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PROTOCOL

In Vitro Cytoprotective Study Against Blue Light Exposure

Laboratory : Skinovation Centre, Indonesia International Institute for Life Sciences
Authors : apt. Pietradewi Hartrianti, Ph.D
Richard Sutejo, Ph.D
Marsia Gustiananda, Ph.D
Elizabeth Chrestella Wibowo
Shakila Angjaya Adiyanto

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Elizabeth Chrestella Wibowo

Shakila Angjaya Adiyanto

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FOREWORD

This protocol book was written to serve as a working guideline for determining the cytoprotective activity of a certain compound or product against blue light exposure. This protocol was written following the recent trend of increased exposure of blue light from digital devices and how it will affect the health. Modern digitality as well as the pandemic situation has led a lot of people to spend most of their job-related activities and free time in front of their digital devices. However, these digital devices could exert some unwanted effects due to increased exposure to the eyes and skin. In relation to the effect on the skin, blue light exposure has been a well-known issue among users.

The information and guidelines presented in the protocol focus on the study and assessment of compounds and products for their blue light-cytoprotective activity. In addition, several basics related to cell culture, cell seeding, optimization, and cytotoxicity assay are also provided to further aid the experiment.

It's necessary to note that although the basics of cell culture experiments have some similarities, the conditions and cell lines can vary according to the culture condition, as well as the product type. Therefore, it is recommended to familiarize yourself with cell culture and skincare products of interest, to achieve the best results of the objectives.

Date : 15 May 2022

Sincerely,

Authors

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1. INTRODUCTION

Sun exposure has become one of the main sources of negative effects on human skin. This is due to the sunlight consisting of 5%-7% of ultraviolet (UV) radiation, 54% of infrared radiation, and 44% of visible light. Among these three, UV radiation has been known to be the main cause of skin damage. However, nowadays, the high energy visible (HEV) light has been studied to also play a significant role in causing skin damage (Bernstein, Sarkas, and Boland, 2021). The author depicted that throughout several wavelengths of visible light, shorter wavelengths that are more energetic than the longer wavelengths could exert a higher effect on the skin, and those shorter wavelengths include the violet/blue or high-energy visible (HEV) light.

In relation to its effect on the human skin, blue light, which possesses a wavelength in the range of 400 to 500 nm, is closely related to the devices that have been used daily by people. Blue light can be emitted from any electronic device such as smartphones, tablets, computers, and any other devices with an LED screen, with the intensity of blue light increasing from cell phone, computer, TV, and sun increasing in order (Coats *et al.*, 2021). Despite these devices exerting negative effects, previous study reported that their usage has been increasing alongside the current rise in online activity, which is affected by the COVID-19 pandemic situation (Jakhar and Kaur, 2020).

These prolonged exposures to blue light will contribute to various negative effects on the skin, including photoaging, whose effects include increasing skin wrinkles, worsening skin laxity, and especially causing hyperpigmentation (Bernstein, Sarkas, and Boland, 2021). These effects were hypothesized to be caused by the formation of ROS and RNS in the skin, due to the blue light exposure, based on past studies (Bernstein, Sarkas, and Boland, 2021; Coats *et al.*, 2021). The same author also depicted that ROS and RNS could lead towards oxidative stress in the skin, thus further affecting the melatonin precursors, resulting in the aforementioned effects. In addition, these studies also depicted that ROS affect collagen and elastin fibers degradation, as well as extracellular matrix degradation, leading to early aging.

In addition to those studies, a study by Sadowska, Narbutt, and Lesiak (2021) stated that blue light irradiation can increase 53% the chance of DNA damage and ROS (Reactive Oxygen Species) by 147%. The study investigated the mechanism of blue light oxidative damage in which these unwanted effects might happen due to the skin photoreceptors activation, such as the flavins, porphyrin-containing enzymes, cytochromes, and flavoproteins, which could absorb the blue light (Jakhar and Kaur, 2020; Liebmann *et al.*, 2010). In this case, Flavin plays a role in generating ROS. Upon prolonged blue light exposure, the nitric oxide (NO) that is produced and superoxide will react with each other, thus causing a peroxynitrite production, and leading to DNA damage (Jakhar and Kaur, 2020). In addition, as these molecules reach their excited state, they will affect the secondary targets located inside the cells, transducing the light signal into a molecular response (Liebmann *et al.*, 2010).

The results of these events can damage the skin dermis layer, leading to epidermal barrier disruption (Sadowska, Narbutt, and Lesiak, 2021; Ngoc *et al.*, 2019). Additionally, the blue light can also cause excited chromophores, thus oxidizing DNA bases formation which is genotoxic and may lead to the DNA strand breaks, oxidized pyrimidines, and the formation of 8-oxoguanine (Chamayou-Robert *et al.*, 2021). Furthermore, the activation of these photoreceptors could also affect cell growth, cell survival, as well as succinate dehydrogenase activity (Becker *et al.*, 2015).

Moreover, in association with hyperpigmentation, the oxidative stress towards the melatonin precursors will induce the photooxidative agent production which leads to immediate pigment darkening (IPD) and persistent pigment darkening (PPD). IPD is a grayish-look-alike-darkening that takes place directly after exposure and will fade in a short

time afterward, while PPD is a brownish-black pigmentation that can occur without any involvement in the melanogenesis process (Campiche *et al.*, 2020).

It has been studied that the skin has its own protective mechanism against these damaging effects, in which the presence of ROS due to blue light exposure is associated with the antioxidant level in the skin (Coats *et al.*, 2021). The authors reported that the antioxidant that is produced in the skin has a role in removing these free radical species. Thus, upon blue light exposure, the number of antioxidants in the skin will be depleted as it removes the free radicals species. After the depletion of antioxidants, it could be restored endogenously, but it takes up to 24 hours. Hence, the application of exogenous antioxidants and other blue light protecting agents has been studied these days, as the protection of the endogenous antioxidant may not suffice (Coats *et al.*, 2021)

In regards to it, some ingredients/agents were shown to have shown the skin protection effect against ROS, although no study has proved its significant effect in protecting the skin against blue light exposure. This is due to the action of blue light in exerting damage against the skin, in which it does not directly cause the production overwhelming amount of ROS which could not be handled by the cellular antioxidant defense, but it could also continuously produce a low level of the radicals species, which could evade the skin defense mechanism. This event could eventually lead to permanent DNA damage (Coats *et al.*, 2021). In protecting the skin against this mechanism, several ingredients were studied and shown to exhibit antioxidant and/or blue light-absorbing properties Those ingredients include the antioxidants such as vitamin C, which are known for their capabilities in protecting the skin against damage from free radicals. In addition, the UV-absorbing compound including avobenzone, oxybenzone, octocrylene, octinoxatem, and homosalate are also studied for their potential against blue light irradiation, although there are still limited to no data regarding its protection ability (Bernstein, Sarkas, and Boland, 2021).

Furthermore, responding to these known effects, various skincare companies also have investigated and claimed that some skincare products, especially sunscreen, possess the photoprotective capability, which is not only limited to protecting against ultraviolet exposure but also against blue light. The claim was based on the ability of some sunscreen in supplying a physical barrier to the skin by reflecting and disseminating the light, thus protecting it from photooxidation. There are two types of photoprotection including primary and secondary photoprotection. The primary photoprotection is more toward all ingredients that contain a physical filter and has the ability to reflect and scatter the spectrum of visible light (Campiche *et al.*, 2020). These kinds of ingredients that includes in the primary photoprotection are suggested to hold a high refractive index as they may contribute to the sunscreen effectiveness, although the white appearance has become their deficiency because in cosmetics they are less appealing to the user (Morabito *et al.*, 2011). The secondary photoprotection is addressed to the ingredients that contain DNA-repair enzymes and antioxidants. These kinds of ingredients will be taking part in the photochemical cascade disruption that occurs during the light exposure hence reducing the skin damage (Morabito *et al.*, 2011). In comparison, a study by Morabito *et al.,* (2011) depicted that between these two photoprotection mechanisms, the primary photoprotection is more effective in giving the skin protection than the secondary photoprotection, which absorbs the radiation lights. These photoprotection abilities are further studied by Bernstein, Sarkas, and Boland (2020), in which it was shown that with the sunscreen product, there are up to 71.9 - 85.6% of HEV alleviation for the skin, while in the unprotected skin there are only 3.9 - 4.9% showed for HEV alleviation.

The rise of the skincare claim for blue light protection ability has led to the scientifically-proven method demand and to validate the ability. One of the many ways in strengthening these claims is by measuring the skincare product's capability of blue light protection through *In vitro* HaCat cells test. HaCat is an immortalized keratinocyte cell in which

the keratinocyte itself has a responsibility to aid the epidermis supply the skin structure by generating cytokines that are substantial in cell communication arrangement for skin barrier functioning. Aside from that, this *in vitro* method with HaCat cells has been generally used in past studies to verify the blue light exposure effect on cell aging and cell viability. A study discovered that for keratinocytes, blue light is toxic as there was a cell decline after irradiation for three consecutive days every 24 hours (Sadowska, Narbutt, and Lesiak, 2021). With this basis, the proposed methodology using immortalized keratinocyte cells is chosen to prove that the sunscreen product tested may provide cytoprotective ability against blue light.

The *in vitro* method used in this experiment has been considered for not only being more practical in comparison to clinical trials but also it has no ethical concerns that can come up from animal testing. Moreover, previous research has shown that the blue light detrimental effect that comes from irradiation can be observed through *in vitro* cultured cell assays (Avola *et al.*, 2019). Prior to the *in vitro* test, the blue light exposure optimization protocol will be conducted. After that, following the optimization, the cytotoxicity and the cell viability study will be implemented by looking at the viability difference between the control cells without product treatment towards the HaCat cells treated with the product against the blue light exposure.

AIM OF STUDY : to prove the claims of the tested sunscreen product for its protective ability against blue light through in vitro cell viability measurement using HaCat cells.

2. MATERIALS AND METHODS

a. In vitro model/Subject

- Model : HaCat (Immortalized human keratinocyte) cells
- Culture conditions : 37°C, 5% CO_2
- Culture medium : DMEM supplemented with
  L-glutamine 2mM
  Penicillin 1% - Streptomycin 1%
  Fetal Bovine Serum (FBS) 10%

b. Test Compound

1. Product tested
2. Active ingredients (API)
3. Base
4. Negative control

Note: these test groups can be adjusted as needed.

c. Methods

c.1 Method origin

The *in vitro* assay using HaCat cells for determining the cytoprotective ability of sunscreen against blue light was chosen in this experiment as the previous research study from Sadowska, Narbutt, and Lesiak (2021) found that blue light is toxic for keratinocyte cells as there was a decrease in the cells after irradiation every 24 hours in three consecutive days. The duration and distance tested in this experiment for blue light optimization are according to Togni *et al.* (2019), which then be adjusted according to the available equipment in the lab. Besides that, the utilization of UV lamps for sample sterilization was also used based on the previous experiment that studied the effectiveness of UV lamps in inactivating various bacteria and can become one of the methods for sterilization (Mori *et al*., 2007). Then the cytoprotective method especially the one that does not make direct contact between the cells and the sample was initiated by Tyagi *et al*., 2016.

In this experiment, the samples tested might contain materials that are insoluble in water. Therefore, a miscibility test was necessary to be conducted. According to Mizuno et al., 2016, the recommended products used to the face are usually in the amount of 2 mg/cm2. When converted into a 96-well plate scale, the requirement for product to be used is 0.6 mg. Hence the sample concentration that was prepared in this experiment was 10 mg/ml, 5 mg/ml, 2.5 mg/ml, 1.25 mg/ml, 0.625 mg/ml, 0.3125 mg/ml.

c.2 Method Principle

The whole experiment was utilizing *in vitro* cell culture method using HaCat cell. Each part of the experiment was conducted by triplication with 3 wells in the same well plate.In the first step of the experiment, blue light optimization was conducted to determine at which

distance and duration the blue light exposure to the cells may result in the decrease of cell viability by about 50%. Besides that, the cytotoxicity test with various concentrations of the sunscreen product, base, and API was also done to find the highest concentration of the sample that is not cytotoxic to the cells, which is marked by having cell viability of more than 70%. After all requirement parameters were determined, the cytoprotective assay was done to determine at which concentration the sunscreen product was able to provide the cytoprotective ability to the HaCat cells.

c.3 Description of the method

c.3.1 HaCat Cell Culture

1. Before subculturing, the confluence of the cells was checked under the inverted microscope.
2. The old media was discarded into the waste container.
3. The cells were washed two times using 2 mL DMEM only.
4. Approximately 1 mL trypsin was then added to the T25 flask and incubated for 6 minutes or until the proper cell detachment was observed
5. After the proper cell detachment was observed under the microscope, the trypsin was inactivated with 1 mL cDMEM and resuspended gently.
6. The cell suspension was transferred to the new 2 pieces of 1.5 mL microcentrifuge tubes and centrifuged at 2,000 rpm for 4 minutes.
7. The supernatant was discarded gently and the cell pellet was resuspended with 1 mL total volume of cDMEM.
8. Take 250 uL of the cell suspension then seed it to a new T25 flask and 5 mL cDMEM was added to the flask.
9. The cells were incubated in the 5% CO2 incubator at 37°C.
10. In 2-3 days until the confluency of the cells had already reached 80-90%, the cells will be subcultured again.

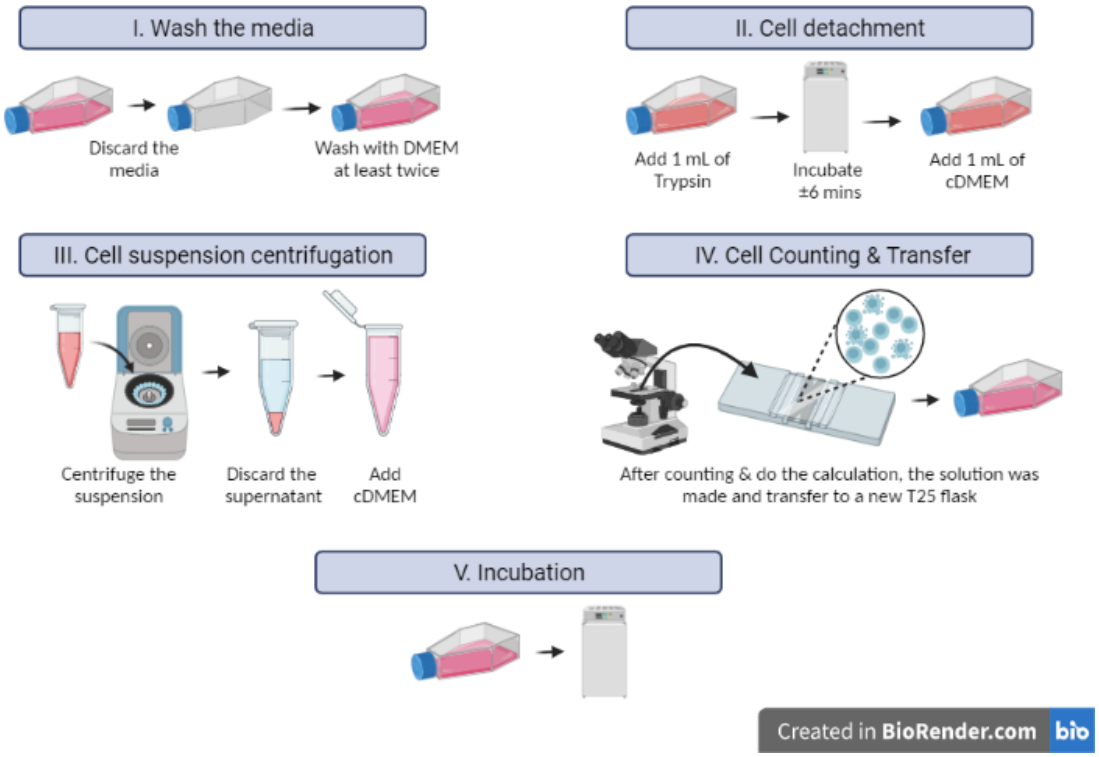

Figure 1. The Method Flow for Cells Subculture

c.3.2 Cell Seeding

1. The cell seeding was conducted one day before the assay was taken.

2. Before the cell seeding, the confluence of the cells was checked under the inverted microscope.
3. The old media was discarded into the waste container.

4. The cells were washed once using 2 mL DMEM twice.
5. 1 mL trypsin was then added to the T25 flask. Proper cell detachment was observed after 4 minutes of incubation.
6. 1 mL cDMEM was added to the T25 flask to inactivate the trypsin.
7. The cell suspension was resuspended gently and then transferred to two 1.5 mL microcentrifuge tubes (1 mL each).
8. The tubes were centrifuged at 2,000 rpm for 4 minutes.
9. The supernatant from the tube was discarded and the cell pellet for each tube was resuspended with 1 mL total volume of cDMEM. After that, to seeding the cells in 96 well plate, the 10ul of cell suspension was taken and placed in the clean parafilm
10. Then 10ul of trypan blue was added and resuspend together with the cell suspension
11. A 10ul of the mixture was taken and placed in the hemacytometer
12. The cells were counted using a microscope and then being calculated to make a density of $1x10^4$ cells per100 uL for each well
13. After the mixture was made and it already transferred into a well plate, the cells were incubated in the 5% CO2 incubator at 37°C overnight

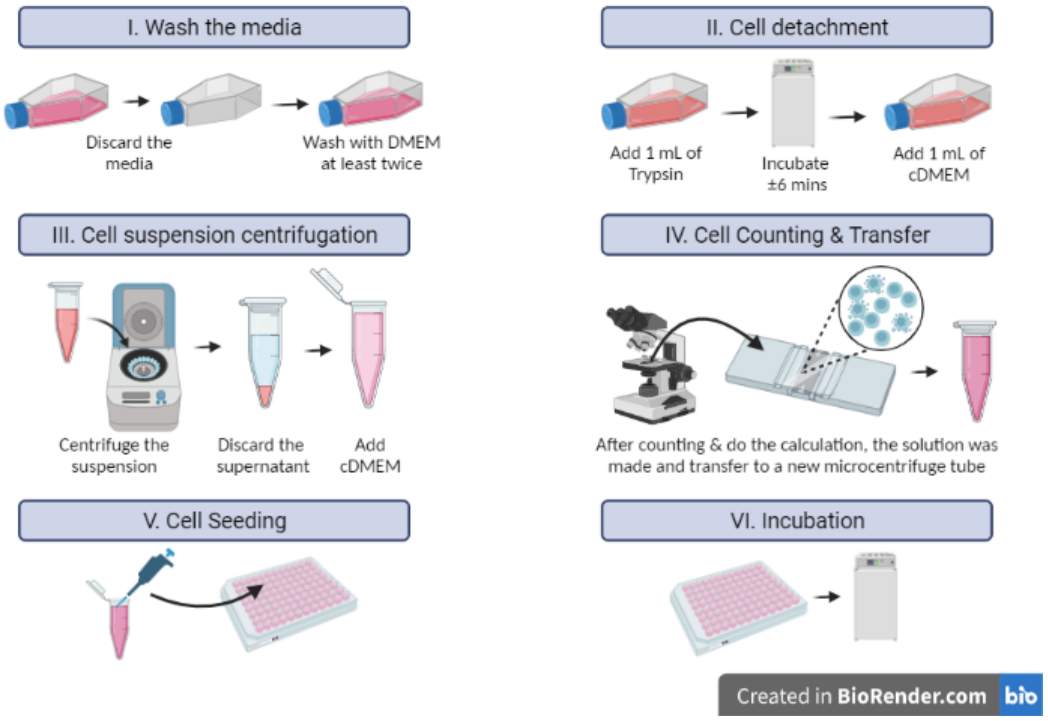

Figure 2. The Method Flow for Cell Seeding

c.3.3 Miscibility Test

1. The miscibility test is conducted as needed, in which the sample or product should be soluble in the cell media

c.3.4 Blue light Optimization

1. The HaCat cells are then seeded and cultured in a 96-well plate. When it reaches a confluency of 60-80%, the cells are incubated inside the blue light box inside the incubator at 37°C with 5% CO_2. The blue light box is facilitated with 6 Watt of blue light.

2. After the designated time of incubation (6 and 24 hours) and distance of irradiation (10 cm and 15 cm), the cells' confluency is observed using MTS assay.

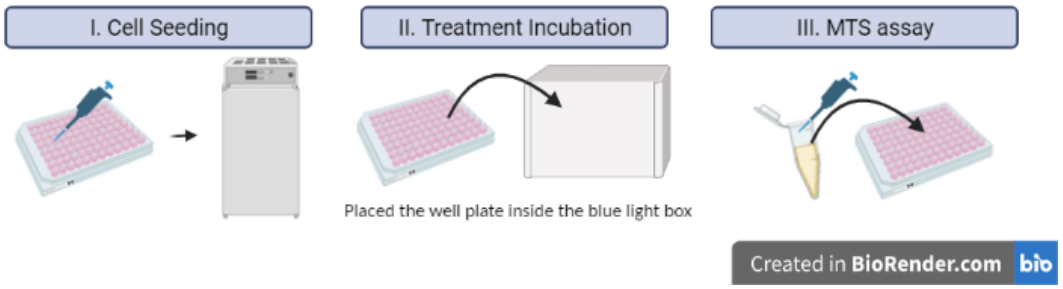

Figure 3. The Method Flow for Blue light Optimization

c.3.5 Cytotoxicity test of samples

1. Seed the HaCaT cells inside 96 well plates. Each well contains 1 x 10^4 cells in 100 µl of DMEM medium. Close the lid and incubate the plate for 24h at 37°C in a 5% CO_2 humidified atmosphere.
2. When it reaches 60-80% of confluency, wash the cells, then add 100uL of the product tested, active ingredients (API), base, and negative control to each well. Several concentrations of these groups are preferable, as comparison to each other.
3. The 96-well plate is then incubated at 37°C with 5% CO_2 for 6 hours or 24 hours, based on the result of Blue Light Optimization.
4. After the incubation, the cell confluency is assessed with MTS assay.

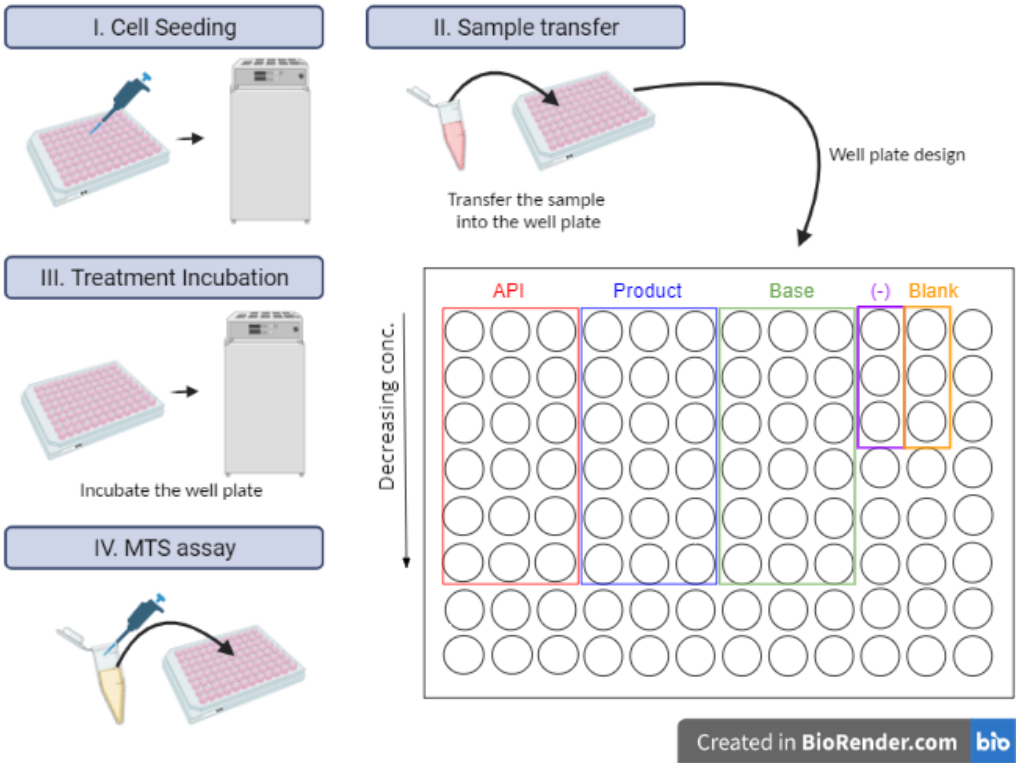

Figure 4. The Method Flow for Cytotoxicity Test assay

c.3.6 Cytoprotective ability measurement against blue light exposure

1. Seed the HaCaT cells inside 96 well plates. Each well contains 1 x 10^4 cells in 100 µl of DMEM medium. Close the lid and incubate the plate for 24h at 37°C in a 5% CO_2 humidified atmosphere.
2. When it reaches 60-80% of confluency, wash the cells, then add 100uL of the product tested, active ingredients (API), base, and negative control to each well.
3. Place the treatment plate inside the blue light box, then place the control plate outside the blue light box. Both of the plates were incubated for 6 hours or 24 hours, at 10 cm or 15 of distance, based on the results for blue light optimization
4. After the incubation, measure the protective ability of the product against blue light using MTS assay and cell viability test with trypan blue

5. An alternative method can be used for semi-solid product, or product that can be applied to the back lid of the well plate to avoid contact with the cells that could lead to death.

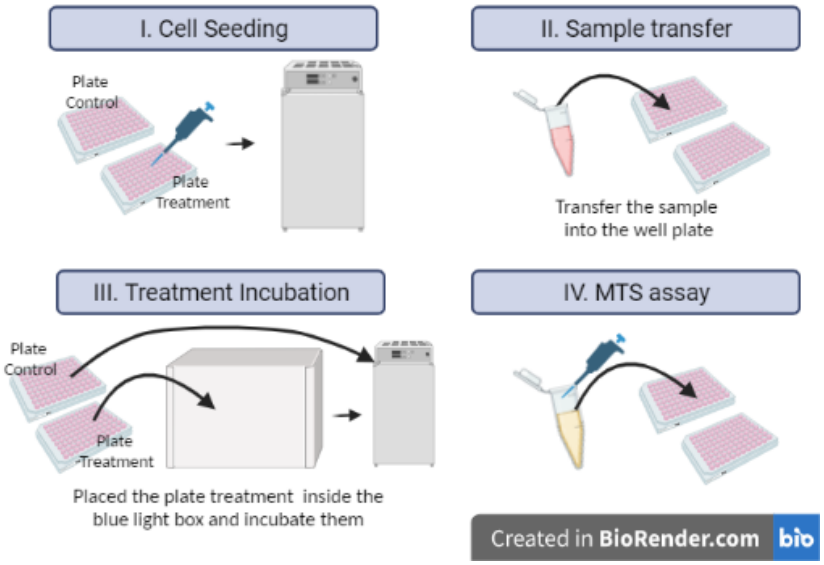

Figure 5. The Method Flow for Cytoprotective Assay

For the second method, after the HaCat cells were seeded into a well plate and incubated for 24 hours, the sunscreen product and base were applied with a dosage of 2 mg/cm^2 on the back lid of the well plate. Then step 4 and 5 were same as the first method.

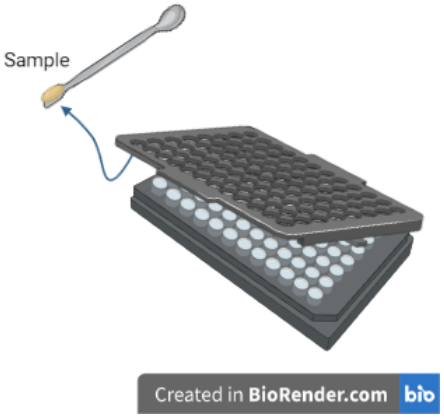

Figure 6. The Sample Application in Second Cytoprotective assay

c.3.7 MTS Assay

1. After the incubation of the treatment assay, the media in each well was discarded and washed with DMEM once
2. Then a 100uL of cDMEM was added into each well
3. A 15ul of MTS reagent in cDMEM was added afterward under dark conditions
4. Cells were then incubated at 37°C for 3 hours in a 5% CO2 humidified atmosphere
5. After incubation, the result was measured with a plate reader to record the absorbance at 490 nm.

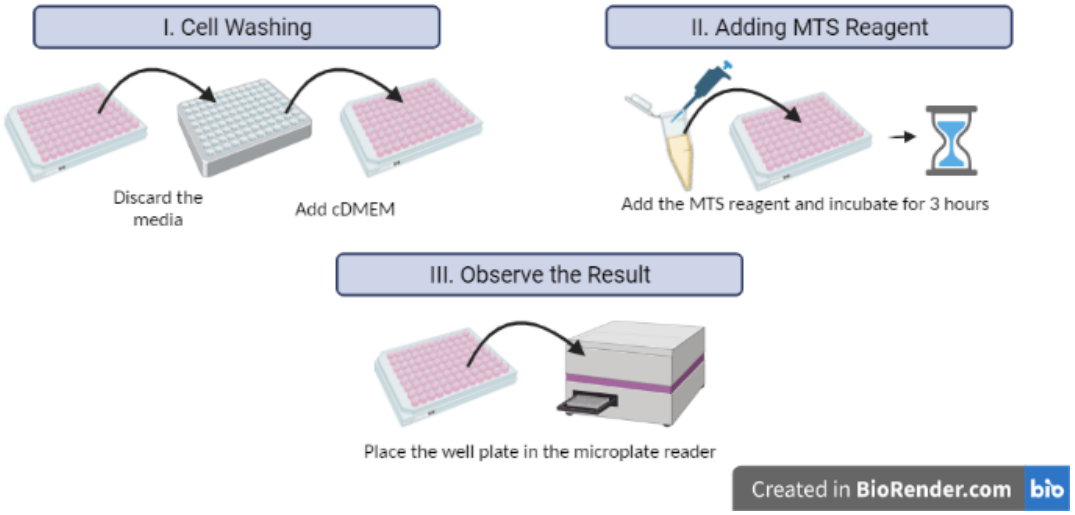

Figure 7. The Method Flow for MTS Assay

c.3.8 Cell Viability Test with Trypan Blue

1. After the incubation of the treatment assay, the media in each well was discarded and washed with DMEM once
2. The trypan blue was added to each well for 100 uL and was left for approximately 3 minutes before being discarded
3. After staining part, each well was filled with another 100uL DMEM to ensure the leftover living cells were not in dry conditions
4. The stained wells were then observed under the inverted microscope and in each well, there will be 4 photos collected from different areas in the wells.
5. The cell viability percentage was determined by using an ImageJ application by calculating the % area average of the live cells from each well.

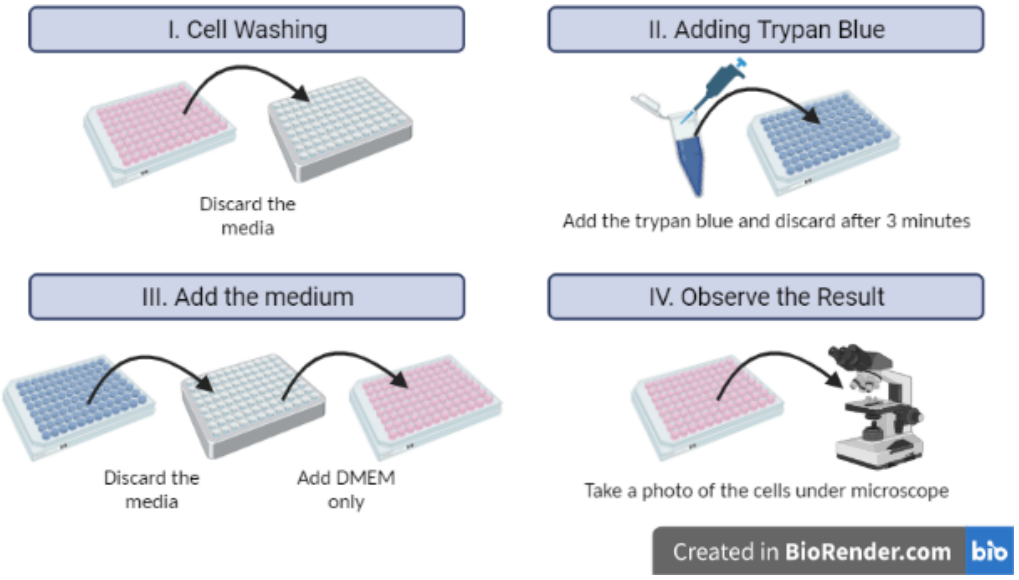

Figure 8. The Method Flow for Trypan Blue Assay

c.4 Materials and Equipment used

1. Materials:
    a. HaCat cell from Prof Ng Kee Woei from the School of Materials Science and Engineering, Nanyang Technological University
    b. DMEM only
        i. Sodium bicarbonate
        ii. Penicillin 1% - Streptomycin 1%
    c. Complete DMEM (cDMEM): DMEM only with an addition of Fetal Bovine Serum (FBS) 10%
    d. Sunscreen product
    e. Sunscreen base
    f. Sunscreen API only, containing: Bifilda Ferment Lysate, Bisabolol, Pongamia Glabra, Propanediol, Water, Artemisia Capillaris Flower extract, Saccharide Isomerate, Aqua, Citric Acid, Sodium Citrate, Titanium Dioxide, Dimethicone, Silica, Zinc Oxide, and Triethoxycaprylylsilane.
    g. MTS reagent: CellTiter 96® AQueous One Solution Cell Proliferation Assa
    h. Trypan blue
2. Equipments:
    a. A 37°C CO_2 incubator with a 5% humidified atmosphere
    b. Ultrasonic bath
    c. 10 watt UVA lamp with a wavelength of 352 nm
    d. Inverted microscope
    e. Optical Microscope
    f. Haemocytometer
    g. Microplate reader

3. DATA ANALYSIS

The absorbance data from the cytotoxicity and cytoprotective study which used MTS assay will be calculated using the formula below, to know the % Cell viability of the HaCaT cells. (Sjafaraenan, Johannes, and Wulandari, 2019).

$$\%\ Cell\ Viability\ = \frac{a-b}{b-c}\ x\ 100\%$$

A = Absorbance of treatment cell
B = Absorbance of control media (DMEM only)
C = Absorbance of control cell (cell + DMEM only)

After that, the statistical analysis should be performed to assess the significance, based on the preferable comparison. If the result is normally distributed, it is suggested to be assessed using GraphPad Prism 8.0.1 by using the paired T-test for the blue light optimization data, one-way ANOVA in cytotoxicity results data, and two-way ANOVA method in cytoprotective results. On the other hand, if the data result is not normally distributed or when the one-way ANOVA assumptions are not met, a nonparametric test called the Kruskal-Wallis test can be used instead.

In addition, for the cytoprotective study which uses the trypan blue method, the photos that were taken from four different areas per 1 well, will be processed in the ImageJ application. Using ImageJ, the % area will be calculated and averaged among the four areas. Then, the

average % area from each well with the same treatment group will be accumulated and averaged, to determine the final value, which could be inferred as the % amount of living cells.

4. RESULT INTERPRETATION

In the blue light optimization, the aim is to determine the suitable distance and duration used under blue light exposure for the experiment. The main observation of this experiment was to find at which time duration and distance of the cell exposure to blue light will result in around 50% of cell viability of the HaCat cell. The resulting cell viability of around 50% is preferable as it may show a more significant difference between the treated and non-treated cells in the cytoprotective assay, taking the standard deviation into consideration. However, the cell viability should not be too close to 0%, which could indicate that the cells are unhealthy, thus not preferable to be used in the following assay.

For the cytotoxicity assay, the aim is to find the highest concentration of the product, base, and API of the sunscreen that is non-cytotoxic (more than 70% cell viability) to the cell.

Following the cytotoxicity assay, the cytoprotective assay will be conducted with the aim of determining the cytoprotective ability of the sunscreen product for HaCat cells, against blue light irradiation. A significant increase in the cell viability of the cells treated with sunscreen product compared to the internal control (cells irradiated to blue light, but no treatment solution given) may be interpreted as the product having cytoprotective properties against blue light irradiation.

For all of the experiments conducted above. all samples were done triplicate to ensure the accuracy of the result obtained in each assay.

5. REFERENCES

Avola, R., Graziano, A. C. E., Pannuzzo, G., Bonina, F., & Cardile, V. (2019). Hydroxytyrosol from olive fruits prevents blue-light-induced damage in human keratinocytes and fibroblasts. Journal of cellular physiology, 234(6), 9065-9076.

Becker, A., Sticht, C., Dweep, H., van Abeelen, F. A., Gretz, N., & Oversluizen, G. (2015, March). Impact of blue LED irradiation on proliferation and gene expression of cultured human keratinocytes. In *Mechanisms for Low-Light Therapy X* (Vol. 9309, pp. 58-69). SPIE.

Bernstein, E. F., Sarkas, H. W., & Boland, P. (2021). Iron oxides in novel skin care formulations attenuate blue light for enhanced protection against skin damage. Journal of Cosmetic Dermatology, 20(2), 532-537.

Campiche, R., Curpen, S. J., Lutchmanen-Kolanthan, V., Gougeon, S., Cherel, M., Laurent, G., ... & Schuetz, R. (2020). Pigmentation effects of blue light irradiation on skin and how to protect against them. International journal of cosmetic science, 42(4), 399-406.

Chamayou-Robert, C., DiGiorgio, C., Brack, O., & Doucet, O. (2021). Blue light induces DNA damage in normal human skin keratinocytes. Photodermatology, Photoimmunology & Photomedicine.

Coats, J. G., Maktabi, B., Abou-Dahech, M. S., & Baki, G. (2021). Blue Light Protection, Part I—Effects of blue light on the skin. Journal of cosmetic dermatology, 20(3), 714-717. doi:10.1111/jocd.13837

Jakhar, D., Kaul, S., & Kaur, I. (2020). Increased usage of smartphones during COVID-19: Is that blue light causing skin damage?. Journal of Cosmetic Dermatology, 19(10), 2466-2467.

Liebmann, J., Born, M., & Kolb-Bachofen, V. (2010). Blue-light irradiation regulates proliferation and differentiation in human skin cells. *Journal of Investigative Dermatology*, *130*(1), 259-269.

Mizuno, M., Kunimoto, K., Naru, E., Kameyama, K., Furukawa, F., & Yamamoto, Y. (2016). The effects of continuous application of sunscreen on photoaged skin in Japanese elderly people–the relationship with the usage. Clinical, cosmetic and investigational dermatology, 9, 95.

Morabito, K., Shapley, N. C., Steeley, K. G., & Tripathi, A. (2011). Review of sunscreen and the emergence of non-conventional absorbers and their applications in ultraviolet protection. International journal of cosmetic science, 33(5), 385-390.

Mori, M., Hamamoto, A., Takahashi, A., Nakano, M., Wakikawa, N., Tachibana, S., ... & Kinouchi, Y. (2007). Development of a new water sterilization device with a 365 nm UV-LED. Medical & biological engineering & computing, 45(12), 1237-1241.

Ngoc, L. T. N., Tran, V. V., Moon, J. Y., Chae, M., Park, D., & Lee, Y. C. (2019). Recent trends of sunscreen cosmetic: An update review. Cosmetics, 6(4), 64. doi:10.3390/cosmetics6040064

Togni, S., Maramaldi, G., Cavagnino, A., Corti, A., & Giacomelli, L. (2019). Vitachelox: Protection of the Skin Against Blue Light-Induced Protein Carbonylation. Cosmetics, 6(3), 49.

Sadowska, M., Narbutt, J., & Lesiak, A. (2021). Blue Light in Dermatology. Life, 11(7), 670. doi:10.3390/life11070670

Sjafaraenan, S., Johannes, E., & Wulandari, S. N. (2019). Pengaruh Interval Dosis 2, 44-19, 53 µG/ml Ekstrak N-heksana Dari Hydroid Aglaopheniacupressinalamoureoux Terhadap Aktivitas Pertumbuhan Selhela. BIOMA: JURNAL BIOLOGI MAKASSAR, 4(1), 11-19.

Tyagi, N., Srivastava, S. K., Arora, S., Omar, Y., Ijaz, Z. M., AL-Ghadhban, A., Singh, S. (2016). Comparative analysis of the relative potential of silver, Zinc-oxide and titanium-dioxide nanoparticles against UVB-induced DNA damage for the prevention of skin carcinogenesis. Cancer Letters, 383(1), 53–61. doi:10.1016/j.canlet.2016.09.026

6. APPENDIX

Figure 6.1 Blue light box (size 30x10x15 cm)