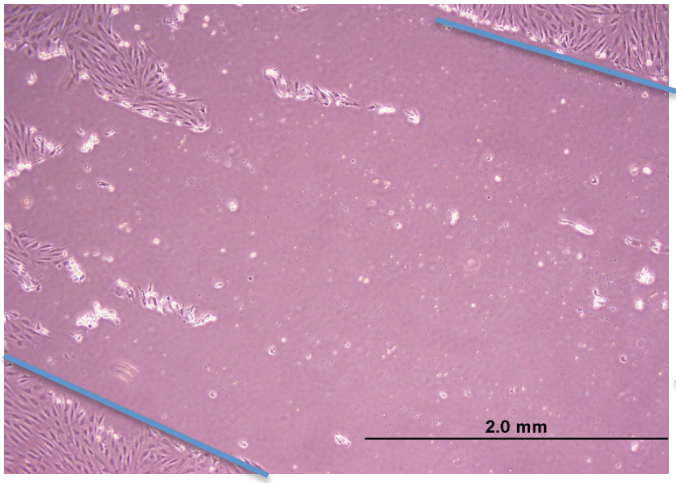


CROSS WOUND 4 DAY 3 @ 0.17 J/cm^2

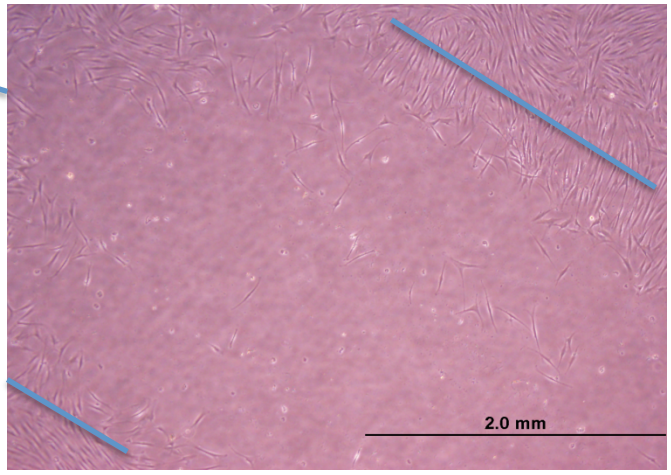


CROSS WOUND 4 DAY 6 @ 0.17 J/cm^2

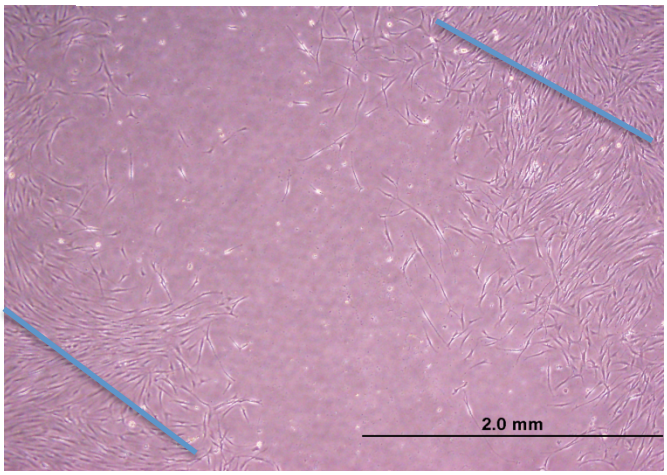
Figure 17. Wound healing assay as cross wound of treated fibroblast group with 0.17 J/cm^2. Blue lines represent the edge of the wound on day 1.



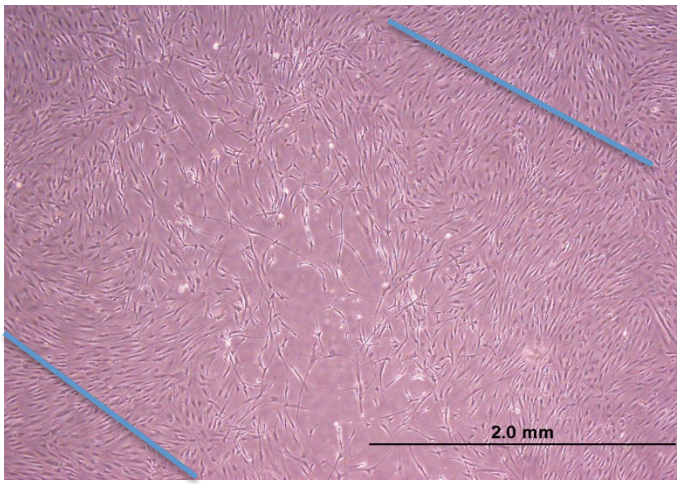
WILD LINE WOUND 3 DAY 1 @ 0.34 J/cm^2



WILD LINE WOUND 3 DAY 2 @ 0.34 J/cm^2

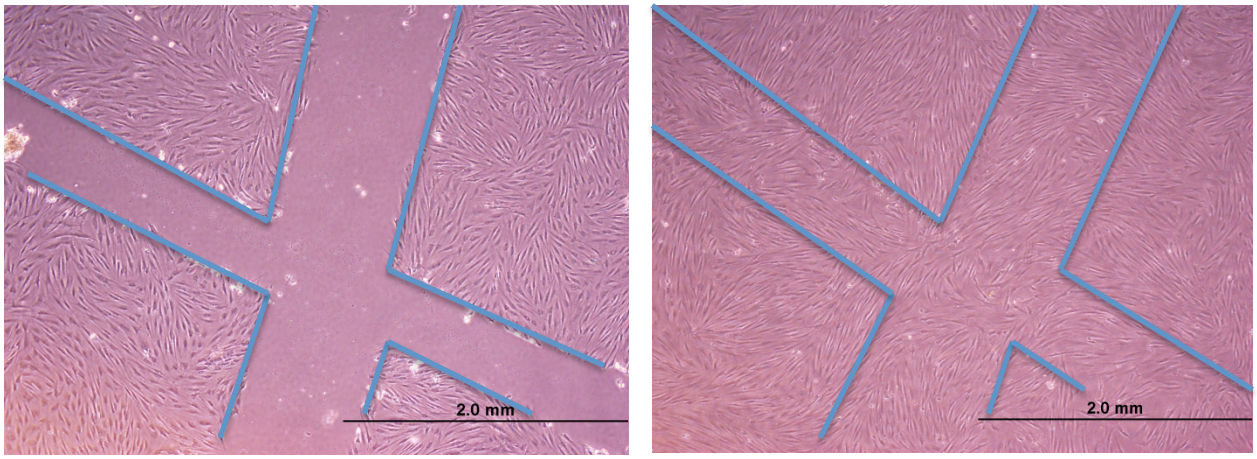


WILD LINE WOUND 3 DAY 3 @ 0.34 J/cm^2



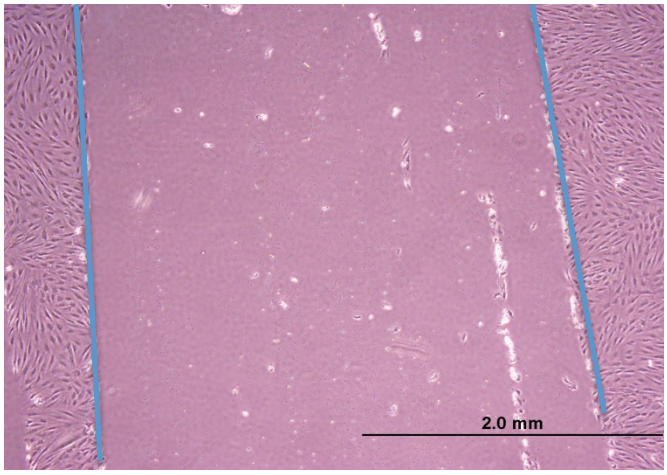
WILD LINE WOUND 3 DAY 6 @ 0.34 J/cm^2

Figure 18. Wound healing assay as wide line wound of treated fibroblast group with 0.34 J/cm^2. Blue lines represent the edge of the wound on day 1.

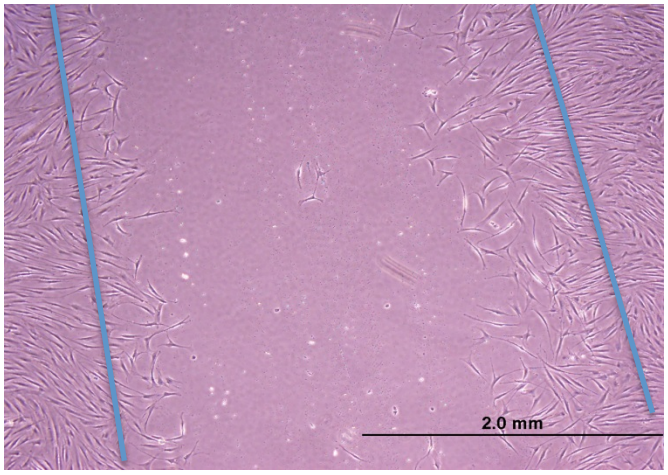


CROSS WOUND 4 DAY 1 @ 0.34 J/cm^2 CROSS WOUND 4 DAY 2 @ 0.34 J/cm^2

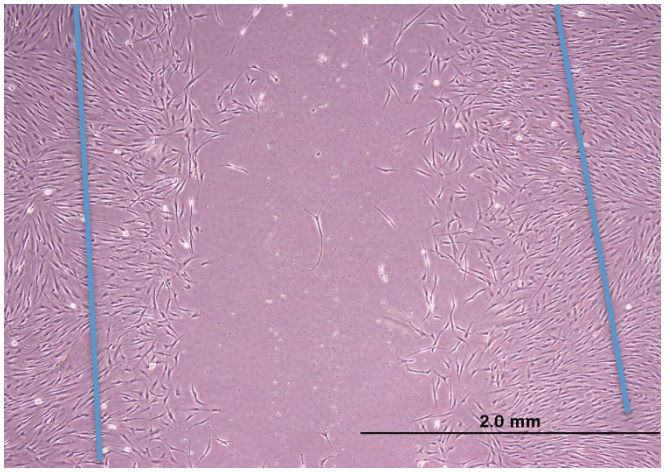
Figure 19. Wound healing assay as cross wound of treated fibroblast group with 0.34 J/cm^2. Blue lines represent the edge of the wound on day 1.



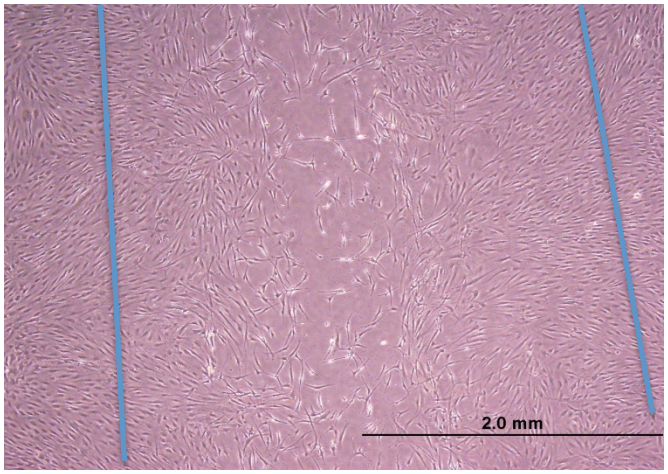
WILD LINE WOUND 6 DAY 1 @ 0.67 J/cm^2



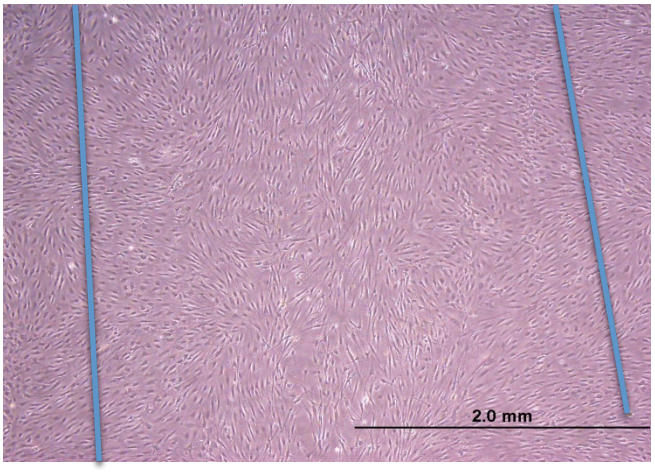
WILD LINE WOUND 6 DAY 2 @ 0.67 J/cm^2



WILD LINE WOUND 6 DAY 3 @ 0.67 J/cm^2



WILD LINE WOUND 6 DAY 6 @ 0.67 J/cm^2



WILD LINE WOUND 6 DAY 8 @ 0.67 J/cm^2

Figure 20. Wound healing assay as wide line wound of treated fibroblast group with 0.67 J/cm^2. Blue lines represent the edge of the wound on day 1.

After picture analysis with ImageJ, the distances of cell migration were recorded in Table 2. The average rate of migration comparing with control was found that fibroblast was able to migrate fastest at the power of 0.34 J/cm^2 (66% more than control) and 33% more at 0.67 J/cm^2. However, there was no significant difference of cell migration between 0.17 J/cm^2 treatment group and control group. Therefore, at the power of 0.34 J/cm^2 is the optimum condition for fibroblast cell lines, which this condition will be used for the next step of this study, which is Gene Expression.

Table 2. Wound healing assay results: The speed of wound closing (millimeters per day) of both normal and diabetic fibroblast cells that were exposed to different power of red LED light.

Power	Control	0.17 J/cm^2	0.34 J/cm^2	0.67 J/cm^2
Normal Fibroblast	0.314 0.343 0.356 0.352	0.372 0.345 0.378 0.381	0.512 0.525 0.529 0.511	0.478 0.435 0.455 0.462
Average±SD	0.341 ± 0.018	0.369 ± 0.016	0.519 ± 0.009	0.457 ± 0.017
Diabetic Fibroblast	0.295 0.287 0.312 0.306	0.324 0.343 0.312 0.323	0.498 0.510 0.498 0.509	0.434 0.455 0.467 0.423
Average±SD	0.3 ± 0.011	0.325 ± 0.012	0.501 ± 0.009	0.444 ± 0.019

3. Gene expression with real-time PCR results

After taking both cell lines from Coriell Institute for Medical Research, they were sub-culture to DMEM with 10% FBS, 1 % penicillin and 1% streptomycin. After sub-culturing into 10-centimeter plates with 2,000,000 cells seeding density, treatment groups were treated with red, blue, green LED at the power of 0.34 J/cm^2 for 0, 5, 10, 20, 40 minutes. Then, they were taken back to

the incubator for 24 hours. On the following day, all samples were harvested and trypsinized with 0.5 % trypsin EDTA. Next, mRNA extraction and cDNA conversion were preceded. This step was repeated for 4 days. After that, all samples were gone through 8 gene expression assay with real-time PCR. These 8 genes are ACTB, CDH1, FGA, GAPDH, HGF, IL8, LEPTIN, and VEGFA. ACTB and GAPDH are housekeeping genes. Thus, they are practically served as control genes or reference genes for delta delta Cq quantitative analysis and here are the results.

For blue light treated groups

For normal fibroblast, gene expression results show that Leptin (fat burning protein) and VEGFA expressed around 80 and 60 folds higher than control groups respectively, shown in Figure 21.

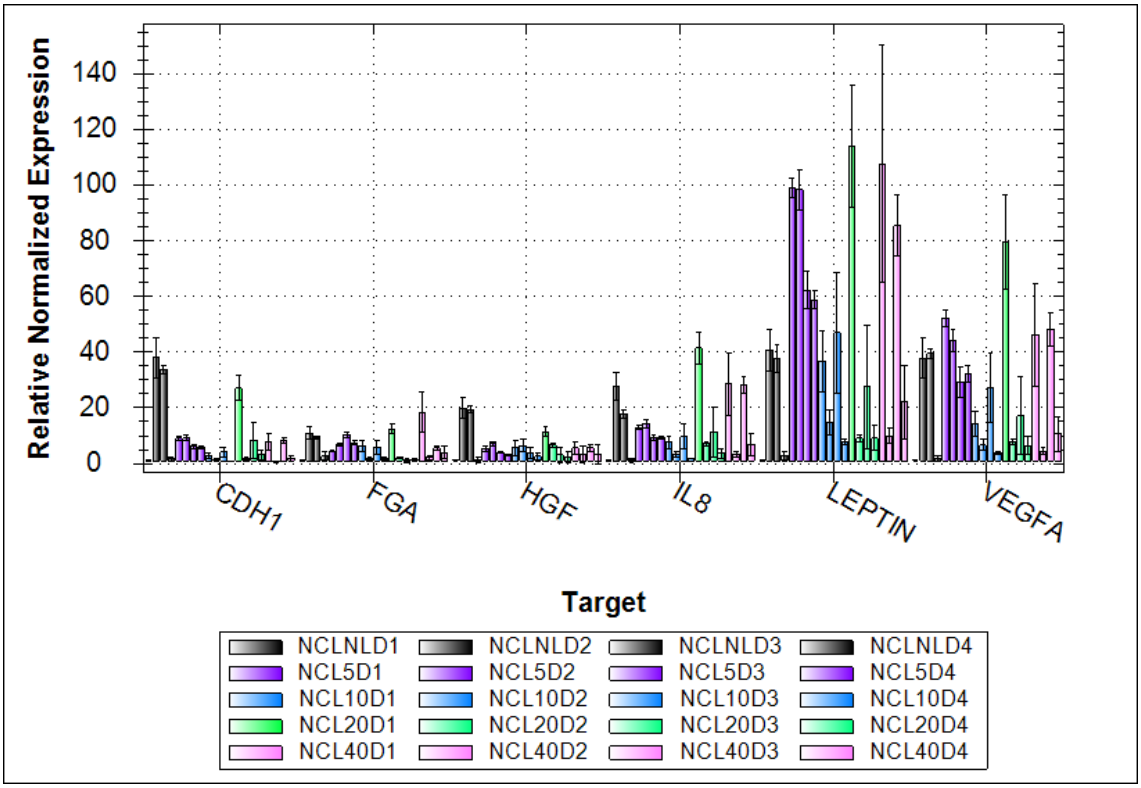


Figure 21. Real-time PCR results of 8 gene expression from normal fibroblast cell lines that were treated with blue light shown by target gene categories (NCL is normal fibroblast cell line, NL is no-light condition, 5 is 5 minutes under irradiation, and D1 is one day after treatment)

For diabetic fibroblast groups, HGF (a cell's morphogenic development gene) were significantly up regulated between 2 to 10 folds higher than control groups. In addition, IL8 (an inflammation signaling gene) were significantly up regulated between 10 to 120 folds higher than control groups, shown in Figure 22.

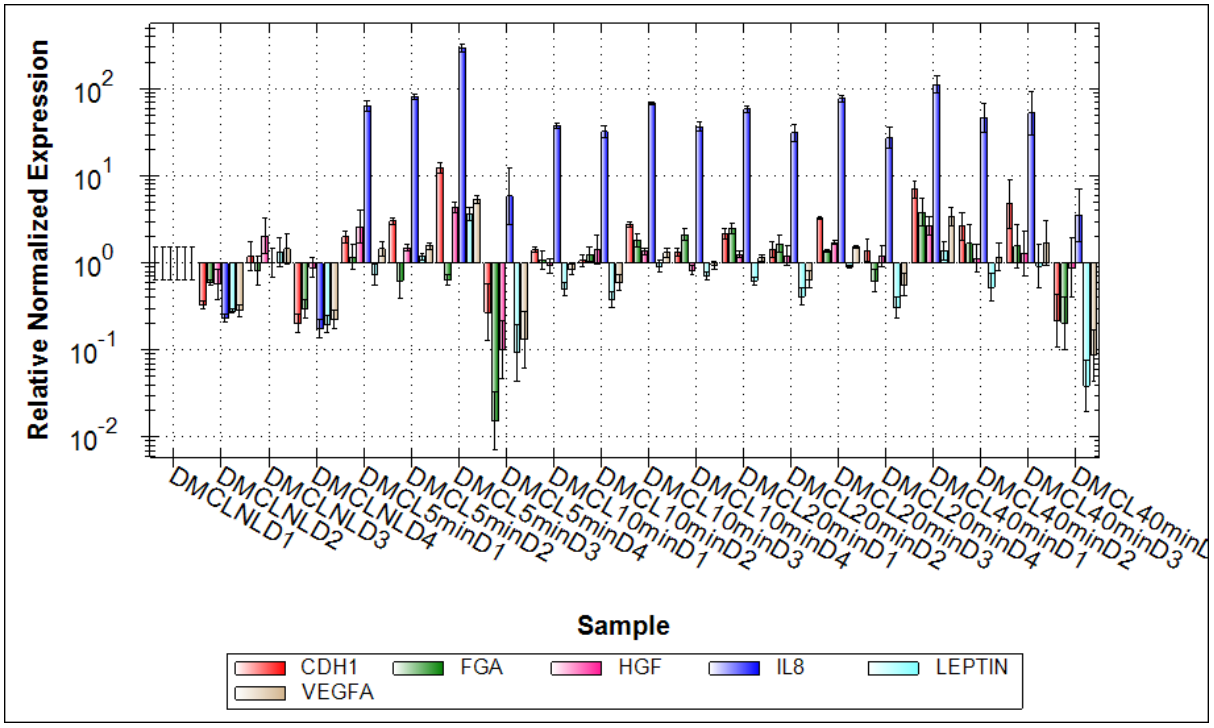


Figure 22. Real-time PCR results of 8 gene expression from diabetic cell lines that were treated with blue light shown by samples (DMCL is diabetic fibroblast cell line, NL is no-light condition, 5 is 5 minutes under irradiation, and D1 is one day after treatment)

For green light treated groups

For normal fibroblast, gene expression results show that IL8 (an inflammation signaling gene) was strongly up regulated in the range between 1,000 to around 1 million folds higher than control groups, shown in Figure 23.

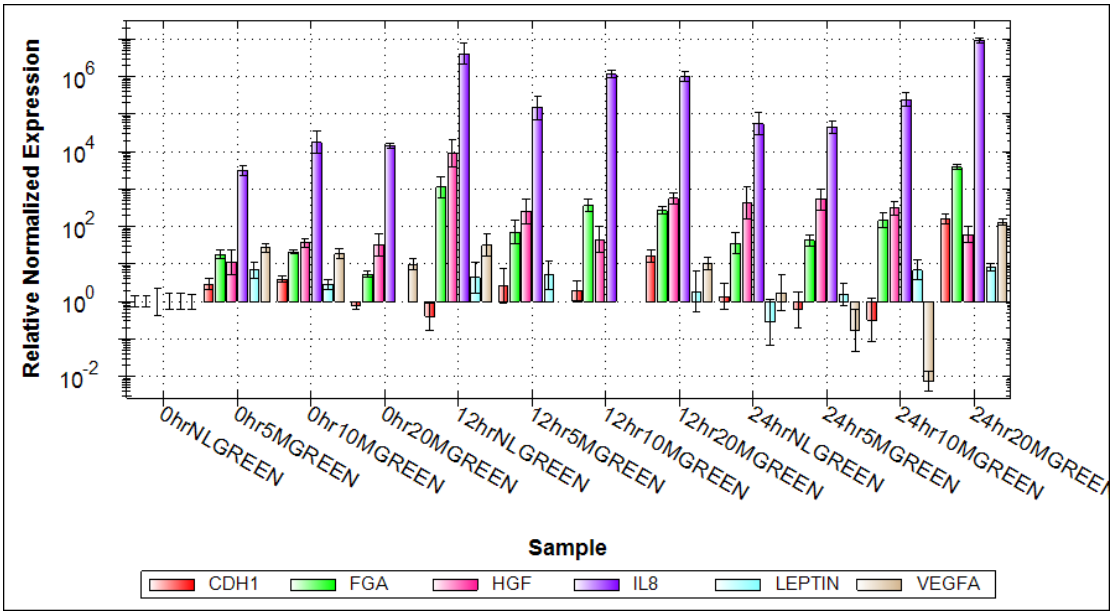


Figure 23. Real-time PCR results of 8 gene expression from normal fibroblast cell lines that were treated with green light shown by samples (GREEEN is normal fibroblast cell lines that were treated with green light, 5M is 5 minutes under irradiation, 0hr is expression level right after treatment, and 24hr is expression after treatment for 24 hours)

For diabetic fibroblast groups, FGA (a blood clotting cascade gene) was immediately expressed from 50 to 1,00 folds higher after 24 hours of irradiation. Besides, HGF (a cell's morphogenic development gene) were up regulated for 100 to 1,000 folds higher. Surprisingly, IL8 (Interleukin8 or an inflammation signaling gene) shows the highest expression after 24 hours of irradiation, shown in Figure 24.

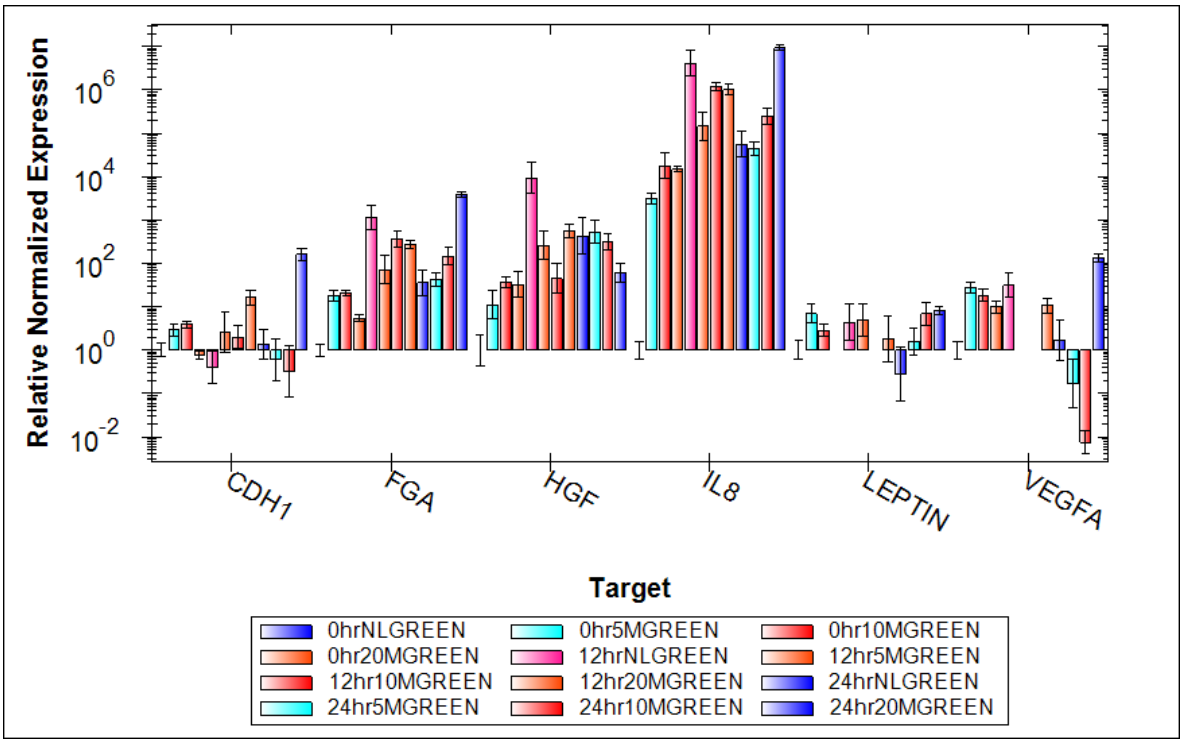


Figure 24. Real-time PCR results of 8 gene expression from diabetic cell lines that were treated with green light shown by target gene categories (GREEEN is diabetic fibroblast cell lines that were treated with green light, 5M is 5 minutes under irradiation, 0hr is expression level right after treatment, and 24hr is expression after treatment for 24 hours)

For red light treated groups

For normal fibroblast, gene expression results show that FGA (a blood clotting cascade gene), HGF (a cell's morphogenic development gene), and IL8 (Interleukin8 or an inflammation signaling gene) were suddenly expressed up to a thousand folds higher after 24 hours of irradiation, shown in Figure 25.

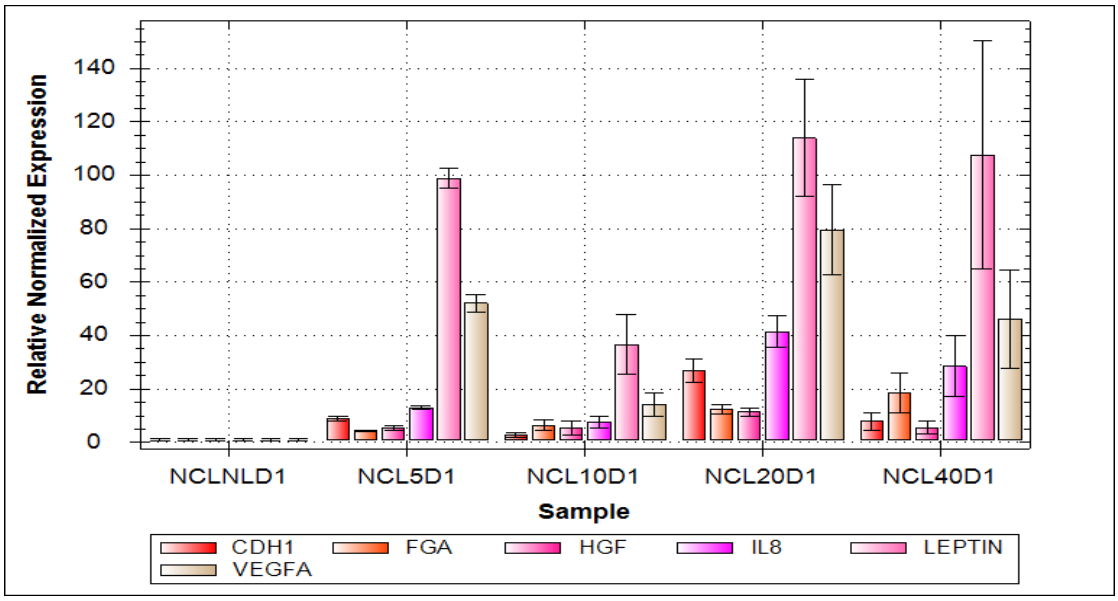


Figure 25. Real-time PCR results of 8 gene expression from normal fibroblast cell lines that were treated with red light shown by target gene categories (NCL is normal fibroblast cell line, NL is no-light condition, 5 is 5 minutes under irradiation, and D1 is one day after treatment)

For diabetic fibroblast groups, CDH1, FGA, HGF, LEPTIN, VEGFA immediately express from 3-20 folds higher after 24 hours of irradiation. In addition, IL8 (Interleukin8 or an inflammation signaling gene) shows around 10 folds higher after 24 hours of irradiation, shown in Figure 26.

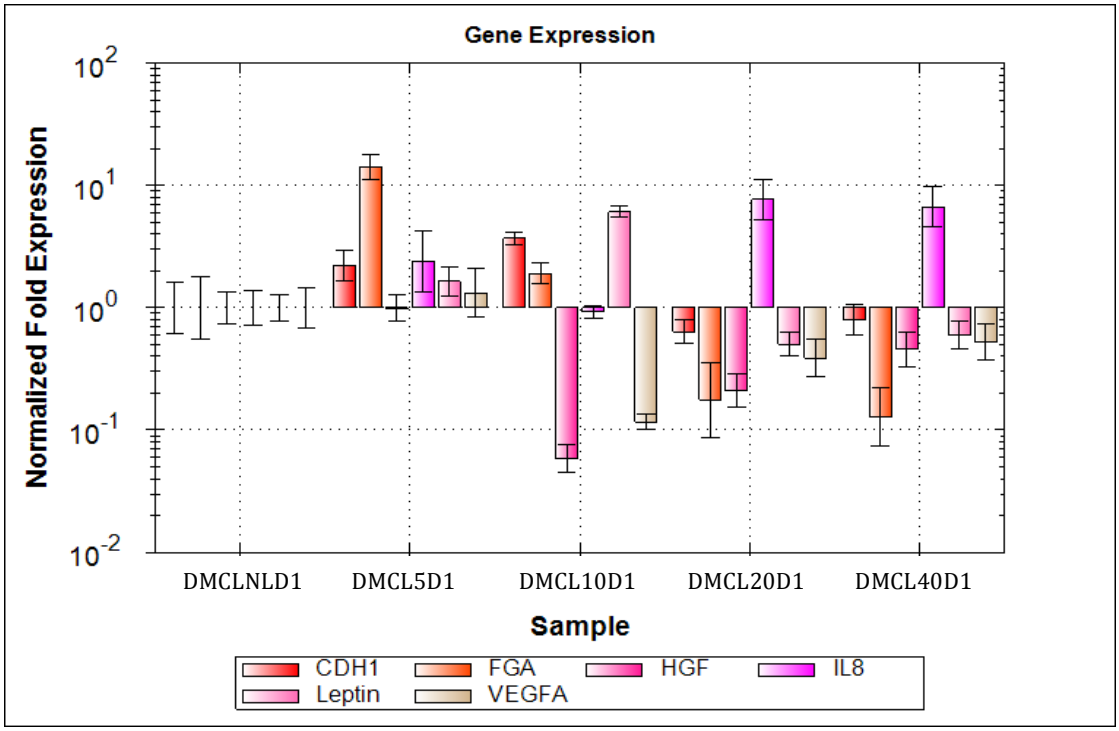


Figure 26. Real-time PCR results of 8 gene expression from diabetic cell lines that were treated with green light shown by target gene categories (DMCL is diabetic fibroblast cell line, NL is no-light condition, 5 is 5 minutes under irradiation, and D1 is one day after treatment)

In summary, it can be briefly categorized the expression of these 8 genes into the groups corresponding to each light condition, shown in Table 3. As you can see, there are a couple of gene expressions changing in very high levels after the irradiation especially in green light and red light treated groups. In contrast, just only little change was observed in blue light treated group. This evidence could be implied that green and red light can affect higher number of genes than what blue light treated group does. Nevertheless, unlike green light and red light treated groups, it is clearly see that all 8 genes can be affected from blue light irradiation as well.

Table 3. The summary of 8 gene expression results from real-time PCR when each condition was compared with no-light (untreated) normal fibroblast and no-light (untreated) diabetic fibroblast respectively.

Changes in Folds	CDH1	FGA	HGF	IL8	LEPTIN	VEGFA
NCL	10	14	5	30	100	50
NCL	2	500	1,000	500,000	<0	30
NCL	<0	1,000	<0	<0	<0	<0
DMCL	2	2	2	70	<0	2
DMCL	<0	100	500	20,000	<0	20
DMCL	<0	10	<0	8	8	<0

4. Whole gene expression results with microarray data

Both normal and diabetic fibroblast cell lines from Coriell Institute for Medical Research were cultured in DMEM with 10% FBS, 1 % penicillin and 1% streptomycin. After sub-culturing into 10-centimeter plates with 2,000,000 cells seeding density, treatment groups were treated with red, blue, green LED at the power of 0.34 J/cm^2 (for 10 minutes). Then, right after irradiation finished, all samples were harvested and trypsinized with 0.5 %trypsin EDTA. Next, mRNA extraction step was proceeded with GeneJet Purification kit from ThermoScientific. Within next 24 hours, all mRNA samples were shipped to Agilent microarray service facility in India for RNA purity confirmation and Microarray analysis. Here are the results:

In Appendix A, it shows the purity of RNA samples before proceeding Microarray step. All 8 samples were measured the purity with Bioanalyzer. To measure the purity and quality of RNA samples, RNA Integrity Number (RIN) was used. If RIN value is more than 8.0, it represents good purity and quality of samples. All mRNA samples show RIN value more than 9.20 as shown in Appendix A. Thus, they are all good for microarray analysis and all mRNA samples were then hybridized with Agilent SurePrint 8x60K mRNA

expression microarray. After that, raw photos were analyzed the brightness of each dots on microarray slides. GeneSpring 13 from Agilent later analyzed these raw data in Thailand.

After receiving raw data, they were further analyzed by GeneSpring 13 software. It was found that when all samples were compared with the control group, the 2-fold different changes of the whole 50,739 genes were filtered and selected only the ones with P value is less than 0.05. Then, in order to explore for more details about the relationship among gene ontology and their relating pathways, the selected samples were further analyzed against the database from NCBI and grouped them based on their cellular functions. According to Table 4, after analysis, it found that the percentages of affected genes were in the range between 6.05 to 12.02 percent. Although the percentages of gene expression changes are roughly the same among groups, the affected genes were different when the data were further analyzed into their functions, gene ontology, and pathways.

Table 4. The number of genes in each treated group which was significantly up and down for 2 folds when were compared with normal fibroblast and diabetic fibroblast cell lines.

From total of detected 50739 genes		NCL BLUE	NCL GREEN	NCL RED
NCL control	UP	1743 (3.44%)	2606 (5.14%)	2459 (4.85%)
	DOWN	5222 (10.29%)	5234 (10.32%)	5075 (10.00%)
DMCL control	UP	4778 (9.42%)	5753 (11.34%)	5590 (11.02%)
	DOWN	4887 (9.63%)	5066 (9.98%)	4814 (9.49%)
From total of detected 50739 genes		DMCL BLUE	DMCL GREEN	DMCL RED
NCL control	UP	3072 (6.05%)	3545 (6.99%)	3395 (6.69%)
	DOWN	5478 (10.08%)	6101 (12.02%)	5300 (10.45%)
DMCL control	UP	3890 (7.67%)	4288 (8.45%)	4071 (8.02%)
	DOWN	3244 (6.39%)	3383 (6.67%)	2934 (5.78%)

After analyzing and grouping the filtered results with p value less than 0.001 against NCBI database, we found that when the treated group of normal fibroblast was compared against the control normal fibroblast group, blue light affected the least number of cellular functions, as shown in Table 5. Meanwhile, green and red light seems to promote wound healing because it showed an up regulation of inflammatory respond, anti-viral response, and cell development in both groups. For treated diabetic groups, it seemed that all 3 color of light can promote wound healing because they all activate collagen synthesis, mitotic cell cycle,

and cell proliferation. However, the best condition for diabetic wound healing would be the red light because it can express key elements to promote wound healing, such as inflammatory response, blood clotting, vascular endothelial growth factor (VEGF), and oxidative stress, which will trigger the wound to heal faster. Complete lists of gene ontology (GO) comparing among groups are presented in Appendix C.

Table 5. Summary of up regulate and down regulate effects from different wavelengths in both normal and diabetic fibroblast cells.

	NCL			DMCL		
	BLUE	GREEN	RED	BLUE	GREEN	RED
Up regulation	· GPCR · cancer	· GPCR · cancer · inflammatory · mitotic cycle · antiviral response · stem cell development · hematopoietic stem cell development	· GPCR · inflammatory · antiviral response · stem cell development · hematopoietic stem cell development	· GPCR · Ca+ channel · Collagen synthesis · mitotic cycle · cell adhesion · VEGF · cell proliferation · extracellular matrix	· GPCR · Ca+ channel · Collagen synthesis · mitotic cycle · inflammatory · blood clotting · sexual reproduction · cell proliferation	· GPCR · Ca+ channel · Collagen synthesis · mitotic cycle · inflammatory · blood clotting · VEGF · oxidative stress
Down regulation	· perception	· blood clotting · perception	· blood clotting · cell migration · ovarian infertility · adipogenesis · perception	· stem cell development · adipogensis	· stem cell development · perception	· angiogenesis · allograft rejection · adipogensis

CHAPTER 4

DISCUSSION

After being exposed with LED light, both proliferation rate and gene expressions respond in the same direction, which can promote the wound healing process via inflammatory responses, especially with red or green LED light. These lead to a potential and practical wound healing treatment in both normal and diabetic patient.

According to 8 gene expression results from Real-time PCR, CDH1(cadherin 1) is one of extracellular matrix (ECM) protein promoting intercellular communication. FGA (fibrinogen alpha chain) facilitate blood clotting. HGF (Hepatocyte growth factor) is a paracrine serving for growth and morphogenesis. LEPTIN is a hormone that control energy intake and expenditure, including metabolism, appetite, behavior, and the expression of VEGFA. These genes significantly facilitate the wound healing. These lead to a potential and practical wound healing treatment in both normal and diabetic patient. The optimum energy for wound healing is 0.34 J/cm^2 at 630 nm.

Different whole gene expression between normal and diabetic cells

The differences of whole gene expression between DMCL and NCL are shown in Table 4. It was found that when DMCL was compared against NCL, 3375 genes were significantly up which can be grouped into 124 gene ontology terms. In addition, 6587 genes were down which can be grouped into 94 gene ontology terms. After analysis, we found that DMCL responses to wounding better than NCL. Nevertheless, it lacks other functions to help it close its wounds. For example, as shown in Table 5, although there are many up regulated pathways about inflammatory response, response to wounding, ossification, cell differentiation & development, and cell migration pathways, regulation of mitosis and meiosis, G-protein receptor activity, cell adhesion, and hair cycle pathways were significantly down.

Up and down expression of each treated group

Red light irradiation Group

By comparing with normal control group, only genes relating to response to virus and defense response to virus from red light-treated normal cells (NCL RED) group were significantly up regulated shown in Appendix B. However, calcium ion binding, regulation of acute

inflammatory response, and blood clot formation genes were greatly surged in red light-treated diabetic cells (DM RED) group as shown in Appendix B. In addition, when DM RED was compared with DMCL control group, we found that mitotic cell cycle, cell division, response to DNA damage, motor activity, and DNA repair genes were significantly up regulated. Yet, it is interesting that there is no significant down expression in the comparison of DM RED and DMCL control group. Nevertheless, when DM RED was compared with NCL, angiogenesis, cell adhesion, growth factor activity, extracellular matrix, and hair cycle were obviously down. In contrast, most of other important pathways relating to wound healing process were down regulated, such as response to wounding, collagen fibril assembly, extra cellular matrix, elastic fiber formation, and formation of fibrin clot in NCL RED.

Blue light irradiation

For the groups which were irradiated with blue light and compared with NCL, only G-protein coupled receptor activity was strongly escalated in blue light-treated normal cells (NCL BLUE) as shown in Appendix B. Cell adhesion, ion channel activity, and calcium ion binding were down. For blue light-treated diabetic cells (DM BLUE) shown in Appendix B. positive regulation of cell proliferation, hair follicle

development, cell proliferation, epithelial cell differentiation, degradation of collagen, endoderm differentiation were notably boosted. Interestingly, collagen assembly, extra cellular matrix, potassium channels, alpha cells, immune regulatory, and IL-7 signaling were greatly down regulated. However, when DM BLUE was compared with DMCL control group, it shows that mitotic cell cycle, meiotic cell cycle, cell proliferation, and reproduction were strongly up regulated. In contrast, cell adhesion, extracellular matrix, hair cycle, and ion channel activity were down.

Green light irradiation

Green light treated groups compared with NCL control group are shown in Table 5. For NCL GREEN, the expression of cell division, mitotic cell division, and regeneration were raised. And, it was surprising that immune system process, cellular response to type I interferon, and defense response to virus were also up. On the other hand, extracellular matrix, response to wounding, and cell morphogenesis were apparently down. For DMCL GREEN, like the expression of DM RED represented in Table 4, the expressions of calcium ion binding, regulation of acute inflammatory response, and inflammatory response were also increased. However, when DMCL GREEN were compared with DMCL control group, cell division, mitotic cell division, endochondral ossification,

metabolism of angiotensinogen to angiotensins, and cell proliferation were up regulated.

Gene expression that significantly affect the wound healing pathway

According to Gould L, Abadir P, Brem H et al. in 2015, wound healing process consists of the processes of angiogenesis, vascular development, immune response, inflammatory response, blood coagulation, cell adhesion, cell division, cell proliferation, cell migration, cell differentiation, and extracellular matrix as shown in Figure 27. Their details will be described in the following page. However, each process is differently expressed in different wavelength as shown in Appendix B.

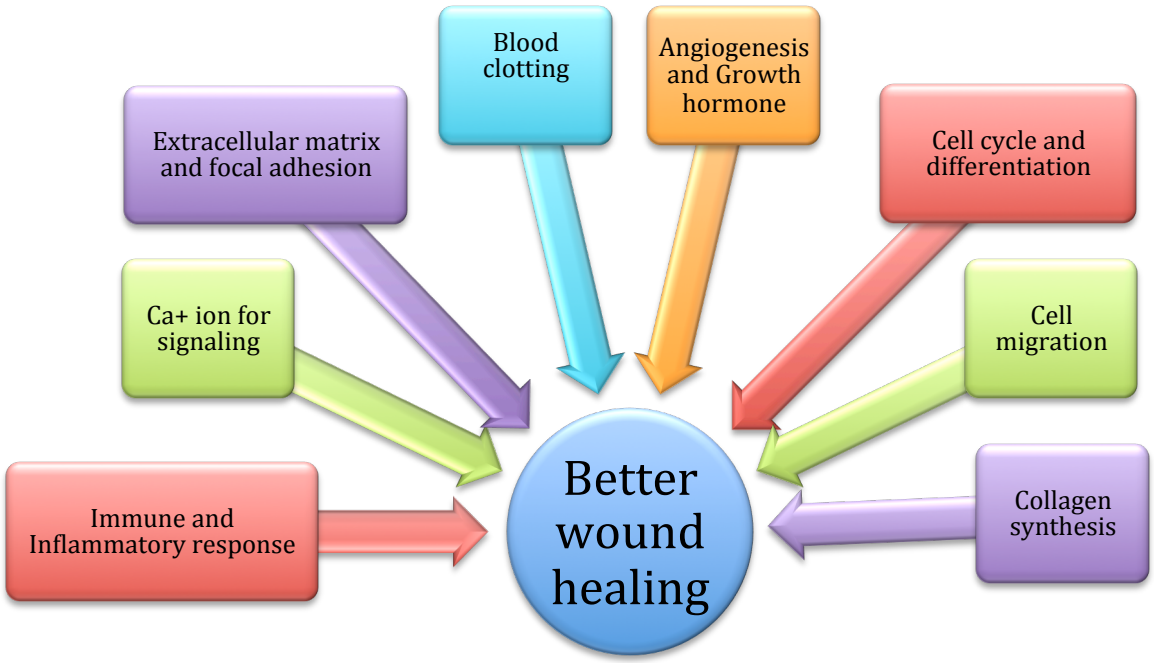


Figure 27. Summary of pathways that were activated to promote wound healing

The expressions of angiogenesis and cardiovascular development are significantly down in DMCL RED group comparing with NCL control. After exploring their relating pathways, we found that there are 62 genes were suppressed which affect 69 pathways. Most of them significantly affect hair follicle development pathway and assembly of collagen fibrils pathway. In addition, focal adhesion, differentiation, extracellular matrix organization, TGF beta signaling, PIP3 activates AKT signaling, adipogenesis, and signaling by FGFR pathway were also significantly affected. This suggests that red light irradiation may not suitable for diabetic patients to get an angiogenesis effect.

Immune response and inflammatory response were significantly up in NCL GREEN comparing with NCL control. This expression also links to the cellular response to viral infection which was also highly active in NCL RED and NCL GREEN group. Green light tends to actively trigger more number of genes than red light does. After pathway analysis, we found that kinesins, interferon alpha-beta signaling, type I & II & III interferon, MHC class II antigen presentation, toll-like receptor, Nitric oxide dioxygenase (NOD), apoptosis, and DNA damage response pathways were significantly up regulated. In contrast, this antiviral response was not significantly different when the results were compared

between treated diabetic cells and healthy normal cells. However, red and green light still can significantly induce inflammatory response in the comparison of DM RED or DM GREEN and diabetic control group.

Focal adhesion, blood clotting, cell surface interaction with vascular wall, ECM, and angiogenesis are also the key role in wound healing. Typically, the expression of cell adhesion in diabetic cells is a lot lower than what normal cells do. However, only DM BLUE significantly shows positive expression of cell-cell adhesion. This cell-cell adhesion process mainly links to cell surface interaction at vascular wall pathway and integrin cell surface interaction pathway.

Blood clotting cascade, which is also a part of inflammatory response, was highly activated in DM RED comparing with NCL control. In addition, formation of fibrin clot pathway was also up in both DM RED and DM GREEN comparing with NCL control. However, a significant reduction of such expression pathway was found in NCL RED and NCL GREEN. This means that blood clotting pathway can particularly be reactivated with red or green light in only diabetic cells.

For cell proliferation, green and blue light can highly stimulate the expression of cell proliferation in diabetic fibroblast cell line comparing with DMCL control. Moreover, DMCL blue group also strongly shows a

significant expression of epithelium cell proliferation when it was compared with NCL control group. In details, green light can affect 111 genes which directly link to 98 pathways. Unfortunately, 5 of them are integrated pancreatic cancer, integrated breast cancer, role of retinoblastoma cancer, prostate cancer, and gastric cancer pathways. However, protein expression and *in vivo* experiments are required in the future investigation. Proper doses of green light irradiation may reduce the risk of turning a normal cell to a cancer cell. For DM BLUE which can induce the epithelium cell proliferation & cell differentiation comparing with NCL control, we found that there are 25 genes inducing epithelium cell proliferation and 11 genes activating cell differentiation respectively. These genes mainly link to hair follicle development, endochondral ossification, wnt signaling angiogenesis, signaling by VEGF, heart development, and IL-2,5,6,7 pathways.

For a signaling pathway relating to vascular endothelium growth factor (VEGF), which is believed to be a marker gene to indicate the initiation of angiogenesis, only KDR and NRP2 were highly expressed after irradiating with red and blue light in diabetic cells comparing with NCL control. However, the expression of VEGF itself was not significantly different.

Mitotic cell cycle is significantly up regulated in NCL GREEN comparing with NCL control. In addition, all treated groups in diabetic fibroblast cells shows very high expression of mitotic cell cycle process when they were compared with DMCL control, but not when they were compared with NCL control. This means that phototherapy with red, blue, and green light can restore the function of mitotic cell cycle in diabetic cells to be like normal cells.

Cell migration is negatively regulated when a normal fibroblast cell was treated with red light (NCL RED) comparing with NCL control. Moreover, this NCL RED group even shows lower expression than DMCL control. Besides, there was no significant difference in other treated groups which means that red light can worsen cell migration process especially in normal cells.

Extracellular matrix organization pathway, blue light in diabetic cells can trigger this pathway to significantly up its expression higher than diabetic control; however, it is still lower than the expression of normal control group. 14 genes were down (DMD, NTN4, TTR, PDGFA, C4S-BCAN, CS4-ACAN, LAMA1, MATN4, LAMB1, NCAM1, ITGA2B, ITGB3, ITGB6, and MATN3). And 4 genes were up (LAMA4, LAMA5, TNC, and VTN).

For collagen biosynthesis, there is no significant difference between all treated normal cell groups and normal control. However, this pathway is down regulated in only all treated diabetic cell groups compared with normal control. 14 genes were down (COL2A1, COL4A1, COL4A2, COL4A5, COL4A6, COL7A1, COL8A1, COL8A2, COL11A1, COL15A1, COL21A1, COL23A1, COL24A1 and LEPRPEL1). And, 5 genes were up (COL4A3, COL9A3, COL17A1, COL22A1, and COL 28A1). These results can be assumed that red, green, and blue lights do not affect both normal and diabetic cells.

However, for wound healing response, DMCL already up regulate this process when they were compared with NCL. According to the results, no light can significantly up regulate this response. NCL GREEN shows the worst wound healing response because its expression is significantly lower than normal level of NCL's expression.

For calcium ion binding channel, all treated groups in diabetic fibroblast cells shows very high expression when they were compared with NCL control. There are 84 genes are responsible for this calcium ion binding activities. These genes also directly link to 18 other pathways, such as human complement system, coagulation cascade, PTM gamma

carboxylation, hypusine formation, arylsulfatase activation, and degradation of collagen pathways.

In conclusion, according to figure 27, there are eight major factors that are related to this wound healing process. From these results, it clearly shows that 5 of such factors, which are immune & inflammatory response, Ca+ ion for signaling, Extracellular matrix & focal adhesion, Blood clotting, and Cell cycle & Cell differentiation, were up regulated by red and green LED lights. Therefore, such phototherapy will be a useful technique if proper selection of wavelength matches with types of target cells and the purpose for each application. For example, if one wants induce antiviral infection process, green or red light irradiation will be a good choice. This condition only works well in normal cells, not in diabetic cells. In addition, although a particular wavelength can restore the expression of some crucial pathways in diabetic cells, its expression does not still significantly improved in treated normal cells, such as cell migration, extracellular matrix, and collagen biosynthesis.

Agreement with the previous studies

For inflammatory response, which is one of the important steps in wound healing process, we found that both acute inflammatory response genes and inflammatory response genes were strongly up regulated

($p<0.05$) in only DM GREEN and DM RED. However, no significant sign of inflammatory genes critically changes in the NCL blue group.

However, for blue light treated group in NCL, only GPCR pathway and oxidation of cytochrome P450 were significantly upregulated. However, the activity of P450 was previously observed only in UVA irradiation.

For hair follicle development, there are 3 parts about this pathway – 1) induction 2) organogenesis 3) cytodifferentiation. Diabetic cells treated with blue light can trigger this pathway the best because it can stimulate all 3 parts of the pathway whereas red and green can trigger only the third part of this pathway.

Prospective experiment

To expand the knowledge about how this phototherapy affecting the cells, other irradiating wavelengths, such as infrared, far infrared, UV-A, and UV-B, are needed to explore with more comprehensive studies, such as mRNA expression array, miRNA study, other protein studies, and multi-omic analysis. In addition, *in vivo* and clinical trial studies are also essential. The researchers believe that each color of light can activate

certain groups of cellular molecules that some molecules intersect and can be grouped to fully understand the relationship between wavelengths and cellular responses.

In addition, the changes of mRNA and protein expression before, during, and after the treatment would be the best solution to understand any unclear conditions for diabetic wound healing process with this kind of phototherapy technique.

CHAPTER 5

CONCLUSION

According to the results we can conclude that:

1. Red LED irradiation with the power at 0.34 J/cm^2 can best promote wound healing in fibroblast cells. Because, according to the results of wound healing assay, red LED irradiation can help the wound the close 66% percent faster than control group. In addition, from MTT results, red LED can promote 70 % more proliferation rate than control groups do.

2. According to real-time PCR results, red LED irradiation can strongly affect the Interleukin8 (IL8), which is one of inflammatory respond genes, and fibrinogen alpha chain (FGA), which is one of remodeling enzymes that facilitate cells to form correctly, in normal fibroblast cells. However, in diabetic fibroblast cells, CDH1, FGA, HGF, LEPTIN, VEGFA are also little promoted from red LED irradiation. This concludes that red LED can significantly promote wound healing in diabetic fibroblast cells at a lower level than what red LED does in normal fibroblast cells.

3. According to whole gene expression results, the advantages of red light irradiation is that red LED still best promotes wound healing with less side effects as shown in Table 5. For example, red LED irradiation in diabetic cells can promote many cellular functions relating to wound healing (such as Ca+ channel, Collagen synthesis, mitotic cycle, inflammatory respond, blood clotting, VEGF, and oxidative stress). On the other hand, blue and green light irradidation may significnatly activate some cancer genes.

4. Some disadvantages are:

    a. Red light irradiation in normal fibroblsat cells can down regulate blood clotting. Therefore, this treament might be utilized after the second step of wound healing process is completed. In addition, in diabetic groups, red light irraidaiton down regulates angiogenesis a bit. However, an up regulation of VEGF may compensate this function.

    b. Blue and green light may cause some cancer.

In summary, red light (630 nm) at 0.34 J/cm^2 would be an ideal wavelength to treat diabetic wound because it can significantly promote many pathways relating to wound healing with less side effects.

BIBLIOGRAPHY

Aggarwal BB, Vijayalekshmi RV, Sung B. (2009) Targeting inflammatory pathways for prevention and therapy of cancer: short-term friend, long- term foe. *Clin Cancer Res* 15:425–30

Andrew J. Murphy, Laurent Yvan-Charvet (2015) Adipose Modulation of ABCG1 Uncovers an Intimate Link Between Sphingomyelin and Triglyceride Storage. *Diabetes* 64:3 689-692

Avci P, Gupta A, Sadasivam M et al. (2013) Low-level laser (light) therapy (LLLT) in skin: stimulating, healing, restoring. A review. *Semin Cutan Med Surg* 32(1):41-52

Bettahi I, Sun H, Gao N et al. (2014) Genome-wide transcriptional analysis of differentially expressed genes in diabetic, healing corneal epithelial cells: hyperglycemia-suppressed TGFβ3 expression contributes to the delay of epithelial wound healing in diabetic corneas. *Diabetes* 63(2):715-27

Bossini PS, Fangel R, Habenschus RM et al. (2009) Low-level laser therapy (670 nm) on viability of random skin flap in rats. *Lasers Med Sci* 24(2):209-13

Butler GJ, Neale R, Green AC et al. (2005) Nonsteroidal anti-inflammatory drugs and the risk of actinic keratoses and squamous cell cancers of the skin. *J Am Acad Dermatol* 53:966–72

Deeni YY, Ibbotson SH, Woods JA et al. (2013) Cytochrome P450 CYP1B1interacts with 8-methoxypsoralen (8-MOP) and influences psoralen-ultraviolet A(PUVA) sensitivity. *PLoS One* 23;8(9):e75494

Eells JT, Henry MM, Summerfelt P et al. (2003) Therapeutic photobiomodulation for methanol-induced retinal toxicity. *Proc Natl Acad Sci U S A* 18;100(6):3439-44

Erdle BJ, Brouxhon S, Kaplan M et al. (2008) Effects of continuous-wave (670-nm) red light on wound healing. *Dermatol Surg* 34(3):320-5

Evans DH, Abrahamse H (2008) Efficacy of three different laser wavelengths for in vitro wound healing. *Photodermatol Photoimmunol Photomed* 24(4):199-210

Fushimi T, Inui S, Nakajima T et al. (2012) Green light emitting diodes accelerate wound healing: characterization of the effect and its molecular basis in vitro and in vivo. *Wound Repair Regen* 20(2):226-35

Gould L, Abadir P, Brem H et al. (2015) Chronic wound repair and healing in older adults: current status and future research. *J Am Geriatr Soc* 63(3):427-38

Greenhalgh DG (2003) Tissue repair in models of diabetes mellitus. A review. *Methods Mol Med* 78:181-9

Hamblin, Ronald W. Waynant, Juanita Anders (2006) Mechanisms for Low-Light Therapy. *Proc. of SPIE* Vol. 6140, 614001

Hausch F (2013) Structures of class B G protein-coupled receptors: prospects for drug discovery. *Angew Chem Int Ed Engl* 52(49):12783-5

Hawkins DH, Abrahamse H (2006) The role of laser fluence in cell viability, proliferation, and membrane integrity of wounded human skin fibroblasts following helium-neon laser irradiation. *Lasers Surg Med* 38(1):74-83

Hong WX, Hu MS, Esquivel M et al. (2014) The Role of Hypoxia-Inducible Factor in Wound Healing. *Adv Wound Care (New Rochelle)* 3(5):390-399

Houreld NN, Abrahamse H (2008) Laser light influences cellular viability and proliferation in diabetic-wounded fibroblast cells in a dose- and wavelength-dependent manner. *Lasers Med Sci* 23(1):11-8

Jahangiri Noudeh Y, Shabani M, Vatankhah N et al. (2010) A combination of 670 nm and 810 nm diode lasers for wound healing acceleration in diabetic rats. *Photomed Laser Surg* 28(5):621-7

Karu TI, Kolyakov SF (2005) Exact action spectra for cellular responses relevant to phototherapy. *Photomed Laser Surg* 23(4):355-61

Kaviani A, Djavid GE, Ataie-Fashtami L et al. (2011) A randomized clinical trial on the effect of low-level laser therapy on chronic diabetic foot wound healing: a preliminary report. *Photomed Laser Surg* 29(2):109-14

Kavitha KV, Tiwari S, Purandare VB et al. (2014) Choice of wound care in diabetic foot ulcer: A practical approach. *World J Diabetes* 5(4): 546-556

Lee SY, You CE, Park MY (2007) Blue and red light combination LED phototherapy for acne vulgaris in patients with skin phototype IV. *Lasers Surg Med* 39(2):180-8

Lei zhang, Shen qu, Aibin liang et al. (2013) Gene expression microarray analysis of the sciatic nerve of mice with diabetic neuropathy.

International Journal Of Molecular Medicine 35: 333-339

Li X, Hou W, Wu X et al. (2014) 660 nm red light-enhanced bone marrow mesenchymal stem cell transplantation for hypoxic-ischemic brain damage treatment. *Neural Regen Res* 9(3):236-42

Mirza RE, Fang MM, Weinheimer-Haus EM et al. (2014) Sustained inflammasome activity in macrophages impairs wound healing in type 2 diabetic humans and mice. *Diabetes* 63(3):1103-14

Natoli R, Zhu Y, Valter K et al. (2010) Gene and noncoding RNA regulation underlying photoreceptor protection: microarray study of dietary antioxidant saffron and photobiomodulation in rat retina. *Mol Vis* 16:1801-22

Nicolette N. Houreld (2014) Shedding Light on a New Treatment for Diabetic Wound Healing: A Review on Phototherapy. *The Scientific World Journal* Article ID 398412, 13 pages

Nowak-Sliwinska P, van Beijnum JR, van Berkel M et al. (2010) Vascular regrowth following photodynamic therapy in the chicken embryo chorioallantoic membrane. *Angiogenesis* 13(4):281-92

Ohtani, K., Usuda, J., Ichinose, S. (2008) High expression of GADD-45α and VEGF induced tumor recurrence via upregulation of IL-2 after photodynamic therapy using NPe6. *International Journal of Oncology* 32: 397-403

Rácz E, Prens EP, Kurek D et al. (2011) Effective treatment of psoriasis with narrow-band UVB phototherapy is linked to suppression of the IFN and Th17 pathways. *J Invest Dermatol* 131(7):1547-58

Reddy GK, Stehno-Bittel L, Enwemeka CS (2001) Laser photostimulation accelerates wound healing in diabetic rats. *Wound Repair Regen* 9(3):248-55

Shaw TJ, Martin P (2009) Wound repair at a glance. *J Cell Sci* 15;122(Pt18):3209-13

Tatli MM, Minnet C, Kocyigit A et al. (2008) Phototherapy increases DNA damage in lymphocytes of hyperbilirubinemic neonates. *Mutat Res* 30;654(1):93-5

Vatansever F, Ferraresi C, de Sousa MV et al. (2013) Can biowarfare agents be defeated with light? *Virulence* 4(8):796-825

Whelan HT, Smits RL Jr, Buchman EV et al. (2001) Effect of NASA light-emitting diode irradiation on wound healing. A Review. *J Clin Laser Med Surg* 19(6):305-14

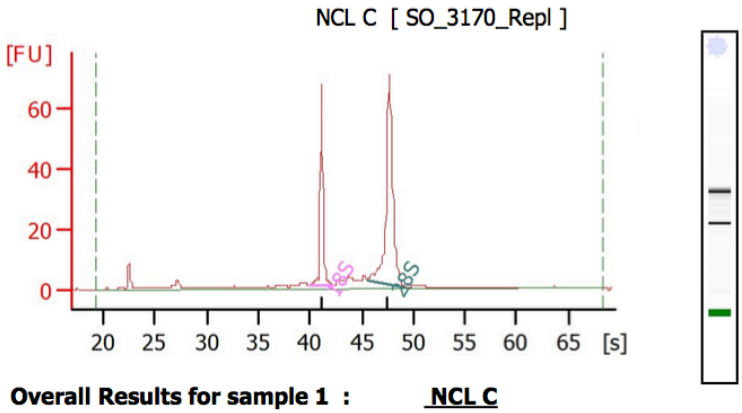
Wu YH, Wang J, Gong DX, et al. (2012) Effects of low-level laser irradiation on mesenchymal stem cell proliferation: a microarray analysis. *Lasers Med Sci* 27(2):509-19

Yin R, Dai T, Avci P et al. (2013) Light based anti-infectives: ultraviolet C irradiation, photodynamic therapy, blue light, and beyond. *Curr Opin Pharmacol* 13(5):731-62

Zhang Y, Song S, Fong CC et al. (2003) cDNA microarray analysis of gene expression profiles in human fibroblast cells irradiated with red light. *J Invest Dermatol* 120(5):849-57

APPENDIX A

RIN value of all samples which were analyzed by Bio analyzer machine

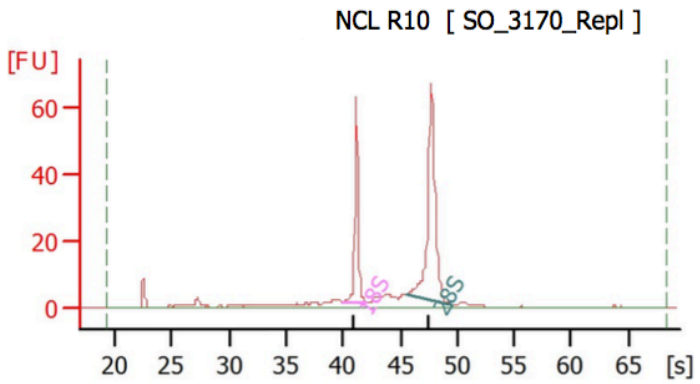


Overall Results for sample 1 : NCL C

RNA Area:	244.9
RNA Concentration:	263 ng/µl
rRNA Ratio [28s / 18s]:	2.1
RNA Integrity Number (RIN):	9.6 (B.02.08)
Result Flagging Color:	
Result Flagging Label:	RIN: 9.60

Fragment table for sample 1 : NCL C

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	39.99	42.22	51.6	21.1
28S	45.59	49.31	106.7	43.5

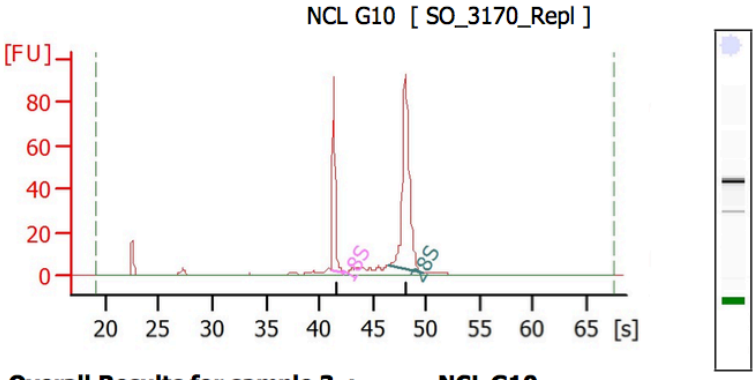


Overall Results for sample 2 : NCL R10

RNA Area:	253.5
RNA Concentration:	272 ng/µl
rRNA Ratio [28s / 18s]:	2.1
RNA Integrity Number (RIN):	9.5 (B.02.08)
Result Flagging Color:	
Result Flagging Label:	RIN: 9.50

Fragment table for sample 2 : NCL R10

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	39.99	42.18	49.6	19.6
28S	45.59	49.51	106.0	41.8

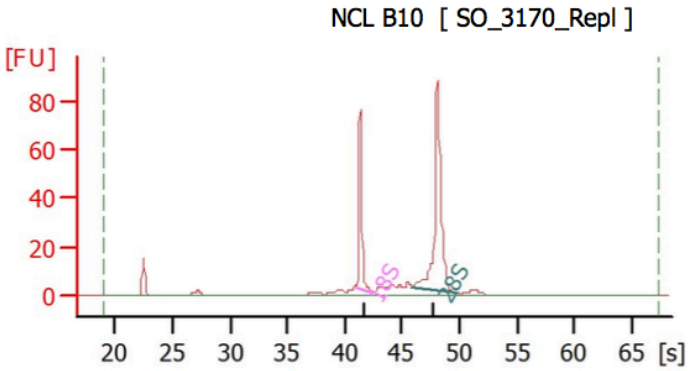


Overall Results for sample 3 : NCL G10

RNA Area:	304.0
RNA Concentration:	186 ng/µl
rRNA Ratio [28s / 18s]:	2.1
RNA Integrity Number (RIN):	9.8 (B.02.08)
Result Flagging Color:	
Result Flagging Label:	RIN: 9.80

Fragment table for sample 3 : NCL G10

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.03	42.40	68.6	22.5
28S	46.47	49.75	140.7	46.3

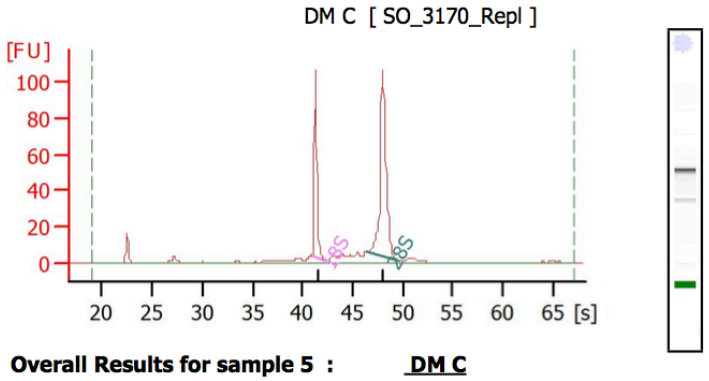


Overall Results for sample 4 : NCL B10

RNA Area:	262.8
RNA Concentration:	161 ng/µl
rRNA Ratio [28s / 18s]:	2.2
RNA Integrity Number (RIN):	9.7 (B.02.08)
Result Flagging Color:	
Result Flagging Label:	RIN: 9.70

Fragment table for sample 4 : NCL B10

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.05	42.36	54.8	20.9
28S	45.83	49.69	119.4	45.4



Overall Results for sample 5 : DM C

RNA Area:	342.1
RNA Concentration:	209 ng/µl
rRNA Ratio [28s / 18s]:	1.9
RNA Integrity Number (RIN):	9.7 (B.02.08)
Result Flagging Color:	
Result Flagging Label:	RIN: 9.70

Fragment table for sample 5 : DM C

Name	Start Time [s]	End Time [s]	Area	% of total Area
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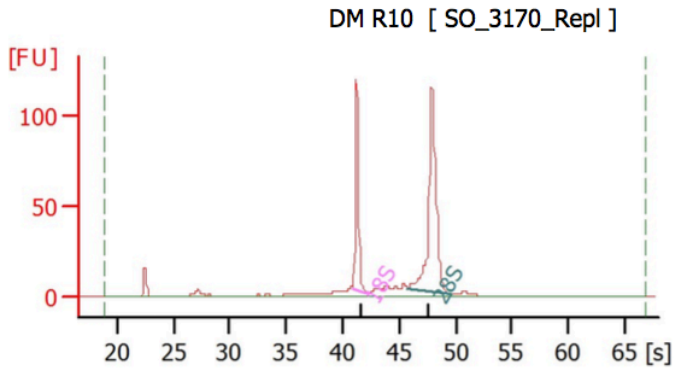
28S	46.51	49.63	153.3	44.8

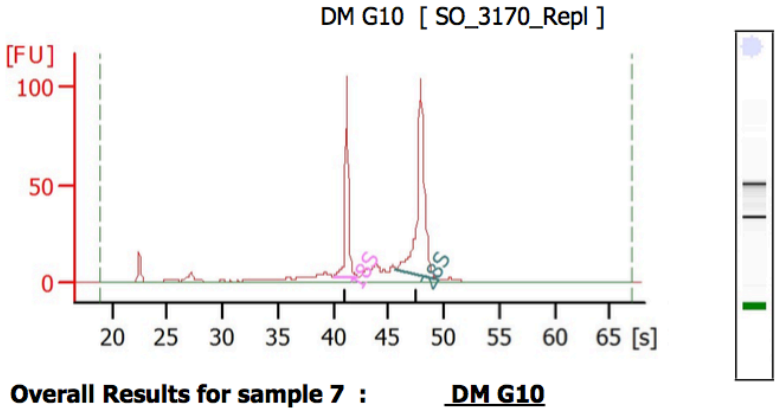


Overall Results for sample 6 : DM R10

RNA Area:	396.4
RNA Concentration:	242 ng/µl
rRNA Ratio [28s / 18s]:	2.1
RNA Integrity Number (RIN):	9.7 (B.02.08)
Result Flagging Color:	
Result Flagging Label:	RIN: 9.70

Fragment table for sample 6 : DM R10

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	40.98	42.28	87.4	22.0
28S	45.82	49.56	181.9	45.9

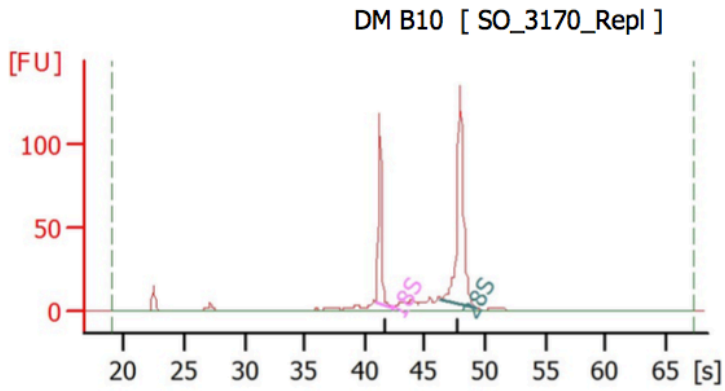


Overall Results for sample 7 : DM G10

RNA Area:	438.9
RNA Concentration:	268 ng/µl
rRNA Ratio [28s / 18s]:	1.9
RNA Integrity Number (RIN):	9.2 (B.02.08)
Result Flagging Color:	
Result Flagging Label:	RIN: 9.20

Fragment table for sample 7 : DM G10

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	39.99	42.28	84.5	19.3
28S	45.68	49.52	163.3	37.2

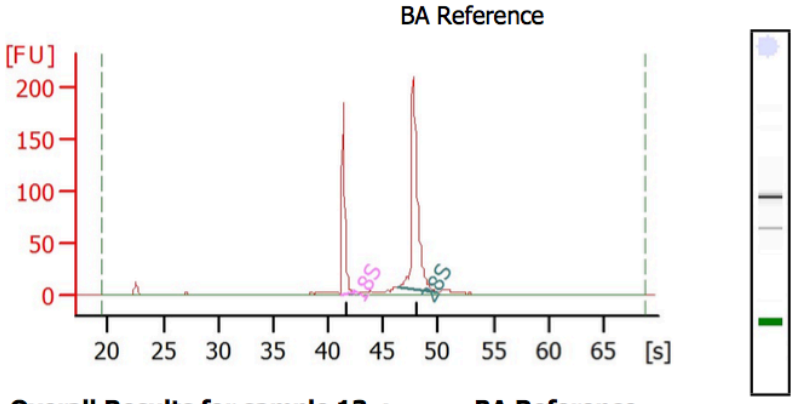


Overall Results for sample 8 : DM B10

RNA Area:	420.8
RNA Concentration:	257 ng/µl
rRNA Ratio [28s / 18s]:	2.0
RNA Integrity Number (RIN):	9.6 (B.02.08)
Result Flagging Color:	
Result Flagging Label:	RIN: 9.60

Fragment table for sample 8 : DM B10

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	40.95	42.27	87.0	20.7
28S	46.37	49.25	169.9	40.4



Overall Results for sample 13 : BA Reference

RNA Area:	533.0
RNA Concentration:	489 ng/µl
rRNA Ratio [28s / 18s]:	1.9
RNA Integrity Number (RIN):	10 (B.02.08)
Result Flagging Color:	
Result Flagging Label:	RIN:10

Fragment table for sample 13 : BA Reference

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	41.06	42.31	140.5	26.4
28S	46.35	49.89	269.8	50.6

APPENDIX B

Raw data of gene ontology analysis from whole gene expression array

Table 6, 7 and 8 illustrate the groups of expression profiles of different treated groups categorized as gene ontology (GO) and compared with normal fibroblast cell line control group (NCL) and diabetic control fibroblast control group (DMCL). Table 6 is the comparison of expression results relating to wound healing process between diabetic fibroblast control group (DMCL) and normal fibroblast control group (NCL), which were grouped into their gene ontology (GO) terms. Table 7 is the comparison of expression results relating to wound healing process between normal fibroblast control group (NCL) and LED-treated normal fibroblast groups, which are NCL RED, NCL BLUE, and NCL GREEN. Table 8 is the comparison of expression results of LED-treated diabetic fibroblast groups, which are DM RED, DM BLUE, and DM GREEN, relating to wound healing process against both normal fibroblast control group (NCL) and diabetic fibroblast control group (DMCL).

TABLE 6. Summary of very significant gene ontology (G0) profiles relating to wound healing pathway ($p<0.001$) when diabetic fibroblast control group (DMCL) were compared with normal fibroblast control group (NCL)

DMCL expression compared against NCL			
UP (3375 genes = 124 GO terms)		DOWN (6587 genes = 94 GO terms)	
• calcium ion binding • cell migration • cell motility • cell surface receptor signaling pathway • cellular developmental process • cellular response to chemical stimulus • chemokine activity • chordate embryonic development • complement activation • cytokine activity • developmental growth involved in morphogenesis • digestive system development • digestive tract development • digestive tract morphogenesis • embryo development • embryo development ending in birth or egg hatching • embryonic digestive tract development • embryonic morphogenesis • embryonic organ development • embryonic organ morphogenesis • embryonic skeletal system development • embryonic skeletal system morphogenesis • enzyme linked receptor protein signaling pathway • epithelium development • extracellular region • extracellular space • face morphogenesis • female pregnancy • gland development • glycosaminoglycan binding • growth factor activity • heparin binding • inflammatory response • kidney development • localization of cell • lung development • lung-associated	• palate development • pattern specification process • positive regulation of cell communication • positive regulation of cell differentiation • positive regulation of cell proliferation • positive regulation of response to stimulus • positive regulation of signal transduction • positive regulation of signaling • positive regulation of transcription from RNA polymerase II promoter • primary alcohol metabolic process • protein activation cascade • proteinaceous extracellular matrix • receptor binding • regulation of acute inflammatory response • regulation of cell differentiation • regulation of cell proliferation • regulation of developmental process • regulation of inflammatory response • regulation of ossification • regulation of osteoblast differentiation • regulation of response to stimulus • regulation of response to wounding • regulation of stem cell differentiation • renal system development • reproductive process • respiratory system development • respiratory tube development • response to chemical • response to lipid • response to steroid	• angiogenesis • basolateral plasma membrane • biological regulation • blood vessel development • blood vessel morphogenesis • cell cycle • cell cycle process • cell development • cell division • cell proliferation • cell-cell signaling • cellular component movement • cellular process involved in reproduction • cellular response to stimulus • centromere complex assembly • chromosome segregation • chromosome, centromeric region • condensed chromosome • condensed chromosome kinetochore • condensed chromosome outer kinetochore • condensed chromosome, centromeric region • condensed nuclear chromosome kinetochore • condensed nuclear chromosome, centromeric region • cytoskeletal protein binding • cytoskeleton organization • DNA replication • epidermis development • G-protein coupled receptor activity • hair cycle • histone exchange • I band	• protein-DNA complex • regulation of cell division • regulation of chromosome segregation • regulation of mitosis • regulation of nuclear division • regulation of organelle organization • regulation of phosphate metabolic process • reproduction • response to stimulus • signaling receptor activity • single-organism cellular process • single-organism process • sister chromatid segregation • spindle organization • spindle pole • transmembrane signaling receptor activity

mesenchyme development • mammary gland development • mesenchyme development • morphogenesis of an epithelium • negative regulation of cell proliferation • negative regulation of multicellular organismal process • negative regulation of secretion • negative regulation of transport • neurological system process • odontogenesis • organ morphogenesis • ossification	hormone • response to wounding • skeletal system development • skeletal system morphogenesis • system process • thymus development • tissue development • tissue morphogenesis • trachea morphogenesis • transmembrane receptor protein kinase activity • transmembrane receptor protein tyrosine kinase activity • tube development • tube morphogenesis • urogenital system development	• kinetochore • meiotic cell cycle • meiotic nuclear division • membrane • membrane part • mitotic cell cycle • mitotic cell cycle process • mitotic nuclear division • mitotic sister chromatid segregation • molecular transducer activity • molting cycle • nuclear division • organelle fission • plasma membrane part • plasma membrane region	

TABLE 7. Summary of very significant gene ontology profiles relating to wound healing pathway ($p<0.001$) when all treated groups were compared with human normal fibroblast cell line (NCL). NCL RED, NCL BLUE, and NCL GREEN are human normal fibroblast cell line which was irradiated with 630(red) nm, 465(blue) nm, and 520(green) nm LED for 10 minute respectively.

Control group	Expression	NCL BLUE	NCL GREEN	NCL RED
NCL	Up	• G-protein coupling activity	• mitotic cell cycle • nuclear division • immune system process • cell cycle • mitotic nuclear division • response to virus • cell division • defense response to virus • regulation of nuclear division • type I interferon signaling pathway • cellular response to zinc ion • cellular response to type I interferon • cell periphery	• receptor activity • response to virus • signaling receptor activity • membrane part • defense response to virus • cell periphery
	Down	• ion channel activity • calcium ion binding • extracellular space • cell adhesion • cell-cell signaling • sensory perception • sensory perception of sound • sensory perception of chemical stimulus • sensory perception of smell • molecular transducer activity • cell periphery	• cell morphogenesis involved in differentiation • olfactory receptor activity • cytokine activity • calcium ion binding • collagen trimer • extracellular space • cell adhesion • cell-cell signaling • sensory organ development • response to wounding • ion transmembrane transporter activity • neuron differentiation • collagen catabolic process • extracellular matrix • regulation of insulin-like growth factor receptor signaling pathway • ear development • cell development • organ development • neuron development • inner ear development • synapse organization • neurological system process • axon development • cell periphery	• extracellular matrix • sensory perception • cell adhesion • cell-cell signaling • calcium ion binding • nervous system development • cell differentiation • collagen trimer • response to wounding • ion channel complex • localization of cell • channel activity • regulation of insulin-like growth factor receptor signaling pathway • ion channel activity • heparin binding • reproductive process • neuron differentiation • neuron development • cytokine activity • fibronectin binding • neurogenesis • positive regulation of osteoblast differentiation • cell migration • collagen fibril organization • cellular response to cAMP

TABLE 8. Summary of very significant gene ontology profiles relating to wound healing pathway ($p<0.001$) when DMCL treated groups were compared with human normal fibroblast cell line (NCL) and human diabetic fibroblast cell line (DMCL). DM RED, DM BLUE, and DM GREEN are human diabetic fibroblast cell line which was irradiated with 630(red) nm, 465(blue) nm, and 520(green) nm LED for 10 minute respectively.

Control group	Expression	DM BLUE	DM GREEN	DM RED
NCL	Up	• signal transducer activity • calcium ion binding • cAMP catabolic process • positive regulation of cell proliferation • tissue development • biological adhesion • positive regulation of epithelial cell differentiation • cell periphery • positive regulation of kidney development • cell-cell adhesion	• regulation of acute inflammatory response • calcium ion binding • extracellular space • integral component of plasma membrane • inflammatory response • cell-cell signaling • developmental process • anatomical structure development	• regulation of acute inflammatory response • calcium ion binding • inflammatory response • developmental process • membrane part
	Down	• signal transducer activity • receptor activity • ion channel activity • chemotaxis • cell adhesion • cell-cell signaling • peripheral nervous system development • epidermis development • calcium ion transmembrane transporter activity • extracellular matrix • locomotion • molting cycle • taxis • hair cycle • skin development • synapse • axon development • cell periphery • synaptic membrane • basement membrane collagen trimer	• signal transducer activity • receptor activity • ion channel activity • calcium ion binding • cell communication • cell adhesion • cell-cell signaling • response to external stimulus • channel activity • biological adhesion • molting cycle • hair cycle • synapse part • cell periphery • cell-cell • adhesion	• cell periphery • receptor activity • cell adhesion • biological adhesion • molting cycle • hair cycle • extracellular matrix • cell-cell signaling • angiogenesis • cardiovascular system development • circulatory system development • vasculature development • growth factor activity • cell-cell adhesion • response to external stimulus • cell projection • basement membrane collagen trimer • extracellular region
Control group	Expression	DM BLUE	DM GREEN	DM RED
DMCL	Up	• mitotic cell cycle • cell cycle • cell cycle process • nuclear division • cell division • DNA replication • reproduction • DNA repair • meiotic cell cycle • cell proliferation • regulation of spindle organization	• mitotic cell cycle • cell cycle process • nuclear division • cell division • DNA replication • DNA repair • reproduction • cell proliferation • microtubule • cell differentiation • motor activity	• mitotic cell cycle • cell cycle • nuclear division • cell cycle process • cell division • DNA replication and repair • meiotic cell cycle • motor activity • cell cycle checkpoint • cytoskeleton • reproduction
	Down	• extracellular space • cell periphery • plasma membrane • cell adhesion • receptor activity • extracellular matrix • regulation of acute inflammatory response	• cytokine activity • extracellular space • complement activation	None

		• transmembrane signaling receptor activity • signaling receptor activity • neurological system process • heparin binding • cytokine activity		

APPENDIX C

These are the complete lists of gene ontology which were collected from microarray analysis with p-value less than 0.001

Up DMCL compared with control NCL

GO ID	GO ACCESSION	GO Term	p-value
14822	GO:0032501|GO:0050874	multicellular organismal process	8.02E-13
21688	GO:0044707	single-multicellular organism process	1.31E-12
3978	GO:0005615	extracellular space	2.26E-11
14823	GO:0032502	developmental process	1.03E-09
21747	GO:0044767	single-organism developmental process	1.56E-09
5293	GO:0007275	multicellular organismal development	5.30E-09
25105	GO:0048598|GO:0048828	embryonic morphogenesis	1.12E-08
25229	GO:0048731	system development	1.67E-08
7133	GO:0009888	tissue development	2.58E-08
13401	GO:0031012	extracellular matrix	4.48E-08
895	GO:0001501	skeletal system development	4.55E-08
28273	GO:0055123	digestive system development	5.02E-08
25349	GO:0048856	anatomical structure development	9.57E-08
25076	GO:0048565	digestive tract development	1.04E-07
6914	GO:0009653	anatomical structure morphogenesis	1.08E-07
25204	GO:0048706	embryonic skeletal system development	1.08E-07
12820	GO:0030323	respiratory tube development	1.19E-07
1002	GO:0001655	urogenital system development	1.41E-07
28298	GO:0060021	palate development	1.94E-07
5042	GO:0006954	inflammatory response	2.34E-07
12821	GO:0030324	lung development	2.40E-07
12440	GO:0022610	biological adhesion	3.36E-07
25077	GO:0048566	embryonic digestive tract development	3.46E-07
5200	GO:0007155	cell adhesion	5.10E-07
25028	GO:0048513	organ development	5.19E-07
17537	GO:0035295	tube development	5.86E-07
19262	GO:0042127	regulation of cell proliferation	6.58E-07
3683	GO:0005102	receptor binding	7.51E-07
11158	GO:0019199	transmembrane receptor protein kinase activity	7.60E-07
1979	GO:0002673	regulation of acute inflammatory response	7.66E-07

28815	GO:0060541	respiratory system development	1.19E-06
25202	GO:0048704	embryonic skeletal system morphogenesis	1.45E-06
7132	GO:0009887	organ morphogenesis	1.57E-06
5574	GO:0007610	behavior	1.59E-06
2259	GO:0003008	system process	2.12E-06
25230	GO:0048732	gland development	2.23E-06
26646	GO:0051241	negative regulation of multicellular organismal process	2.37E-06
31877	GO:0072001	renal system development	2.51E-06
9565	GO:0016477	cell migration	3.35E-06
5814	GO:0008285	negative regulation of cell proliferation	3.44E-06
3942	GO:0005576	extracellular region	3.85E-06
3700	GO:0005125	cytokine activity	3.97E-06
25203	GO:0048705	skeletal system morphogenesis	4.23E-06
26644	GO:0051239	regulation of multicellular organismal process	4.26E-06
25227	GO:0048729	tissue morphogenesis	4.31E-06
3944	GO:0005578	proteinaceous extracellular matrix	4.44E-06
25079	GO:0048568	embryonic organ development	5.26E-06
30779	GO:0070887	cellular response to chemical stimulus	5.51E-06
12546	GO:0023052|GO:0023046	signaling	6.10E-06
21682	GO:0044700	single organism signaling	6.10E-06
25095	GO:0048584	positive regulation of response to stimulus	7.18E-06
25051	GO:0048538	thymus development	8.01E-06
5662	GO:0008083	growth factor activity	8.43E-06
12668	GO:0030154	cell differentiation	9.31E-06
26144	GO:0050727	regulation of inflammatory response	1.02E-05
25073	GO:0048562	embryonic organ morphogenesis	1.06E-05
25362	GO:0048870	cell motility	1.06E-05
27040	GO:0051674	localization of cell	1.12E-05
5199	GO:0007154	cell communication	1.28E-05
7045	GO:0009790|GO:0009795	embryo development	1.38E-05
13584	GO:0031224	intrinsic component of membrane	1.54E-05
16278	GO:0033993	response to lipid	1.56E-05
5044	GO:0006956	complement activation	1.61E-05
5212	GO:0007167	enzyme linked receptor protein signaling pathway	1.73E-05
19129	GO:0040011	locomotion	1.87E-05
32230	GO:0072358	cardiovascular system development	1.92E-05
32231	GO:0072359	circulatory system development	1.92E-05
19346	GO:0042221	response to chemical	2.10E-05
25361	GO:0048869	cellular developmental process	2.12E-05
4177	GO:0005886|GO:0005904	plasma membrane	2.32E-05

20079	GO:0043009	chordate embryonic development	2.35E-05
3378	GO:0004714	transmembrane receptor protein tyrosine kinase activity	2.54E-05
38144	GO:2000026	regulation of multicellular organismal development	2.72E-05
26502	GO:0051094	positive regulation of developmental process	3.02E-05
1326	GO:0002009	morphogenesis of an epithelium	3.04E-05
6881	GO:0009611|GO:0002245	response to wounding	3.22E-05
7047	GO:0009792	embryo development ending in birth or egg hatching	3.33E-05
897	GO:0001503	ossification	3.37E-05
13278	GO:0030879	mammary gland development	3.47E-05
25059	GO:0048546|GO:0048547	digestive tract morphogenesis	3.59E-05
16589	GO:0034308	primary alcohol metabolic process	3.71E-05
28713	GO:0060439	trachea morphogenesis	3.80E-05
9248	GO:0016021	integral component of membrane	3.93E-05
1146	GO:0001822	kidney development	3.94E-05
22433	GO:0045667	regulation of osteoblast differentiation	3.99E-05
26288	GO:0050877	neurological system process	4.30E-05
12547	GO:0023056	positive regulation of signaling	4.72E-05
31822	GO:0071944	cell periphery	4.94E-05
28703	GO:0060429	epithelium development	5.24E-05
22361	GO:0045595	regulation of cell differentiation	5.28E-05
26461	GO:0051051	negative regulation of transport	5.29E-05
5545	GO:0007565	female pregnancy	5.42E-05
7207	GO:0009967|GO:0035468	positive regulation of signal transduction	5.46E-05
28758	GO:0060484	lung-associated mesenchyme development	5.46E-05
7850	GO:0010647	positive regulation of cell communication	5.56E-05
5210	GO:0007165|GO:0023033	signal transduction	5.84E-05
25145	GO:0048646	anatomical structure formation involved in morphogenesis	6.68E-05
5747	GO:0008201	heparin binding	6.73E-05
5211	GO:0007166	cell surface receptor signaling pathway	6.75E-05
22363	GO:0045597	positive regulation of cell differentiation	7.03E-05
25058	GO:0048545	response to steroid hormone	7.29E-05
19579	GO:0042476|GO:0042477	odontogenesis	7.39E-05
28759	GO:0060485	mesenchyme development	7.77E-05
13586	GO:0031226	intrinsic component of plasma membrane	8.39E-05
26208	GO:0050793	regulation of developmental process	8.94E-05
22670	GO:0045944|GO:0010552|GO:	positive regulation of transcription from RNA polymerase II promoter	9.04E-05

	0045817		
3519	GO:0004872|GO:0019041	receptor activity	9.25E-05
38839	GO:2000736	regulation of stem cell differentiation	9.42E-05
4178	GO:0005887	integral component of plasma membrane	1.00E-04
32248	GO:0072376	protein activation cascade	1.01E-04
12427	GO:0022414	reproductive process	1.02E-04
12778	GO:0030278	regulation of ossification	1.11E-04
25094	GO:0048583	regulation of response to stimulus	1.11E-04
28831	GO:0060560	developmental growth involved in morphogenesis	1.11E-04
3930	GO:0005539	glycosaminoglycan binding	1.13E-04
26458	GO:0051048	negative regulation of secretion	1.27E-04
5606	GO:0008009	chemokine activity	1.32E-04
17482	GO:0035239	tube morphogenesis	1.34E-04
37668	GO:1903034	regulation of response to wounding	1.40E-04
1262	GO:0001944	vasculature development	1.42E-04
28599	GO:0060325	face morphogenesis	1.42E-04
5813	GO:0008284	positive regulation of cell proliferation	1.43E-04
3913	GO:0005509	calcium ion binding	1.44E-04
5389	GO:0007389	pattern specification process	1.49E-04

DOWN DMCL compared with control NCL

GO ID	GO ACCESSION	GO Term	p-value
31822	GO:0071944	cell periphery	8.07E-16
207	GO:0000280	nuclear division	1.09E-14
4177	GO:0005886|GO:0005904	plasma membrane	1.29E-14
24833	GO:0048285	organelle fission	6.97E-14
14822	GO:0032501|GO:0050874	multicellular organismal process	2.24E-13
21688	GO:0044707	single-multicellular organism process	2.79E-12
5131	GO:0007067	mitotic nuclear division	2.85E-11
21442	GO:0044459	plasma membrane part	3.02E-10
205	GO:0000278	mitotic cell cycle	5.24E-10
26701	GO:0051301	cell division	5.31E-10
5124	GO:0007059	chromosome segregation	1.34E-09
5114	GO:0007049	cell cycle	1.55E-09
472	GO:0000777	condensed chromosome kinetochore	7.72E-09
474	GO:0000779	condensed chromosome, centromeric region	7.78E-09
12415	GO:0022402	cell cycle process	1.19E-08
26702	GO:0051302	regulation of cell division	1.33E-08
21743	GO:0044763	single-organism cellular process	3.72E-08
25229	GO:0048731	system development	4.08E-08
3519	GO:0004872|GO:0019041	receptor activity	5.06E-08
21681	GO:0044699	single-organism process	5.78E-08
37681	GO:1903047	mitotic cell cycle process	6.97E-08
513	GO:0000819	sister chromatid segregation	7.25E-08
18704	GO:0038023	signaling receptor activity	7.29E-08
488	GO:0000793	condensed chromosome	1.34E-07
5293	GO:0007275	multicellular organismal development	1.71E-07
471	GO:0000776|GO:0005699	kinetochore	1.91E-07
21747	GO:0044767	single-organism developmental process	2.03E-07
25349	GO:0048856	anatomical structure development	2.13E-07
14823	GO:0032502	developmental process	2.54E-07
50	GO:0000070|GO:0016359	mitotic sister chromatid segregation	2.75E-07
27142	GO:0051783	regulation of nuclear division	2.93E-07
34623	GO:0098590	plasma membrane region	3.00E-07
3533	GO:0004888|GO:0004926	transmembrane signaling receptor activity	3.72E-07
5286	GO:0007267	cell-cell signaling	3.80E-07
913	GO:0001525	angiogenesis	4.27E-07
25029	GO:0048514	blood vessel morphogenesis	8.86E-07
470	GO:0000775|G	chromosome, centromeric region	9.02E-07

	O:0097521		
1262	GO:0001944	vasculature development	1.37E-06
12546	GO:0023052|GO:0023046	signaling	1.50E-06
21682	GO:0044700	single organism signaling	1.50E-06
5200	GO:0007155	cell adhesion	1.72E-06
32230	GO:0072358	cardiovascular system development	1.87E-06
32231	GO:0072359	circulatory system development	1.87E-06
9478	GO:0016323	basolateral plasma membrane	2.09E-06
5084	GO:0007010	cytoskeleton organization	2.11E-06
26306	GO:0050896|GO:0051869	response to stimulus	2.14E-06
12440	GO:0022610	biological adhesion	2.17E-06
950	GO:0001568	blood vessel development	2.67E-06
26644	GO:0051239	regulation of multicellular organismal process	2.91E-06
6914	GO:0009653	anatomical structure morphogenesis	3.03E-06
25111	GO:0048610	cellular process involved in reproduction	3.47E-06
4178	GO:0005887	integral component of plasma membrane	3.71E-06
13584	GO:0031224	intrinsic component of membrane	3.92E-06
25028	GO:0048513	organ development	4.31E-06
4492	GO:0006260|GO:0055133	DNA replication	4.62E-06
25145	GO:0048646	anatomical structure formation involved in morphogenesis	5.93E-06
3518	GO:0004871|GO:0005062|GO:0009369|GO:0009370	signal transducer activity	6.06E-06
28366	GO:0060089	molecular transducer activity	6.06E-06
5199	GO:0007154	cell communication	6.91E-06
19418	GO:0042303	molting cycle	7.56E-06
19722	GO:0042633	hair cycle	7.56E-06
13586	GO:0031226	intrinsic component of plasma membrane	7.86E-06
21409	GO:0044425	membrane part	8.76E-06
5178	GO:0007126	meiotic nuclear division	9.11E-06
19129	GO:0040011	locomotion	9.57E-06
3	GO:0000003|GO:0019952|GO:0050876	reproduction	1.05E-05
20500	GO:0043486	histone exchange	1.05E-05
5116	GO:0007051	spindle organization	1.22E-05
5149	GO:0007088	regulation of mitosis	1.51E-05
9248	GO:0016021	integral component of membrane	1.74E-05
555	GO:0000922|GO:0030615	spindle pole	2.03E-05

12668	GO:0030154	cell differentiation	2.39E-05
475	GO:0000780	condensed nuclear chromosome, centromeric region	2.51E-05
24985	GO:0048468	cell development	2.66E-05
5812	GO:0008283	cell proliferation	2.68E-05
38144	GO:2000026	regulation of multicellular organismal development	2.93E-05
13401	GO:0031012	extracellular matrix	3.06E-05
26718	GO:0051321	meiotic cell cycle	3.10E-05
5023	GO:0006928	cellular component movement	3.25E-05
15306	GO:0032993	protein-DNA complex	3.36E-05
6017	GO:0008544	epidermis development	3.78E-05
29898	GO:0065007	biological regulation	4.05E-05
5670	GO:0008092	cytoskeletal protein binding	4.27E-05
27081	GO:0051716	cellular response to stimulus	4.73E-05
26502	GO:0051094	positive regulation of developmental process	4.88E-05
570	GO:0000940	condensed chromosome outer kinetochore	5.02E-05
473	GO:0000778	condensed nuclear chromosome kinetochore	5.25E-05
16785	GO:0034508	centromere complex assembly	5.39E-05
3561	GO:0004930|GO:0001622|GO:0001623|GO:0001624|GO:0001625|GO:0016526	G-protein coupled receptor activity	6.69E-05
14019	GO:0031674	I band	6.74E-05
27320	GO:0051983	regulation of chromosome segregation	7.31E-05
15355	GO:0033043	regulation of organelle organization	8.39E-05
11177	GO:0019220	regulation of phosphate metabolic process	9.05E-05
9247	GO:0016020	membrane	9.49E-05

Up NCL red compared with control NCL

GO ID	GO ACCESSION	GO Term	p-value
31822	GO:0071944	cell periphery	1.23E-09
4177	GO:0005886|GO:0005904	plasma membrane	2.10E-09
3519	GO:0004872|GO:0019041	receptor activity	1.18E-07
26977	GO:0051607	defense response to virus	1.70E-07
18704	GO:0038023	signaling receptor activity	3.45E-07
21409	GO:0044425	membrane part	3.66E-07
3533	GO:0004888|GO:0004926	transmembrane signaling receptor activity	7.08E-07
13584	GO:0031224	intrinsic component of membrane	1.08E-06
9247	GO:0016020	membrane	1.53E-06
9248	GO:0016021	integral component of membrane	1.68E-06
6883	GO:0009615	response to virus	8.82E-06

Down NCL red compared with control NCL

GO ID	GO ACCESSION	GO Term	p-value
14822	GO:0032501|GO:0050874	multicellular organismal process	1.47E-19
31822	GO:0071944	cell periphery	1.06E-18
21688	GO:0044707	single-multicellular organism process	1.10E-18
4177	GO:0005886|GO:0005904	plasma membrane	2.16E-18
13401	GO:0031012	extracellular matrix	3.25E-18
3944	GO:0005578	proteinaceous extracellular matrix	3.66E-16
3978	GO:0005615	extracellular space	1.42E-14
9248	GO:0016021	integral component of membrane	1.05E-13
13584	GO:0031224	intrinsic component of membrane	1.70E-13
12703	GO:0030198	extracellular matrix organization	1.49E-12
20129	GO:0043062	extracellular structure organization	1.74E-12
21442	GO:0044459	plasma membrane part	9.10E-12
2259	GO:0003008	system process	3.99E-11
26288	GO:0050877	neurological system process	4.35E-11
9247	GO:0016020	membrane	5.14E-11
21409	GO:0044425	membrane part	6.87E-11
5566	GO:0007600	sensory perception	9.39E-11
3519	GO:0004872|GO:0019041	receptor activity	2.45E-10
18704	GO:0038023	signaling receptor activity	4.63E-10
3533	GO:0004888|GO:0004926	transmembrane signaling receptor activity	7.98E-10
21747	GO:0044767	single-organism developmental process	1.66E-09
5293	GO:0007275	multicellular organismal development	2.97E-09
25229	GO:0048731	system development	4.57E-09
14823	GO:0032502	developmental process	6.04E-09
12440	GO:0022610	biological adhesion	6.43E-09
5200	GO:0007155	cell adhesion	9.36E-09
19129	GO:0040011	locomotion	1.71E-08
5228	GO:0007186	G-protein coupled receptor signaling pathway	3.47E-08
4178	GO:0005887	integral component of plasma membrane	4.75E-08
25349	GO:0048856	anatomical structure development	6.20E-08
13586	GO:0031226	intrinsic component of plasma membrane	7.67E-08
3942	GO:0005576	extracellular region	1.13E-07
25028	GO:0048513	organ development	2.08E-07
25332	GO:0048839	inner ear development	3.14E-07
5286	GO:0007267	cell-cell signaling	4.11E-07
3758	GO:0005201	extracellular matrix structural constituent	4.25E-07
3518	GO:0004871|GO:0005062|GO:	signal transducer activity	4.94E-07

	0009369|GO:0009370		
28366	GO:0060089	molecular transducer activity	4.94E-07
12546	GO:0023052|GO:0023046	signaling	5.38E-07
21682	GO:0044700	single organism signaling	5.38E-07
26306	GO:0050896|GO:0051869	response to stimulus	5.96E-07
3913	GO:0005509	calcium ion binding	6.44E-07
5398	GO:0007399	nervous system development	7.15E-07
12668	GO:0030154	cell differentiation	7.80E-07
24985	GO:0048468	cell development	1.17E-06
5199	GO:0007154	cell communication	1.52E-06
5023	GO:0006928	cellular component movement	1.64E-06
20594	GO:0043583	ear development	1.83E-06
12477	GO:0022838	substrate-specific channel activity	2.22E-06
3930	GO:0005539	glycosaminoglycan binding	2.44E-06
3788	GO:0005249	voltage-gated potassium channel activity	2.61E-06
25361	GO:0048869	cellular developmental process	3.25E-06
21404	GO:0044420	extracellular matrix part	3.42E-06
26644	GO:0051239	regulation of multicellular organismal process	3.47E-06
12517	GO:0022891	substrate-specific transmembrane transporter activity	3.78E-06
8477	GO:0015075	ion transmembrane transporter activity	4.04E-06
3946	GO:0005581	collagen trimer	4.06E-06
6881	GO:0009611|GO:0002245	response to wounding	5.01E-06
26316	GO:0050906	detection of stimulus involved in sensory perception	5.17E-06
26976	GO:0051606	detection of stimulus	5.59E-06
23505	GO:0046873	metal ion transmembrane transporter activity	6.57E-06
25362	GO:0048870	cell motility	6.69E-06
12482	GO:0022843	voltage-gated cation channel activity	6.71E-06
16973	GO:0034702	ion channel complex	6.82E-06
6914	GO:0009653	anatomical structure morphogenesis	7.09E-06
27040	GO:0051674	localization of cell	7.13E-06
8642	GO:0015267|GO:0015249|GO:0015268	channel activity	7.33E-06
12459	GO:0022803	passive transmembrane transporter activity	7.33E-06
12447	GO:0022617	extracellular matrix disassembly	7.42E-06
20578	GO:0043567	regulation of insulin-like growth factor receptor signaling pathway	7.48E-06
12491	GO:0022857|GO:0005386|GO:	transmembrane transporter activity	8.52E-06

	0015646		
26364	GO:0050954	sensory perception of mechanical stimulus	9.82E-06
3561	GO:0004930|GO:0001622|GO:0001623|GO:0001624|GO:0001625|GO:0016526	G-protein coupled receptor activity	1.12E-05
19575	GO:0042472	inner ear morphogenesis	1.14E-05
29899	GO:0065008	regulation of biological quality	1.21E-05
2257	GO:0003006	developmental process involved in reproduction	1.30E-05
3763	GO:0005216	ion channel activity	1.33E-05
5747	GO:0008201	heparin binding	1.44E-05
16502	GO:0034220	ion transmembrane transport	1.54E-05
12427	GO:0022414	reproductive process	1.67E-05
37137	GO:1902495	transmembrane transporter complex	1.89E-05
12692	GO:0030182	neuron differentiation	1.93E-05
25165	GO:0048666	neuron development	2.01E-05
26502	GO:0051094	positive regulation of developmental process	2.27E-05
12476	GO:0022836	gated channel activity	2.32E-05
4113	GO:0005796	Golgi lumen	2.49E-05
3790	GO:0005251	delayed rectifier potassium channel activity	2.62E-05
34623	GO:0098590	plasma membrane region	2.82E-05
38075	GO:1990351	transporter complex	3.10E-05
5211	GO:0007166	cell surface receptor signaling pathway	3.16E-05
3700	GO:0005125	cytokine activity	3.20E-05
5571	GO:0007605	sensory perception of sound	3.58E-05
8648	GO:0015276	ligand-gated ion channel activity	4.01E-05
12474	GO:0022834	ligand-gated channel activity	4.01E-05
5574	GO:0007610	behavior	4.06E-05
36334	GO:1901681	sulfur compound binding	4.55E-05
7132	GO:0009887	organ morphogenesis	4.61E-05
1285	GO:0001968	fibronectin binding	5.44E-05
5418	GO:0007423	sensory organ development	6.15E-05
12382	GO:0022008	neurogenesis	6.37E-05
25370	GO:0048878	chemical homeostasis	6.38E-05
5862	GO:0008344	adult locomotory behavior	6.56E-05
19346	GO:0042221	response to chemical	6.77E-05
22435	GO:0045669	positive regulation of osteoblast differentiation	6.85E-05
5030	GO:0006935	chemotaxis	7.00E-05
19443	GO:0042330	taxis	7.00E-05
12961	GO:0030534	adult behavior	7.37E-05
21430	GO:0044447	axoneme part	8.26E-05
26317	GO:0050907	detection of chemical stimulus involved in	8.34E-05

		sensory perception	
4937	GO:0006811	ion transport	8.53E-05
21681	GO:0044699	single-organism process	8.96E-05
10230	GO:0018149	peptide cross-linking	9.33E-05
8570	GO:0015175	neutral amino acid transmembrane transporter activity	9.95E-05
21743	GO:0044763	single-organism cellular process	1.03E-04
4155	GO:0005858	axonemal dynein complex	1.03E-04
12778	GO:0030278	regulation of ossification	1.04E-04
5988	GO:0008509	anion transmembrane transporter activity	1.05E-04
5201	GO:0007156	homophilic cell adhesion	1.07E-04
9565	GO:0016477	cell migration	1.10E-04
9054	GO:0015804	neutral amino acid transport	1.22E-04
12704	GO:0030199	collagen fibril organization	1.24E-04
31212	GO:0071320	cellular response to cAMP	1.24E-04
5042	GO:0006954	inflammatory response	1.34E-04
3968	GO:0005604	basement membrane	1.35E-04
12518	GO:0022892	substrate-specific transporter activity	1.41E-04
5657	GO:0008076	voltage-gated potassium channel complex	1.45E-04
5572	GO:0007606	sensory perception of chemical stimulus	1.51E-04

Up DMCL red compared with control DMCL

GO ID	GO ACCESSION	GO Term	p-value
205	GO:0000278	mitotic cell cycle	1.01E-32
5114	GO:0007049	cell cycle	1.41E-28
207	GO:0000280	nuclear division	1.14E-27
24833	GO:0048285	organelle fission	6.47E-26
12415	GO:0022402	cell cycle process	1.09E-25
5131	GO:0007067	mitotic nuclear division	1.19E-25
37681	GO:1903047	mitotic cell cycle process	3.58E-25
26701	GO:0051301	cell division	9.62E-21
474	GO:0000779	condensed chromosome, centromeric region	6.99E-19
472	GO:0000777	condensed chromosome kinetochore	7.96E-19
488	GO:0000793	condensed chromosome	2.27E-18
5124	GO:0007059	chromosome segregation	5.09E-18
470	GO:0000775|GO:0097521	chromosome, centromeric region	2.38E-17
471	GO:0000776|GO:0005699	kinetochore	3.55E-16
15306	GO:0032993	protein-DNA complex	1.45E-15
4033	GO:0005694	chromosome	7.58E-15
4492	GO:0006260|GO:0055133	DNA replication	1.56E-14
21411	GO:0044427	chromosomal part	2.28E-14
12446	GO:0022616	DNA strand elongation	1.50E-13
4491	GO:0006259|GO:0055132	DNA metabolic process	2.44E-13
4501	GO:0006271	DNA strand elongation involved in DNA replication	4.62E-13
513	GO:0000819	sister chromatid segregation	3.06E-12
4124	GO:0005819	spindle	7.79E-12
50	GO:0000070|GO:0016359	mitotic sister chromatid segregation	3.51E-11
4493	GO:0006261|GO:0006262|GO:0006263	DNA-dependent DNA replication	9.02E-11
5116	GO:0007051	spindle organization	9.43E-10
37231	GO:1902589	single-organism organelle organization	1.42E-09
555	GO:0000922|GO:0030615	spindle pole	2.15E-09
7769	GO:0010564	regulation of cell cycle process	2.19E-09
4537	GO:0006312	mitotic recombination	4.74E-09
21750	GO:0044770	cell cycle phase transition	5.39E-09
21752	GO:0044772	mitotic cell cycle phase transition	5.39E-09
5149	GO:0007088	regulation of mitosis	8.70E-09

27091	GO:0051726|GO:0000074	regulation of cell cycle	1.06E-08
167	GO:0000226	microtubule cytoskeleton organization	1.42E-08
4169	GO:0005876	spindle microtubule	1.50E-08
5088	GO:0007017	microtubule-based process	1.50E-08
27142	GO:0051783	regulation of nuclear division	3.39E-08
26702	GO:0051302	regulation of cell division	4.40E-08
27320	GO:0051983	regulation of chromosome segregation	4.74E-08
4510	GO:0006281	DNA repair	5.88E-08
5349	GO:0007346	regulation of mitotic cell cycle	7.30E-08
14526	GO:0032201	telomere maintenance via semi-conservative replication	7.67E-08
4500	GO:0006270|GO:0042024	DNA replication initiation	1.47E-07
16364	GO:0034080|GO:0034509	centromere-specific nucleosome assembly	1.47E-07
58	GO:0000082	G1/S transition of mitotic cell cycle	2.13E-07
21822	GO:0044843	cell cycle G1/S phase transition	2.13E-07
16785	GO:0034508	centromere complex assembly	2.22E-07
20500	GO:0043486	histone exchange	2.22E-07
4542	GO:0006323	DNA packaging	3.96E-07
30995	GO:0071103	DNA conformation change	4.36E-07
13442	GO:0031055	chromatin remodeling at centromere	4.77E-07
15566	GO:0033260	nuclear cell cycle DNA replication	4.77E-07
475	GO:0000780	condensed nuclear chromosome, centromeric region	5.11E-07
5178	GO:0007126	meiotic nuclear division	5.11E-07
4535	GO:0006310	DNA recombination	7.33E-07
5613	GO:0008017	microtubule binding	7.53E-07
4003	GO:0005657	replication fork	8.00E-07
169	GO:0000228	nuclear chromosome	8.09E-07
420	GO:0000722	telomere maintenance via recombination	8.12E-07
21766	GO:0044786	cell cycle DNA replication	8.12E-07
21414	GO:0044430	cytoskeletal part	1.07E-06
30401	GO:0070507	regulation of microtubule cytoskeleton organization	1.12E-06
19652	GO:0042555	MCM complex	1.47E-06
570	GO:0000940	condensed chromosome outer kinetochore	1.54E-06
26718	GO:0051321	meiotic cell cycle	1.58E-06
4167	GO:0005874	microtubule	1.59E-06
21743	GO:0044763	single-organism cellular process	1.65E-06
5117	GO:0007052	mitotic spindle organization	1.69E-06
5073	GO:0006996	organelle organization	2.12E-06
2716	GO:0003777	microtubule motor activity	2.29E-06
8890	GO:0015630	microtubule cytoskeleton	2.33E-06
4547	GO:0006336	DNA replication-independent nucleosome	2.56E-06

		assembly	
16994	GO:0034724	DNA replication-independent nucleosome organization	2.56E-06
15199	GO:0032886	regulation of microtubule-based process	3.20E-06
2715	GO:0003774	motor activity	3.20E-06
52	GO:0000075|GO:0031576|GO:0071779	cell cycle checkpoint	3.25E-06
473	GO:0000778	condensed nuclear chromosome kinetochore	3.46E-06
489	GO:0000794	condensed nuclear chromosome	3.63E-06
59	GO:0000083	regulation of transcription involved in G1/S transition of mitotic cell cycle	4.22E-06
13923	GO:0031577	spindle checkpoint	5.47E-06
8891	GO:0015631	tubulin binding	5.51E-06
26678	GO:0051276|GO:0007001|GO:0051277	chromosome organization	6.77E-06
21681	GO:0044699	single-organism process	9.65E-06
5058	GO:0006974|GO:0034984	cellular response to DNA damage stimulus	1.03E-05
12598	GO:0030071	regulation of mitotic metaphase/anaphase transition	1.05E-05
36750	GO:1902099	regulation of metaphase/anaphase transition of cell cycle	1.05E-05
33520	GO:0090224	regulation of spindle organization	1.15E-05
8141	GO:0010948	negative regulation of cell cycle process	1.30E-05
4154	GO:0005856	cytoskeleton	1.90E-05
31186	GO:0071294	cellular response to zinc ion	2.53E-05
26131	GO:0050714	positive regulation of protein secretion	3.27E-05
4006	GO:0005663	DNA replication factor C complex	3.30E-05
21442	GO:0044459	plasma membrane part	3.58E-05
8029	GO:0010833	telomere maintenance via telomere lengthening	4.48E-05
21437	GO:0044454	nuclear chromosome part	4.53E-05
29895	GO:0065004	protein-DNA complex assembly	4.82E-05
3	GO:0000003|GO:0019952|GO:0050876	reproduction	4.88E-05
14822	GO:0032501|GO:0050874	multicellular organismal process	5.03E-05
422	GO:0000724|GO:0016924	double-strand break repair via homologous recombination	5.80E-05
4524	GO:0006297	nucleotide-excision repair, DNA gap filling	6.50E-05
26703	GO:0051303	establishment of chromosome localization	6.50E-05
13620	GO:0031262	Ndc80 complex	6.61E-05
423	GO:0000725	recombinational repair	7.21E-05

36640	GO:1901988	negative regulation of cell cycle phase transition	7.23E-05
13848	GO:0031497	chromatin assembly	7.71E-05
27325	GO:0051988	regulation of attachment of spindle microtubules to kinetochore	8.16E-05
25111	GO:0048610	cellular process involved in reproduction	9.19E-05
31065	GO:0071173	spindle assembly checkpoint	9.30E-05
27143	GO:0051784	negative regulation of nuclear division	9.35E-05
5084	GO:0007010	cytoskeleton organization	1.06E-04
36639	GO:1901987	regulation of cell cycle phase transition	1.07E-04
4164	GO:0005871	kinesin complex	1.18E-04
26125	GO:0050708	regulation of protein secretion	1.21E-04
4545	GO:0006334	nucleosome assembly	1.23E-04
15355	GO:0033043	regulation of organelle organization	1.26E-04
22582	GO:0045841	negative regulation of mitotic metaphase/anaphase transition	1.26E-04
36751	GO:1902100	negative regulation of metaphase/anaphase transition of cell cycle	1.26E-04
36643	GO:1901991	negative regulation of mitotic cell cycle phase transition	1.35E-04
31710	GO:0071824	protein-DNA complex subunit organization	1.36E-04
12762	GO:0030261|GO:0000068	chromosome condensation	1.40E-04
25434	GO:0050000	chromosome localization	1.45E-04

DOWN DMCL red compared with control DMCL

No gene ontology analysis results when P value is less than 0.01

UP DMCL red compared with control NCL

GO ID	GO ACCESSION	GO Term	p-value
14822	GO:0032501|GO:0050874	multicellular organismal process	5.77E-08
21688	GO:0044707	single-multicellular organism process	2.95E-07
3913	GO:0005509	calcium ion binding	4.46E-07
25349	GO:0048856	anatomical structure development	9.90E-07
13584	GO:0031224	intrinsic component of membrane	1.49E-06
1979	GO:0002673	regulation of acute inflammatory response	1.96E-06
9248	GO:0016021	integral component of membrane	2.69E-06
21442	GO:0044459	plasma membrane part	3.84E-06
14823	GO:0032502	developmental process	4.10E-06
26644	GO:0051239	regulation of multicellular organismal process	5.75E-06
5042	GO:0006954	inflammatory response	5.93E-06
21747	GO:0044767	single-organism developmental process	6.05E-06
5293	GO:0007275	multicellular organismal development	7.92E-06
21409	GO:0044425	membrane part	1.27E-05

DOWN DMCL red compared with control NCL

GO ID	GO ACCESSION	GO Term	p-value
31822	GO:0071944	cell periphery	4.34E-17
4177	GO:0005886|GO:0005904	plasma membrane	7.97E-17
13584	GO:0031224	intrinsic component of membrane	1.21E-12
21442	GO:0044459	plasma membrane part	2.33E-11
9248	GO:0016021	integral component of membrane	3.34E-11
21409	GO:0044425	membrane part	8.12E-11
3519	GO:0004872|GO:0019041	receptor activity	3.88E-10
18704	GO:0038023	signaling receptor activity	1.42E-09
4178	GO:0005887	integral component of plasma membrane	2.35E-09
9247	GO:0016020	membrane	3.84E-09
13586	GO:0031226	intrinsic component of plasma membrane	5.49E-09
3533	GO:0004888|GO:0004926	transmembrane signaling receptor activity	6.14E-09
5200	GO:0007155	cell adhesion	3.48E-08
12440	GO:0022610	biological adhesion	4.40E-08
14822	GO:0032501|GO:0050874	multicellular organismal process	2.45E-07
21688	GO:0044707	single-multicellular organism process	3.65E-07
3518	GO:0004871|GO:0005062|GO:0009369|GO:0009370	signal transducer activity	1.98E-06
28366	GO:0060089	molecular transducer activity	1.98E-06
19418	GO:0042303	molting cycle	2.30E-06
19722	GO:0042633	hair cycle	2.30E-06
13401	GO:0031012	extracellular matrix	2.71E-06
6914	GO:0009653	anatomical structure morphogenesis	4.06E-06
5286	GO:0007267	cell-cell signaling	5.90E-06
913	GO:0001525	angiogenesis	9.42E-06
32230	GO:0072358	cardiovascular system development	1.14E-05
32231	GO:0072359	circulatory system development	1.14E-05
3944	GO:0005578	proteinaceous extracellular matrix	1.24E-05
12546	GO:0023052|GO:0023046	signaling	1.40E-05
21682	GO:0044700	single organism signaling	1.40E-05
25145	GO:0048646	anatomical structure formation involved in morphogenesis	1.47E-05
26502	GO:0051094	positive regulation of developmental process	1.61E-05
1262	GO:0001944	vasculature development	1.68E-05
5662	GO:0008083	growth factor activity	1.71E-05

20075	GO:0043005	neuron projection	2.13E-05
25229	GO:0048731	system development	2.14E-05
19346	GO:0042221	response to chemical	2.63E-05
5199	GO:0007154	cell communication	2.68E-05
14019	GO:0031674	I band	3.17E-05
34642	GO:0098609	cell-cell adhesion	3.23E-05
6875	GO:0009605	response to external stimulus	3.74E-05
20065	GO:0042995	cell projection	4.11E-05
34684	GO:0098651	basement membrane collagen trimer	4.50E-05
3942	GO:0005576	extracellular region	4.63E-05
21432	GO:0044449	contractile fiber part	4.78E-05
12564	GO:0030018	Z disc	4.79E-05
22002	GO:0045202	synapse	4.94E-05
12703	GO:0030198	extracellular matrix organization	5.15E-05

UP NCL Blue compared with control NCL

GO ID	GO ACCESSION	GO Term	p-value
3561	GO:0004930|GO:0001622|GO:0001623|GO:0001624|GO:0001625|GO:0016526	G-protein coupled receptor activity	5.95E-07
5228	GO:0007186	G-protein coupled receptor signaling pathway	1.94E-06
18704	GO:0038023	signaling receptor activity	2.17E-06

DOWN NCL Blue compared with control NCL

GO ID	GO ACCESSION	GO Term	p-value
2259	GO:0003008	system process	5.88E-13
3518	GO:0004871|GO:0005062|GO:0009369|GO:0009370	signal transducer activity	1.55E-11
3519	GO:0004872|GO:0019041	receptor activity	2.69E-16
3533	GO:0004888|GO:0004926	transmembrane signaling receptor activity	5.90E-14
3561	GO:0004930|GO:0001622|GO:0001623|GO:0001624|GO:0001625|GO:0016526	G-protein coupled receptor activity	5.25E-12
3610	GO:0004984	olfactory receptor activity	9.65E-08
3763	GO:0005216	ion channel activity	5.34E-05
3795	GO:0005261|GO:0015281|GO:0015338	cation channel activity	1.56E-05
3913	GO:0005509	calcium ion binding	1.76E-05
3978	GO:0005615	extracellular space	3.30E-06
4177	GO:0005886|GO:0005904	plasma membrane	1.49E-17
4178	GO:0005887	integral component of plasma membrane	4.25E-05
5199	GO:0007154	cell communication	9.17E-08
5200	GO:0007155	cell adhesion	3.46E-07
5211	GO:0007166	cell surface receptor signaling pathway	9.36E-06
5228	GO:0007186	G-protein coupled receptor signaling pathway	2.43E-12
5286	GO:0007267	cell-cell signaling	2.32E-08
5287	GO:0007268	synaptic transmission	2.27E-05
5566	GO:0007600	sensory perception	1.29E-16
5571	GO:0007605	sensory perception of sound	5.26E-07
5572	GO:0007606	sensory perception of chemical stimulus	3.57E-09
5573	GO:0007608	sensory perception of smell	6.94E-08
5574	GO:0007610	behavior	4.98E-05
6866	GO:0009593	detection of chemical stimulus	2.09E-08
9247	GO:0016020	membrane	1.97E-08
9248	GO:0016021	integral component of membrane	2.56E-12
12440	GO:0022610	biological adhesion	4.20E-07
12477	GO:0022838	substrate-specific channel activity	3.18E-05
12546	GO:0023052|GO:0023046	signaling	1.27E-07

12961	GO:0030534	adult behavior	1.10E-05
13584	GO:0031224	intrinsic component of membrane	1.19E-12
14822	GO:0032501|GO:0050874	multicellular organismal process	5.22E-11
18704	GO:0038023	signaling receptor activity	6.33E-16
20317	GO:0043269	regulation of ion transport	1.84E-05
21409	GO:0044425	membrane part	1.42E-09
21442	GO:0044459	plasma membrane part	1.12E-05
21682	GO:0044700	single organism signaling	1.27E-07
21688	GO:0044707	single-multicellular organism process	9.59E-11
26288	GO:0050877	neurological system process	1.05E-16
26306	GO:0050896|GO:0051869	response to stimulus	5.30E-07
26316	GO:0050906	detection of stimulus involved in sensory perception	4.06E-12
26317	GO:0050907	detection of chemical stimulus involved in sensory perception	1.27E-09
26321	GO:0050911	detection of chemical stimulus involved in sensory perception of smell	9.65E-08
26364	GO:0050954	sensory perception of mechanical stimulus	1.11E-07
26976	GO:0051606	detection of stimulus	7.74E-11
28366	GO:0060089	molecular transducer activity	1.55E-11
31822	GO:0071944	cell periphery	1.93E-17

UP NCL Green compared with control NCL

GO ID	GO ACCESSION	GO Term	p-value
50	GO:0000070|GO:0016359	mitotic sister chromatid segregation	1.10E-05
205	GO:0000278	mitotic cell cycle	5.43E-12
207	GO:0000280	nuclear division	4.52E-14
470	GO:0000775|GO:0097521	chromosome, centromeric region	1.43E-08
471	GO:0000776|GO:0005699	kinetochore	1.04E-07
472	GO:0000777	condensed chromosome kinetochore	2.55E-08
473	GO:0000778	condensed nuclear chromosome kinetochore	1.48E-05
474	GO:0000779	condensed chromosome, centromeric region	1.16E-08
475	GO:0000780	condensed nuclear chromosome, centromeric region	3.61E-07
488	GO:0000793	condensed chromosome	4.51E-09
513	GO:0000819	sister chromatid segregation	4.05E-07
570	GO:0000940	condensed chromosome outer kinetochore	3.14E-05
1682	GO:0002376	immune system process	1.77E-05
4124	GO:0005819	spindle	2.05E-08
4164	GO:0005871	kinesin complex	3.74E-05
4177	GO:0005886|GO:0005904	plasma membrane	1.02E-05
5114	GO:0007049	cell cycle	2.48E-08
5124	GO:0007059	chromosome segregation	2.87E-09
5131	GO:0007067	mitotic nuclear division	4.76E-14
5149	GO:0007088	regulation of mitosis	6.82E-06
6883	GO:0009615	response to virus	8.32E-07
12415	GO:0022402	cell cycle process	2.34E-08
12762	GO:0030261|GO:0000068	chromosome condensation	3.32E-05
13484	GO:0031099	regeneration	6.05E-05
13923	GO:0031577	spindle checkpoint	1.62E-05
14822	GO:0032501|GO:0050874	multicellular organismal process	2.60E-06
15306	GO:0032993	protein-DNA complex	1.15E-06
16619	GO:0034340	response to type I interferon	3.50E-06
21688	GO:0044707	single-multicellular organism process	1.30E-05
24833	GO:0048285	organelle fission	2.61E-14
25038	GO:0048525	negative regulation of viral process	1.73E-05
26701	GO:0051301	cell division	4.18E-12
26702	GO:0051302	regulation of cell division	2.89E-05
26977	GO:0051607	defense response to virus	1.30E-08
27142	GO:0051783	regulation of nuclear division	1.48E-05

27320	GO:0051983	regulation of chromosome segregation	3.32E-05
28611	GO:0060337	type I interferon signaling pathway	2.82E-06
31186	GO:0071294	cellular response to zinc ion	3.14E-05
31249	GO:0071357	cellular response to type I interferon	2.82E-06
31822	GO:0071944	cell periphery	7.65E-06
37231	GO:1902589	single-organism organelle organization	5.15E-05
37681	GO:1903047	mitotic cell cycle process	1.02E-10

DOWN NCL Green compared with control NCL

GO ID	GO ACCESSION	GO Term	p-value
14822	GO:0032501|GO:0050874	multicellular organismal process	6.59E-18
21688	GO:0044707	single-multicellular organism process	3.14E-17
13401	GO:0031012	extracellular matrix	4.37E-17
3944	GO:0005578	proteinaceous extracellular matrix	1.09E-15
3978	GO:0005615	extracellular space	1.22E-14
31822	GO:0071944	cell periphery	1.32E-14
4177	GO:0005886|GO:0005904	plasma membrane	4.97E-14
13584	GO:0031224	intrinsic component of membrane	9.77E-12
9248	GO:0016021	integral component of membrane	2.26E-11
26288	GO:0050877	neurological system process	1.72E-10
5566	GO:0007600	sensory perception	3.74E-10
5200	GO:0007155	cell adhesion	7.51E-10
21409	GO:0044425	membrane part	9.32E-10
12440	GO:0022610	biological adhesion	9.70E-10
3519	GO:0004872|GO:0019041	receptor activity	1.05E-09
3533	GO:0004888|GO:0004926	transmembrane signaling receptor activity	1.06E-09
12703	GO:0030198	extracellular matrix organization	1.06E-09
20129	GO:0043062	extracellular structure organization	1.22E-09
5228	GO:0007186	G-protein coupled receptor signaling pathway	1.42E-09
2259	GO:0003008	system process	1.50E-09
18704	GO:0038023	signaling receptor activity	2.19E-09
5286	GO:0007267	cell-cell signaling	3.99E-09
21442	GO:0044459	plasma membrane part	2.10E-08
25332	GO:0048839	inner ear development	3.21E-08
20594	GO:0043583	ear development	3.32E-08
19575	GO:0042472	inner ear morphogenesis	3.84E-08
3942	GO:0005576	extracellular region	4.99E-08
9247	GO:0016020	membrane	5.80E-08
24985	GO:0048468	cell development	5.93E-08
25229	GO:0048731	system development	9.55E-08
26976	GO:0051606	detection of stimulus	9.66E-08
3758	GO:0005201	extracellular matrix structural constituent	1.86E-07
4178	GO:0005887	integral component of plasma membrane	2.80E-07
5293	GO:0007275	multicellular organismal development	2.84E-07
19574	GO:0042471	ear morphogenesis	3.41E-07
25349	GO:0048856	anatomical structure development	5.37E-07
25028	GO:0048513	organ development	5.38E-07
8566	GO:0015171|G	amino acid transmembrane transporter	6.12E-07

	O:0015359	activity	
21747	GO:0044767	single-organism developmental process	6.14E-07
13586	GO:0031226	intrinsic component of plasma membrane	7.22E-07
19129	GO:0040011	locomotion	9.02E-07
6881	GO:0009611|GO:0002245	response to wounding	1.01E-06
14823	GO:0032502	developmental process	1.41E-06
26316	GO:0050906	detection of stimulus involved in sensory perception	1.66E-06
5287	GO:0007268	synaptic transmission	1.76E-06
3561	GO:0004930|GO:0001622|GO:0001623|GO:0001624|GO:0001625|GO:0016526	G-protein coupled receptor activity	2.21E-06
537	GO:0000904	cell morphogenesis involved in differentiation	2.80E-06
5418	GO:0007423	sensory organ development	3.45E-06
12995	GO:0030574	collagen catabolic process	4.00E-06
21231	GO:0044243	multicellular organismal catabolic process	4.57E-06
23573	GO:0046943	carboxylic acid transmembrane transporter activity	4.71E-06
15302	GO:0032989	cellular component morphogenesis	4.88E-06
3518	GO:0004871|GO:0005062|GO:0009369|GO:0009370	signal transducer activity	5.28E-06
28366	GO:0060089	molecular transducer activity	5.28E-06
26644	GO:0051239	regulation of multicellular organismal process	6.39E-06
17042	GO:0034774	secretory granule lumen	6.47E-06
3946	GO:0005581	collagen trimer	7.83E-06
5574	GO:0007610	behavior	7.95E-06
21224	GO:0044236	multicellular organismal metabolic process	8.29E-06
3840	GO:0005342	organic acid transmembrane transporter activity	8.62E-06
15276	GO:0032963	collagen metabolic process	9.01E-06
2571	GO:0003333	amino acid transmembrane transport	9.18E-06
5993	GO:0008514	organic anion transmembrane transporter activity	1.01E-05
3913	GO:0005509	calcium ion binding	1.09E-05
12447	GO:0022617	extracellular matrix disassembly	1.52E-05
6914	GO:0009653	anatomical structure morphogenesis	1.58E-05
8570	GO:0015175	neutral amino acid transmembrane transporter activity	1.82E-05
535	GO:0000902|G	cell morphogenesis	1.85E-05

	O:0007148|GO:0045790|GO:0045791		
25166	GO:0048667	cell morphogenesis involved in neuron differentiation	1.93E-05
21404	GO:0044420	extracellular matrix part	2.08E-05
8571	GO:0015179	L-amino acid transmembrane transporter activity	2.15E-05
37117	GO:1902475	L-alpha-amino acid transmembrane transport	2.15E-05
26317	GO:0050907	detection of chemical stimulus involved in sensory perception	2.52E-05
9054	GO:0015804	neutral amino acid transport	2.77E-05
5988	GO:0008509	anion transmembrane transporter activity	2.90E-05
5745	GO:0008199	ferric iron binding	3.06E-05
21247	GO:0044259	multicellular organismal macromolecule metabolic process	3.11E-05
5023	GO:0006928	cellular component movement	3.46E-05
12886	GO:0030414	peptidase inhibitor activity	4.58E-05
12692	GO:0030182	neuron differentiation	4.80E-05
3021	GO:0004175|GO:0016809	endopeptidase activity	5.57E-05
29824	GO:0061564	axon development	5.59E-05
8477	GO:0015075	ion transmembrane transporter activity	5.80E-05
6866	GO:0009593	detection of chemical stimulus	6.10E-05
3841	GO:0005343	organic acid:sodium symporter activity	6.31E-05
25145	GO:0048646	anatomical structure formation involved in morphogenesis	6.48E-05
3032	GO:0004252	serine-type endopeptidase activity	6.65E-05
26222	GO:0050808	synapse organization	7.20E-05
3700	GO:0005125	cytokine activity	7.50E-05
3610	GO:0004984	olfactory receptor activity	8.31E-05
26321	GO:0050911	detection of chemical stimulus involved in sensory perception of smell	8.31E-05
1010	GO:0001664	G-protein coupled receptor binding	8.43E-05
20578	GO:0043567	regulation of insulin-like growth factor receptor signaling pathway	8.44E-05
10071	GO:0017171	serine hydrolase activity	8.61E-05
12517	GO:0022891	substrate-specific transmembrane transporter activity	8.66E-05
5572	GO:0007606	sensory perception of chemical stimulus	9.66E-05
25165	GO:0048666	neuron development	9.69E-05
26502	GO:0051094	positive regulation of developmental process	1.12E-04

UP DMCL Blue compared with control NCL

GO ID	GO ACCESSION	GO Term	p-value
18704	GO:0038023	signaling receptor activity	8.39E-08
4177	GO:0005886|GO:0005904	plasma membrane	1.63E-07
3519	GO:0004872|GO:0019041	receptor activity	1.85E-07
3533	GO:0004888|GO:0004926	transmembrane signaling receptor activity	3.68E-07
14822	GO:0032501|GO:0050874	multicellular organismal process	4.66E-07
31822	GO:0071944	cell periphery	6.40E-07
21688	GO:0044707	single-multicellular organism process	7.15E-07
5574	GO:0007610	behavior	8.84E-07
21442	GO:0044459	plasma membrane part	1.70E-06
33480	GO:0090184	positive regulation of kidney development	2.70E-06
38144	GO:2000026	regulation of multicellular organismal development	3.04E-06
26644	GO:0051239	regulation of multicellular organismal process	3.34E-06
3518	GO:0004871|GO:0005062|GO:0009369|GO:0009370	signal transducer activity	3.64E-06
28366	GO:0060089	molecular transducer activity	3.64E-06
3913	GO:0005509	calcium ion binding	5.87E-06
5813	GO:0008284	positive regulation of cell proliferation	8.50E-06
22783	GO:0046058	cAMP metabolic process	1.09E-05
34642	GO:0098609	cell-cell adhesion	1.53E-05
7133	GO:0009888	tissue development	1.95E-05
26502	GO:0051094	positive regulation of developmental process	1.95E-05
13260	GO:0030858	positive regulation of epithelial cell differentiation	2.01E-05
26096	GO:0050679	positive regulation of epithelial cell proliferation	2.40E-05
12440	GO:0022610	biological adhesion	2.49E-05
4439	GO:0006198	cAMP catabolic process	2.94E-05

DOWN DMCL Blue compared with control NCL

GO ID	GO ACCESSION	GO Term	p-value
31822	GO:0071944	cell periphery	2.94E-17
13584	GO:0031224	intrinsic component of membrane	2.10E-16
4177	GO:0005886|GO:0005904	plasma membrane	3.18E-16
9248	GO:0016021	integral component of membrane	2.11E-14
21409	GO:0044425	membrane part	6.19E-14
21442	GO:0044459	plasma membrane part	9.81E-14
3519	GO:0004872|GO:0019041	receptor activity	6.37E-10
9247	GO:0016020	membrane	3.17E-09
13586	GO:0031226	intrinsic component of plasma membrane	5.83E-09
4178	GO:0005887	integral component of plasma membrane	1.12E-08
18704	GO:0038023	signaling receptor activity	1.81E-08
5200	GO:0007155	cell adhesion	2.54E-08
12440	GO:0022610	biological adhesion	3.21E-08
3533	GO:0004888|GO:0004926	transmembrane signaling receptor activity	7.99E-08
3978	GO:0005615	extracellular space	9.06E-08
13401	GO:0031012	extracellular matrix	1.36E-07
12703	GO:0030198	extracellular matrix organization	1.82E-07
12477	GO:0022838	substrate-specific channel activity	1.89E-07
20129	GO:0043062	extracellular structure organization	2.04E-07
3795	GO:0005261|GO:0015281|GO:0015338	cation channel activity	2.45E-07
8642	GO:0015267|GO:0015249|GO:0015268	channel activity	4.21E-07
12459	GO:0022803	passive transmembrane transporter activity	4.21E-07
6875	GO:0009605	response to external stimulus	7.53E-07
14822	GO:0032501|GO:0050874	multicellular organismal process	9.93E-07
3763	GO:0005216	ion channel activity	1.11E-06
23505	GO:0046873	metal ion transmembrane transporter activity	1.14E-06
21688	GO:0044707	single-multicellular organism process	1.50E-06
37137	GO:1902495	transmembrane transporter complex	1.87E-06
16973	GO:0034702	ion channel complex	2.33E-06
3944	GO:0005578	proteinaceous extracellular matrix	2.61E-06
38075	GO:1990351	transporter complex	3.37E-06
19129	GO:0040011	locomotion	4.20E-06
6017	GO:0008544	epidermis development	4.27E-06
25229	GO:0048731	system development	5.70E-06
12552	GO:0030001	metal ion transport	6.17E-06

33946	GO:0097060	synaptic membrane	6.41E-06
19418	GO:0042303	molting cycle	7.09E-06
19722	GO:0042633	hair cycle	7.09E-06
12476	GO:0022836	gated channel activity	7.59E-06
6914	GO:0009653	anatomical structure morphogenesis	1.40E-05
20599	GO:0043588	skin development	1.46E-05
3942	GO:0005576	extracellular region	1.63E-05
5286	GO:0007267	cell-cell signaling	1.66E-05
5030	GO:0006935	chemotaxis	1.86E-05
19443	GO:0042330	taxis	1.86E-05
3518	GO:0004871|GO:0005062|GO:0009369|GO:0009370	signal transducer activity	2.36E-05
28366	GO:0060089	molecular transducer activity	2.36E-05
19346	GO:0042221	response to chemical	2.60E-05
3758	GO:0005201	extracellular matrix structural constituent	3.29E-05
8484	GO:0015085	calcium ion transmembrane transporter activity	3.53E-05
34684	GO:0098651	basement membrane collagen trimer	4.37E-05
10230	GO:0018149	peptide cross-linking	4.48E-05
25028	GO:0048513	organ development	4.60E-05
26502	GO:0051094	positive regulation of developmental process	4.97E-05
29824	GO:0061564	axon development	5.56E-05
5417	GO:0007422	peripheral nervous system development	5.89E-05
26644	GO:0051239	regulation of multicellular organismal process	6.11E-05
26288	GO:0050877	neurological system process	6.23E-05
12546	GO:0023052|GO:0023046	signaling	6.84E-05
21682	GO:0044700	single organism signaling	6.84E-05
22002	GO:0045202	synapse	6.84E-05

UP DMCL Green compared with control NCL

GO ID	GO ACCESSION	GO Term	p-value
14822	GO:0032501|GO:0050874	multicellular organismal process	2.77E-09
21688	GO:0044707	single-multicellular organism process	2.65E-08
21442	GO:0044459	plasma membrane part	1.81E-07
5286	GO:0007267	cell-cell signaling	2.20E-06
3913	GO:0005509	calcium ion binding	2.74E-06
1979	GO:0002673	regulation of acute inflammatory response	2.92E-06
5042	GO:0006954	inflammatory response	3.12E-06
14823	GO:0032502	developmental process	4.24E-06
25349	GO:0048856	anatomical structure development	4.43E-06
3978	GO:0005615	extracellular space	5.72E-06
21747	GO:0044767	single-organism developmental process	6.05E-06
5574	GO:0007610	behavior	7.24E-06
2259	GO:0003008	system process	9.55E-06
5293	GO:0007275	multicellular organismal development	1.01E-05
13586	GO:0031226	intrinsic component of plasma membrane	1.58E-05
4178	GO:0005887	integral component of plasma membrane	1.93E-05

DOWN DMCL Green compared with control NCL

GO ID	GO ACCESSION	GO Term	p-value
31822	GO:0071944	cell periphery	5.47E-22
4177	GO:0005886|GO:0005904	plasma membrane	1.92E-21
13584	GO:0031224	intrinsic component of membrane	3.61E-16
9248	GO:0016021	integral component of membrane	2.25E-14
21442	GO:0044459	plasma membrane part	4.23E-14
21409	GO:0044425	membrane part	7.22E-14
3519	GO:0004872|GO:0019041	receptor activity	6.29E-12
18704	GO:0038023	signaling receptor activity	1.35E-11
3533	GO:0004888|GO:0004926	transmembrane signaling receptor activity	2.16E-11
9247	GO:0016020	membrane	8.93E-11
13586	GO:0031226	intrinsic component of plasma membrane	2.62E-09
4178	GO:0005887	integral component of plasma membrane	4.08E-09
21688	GO:0044707	single-multicellular organism process	3.88E-08
14822	GO:0032501|GO:0050874	multicellular organismal process	4.98E-08
3518	GO:0004871|GO:0005062|GO:0009369|GO:0009370	signal transducer activity	6.56E-08
28366	GO:0060089	molecular transducer activity	6.56E-08
5200	GO:0007155	cell adhesion	2.46E-07
5228	GO:0007186	G-protein coupled receptor signaling pathway	2.51E-07
12440	GO:0022610	biological adhesion	3.08E-07
3561	GO:0004930|GO:0001622|GO:0001623|GO:0001624|GO:0001625|GO:0016526	G-protein coupled receptor activity	6.33E-07
5199	GO:0007154	cell communication	7.87E-07
5211	GO:0007166	cell surface receptor signaling pathway	1.04E-06
26288	GO:0050877	neurological system process	1.27E-06
12546	GO:0023052|GO:0023046	signaling	1.45E-06
21682	GO:0044700	single organism signaling	1.45E-06
12477	GO:0022838	substrate-specific channel activity	2.43E-06
3913	GO:0005509	calcium ion binding	2.75E-06
5286	GO:0007267	cell-cell signaling	2.94E-06
5566	GO:0007600	sensory perception	3.17E-06
20285	GO:0043235	receptor complex	3.78E-06

13401	GO:0031012	extracellular matrix	6.49E-06
2259	GO:0003008	system process	6.58E-06
3978	GO:0005615	extracellular space	6.66E-06
12703	GO:0030198	extracellular matrix organization	7.89E-06
20129	GO:0043062	extracellular structure organization	8.72E-06
8642	GO:0015267|GO:0015249|GO:0015268	channel activity	9.69E-06
12459	GO:0022803	passive transmembrane transporter activity	9.69E-06
3763	GO:0005216	ion channel activity	1.16E-05
6875	GO:0009605	response to external stimulus	1.57E-05
3795	GO:0005261|GO:0015281|GO:0015338	cation channel activity	1.88E-05
19418	GO:0042303	molting cycle	2.16E-05
19722	GO:0042633	hair cycle	2.16E-05
5201	GO:0007156	homophilic cell adhesion	2.23E-05
3758	GO:0005201	extracellular matrix structural constituent	2.45E-05
12476	GO:0022836	gated channel activity	2.83E-05
19129	GO:0040011	locomotion	3.09E-05
22002	GO:0045202	synapse	3.52E-05
21439	GO:0044456	synapse part	3.85E-05
34642	GO:0098609	cell-cell adhesion	5.35E-05
26316	GO:0050906	detection of stimulus involved in sensory perception	5.57E-05

UP DMCL Blue compared with control DMCL

GO ID	GO ACCESSION	GO Term	p-value
205	GO:0000278	mitotic cell cycle	1.03E-36
5114	GO:0007049	cell cycle	8.42E-35
12415	GO:0022402	cell cycle process	8.13E-31
37681	GO:1903047	mitotic cell cycle process	2.45E-27
207	GO:0000280	nuclear division	4.78E-26
5131	GO:0007067	mitotic nuclear division	4.08E-25
24833	GO:0048285	organelle fission	6.24E-25
26701	GO:0051301	cell division	7.87E-22
488	GO:0000793	condensed chromosome	3.73E-19
4492	GO:0006260|GO:0055133	DNA replication	2.59E-18
470	GO:0000775|GO:0097521	chromosome, centromeric region	3.83E-18
5124	GO:0007059	chromosome segregation	4.33E-18
474	GO:0000779	condensed chromosome, centromeric region	4.99E-18
472	GO:0000777	condensed chromosome kinetochore	5.90E-18
15306	GO:0032993	protein-DNA complex	6.27E-18
4491	GO:0006259|GO:0055132	DNA metabolic process	2.60E-16
471	GO:0000776|GO:0005699	kinetochore	1.92E-15
4033	GO:0005694	chromosome	2.39E-15
21411	GO:0044427	chromosomal part	2.82E-15
4501	GO:0006271	DNA strand elongation involved in DNA replication	2.78E-14
4493	GO:0006261|GO:0006262|GO:0006263	DNA-dependent DNA replication	9.66E-14
12446	GO:0022616	DNA strand elongation	1.40E-13
37231	GO:1902589	single-organism organelle organization	5.99E-13
4124	GO:0005819	spindle	2.07E-12
513	GO:0000819	sister chromatid segregation	2.81E-12
21750	GO:0044770	cell cycle phase transition	9.51E-12
21752	GO:0044772	mitotic cell cycle phase transition	9.51E-12
50	GO:0000070|GO:0016359	mitotic sister chromatid segregation	3.25E-11
30995	GO:0071103	DNA conformation change	2.15E-10
555	GO:0000922|GO:0030615	spindle pole	4.41E-10
4542	GO:0006323	DNA packaging	9.20E-10
27091	GO:0051726|GO:0000074	regulation of cell cycle	2.31E-09
20500	GO:0043486	histone exchange	2.52E-09

4537	GO:0006312	mitotic recombination	4.49E-09
58	GO:0000082	G1/S transition of mitotic cell cycle	5.79E-09
21822	GO:0044843	cell cycle G1/S phase transition	5.79E-09
4500	GO:0006270|GO:0042024	DNA replication initiation	1.16E-08
16364	GO:0034080|GO:0034509	centromere-specific nucleosome assembly	1.16E-08
167	GO:0000226	microtubule cytoskeleton organization	1.25E-08
5088	GO:0007017	microtubule-based process	1.28E-08
26678	GO:0051276|GO:0007001|GO:0051277	chromosome organization	1.59E-08
5116	GO:0007051	spindle organization	2.14E-08
5073	GO:0006996	organelle organization	2.32E-08
16785	GO:0034508	centromere complex assembly	2.45E-08
21414	GO:0044430	cytoskeletal part	3.03E-08
13442	GO:0031055	chromatin remodeling at centromere	4.42E-08
15566	GO:0033260	nuclear cell cycle DNA replication	4.42E-08
7769	GO:0010564	regulation of cell cycle process	4.75E-08
14526	GO:0032201	telomere maintenance via semi-conservative replication	7.35E-08
21766	GO:0044786	cell cycle DNA replication	8.11E-08
8890	GO:0015630	microtubule cytoskeleton	1.32E-07
4535	GO:0006310	DNA recombination	2.39E-07
21743	GO:0044763	single-organism cellular process	2.60E-07
169	GO:0000228	nuclear chromosome	3.22E-07
4154	GO:0005856	cytoskeleton	3.29E-07
4547	GO:0006336	DNA replication-independent nucleosome assembly	3.38E-07
16994	GO:0034724	DNA replication-independent nucleosome organization	3.38E-07
475	GO:0000780	condensed nuclear chromosome, centromeric region	4.95E-07
4003	GO:0005657	replication fork	7.57E-07
5084	GO:0007010	cytoskeleton organization	7.58E-07
420	GO:0000722	telomere maintenance via recombination	7.80E-07
26702	GO:0051302	regulation of cell division	7.95E-07
30401	GO:0070507	regulation of microtubule cytoskeleton organization	1.05E-06
3	GO:0000003|GO:0019952|GO:0050876	reproduction	1.10E-06
4164	GO:0005871	kinesin complex	1.14E-06
12416	GO:0022403	cell cycle phase	1.26E-06
27142	GO:0051783	regulation of nuclear division	1.26E-06
15355	GO:0033043	regulation of organelle organization	1.36E-06

5178	GO:0007126	meiotic nuclear division	1.43E-06
19652	GO:0042555	MCM complex	1.43E-06
570	GO:0000940	condensed chromosome outer kinetochore	1.49E-06
5149	GO:0007088	regulation of mitosis	1.84E-06
4510	GO:0006281	DNA repair	2.31E-06
31710	GO:0071824	protein-DNA complex subunit organization	2.67E-06
13848	GO:0031497	chromatin assembly	2.85E-06
27320	GO:0051983	regulation of chromosome segregation	3.25E-06
473	GO:0000778	condensed nuclear chromosome kinetochore	3.38E-06
489	GO:0000794	condensed nuclear chromosome	3.42E-06
4169	GO:0005876	spindle microtubule	3.53E-06
59	GO:0000083	regulation of transcription involved in G1/S transition of mitotic cell cycle	4.07E-06
26718	GO:0051321	meiotic cell cycle	4.18E-06
21681	GO:0044699	single-organism process	5.45E-06
29895	GO:0065004	protein-DNA complex assembly	5.64E-06
14822	GO:0032501|GO:0050874	multicellular organismal process	5.71E-06
16998	GO:0034728	nucleosome organization	6.59E-06
4544	GO:0006333	chromatin assembly or disassembly	6.61E-06
5349	GO:0007346	regulation of mitotic cell cycle	6.77E-06
8029	GO:0010833	telomere maintenance via telomere lengthening	7.65E-06
2716	GO:0003777	microtubule motor activity	8.43E-06
15199	GO:0032886	regulation of microtubule-based process	9.65E-06
20111	GO:0043044	ATP-dependent chromatin remodeling	1.01E-05
5117	GO:0007052	mitotic spindle organization	1.09E-05
33625	GO:0090329	regulation of DNA-dependent DNA replication	1.09E-05
4545	GO:0006334	nucleosome assembly	1.35E-05
62	GO:0000086	G2/M transition of mitotic cell cycle	1.37E-05
21818	GO:0044839	cell cycle G2/M phase transition	1.37E-05
5613	GO:0008017	microtubule binding	1.40E-05
5812	GO:0008283	cell proliferation	1.60E-05
21437	GO:0044454	nuclear chromosome part	2.01E-05
12762	GO:0030261|GO:0000068	chromosome condensation	2.25E-05
25111	GO:0048610	cellular process involved in reproduction	2.40E-05
26775	GO:0051383	kinetochore organization	2.47E-05
4167	GO:0005874	microtubule	2.52E-05
491	GO:0000796|GO:0005676|GO:0008620	condensin complex	3.24E-05
4006	GO:0005663	DNA replication factor C complex	3.24E-05
21688	GO:0044707	single-multicellular organism process	3.29E-05
61	GO:0000085	mitotic G2 phase	3.46E-05
26716	GO:0051319	G2 phase	3.46E-05

5140	GO:0007076	mitotic chromosome condensation	4.94E-05
8891	GO:0015631	tubulin binding	6.10E-05
4524	GO:0006297	nucleotide-excision repair, DNA gap filling	6.31E-05
26703	GO:0051303	establishment of chromosome localization	6.31E-05
26722	GO:0051325	interphase	6.31E-05
26726	GO:0051329	mitotic interphase	6.31E-05
13620	GO:0031262	Ndc80 complex	6.51E-05
13203	GO:0030801	positive regulation of cyclic nucleotide metabolic process	6.62E-05
26306	GO:0050896|GO:0051869	response to stimulus	6.68E-05
4119	GO:0005813	centrosome	7.45E-05
4498	GO:0006268	DNA unwinding involved in DNA replication	7.99E-05
27325	GO:0051988	regulation of attachment of spindle microtubules to kinetochore	7.99E-05
31708	GO:0071822	protein complex subunit organization	8.97E-05
4121	GO:0005815	microtubule organizing center	9.35E-05
13218	GO:0030816	positive regulation of cAMP metabolic process	9.70E-05
29899	GO:0065008	regulation of biological quality	9.76E-05
26536	GO:0051128	regulation of cellular component organization	9.77E-05
22706	GO:0045981	positive regulation of nucleotide metabolic process	1.12E-04
35220	GO:1900544	positive regulation of purine nucleotide metabolic process	1.12E-04
4168	GO:0005875	microtubule associated complex	1.20E-04
12979	GO:0030554	adenyl nucleotide binding	1.33E-04
7169	GO:0009925	basal plasma membrane	1.36E-04
13923	GO:0031577	spindle checkpoint	1.37E-04
25434	GO:0050000	chromosome localization	1.41E-04
13206	GO:0030804	positive regulation of cyclic nucleotide biosynthetic process	1.42E-04
26874	GO:0051493	regulation of cytoskeleton organization	1.48E-04
33603	GO:0090307	spindle assembly involved in mitosis	1.58E-04
33520	GO:0090224	regulation of spindle organization	1.62E-04

DOWN DMCL Blue compared with control DMCL

GO ID	GO ACCESSION	GO Term	p-value
3978	GO:0005615	extracellular space	3.90E-10
13584	GO:0031224	intrinsic component of membrane	2.33E-09
9248	GO:0016021	integral component of membrane	1.25E-08
31822	GO:0071944	cell periphery	4.27E-08
4177	GO:0005886|GO:0005904	plasma membrane	1.19E-07
21409	GO:0044425	membrane part	3.22E-07
5200	GO:0007155	cell adhesion	7.83E-07
4178	GO:0005887	integral component of plasma membrane	8.43E-07
12440	GO:0022610	biological adhesion	9.22E-07
13586	GO:0031226	intrinsic component of plasma membrane	1.54E-06
3519	GO:0004872|GO:0019041	receptor activity	1.58E-06
13401	GO:0031012	extracellular matrix	2.70E-06
1979	GO:0002673	regulation of acute inflammatory response	3.45E-06
3533	GO:0004888|GO:0004926	transmembrane signaling receptor activity	3.50E-06
21442	GO:0044459	plasma membrane part	4.70E-06
18704	GO:0038023	signaling receptor activity	6.04E-06
26288	GO:0050877	neurological system process	6.63E-06
5747	GO:0008201	heparin binding	8.57E-06
3700	GO:0005125	cytokine activity	1.33E-05
2259	GO:0003008	system process	1.50E-05
21688	GO:0044707	single-multicellular organism process	1.94E-05
5046	GO:0006958	complement activation, classical pathway	2.09E-05
5044	GO:0006956	complement activation	2.43E-05
3930	GO:0005539	glycosaminoglycan binding	3.06E-05

UP DMCL Green compared with control DMCL

GO ID	GO ACCESSION	GO Term	p-value
205	GO:0000278	mitotic cell cycle	9.58E-35
5114	GO:0007049	cell cycle	2.68E-31
12415	GO:0022402	cell cycle process	6.44E-29
207	GO:0000280	nuclear division	1.08E-27
24833	GO:0048285	organelle fission	1.78E-27
37681	GO:1903047	mitotic cell cycle process	8.91E-27
26701	GO:0051301	cell division	2.66E-22
5131	GO:0007067	mitotic nuclear division	7.91E-22
488	GO:0000793	condensed chromosome	2.66E-16
4492	GO:0006260|GO:0055133	DNA replication	5.46E-16
4491	GO:0006259|GO:0055132	DNA metabolic process	7.17E-16
474	GO:0000779	condensed chromosome, centromeric region	1.39E-15
472	GO:0000777	condensed chromosome kinetochore	1.58E-15
5124	GO:0007059	chromosome segregation	1.95E-15
12446	GO:0022616	DNA strand elongation	5.75E-15
470	GO:0000775|GO:0097521	chromosome, centromeric region	8.60E-15
4501	GO:0006271	DNA strand elongation involved in DNA replication	1.52E-14
15306	GO:0032993	protein-DNA complex	2.38E-14
21411	GO:0044427	chromosomal part	1.74E-13
4033	GO:0005694	chromosome	3.08E-13
471	GO:0000776|GO:0005699	kinetochore	3.76E-13
21750	GO:0044770	cell cycle phase transition	5.97E-13
21752	GO:0044772	mitotic cell cycle phase transition	5.97E-13
4493	GO:0006261|GO:0006262|GO:0006263	DNA-dependent DNA replication	1.24E-11
37231	GO:1902589	single-organism organelle organization	3.41E-11
513	GO:0000819	sister chromatid segregation	2.16E-10
4124	GO:0005819	spindle	5.01E-10
5116	GO:0007051	spindle organization	1.91E-09
50	GO:0000070|GO:0016359	mitotic sister chromatid segregation	1.96E-09
5178	GO:0007126	meiotic nuclear division	5.91E-09
30995	GO:0071103	DNA conformation change	8.45E-09
167	GO:0000226	microtubule cytoskeleton organization	1.09E-08
58	GO:0000082	G1/S transition of mitotic cell cycle	1.13E-08
21822	GO:0044843	cell cycle G1/S phase transition	1.13E-08
20500	GO:0043486	histone exchange	1.21E-08

4537	GO:0006312	mitotic recombination	2.13E-08
5073	GO:0006996	organelle organization	2.30E-08
26718	GO:0051321	meiotic cell cycle	2.45E-08
25111	GO:0048610	cellular process involved in reproduction	3.34E-08
4500	GO:0006270|GO:0042024	DNA replication initiation	4.26E-08
16364	GO:0034080|GO:0034509	centromere-specific nucleosome assembly	4.26E-08
26702	GO:0051302	regulation of cell division	4.78E-08
21442	GO:0044459	plasma membrane part	5.02E-08
4542	GO:0006323	DNA packaging	6.34E-08
19652	GO:0042555	MCM complex	8.18E-08
7769	GO:0010564	regulation of cell cycle process	9.36E-08
16785	GO:0034508	centromere complex assembly	1.04E-07
4510	GO:0006281	DNA repair	1.53E-07
13442	GO:0031055	chromatin remodeling at centromere	1.60E-07
4535	GO:0006310	DNA recombination	2.04E-07
5088	GO:0007017	microtubule-based process	2.31E-07
14526	GO:0032201	telomere maintenance via semi-conservative replication	2.43E-07
489	GO:0000794	condensed nuclear chromosome	2.73E-07
3	GO:0000003|GO:0019952|GO:0050876	reproduction	2.78E-07
169	GO:0000228	nuclear chromosome	2.78E-07
5149	GO:0007088	regulation of mitosis	3.24E-07
555	GO:0000922|GO:0030615	spindle pole	3.45E-07
26678	GO:0051276|GO:0007001|GO:0051277	chromosome organization	4.44E-07
14822	GO:0032501|GO:0050874	multicellular organismal process	4.73E-07
21743	GO:0044763	single-organism cellular process	7.54E-07
27091	GO:0051726|GO:0000074	regulation of cell cycle	8.80E-07
9265	GO:0016043|GO:0044235|GO:0071842	cellular component organization	9.24E-07
27142	GO:0051783	regulation of nuclear division	1.19E-06
475	GO:0000780	condensed nuclear chromosome, centromeric region	1.25E-06
4547	GO:0006336	DNA replication-independent nucleosome assembly	1.26E-06
16994	GO:0034724	DNA replication-independent nucleosome organization	1.26E-06
5812	GO:0008283	cell proliferation	1.27E-06

5349	GO:0007346	regulation of mitotic cell cycle	1.37E-06
27320	GO:0051983	regulation of chromosome segregation	1.42E-06
59	GO:0000083	regulation of transcription involved in G1/S transition of mitotic cell cycle	1.47E-06
15566	GO:0033260	nuclear cell cycle DNA replication	1.47E-06
420	GO:0000722	telomere maintenance via recombination	2.48E-06
21766	GO:0044786	cell cycle DNA replication	2.48E-06
4169	GO:0005876	spindle microtubule	2.60E-06
52	GO:0000075|GO:0031576|GO:0071779	cell cycle checkpoint	3.18E-06
5613	GO:0008017	microtubule binding	3.20E-06
570	GO:0000940	condensed chromosome outer kinetochore	3.41E-06
4164	GO:0005871	kinesin complex	5.05E-06
4167	GO:0005874	microtubule	5.21E-06
21437	GO:0044454	nuclear chromosome part	5.38E-06
5117	GO:0007052	mitotic spindle organization	5.44E-06
62	GO:0000086	G2/M transition of mitotic cell cycle	5.95E-06
21818	GO:0044839	cell cycle G2/M phase transition	5.95E-06
5084	GO:0007010	cytoskeleton organization	6.35E-06
473	GO:0000778	condensed nuclear chromosome kinetochore	6.41E-06
21414	GO:0044430	cytoskeletal part	6.73E-06
8890	GO:0015630	microtubule cytoskeleton	8.65E-06
4498	GO:0006268	DNA unwinding involved in DNA replication	9.12E-06
8891	GO:0015631	tubulin binding	1.37E-05
21681	GO:0044699	single-organism process	1.46E-05
21688	GO:0044707	single-multicellular organism process	1.56E-05
4003	GO:0005657	replication fork	1.58E-05
31726	GO:0071840|GO:0071841	cellular component organization or biogenesis	1.97E-05
13848	GO:0031497	chromatin assembly	2.18E-05
5293	GO:0007275	multicellular organismal development	2.31E-05
31708	GO:0071822	protein complex subunit organization	2.36E-05
5058	GO:0006974|GO:0034984	cellular response to DNA damage stimulus	2.48E-05
31710	GO:0071824	protein-DNA complex subunit organization	2.57E-05
4154	GO:0005856	cytoskeleton	3.19E-05
20111	GO:0043044	ATP-dependent chromatin remodeling	3.87E-05
2716	GO:0003777	microtubule motor activity	4.09E-05
29895	GO:0065004	protein-DNA complex assembly	4.34E-05
13586	GO:0031226	intrinsic component of plasma membrane	4.40E-05
4178	GO:0005887	integral component of plasma membrane	4.87E-05
16998	GO:0034728	nucleosome organization	5.01E-05
4544	GO:0006333	chromatin assembly or disassembly	5.25E-05
4006	GO:0005663	DNA replication factor C complex	5.51E-05
36639	GO:1901987	regulation of cell cycle phase transition	5.60E-05

21747	GO:0044767	single-organism developmental process	5.75E-05
61	GO:0000085	mitotic G2 phase	6.43E-05
25213	GO:0048715	negative regulation of oligodendrocyte differentiation	6.43E-05
26716	GO:0051319	G2 phase	6.43E-05
25229	GO:0048731	system development	6.46E-05
25349	GO:0048856	anatomical structure development	6.70E-05
14823	GO:0032502	developmental process	6.92E-05
37680	GO:1903046	meiotic cell cycle process	7.28E-05
4636	GO:0006461	protein complex assembly	8.00E-05
34623	GO:0098590	plasma membrane region	8.01E-05
4545	GO:0006334	nucleosome assembly	8.18E-05
13923	GO:0031577	spindle checkpoint	8.76E-05
36642	GO:1901990	regulation of mitotic cell cycle phase transition	9.57E-05
30165	GO:0070271	protein complex biogenesis	9.78E-05
3884	GO:0005452	inorganic anion exchanger activity	9.93E-05
13494	GO:0031109	microtubule polymerization or depolymerization	9.93E-05
13620	GO:0031262	Ndc80 complex	1.00E-04
7920	GO:0010721	negative regulation of cell development	1.07E-04
26536	GO:0051128	regulation of cellular component organization	1.07E-04
11816	GO:0019953	sexual reproduction	1.16E-04
12416	GO:0022403	cell cycle phase	1.17E-04
24789	GO:0048232	male gamete generation	1.17E-04
8029	GO:0010833	telomere maintenance via telomere lengthening	1.24E-04
8141	GO:0010948	negative regulation of cell cycle process	1.38E-04
4524	GO:0006297	nucleotide-excision repair, DNA gap filling	1.47E-04
26722	GO:0051325	interphase	1.47E-04
26726	GO:0051329	mitotic interphase	1.47E-04
27325	GO:0051988	regulation of attachment of spindle microtubules to kinetochore	1.47E-04
7142	GO:0009897	external side of plasma membrane	1.48E-04
12598	GO:0030071	regulation of mitotic metaphase/anaphase transition	1.54E-04
36750	GO:1902099	regulation of metaphase/anaphase transition of cell cycle	1.54E-04
12668	GO:0030154	cell differentiation	1.56E-04
2715	GO:0003774	motor activity	1.62E-04

DOWN DMCL Green compared with control DMCL

GO ID	GO ACCESSION	GO Term	p-value
3978	GO:0005615	extracellular space	3.57E-08
5044	GO:0006956	complement activation	3.51E-06
3700	GO:0005125	cytokine activity	3.54E-06

VITAE

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