

Near-infrared photobiomodulation stimulates viability and cartilage matrix synthesis in human chondrocytes

Supplementary Material

Supplementary Table S1. Reasons for selection of the most promising conditions for further optimization

Experimental conditions with statistically significant differences compared to control	p-value	Selected	Reason for selection
850 nm, 4 mW/cm ² , pulsed, daily	0.001		Selected due to the lowest p-value (0.000) for every other day, indicating a statistically robust improvement in metabolic activity compared to daily application, i.e., the strongest biological response. Although significant, the every-other-day protocol demonstrated superior statistical results compared to daily.
850 nm, 4 mW/cm ² , pulsed, every other day	0.000	x	
940 nm, 4 mW/cm ² , continuous, daily	0.006	x	N/A
850 nm, 7 mW/cm ² , continuous, daily	0.000	x	p-value of continuous mode is lower than pulsed mode with daily application, thus this protocol was selected in continuous mode daily.
850 nm, 7 mW/cm ² , pulsed, daily	0.001		
850 nm, 7 mW/cm ² , pulsed, every other day	0.000		
850 nm, 10 mW/cm ² , continuous, daily	0.000	x	N/A
940 nm, 14 mW/cm ² , pulsed, daily	0.003	x	N/A
810 nm, 17 mW/cm ² , continuous, every other day	0.009	x	N/A
940 nm, 17 mW/cm ² , continuous, daily	0.000	x	Continuous mode yielded better outcomes compared to pulsed mode for the same wavelength since the p-value of continuous mode is lower than pulsed daily.
940 nm, 17 mW/cm ² , pulsed, daily	0.009		

N/A: Not applicable

Supplementary Table S2. Temperature variation, in °C, during PBM for all parameters

Wavelength (nm)											
		600*		655		810**		850**		940**	
		C	P	C	P	C	P	C	P	C	P
Power density (mW/cm²)	4	0.1	0.1	0.2	0.1	0.7	0.3	0.4	0.3	0	0.1
	7	0.2	0.2	0	0.1	0.3	0	0.4	0.3	0.3	0.2
	10	0.2	0.2	0.2	0.1	0.1	0.2	0.1	0.4	0.1	0
	14	0.8	0.3	0.5	0.2	0	0.1	0.6	0.4	0.3	0
	17	1.6	0.9	0.7	0.3	0	0.1	0.7	0.2	0.3	0.1

C: Continuous for 1 min; P: pulsed for 2 min.

*There was a significant temperature rise for 600 nm, being not ideal for clinical applications;

**For most of the experimental conditions, temperature increase is limited to 0.4°C, demonstrating minimal thermal impact and ensuring safe conditions for cellular stimulation.

Supplementary Table S3. Effect of PBM protocols on each biological outcome

PBM protocols	Biological outcomes						
	COL II		ACAN		COL I		GAGs
	WB	Immunostaining	WB	Immunostaining	WB	Immunostaining	Alcian blue
4 mW/cm ² , 850 nm, pulsed, every other day	↓	↑	=	=	↓	↓	=
4 mW/cm ² , 940 nm, continuous, daily	↓	↑	=	↑	=	=	=
7 mW/cm ² , 850 nm, continuous, daily	=	↑	=	↑	=	↓	=
10 mW/cm ² , 850 nm, continuous, daily	=	↓	↑	↑	=	↑	=
17 mW/cm ² , 940 nm, continuous, daily	=	↑	↑	↑	=	=	↑

WB: western blot, ACAN: aggrecan: COL I: collagen type I, COL II: collagen type II, GAGs: glycosaminoglycans

Green ascending arrow indicates significant increase; red descending arrow indicates significant decrease, and equal sign indicates no significant difference compared to control.

Supplementary Figure S1. Uncropped western blots from Figure 6.

