MedChem Laboratory BIOCEV UNIVERZITA KARLOVA 1. lékařská fakulta	**RDPM30**	**Name:** RD measurement record **Date:** 19/10/2021 Address: Průmyslová 595, 252 50 Vestec, Czech Republic

R&D measurement record

Performed by:	Ing. Zdeněk Kejík, Ph.D.	Signature :		Date:	13/10/2021
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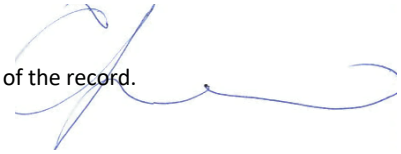
Received	Ing. Petra Gottwald	Signature		Date:	21/10/2021
Note: Handover record. I agree with the measurement and confirm receipt of the record. 					

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Objective

Experimental comparison of the effect of commercial lighting units with the Spectrasol pro-cognitive LED lighting unit with a red reparation light component on the human eye by testing on the R28 cell line (Retinal Cell Line, Kerafast).

Devices

- LUNA II, Automated cell counter (Logos)
- CO2-regulated incubator
- LUNA slide
- Culture bottles, common laboratory material and accessories
- Laminar box
- Thorlabs s130c light sensor

Chemicals

- **Trypan Blue Stain 0.4%**
- **R28 cell line, CVCL_5I35** (R28 is an adherent retinal precursor cell line derived from the retina of a postnatal 6-day-old Sprague-Dawley rat retina immortalised by the adenovirus gene 12S E1A. Gene 12S E1A was introduced via an incompetent retrovirus vector; thus, no infectious virus is produced by R28 cells. Cells have been passaged 200 times to date and show no signs of ageing).
- **DMEM+** (420 ml of incomplete DMEM D5523 (Sigma); 15 ml of sodium bicarbonate (7.5% stock solution, w/v) (Sigma S5761); 50 ml of foetal bovine serum (HyClone, SH30073.03HI); 5 ml of non-essential amino acids MEM (GIBCO, 11140-019); 5 ml of L-glutamine (200 mM stock) (GIBCO, 25030-024); 0.625 ml of gentamicin (stock solution is 80 mg/ml)
- **CMF-EDTA** (CMF was created at first: NaCl 8.77 g; Na2HPO4.7H2O 2.28 g; KCl 0.2 g; KH2PO4 0.2 g; NaHCO3 2.18 g; These compounds were dissolved in 900 ml of dH2O. PH solution standardised to 7.4. Volume is subsequently increased to one litre with sterile H2O. Sterilisation with a filter and autoclave. CMF-EDTA: the following substances were added per each 100 ml of sterile CMF prepared above: 1 ml of sterile 2% EDTA; 1 ml of sterile 10% glucose, 125 µl of gentamicin (final concentration 50 µg/ml).

Light sources – technical specification

The individual tested LED light sources were mounted on a PCB with a size of approximately 20x250 mm, for suitable placement into a culture incubator. Sources were placed into a culture box and fixed at a distance of 400 mm from the testing area of culture well microplates. Microplates were closed during exposure, and exposure of retinal cell samples was uniform. Each light source was equipped with its own power supply with regulation, for precise adjustment of energy radiated at a reference wavelength of 480 nm (blue). Reparation energy of light at a wavelength of 670 nm (NIR) was also measured for control for all light sources adjusted according to the reference energy of 480 nm. Detailed specification of light sources, see the table below. Exposure was performed in a closed dimmed incubator, one type of light source each, where each set of culture microplates with R28 tissue culture was irradiated separately with shielding against any external light source, to eliminate any error.

Designation and technical specification of light sources used:

Source designation		LED type	LED technical description	Power density [µW/cm²]		Source distance from the experimental area
number	internal designation			λ [nm]	[µW/cm²]	
5	NS	Cold white LED with high intensity of blue primary energy	LED 6500K CRI 93 (primary energy λ≈450 nm)	480	239	400
6	SLK	Neutral white LED with purple primary energy	LED 4000K CRI 95 (primary energy λ≈420 nm)	480	240	400
7	STND	Neutral white LED with blue primary energy, standard CRI	LED 4000K CRI 80 (primary energy λ≈450 nm)	480	240	400
8	SPPK	Pro-cognitive LED Spectrasol with red reparation energy	LED 4900K CRI 95 with 670 nm (primary energy λ≈multi)	480	240	400

Spectral diagrams of light sources tested:

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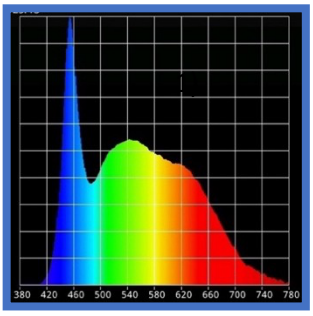

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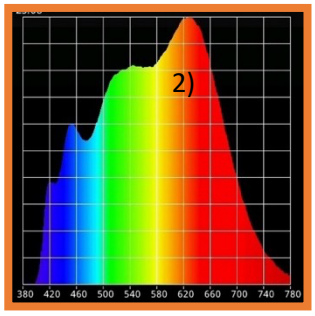

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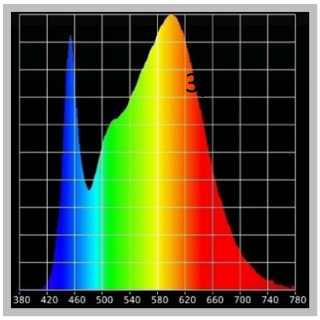

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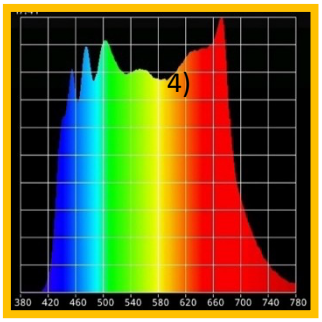


Experimental part

Cells were thawed at room temperature (for approximately 15 min.). Cell content was subsequently pipetted into 5 ml of the DMEM+ medium and centrifuged for a period of 5 minutes. The cell pellet was dispersed in 10 ml of the DMEM+ medium (vortex) and incubated in an incubator (37°C) for 2 days.

Cells were passaged after 2 days of incubation. Cells were rinsed with 1 ml of EDTA. 1 ml of EDTA and 1 ml of trypsin were further added, and the prepared culture bottle was incubated for 5 minutes. 5 ml of a fresh DMEM+ medium was added and the solution was stirred (vortex). One half of the solution was pipetted into a new culture bottle and a new DMEM+ medium was added to both bottles to a final volume of 20 ml. Culture bottles prepared this way were again placed into the incubator and incubated for a period of 3 days. Initial concentration after culture was approximately 519,000-579,000 cells per ml.

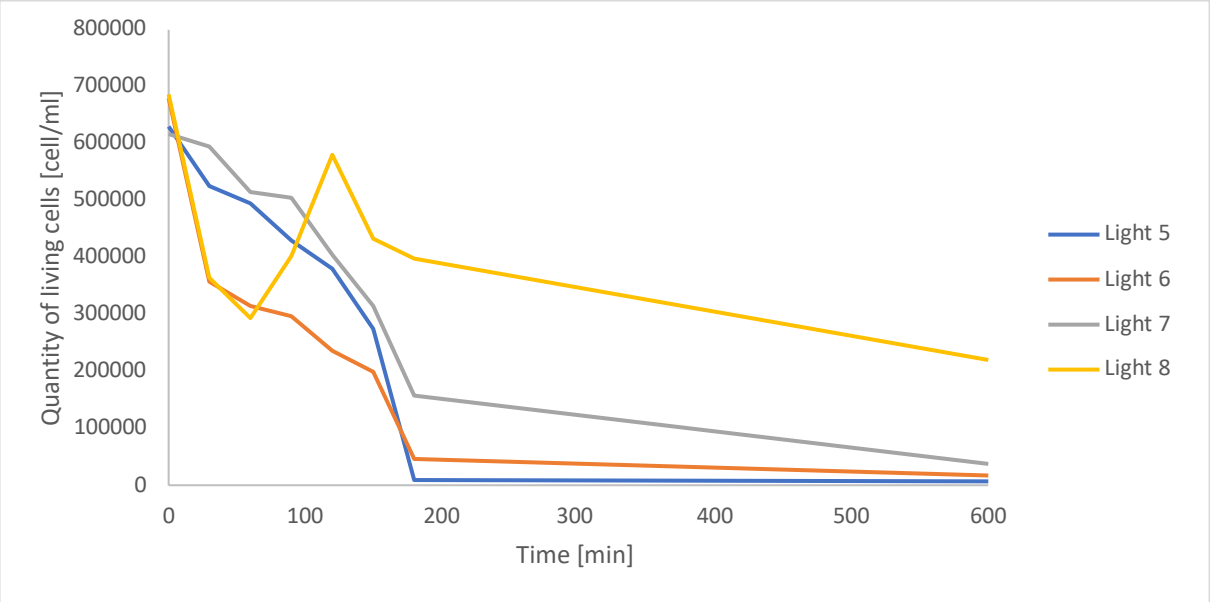
Cells were checked every day under a microscope from the perspective of their growth. Cells were then pipetted into the individual wells of a 12-well microplate. Subsequently, after three days, cells were placed into an incubator with temperature of 37°C and with 5% CO2 and exposed to light source radiation according to the technical specification. The first collection was performed before initiation of irradiation; the light source was subsequently switched on after the incubator was closed. Collection was more frequent for the first three hours, particularly every 30 minutes, when the greatest progress in the tissue culture growth was expected, while the last collection was performed after 10 hours, i.e., after 600 minutes of irradiation. At each time point of collection, cells were collected from two wells, processed, and their concentration (number of living cells) was measured. The results were transformed into a graphic form.

Measured results

Living cell values collected from irradiation at a given time

LIVE	Light 5	Light 6	Light 7	Light 8
0	630000	680000	617000	686500
30	525000	357500	595000	364500
60	494500	315000	515000	293500
90	430000	297000	505000	402500
120	380000	236000	405000	580000
150	275000	199000	314500	433500
180	9500	46400	158000	398000
600	6500	17050	37400	220000

Graphical dependence of the duration of tissue culture irradiation with light sources on the number of living cells



Conclusion

As with the light sources in the first experiment, the current experiment has also demonstrated a significant difference in the effect of radiation on opposite sides of the visible spectrum part, particularly short wavelengths of blue and purple light compared to radiation of long wavelengths of red light, on the growth itself, or cell division of the R28 retinal line.

The quantitative difference of living cells was observed over time under the effect of radiation, this time with white LED lights commonly used in interiors. Compared commercial light sources 5 to 7 caused cell apoptosis after approximately 200 minutes of exposure; only light source 8 showed growth after 60 minutes of exposure from $2.9*10^5$ to $5.8*10^5$ (almost double) following an initial reduction of living cells. The subsequent drop was probably due to nutrient depletion (the experiment was performed in batches).

The result of the experiment indicates that irradiation of R28 retinal cells (retinal cells of the eye), particularly by electromagnetic radiation of visible light of short wavelengths with a maximum of blue light at 450 nm (light source 5), or a light source with an even shorter maximum wavelength of 420 nm for the purple part of the light spectrum (light source 6) results in cell apoptosis.

The positive effect of photobiomodulation energy of visible radiation at 670 nm contained in light source 8 on vitality of R28 retinal cells was observed at the same time. Thus, it is likely that the white LED light source 8 with a red LED chip with a maximum radiation at 670 nm neutralises the adverse effect of blue light on retinal cells of the eye and has a positive effect on their vitality by its simple use. These findings need to be verified and analysed in detail by further research in order to repeatedly demonstrate the reported processes and describe them in more detail.